Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-bromo-3,3,3-trifluoroprop-1-ene

EC Number: -
CAS Number: 1514-82-5

CLH-O-0000007363-75-01/F

Adopted

14 September 2023

14 September 2023

CLH-O-0000007363-75-01/F

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

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **14 September 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2-bromo-3,3,3-trifluoroprop-1-ene**

EC Number: **627-872-0**

CAS Number: **1514-82-5**

Rapporteur, appointed by RAC: **Wendy Rodriguez**

Administrative information on the opinion

Spain has submitted on **28 September 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 November 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 January 2023**.

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

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry, if agreed by the Commission.

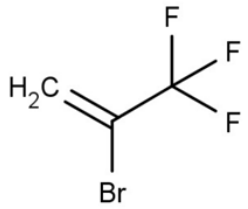
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-bromo-3,3,3-trifluoroprop-1-ene	-	1514-82-5	Repr. 1B STOT SE 3 STOT SE 3	H360FD H335 H336	GHS08 GHS07 Dgr	H360FD H335 H336			
RAC opinion	TBD	2-bromo-3,3,3-trifluoroprop-1-ene	-	1514-82-5	Repr. 1B STOT SE 3 STOT SE 3	H360FD H335 H336	GHS08 GHS07 Dgr	H360FD H335 H336			
Resulting Annex VI entry if agreed by COM	TBD	2-bromo-3,3,3-trifluoroprop-1-ene	-	1514-82-5	Repr. 1B STOT SE 3 STOT SE 3	H360FD H335 H336	GHS08 GHS07 Dgr	H360FD H335 H336			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-Bromo-3,3,3-trifluoroprop-1-ene (2-BTP) is a clear volatile liquid (at 20°C), soluble in water (1000 mg/L at 20°C), with a small molecular weight (174.95) and a partition coefficient n-octanol/water of 2.7 (at 25°C). The uses of this substance include filling of hand-held fire extinguisher and emergency discharge of fire extinguishers within the aviation industry. 2-BTP is manufactured in and/or imported to the EEA at ⩾ 10 to < 100 tonnes per annum.



There is no specific toxicokinetic study performed with 2-BTP. Nevertheless, a toxicokinetic assessment was provided based on the physicochemical properties of the substance, the partition coefficients determined *in vitro* (Anonymous, 2013a, the study and results not detailed in the CLH report) and the *in vivo* toxicological studies included in the REACH registration dossier. According to the physicochemical properties, 2-BTP will be readily absorbed across biological membranes via all routes. Nevertheless, no data was available on absorption via oral route. In addition, the boiling point (34.4°C) close to body temperature suggest that the inhalation route will be the major route of exposure compared to dermal route. Systemic distribution to liver, spleen, heart, and reproductive organs in rats or dogs is supported by the toxicity studies and/or the partition coefficient values. 2-BTP is expected to have a high bioavailability. No data are available on metabolism in the existing toxicity studies, but metabolization of 2-BTP is supported by microscopic changes in liver. There is no data on excretion either; however, rapid excretion and a lack of bioaccumulation were supported by its partition coefficient as well as a post-exposure quick blood concentration decrease and rapid recovery of the clinical signs observed in dogs (Anonymous, 2013b, the study and results not detailed in the CLH report).

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Two acute inhalation (nose only) toxicity studies were available. Anonymous (2004), considered as the key study (compliant with OECD TG 403 and GLP), was performed on Sprague-Dawley rats. The animals showed clinical symptoms associated with respiratory tract irritation (clear or red nasal discharge, red discolorations and fluid in the lungs, bronchiolar lesions with desquamated epithelium, bronchiolar/peribronchiolar acute/subacute inflammation) and decreased activity in the highest dose. In the second acute toxicity study available, Anonymous (1999), F344 rats showed temporary anesthesia and relaxed breathing shortly after exposure. This study did not follow any guideline and was considered as supportive.

In addition, one 90-day inhalation toxicity study (whole body exposure, Anonymous 2013d), compliant with OECD TG 413 and GLP, was considered relevant in the CLH report for the current

classification proposal. Shallow breathing, piloerection, grinding teeth and hunched posture (related to the inhalation of an irritant material according to the DS) were visible during and after the exposure. Underactivity and partially closed eyelids (associated with possible CNS effect according to the DS) were also described from the beginning of the exposure.

Therefore, the DS concluded that a classification for STOT SE3, H335 (may cause respiratory irritation) and H336 (may cause drowsiness or dizziness) is warranted.

Comments received during consultation

Two MSCAs commented the STOT SE3 classification proposal during the consultation. Whereas the first supported the DS proposal, the other questioned if the effects were sufficient to justify a classification for narcotic effects (according to the commenting MSCA the effects reported on reactivity may be related to a general toxicity) and if STOT RE classification should rather be foreseen for respiratory tract effects. One National Authority commented the dossier and raised uncertainties related to the effects seen at the top doses in acute toxicity studies (top doses in both acute studies were above the limit dose of 20 mg/L for vapours stated in OECD GD 39 and in the OECD TG 403 the top dose caused 100% mortality) and considered the effects seen in a 90-day study as not relevant for STOT SE classification. In his answer, the DS mentioned that clinical signs were observed from the first day of exposure in the 90-day study, the effects were transient or the animals partially recovered remaining with minimal or slight degree of severity and the effects did not justify a classification for STOT RE. In addition, some effects reported in Anonymous (2004) were seen in absence of general toxicity. One Industry also commented during the consultation and supported the DS proposal.

Assessment and comparison with the classification criteria

Acute inhalation toxicity studies (nose only)

In **Anonymous (2004)**, 5 Sprague Dawley rats/sex/dose were exposed to 5173 and 26580 ppm of 2-BTP (purity: 99.5%) by inhalation (nose only exposure) for 4 hours. No control group was available. The animals were then observed up to 14 days post-exposure. All rats from the high dose group died or were euthanized by day 2 post exposure, whereas all the animals from the lower dose group survived. In both exposure groups, animals exhibited laboured breathing and gasping during the last hour of each exposure. Immediately following exposure, clear or red nasal discharge, excessive salivation, laboured breathing and moist rales were seen in both exposure groups. These effect at low dose are relevant for classification, as not mortality occurs in that group. The severity and frequency of the signs increased with the dose. Gasping and decreased motor activity were also noted following exposure at the higher concentration. Occurrences of red/ discoloured lungs were seen in both exposure groups (one case in low dose group). Nevertheless, according to the study authors, discoloration was due to vascular congestion which is commonly seen in rats found dead (not exsanguinated) or moribund (inadequately exsanguinated) prior to post-mortem examination, therefore the relevance of this finding for the current proposal is considered uncertain by RAC. Fluid in the lungs as well as bronchiolar lesions with desquamated epithelium, bronchiolar/peribronchiolar acute/subacute inflammation and macrophage in lungs (alveolar and intra-alveolar) were observed only in high dosed animals. As these histological findings in lungs and decreased motor activity were only seen at the dose with 100% of mortality, they do not support a classification for STOT SE, H335 and H336, respectively. No apparent effects on organ weights were reported. An increase of body weight was mentioned in surviving animals during observation period.

In **Anonymous (1999)**, 5 Fischer 344 rats/sex were exposed to 0 and 44000 ppm of 2-BTP (purity not reported) by inhalation (nose only exposure) for 30 minutes. This concentration,

which is reported as 5% v/v (nominal) in the CLH dossier, was provided within a summary provided by the DS (Summary of Toxicological Testing on BTP_July2019). The animals were observed only for 2h after the exposure. The rats exhibited relaxed breathing and appeared to be anesthetized shortly after the beginning of the exposure, but these effects returned to normal within 10-minutes post-exposure (occurrences not mentioned). The animals appeared normal at necropsy.

90-day inhalation toxicity study (whole body)

In **Anonymous (2013d)**, 10 Crl:CD (SD) rats/sex/dose were exposed to 0, 199, 505 and 2876 ppm of 2-BTP (purity: 99.6%) by inhalation (whole body exposure), 6 hours/day, 5 days/week for 90 days. Extra 4-week recovery groups (10 animals/sex/dose for control and high dose group) were also constituted. In his analysis for current classification, the DS mainly focused on the transient clinical signs that were evident already after the single exposure and on the histopathological treatment related findings (also results regarding other parameters such as body weight gain, motor activity, sensory reactivity, grip strength, food consumption, haematology, biochemistry, relative organ weight and gross pathology were summed up in table 40 of the CLH report). As STOT RE is not under the scope of this CLH proposal, RAC shall only form an opinion whether STOT SE classification is warranted. No mortality that were considered as a direct result of exposure was reported. Transient slow/shallow breathing, grinding teeth, piloerection, hunched posture, underactivity, unresponsiveness and partially closed eyelids (closed eyelids on day 1) were seen in all exposed groups during and after exposure (over the 13 weeks). According to the DS, taking into account the individual data provided (mainly in the mid- and high-dose groups), the transient clinical signs associated with dosing were observed from the first day of exposure and approximately two hours and four hours during exposure, not being observed one or two hours after completion of dosing or at the end of the working day. These clinical effects were described as dose-dependent (in number and prevalence) in the full study (taking into account the full exposure period).

A statistically significant and dose-dependent decrease of body weight gain (no significant effects on body weight) was observed in both sexes. Several changes were seen in organ weights, but only the increase in adjusted mean weight of lung as well as the decrease in adjusted mean weights of pituitary and thymus in females were dose-dependent and statistically significant (in the highest dose at least, see the table below). Macroscopic examination performed at week 13 revealed a dose-dependent increase in pale teeth and the spleen capsule was thickened in both sexes (Table 40 of the CLH report). Histopathological treatment-related changes included nasal turbinate findings and larynx squamous ventral metaplasia (table below). These changes were fully (larynx metaplasia) or partially (atrophy/disorganisation/vacuolation of the olfactory epithelium and nasolacrimal duct inflammation) reversible following the 4-week recovery period. Other histopathological changes, dose-dependent in occurrence and/or severity, were recorded. These include acinar cell degranulation in pancreas, heart chronic inflammation and spleen capsular inflammation/thickening (table below), whereas other findings were seen in high dose group only (liver centrilobular hypertrophy and involution/atrophy of the thymus in both sexes, Table 40 of the CLH report).

***Table**: Summary of relevant information (Anonymous, 2013d)*

	0 ppm	199 ppm	505 ppm	2876 ppm
Bodyweight gain (week 0-13) as % of control (m-f)	-/-	**-22.01%*/ -**5%	**-27.58%*/ -21.06%**	-**47.64%*/ -44.74%**
Lung (+ bronchi) mean adjusted weight (f)	1.192	1.195	**1.279* (7.29%)**	**1.330* (11.58%)**
Pituitary mean adjusted weight (f)	0.017	0.016	0.015	**0.011*** **(-35%)**
Thymus mean adjusted weight (f)	0.207	0.181	0.167	**0.111*** **(-46%)**
Larynx squamous metaplasia (m-f)	0/10-0/9	0/10-0/10	0/10-0/10	5/10-2/10
Nasal turbinates, Olf. Epith., Atrophy/disorg./vacuolation (m-f)	1/10-1/9	2/10-0/10	8/10-5/10	10/10-10/10
Nasolacrimal duct inflammation (n/tissues; m-f), *Total* - Minimal - Slight - Moderate	*5/10-2/9* 1/10-2/9 4/10-0/9 0/10-0/9	*8/10-4/10* 3/10-1/10 5/10-3/10 0/10-0/10	*4/10-7/10* 1/10-1/10 3/10-5/10 0/10-1/10	*8/10-9/10* 2/10-0/10 5/10-5/10 1/10-4/10
Pancreas acinar cell degranulation (m-f)	0/10-1/9	3/10-4/10	5/10-5/10	7/10-6/10
Heart chronic inflammation (m-f)	0/10-0/9	4/10-0/10	9/10-4/10	8/10-10/10
Spleen capsular thickening (m-f)	1/10-1/9	1/10-2/10	3/10-3/10	6/10-8/10

* Statistically significant ($p<0.05$ or $p<0.01$).

OECD TG 421 study (Anonymous, 2013c)

Concentrations of 0, 198, 505 and 2900 ppm were tested. During the 6-hour daily exposure, clinical findings such as underactivity, unresponsiveness, piloerection, partially closed eyelids and shallow and/or slow breathing were occasionally observed in males and females at 505 and 2900 ppm, as well as hunched posture in females at 2900 ppm. These signs associated with dosing were reversible after the daily 6-hour exposure or before the end of the working day. Underactivity, unresponsiveness, piloerection and partially closed eyelids were occasionally noted in 198 ppm as well, but at a more reduced incidence than those observed at higher doses (taking into account the whole exposure period). Only underactivity and piloerection were noted immediately after exposure during gestation. According to the DS, the transient clinical signs such as underactivity and unresponsiveness were observed already after the first days of daily 6-hour exposure at all doses tested. Mortality was only observed at 505 and 2900 ppm in females around the delivery date and shortly after and is considered as a sign of dystocia rather than general toxicity.

OECD TG 421 study (Anonymous, 2014)

Concentrations of 0, 50, 100, 175 ppm were tested. In an additional acute exposure group (10000 ppm), 2-BTP was administered via whole-body inhalation exposure for 5 minutes per day to Crl:CD(SD) rats and these animals were exposed to filtered air for the remainder of the 6-hour exposure period. In this group adverse clinical signs (hypoactivity, decreased respiration, completely shut eyelids, and lacrimation) were noted for all males and females on the first day of test substance exposure only. These findings were noted at approximately 15 minutes

following test substance exposure and were resolved by approximately 1 hour following exposure. Salivation and red and/or clear material around the mouth and/or nose were noted for males and females in the 10,000 ppm group throughout the respective exposure periods at approximately 15 minutes and/or 1 hour following exposure and were considered test substance-related. No other test substance-related clinical findings were noted at any exposure level at the weekly detailed physical examinations or 1 hour following the 6-hour exposure period. However, the dose levels in the 6-hour daily exposure groups were lower than those in Anonymous (2013c) and Anonymous (2013d). There were no treatment-related mortalities.

Conclusions

Respiratory tract irritation (RTI)

Recapitulative table*: effects seen at non-lethal doses, reversible and visible at the first exposure*

Study	**Non-lethal dose**	**Clinical effects indicative of RTI**
Anon. (2004) - inhal. (nose only)	5173 ppm	Labored breathing (1F), clear or red nasal discharge (3F and 1M) and moist rales (3M)
Anon. (1999) - inhal. (nose only)	44000 ppm (with uncertainties)	None
Anon. (2013d) - inhal. (whole body) - 90d study	No lethal dose	From 199 ppm: Piloerection (all animals) 2876 ppm: Shallow breathing, hunched posture (all animals)
Anon. (2013c) - inhal. (whole body)	No lethal dose	From 505 ppm: Piloerection (all animals) 2900 ppm: Shallow breathing (all animals)
Anon. (2014) - inhal. (whole body)	No lethal dose	10000 ppm: Lacrimation (all animals), red and/or clear material around the mouth and/or nose (throughout exposure period, all animals)

In Anonymous (2004), clinical effects that could be linked with RTI (laboured breathing, clear or red nasal discharge, and moist rales) were seen. They are described as increased in highest dose (frequency and severity). The histopathological findings, consisting of bronchiolar lesions with desquamated epithelium, bronchiolar/peribronchiolar acute/subacute inflammation, were identified only at the lethal top dose. No effects that could be related to RTI were described in the second acute toxicity study available, Anonymous (1999), although the reliability of this study appear low (30 minutes of exposure, only 2h of observation before sacrifice, only one very high dose tested).

In Anonymous (2013d) repeated exposure toxicity study, dose-dependent increase in clinical signs that could be related to RTI were reported. Histopathological examination revealed larynx metaplasia and nasal turbinates epithelium atrophy/disorganisation/vacuolation. These effects appeared, at least partially, transient after the 4-week recovery period. The CLP Regulation acknowledges: *"there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation*." Nevertheless, these specific histopathological findings could also point toward sub chronic effects, therefore more weight is given to clinical signs for current classification proposal.

In Anonymous (2014) OECD TG 421 study, completely shut eyelids and lacrimation were described in the first day of exposure, as well as salivation and red and/or clear material around the mouth and/or nose throughout the respective exposure period (10000 ppm dose group).

Overall, clinical signs related to RTI were seen in the key acute toxicity study, and they were supported by clinical signs in well performed repeated exposure toxicity studies. Altogether, RAC considers that the weight of evidence supports the proposal of the DS and, therefore, a **classification for STOT SE 3, H335 is warranted.**

Narcotic effects

***Recapitulative table**: effects seen at non-lethal doses, reversible and visible at the first exposure*

Study	Non-lethal dose	Clinical effects indicative of narcotic effects
Anon. (2004) - inhal. (nose only)	5173 ppm	None
Anon. (1999) - inhal. (nose only)	44000 ppm (with uncertainties)	Relaxed breathing and animals appeared to be anesthetized. After exposure, impaired motor activity was seen but returned to normal after 10 min. Occurrences not mentioned
Anon. (2013d) - inhal. (whole body) - 90d study	No lethal dose	From 505 ppm: Transient underactivity, unresponsiveness and closed eye lids (partially at MD, fully at HD) seen in all animals.
Anon. (2013c) - inhal. (whole body)	No lethal dose	From 198 ppm: Underactivity and unresponsiveness (all animals) From 505 ppm: partially closed eyelids (all animals).
Anon. (2014) - inhal. (whole body)	No lethal dose	10000 ppm: Transient hypoactivity, decreased respiration, completely shut eyelids (all animals)

According to CLP Regulation, "*narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure."* Guidance values do not apply to STOT SE 3 unlike to STOT SE 1 and 2.

Signs of sedation were reported in Anonymous (2004), but only after exposure to a dose leading to 100% lethality (highest dose - 26580 ppm). In Anonymous (1999), temporary anaesthesia and relaxed breathing were reported shortly after the single 30-min exposure and returned to normal within 10-minutes post-exposure. In Anonymous (2013c) OECD TG 421 study, transient clinical signs such as underactivity and unresponsiveness were observed at all doses after the first day of treatment. These effects were reversible at the end of the daily 6-hour exposure period or before the end of the working day. In Anonymous (2013d) 90-day repeated exposure toxicity study, transient underactivity, unresponsiveness and partially closed eyelids were described from day 1 in exposed animals at non-lethal doses. In Anonymous (2014) OECD TG 421 study, in which lower doses were tested in the standard 6-hour daily exposure groups than in the other available repeated dose studies, hypoactivity and decreased respiration were described on the first day of exposure in the "special acute exposure group" (daily 5-minute exposure to 10000 ppm). Although no narcotic effect at the non-lethal dose in the key acute toxicity study was recorded, consistent evidence of potential narcotic effects was found in repeated-dose toxicity studies. Altogether, the overall data set support the DS proposal, therefore, **a classification STOT SE 3, H336 is warranted.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

Two inhalation (whole body) reproduction/developmental toxicity screening tests, performed on Sprague-Dawley rats, were available. Both of them were compliant with OECD TG 421 and GLP.

In Anonymous (2013c), longer estrus cycles in females and abnormal sperm parameters in males were described, as well as a decrease of copulation plugs, fertility index and mating percentage. In addition, a shift toward a longer gestation duration was seen. In high dose group, only one female littered, leading to a strong gestation index decrease (considered in development part in RAC opinion). From the DS point of view, the value given in the full study report for implantation count for the mid dose group was not correctly derived, since implantations from one female showing total resorption was not included in the mean calculation. After recalculation, the DS also noted a dose-dependent decrease of implantation counts (significance unknown). Sacrifice due to poor condition around the delivery date and shortly after was necessary in some females from mid and high dose groups. According to the DS, linked to an increase in the gestation length, these mortalities could be associated with dystocia. Altogether, the DS considered that the effects on sexual function and fertility were related to treatment and not secondary consequences of systemic toxicity. Some of the findings identified in the first study were confirmed in Anonymous (2014), in which lower dose levels were tested, mainly longer gestation duration, lower pituitary weights and longer mean pre-coital interval (without statistical significance). These effects were not co-occurring with mortality or marked systemic toxicity.

Therefore, the DS concluded that there was clear evidence of adverse effects on sexual function and fertility and proposed a classification Repr. 1B, H360F.

Development

The same two inhalation studies that were described in sexual function and fertility section were available for the assessment of developmental toxicity in the CLH dossier.

From the DS point of view, the values given in Anonymous (2013c) were not correctly derived, and new calculated values (total litter size per dose group, live litter size on days 1, 5 and 10 per dose group, post-implantation survival index, live birth index and viability index), were provided in the CLH dossier. A reduction in total litter size and live litter size (in all exposed groups) was reported. A decrease in post-implantation survival, viability and live birth indices were also reported. As these indices were recalculated, their statistical significances were not determined, but the effects were clear and dose dependent. Several pups were sacrificed due to poor condition (reduced activity and body temperature). The DS concluded that all these developmental effects occurred in the absence of a clear maternal systemic toxicity. Therefore, the reported effects on development were considered treatment-related and not secondary consequences of maternal systemic toxicity. In Anonymous (2014), a reduced postnatal survival (not statistically significant) was also observed at the two highest doses. Additionally, the DS noted a dose-dependent increase in interventricular septal defect (VSD), which was also considered treatment related and a severe effect. Both adverse effects occurred in absence of systemic maternal toxicity.

Therefore, the DS concluded that there was clear evidence of developmental toxicity and proposed a classification Repr. 1B, H360D.

Lactation

The results obtained in the two screening toxicity studies were considered inconclusive for adverse effect on or via lactation. Therefore, no classification was proposed.

Comments received during consultation

One MSCA supported the classification proposed by the DS (Repro. 1B; H360FD). Another MSCA considered that the case was a borderline between classification category 2 (the sperm effects were observed only in one study, reduced fertility index at high dose only was associated with general toxicity) and category 1B (gestation length and effect on oestrus cycles) for sexual function and fertility but supported a classification category 1B for development. One National Authority commented the dossier and raised uncertainties: the high number of sacrifices in Anonymous (2013c), the whole-body exposure (exposure via grooming) and the possibility that narcotic effect could have had an impact on mating performance. DS replied that only two from the three dams that failed to mate showed clinical signs and that the fourth dam that successfully mated but failed to become pregnant showed no clinical signs. In addition, the reported clinical signs were reversible by the end of the working day. The poor clinical condition described in sacrificed animals seemed to be clearly related to pregnancy at mid and top doses. It was mainly observed prior to or around the delivery date and was thus considered to be related to dystocia rather than to general poor condition of dams. This conclusion was supported also by the observation that the 'poor condition' was not observed in males and none of the males was sacrificed at any tested dose.

Several comments were provided by Industry. Most of them supported a classification as Repr. 2 (H361fd), based on several arguments. Namely, clinical observations in females from Anonymous (2013c), acute toxicity (Anonymous, 2004) and sub-chronic (13-week, Anonymous, 2013d) inhalation studies were considered to suggest parental toxicity consisting in CNS depression, respiratory irritation (inducing a post-treatment general malaise), body weight decrease, stress (reference to Everds *et al.*, 2013) and lack of maternal care. These effects would cause most of the findings identified on fertility and development as secondary non-specific effects. In addition, Industry indicated that the mode of action was unlikely to be through endocrine disruption as functions regulated by sex hormones both increased and decreased in the same dose groups. Industry did not provide much weight to VSD identified in dead pups at or near birth in Anonymous (2014) because it was considered to be caused by autolysis of the membranous tissue closing the septum or by retardation of fetal growth in utero. Finally, Industry mentioned the inter-species variability: rats tend to be more susceptible than humans to RTI because they have more convoluted nasal turbinates and are obligate nose breathers. DS responded that clinical observations were reversible after the 6-hour exposure or before the end of the working day and that narcotic effects did not induce death as no deaths occurred in males nor in the sub-chronic study. In females, reductions in body weight gain were accompanied only by slight decreases in mean body weights and, although the decrease in body weight was more pronounced in males, none of the males showed poor clinical condition. RAC stresses that no mechanistic data was provided to confirm or infirm any mode of action. In addition, a detailed assessment of toxicity in pregnant animals cannot be extrapolated from studies with non-pregnant animals. VSD will be discussed later in the opinion.

Assessment and comparison with the classification criteria

Sexual function and fertility

Anonymous, 2013 c

10 Crl:CD (SD) rats/sex/dose were exposed to 0, 198, 505 and 2900 ppm of 2-BTP (purity: 99.9%) by inhalation (whole-body exposure). Exposure took place 6 hours/day, 7 days/week from 15 days before pairing to day 10 of lactation. Females were untreated from Day 20 after mating until Day 4 after birth (inclusive). No historical control data were available for this study (except for copulation plugs, see below).

Underactivity, unresponsiveness, piloerection and partially closed eyelids were described in all exposed groups (both sexes), with low incidence in low dose group. Shallow and/or slow breathing were also seen in males and females from mid and high dose groups. These effects lasted occasionally after exposure completion in females, mainly from the highest doses groups, but ended before the end of the working day (up to 2h later).

All females from high dose group and half of the mid dose group were killed before study termination as they failed to mate (sacrificed on Day 25 after the last paring), failed to litter (sacrificed on Day 25 after mating), had a total litter loss post-partum (sacrificed on the day the last pup died, between pre-LD1 and LD2) or were sacrificed for welfare reasons (GD24 and LD1, see table 2). The latter condition concerned 3 dams, two from highest dose (276 and 279) and one in the mid dose group (262). In the high dose group, both animals were killed on GD24, and one of them was noted to be attempting parturition. This poor condition could be related to dystocia. The animal from the mid dose group had a total litter loss, in addition to a poor condition, and no evidence of lactating was collected. No specific clinical signs were seen in that animal before parturition, and no macroscopic finding that could explain the death was identified. One animal from the low dose group (257) was not pregnant and therefore was killed on Day 25 after the last paring.

In males, a statistically significant decrease in mean body weight gain compared to control was seen in all doses tested at study termination, leading to a lower mean body weight (table below). No significant changes in food or water consumption were seen. In females, a statistically significant decrease in mean body weight and body weight gain were observed at the highest dose prior to pairing. During gestation, a statistically significant decrease in body weight and body weight gain was seen in mid dose group (no statistical analysis performed for the high dose group). This could be linked with the dose-dependent decrease in litter size noted in exposed females (see development part of this opinion). During lactation, the body weights were not recorded in the high dose group (no litters survived), but a statistically significant decrease in body weight gain in low and mid dose groups was described from LD1 to LD10 (see table 10 of CLH report). A statistically significant lower body weight in mid dose group compared to control was also visible, but at LD10 only (not at LD1 and LD5, see table 10 of the CLH report). This could reflect the reintroduction of the treatment at LD5, but also the decrease in food consumption that was already visible at the beginning of the lactation phase in the lowest and mid dose groups. A statistically significant decrease in food consumption was visible during gestation, but without clear dose dependency (see table 10 of CLH report).

A dose-dependent increase in pale teeth was described in males (1/10, 0/10, 6/10 and 10/10 cases in 0, 198, 505 and 2900 ppm, respectively) and females (0/10, 0/10, 3/10 and 8/10 cases in 0, 198, 505 and 2900 ppm, respectively). The potential causes were not discussed in the full study report.

No organ weight evaluation was provided for females. A significant decrease in relative pituitary weight was reported in males, but the pituitary was not examined histopathologically. Capsular

thickening and adhesions in spleen were seen in both treated males and females at the macroscopic examination (tables below), whereas no case was reported in control animals. Some histological findings were mentioned in spleen in both sexes (adhesions/inflammation/fibrosis, capsular thickening or inflammation, see tables 24 and 25 of the CLH report), but as no controls were examined microscopically (both sexes), the significance of these changes is unclear. Microscopic examination of females revealed a reduction in the corpora lutea size at highest dose, but without dose dependency (Table 25 of the CLH report).

***Table**: Summary of relevant information: Females (Anonymous, 2013c)*

	0 ppm	198 ppm	505 ppm	2900 ppm
Killed before LD10: Welfare/ Poor condition TLL No litter *** Fail to mate	 0/10 0/10 0/10 0/10	 0/10 0/10 1/10 0/10	 1/10 (LD1) 4/10 (PLD1-LD2) 1/10 0/10	 2/10 (GD24) 1/10 (LD2) 4/10 (D25 post mating) 3/10 (D25 post pairing)
Body weight before pairing / mean BW change **D 1-15** (% ctrl)	245g/ 20g	252g/ 24g	244g/ 19g	**232g*/ 4g*** (-5.3%/-80%)
Body weight at GD20 / mean BW change **GD 0-20** (% ctrl)	410g/ 154g	397g/ 142g	**377g*/128g*** (-8.1%/-16.9%)	305g/66g (only 6 pregnant females)
Body weight at LD10 / mean BW change **LD 5-10** (% ctrl)	341g/ 20g	322g/ 11g	**297g*/ -2g*** (-12.9%/ -)	N/A
Macroscopic and microscopic observations				
Spleen adhesions	0/10	0/10	1/10	5/10
Spleen capsule thickened	0/10	0/10	2/10	3/10
Spleen enlarged	0/10	0/10	0/10	1/10
Sexual function and fertility				
Oestrus (%)**: 2-3 days/4 days/5 days/6-10 days/11-20 days	0%/82%/18%/0%/0%	0%/**30%/70%**/0%/0%*	0%/**18%/61%/18%/3%***	**4%/4%/29%/46%/18%***
Pre-coital interval in days (% of animals)	1-4 d: 90% 5-8 d: 10%	1-4 d: 90% 5-8 d: 0% **9-12 d: 10%**	1-4 d: 70% **5-8 d: 30%**	**1-4 d: 57%** **5-8 d: 29%** **9-12 d: 14%**
% Mating	100%	100%	100%	**70%***
Fertility index	100%	90%	100%	**60%***
Gestation length in days (% of live litter born)	22-22.5 d: 90% 23 d: 10%	**23-23.5 d: 100%**	**23-23.5 d: 44%** **24.5-25 d: 33%** **25.5 d: 22%**	**25.5 d: 100% (1F)**
Implantation count (*Value recalculated by the DS*)	15.9	**14.1**	14.1 ***(13.0)***	**9.5**

*Statistically significant ($p<0.05$ or $p<0.01$). **Oestrus cycle %: number of cycles in category. ***No litter: total resorption and/or not pregnant.

Regarding sexual function and fertility, a dose-dependent (in occurrence and severity) and statistically significant increase in longer oestrus cycles was observed at 505 and 2900 ppm compared to controls. In addition, more females had irregular cycles (shorter cycles of 2-3 days or longer cycles of 6-10 days) or were acyclic (at least 10 days without oestrus) in these dose groups. Although the cycles were regular, a clear and statistically significant increase of oestrus cycle duration (5 days) was already visible from low dose group (table above). No effect on body weight or body weight gain in females from mid and high doses groups were reported before pairing.

A longer pre-coital interval was observed in mid and high dose groups but without statistical significance (table above). According to the author, except in 2 cases (one in mid dose and one in high dose group), females mated at the first recorded oestrus. Therefore, this finding could reflect the reduction in regular oestrous cycles. A statistically significant decrease in mating percentage in the high dose group (7/10 females mated, see table above) as well as a statistically significant trend in the decrease in copulation plugs in the mid and high dose groups was noted, although the latter finding did not appear clearly dose dependent in occurrence and severity (see table 16 of the CLH report). The HCD was provided solely for this finding (Table 16 of the CLH report) and the number of animals with only one copulation plug was outside of the HCD range for low and mid doses only (and not for high dose group). Therefore, the toxicological relevance of this finding appears unclear. A non-significant decrease in sperm count in the vaginal smear (Table 17 of CLH report) was also reported. Of the 3/10 females (272, 275, 277) that failed to mate in the high dose group, one did not show evidence of systemic effects (two others showed occasionally hunched posture or piloerection). All animals from low and mid dose groups succeeded to mate. A decrease in fertility index (number of animals reaching pregnancy/animals pairing) was reported in the high and low dose groups, although the decrease was significant only in the high dose group. Two females (from the low and high dose groups, 257 and 280, respectively) mated but did not reach pregnancy and did not show systemic effects after exposure cessation. The mean body weights were not provided for females during pairing, but they were statistically significantly decreased (between -5.3% and -6%) between the end of the pre-pairing period and the beginning of the gestation in high dose group (see table 10 of the CLH report).

A statistically significant increase in gestation duration was described in all exposed groups. The increase in occurrence and severity appeared dose-related. This finding occurred also at doses with low (mid dose) or no (low dose) change in body weight in dams. A dose-dependent decrease in mean implantation counts was seen (table above) after recalculation of the mid dose value by the DS. In all dose groups, the values reported in the table below were obtained considering pregnant animals only, including animals with total resorption or total litter loss. Nevertheless, the statistical significance is unknown for mid- (as recalculated) and high dose groups (not included in statistical evaluation analysis).

***Table**: Summary of relevant information: Males (Anonymous, 2013c)*

	0 ppm	198 ppm	505 ppm	2900 ppm
Body weight at D50 / mean BW change D1-50 (% ctrl)	542g/ 212g	**483g*/ 164g*** (-10.9%/ -22.6%)	**455g*/ 133g*** (-16.1%/ -37.3%)	**424g*/ 99g*** (-21.8%/ -53.3%)
Organ weight and macroscopic observations				
Spleen adhesions	0/10	2/10	3/10	4/10
Spleen capsule thickened	0/10	8/10	6/10	9/10
Pituitary weight Absolute (g)/ Relative	0.015/ 0.013	0.011 (-26%)/ **0.011*** (-15.39%)	0.011 (-26%)/ **0.011*** (-15.39%)	0.009 (-40%)/ **0.010*** (-23.08%)

Prostate small	0/10	1/10	7/10	10/10
Prostate weight Absolute (g)/ Relative	1.364/ 1.297	1.008 (-26.10%)/ **1.000*** (-22.90%)	0.937 (-31.30%)/ **0.960*** (-25.98%)	0.625 (-54.18%)/ **0.677*** (-47.81%)
Seminal ves. Weight Absolute (g)/ Relative	1.667/ 1.627	1.357 (-18.60%)/ 1.352 (-16.91%)	1.128 (-32.33%)/ **1.142*** (-29.81%)	1.125 (-32.51%)/ **1.156*** (-28.95%)
Epididymis weight (Absolute) (g)	1.337	1.269 (-5.09%)	1.214 (-9.20%)	**1.164* (-12.94%)**
Total abnormal sperm	1.5±0.9%	**3.4±2.2%***	**4.2±1.9%***	**9.8±6.1%***
VAP (µm/s)**	138	**126***	**119***	**125***
VSL (µm/s)**	102	92	**85***	**90***
VCL (µm/s)**	299	**265***	**240***	**260***
Progressively motile sperm (%)	59%	48%	44%	**43%***
Total sperm in cauda epipidymis (millions)	281	269	223	**197***
BCF (Hz)	25	27	26	**28***

* Statistically significant ($p<0.05$ or $p<0.01$). ** VAP: Average path velocity; VSL: Progressive/straight line velocity; VCL: curvilinear velocity or track speed.

A dose-dependent increase in small prostates was seen in all exposed males. Decreases in relative prostate and seminal vesicles weights, compared to the control group, were also observed in all exposed groups. Except the seminal vesicles weight in the low dose group, all these findings were statistically significant (Table 3). In addition, statistically significantly reduced absolute epididymis weight was noted at the high dose (relative values not provided).

A statistically significant and dose-dependent increase in total abnormal sperm was seen in all exposed groups (table above). Observed abnormalities were primarily breakages, and more specifically detached/broken neck/midpiece/tail number, decapitate sperm and abnormal head shape (Table 14 of the CLH report). No body weight loss was observed in exposed males (Table 9 of the CLH report). In males in the low dose group, only a slight (not marked) decrease in body weight compared to control (<11%) was seen. Clinical observations were of low severity in the low dose group and did not persist after exposure completion (all doses). Statistically significant reductions in sperm velocity were seen from low (VAP and VCL) and mid dose groups (VSL). In addition, reductions in progressively motile sperm and total sperm in the cauda epididymis as well as an increase in BCF were described in the highest dose group (table above).

Anonymous, 2014

12/sex/dose Sprague Dawley rats were exposed to 0, 50, 100 and 175 ppm of 2-BTP (purity 99.4%) by inhalation (whole body exposure). Exposure took place 6 hours/day, 7 days/week, from 14 days before pairing to GD20 (35-46 days) in females and until the end of the mating period (28-29 days) in males. An extra group (Group 5), exposed to 10000 ppm of 2-BTP during 5 minutes/day, was included to mimic the worst case scenario of human exposure according to the study sponsor (complete discharge of fire extinguisher in confined space or aircraft). All females were allowed to give birth and to nurse their pups until sacrifice (pups and dams) at lactation day 4.

At 10000 ppm, clinical findings such as hypoactivity, decreased respiration, completely shut eyelids and lacrimation were described, but only on the first day of exposure and were resolved by 1 hour following exposure. In addition, salivation and red and/or clear material around the mouth and/or nose were also noted for both sexes, 15 minutes and/or 1 hour following exposure,

throughout the respective exposure period. No clinical findings were observed in the other groups and all animals survived to study termination, with the exception of two females from the 175 ppm dose group: one (91654) was killed because of a total litter loss and another (91623) because it was not pregnant/ did not mate.

A decrease in mean body weight gains was noted in males from the 10000 ppm group. This decrease was generally statistically significant during the entire period (table 26 of the CLH report) and resulted in a slight but significant mean body weight decrease (less than 10%, see the table below) compared to the control group at day 28. Considering the entire treatment period, a statistically significant decrease in body weight gain was seen in males from the 100 ppm exposure group, but without significant effect on body weight (table 26 of the CLH report). A statistically significant decrease in food consumption was visible in the same group. As no dose dependency was seen for these changes, the toxicological relevance is uncertain. Only sporadic decrease in body weight gain, compared to controls, was seen in females from 175 ppm and 10000 ppm dose groups during gestation (table 27 of the CLH report). Isolated and slight, but statistically significant decrease in food consumption was seen during the beginning of premating and gestation in females from the 175 ppm dose group (below 10 and 15%, respectively), whereas a significant increase in water consumption throughout gestation period, followed by a significant decrease during lactation in the same group (table 27 of the CLH report).

A statistically significant decrease in pituitary weight was seen in both sexes from 100, 175 and 10000 ppm dose groups (table below). Nevertheless, the values were within or just below HCD and not dose-dependent. In the 175 ppm group males, a statistically significant increase in mean relative weight of lung was described (table below), but according to the statement in the full study report, the values were within the HCD ranges and the decrease was not dose-dependent. For both organs, no associated histological findings were seen (microscopical evaluation performed for high dose group). For these reasons, these findings were considered of low toxicological relevance.

No evidence of mating was found for one pair of animals from the 175 ppm dose group (female 91623 and male 91568). Accordingly, no litter was sired and the fertility index was slightly decreased (table below) but without statistical significance and the index was still within HCD range (70-100%). In addition, from the 11 pairs of animals who succeeded to mate in the 175 ppm group, a longer mean pre-coital interval was mentioned, but without statistical significance (table below) and the effect was within the HCD range (1.8-4.7). Nevertheless, it has to be noted that, in addition to the pair that did not mate in the high dose, 2/11 pairs had 10 days or more before the coitus and other 2/11 had between 6 and 7 days before coitus. This finding was considered treatment related and adverse. All pairs mated ≤ 5 days after pairing in control and low dose groups (table below). Oestrus cycles were not affected by treatment. All males that mated sired a litter. A dose-dependent (for females exposed 6h/d) increase in mean gestation length was observed in all exposed groups. This increase was statistically significant for the females exposed to 100 and 175 ppm (table below), and outside the HCD range (21.5 – 22.3 days) in the 175 ppm dose group. This finding is a reflection of the increase in the number of individuals with a gestation length of 23 days, seen in all the exposed groups (no occurrence in control), with clear dose dependency (for females exposed 6h/d). Therefore, this effect is considered test substance related and adverse. No other significant changes were seen in females.

In the 10000 ppm group, a statistically significant increase in relative weight of left epididymis as well as relative weight of right testis were seen (Table 30 of the CLH report). However, in the absence of absolute weight changes or histological findings, these were considered a result of the low mean final body weight. No significant other effects were observed in F0 males.

***Table**: Summary of relevant information: Males and females (Anonymous, 2014)*

	0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
Females					
Killed before LD 4: TLL Not pregnant	 0/12 0/12	 0/12 0/12	 0/12 0/12	 1/12 1/12 (D25)	 0/12 0/12
Pituitary weight Absolute/Relative	0.0207/ 0.006	0.019/ 0.006	**0.0156*(-24.64%)/ 0.005*(-16.67%)**	**0.0175*(-15.46%)/ 0.005*(-16.67%)**	**0.0175*(-15.46%)**/ 0.005
Oestrus cycle length (days)	5.2±2.41	4.6±0.50	4.9±0.42	5.0±1.05	5.1±1.67
Pre-coital interval in days	2.9±1.16	2.9±1.62	2.4±1.62	4.5±3.78	2.0±1.04
N pairs with ⩾6 days pre-coitus	0	0/12	1/12	4/12	0/12
Fertility index (%)	100% (12/12)	100% (12/12)	100% (12/12)	91.7% (11/12)	100% (12/12)
Gestation length in days	21.7 ±0.49	22.1 ±0.29	**22.3 ±0.49***	**22.6 ±0.50***	22.1 ±0.29
Gestation length of 23 days	0/12	1/12	**4/11**	**7/12**	1/12
Males					
BW at D28 / mean BW change D0-28 (% ctrl)	448g/ 78g	456g/ 84g	431g/ **57g***	438g/ 68g	**418g*/ 48g*** (-6.7%/-38.5%)
Pituitary weight Absolute (g)/Relative	0.0156/ 0.0033	0.0146/ 0.0031	**0.0126* (-19.23%)/ 0.0029*(-12.5%)**	**0.0127*(-18.59%)/ 0.003*(-9.09%)**	**0.0133*(-14.74%)**/ 0.0031
Lung weight Absolute (g) /Relative	1.66/0.369	1.71/0.377	1.69/0.393	1.8/**0.412* (11.65%)**	1.64/0.392

* Statistically significant ($p<0.05$ or $p<0.01$).

Conclusion on sexual function and fertility

Both studies provided by the dossier submitter are relevant for the classification proposal. No general toxicity was detected in Anonymous (2014). In Anonymous (2013c), general toxicity was seen after 2-BTP exposure. In females, the poor general condition leading to sacrifice of two animals from the highest dose group seem to be linked to dystocia. No clinical signs (except during exposure) or abnormalities were detected before LD1 in the dam from mid dose group that was sacrificed for ethical reasons and had a total litter loss. No macroscopic findings were recorded for this animal, and it cannot be excluded that its poor condition was also caused by dystocia. This is supported by the fact that no mortalities, considered as a direct consequence of exposure, occurred in the subchronic toxicity studies from Anonymous (2013d) despite the doses being similar and the exposure longer. Only a slight decrease in body weight in females from the mid dose group was detected during gestation (without body weight loss) and lactation (-8.05% and -12.9% compared to control, respectively). This effect could also be explained by the fact that the litters from these dams were smaller than in the control group (see section on developmental toxicity). In addition, other clinical signs were transient and were not present at the end of the working day (2h post exposure). Such effects were not identified or were of lower severity in animals from the low exposure group. The males survived until study termination, and the clinical signs were not apparent after the end of exposure. A body weight decrease compared to control was seen in all dose groups but remained moderate (not marked) in the low

dose group (-10.9%). No body weight loss was observed. In conclusion, the reprotoxic effects recorded in Anonymous (2013c) are considered relevant for classification purposes.

A statistically significant and dose dependent increase in gestation duration was seen in both studies. In both studies a tendency for a longer pre-coital interval was observed, and in some animals of the highest dose even an absence of mating was reported. When higher doses were tested (Anonymous, 2013c), a statistically significant and dose-dependent increase in oestrus duration was noted. A concerning increase in dystocia was also seen at mid and highest doses (Anonymous, 2013c). In that study, adverse effects on fertility were also noted in males, with a statistically significant and dose-dependent increase in total abnormal sperm, as well as a dose dependent increase in the number of animals with small prostate and a decrease in relative prostate weight. These effects are already visible in the lowest dose group. Altogether, these findings provide clear evidence of an adverse effect on sexual function and fertility following 2-BTP exposure at doses not causing marked systemic effects. No mechanistic evidence was provided that could raise doubts on the human relevance of these observations. Altogether, RAC concludes that **a classification as Repr. 1B (H360F) is warranted**.

Development

Anonymous 2013c

10 Crl:CD (SD) rats/sex/dose were exposed to 0, 198, 505 and 2900 ppm of 2-BTP by inhalation (whole body exposure). Exposure took place from 15 days before pairing to day 10 of lactation, but females were untreated from Day 20 after mating until Day 4 after birth (inclusive). In a few females from the two highest doses, poor general condition leading to early sacrifice surrounding the parturition was seen. Systemic effects (body weight, clinical condition) were described, but cannot be considered as marked, especially in the low and mid dose groups (see Table 2 and the fertility section of the opinion for general toxicity).

Only six animals from the highest dose group were pregnant. A dose-dependent increase in dams with total resorption (implantation visible, litter loss in utero) was visible, with one case in the mid dose group (263) and three others (271, 274 and 278) in the high dose group (table below). Only one (274) of these dams was described as having a general poor condition at the moment of the sacrifice. In the 505 ppm dose group, a clear increase in total litter loss was detected (at LD2 the latest). The majority of the pups from this group were noted to be cold on day 1. The pups that died or were from the litters that were sacrificed early were frequently being noted as dark in colour or inactive. In the surviving offspring, no later signs were generally noted. Absence of milk in the stomach was also frequently observed in pups from the mid dose group. A strong and statistically significant decrease in gestation index (number of live litters born/number pregnant females) was seen in high dose group, where only one dam succeeded to have a live litter. This value reflects a combination of several adverse events, among other, total resorption and total litter loss. This decrease in gestation index was also observed in the mid dose group, but statistical significance was not reached (table below).

A dose-dependent decrease in total and live mean litter size at day 1 as well as live mean litter size at day 10 (mean) was seen. These changes were statistically significant from the lowest dose group. These values were calculated based on all pregnant females or females that littered where applicable (Table 32a from the CLH report). According to the DS, the values given in the full study report for post-implantation survival index (total number of offspring born/ total number of uterine implantation sites), live birth index (number of live offspring on day 1 after littering/ total number of offspring born) and viability index (number of live offspring on day 10 after littering/ number of live offspring on day 1 after littering) were incorrectly derived and therefore new calculated values were provided in table 33b of the CLH report. Nevertheless, it seems that the female with total resorption (263) was not included in the calculation of post-

implantation survival index made by the DS for the mid dose group, leading to a small overestimation (63% instead of 61.5%, see table below). The post-implantation survival, live birth and viability indices calculated for the high dose group considered only the female that littered. Nevertheless, a dose-dependent decrease in post implantation survival index, live birth index and viability index was visible from the calculations by both DS and the authors of the full study report (table below). The statistical significance was reached in the full study report at mid dose (the values recorded for high dose were not included in the statistical evaluation). In low dose group, 3/9 litters (255, 258, 259) showed lower post implantation survival (63-74%), and 2 (252, 255) showed lower viability indices (54 and 82%), which contributed to a lower total and live litter sizes on day 1 of lactation. A relationship to treatment was considered likely. It can be reminded that the dams were not exposed during the last days of gestation, parturition and first days of lactation. Therefore, the hypothesis that the decreased offspring viability index was secondary to lack of maternal care due to stress of direct exposure or to transient CNS/RTI effect seems less plausible.

A slight but statistically significant increase in body weight at PND1 was seen in female pups from low dose group compared to controls. In male pups, a statistically significant decrease in body weight gain (compared to controls) was detected in the mid dose group (table below). As these findings were not dose dependent and they were recorded in one sex only, their toxicological relevance remains unsure.

***Table**: Summary of relevant information: Dams and pups (Anonymous, 2013c)*

	0 ppm	198 ppm	505 ppm	2900 ppm
Post-implantation survival index *(Value recalculated by the DS or RAC)*	94.5% *(94.3%)*	86.0% *(86.6%)*	**56.6%*** *(61.5%)*	25% (one litter)
Pregnant dams	10/10	9/10	10/10	6/10
With total litter loss	0/10	0/10	**4/10**	**1/6**
With total resorption	0/10	0/10	**1/10**	**3/6**
Gestation index	100%	100%	90%	**17%***
Total litter size day 1 (calculated by DS)	150	110	80	3
Live litter size day 1 (calculated by DS)	149	106	57	1
Live litter size day 10 (calculated by DS)	147	95	33	-
Total litter size D1 (mean)	15.0±1.2	**12.2±3.3***	**8.9±4.3*/****	3.0 (one litter)
Live litter size D1 (mean)	14.9±1.2	**11.8±3.1***	**11.4±2.2*/****	1.0 (one litter)
Live litter size D10 (mean)	14.7±1.3	**10.6±3.3***	**8.3±2.1*/****	- (no pups alive)
Live birth index *(calculated by DS)*	99.4% *(99.3%)*	96.8% *(96.4%)*	**51.7%*** *(71.2%)*	33.3% (one litter)
Viability index D1-10 *(calculated by DS)*	98.6%	90.3% *(89.6%)*	**62.6%*** *(57.9%)*	0% (one litter)
Offspring body weight PND1				
Males	6.8±0.3	7.4±0.6	6.9±0.7	- (no pups alive)
Females	6.4±0.4	**6.9±0.4***	6.2±0.2	5.5 (1 pup)***
Offspring body weight gain				
Males (D1-10)	10.7±0.9	9.6±0.8	**9.1±2.2***	- (no pups alive)
Females (D1-10)	10.3±0.9	9.3±1.4	8.8±2.4	-

* Statistically significant ($p<0.05$ or $p<0.01$). **For statistics (mean and significance): values calculated with all pregnant females/females that littered where applicable. *** Not included in statistical evaluation.

Anonymous, 2014

12 Crl:CD (SD) rats/sex/dose were exposed to 0, 50, 100, 175 and 10000 ppm of 2-BTP by inhalation (whole body exposure). Exposure took from 14 days before pairing to GD20 in females and until the end of the mating period in males. Two females from the 175 ppm dose group were killed before study termination (one with total litter loss and the other was not pregnant/ did not mate). Except slight systemic effects at 10000 ppm, resolved by 1 hour following exposure, no severe clinical effect or strong body weight decrease compared to controls were seen (see the fertility section of the opinion).

A slight but dose-dependent decrease in the mean number of pups born and live litter size (PND 0) was observed at all doses tested, although without statistical significance. A dose-dependent decrease in post-natal survival (from birth to PND4) was seen, and the values were below the HCD range (83.8-98.7%) in 175 ppm dose group. In the same group, one female had a total litter loss (HCD: 14 females with a total litter loss on 1868 females selected to deliver). No significant clinical observations were mentioned for this female. This decrease in post-natal survival was considered test substance-related and adverse by the study author, although not statistically significant.

At PND1, a statistically significant increase in pup body weight (compared to controls) was noted in males from the 50 ppm, 100 ppm and 10000 ppm dose groups. However, this increase was not dose dependent and it was not statistically significant in the 175 ppm dose group. In addition, pup body weights on PND 4 and mean body weight gains during PND 1-4 were similar to the control group and no statistically significant changes were seen for body weight and body weight gain in female pups compared to controls (table below, see also table 38 from the CLH report).

A dose-dependent increase in VSD (an opening in the anterior portion of the septum of 1 or 2 mm in diameter) was seen at 100 and 175 ppm dose groups. In the 175 ppm dose group, 15.2% of the pups were affected. This investigation was not included in Anonymous (2013c), and only pups found dead were necropsied in Anonymous (2014). Although interventricular defects could be considered as a developmental delay in the cardiac system, they may be considered adverse depending on the size of the opening and the impact on viability and growth of the pups. Therefore, associated with the reduction of postnatal survival in high dose group, this finding is considered test substance-related and adverse. Milk was found in the stomach in one of these pups (the other pups were too autolysed to be examined for this issue). Vertebral agenesis, anal atresia, fused kidney (defined as malformation), as well as major blood vessel variation, were found in one pup of the 175 ppm dose group, whereas another pup showed dark red contents in the thoracic cavity (see table 39 of the CLH dossier).

***Table**: Summary of relevant information: Dams and pups (Anonymous, 2014)*

	0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
Dams with total litter loss/ Dams pregnant	0/12	0/12	0/12	1/11	0/12
Mean number of pups born	14.3 ± 1.78	13.8 ± 2.12	13.7 ± 2.96	13.3 ± 2.10	13.9 ± 1.24
Mean Live litter size D0	13.9 ± 1.98	13.6 ± 2.39	13.0 ± 2.98	12.7 ± 1.74	13.5 ± 1.51
Postnatal survival** – Birth to PND4	92.8%	92.1%	84.1%	**67.9%**	95.7%
Offspring body weight (g) PND1 – Males	6.8	**7.9* (+16.17%)**	**7.6* (+11.76%)**	7.3	**7.8*% (+14.7)**
Offspring body weight (g) PND4 – Males	9.6	10.2	9.7	9.7	10.2
Interventricular septal defect: n cases/ n examined per dose (%) - n litter concerned/ n litter examined per dose (%)	0/11 (0%) – 0/8 (0%)	0/11 (0%) – 0/6 (0%)	1/16 (6.2%) – 1/9 (11%)	5/33 (15.2%) – 2/10 (20%)	0/7 (0%) – 0/4 (0%)

* Statistically significant ($p<0.05$ or $p<0.01$). **Percentage per litter.

Conclusion on development

Both studies provided by the dossier submitter are relevant for the classification proposal. No maternal toxicity was observed in Anonymous (2014), whereas in Anonymous (2013c), general toxicity after 2-BTP exposure was seen in some females from the high dose group. Nevertheless, this toxicity does not correspond to such severe systemic effects that it would be reasonable to assume that developmental toxicity was produced solely as a secondary consequence of maternal toxicity as defined in the CLP 3.7.2.4.3 of Annex I (when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups – see "conclusion on sexual function and fertility" part of this opinion)

A dose-dependent decrease in post-natal survival was seen in both studies. This decrease in survival is reflected by a dose-dependent decrease in the viability index, life birth index and a statistically significant and dose-dependent decrease in the mean live litter size at D1 and D10 (compared to control) in Anonymous (2013c). Several of these findings are already significant at the lowest dose. In Anonymous (2014), although not statistically significant, a dose dependent decrease in postnatal survival (birth to PND4) was seen, and it was well below the HCD range provided for the highest dose. In both studies, case(s) of total litter loss were observed, despite very low occurrence reported in HCD available (Anonymous, 2014). In addition, a dose-dependent increase in total resorptions as well as a dose-dependent decrease in post-implantation survival and live birth index in Anonymous (2013c) were identified, which could indicate that 2-BTP is also lethal in utero after higher exposure. A dose-dependent increase in VSD (outside of the HCD range) was considered adverse in combination with the viability index decrease and it was observed in several litters from the Anonymous (2014) study.

Altogether, these findings provide clear evidence of an adverse effect on development after 2-BTP exposure, already visible at doses without marked systemic effects. No mechanistic evidence raising doubt on human relevance of these observations were provided, and RAC concludes that a **classification as Repr. 1B (H360D) is warranted**.

Lactation

No toxicokinetic data indicating the likelihood of the substance presence in milk is available.

Two screening toxicity studies were presented in the CLH report. In Anonymous (2013c), the females were untreated from day 20 after mating until lactation day 4 (inclusive) and sacrificed at lactation day 10 at the latest (see above). This treatment interruption increases the uncertainties regarding pups exposure to 2-BTP via milk, especially in the first days of lactation. In Anonymous (2014), females were not exposed during lactation phase, as they were exposed until GD20. No other studies were available to investigate potential adverse effects on lactation.

Therefore, RAC considers **classification is not warranted for adverse effects on or via lactation**, **due to lack of data** (as proposed by the DS).

Additional references

Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, Rosol TJ, Sellers T. 2013. Interpreting Stress Responses during Routine Toxicity Studies: A Review of the Biology, Impact, and Assessment. Toxicologic Pathology. Vol. 41: 560-614.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable).

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