Committee for Risk Assessment

RAC

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

Adopted

8 October 2020

8 October 2020

CLH-O-0000006899-51-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Ammonium bromide**

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

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

The proposal was submitted by **Sweden** and received by RAC on **22 February 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **15 April 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **14 June 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Normunds Kadiķis**

Co-Rapporteur, appointed by RAC: **Ivan Dobrev**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 October 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	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)			
RAC opinion	TBD	Ammonium bromide	235-183-8	12124-97-9	Repr. 1B Lact. Eye Irrit. 2 STOT SE 3 STOT RE 1	H360FD H362 H319 H336 H372 (nervous system)	GHS07 GHS08 Dgr	H360FD H362 H319 H336 H372 (nervous system)			
Resulting Annex VI entry if agreed by COM	TBD	Ammonium bromide	235-183-8	12124-97-9	Repr. 1B Lact. Eye Irrit. 2 STOT SE 3 STOT RE 1	H360FD H362 H319 H336 H372 (nervous system)	GHS07 GHS08 Dgr	H360FD H362 H319 H336 H372 (nervous system)			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Ammonium bromide is a substance with widespread use, registered under REACH at 1000 - 10000 tonnes per annum, with carcinogenic, mutagenic and/or reprotoxic properties and was therefore prioritized for harmonised classification and labelling. Industrial uses identified for ammonium bromide include flame retardants and as an oxidizing/reducing agent (EPA Chemical Data Report). Ammonium bromide has consumer uses in fabrics; textile and leather products, as well as in photographic supplies. Ammonium bromide is also used as a precursor along with sodium hypochlorite 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.

HUMAN HEALTH HAZARD EVALUATION

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 on 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 the 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 the combined sexes. As the LD_{50} values are

above the oral acute toxicity estimate (ATE) 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 reliable study with SD rats performed according to OECD TG 402, in which five male and five female rats were exposed to a 24 h dermal application of 2000 mg/kg bw of ammonium bromide 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 reliable animal study performed according to OECD TG 403 in which five male and five female rats were exposed to aerosolized ammonium bromide (dust) at 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. 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 concentrations of ammonium bromide higher than 0.1 mg/L could not be generated due to rapid sedimentation of the relatively heavy ammonium bromide particles. As 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**.

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, the DS included one oral acute toxicity study on ammonium bromide in rats, one oral acute toxicity study on sodium bromide in rats as well as human studies describing overdosing of bromides, the latter reported as supporting studies in the REACH Registration dossier.

In the oral acute toxicity study on ammonium bromide in rats 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 a recent bromism case in a 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 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).**

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**.

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**.

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**.

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 as STOT RE 2, H373 (nervous system), but disagreed with the inclusion of the thyroid as a target organ.

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

The 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 (F)	0/25 (M) 0/25 (F)	2/15 (M) 0/15 (F)	12/15 (M)	11/15 (F)
Nasal bleeding	0/25 (M) 0/25 (F)	0/25 (M) 0/25 (F)	0/25 (M) 0/25 (F)	5/15 (M)	8/15 (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 ($p \leq 0.01$) 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

Thyroid

RAC considers that the severity of the effects on thyroid is not sufficient to include it 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 Annex I, 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 available human evidence, with the animal studies providing supporting evidence.

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**.

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 due to inconclusive data**.

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, $p \leq 0.01$) and in the percent motile sperm from vas deferens (75.3% of control, $p \leq 0.05$) 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 $\geqslant$ 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 ($p \leq 0.05$); ** Significantly different from control value ($p \leq 0.01$). 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, $p \leq 0.01$).

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%, $p \leq 0.01$). 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 retention			9/23	17/20

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 ($p \leq 0.05$); ** Significantly different from control value ($p \leq 0.01$). 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 as Repr. 1B, H360F is warranted for** fertility and sexual function.

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 (2/22)	2/247 (1/22)	4/188 [2%] (4/22)	9/208 [4%] (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 (0/20)	n.a.	1/213 (1/18)	n.a.	n.a.
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 (↓ 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 (0/23)	1/149 (1/21)	1/154 (1/24)	8/137 (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%, $p \leq 0.01$) 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**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).