Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Ammonium bromide

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

CLH-O-0000006899-51-01/F

Adopted

8 October 2020

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: ammonium bromide
CAS number: 12124-97-9
EC number: 235-183-8
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	United Kingdom	<confidential>	Company-Downstream user	1

Comment received

• Chemical: Ammonium Bromide
• Organisation: On behalf of an Organisation; Date: 14.06.2019
Comment: General appraisal of the proposed classification of Ammonium Bromide to Repr 1B H360FD

As a global Supplier of Water and Process Treatments to the Paper Industry, amongst others, we have been using Ammonium Bromide as a key component in microbiological control (MB control) for over 20 years. Before oxidising products of this type were developed, then the main on-machine MB control was carried out using traditional organic biocides, most of which have a very poor safety and environmental profile, when compared to the ammonium bromide type biocides. In fact as we progress through the BPR many actives are no longer available for the exact reason of their environmental profile.
In fact today, after these 20 or so years, the profile of the type of products in use in the Paper Industry has dramatically changed such that we have moved from 100% organic to 66% oxidising (of the ammonium bromide type):33% organic. This switch is down entirely to on-machine performance, (far superior control at far lower addition levels) extremely fast acting, very short half-life and by far, ''safer'', breakdown products which ultimately end up in the secondary effluent plants.
The use of Ammonium bromide technology in Paper Mills, is only carried out by highly trained chemical professionals. The dosing units are secure, any product testing or equipment repairs are again, only carried out by trained professionals and in fact the general workforce of a Paper Mill rarely comes into contact with either the product or the dosing system. The chemical itself is fully bunded with the dosing system also located inside the bund, again minimising any contact with the general work force.
The actual dosing system incorporates a water flush system so that the product is dosed

into a water line which then transports the chemical to the addition point. After the dosing cycle is complete the water remains flushing so that no active chemical is left in any dosing lines.
A classification of Ammonium Bromide as Repr. 1B would undoubtedly result in the use of this excellent MB programme being reviewed with the likely impact that Customers would/could choose not to use this type of chemistry, simply as a point of principle, even though there is no evidence as far as we are aware, that the use of this chemical, in this way, is actually having an impact on the worker population as outlined by the proposed classification, however justified by the testing.
A switch to a less effective, possibly more expensive, possibly more hazardous treatment programme would not seem to be the desired outcome of this updated classification.

The classification of Ammonium Bromide as Repr. 1B is, in our opinion, not the most effective answer for better worker protection, as a greater level of protection can be achieved by using more targeted directives on occupational safety and health (OSH) and effective and appropriate risk management measures for worker protection with less administrative costs and far less negative socioeconomic consequences.

Conclusion: we hope that this proposed classification is reviewed in light of the advances that the chemistry as allowed us to make in the area of microbiological control when compared to the traditional organic biocides that are available and how the product is actually used. In addition, considering the length of time the product has been in use, not only for downstream users, but in terms of the production and processing of the material at source and the lack of evidence on the population that has been exposed to this material then more targeted directives for risk management of chemical handling would seem more appropriate.

I have also attached these comments as a word document in the section below

ECHA note – An attachment was submitted with the comment above. Refer to public attachment AmBr classification ECHA.docx

Dossier Submitter's Response

Thank you for your comments.

Lack of reported adverse effects of workers exposed to ammonium bromide is not sufficient evidence to exclude potential adverse effects of ammonium bromide and does not negate positive results from animal studies.

Moreover, we also would like to remind you that exposure is not taken into consideration in the classification, since classification is based on the intrinsic hazardous properties of the substance.

In addition, socio-economic consequences are not taken into consideration in classification that is solely based on the intrinsic hazardous properties of the substance.

Consequences in downstream legislation are also not taken into consideration and we consider that this issue would be more appropriately dealt with independently through other European legal instruments.

Discussion of the most appropriate risk management, and alternatives other than CLH is out of the scope of the current public consultation.

RAC's response

RAC agrees with the answer provided by the DS.

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	2
Comment received				
These comments are submitted by BSEF aisbl, the international bromine council, on behalf of its member companies Albemarle, Lanxess, ICL and Tosoh. This is the third, and last, part of our comments. We have provided comments on the endpoints reproductive toxicity and STOT RE in separate submissions. In order to make sure that the formatting of our comments is not lost in the ECHA web-interface (our comments included some tables, for example) we also attach the pdf files for all safety. ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF comments reprotox 20190612.pdf				
Dossier Submitter's Response				
Noted. Please see response to comments no. 8.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	3
Comment received				
These comments are submitted by BSEF aisbl, the international bromine council, on behalf of its member companies Albemarle, Lanxess, ICL and Tosoh. This is part 2 of our comments.				
Dossier Submitter's Response				
Noted. Please see response to comments no. 8 and 12.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2019	Belgium	BSEF aisbl	Industry or trade association	4
Comment received				
These comments are submitted by BSEF aisbl, the international bromine council, on behalf of its member companies Albemarle, Lanxess, ICL and Tosoh. This is part 1 of our comments. 1. Introductory Overview of BSEF Comments New, carefully designed and detailed 90 day and 2 generation toxicity studies, and toxicokinetic studies have fully characterised the effects of sodium bromide and the relationship between systemic and reproductive toxicity and exposure. In the light of these new data and their impact on previously submitted data the registrant concludes that the proposed classification of Category1b is not appropriate for the Developmental or Reproductive Toxicity of ammonium bromide.				

This conclusion is based on the detailed comments submitted by BSEF which are summarized in the following points:
• The assessment should be based on pivotal GLP compliant, OECD guideline studies on sodium and ammonium bromide as previous published work does not meet the standard required for classification purposes, having major flaws which preclude inclusion even as weight of evidence data
• In particular, the older published studies were conducted using small group sizes (5 to 12 animals at termination) at high dose levels (nominal 19200 ppm NaBr in the diet) which elicited motor inco-ordination, lack of grooming and significant growth retardation (20 - 24% lower bodyweight than controls after 12 weeks at 19200 ppm, Loeber 1983, van Logten 1974). In OECD guidance on recognition assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation [ENV/JM/MONO(2000)7] failure to groom is recognised as an indication that 'the animal is definitely ill, and may be in severe pain and discomfort'
• Calculation of the Sodium Bromide intakes based on the food intake and body weight data available in van Logten 1973 indicate exposures for females ranged from 1113-3840 mg/kg bw/day for the 19200 ppm dose groups, respectively, with the highest exposure at the start of the study. For males, the corresponding intakes ranged from 1190-3360 mg/kg bw/day. The high dose level in this series of studies therefore exceeded the limit dose of 1000 mg/kg/day defined OECD guidance, by up to 380%.
• CLP Guidance, Annex 1 Section 3.7.2.5.8 states: "In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, extensive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate", therefore these high dose effects should not be considered for classification for reproductive effects. [For full evaluation of published studies see Addendum 1]
• In addition, assessment of systemic toxicity should not be limited to body weight and food intake but should take full account of the known sedative effects of bromide which arise at lower dosages, increase with duration of dosing and have been demonstrated to cause marked impairment of behaviours such as ataxia, loss of reflexes etc, which lead to interference with mating, parturition and postnatal care of offspring. The outcome of these studies is considered not to be impaired reproductive function per se but secondary to the disruption of natural behaviours by characteristic clinical responses such as sedation, ataxia and impaired movement, as recognised by the proposed STOT-SE 3 H336: (May cause drowsiness or dizziness), based on narcotic effects.
In rats, clinical signs such as sedation and impaired movement are notoriously difficult to record in standard assessments of clinical condition, particularly when these signs are only recorded once daily, pre-dose. Response to treatment is therefore likely to have been underestimated in any study which relies on cage-side observations as a primary indicator of post-dose effects, and particularly so in published studies where the frequency of observations is not reported. In the 90 day study with ammonium bromide (Barton 2000), detailed neurological assessments detected adverse effects at dosage as low as 100 mg/kg/day, that were not evident when clinical condition was assessed using routine methodologies. This indicates that adverse physical effects may have been underreported in any study relying on standard observations and therefore likely present at dose levels lower than those reported.
2. Proposed Classification for Adverse Effects on Sexual Function and Fertility
• CLH Guidance (2017) in section 3.7.2.2.1.1 specifically indicates that adverse effects on fertility seen only at dose levels causing marked systemic toxicity (eg lethality, dramatic reduction in body weight, coma) are not relevant for classification purposes. The guidance also acknowledges that mating behaviour can be influenced by parental effects not directly related to reproduction eg sedation and such effects on mating behaviour may

not warrant classification. Lethality and severe sedation were observed in the 2 generation study on sodium bromide (Hoberman 2016b) in males at 350 mg/kg/day and in females at 500 mg/kg/day, in the 90 day toxicity study with sodium bromide (Hoberman 2016a) at 500 mg/kg/day, and in the 90 day toxicity study with ammonium bromide (Barton 2000) at the high dose level (500 mg/kg bw /day males, 750 mg/kg bw/day females). The reproductive outcome at these dosages should not therefore be considered relevant for classification

• At lower dosages, with more modest effects on clinical condition, effects were insufficient to warrant a 1b classification, which requires clear evidence of an adverse effect on sexual function and fertility:

At 175 mg/kg/day on the 90 day toxicity study with sodium bromide (Hoberman 2016a), there was no effect on reproductive organ weight or histopathology. No adverse effect on oestrous cycles or on sperm count, density, motility or morphology was concluded, although there was evidence of spermatid retention and epididymal changes - but only in 2/10 males, and at minimal-mild severity.

At 175 mg/kg/day on the 2 generation reproductive toxicity study on sodium bromide (Hoberman 2016b) the P generation male mating index was 95.8% with all females and 91.7% with treated females, and the fertility index was 73.9%. In the second cohabitation period, the mating index was 86.4% and the fertility index was 73.7% which although lower than concurrent controls, was only slightly lower than the historical control range. In females, although the pregnancy incidence was low (as described above), there were no adverse effects on oestrous cycles, duration of gestation, gestation index, number of pups born, pup viability, sex ratio, anogenital distance, growth or physical development (as assessed by pinna unfolding, hair growth, tooth eruption and eye opening) in either the F1a or F1b litters.

In the ammonium bromide 90 day study (Barton 2000), no adverse effect on reproductive organs of males or females was determined at histopathology at 225 mg/kg/day.

The above effects are considered insufficient to demonstrate the 'clear evidence of an adverse effect' required for classification as 1b, but it is acknowledged that they may indicate 'some evidence of an adverse effect'

3. Proposed Classification for Effects on Development

CLH guidance (2017) (Annex I: 3.7.2.4.3) indicates 'when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity'. In the developmental toxicity studies with ammonium and sodium bromide, the high dose level was associated with severe effects in the pregnant females. In the ammonium bromide study ([Irvine and Hallmark 2000), all high dose (1000 mg/kg/day) females showed characteristic clinical signs of abnormal gait, lack of body tone and abnormal respiration from the first 24h after treatment until termination, and there was a significant reduction in weight gain. One female was euthanised owing to the severity of clinical signs. In the sodium bromide developmental toxicity study (Myers 1995), effects at 1000 mg/kg/day were similar with uncoordinated movements, feet falling through the cage grid floors, reduced body tone and one animal killed because of severity of signs. The clinical effects at this limit dose level in both studies are considered so severe as to preclude the use of these data for classification purposes. At the intermediate dose level of 300 mg/kg/day of sodium and ammonium bromide in both studies, effects of treatment were limited to lower maternal body weight gain in late pregnancy which, not unsurprisingly, was associated with minor developmental delays such as reduced ossification in the offspring.

These effects are generally considered as transient (Chahoud 2005, Carney 2007, Kimmel 2014, Chahoud 2015, De Sesso 2018) and a subsequent developmental toxicity study on ammonium bromide (Barton 2007) in which the study design was modified to include a littering phase at 300 mg/kg/day, demonstrated that these effects were entirely reversible, with no evidence of developmental delays at Day 21 post-partum. It should also be noted that this study incorporated additional dose levels of 600 and 800 mg/kg/day ammonium bromide in which the characteristic clinical signs of staggering, abnormal gait and abnormal respiration (including one female terminated early owing to severity of signs), were observed in maternal females but foetal effects were limited to reversible effects on foetal ossification. Classification for Developmental Toxicity as Category 1b is considered not justified, therefore, as developmental effects observed at dose levels below the severely toxic limit dose have been demonstrated as reversible. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Charles RiverReproductiveToxicologyHistoricalControlDatainRats.pdf
Dossier Submitter's Response
Thank you for your comments. Since these comments are a summary/overview of the comments submitted in comment number 8, please refer to the response in no. 8
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
12.06.2019	Israel	ICL Europe Coöperatief U.A.	Company-Importer	5
Comment received				
This confidential document is submitted as a reference to the comments submitted by BSEF, the International Bromine Council, on behalf of its member companies, including ICL and its EU affiliate ICL Europe Coöperatief U.A. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment ICL blood samples 12 June 2019.pdf				
Dossier Submitter's Response				
Thank you, noted. Please see response to comment no. 8.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.06.2019	Germany		MemberState	6
Comment received				
The German CA agrees with the proposed harmonised classification of Ammonium bromide. In section 7 of the CLH report there is a water solubility of 145.6 g/l at 100 °C given. In the reference Smith and Eastlack (1916) it is reported that 145.6 g are soluble in 100 g of water and not per litre.				
Dossier Submitter's Response				
Thank you for your support and for noticing the editorial mistake. We agree that the water solubility is 145.6 g in 100 g of water.				
RAC's response				

Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	7
Comment received				
see comments submitted separately				
Dossier Submitter's Response				
Noted. Please see response to comment no. 8.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2019	Belgium	BSEF aisbl	Industry or trade association	8
Comment received				

Effects on Sexual Function and Fertility:

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

CLH comment: Page 47

'The studies available to assess reproductive toxicity of ammonium bromide include one dose-range finding reproductive toxicity study, one oral 90-day repeated dose toxicity study and one four-week dose-range finding study for oral repeated dose toxicity.'

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

BSEF Comment: In general, we concur with the CLH Summary of the effects observed, and in particular with the decision that most important studies for classification are the Guideline Compliant GLP studies, and that the older published studies are not relevant for classification. We also agree with the observation that the effects observed at the highest dose levels in the Guideline studies produced such severe toxicity in both males and females, that the results at the top dose level very likely secondary to the systemic toxicity and not relevant for classification. This means that the only results relevant for classification are a slight decrease in fertility in the Parental generation to 74%, which was only insignificantly lower than the historical control range (75 to 100%), and showed inconsistency between the affected individuals at each pairing. No effect on fertility was apparent in the F1 or F2 generations, or at the lowest dose level of 50 mg/kg/day. In the 90 day toxicity study at 175 mg/kg/day the number of sperm with detached/no head was higher than the concurrent control but considered unlikely to have been an effect of treatment as it was closer to the expected (historical control mean) value than was the unusually low control value, and there were no clear adverse effects in females, so no adverse effect of treatment on fertility is concluded.

Several non-guideline studies are also reviewed in the CLH Report, but BSEF consider that these are not relevant for classification, not even as' supportive', owing to uncertainties resulting from non-standard methodologies, unconfirmed dosages, small sample sizes and/or limited reporting.

Ammonium bromide - non-guideline reproductive toxicity studies

Dose-range finding study for reproductive toxicity of ammonium bromide in rat

(Study report, 2001).
CLH comment: Page 47
'Ammonium bromide was administered to rats (10/sex/group) via food at concentrations of 0, 1600, 3200 and 6400 ppm (corresponding to 0, 127, 242 and 503 mg/kg bw/day in males; 0, 228, 454, 651 mg/kg bw/day in females). Animals were treated from two weeks prior to mating until the first generation had been weaned. No statistical analysis was performed due to the small group size. "
Conclusion
CLH Comment: Page 48
'The effects on fertility are considered as being treatment related. The slightly reduced fertility index in the mid dose group and the markedly decreased fertility index in high dose groups (only one dam became pregnant in the high dose group) are not considered as being secondary to adverse general toxicity. The general toxicity was not significant in terms of effects on body weight and body weight gains, but there were clinical observations (including rolling gait) in high dose group throughout the treatment period. However, the observed neurotoxicity is not expected to impact on fertility, but probably impacted the mating performance in the high dose group.'
BSEF comment:
This study is considered not suitable for classification purposes for the following reasons: Although GLP compliant, this is a dose-range finding study, falls short of an OECD Guideline 421 compliant screening study (which ECHA indeed define as 'not meant to provide complete information on all aspects of reproduction and development') and the small group size precludes any definitive assessment of reproductive capacity.

We disagree that the general toxicity was not significant. Reductions in body weight were reported in males at 6400 ppm and 3200 ppm, but throughout the toxicology programme it is clear that clinical signs are the most sensitive marker of toxicity, and the CLH report acknowledges this in the proposed classification for sedative effects. In this study, even though clinical signs were only recorded at the minimum of once daily, signs in males included the typical rolling gait, piloerection and hunched posture and females showed signed of hyperactivity. Both sexes also showed staining and unkempt appearance – attributed to their 'generally ill condition'. Collectively, these signs are clear evidence of clinical condition so perturbed as to interfere with mating and reproductive performance, including litter loss due to poor maternal care.

We would also like to draw your attention to the fact that the target dosages of Ammonium Bromide for the study were 120, 240 and 480 mg/kg bw /day but actual dosages generally exceeded the target, especially in pregnant and lactating females:

In males actual dosage was up to 28% higher than the target at the start of the dosing period and after week 6 was up to 14% lower, with the difference generally higher in the high dose group.
Achieved dosage in mg Ammonium Bromide/kg/day: Males
Target dose level 1600 ppm
120 mg/kg/day 3200 ppm
240 mg/kg/day
6400 ppm
480 mg/kg/day
Week of treatment
1 154 275 617
2 148 280 565
6 114 225 473
7 112 222 440
8 107 207 418

% of nominal 89 – 128 86 – 117 87 – 129

In females, actual dosage was about 50% higher than target in all dose groups at the start of treatment, and in the range 116 – 127% of target over the gestation period. In the first and second weeks of lactation, values were up to 3-fold higher than target values and in the 3rd week this rose to 3.5 to 3.7 fold target values, although the latter value may have been increased by consumption of diet by the weaning pups.

Achieved dosage in mg Ammonium Bromide/kg/day: Females
Target dose level 1600 ppm
120 mg/kg/day 3200 ppm
240 mg/kg/day
6400 ppm
480 mg/kg/day
Week of treatment
1 178 361 737
2 175 333 697
Week of gestation
1 176 335 558
2 158 306 631
3 144 305 631
Week of lactation
1 217 431 -
2 355 676 -
3 423 882 -
% of nominal 120 - 353 127 - 368 117 - 154

These values emphasise the variation in exposure consequent to administration of the test material as a fixed concentration in the diet, the potential for overdose in pregnant and lactating animals. The achieved high dose for females is well within the range known to cause severe maternal toxicity so the outcome for this group is not unexpected. At 3200 ppm, fertility index was lower than control values as 2 males did not sire a pregnancy but, as non-mated males were replaced with proven males, only one female was not pregnant. The Study Director considered the difference from control too small to indicate an effect of treatment. It is of note that the fertility index was calculated as the percentage of pairings that resulted in pregnancy, which emphasises the difference whereas conventionally this is divided into mating index (percentage of pairings that result in matings) and fertility index (percentage of matings that result in pregnancy).

The variation in dosage over the treatment period, which did not follow the same pattern in each sex/dose group, is considered to affect the interpretation of results, given the known steep dose response curve. We therefore consider that no firm conclusions on treatment-related reproductive effects can be drawn from this inadequate dose-range finding study.

Sodium Bromide – test guideline reproductive toxicity studies

Two generation reproductive toxicity study of sodium bromide (Study Report 2016)
P generation – fertility, parturition and sexual function
CLH Comment: Page 50
In the first cohabitation period (P generation to produce F1a) there was no effect of treatment on male or female mating performance or fertility at 50 mg/kg bw/day. At

intermediate dose the mating index was 95.8% with all (treated + untreated) females, and 91.7% for males with treated females. The fertility index was 73.9% with all females and 72.7% with treated females, significantly lower than controls ($p \leq 0.05$)"
CLH Comment: Page 51
'However, the decreased fertility index may be concluded to be treatment related and not secondary to general toxicity since in the intermediate dose group this effect was statistically significantly lower than control and observed in absence of any marked general toxicity.'

BSEF comment: It should be noted that the fertility index in the mid dose group, although significantly lower than concurrent controls was very close to the historical control range, as is demonstrated in the report These historical control data have been collated from 187 studies conducted in the crl:CD(SD)rat the conducting laboratory since 2008
(https://www.criver.com/sites/default/files/resources/ReproductiveToxicologyHistoricalControlDatainRats.pdf) and therefore we disagree with the comment that the validity of the statements referencing these data is low.
In total, over both cohabitation periods, all males in Groups 1, 2 and 3 mated at least one female. All control males impregnated at least one female and there was only one male in the 50 mg/kg/day group which did not achieve a pregnancy.
At 175 mg/kg/day, although reduced pregnancy rates were observed at both cohabitation periods compared to concurrent controls, unusually, the affected animals differed and in total only 2 males did not impregnate a female, giving an overall male fertility index of 91.7% (within the expected range). This was likely a deficit in these males, as neither of the treated females allocated to one male became pregnant at the alternative pairing and the allocated untreated female was not mated, but the females allocated to the other male both became pregnant with alternative males.
All females in Groups 1, 2 and 3 mated during either the first or second cohabitation periods. Five females in the 175 mg/kg/day group did not get pregnant from either pairing (with treated males only), giving an overall female fertility index of 77.3% (17/22), below the concurrent control value but within the expected range. Only two of these females showed marked depletion of corpora lutea at histopathology and no corpora lutea at ovarian follicle examination. One of these females had also shown extended estrus (9 days) and a further female, showed extended periods of diestrus (which may indicate pseudopregnancy). Another female which had marked depletion of corpora lutea at histopathology and no corpora lutea at follicle counting, was pregnant at the first pairing (but not at the second).Therefore, the conclusion of 'clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects' cannot unequivocally be drawn from these findings, owing to the potential for adverse effects on reproductive performance consequent to treatment-related effects on condition (including sedation), and to the difference in performance of individuals in both cohabitation periods.
P generation – reproductive organ weights and histopathology
CLH comment: Page 53
'No significant differences were noted in the number of primordial follicles in either left, right or both ovaries for animals treated with 50, 175 or 500 mg/kg bw/day sodium bromide compared to the control group. Corpora lutea were present for all control females at the terminal kill, and one animal in each of 50 mg/kg bw/day and 175 mg/kg bw/day dose groups had no corpora lutea present. Two further females from the 175 mg/kg/day group (mated but not pregnant at the second cohabitation and killed on gestation day 25) had no corpora lutea present and another female (killed gestation day 25) had corpora lutea present but they were largely regressing, although there was no effect on the overall number of follicles in these females. There were, however, 3 other not mated/not pregnant females which did have corpora lutea present. In the high dose

group, 10 females had depleted corpora lutea (although there was no effect on ovarian follicle counts and six of these females became pregnant): 5/8 terminal kill females evaluated had no corpora lutea present, and there were 5 further females terminated early which also had no corpora lutea. This finding is considered to be likely related to the test substance since 3/20 females treated at 500 mg/kg bw/day in the recent 90-day dose toxicity study of sodium bromide (Study report 2016b) had no corpora lutea present, and in addition, the number of corpora lutea per female was reduced at the high dose level of 19200 ppm/kg diet (in excess of 1000 mg/kg body weight/day) in the study by van Logten et al (1974).'

BSEF comments:

Determination of the number of corpora lutea was variable and depends on the oestrous stage at termination. Ovarian follicle counts seem to be a more reliable endpoint. We recommend to not draw conclusions on any possible substance-related effect from these variable counts of corpora lutea, as variation in numbers is expected within a normal cycle (Yoshida 2009). The additional reference to effects reported in the van Logten et al, 1974 publication is equally uncertain, and was reported only at severely toxic dose levels that should not be used for the evaluation of adverse reproductive effects.

CLH Comment: Page 54

'All males (20) at 350 mg/kg bw/day showed retained spermatid heads of minimal to moderate severity. As these findings were also observed in males which died or were killed in week 12 they were likely present during the mating period. There was no apparent correlation, however, between the severity of the findings and pregnancy outcome of the pairings with treated or untreated females. At 175 mg/kg/day 11/23 males were affected, with the majority showing only minimal changes and only 4 showing epididymal debris.'

CLH comment: Page 53

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

BSEF comment: It should be noted that all findings in the 175 mg/kg/day dose group that were flagged as statistically significant compared to the concurrent control group where well within the historical control range and therefore these changes are not toxicologically relevant. As in many laboratories, the variation observed in control males for these parameters is relatively high and we do not think it is justified to conclude a clearly substance-related effect from these findings. As acknowledged in the CLH report, the findings did also not correlate with the pregnancy outcome of the pairing.

Conclusion

CLH Comment: Page 57

'In conclusion, adverse effects on reproductive capacity were observed in the P-generation at 350/500 mg/kg bw/day with reduced male and female fertility, adverse effects on sperm count and morphology. All males also showed retained spermatid heads of minimal to moderate severity, and 10 females had depleted corpora lutea (although there was no effect on ovarian follicle counts, and no direct correlation with infertility). Litter size at birth was lower and pup viability was very poor, and a selection for a second generation was therefore not possible. Since the observed adverse effects on fertility and reproductive system of both males and females occurred in presence of significant generalised toxicity (including neurotoxic effects, mortality, marked decreases in body weight gain and food intake), these findings are not considered sufficient as a basis for classification. However, at the next lower dose level where significant generalised toxicity is absent similar effects are seen that can be used as a basis for classification. At 175 mg/kg/day there were decreases in fertility (approx. 73-74% of control values) in the P-generation in both pairings but less marked effects on clinical condition, body weight and food intake were observed in males and females. During the premating and mating there were no marked or statistically significant increases of clinical observations in males or

females, and no statistically significant effects on body weights or body weight gains in females that impacted the reproductive performance. In males, the body weight was >10% lower than control during second mating and there were body weight loss recorded during the first week of mating in both pairings. However, at least in females, the adverse effets on fertility is clearly not considered as being secondary to general toxicity at 175 mg/kg bw/day.'

BSEF comments:

We concur with the CLH report that the findings in the high dose group in the presence of significant generalised toxicity are not considered sufficient as a basis for classification. However, in BSEF's opinion, as the fertility rates in the P generation (including all related findings) were within or only slightly lower than the historical control ranges and as there were no adverse effects in the F1 and F2 generation, the study findings cannot be considered 'clear evidence' of effects on sexual function and fertility and thus supportive of a category 1B classification. BSEF consider that, at most, there is 'some evidence' of an effect and hence a category 2 classification is more appropriate for this substance.

Sodium bromide – non-guideline reproductive toxicity studies

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

Fertility, parturition and sexual function

CLH comment: Page 58

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

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

BSEF Comment

This publication contains so little detail on experimental method that it is considered unreliable data. In particular, there is obvious confusion in terminology where pairing of animals is reported as 'mating'. In the absence of data on actual mating at each pairing and on the systemic toxicity observed, no conclusions on fertility impairment can be drawn from this study. Any further assumptions as to what sex might have been affected are speculative since the practices of using 4 month old, proven males at the study start, of breeding at least 3 times in the first generation and of combining litter data from different pairings are all confounding factors. In addition, although the age and reproductive status of animals used in the cross-over study are not reported, it would appear that this was a fourth mating for the treated animals and thus, since reproductive senescence in mature rats can begin as early as 6 months of age (Seller 2007) any effect on fertility cannot reasonably be deduced.

It is of note that levels of bromide reported in maternal plasma after 7 months of treatment in this publication range from 0.5 mmol/L at 75 ppm to 7.8 mmol/L at 1200ppm (the NOAEL for reproductive effects) and compare to human reference values from background levels up to the therapeutic range.

However, at the 4800ppm LOAEL, maternal plasma levels were 27.6 mmol/L ie in excess

of human exposure levels referenced as 'severely toxic' (>12.5 mmol/L), and within the range eliciting 'possible coma'(25 – 37.5 mmol/L). It is evident, therefore that any reproductive effects occur at exposure levels which are not relevant for human exposures.
Conclusion
CLH Comment: Page 59
'In this study there are indications of impaired fertility in F1 and F2 at 1200 ppm (108 mg/kg bw/day) and at 4800 ppm (432 mg/kg bw/day) and 19200 (1728 mg/kg bw/day) in F0, however since the fertility indices were low also in control groups in all three generations (52-70%), and there is no information on clinical observations for any of the animals, and no information on body weight of animals in the dose group of 19200 ppm the quality of the study and the relevance of the findings may be questioned. Moreover, the dose 1728 mg/kg bw/day may be considered to be in excess.'

BSEF Comments

In addition to the weaknesses quoted above, BSEF would like to point out the following shortcomings in this publication:
BSEF contends that the published studies are not of the quality expected for safety evaluation and have been superseded by new GLP compliant studies, which should form the basis of the assessment. A full critique of the publications is presented as Appendix 1, but the salient points are:

- The publications would warrant a Klimisch score of 3 at best and as such should not be the basis of classification
- The van Leeuwen paper is a review article and too brief to provide detail on the study design for the multigeneration study or robust data for analysis
- The studies do not comply with any OECD guidance on study design: the multigeneration study appears to be based on concurrent US FDA guidance (3 generation/2 litters per generation)
- The number of animals in the multigeneration study is unclear and seems to vary in each generation: data from different generations have been combined in some cases, yet the exposure profile differs (the parental generation were not exposed in utero)
- It is unclear whether mating was monitored directly or presumed from pregnancy status, and if littering was observed directly – and hence whether the absence of any pregnancies in the high dose group and reduced pregnancy rate at 4800 ppm was due to mating performance (possibly secondary to severe clinical response to treatment), infertility post-mating or total litter loss pre- or early post-partum
- It cannot be confirmed whether the studies were GLP compliant but from the date and the performing laboratory it is considered unlikely
- No diet analysis was conducted to verify the achieved concentrations and consistency of formulations
- Fixed target dosages of 0, 75, 300, 1200, 4800 and 19200 mg Sodium Bromide per kilogramme of diet (ie ppm) reported as corresponding to (3.75), 15, 60, 240 and 960 mg/kg bw/day but likely to have exceeded these levels at critical stages eg late gestation and lactation where food intake increases. Dietary administration also brings pups into direct contact with test material in the diet from birth, as well as early exposure as soon as pups begin to consume solid food. Given the steep dose response curve, this has likely affected the outcome of the study and interpretation of results (the new studies have been conducted by gavage to prevent this drift from target dose).
- The high dose level in the multigeneration study elicited clinical signs and body weight changes at the higher dosages which exceed levels of toxicity currently considered acceptable. As clinical signs define the response to treatment for Bromides (rather than the conventional markers of toxicity ie body weight gain and food intake) and since Bromide has known sedative effect, it is considered that the more subtle effects at lower dosages are unlikely to have been fully characterised: By their nature, signs such as ataxia, prostration, lethargy, abnormal gait require diligent and frequent observation to assess in nocturnal animals.

Although the CLH report only speaks about 'indications of impaired fertility' BSEF contends that the weaknesses of the study preclude any conclusions on fertility, even as 'indications'. Therefore, in the context of regulatory decision making, when GLP studies compliant with current guidance are available, these data are inadequate and too unreliable for consideration.

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

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

Organ weights and Histopathology

CLH Comment: Page 59

'Microscopic examination of testes revealed treatment related findings of retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm in 2/10 in the 175 mg/kg and in 9/9 500 mg/kg terminal euthanasia animals. All 4 early deaths (two of which were recovery group animals that died either during the dosing period or shortly thereafter) in the 500 mg/kg dose groups had treatment related findings consisting of minimal to moderate retained spermatids at the lumen of the seminiferous tubule epithelium-primarily at Stages X-XII and minimal to moderate retained spermatid heads in the Sertoli cell near the basement membrane-primarily at Stages XI-XII. A secondary change in the epididymis, originating from the corresponding testis, was increased cellular debris in the lumen."

BSEF comments

Retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm is a subtle change which can occur in isolation or can be associated with abnormalities in sperm parameters (number, motility, and/or morphology) or with other degenerative changes seen in the seminiferous tubule epithelium. These changes can affect fertility and can be associated with testosterone deficiency but there was no evidence of this in any other parameters measured on this study. Retained spermatids are not unknown in untreated animals, so the observation is recorded when an increase over the control incidence is identified. It should also be noted that these findings were not apparent in recovery animals

Conclusions

CLH Comment:Page 62

'However, similar effects were also observed at lower dose level. At 175 mg/kg/day, the number of sperm with detached/no head and the percent abnormal sperm was slightly (and statistically significantly) increased compared to control but the clinical signs were less severe compared to the high dose group and there were no deaths or effects on body weights or body weight gains. The effect on sperm parameters at this dose level cannot be considered as secondary consequences of significant generalised toxicity and they furthermore point to a dose related trend in increase in incidences.'

BSEF comments

In accordance with the authors of the study, BSEF do not consider the effects on the sperm parameters in the 175 mg/kg dose group as treatment related, as the number was well within the historical control range and the control values were at the low end of the historical control or below (for detached heads, see table at p. 61 of the CLH report). Thus although there was statistical significance when compared to concurrent controls these findings are not considered biologically significant.

CLH Comment: Page 62

'In females, three out of 10 animals in the 500 mg/kg/day dose group had no corpora lutea in the ovary, but overall follicle counts were not affected. No other adverse effects on reproductive organ weight, histopathology or estrous cycle were recorded in females in this study. The depletion of corpora lutea in female is considered a substance related

effect not secondary to general toxicity.'

BSEF comments:

We do not consider the isolated finding of no corpora lutea in the ovaries of 3 females of the high dose group as indicative of a substance related effect for the following reasons: there were no other effects on female reproductive organs and there were technical issues with sectioning of the ovaries. This finding can, at most, be regarded as inconclusive.

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

Conclusion

CLH Comment: Page 63

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

BSEF Comments

The conclusion deviates from the principle that studies at excessively high doses should not be used in the assessment when other studies are available. We have further analysed the study and would like to draw your attention to the fact that, as this was a dietary study, dose levels had a wide range: Calculation of the Sodium Bromide intakes based on the food intake and body weight data available indicate that these data show that exposures for females ranged from 4-15, 17-60, 70-240, 278-960 and 1113- 3840 mg/kg bw/day for the 75, 300, 1200, 4800 and 19200 ppm dose groups, respectively, with the highest exposure at the start of the study. For males, the corresponding intakes ranged from 4-14, 15-55, 60-220, 247-878, 1190-3360 mg/kg bw/day for the low to high doses. The high dose level was therefore well in excess of the limit dose of 1000 mg/kg/day defined OECD guidance. Decreased prostate weight was observed in males at 4800 and 19200 ppm but there was no significant change in relative testes or ovarian weight, which is pertinent for the assessment of reproductive effects since testis weight is the primary indicator of testicular damage. The description of the other observations is ill-defined, vague and only reported at excessive dose levels. BSEF considers, therefore, that this study should not be included in the assessment.

Summary

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

Females:

CLH Comment: Page 64

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

Similar effects on the female reproductive organs were observed in the 90-day repeat dose toxicity study on sodium bromide. Depletion of corpora lutea was observed in 3/10 females in the 500 mg/kg/day group, which were all in estrus at termination, and in absence of severe general toxicity.
Also in a non-guideline 90-day repeated dose toxicity study of sodium bromide in rats the number of corpora lutea in the ovaries was found to be decreased, however at a very high dose level (1728 mg/kg bw/day) (Van Logten et al., 1974). These findings were seen in absence of severe general toxicity and are considered as supportive evidence for classification.'

BSEF comments

There was no adverse effect on female gonads in the 90-day study with Ammonium Bromide (Barton 2000). In the 90 day with sodium bromide (Hoberman 2016b), 3 females in the 500 mg/kg/day group showed depletion of corpora lutea (one of which was based only on an incomplete set of partial sections) but no effect on the oestrous cycles. In the 2 generation study (Hoberman 2016b 5/15 females at 500 mg/k/day had no corpora lutea, but at 175 mg/kg/day there was only 1/24 females with no corpora lutea at terminal kill. There were, however, 3 in various groups on the study were reported to have corpora lutea, even though they were not mated or pregnant. We suggest therefore that the assessment of corpora lutea may have been less than rigorous and therefore the interpretation of the findings is not clear. This should not be taken as evidence for impairment of female fertility leading to a classification.

Males

CLH Comment: Page 64

'In the two-generation reproductive toxicity study of sodium bromide at the high dose level (350 mg/kg bw/day) adverse effects on sperm count and morphology were reported. All P males at 350 mg/kg bw/day showed minimal-moderate cellular debris in the epididymis and/or spermatid head retention in the testis and 11/23 males in the 175 mg/kg/day group showed similar but, for the majority, minimal changes. Statistically significantly lower count of motile sperm in vas deferens was recorded in both 350 mg/kg bw/day and 175 mg/kg bw/day dose groups compared to control ($p \leq 0.01$ and $p \leq 0.05$) and the percentage of sperm with abnormal morphology in epididymis was increased (7.6% and 21.3%, $p \leq 0.01$ and $p \leq 0.05$ respectively, compared to control). Spermatid head retention in testis of F1 males (175 mg/kg bw/day; highest dose tested) were not as clear (3 animals) as in P generation, and the total count and number of motile sperm in the vas deferens was lower than controls.
Also in the 90-day repeated dose toxicity study of sodium bromide treatment related findings of retained spermatids in testes in 2/10 in the 175 mg/kg bw/day group and in 9/9 of the 500 mg/kg terminal euthanasia animals were recorded. Four early deaths (of which two from the recovery group) in the 500 mg/kg dose groups had treatment related findings consisting of minimal to moderate spermatid retention in the seminiferous tubule epithelium and in Sertoli cells. Moreover, at 500 mg/kg/day there was a reduction (88.6% of control, $p \leq 0.01$) in the number of normal sperm and percent motile sperm from the vas deferens (75.3% of control, $p \leq 0.05$). At both 500 mg/kg bw/day and 175 mg/kg bw/day the mean number of sperm with detached head or no head were increased compared to the control group values.
The histopathological changes in the gonads observed in the high dose groups of the two-generation reproductive toxicity study and the 90-day repeated dose toxicity study occur in the presence of significant and severe generalised toxcicity and may not be used as a basis of classification, but as supportive information in the total weight of evidence. It could be noted however, that at the intermediate doses (175 mg/kg bw/kg), less adverse changes in the gonads were observed in absence of significant generalised toxicity indicating a treatment-related dose-dependent increase.'

BSEF comments:

We do not agree that the effects seen at the 175 mg/kg bw dose group are indicative of

an adverse effect on fertility and given the steep dose response for general toxicity the postulation of evidence of a dose-dependent changes is not appropriate in our opinion.
Fertility
CLH Comment: Page 65
'The dose-range finding study of ammonium bromide reported slightly reduced fertility indices at 242/454 mg/kg bw/day (male fertility index: 80%, female fertility index: 90%) and markedly reduced male and female fertility indices at 503/651 mg/kg bw/day (10% compared to 100% in control). Only one female became pregnant at 651 mg/kg bw/day and the litter produced was dead before day 4 of lactation. At this dose level clinical observations, including rolling gait, was observed but the reduced fertility is not considered to be a secondary consequence of this toxicity.'

BSEF Comment

This study is considered not suitable for classification purposes for the reasons indicated on comments on Pag 48. Although GLP compliant, this is a dose-range finding study, falls short of an OECD Guideline 421 compliant screening study (which ECHA indeed define as 'not meant to provide complete information on all aspects of reproduction and development') and the small group size precludes any definitive assessment of reproductive capacity.

The achieved high dose for females is well within the range known to cause severe maternal toxicity so the outcome for this group is not unexpected. The Study Director considered the difference from control too small to indicate an effect of treatment. It is of note that the fertility index was calculated as the percentage of pairings that resulted in pregnancy, which emphasises the difference whereas conventionally this is divided into mating index (percentage of pairings that result in matings) and fertility index (percentage of matings that result in pregnancy).

The variation in dosage over the treatment period, which did not follow the same pattern in each sex/dose group, is considered to affect the interpretation of results, given the known steep dose response curve. BSEF consider that no firm conclusions on treatment-related reproductive effects can be drawn from this inadequate dose-range finding study.

CLH Comment: Page 66
'In the two-generation reproductive toxicity study of sodium bromide fertility was statistically significantly reduced in both cohabitation periods of P generation at 175 mg/kg bw/day (approx. 73% compared to 100% in control) in absence of severe general toxicity. Fertility was also severly reduced at 500 mg/kg/bw/day (approx. 60% compared to 100% in control) in presence of excessive general toxicity (mortality, adverse clinical signs and effects on body weights). In the F1 generation, there was no effect on mating or fertility'

BSEF Comment:

The results in the high dose group, in conjunction with excessive toxicity, should not be used for the evaluation. The slight decrease in fertility in the Parental generation at 175 mg/kg bw/day to 74% of the control was only marginally lower than the historical control range (75 to 100%) and showed inconsistency between the affected individuals at each pairing. No effect on fertility was apparent in the F1 or F2 generations, or at the lowest dose level of 50 mg/kg/day. In the 90 day toxicity study at 175 mg/kg/day the number of sperm with detached/no head was marginally higher than the concurrent control but considered unlikely to have been an effect of treatment as it was closer to the expected (historical control mean) value than was the unusually low control value, and there were no clear adverse effects in females. An adverse effect of treatment on fertility cannot therefore be concluded with any certainty.
CLH Comment: Page 66

'In the three-generation reproductive toxicity study of sodium bromide impaired reproductive capacity was also reported for males and females. F0 animals treated at 1728 mg/kg bw/day were not fertile (0%), and fertility of the 432 mg/kg bw/day dose group was 25% compared to 70% in control (unclear if statistical significantly different). In F1 fertility index was 70% at 27 mg/kg bw/day and 85% compared to 62% in control at 108 mg/kg bw/day. In F2 the lowest fertility index was 87% at 108 mg/kg bw/day. It is noted that the fertility index in the controls of each generation were unusually low (70, 62 and 52% in F0, F1 and F2 respectively). Moreover, since there is no information on clinical observations for any of the animals, and no information on body weight of animals in the dose group of 19200 ppm the quality of the study and the relevance of the findings may be questioned.
Results from the cross-mating study of the 1728 mg/kg bw/day dose group, indicate that the observed effects were due to infertility of male as well as female rats and results from the reversibility study indicate that there is some recovery from the effects on fertility.'
BSEF Comments:
Given the deficiencies of the study as indicated in the CLH report and our comments above (referring to Page 59), BSEF contends that the weaknesses of the study preclude any conclusions on fertility, even as 'indications'. This study should not therefore be used in the context of regulatory decision making, particularly as other, robust GLP and current guideline-compliant studies are available.

Section 10.10.3 Comparison with the CLP Criteria
CLH comment: Page 66
'The criteria for classification in Repr. 1B for adverse effects on sexual function and fertility are considered fulfilled since:
- There are clear evidence of effects on impaired fertility noted in the rat in studies of ammonium bromide and sodium bromide. The effects were severe, dose related and not solely a secondary consequence of general systemic toxicity. Moreover, the observed effects appears to be due to infertility of both male and female rats'.

BSEF Comment: We do not agree with the first statement since there are no guideline compliant studies on fertility with ammonium bromide, only with sodium bromide. The GLP and OECD guideline compliant 90 day study with Ammonium Bromide (Barton 2000) showed no adverse pathology of reproductive organs, even at a high dose of 500/750 mg/kg/day where significant toxicity was observed.
We do not agree that there is 'clear evidence' of impaired fertility or that the effects can be considered as severe since at the 175 mg/kg/day level in the 2 generation Sodium Bromide study there was only a slight decrease in fertility in the Parental generation (to 73% of the controls), but no effect on fertility in the F1 or F2 generations, or at the lowest dose level. The reduction in fertility was statistically significant when compared to the concurrent controls, but very close to the Historical Control values. It cannot be determined whether the reduced fertility was a male or female effect, and the affected individuals were not consistent between the two pairings. We consider therefore that the outcome can only be interpreted as 'some evidence' of adverse effect which does not meet the criteria for classification as Repro 1B.
CLH comment: Page 66
There are some evidence of effects on the male reproductive organs seen in studies of ammonium bromide and sodium bromide in the rat: decreased organ weights, histopathological changes, adverse effects on sperm count, morphology, and motility. Moreover, there were some evidence indicating reduced spermatogenesis and reduced secretory activity of prostate. These effects were considered not being solely secondary non-specific consequences of systemic toxicity
BSEF Comment:
These effects were only clearly observed at the highest, toxic dose levels, so not relevant

for classification, and the effects at the mid dose level were much less marked, often within the historical control levels and not considered biologically relevant (please also see above comments on each study evaluation in the CLH report.
CLH Guidance (2017) in section 3.7.2.2.1.1 specifically indicates that adverse effects on fertility seen only at dose levels causing 'marked systemic toxicity (eg lethality, dramatic reduction in body weight, coma) are not relevant for classification purposes'.
CLH Comment: Page 67
- There are some evidence for effects on female gonads: a decreased number of corpora lutea were noted in the subchronic toxicity studies of sodium bromide (both guideline and non-guideline) and in the two-generation reproductive toxicity study of sodium bromide. These effects were observed in the absence of severe systemic toxicity in females in the OECD TG 90-day repeated dose toxicity study, and in P females of the intermediate dose group in the two-generation reproductive toxicity study. In females of the high dose group in the two-generation reproductive toxicity study theses effects were seen in presence of excessive maternal toxicity."

BSEF Comment: There was no adverse effect on female gonads in the 90 day study with Ammonium Bromide (Barton 2000). In the 90 day with sodium bromide (Hoberman 2016b), 3 females in the 500 mg/kg/day group showed depletion of corpora lutea (one of which was based only on an incomplete set of partial sections) but no effect on the oestrous cycles. In the 2 generation study (Hoberman 2016a) 5/15 females at 500 mg/k/day had no corpora lutea, but at 175 mg/kg/day there was only 1/24 females with no corpora lutea at terminal kill. Variation in the number of corpora lutea during the oestrous cycle is expected and the stage of oestrous of each animal at terminal kill is a possible confounding factor.
We suggest therefore that the assessment of corpora lutea may have been less than rigorous and therefore the interpretation of the findings is not straightforward. This should not, therefore, be considered as sufficiently robust evidence for impairment of female fertility, or justification for classification for reproductive effects.
CLH Comment: Page 67
'Thus, in a total weight of evidence the available data provide clear evidence of an adverse effect on both male and female sexual function and fertility and there is no mechanistic evidence to indicate that the observed effects are not relevant for humans. Classification in Repr. 1B, H360F is therefore warranted.'
BSEF Comment: We agree with the earlier CLH statement that the effects at the highest dose levels are too systemically toxic to be used for classification purposes. As the CLH report agrees that the non-compliant studies are either not relevant for classification or are at best "supportive" (pages 63, 64), these should not be used in this circumstance to provide a "weight of evidence" to support classification as Category 1B.
The Guideline GLP compliant studies indicate that the effect on fertility is only marginally significant, often within the historical control levels and considered not biologically relevant (please also refer to comments on each study evaluation in the CLH report), as are the sperm observations in some males at the 175 mg/kg/day dose levels.
BSEF suggests that Classification as Repro 1B is not warranted as there is only 'some evidence' of adverse findings and therefore Repro 2 would be more appropriate based on these data;
Furthermore, recent rat gavage toxicokinetic data have demonstrated that exposure levels at which rats demonstrate minor effects far exceed tolerable human exposures. Data from a recent rat gavage toxicokinetic study (Barnett 2019) demonstrate that mean plasma exposure levels for rats treated at the same dose (ie 175 mg/kg/day for 6 weeks) are 33.4 mmol/L for males (range 18.9 – 40.4) and 30.2 (range 23.6 – 38.5) for females. These plasma levels are well in excess of human exposure levels referenced as severely toxic (>12.5 mmol/L), being within the range of 'possible coma'(25 – 37.5 mmol/L) and some individuals within these groups occasionally achieved blood levels which would be

classed as 'possibly fatal' (>37.5 mmol/L) in humans (Ellenhorn 1997).
By comparison, serum bromide concentration above 6.3 mmol/L in humans is confirmatory for bromism (Carney 1973), and levels of 21.5 mmol/L (Frances 2003) and 39.8 mmol/L (Horowitz 1997) in human case studies demonstrated neuropsychological manifestations of confusion, disorientation, auditory and visual hallucinations and ataxia. Clinical trials report subjects receiving 1 mg Br-/kg daily for 8 weeks (Sangster 1982) had mean plasma bromide concentrations of ~ 0.9 mmol/L, and in a further 12 week study at 0, 4 and 9 mg Br/kg bw/day, bromide levels were 0.08, 2.14 and 4.30 mmol/L, respectively, for males and 0.07, 3.05 and 4.93 mmol/L for females (Sangster 1983), without effect of treatment on the endocrine and neurological parameters assessed. A subsequent randomised double-blind study (van Gelderen 1992) with sodium bromide administered to 48 young (20-28 years of age) females over 3 menstrual cycles provided mean plasma bromide levels of 3.22 mmol/L and 7.99 mmol/L (at 4 and 9 mg/kg body weight/day, respectively) with no effect on T4, FT4,TBG, T3,or TSH or on the outcome of clinical neurological examinations.
Data from untreated males and females in human studies indicate serum/plasma levels of bromide of 0.04 to 0.09 mmol/L (Sangster 1983, 1986, van Gelderen 1993, Cuenca 1988, Nagamine 1988, Olszowy 1998, Campbell 1986), over 300-fold less than exposure levels associated with the minor reproductive effects in the rat studies referenced above. Furthermore, worker exposure data collected over a 5 year period (ICL Europe Coöperatief U.A., 2019, confidential) indicate mean annual serum values of 0.042 to 0.050 mmol/L, comparable to control values in clinical studies and therefore also several hundred-fold less than the exposure levels associated with minimal reproductive effects in rat studies.

It can be concluded, therefore, that reproductive and developmental effects in animals occur only at dose levels which produce plasma exposure levels which would elicit severe toxicity (or even death) in humans, such that prolonged exposure at these levels would be prevented. and/or moderated by medical intervention. Any effects occurring in rats after ≥ 10 weeks of dosages eliciting high exposure levels (including the 175 mg/kg bw mid dose in the most recent rat studies) do not represent relevant human exposures due to the high systemic toxicity in humans, and are therefore not relevant for classification purposes.

Effects on Development

Section 10.10.5: Short summary and overall relevance of the provided information on adverse effects on development.
CLH Comment: Page 75
'Available studies of developmental toxicity are two pre-natal developmental toxicity studies (OECD TG 414) of ammonium bromide in rat, two pre-natal developmental toxicity studies (OECD TG 414) of sodium bromide in rabbit and rat respectively, and one dose range finding study on sodium bromide in rabbit. Moreover, there is one dose-range finding study for reproductive toxicity of ammonium bromide in rat, one two-generation reproductive toxicity study (OECD TG 416) and one non-guideline multigeneration reproductive toxicity study of sodium bromide in rat. Finally therer are two non-guideline studies of developmental neurotoxicity of sodium bromide in rat and a number of human case reports of infants exposed to bromide during the pregnancy'.

Ammonium bromide– test guideline developmental toxicity studies
Pre-natal developmental toxicity study of ammonium bromide in rat (Study report, 2000b)
Conclusion
CLH Comment: Page 76

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."

BSEF Comment: We refer to CLH guidance (Annex I: 3.7.2.4.3) which indicates 'when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity'.

We consider that the toxicity observed at the high (limit) dose level are so severe that this dose level should be omitted from CLP assessment, as indicated in CLP Guidance, Annex 1 Section 3.7.2.5.8. We disagree with the conclusion that the major and minor malformations are a direct effect of the substance and not secondary to the effects of maternal toxicity. The maternal toxicity was excessive at the top dose level of 1000 mg/kg/day (with severe neurological signs and one death) and such disruption of maternal homeostasis could well have been the cause of the major malformations observed. The minor skeletal effects with reduced ossification are a common consequence of maternal toxicity (Chahoud 2005, Carney 2007, Kimmel 2014, Chahoud 2015, De Sesso 2018) and are reversible, as is demonstrated in the subsequent study discussed below.

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

Adverse effects on the offspring (pre-natal phase)

CLH Comment: Page 77

'At 300, 600 and 800 mg/kg bw/day there were increased incidences of foetuses with kinked and/or slightly kinked ribs and of curved scapula. There was also indications of effects on ossification at these dose levels. Similar findings were seen in the previous prenatal toxicity study of ammonium bromide from year 2000 in rat (Study report, 2000b)'….

'However, there was no dose-related increase in incidence of kinked ribs in foetuses and the kinked ribs were no longer present in weanlings of the recovery group. Therefore, the level of concern is considered by the dossier submitter to be less serious. For the observed curved scapula in foetuses there was a dose-related increase in incidence at 300, 600 and 800 mg/kg bw/day with no findings recorded at 50 mg/kg bw/day and in control group, but no reported incidence in weanlings of the recovery group. Thus, the level of concern for classification is considered to be moderate for this finding.'

BSEF Comment:

The second study used 0, 50, 300, 600 and 800 mg/kg/day, ie only a slightly lower top dose on Days 6-19 of gestation. The study design was adapted to include extra animals to allow some litters from the control and 300 mg/kg dose group to litter and raised to weaning to study any persistence of fetal effects. Severe maternal toxicity was observed at the two top dose levels and one animal at 600 mg/kg/day was euthanised on Day 11 due to the severity of the effects. Increased incidences of kinked ribs, curved scapulae and other indicators of retarded ossification were observed at the top three dose levels compared with the controls. In the 300 mg/kg/day group which was allowed to litter and raise pups to weaning, the incidence of abnormalities of the ribs, scapulae and pelvis was the same as in the controls.

We suggest that this study supports our comment above that the irreversible abnormalities observed in the previous 2000b study were related to the severe toxicity at 1000 mg/kg/day, and they were not present in this study. Furthermore, it provides

evidence for the minor and reversible nature of the rib, scapulae and other ossification effects observed at the lower dose levels.

Sodium bromide– test-guideline developmental toxicity studies

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

Conclusion

CLH Comment: Page 79

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

BSEF Comments:

Malformations in foetuses, observed at the top dose level of 1000 mg/kg/day, were of a very similar type to those observed in the ammonium bromide study (Barton 2000b), affecting the urogenital system and ribs, but with a lower incidence. At 300 mg/kg/day, reduced ossification and skeletal variants were observed. Unlike the ammonium bromide study, however, there was no effect on fetal weight at any dose level.

Maternal toxicity at 1000 mg/kg/day was very severe and similar to that observed with ammonium bromide at the same dosage with one animal having to be euthanised on Day 11 due to the severity of the effects, and maternal bodyweights were reduced in the 300 and 1000 mg/kg dose groups by 15% and 16% compared with controls.

The fetal abnormalities seen at 1000 mg/kg/day in this study were very similar to those observed in the ammonium bromide study at the same dosage, and the minor, reversible effects on ossification at the lower dose levels were also very similar. It is very likely that the absence of any effects on fetal bodyweight in this study are related to the fact that dosing stopped on Day 15 of gestation (as was the practice in the concurrent OECD guideline), and the majority of fetal growth occurs in the later stages of gestation. We therefore consider that this study does not raise the level of concern, and therefore does not support classification.

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

Conclusion

CLH Comment: Page 80

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

BSEF Comment: We agree with this conclusion.

Sodium bromide– test guideline generation reproductive toxicity studies

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

Conclusion

CLH Comment: Page 82

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

BSEF Comment: We agree with this conclusion.

Developmental neurotoxicity studies
Postnatal Growth and Brain Development study (no guideline) of ammonium bromide (Disse et al.,1996)
Conclusion: Page 84
'In the study by Disse et al (1996) it was shown that bromide cross the placenta and causes changes in the brain (reduced protein content) and olfactory tract (increased size of olfactory glomeruli) in rats following administration of sodium bromide in dams during gestation days 5-15 at a dose level of 200 mg/kg bw/day (156 mg bromide/kg bw/day). These effects persisted in the offsprings after completed excretion of bromide and showed periods of partial compensation and decompensation.'
BSEF comment: This is a non-standard, non GLP study apparently using small numbers (n= not reported) of samples per time point and investigation, with no historical data or paired body weight control data to determine whether variations in measured parameters such as brain weight, protein content and number of olfactory glomeruli were within expected variations or true effects of maternal bromide treatment. BSEF consider that the limitations of the study are such that it warrants only a Klimisch 3 score and therefore should not be considered even 'indicative' of developmental neurotoxicity.

Pre-natal developmental toxicity study (no guideline) of sodium bromide in rats (Harned et al., 1944)
Conclusion: Page 84
'Reduced learning ability was noted in rats following administration of sodium bromide in dams during gestation days 3-20. This effect was noted at a dose level of 80 mg/kg bw/day (62 mg bromide/kg bw/day) and at 120 mg/kg bw/day (93 mg bromide/kg bw/day). Dose-dependent increased pup mortality was noted starting from a dose level of 40 mg/kg bw/day (31 mg bromide/kg bw/day).'
BSEF Comment:
'Reduced learning' was determined by assessment of speed and errors in a 5-unit U maze in rats from Days 61 to 85 of age. Pups from dams in the high dose group showed more errors and were slower than those in other groups at the start of monitoring on Day 61 but were equivalent by Day 85, suggesting that growth impairment may have been the underlying cause, and differing sex ratios between each group will also have been a confounding factor. Maternal response to treatment was not reported but the pup survival rates indicate lack of maternal care which may also have contributed. BSEF consider that this very old, non-standard, non GLP study, with such limited reporting warrants a Klimisch 3 (unreliable) score and should be excluded from any assessment of developmental neurotoxicity.
We also disagree with the comment on Page 88 that there are 'some indications of developmental neurotoxicity' as detailed clinical observations in the 2 generation study (Hoberman 2016a) revealed no adverse effects on the F1 generation, and there was no effect on growth, mating performance of fertility.
Therefore we support the assessment on Page 88 which indicates that data on functional deficiency are 'not conclusive'.

Human studies of bromide
Conclusion: Page 86
There are human case reports indicating developmental growth retardation (height, weight and skull circumference) in infants exposed to bromide during the entire pregnancy. Moreover, some of the studies only report effects on the infant but no effects in the mother pointing to a higher susceptibility to bromide in the child compared to the mother.
BSEF Comment:
There are no robust clinical data on effects of bromide in human infants.
No report of single case studies can determine causality for developmental growth

retardation, not least because of uncertain exposure scenarios of both bromide products and any co-exposures, or other confounding factors such as smoking, alcohol consumption, diet or maternal age, nutritional status and health status. The authors of several papers clearly state that any association between maternal exposure to bromide and the outcome for the infant is circumstantial or may be co-incidental.

BSEF Comment on Developmental Toxicity Assessments reported on Pages 86-88:
No other Guideline studies were reviewed in the CLH report and the uncertainties in the procedure and reporting of non-guideline studies preclude their consideration for classification.
The important developmental toxicity effects are summarised in pages 86-88. The most relevant results relating to structural abnormalities are the following:
- In rats, major structural and visceral malformations were only observed at the highest dose level of 1000 mg/kg/day ammonium bromide
- At lower dose levels abnormalities/variants such as kinked ribs, curved scapulae, and other signs of retarded ossification were observed
- These effects were not observed in a follow-up study at 300 mg/kg/day when the pups were delivered and raised to weaning, clearly demonstrating that the effects were transient/reversible
- No embryofoetal effects were observed in rabbit studies
- No conclusive studies on functional deficiencies were reported

Section 10.10.6 Comparison with CLP Criteria
CLH Comment: Page 88
'The criteria for classification in Repr. 1B for adverse effects on the development of the offspring are considered to be fullfilled since there is clear evidence of adverse effects on the development of the offspring recorded in studies of ammonium and sodium bromide. The effects were of high concern, dose related and evident also at dose levels where there was no overt maternal toxicity:
- A dose-related increased incidence of displaced testis was noted at 100 and 300 mg/kg bw/day (dose levels without maternal toxicity) and 1000 mg/kg bw/day (dose level with maternal toxicity) in the pre-natal developmental toxicity study of ammonium bromide in rat.
- A statistically significant increase in incidences of visceral malformations (reduction or absence) were seen at a dose level of 1000 mg/kg bw/day in studies of ammonium bromide and sodium bromide in rats. These defects observed in the urogenital system, uterine, spleen and thyroid at a high dose level in two studies are considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female.
- A dose-dependent increase in incidences of skeletal abnormalities and variants (kinked ribs, curved scapulae and incomplete ossification of ribs) were observed at lower dose levels (from 100 mg/kg bw/day) without associated reductions in foetal weights and without maternal toxicity in two studies of ammonium bromide in rat. In the pre-natal developmental study of sodium bromide in rat skeletal malformations (ribs) were recorded at higher doses (1000 mg/kg bw/day) with maternal toxicity (clinical sign of neurotoxicity) and skeletal anomalies (ribs, cranial centres and sternebrae) were recorded at lower doses (300 mg/kg bw/day) without maternal toxicity. These skeletal abnormalities are also considered to reflect a selective effect on embryofoetal development and not a secondary effect resulting from toxicity to the parent female.
BSEF Comments: The criteria for Classification in Repr.1B for developmental effects are not fulfilled because the severe irreversible malformations were only observed at the highest dose level of 1000 mg/kg/day of ammonium and sodium bromide which is severely toxic to the dams (including deaths) and should therefore not be considered for

classification. The other minor abnormalities/variants in ribs, scapulae and other incomplete ossifications, observed at the lower dose levels, are commonly observed in developmental toxicity studies at doses producing minor toxicity and are normally reversible before the age of weaning, as was demonstrated in the follow-up study (Barton 2007a) and do not therefore warrant classification.
We question that the first point to justify classification as Category 1B was the finding of displaced testes at 100, 300 and 1000 mg/kg/day ammonium bromide, since this effect was only observed in the first study (Study 2000b) at a low incidence, and is a common finding in this laboratory and in others (Lang 1993) since the testes descend through the abdomen during normal development (Fiegel 2011). As reported in Page 76 of the CLH report, the incidence of the defect was 4%, 8% and 10% in the 100, 300 and 1000 mg/kg/day groups respectively, compared with 1.6% in the controls. The lowest incidence of 4% was within the historical control range of 0 - 4.1%, and the statement that the 300 mg/kg dose level was "without maternal toxicity" is inconsistent since page 76 states "Clinical signs (piloerection) were also noted in dams at 300 mg/kg bw/day." Displaced testes were not observed in any of the other developmental toxicity studies in rats, or in the 2 generation study in rats (Hoberman 2018b). Since it also states on page 68 of the CLH report that no statistical analysis was performed, it is not clear if the incidences were statistically significant but they are considered not toxicologically significant.
The second point to justify the classification refers only to the malformations observed at the top dose level of 1000 mg/kg/day which has already been shown to be a severely toxic dose level to the dams, and so does not support the classification.
The third point to justify the classification relates to the dose related abnormalities/variants of kinked ribs, curved scapulae and other incomplete ossifications eg of the ribs. As discussed above, these are commonly observed variations in ossification, and were shown in study 2007a to be reversible by the age of weaning. They do not justify classification as Category 1B.
The subsequent 5 points made in the justification do not provide any strong supportive evidence and do not add to the weight of evidence as claimed in page 89 to justify the classification as Category 1B.
BSEF are of the opinion that the developmental toxicity studies and the 2 generation toxicity study show that major irreversible developmental toxic effects are only observed at the severely toxic dose level of 1000 mg/kg /day of both sodium and ammonium bromide and are secondary to the disruption of maternal homeostasis elicited by severe toxicity. The minor reversible effects on ossification do not warrant classification at all, and certainly not higher than Category 2.
In the recent rat gavage toxicokinetic study (Barnett 2019), the mean plasma exposure level for female rats treated at 175 mg/kg/day for 6 weeks was 30.2 (range 23.6 – 38.5). This level is in excess of human exposure levels referenced as 'severely toxic' (>12.5 mmol/L), and within the range eliciting 'possible coma'(25 – 37.5 mmol/L), yet it is still 40% lower than the 300 mg/kg/day level associated with transient ossification delays in the studies described above. It is considered therefore that the effects observed in rat developmental and reproductive toxicity studies have no relevance for human safety assessment and do not support classification.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation
Postnatal study of sodium bromide and potassium bromide in rat (Vobecký et al., 2005)
CLH Comment: Page 92
'Thus, bromide can be transferred not only to the foetus but also via mothers milk to their pups. The reduced body weight and survival of the pups in the high dosage level is likely to be a consequence of reduced milk production in dams resulting in a state of malnutrition and lowered viability in pups.'

BSEF Comment:
The non-guideline study reported only indicates that bromide in the milk is 'one possible reason for the reduced growth and survival of pups'. This study and the Pavelka study (also on Page 92) from the same institute rely on maternal weight change (over a 50-80 minute window to nurse their litter after a 10-11 hour separation and a dose of oxytocin) as a proxy for milk production and do not account for other possible changes in maternal body weight and food intake. The 10-11 hour separation between dam and pups alone is likely to affect hydration, growth and survival of pups, The publication also fails to address the maternal toxicity at the 1g/L (limit) and 5g/L dose used and the resulting maternal toxicity/behavioural changes which would also have adversely affected maternal care.

BSEF Comment on Classification for Lactation:
The high dose levels, small group size and non-standard procedures render the quoted animal studies unsuitable for the purposes of classification.
The clear lack of any adverse effects on the F1 or F2 generations in the guideline compliant 2-generation study on sodium bromide provides strong evidence that ammonium and sodium bromide should not be classified for effects on lactation H362.
The human data is from a single 1938 case study (Tyson et al 1938) which cannot determine causality (Richason 2009). The American Academy of Paediatrics classifies bromides as compatible with breast feeding (AAP 2001).

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ECHA note – An attachment was submitted with the comment above. Refer to public attachment Charles RiverReproductiveToxicologyHistoricalControlDatainRats.pdf

Dossier Submitter's Response

Thank you for your comments.

Most of your concerns for classification in reproductive toxicity are already discussed in the CLH-report. Here we respond to and try to elaborate further on your main concerns.

Adverse effects on sexual function and fertility

Section 10.10.2: Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility:
Regarding your comment: *"This means that the only results relevant for classification are a slight decrease in fertility in the Parental generation to 74%, which was only insignificantly lower than the historical control range (75 to 100%), and showed inconsistency between the affected individuals at each pairing. No effect on fertility was apparent in the F1 or F2 generations, or at the lowest dose level of 50 mg/kg/day."*

We consider that the most important control is the concurrent control and in this case the fertility index in the Parental generation was decreased to 73% (first cohabitation, $p \leq 0.05$) and 74% (second cohabitation, $p \leq 0.01$) at 175 mg/kg bw/day compared to control. Compared to the available HCD this is still outside the range (75-100%). Moreover, the study period of the HCD (2008-2016) is much wider than what is recommended in OECD GD 43 (*If historical control data are used, the most appropriate of these are from studies conducted in the same laboratory, within a reasonable amount of time prior to the study being interpreted (e.g., ± 2 years) in order to avoid genetic drift in the laboratory animal population, and under the same study conditions (e.g., identical species, strain, source, age, vehicle, route and duration of administration, technical personnel, etc.)*). Thus, comparison with the submitted HCD is of less relevance.

In your comment you make a point that there is an inconsistency in fertility index between affected individuals in the two cohabitations of the P generation. Although there were 2 out of 22 males and 5 out of 22 females that were reported to have decreased reproductive capacity in both cohabitations, there were additionally 11/22 affected males and 5/22 affected females in either one of the cohabitations (excluding those animals that were not paired in the second cohabitation or animals that were euthanized before second cohabitation). It is well known that rats have a very high reproductive capacity, and for that reason the observed effects on fertility still demonstrates that the fertility is disturbed but not completely diminished. We therefore consider that it is not contradictory that different individuals are affected in the two cohabitations of the P generation.

The study did not however reveal this disturbance in the successive generation F1. Why there was no effect on fertility index at the same dose level in F1 generation, where only 1 out 15 females did not get pregnant (fertility index 93.3% for both males and

females), we do not have a clear answer. Nevertheless, we consider that the effects on fertility in P generation cannot be disregarded.

Regarding your comment: *"In the 90 day toxicity study at 175 mg/kg/day the number of sperm with detached/no head was higher than the concurrent control but considered unlikely to have been an effect of treatment as it was closer to the expected (historical control mean) value than was the unusually low control value, and there were no clear adverse effects in females, so no adverse effect of treatment on fertility is concluded."*

We note that the study period of the HCD (1996-2012) for morphology of sperm is much wider than what is recommended in OECD GD 43 (see above) and is thus of less relevance for comparison. Moreover, the median value (and not mean) for this parameter would be more appropriate for comparison since this better reflects the actual data and the influence of large variations in one or a few animals.

Regarding your comment: *"Several non-guideline studies are also reviewed in the CLH Report, but BSEF consider that these are not relevant for classification, not even as' supportive', owing to uncertainties resulting from non-standard methodologies, unconfirmed dosages, small sample sizes and/or limited reporting."*

Non-guideline studies are indeed relevant to review and present for transparency in the hazard assessment. Morover, findings in these studies point to similar effects and thus give a consistent picture of potential adverse effects of bromide that is relevant to consider in a total weight of evidence assessment.

Ammonium bromide - non-guideline reproductive toxicity studies

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

Regarding your comment: *"This study is considered not suitable for classification purposes for the following reasons: Although GLP compliant, this is a dose-range finding study, falls short of an OECD Guideline 421 compliant screening study (which ECHA indeed define as 'not meant to provide complete information on all aspects of reproduction and development') and the small group size precludes any definitive assessment of reproductive capacity."*

We agree that a OECD TG 421 study does not provide complete information on all aspects of reproduction and development, and that the dose-range finding study has a small group size precluding statistical analysis of findings etc. However, this does not preclude to use observed adverse findings from such studies as (supporting) evidence for classification. In particular when they are in line with findings from other studies, which is the case for the dose-range finding study for reproductive toxicity of ammonium bromide in rat (Study report, 2001).

Regarding your comment: *"We disagree that the general toxicity was not significant. Reductions in body weight were reported in males at 6400 ppm and 3200 ppm, but throughout the toxicology programme it is clear that clinical signs are the most sensitive marker of toxicity, and the CLH report acknowledges this in the proposed classification for sedative effects. In this study, even though clinical signs were only recorded at the minimum of once daily, signs in males included the typical rolling gait, piloerection and hunched posture and females showed signed of hyperactivity. Both sexes also showed staining and unkempt appearance – attributed to their 'generally ill condition'. Collectively, these signs are clear evidence of clinical condition so perturbed as to interfere with mating and reproductive performance, including litter loss due to poor*

maternal care."

In the dose-range finding study for reproductive toxicity of ammonium bromide in rat (Study report, 2001) body weights in males at 6400 ppm were 94% of control during premating (weeks 1 and 2) and body weight gains were 66% of control (29 g versus 44 g in control) during this time period. Other than that there were no differences in body weights compared to control that would indicate marked general toxicity during premating that would interfere with mating and reproductive performance. There were also no significant effects on body weight or body weight gains in females during premating.
With regards to the clinical condition of the animals and interference with mating and reproductive performance it could be noted that at 6400 ppm 4/10 females were observed with hyperactive behaviour and 10/10 females with rolling gait (weeks 2-11). Nevertheless, 7/10 females had signs of mating. In contrast, only one became pregnant. At 3200 ppm there were 9/10 males with rolling gait, but only two males did not sire. At the same dose, 6/10 females were observed with rolling gait and only one was not pregnant. Is is therefore clear that mating did occur, as evidenced by "vaginal plug" or sperm, and consequently we do not consider that the inability to conceive is secondary to the observed clinical signs.

We do agree with the comment that litter loss due to poor maternal care is probably caused by perturbed clinical condictions of the dams.

Regarding your comment: *"We would also like to draw your attention to the fact that the target dosages of Ammonium Bromide for the study were 120, 240 and 480 mg/kg bw /day but actual dosages generally exceeded the target, especially in pregnant and lactating females"*

We note the actual achieved dosages of ammonium bromide in the dose-range finding study for reproductive toxicity (Study report, 2001) as pointed out in your comment. In this study, findings of decreased fertility index was used in the total weight of evidence for assessment of adverse effects on sexual function and fertility. Thus, information from lactating females are not taken into consideration.
Since classification in reproductive toxicity is based on the strength of evidence and is not potency based there are no guidance values for classification and therefore this information does not influence the outcome of the classification. Moreover, doses are still below the "limit dose" as recommended in OECD TG 421.

Regarding your comment: *"At 3200 ppm, fertility index was lower than control values as 2 males did not sire a pregnancy but, as non-mated males were replaced with proven males, only one female was not pregnant. The Study Director considered the difference from control too small to indicate an effect of treatment."*

We still think that 2/10 animals not siring a litter points to an effect in treated males since at the next higher dose level only 1/10 males sired a litter.

Regarding your comment: *"It is of note that the fertility index was calculated as the percentage of pairings that resulted in pregnancy, which emphasises the difference whereas conventionally this is divided into mating index (percentage of pairings that result in matings) and fertility index (percentage of matings that result in pregnancy)."*

When assessing the individual data on the number of females with clear indication of mating it can be seen that only one female out of seven with clear indication of mating became pregnant at 6400 ppm, thus female fertility index (calculated as percentage of matings that result in pregnancy) is 14%.

Regarding your comment. *"The variation in dosage over the treatment period, which did not follow the same pattern in each sex/dose group, is considered to affect the interpretation of results, given the known steep dose response curve. We therefore consider that no firm conclusions on treatment-related reproductive effects can be drawn from this inadequate dose-range finding study."*

Since the classification for reproductive toxicity is not dependent on actual used doses we do not consider that this variation in doses preclude the inclusion of the findings of decreased fertility index in the total weight of assessment for classification.

Sodium Bromide – test guideline reproductive toxicity studies

Two generation reproductive toxicity study of sodium bromide (Study Report, 2016)

Regarding your comment: "It should be noted that the fertility index in the mid dose group, although significantly lower than concurrent controls was very close to the historical control range, as is demonstrated in the report These historical control data have been collated from 187 studies conducted in the crl:CD(SD)rat the conducting laboratory since 2008 (https://www.criver.com/sites/default/files/resources/ReproductiveToxicologyHistoricalControlDatainRats.pdf) and therefore we disagree with the comment that the validity of the statements referencing these data is low."

In OECD GD 43 it is stated: *If historical control data are used, the most appropriate of these are from studies conducted in the same laboratory, within a reasonable amount of time prior to the study being interpreted (e.g., ± 2 years) in order to avoid genetic drift in the laboratory animal population, and under the same study conditions (e.g., identical species, strain, source, age, vehicle, route and duration of administration, technical personnel, etc.)*.
The period for collection of historical control data submitted spans 8 years (2008-2016) and therefore a lot wider than what is recommended in the OECD guideline. Thus, this makes the comparison with historical control data less relevant (also stated earlier in respononse to comment no. 8).

In addition, there is no median value of the HCD presented for comparison as recommended in the GD. This would describe the varation of the data better.

Regarding your comment: "*In total, over both cohabitation periods, all males in Groups 1, 2 and 3 mated at least one female. All control males impregnated at least one female and there was only one male in the 50 mg/kg/day group which did not achieve a pregnancy. At 175 mg/kg/day, although reduced pregnancy rates were observed at both cohabitation periods compared to concurrent controls, unusually, the affected animals differed and in total only 2 males did not impregnate a female, giving an overall male fertility index of 91.7% (within the expected range). This was likely a deficit in these males, as neither of the treated females allocated to one male became pregnant at the alternative pairing and the allocated untreated female was not mated, but the females allocated to the other male both became pregnant with alternative males."*

Please refer to our response earlier in this comment on the observation that affected indivual animals differed between cohabitations.

Regarding your comment: *"All females in Groups 1, 2 and 3 mated during either the first or second cohabitation periods. Five females in the 175 mg/kg/day group did not get pregnant from either pairing (with treated males only), giving an overall female fertility index of 77.3% (17/22), below the concurrent control value but within the expected range. Only two of these females showed marked depletion of corpora lutea at histopathology and no corpora lutea at ovarian follicle examination. One of these females had also shown extended estrus (9 days) and a further female, showed extended periods of diestrus (which may indicate pseudopregnancy). Another female which had marked depletion of corpora lutea at histopathology and no corpora lutea at follicle counting, was pregnant at the first pairing (but not at the second).Therefore, the conclusion of 'clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects' cannot unequivocally be drawn from these findings, owing to the potential for adverse effects on reproductive performance consequent to treatment-related effects on condition (including sedation), and to the difference in performance of individuals in both cohabitation periods."*

See also our reaction to similar statement earlier in this comment 8.

Regarding the individual female with marked depletion of corpora lutea at histopathology and no corpora lutea at follicle counting being pregnant in the first cohabitation but not the second, we would like to point to the fact that, obviously, counting of follicles can only be performed at termination, i.e. after second cohabitation. And indeed, this female did not get pregnant in second cohabitation.

The following points regarding reduction in number of corpora lutea can be noted from the Study report (extracts from the original study report):

- *At 500mg/kg/day, 5/8 terminal kill females evaluated had no corpora lutea present, and there were 5 further females terminated early which also had no corpora lutea. As 3/20 females treated at 500 mg/kg/day in the recent 90 day study [Study report 2016b] had no corpora lutea present, and as a reduction in the number of corpora lutea per female was observed at the high dose level of 19200 ppm/kg diet (in excess of 1000 mg/kg body weight/day) in a published study (van Logten et al (197412), this finding may be related to administration of sodium bromide*
- *As the 175 mg/kg/day dose group females in the F1-Generation appeared to have fewer atretic follicles and follicular types were not as well represented, the possibility of an effect of sodium bromide treatment cannot be discounted*
- *With regard to Group 3 F1-generation female rats, while corpora lutea were present in all animals evaluated, a qualitative notation was made in the data that for this group in general, corpora lutea were largely regressed. Since depletion of corpora lutea is commonly associated with this test article, an effect of administration of Sodium Bromide should not be ruled out*
- *Therefore, under the conditions of the protocol, although administration of Sodium Bromide did not appear to affect the development of primordial follicles, there appeared to be an effect of this test article upon the development of copora lutea in both P and F1-generation female rats.*

Regarding your comment: *"Determination of the number of corpora lutea was variable and depends on the oestrous stage at termination. Ovarian follicle counts seem to be a more reliable endpoint. We recommend to not draw conclusions on any possible substance-related effect from these variable counts of corpora lutea, as variation in numbers is expected within a normal cycle (Yoshida 2009)."*

See also our response above.

We agree that histopathological evaluation of ovaries is complicated and not straight forward for several reasons.
We also note in OECD GD 43 that "multiple litters produced by a single dam will compromise corpora lutea count; in dams after multiple pregnancies, corpora lutea counts are not likely to be reliable, since corpora lutea remnants from previous pregnancies may be included in the count." Consequently, due to the design of this two-generation reproductive toxicity study with two cohabitations, the number of corpora lutea may have been an overestimation, and therefore the toxicity of the female gonads may have been underestimated.

Nevertheless, since depletion of corpora lutea is seen in more than one study (both guideline and non-guideline) these findings cannot be disregarded.

Regarding your comment: *"It should be noted that all findings in the 175 mg/kg/day dose group that were flagged as statistically significant compared to the concurrent control group where well within the historical control range and therefore these changes are not toxicologically relevant. As in many laboratories, the variation observed in control males for these parameters is relatively high and we do not think it is justified to conclude a clearly substance-related effect from these findings. As acknowledged in the CLH report, the findings did also not correlate with the pregnancy outcome of the pairing"*

Concerning findings being within the range of historical control data, see our earlier respone above.
We consider that statistically significant changes compared to control in sperm parameters still gives an indication that the male reproductive organ may be a target organ for the test substance and histopathology of gonads may be among the most sensitive parameters to detect adverse effects on male fertility. Furthermore, changes in sperm parameters may provide important information because in humans even a slight reduction in sperm quality/count may be critical for fertility.
We agree that there were no correlation to the functional reproductive performance, but would like to emphasize that mechanistic evidence is not needed to explain deficit functional performance for classification for adverse effects on sexual function and fertility.

Sodium bromide – non-guideline reproductive toxicity studies

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

Regarding your comment: *"It is of note that levels of bromide reported in maternal plasma after 7 months of treatment in this publication range from 0.5 mmol/L at 75 ppm to 7.8 mmol/L at 1200ppm (the NOAEL for reproductive effects) and compare to human reference values from background levels up to the therapeutic range.*
However, at the 4800ppm LOAEL, maternal plasma levels were 27.6 mmol/L ie in excess of human exposure levels referenced as 'severely toxic' (>12.5 mmol/L), and within the range eliciting 'possible coma'(25 – 37.5 mmol/L). It is evident, therefore that any reproductive effects occur at exposure levels which are not relevant for human exposures"

Classification is based on identification of hazard. Exposure considerations are not relevant for classification. Nevertheless, it is noted that the very high dose levels used in this rat study give rise to plasma levels achievable in humans. In the publication by Lugassy and Nelson (2009), toxic concentrations of bromide for humans were now

considered to be >50 mg/dL (50 mg/dl = 46 mmol/L) or 6.3 mEq/L, and severe systemic toxicity can occur at concentrations >200 mg/dL (200 mg/dl = 185 mmol/L) or 25 mEq/L.

Regarding your comment: *"BSEF contends that the published studies are not of the quality expected for safety evaluation and have been superseded by new GLP compliant studies, which should form the basis of the assessment. A full critique of the publications is presented as Appendix 1, but the salient points are:*
• The publications would warrant a Klimisch score of 3 at best and as such should not be the basis of classification"

We do agree that this study has limitations and that it is not robust enough to draw a firm conclusion from findings on reproductive toxicity by its own. Hence, we have, as you rightly have pointed to, only concluded that there were indications of effects on fertility in this study and added this information to the total weight of evidence evaluation.

It could be noted that in the REACH Registration a Klimisch score of 2 is given to this study.

Regarding your comment: *"Although the CLH report only speaks about 'indications of impaired fertility' BSEF contends that the weaknesses of the study preclude any conclusions on fertility, even as 'indications'. Therefore, in the context of regulatory decision making, when GLP studies compliant with current guidance are available, these data are inadequate and too unreliable for consideration"*

As written in the report, we do not consider that any reliable/definitive conclusion can be made from this study but persist in that the study gives "indications" of an adverse effect on sexual function and fertility.

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

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

Regarding your comment: *"Retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm is a subtle change which can occur in isolation or can be associated with abnormalities in sperm parameters (number, motility, and/or morphology) or with other degenerative changes seen in the seminiferous tubule epithelium. These changes can affect fertility and can be associated with testosterone deficiency but there was no evidence of this in any other parameters measured on this study. Retained spermatids are not unknown in untreated animals, so the observation is recorded when an increase over the control incidence is identified. It should also be noted that these findings were not apparent in recovery animals"*

From the study report (2016b) it is noted that the study author states:
"Although a few retained spermatids can be seen normally, this finding occurred in an obvious increase over controls in 2/10 in the 175 and in 9/9 500 mg/kg terminal euthanasia animals. This change can occur in isolation (e.g., boric acid, 2, 5-hexanedione) or can be one of many other degenerative changes (e.g., ethylmethanesulphonate) seen in the seminiferous tubule epithelium."

The incidence of retained spermatids in control was 0/10.

We would like to make clear that hormone levels of testosterone has not been determined in this study, thus it is not possible to make a conclusion on the correlation of retained spermatids with testosterone levels.

Regarding your comment: *"In accordance with the authors of the study, BSEF do not consider the effects on the sperm parameters in the 175 mg/kg dose group as treatment related, as the number was well within the historical control range and the control values were at the low end of the historical control or below (for detached heads, see table at p. 61 of the CLH report). Thus although there was statistical significance when compared to concurrent controls these findings are not considered biologically significant"*

In the study report, the study author states: *"At 175 mg/kg/day, the number of sperm with detached/no head was higher than the concurrent control but considered unlikely to have been an effect of treatment as it was closer to the expected (historical control mean) value than was the unusually low control value"*.

The dossier submitter notes that the historical control data spans years 1996-2012 which is considered to be a much too long time period and too far off from when the current study was performed than what is recommended in OECD GD 43 and therefore (as stated in the CLH-report), less relevant for comparision. The submitted HCD (from 1996-2012) for detached head was mean: 6.0 and range: 1.0-19.4. The incidences of detached heads in the current study from 2016 were: 0.8, 1.0, 5.0* and 20.6** in 0, 50, 175 and 500 mg/kg bw/day dose groups respectively.

Also, in the the two-generation reproductive toxicity study (2016a), the incidences of detached head were increased: 4.4, 6.0, 7.3*, 23.7** at 0, 50, 175 and 350 mg/kg bw/day respectively, pointing to a consistent picture and an effect difficult to disregard.

Regarding your comment: *"We do not consider the isolated finding of no corpora lutea in the ovaries of 3 females of the high dose group as indicative of a substance related effect for the following reasons: there were no other effects on female reproductive organs and there were technical issues with sectioning of the ovaries. This finding can, at most, be regarded as inconclusive."*

See our earlier reasoning for this above in this comment 8.

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

Regarding your comment: *"The conclusion deviates from the principle that studies at excessively high doses should not be used in the assessment when other studies are available. We have further analysed the study and would like to draw your attention to the fact that, as this was a dietary study, dose levels had a wide range: Calculation of the Sodium Bromide intakes based on the food intake and body weight data available indicate that these data show that exposures for females ranged from 4-15, 17-60, 70-240, 278-960 and 1113- 3840 mg/kg bw/day for the 75, 300, 1200, 4800 and 19200 ppm dose groups, respectively, with the highest exposure at the start of the study. For males, the corresponding intakes ranged from 4-14, 15-55, 60-220, 247-878, 1190-3360 mg/kg bw/day for the low to high doses. The high dose level was therefore well in excess of the limit dose of 1000 mg/kg/day defined OECD guidance. Decreased prostate weight was observed in males at 4800 and 19200 ppm but there was no significant change in relative testes or ovarian weight, which is pertinent for the assessment of reproductive effects since testis weight is the primary indicator of testicular damage. The description of the*

other observations is ill-defined, vague and only reported at excessive dose levels. BSEF considers, therefore, that this study should not be included in the assessment"

As stated in the CLH-report, we agree that the histopathological findings relevant for reproductive toxicity are observed at very high dose levels and are thus only considered as supporting evidence for classification.

Section 10.10.3 Comparison with the CLP Criteria

Regarding your comment: *"There was no adverse effect on female gonads in the 90-day study with Ammonium Bromide (Barton 2000). In the 90 day with sodium bromide (Hoberman 2016b), 3 females in the 500 mg/kg/day group showed depletion of corpora lutea (one of which was based only on an incomplete set of partial sections) but no effect on the oestrous cycles. In the 2 generation study (Hoberman 2016b 5/15 females at 500 mg/k/day had no corpora lutea, but at 175 mg/kg/day there was only 1/24 females with no corpora lutea at terminal kill. There were, however, 3 in various groups on the study were reported to have corpora lutea, even though they were not mated or pregnant. We suggest therefore that the assessment of corpora lutea may have been less than rigorous and therefore the interpretation of the findings is not clear. This should not be taken as evidence for impairment of female fertility leading to a classification"*

You indicate in your comment that there is a discrepancy between the 90-day repeated dose toxicity study in rat of ammonium bromide compared with 90-day repeated dose toxicity study of sodium bromide in rat and the the two-generation reproductive toxicity study of sodium bromide in rat.

First we would like to repeat that we consider the read-across from sodium bromide for reproductive toxicity as valid. Second, if there are contradictory findings between a repeated dose toxicity study and a reproductive toxicity study the findings from the reproductive toxicity studies are considered as more relvant than the repeated dose toxicity study.

The findings on ovaries are consistently seen in the two guideline studies on sodium bromide from 2016. It is to be noted also that in these two studies the test substance is administered via oral gavage, whereas in the 90-day repeated dose toxicity study of ammonium bromide the test substance is administered orally via the diet.

Regarding your comment: *"We do not agree with the first statement since there are no guideline compliant studies on fertility with ammonium bromide, only with sodium bromide. The GLP and OECD guideline compliant 90 day study with Ammonium Bromide (Barton 2000) showed no adverse pathology of reproductive organs, even at a high dose of 500/750 mg/kg/day where significant toxicity was observed."*

Again, we would like to stress that we consider the read-across from sodium bromide as valid and justified for ammonium bromide. Secondly, the robustness of assessing effects on reproductive organs is higher in generational studies were the entire course of spermatogenesis and folliculogenesis is covered, compared to repeated dose toxicity or reproductive screening studies.

Regarding your comment: "*These effects were only clearly observed at the highest, toxic dose levels, so not relevant for classification, and the effects at the mid dose level were much less marked, often within the historical control levels and not considered biologically relevant (please also see above comments on each study evaluation in the CLH report. CLH Guidance (2017) in section 3.7.2.2.1.1 specifically indicates that adverse effects on*

fertility seen only at dose levels causing 'marked systemic toxicity (eg lethality, dramatic reduction in body weight, coma) are not relevant for classification purposes'."

A steep dose response for general toxicity may mask adverse effects on fertility and sexual function. Therefore it may be important to note evidence of dose-dependent changes, even if they are mild at lower doses where general toxicity is not adverse when marked effects are seen at high dose in presence of severe general toxicity.

Regarding your comment: *"As the CLH report agrees that the non-compliant studies are either not relevant for classification or are at best "supportive" (pages 63, 64), these should not be used in this circumstance to provide a "weight of evidence" to support classification as Category 1B."*

According to the Guidance on the Application of the CLP Criteria, 3.7.2.3: "Appropriate classification will always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach". Thus, supportive evidence from non-guideline studies contributes to the total weight of evidence. Sufficient evidence from several independent sources of information giving a consistent picture may support classification in category 1B.

Regarding your comments: *"Data from a recent rat gavage toxicokinetic study (Barnett 2019) demonstrate that mean plasma exposure levels for rats treated at the same dose (ie 175 mg/kg/day for 6 weeks) are 33.4 mmol/L for males (range 18.9 – 40.4) and 30.2 (range 23.6 – 38.5) for females.*
These plasma levels are well in excess of human exposure levels referenced as severely toxic (>12.5 mmol/L), being within the range of 'possible coma'(25 – 37.5 mmol/L) and some individuals within these groups occasionally achieved blood levels which would be classed as 'possibly fatal' (>37.5 mmol/L) in humans (Ellenhorn 1997).
By comparison, serum bromide concentration above 6.3 mmol/L in humans is confirmatory for bromism (Carney 1973), and levels of 21.5 mmol/L (Frances 2003) and 39.8 mmol/L (Horowitz 1997) in human case studies demonstrated neuropsychological manifestations of confusion, disorientation, auditory and visual hallucinations and ataxia. Clinical trials report subjects receiving 1 mg Br-/kg daily for 8 weeks (Sangster 1982) had mean plasma bromide concentrations of ~ 0.9 mmol/L, and in a further 12 week study at 0, 4 and 9 mg Br/kg bw/day, bromide levels were 0.08, 2.14 and 4.30 mmol/L, respectively, for males and 0.07, 3.05 and 4.93 mmol/L for females (Sangster 1983), without effect of treatment on the endocrine and neurological parameters assessed. A subsequent randomised double-blind study (van Gelderen 1992) with sodium bromide administered to 48 young (20-28 years of age) females over 3 menstrual cycles provided mean plasma bromide levels of 3.22 mmol/L and 7.99 mmol/L (at 4 and 9 mg/kg body weight/day, respectively) with no effect on T4, FT4,TBG, T3,or TSH or on the outcome of clinical neurological examinations"

"It can be concluded, therefore, that reproductive and developmental effects in animals occur only at dose levels which produce plasma exposure levels which would elicit severe toxicity (or even death) in humans, such that prolonged exposure at these levels would be prevented. and/or moderated by medical intervention. Any effects occurring in rats after ≥ 10 weeks of dosages eliciting high exposure levels (including the 175 mg/kg bw mid dose in the most recent rat studies) do not represent relevant human exposures due to the high systemic toxicity in humans, and are therefore not relevant for classification purposes"

The classification criteria do not consider exposure assessments. Rather, it is the inherent toxicological properties of the substances that lead to classification.

We note that in the publication by Lugassy and Nelson (2009) that toxic concentrations of bromide in humans are now considered to be >50 mg/dL (46 mmol/l) or 6.3 mEq/L, and severe systemic toxicity can occur at concentrations >200 mg/dL (185 mmol) or 25 mEq/L. This means that dose levels of 175 mg/kg bw/day used in rat studies give plasma levels below dose values.

According to CLP AnnexI, 3.7.2.3.2 and 3.7.2.5.5, when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans, then the substance that produces an adverse effect on reproduction in experimental animals should not be classified. In this case, bromide has been observed to cause adverse effects on reproduction in experimental animals, and there is no data showing that the hazardous property will not be expressed in humans.

Human clinical studies included in this CLH-report (Sangster 1982, Sangster 19833, van Gelderen, 1992) measured bromide levels in serum and effects on CNS and the endocrine system (serum levels of hormones). In these studies there is no assessment of adverse effects on reproductive organs or on sexual function or fertility and no correlation to reproductive outcome was performed.

Furthermore, dose effect levels do not need to be the same in humans and animals. Humans appear to have a higher sensitivity with regards to general systemic toxicity of bromide than rat, and could as well have a lower dose effect level for reproductive toxicity than rat.

There are a no epidemiological studies available on the potential effects of bromide exposure on parameters related to fertility or pregnancy outcomes.

Adverse effects on the development of the offspring

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

Regarding your comment: *"We consider that the toxicity observed at the high (limit) dose level are so severe that this dose level should be omitted from CLP assessment, as indicated in CLP Guidance, Annex 1 Section 3.7.2.5.8. We disagree with the conclusion that the major and minor malformations are a direct effect of the substance and not secondary to the effects of maternal toxicity. The maternal toxicity was excessive at the top dose level of 1000 mg/kg/day (with severe neurological signs and one death) and such disruption of maternal homeostasis could well have been the cause of the major malformations observed. The minor skeletal effects with reduced ossification are a common consequence of maternal toxicity (Chahoud 2005, Carney 2007, Kimmel 2014, Chahoud 2015, De Sesso 2018) and are reversible, as is demonstrated in the subsequent study discussed below."*

The maternal toxicity at 1000 mg/kg bw/day was reported as adverse clinical observations and marked reduction of body weight gain (-18%) over the dosing period. The mortality was 1 out 24 dams in this dose group, i.e. <10% and should thus not automatically be discounted for further evaluation (CLP Annex I, 3.7.2.4.4.). Moreover, in CLP Annex I, 3.7.2.4.2. it is stated that "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by- case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be*

considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.".

From the individual data it is clear that all animals at 1000 mg/kg bw/day display clinical signs (including neurotoxicity) as also indicated in the CLH-report. There was a reduction in body weight gain (43% of control) during the first six days of treatment (Days 6-12 of gestation), and there was a notable weight loss for two of the animals of this dosage group during this period. However, at GD 18 the body weight in high dose group was similar (94%) to control body weight. Moreover, looking at individual data of the dams there is no clear correlation between severity of maternal toxicity and litter incidence of fetal abnormalities and variants. The overall litter incidence of major abnormalities was 20/22 compared to 5/22 in control. In the 100 and 300 mg/kg bw/day dose groups, there were no adverse clinical signs reported and body weights and body weight gains were similar to control. In these two dose groups the litter incidences of major foetal abnormalitites were 11/22. Curved scapula and kinked ribs were seen in all dose groups, including control but it could be noted that bilateral curved scapula was seen only in substance treated groups (litter incidence 1/22, 1/22 and 6/22 in 100, 300 and 1000 mg/kg bw/day). The litter incidence of incomplete ossification of ribs was dose-related increased from 100 mg/kg bw/day and was 0 in control.

Incidences of foetuses (litters) with major foetal abnormalities [only showing abnormalities with the highest litter incidence]

	0	**100**	**300**	**1000**
Total numbers examined	191 (22)	247 (22)	188 (22)	208 (22)
Number with major abnormality	5 (5)	17 (11)	23 (11)	85 (20)
Curved scapula	1 (1) (Litter based: 4.5%)	2 (2) (Litter based: 9%)	2 (2) (Litter based: 9%)	18 (8) (Litter based: 36%)
Kinked ribs	3 (3) (Litter based: 14%)	11 (7) (Litter based: 32%)	17 (9) (Litter based: 41%)	52 (18) (Litter based: 82%)
Incomplete ossification of ribs	0	5 (3) (Litter based: 14%)	17 (8) (Litter based: 36%)	34 (14) (Litter based: 64%)
Left kidney absent/small/displaced, with/withour absent left adrenal and absent left ureter	0	0	0	26 (7) (Litter based: 32%)
Spleen flattened and/or reduced in size	0	1 (1) (Litter based: 4.5%)	0	19 (7) (Litter based: 32%)
Narrow left uterine horn with flattened ovarian end, and displaced from ovary	0	0	0	14 (5) (Litter based: 23%)

As stated on the CLH-report, we do not consider the observed major and minor fetal abnormalities in this study as being unspecific and secondary to maternal toxicity. Moreover, in presence of some maternal toxicity there were irreversible effects such as structural malformations in foetuses in this study, i.e. absence of organs (kidney, ureter, adrenal, thyroid) that cannot be disregarded as being secondary to maternal toxicity.

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

Regarding your comment: *"The second study used 0, 50, 300, 600 and 800 mg/kg/day, ie only a slightly lower top dose on Days 6-19 of gestation. The study design was adapted to include extra animals to allow some litters from the control and 300 mg/kg*

dose group to litter and raised to weaning to study any persistence of fetal effects. Severe maternal toxicity was observed at the two top dose levels and one animal at 600 mg/kg/day was euthanised on Day 11 due to the severity of the effects. Increased incidences of kinked ribs, curved scapulae and other indicators of retarded ossification were observed at the top three dose levels compared with the controls. In the 300 mg/kg/day group which was allowed to litter and raise pups to weaning, the incidence of abnormalities of the ribs, scapulae and pelvis was the same as in the controls.
We suggest that this study supports our comment above that the irreversible abnormalities observed in the previous 2000b study were related to the severe toxicity at 1000 mg/kg/day, and they were not present in this study. Furthermore, it provides evidence for the minor and reversible nature of the rib, scapulae and other ossification effects observed at the lower dose levels."

As stated in our comments above on the PNDT study (2000b), it cannot be unequivocally demonstrated that the observed malformations manifested as absence of organs (kidney, ureter, adrenal), which are considered as irreversible effects, are secondary and unspecific effects to maternal toxicity/disturbed maternal homestasis. The dose-respone curve of bromide appears to be steep and since the dose of 1000 mg/kg bw/day was not tested in the current study, the absence of findings of major structural visceral malformations does not contradict the earlier PNDT study. On the other hand, increased incidences of curved scapula and kinked ribs, seen from 100/300 mg/kg bw/day in the earlier PNDT study (Study report, 2000b) are also seen from 300 mg/kg bw/day in this study.

It is noted that the reversibility assessment of the ribs and scapulae was only done in the 300 mg/kg bw/day dose group compared to control and not in the two higher dose groups.

Pre-natal developmental toxicity study of sodium bromide in rat (Study report, 1995)
Regarding your comment: *"The fetal abnormalities seen at 1000 mg/kg/day in this study were very similar to those observed in the ammonium bromide study at the same dosage, and the minor, reversible effects on ossification at the lower dose levels were also very similar. It is very likely that the absence of any effects on fetal bodyweight in this study are related to the fact that dosing stopped on Day 15 of gestation (as was the practice in the concurrent OECD guideline), and the majority of fetal growth occurs in the later stages of gestation. We therefore consider that this study does not raise the level of concern, and therefore does not support classification."*

In this study, findings of structural visceral malformations and skeletal abnormalities are consistent with findings in the PNDT studies of ammonium bromide in rat. None of these findings were seen in control animals or in lower dose groups. Litter incidences were:
- Absent kidney 13.6% (3/22)
- Absent ureter 18.2% (4/22)
- Absent uterine horn 9% (2/22)
- Distorted/minimally distorted/ossification irregularitites ribs 18.2% (4/22)

These effects were seen in presence of maternal toxicity manifested as clinical neurotoxicity including signs of unsteady gait (nearly all animals at each day of treatment), feet falling through cage grid floor during ambulation, poorly coordinated movements and reduced bodytone. These clinical signs were not seen in control or the lower dose groups. Maternal bodyweights were reduced in the 300 and 1000 mg/kg dose groups by 15% and 16% compared with controls, but there were no effects on fetal weights in these groups.

As stated in the CLH-report, the observed major malformations that are consistent with findings in a later PNDT study of ammonium bromide in rat, cannot be considered as being secondary to maternal toxicity and should be taken into account for the toal weight of evidence assessment for classification.

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

Regarding your comment: *"BSEF consider that the limitations of the study are such that it warrants only a Klimisch 3 score and therefore should not be considered even 'indicative' of developmental neurotoxicity."*

Indeed there are limitations of this study, and thereforewe we have stated in the CLH-report that findings are indicative but not conclusive. It could be noted that in the REACH registration this study is rated with a Klimisch score of 3 and indicated as a supporting study.

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

Regarding your comment: *"BSEF consider that this very old, non-standard, non GLP study, with such limited reporting warrants a Klimisch 3 (unreliable) score and should be excluded from any assessment of developmental neurotoxicity.*
We also disagree with the comment on Page 88 that there are 'some indications of developmental neurotoxicity' as detailed clinical observations in the 2 generation study (Hoberman 2016a) revealed no adverse effects on the F1 generation, and there was no effect on growth, mating performance of fertility.
Therefore we support the assessment on Page 88 which indicates that data on functional deficiency are 'not conclusive'."

Again, it could be noted in the REACH registration that this study is stated as being a supportive study for neurotoxicity, and is even given a Klimisch score of 2.

With regards to absence of effects in the F1 generation in the two-generation reproductive toxicity study, we would like to point to the difference in endpoints for neurotoxitiy being assessed. There were no such meaurements of maze navigation speed etc in the two-generation reproductive toxicity study as were done in the Harned study. Nevertheless, we agree that the study has limitations that prevent any firm conclusion on developmental neurotoxicity and thus our overall conclusion on developmental toxicity in the CLH-report is as you point out "not conclusive".

Human studies of bromide

Regarding your comment: *"No report of single case studies can determine causality for developmental growth retardation, not least because of uncertain exposure scenarios of both bromide products and any co-exposures, or other confounding factors such as smoking, alcohol consumption, diet or maternal age, nutritional status and health status. The authors of several papers clearly state that any association between maternal exposure to bromide and the outcome for the infant is circumstantial or may be co-incidental."*

We agree that there are no robust human studies on developmental toxicity of bromide and that there are no proven causality for developmental growth retardation. For this reason, the information included in the CLH-report from human case studies are not used in the total weight of evidence and comparison with criteria, but briefly summarised for

completeness. In any event, there are no indications that this information contradicts the available animal data.

Adverse effects on or via lactation

Regarding your comment: "*The high dose levels, small group size and non-standard procedures render the quoted animal studies unsuitable for the purposes of classification. The clear lack of any adverse effects on the F1 or F2 generations in the guideline compliant 2-generation study on sodium bromide provides strong evidence that ammonium and sodium bromide should not be classified for effects on lactation H362. The human data is from a single 1938 case study (Tyson et al 1938) which cannot determine causality (Richason 2009). The American Academy of Paediatrics classifies bromides as compatible with breast feeding (AAP 2001).*"

We agree that there is no evidence from the OECD TG 416 study on adverse effects on or via lactation. We also agree that there are some limitaitons of the Vobecký et al. (2005) and Pavelka et al. (2002) studies and that (as stated in CLH-report) the Tyson study (1938) only gives a weak indication. All in all, we agree that the evidence for effects on or via lactation may not be suffiently clear for classification and look forward to the discussion in RAC.

RAC's response

RAC acknowledges the review of the studies and detailed data analysis provided by BSEF. With respect to the comments on validity and relevance of HCD expressed in ***Section 10.10.2: Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility,*** RAC agrees with DS response. The requirements on HCD collection are clearly specified in OECD GD 43. Further, the effects on fertility in the P generation cannot be disregarded due to inconsistency in fertility index between the two cohabitations. RAC agrees with DS that also non-guideline studies can contribute to the hazard assessment, especially if results are consistent with those from guideline tests.

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

RAC notes that the study results are used as supporting evidence for classification in an overall weight of evidence approach. The role of maternal toxicity for evaluation of study results is addressed adequately by DS and considered in the RAC assessment. RAC further notes that variation in dose levels does not invalidate the results of the study as long as they do not significantly exceed the "limit dose".

Two generation reproductive toxicity study of sodium bromide (Study Report, 2016)

Comments on HCD and different reproductive outcome between cohabitations were addressed earlier. Regarding the assessment of reduced numbers of corpora lutea, RAC notes that depletion of corpora lutea observed across more than one study is an effect on fertility that should not be disregarded in a weight of evidence evaluation. It is further noted that the lack of correlation between effects on sperm parameters and the pregnancy outcome does not eliminate the concern of these effects.

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

Comments on quality of the study and exposure considerations are noted.

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

RAC notes that the increased incidence of retained spermatids at the luminal surface or in basal Sertoli cell cytoplasm appears to be dose-dependent, and thus cannot be seen as a chance finding.

Non-guideline study: 90-day oral repeated dose toxicity study (Van Logten et al., 1974)
Comment on the use of data from studies at levels in excess of limit dose is noted.
Section 10.10.3 Comparison with the CLP Criteria
RAC notes the comparative assessment of toxic bromide plasma levels in both animals and humans. RAC agrees that classification criteria do not include exposure considerations. Further, there is no specific data indicating that different toxicokinetics in humans will result in lack of reproductive toxicity.
Pre-natal developmental toxicity study of ammonium bromide in rat (Study report, 2000b)
Severity of maternal toxicity at the limit dose of 1000 mg/kg bw/day has been considered in the CLH report. RAC agrees with DS assessment that irreversible effects such as structural malformations in foetuses cannot be disregarded as being secondary to maternal toxicity.
Pre-natal developmental toxicity study of ammonium bromide in rat (Study report, 2007a)
RAC notes that the top dose of 800 mg/kg bw/day is lower than the limit dose used in the previous study, and thus does not consider the absence of major visceral malformations as contradictory. It is further noted that several skeletal effects were seen in the present study as well.
Pre-natal developmental toxicity study of sodium bromide in rat (Study report, 1995)
Comment noted.
Postnatal Growth and Brain Development study (no guideline) of ammonium bromide (Disse et al., 1996)
Comment on quality and limitation of the study is noted and addressed in ODD.
Pre-natal developmental toxicity study (no guideline) of sodium bromide in rats (Harned et al., 1944)
Comment noted.
Human studies of bromide
Discussion on the robustness of the human data and their value for the overall conclusion on classification is included in the report.
Adverse effects on or via lactation
RAC notes the lack of clear evidence for effects on or via lactation from the 2-generation study. A conclusion on classification for effects on or via lactation should be reached in plenary.

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2019	Netherlands		MemberState	9
Comment received				
The NL CA agrees with the proposed classification for reproductive toxicity as Repr. 1B (H360FD). Fertility With respect to adverse effects on fertility and sexual function, a discussion on the mode of action is welcomed. Related to this, the NL CA points to the following studies that might be also relevant for this and which could be taken into consideration: - Sangster B., Krajnc E.I., Loeber J.G., Rauws A.G. and van Logten M.J. (1982) Study of Sodium Bromide in Human Volunteers, with Special Emphasis on the Endocrine System, Human Toxicol., 1982, 1, 393-402. - Sangster B., Blom J.L., Sekhuis V.M., Loeber J.G., Rauws A.G. and Koedam J.C. (1983) The Influence of Sodium Bromide in Man: a Study in Human Volunteers with Special Emphasis on the Endocrine and the Central Nervous System, Fd. Chem. Toxic., 1983, Vol				

21 no. 4, 409-419.
- Van Leeuwen FXR, Hanemaaijer R, Loeber JG. (1987b) Mechanism of action of sodium bromide in rat thyroid [in Dutch: Het werkingsmechanisme van natriumbromide in de schildklier van de rat]. RIVM report nr 618211002.

Suggestions for discussion of the mode of action:
- The mechanism of the bromide-induced alterations in gonads is unresolved. It is feasible that the effects are secondary effects due to thyroid dysfunction. However a direct action of bromide on these organs could not be excluded (Van Leeuwen and Sangster, 1987a).
- In a 14 day study a dose of 19 200 mg NaBr/kg feed inhibits the absorption of iodine in the thyroid gland. There was an increase of TSH, decrease in T4, decrease in TPO activity and increase in relative thyroid gland weight (van Leeuwen et al., 1987b).
- Some effects on the thyroid function were found in humans. At a dose level of 9 mg bromide/kg bw/day statistically significant increased serum thyroxine and triiodothyronine concentrations were noted in female volunteers although within normal limits (Sangster et al., 1983). However, in a follow up study (Sangster et al., 1986) no significant differences were found in thyroxine concentrations in all treated groups. Also, no changes in the thyroid gland or central nervous system were found. These different effects could be partly explained due to differences in the test-persons groups. Also individual variability in reaction on the administered bromide could explain part of the differences found in these 2 studies.
- Furthermore, there are data in the open literature showing that bromine values of more than 6 mg/l could potentiate an increase in plasma TSH concentration in human, probably as a consequence of a minor inhibitory effect on thyroid activity (Allain et al., 1993).
- It is difficult to know the precise risk of sexual dysfunction associated with the pharmaceutical use of bromides since there are no adequately designed studies examining sexual dysfunction. In the open literature there are some data reporting an increased incidence of sexual side effects in patients taking antidepressant drugs containing bromide (such as impotence, decrease libido, ejaculatory delay), and it is stated that physicians should routinely inquire about such possible side effects. In humans, thyroid hormone disorders including hypothyroidism are associated with subfertility (Vissenberg et al., 2015).

Taking together this points towards that the exact mechanism of the bromide induced fertility effects is unresolved, it is not known whether the effects are due to a direct action on these organs or due to an indirect effect, but could probably mediated via thyroid dysfunction.

Developmental toxicity
With respect to adverse effects on development, the NL CA has no additional comments. The NL CA agrees with the proposed classification for reproductive toxicity as Repr. 1B (H360FD).

Lactation
With respect to adverse effects on or via lactation (H362), the NL CA does not agree with the proposed classification. The only study with exposure only via lactation (Vobecky et al 2005) showed a clear reduction of milk production, increased bromide content of the milk and effects on pup growth and survival at the highest dose. However, there were clear maternal effects including reduced water and food consumption, weight reduction of approximately 15-20% and mortality (4%). As the reduction in water consumption did not start with the first exposure, the effect is not related to the taste. The study by Carney et al. from 2004 (Toxicological sciences 82, 237-249) shows that feed restriction during lactation results in a clear reduction in pup weight. No pup mortality was observed in the study by Carney. However, this difference with the other study (Vobecky et al

2005) might be caused by the culling of the pups on day 4 in the Carney study. Therefore, the observed effects during lactation are considered more likely to be secondary to the maternal toxicity.

The NL CA points to the following studies that might be also relevant and could be taken into consideration:
- A6.8.2/01 (Dose range-finder for OECD 416; 2-generation reproduction study; CLH-report Annex I 3.10.1.1)
- Van Leeuwen et al 1983. Fd. Chem. Toxic., 1983, Vol. 21 no 4, 383-389.

These other studies also show effects during lactation, however it is unclear whether the effects on the pups were due to the in utero exposure. Therefore taken all together, the NL CA proposes no classification for effects on or via lactation.

Dossier Submitter's Response

Thank you for your support to classify ammonium bromide for reproductive toxicity as Repr. 1B (H360FD).

We also thank you for providing references and discussion on the mode of action of ammonium bromide on the observed adverse effects on sexual function and fertility. We do agree that thyroid dysfunction is one plausible mode of action for these effects.

We note that you do not agree to classify ammonium bromide for adverse effects on or via lactation and we concur that it may be discussed if evidence are sufficiently clear to warrant classification due to concerns on the robustness of data.

Regarding findings in the Dose range-finder for OECD 416; 2-generation reproduction study of ammonium bromide it is not clear from this study if the decreased mean litter/pup weights and decreased pup viability during lactation at 454 mg/kg bw/day (3200 ppm) were due to poor maternal care beacause of the observed clinical signs of neurotoxicity in the dams or if there is a direct effect of bromide on the pups. Moreover, the viability indices in the control group were unexpectedly low and therefore it is difficult to conclude on a clear direct effect of ammonium bromide on pup viability.

In the Van Leeuwen et al study (1983) the viability of the F1 pups in the 432 mg/kg bw/day dose group was markedly reduced during both postnatal days 1-4 and during post-natal days 5-21. Maternal body weights were not affected at this dose level but the clinical conditions were not reported, and thus it is not possible to conclude if the pup mortality was a consequence of poor maternal care or a direct effect not secondary to maternal toxicity

RAC's response

Thank you for your comment on the possible mode of bromide action on adversity to sexual function and fertility. RAC agrees that the data is not sufficient to conclude whether the effects are due to a direct bromide action on the reproductive organs, or are rather mediated via thyroid dysfunction. The clinical studies included in this CLH report establish a link between serum levels of bromide and effects on the endocrine system, however there is no assessment of adverse effects on the reproductive outcome.

With respect to classification for adverse effects on or via lactation, RAC agrees with the DS assessment of the evidence from the dose-range finding study and the 2-generation reproduction study with ammonium bromide. However, milk production was affected in dams of the low dose group in Vobecky *et al.* (2005) and at both doses in Pavelka *et al.*

(2002), without significant maternal body weight changes reported. Overall, it has been demonstrated that bromide can be transferred via milk to the pups, and milk production and its elementary composition was changed in dams receiving bromide during lactation. There are also some weak indications on possible effects on the central nervous system in infants following maternal intake of bromide during lactation. Therefore, RAC concludes that classification for effects on or via lactation is justified.

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	10
Comment received				
see attached pdf document In our comments submitted on 13 June 2019 via the web form, a reference was missing: Barnett, JF. (2019). 6 Week Oral (Gavage) Repeat Dose Toxicokinetic Study of Sodium Bromide in Rats, CR report no.20136246. Charles River, Horsham PA, USA. This is been added to the pdf file which is attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF comments reprotox 20190612.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
12.06.2019	Germany		MemberState	11
Comment received				
We agree with the proposed classification as Eye Irrit. 2, H319 based on conjunctival redness (mean score ≥ 2) in 4 out of 6 animals observed in an eye irritation study.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	12
Comment received				
Section 10.12 Specific target organ toxicity repeated exposure 10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure CLH report p.130 and p. 140 table 58 Loeber et al., 1983 "Reduced bodyweight was noted at 19200 ppm. Animals of this high dose group also showed a statistical significant increase in thyroid weight. Increased thyroid weight was				

also noted in animals of the 1200 ppm group after 4 weeks but not after 12 weeks. In the pituitary gland of the 19200 ppm group rats only a slight tendency towards less GH immunoreactivity was observed in comparison with the control animals. On the other hand there was distinctly more immunoreactivity for TSH and ACTH but only after 12 weeks. Histopathological changes were noted in thyroidea (increase of follicles and a decrease in their size) and in testes (reduction of tubule diameter) in animals of the 19200 ppm group, and decreased spermatogenesis were noted in this dose group animals after 12 weeks. The concentration of thyroxin, testosterone and corticosteron in the serum appeared to be decreased at the highest dose level. Reduced T4 level was also noted in animals of the 1200 ppm group (at 4 weeks only). FSH level was increased at 19200 ppm (at 4 and 12 weeks) and at 1200 ppm (at 12 weeks only). In addition TSH levels and increased insulin levels were noted at 19200 ppm. No histopathological changes could be detected in the haematoxylin/eosin stained sections of the pituitary glands in neither dosage group."

BSEF comment:

In this study statistically significant effects on T4 were noted after 4 week exposure to about 108 mg/kg bw/day. An increase in thyroid weight at this dose was noted after 4 but not after 12 weeks. No histopathological changes were noted at this dose either after 4 nor 12 weeks. (This is in contrast to the evaluation given in the table 58, page 140 of the CLH report). Without historical control data he elevated T4 value is difficult to interpret especially as the next higher test dose was about 15 times higher and clearly toxic. In contrast at the massive toxic dose of 1728 mg/kg bw/day effects on the thyroid (increase of weight and histopathological changes) were noted. Similarly, the noted effects on the thyroid (statistically different after 4 but not after 12 weeks treatment) indicated rather an accidental finding then a substance related effect.

10.12.2. Comparison with GLP criteria

CLH page 143:

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

- Statistical significant reduction in T3 (males only) and in T4 (males and females) from 175 mg sodium bromide /kg/day (corresponding to 167 mg ammonium bromide/kg bw/day) (week 4) in the 90-day repeated dose toxicity study in rat (Study report, 2016b). Single animals in these groups also showed depletion (mild/moderate) of colloid in the thyroid at histopathology (2 males and 2 females in each group) but there was generally no correlation between this finding, hormone levels or thyroid weight in individuals."

BSEF comment:

BSEF notes that there are several studies relevant for an assessment of the classification of ammonium bromide for thyroid effects. They include studies on ammonium bromide and studies on sodium bromide in different species and humans. There are three reliable guideline compliant studies employing ammonium bromide and sodium bromide that investigated effects on the thyroid besides histopathology of the organ alone. These include two studies using ammonium bromide namely an OECD Test Guideline No 408: Repeated Dose 90-Day Oral Toxicity Study in rats (Study report, 2010a), and an OECD Test Guideline No 414: Prenatal Developmental Toxicity Study in rats (Study report, 2007a), both showing no effects on the thyroid up to the highest dose tested, and one study using sodium bromide according to OECD Guideline 408: Repeated Dose 90-day Oral Toxicity Study in rats (Study report, 2016b). The findings in the only study that

showed some, however inconsistent and not clearly adverse effects on the thyroid was the Hoberman et. al, 2016 study (Study report, 2016b). Even if these effects were considered for classification, they occurred at dose levels above the cut off values for STOT-SE classification.
CLH report P. 144:
Non-guideline studies:
- Statistical significant increase in thyroid weight noted at ≥ 108 mg sodium bromide /kg bw/day (corresponding to 103 mg ammonium bromide/kg bw/day), histopathological changes in thyroidea noted at 1728 mg/kg bw/day, and changes in hormone levels in serum noted at ≥108 mg/kg bw/day (decreased thyroxin) after four weeks administration of sodium bromide in rat (Loeber et al., 1983). This effect supports classification in STOT RE 2 since it is below the guidance value of 300 mg/kg bw/day (28-day).

BSEF comments:
It should be noted that the authors report effects after 28-days and 12 weeks and at 12 weeks of exposure no effects on the thyroid were observed. This puts the relevance and reliability of the thyroid hormone measurements into question. We do not agree that this study should be used for classification.
Statistically significant effects on T4 were noted after 4 week exposure to about 108 mg/kg bw/day. An increase in thyroid weight at this dose was noted after 4 but not after 12 weeks. No histopathological changes were noted at this dose either after 4 nor 12 weeks. (This is in contrast to the evaluation given in the table 58, page 140 of the CLH report). Without historical control data he elevated T4 value is difficult to interpret especially as the next higher test dose was about 15 times higher and clearly toxic. In contrast at the massive toxic dose of 1728 mg/kg bw/day effects on the thyroid (increase of weight and histopathological changes) were noted. Similarly, the noted effects on the thyroid (statistically different after 4 but not after 12 weeks treatment) indicated rather an accidental finding then a substance related effect.
CLH report p. 114:
- "Statistical significant increase in thyroid weight from 108 mg sodium bromide /kg bw/day (corresponding to 103 mg ammonium bromide/kg bw/day) in females, decreased size of follicles in thyroidea in females at ≥ 432 mg/kg bw/day; increased activity of thyroids in females at ≥ 432 mg/kg bw/day in the 90-day repeated dose toxicity study of sodium bromide in rats (Van Logten et al., 1974).
- Supporting data pointing to effects on the thyroid and the specific profile of toxicity, but not sufficient for classification, was also found in the three-generation reproductive toxicity study of sodium bromide in rats (van Leeuwen, 1983) where reduced thyroid hormone (T4) in males at ≥108 mg/kg bw/day (corresponding to 103 mg ammonium bromide/kg bw/day) and in females at ≥ 432 mg/kg bw/day were reported."

BSEF comment:
The high dose levels of the van Logten 1973 & 1974, van Leeuwen 1983 and Loeber 1983 studies and the two highest dosages in the Buchberger 1990 study were all above the limit dose , and the highest dose in the van Leeuwen 1983 study which has been used in the CLH report to define the mechanism of action on the thyroid gland was approximately 1728 mg/kg bw/day. The effects observed at these high doses causing massive systemic toxicity are not relevant for the C&L purposes nor for concluding a mechanism of action in humans, as such doses are massive toxic/lethal for humans due to the neurotoxic effects of bromide in humans.
It is also important to recognise that these dose levels in the rat could not be sustained by and are therefore not relevant for humans. Data from a recent rat gavage toxicokinetic study (Barnett, 2019) demonstrate that mean plasma exposure levels for rats treated with ammonium bromide at a dose of 175 mg/kg bw/day for 6 weeks are 33.4 mmol/L for males (range 18.9 – 40.4) and 30.2 (range 23.6 – 38.5) for females. These results are well in excess of human exposure levels referenced as severely toxic (>12.5 mmol/L), being within the range of 'possible coma (25 – 37.5 mmol/L) and some

individuals within these groups occasionally achieved blood levels which would be classed as 'possibly fatal' (>37.5). Thus effects observed in rats at dose levels above 175 mg/kg bw should not be regarded as relevant for humans, as humans would suffer from other effects at lower dose levels and thyroid is unlikely to be adversely affected at lower doses.
CLH report p. 144:
"In human studies the following indications of effects on the thyroid were reported in the study by Sangster et al (1982): slight but significant increase in T3 and T4 in females only (20% and 14%, p,<0.01) at 9 mg bromide/kg bw/day after 3 months intake of oral capsules (Sangster et al., 1982). However, since the levels of both T3 and T4 were within normal limits at the start and the end of the investigation they are considered by the dossier submitter to be of less significant toxicological relevance and as supportive information for classification in STOT RE with the thyroid as target organ."

BSEF comments

The human data base is greater than this single study and all indicate that in humans no relevant adverse effects on the thyroid are observed. Also it should be noted that in humans diagnostic data are mostly based on TSH levels being more reliable than T3 and T4 levels that also undergo a considerable circardiane variability for example.
The first human studies by Sangster (1982) used a dose of sodium bromide that was equivalent to the World Health Organization's acceptable daily intake value developed based on a minimum pharmacologically effective dosage in humans of about 900 mg of potassium bromide, equivalent to 600 mg of bromide ion (Sangster et al 1982; FAO/WHO Pesticides Committees, 1967). In this study there were no changes in serum concentrations of thyroxine, free thyroxine, thyroxin binding globulin, triiodothyronine, cortisol, testosterone, oestradiol and progesterone. Also, no changes were observed in serum concentrations of thyroid stimulating hormone (TSH), prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) before and after administration of thyroid stimulating hormone releasing hormone (TRH) and luteinizing hormone releasing hormone (LHRH). The second human study (Sangster et al, 1983) employed slightly higher concentrations of sodium bromide and exposure lasted longer than the previous study. A slight but statistically significant increase in thyroxine (T4) and triiodothyronine (T3) in females only was noted. However the individual concentrations of T4 and T3 in this group were well within normal limits at the start and the end of the investigation. Thus the statistical differences seem to be an accidental finding. A third human study (van Gelderen 1993) in which sodium bromide was orally administered in capsules to healthy female volunteers (45 volunteers/dose) at doses of 0, 4 or 9 mg Br /kg bw for three menstrual cycles reported no significant differences in the levels of thyroid hormones (T4, FT4, TBG, T3 and TSH) between the start and the end of the study period. All these studies show that at the doses employed for the respective exposure duration there was no effect on the thyroid parameters monitored.
To elaborate, there are three investigations on sodium bromide with human volunteers available (an 8-week study and two 3-month studies) (Sangster et al 1982, 1983; van Gelderen et al 1993). The exposures achieved at the high dose level during these studies (4.30 mmol/L for males and 4.93 mmol/L for females) were not insignificant as therapeutic doses are generally in the range 9.6-24 mmol/L. Doses giving plasma levels in humans of ≥12 mmol/L (96 mg Br/L) have produced bromism and plasma levels of > 40 mmol/L (320 mg Br/L) have been fatal (Ellenhorn and Barceloux, 1997).
The study by Sangster et al (1983) evaluated effects on the thyroid in humans after 3 months intake of oral capsules at dose levels of 9 mg bromide/kg bw/day. At the dose level tested in human volunteers (9 mg bromide/kg bw/day) no adverse effects on the thyroid or thyroid function was observed (Sangster et al 1983). In the females taking 9 mg Br/kg bw/day (but in no other group) there was a significant ($P < 0.01$) increase in serum thyroxine and triiodothyronine between the start and end of the study but all concentrations remained within normal limits. No changes were observed in serum concentrations of free thyroxine, thyroxine-binding globulin, cortisol, oestradiol,

progesterone or testosterone, or of thyrotropin, prolactin, luteinizing hormone (LH) and follicle-stimulating hormone before or after the administration of thyrotropin-releasing hormone and LH-releasing hormone. The most sensitive indicator of bromide toxicity this study was an effect on EEG measurements. Other side-effects were limited to the skin (dermatosis) and gastrointestinal tract (nausea).
Page 144 of the CLH report notes indications of effects on the thyroid ("slight but significant increase in T3 and T4 in females only (20% and 14%, p <0.01") were reported in the study but also states that although there is a significant increase in thyroid hormone levels, the levels are in fact within the normal range. Moreover, KEMI recognises two additional human studies (Sangster et al 1982; van Gelderen 1992) report no significant differences in the levels of thyroid hormones. The authors of these two additional studies also indicate that repeated exposure to bromide over 3 months in humans influences (increases) production of T4 and T3, albeit 'within normal limits'.
In fact, levels of these hormones are controlled by both internal and external factors (e.g. diurnal variation and environmental factors respectively), so it is not likely based on available data that the slight increase in T3 and T4 (within normal range) reported by Sangster is a real effect relating to bromide exposure or a normal variation in circulating concentrations of thyroid hormone levels. Bromide is a natural component of a healthy human diet. The presence of bromide in the diet may influence the concentration of iodide available physiologically, but this is typically within the normal range of functionality of the thyroid and endocrine system. Indeed, the endocrine system as a whole effectively manages such variations in concentration within a certain range and the role of the thyroid as part of the endocrine system is to maintain homeostasis by regulating changes in the organism's environment following exposure to xenobiotics.. Therefore, in the Sangster study (1983) the fluctuations in thyroid hormone levels is to be expected as a likely effect of exposure to a xenobiotic such as bromide, as well as other external factors, but is in the range of the physiological normal function and cannot be considered an adverse effect.
Subsequent publications which refer to these human studies also indicate a general consensus that no significant effect on thyroid hormones was observed (Allain et al, 1993, IPCS INCHEM Monograph 1988). Further indications of no significant effect are reported in a study of patients with thyroid disorders: the percentage with normal, low, and high T4 or TSH plasma activities did not differ between patients with low and high bromine concentrations (Allain et al 1993).
In summary there is no evidence from human studies nor from pharmacovigilance, that bromide might have a negative impact on the thyroid at pharmacological doses. In contrast, neurotoxic effects are noted in the humans and are considered as sensitive endpoint for chronic exposure towards bromide. This is also recognised in the CLH report (page 143) as effects are noted here at significantly lower doses as in rodents (see also above about the difference in toxicity between rats and humans based on plasma levels)
CLH report p. 144
"Based on significant changes, which clearly indicate functional disturbance and morphological changes which are toxicologically relevant, at dose levels below the guidance values for classification both for category 1 (Velický et al., 1997a, Velický et al., 1998) and 2 (Loeber et al., 1983) seen in three nonguideline studies in rat with a focus on alterations in the endocrine system, and considering the limitations of the studies, using expert judgement and a total weight of evidence assessment of all available information, a classification of ammonium bromide in STOT RE 2 for effects on the thyroid is considered warranted''.

BSEF comment:

The findings of the group of Velicky are difficult to interpret as they report effects in the thyroid at doses of 0.5 mg/kg bw/day which have not been reported in any other older or new state of the art guideline compliant study at 20 or even 50 times higher doses although a comprehensive hormone level determination or at least a detailed

histopathology of the thyroid was done in these studies.
In the second study referred to in the CLH report to justify STOT RE 2 (Loeber at al 1983), statistically significant effects on T4 were noted after 4 week exposure to about 108 mg/kg bw/day. An increase in thyroid weight at this dose was noted after 4 but not after 12 weeks. No histopathological changes were noted at this dose either after 4 nor 12 weeks. (This is in contrast to the evaluation given in the table 58, page 140 of the CLH report). Without historical control data he elevated T4 value is difficult to interpret especially as the next higher test dose was about 15 times higher and clearly toxic. In contrast at the massive toxic dose of 1728 mg/kg bw/day effects on the thyroid (increase of weight and histopathological changes) were noted. Similar the noted effects on the thyroid (statistically different after 4 but not after 12 weeks treatment) indicated rather an accidental finding then a substance related effect.
For all other even older studies, relevant effects if noted at all) were noted above the trigger value of 100 mg/kg bw/day as also noted by KEMI (see above).
CLH Report
10.12.3 Conclusion on classification and labelling STOT RE
"Classification of Ammonium bromide in STOT-RE 2; H373 (nervous system, thyroid) is warranted."
In the CLH report on page 139 it is stated. "With regards to differences between rats and humans it is noted that in the 'Guidance for the identification of endocrine disruption in biocides and pesticides, Appendix A – Additional considerations on how to assess the potential for thyroid disruption for human health' when interpreting data from experimental animals "Substances inducing histopathological changes (i.e. follicular hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of thyroid hormones, would pose a hazard for human thyroid insufficiency in adults as we all as pre- and post-natal neurological development of offspring." In this case of ammonium bromide and bromide salts, histopathological changes (i.e. follicular hypertrophy and/or hyperplasia) in the thyroid, and changes in the circulating levels of thyroid hormones have been reported. Thus, human relevance of thyroid disruption of ammonium bromide cannot be ruled out.

We support the CLH considerations on p. 139 that the species difference between the rat and humans needs to be taken into account However, based on the above rational our interpretation of the data set is different as the conclusion as the CLH report:
1) Taken all data in rats together a WOE approach should be taken into account for the C&L proposal of bromide for potential thyroid effect, with emphasis on the most recent guideline compliant and well documented studies and not be based on the lowest figure obtained in at least questionable studies.
2) Relevant effects as histopathological changes were not reported in well documented older studies by different groups nor seen in newer state of the art studies below or at the trigger value for STOT RE classification of 100 mg/kg bw/day for a 90 day exposure. Even at higher doses hardly effects on the thyroid were noted. This also holds true for the reproductive toxicity studies in which the thyroid was also investigated.
3) T3/T4 effects in the rat noted in isolation cannot considered be relevant for humans due to different kinetics (see below) and should therefore not be taken as a reliable basis for classification and labelling.
4) If effects on the thyroid are noted at all in rats, this is often accompanied by massive systemic toxicity. Such effects should therefore neither taken into account for C&L purposes nor to conclude on a mechanism of action in humans.
The less reliable non-guideline studies are not considered by BSEF to be relevant for classification (reasons see below), or are at best supportive and therefore should not be given a similar weighting as the reliable OECD Guideline GLP studies used to provide a "weight of evidence" to support classification as STOT RE 2. It seems the CLH report has afforded greater weight to the less reliable older studies that report effects on the thyroid

and seems to have discounted the reliable studies in which effects were absent or not noted at all below the relevant trigger value for STOT RE2 with regard to the thyroid. We consider that the threshold for potentially relevant effects noted in rats were all above the trigger value of 100 mg/kg bw/day based on a 90 day or longer exposure if well documented and state of the art repeated dose studies are taken into account.
The CLH report attributed reliability of K2 to several relatively older studies which should be scored as K4 or even K3 because they employed dietary or drinking water administration of the substance and substances administered but those routes leads to uncertainty about the exact amount of substance that has been ingested by each animal and such information is not reported in the respective publications. The older studies did not follow OECD guidelines because the guidelines were not available at time of the study. The non-guideline studies were conducted under GLP at the time of the studies. For these reasons the older studies were considered by BSEF to be unreliable or have to be at least interpreted with care as the determination of T3, T4 or TSH was not a routine parameter at the time the studies were performed. There are still today a lot of difficulties to standardize and establish reliable and repeatable methods to determine thyroid hormones. Thus thyroid hormone level changes alone, in particular in older studies should be interpreted with care and cannot be used as such for regulatory pruposes.
Furthermore, additional studies (e.g in dogs) and interpretation relevant for the assessment of thyroid effects in humans not considered in the CLH report must be taken into account.

Evidence in Dogs
The CLH report notes on page 138 that 'In contrast to rats, investigations in dogs did not reveal effects on the thyroid gland (with exception of a decrease in serum total thyroxine and free thyroxine over time which were within the reference ranges and was observed both in control and treated animals and therefore not clearly attributable to treatment), at a dosages of 240 mg/kg bw/day (Paull et al 2003)'.
The report attributes this difference in the increased sensitivity of rats compared to dogs to bromide treatment to differences in the regulation, plasma protein binding and half-lives of thyroid hormones between rats and dogs.
BSEF considers that although the dog study (Paull et al 2003) was non-guideline and non-GLP, given that the study employed five 'Laboratory hound' dogs of mixed sex/group, study conducted in a USFDA approved lab and histopathology was conducted by a board-certified pathologist that the study was conducted under robust scientific conditions and should be considered reliable with restrictions (K2). No effects were noted with regard to the thyroid at clearly pharmacologically active serum doses. This is even more important as the dog similar to humans has TBG, which has a great impact on kinetics/effects to be expected in humans.
The CLH report also states on page 134 that 'it is concluded that potassium bromide administration for 6 months to young, healthy adult dogs did not have a significant effect on the function or morphology of the canine thyroid gland compared to the control group'. BSEF agrees with the conclusion that there were no thyroid effects in dogs following administration of potassium bromide.

Thyroid toxicity depends on species specific physiology
The most obvious species difference between rodents versus humans and dogs is the lack of thyroid binding globulin (TBG) in the rodent, as is the case for other species including birds and reptiles (Vranckx et al 1990). TBG is the predominant plasma protein that binds and transports thyroid hormone in the blood (Robbins 2000). In humans, thyroxine binds to three plasma proteins, TBG, pre-albumin and albumin, with binding constants of 10-10, 10-7 and 10-5, respectively (Choksi et al 2003). This means that thyroxine has a far greater binding affinity with TBG than pre-albumin or albumin. In dogs, the majority of thyroxine is bound to TBG; the binding-affinity of thyroxine to this thyroid hormone

binding protein is high and comparable to that in humans, but plasma concentrations of TBG in the dog are only 15% of those observed in humans which could explain why the unbound or free fraction of circulating T4 is higher, and hormone metabolism is more rapid in most domestic animals such as dogs than in humans (Daminet and Ferguson 2003).
It is known that rats are more susceptible to a decline in circulating thyroid hormone levels which results in stimulation of thyroid proliferation and which is considered to be a result of a lower binding of hormones to plasma proteins in the rat (Dubowitz 1962). In rodents, the lack of TGB with a binding affinity of 3 and 5 orders of magnitude more than albumin and pre-albumin may be a factor responsible for species differences in thyroid gland function.
A marked difference in the plasma half-lives of thyroid hormones could also be explained by the lower protein-binding affinity in the plasma of rats. The half-life of thyroxine (T4) is 12 hours in the rat compared to 5-9 days in humans and serum TSH is 25-times higher in the rodent as compared to man (Brown-Grant 1963, Nicoloff 1972). Mild to moderate increase in TSH in the rodent for instance will exhibit an increase in thyroid gland neoplasia which could not be established in humans. In the dog, the plasma half-life of T4 has been estimated to be approximately 8–16 hours compared to about 7 days in humans, resulting in relatively higher levels of unbound or free circulating T4 relating to lower levels of TBG in plasma in the dog than in humans. Similarly, the plasma half-life of T3 in the dog has been estimated to be 5–6 hours, compared to 24–36 hours in humans (Daminet and Ferguson 2003).
These findings indicate a much higher level of functional activity in the rodent thyroid gland as compared to humans. Therefore findings noted in rats especially with regard to plasma T3/T4 levels cannot be transferred 1:1 or considered equally be relevant for humans.

Evidence for physiological basis for thyroid effects in rats and lack of effects in humans
The results of various investigations with known inhibitors of the thyroid hormone synthesis (sulphonamides and thioureylenes) demonstrate a profoundly different pattern in the regulation of thyroid hormone synthesis, the binding of thyroid hormones to plasma protein and their half-life in the blood between rodents, versus primates and humans, indicating that the rat is not a good model for the assessment and evaluation of such effects in humans. The rodent thyroid reveals a much higher functional activity and operates at a considerably higher level with respect to thyroid hormone turnover as compared to the primate, a conclusion also supported by the histological appearance of the thyroid. These examples support the conclusion that there are marked differences between rodents and primates in thyroid gland physiology.
The greater sensitivity of the rodent thyroid is related to the shorter plasma half-life of thyroxine than in humans due to the considerable differences in the transport proteins for thyroid hormones between these species (Isobe et al 2016). This demonstrates that there is a profound difference in the regulation of thyroid hormones and the functional activity in the thyroid between rodents and humans. Due to these differences in the physiology and regulation of the thyroid and thyroid hormones, rodents appear to be more sensitive to the effect of chemicals on the thyroid function as compared to non-rodents, primates and humans.

Conclusions on weight of evidence for human thyroid toxicity of bromide
In summary, these findings demonstrate that the mechanism by which bromide acts on the rat thyroid cannot be transferred 1:1 to the situation in humans. As a consequence effect values obtained in rat studies need to be interpreted with care and focus on relevant effects as indicated in the guidance document 'Guidance for the identification of endocrine disruption in biocides and pesticides, Appendix A – Additional considerations on

how to assess the potential for thyroid disruption for human health' ,. This is very relevant for bromide as in available human volunteer studies with therapeutic bromide levels no adverse effect on thyroid hormone levels was noted. Rats obviously show a greater sensitivity to derangement by pharmaceutical drugs, certain chemicals and physiologic perturbations than humans regarding the thyroid gland. This is due to the absence of TBG in rodents, which is the major thyroxine and triiodothyronine binding protein in man with a much higher binding activity than albumin. Another cause for the greater sensitivity of rodents is the shorter plasma half-life of T4 compared to man - 12-24 hours in rats compared to 5-9 days in humans - and this is in part due to the considerable differences in thyroid hormone transport proteins between these species, demonstrating a much higher functionality of the rat thyroid which operates at a considerably higher level with respect to thyroid hormone turnover.. These findings, thus, strongly indicate that the regulation of thyroid hormones in rats and humans is different and therefore xenobiotic-induced effects on the thyroid or thyroid hormones of rats are of questionable relevance for humans and need to be interpreted very carefully. The overall effect is that humans are relatively better equipped to compensate for sudden changes in thyroid hormone balance as compared to rodents.

Implications for Classification

According to the CLH report, bromide meets the criteria for classification as STOT RE 2 under CLP for effects on the thyroid. However, effects on the thyroid have only been demonstrated in less reliable rodent studies with no clear effects evident in guideline studies in rats and no evidence for a thyroid hormone mediated adverse effect in dogs and humans. Reliable rat studies conducted according to GLP do not show an effect on the thyroid parameters investigated below or around the trigger values for STOT classification Furthermore, available evidence indicates important physiological differences between the rat and other species, notably humans, that suggest the mechanism for observed effects in rodents is not adequately relevant for humans, thus certain xenobiotic-induced thyroid gland effects are not considered to be of human health concern (McClain 1995).

The CLP guidance for classification as STOT RE 2 states that 'These adverse health effects include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health'.

Some less reliable studies on the rat have reported biochemical and at higher doses morphological effects in the thyroid, but the relevance of this is questionable based on non-observation of these effects at higher dose levels not resulting in excessive toxicity in GLP guideline studies. The lack of such effects in available human studies supports the fact, that the substance should not be classified as STOT RE 2 for thyroid effects as no changes relevant for human health have been demonstrated. In addition the clear differences in physiology of the thyroid in rodents and other species indicate rats are more susceptibility to thyroid effects than other species.

The CLP guidance also clearly states that 'Classification for target organ toxicity (repeated exposure) identifies the substance or mixture as being a specific target organ toxicant and, as such, it may present a potential for adverse health effects in people who are exposed to it' and 'These adverse health effects include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health'. However, the criteria for STOT RE2 classification is written 'Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat

exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
The data at hand does not show effects potentially be relevant for humans below or at the trigger value for STOT RE2 even for rats in reliable studies. Changes to the thyroid hormones alone, without histological evidence in the rat lacking of TBG and revealing therefore a quite different physiology and kinetics cannot directly be transferred to humans especially as no adverse effect in humans following bromide exposure with therapeutic (pharmacological) effects have been noted until today. This conclusion is further supported by the fact that no effects of bromide in the dog were noted, which possesses TBG.In summary, studies of relatively less reliability in which exposure to bromide in rats resulted in changes in thyroid hormone levels accompanied especially at high and systemic toxic doses with histological changes in the thyroid, but these effects are neither seen in the available human studies nor in the reliable rat studies or in dogs. Furthermore the interpretation and the assessment of findings in the rat for the human situation in relation to thyroid function should be done with caution given there is a fundamental difference in thyroid metabolism and function between rats and humans.
Taken all data together:
• Based on a WOE consideration, no relevant effects with regard to thyroid noted in the questionable model rat at or below the trigger value for STOT RE2
• Significant species differences in kinetics and physiology regarding the thyroid (especially between rat and man)
• No effects noted in the dog
• No effects noted in humans at pharmacological doses
• Neurotoxicity being a prominent and sensitive effect in humans and covered by the proposed STOT RE2 (nervous system)
STOT RE 2 with regard to the thyroid is not triggered.

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Dossier Submitter's Response

Thank you for your comments.

Thyroid as target organ

There are no doubt that bromide affects the thyroid function to some extent as observed in available animal studies. BSEF questions if there is sufficient evidence in animals studies at or below the guidance values for STOT RE 2 classification, and if the findings are relevant for humans.

Below we have tried to address your main points of concern.

Loeber et al (1983) study

You write in your comment: *"Statistically significant effects on T4 were noted after 4 week exposure to about 108 mg/kg bw/day. An increase in thyroid weight at this dose was noted after 4 but not after 12 weeks. No histopathological changes were noted at this dose either after 4 nor 12 weeks. (This is in contrast to the evaluation given in the table 58, page 140 of the CLH report)."*

We agree, in table 58, page 140 of the CLH report there was a mistake in the reporting of the Loeber et al., 1983 study: at 108 mg/kg bw/day there were no histopathological changes in thyroidea. But in the table it is correct that a statistically significant decrease (23%) in T4 levels compared to control was seen after 4 weeks. Histophatological changes were only seen in the high dose group (1728 mg/kg bw/day) in this study.

Regarding the comment: *"It should be noted that the authors report effects after 28-days and 12 weeks and at 12 weeks of exposure no effects on the thyroid were observed. This puts the relevance and reliability of the thyroid hormone measurements into question. We do not agree that this study should be used for classification."*

In your comment on the Loeber et al (1983) study you question the relevance and reliability of thyroid hormone measurements since the decrease in thyroxin in serum noted at ≥108 mg/kg bw/day (corresponding to 1200 ppm, mg/kg bw/day calculated using default conversion factor 0.09 for subchronic studies) after four weeks administration of sodium bromide in rat was not statistically significantly decreased after 12 weeks.

Since sodium bromide is administered via diet at set concentrations mg sodium bromide/kg diet and the corresponding dose in mg/kg bw/day is a mean value, there is likely higher exposure to sodium bromide during the first four weeks of the study compared to the remaining eight weeks. Comparing the body weight at 4 weeks compared to 12 weeks for animals in the 1200 ppm group (108 mg/kg bw/day) there is an increase of 63% in body weight. Thus, the dose per kg of body weight is probably lower during the later part of the study and may at least partly explain why there is less marked effects on the hormone levels at 12 weeks compared to 4 weeks.

If instead using the default conversion factor 0.117 for subacute studies to calculate the dose in mg/kg bw/day for the first four weeks of the study, the corresponding dose would be 140 mg/kg bw/day and would still be below the GV for 28 day studies for STOT RE 2 classification.

Human relevance

You write in your comment: "*As a consequence effect values obtained in rat studies need to be interpreted with care and focus on relevant effects as indicated in the guidance document 'Guidance for the identification of endocrine disruption in biocides and pesticides, Appendix A – Additional considerations on how to assess the potential for thyroid disruption for human health' ,. This is very relevant for bromide as in available human volunteer studies with therapeutic bromide levels no adverse effect on thyroid hormone levels was noted.*"

We would like to point to the fact that hazard assessment aims to identify hazards, and should not be limited to focus on adverse effects only at "human therapeutic doses".

According to the Guidance application of CLP criteria 1.2.2: *Hazard classification is based on the intrinsic properties of a substance or mixture and does not take into account exposure. Reasonably expected use summarises all physical forms and states of a substance or mixture that may occur during intended use or reasonably foreseeable conditions of misuse.* And for reasonable expected use this includes reasonably foreseeable accidental exposure, but not abuse such as criminal or suicidal uses.

You state in your comment that the highest dose used in the human studies available in the CLH-report (Sangster et al., 1982 and 1983, van Gelderen er al., 1993) corresponds to 4.30 mmol/L for males and 4.93 mmol/L for females and that the therapeutic doses for bromides are generally in the range 9.6-24 mmol/L. Further, you state that plasma levels in humans of ≥12 mmol/L (96 mg Br/L) have produced bromism and plasma levels of >40 mmol/L (320 mg Br/L) have been fatal.
The plasma levels after 3 months administration of 9 mg/kg bw/day was 7.99 mmol/L in the study by van Gelderen et al., 1993. This is still below the plasma levels for observed bromism (12 mmol/L) and mortality (>40 mmol/L). Thus, the absence of effects on thyroidea or hormone levels after repeated exposure does not prove that bromide does not have these effects in humans and that effects observed in studies with rats at higher concentrations are not relevant for humans. The absence of effects can rather be explained by low systemic exposure.

You comment that: *Further indications of no significant effect are reported in a study of patients with thyroid disorders: the percentage with normal, low, and high T4 or TSH plasma activities did not differ between patients with low and high bromine concentrations (Allain et al 1993).*
We would like to clarify that in this study "high bromine" is characterized by mean plasma bromine concentration of ≥ 6 mg/L, corresponding to 0.56 mmol/L. Thus, this level is far below plasma levels where "bromism" is seen in humans. Further, this study does not demonstrate any evidence of lack of effects on the thyroid in humans due to bromine.

In response to your arguments for "*Evidence for physiological basis for thyroid effects in rats and lack of effects in humans"* we would like to refer to the recent RAC opinion on the CLH-proposal for mancozeb regarding STOT RE classification for effects on thyroidea and relevance for humans, where the following statement is noted: "*Humans do have a larger reservoir of thyroid hormones compared to rats, but if thyroid hormone synthesis is reduced for prolonged periods of time, the reservoir will ultimately be depleted and thyroid hormone levels will decrease."*

Furthermore, in the same opinion, RAC states that only a reduction in hormone levels is considered by RAC to be an adverse effect for the purpose of STOT RE classification.

Evidence in dogs

With regards to absence of effects on the thyroid or thyroid hormone levels in dogs (having comparable binding-affinity of thyroxine to TBG to humans, but plasma concentrations of TBG being only 15% of those observed in humans) we would like to clarify that in the studies by Rosenblum, 1958; March et al., 2002, and Paull et al., 2003 no hormonal analysis were performed (in first two studies) or were flawed by unstable measurements/function in control animals (in the latter study). Also, in the Rosenblum study, there were no histopathological examination done and in March et al (200) there was no histopathological examination of the thyroid. In Paull et al 2003, neither signs of hypothyroidism nor evidence of bromism were identified in any of the dogs after treatment for 180 days at a loading dose of 100 mg/kg bw/day for two days and maintaining dose of 30 mg/kg bw/day. However, there was a marked (approx. 50%) decrease in T4 levels (both mean value and individual values) in both control and treated dogs over time. Therefore, as mentioned above, thyroidea function of the animals in the this study appeared not to be stable and it is therefore not possible to make a robust conclusion from this study for this end point.

Moreover, all three dog studies are very small in size (2-4 animals). Consequently, it is not possible based on available studies to firmly conclude on an absence of effects on the thyroid function in dogs.

Relevance of findings in non-guideline studies in comparison with absence of findings in more recent guideline studies

Regarding your comment: *"The high dose levels of the van Logten 1973 & 1974, van Leeuwen 1983 and Loeber 1983 studies and the two highest dosages in the Buchberger 1990 study were all above the limit dose , and the highest dose in the van Leeuwen 1983 study which has been used in the CLH report to define the mechanism of action on the thyroid gland was approximately 1728 mg/kg bw/day. The effects observed at these high doses causing massive systemic toxicity are not relevant for the C&L purposes nor for concluding a mechanism of action in humans, as such doses are massive toxic/lethal for humans due to the neurotoxic effects of bromide in humans."*
We would like to clarify that the study by van Leeuwen et al (1988) was referred to as a mechanistic study in the CLH-report but we did not intend to give the impression that this study alone was used to define the mechanism of action on the thyroid gland. Moreover, with regards to your comment that doses used in van Logten 1973 & 1974, van Leeuwen 1983 and Loeber 1983 we agree that the doses used are excessive for classification purposes. Nevertheless, the picture of bromide toxicity in these studies is quite consistent in effects on thyroidea and same observations are made at lower concentrations as well.

Regarding your comment *"The CLH report attributed reliability of K2 to several relatively older studies which should be scored as K4 or even K3 because they employed dietary or drinking water administration of the substance and substances administered but those routes leads to uncertainty about the exact amount of substance that has been ingested by each animal and such information is not reported in the respective publications"* we note that the reliability score set for the non-guideline studies are the same score used in the REACH registration and CAR, including Loeber et al., 1983 and Velicky et al., 1998 (Velicky et al., 1997a not included in the REACH registration dossier for ammonium

bromide). Thus, there seems to be a discrepany in the current evalution of reliability by BSEF compared to the registrants previous evalution.

Regarding your comment: *"It seems the CLH report has afforded greater weight to the less reliable older studies that report effects on the thyroid and seems to have discounted the reliable studies in which effects were absent or not noted at all below the relevant trigger value for STOT RE2 with regard to the thyroid. We consider that the threshold for potentially relevant effects noted in rats were all above the trigger value of 100 mg/kg bw/day based on a 90 day or longer exposure if well documented and state of the art repeated dose studies are taken into account."* we provide below some clarifications of study details in an attempt to also provide some explanation as to why are effects on thyroidea not seen in (all) guideline RDT studies in rat.

<u>90-day oral repeated dose toxicity study of ammonium bromide in rats (Study report, 2000a)</u>
Up the to highest dose tested (500/750 mg/kg bw/day) there were no effects on thyroidea (weights, histopathological findings) but it should be noted that hormones were not measured.

<u>90-day oral repeated dose toxicity study of sodium bromide in rats, including recovery assessments (Study report, 2016b)</u>
Thyroid hormone analysis in serum was conducted on a single occasion in Week 4 and demonstrated an apparent dose-related statistically significant reduction in T3 (males only) from 60 mg/kg bw/day and in T4 (males and females) from 175 mg/kg/day. There were reductions in T4 already at 60 mg/kg bw/day in both males and females, but not statistically significant compared to control. The author of the study report states that at 175 mg/kg/day, differences from control in T3 ($p \leq 0.05$, males) and T4 ($p \leq 0.01$, males and females) were comparable to historical control values, however, this HCD was not available to the DS and does not appear to be robust for comparison. It is stated in the report: *Interpretation of thyroid hormone data was constrained by the limited amount of historical control data (2 studies using the same test kit), the consequent variation of some control values from this study in comparison to these historical values and the high number of values below the LLOQ*. Of note, for T4, there were no samles below LLOQ of the assay.

Mean TSH levels were 36% and 74% higher than controls in the 175 and 500 mg/kg/day male groups, respectively but not statistically significantly different. All mean values, including controls were, however, markedly higher (~2 to 6 fold) than the historical control range. There was no significant effect on TSH in females.

All in all, the DS considers that changes in thyroid hormone levels are seen starting from 60 mg/kg bw/day that is below the guidance values for STOT RE 2 classification, but were only statistically significant different compared with control for T3 in males at this dose level and for T4 in females. Thus, since dose-related changes are observed for T4 in both males and females, and for T3 in males, these observed effects indeed supports classification in STOT RE 2 for effects on the thyroid.

		Males				Females			
	mg/kg/day	0	60	175	500	0	60	175	750
T3	ng/dL	99.47	72.67*	71.77*	62.42**	100.98	103.67	95.44	101.85
	Compared to ctrl		↓ 27%	↓ 28%	↓ 37%		↑ 3%	↓ 5%	↑ 1%
T4	ng/dL	5.027	4.34	3.629**	2.394**	3.684	2.734*	2.438**	1.968**
	Compared to ctrl		↓ 14%	↓ 28%	↓ 52%		↓ 26%	↓ 34%	↓ 47%
TSH	ng/dL	5.717	6.377	7.779	9.952	3.215	5.137	3.639	4.958

	Compared to ctrl		↑ 12%	↑ 36%	↑ 74%		↑ 60%	↑ 13%	↑ 36%

Of note is that there were excessive general toxicity only observed in males at 500 mg/kg bw/day where there were 4 animals euthanized before the termination of the study.

With regards to organ weights and histopathology: Single animals in the intermediate and high dose group groups also showed depletion (mild/moderate) of colloid in the thyroid at histopathology (2 males and 2 females in each group) but there was generally no correlation between this finding, hormone levels or thyroid weight in individuals. There were no statistically significant differences in absolute thyroid weight and increases relative to body weight were only significant ($p \leq 0.05$) in females treated at 500 mg/kg/day. However, it should be noted that the author of the study report do indicate the colloid depletion as being test-substance related.

Two-generation reproductive toxicity study of sodium bromide in rat (Study report, 2016a)
In this study there were no effects on thyroidea with regards to weight or histopathological changes. Measurement of thyroid hormones were not performed, but blood samples were collected and stored for possible TSH and T4 analysis.

Although not being a guideline study (but performed according to GLP and tested on ammonium bromide itself) the Dose-range finding study (4-weeks) for a 90-day oral repeated dose toxicity study of ammonium bromide in rats (Study report, 1999) at concentrations up to 1000 mg/kg bw/day mixed with the diet there were no histopathological examination of thyroidea or analysis of hormone levels performed.

OECD Test Guideline No 414: Prenatal Developmental Toxicity Study of ammonium bromide in rats (Study report, 2007a)
In this study there were no histopathological examination of the thyroid and no analysis of hormones performed.

Consequently, the above guideline studies in rat of ammonium bromide and sodium bromide do not contradict the findings of changes in thyroid hormone levels in non-guideline studies. On the contrary, we consider that the observed effects the 90-day repeated dose toxicity study of sodum bromide indeed supports classification in STOT RE 2 for effects on the thyroid.

As stated in the CLH-report, our conclusion on classification in STOT RE 2 is thus based on an overall weight of evidence evaluation and expert judgement taking into account histopathological findings in thyroidea and changes in hormone levels in non-guideline studies at dose levels below guidance values for both STOT RE 1 and 2, and effects seen in a guideline study around guidance values for category 2 classification.

We agree that it is difficult to assess the toxicological significance of effects mainly seen in non-guideline studies and most profound in rats. However, taking into account the limitations of studies in dogs, human data being restricted to therapeutic dose levels and that effects on the thyroid indeed is seen also in guideline studies but only significant at higher doses, we maintain our view that effects on thyroid cannot be dismissed and classification STOT RE should thus be considered by RAC.

Nervous system as target organ
We read your comments as you support STOT RE 2 classification with the nervous system as target organ. We thank you for this support.

RAC's response
Noted with respect to STOT RE 2 classification with the nervous system as target organ. With respect to thyroid effects, RAC considers that weight of evidence from all studies available (animal guideline, animal non-guideline, human studies) as well as considerations about differencies in metabolism of thyroid hormones in rats, other animals and humans must be taken into account.

Date	Country	Organisation	Type of Organisation	Comment number
13.06.2019	Belgium	BSEF aisbl	Industry or trade association	13
Comment received				
Comments on this endpoint will be provided in a separate submission. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Charles RiverReproductiveToxicologyHistoricalControlDatainRats.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
05.06.2019	Netherlands		MemberState	14
Comment received				
The NL CA does not agree with the proposed STOT RE 2 (H373 (nervous system, thyroid)) classification. Instead a classification as STOT RE 1 (H372 (nervous system)) is considered more appropriate. The NL CA points to the following studies that might be also relevant for this and which could be taken into consideration: - Lugassy DM, Nelson LS (2009). Case files of the Medical Toxicology Fellowship at the New York City Poison Control: Bromism: Forgotten, but not gone. Toxicology Case Files. - Mitsumori K., Maita K., Kosaka T., Miyaoka T. and Shirasu Y. (1990) Two Year Oral Chronic Toxicity and Carcinogenicity Study in Rats of Diets Fumigated with Methyl Bromide, Fd. Chem. Toxic., Vol. 28, No. 2, pp. 109-119. Although effects on the thyroid in the available 90-day repeated dose toxicity studies with rats were seen at dose levels < 100 mg/kg bw/day, they were limited to the reduction of serum thyroxine levels and increase in FSH level and thyroid weight only, unaccompanied by histopathological changes. Regarding neurotoxic effects slight limpness was observed in male rats at the dose level of 100 mg/kg bw/day; however, this effect was limited to only a few animals. Based on these considerations the assignment of STOT RE based on animal data is considered to be not justified. However, the available human data clearly show that exposure to bromide containing substances results in accumulation and adverse neurological effects. Therefore, classification as STOT RE 1 is considered justified. During prolonged exposure bromide accumulation may occur giving rise to bromide intoxication or bromism. Symptoms include nausea and vomiting, anorexia, confusion, behavioural disturbances, slurred speech, memory impairment, drowsiness, irritability, ataxia, tremors, hallucinations,				

mania, delirium, psychoses, stupor, coma and other manifestations of CNS depression. Bromides are also used in multi-ingredient preparations for treatment of coughs. Bromides are readily absorbed from the gastrointestinal tract. They displace chloride in extracellular body fluids and have a half-life time in the body of about 12 days. They may be distributed into breast milk and cross the placenta (Martindale: The Complete Drug Reference) Based on the intended use (sedation) and the observed adverse effects and the potential to accumulate upon repeated exposure within the human body, classification with STOT RE 1 should be considered.

Dossier Submitter's Response

Thank you for your comments.

Thyroid as target organ
First we would like to call your attention to that in previous assessments of STOT RE classification for effects on thyroidea by RAC, it has been stated (e.g. in the recent RAC opinion for Mancozeb) that only a reduction in hormone levels is considered by RAC to be an adverse effect for the purpose of STOT RE classification.

In addition, in CLP Annex I, 3.9.2.7.3 effects warranting classification in STOT RE are listed, but it should be noted that is stated that the list is not limited to the these effects. Thus, there is no requirement of histopthological findings accompanying other relevant effects in target organs for classification in this hazard class.

Moreover, both irreversible and reversible effects are relevant for STOT RE classification (CLP, Annex I, 3.9.1.1).

Consequently, statistically significant dose-related reduction of serum thyroxine levels in both males and females starting from 60 mg/kg/day (only stat sign for females at this dose) and dose-related increase in TSH (only in males) in the available 90-day repeated dose toxicity study of sodium bromide in rats (see also table in comment no. 12) together with findings of changes in hormone levels and histopathological changes in non-guideline repeated dose toxicity studies in rat at dose levels below the guidance values for classification, are considered as adverse effects sufficient for classification.

Nervous system as target organ
We agree that there are many human case reports (with low levels of details) of adverse health effects (e.g. as cited in Lugassy and Nelson, 2009), including adverse neurological effects, after bromide exposure which appears to be consistent. Together with animal data also consistently showing neurotoxic effects at or around the guidance value 100 mg/kg bw/day a classification in category 2 is considered justified. A discussion by RAC whether the human data is sufficient to justify a classification in STOT RE 1 is welcomed.

RAC's response

RAC notes that animal data are showing neurotoxic effects at or around the CLP guidance value 100 mg/kg bw/day which is the borderline between STOT RE 2 and no classification after a 90-day exposure. Existing human case studies indicating bromism are considered to justify classification as STOT RE 1 with the nervous system as target organ. Positive human data, regardless of the dose, predominates over animal data.

With respect to thyroid effects, RAC considers that weight of evidence from all studies available (animal guideline, animal non-guideline, human studies) as well as considerations about differencies in metabolism of thyroid hormones in rats, other animals and humans must be taken into account.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON AMMONIUM BROMIDE

Date	Country	Organisation	Type of Organisation	Comment number
14.06.2019	Belgium	BSEF aisbl	Industry or trade association	15
Comment received				
see attached pdf document ECHA note – An attachment was submitted with the comment above. Refer to public attachment BSEF comments reprotox 20190612.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. AmBr classification ECHA.docx [Please refer to comment No. 1]
2. BSEF comments reprotox 20190612.pdf [Please refer to comment No. 2, 10, 15]
3. Charles RiverReproductiveToxicologyHistoricalControlDatainRats.pdf [Please refer to comment No. 4, 8, 13]

CONFIDENTIAL ATTACHMENTS
1. ICL blood samples 12 June 2019.pdf [Please refer to comment No. 5]