CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

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

EC / List Number: -

CAS Number: 1514-82-5

Index Number: n.a.

Contact details for dossier submitter: **Ministry of Health
Paseo del Prado, 18-20
28071 - Madrid
Spain**

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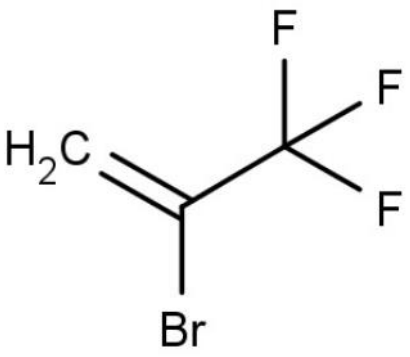
CONTENTS

TABLES

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*2-bromo-3,3,3-trifluoroprop-1-ene*
Other names (usual name, trade name, abbreviation)	*Halotron BRX*
ISO common name (if available and appropriate)	*n.a.*
EC number (if available and appropriate)	*n.a.*
EC name (if available and appropriate)	*n.a.*
CAS number (if available)	*1514-82-5*
Other identity code (if available)	*n.a.*
Molecular formula	*C3H2BrF3*
Structural formula	
SMILES notation (if available)	*n.a.*
Molecular weight or molecular weight range	*174.947*
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*n.a.*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*n.a.*
Degree of purity (%) (if relevant for the entry in Annex VI)	*n.a.*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
2-bromo-3,3,3-trifluoroprop-1-ene (CAS no. 1514-82-5)	Mono-constituent	n.a.	Repr. 2 (H361: Suspected of damaging fertility or the unborn child) STOT SE 3 (H335: May cause respiratory irritation) STOT SE 3 (H336: May cause drowsiness or dizziness)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	**harmonised classification proposed (Repr. 1B; H360FD)**	Yes
Specific target organ toxicity-single exposure	**harmonised classification proposed (STOT SE 3; H335, H336)**	Yes
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

2-bromo-3,3,3-trifluoroprop-1-ene is not listed in Annex VI of CLP and has not been considered for harmonised classification and labelling previously.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

For the hazard class Toxicity for Reproduction there is no requirement for justification that action is needed at Community level.

Reason for need for action at Community level for the classification as STOT SE:

Differences in self-classification

2-BTP is self-classified by several notifiers. As reported on the ECHA dissemination site, there are in total a number of 50 notifiers in the C&L inventory (July 2022).

STOT SE 3 - H335 (Respiratory System; inhalation): 1/50 notifiers

STOT SE - H335 (Nervous System; inhalation, oral): 1/50 notifiers

STOT SE – H336 (Central Nervous System; inhalation): 1/50 notifiers

5 IDENTIFIED USES

According to the information from REACH registration dossier, uses of 2-bromo-3,3,3-trifluoroprop-1-ene (2-BTP) include: filling of hand-held fire extinguisher and emergency discharge of fire extinguishers within the aviation industry.

The substance is imported into the EU. It is described in the registration to be transferred to fire extinguisher cylinders (formulation) via a closed system at dedicated facilities. Professional and consumer use are flagged in the registration dossier regarding the very rare situation of an emergency discharge of fire extinguishers.

6 DATA SOURCES

The following data sources have been taking into account for the compilation of this CLH report:

- REACH registration data
- The ECHA dissemination website.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid	(ECHA Dissemination, 2021)	Visual assessment Purity of test substance: 99.9%
Melting/freezing point	-111.2 ºC	(ECHA Dissemination, 2021)	OECD 102/EU A.1 Purity of test substance: 99.9%
Boiling point	34.4°C at 1013 mbar	(ECHA Dissemination, 2021)	OECD 103/EU A.2 Purity of test substance: 99.9%
Relative density	1.65 at 20°C	(ECHA Dissemination, 2021)	OECD 109/EU A.3 Purity of test substance: 99.9%
Vapour pressure	82000 Pa at 25°C	(ECHA Dissemination, 2021)	OECD 104/EU A.4 Purity of test substance: 99.9%
Surface tension	72 mN/m at 20ºC	(ECHA Dissemination, 2021)	OECD 115/EU A.5 Purity of test substance: 99.9%

Property	Value	Reference	Comment (e.g. measured or estimated)
Water solubility	1.01 g/L at 20 °C	(ECHA Dissemination, 2021)	OECD 105/EU A.6 Purity of test substance: 99.9%
Partition coefficient n-octanol/water	2.7 at 25 °C	(ECHA Dissemination, 2021)	OECD 117/EU A.8 Purity of test substance: 99.9%
Flash point	No flashpoint observed below the boiling point	(ECHA Dissemination, 2021)	
Flammability	Non flammable	(ECHA Dissemination, 2021)	The study does not need to be conducted because the substance is a liquid at room temperature
Explosive properties	Non explosive	(ECHA Dissemination, 2021)	Non-explosive on the basis of a theoretical assessment of the chemical structure
Self-ignition temperature	No value available		
Oxidising properties	No oxidising properties	(ECHA Dissemination, 2021)	Not oxidising on the basis of an assessment of the chemical structure
Granulometry	Not relevant	(ECHA Dissemination, 2021)	The substance is a liquid

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There is no specific toxicokinetic study performed with 2-BTP.

A toxicokinetic assessment was provided based on the physicochemical properties of the substance, the available data from an *in vitro* method to determine partition coefficients (Anonymous, 2013a), and the *in vivo* toxicological studies included in the registration dossier.

Accordingly, 2-BTP is readily absorbed via the lungs. Also it is considered likely that 2-BTP will cross the skin barrier, although dermal exposure will be limited by the compound volatility and boiling point close to body temperature. There is no available information regarding absorption via the oral route. Systemic distribution to liver, spleen, heart and reproductive organs in rats or dogs is supported by the toxicity studies and the partition coefficient values. Although no data are available on metabolism in the toxicity studies available, histopathological changes in the liver observed in the subchronic toxicity study suggest some metabolic activity. Similarly there is no data on excretion; however, rapid excretion and a lack of bioaccumulation were supported by a post-exposure quick blood concentration decrease and a rapid recovery of the clinical signs observed in a study in dogs (Anonymous, 2013b) as well as by its partition coefficient (Anonymous, 2013a).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not assessed in this dossier.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Not assessed in this dossier.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

10.6 Respiratory sensitisation

Not assessed in this dossier.

10.7 Skin sensitisation

Not assessed in this dossier.

10.8 Germ cell mutagenicity

Not assessed in this dossier.

10.9 Carcinogenicity

Not assessed in this dossier.

10.10 Reproductive toxicity

The influence of 2-BTP on reproduction has been investigated in two well conducted, guideline- and GLP-compliant inhalation reproductive/developmental toxicity screening studies (according to OECD TG 421) in SD rats (Table 8).

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Inhalation reproduction/ developmental toxicity screening test (OECD 421) GLP: Yes Rat/Crl:CD (SD) 10 animals/sex/dose	2-BTP (purity: 99.9%) Inhalation: vapour (whole body) Concentrations: 0, 198, 505, 2900 ppm Exposure: from 15 days before pairing to Day 10 of lactation.	**F0 - Parental generation** **General toxicity** <u>Mortality and general clinical observations</u> *198 ppm* Underactivity, unresponsiveness, piloerection, and partially closed eyelids (occasionally observed and reversible after 6 hour exposure or before the end of the working day). *505 ppm* Five females and litters sacrificed due to poor condition during lactation. Underactivity, unresponsiveness, piloerection, partially closed eyelids, shallow and/or slow breathing (ocassionally and reversible after 6 hour exposure or before the end of the working day). *2900 ppm* Two females sacrificed on Day 24 after mating due to poor condition. Another female with a live litter born was killed for reasons of animal welfare after parturition on Day 25 following total litter loss. Three females were sacrified on Day 25 after mating as parturition had not started and there was no indication of this occurring. Underactivity, unresponsiveness, piloerection, partially closed eyelids, hunched posture, shallow and/or slow breathing (reversible after 6-hour exposure or before the end of the working day). <u>Body weight, food and water consumption (Tables 9-10)</u> *198 ppm* Males: ↓ Body weight gain throughout study (Days 1-50) (22.64%, $p<0.01$). ↓ Mean body weight (<10%). Females: ↓ Body weight gain during lactation (Days 1-10) (40.54%, $p<0.05$). No statistically significant changes in mean body weight. Males: ↓ Food consumption prior to pairing (mean Weeks 1-2) (11.58%, not statistically significant). Females: ↓ Food consumption prior to pairing (mean Weeks 1-2) (8.34%, not statistically significant), during gestation (GD 0-19) (11.11%, $p<0.05$) and during lactation (Days 1-9) (28.57%, $p<0.01$). Females: ↑ Water consumption prior to pairing (Week 2) (17.24%, not statistically significant), during gestation (14.47%, not statistically significant) and ↓ during lactation (Days 1-3: 5.8%, not statistically significant) (Days 4-9: 17.39%, not statistically significant).	Anonymous, 2013c

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		505 ppm Males: ↓ Body weight gain throughout study (Days 1-50) (37.27%, p<0.01). ↓ Mean body weight (<20%). Females: ↓ Body weight gain during gestation (GD 0-20) (16.88%, p<0.05) and lactation (Days 1-10) (86.49%, p<0.01). ↓ Mean body weight during gestation (<10%) and lactation (<15%). Males: ↓ Food consumption prior to pairing (mean Weeks 1-2) (24.08%, not statistically significant). Females: ↓ Food consumption prior to pairing (mean Weeks 1-2) (18.18%, not statistically significant), during gestation (GD 0-19) (11.11%, p<0.01) and during lactation (Days 1-9) (40.82%, p<0.01). Females: ↑ Water consumption prior to pairing (Week 2) (58.62%, not statistically significant) and during gestation (GD 0-17) (16.35%, not statistically significant) and ↓ during lactation (Day 5) (39.78%, not statistically significant). *2900 ppm* Males: ↓ Body weight gain throughout study (Days 1-50) (53.30%, p<0.01). ↓ Mean body weight (up to 20.27%). Females: ↓ Body weight gain prior to pairing (Days 1-15) (80.00%, p<0.01) and during gestation (GD 0-20) (57.14%, not statistically significant). ↓ Mean body weight on Days 8 and 15 pior to pairing (<10%). No statistically significant changes in mean body weight during gestation. Males: ↓ Food consumption prior to pairing (mean Weeks 1-2) (24.08%, not statistically significant). Females: ↓ Food consumption prior to pairing (mean Weeks 1-2) (30.30%, not statistically significant) and during gestation (GD 0-19) (22.22%, not statistically significant). Females: ↑ Water consumption prior to pairing (Week 2) (72.41%, not statistically significant) and during gestation (GD 0-16) (15.46%, not statistically significant). <u>Organ weights (Table 21)</u> *198 ppm* ↓ Relative prostate (22.90%, p<0.01), seminal vesicles (16.91%, not statistically significant) and pituitary (15.39%, p<0.05) weights. *505 ppm* ↓ Relative prostate (25.98%, p<0.01), seminal vesicles (29.81%, p<0.05) and pituitary (15.39%, p<0.05) weights. *2900 ppm* ↓ Relative prostate (47.81%, p<0.01), seminal vesicles (28.95%, p<0.05), and pituitary (23.08%, p<0.05) weights and absolute epididymis (12.94%, p<0.01) weights. <u>Gross pathology (Tables 22-23)</u>	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		198 ppm Males: Small prostates (1/10), spleen capsular thickening (8/10) and adhesions (2/10). *505 ppm* Males: Small prostates (7/10), spleen capsular thickening (6/10) and adhesions (3/10), pale incisor teeth (6/10). Females: Spleen capsular thickening (2/10) and adhesions (1/10), pale incisor teeth (3/10). *2900 ppm* Males: Small prostates (10/10), spleen capsular thickening (9/10) and adhesions (4/10), pale incisor teeth (10/10). Females: Spleen capsular thickening (3/10) and adhesions (5/10), pale incisor teeth (10/10). <u>Histopathology (Tables 24-25)</u> *198 ppm* Males: Spleen capsular/subcapsular inflammation (8/8), capsular thickening (4/8), adhesions/inflammation/fibrosis (5/5). Females: Reduced size of corpora lutea (1/10). *505 ppm* Males: Spleen capsular/subcapsular inflammation (6/7), capsular thickening (6/7), adhesions/inflammation/fibrosis (4/7). Females: Spleen capsular/subcapsular inflammation (1/2), capsular thickening (2/2), adhesions/inflammation/fibrosis (2/2). *2900 ppm* Males: Spleen capsular/subcapsular inflammation (7/9), capsular thickening (7/9), adhesions/inflammation/fibrosis (6/9). Females: Spleen capsular/subcapsular inflammation (4/7); capsular thickening (7/7); adhesions/inflammation/fibrosis (5/7). Reduced size of corpora lutea (4/10). **Sexual function and fertility** <u>Oestrus cycle length (Table 11)</u> *0 ppm (control group)* 82% of all cycles of 4 days and 18% of 5 days. *198ppm* Longer oestrus cycles: 70% of all cycles of 5 days, 30% of all cycles of 4 days ($p<0.01$). *505ppm* Longer oestrus cycles: 18% of cycles of 6 days or longer, 3% of 11 days or longer ($p<0.01$) and 79% of the total cycles being regular (4-5 days).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		2900 ppm Longer oestrus cycles: 46% of all cycles of 6 days or longer and 18% of 11 days or longer ($p<0.01$). Only 33% of the total cycles being regular (4-5 days). <u>Sperm measures (Tables 12-13-14)</u> *198 ppm* ↓ Sperm velocity (↓ VAP: 8.70%, $p<0.05$; ↓ VCL: 11.37%, $p<0.01$). ↑ Total abnormal sperm (3.40% *vs* 1.50% in controls, $p<0.01$). *505 ppm* ↓ Sperm velocity (↓ VAP: 13.77%, $p<0.01$; ↓ VCL: 19.74%, $p<0.01$; ↓ VSL: 16.67%, $p<0.05$). ↑ Total abnormal sperm (4.20% *vs* 1.50% in controls, $p<0.01$). *2900 ppm* ↓ Progressively motile sperm (43.00% *vs* 59.00% in controls, $p<0.05$). ↓ Sperm velocity (↓ VAP: 9.42%, $p<0.01$; ↓ VCL: 13.04% $p<0.01$; ↓ VSL: 11.77%, $p<0.01$). ↓ Sperm number in the cauda epididymis (29.89%, $p<0.01$). ↑ BCF (Beat Cross Frequency) (12.00%, $p<0.01$). ↑ Total abnormal sperm (9.80% *vs* 1.50% in controls, $p<0.01$). <u>Pre-coital interval (Table 15)</u> *0 ppm (control group)* 90% (9/10) animals with intervals being of 1-4 days, 10% (1/10) animals of 5-8 days. *198 ppm* All pairings showed evidence of mating. 90% (9/10) animals with intervals being of 1-4 days, 10% (1/10) animals of 9-12 days. *505 ppm* All pairings showed evidence of mating. 70% (7/10) animals with intervals being of 1-4 days, 30% (3/10) animals of 5-8 days. *2900 ppm* Only 7 of the pairings with evidence of mating. 57% (4/7) animals with intervals being of 1-4 days, 29% (2/7) animals of 5-8 days, 14% (1/7) animals of 9-12 days. <u>Copulation plugs at mating (Table 16)</u> *0 ppm (control group)* 80% (8/10) animals with 4-6 copulation plugs, 20% (2/10) animals with 3 copulation plugs. *198 ppm* 70% (7/10) animals with 4-6 copulation plugs, 10% (1/10) animals with 3 copulation plugs, 10% (1/10) animals with 2 copulation plugs and 10% (1/10) animals with 1 copulation plug.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		505 ppm 20% (2/10) animals with 4-6 copulation plugs, 60% (6/10) animals with 3 copulation plugs, 10% (1/10) animals with 2 copulation plugs and 10% (1/10) animals with 1 copulation plug. Statistically significant decrease ($p<0.05$). *2900 ppm* 29% (2/7) animals with 4-6 copulation plugs, 43% (3/7) animals with 3 copulation plugs, 29% (2/7) animals with 2 copulation plugs. Statistically significant decrease ($p<0.05$). <u>Sperm counts in the vaginal smear (Table 17)</u> *0 ppm (control group)* 60% solid masses of sperm, 20% many scattered sperm, 10% continuous few sperm, 10% no sperm. *198 ppm* ↓ Sperm count: 10% solid masses of sperm, 40% many scattered sperm, 20% continuous few sperm, 30% occasional sperm, 0% no sperm. *505 ppm* ↓ Sperm count: 20% solid masses of sperm, 20% many scattered sperm, 10% continuous few sperm, 10% occasional sperm, 40% no sperm. *2900 ppm* ↓ Sperm count: 14% solid masses of sperm, 57% many scattered sperm, 29 % continuous few sperm, 0% occasional sperm, 0% no sperm. <u>Mating performance and fertility (Table 18)</u> *2900 ppm* ↓ Fertility index (60% *vs* 100% in controls) ($p<0.05$) and percentage of mating (70% *vs* 100% in controls) ($p<0.05$). <u>Duration of gestation and gestation index (Table 19)</u> *198 ppm* ↑ Duration of gestation (23-23.5 days *vs* 22-23 days in controls) ($p<0.01$). *505 ppm* ↑ Duration of gestation (23-25.5 days *vs* 22-23 days in controls) ($p<0.01$). *2900 ppm* ↑ Duration of gestation (25.5 days with only one female littering *vs* 22-23 days in controls) ($p<0.01$). ↓ Gestation index (17% *vs* 100% in controls) ($p<0.01$). <u>Implantation counts (Table 20)</u> *198 ppm* ↓ Implantations counts (14.1 *vs* 15.9 in controls). *505 ppm* ↓ Implantation counts (13 *vs* 15.9 in controls).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		2900 ppm ↓ Implantation counts (9.5 *vs* 15.9 in controls). **NOAEC for fertility and reproductive effects was established below 198 ppm, based on reproductive effects observed in parental animals.**	
Inhalation reproduction/ developmental toxicity screening test (OECD 421) GLP: Yes Rat/Sprague-Dawley 12 animals/sex/dose	2-BTP (purity: > 99.4%) Inhalation: vapour (whole body) Concentrations: 0, 50, 100, 175 ppm Special acute exposure group: 10000 ppm Exposure: Males 14 days prior to mating and throughout the mating period for a total of 28-29 days of exposure. Females 14 days prior to pairing and until gestation Day 20 (total of 35-46 days). Females that failed to deliver dosed through the day prior to euthanasia for a total of 52 days.	**F0 - Parental generation** **General toxicity** <u>Mortality and general clinical observations</u> No mortality at any exposure concentration. <u>Body weight, food and water consumption (Tables 26-27)</u> *100 ppm* Males: ↓ Body weight gain (Days 0-28) (26.92%, p<0.05). No changes in mean body weights. No changes in females. Males: ↓ Food consumption (Days 7-13) (6.78%, p<0.05) No changes in females. Females: ↑ Water consumption during gestation (GD 4-7) (22.80%, p<0.01). ↓ Water consumption during lactation (Days 1-4) (19.13%, p<0.05). *175 ppm* Males: ↓ Body weight gain (Days 21-28) (37.50%, p<0.05). No changes in mean body weights. Females: ↓ Body weight gain during gestation days 0-4 (32.00%, p<0.05) and during gestation days 11-14 (33.33%, p<0.01). Females: ↓ Food consumption during pre-mating (Days 0-7) (9.86%, p<0.01) and during gestation (GD 0-4) (14.29%, p<0.01) No changes in males. Females: ↑ Water consumption throughout gestation (GD 0-20) (19.66%, p<0.01). ↓ Water consumption during lactation (Days 1-4) (24.04%, p<0.01). <u>Organ weights (Table 30)</u> *100 ppm* Males: ↓ Pituitary weight (absolute: 19.23%, p<0.01; relative: 12.50%, p<0.01). Females: ↓ Pituitary weight (absolute: 24.64%, p<0.01; relative: 16.67%, p<0.01). Within the range of historical control data. *175 ppm* Males: ↓ Pituitary weight (absolute: 18.59%, p<0.01; relative:	Anonymous, 2014

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		9.09%, p<0.01). ↑ Mean relative lungs weight (11.65%, p<0.01). Females: ↓ Pituitary weight (absolute: 15.46%, p<0.05; relative: 16.67%, p<0.05). Within the range of historical control data. **Sexual function and fertility** <u>Reproductive performance (Tables 28-29)</u> *100 ppm* ↑ Mean gestation length (22.3 days *vs* 21.7 days in controls) (p<0.01) but within the range of historical control data. *175 ppm* ↑ Mean gestation length (22.6 days *vs* 21.7 days in controls) (p<0.01) and out of the range of historical control data. ↑ Mean pre-coital interval (4.5 days *vs* 2.9 days in controls), not statistically significant and within the range of historical control data. *Acute exposure group (10000 ppm)* Hypoactivity, decreased respiration, completely shut eyelids, lacrimation in males and females only the first day of exposure. Salivation and red and/or clear material around the mouth and/or nose in males and females throughout 15 minutes and 1 hour post-exposure. Males: ↓ Mean body weight gain throughout exposure period (Days 0-28) (38.46%, p<0.01) resulted in ↓ mean body weight on Day 28 (6.70%, p<0.05). ↓ Food consumption during pre-mating period (Days 7-13) (8.48%, p<0.01). ↑ Relative left epididymis weight (9.92%, p<0.01), right testis weight (9.94%, p<0.01) and absolute pituitary weight (14.74%, p<0.05) Females: ↓ Mean body weight gain during gestation (Days 11-14) (22.22%, p<0.05). ↓ Food consumption during pre-mating (Days 0-7) (8.45%, p<0.05). ↓ Absolute pituitary weight (15.46%, p<0.05) but within the range of historical control data. **NOAEC for systemic, fertility and reproductive effects was established at 100 ppm, based on increases in mean water consumption for females during gestation, longer mean pre-coital intervals and longer mean gestation length.**	

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

Two inhalation reproductive/developmental toxicity screening studies are available to assess the effect of 2-BTP on sexual function and fertility.

Inhalation reproduction/developmental toxicity screening test (OECD TG 421) (Anonymous, 2013c)

A GLP reproduction/developmental toxicity screening test in Crl:CD (SD) rats, flagged in the IUCLID as supporting study, was conducted with 2-BTP according to OECD TG 421 (Anonymous, 2013c). A summary of the study results is included in Table 8.

The substance was administered, by whole-body inhalation exposure, to groups of 10 animals per sex and dose level, at aerosol concentrations of 198, 505 and 2900 ppm (achieved chamber concentrations). Control animals were exposed to air only, using the same dosing procedure. Animals were exposed from 15 days before pairing to day 10 of lactation, 6 hours/day, 7 days/week. Animals of the F1 generation were exposed via mothers during gestation and lactation.

The selection of these concentrations was based on the results of two-week dose-range finding studies. It has to be noted that the target exposure levels considered for this screening study were the same as those used in the sub-chronic inhalation toxicity study (Anonymous, 2013d). The high exposure concentration was selected to allow assessment of reproductive effects at an exposure concentration anticipated to produce evidence of systemic toxicity. Lower concentrations were chosen to assess any possible effect observed.

Mortality was observed at the two highest doses. At 2900 ppm only one out of six pregnant females littered on Day 25 after mating. This single dam with a live litter born was killed for reasons of animal welfare following total litter loss. Two other females were sacrificed on day 24 after mating with signs possibly associated with stress involved in commencement of parturition. One of these dams had underactive, reduced body temperature, hunched posture and piloerection, and the other one had piloerection and perigenital staining. The remaining 3 females were sacrificed on Day 25 after mating as parturition had not commenced and there was no indication of this occurring. These females were found to have been pregnant but had no viable conceptuses at necropsy.

In addition, at 505 ppm, five females and litters were sacrificed during lactation. One of these dams showed total litter loss post partum associated with general poor condition of the dam. The remaining four were sacrified before lactation Day 2 due to total litter loss.

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. In addition, hunched posture was also occasionally observed 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.

At 198 ppm, underactivity, unresponsiveness, piloerection and partially closed eyelids were occasionally noted as well, being reversible at the end of the daily exposure period or before the end of the working day, even though these effects occurred at a much reduced incidence than those observed at higher doses. Only underactivity and piloerection were noted immediately after exposure during gestation.

Lower mean body weight gain was observed in males at all doses tested throughout the study. In females, lower mean body weight gain was observed at 2900 ppm prior to pairing, at 505 and 2900 ppm during gestation and at 198 and 505 ppm during lactation (at this stage, no body weights were recorded at 2900 ppm since no litters survived at this dose). Nevertheless, these changes in body weight gain were only accompanied by slight decreases in mean body weight values. In males, decreases in mean body weight values were lower than 20% at all doses throughout the study. In females, decreases lower than 10% were observed at 2900 ppm on Days 8 and 15 pior to pairing. At 505 ppm decreases lower than 10% and 15% were observed during gestation and at the end of lactation, respectively.

Despite the statistically significant changes in body weight gain, the slight decreases observed in mean body weights (<20% for males and <15% for females) cannot be considered as a marked systemic toxicity.

In addition, lower food intake was observed in both sexes at all treated doses prior to pairing and in females during the gestation phase. During lactation, this decrease was noted at 198 and 505 ppm (no litters survived at the highest dose). A non-statistically significant increase in water intake was noted for females prior to mating and during gestation for all groups but decreasing during lactation at 198 and 505 ppm (Tables 9-10).

Table 9: Body weight (g), body weight change (g), food consumption (g/animal/day) and water consumption (mL/animal/day) data for F0 male animals from the screening test (Anonymous, 2013c)

Doses (ppm)		0 ppm	198 ppm	505 ppm	2900 ppm
F0 - Mean body weight (entire period)	Day 1	329	319	322	325
	Day 8	380	**361* (-5.00%)**	**349** (-8.16%)**	**342** (-10.00%)**
	Day 11	395	**375* (-5.07%)**	**357** (-9.62%)**	**355** (-10.13%)**
	Day 15	420	**393* (-6.43%)**	**376** (-10.48%)**	**368** (-12.38%)**
	Day 22	444	420	**398** (-10.36%)**	**386** (-13.06%)**
	Day 29	471	**443* (-5.95%)**	**415** (-11.89%)**	**406** (-13.80%)**
	Day 33	487	**452* (-7.19%)**	**426** (-12.53%)**	**409** (-16.02%)**
	Day 36	498	**460* (-7.63%)**	**434** (-12.85%)**	**414** (-16.87%)**
	Day 39	503	**465* (-7.56%)**	**437** (-13.12%)**	**415** (-17.50%)**
	Day 43	517	**469** (-9.29%)**	**441** (-14.70%)**	**419** (-18.96%)**
	Day 46	528	**476** (-9.85%)**	**449** (-14.96%)**	**421** (-20.27%)**
F0 - Mean body weight change (entire period)	D 1-15	91	74	**54** (-40.66%)**	**43** (-52.75%)**
	D 15-50	121	**90** (-25.62%)**	**79** (-34.71%)**	**56** (-53.72%)**
	D 1-50	212	**164** (-22.64%)**	**133** (-37.27%)**	**99** (-53.30%)**
F0 - Mean food consumption (before pairing)	Week 1	220	191	162	149
	Week 2	212	190	165	179
	Mean weeks 1-2	216	191 (-11.57%)	164 (-24.07%)	164 (-24.07%)
F0 - Mean water consumption (before pairing)	Week 2	45	43	44	47
	Week 5	41	38	40	39
	Week 6	42	40	42	39
	Week 7	42	37	40	39

*: $p<0.05$; **: $p<0.01$

Table 10: Body weight (g), food consumption (g/animal/day) and water consumption (mL/animal/day) data for F0 female animals from the screening test (Anonymous, 2013c)

Doses (ppm)		0 ppm	198 ppm	505 ppm	2900 ppm
F0 - Mean body weight (before pairing)	Day 1	225	227	225	228
	Day 8	237	239	232	**227* (-4.22%)**
	Day 11	239	244	236	231
	Day 15	245	252	244	**232** (-5.31%)**
F0 - Mean body weight change (before pairing)	D 1-15	20	24	19	**4** (-80.00%)**
F0 - Mean body weight (gestation)	Day 0	256	255	249	239
	Day 3	273	272	265	254
	Day 7	294	286	**277* (-5.78%)**	260
	Day 10	304	296	**287* (-5.59%)**	272
	Day 14	327	317	312	285
	Day 17	360	347	**333* (-7.50%)**	293
	Day 20	410	397	**377* (-8.05%)**	305
F0 - Mean body weight gain (gestation)	D 0-10	49	41	38	33
	D 10-20	106	100	90	33
	D 0-20	154	142	**128* (-16.88%)**	66 (-57.14%) Only 6 females
F0 - Mean body weight (lactation)	Day 1	304	300	292	**No litters**
	Day 5	322	311	299	
	Day 10	341	322	**297* (-12.90%)**	
F0 - Mean body weight gain (lactation)	D 1-5	18	11	7	**No litters**
	D 5-10	20	11	**-2** (-110.00%)**	
	D 1-10	37	**22* (-40.54%)**	**5** (-86.49%)**	
F0- Mean food consumption (before pairing)	Week 1	133	117	101	86
	Week 2	130	124	114	98
	Mean weeks 1-2	132	121 (-8.33%)	108 (-18.18%)	92 (-30.30%)
F0- Mean food consumption (gestation)	Day 0-2	24	22	21	19
	Day 3-6	26	**21** (-19.23%)**	**22** (-15.39%)**	19
	Day 7-9	26	**21** (-19.23%)**	**22** (-15.39%)**	20
	Day 10-13	27	**25* (-7.41%)**	**24** (-11.11%)**	21
	Day 14-16	28	25	**24** (-14.29%)**	21
	Day 17-19	29	30	29	24
	Mean days 0-19	27	**24* (-11.11%)**	**24**(-11.11%)**	21 (-22.22%)
F0-Mean food consumption (lactation)	Day 1-4	40	**31** (-22.5%)**	**28** (-30.00%)**	No litters
	Day 5-9	56	**39** (-30.36%)**	**30** (-46.43%)**	
	Mean D 1-9	49	**35** (-28.57%)**	**29** (-40.82%)**	
F0-Mean water consumption (before pairing)	Week 2	29	34 (+17.24%)	46 (+58.62%)	50 (+72.41%)
F0-Water consumption (gestation)	Days 0-2	60	55	61	66
	Days 3-5	54	64	62	65
	Days 6-8	57	67	63	65
	Days 9-11	64	71	76	75
	Days 12-15	67	76	82	77
	Days 16-17	70	79	91	77
F0-Water consumption (lactation)	Days 1-3	69	65 (-5.8%)	44	No litters
	Days 4-9	92	76 (-17.39%)	71	

*: $p<0.05$ **: $p<0.01$

In relation to the effects on sexual function and fertility, statistically significant longer oestrus cycles (6 days or longer) with more females having irregular cycles or being acyclic were observed at 505 and 2900 ppm, compared to the control group. At 198 ppm, regular cycles were observed but with a tendency to be longer than controls (5 days) (Table 11).

Table 11: Oestrus cycle evaluation for F0 generation females from the screening test (Anonymous, 2013c)

Oestrous cycles - incidence of specific cycle lengths - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of animals	Number of cycles		Cycle length (days) 2	3	4	5	6-10	11-20
Statistical test: Lt									
1	10	39	n	0	0	32	7	0	0
			(%)			(82)	(18)		
2**	10	33	n	0	0	10	23	0	0
			(%)			(30)	(70)		
3**	10	33	n	0	0	6	20	6	1
			(%)			(18)	(61)	(18)	(3)
4**	10	28	n	1	0	1	8	13	5
			(%)	(4)		(4)	(29)	(46)	(18)

n Number of cycles in category

Sperm measures showed statistically significant reductions in percent progressively motile sperm, sperm velocity, sperm count in the cauda epididymis and increases in BCF (Beat Cross Frequency) and in abnormal sperm (breakages and abnormal head shape) at the highest dose. Statistically significant reductions in sperm velocity and increases in abnormal sperm were observed at mid and low doses (Tables 12-14).

Table 12: Sperm analysis for F0 generation males from the screening test (Anonymous, 2013c)

Sperm analysis - group mean values

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group		Motile sperm (%)	Progressively motile sperm (%)	------ Cauda epididymis ------ Weight (g)	Sperm count (millions/g)	Total (million)	-------- Testis -------- Weight (g)	Sperm count (millions/g)	Total (million)
Statistical test:		Sh	Wi	Wi	Wi	Wi	Sh	Wi	Wi
1	Mean	94	59	0.240	1174	281	1.80	199	359
	SD	3	15	0.026	160	62	0.13	37	71
	n	10	10	10	9	9	10	10	10
2	Mean	95	48	0.237	1145	269	1.84	195	362
	SD	4	19	0.016	187	37	0.10	65	135
	n	10	10	10	10	10	10	10	10
3	Mean	92	44	0.219	957	223	1.66	148	270
	SD	11	21	0.046	396	94	0.49	75	157
	n	9	9	10	10	10	10	10	10
4	Mean	92	43*	0.199**	1003	197**	1.74	179	312
	SD	4	11	0.027	209	37	0.11	43	85
	n	10	10	10	10	10	10	10	10

Table 13: Sperm motility for F0 generation males from the screening test (Anonymous, 2013c)

Sperm motion data - group mean values

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group		VAP (um/s)	VSL (um/s)	VCL (um/s)	ALH (um)	BCF (Hz)	STR (%)	LIN (%)	Elongation (%)	Area (um sq)	Rapid (%)	Medium (%)	Slow (%)	Static (%)
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Sh
1	Mean	138	102	299	22	25	74	35	24	487	67	3	24	6
	SD	8	9	24	2	2	4	2	5	95	15	2	15	3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10
2	Mean	126*	92	265**	22	27	73	36	25	506	54	5*	36	5
	SD	13	10	22	1	2	4	2	4	124	19	2	19	4
	n	10	10	10	10	10	10	10	10	10	10	10	10	10
3	Mean	119**	85*	240**	21	26	71	37	25	445	52	5*	36	8
	SD	14	13	29	2	3	3	2	2	87	25	2	22	11
	n	9	9	9	9	9	9	9	9	9	9	9	9	9
4	Mean	125**	90**	260**	22	28**	72	37	26	525	51	7**	35	8
	SD	13	13	31	1	1	4	2	4	155	13	2	10	4
	n	10	10	10	10	10	10	10	10	10	10	10	10	10

Table 14: Sperm morphology for F0 generation males from the screening test (Anonymous, 2013c)

Sperm morphology - group mean values

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of animals	Total number of sperm examined		Normal		Total abnormal		Decapitate		Head abnormal		Detached/broken neck/midpiece/tail		Other abnormality	
				number	%	number	%	number	%	number	%	number	%	number	%
Statistical test:					Sh		lWi		Sh		Sh		Sh		Wi
1	10	2000	Mean	197	98.6	3	1.5	1.4	0.7	1.0	0.5	0.2	0.1	0.4	0.2
			SD	2	0.9	2	0.9	2.1	1.0	0.8	0.4	0.6	0.3	0.7	0.3
2	10	2013	Mean	194	96.6	7	3.4**	3.0	1.5	3.0	1.5	0.6	0.3	0.4	0.2
			SD	5	2.2	5	2.2	2.9	1.5	3.6	1.8	0.7	0.3	0.5	0.3
3	8	1600	Mean	192	95.8*	8	4.2**	4.8	2.4	2.6	1.3	1.3	0.6*	0.1	0.1
			SD	4	1.9	4	1.9	4.4	2.2	2.7	1.4	1.2	0.6	0.4	0.2
4	10	2000	Mean	181	90.3**	20	9.8**	10.3	5.2**	6.8	3.4**	2.7	1.4**	1.2	0.6**
			SD	12	6.1	12	6.1	8.9	4.5	4.8	2.4	2.3	1.2	0.8	0.4

At 2900 ppm, effects on mating performance such as longer pre-coital interval (Table 15), decreases in the percentage of mating (70% *vs* 100% in controls, $p<0.05$), fewer copulation plugs (Table 16) and lower but not statistically significant sperm count in the vaginal smear (Table 17) were recorded. Fertility index is reduced to 60% *vs* 100% in controls ($p<0.05$) (Table 18) due to the fact that three females failed to mate, two of them with apparent clinical signs and the other one with no evicence of systemic effects. A fourth female successfully mated showed no evidence of pregnancy in the absence of clinical signs. In addition, extended duration of gestation (25.5 days) with only one female littering on Day 25 of gestation were noted (Table 19). For this reason, the gestation index was reduced to 17%, reflecting the single litter born.

At 505 ppm, the same effects but slightly less pronounced were reported; in this case, the duration of gestation was between 23 and 25.5 days. At 198 ppm, a slightly lower sperm count in the vaginal smear and

a shift to a longer duration of gestation (23-23.5 days) were also observed. No changes in the fertility index were reported at mid and low doses.

Table 15: Pre-coital intervals for F0 generation animals from the screening test (Anonymous, 2013c)

Pre-coital interval - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of animals		Pre-coital interval (days) 1-4	5-8	9-12	13-14
Statistical test: Lt						
1	10	n	9	1	0	0
		(%)	(90)	(10)		
2	10	n	9	0	1	0
		(%)	(90)		(10)	
3	10	n	7	3	0	0
		(%)	(70)	(30)		
4	7	n	4	2	1	0
		(%)	(57)	(29)	(14)	

Table 16: Copulation plugs in F0 generation females from the screening test (Anonymous, 2013c)

Number of copulation plugs at mating - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of animals		Number of copulation plugs 1	2	3	4 - 6
Statistical test: Lt						
1	10	n	0	0	2	8
		(%)			(20)	(80)
2	10	n	1	1	1	7
		(%)	(10)	(10)	(10)	(70)
3*	10	n	1	1	6	2
		(%)	(10)	(10)	(60)	(20)
4*	7	n	0	2	3	2
		(%)		(29)	(43)	(29)
Historical control data [a]						
	288	%	0.4	7.6	15.6	72.7

a From 29 studies of similar type and age at pairing

Table 17: Sperm counts in the vaginal smear in F0 generation females from the screening test (Anonymous, 2013c)

Sperm count estimates from vaginal smears at mating - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of animals		Sperm count category ϖ 0	1	2	3	4
Statistical test: Lt							
1	10	n	1	0	1	2	6
		(%)	(10)		(10)	(20)	(60)
2	10	n	0	3	2	4	1
		(%)		(30)	(20)	(40)	(10)
3	10	n	4	1	1	2	2
		(%)	(40)	(10)	(10)	(20)	(20)
4	7	n	0	0	2	4	1
		(%)			(29)	(57)	(14)

ϖ 0 No sperm
1 Occasional sperm
2 Continuous few sperm
3 Many scattered sperm
4 Solid masses of sperm

Table 18: Mating performance and fertility for F0 animals from the screening test (Anonymous, 2013c)

Mating performance and fertility - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
Statistical test:				Ca	Ca	Ca
1M	10	10	10	100	100	100
2M	10	10	9	100	90	90
3M	10	10	10	100	100	100
4M	10	7	6	70*	86	60*
Statistical test:				Ca	Ca	Ca
1F	10	10	10	100	100	100
2F	10	10	9	100	90	90
3F	10	10	10	100	100	100
4F	10	7	6	70*	86	60*

Table 19: Gestation length and gestation index for F0 females from the screening test (Anonymous, 2013c)

Gestation length and gestation index - group values - F0 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group	Number of pregnant animals		Gestation length (days) 22	22.5	23	23.5	24	24.5	25	25.5	Number of live litters born	Gestation index (%)
Statistical test:						Lt						Ca
1	10	n	6	3	1	0	0	0	0	0	10	100
		(%)	(60)	(30)	(10)							
2**	9	n	0	0	4	5	0	0	0	0	9	100
		(%)			(44)	(56)						
3**	10	n	0	0	1	3	0	2	1	2	9	90
		(%)			(11)	(33)		(22)	(11)	(22)		
4**	6	n	0	0	0	0	0	0	0	1	1	17**
		(%)								(100)		

Regarding implantation counts (Table 20), from the dossier submitter point of view, the mean value given in the full study report for the mid dose group is not correctly derived, since implantations from one female showing total resorption was not included in the mean calculation. Therefore, taking into account the new calculation, mean values show a clear dose-dependent decrease, being 14.1, 13 and 9.5 at 198, 505 and 2900 ppm, respectively, *vs* 15.9 for the control group.

Table 20: Implantation counts (Anonymous, 2013c and corrected values by the dossier submitter)

Doses (ppm)	0 ppm	198 ppm	505 ppm	2900 ppm
Implantations (original data from the Full Study Report)	15.9	14.1	14.1	9.5
Implantations (corrected values)	15.9	14.1	13.0	9.5

In relation to organ weights of males, dose-dependent reductions in absolute and relative values were reported at all doses tested. Decreases in relative prostate (47.81%, 25.98%, 22.90%), seminal vesicles (28.95%, 29.81%, 16.91%), and pituitary (23.08%, 15.39%, 15.39%) weights, compared to the control group, were observed at 2900 ppm, 505 ppm and 198 ppm, respectively. In addition, at the highest dose tested, reduced absolute epididymis weight (12.94%) was also noted. All these decreases were statistically significant with the exception of seminal vesicles weight at 198 ppm (Table 21). No changes in organ weights of females were observed.

Table 21: Mean organ weights (g) for F0 males from the screening test (Anonymous, 2013c)

Doses (ppm)			0 ppm	198 ppm	505 ppm	2900 ppm
♂	Terminal body weight		543	**483** (-11.05%)**	**453** (-16.58%)**	**423** (22.10%)**
	Epididymis weight	Absolute	1.337	1.269 (-5.09%)	1.214 (-9.20%)	**1.164** (-12.94%)**
		Relative	-	-	-	-
	Pituitary weight	Abosulte	0.015	0.011 (-26%)	0.011 (-26%)	0.009 (-40%)
		Relative	0.013	**0.011*** (-15.39%)**	**0.011*** (-15.39%)**	**0.010*** (-23.08%)**
	Prostate weight	Absolute	1.364	1.008 (-26.10%)	0.937 (-31.30%)	0.625 (-54.18%)
		Relative	1.297	**1.000** (-22.90%)**	**0.960** (-25.98%)**	**0.677** (-47.81%)**

	Seminal vesicles weight	Absolute	1.667	1.357 (-18.60%)	1.128 (-32.33%)	1.125 (-32.51%)
		Relative	1.627	1.352 (-16.91%)	**1.142* (-29.81%)**	**1.156* (-28.95%)**
	Testes weight	Absolute	3.59	3.68	3.50	3.46
		Relative	3.55	3.67	3.52	3.49

*: p<0.05 **: p<0.01

Gross pathology revealed intergroup differences in the prostate, spleen, incisor teeth and skin. Small prostates were seen in all males exposed to 2900 ppm, in the majority of males exposed to 505 ppm and only in one male at 198 ppm. Effects on spleen were related to capsular thickening observed in the majority of males of the three doses tested and in occasional females at the two highest doses. Capsular adhesions were also observed in occasional treated males in all groups, in a few females at 2900 ppm and in one female at 505 ppm. Pale incisor teeth were noted in males and females at the two highest doses tested (Tables 22-23).

Table 22: Macroscopic observations for F0 males from the screening test (Anonymous, 2013c)

Macropathology - group distribution of findings for males - F0 generation

Group	:	1	2	3	4			
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene			
Exposure level (ppm)	:	0	198	505	2900			
					Group/Sex:	1M	2M	3M	4M
Tissue and Finding					Number Examined:	10	10	10	10
Prostate									
Small						0	1	7	10
Lt. epididymis									
Small						0	0	1	0
Lt. testis									
Prominent tubules						0	0	1	0
Small						0	0	1	0
Teeth									
Incisor(s) pale						1	0	6	10
Spleen									
Adhesions						0	2	3	4
Capsule thickened						0	8	6	9
Skin									
Hair loss						0	1	2	4
Scab(s)						0	0	1	0

Table 23: Macroscopic observations for F0 females from the screening test (Anonymous, 2013c)

Macropathology - group distribution of findings for all females - F0 generation

Group	:	1	2	3	4			
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene			
Exposure level (ppm)	:	0	198	505	2900			
				Group/Sex:	1F	2F	3F	4F
Tissue and Finding				Number Examined:	10	10	10	10
Ovaries								
Cyst(s)					0	0	1	0
Vagina								
Abnormal contents					0	0	0	1
Teeth								
Incisor(s) pale					0	0	3	10
Spleen								
Adhesions					0	0	1	5
Capsule thickened					0	0	2	3
Enlarged					0	0	0	1
Skin								
Hair loss					2	2	0	0
Kidneys								
Irregular surface					0	0	0	1
Pale area(s)					0	0	0	2
Pelvic dilatation					0	1	0	2

Microscopic examination confirmed spleen capsular/subcapsular inflammation, capsular thickening and/or adhesions/inflammation/fibrosis in the majority of males treated at all doses tested and in a few females treated at 505 and 2900 ppm. It has to be highlighted that no microscopic examination of the spleen was performed in the control group. Reduced size of corpora lutea were observed in 4 females treated at 2900 ppm and in 1 female at 198 ppm. No changes were observed in testes, prostate and epididymis (Tables 24-25).

Table 24: Microscopic observations for F0 males from the screening test (Anonymous, 2013c)

Histopathology - group distribution of findings for males - F0 generation

Group	:	1	2	3	4			
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene			
Exposure level (ppm)	:	0	198	505	2900			
				Group/Sex:	1M	2M	3M	4M
Tissue and Finding				Number:	10	10	10	10
Rt. Epididymis				Number Examined:	10	0	0	10
Rt. Testis				Number Examined:	10	0	0	10
Spermatocytes Degeneration					0	0	0	1
Spleen				Number Examined:	0	8	7	9
Adhesions/Inflammation/Fibrosis					0	5	4	6
Capsular Thickening					0	4	6	7
Capsular/Subcapsular Inflammation					0	8	6	7
Prostate				Number Examined:	10	10	10	10
Abscessation					0	0	1	1
Acinar Cell Atrophy					1	0	2	0
Interstitial Inflammation					2	2	3	3
Lt. Epididymis				Number Examined:	0	0	1	0
Lt. Testis				Number Examined:	0	0	1	0
Skin				Number Examined:	0	0	1	0
Epidermal Hyperplasia					0	0	1	0
Scab(s)					0	0	1	0

Table 25: Microscopic observations for F0 females from the screening test (Anonymous, 2013c)

Group	:	1	2	3	4			
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene			
Exposure level (ppm)	:	0	198	505	2900			
				Group/Sex:	1F	2F	3F	4F
Tissue and Finding				Number:	10	10	10	10
Ovaries				Number Examined:	10	10	10	10
Cyst(s)					0	0	1	0
Reduced Size of Corpora Lutea					0	1	0	4
Spleen				Number Examined:	0	0	2	7
Adhesions/Inflammation/Fibrosis					0	0	2	5
Capsular Thickening					0	0	2	7
Capsular/Subcapsular Inflammation					0	0	1	4
Extramedullary Haemopoiesis					0	0	1	3
Kidneys				Number Examined:	0	1	0	3
Cortical Tubular Basophilia					0	1	0	2
Cortical Tubular Dilatation					0	1	0	3
Cortical Tubular Necrosis					0	0	0	1
Pelvic Dilatation					0	1	0	2
Liver				Number Examined:	0	0	0	1
Stomach				Number Examined:	0	1	0	0
Squamous Cyst - Limiting Ridge					0	1	0	0

Taking into account all the effects previously assessed, a NOAEC for reproductive performance was considered to lie below 198 ppm based on male and female reproductive effects (effects on oestrous cycles, sperm measures, longer duration of gestation, implantation counts and male reproductive organ weights) observed in parental animals.

The registrant considered that it was not possible to assess the toxicity of the substance to reproduction as toxic effects on the reproductive performance and development were accompanied by general parental toxic effects partly related to narcotic and irritant properties.

Regarding general toxicity, it has to be noted that mortality was only observed at 505 and 2900 ppm in females around the delivery date and shortly after. This fact linked to an increase in the gestation length are considered signs of dystocia. It is noted that not in all cases dystocia was accompanied by evidence of systemic toxicity.

In relation to clinical signs, underactivity, unresponsiveness, piloerection and partially closed eyelids, were observed in males and females at the two highest doses. These effects were also observed in the low dose but to a much lesser extent. All these effects were reversible immediately after the daily 6-hour exposure or before the end of the working day.

A reduction in mean body weight gain was mainly observed in males at 505 and 2900 ppm. For females, this reduction was smaller and mainly affected gestation and lactation. Nevertheless, these changes in body weight gain were only accompanied by slight decreases in mean body weight values. Consequently, the repoted effects are not considered toxicologically relevant.

Taking into account these observations and in the absence of any other marked systemic effects (such as letality, dramatic reductions in absolute body weight, organ toxicity, histopathological findings) it cannot be considered that the substance causes a marked systemic toxicity. Therefore, effects on reproductive performance and fertility reported at all dose levels should be considered related to treatment and not a secondary consequence of systemic toxicity.

Inhalation reproduction/developmental toxicity screening test (OECD TG 421) (Anonymous, 2014)

Since the above described study was considered by the registrant as inconclusive, another reproductive/developmental toxicity screening study at lower doses was performed. Thus, the reproductive toxicity of 2-BTP was evaluated in an additional GLP inhalation reproduction/developmental toxicity

screening test performed according to OECD TG 421 and reported as the key study in the IUCLID dossier (Anonymous, 2014). A summary of the study results is included in Table 8.

2-BTP was administered daily, via the inhalation route (whole body exposure), to groups of 12 Sprague-Dawley rats per sex and dose level at concentrations of 0, 50, 100 and 175 ppm, 6 hour/day. A special acute 5-minute exposure group of 10000 ppm was included to mimic and assess the effects of a single maximum exposure in humans since the test substance is intended to be used as a fire extinguishing agent. In this group, males and females were observed 15 minutes and 1 hour following the acute exposure.

During the study, no mortality occurred at any dose group after 2-BTP exposure. At 10000 ppm, clinical findings such as hypoactivity, decreased respiration, completely shut eyelids and lacrimation were observed only on the first day of exposure and were resolved by 1 hour following exposure. Additionally, salivation and red and/or clear material around the mouth and/or nose were noted for both sexes at 15 minutes and/or 1 hour following exposure. No clinical findings were observed in the other treatment groups.

Lower mean body weight gains were observed throughout the exposure period in males dosed with 10000 ppm, resulted in a lower mean body weight on Day 28. Lower mean food consumption was also noted for this group during the pre-mating period. Both effects were considered test substance-related and adverse. Nevertheless the registrant has considered that, since this exposure level was intended to mimic and assess the effects of a single maximum exposure in humans, reduction in mean body weight gain only after 28 days of exposure would not be relevant to a single exposure scenario at the same exposure level. For females, lower mean body weight gain was observed during gestation days 11 to 14 and reduced food consumption during premating days 0 to 7.

Lower mean body weight gain was also noted in males of the 100 and 175 ppm groups during the latter half of the exposure period (days 21-28) leading to slightly lower mean body weight gains during the entire exposure period of 28 days at 100 ppm. Although these effects were generally significant they were not of sufficient magnitude to affect mean body weights, and therefore were considered non-adverse. For females at 175 ppm, lower mean body weight gains were observed during gestation but only on Days 0-4 and 11-14 and returning to normal values at the end of this period and not affecting mean body weights. Test substance-related, higher mean maternal water consumption was noted in females at 175 ppm throughout gestation, and was considered by the authors of the study as adverse (Tables 26-27).

Table 26: Body weight (g), body weight change (g), food consumption (g/kg/day) and water consumption (g/kg/day) data for F0 male animals from the screening test (Anonymous, 2014)

Doses (ppm)		0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
F0 - Mean body weight (entire period)	Day 0	371	372	374	370	370
	Day 7	387	393	386	384	375
	Day 13	407	417	401	406	387
	Day 21	424	436	415	423	404
	Day 28	448	456	431	438	**418*(-6.70%)**
F0 - Mean body weight change	D 0-7	16	20	12	14	**5*(-68.25%)**
	D 7-13	20	24	16	22	**12*(-40.00%)**
	D 13-21	18	20	14	17	17
	D 21-28	24	20	**16* (-33.33%)**	**15*(-37.50%)**	**13**(-45.83%)**
	D 0-13 (premating	36	44	27	36	**17**(-52.78%)**
	Day 0-28 (entire treatment)	78	84	**57*(-26.92%)**	68	**48**(-38.46%)**

F0-Mean food consumption (pre-mating)	D 0-7	63	62	60	59	59
	D 7-13	59	59	**55* (-6.78%)**	58	**54**(-8.48%)**
F0 - Mean water consumption (pre-mating)	D 0-7	111	104	101	119	103
	D 7-13	94	93	92	105	98

*: p<0.05; **: p<0.01

Table 27: Body weight (g), food consumption (g/kg/day) and water consumption (g/kg/day) data for F0 female animals from the screening test (Anonymous, 2014)

Doses (ppm)		0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
F0 - Mean body weight (pre-mating)	Day 0	252	246	243	252	246
	Day 7	265	262	261	264	257
	Day 13	276	275	278	282	269
F0 - Mean body weight change (pre-mating)	D 0-7	14	16	18	12	12
	D 7-13	10	13	16	18	11
	D 0-13	24	29	35	31	23
F0 - Mean body weight (gestation)	Day 0	277	276	279	289	269
	Day 4	302	301	302	306	292
	Day 7	314	314	314	318	303
	Day 11	336	332	332	338	323
	Day 14	354	346	348	349	336
	Day 17	385	378	378	380	369
	Day 20	427	425	427	430	415
F0 - Mean body weight gain (gestation)	D 0-4	25	24	22	**17* (-32.00%)**	24
	D 4-7	12	13	12	12	10
	D 7-11	21	18	18	19	20
	D 11-14	18	14	16	**12** (-33.33%)**	**14*(-22.22%)**
	D 14-17	32	31	31	31	33
	D 17-20	42	48	49	51	46
	D 0-20	150	149	147	141	146
F0 - Mean body weight (lactation)	Day 1	326	316	317	326	307
	Day 4	342	327	326	337	326
F0 - Mean body weight gain (lactation)	D 1-4	16	11	8	10	20
F0- Mean food consumption (pre-mating)	D 0-7	71	68	68	**64**(-9.86%)**	**65*(-8.45%)**
	D 7-13	70	67	70	68	
F0- Mean food consumption (gestation)	D 0-4	70	69	66	**60**(-14.29%)**	66
	D 4-7	72	71	73	68	70
	D 7-11	73	70	70	67	68
	D 11-14	71	70	68	67	68
	D 14-17	71	70	70	68	71
	D 17-20	63	66	65	67	64
	D 0-20	69	68	67	65	67
F0-Mean food consumption (lactation)	Days 1-4	96	92	91	83	112
F0-Mean water consumption (pre-mating)	D 0-7	129	132	140	143	123
	D 7-13	131	130	147	138	125
F0-Water consumption (gestation)	D 0-4	112	118	128	126	114
	D 4-7	114	119	**140** (+22.80%)**	**139**(+21.93%)**	116
	D 7-11	121	115	133	139	113
	D 11-14	123	124	143	**152**(+23.58%)**	126
	D 14-17	129	130	147	**160**(+24.03%)**	130
	D 17-20	113	118	126	**143**(+26.55%)**	116
	D 0-20	117	119	133	**140**(+19.66%)**	117
F0-Water consumption (lactation)	Days 1-4	183	150	**148*(-19.13%)**	**139**(-24.04%)**	136

*: $p<0.05$ **: $p<0.01$

Higher mean pre-coital interval and longer mean gestation length were observed in females at 175 ppm, compared to the control group. These effects were considered test-substance related and adverse. At 100 ppm, a longer mean gestation length (22.3 days) was also noted and considered test substance-related. Although it was not considered as an adverse effect by the study authors since the value was within the range of historical control data, it is noted that it was close to the upper limit of the HCD range (21.5-22.3 days) (Tables 28-29).

Table 28: Reproductive performance for F0 animals from the screening test (Anonymous, 2014)

	Dosage Level (ppm)					WIL HC[a]
Parameter	0	50	100	175	10,000	Mean (Range)
Male Mating Index	100.0	100.0	100.0	91.7	100.0	95.5 (86.7-100.0)
Female Mating Index	100.0	100.0	100.0	91.7	100.0	96.3 (86.7-100.0)
Male Fertility Index	100.0	100.0	100.0	91.7	100.0	87.5 (70.0-100.0)
Female Fertility Index	100.0	100.0	100.0	91.7	100.0	88.0 (70.0-100.0)
Male Copulation Index	100.0	100.0	100.0	100.0	100.0	92.4 (70.0-100.0)
Female Conception Index	100.0	100.0	100.0	100.0	100.0	92.3 (70.0-100.0)
Estrous Cycle Length (days)	5.2	4.6	4.9	5.0	5.1	4.5 (4.0-5.8)
Pre-Coital Interval (days)	2.9	2.9	2.4	4.5	2.0	3.0 (1.8-4.7)

[a] = WIL historical control data

Table 29: Gestation lengths (days) for F0 females from the screening test (Anonymous, 2014)

TABLE S35 (F0 FEMALES)
PROJECT NO.:WIL-65501F RAT INHAL REPRO/ DEV/TOX STUDY OF 2-BROM-3,3,3-TRIFLUOROPROPENE PAGE
SPONSOR:AMERICAN PACIFIC CORP. SUMMARY OF GESTATION LENGTHS [DAYS]

GROUP:	0 PPM	50 PPM	100 PPM	175 PPM	10000 PPM
GESTATION LENGTH (DAYS)					
MEAN	21.7	22.1	22.3**	22.6**	22.1
S.D.	0.49	0.29	0.49	0.50	0.29
S.E.	0.14	0.08	0.14	0.15	0.08
N	12	12	12	11	12

** = Significantly different from the control group at 0.01 using Dunnett's test

Statistically significant lower pituitary weights (absolute and relative) were observed at 100, 175 and 10000 ppm in males and females but within historical control ranges. Additionally, sligh increases in relative (left and right) epididymis weights were observed at 10000 ppm, probably due to the decrease observed in terminal body weight (Table 30).

Table 30: Organ weigths (g) for F0 animals from the screening test (Anonymous, 2014)

Doses (ppm)			0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
♂	Terminal body weight		450	456	431	438	**418* (-7.11%)**
	Pituitary weight	Absolute	0.0156	0.0146	**0.0126** (-19.23%)**	**0.0127** (-18.59%)**	**0.0133* (-14.74%)**
		Relative	0.0033	0.0031	**0.0029* (-12.50%)**	**0.003* (-9.09%)**	0.0031
	Lungs weight	Absolute	1.66	1.71	1.69	1.80	1.64
		Relative	0.369	0.377	0.393	**0.412** (+11.65%)**	0.392
	Left epididymis weight (relative)		0.131	0.135	0.137	0.139	**0.144** (+9.92%)**
	Right testis weight (relative)		0.372	0.371	0.388	0.385	**0.409** (+9.94%)**
♀	Terminal body weight		342	327	326	337	326
	Pituitary weight	Asolute	0.0207	0.0190	**0.0156** (-24.64%)**	**0.0175* (-15.46%)**	**0.175* (-15.46%)**
		Relative	0.006	0.006	**0.005* (-16.67%)**	**0.005*(-16.67%)**	0.005

*: p<0.05 **: p<0.01

Fertility, sperm measures, oestrus cycles, parturition, histopathology and gross pathology were unaffected by the treatment with 2-BTP.

In the IUCLID dossier, a NOAEC of 100 ppm was reported for female systemic and reproductive toxicity, based on the increase in mean water consumption, longer mean pre-coital interval and longer mean gestation length observed in the 175 ppm group. For male toxicity, the NOAEC was considered to be 175 ppm, based on the lack of adverse effects.

10.10.3 Comparison with the CLP criteria

According to the CLP criteria, adverse effects on sexual function and fertility include those that interfere with the reproductive system. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

Substances are either classified in Category 1 (1A or 1B; known or presumed human reproductive toxicant) or Category 2 (suspected human reproductive toxicant). A substance known to have produced an adverse effect on sexual function or fertility is classified in Category 1A and the data are mainly based on evidence from humans. If the data are largely derived from animal studies, a substance is either classified Category 1B or Category 2 based on the strength of the evidence and the relevance of the effect for humans.

There are no relevant data on adverse effect on sexual function and fertility in humans, hence classification for Category 1A is not proposed.

The classification of a substance in Category 1B is largely based on data from animal studies. According to CLP criteria, such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. When there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. It has to be highlighted that such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

A concern for sexual function and fertility arises from the results obtained in the two inhalation reproduction/developmental toxicity screening tests (OECD TG 421) performed with 2-BTP.

In the first study (Anonymous, 2013c), the systemic toxicity observed was limited to clinical signs such as underactivity, unresponsiveness, piloerection and partially closed eyelids. These signs associated with dosing were reversible after the daily 6-hour exposure or before the end of the working day. Furthermore, lower body weight gain in males and females and food intake were also recorded. Nevertheless, these changes in body weight gain were only accompanied by slight decreases in mean body weight values, mainly in males.

At the top dose, two females were sacrificed on Day 24 after mating. One of these dams had partially closed eyes, hunched posture and piloerection and the other one had piloerection and perigenital staining and appeared distressed and attempting parturition. A third female with a live litter born showed a general poor condition and total litter loss on lactation Day 2 and was killed for reasons of animal welfare. The remaining 3 females were sacrificed on Day 25 after mating as parturition had not commenced and there was no indication of this occurring.

In addition, at 505 ppm, five females and litters were sacrificed during lactation. One of these dams showed total litter loss post partum associated with general poor condition of the dam. The remaining four were sacrified before lactation Day 2 due to total litter loss.

It is highlighted that mortality was only observed at 505 and 2900 ppm in females around the delivery date and shortly after. This fact linked to an increase in the gestation length are considered signs of dystocia.

In relation to adverse effects on sexual function and fertility, statistically significant longer oestrus cycles at the two highest doses were observed. Abnormal sperm parameters such as significant reductions in percent progressively motile sperm, sperm velocity, sperm count in the cauda epididymis and increases in Beat Cross Frequency (BCF) and in abnormal sperm (breakages and abnormal head shape) were recorded at all doses, being more pronounced at the highest one. These effects were seen jointly with decreases in prostate, seminal vesicles, pituitary and epididymis weights.

Longer pre-coital intervals were observed at all doses. Mating performance was affected at the two highest dosis (only statistically significant at the highest dose) with a reduction in the number of pairings with evidence of mating and, on the day of mating, with a reduced presence of copulation plugs or sperm in the vaginal smear. Nevertheless, mating index was only reduced at 2900 ppm.

At the highest dose, fertility index was reduced to 60% due to the fact that three females failed to mate, two of them with apparent clinical signs and the other one with no evicence of systemic effects. A fourth female successfully mated showed no evidence of pregnancy in the absence of clinical signs. Nevertheless, no changes in the fertility index were reported at mid and low doses.

In addition, at 2900 ppm, extended duration of gestation with only one female littering on Day 25 of gestation were noted. For this reason, the gestation index was reduced to 17%, reflecting the single litter born. The same effects but slightly less pronounced were reported at mid and low doses.

A dose-related reduction in implantation counts was observed at all doses tested, being only statistically significant at 2900 ppm.

All the effects in sexual function and fertility parameters lead to a clear reduction in the fertility and gestation indices. Since these alterations are observed in the absence of any other marked systemic effects (such as letality, dramatic reductions in absolute body weight, organ toxicity, histopathological findings), they can be considered related to treatment and not a secondary consequence of systemic toxicity.

On the other hand, some of the findings reported in the first OECD TG 421 were consistently observed in the second study (Anonymous, 2014) at the two highest doses tested (only statistically significant at the high dose), i.e. lower pituitary weights, longer mean pre-coital interval and duration of gestation.

Consequently, data show a concern related to sexual function and fertility since common effects on both endpoints are noted in the two OECD TG 421 studies, such as longer mean pre-coital interval and a longer duration of gestation. Besides these common effects, several adverse effects on sexual function and fertility, such as longer estrous cycles, decreases in mating index, copulation plugs, sperm counts, fertility and gestation indices and number of implantations, and changes in male reproductive organ weights are clearly observed in the first screening study.

Based on the data, there is clear evidence of adverse effects on sexual function and fertility and classification as Repr. 1B (H360F) is proposed.

10.10.4 Adverse effects on development

Table 31: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Inhalation reproduction/ developmental toxicity screening test (OECD TG 421) GLP: Yes Rat/Crl:CD (SD) 10 animals/sex/dose	2-BTP (purity: 99.9%) Inhalation: vapour (whole body) Concentrations: 0, 198, 505, 2900 ppm Exposure: from 15 days before pairing to Day 10 of lactation.	See general toxicity and effects on sexual function and fertility in Table 8 (Section 10.10.1) **Developmental toxicity** <u>Offspring viability (Tables 32-33(a and b))</u> *198 ppm* ↓ Post-implantation survival (86.00% *vs* 94.50% in controls, not statistically significant) resulting in a lower total litter size (12.20 *vs* 15.00 in controls) (18.67%, p<0.05) and live litter size (Day 10: 10.6 *vs* 14.7 in controls) (27.89%, p<0.01). ↓ Group mean survival from birth to day 10 post-partum. *505 ppm* ↓ Post-implantation survival (56.60% *vs* 94.50% in controls, p<0.05), live birth index (91.30% *vs* 99.40% in controls, not statistically significant) and viability index (78.20% *vs* 98.60% in controls, p<0.05), resulting in a lower total litter size (11.8 *vs* 15 in controls) (21.33%, p<0.01) and live litter size (Day 10: 8.3 *vs* 14.7 in controls) (43.54%, p<0.01). Nine pups in four litters sacrificed due to poor condition. In another dam, total litter loss was observed at the completion of parturition check (pre-Day 1). *2900 ppm* Only one female produced a litter showing ↓ post-implantation survival (4.16% *vs* 94.50 % in controls) and ↓ live birth index (33.30% *vs* 99.40% in controls) with only one pup alive and sacrificed due to poor condition. <u>Offspring body weight (Table 34-35)</u> *198 ppm* Male pups: ↑ Mean pup weight (slight) on PND 1 (8.82%, not statistically significant). ↓ Body weight gain from days 1-10 (10.28%, not statistically significant). Female pups: ↑ Mean pup weight on PND 1 (7.81%, p<0.01). *505 ppm* Male pups: ↓ Body weight gain from days 1-10 (14.96%, p<0.05). Female pups: No body weight changes.	Anonymous, 2013c

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Offspring gross pathology *505 ppm* No milk in the stomach frequently observed in the offspring died or sacrificed in the very early days of lactation. **NOAEC for developmental effects in the offspring was established below 198 ppm due to lower post-implantation survival and viability indices leading to lower litter size.**	
Inhalation reproduction/ developmental toxicity screening test (OECD TG 421) GLP: Yes Rat/Sprague-Dawley 12 animals/sex/dose	2-BTP (purity: > 99.4 %) Inhalation: vapour (whole body) Concentrations: 0, 50, 100, 175 ppm Special acute exposure group: 10000 ppm Exposure: Males 14 days prior to mating and throughout the mating period for a total of 28-29 days of exposure. Females 14 days prior to pairing and until gestation Day 20 (total of 35-46 days). Females that failed to deliver dosed through the day prior to euthanasia for a total of 52 days.	See general toxicity and effects on sexual function and fertility in Table 8 (Section 10.10.1) **Developmental toxicity** Offspring viability (Table 36) *100 ppm* ↓ Postnatal survival from birth to PND 4 (84.10% *vs* 92.80% in controls, not statistically significant and within the range of historical control data). *175 ppm* ↓ Postnatal survival from birth to PND 4 (67.90% *vs* 92.80% in controls, not statistically significant but below the range of historical control data). Offspring body weight (Table 38) *50 ppm* Male pups: ↑ Mean birth weights only on PND 1 (16.17%, p<0.01). Female pups: No body weight changes. *100 ppm* Male pups: ↑ Mean birth weights only on PND 1 (11.76%, p<0.01). Female pups: No body weight changes. *175 ppm* No effects on pup weights. *Acute exposure group (10000 ppm)* Male pups: ↑ Mean birth weights only on PND 1 (14.70%, not statistically significant). Female pups: No body weight changes. Offspring necropsy findings (Table 39) *100 ppm*	Anonymous, 2014

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Increase in the incidence of interventricular septal defect (one pup in one litter). *175 ppm* Adverse increase in the incidence of interventricular septal defect (five pups in two litters). **NOAEC for developmental effects in the offspring was established at 100 ppm based on the reduced postnatal survival at 175 ppm.**	

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The potential of 2-BTP to adversely affect development has been assessed in the two same inhalation reproductive/developmental toxicity screening studies described for effects on sexual function and fertility (Table 31).

Inhalation reproduction/developmental toxicity screening test (OECD TG 421) (Anonymous, 2013c)

In this first inhalation reproduction/developmental toxicity screening test, F1 litters were exposed to the substance through their mothers who were dosed with aerosol concentrations of 198, 505 and 2900 ppm during pregnancy and until postnatal day (PND) 10. A summary of the study results is included in Table 31.

At 2900 ppm only one out of six pregnant females littered on Day 25 after mating. This single dam with a live litter born was killed for reasons of animal welfare following total litter loss. Two other females were sacrificed on Day 24 after mating with signs possibly associated with stress involved in commencement of parturition. One of these dams had underactive, reduced body temperature, hunched posture and piloerection, and the other one had piloerection and perigenital staining. The remaining 3 females were sacrificed on Day 25 after mating as parturition had not commenced and there was no indication of this occurring.

It has to be highlighted that from the dossier submitter point of view, the values given in the full study report for developmental toxicity are not correctly derived. For this reason, new calculated values based on individual data are referred below.

At 2900 ppm, the only one female that was able to produce a litter showed a low post-implantation survival (25.0% *vs* 94.3%) and low birth index (33.3% *vs* 99.4%), which led to only one pup being alive out of the three pups born on PND 1 (indicating that 2 pups died during parturition or shortly after). The only pup alive was sacrificed due to poor condition.

At 505 ppm, post-implantation survival was reduced (63.0% *vs* 94.3%). The total number of pups born was 80 and the total number of pups born alive was 57 on PND 1 which is clearly indicating a reduction in the survival of the offspring, being reflected on live birth index (71.2% *vs* 99.3%). In addition, lower total (8.9 *vs* 15.0) and live litter size (11.4 *vs* 14.9) on PND 1 and lower litter size (8.3 *vs* 14.7) on PND 10, compared to the control group were recorded, leading to a reduction in the viability index (57.9% *vs* 98.6%). It is noted that several offsprings were sacrificed due to poor condition (reduced activity and body temperature) (Tables 32a and 32b).

At 198 ppm, a reduced post-implantation survival (86.6% *vs* 94.3%) was also observed. The total number of pups born on PND 1 was 110 and the total number of pups born alive was 106, which leads to a lower total (12.2 *vs* 15.0) and live litter size (11.8 *vs* 14.9). Total litter size on PND 10 was also lower (10.6 *vs* 14.7) and therefore, viability index was reduced from 98.6% to 89.6% for control and treatment group, respectively (Tables 33a and 33b).

Table 32a: Summary table of litter data (Anonymous, 2013c)

Litter size - group mean values for litters that survived to Day 10 of lactation - F1 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group		Implantations	Total litter size Day 1	Live litter size on Day 1	Live litter size on Day 5	Live litter size on Day 10
Statistical test:		Wi	Sh	Wi	Wi	Wi
1	Mean	15.9	15.0	14.9	14.8	14.7
	SD	1.3	1.2	1.2	1.4	1.3
	n	10	10	10	10	10
2	Mean	14.1	12.2*	11.8**	10.6**	10.6**
	SD	3.1	3.3	3.1	3.3	3.3
	n	9	9	9	9	9
3	Mean[a]	15.5	11.8**	10.8**	8.5**	8.3**
	SD	1.7	1.0	1.9	2.1	2.1
	n	4	4	4	4	4
	Mean[b]	14.1	8.9**	11.4*	8.5**	8.3**
	SD	2.6	4.3	2.2	2.1	2.1
	n	9	9	5	4	4
4	Mean[a]	9.5				
	n	6				
	Mean[b]	12.0	3.0	1.0		
	n	1#	1	1		

Implantations only recorded for one female, not included in statistical evaluation. Group 4 not included in statistical analysis.
a Females surviving to Day 10 post partum
b All pregnant females/females that littered where applicable

Table 32b: Summary table of litter data (calculated by the dossier submitter)

Group	Implantations	Total litter size Day 1		Live litter size on Day			
					1	5	10
0 ppm	15.9	Total number	150	Total number	149	135	147
		Mean number	15	Mean number	14.9	13.5	14.7
198 ppm	14.1	Total number	110	Total number	106	95	95
		Mean number	12.2	Mean number	11.8	10.5	10.5
505 ppm	13	Total number	80	Total number	57	34	33
		Mean number	8.9	Mean number	11.4	8.5	8.25
2900 ppm	9.5	Total number	3	Total number	1	---	---
		Mean number	0.75	Mean number	0.25	---	---

Table 33a: Summary of survival indices in the offspring (Anonymous, 2013c)

Offspring survival indices - group mean values for litters that survived to Day 10 of lactation - F1 generation

Group	:	1	2	3	4
Compound	:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)	:	0	198	505	2900

Group		Post implantation survival index (%)	Live birth index (%)	Viability index (%)
Statistical test:		Wi	Sh	Sh
1	Mean	94.5	99.4	98.6
	n	10	10	10
2	Mean	86.0	96.8	90.3
	n	9	9	9
3	Mean[a]	76.8*	91.3	78.2*
	n	4	4	4
	Mean[b]	56.5**	51.7**	62.6**
	n	10	9	5
4	Mean[a]	25.0	33.3	
	n	1#	1#	
	Mean[b]	4.2**		
	n	6		

Implantations only recorded for one female, not included in statistical evaluation
a Females surviving to Day 10 post partum
b All pregnant females/females that littered where applicable

Table 33b: Summary of survival indices in the offspring (calculated by the dossier submitter)

	Post-implantation survival index (%)	Live birth index (%)	Viability index (%)
0 ppm	94.3	99.3	98.6
198 ppm	86.6	96.4	89.6
505 ppm	63.0	71.2	57.9
2900 ppm	25	33.3	---

A slight increase in pup body weight was observed at 198 ppm only on PND 1, being statistically significant only for females. In males, body weight gain from PND 1 to PND 10 was slightly lower at 505 ppm, compared to controls (Tables 34-35).

Table 34: Summary of body weights and body weight change (g) for male offspring (Anonymous, 2013c)

Bodyweight and bodyweight change - group mean values (g) for male offspring from litters surviving to Day 10 of lactation - F1 generation

Group		:	1	2	3	4
Compound		:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)		:	0	198	505	2900

Group		Day of age			Change	
		1	5	10	1 to 5	1 to 10
Statistical test:		Wi	Wi	Wi	Wi	Wi
1	Mean	6.8	10.9	17.5	4.1	10.7
	SD	0.3	0.6	1.0	0.4	0.9
	n	10	10	10	10	10
2	Mean	7.4	11.1	17.0	3.8	9.6
	SD	0.6	1.0	1.2	0.6	0.8
	n	9	9	9	9	9
3	Mean	6.9	10.6	16.0	3.7	9.1*
	SD	0.7	1.8	2.7	1.3	2.2
	n	4	4	4	4	4

Table 35: Summary of body weights and body weight change (g) for female offspring (Anonymous, 2013c)

Bodyweight and bodyweight change - group mean values (g) for female offspring from litters surviving to Day 10 of lactation - F1 generation

Group		:	1	2	3	4
Compound		:	Control	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene	2-bromo-3,3,3-trifluoropropene
Exposure level (ppm)		:	0	198	505	2900

Group		Day of age			Change	
		1	5	10	1 to 5	1 to 10
Statistical test:		Du	Wi	Wi	Sh	Wi
1	Mean	6.4	10.4	16.7	4.0	10.3
	SD	0.4	0.6	1.1	0.3	0.9
	n	10	10	10	10	10
2	Mean	6.9**	10.9	16.2	3.9	9.3
	SD	0.4	1.0	1.6	0.7	1.4
	n	9	9	9	9	9
3	Mean	6.2	9.7	15.1	3.4	8.8
	SD	0.2	1.5	2.5	1.4	2.4
	n	4	4	4	4	4
4@	Mean	5.5				
	n	1				

@ Not mean value, presented for information only, weight of single pup on Day 1. Not included in statistical evaluation

No macroscopic effects related to treatment were observed on the offspring sacrificed on PND 10. However, no milk in the stomach was recorded in some offspring which died or were sacrificed prior to PND 2, especially at 505 ppm.

Considering all these observations, a NOAEC below 198 ppm was established for developmental effects due to lower post-implantation survival, live birth and viability indices leading to lower litter size.

Inhalation reproduction/developmental toxicity screening test (OECD TG 421) (Anonymous, 2014)

In the key screening study (Anonymous, 2014), F1 pups were also indirectly exposed during gestation and lactation until PND 4 to doses of 50, 100, 175 and 10000 ppm of 2-BTP.

Lower postnatal survival was observed at 100 and 175 ppm form birth to PND 4, compared to the control group. Only at 175 ppm, values were below historical control data and for this reason, considered adverse (Table 36).

Table 36: Summary of postnatal survival (% per litter) (Anonymous, 2014)

Parameter	Dosage Level (ppm) 0	50	100	175	10,000	WIL HC[a] Mean (Range)
PND 0 (relative to number born)	96.5	98.0	95.2	95.4	97.0	98.0 (93.3-100.0)
PND 0 to PND 1	99.2	98.1	96.1	91.7	99.4	98.7 (94.6-100.0)
PND 1 to PND 4	97.1	95.9	91.7	76.5	99.3	98.4 (87.0-99.8)
Birth to PND 4	92.8	92.1	84.1	67.9	95.7	95.3 (83.8-98.7)

[a] = WIL historical control data

A slight, dose-dependent but not statistically significant decrease in the mean number of pups born and live litter size (PND 0) was observed at all doses tested. Pup sex ratio was unaffected by 2-BTP exposure of parental animals (Table 37).

Table 37: Summary of litter data on PND 0 (Anonymous, 2014)

TABLE S51 (F1)
PROJECT NO.:WIL-65501F RAT INHAL REPRO/ DEV/TOX STUDY OF 2-BROM-3,3,3-TRIFLUOROPROPENE PAGE 1
SPONSOR:AMERICAN PACIFIC CORP. SUMMARY OF PND 0 LITTER DATA

GROUP :	1	2	3	4	5
NUMBER BORN					
MEAN	14.3	13.8	13.7	13.3	13.9
S.D.	1.78	2.12	2.96	2.10	1.24
S.E.	0.51	0.61	0.86	0.63	0.36
N	12	12	12	11	12
SEX AT BIRTH (% MALES PER LITTER)					
MEAN	57.3	47.7	48.9	54.6	52.6
S.D.	12.61	11.09	11.29	14.17	14.79
S.E.	3.64	3.20	3.26	4.27	4.27
N	12	12	12	11	12
LIVE LITTER SIZE (PND 0)					
MEAN	13.9	13.6	13.0	12.7	13.5
S.D.	1.98	2.39	2.98	1.74	1.51
S.E.	0.57	0.69	0.86	0.52	0.44
N	12	12	12	11	12

1- 0 PPM 2- 50 PPM 3- 100 PPM 4- 175 PPM 5- 10000 PPM
SEX COMPARED USING DUNN'S TEST, NUMBER BORN, AND LIVE LITTER SIZE COMPARED USING DUNNETT'S MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

Higher mean pup body weights were noted in males on PND 1 in the 50, 100 and 10000 ppm groups. However, pup body weights on PND 4 and mean body weight gains during PND 1-4 were similar to the control group. For this reason, it was not considered as an adverse effect. Overall, no relevant effects were detected on body weights and body weight gains (Table 38).

Table 38: Mean pups body weight and body weight change (g) from the screening test (Anonymous, 2014)

Doses (ppm)			0 ppm	50 ppm	100 ppm	175 ppm	10000 ppm
F1 - Mean pup body	♂	PND 1	6.8	**7.9** (+16.17%)**	**7.6** (+11.76%)**	7.3	**7.8** (+14.70)**

weight		PND 4	9.6	10.2	9.7	9.7	10.2
F1 – Pup body weight change		PND 1-4	2.7	2.3	2.0	2.3	2.5
F1 – Mean pup body weight	♀	PND 1	6.6	7.3	7.2	6.9	7.2
		PND 4	9.1	9.5	9.4	9.3	9.5
F1 – Pup body weight change		PND 1-4	2.5	2.2	2.1	2.3	2.3

*: p<0.05 **: p<0.01

Necropsy of pups that were found dead at 175 ppm, showed a substance-related and adverse increase in the incidence of an interventricular septal defect (a 1 or 2 mm in diameter opening in the anterior portion of the septum). Five pups (15% of litters) were affected at this dose level. At 100 ppm, this effect was only observed in a single pup (6.25% of litters) and postnatal survival was within the historical control ranges, therefore it was not considered test substance-related by the registrants (Table 39). Nevertheless, from the dossier submitter point of view, both effects are considered related to treatment since they are observed in a dose-dependent manner.

Table 39: Summary of pups necropsy findings (Anonymous, 2014)

TABLE S56 (F1 - UNSCHEDULED DEATHS)
PROJECT NO.:WIL-65501F RAT INHAL REPRO/ DEV/TOX STUDY OF 2-BROM-3,3,3-TRIFLUOROPROPENE PAGE 1
SPONSOR:AMERICAN PACIFIC CORP. SUMMARY OF PUP NECROPSY FINDINGS

FOUND DEAD OR EUTHANIZED MORIBUND OR IN EXTREMIS

	PUPS					LITTERS				
DOSE GROUP:	1	2	3	4	5	1	2	3	4	5
NUMBER EXAMINED VISCERALLY	11	11	16	33	7	8	6	9	10	4
STOMACH										
MILK NOT PRESENT	3	3	8	10	3	3	2	6	6	3
MILK PRESENT	1	0	0	2	0	1	0	0	2	0
MALFORMATION										
INTERVENTRICULAR SEPTAL DEFECT	0	0	1	5	0	0	0	1	2	0
VERTEBRAL AGENESIS	0	0	0	1	0	0	0	0	1	0
ANAL ATRESIA	0	0	0	1	0	0	0	0	1	0
KIDNEY(S)- FUSED	0	0	0	1	0	0	0	0	1	0
CONJOINED TWIN	1	0	0	0	0	1	0	0	0	0
VARIATION										
MAJOR BLOOD VESSEL VARIATION	0	0	0	1	0	0	0	0	1	0
THORACIC CAVITY- CONTENTS, DARK RED	0	0	0	1	0	0	0	0	1	0

1- 0 PPM 2- 50 PPM 3- 100 PPM 4- 175 PPM 5- 10000 PPM

For developmental toxicity, a NOAEC of 100 ppm was established in this study based on the reduced postnatal survival noted in the 175 ppm group.

10.10.6 Comparison with the CLP criteria

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency.

Substances are either classified in Category 1 (1A or 1B; known or presumed human reproductive toxicant) or Category 2 (suspected human reproductive toxicant). A substance known to have produced an adverse effect on development in humans is classified in Category 1A and the data are mainly based on evidence from humans. If the data are largely derived from animal studies, a substance is either classified Category 1B or Category 2 based on the strength of the evidence and the relevance of the effect for humans.

There are no relevant data on adverse effect on development in humans, hence classification for Category 1A is not proposed.

The classification of a substance in Category 1B is largely based on data from animal studies. According to CLP criteria, such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. When there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. It has to be highlighted that such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

In the case of 2-BTP, a concern for development arises from the results obtained in the two screening toxicity studies. Both studies showed a reduction in post-natal survival with clear dose-dependency.

In the first study (Anonymous, 2013c), a dose-dependent reduction in the post-implantation survival index was observed at all doses tested, being statistically significant at the two highest doses. A statistically significant and dose-dependent reduction in total litter size and live litter size were also reported in all treatment groups. According to these findings, dose-dependent decreases in viability and live birth indices were recorded as well. These decreases were only statiscally significant at the mid dose since they were not calculated for the high dose, due to the reduced number of pups (only one pup alive). All these described effects occurred in the absence of a clear maternal systemic toxicity (such as letality, dramatic reductions in absolute body weight, organ toxicity, histopathological findings). Therefore, the reported effects on development can be considered treatment-related and not a secondary consequence of maternal systemic toxicity.

In the second study (Anonymous, 2014), a statistically significant decrease in postnatal survival from birth to PND 4 was observed at the two highest doses. Additionally, an increase in the incidence of interventricular septal defect, which is a severe effect, was reported at the high-dose level. Both adverse effects occurred in absence of systemic maternal toxicity. While the septal defect may have been related to the apparent developmental delay noted in the 175 ppm group, it was considered test substance-related and adverse when coupled with the reduction in postnatal survival noted in this group. In addition, this effect was also observed at 100 ppm with a lower incidence. The available information in this study shows that 2-BTP has an adverse effect on the development of rats.

According to the CLP criteria, developmental effects that occur 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. No marked maternal toxicity can be observed in the first study and, in the second study effects are observed without maternal toxicity. In addition, a reduction in postnatal survival was consistently reported in both studies. For this reason, the developmental toxicity findings should therefore be considered as treatment-related effects which have not been demonstrated to be secondary to maternal toxicity.

In conclusion, based on the data, there is a clear evidence of developmental toxicity and classification as Repr. 1B for development is proposed for 2-BTP.

10.10.7 Adverse effects on or via lactation

According to CLP Regulation effects on or via lactation are allocated to a separate single category. Substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period, and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic leves in breast milk.

No data are available to conclude on 2-BTP adverse effect on or via lactation. Therefore, no classification is proposed.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

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

10.10.9 Comparison with the CLP criteria

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

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification for adverse effects on sexual function and fertility and on development of the offspring ‘Repr. 1B (H360FD)’ is considered warranted.

10.11 Specific target organ toxicity-single exposure

The specific target organ toxicity (single exposure) of 2-BTP has been investigated in two acute inhalation toxicity studies (one key study study according to OECD TG 403 and a non-guideline supporting study) and in a subchronic (90 days) inhalation toxicity study in SD rats (Table 40).

Table 40: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD TG 403 Acute Inhalation Toxicity Study Deviations: no Rat Sprague Dawley Male/female 5 animals/sex/dose	2-BTP (purity ≥ 99.5%) Inhalation vapour (nose only) 4-hour exposure Concentrations: 26580 ppm and 5173 ppm (analytical concentrations)	<u>Mortality</u> *5173 ppm* 0/10 animals died. *26580 ppm* 10/10 animals died by day 2 post-exposure. <u>Clinical signs</u> *26580 ppm* Labored breathing or gasping during the last hour of each exposure. Decrease motor activity. Clear or red nasal discharge, excessive salivation. <u>Gross pathology</u> *5173 ppm* Discoloration of the lungs due to vascular congestion. *26580 ppm* Fluid in the lungs of one male. Discoloration of the lungs due to vascular congestion. Bronchiolar lesions such as desquamated epithelium, bronquiolar/peribronchiolar acute/subacute inflammation and/or intraluminal debris. Minimal to moderate alveolar/intralveolar macrophages in lungs. **LC_{50} (4h): 11726 ppm (male/female) based on test material.**	Anonymous, 2004
No guideline followed Deviations: exposure duration only 30 minutes Fischer 344 rats Male/female 5 animals/sex/dose	2-BTP (no purity reported) Inhalation (nose only) 30 minutes exposure Concentration: 5% (v/v) (nominal)	<u>Mortality</u> No mortality observed. <u>Clinical signs</u> Relaxed breathing shortly after exposure. Anesthesized appearance of animals for a few minutes. **LC_{50} (30 minutes): > 5% (v/v) (male/female).**	Anonymous, 1999
90-day inhalation study (whole body) OECD TG 413/EU B.29 GLP: Yes Deviations: no Rat/Crl:CD (SD)	2-BTP (purity > 99.6%) Inhalation: vapour (whole body) Concentrations: 0, 199, 505, 2876 ppm Duration of exposure: 13 weeks	<u>Mortality</u> No mortality observed at any of the doses tested. <u>Clinical signs</u> *199 ppm* Underactivity, unresponsiveness, slow breathing, piloerection, partially closed eyelids. *505 ppm* Underactivity, unresponsiveness, shallow and slow breathing, piloerection, partially closed eyelids, grinding	Anonymous, 2013d

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
10 animals/sex/dose	+ 4 weeks of recovery	teeth, hunched posture. *2876 ppm* Underactivity, unresponsiveness, shallow and slow breathing, piloerection, partially closed eyelids, hunched posture. <u>Sensory reactivity and grip strength</u> *199 ppm* No observations. *505 ppm* Sensory reactivity unaffected. Males: ↓ Hindlimb grip strength (11.48%, p<0.05). No changes in females. *2876 ppm* Sensory reactivity unaffected. Males: ↓ Forelimb grip strength (15.00%) and hindlimb grip strength (19.67%) (p<0.01). Females: ↓ Forelimb grip strength (22.73%) and hindlimb grip strength (18.18%) (p<0.01). Week 4 of recovery: Forelimb grip strength values similar to controls. <u>Motor activity</u> *199 ppm* Unaffected. *505 ppm* Males: ↓ High beam breaks (23.91%, p<0.05). Females: ↓ High beam breaks (26%, p<0.05). *2876 ppm* Males: ↓ High beam breaks (36.75%) and ↓ low beam breaks (34.45%) (p<0.01). Females: ↓ High beam breaks (31.97%) and ↓ low beam breaks (39.12%) (p<0.01). <u>Body weight gain</u> *199 ppm* Males: ↓ Mean body weight gain from week 0 to week 13 (22.01%, p<0.01). Females: No changes. *505 ppm* Males: ↓ Mean body weight gain from week 0 to week 13 (27.58%, p<0.01). Females: ↓ Mean body weight gain from week 0 to week 13 (21.06%, p<0.01). *2876 ppm* Males: ↓ Mean body weight gain from week 0 to week 13 (47.64%, p<0.01). Females: ↓ Mean body weight gain from week 0 to	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		week 13 (44.74%, p<0.01). Week 4 of recovery: In males the mean body weight gain was similar to control values. In females, the body weight gain and the overall body weight were still reduced compared with controls. <u>Food consumption</u> *199 ppm* Males: ↓ Mean food consumption over the 13 weeks of treatment (10.00%). *505 ppm* Males: ↓ Mean food consumption over the 13 weeks of treatment (15.00%). *2876 ppm* Males: ↓ Mean food consumption over the 13 weeks of treatment (26.00%). Females: ↓ Mean food consumption over the 13 weeks of treatment (19.00%). Week 4 of recovery: Similar to control values in both sexes. <u>Haematology</u> *199 ppm* Males: ↑ Mean red blood cell counts (RBC) (5.49%, p<0.05), ↓ mean corpuscular haemoglobin (MCH) (3.65%, p<0.01), ↓ eosinophils counts (52.64%, p<0.01), ↓ APTT (9.63%, p<0.05). Females: ↓ Eosinophils counts (36.33%, p<0.05), ↑ PTP (14.21%, p<0.05), ↑ APTT (9.19%, p<0.01). *505ppm* Males: ↑ Mean red blood cell counts (RBC) (7.32%, p<0.01), ↑ monocytes counts (74.19%, p<0.01), ↓ mean corpuscular haemoglobin (MCH) (4.69%, p<0.01), ↓ mean corpuscular volume (MCV) (3.25%, p<0.05), ↓ eosinophils counts (73.69%, p<0.01), ↓ APTT (12.97%, p<0.05). Females: ↓ Eosinophils counts (36.33%, p<0.05), ↑ monocytes counts (115.78%, p<0.01), ↑ PTP (10.90%, p<0.05), ↑ APTT (10.91%, p<0.01). *2876 ppm* Males: ↑ Mean haematocrit (5.82%, p<0.05), ↑ haemoglobin (5.73%, p<0.05), ↑ mean red blood cell (RBC) (10.62%, p<0.01), ↑ monocytes counts (83.87%, p<0.01), ↑ large unstained cells (LUC) (150.00%, p<0.01), ↓ mean corpuscular haemoglobin (MCH) (4.69%, p<0.01), ↓ mean corpuscular volume (MCV) (4.39%, p<0.05), ↓ leukocytes counts (31.81%, p<0.01), ↓ eosinophils counts (73.69%, p<0.01), ↓ APTT (7.53%, p<0.05). Week 4 of recovery: ↓ Leukocytes counts (27.41%,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		p<0.01), ↓ monocytes counts (32.14%, p<0.05). Females: ↓ Hemoglobin (7.10%, p<0.01), ↓ MCH (8.16%, p<0.01), ↓ MCHC (5.44%, p<0.01), ↓ WBC (32.32%, p<0.05), ↓ leukocytes counts (34.06%, p<0.01), ↓ eosinophils counts (72.73%, p<0.01). ↑ RETA (108.22%, p<0.01), ↑ monocytes counts (68.42%, p<0.01), ↑ PTP (25.11%, p<0.01), ↑ APTT (12.06%, p<0.01). Week 4 of recovery: ↓ WBC (28.71%, p<0.05), ↓ leukocytes counts (33.84%, p<0.01). <u>Biochemistry</u> *199 ppm* Males: ↑ ALT (29.03%, p<0.05), ↑ AST (130.66%, p<0.01), ↑ Cl (1.96%, p<0.01). Females: ↑ Bilirrubin (200.00%, p<0.01), ↑ urea (17.96%, p<0.05), ↓ Cl (1.96%, p<0.05), ↓ phosphatase (42.37%, p<0.05), ↓ total protein (5.72%, p<0.01). *505 ppm* Males: ↑ ALT (25.81%, p<0.05) ↑ AST (200.00%, p<0.01), ↑ urea (25.24%, p<0.01), ↑ K (14.28%, p<0.05), ↑ Cl (2.94%, p<0.01), ↑ A/G ratio (10.81%, p<0.01), ↓ total protein (7.58%, p<0.01). Females: ↑ AST (172.06%, p<0.01), ↑ urea (23.84%, p<0.01), ↑ Cl (2.94%, p<0.01), ↑ phosphatase (75.42%, p<0.01), ↓ total protein (14.29%, p<0.01), ↓ albumin (10.26%, p<0.01). *2876 ppm* Males: ↑ ALP (31.94%, p<0.01), ↑ ALT (25.81%, p<0.05), ↑ AST (188.00%, p<0.01), ↑ urea (63.60%, p<0.01), ↑ K (7.14%, p<0.05), ↑ Cl (5.88%, p<0.01), ↑ phosphatase (30.16%, p<0.01), ↑ A/G ratio (22.52%, p<0.01), ↓ cholesterol (40.81%, p<0.01), ↓ total protein (9.09%, p<0.01). Week 4 of recovery: ↑ ALP (22.36%, p<0.05), ↑ K (7.14, p<0.05) , ↑ Cl (1%, p<0.05), ↑ A/G ratio (7.48%, p<0.05), ↓ glucose (17.95%, p<0.01), ↓ triglycerides (38.24%, p<0.01). Females: : ↑ ALP (42.86%, p<0.01), ↑ urea (54.02%, p<0.01), ↑ triglycerides (70.58%, p<0.05), ↑ Cl (2.94%, p<0.01), ↑ phosphatase (138.13%, p<0.01), ↓ ALT (25.00%, p<0.05), ↓ glucose (31.07%, p<0.01), ↓ cholesterol (46.48%, p<0.01), ↓ Ca (4.44%, p<0.01), ↓ total protein (12.86%, p<0.01), ↓ albumin (10.26%, p<0.01). Week 4 of recovery: ↑ ALP (33.33%, p<0.05), ↑ Na (0.71%, p<0.05), ↑ Cl (2.00%, p<0.01), ↓ Ca (5.54%, p<0.01), ↓ total protein (5.63%, p<0.01). <u>Relative organ weights</u> *199 ppm* Females: ↑ Mean thyroid weight (46.67%, p<0.05), ↓ mean salivary glands (18.72%, p<0.01).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		505 ppm Females: ↑ Mean lungs weight (7.29%, p<0.05) and thyroid weight (20.00%, p< 0.05). ↓ Mean salivary glands (17.81%, p<0.01). *2876 ppm* Males: ↓ Mean thymus weight (36.99%, p<0.05). Females: ↑ Mean liver weight (16.00%, p<0.01), lungs weight (11.58%, p<0.05) and thyroid weight (53.33%, p<0.01). ↓ Mean pituitary weight (35.3%, p<0.01) salivary gland weight (15.53%, p<0.01) and thymus weight (46.38%, p<0.01). <u>Gross pathology</u> *199 ppm* Males: Teeth pallor (3/10), capsular thickening of spleen (1/10). Females: Teeth pallor (3/10), capsular thickening of spleen (1/10). *505 ppm* Males: Teeth pallor (7/10), capsular thickening of spleen (4/10). Females: Teeth pallor (9/10), capsular thickening of spleen (2/10) and adhesions (2/10). *2876 ppm* Males: Teeth pallor (10/10) and thickening (1/10), capsular thickening of spleen (8/10). Females: Teeth pallor (3/10) and thickening (8/10), capsular thickening of spleen (5/10) and adhesions (5/10). <u>Histopathology</u> *199 ppm* Males: ↑ Acinar cell degranulation of pancreas (minimal 3/10), chronic inflammation of heart (minimal 4/10), capsular thickening of spleen (minimal 1/10), atrophy/disorganisation/vacuolitation of the olfactory epitelium (minimal 2/10), nasolacrimal duct inflammation (minimal 3/10, slight 5/10). Females: ↑ Acinar cell degranulation of pancreas (minimal 2/10, slight 2/10), capsular inflammation (minimal 1/10) and capsular thickening (minimal 2/10) of spleen, nasolacrimal duct inflammation (minimal 1/10, slight 3/10). *505 ppm* Males: ↑ Acinar cell degranulation of pancreas (minimal 2/10, slight 3/10), chronic inflammation of heart (minimal 2/10, slight 7/10), capsular inflammation (minimal 1/10, slight 1/10, moderate 1/10) and capsular thickening (minimal 1/10, slight 2/10) of spleen,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		atrophy/disorganisation/vacuolitation of the olfactory epitelium (minimal 5/10, slight 3/10), nasolacrimal duct inflammation (minimal 1/10, slight 3/10). Females: centrilobular hepatocyte hipertrophy (minimal 1/10), ↑ acinar cell degranulation of pancreas (minimal 3/10, slight 2/10), chronic inflammation of heart (minimal 4/10), capsular inflammation (slight 1/10), capsular thickening (minimal 2/10, slight 1/10) and adhesions (1/10) of spleen, atrophy/disorganisation/vacuolitation of the olfactory epitelium (minimal 2/10, slight 3/10), nasolacrimal duct inflammation (minimal 1/10, slight 5/10, moderate 1/10). *2876 ppm* Males: Centrilobular hepatocyte hipertrophy (minimal 2/10), ↑ acinar cell degranulation of pancreas (minimal 1/10, slight 5/10, moderate 1/10), chronic inflammation of heart (minimal 1/10, slight 3/10, moderate 4/10), capsular inflammation (minimal 2/10, moderate 3/10) and capsular thickening (minimal 4/10, slight 2/10) of spleen, thymus involution/atrophy (moderate 1/10), ventral squamous metaplasia of larynx (minimal 5/10), atrophy/disorganisation/vacuolitation of the olfactory epitelium (slight 8/10, moderate 2/10), nasolacrimal duct inflammation (minimal 2/10, slight 5/10, moderate 1/10). Females: Centrilobular hepatocyte hipertrophy (minimal 4/10), ↑ acinar cell degranulation of pancreas (slight 6/10), chronic inflammation of heart (minimal 7/10, slight 2/10, moderate 1/10), capsular inflammation (minimal 1/10, slight 3/10, moderate 2/10), capsular thickening (minimal 2/10, slight 2/10, moderate 4/10) and adhesions (3/10) of spleen., thymus involution/atrophy (minimal 2/10, slight 1/10, moderate 2/10), ventral squamous metaplasia of larynx (minimal 2/10),atrophy/disorganisation/vacuolitation of the olfactory epitelium (slight 4/10, moderate 6/10), nasolacrimal duct inflammation (slight 5/10, moderate 4/10), pulp cavity necrosis (slight 4/10, moderate 1/10). **NOAEC was established at 199 ppm based on the adverse effects observed related to chronic inflammation of the heart, transient clinical signs and histopathology changes related to irritation of the respiratory tract, lower body weight gain and food consumption and CNS effects (grip strength and motor activity).**	

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

The specific target organ toxicity (single exposure) of 2-BTP has been investigated in two acute inhalation toxicity studies (one key study study according to OECD TG 403 and a non-guideline supporting study) and in a subchronic inhalation toxicity study in SD rats. A summary of these studies is included in Table 40.

The acute toxicity of 2-BTP has been investigated via the inhalation route. No information regarding oral or dermal administration of the substance is available, since the registrant waived these information requirements based on the high volatility of the substance. 2-BTP is a liquid at room temperature, boiling at approximately physiological temperature (34 °C at 1013 mbar). Therefore, it is anticipated that under any foreseeable use conditions inhaled exposure will involve the substance in a vapour state.

In a well-conducted toxicity study included in the registration dossier as the key study (Anonymous, 2004), ten SD rats (5 rats per sex) were initially exposed to a target concentration of 25000 ppm (analytical concentration of 26580 ppm). A subsequent group of ten rats were exposed to a target concentration of 5000 ppm (analytical concentration of 5173 ppm). All animals exposed at 26580 ppm were found dead or were euthanized due to poor condition on the day after exposure. All animals at 5173 ppm survived to the end of the 14-day post-exposure observation period. Clear or red nasal discharge were noted at the two concentrations tested immediately following the exposure to 2-BTP. Rats from both exposure levels had red discolorations of the lungs and fluid was present in the lungs of one male from the highest exposure level. Bronchiolar lesions with desquamated epithelium, bronchiolar/peribronchiolar acute/subacute inflammation were also observed at the highest dose level. In addition, decrease activity was observed at the highest concentration tested.

A LC_{50} of 11726 ppm (= 83900 mg/m^3) was determined for 2-BTP following a 4-hour inhalation exposure in rats.

Additionally, in the subchronic toxicity study (Anonymous, 2013d), transient clinical signs (shallow breathing, piloerection, grinding teeth and hunched posture) related to inhalation of an irritant material were evident during and after exposure at the three exposure levels tested. Histopathological treatment-related changes were also observed in the nasal turbinates (findings related to minor local irritants) and larynx (ventral squamous metaplasia) at the two highest doses. Following the 4-week recovery period, histopathological changes seen in the larynx were fully reversible but only partial recovery was seen in the nasal turbinates (atrophy/disorganisation/vacuolation of the olfactory epithelium and nasolacrimal duct inflammation).

Regarding narcotic effects, the acute inhalation study (Anonymous, 2004) showed depression of the central nervous system, noted by the decrease motor activity, at the high dose. In addition, temporary anesthesia and relaxing breathing were seen in the supporting study shortly after exposure (Anonymous, 1999). In the subchronic toxicity study (Anonymous, 2013d), possible effects on tne central nervous system (underactivity and partially closed eyelids) were evident from the beginning of the exposure. Transient clinical signs, such as underactivity and unresponsiveness were observed after the first days of a daily 6-hour exposure in the OECD TG 421 study (Anonymous, 2013c) at all doses tested (2900, 505 and 198 ppm).

Inhalation exposure to 2-BTP appears to induce temporary depression of the central nervous system, noted by the decrease activity observed in the key study at the highest concentration tested, and the temporary anesthesia seen in the supporting study (Anonymous, 1999). According to this, the registrant considered that a classification for STOT SE 3 (H336: may cause drowsiness or dizziness) should be applied. Furthermore, based on the irritation observed in the respiratory tract in the acute and subchronic studies, the substance is also self-classified by the registrant as STOT SE 3 (H335: May cause respiratory irritation).

10.11.2 Comparison with the CLP criteria

According to CLP criteria, classification for STOT SE is appropriate when it has been demonstrated form human or animal data that specific non-lethal target organ toxicity arises form a single exposure to a substance. Category 1 and 2 cover non letal “significant and/or severe toxic effects”, and they reflect the dose level required to cause the effect.

Category 3 covers “transient target organ effects” occurring after a single exposure, specifically respiratory tract irritation (RTI) and narcotic effects (NE) for which a substance does not meet the criteria to be classified in Categories 1 or 2.

The criteria for classifying substances as Category 3 for respiratory tract irritation, taking into account animal studies, are:

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

Additionally, the criteria for classifying substances as Category 3 for narcotic effects, taking into account animal studies, are:

Narcotic effects observed in animal studies such as lethargy, lack of coordination, loss of righting reflex, and ataxia if they are transient in nature.

Clinical symptoms associated with RTI, such as clear or red nasal discharge, red discolorations and fluid of the lungs, bronchiolar lesions with desquamated epithelium and bronchiolar/peribronchiolar acute/subacute inflammation were observed in the acute inhalation study (Anonymous, 2004). In addition, in the subchronic inhalation study (Anonymous, 2013d), transient clinical signs related to inhalation of an irritant material were evident, during and after exposure. Over the 13-week treatment period, histopathological treatment-related changes were observed in the nasal turbinates and larynx

Regarding NE, the acute inhalation study (Anonymous, 2004) showed depression of the central nervous system, noted by the decrease motor activity, at the high dose observed. In addition, temporary anesthesia and relaxing breathing were seen in the supporting study shortly after exposure (Anonymous, 1999).

Based on the data, RTI and NE effects were clearly observed in the acute and subchronic studies included in the registration dossier and classification for STOT SE 3; H335 and H336 according to CLP regulation is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification as STOT SE 3 (H335: may cause respiratory irritation) and as STOT SE 3 (H336: may cause drowsiness or dizziness) are considered warranted.

10.12 Specific target organ toxicity-repeated exposure

Not assessed in this dossier.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

Anonymous (1999). Acute inhalation toxicity of candidate halon replacement compounds in rats. Report nº. FY99003.

Anonymous (2004). 2-Bromo-3,3,3-trifluoropropene, E-1-bromo-3,3,3,-trifluoropropene and 1-bromo-2-trifluoromethyl-3,3,3-trifluoropropene: an acute (4-hour) inhalation toxicity study in the rat via nose only. Report nº. 02-5449.

Anonymous (2013a). Determination of tissue to air partition coefficients for 2-bromo-3,3,3-trifluoropropene (Halotron BrX).

Anonymous (2013b). 2-Bromo-3,3,3-trifluoropropene: measurement of arterial blood levels following inhalation administration to beagle dogs. Report No. WAG0016

Anonymous (2013c). 2-Bromo-3,3,3-trifluoropropene: reproductive/developmental toxicity screening study in the CD rat by inhalation administration. Report No. WAG0015.

Anonymous (2013d). 2-Bromo-3,3,3-trifluoropropene: toxicity study by inhalation administration to CD rats for 13 weeks followed by a four-week recovery period. Report No. WAG0014.

Anonymous (2014). An inhalation reproduction/developmental toxicity screening study of 2-bromo-3,3,3-trifluoropropene in rats. Report No. WIL-65501.

15 APPENDIX. HISTORICAL CONTROL DATA

Historical control data only reported in one of the two inhalation reproduction/developmental toxicity studies included in the IUCLID file (Anonymous, 2014).

Reproductive performance

Sex: Male/Female		Species: Rat			Number of Studies/Data Sets: 35 / 65		
Study Type: 1-/2-Gen		Strain: Crl:CD(SD)			Range of Study Dates: 10/00 - 05/11		
	Total	**Mean**	**S.D.**	**N**	**Mean -/+ 2 S.D.**	**Mean -/+ 3 S.D.**	**Min/Max Value**
Number of Males in Control Group	1837						
Male Mating Index (%)		95.5	4.05	63	87.40 / 100.00	83.35 / 100.00	86.7 / 100.0
Male Fertility Index (%)		87.5	7.30	63	72.90 / 100.00	65.60 / 100.00	70.0 / 100.0
Male Copulation Index (%)		92.4	6.72	49	78.96 / 100.00	72.24 / 100.00	70.0 / 100.0
Number of Females in Control Group	1903						
Female Mating Index (%)		96.3	3.80	65	88.70 / 100.00	84.90 / 100.00	86.7 / 100.0
Female Fertility Index (%)		88.0	7.25	65	73.50 / 100.00	66.25 / 100.00	70.0 / 100.0
Female Conception Index (%)		92.3	6.73	48	78.84 / 100.00	72.11 / 100.00	70.0 / 100.0
Number of Females Selected to Deliver	1868						
Number that Delivered	1624						
Number that did not Deliver	225						
Number that Died Prior to Delivery	7						
Number with Total Litter Loss	14						
Number with Dystocia	2						

Sex: Female	Species: Rat	Number of Studies/Control Groups: 35 / 65
Study Type: 1-/2-Gen	Strain: Crl:CD(SD)	Range of Study Dates: 10/00 - 05/11

	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
Mean Estrous Cycle Length (days)	4.5	0.39	65	3.72 / 5.28	3.33 / 5.67	4.0 / 5.8
Mean Pre-Coital Interval (days)	3.0	0.50	65	2.00 / 4.00	1.50 / 4.50	1.8 / 4.7
Mean Gestation Length (days)	22.0	0.16	65	21.68 / 22.32	21.52 / 22.48	21.5 / 22.3
Mean Number of Pups Born	13.8	0.81	65	12.18 / 15.42	11.37 / 16.23	11.7 / 15.1
Mean Viable Litter Size (PND 0)	13.6	0.82	65	11.96 / 15.24	11.14 / 16.06	11.6 / 15.0
Mean % Males/ Litter	50.4	2.78	65	44.84 / 55.96	42.06 / 58.74	44.2 / 57.3
Mean % Females/ Litter	49.7	2.65	65	44.40 / 55.00	41.75 / 57.65	43.4 / 55.8
Mean No. of Former Implantation Sites at Weaning	14.7	0.78	58	13.14 / 16.26	12.36 / 17.04	12.6 / 16.0
Mean No. of Sites Unaccounted for at Weaning	0.8	0.26	56	0.28 / 1.32	0.02 / 1.58	0.3 / 1.4
Mean Post-Implantation Loss (%/Litter)	6.2	3.46	2	0.00 / 13.12	0.00 / 16.58	3.8 / 8.7
Mean Primordial Follicle Count of 10 Sections/Dam	121.7	36.37	30	48.96 / 194.44	12.59 / 230.81	50.4 / 184.4

Organ weights (necropsy)

Sex: Male/Female	Species: Rat	Number of Studies/Number Data Sets: 35 / 65
Study Type: 1-/2-Gen	Strain: Crl:CD(SD)	Range of Study Dates: 10/00 - 05/11

Males	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
Mean Age of Males at Necropsy (weeks)	24.4	2.62	63	19.16 / 29.64	16.54 / 32.26	17.0 / 31.0
Mean Seminal Vesicle/Coagulating Gland Weight (g)	2.26	0.152	51	1.956 / 2.564	1.804 / 2.716	1.88 / 2.59
Mean Prostate Weight (g)	1.07	0.095	61	0.880 / 1.260	0.785 / 1.355	0.85 / 1.29
Mean Right Testis Weight (g)	1.82	0.062	59	1.696 / 1.944	1.634 / 2.006	1.67 / 1.96
Mean Left Testis Weight (g)	1.83	0.068	59	1.694 / 1.966	1.626 / 2.034	1.70 / 2.09
Mean Right Epididymis Weight (g)	0.75	0.033	59	0.684 / 0.816	0.651 / 0.849	0.66 / 0.83
Mean Left Epididymis Weight (g)	0.72	0.030	59	0.660 / 0.780	0.630 / 0.810	0.63 / 0.77
Mean Right Cauda Epididymis Weight (g)	0.3439	0.02157	58	0.30076 / 0.38704	0.27919 / 0.40861	0.2972 / 0.3939
Mean Left Cauda Epididymis Weight (g)	0.3348	0.01593	58	0.30294 / 0.36666	0.28701 / 0.38259	0.3028 / 0.3726
Mean Brain Weight (g)	2.15	0.036	59	2.078 / 2.222	2.042 / 2.258	2.06 / 2.22
Mean Pituitary Weight (g)	0.016	0.0013	55	0.0134 / 0.0186	0.0121 / 0.0199	0.0129 / 0.0219
Mean Adrenal Weight (g)	0.061	0.0040	54	0.0530 / 0.0690	0.0490 / 0.0730	0.0528 / 0.0776
Females						
Mean Age of Females at Necropsy (weeks)	24.5	2.45	63	19.60 / 29.40	17.15 / 31.85	17.0 / 31.0
Mean Combined Ovary/Oviduct Weight (g)	0.1348	0.02491	31	0.08498 / 0.18462	0.06007 / 0.20953	0.0991 / 0.1705
Mean Combined Ovary w/o Oviduct Weight (g)	0.1192	0.01057	22	0.09806 / 0.14034	0.08749 / 0.15091	0.0957 / 0.1519
Mean Brain Weight (g)	1.98	0.033	59	1.914 / 2.046	1.881 / 2.079	1.90 / 2.05
Mean Pituitary Weight (g)	0.021	0.0220	55	0 / 0.0650	0 / 0.0870	0.0138 / 0.1810
Mean Adrenal Weight (g)	0.087	0.0867	54	0 / 0.2604	0 / 0.3471	0.0581 / 0.7110

Sperm concentration and morphology

Sex: Male/Female | Species: Rat | Number of Studies/Control Groups: 35 / 65
Study Type: 1-/2-Gen | Strain: Crl:CD(SD) | Range of Study Dates: 10/00 - 05/11

Mean Sperm Concentration (millions/ gram of tissue)	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
Left Testis	78.5	8.76	58	60.98 / 96.02	52.22 / 104.78	53.6 / 96.8
Left Epididymis	405.0	56.17	52	292.66 / 517.34	236.49 / 573.51	302.5 / 548.6
Left Cauda Epididymis	662.3	62.52	6	537.26 / 787.34	474.74 / 849.86	568.0 / 738.9
Mean Sperm Production Rate (millions/ gram of tissue/ day)	12.9	1.43	58	10.04 / 15.76	8.61 / 17.19	8.8 / 15.9
Mean Sperm Motility (%)	87.3	2.82	58	81.66 / 92.94	78.84 / 95.76	79.0 / 93.0
Mean Progressive Sperm Motility (%)	74.5	3.79	49	66.92 / 82.08	63.13 / 85.87	62.0 / 81.0
Sperm Morphology Differential Count (%)						
Normal	99.4	0.78	58	97.84 / 100.00	97.06 / 100.00	96.1 / 99.9
Normal Head Separated from Flagellum	0.3	0.28	58	0.00 / 0.86	0.00 / 1.14	0.0 / 1.6
Head Absent w/ Normal Flagellum	0.3	0.35	58	0.00 / 1.00	0.00 / 1.35	0.0 / 2.3
Head Absent w/ Abnormal Flagellum	0.0	0.00	58	0.00 / 0.00	0.00 / 0.00	0.0 / 0.0
Misshapen Head w/ Normal Flagellum	0.0	0.00	58	0.00 / 0.00	0.00 / 0.00	0.0 / 0.0
Misshapen Head w/ Abnormal Flagellum	0.0	0.01	58	0.00 / 0.02	0.00 / 0.03	0.0 / 0.1
Degenerative Flagellar Defects w/ Normal Head	0.0	0.00	58	0.00 / 0.00	0.00 / 0.00	0.0 / 0.0
Other Flagellar Defects w/ Normal Head	0.0	0.00	58	0.00 / 0.00	0.00 / 0.00	0.0 / 0.0

Pup survival indices (%)

Sex: Male/Female | Species: Rat | Number of Studies/Data Sets: 35 / 65
Study Type: 1-/2-Gen | Strain: Crl:CD(SD) | Range of Study Dates: 10/00 - 05/11
Age Range: 0 - 21 Days

	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
Mean Number of Pups Born	13.8	0.81	65	12.18 / 15.42	11.37 / 16.23	11.7 / 15.1
Survival (%)						
PND 0 (Relative to No. Born)	98.0	1.34	65	95.32 / 100.00	93.98 / 100.00	93.3 / 100.0
PND 0 to PND 1	98.7	1.25	65	96.20 / 100.00	94.95 / 100.00	94.6 / 100.0
PND 1 to PND 4 (Before Selection)	98.4	1.93	65	94.54 / 100.00	92.61 / 100.00	87.0 / 99.8
PND 4 (After Selection) to PND 7	98.9	1.29	65	96.32 / 100.00	95.03 / 100.00	93.5 / 100.0
PND 7 to PND 14	99.4	1.18	65	97.04 / 100.00	95.86 / 100.00	93.5 / 100.0
PND 14 to PND 21	99.6	0.92	65	97.76 / 100.00	96.84 / 100.00	94.7 / 100.0
Birth to PND 4 (Before Selection)	95.3	2.63	65	90.04 / 100.00	87.41 / 100.00	83.8 / 98.7
PND 4 (After Selection) to PND 21	98.0	1.95	60	94.10 / 100.00	92.15 / 100.00	91.1 / 100.0

Pup body weights (g)

Sex: Male/Female
Study Type: 1-/2-Gen
Species: Rat
Strain: Crl:CD(SD)
Age Range: 1 - 28 Days
Number of Studies/Data Sets: 35 / 65
Range of Study Dates: 10/00 - 05/11

Males (g)	PND	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
	PND 1	7.1	0.23	65	6.64 / 7.56	6.41 / 7.79	6.7 / 7.6
	PND 4	10.0	0.49	65	9.02 / 10.98	8.53 / 11.47	9.1 / 11.0
	PND 7	15.4	1.20	65	13.00 / 17.80	11.80 / 19.00	11.9 / 17.6
	PND 11	23.1	1.88	3	19.34 / 26.86	17.46 / 28.74	22.0 / 25.3
	PND 14	30.9	3.28	63	24.34 / 37.46	21.06 / 40.74	20.2 / 36.4
	PND 17	32.2	0.49	2	31.22 / 33.18	30.73 / 33.67	31.9 / 32.6
	PND 21	48.6	4.98	65	38.64 / 58.56	33.66 / 63.54	32.8 / 57.8
	PND 28	79.3	4.93	8	69.44 / 89.16	64.51 / 94.09	72.0 / 88.4
Females (g)	**PND**	**Mean**	**S.D.**	**N**	**Mean -/+ 2 S.D.**	**Mean -/+ 3 S.D.**	**Min/Max Value**
	PND 1	6.7	0.22	65	6.26 / 7.14	6.04 / 7.36	6.4 / 7.1
	PND 4	9.5	0.48	65	8.54 / 10.46	8.06 / 10.94	8.5 / 10.4
	PND 7	14.6	1.14	65	12.32 / 16.88	11.18 / 18.02	11.3 / 16.7
	PND 11	22.1	2.02	3	18.06 / 26.14	16.04 / 28.16	20.9 / 24.4
	PND 14	29.7	3.10	63	23.50 / 35.90	20.40 / 39.00	20.3 / 34.8
	PND 17	30.9	0.64	2	29.62 / 32.18	28.98 / 32.82	30.5 / 31.4
	PND 21	46.5	4.55	65	37.40 / 55.60	32.85 / 60.15	32.8 / 54.9
	PND 28	73.4	4.58	8	64.24 / 82.56	59.66 / 87.14	66.7 / 82.4

Pup organ weights (PND 21)

Sex: Male/Female
Study Type: 1-/2-Gen
Species: Rat
Strain: Crl:CD(SD)
Age: 21 Days
Number of Studies/Number Data Sets: 35 / 65
Range of Study Dates: 10/00 - 05/11

Males	Mean	S.D.	N	Mean -/+ 2 S.D.	Mean -/+ 3 S.D.	Min/Max Value
Brain (g)	1.4556	0.04067	50	1.37426 / 1.53694	1.33359 / 1.57761	1.3451 / 1.5436
Spleen (g)	0.2210	0.04129	50	0.13842 / 0.30358	0.09713 / 0.34487	0.1471 / 0.3641
Thymus (g)	0.2061	0.03452	50	0.13706 / 0.27514	0.10254 / 0.30966	0.1617 / 0.3541
Females						
Brain (g)	1.4063	0.03764	50	1.33102 / 1.48158	1.29338 / 1.51922	1.3142 / 1.5082
Spleen (g)	0.2161	0.03828	50	0.13954 / 0.29266	0.10126 / 0.33094	0.1401 / 0.3099
Thymus (g)	0.2079	0.03517	50	0.13756 / 0.27824	0.10239 / 0.31341	0.1669 / 0.3483

Developmental landmarks

Sex: Male/Female	Species: Rat			Number of Studies/Control Groups: 35 / 65		
Study Type: 1-/2-Gen	Strain: Crl:CD(SD)			Range of Study Dates: 10/00 - 05/11		
	Mean	**S.D.**	**N**	**Mean -/+ 2 S.D.**	**Mean -/+ 3 S.D.**	**Min/Max Value**
Mean Male Anogenital Distance (PND1) (mm)	4.23	0.364	12	3.502 / 4.958	3.138 / 5.322	3.5 / 4.7
Mean Female Anogenital Distance (PND1) (mm)	2.30	0.218	12	1.864 / 2.736	1.646 / 2.954	1.8 / 2.6
Mean Day of Balanopreputial Separation	45.3	1.29	31	42.72 / 47.88	41.43 / 49.17	43.0 / 49.0
Mean Body Weight at Aquisition (g)	229.9	12.85	31	204.20 / 255.60	191.35 / 268.45	203.7 / 251.7
Mean Day of Vaginal Patency	33.6	1.17	31	31.26 / 35.94	30.09 / 37.11	31.3 / 37.0
Mean Body Weight at Aquisition (g)	111.6	5.26	31	101.08 / 122.12	95.82 / 127.38	100.0 / 123.2
Mean Day of Surface Righting Response						
Males	5.0	0.07	2	4.86 / 5.14	4.79 / 5.21	5.0 / 5.1
Females	5.1	0.07	2	4.96 / 5.24	4.89 / 5.31	5.1 / 5.2
Mean Day of Pinnal Detachment						
Males	4.1	0.12	6	3.86 / 4.34	3.74 / 4.46	4.0 / 4.3
Females	4.0	0.08	6	3.84 / 4.16	3.76 / 4.24	4.0 / 4.2
Mean Day of Eye Opening						
Males	16.0	1.05	6	13.90 / 18.10	12.85 / 19.15	15.1 / 17.8
Females	15.9	1.13	6	13.64 / 18.16	12.51 / 19.29	15.1 / 17.8
Mean Day of Hair Growth						
Males	13.5	1.43	4	10.64 / 16.36	9.21 / 17.79	11.7 / 15.0
Females	13.6	1.51	4	10.58 / 16.62	9.07 / 18.13	11.8 / 15.2
Mean Day of Incisor Eruption						
Males	10.3	0.78	6	8.74 / 11.86	7.96 / 12.64	9.3 / 11.4
Females	10.2	0.70	6	8.80 / 11.60	8.10 / 12.30	9.3 / 11.1