Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

3 bromide substances

Sodium bromide	**231-599-9**	**7647-15-6**
Potassium bromide	**231-830-3**	**7758-02-3**
Calcium bromide	**232-164-6**	**7789-41-5**

CLH-O-0000007428-67-01/F

Adopted
14 March 2024

14 March 2024

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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 on **14 March 2024** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

No	Chemical name	EC number	CAS number
1	**Sodium bromide**	**231-599-9**	**7647-15-6**
2	**Potassium bromide**	**231-830-3**	**7758-02-3**
3	**Calcium bromide**	**232-164-6**	**7789-41-5**

Rapporteur, appointed by RAC: **Benjamin Piña**

Administrative information on the opinion

Sweden has submitted on **10 March 2023** a CLH dossier for each of the three substances above containing a proposal together with the justification and background information documented in a CLH report.

The CLH reports were made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 April 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 June 2023**.

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 following tables provide a summary of the Current Annex VI entries, Dossier submitter proposals, RAC opinions and potential Annex VI entries if agreed by the Commission.

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	Sodium bromide	231-599-9	7647-15-6	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372	GHS08 GHS07 Dgr	H360FD H362 H336 H372			Note on additive effects[1]
RAC opinion	TBD	Sodium bromide	231-599-9	7647-15-6	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]
Resulting Annex VI entry if agreed by COM	TBD	Sodium bromide	231-599-9	7647-15-6	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
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Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Potassium bromide	231-830-3	7758-02-3	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372	GHS08 GHS07 Dgr	H360FD H362 H336 H372			Note on additive effects[1]
RAC opinion	TBD	Potassium bromide	231-830-3	7758-02-3	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]
Resulting Annex VI entry if agreed by COM	TBD	Potassium bromide	231-830-3	7758-02-3	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]

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Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Calcium bromide	232-164-6	7789-41-5	Repr. 1B, Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372	GHS08 GHS07 Dgr	H360FD H362 H336 H372			Note on additive effects[1]
RAC opinion	TBD	Calcium bromide	232-164-6	7789-41-5	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]
Resulting Annex VI entry if agreed by COM	TBD	Calcium bromide	232-164-6	7789-41-5	Repr. 1B Lact. STOT SE 3 STOT RE 1	H360FD H362 H336 H372 (nervous system, thyroid)	GHS08 GHS07 Dgr	H360FD H362 H336 H372 (nervous system, thyroid)			Note on additive effects[1]

[1] "The classification of mixtures as reproductive toxicant category 1 is necessary if the sum of the concentrations of individual bromide salts that are classified as category 1 reproductive toxicant in the mixture as placed on the market ≥ 0.3 %"

RAC general comment

The CLH dossiers for calcium bromide, potassium bromide and sodium bromide were submitted and processed at the same time, with identical proposals for classification, as they were essentially based on the same set of data. In fact, these dossiers have been developed based on the CLH dossier for ammonium bromide, which resulted in the harmonized classification as Repr. 1B (H360FD), Lact. (H362), STOT RE 1 (H372, nervous system), STOT SE 3 (H336) and Eye Irrit. 2 (H319) (added to Table 3, Annex VI of the CLP Regulation via Commission Delegated Regulation (EU) 2022/692 of 16 February 2022). Therefore, the first step on the analysis was to conclude on the applicability of read across between the different bromide salts. Ammonium, sodium, potassium and calcium bromide salts are ionic compounds which readily dissociate in water to their constitutive ions. Sodium, potassium, and calcium cations do not present any additional hazard, at least at toxicologically relevant concentrations, so all toxicity analysis should focus on the bromide anion. In contrast, ammonium salts do represent some intrinsic hazards irrespectively the counter anion; for example, ammonium chloride is classified as Acute Tox. 4 (H302) and Eye Irrit. 2 (H319). This is likely the reason for the no self-classification of sodium, potassium and calcium bromide salts as eye irritants, as the read across from ammonium bromide is presumably not justified for this particular hazard class. However, acute toxicity and serious eye damage/eye irritation were not under the scope of the current CLH proposals and RAC opinions for calcium bromide, potassium bromide and sodium bromide.

Chloride, bromide, and iodine anions belong to the same group 7A of the periodic table, and therefore sharing multiple physicochemical properties. Bromide cation lies between the other two in terms of both ionic radius (ascendent order) and electronegativity (descendent order). These characteristics are likely at the root of its toxic mode of action, as it is able to interact with both cellular chlorine and iodine transporters, and therefore, to alter biological functions that rely on them. Following this theoretical deduction, the nervous and the neuroendocrine systems should be considered as putative targets for the disruption of the chloride-based cellular signalling, whereas the thyroid system could be in principle affected by the potential disruption of iodine uptake and signalling by bromide anions. Both systems, together with other chlorine ion-related signalling mechanisms, are also involved in gonad regulation and embryo development and relevant for the reproductive toxicity hazard class that is under the scope of this CLH dossier and RAC assessment.

Bromide salts (mainly sodium and lithium) were clinically used for decades as sedatives and to treat epilepsy, until the development of more selective and efficient medication. There is also a rich medical literature on bromide intoxication, a condition termed "bromism" that includes symptoms from sedation, rashes, and other neurological and dermal effects to coma. As these symptoms usually appear in patients with multiple treatments and neurological syndromes, in many cases it was difficult to link them exclusively to bromide intoxication.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Bromine sedatives have known acute sedating effects in humans. Several case studies of bromide poisoning, resulting in central nervous system depression in mothers and new-borns were presented in the CLH report under 10.10.5 and 10.10.8, respectively (Finken and Robertsson, 1963; Mangurten and Ban, 1974; Pleasure and Blackburn, 1975; Mangurten and Kaye, 1982; Lugassy and Nelson, 2009; Tyson et al. 1938). Neurological symptoms reported in the mothers included delirium, semiconsciousness, and hyperreflexia. According to the DS, the acute effects of sedatives and early manifestation of central nervous system depression in the human case studies warranted classification in STOT SE 3, H336.

A variety of narcotic effects were observed (including lethargy, ataxia and decreased activity) in test guideline studies of acute toxicity of calcium bromide, ammonium bromide, potassium bromide and sodium bromide in rats. These effects occurred from the lowest oral doses (2000 mg/kg bw) tested. These effects were deemed to be transient in nature. The oral LD_{50} for the different bromide salts ranged from 2210 to >5000 mg/kg bw. Transient narcotic effects observed in the oral acute toxicity studies of bromide salts were considered to support classification in STOT SE Category 3, H336.

Furthermore, the DS noted that in their evaluation of the CLH proposal for ammonium bromide, RAC had previously concluded that the central nervous system (CNS) depressing effects in humans after a single exposure were transient and justified classification as STOT SE 3, H336. This was further supported by the narcotic effects observed in the oral acute toxicity study of ammonium bromide in rats (ECHA, 2020).

Based on evidence of acute narcotic effects in humans, and with support of transient narcotic effects reported in several acute toxicity studies in rats, classification in STOT SE 3, H336 (May cause drowsiness or dizziness) is proposed by the DS.

Comments received during consultation

Two identical comments were received (2 MSCA) for calcium bromide, potassium bromide and sodium bromide. Both supported the classification for STOT SE 3, H336.

Assessment and comparison with the classification criteria

RAC considered that criteria for Categories 1 and 2 were not fulfilled, as transient depression of nervous system is covered by STOT SE 3, H336, and because there were no other effects in humans or in animals that fulfil the requirement for 'significant' or 'severe' toxicity after a single dose. Results from animal experiments (rats) by oral gavage showed relevant, transient effects indicative of nervous system depression (decreased activity, decreased muscle tone, decreased reflexes, ataxia, lacrimation, piloerection, tip toe gait) only at concentrations (2000-4000 mg/kg bw) close to the experimental LD_{50} (2210 to >5000 mg/kg bw) (Study report, 1996; Study report, 1980; Study report, 1988; Study report, 1992; Study report, 1994). One of the two inhalation studies showed transient lethargy at 203.3 mg/L, with complete recovery at 8 days post-

exposure, and no relevant general toxicity (Study report, 1978d). The other inhalation study (Study report, 1978c) found no signs of toxicity at 240 mg/L (aerosol, 1h exposure in both cases).

The most relevant information for classification as narcotic effects is the clinical data derived from the (former) use of bromides as sedatives. Overdosage with bromides causes slurring of speech, unsteadiness, depression or stupor, and psychosis. Delirium, transitory schizophrenia and hallucinations are characteristic (Grant, 1974). As these clinical effects are transient, RAC concludes that **STOT SE 3, H336** (May cause drowsiness or dizziness) is warranted for calcium bromide, potassium bromide and sodium bromide.

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

Summary of the Dossier Submitter's proposal

Nervous system

Long-term intake of bromide salts is known to elicit a defined condition termed bromism. Symptoms of bromism include neurological depression, tremor, ptosis, hypotonia, alteration of deep tendon reflexes and behavioural effects, and they occur in both adults and new-borns (Finken and Robertsson, 1963; Mangurten and Ban, 1974; Pleasure and Blackburn, 1975; Mangurten and Kaye, 1982; Lugassy and Nelson, 2009; Tsutaoka et al. 2004; Yu et al. 2004; Tyson et al., 1938).

Experimental repeated administration of bromide to volunteers resulted in noticeable, but mild alteration in electroencephalogram (EEG) and other minor symptoms of neurotoxicity (Sangster et al., 1983; Van Gelderen et al., 1993). These effects were considered as not adverse by the European Medicines Agency. The DS considered them as supportive information for STOT RE classification with the nervous system as target organ after repeated exposure.

Administration of bromide salts to experimental animals (rats, dogs, mice, rabbits) resulted in a depressive effect on the CNS by bromide and formed the principal basis for the former use of bromide compounds as antidepressants and anticonvulsants in humans and their sustained application for the treatment of seizures in dogs. The underlying mechanism of the anticonvulsant and antidepressant activity of bromide is hypothesised to be due to the disturbance of the active as well as the passive transport of chloride across nerve cell membranes, leading to hyperpolarisation of these cells (e.g. Van Leeuwen and Sangster, 1987).

However, the relevance of the neurofunctional changes in the animal studies was considered unclear due to the presence of general toxicity in some cases, and due to the absence of neuropathological changes. Nevertheless, the effects (clinical signs of depression of the central nervous system, neurofunctional effects and neurobehavioural effects including effects on motor activity) were considered clear and consistent and they followed a dose-response curve when increasing exposure and duration.

The following neurotoxic effects were detected within the guidance value (GV) range for STOT RE in animal studies after repeated dosing:

- Slight limpness in three males (of these, only one showed the finding on more than one occasion) in a 90-day study in rats at 100 mg/kg bw/day ammonium bromide (Study report, 2000a).

- Decrease in evasion time in mice at a dose level of 240 mg/kg bw/day sodium bromide after a 36-day exposure (Hansen and Hübner, 1983).
- Ataxia, shivering and coma in dogs at the dose level of 200 mg/kg bw/day sodium bromide after a 42-day exposure (Rosenblum, 1958).
- Staggering, rolling gait, and subdued behaviour in rats at 600 mg/kg bw/day ammonium bromide after a 13-day exposure accompanied by 1/22 deaths (Study report, 2007).

In conclusion, the available human data on bromism after repeated exposure to bromide salts was considered to provide evidence of significant effects on the nervous system and justify classification in STOT RE 1 with the nervous system as the target organ. In addition, the effects on the nervous system in animal studies were considered as supportive evidence to this classification. Although the available animal studies were not considered to warrant STOT RE 1 on their own, the clear evidence of neurotoxic effects from human data, regardless of the dose, predominates over animal data (CLP Annex I, 3.9.2.10.2) and was considered by the DS to justify STOT RE 1.

Thyroid

Guideline studies on sodium bromide in rats showed statistically significant effects either on thyroid hormone levels (Study report, 2016b) or thyroid weights (Study report, 2016a), starting from 175 mg/kg bw/day. However, these effective doses were above the guidance value range for STOT RE 2 and they did not correlate with histopathological findings. A third guideline study at doses up to 500/750 mg/kg bw/day of ammonium bromide did not show any effect on thyroid parameters (Study report, 2000a). In contrast, older studies on potassium bromide and sodium bromide did show significant changes at dose levels within the guidance value ranges for classification for Category 1 (Velický et al., 1997a; Velický et al., 1998) and 2 (Loeber et al., 1983), respectively.

According to the DS, the relevance of the thyroid effects in rats for human health was dubious, as no severe thyroid effects were linked to repeated administration of bromide in humans (Sangster et al., 1983) or dogs (Paull et al., 2003). Furthermore, and based on the same studies presented above, the DS noted that RAC had previously concluded not to have thyroid as the target organ for ammonium bromide, as the severity of effects was considered not to fulfil the CLP criteria for STOT RE at that time (ECHA, 2020).

Adrenals

Short term toxicity studies on bromides in rats showed different mild effects on the adrenal gland at low dose levels. In a 90-day study, histopathological changes (decreased vacuolisation of zona fasciculate in both sexes) from a dose level of 6.75 mg sodium bromide/kg bw/day (corresponding to 4.4 mg bromide/kg bw/day) were reported (Van Logten et al., 1974). In another 90-day study in rats decreased vacuolisation of the zona fasciculate in both sexes was reported from 45 mg sodium bromide/kg bw/day (Van Logten et al., 1976). At 108 mg/kg bw/day in a 90-day study in rats (Van Logten et al., 1974) the effects on the adrenals were considered significant/severe, but the lack of comparable effects in both humans and dogs brought the DS to consider these findings as non-relevant for human health. Glucocorticoid levels were reduced in a 4-12 week study with sodium bromide in rats, but only at the top dose (19200 mg/kg diet, corresponding to 1728 mg/kg bw/day) (Loeber et al., 1983).

Conclusion of the DS

Based in human and animal data, classification as STOT RE 1; H372 (nervous system) was proposed by the DS for calcium bromide, potassium bromide and sodium bromide.

The DS also concluded that there was no need for setting a specific concentration limit.

Comments received during consultation

Three identical comments were received for calcium bromide, potassium bromide and sodium bromide. One was supportive (MSCA Germany), a second one (MSCA France) supportive but requesting a clarification for not including thyroid as a target organ, and a third one from the International Bromine Council BSEF (Austria) stated that the doses inducing neurotoxicity were too high for being considered for classification in STOT RE 1.

Regarding the comment to include thyroid as a target organ, the DS replied by referring to the former RAC opinion on ammonium bromide (ECHA, 2020).

The DS replied to the International Bromine Council BSEF comment stating that cut off limits for STOT RE are not applicable to human data, and cited RAC opinion on ammonium bromide (ECHA, 2020).

Assessment and comparison with the classification criteria

Nervous system

Clinical data in humans is considered by RAC to justify the classification for STOT RE 1, H372 with nervous system as a target organ for calcium bromide, potassium bromide and sodium bromide based on the well stablished description of the bromism syndrome in humans associated with nervous system impairment after repeated exposure.

Thyroid

Studies in rats reported statistically significant effects either on thyroid hormone (TH) levels or thyroid weights, at doses within GV range for STOT RE 2 or STOT RE 1 classification (table below). Statistically significant decreases in TH were recorded in all studies in which they were measured. The decrease was dose-dependent in all these studies except in Loeber et al. (1983). In the only test guideline study in which TH levels were measured, a significant (and dose dependant) decrease of TH was found within GV for STOT RE 2. In addition, histopathological changes in the thyroid (mild/moderate depletion of colloid in the thyroid) were reported in this study, but these occurred above GV range for classification. In Velicky et al. (1997a and 1998), TH effects were concomitant with histopathological findings (including marked growth activation of the follicular epithelial component, frequent mitoses in the follicular cells, microfollicular tissue rearrangement, lowering in the portion of colloid in the thyroid tissue, slight to moderate thyroglobulin-positivity of colloid tissue) within GV range for STOT RE 1 (Velicky et al, 1997a and 1998). In Velicky et al. (2004), the most important finding in the cytoplasm of thyrocytes was the hypertrophy and hyperplasia of the endoplasmic reticulum, combined with dilated cisterns and tubules not only in the central and basal but also in the apical cytoplasm, where, in addition, the dilated cisterns were often of ovoid shape. In Loeber et al. (1983), Van Leeuwen et al. (1988) and Buchberger et al. (1990) increases in thyroid weight were reported in addition to the changes in TH levels although the evidence for these findings was less robust (due to e.g. no dose dependency or only one dose being tested). Several workshop members of the 6th European Society of Toxicologic Pathology (ESTP) International Expert Workshop on thyroid alterations noted that colloid alteration did not necessarily reflect thyroid dysfunction, considering the widespread occurrence of this change in healthy control rat, but that the overall spectrum of findings was considered to provide important context for adversity decisions (Huisinga et al., 2020).

Table: *Summary of thyroid effects in rats. LOEC (lowest effect concentration) for the tested bromide salt and GV values as mg/kg bw/day*

Reference	TG	Effects	LOEC	days	GV STOT-RE1	GV STOT-RE2
Study report, 2016a	Yes	low thyroid/parathyroid weight	175	181-186	5	50
Study report, 2016b	Yes	high thyroid weight	500	90	10	100
Study report, 2016b	Yes	↓ T3 in males (27%, p≤ 0.05), ↓ T4 in females (26%, p≤ 0.05), ↑ mean TSH in females (60%)	60	90	10	100
Buchberger et.al., 1990	No	low T4, T3, rT3, high thyroid weight	200	28	30	300
Loeber et al., 1983	No	108 mg/kg bw/day ↑ thyroid weight (38%, p<0.01 at 4 weeks), ↓ T4 level (23%, p<0.01 at 4 weeks)	108	28/90	30/10	300/100
Van Leeuwen et al., 1983	No	↓ thyroxine (T4) serum concentrations in F0 males: approx. 10-16% at 6.25 and 27 mg/kg bw/day, 25% at 108 mg/kg bw/day	6.75	90	10	100
Van Leeuwen et. al, 1988	No	low T4, high TSH, thyroid weight	950	14	60	600
Van Logten et al., 1976	No	thyroid activity	45	90	10	100
Van Logten et al., 1974	No	↑ relative thyroid weight (32%, p≤0.01)	108	90	10	100
Velický et al., 1997a	No	thyroid histopathological changes, low T4	0.5	16/66	60/20	60/200
Velický et al., 1997b	No	thyroid histopathological changes	0.5	16/66/133	60/20/6.8	600/200/68
Velický et al., 2004	No	thyroid histopathological changes	0.5	16/66/133	60/20/6.8	600/200/68
Velický, 1998	No	thyroid histopathological changes, low T4	3.3-5	133	10	100

According to the CLH report, Velicky et al. (2004) suggested that the changes caused by bromide treatment of rats in the thyrocytes point to a defect in transport and probably also synthesis of thyroidal hormones caused by increased bromide levels which inhibit active absorption of iodide by the thyroid, whereas Van Leeuwen et al. (1983) concluded that bromide not only inhibits the uptake of iodide by the thyroid gland but also inactivates thyroid peroxidase, thus inhibiting iodide incorporation in tyrosine residues and coupling of iodotyrosine residues to iodothyronine. The modes of actions initiated by competition for the Na+/I− symporter (NIS) and decrease of thyroid peroxidase (TPO) activity are considered relevant to human.

The thyroid effects in rats were considered to represent significant toxic effects and relevant to human health, even though no severe thyroid effects were reported after repeated administration of bromide in humans (Sangster et al., 1983) or dogs (Paull et al., 2003). However, the highest exposure reported in human was 9 mg/kg bw/d whereas in guideline studies the dose causing TH decrease in rats was 60 mg/kg bw/d. Despite the low exposure levels in humans, some effects on TH could be detected at 9 mg/kg bw/d in Sangster et al. (1983): "*At 9 mg/kg bw/day, a slight but significant increase in T4 and T3 in females only (individual concentrations of T4 and T3 in this group within normal limits at the start and the end of the investigation)*". It was also unclear to what extent thyroid effects (including hormone measurements) had been monitored in humans despite the wide use of bromides as therapeutic agents. In addition, humans were exposed to relatively low concentrations, and it must be stressed that hazard classification does not take exposure scenarios into consideration unlike risk assessment. According to the CLP guidance: "*If there is human data indicating no classification but there is also non-human data indicating classification then the classification is based on the non-human data* ***unless*** *it is shown that the human data cover the exposure range of the non-human data and that the non-human data are not relevant for humans*."

RAC also considers that the lack of reported effects in dog (decrease in serum total thyroxine and free thyroxine over time were reported but according to the CLH dossier these values were within the reference ranges and observed both in control and treated animals and therefore not clearly attributable to treatment) does not overrule the relevance of the findings in rats. Only a limited number of animals was investigated in the dog study (Paull et al., 2003). RAC notes that also reversible significant health effects should be considered for STOT RE classification according to the CLP Regulation: "*Annex I: 3.9.1.1. Specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included*."

RAC concludes that thyroid is considered as a STOT RE target organ for calcium bromide, potassium bromide and sodium bromide as the combined decrease in T3 and T4 hormones and the increase in TRH indicates a negative effect to the whole thyroid system that represent a significant toxic effect of relevance to human health. Histopathological findings were not considered determinants for classification, as they can be regarded as consistent with the natural variation of the thyroid gland. However, the observed histopathological findings in thyroid and effects on thyroid weight contributed to the weight of evidence assessment as a further indication of the general alteration of the thyroid by bromide salts that supports classification. It is also noted that according to Noyes (2019), the molecular initiating events in adverse outcome pathway (AOP) network for chemically induced thyroid activity include inhibition of NIS and TPO and lead to learning and memory deficits, hearing loss and visual dysfunction as adverse outcomes via TH decrease as a key event. Histopathological changes in thyroid are not included as the key events in the AOPs leading to such neurological adverse outcomes.

Once all these considerations are taken into account, RAC concludes that **STOT RE 2, H372** classification for thyroid as a target organ is warranted for calcium bromide, potassium bromide and sodium bromide.

Adrenals

Regarding effects on adrenals, decreased vacuolisation of zona fasciculate occurred within GV range for STOT RE 1 in Van Logten et al. (1974) and STOT RE 2 in Van Logten et al. (1976). However, these alterations did not result in changes in glucocorticoid levels, which only occurred at doses considerably above GV limits (at 1728 mg/kg bw/day) (Loeber et al., 1983), and therefore were not considered relevant for classification.

RAC conclusion on classification

RAC concludes that **classification STOT RE 1, H371 (nervous system) is warranted** based on human data, supported by animal data. In addition, **STOT RE 2, H372 (thyroid)** is considered warranted, based mainly on animal data on thyroid hormone effects, but supported by histopathological findings in thyroid and effects on thyroid weight.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

There were no human data on the effects of bromine on 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 summarised in the CLH report.

The DS concluded that there were effects on sexual function and fertility that were severe, dose related, and not solely secondary consequences of general systemic toxicity. At the mid dose of the two-generation study (Study report, 2016a) decreases in fertility by approximately 73-74% of control values in the P-generation in both pairings were reported; of the five P0 females that were not pregnant at either pairing, two had no corpora lutea; an increase in the incidence of spermatid retention in P0 was observed; minimal/mild spermatid head retention or irregularity of oestrous cycle/differences in follicle counts in F1 were recorded. At the mid dose of the 90-day oral repeated dose toxicity study on sodium bromide (Study report 2016b), the number of sperm with detached/no head and the percent abnormal sperm was slightly (and statistically significantly) increased compared to control. In addition, the depletion of corpora lutea in top dose females was considered a substance related effect that was not secondary to general toxicity. In the 90-day oral repeated dose toxicity study (Van Logten et al., 1974), the reduced secretory activity of prostate, decreased spermatogenesis, decreased number of corpora lutea and reduced size of tubuli were observed at the top dose of 1728 mg/kg bw/day in the absence of severe general toxicity, but due to high dose level they were considered as supportive evidence for classification. The slightly reduced fertility index in the mid dose group and the markedly decreased fertility index in high dose group were not considered as being secondary to general toxicity in dose-range finding study for reproductive toxicity of ammonium bromide in rat (Study report, 2001). The reductions of weights of epididymis and testis in the oral 90-day repeated dose toxicity study of ammonium bromide in rat (Study report, 2000a).

The available data was considered to provide clear evidence of an adverse effect on both male and female sexual function and fertility. There was no mechanistic evidence to indicate that the observed effects were not relevant for humans. Therefore, classification in Repr. 1B, H360F was considered warranted for calcium bromide, potassium bromide and sodium bromide.

In conclusion, available data was considered to provide clear evidence of an adverse effect on both male and female sexual function and fertility. There was no mechanistic evidence to indicate that the observed effects were not relevant for humans. Therefore, classification in Repr. 1B, H360F was considered warranted for calcium bromide, potassium bromide and sodium bromide.

Development

The DS summarised human case reports indicating developmental growth retardation (height, weight and skull circumference) in infants exposed to bromide during the entire pregnancy. In addition, there were several case studies of prenatally exposed infants showing hyporeflexia, hypoactivity, and weak cry, suck and grasp. Similar effects were also observed in a 22-day-old infant who had been given a bromide-containing medicine for about two weeks. These human studies were either considered as not robustly linked to the bromine maternal ingestion or not severe enough to constitute an adverse effect on human development by the DS. All these perinatal bromism cases were associated to multiple treatments and neurological syndromes, and it was considered difficult to link them exclusively to bromide intoxication.

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 summarised in the CLH report. Two developmental neurotoxicity studies on sodium bromide in rat were considered as well.

In the pre-natal developmental study of sodium bromide in rat, skeletal malformations (ribs) were recorded at higher doses (1000 mg/kg bw/day) with maternal toxicity (clinical sign of neurotoxicity) and skeletal anomalies (ribs, cranial centres and sternebrae) were recorded at lower doses (300 mg/kg bw/day) without maternal toxicity. These skeletal abnormalities were considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female. In addition, a dose-dependent increase in incidences of skeletal abnormalities and variants (kinked ribs, curved scapulae and incomplete ossification of ribs) were observed at lower dose levels (from 100 mg/kg bw/day) without associated reductions in foetal weights and without maternal toxicity in two studies of ammonium bromide in rat. A statistically significant increase in incidences of visceral malformations (reduction or absence) were seen at a dose level of 1000 mg/kg bw/day in studies of sodium bromide and ammonium bromide in rats. These defects observed in the urogenital system, uterine, spleen and thyroid at a high dose level in two studies were considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female. A dose-related increased incidence of displaced testis was noted at 100 and 300 mg/kg bw/day (dose levels without maternal toxicity) and 1000 mg/kg bw/day (dose level with maternal toxicity) in the pre-natal developmental toxicity study of ammonium bromide in rat. In the two-generation reproductive toxicity study of sodium bromide at 500 mg/kg/day, the number of P generation dams with stillborn pups and number of dams with all pups dying before day 4 postpartum were significantly increased ($p \leq 0.01$). Delivered litter size, surviving pups per litter and live litter size were also reduced and the number of liveborn pups was reduced ($p \leq 0.01$). However, in this dose group severe generalised toxicity was evident. Effects on the pup viability were also seen

in the three-generation reproduction toxicity study of sodium bromide where both the viability index and lactation index of the F1 pups in the 432 mg/kg bw/day dose group was significantly reduced in the first litter compared to control but survival was shown to be greater in the second litter when compared to the first litter. Maternal body weights were not affected at these dose levels but the clinical condition of the dams was not reported in this study and therefore these findings are difficult to evaluate. A dose-related increase in pup mortality was noted starting from a dose level of 40 mg/kg bw/day (31 mg bromide/kg bw/day) in the pre-natal developmental toxicity study of sodium bromide in rat (Harned et al., 1944). However, pup-killing/cannibalism of the pups by the dams (unclear to what extent) were also indicated in the 80 and 120 mg/kg bw/day dose groups. Maternal body weights and the clinical condition of the dams were not reported in this study and therefore the observed pup mortality cannot be concluded as not being a secondary consequence of maternal toxicity. Marked effects on rat pup viability and survival were seen in the dose range finding reproductive toxicity study of ammonium bromide at doses (454 and 651 mg/kg bw/day) where moderate (454 mg/kg bw/day) and severe (651 mg/kg bw/day) maternal toxicity (clinical signs of neurotoxicity) was also seen. At 651 mg/kg bw/day there was only one litter produced and all pups were dead before day 4 of lactation, and at 454 mg/kg bw/day all pups in 4 out of 9 litters died before day 21 of lactation (found dead days 4 and 14) and included one litter where all pups were born dead. It was considered not clear from this study if the decreased pup viability during lactation at 454 mg/kg bw/day were due to poor maternal care because of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups. Moreover, since both the viability index and the survival index were unusually low in the control group the significance of the findings is difficult to evaluate. A reduction (>10%) of mean litter weights (from lactation day 1) and female pup weights (from lactation day 7) without any effect on maternal body weight at 454 mg/kg bw/day were reported in the dose range finding reproduction toxicity study of ammonium bromide (Study report, 2001). However, it was considered not clear from this study if the decreased mean litter/pup weights during lactation at 454 mg/kg bw/day were due to poor maternal care because of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups.

Based on the overall weight of evidence, the DS considered that there was clear evidence of structural abnormalities and some evidence of both increased mortality and retarded growth. Moreover, the recorded effects were considered relevant for humans, and were not considered to be secondary to maternal toxicity. Therefore, the DS proposed classification as Repr. 1B, H360D for calcium bromide, potassium bromide and sodium bromide.

Lactation

In humans, maternal intake of sodium bromide of 5.4 g/day for 3-5 days (beginning on the sixth day following delivery) resulted in irritability, drowsiness, sleepiness, absence of cry and rash on face in the babies (Tyson et al., 1938). These effects were attributed to alterations in the maternal milk due to the bromide administration.

A two-generation study with sodium bromide or the dose range finding studies with ammonium or sodium bromide showed no clear evidence of adverse effects in the offsprings due to transfer in the milk or adverse effect on the quality of the milk.

The available non-guideline studies with sodium or potassium bromide, (Vobecký et al, 2005; Pavelka et al., 2002) showed that bromide could be transferred via mothers' milk to their pups. The milk production was decreased and the elementary composition of the milk was changed (about 54% of the chloride in mother´s milk was replaced by bromide) in dams administered 900 mg bromide/kg bw/day during lactation (Vobecký et al, 2005). In the same dose group, the reduced milk production in dams resulted in a state of malnutrition and lowered viability in pups.

The DS concluded that the available evidence indicated that bromide may cause harm in rats due to its effects on and via lactation. In addition, The DS considered there was a weak indication from a human case report on possible effects on the central nervous system of the infant/child after maternal intake of sodium bromide during lactation. Thus, in an overall weight of evidence assessment, classification for effects on or via lactation, Lact., H362 was considered justified. Finally, the DS noted that RAC had previously concluded that classification for effects on or via lactation for ammonium bromide was warranted based on the same data.

The overall DS conclusion on classification for reproductive toxicity

Considering all available evidence, the DS proposed to classify calcium bromide, potassium bromide and sodium bromide for adverse effects on sexual function and fertility, adverse effects on development, and adverse effects on or via lactation as Repr. 1B, H360FD and Lact., H362

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

Comments received during consultation

The dossiers for calcium bromide, potassium bromide and sodium bromide received identical comments during the consultation.

Two MSCAs provided supportive comments. The latter required some further clarification about the calculation of ED10, which was provided by DS.

A governmental organisation noted that malformations were reported at doses that also caused maternal toxicity in many studies and wondered about the impact of maternal toxicity on the relevance of the malformations for the proposed classification. In their reply, the DS pointed out that several other reports described different malformations at concentrations at which little or no maternal toxicity was observed. The DS also referred to the ammonium bromide RAC opinion (2020). RAC supports the DS's reply about the sufficient data showing clear dose-related toxic effects in both sexual function and fertility and development at concentrations with low or no maternal toxicity.

Industry produced a very exhaustive comment indicating weak points in most studies proposed to substantiate the classification for reproductive toxicity. In addition, they argued that fertility and developmental toxicity occurred at doses too high to be considered for classification and, in any case, that toxicity occurred in concurrence with (and, arguably, motivated by) maternal toxicity. The DS replied that there were sufficiently GLP, well-conducted experiments to support the proposed classification. RAC supports the DS's reply and agrees that the evidence is sufficient to warrant the Repr. 1B, H360FD classification (see next section).

In the same comment, industry pointed out that "*There are no robust clinical data on effects of bromide in human infants*", and that therefore there is no proof that rat reprotoxic effects could be extrapolated to humans. The DS replied that human cases were taken as supportive, not as determinant for the classification. RAC agrees with DS's analysis and noted that the absence of adverse effects in human does not contradict finding in animals.

Assessment and comparison with the classification criteria

Summary of studies on sexual function and fertility

There were no human data on the effects of bromine on sexual function and fertility.

Three guideline studies with bromide salts were available and assessed for sexual function and fertility: a two-generation reproductive toxicity study (Study report, 2016a) and a 90-day repeated dose toxicity study (Study report, 2016b) with sodium bromide and an oral 90-day repeated dose toxicity study with ammonium bromide (Study report, 2000a). In addition, there were five relevant non-guideline studies; a three-generation reproductive toxicity study (Van Leeuwen et al, 1983) and two older 90-day repeated dose toxicity studies (Van Logten, 1974 and 1976) with sodium bromide, and a dose-range finding reproductive toxicity study (Study report, 2001) and a four-week dose-range finding study for oral repeated dose toxicity (Study report, 1999) with ammonium bromide. Rat studies with sodium and ammonium bromide provided evidence for impaired sexual function and fertility.

The OECD TG 416 on *sodium* bromide (Study report, 2016a)

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 prior to mating, 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 the P generation was approximately 183 days, whereas the 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. Four males and nine females died or were terminated earlier resulting in mortality rates of > 10%. 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.

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 ($p \leq 0.05$).

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 statistically significantly lower than control values ($p \leq 0.01$-0.05). In high dose females, the fertility index was 60% (6/10) for females mated with treated males ($p \leq 0.01$), 90% (9/10) for females mated with untreated males and 75% (15/29) including both treated and untreated males, compared to 100% in control females.

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 were paired with untreated males compared to treated males paired with untreated females (90% versus 60%) suggested 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%.

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 were 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 in ovary weight was noted, expressed both as absolute and relative to body/brain weight 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 organ weights to terminal body weight values were comparable or higher than control, while organ weights 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.

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. 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 was noted at 175 mg/kg bw/day (2.7 vs 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, neither in absolute nor 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 could 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 could not 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. 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.

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

A 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).

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 was 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 weights 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 39 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 of 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.

<u>Oral 90-day repeated dose toxicity study on ammonium bromide in rats (Study report, 2000a)</u>

In an oral (feed) repeated dose toxicity study (OECD TG 408) on ammonium bromide in rat, 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 at the end 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.

At 500 mg/kg bw/day, there was a slight increase in body weight gain to day 6; from day 6-13 body weight gain was similar to control, but by day 28 mean body weights were lower than control, with differences from day 35 attaining statistical significance (-23% on day at the end 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.

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 was 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. Since no clinical observations were reported, and information on body weight changes at the top dose was lacking, the relevance of these findings for classification was questionable. Some statistically significant changes in the relative weights of uterus, ovary, testis, pituitary and adrenals were reported, however without consistency and dose relation.

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.

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.

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

In the dose-range finding study for reproduction toxicity, 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 (fertility index 10% in both sexes), and none of the pups of the single litter produced survived to day 4 of lactation. 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. Fertility index at was 80% at 3200 ppm in males and 90% in females. Individual body weights of the dams losing their litters were not severely affected.

Dose-range finding study for a 90-day oral repeated dose toxicity of ammonium bromide in rats (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) were 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.

RAC assessment and conclusion on sexual function and fertility

There is no human data investigating effects on sexual function and fertility and thus classification as Repr. 1A, H360F is not warranted.

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.

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 (Study report, 2001). 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 (Study report 2016), 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 (Van Leeuwen et al., 1983) 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 (Study report 2016), 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 (Study report 2016b) 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) (Van Logten et al., 1974). In the two-generation study on sodium bromide (Study report 2016), 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. In the two-generation study on sodium bromide (Study report 2016), 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 (Study report 2016b). 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) (Van Logten et al., 1974), statistically significant reduction in the adjusted prostate weight (33-50%) and

secretory activity was recorded at doses starting from 432 mg/kg bw/day. Effects on testes (decreased spermatogenesis, reduction of tubules, decreased serum testosterone) and reduced epididymides weight were observed at very high doses (1728 mg/kg bw/day) and these effects are regarded only as supportive evidence. In a weight of evidence approach, RAC considers that there is clear evidence of an adverse effect on both male and female sexual function and fertility, also in the absence of severe general toxicity. No mechanistic information is available to indicate that the observed effects are not relevant for humans.

RAC concludes that **classification in Repr. 1B, H360F is warranted** for calcium bromide, potassium bromide and sodium bromide.

Developmental toxicity

Summary of studies on developmental toxicity

Bromine anions are able to cross the human placenta and accumulate in the foetus (Pleasure and Blackburn, 1975; Mangurten and Ban, 1974; Finken and Robertsson, 1963). Several reports described symptoms of neonatal bromism in prenatally exposed infants. In Finken and Robertsson (1963) and Pleasure and Blackburn (1975), these infants were postnatally treated with extra salt or by intravenous antibiotics, salt and glucose in an attempt to hasten renal excretion of bromide. In Finken and Robertsson (1963), the serum bromide concentration was reported to persist in the "potentially lethal" range until the eighth day of life. The reversible effects (by 1-5 months of age) in these children included the absence or poor reflexes, marked hypoactivity, and weak cry, suck and grasp (Finken and Robertsson, 1963; Pleasure and Blackburn, 1975; Mangurten and Ban, 1974, Mangurten and Kaye, 1982). Similar effects (lethargy, hypotonia, slightly diminished reflexes, slight response to peripheral intravenous catheter placement and no crying during examination) were also observed in a 22-day-old infant who had been given bromide-containing medicine for about two weeks. The girl was hydrated with chloride-containing i.v. fluids, and there were no remaining symptoms reported three months later (Lugassy and Nelson, 2009). However, one of the case studies did report (in addition to reversible neurotoxic effects) severe, apparently irreversible developmental effects in the form of dysmorphic facial features (head which appeared large in relationship to the trunk, frontal bossing, broadening of the nasal bridge and a dull fixed facial expression) suggesting a possible teratogenic effect derived from maternal bromide-exposure during the pregnancy. In this child, there was also still some residual mild hypotonia of the neck musculature at 9½ months, and it was not reported if this effect was eventually reversible or not. In addition to being exposed to photographic chemicals, containing sodium and potassium bromide amongst others from the beginning of the pregnancy, the mother of this child had had two episodes of respiratory infection during pregnancy, both accompanied by fever to 39°C, and she had smoked approximately one package of cigarettes per day, with no alcohol intake (Mangurten and Kaye, 1982). In another case study, an extremely severe case (microcephalia and congenital heart disease) was reported (together with growth retardation still evident at age of 7 – 8 years) in a child coincidental with a period of maternal abuse of bromide medication during pregnancy. The other child also born during the bromide abuse period of this mother also showed similar growth retardation as his brother. Other two children born prior to mother's bromide abuse and the one born after the maternal bromide abuse had halted were of normal health (Opitz et al., 1972). In a third case report, a child prenatally exposed to bromide-containing medication of the mother during the entire pregnancy showed growth retardation, extreme irritability, a high-pitched cry and being very difficult to feed until 9 weeks of age after which the child started to improve. At the age of 2.5 years, the baby had only just started to walk, had very brisk knee and adductor jerks, and had not spoken. No follow-up was reported (Rossiter and Rendle-Short, 1972).

Available guideline studies of developmental toxicity of bromide salts were two prenatal developmental toxicity studies (OECD TG 414) on sodium bromide in rabbits (Study report, 2008b) and rats (Study report, 1995) and two prenatal developmental toxicity studies (OECD TG 414) on ammonium bromide in rats (Study report, 2000b, Study report, 2007a). Moreover, there was one two-generation reproductive toxicity study (OECD TG 416) on sodium bromide in rats. Available non-guideline studies relevant for developmental toxicity were one multi-generation reproductive toxicity study on sodium bromide in rats (Van Leeuwen et al., 1983), one dose-range finding study for reproductive toxicity on ammonium bromide in rats (Study report, 2001) and one dose range finding study on sodium bromide in rabbits (Study report, 2008a). Finally, there were two non-guideline studies of developmental neurotoxicity on sodium bromide in rats.

Prenatal developmental toxicity study of sodium bromide in rabbits (Study report, 2008b)

In a prenatal 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 gestation day (GD) 6-28. 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. 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. There was no maternal toxicity in this study and no significant developmental effects warranting classification according to DS.

Prenatal developmental toxicity study of sodium bromide in rats (Study report, 1995)

In a prenatal developmental toxicity study (OECD TG 414), 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 GD 11. 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.

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. 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.

Prenatal developmental toxicity study on ammonium bromide in rats (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. 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.

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). 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.

The major malformations at high dose and the minor abnormalities and variants that were observed from lower dose levels with a dose-dependent increase in incidences are considered as direct effects of the test substance and not secondary effects to maternal toxicity.

Prenatal developmental toxicity study of ammonium bromide in rats (Study report, 2007a)

In a second prenatal developmental toxicity study, 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 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).

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. Postnatal evaluation revealed no effect on litter size and survival at 300 mg/kg bw/day. Mean litter weights on prenatal day (PND) 21 were slightly lower (ca. 10%) than in the control group.

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

In a two-generation reproduction toxicity study (similar to OECD TG 416), sodium bromide was administered by gavage to Crl:CD(SD) rats at dose levels of 0, 50, 175, 350/500 (male/female) mg/kg bw/day. 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 were not considered for further evaluation.

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.

Dose range finding study of a prenatal developmental toxicity study of sodium bromide in rabbits (Study report, 2008a)

In a preceding developmental dose range finding study, 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 provided 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)

In the dose-range finding study for reproduction toxicity, 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% lower 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.

Postnatal growth and brain development study (no guideline) of ammonium bromide (Disse et al., 1996)

In a published study, rats were orally treated with sodium bromide (0.25%) in drinking water (corresponding 200 mg/kg bw/day) during GD 5-15. Controls received either tap water or NaCl 0.25% solution.

This study showed that bromide crossed the placenta and caused changes in the brain (reduced protein content) and olfactory tract (increased size of olfactory glomeruli) at a dose level of 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.

Prenatal developmental toxicity study (no guideline) of sodium bromide in rats (Harned et al., 1944)

In a published study, the effects of prenatal administration of sodium bromide to rats was investigated by means of tests designed to detect functional damage in the central nervous system of the offspring by studying the learning ability after birth. Pregnant rats were treated with 0, 40, 80 or 120 mg sodium bromide/kg bw/day from Day 3 to 20 of gestation. Pups born on gestation day 22 received bromide only via the milk of their mothers and were weaned by 20 days of age. At the age of 57-60 days the offspring was prepared for learning in the maze and on days 61-85 each animal was given two trials per day in a five cul-de-sac u-maze.

Reduced learning ability was noted at a dose level of 80 mg/kg bw/day (62 mg bromide/kg bw/day) and 120 mg/kg bw/day (93 mg bromide/kg bw/day). Dose-dependent increased pup mortality was noted: 2.43, 27, 42 and 58% from control to high dose, respectively. Maternal toxicity (body weight and clinical conditions) was not reported in the publication, but pup-killing/cannibalism of the pups by the dams were reported in the 80 and 120 mg/kg bw/day dose groups.

RAC assessment and conclusion on developmental toxicity

RAC concludes that Repr. 1A, H360D is not warranted because the available human data is not robust enough, but it supports the animal data. As regards animal data, a dose-related increased incidence of displaced testis was noted in the range of 100-1000 mg/kg bw/day in the prenatal developmental toxicity study on ammonium bromide in rats (Study report 2000b). 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 (Study report 2000b) and sodium bromide (Study report 1995). 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.

Effects on pup viability and survival were reported in several (multi-)generation studies. In the dose range finding reproductive toxicity study of ammonium bromide (Study report, 2001), both the viability and the survival index were severely reduced at 651 mg/kg bw/day (none of the pups in the single litter produced survived 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 with sodium bromide (Study report, 2016a), 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 with sodium bromide (Van Leeuwen, et al., 1983), 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 prenatal developmental toxicity study with sodium bromide in rats

(Harned et al., 1944), however, due to pup-killing/cannibalism and poor reporting, the interpretation of these findings is difficult.

Considering all available information, RAC concludes that in animal studies 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. Therefore, RAC concludes that **classification is warranted for Repr. 1B, H360D** for calcium bromide, potassium bromide and sodium bromide.

Effects on or via lactation

There was no clear evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk in the two-generation study of sodium bromide or the dose range finding studies of ammonium bromide and sodium bromide. However, there is sufficient evidence that bromide alter the composition of mother's milk, both in humans and in rats (Tyson et al., 1938; Vobecký et al, 2005) and that it can be transferred via milk to rat pups (Vobecký et al, 2005; Pavelka et al., 2002). There is also evidence that bromide may cause harm due to its effects on and via lactation, including malnutrition and lowered viability in rat pups (Vobecký et al, 2005; Pavelka et al., 2002). Moreover, there is a weak indication from a human case report on possible effects on the central nervous system of the infant/child after maternal intake of sodium bromide during lactation (Tyson et al., 1938), weakly indicating a hazard to babies during the lactation period. All this evidence fulfils the criteria "*Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk*" in the CLP Regulation (Table 3.7.1(b), point (c)); therefore, RAC concludes that **classification for effects on or via lactation Lactation, H362 is warranted** for calcium bromide, potassium bromide and sodium bromide.

Specific concentration limits

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

Additional references

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ANNEXES:

Annex 1 The Background Documents (BD) give the detailed scientific grounds for the opinion. Each BD is based on the CLH report prepared by the Dossier Submitter on the relevant bromide.

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

Annex 3 Comments received on the CLH report on potassium bromide, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 4 Comments received on the CLH report on calcium bromide, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).