European Chemicals Agency (EChA), Helsinki

To support the DNEL setting for 1-bromopropane on its toxicity for reproduction, related to the use in Application for Authorisation

Service Request
under the Multiple Framework Contract with re-opening of competition
for scientific services for ECHA

CONTRACT NUMBER ECHA/2015/368 – SC-30 (LOT 2)

FINAL REPORT

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Consortium ETeSS
Expert Team providing scientific support for ECHA

CONTENTS

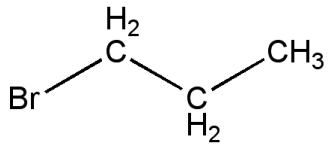
1. Introduction

1-Bromopropane (1-BP) has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The purpose of this review is to evaluate the available information relevant to deriving DNELs for the reproductive toxicity of 1-bromopropane.

The contractor has been unable to identify a document that describes the specific data driving the classification, but a number of reproductive and developmental effects have been described in the reviews and literature summarised in this report.

The contractor has identified 3 detailed reviews of the toxicity of 1-Bromopropane, from internet searches and requests to other regulatory agencies, these form the basis of much of this first report (Section 5). The contractor has performed an extensive literature search for 1-bromopropane (see section 13) and identified 13 additional, relevant publications, which have been reviewed and summarised. The contractor has also reviewed registration dossiers for 1-bromopropane; some of these have provided more detailed summaries of information in the main reviews but no copies of the critical study reports. There was no new information in the registration reports that was considered relevant to the derivation of reproductive DNELs for 1-BP.

2. Chemical properties of 1-bromopropane



Chemical structure of 1-bromopropane

Property	**Information**
CAS Registry number	106-94-5
Molecular formula	C3H7Br
Synonyms	1-BP; Propyl bromide; *n*-Propyl bromide; Propane, 1-bromo; *normal* propyl bromide; nPB
Molecular weight	123.0
Melting point	-110C
Boiling point	64.7C
Vapor pressure (mm Hg)	110.8 at 20C
Vapor density	4.25
Specific gravity	1.353 at 20C
Solubility in water (20°C)	2.45 g/L
Octanol/water partition coefficient (log Kow)	2.10
Conversion factors (1-bromopropane in air)	
parts per million (ppm) to mg/m3	mg/m3 = 5.03 x (ppm)
mg/m3 to parts per million (ppm)	ppm = 0.2 x (mg/m3)

1-Bromopropane is a pale yellow, volatile liquid with a strong odour. It has uses in cleaning and degreasing and as an intermediate. The registration dossiers indicate it is used mainly in closed systems.

3. Absorption, distribution, metabolism and excretion of 1-Bromopropane.

This section is based primarily on the review by the US National Toxicology Program (NTP, 2011; 2013), supplemented by information in the registration dossiers. A recent published paper provides information on dermal absorption.

Studies in humans and laboratory animals indicate that 1-bromopropane can be absorbed following inhalation, ingestion, or dermal exposure. Occupational exposure occurs primarily by inhalation and dermal contact and studies of workers show a good correlation between urinary concentrations of 1-bromopropane, bromide ion, and *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) with their 1-bromopropane breathing zone air concentrations. Several studies have monitored urine and blood samples in workers to establish biomarkers of exposure. These studies also indicate that unmetabolized 1-bromopropane is excreted in the urine in humans but has not been reported in animal studies. The four urinary mercapturic conjugates identified from 1-bromopropane-exposed workers have also been reported as urinary metabolites from studies in rodents, including AcPrCys, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, and *N*-acetyl-*S*-(3-hydroxy-*n*-propyl)-L-cysteine. The oxidative metabolites that likely lead to the conjugates have not been reported in human studies; however, no publications were identified that actually tested for them.

Experimental animal studies have shown that at relatively high concentrations 1-bromopropane is absorbed, rapidly distributed, and predominantly eliminated by exhalation (approximately 40% to 70%), but is also excreted in the urine and faeces. In rats and mice, most of the 1-bromopropane administered by i.v. injection was exhaled unchanged or as CO2 within 4 hours of exposure. Urinary metabolites accounted for 13% to 23% of the administered dose after 48 hours. The available studies on 1-bromopropane metabolism show that CYP catalyzed oxidation (primarily via CYP2E1) reactions and glutathione conjugation are the primary metabolic pathways. At least 16 urinary metabolites have been identified in rodent studies (either rats or mice), including several reactive intermediate metabolites (bromoacetone, glycidol, and -bromohydrin).

Although there are differences in results between rodents and humans the database is limited for humans and some differences appear to be related to the extent of investigation. The contractor considers there is no reason to assume humans are markedly different from animals in the kinetics of 1-bromopropane.

There are no reliable data on the relative absorption via oral, dermal and inhalation routes, particularly at levels relevant to likely human exposures. The contractor

proposes to use the default assumptions in guidance document R8 for inhalation and oral absorption. A published study on the *in vitro* dermal absorption of 1-BP is summarised below.

Dermal absorption

Frasch et al (2011) studied the dermal absorption of 1-BP using finite and infinite dose exposures of rat and human skin samples. The human data are considered most appropriate and are summarised below. There was also an investigation of the evaporation rate of 1-BP from a petri dish to simulate evaporation following splashes on the skin. The investigations used non-radiolabelled material with analyses performed by GC-FID.

- The evaporation flux at 23°C was determined to be 470 mg/cm^2/h, with a half time for evaporation of <100 seconds. Extrapolating to 32°C to match the temperature of the *in vitro* dermal data gave a flux of approximately 600 mg/cm^2/h.

- The dermal absorption investigations are summarised in Table 1 below. Human epidermis samples from female cosmetic surgery procedures were prepared by heat separation. Each element of the study used 9 replicates with samples from 3 donors. The receptor fluid in the static (Franz) cells was HEPES buffered Hanks balanced salt solution. 1-BP is moderately water soluble (*ca* 2g/L) and solubility in the receptor fluid is not seen as a rate limiting factor. Infinite dose tests had the wells covered with Para film, the finite dose test was left unoccluded resulting in extensive evaporation. Results were presented for 3 hours even though total exposures were for 24 hours in most tests.

- For the infinite dose experiments the absorption: time profile was almost linear. For the finite dose test the absorption was complete within 10 - 15 minutes, presumably due to evaporation of 1-BP. For the 10 minute exposure, absorption stopped a few minutes after the swabbing procedure.

- These data show that due to evaporation, dermal exposures to 1-BP should result in minimal systemic exposure (the evaporation flux is approximately 500 times that of the dermal absorption flux). In the event that the dermal exposures result in occluded conditions (e.g. under protective gloves) or are repeated (e.g. handling wet items) a conservative dermal absorption value has been calculated based on the finite dose data. Absorption under unoccluded, finite dose conditions was 0.16% of the applied dose all within approximately 10 minutes. Extrapolating this to a 10h exposure to represent a long shift gives a value of 10%.

Table 1: *in vitro* dermal absorption data for 1-BP (mean +/- SD)

Test material	Dose	Duration	Flux (µg/cm^2/h)$^{\#}$	% absorbed
Pure 1-BP	Infinite	24 hours	625 ± 176	
Saturated aqueous solution of 1-BP	Infinite	24 hours	585 ± 320	
Pure 1-BP	Finite (10ul/cm^2)	24 hours	Approximately 1	0.16
Pure 1-BP	Infinite	10 minutes	Approximately 750	
Pure Drysolv*	Infinite	24 hours	441 ± 116	
Saturated aqueous solution of Drysolv	infinite	24 hours	644 ± 255	

* Drysolv is a commercial dry-cleaning solution (95% 1-BP)
# Flux is 0-3 hours except for the 10 minute exposure where it is the peak flux over 15 minutes corrected to 1 hour

It is proposed to use 10% as a conservative value for the dermal absorption of 1-BP.

Transfer across placenta and blood:testis barrier

There are no data on the potential for 1-bromopropane or its metabolites to cross the placenta or blood:testis barrier. Effects on the fetus have been reported but it is not clear if these are due to direct exposure or external factors such as reductions in blood supply or nutrient levels. Effects on sperm are consistent findings with 1-BP but there is no information on whether 1-BP itself, or a metabolite such as bromide, acts directly on the testicular cells / sperm or whether there is an indirect mode of action.

4. Summary of the non-reproductive toxicity on 1-Bromopropane

A more extensive description is presented in Annex 1.

Acute toxicity

The acute oral and dermal LD50s for 1-bromopropane are reported in the registration dossier as >2000 mg/kg bw in rats. The acute inhalation LC50 (4h exposure) in rats is 7000 ppm (35,000 mg/m^3), with no deaths at 6000 ppm. 1-Bromopropane is classified for narcotic effects (H336).

1-Bromopropane has a harmonised classification as a skin irritant (H315), as a severe eye irritant (H317) and a respiratory irritant (H335). The skin sensitisation is equivocal, in a maximization test in guinea pigs positive reactions in above 30% of animals are reported but the interpretation in the registration dossier is that 1-bromopropane is not a skin sensitiser.

Repeat dose toxicity

The target organs for 1-BP following inhalation exposures are the central nervous system and liver. The lowest NOAECs are in the region of 250 – 500 ppm (8h/day). Repeat dose oral and dermal studies have not been identified.

Carcinogenicity

Two year inhalation toxicity studies in F344 rats (125, 250 or 500 ppm) and B6C3F1 mice (62.5, 125 or 250 ppm) found that 1-bromopropane caused increases in the incidence of malignant or benign tumors of the skin (keratoacanthoma; keratoacanthoma or squamous-cell carcinoma combined; and keratoacanthoma, squamous-cell carcinoma, basal-cell adenoma, or basal-cell carcinoma combined) in male rats. Increases in benign large intestine tumors (adenoma of the colon and rectum) were seen in female and male rats, and benign or malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma combined) in female mice. Increases in skin tumors in male rats, intestinal tumors in female rats, and lung tumors in female mice were statistically significant and dose related. The tumors in the large intestine of male rats, although not statistically significant, were considered to be of biological significance due to their rarity (less than 0.2% incidence in historical controls). Additionally, tumors observed that may have been related to 1-bromopropane exposure included malignant mesothelioma of the abdominal cavity and pancreatic islet tumors (adenoma) in male rats and skin tumors (keratoacanthoma, basal-cell adenoma, or basal-cell carcinoma combined) in female rats (NTP 2011; 2013).

Although clear dose response relationships were not seen for all the tumours, significant increases in one or more tumours were seen at all tested concentrations.

No studies were found evaluating modes of action for the tumor sites found in experimental animals: skin, large intestine, and lung. However, 1-bromopropane, either directly or via reactive metabolites, causes molecular alterations that have been associated with carcinogenesis, including adduct formation, oxidative stress, glutathione depletion, immunosuppression, and inflammation.

The cancer NOAEC is <62.5 ppm in mice.

Genotoxicity

Studies *in vivo* show that 1-bromopropane can covalently bind to protein in exposed rats and occupationally exposed workers. The available data provide some indication that 1-bromopropane has genotoxic potential as it has been reported to induced mutations in bacterial and mammalian cells and produce DNA damage in human cells. However, the NTP reported that 1-BP was not genotoxic in an Ames test at concentrations up to 5000 µg/plate, with or without metabolic activation. There is limited evidence that DNA damage was induced in leukocytes from 1-bromopropane workers. 1-Bromopropane did not induce chromosomal damage in mice (micronucleus induction assay) following 3 month's exposure at up to 500 ppm and did not induce gene-cell mutations (dominant lethal mutation assay) in rats exposed to 400mg/kg bw/d for 5 days (oral gavage).

The contractor considers that whilst 1-BP binds to macromolecules the genotoxicity database is inconsistent with no convincing evidence of genotoxicity *in vivo*. The effects of 1-BP on parameters related to reproduction show dose response relationships with thresholds identified in all but one study (Liu et al). The overall weight of evidence is considered to support a conclusion that the effects of 1-BP on fertility are unlikely to be produced by a genotoxic mode of action.

Neurotoxicity

Neurotoxicity has been seen in both experimental animals and occupationally exposed humans. Although the molecular mechanisms of the neurotoxicity are not completely understood, recent studies show that the hippocampus is especially susceptible to 1-bromopropane-induced effects and involves oxidative stress, loss of ATP production, altered GABA metabolism and reduced GABAergic feedback inhibition, inhibition of the ubiquitination-proteosome system, changes in neurotransmitter receptor expression, and modifications of intracellular signaling cascades.

The CERHR review (NTP, 2003) concluded that the NOAEC for neurotoxicity was 200 ppm.

5. Reviews of the reproductive toxicity data on 1-Bromopropane

The contractor has identified 3 major reviews with significant information on reproductive and developmental effects:

- US NTP CERHR (2003);
- Toxicology Excellence for Risk Assessment (TERA; 2004) and
- US NTP Carcinogenicity evaluations (2011, 2013).

Colleagues in the US, Canada, Australia, Germany and Japan have been contacted to determine if any additional national reviews are available. One of the authors of the TERA report has been contacted to ascertain if there has been any subsequent work by this group.

- The Australian review contains limited information.
- No Canadian review has been found.
- No additional, unpublished US reviews have been identified.
- The Japanese summary database contains surprisingly little information, given the amount of investigative work on 1-BP performed by Japanese scientists.
- TERA have no updated evaluations available.

These reviews are summarised in Table 2.

Table 2: Reviews of the reproductive toxicity of 1-bromopropane

Review purpose	Conclusions on reproductive toxicity	Reference
Specific and comprehensive review of reproductive toxicity.	Concluded that there was serious concern for adverse effects on development and reproduction for exposures at the upper end of the reported occupational exposure range (18 – 381 ppm). For intermittent and well controlled exposures (0.04 – 0.63 ppm) there was minimal concern for reproductive effects. Available human data are insufficient to draw conclusions on the potential for reproductive or developmental toxicity. Available data are sufficient to conclude that 1-BP exposure can induce developmental and reproductive toxicity in rats. In evaluating the potential effects on human reproduction, the rat data are assumed to be relevant for humans. Accordingly, dose levels were identified from animal studies to use in this evaluation. **• A benchmark concentration 95th percentile lower confidence limit of 305 ppm (1,534 mg/ m^3) was identified from a rat inhalation developmental toxicity.** **• A LOAEC of 250 ppm (1,257 mg/ m^3) for female reproduction (NOAEC=100 ppm [503 mg/ m^3] was identified from an inhalation, two-generation reproductive toxicity study.** **• A LOAEC of 200 ppm (1,006 mg/ m^3) for male reproduction (NOAEC=100 ppm [503 mg/ m^3] was identified from the Ichihara et al. study and WIL Research Laboratories study.**	CERHR, 2003
Main focus was investigation of carcinogenicity. Included review of available toxicity data including reproductive toxicity. Reports of	No changes in reproductive organs or tissues in 2, 14 or 104 week segments. Reduced sperm counts and motility, and altered oestrous cycling seen in rats after 14 week exposures at 250 ppm (lowest dose investigated) and above. In mice, reduced sperm counts and motility were seen at 250 ppm and above **(NOAEL = 125 ppm),** with altered oestrous cycling at 500 ppm and above.	NTP, 2011 NTP, 2013

studies included non-neoplastic end-points including reproductive organs and sperm investigations.		
Review of toxicity for setting inhalation OELs. Main focus was reproductive toxicity. Included derivation of a number of BMDs and NOAECs for reproductive and developmental toxicity endpoints. Conclusions based on $BMDL_{1SD}$ for continuous data or $BMDL_{10}$ for dichotomous data.	Reviewed neurotoxicity, hepatotoxicity and reproductive / developmental toxicity end points. A number of BMD analyses were performed:- **Table 2** **BMD and BMDL Estimates*** **Endpoint** / **Stelljes and Wood** BMD (ppm), BMDL (ppm) / **TERA** BMR, BMD (ppm), BMDL (ppm), **Model**, **Variance** Hindlimb strength: 286, 214, 1 sd, 290, 210, Linear, Homogeneous Minimal centrilobular vacuolization males: 345, 226, 10%, 290, 200, Multistage-2 Fetal body weight: 1 sd, 510, 310, Poly-2, Non-homogeneou F_0 sperm motility: 343, 263, 1 sd, 380, 270, Linear, Homogeneous F_1 sperm motility: 261, 156, 1 sd, 260, 150, Power, Non-homogeneou F_0 prostate weight: 1 sd, 740, 190, Power, Homogeneous F_0 Estrous Cycle Length: 1 sd, 290, 210, Power, Non-homogeneou F_1 Estrous Cycle Length: 1 sd, 810, 400, Linear, Non-homogeneou F_0 No Estrous Cycle Incidence: 10%, 670, 480, Multistage-2 F_1 No Estrous Cycle Incidence: 10%, 360, 180, Quantal Linear Maternal GD20 body weight: 1 sd, 1000, 690, Linear, Homogeneous F_1 litter viability index: No dose-response F_1 pup weight gain PND 21 to 28: 1 sd, 240, 180, Linear, Homogeneous **F_1 decreased live litter size: 280, 188, 1 sd, 280, 190, Linear, Non-homogeneo** F_2 decreased live litter size: 238, 169, 1 sd, 240, 170, Linear, Non-homogeneou *See text for additional details The conclusion was for an OEL to be based on the BMDL of 190 ppm for reduced litter size in the F1 generation. This was considered to be more relevant than the slightly lower BMDL of 170 ppm from the F2 generation.	TERA 2004

General review	No specific mention of reproductive toxicity hazards	Finland , National Board of Labour Protection, 1989
General review	Identified effects on reproduction and development. No specific recommendations for NOAECs or exposure limits.	Australian NICNAS, (2013).
General review	Summarises the reproductive studies covered by the reviews presented above. No specific conclusions on reproduction NOAECs.	HSE, 2002

5.1 Main aspects of the CERHR (2003) review.

The text below is taken from the CERHR report, the contractor has performed only minor editorial changes.

Reproduction / Fertility

In 1998, NIOSH conducted a health hazard evaluation at a plant where a 1-BP-containing spray adhesive was used in the manufacture of seat cushions. Forty-three employees (34 females and 9 males), whose exposure levels were classified as 'low' (117 ppm [585 mg/m3]), 'medium' (170 ppm [850 mg/m3]), or 'high' (197 ppm [985 mg/m3]), were asked about reproductive problems. One employee (sex not specified) in the low exposure group reported seeing a doctor for reproductive/fertility problems and two males and one female in the low or mid exposure groups said they could not have a child after attempting to conceive for 1 year. NIOSH noted that their ability to detect reproductive or fertility problems was limited by the small sample size and personal nature of the questions asked.

Strength/Weaknesses: The NIOSH case report is very limited in content. The survey analysis was based on only 43 of 70 workers. According to the National Center for Health Statistics, about 10% of couples in the US seek medical attention for infertility. Therefore, 3 of 42 workers reporting possible fertility problems is not unexpected.

Utility (adequacy) for CERHR Evaluation Process: The study is not useful except to point out the need for a well-designed human study with adequate exposure information and adequate power to detect an effect, i.e., one that monitors menstrual cycles and examines semen quality and serum hormones.

Reproductive toxicity study in rats

In a study sponsored by the BSOC, WIL Research Laboratories evaluated the potential adverse effects of 1-BP whole-body inhalation exposure in F0 and F1 parental rats; reproductive capabilities were examined in the F0 and F1 generations and neonatal survival, growth and development were evaluated in F1 and F2 offspring.

In this two-generation reproductive toxicity study, groups of 25 male and female Crl:CD(SD)IGS BR rats were exposed to filtered air or 100, 250, 500, or 750 ppm[0, 503, 1,257, 2,514, 3,771 mg/m3] 1-BP vapors (99.8% purity) for 6 hours/day, 7 days/week. Exposure concentrations within each chamber were measured 9–10 times during each exposure period by a validated GC method. Exposure of F0 rats commenced at 7 weeks of age and F1 rats began direct exposure at weaning. Exposures were conducted for at least 70 days prior to mating. Females were not exposed on post-natal day (pnd) 0–4 and only they, not their litters, were exposed during pnd 5–21. Therefore, offspring (litters) were indirectly exposed to the test chemical *in utero* and through nursing. In addition, the F1 pups selected randomly for propagation of F2 litters were directly exposed from pnd 22 forward. Results in treated animals were compared to both air control and historical control data from WIL Research Laboratories. The study was stated to have been conducted in

compliance with GLP.

The main reproductive findings are summarised in Table 3

Table 3: Reproductive findings in Sprague Dawley rats exposed to 1-BP

Number[a]	*Dose in ppm (mg/m³)*	*Effects in F_0 Parents*[b]	*Effects in F_1 Offspring [F_2 Offspring]*[b]
25	0		
25	100 (503)	NOAEC	NOAEC
25	250 (1,257)	↓Prostate weight	↓F_1 weight gain on pnd 21–28 (M) ↑Estrous cycle length (49 vs 45 days)[d]
25	500 (2,514)	↑Precoital interval (43 vs 34 days)[c] ↑Estrous cycle length (55 vs 42 days)[d] ↓Fertility (52 vs 92%) ↓Implantation sites (90 vs 153) ↓Litter size (n=83 vs 144) ↓Normal sperm (982 vs 997%) ↓Sperm motility (72 vs 87%) ↓Cauda epididymis and prostate weights No effects on gestation length or parturition, testicular weight or sperm counts, ovarian weight	↓F_1 weight gain through pnd 28 (M) and pnd 21–28 (F) ↑Estrous cycle length (51 vs 45 days)[c] ↓Implantation sites (98 vs 155) ↓Litter size (86 vs 145) ↓Normal sperm (953 vs 995%) ↓Sperm motility (74 vs 89%) ↓F_1 cauda epididymis and pituitary weight [↓F_2 postnatal weight gain on pnd 4–21] No effect on F_1 or F_2 postnatal survival, F_1 age at vaginal patency, F_1 age at balanopreputial separation, mating indices, gestation length, parturition, or testicular lesions
25	750 (3,771)	↓Weight gain (M) ↑Estrous cycle length (56 vs 42 days)[d] ↓Mating (68 vs 96%) ↑Pre-coital interval (48 vs 34 days)[c] No conceptions ↓Ovary weight ↓Corpora lutea ↑Ovarian cysts ↓Normal sperm (906 vs 997%) ↓Sperm motility (53 vs 87%) ↓Epididymal sperm count (370 vs 472x10^6/gram tissue) ↓Epididymis, prostate, seminal vesicle, and pituitary weight	No F_1 rats available due to complete infertility in F_0 rats

Protocol: Inhalation exposure to 1-BP from 70 days prior to mating, during gestation and most of lactation in F_0 and F_1. Reproductive function evaluated in F_0 and F_1; postnatal mortality and growth evaluated in F_1 and F_2 litters.

Notes: M=Male; F=Female; ↑,↓=Statistically significant increase, decrease.
[a]Number of F_0 and F_1 male and female pairs, except that no F_1 offspring were available at 750 ppm.
[b]See synopses for details about systemic effects.
[c]Not statistically significant but above historical control value.
[d]No statistical analyses conducted, but considered test article related (see text).

Prior to mating, the F0 female rats exhibited increased estrous cycle length. While this effect appeared to be dose-related, statistical analysis of the data was not conducted, in part because several animals in each of the high dose groups did not cycle at all. However, the study authors considered values for the 500 and 750 ppm groups to be test agent related since they exceed the range of their historic control data for this end point (4.1–5.1 days). Reproductive performance was impaired in the higher dosage F0 groups as evidenced by significant decreases in male/female mating index in the 750 ppm group, and in the male/female fertility index in the 500 and 750 ppm groups. An increased time to coitus in the F0 500 and 750 ppm groups was not statistically significant but was considered test agent related since it exceeded historical control values. None of the females in the F0 750 ppm group became pregnant. In contrast, 1-BP treatment had no effect on gestation length or complications during delivery at

500 ppm. However, numbers of implantation sites and pups born to F_0 females were significantly reduced in the 500 ppm group.

At necropsy, significant reductions in F_0 absolute reproductive organ weights were observed for ovary (750 ppm), cauda epididymis (500 and 750 ppm), prostate (≥250 ppm, but did not decrease with increasing dose), seminal vesicles (750 ppm), and pituitary (750 ppm). Significant decreases in relative weights of these organs were only observed in the 750 ppm group for caudae epididymides and ovaries. Ovarian histologic analysis in F_0 rats in the 750 ppm group revealed a significant increase in the incidence of ovaries with reduced numbers of corpora lutea and with follicular luteinized cysts.

In males, a slightly increased incidence of seminiferous tubule degeneration was not considered treatment related by the study authors since lesions in 4 of 6 affected rats were of minimal severity. Also, testicular sperm counts (absolute or per gram testis) were not significantly altered by treatment. An analysis of cauda epididymis spermatozoa from F_0 rats revealed significant reductions in morphologically normal sperm at ≥ 250 ppm. However the decrease from 99.7% normal sperm in controls to 99.3% at 250 ppm was not considered by the authors to be treatment related because this value is above historical control value of 99.0%. Cauda epididymis sperm numbers were significantly reduced at 750 ppm and the percentage of motile sperm was significantly reduced at 500 and 750 ppm.

A statistically significant decrease in implantation sites and in the number of offspring at birth was seen at the 500 ppm dose in both generations. The F_1 rats were evaluated for postnatal growth, development, and survival. A slight, but significant, reduction in pup viability on pnd 14–21 in the F_1 500 ppm group (97.7% vs. 100% in controls) was not considered of sufficient magnitude to be treatment related, especially because postnatal survival calculated from pnd 4 to pnd 21 was not different by treatment. Therefore, the authors concluded that there were no effects on pup survival.

Mean offspring weights (litter as experimental unit) were lower at the 500 ppm dose in both generations. Significant reductions in F_1 litter weight gain were found in males of the 250 ppm group (pnd 21–28) and 500 ppm group (pnd 4–7, 7–14, and 21–28). A significant reduction in F_1 female weight gain was only noted in the 500 ppm group on pnd 21–28. The age of balanopreputial separation was significantly increased in the F_1 500 ppm group but authors attributed that effect as secondary to reduced weight gain in that group. The age at which female offspring attained of vaginal patency was not significantly different in treated F_1 offspring.

1-BP exposure in the F_1 animals was initiated on pnd 22. Twenty-five rats/sex/group in control and 100–500 ppm treatment groups were selected for mating. The mating experiment was conducted as described for the F_0 rats. Increased estrous cycle lengths in the 250 and 500 ppm F_1 groups (4.9 and 5.1 days) were within ranges of historical controls (4.1–5.1) but were nevertheless attributed by the authors to be related to 1-BP treatment. This judgement was based on the fact that 3 and 4 animals, in the 250 and 500 ppm groups, respectively, had no complete estrous cycles (versus only 1 each in the control and 100 ppm groups). Again, no statistical analysis was performed for this endpoint. No significant effects were noted for F_1 fertility or mating indices, days to mating, gestation length, or birthing complications. However, authors noted that non-

significant and non-dose-related reductions in fertility indices in the F_1 100, 250, and 500 ppm groups (68, 64, 72%, respectively) were below fertility indices of historical controls (~90%). Mean numbers of implantation sites were reduced in the F_1 dams in the 250 and 500 ppm groups with statistical significance achieved at the higher dose level. Live litter size was significantly decreased at 500 ppm. Apparent increases in the incidence of ovarian follicular cysts and interstitial cell hyperplasia (mild) in F_1 females in the 500 ppm group were not statistically significant. Absolute (but not relative) epididymis and pituitary weights were significantly reduced in the F_1 500 ppm males. Lesions observed in testes were considered minimal and their incidence was not altered significantly by treatment, although there appeared to be a trend. Other male reproductive organs were histologically normal. The percentage of motile sperm was slightly, but significantly, reduced in the F_1 males (from 89% in controls to 85%) at 250 ppm. The study authors did not consider this treatment-related since this value exceeds that of historic controls. However, the percentage of motile sperm was further (and significantly) reduced to 74% in the 500 ppm group. The percentages of morphologically normal sperm were significantly reduced at 500 ppm. A slight but statistically significant reduction from 99.5% normal sperm in controls to 98.9% in the 100 ppm group was not considered by the study authors to be test article related because the difference was very small, and no significant changes were seen in the 250 ppm group. [The CERHR ExpertPanel agreed with this interpretation.]

F_2 rats were only evaluated for postnatal growth and survival to pnd 21. Postnatal weight gain in males and females was significantly reduced in the F_2 500 ppm group. Survival was unaffected.

[The CERHR Expert Panel identified 100 ppm as a NOAEC in this study, and 250 ppm as a LOAEC, based on decreased prostate weight in the F_0 males and increased estrous cycle length in the F_1 female offspring. From the perspective of the LOAECs observed, both sexes are equally sensitive to 1-BP. Alterations in male and female reproductive outcomes at 500 ppm may contribute to the altered fertility and reduced litter size seen at this concentration, and the infertility seen at 750 ppm.]

Strength/Weaknesses: This is a comprehensive study conducted under GLP and it meets specifications of EPA's harmonized reproductive test guidelines. It includes indices of puberty as measures for reproductive development, and sperm measures as indices of testicular and epididymal function. This allows effects on reproductive organ function to be detected in the absence of an effect on reproductive performance at lower doses. Results provide convincing evidence that 1-BP is a reproductive toxicant in both male and female rats, with neither sex being obviously more sensitive than the other. Adverse effects on litter size and sperm measures at 500 and 750 ppm were consistent across generations, suggesting a lack of a transgenerational effect, or increased susceptibility during perinatal or pubertal stages. Apparent increase in age at balanopreputial separation appears to be related to reduced bodyweights in offspring in the highest dosage group rather than to direct effects of the test agent on puberty. Despite significant (though not dramatic) reductions in epididymis weight, sperm morphology, sperm motility, and epididymal sperm counts, there were no effects on testicular histology or testicular sperm counts. Likewise, in the F_0 females, alterations in estrous cyclicity and litter size were found in the absence of significant decreases in ovarian weight or significantly abnormal ovarian histology at 500 ppm. Criteria for scoring histology were not provided (p. 55 of study). Some animals at 500

and 750 ppm were apparently more than "minimally" affected, especially as the testes contained at least some tubules with "Sertoli cell only." One might expect to see histologic evidence of abnormal spermatogenesis based on significant reduction of epididymal sperm counts in the 750 ppm group. The Panel suggested that study authors may want to reconsider the statistical analyses for testicular pathology.

Utility (adequacy) for CERHR Evaluation Process: This is an excellent study for hazard identification and is adequate for the CERHR evaluation process. The wide array of endpoints provides a comprehensive picture of alterations in both the male and female reproductive system that together appear to account for the subfertility at 500 ppm and infertility at 750 ppm. Effects on many endpoints at 500 ppm, in the absence of significantly decreased bodyweight or other pathology, provide strong evidence for specificity of the reproductive toxicity.

Investigation of testicular toxicity

A study by Ichihara et al. examined the dose response of 1-BP-induced testicular toxicity including sperm measures (motility/morphology) and detailed testicular histology (testes fixed in Bouin's and stained with period acid Schiff's reagent). In the examination of testicular histology, subtle changes in seminiferous tubule cell associations, similar to those recommended by Creasy (*71*), were evaluated. These included enumeration of spermatogenic cells in stage VII tubules and elongated spermatids retained in stage IX−XI tubules (normally released at stage VIII). The rationale for this study included the increased use of 1-BP in industry and the previously reported reproductive toxicity induced by its isomer, 2-BP. Eight-to-nine, 10-week-old male Wistar rats (from the Shizuoka Laboratory Animal Center) were exposed to air or 200, 400, or 800 ppm [1,006, 2,012, or 4,025 mg/m3] 1-BP vapors (99.81% purity) for 8 hours/day for 12 weeks. The maximum dose in this study was selected based on observations in previous studies that exposure to 1,000 ppm resulted in debilitation. Chamber concentrations of 1-BP were measured by GC and reported. At the end of the exposure period the rats were sacrificed and necropsied. Data were evaluated by one-way ANOVA followed by Dunnett's method. Table 4 lists findings of this study.
Significant reductions in absolute organ weights were observed for seminal vesicles (≥200 ppm), epididymides and pituitary (≥400 ppm), and prostate (800 ppm). Significant reductions in relative organ weights were noted in seminal vesicles (≥200 ppm) and epididymides (800 ppm). Bodyweight gain was reduced in the 400 and 800 ppm groups. Histopathological changes were observed in the epididymides, prostate, and seminal vesicles of the 800 ppm group. Epididymides had reduced duct cavity diameter, wider interstitial space, increased epithelial cell height and contained neutrophils or degenerated epithelial cells. Prostate and seminal vesicles had reduced alveoli size and degenerated cells were observed in the seminal vesicle cavity. Histological evaluation of testes revealed vacuolated seminiferous epithelium in 2 of 9 rats of the 800 ppm group. The numbers of retained elongated spermatids in stages IX, X, and XI were significantly increased in 400 and 800 ppm groups and a significant increase in degenerating spermatocytes in stage VII was seen in the 800 ppm group. Sperm quality was also affected as observed by significant reductions in sperm count and motility and increases in tailless sperm at ≥400 ppm. At 800 ppm a significant increase in sperm with abnormal heads (banana-like or straight) was observed. Table 4 includes values for sperm parameters. Plasma testosterone level

was significantly reduced in the 800 ppm group, but there were no changes in follicle stimulating hormone (FSH) or luteinizing hormone (LH) levels. The presence of retained elongated spermatid during the post-spermiation periods (stages IX−XI) led authors to conclude that the likely mode of 1-BP toxicity results in failure of spermiation. Authors stated that this pattern of toxicity differs from that of 2-BP which has been reported to target spermatogonia.

Table 4: Major reproductive findings in the study of Ichihara et al.

Number/ Dose	*Dose in ppm (mg/m^3)*	*Effects*
8	0	
9	200 (1,006)	↓Absolute seminal vesicle weight ↓Relative seminal vesicle weight
9	400 (2,012)	↓Absolute seminal vesicle, epididymides, and pituitary weight ↓Relative seminal vesicle weight ↑Retained elongated spermatids (13 vs 049/tubule) ↓Sperm count (588 vs 792x10^6/g cauda) ↓Motile sperm (67 vs 83%) ↑Tailless sperm (18 vs 4%) ↓Bodyweight gain ↑Relative liver weight ↓Mean corpuscular hemoglobin concentration
9	800 (4,025)	↓Absolute seminal vesicle, epididymides, pituitary, and prostate weight ↓Relative seminal vesicle and epididymides weight ↑Histological changes in epididymides, prostate, and seminal vesicles ↑Retained elongated spermatids (48 vs 049/tubule) ↑Degenerating spermatocytes (06 vs 004/tubule) ↓Sperm count (240 vs 792x10^6/g cauda) ↓Motile sperm (25 vs 83%) ↑Tailless sperm (36 vs 4%) ↑Abnormal sperm (100 vs 1%) Vacuolated seminiferous epithelium in 2/9 rats ↓Plasma testosterone (29 vs 45 ng/mL) with no change in LH or FSH ↓Bodyweight gain ↑Relative and absolute liver weight; ↓absolute spleen weight ↑Histological changes in liver ↑Mean corpuscular volume ↓Mean corpuscular hemoglobin concentration

Protocol: 10-week-old male rats exposed to 1-BP vapors for 8 hours/day for 12 weeks.
Notes: ↑,↓: Statistically significant increase, decrease.

Strength/Weaknesses: A strength of this study is the thorough evaluation of testicular effects of 1-BP including detailed histology, sperm measures, and serum hormones. The exposure period is sufficiently long to see effects on all spermatogenic stages, and the range of doses is sufficiently wide to determine a no effect level and begin to see systemic effects on bodyweight. Enumeration of spermatogenic cell types in seminiferous tubule cross sections allowed conclusions about sensitive cell types and/or stages. The conclusion that the main effect in testis is spermatid retention beyond Stage VIII is consistent with a possible effect on Sertoli cell function and/or possible effect on the endocrine support of spermatogenesis. Decreased testosterone levels in the high-dose group coupled with decreased weights of testosterone-dependent organs (most consistently the seminal vesicles) are consistent with the latter hypothesis, as is observed decrease in sperm quality (motility/morphology).

Retained spermatids may account for the decreased numbers of sperm in the epididymides. A weakness of the study is that relatively low numbers of animals per group (9–10) limits the power of the study to detect an effect. For example, lower and more variable serum testosterone level might obtain statistical significance if more animals were assessed. Also, the significance of the adverse effects on testicular and sperm measures is hard to interpret without fertility data.

Utility (adequacy) for CERHR Evaluation Process: This study is particularly useful for characterizing effects of 1-BP in males since it includes detailed histology with quantification of germ cells and serum hormones. It has limited usefulness for hazard identification since it does not include a fertility assessment, but is a valuable adjunct to 1-BP weight of evidence considerations.

Testicular effects in rats

Kim et al. and Yu et al. examined testes microscopically and no adverse effects were reported. In the Kim et al. study, Sprague-Dawley rats inhaled 1,800 ppm 1-BP for 6 hours/day, 5 days/week, for 8 weeks. Testes were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-Eosin and/or PAS hematoxylin. In the Yu et al. (*51*) study, male Wistar rats were exposed to 1,000 ppm 1-BP vapors (99.4% purity) for 8/hours/day for 5 or 7 weeks. Kim et al. reported an increase in relative weight of ovaries, but no ovarian lesions were observed.

Strength/Weaknesses: The experimental designs of the Kim et al. and Yu et al. studies could allow comparison of relative effects on gonads and blood. However, since there is no indication that testes (or ovaries) were examined for subtle effects such as retained spermatids or vacuolated Sertoli cells, it is doubtful that testes were evaluated in sufficient detail to detect changes seen in the other studies. Lack of effect could also be due to the shorter duration of exposure, but 7–8 weeks should be sufficient to detect changes in spermiation and sperm counts. Increased relative weights of testes and ovaries could simply be due to bodyweight depression without change in absolute gonad weights.

Utility (adequacy) for CERHR Evaluation Process: These studies by Kim et al. and Yu et al. are not useful for evaluating reproductive effects. They may be useful for comparing blood measures with other studies.

Two unpublished general toxicity studies also included evaluation of the testes. In a 28-day inhalation study, testicular hypo/aspermatogenesis was seen in two surviving Sprague-Dawley rats after exposure to 8,000 mg/m3 or about 1,600 ppm, but this effect could not be specifically related to the exposure due to systemic toxicity at this concentration. On the other hand, no testicular or ovarian lesions were observed in Sprague-Dawley rats exposed to up to 3,000 mg/m3 (~700 ppm) 1-BP vapors for 13 weeks. This finding is consistent with the results of the multigeneration study described above.

Dominant lethal test in rats

Saito-Suzuki et al. conducted dominant lethal studies in rats to determine the structure-activity relationships of 5 halogenated propanes. In addition to 1-BP (>98%

purity) the following compounds were examined: 1,2,3-tribromopropane, 1,2-dibromopropane, 1,2,3-trichloropropane, and 1-chloropropane.
Eleven-week-old male Crl: Sprague Dawley rats (n=15/group) were gavaged with 10% of the acute lethal dose of each compound in olive oil for 5 days. 1-BP was administered at a dose of 400 mg/kg bw. Olive oil was the negative control and 1,2-dibromo-3-chloropropane was the positive control (n=15/group). At 1–8 weeks after treatment, the males were mated weekly with untreated females. 1-BP treatment had no effect on male fertility. Females (n=15/time period) were sacrificed 13–14 days after mating and examined for corpora lutea, implants, live embryos, and early and late embryonic deaths. 1-BP treatment increased the number of mean dead implants at the 8-week mating but had no effect on the dominant lethal mutation index (live embryos per test female/live embryos per control females). The authors concluded that dominant lethal mutations were induced by propanes containing a bromine or chlorine atom on each carbon with bromine comprising two of the atoms.

Strength/Weaknesses: This is a classic dominant lethal protocol showing that a relatively high oral dose of 1-BP (~maximum tolerated dose) does not induce dominant lethality. Other halogenated propanes serve as controls in that they are effective at lower dosages.

Utility (adequacy) for CERHR Evaluation Process: This paper is important since it eliminates 1-BP as a germ cell mutagen, and thereby rules out a mechanism of action exhibited by related halogenated propanes. The study also shows that short-term (5 day) exposure at high levels failed to produce adverse effects sufficient to affect fertility. The protocol neglected specific changes in testis/epididymis function.

OECD 422 screening study

An abstract is available that describes a reproductive study conducted in rats according to OECD Guideline 422. While the abstract reported results similar to those observed by WIL Research Laboratories, the full study report was not available to the Expert Panel during the present review of 1-BP.

Summary of the reproductive toxicity studies reviewed by CERHR

Reproductive studies, including a two-generation study, were conducted in rats. Major findings of these studies are included in Table 5 below.

The two-generation study provides sufficient data to indicate that repeated chronic inhalation exposure of female Sprague-Dawley rats to 1-BP at doses of 250 ppm (1,257 mg/m3) and higher results in reproductive toxicity (*58*). Effects included a dose-related increase in estrous cycle length in F1 females exposed to ≥ 250 ppm (1,257 mg/m3) and F0 females exposed to ≥ 500 ppm. Follicular cysts were seen in ovaries of F0 females exposed to 750 ppm (3,771 mg/m3) and were accompanied by decreased ovarian size and decreased numbers of corpora lutea (*58*). Reduced fertility and litter size were observed in the F0 and F1 generations at ≥500 ppm (2,514 mg/m3), but the experimental design did not permit differentiation as to whether these effects were due to reduced female or male fertility, or both. There are sufficient data to indicate that repeated inhalation exposure of male rats to 1-BP results in reproductive toxicity at doses of 200 ppm (1,006 mg/m3) and higher. Effects in a two-

generation study included decreased prostate weight in F0 males at 250 ppm (1,257 mg/m3), and dose-related decreases in percentages of normal sperm and motile sperm in F0 and F1 generations at ≥500 ppm (2,514 mg/m3) (*58*). Decreased epididymal sperm count, epididymal weight, and seminal vesicle weight were observed at the 750 ppm (3,771 mg/m3) dose. Reduced fertility and litter size were observed in the F0 and F1 generations but the experimental design did not permit differentiation as to whether these effects were due to reduced female or male fertility, or both. Testicular toxicity consistent with the above study was also characterized in a subchronic inhalation study (*57*). Decreased absolute and relative seminal vesicle weights were observed at 200 ppm (1,006 mg/m3). Histopathological changes were observed in epididymides, prostate, and seminal vesicles at a dose of 800 ppm (4,025 mg/m3). The presence of retained elongated spermatids was increased at doses of 400 and 800 ppm (2,012 and 4,025 mg/m3), and reductions in sperm count and motility were observed. Plasma testosterone levels were reduced at 800 ppm (4,025 mg/m3) (*57*). The Expert Panel noted a conclusion by Ichihara et al. (*57*) that the main effect in testis is spermatid retention beyond Stage VIII. The Panel concludes that such effects are consistent with altered Sertoli cell function or impaired endocrine support of spermatogenesis.

The Expert Panel selected a NOAEC of 100 ppm (503 mg/m3) for the two-generation reproductive toxicity study (*58*), and opined that reduced fertility in the two-generation study was due to reproductive toxicity in both males and females. This was based on the observation that exposure to 2,514 mg/m3 (500 ppm) increased estrous cycle length and compromised sperm quality (as discussed above in separate summaries for male and female rats). Further, the Panel noted that the male and female reproductive systems may be equally sensitive since decreased prostate weight at 250 ppm (*58*) and decreased seminal vesicle weight at 200 ppm (*57*), as well as extended estrous cycles in F1 females at 250 ppm occurred at similar concentrations. However, difficulties in analyzing the length of the estrous cycle when some of the animals were not cycling precluded a definitive statistical analysis on this last point. Lastly, the Expert Panel noted consistency of effects across the two generations and stated there was no evidence of increased sensitivity in developing rats exposed *in utero* and indirectly through mother's milk, or during pubertal development.

Summary Statements of the CERHR Panel

- There is insufficient evidence in humans that 1-BP causes reproductive toxicity due to an absence of data.
- There is sufficient evidence in female rats that exposure to 1-BP causes reproductive toxicity manifested as ovarian dysfunction following inhalation at ≥250–500 ppm daily for 6 h/d for 10 weeks.
- Subfertility is observed following inhalation at ≥ 500 ppm under the same conditions. These data are assumed relevant to consideration of human risk.
- There is sufficient evidence in male rats that exposure to 1-BP causes reproductive toxicity manifested as decreased secondary sex organ weights following inhalation at ≥200–500 ppm daily for 6–8 h/day for 10–12 weeks. The data are assumed relevant to consideration of human risk.

Table 5: Summary of reproductive toxicity studies with 1-BP

Concentration in ppm (mg/m^3)	*Exposure Regimen*	*Species/ Strain*	*Concentration: Effect*	*Reference*
100 (503) 250 (1,257) 500 (2,514) 750 (3,771) [F_0 only]	6h/7d/10 wk prior to mating and during gestation and most of lactation. Whole body.	Male and female CD Rat	**NOAEC= 100 ppm (503 mg/m^3** **250 ppm (1,257 mg/m^3):** ↑Estrous cycle length in F_1 ↓Prostate weight (F_0) **500 ppm (2,514 mg/m^3):** ↑Estrous cycle length ↓Normal sperm and sperm motility ↓Epididymis and prostate (F_0) weights ↓Fertility, implantation sites, and litter size ↑Precoital interval **750 ppm (3,771 mg/m^3):** ↑Estrous cycle length ↑Ovarian follicular cysts and ↓ corpora lutea ↓Sperm count, normal sperm and sperm motility ↓Ovary weight and numbers of corpora lutea ↓Epididymis, prostate, seminal vesicle and pituitary weights ↓Mating ↑Precoital interval, and complete infertility	WIL Research Laboratories (*58*)
200 (1,006) 400 (2,012) 800 (4,025)	8h/12 wk Whole body.	Male Wistar Rats	**200 ppm (1,006 mg/m^3):** ↓Absolute and relative seminal vesicle weight **400 ppm (2,012 mg/m^3):** ↑Retained elongated spermatids ↓Sperm count and motility and ↑tailless sperm ↓Absolute seminal vesicle, epididymides, and pituitary weight and relative seminal vesicle weight **800 ppm (4,025 mg/m^3):** ↑Retained elongated spermatids and degenerating spermatocytes ↓Sperm count and motility ↑Tailless sperm and abnormal sperm Vacuolated seminiferous epithelium in 2/9 rats Epididymis, prostate, and seminal vesicle lesions ↓Testosterone ↓Absolute seminal vesicle, epididymides, prostate, and pituitary weight and relative seminal vesicle and epididymides weight	Ichihara et al. (*57*)

h = hours; d = days; wk = week
↑=Increased Effect; ↓=Decreased Effect
F_0=Effects observed only in F_0
F_1=Effects observed only in F_1.

Developmental toxicity

Human Data

No case report or epidemiological data were available for any aspect of potential developmental toxicity induced by exposure to 1-BP.

Experimental Animal Data

Developmental toxicity study in rats (HLS)

The BSOC sponsored a standard developmental toxicity study using Crl: CD (SD) IGS BR Sprague- Dawley rats. Pregnant animals (25/group) were exposed to actual vapor concentrations of 0, 520, 2,530, or 5,060 mg/m3 [0, 103, 503, or 1,005 ppm] 1-BP (99.9% purity) for 6 hours/day from gd 6–19 (day of conception=day 0).

Exposures were conducted in whole-body chambers under dynamic conditions. Concentrations were monitored by infrared (IR) spectrometry. Nominal chamber concentrations were selected to produce a gradation of effects from the lowest to the highest dose. Pregnancy was terminated on gd 20 and the fetuses removed by cesarean section. The uterine contents were weighed and one-half the fetuses preserved in Bouin's fluid for soft-tissue evaluation, while the other half were eviscerated and processed for skeletal evaluation using Alizarin Red-S and Alcian Blue.

The study was conducted according to GLP. Results of this study are summarised in Table 6.

Table 6: Major effects observed in the pre-natal toxicity performed by HLS

Concentration in ppm (mg/m³)	*Exposure Regimen*	*Species/ Strain*	*Concentration: Effect*	*Reference*
100 (503) 250 (1,257) 500 (2,514) 750 (3,771) [F_0 only]	6h/7d/10 wk prior to mating and during gestation and most of lactation. Whole body.	Male and female CD Rat	**NOAEC= 100 ppm (503 mg/m³** **250 ppm (1,257 mg/m³):** ↑Estrous cycle length in F_1 ↓Prostate weight (F_0) **500 ppm (2,514 mg/m³):** ↑Estrous cycle length ↓Normal sperm and sperm motility ↓Epididymis and prostate (F_0) weights ↓Fertility, implantation sites, and litter size ↑Precoital interval **750 ppm (3,771 mg/m³):** ↑Estrous cycle length ↑Ovarian follicular cysts and ↓ corpora lutea ↓Sperm count, normal sperm and sperm motility ↓Ovary weight and numbers of corpora lutea ↓Epididymis, prostate, seminal vesicle and pituitary weights ↓Mating ↑Precoital interval, and complete infertility	WIL Research Laboratories (*58*)
200 (1,006) 400 (2,012) 800 (4,025)	8h/12 wk Whole body.	Male Wistar Rats	**200 ppm (1,006 mg/m³):** ↓Absolute and relative seminal vesicle weight **400 ppm (2,012 mg/m³):** ↑Retained elongated spermatids ↓Sperm count and motility and ↑tailless sperm ↓Absolute seminal vesicle, epididymides, and pituitary weight and relative seminal vesicle weight **800 ppm (4,025 mg/m³):** ↑Retained elongated spermatids and degenerating spermatocytes ↓Sperm count and motility ↑Tailless sperm and abnormal sperm Vacuolated seminiferous epithelium in 2/9 rats Epididymis, prostate, and seminal vesicle lesions ↓Testosterone ↓Absolute seminal vesicle, epididymides, prostate, and pituitary weight and relative seminal vesicle and epididymides weight	Ichihara et al. (*57*)

h = hours; d = days; wk = week
↑=Increased Effect; ↓=Decreased Effect
F_0=Effects observed only in F_0
F_1=Effects observed only in F_1.

There was no effect on pregnancy rate. One animal from the 1,005 ppm (5,060 mg/m3) group was sacrificed moribund. Examination of this animal indicated extramedullary hematopoiesis, hepatocellular necrosis, hepatocellular inflammation, and lymphoid cell hyperplasia of the spleen. These findings were not considered to be

treatment related. Lacrimation and salivation were observed in animals exposed to 1,005 ppm (5,060 mg/m3). Mean maternal bodyweight and bodyweight corrected for gravid uterus weight in the 503 ppm (2,530 mg/m3) and 1,005 ppm (5,060 mg/m3) groups were significantly reduced compared to the concurrent controls; maternal bodyweight gain and food consumption were also significantly reduced in these two groups. No signs of embryotoxicity were observed, and no treatment-related visceral or skeletal anomalies were noted. Fetal bodyweight was significantly reduced in all treated groups. A significant treatment-related increase in the litter incidence of bent ribs was seen in the 1,005 ppm (5,060 mg/m3) group, but the authors considered this condition reversible and frequently observed in untreated rats. [The CERHR Panel noted that bent ribs (also referred to as undulating, angulated, bowed or wavy rib) is a term applied to congenital undulations of several ribs and these changes are considered a fetal aberration (deviation) as contrast to a frank malformation.]

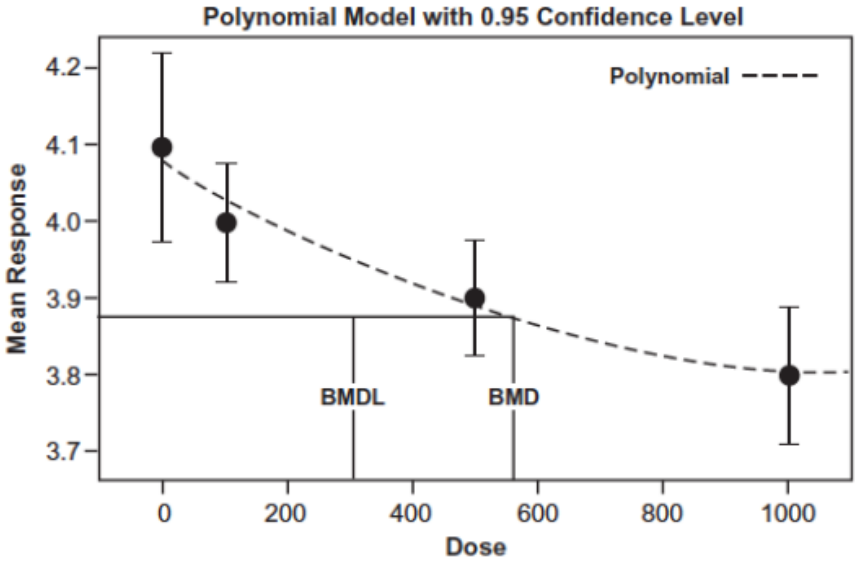
A slight (but not significant) increase in the incidence of wavy ribs was observed in litters exposed to 503 ppm (2,530 mg/m3) 1-BP. No fetuses with wavy ribs were found in either control litters or in those recovered from dams inhaling 103 ppm (520 mg/m3). [The CERHR Panel noted that the incidence of 'wavy' ribs in control rat fetuses is generally low (0 to 2.7%), is bilateral and the condition resolves without sequelae. Bent or wavy ribs are associated with reduced local alkaline phosphatase activity and reduced calcium deposition and are thought to be the result of opposing cervical and abdominal muscular forces acting upon incompletely ossified ribs.] Reduced skull ossification was observed at 503 and 1,005 ppm (2,530 and 5,060 mg/m3), and was associated with maternal toxicity and reduced fetal bodyweight by authors. Thus, reduced ossification of the fetal rat skull occurred after 1-BP exposures less than those required to retard ossification of the ribs (*18*). The authors identify 103 ppm (520 mg/m3) as a NOEC for maternal and fetal toxicity, and 1,005 ppm (5,060 mg/m3) for teratogenicity. The Expert Panel noted that Huntingdon Life Sciences (*18*) did not consider reduced fetal weight at the lowest dose to be treatment-related. Huntingdon Life Sciences (*18*) suggested that the practice of holding "1 or 2" control dams "until the end of the daily cesarean section period" resulted in "many control group fetuses weighing ~0.2 g heavier in bodyweight versus the laboratories' usual 3.9 gram fetuses" (*68*). Huntingdon Life Sciences (*18*) speculated that this practice resulted in apparent bodyweight reductions among treated as compared to concurrent control groups when considered for individual male and female fetuses. There is no question that normal fetal rodent bodyweights increase rapidly near term. However, 23−25 dams/group and their litters were examined (*18*). Therefore, the Panel questioned whether holding one dam/group (from 23-25 total litters) until the end of the workday could result in a statistical distortion of the data that was not related to exposure history.

The CERHR Expert Panel addressed the question of whether reduced fetal bodyweights at the 103 ppm (520 mg/m3) concentration were due to 1-BP treatment by calculating a benchmark dose (BMD). The EPA BMD Software program (BMDS Version 1.3) was used. Initial attempts to model the data indicated that none of the models fit the data adequately. Examination of the raw data suggested that a single litter in the 103 ppm (520 mg/m3) group was aberrantly low (i.e., an outlier): the mean for this litter was 3.2 grams, approximately three SD lower than the mean of 3.9 grams. Therefore, this litter was excluded from subsequent analyses. Doing this changed the mean and SD for this group from 3.9 ± 0.23 to 4.0 ± 0.17 grams. More

importantly, it made the data amenable to dose-response modeling using the BMDS software. The polynomial model provided the best data fit (Figure 1). The BMD was calculated as the concentration at which fetal weight was reduced 5% from the concurrent control mean. This provided a central estimate for the BMD of 561 ppm, and a 95th percentile lower confidence limit (BMDL) of 305 ppm. The CERHR Expert Panel noted that the BMD is consistent with the LOAEC for skeletal variations.

Figure 1

Benchmark Dose Analysis of Fetal CD Rat Bodyweight Following In Utero Exposure to 1-BP (18)



Strength/Weaknesses: The Huntingdon Life Sciences (*18*) bioassay is a well-conducted, GLP study performed in accord with current regulatory guidelines and standard practices using appropriate numbers of animals. Chamber concentrations were determined by weighing the 1-BP vapor generation apparatus prior to and following cessation of exposure, then dividing by the duration and airflow. The purity of the test material was determined "using a modified method provided by the sponsor" and was stated as 99.87%. The chamber air concentrations were measured using a Miran air analyzer, but specific details of that infrared method were not provided. Fetuses were evaluated for signs of developmental toxicity using appropriate methods.

Utility (adequacy) for CERHR Evaluation Process: The Huntingdon Life Sciences (*18*) rat data are directly applicable to the evaluation process in that the protocol conformed to that expected from a standard inhalation bioassay carried out under GLP. It is noteworthy that only rats were included in the protocols available to date. No data for other common laboratory species (e.g., mice, rabbits) as required for standard developmental toxicity risk assessments were found. No pharmacokinetic, disposition, or transplacental transfer data were collected that could be applied in quantitative interspecies extrapolation of dose. Limited information about developmental toxicity associated with exposure to 1-BP is also available from a two-generation reproductive toxicity study in which Crl:CD(SD) IGS BR rats were

exposed to 0, 100, 250, 500, or 750 ppm [0, 503, 1,257, 2,514, 3,771 mg/m3] 1-BP vapors during premating, mating, gestation, and lactation (pnd 5−21) (*58*). Complete details of the WIL Research Laboratories (*58*) study are included in Section 4. Statistically significant reductions in live litter size were observed in F_0 and F_1 females exposed to 500 ppm and no offspring were observed in F_0 females exposed to 750 ppm. Given that there were proportionate decreases in implantation sites in the F_0 dams, the Panel noted the plausible explanation of an effect on fertility rather than adverse effects on development. Significant reductions in neonatal weight gain during nursing occurred in F_1 males and F_2 males and females of the 500 ppm group. There were no treatment-related effects on postnatal survival in either F_1 or F_2 litters. The authors stated that skeletal examinations would be conducted according to the Dawson method if external abnormalities were observed. There were no reports of external malformations in offspring. [The CERHR Expert Panel identified a developmental NOEC of 250 ppm.]

Strength/Weaknesses: This is a well-conducted GLP study performed according to standard practices and guidelines. Adequate numbers of animals were used in the evaluation and all the appropriate endpoints for reproductive toxicity were examined. One limitation is that developmental effects may have been missed because animals were allowed to give birth, which may prevent detection of malformed or otherwise abnormal fetuses.

Utility (adequacy) for CERHR Evaluation Process: This two generation study provides convincing evidence that adverse effects on rat reproductive performance associated with inhaled 1-BP were due to reduced fertility; no evidence of developmental toxicity was found in pregnant females (through 500 ppm groups). This study provides useful indirect evidence that inhaled 1-BP is not a developmental toxicant in rats at up to 500 ppm.

HLS 2-generation reproductive toxicity study – developmental segment

Limited information about potential developmental effects is available from a range-finding one generation GLP study conducted by Huntingdon Life Sciences. Ten individually housed groups of Crl: CD (SD) IGS BR rats were randomly assigned and exposed (whole body) to HEPA-filtered air or 1-BP vapors (99.9% purity) in nitrogen at concentrations of 100, 199, 598, or 996 ppm [503, 1,001, 3,008, 5,010 mg/m3] on gd 6–19 for 6 hours/day. Dams delivered and were exposed to 1-BP together with their litters on pnd 4–20. Concentrations in dynamic inhalation chambers were verified using an unstated infrared method. At birth, pups from 7–9 litters/group were sexed, weighed, and examined for viability and external abnormalities. Pup growth and survival were monitored from birth through weaning. At weaning, 1 pup/sex/litter was randomly selected for a post-weaning growth study and exposed to 1-BP on pnd 22–28. Hematology and clinical chemistry analyses and organ weight measurements were conducted in dams at the end of the lactation period and offspring from the pnd 22–28 day post-weaning study on pnd 29. Data were analyzed by ANOVA, Dunnett's test, and/or the Kruskal-Wallis test. Results in this study are summarised in Table 7. Dams in the 996 ppm group experienced lacrimation and increased salivation. Maternal bodyweight gain was reduced during treatment in the 199, 598, and 996

ppm groups but did not reach statistical significance. Food intake was not affected by treatment. Significant organ weight changes in dams included increases in relative weights (to bodyweight) of the liver and kidneys in the 598 and 996 groups. The authors did not consider any of the changes in maternal hematology or clinical chemistry parameters to be toxicologically significant. No external abnormalities or reduced birth weight were reported. During the period from birth to weaning, no increased pup mortality was observed; pup bodyweights were slightly, but not significantly, reduced in the 996 ppm group. Bodyweight gain during the post-weaning period (pnd 22–29) was significantly lower for males in the 598 and 996 groups and females in the 996 group. Significant organ weight changes in male offspring included increased absolute adrenal (100 and 199 ppm) and reduced brain weight (996 ppm) and increased relative adrenal weight (100, 199, and 996 ppm). In female offspring, absolute brain weight was significantly reduced in the 996 ppm group. The only hematological and biochemical effects in offspring that were considered treatment-related by authors included statistically significant reductions in platelet count in 598 and 996 ppm females and 996 ppm males, elevations in gamma-glutamyl transferase levels in 996 ppm males and females, and reduced circulating glucose levels in the 996 ppm females and in all treated males (significant only at high dose for males). Authors identified a maternal NOEC of 100 ppm but did not identify a developmental NOEC. The results are summarised in Table 7. [The CERHR Expert Panel noted that exposure to 1-BP during the post-weaning period reduced bodyweight gain and may have targeted adrenals, platelets and the liver.]

Table 7

Major Effects Observed in a Developmental Range-Finding Study by Huntingdon Life Sciences (69)

Number[a]	*Dose in ppm (mg/m^3)*	*Maternal Effects*	*Offspring Effects*
10/8	0		
10/9	100 (503)	NOEC	↑Relative adrenal weight (M)
10/8	199 (1,001)	↓Bodyweight gain[b]	↑Relative adrenal weight (M)
10/7	598 (3,008)	↓Bodyweight gain[b] ↑Relative liver and kidney weight	↓Bodyweight gain on pnd 22–25(M) ↓Platelets (F)
10/10	996 (5,010)	↓Bodyweight gain[b] ↑Relative liver and kidney weight Clinical signs No toxicologically significant effects on hematology or blood chemistry	↓Bodyweight gain on pnd 22–29 (M) and 22–25 (F) ↑Relative adrenal weight (M) ↓Absolute brain weight ↓Platelets ↓Glucose levels ↑Gamma-glutamyl transferase No reduced birth weight, external abnormalities or increased mortality

Protocol: CD rats were exposed to 1-BP on gd 6–19. Dams delivered and were then exposed to 1-BP with litters on pnd 4–20. One offspring/sex/litter was exposed on pnd 22–28.

Notes: ↑, ↓=significant increase, decrease.
M, F=Males, Females only.
[a]Number of pregnant dams/litters evaluated.
[b]Although not statistically significant, bodyweight effects were considered by authors in selection of NOEC.

Strength/Weaknesses: One deficiency noted concerned 1-BP purity and stability in that a "modified method provided by the study sponsor" was utilized in those analyses. Chamber nominal concentrations were determined by weighing the 1-BP vapor generation apparatus prior to and following cessation of exposure, then dividing by the duration and airflow. Huntingdon Life Sciences (*69*) indicated that chamber air was collected at four time points over the 6-hour exposures and evaluated using a 'Miran air analyzer.' The report neither presented the details of chamber air sampling and infrared quantification, provided references to published or standardized methods for 1-BP measurement nor was it clear whether the calculated nominal concentrations were confirmed using conventional GC techniques.

Utility (adequacy) for CERHR Evaluation Process: While the protocol is of little utility in hazard identification for teratogens, the investigation is valuable inasmuch as exposures were conducted throughout organogenesis, through neonatal life, and infancy. A rat LOAEC for developmental toxicity of 100 ppm can be identified based on the significant increase in absolute and relative adrenal weights for the F1 males. After exposure to 598 or 996 ppm, adrenal to bodyweight ratios were also increased for the males, but not for the female offspring. Huntingdon Life Sciences identified a maternal NOEC of 100 ppm based on reduced maternal bodyweight gain after exposure to 199, 598, and 996 ppm.

Conclusions of the CERHR panel

No data are available to evaluate potential developmental toxicity of 1-BP exposures in humans.

In rats, appropriate methods were used to evaluate teratogenicity following exposure on gd 6-19. Postnatal growth and survival were evaluated in a two-generation reproductive toxicity assay and in a range-finding reproductive toxicity assay. Dams in the two-generation study were exposed throughout the entire gestation period and from pnd 5 to 21, while dams in the screening study were exposed on gd 6–19 and pnd 4–20. All studies were conducted according to GLP. The data were adequate to evaluate developmental toxicity in rats. No data were available for other common laboratory species such as mice and rabbits. In addition, there are no pharmacokinetic, disposition, or transplacental data that could be applied to quantitative interspecies extrapolation.

CERHR Summary of Developmental Toxicity

There were no studies located that address potential developmental toxicity in humans exposed to 1-BP.

The data are sufficient to indicate that inhalation exposure to 1-BP in rats can induce signs of developmental toxicity.

A prenatal inhalation developmental toxicity study in rats observed reduced ossification of the skull at ≥503 ppm (≥2,530 mg/m3) and increased bent ribs at 1,005 ppm (5,060 mg/m3); decreased maternal bodyweight gain and food consumption also occurred at these concentrations. Significant reductions in fetal bodyweight were

observed at ≥103 ppm (520 mg/m3). No evidence of embryotoxicity (lethality/terata) was seen at concentrations up to 1,005 ppm (5,060 mg/m3) 1-BP on gd 6–19.

There was no NOAEC for fetal weight decrease in the 1-BP developmental toxicity study. The authors of the study speculated on non-treatment-related reasons for the statistically significant effect at the lowest exposure concentration (103 ppm) and suggested that the true LOAEC was the next higher concentration level, 503 ppm. The Expert Panel addressed the question by calculating a BMD. The EPA BMD Software program (BMDS Version 1.3) was used. The BMD was calculated as the concentration at which fetal weight was decreased by 5% from the control mean. This provided a central estimate for the BMD of 561 ppm, and a 95th percentile lower confidence limit (BMDL) of 305 ppm. The CERHR Expert Panel noted that the BMD is consistent with the LOAEC for skeletal variations.

Two studies examined postnatal growth in rat pups whose dams were exposed to 1-BP during gd 6–19 and pnd 4−20 or throughout the entire gestation and majority of the lactation period (pnd 5−21) (*58*). Whereas reduced fetal bodyweights were seen at day 20 after dams inhaled 103 ppm 1-BP from days 6–19 (*18*), no such changes were observed at birth (day 21) in male and female CD neonatal rats born to dams inhaling 100 ppm 1-BP from days 6–19 (*69*). Postnatal pup bodyweight gain was reduced in litters from dams exposed to ≥598 ppm (3,008 mg/m^3) (*69*). F1 and F2 pup weight gain was reduced during the nursing period at 500 ppm (2,514 mg/m^3) in a two-generation study in. Additional relevant effects are listed in Table 8.

The CERHR Panel concluded there is sufficient evidence in rats that inhalation exposure to 1-BP causes developmental toxicity in the form of skeletal variations, consistent with developmental delay, following inhalation at ≥503 ppm, 6 hours/day, gd 6–19. There was also a concentration-related reduction in fetal bodyweight consistent with developmental delay. The data are assumed relevant to consideration of potential risk to human health.

CERHR Summary and Conclusions of Reproductive and Developmental Hazards

Developmental Toxicity

Prenatal developmental toxicity was assessed in Crl:CD rats. The data are sufficient to conclude that 1-BP caused developmental toxicity, in the form of decreased fetal weight and increased incidence of skeletal variations, in rats exposed to the compound by inhalation on a daily basis during the period of *in utero* development. The skeletal effects are typical of those associated with developmental delay and are believed to be reversible. The skeletal effects occurred in pups whose dams were exposed 6 hours/day to concentrations of 503 ppm (2,530 mg/m^3) and higher; a benchmark analysis of the fetal weight data indicated a BMD that detected a 5% change was 561 ppm (central estimate) with a lower 95th percent confidence limit of 305 ppm. These data are assumed relevant for assessing human hazard.

No information was available on developmental outcome after 1-BP exposure in humans.

Table 8: Summary of developmental toxicity studies with 1-BP

Analytical Concentration in ppm (mg/m³)	*Exposure Regimen*	*Species/ Strain*	*Concentration: Effect*	*Reference*
103 (520) 503 (2,530) 1,005 (5,060)	6h/d gd 6–19	CD (SD) IGS BR Rat	***Dams:*** Maternal NOEC=103 ppm (520 mg/m³) 503 ppm (2,530 mg/m³): ↓Weight gain and food intake 1,005 ppm (5,060 mg/m³): ↓Weight gain and food intake Lacrimation, Excessive salivation ***Fetuses[a]:*** 103 ppm (520 mg/m³): ↓Bodyweight 503 ppm (2,530 mg/m³): ↓Bodyweight; ↓Skull ossification 1,005 ppm (5,060 mg/m³): ↓Bodyweight; ↓Skull ossification; ↑Bent ribs	Huntingdon Life Sciences (*18*)
100 (503) 199 (1,001) 598 (3,008) 996 (5,010)	6h/d gd 6–19 and pnd 4–20	CD Rat	***Dams:*** Maternal NOEC=100 ppm (503 mg/m³) 199 ppm (1,001 mg/m³): ↓Bodyweight gain 598 ppm (3,008 mg/m³): ↓Bodyweight gain ↑Relative liver and kidney weight 996 ppm (5,010 mg/m³): ↓Bodyweight gain ↑Relative liver and kidney weight Clinical signs ***Pups:*** 100 ppm (503 mg/m³): ↑Relative adrenal weight (M) 199 ppm (1,001 mg/m³): ↑Relative adrenal weight (M) 598 ppm (3,008 mg/m³): ↓Bodyweight gain (M; pnd 22–25) ↓Platelets (F) 996 ppm (5,010 mg/m³): ↓Bodyweight gain (M: pnd 22–29, F: pnd 22–25) ↓Absolute brain weight (M,F) ↑Relative adrenal weight (M) ↓Platelets (M,F); ↓Glucose (M,F) ↑Gamma-glutamyl transferase (M,F)	Huntingdon Life Sciences (*69*)
100 (503) 250 (1,257) 500 (2,514)	6h/7d/wk for gestation and most of lactation. Whole body.	CD Rat	***Dams:*** See Table 4-3 in Section 4 ***Pups:*** Developmental NOEC=250 ppm (1,257 mg/m³) 500 ppm (2,514 mg/m³): ↓Weight gain during lactation period	WIL Research Laboratories (*58*)[b]

h = hours; d = days; wk = week; M=Male, F=Female; ↑=Increased Effect; ↓=Decreased Effect

[a]**[BMDs for fetal bodyweight: 561 ppm (central estimate); 305 ppm (95th percentile lower confidence limit).]**

[b]Reproductive effects for this study are summarized in Section 4.

Reproductive Toxicity

Reproductive effects of 1-BP were observed in a two-generation inhalation study in Crl:CD rats.

Decreased fertility, decreased numbers of implantation sites and litter size and increased pre-coital interval were observed after exposure at concentrations of 500 ppm (2,514 mg/m^3) and higher. These effects could be attributable to effects on either the male or female parent. Evaluation of other endpoints indicated adverse effects in both sexes. In males, prostate weight was decreased at concentrations of 250 ppm (1,257 mg/m^3) and higher, and there were effects on seminal vesicle weight and sperm quality at higher concentrations. Effects in females included an increase in ovarian follicular cysts at 750 ppm (3,771 mg/m^3). There was also an increase in

estrous cycle length that was judged to be 1-BP-related at 250 ppm (1,257 mg/m^3) and higher. The NOAEC for this study was 100 ppm (503 mg/m^3).

A subchronic inhalation study in male Wistar rats confirms the effect on reproductive organ weights (*57*). These effects were observed at the lowest concentration tested, 200 ppm (1,006 mg/m^3). Histopathologic evidence of inhibited spermiation was also observed in this study at concentrations of 400 ppm (2,012 mg/m^3) and higher.

There is sufficient evidence to conclude that inhaled 1-BP causes reproductive toxicity in male and female rats. The NOAEC for these effects was 100 ppm (503 mg/m^3). These results are assumed relevant for human hazard assessment.

5.2 TERA (2004) Review

The review was initiated as existing occupational exposure limits (OELs) in the USA varied by approximately 16 fold, from 10 ppm to 156 ppm, due to the use of different studies, end points and assessment factors. The review aimed to critically evaluate the underlying basis of the OELs and determine a common approach to deriving the OEL. The critical studies were identified as those chosen by CERHR (WIL Research; HLS; Ichihara).

The approach was to identify the critical effect or effects; derive a benchmark dose (BMD) and apply an appropriate uncertainty factor. The analyses were performed on a number of end points, including neurotoxicity, hepatotoxicity and reproductive / developmental toxicity.

The benchmark dose (BMD) approach was also used in conjunction with the more standard NOAEL/LOAEL technique to analyze the data for 1-BP. The use of both approaches necessitates expert judgment and adds value to the overall assessment. U.S. EPA's BMD software, version 1.3.2 (U.S. EPA, 2001) was used to reproduce each critical benchmark dose (BMD) and lower bound benchmark dose (BMDL) for 1-BP calculated by Stelljes and Wood (2004) and to expand the analysis to additional endpoints of interest for the 2-generation study (WIL Research, 2001). Benchmark responses (BMRs) of 1.0 control standard deviation were used by *TERA* for all continuous data and BMRs of 10% were used for all dichotomous data. These choices reflect standard operating procedure. All the available models in the BMDS software were run for each data set, and BMDs and BMDLs from the best fitting model were selected.

Reproductive and Fetal Effects.

The ACGIH (2004) use of a LOAEL of 100 ppm for decreased fetal weight in the Huntingdon Study (2001) as the critical basis for its OEL derivation is difficult to justify. As a first consideration, BMDL estimates for this endpoint are greater than for other effects. For example, both the NTP expert panel (NTP, 2002) and *TERA* (shown later in this text and in Table 2) identified a BMDL of approximately 300 ppm. Furthermore, questions about the conduct of the Huntingdon (2001) study, including the change in procedure with control animals that lead to higher body weights, lack of related findings of developmental delays in pups in multigeneration studies at similar

concentrations (see WIL Research, 2001), minimal severity of the effect (a maximum of 7% change from control), and potential relatedness to maternal effects (although BMDL for pup fetal weight is lower than for maternal weight) decreases the selection of this endpoint as the most relevant for deriving the OEL.

Stelljes and Wood (2004) argue that the effect level for sperm parameters in the WIL Research (2001) study should be based on the F0 generation results and not those for F1or F2 animals, because the goal of an OEL is to develop a safe exposure level for workers and the exposure patterns for the parental animals more closely resemble occupational exposure scenarios. A counter to this argument is that *in utero* exposure may cause effects manifested as these exposed animals become adult males. However, it is not clear what mechanisms would generate changes in sperm parameters based on the normal turnover in sperm through the cycle of spermatogenesis, in the absence of findings on male reproductive organ histopathology. The BMDL calculated by Stelljes and Wood (2004) for sperm motility in F0 animals was 263 ppm. *TERA* identified a BMDL of 270 ppm (see later in this text). Based on data from Ichihara et al. (2000b), Stelljes and Wood (2004) calculated a BMDL of 232 ppm for sperm count in F0 adult males. Taken together, the effects of 1-BP on male sperm parameters suggests that the male reproductive effect in parental animals occur in the same general range, but are not more sensitive than other relevant effects.

Benchmark dose modelling for several measures of male and female reproductive parameters from the two-generation study correspond well with each other and provide a consistent story indicating that 1-BP can affect reproductive parameters in males (decreased sperm motility and prostate weight), females (increased oestrous cycle length, no oestrous cycle incidence, and maternal body weight at gestation day 20), and functional reproductive performance (litter viability index, pup weight gain at post-natal days 21 to 29, and live litter size). The BMDL values for these latter effects are in the same range, but slightly lower, than for the liver effects, and represent a more serious outcome (Table 2). The BMDL value of 188 ppm from Stelljes and Wood (2004) or of 190 ppm from *TERA* for decreased live F1 litter size is the most appropriate basis for deriving the OEL, since this is the lowest measure related to exposure to F0 animals that is clearly adverse.

The TERA review considers newer studies not in the original OEL evaluations. This included the general toxicity findings in the 90 day studies performed by the NTP (see below), but surprisingly does not mention the specific investigations of sperm parameters and oestrous cycling.

The TERA review concluded: in summary, NOAEL/LOAEL and BMD and BMDL boundaries for experimental animal male and female nervous system, liver toxicity and reproductive and fetal effects are in the same range, suggesting that regardless of endpoint selected the critical effect levels will not vary greatly. These boundaries are generally similar to that seen in humans. This increases confidence the overall OEL value derived will provide adequate coverage for the range of potential endpoints.

Benchmark Dose Modelling for 1-BP

The results of Benchmark Dose (BMD) modelling are summarised in Table 9.

TERA's calculations were generally consistent with those reported by Stelljes and Wood (2004). For example, the BMDL of 263 ppm for F0 sperm motility from Stelljes and Wood (2004) was similar to that of *TERA* of 270 ppm. The BMD and BMDL computed by Stelljes and Wood (2004) for centrilobular vacuolization of 345 and 226 ppm are consistent with the results of using a multistage model of order 4. However, the simpler multistage model of order 2, which is commonly used in BMD modelling, was used to recalculate a BMDL because the 2nd order model has a slightly lower Akaike's Information Criterion (AIC) (38 versus 39) indicating a superior data fit ($p = 0.9$) with fewer parameters. Additional reproductive and developmental effects not considered by Stelljes and Wood *(*2004) were also evaluated by *TERA*. These endpoints include F0 prostate weights, F0 and F1 oestrous cycle lengths, number of F0 and F1 rats not having oestrous cycles, maternal body weight at gestation day 20, F1 litter viability index, F1 pup weight gain data from WIL Research (2001) as well as fetal weight data from Huntingdon Life Sciences (2001), and F1 and F2 live litter size. BMDs and BMDLs are shown for all of these endpoints except for the litter viability index, which exhibited no clear dose-response. The number of rats not having oestrous cycles was analyzed because the cycle length data analysis necessarily omitted these animals that were experiencing a more severe cycle delay that could not be quantified in terms of days.

A BMDL for fetal weight reduction was computed using the data collected by Huntingdon Life Sciences (2001). Following the NTP-CERHR expert panel on reproductive and developmental toxicity of 1-BP (NTP, 2002), one litter in the 100 ppm dose group was excluded because the average fetal weight was more than 3 standard deviations from the dose group mean. A BMDL of 310 ppm was estimated using a BMR of 1.0 control standard deviation from the control mean (Huntingdon Life Sciences, 2001). This estimate is similar to the BMDL of 305 ppm estimated by the NTP-CERHR expert panel (NTP, 2002), and they are higher than the BMDLs for reproductive endpoints; therefore, fetal weight reduction is unlikely to be the most sensitive effect. This conclusion is consistent with the findings of the NTP expert panel. To further evaluate whether developmental effects were the most sensitive basis for deriving an OEL, a BMDL for maternal weight change was also computed by *TERA* using the data collected by Huntingdon Life Sciences (2001). Note that the maternal body weight was calculated as maternal weight on GD20 subtracted by the litter weight at birth. A BMDL of 690 ppm was estimated using a BMR of 1.0 control standard deviation from the control mean. This estimate is higher than the BMDL of 310 ppm estimated by *TERA* for fetal weight reduction, indicating that the change of fetal weight might be due to direct fetal toxicity from exposure to the compound rather than only a secondary effect from maternal toxicity. On the other hand, no consistent dose-related effect on pup weights was observed in the two-generation study, decreasing concern related to the decreased fetal weight finding.

Furthermore, since the BMDL for decreased fetal weight was greater than for other reproductive parameters from the two generation study, this effect should be adequately addressed by an OEL that protects against reproductive effects. Finally, F1 and F2 live litter size was assessed. BMD and BMDL values of 280 and 188 for the F1 generation, were determined by Stelljes and Wood (2004). These values were confirmed by *TERA* where values of 280 and 190 are shown (Table 9). Stelljes and Wood (2004) and *TERA* also found similar BMD and BMDL values for the F2

generation, although these values were lower than that for the F1. This effect, decrease in live litter size, is of sufficient severity to warrant its choice as the critical effect. Although other effects might occur at the same, or slightly lower exposures, they are not as toxicologically significant. The choice of the BMD and BMDL values of 280 and 190 for the F1 generation, rather than lower values from the F2 generation reflects the desire to replicate the likely exposure in a worker population. Specifically, it is not anticipated that any human will have the exposure pattern of an F2 animal. In contrast, the occupational exposure pattern of an F1 animal might occur in humans.

Table 9: BMD and BMDL estimates from the TERA (2004) review

Endpoint	Stelljes and Wood		TERA				
	BMD (ppm)	BMDL (ppm)	BMR	BMD (ppm)	BMDL (ppm)	Model	Variance
Hindlimb strength	286	214	1 sd	290	210	Linear	Homogeneous
Minimal centrilobular vacuolization males	345	226	10%	290	200	Multistage-2	
Fetal body weight			1 sd	510	310	Poly-2	Non-homogeneous
F_0 sperm motility	343	263	1 sd	380	270	Linear	Homogeneous
F_1 sperm motility	261	156	1 sd	260	150	Power	Non-homogeneous
F_0 prostate weight			1 sd	740	190	Power	Homogeneous
F_0 Estrous Cycle Length			1 sd	290	210	Power	Non-homogeneous
F_1 Estrous Cycle Length			1 sd	810	400	Linear	Non-homogeneous
F_0 No Estrous Cycle Incidence			10%	670	480	Multistage-2	
F_1 No Estrous Cycle Incidence			10%	360	180	Quantal Linear	
Maternal GD20 body weight			1 sd	1000	690	Linear	Homogeneous
F_1 litter viability index			No dose-response				
F_1 pup weight gain PND 21 to 28			1 sd	240	180	Linear	Homogeneous
F_1 decreased live litter size	**280**	**188**	**1 sd**	**280**	**190**	**Linear**	**Non-homogeneous**
F_2 decreased live litter size	238	169	1 sd	240	170	Linear	Non-homogeneous

Most organizations that establish OEL's do not have documented approaches for addressing areas of uncertainty, rather a professional judgment approach is used (Haber and Maier, 2002). In order to evaluate potential OELs for 1-BP, TERA structure a discussion around the U.S. EPA's approach that describes five areas of uncertainty. However, in keeping with the existing OEL approach, TERA were not constrained to using EPA's defaults.

Interspecies Variability (UFA). This area accounts for the differences that occur between experimental animals and humans and is composed of sub-factors for toxicokinetics (how the body distributes and metabolizes the chemical) and toxicodynamics (how the body responds to the chemical). The use of these two considerations is standard practice in the context of environmental risk assessment (Dourson et al., 1996), and is gaining acceptance for assessing occupational risk (Naumann and Weidman, 1995). Ideally, a quantitative comparison of the toxicant concentrations (e.g. AUC or Cmax) in the target organ between animal species and humans would allow interspecies variability in toxicokinetics to be calculated. However, for 1-BP the information available is not adequate to allow such estimation. An alternative is to calculate the human equivalent concentrations (HEC) from the animal data based on the chemical's properties and physiological differences between the tested animal species and humans. This dosimetric adjustment generally provides a better estimate of the target organ doses following inhalation exposure than simply dividing the exposure assessment exposure by a default uncertainty factor of 10-fold. If the HEC is used, a toxicokinetic sub factor for interspecies variability is generally not needed because the expected toxicokinetic difference has been considered to some

extent in the HEC calculation. If no information is available on the quantitative differences in the organ response to the toxicant of interest between animals and humans, then a default value of 3 for this toxicodynamic difference is used in environmental assessments. If data are available to adequately describe this variability, then actual data may be used to replace this default value as well (IPCS, 2001).

For 1-BP, dosimetric adjustment to the HEC per EPA's methods (see for example ICF,1998) support using a factor of 1 to account for species differences in toxicokinetics. Toxicodynamic differences, however, also need to be addressed. There appears to be general consistency in effect levels among species for various toxic endpoints. For example, mild CNS effects in humans, as summarised by Rozman and Doull (2002), were observed in a range generally similar to the BMDL for hind limb grip strength in rats (see Table 2) and several of the clinical findings of Wang et al. (2003). Nevertheless, because there is residual concern about relative sensitivity to reproductive effects, and humans might be expected to be more sensitive to reproductive parameters (based on less excess reproductive capacity) a factor to account for toxicodynamic differences appears appropriate. For example, the *in vivo* dose-response information in humans is scant, and therefore comparative sensitivities of humans and animals are hard to define from the available data. Furthermore, *in vitro* bioassays are available for both human and animal cell cultures, including human hepatocytes, mouse lymphoma and bone marrow cells, but no data were obtained from experiments on reproductive system tissues. Moreover, since the critical effect is decreased live litter size, identifying a suite of relevant *in vitro* studies that could be used to compare animal and human responsive sensitivities would be difficult to obtain without a better understanding of the underlying mechanism of this effect. Since the available data do not provide sufficient information for a quantitative estimation of toxicodynamic variation, a default sub factor of 3 is appropriate for this area of uncertainty. Additional studies investigating relative sensitivities to reproductive effects of 1-BP would be helpful to address this area of uncertainty.

Intraspecies Variability (UFH). This factor accounts for the natural differences that occur among human subpopulations and for the fact that some individuals are more sensitive than the average population. This factor is also composed of two sub-factors – one to account for toxicokinetic differences and one to account for toxicodynamic differences.
If no information is available on human variability, then a default value of 10 is generally used in the context of environmental exposures to the general population. If adequate information is available on either toxicokinetic of toxicodynamics variability, then this information is used to develop estimates of variability from the data (IPCS, 2001; Meek et al., 2001). Unfortunately for 1-BP, no quantitative information regarding human variability in terms of toxicokinetics and toxicodynamics was identified, and therefore, data-derived estimates of human variability cannot be calculated. However, for worker populations the degree of variability in toxicokinetic or toxicodynamic variability is expected to be lower than for the general population. Since some degree of variability in response would be expected even among the worker population, a reduced factor of about 3-fold is generally judged to be reasonable. This is similar to what Rozman and Doull (2002) suggest.

Extrapolation from an Effect Level (UFL). A BMDL was used with the critical effect. Generally no additional factor is considered needed in these situations.

Extrapolation from Less than Lifetime to Lifetime Exposure (UFS). This factor is not generally used by groups that establish OELs (Haber and Maier, 2002). The database for1-BP lacks a completed chronic study,3 and therefore the likelihood that effects would progress with longer duration exposures needs further evaluation. However, the critical effect appears to be on a reproduction parameter and the critical study evaluated the period of interest. Moreover, workers have been exposed to 1-BP for more than short term exposures and their results are considered in all of these OEL estimations. Thus, it does not appear to us that a factor is needed for this area.
Adequacy of the Database (UFD). This factor is not overtly used by groups that establish OELs (Haber and Maier, 2002). However, OEL decisions routinely consider whether the overall body of literature determines that the most sensitive effects have been evaluated.
For 1-BP in particular, reproductive toxicity and possibly neurotoxicity and liver toxicity appear to be the most sensitive effects. A decrease in live litter size appears to be the critical effect. TERA do not see the need for a factor for this area of uncertainty.

TERA Determination of OEL

TERA conclude that the critical effect for the purpose of developing an OEL is decreased live litter size in the F1 generation, with a BMDL of 190 ppm.
Dividing this BMDL with an uncertainty factor of 10-fold, which is composed of 3-fold for extrapolation from an experimental animal study to humans for expected toxicodynamic differences and 3-fold for expected human variability in toxicokinetics and toxicodynamics within the worker population, results in an **OEL of 20 ppm.**
This OEL could be potentially lower if results in workers show definitive reproductive or other toxicity at levels lower than about 100 ppm. This OEL could be potentially higher if the expected reproductive response in experimental animals is shown to be similar to humans and at similar levels

5.3 NTP report (2011; 2013)

Reproductive and developmental toxicity

Humans

The NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) evaluated the potential for 1-bromopropane to produce adverse reproductive and developmental effects in humans (NTP, 2003). CERHR concluded that there was convincing evidence for reproductive and developmental toxicity in experimental animals. Evidence in humans was limited, but in the monograph, note was made of a new case that was not available to the expert panel indicating positive findings in women (altered menstruation) occupationally exposed to 1-bromopropane. The overall NTP conclusion was that “there is serious concern for reproductive and developmental effects of 1-bromopropane at the upper end of the human occupational exposure range (18 to 381 ppm).” “Serious concern” is the highest level of NTP

conclusion regarding the possibilities that human development and reproduction might be adversely affected.

Experimental Animals

1-Bromopropane was shown to cause developmental toxicity in rat pups whose dams were exposed during the period of *in utero* development (Huntingdon Life Sciences, 2001). Decreased fetal weights and increased incidences of skeletal variations were observed in pups of dams exposed to 1-bromopropane at 500 ppm or greater for 6 hours/day during gestation days 6 through 19.

A two-generation reproductive toxicity study (WIL Research Laboratories, 2001) showed exposure of rats to 250 ppm or greater altered numerous reproductive endpoints in both females and males. These included decreased sperm motility and percent normal sperm in males and decreased litter size, decreased numbers of implantation sites, increased ovarian follicular cysts, and increased oestrous cycle length in females. Another study in Wistar rats (Ichihara *et al*., 2000a) comparing reproductive toxicities of 1- and 2-bromopropane demonstrated that exposure to 400 or 800 ppm 1-bromo-propane for 12 weeks (8 hours/day, 7 days per week) inhibited sperm count and sperm motility; it was less toxic than 2-bromopropane. Yamada *et al*. (2003) reported that female Wistar rats exposed to 400 ppm 1-bromopropane 8 hours/day for 12 weeks had a significant increase in the number of irregular oestrous cycles with extended dioestrus. Histological examination of the ovary showed a significant reduction in the number of normal antral follicles, and a decrease in the number of normal growing follicles in the 400 ppm group.

In the studies performed as part of the NTP carcinogenicity investigation, the following findings were reported for reproductive end-points:

In the two week study in F344 rats and B6C3F1 mice exposed 6h/day, 5d/week for 16 or 17 days respectively, there were no adverse effects reported on reproductive organs or tissues at concentrations up to and including 2000 ppm.

In the 3 month segment, F344 rats (10/sex/group) were exposed to 0, 62.5, 125, 250, 500 or 1000 ppm for 6h/day, 5d/week for 14 weeks. In addition to organ weights and histopathological examination of reproductive organs and tissues an investigation of sperm parameters was performed after 14 weeks of exposure at 250, 500 and 1000 ppm. Sperm motility was decreased by 28% and sperm count was decreased by 37% in the 1000 ppm group, with statistically significant reductions in motility at the lower dose groups (Table 10). There was also a change in the pattern of oestrous cycling in all treatment groups (Table 11); although this was without any clear dose response. At 1000 ppm there was a significant reduction in body weights but this was not evident at 500 ppm and below. No adverse effects were reported on weights or pathology of reproductive organs and tissues. A similar pattern of effects was seen in B6C3F1 mice, with reductions in sperm counts and motility at 250 ppm and above and altered oestrous cycling at 500 ppm (Tables 12 & 13). In both sexes of mice, 500 ppm was a lethal concentration.

Table 10

Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Inhalation Study of 1-Bromopropane[a]

	Chamber Control	250 ppm	500 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	345 ± 7	350 ± 7	344 ± 10	305 ± 6**
L. Cauda epididymis	0.1865 ± 0.0039	0.1929 ± 0.0038	0.1740 ± 0.0106	0.1603 ± 0.0067*
L. Epididymis	0.4862 ± 0.0081	0.4994 ± 0.0042	0.4622 ± 0.0192	0.4423 ± 0.0083*
L. Testis	1.5128 ± 0.0313	1.4833 ± 0.0252	1.3867 ± 0.0839	1.4104 ± 0.0269
Spermatid measurement				
Spermatid heads (10^6/g testis)	123.6 ± 2.3	129.9 ± 6.1	112.9 ± 12.3	130.5 ± 3.8
Spermatid heads (10^6/testis)	171.8 ± 4.6	172.0 ± 9.2	150.8 ± 16.9	169.5 ± 6.0
Epididymal spermatozoal measurements				
Motility (%)	90.99 ± 0.83	84.87 ± 0.94**	81.84 ± 0.80**[b]	65.82 ± 3.17**
Sperm (10^6/g cauda epididymis)	699 ± 26	638 ± 34	576 ± 63	523 ± 30**
Sperm (10^6/cauda epididymis)	130.27 ± 5.19	122.90 ± 6.63	105.27 ± 12.01	82.39 ± 2.51**

Table 11

Estrous Cycle Characterization for Female Rats in the 3-Month Inhalation Study of 1-Bromopropane[a]

	Chamber Control	250 ppm	500 ppm	1,000 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	202 ± 3	209 ± 2	205 ± 3	190 ± 4
Proportion of regular cycling females[b]	9/10	10/10	10/10	10/10
Estrous cycle length (days)	4.9 ± 0.11[c]	5.0 ± 0.05	4.9 ± 0.07	5.0 ± 0.05
Estrous stages (% of cycle)[d]				
Diestrus	55.8	43.3	40.0	41.7
Proestrus	17.5	5.0	4.2	6.7
Estrus	20.8	32.5	36.7	33.3
Metestrus	5.0	19.2	19.2	18.3
Uncertain diagnoses	0.8	0.0	0.0	0.0

Table 12

Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Inhalation Study of 1-Bromopropane[a]

	Chamber Control	125 ppm	250 ppm	500 ppm
n	10	10	9	6
Weights (g)				
Necropsy body wt	39.6 ± 0.8	39.6 ± 0.6	37.9 ± 0.7	37.4 ± 1.4
L. Cauda epididymis	0.0164 ± 0.0008	0.0170 ± 0.0009	0.0179 ± 0.0005	0.0192 ± 0.0007
L. Epididymis	0.0538 ± 0.0010	0.0492 ± 0.0016*	0.0532 ± 0.0014	0.0535 ± 0.0012
L. Testis	0.1137 ± 0.0020	0.1063 ± 0.0085	0.1104 ± 0.0028	0.1103 ± 0.0010
Spermatid measurement				
Spermatid heads (10^6/g testis)	188.7 ± 8.1	164.9 ± 19.7	180.5 ± 3.7	199.3 ± 8.0
Spermatid heads (10^6/testis)	19.83 ± 0.89	17.57 ± 2.09	18.17 ± 0.48	20.11 ± 0.59
Epididymal spermatozoal measurements				
Motility (%)	91.57 ± 0.73	89.70 ± 0.66[b]	87.78 ± 1.10*	88.13 ± 0.56**
Sperm (10^6/g cauda epididymis)	1,237 ± 60	1,204 ± 66[b]	1,062 ± 70	890 ± 68**
Sperm (10^6/cauda epididymis)	19.93 ± 0.47	19.66 ± 0.69[b]	18.92 ± 0.97	16.93 ± 1.15

Table 13

Estrous Cycle Characterization for Female Mice in the 3-Month Inhalation Study of 1-Bromopropane[a]

	Chamber Control	125 ppm	250 ppm	500 ppm
Number weighed at necropsy	10	10	10	5
Necropsy body wt (g)	30.9 ± 0.7	31.9 ± 0.8	30.2 ± 0.9	31.3 ± 1.4
Proportion of regular cycling females[b]	9/10	8/10	6/10	5/5
Estrous cycle length (days)	4.2 ± 0.11	4.6 ± 0.50	4.2 ± 0.08	4.6 ± 0.10*
Estrous stages (% of cycle)[c]				
Diestrus	27.5	27.5	25.8	41.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	49.2	48.3	52.5	41.7
Metestrus	23.3	24.2	21.7	16.7

In the chronic toxicity phase in which F344 rats and B6C3F1 mice (50/sex/group) were exposed by inhalation to concentrations of up to 500 ppm (rats) or 250 ppm (mice) there were no reported changes in the patterns of non-neoplastic or neoplastic findings on a wide range of reproductive organs and tissues.

Contractor's conclusion on NTP data

The NOAEC for effects related to reproduction in the NTP studies in rats is <250 ppm based on a statistically significant reduction in sperm motility and alterations in oestrous cycling at 250 ppm, the lowest concentration tested. However, the magnitude of the effects in rats (<10%) indicates that the NOAEC is likely to be near to 250 ppm. In mice the NOAEC for findings related to reproduction is 125 ppm based on reduced sperm counts and motility at 250 ppm.

5.4 Other reviews

Reviews from Australia, UK and Finland contained no additional information relevant to DNEL setting.

6. Other studies of the reproductive toxicity of 1-bromopropane

Table 14: Reproductive toxicity studies with 1-bromopropane not covered by the major reviews

Endpoint	Study description	Repro findings	NOAEL (ppm)	LOAEL (ppm)	Reliability	Reference
Offspring survival and development	Rats (10/group). Inhalation 8h/day during pregnancy and lactation. 0, 100, 400 or 800 ppm. Cross fostering element in second segment at 800 ppm.	Reduced survival and lower body weight. Reduced survival seen in pups exposed only during lactation. Reductions in sperm count, motility and increased abnormal sperm	100 ppm	400 ppm	Reliability 2 Contains adequate description and detail of results.	Furahashi et al, 2006
Ovarian dysfunction	Wistar rats (10/group). Inhalation 8h/day for 7 weeks at 800 ppm (excessive toxicity) or 12 weeks at 200 or 400 ppm.	Irregular oestrous cycles and reduced antral follicles and normal follicles. Maturation of follicles inhibited	<200 ppm	200 ppm	Reliability 2 Limited investigations but adequate detail and description	Yamada et al, 2003.
Altered menstrual period	Workplace survey in Taiwan factory using 1-BP to clean golf clubs.	One of 5 female workers reported shortened menstrual period. Range of other signs and symptoms also recorded.			Air levels not provided. Biomarker levels not given by individuals.	Wang et al, 2014
Testicular gene expression	F344 male rats. Inhalation at 1000 ppm 8h single exposure. mRNA analysis of testes	Down regulation of testicular aromatase, calcium binding protein (S100a); creatine kinase;		1000 ppm	Reliability 4 Abstract contradictory regarding up or down regulation of genes. No	Li et al, 2010

	using 5082 genes on genital chip and RT PCR.	myelin and lymphocyte protein.			indication of level of general toxicity. Full paper not in English	
Brain mRNA and FOB in pups	Dams exposed by inhalation at 0, 400 or 700 ppm on days 1-20 of pregnancy. Duration? Cross-fostering segment at 700 ppm	Decreased Nav1 and GluR1. Hyperactivity in open field and lower memory scores.			Reliability 4 Abstract only; unclear in effects at 400 ppm.	Fueta et al, 2009
Sperm count and morphology. Liver enzyme (CYP) levels.	**Three strains of mice (6 males per group). Inhalation 0, 50, 110, 250 ppm 8h/day 28 days.**	**Reduced sperm count; reduced motility; increased sperm with abnormal heads. Indicates mice more sensitive than rats.**	**<50 ppm**	**50 ppm**	**Reliability 2** **Contains adequate detail and description of methodology and results.**	**Liu et al, 2009**
Sperm count and morphology, including reversibility phase.	Wistar rats (24 males/group). Inhalation at 0, 400 or 1000 ppm; 8h/day; 6 weeks.	At 1000 ppm- Reductions in testicular wt., Epididymal wt., sperm count and motility. Increased abnormal sperm and degenerated spermatogenic cells. At 400 ppm - increased retained spermatids and decreased sperm counts. Full recovery in 4 weeks at 400 ppm. Incomplete recovery at 14 weeks after 1000 ppm	<400 ppm (Effects at 400 relatively minor and reversible)	1000 ppm	Reliability 2 Adequate detail and description of methodology and results.	Banu et al, 2007

Oestrous cycling and ovulation	Female F344 rats. Inhalation 8h/d; 3 weeks; 0, 50, 200 or 1000 ppm	Increase in number of oestrous cycles of 6 days or more. No change in ovary wt. or ovulation pattern.	200 ppm	1000 ppm	Reliability 3 Limited information in some aspects.	Sekiguchi et al, 2002.

1. **Yamada et al (2003).** Groups of female Wistar rats (n=10 per group) were exposed to 1-BP at 0, 200, 400 or 800 ppm 8h/day for up to 12 weeks. Due to poor survival the 800 ppm group was terminated at week 7. Investigations of reproductive parameters included oestrous cycling, ovary weight and follicle counts, vagina and uterus weights and luteinising hormone (LH) and follicle stimulating hormone (FSH). At 800 ppm there were a wide range of effects including significant reductions in normal oestrous from the first exposures and reductions in antral and growing follicles at week 7. At 400 ppm oestrous was prolonged after week 7 and the number of antral and growing follicles was reduced (Table Y1). At 200 ppm the only finding was of reduced antral follicles (Table Y1). There were no clear effects on circulating LH or FSH levels. The pattern of findings in the ovary indicates that 1-BP inhibits maturation of the follicles. The LOAEL is the lowest concentration tested, of 200 ppm.

Table Y1 – Ovarian follicle counts in rats exposed to 1-BP

	0 ppm	200 ppm	400 ppm	800 ppm
Primordial	177	158	206	423
Growing	70	53	47*	30
Antral	30	13*	7**	4

*- $p<0.05$; ** - $p< 0.01$

2. **Furahashi et al (2006**). Groups of Wistar-Imamachi rats (n=10) were exposed to 1-BP in two separate investigations. In phase 1, pregnant dams were exposed to 0 (not removed from pups), 0, 100, 400 or 800 ppm for 8h/d (2 x4h) on gestation days (GD) 0-20 and post-natal days (PND) 1-20. There was no exposure on GD 20-22 when the rats gave birth. Litters were reduced to 4/sex on PND 4, wherever feasible. Offspring were sacrificed on PNDs 21; 33 (pre-pubertal); 50 (mature male) and 63 (mature female). In phase 2 dams were exposed to 0 or 800 ppm in a cross fostering design to determine if the effects were associated with exposures pre- or post-birth. Investigations of pups included sperm count, motility and morphology; sexual organ / tissue weights and pathology; litter size and survival. Survival of pups was reduced at 800 and 400 ppm; with poor maternal care noted at 800 ppm. Sperm levels in the cauda epididymis and seminiferous tubules were reduced on PND 50 at 400 and 800 ppm. In the cross fostering phase, reduced growth and survival was evident in pups born to exposed dams fostered to unexposed dams and in pups born to unexposed dams and fostered to exposed dams, indicating that both gestational and lactational exposures of dams to 1-BP can affect offspring. The most significant effect on growth was seen in the latter group. The NOAEL is 100 ppm.

3. **Sekiguchi et al (2002).** Groups of 12 week old, female F344 rats (n=7 or 8) were exposed to 1-BP at 0, 50, 200 or 1000 ppm, 8h/day for 21 – 24 days. Oestrous cycling was monitored; ovary and uterus weights were taken; ova in the oviduct were counted and the mass of cumulus cells was determined. At 1000 ppm there was an increase in the number of rats with cycles of greater than 6 days in duration (7/8 versus 3/7 in controls) and reductions in ova in the oviducts (7.0 ± 1.9 versus 8.8 ± 1.2 in controls). There were no effects on ovary or uterus weights. There were no effects on any measured parameters at 200 ppm, the NOAEL.

4. Banu et al (2007). Groups of 24 male Wistar rats were exposed to 1-BP, 8h/day for 6 weeks followed by a recovery period of 4 or 14 weeks. Investigations included sperm count, motility and morphology, reproductive organ weights. At 1000 ppm there were clear effects after 6 weeks, on testicular and epidymal weights, sperm counts and motility, abnormal sperm and degeneration of spermatogenic cells. During the recovery phase, there was a reversal of the prostate and seminal vesicle weight reductions at week 14, but other changes only showed partial reversibility. At 400 ppm the only effects were an increase in elongated spermatids retained in the seminiferous tubules (1.3 ± 0.8 versus 0.2 ± 0.2 per tubule) and the Sertoli cell nuclei (18.6 ± 23 versus 2.5 ± 2.7 per 100 nuclei) and reduced sperm counts (382 ± 60 versus 494 ± 89 $x10^6$/g) , which reversed after 4 weeks . This study did not show a NOAEL, but demonstrated that the effects of 1-BP on male reproduction could reverse.

5. **Fueta et al (2009).** In a brief abstract, this group reported that exposure of pregnant rats to 1-BP at 700 ppm affected neuronal function in pups. It is unclear if effects were also produced at 400 ppm, the only other concentration cited.

6. **Wang et al (2014).** In an investigation focussed on neurotoxicity, one of 5 females working a factory using 1-BP had reported a shortened menstrual period. No details of exposures are provided.

7. **Ichihara et al (2000).** Groups of male Wistar rats were exposed to 1-BP for 8h/day over 12 weeks at 0, 200, 400, 800 ppm. Investigations included reproductive organ / tissue weights and pathology; pathogenicity of spermatogenic cells; number of retained spermatids; sperm count, motility and morphology ; plasma testosterone, LH and FSH. At 800 ppm many measured parameters were affected, including reduced body weight (12%), degeneration of pachytene spermatocytes, reduced plasma testosterone; reduced seminal vesicle, epididymides and prostate weights and reduced sperm parameters (Table 15). At 400 ppm there were reductions in epididymal and seminal vesicle weights and sperm parameters (Table 15). At 200 ppm the only finding was a reduction in seminal vesicle weight (Table 15). The contractor considers that the decreased seminal vesicle weight at 200 ppm is not adverse in the absence of other findings and considers 200 ppm to be the NOAEL in this study.

Table 15: Findings in male rats exposed to 1-BP for 12 weeks (Ichihara et al, 2000)

	0 ppm	200 ppm	400 ppm	800 ppm
Seminal vesicle wt. (g)	1.9	1.4**	1.3**	1.0**
Epididymidal wt. (g)	1.1	1.1	1.0**	0.8**
Prostate wt. (g)	0.6	0.6	0.6	0.4**
Sperm (x 10^6/g)	792	772	588*	240*
Motile sperm (%)	83	81	67**	25**
Tailless sperm (%)	4	6	17**	36**
Sperm, abnormal head (%, mainly banana)	1	2	3	99**
Retained elongated	0.5	0.8	1.3**	4.8**

spermatid / tubule				
Retained elongated spermatid / 100 Sertoli nuclei	1.7	3.3	5.2**	20**

* p<0.05; ** p<0.01

8. **Liu et al (2009**). Groups of male mice of three different strains (C57BL/6J; DBA/2J and BALB/cA) were exposed to 1-BP to investigate if there was any link between genotype, hepatic enzyme activities and the reproductive toxicity of 1-BP. Exposures were at 0, 50, 110 or 250 ppm, 8h/day for 4 weeks. Investigations included weights of testes, seminal vesicles and liver; sperm count, morphology and motility. BALB/cA mice were the most sensitive to general toxicity, with 2 mice dying; clear hepatotoxicity and a 15% lower body weight at 250 ppm. One C57 mouse died and both C57 and DBA mice had necrotic areas and degeneration of the liver and a 5% lower body weight at 250 ppm. The hepatotoxicity was concentration related, with significant increases in necrotic areas and degeneration reported at all concentrations. At 250 ppm, C57 mice had reductions in testes and seminal vesicle weights; neither of the other strains were affected. Sperm parameters were affected in all strains at all concentrations (Table 16). There was no clear difference between the strains in susceptibility to the effects on sperm parameters. The effects of 1-BP are potentially cumulative in that in addition to a reduction in the number of sperm, those that are present are less motile and are more likely to be abnormal. This study indicates that mice are more susceptible to the effects on 1-BP on sperm parameters than rats. This study does not demonstrate a NOAEL for reproductive parameters, with adverse effects on sperm being seen at 50 ppm, the lowest concentration tested.

Table 16: Effects on sperm parameters in mice exposed to 1-BP (Liu et al, 2009)

	0 ppm	50 ppm	110 ppm	250 ppm
Sperm ($x10^7$/g)				
C57	73 ± 42	46 ± 30*	25 ± 19*	17*
DBA	43 ± 20	22 ± 15*	17 ± 8*	22*
BALB	58 ± 26	37 ± 11*	24 ± 3*	13*
Motile sperm (%)				
C57	86 ± 3	72 ± 13	69 ± 11*	56*
DBA	88 ± 5	71 ± 12*	62* ± 16	58*
BALB	87 ± 2	63 ± 9*	57 ± 8*	48*
Abnormal sperm (%)				
C57	11 ± 1	29 ± 9*	51 ± 5*	61*
DBA	8 ± 2	21 ± 10	23 ± 8*	31
BALB	20 ± 7	37 ± 7*	43 ± 6*	54*

* p<0.05

7. Comparison of Liu et al study and the similar NTP 13 week mouse study

Due to the differences between the results from the NTP mouse data and the Liu et al study; with Liu et al reporting findings at exposure levels producing no effects on the same end-point in the NTP study a comparison of the methodology has been performed. The details are summarised in Tables 17 & 18 below.

Atmosphere levels of 1-BP in the Liu et al study were confirmed using gas chromatography and adjusted in real time to comply with nominal concentrations. There is no evidence to indicate the reported exposure levels are unreliable.

Although there are differences in the range of end-points investigated in the two studies, the most sensitive ones of sperm count and motility are common to both studies. There were differences in methodology, which might have impacted on the absolute values in controls but sperm counts and motility tests are not considered to be highly sophisticated investigations; reproducibility within the studies was good based on the magnitude of the standard deviations.

Differences in exposure duration per day (8h versus 6 h) and number of exposures per week (7 versus 5) are considered not be sufficient to produce an apparent 10 fold difference in response. The longer duration of exposures in the NTP study (13 weeks versus 4 weeks) would be expected to enhance any effects; whereas the opposite was seen.

Strain differences are not considered to be critical as Liu et al obtained consistent results across 3 strains from different pedigrees.

Body weight changes and general toxicity appear to be broadly similar in both studies.

One parameter that might be relevant is the age of the mice at study termination. The NTP study initiated exposure when the mice were younger which would not be expected to reduce the magnitude of any effects. The mice in the NTP study were 19 – 20 weeks old when the terminal examinations were performed but the Liu et al mice were 14 weeks old, it is possible that the younger mice were more sensitive. This age difference and lower maturity of the Liu et al mice might also be related to the lower control sperm counts (1.8 – 2.7 fold) seen in these mice. However, male mice are reported to reach sexual maturity by approximately week 8, therefore both sets of mice would be sexually mature at study termination, and given the duration of the sperm cycle it is considered unlikely that the marked effects seen at 50 ppm by Liu et al would have reversed entirely within 5 or 6 weeks in the NTP study during which exposure continued. Related to this age difference, the Liu et al mice were approximately 10g lighter than the NTP mice.

Liu and co-workers have published a number of related papers over several years.

Contractor's conclusion on the Liu et al versus NTP data in mice

The contractor can see no obvious reason for the difference between the NTP and Liu et al protocols that would explain the markedly different responses seen.
There is no reason to discount the Liu et al results.

Table 17: Comparison of methodology used in NTP mouse study with that of Liu et al.

	Strain	Age at start and finish of exposure (wk.)	Group size	Exposure time (h/day)	Exposure duration	Exposure conc (ppm)	Weight at termination (g)		Reproductive system examinations (males)	Reproductive system examination (females)	NOAEC
							m	f			
NTP	B6C3F1	5 – 6 19 – 20	10/sex	6h/day	5d/wk. 14 wks.	0 62.5 125 250 500	37.4 To 39.6	33.0 To 30.2	Sperm motility Sperm count Testis wt. Epididymal wt. Sperm in cauda epididymis Homogenisation resistant spermatid nuclei	Vaginal cytology Oestrous cycle	125 ppm
Liu	C57BL/6J DBA/2J BALB/cA	10 14 weeks	6 Males	8h/day	7d/wk. 28 days	0 50 110 250	DBA 27.3 – 28.1 C57 28.1 – 29.7 BALB 29.5 – 33.5		Sperm motility Sperm count Sperm morphology (1000/mouse) Testis wt. Epididymal wt. Seminal vesicle wt. Sperm in cauda epididymis	Not applicable	<50 ppm

Table 18: Comparison of results in NTP mouse study with that of Liu et al.

		mortality	Body wt.	Testis wt.	Seminal vesicle wt.	Cauda epididymis wt.	Sperm count (Epididymal)		Motile sperm		Abnormal sperm
								Control (10^8/g)		Control (%)	
NTP	B6C3F1	1m at 250 ppm 4m + 4f at 500 ppm	5% less at 500 ppm (m); f- OK	No effect	Not performed	↓ 5% at 500 ppm	↓ 3% at 125 ↓ 14% at 250 ↓ 28% at 500 ppm	12	↓ 4.3% at 250 ↓ 4% at 500 ppm	92	Not performed
Liu	C57BL/6J	1 at 250 ppm	No effect	↓12% at 250 ppm	↓18% at 250 ppm	No effect	↓≥ 37% at ≥ 50 ppm*	7.3	↓≥ 15% at ≥ 50 ppm*	86	
	DBA/2J	2 at 250 ppm	No effect	No effect	No effect	No effect	↓≥ 48% at ≥ 50 ppm*	4.3	↓≥ 19% at ≥ 50 ppm*	88	
	BALB/cA	0	7 to 12% higher in test groups	No effect	No effect	No effect	↓≥ 37% at ≥ 50 ppm*	5.9	↓≥ 27% at ≥ 50 ppm*	87	

* dose response observed

8. Overview of the testicular toxicity and sperm effects produced by 1-BP exposures

Testicular and sperm effects are sensitive endpoints, which are consistently affected in both rats and mice exposed to 1-BP. The level of investigation of effects on individual elements of female reproduction is less extensive than that for males. Table 19 summarises the testicular / sperm findings in animals exposed to 1-BP. There is evidence of species differences, with mice being more susceptible than rats exposed to the same concentration of 1-BP. However, due to the approximately 2 fold greater respiratory volume of mice on a body weight basis the species variation is generally minimal when considered in terms of systemic dose on a kg bw/day basis. The study reporting sperm effects at the lowest exposures is that of Liu et al (discussed in section 7 above). Some variations between studies are associated with the different investigations performed, but sperm count, motility and appearance are common to most studies and are end-points particularly sensitive to 1-BP exposures.

In rats, the $BMDL_{10}$ (TERA, 2004) for reduced numbers of motile sperm is fractionally below the BMDLs for reduced litter sizes. The total loss of any viable offspring in rats exposed to 750 ppm 1-BP is of interest as the magnitude of the reductions in sperm number and motility at this exposure level were relatively low (21% and 39% respectively), indicating reduced sperm parameters do not appear to be the sole cause of the reduced reproductive performance in rats exposed to 1-BP. In mice there is no reproduction study to provide information on the magnitude of change to sperm parameters correlating with impaired reproductive outcome.

ECHA has considered previously the human relevance of changes in sperm parameters in rodents (e.g. for phthalates):

- Semen quality is determined by several parameters (e.g., sperm counts, sperm motility, sperm concentration, ejaculation volume and other parameters). Effects on individual sperm parameters in experimental animals may provide important information about effects on semen quality because in humans even a slight reduction in sperm quality/count may be critical for fertility (ECHA guidance Chapter R.7a). Rodents are different than humans with regard to semen quality: as much as 90% reduction in sperm count may be needed to affect the fertility index in rodents. Human males on the contrary have highly variable sperm counts, generally lower than in rodents, and many men have sperm concentrations near or below WHO reference values for fertility (OECD 2008). Thus, in a case of human subfertility even a small change in sperm counts or sperm motility may lead to infertility. For this reason, a statistically significant change in sperm count in a rodent study is considered to be indicative of a potential effect on fertility in humans (OECD 2008).

The applicability of animal models to human male reproduction / fertility is uncertain. Although much work has been done to investigate the effects of chemicals, such as anti-cancer drugs, on aspects of male reproduction in animals, there is limited equivalent investigation in exposed humans. There are clear differences in sperm parameters in humans versus rodent models, other than sperm count (e.g. in humans up to 80% of sperm are reported as having an abnormal appearance versus <20% in rats and mice). However sperm parameters such as motility are considered to be an

early and sensitive marker for the assessment of male reproductive toxicity of chemicals (Hales & Robaire, 2012).

The contractor therefore proposes to base the reproductive DNELs for 1-BP on sperm effects.

Table 19: Testicular / sperm effects of 1-BP – ordered by exposure concentration

Dose level (ppm)	Exposure duration	Effect	Magnitude of change	Species / strain	Study reference
50 ppm	8h/day; 4 weeks	↓ sperm count ↓ motile sperm ↑ abnormal sperm	37 – 48% 15 – 27% 2 – 3 fold	Mouse C57; DBA; BALB	Liu et al, 2009
100 ppm	8h/day; 7 weeks as pups	NOAEL	-	Rats, Wistar – Imamachi - pups	Furahashi et al, 2006
110 ppm	8h/day; 4 weeks	↓ sperm count ↓ motile sperm ↑ abnormal sperm	60 % 20 – 35% 2 – 5 fold	Mouse C57; DBA; BALB	Liu et al, 2009
125 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm	3% NOAEC	Mouse, B6C3F1	NTP, 2011, 2013
150 ppm	6h/day; 7d/wk.; 10 weeks	↓ motile sperm (F_1)	BMDL	Rat CD	TERA, 2004 (based on WIL study)
170 ppm	6h/day; 7d/wk.; 10 weeks	↓ Live litter size (F_2)	BMDL	Rat CD	TERA, 2004 (based on WIL study)
190 ppm	6h/day; 7d/wk.; 10 weeks	↓ Live litter size (F_1)	BMDL	Rat CD	TERA, 2004 (based on WIL study)
200 ppm	8h/day; 12 weeks	↓seminal vesicle wt.	26% NOAEC	Rats, Wistar	Ichihara et al, 2000
250 ppm	8h/day; 4 weeks	↓ sperm count ↓ motile sperm ↑ abnormal sperm	50 - 80 % 35 - 45% 2.7 – 5.5 fold	Mouse C57; DBA; BALB	Liu et al, 2009
250 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm ↓ sperm count	4.3% 14%	Mouse, B6C3F1	NTP, 2011, 2013
250 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm ↓ sperm count	7% 9%	Rat, F344	NTP, 2011, 2013
250 ppm	6h/day; 7d/wk.; 10 weeks	↓ prostate wt.	F_0 NOAEC	Rat SD	WIL, 2001
270 ppm	6h/day; 7d/wk.; 10 weeks	↓ motile sperm (F_0)	BMDL	Rat CD	TERA, 2004 (based on WIL study)
400 ppm	8h/day; 12 weeks	↓seminal vesicle wt.	32% 26%	Rats, Wistar	Ichihara et al, 2000

		↓ sperm count ↓ motile sperm ↑ tailless sperm ↑ retained spermatids	19% 4 fold 3 fold		
400 ppm	8h/day; 7 weeks as pups	No sperm in cauda epididymidis at day 50 Reduced cells in seminiferous tubules	0/6 vs 6/8 in controls Not quantified	Rats, Wistar – Imamachi - pups	Furahashi et al, 2006
400 ppm	8h/day; 6weeks	↓ sperm count ↑ retained spermatids Reversible after 4 weeks recovery	22% 6 fold	Rats, Wistar	Banu et al, 2007
500 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm ↓ sperm count Deaths	4% 28%	Mouse, B6C3F1	NTP, 2011, 2013
500 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm ↓ sperm count	15% 18%	Rat, F344	NTP, 2011, 2013
500 ppm	6h/day; 7d/wk.; 10 weeks	↓ motile sperm ↓litter size ↓ normal sperm ↓cauda epididymis wt. ↓ prostate wt.	13% (F_0) 17% (F_1) 42% (F_0) 40% (F_1) 2% (F_0) 4% (F_1) F_0 & F_1 F_0	Rat SD	WIL, 2001
750 ppm	6h/day; 7d/wk.; 10 weeks (F_0)	↓ motile sperm ↓sperm count ↓normal sperm ↓epididymal, prostate & seminal vesicle wt. No F1 pups produced.	39% 21% 9%	Rat SD	WIL, 2001
800 ppm	8h/day; 12 weeks	↓seminal vesicle wt. ↓ sperm count ↓ motile sperm	47% 70% 70% 9 fold	Rats, Wistar	Ichihara et al, 2000

		↑ tailless sperm ↑ retained spermatids	10 fold		
1000 ppm	6h/day; 5d/wk.; 13 weeks	↓ motile sperm ↓ sperm count ↓cauda epi wt. ↓epididymis wt. ↓ Testis wt.	28% 25% 14% 9% 7%	Rat, F344	NTP, 2011, 2013
1000 ppm	8h/day; 6weeks + 14 weeks recovery	↓ sperm count ↓ sperm motility ↓ sperm normal head Limited reversibility	90% (wk6), 98% (wk20) 23% (wk6), 40% (wk20) 99% (wk6), 74% (wk20)	Rats, Wistar	Banu et al, 2007

9. Contractor's summary of the reproductive toxicity of 1-BP

Inhalation exposures of 1-bromopropane have produced adverse effects on fertility and pup survival in studies of reproductive toxicity in rats and developmental toxicity in fetuses of rats exposed by inhalation. Individual elements of the reproductive cycle have been investigated in both rats and mice and a range of effects have been observed.

Evaluation of specific aspects of end-points related to reproductive performance has consistently shown effects on both males (particularly sperm counts, motility and abnormal sperm) and females (altered oestrous cycling and reduced maturation of ovarian follicles). The effects were seen in both rats and mice. Investigations in males have been more extensive than in females. The most sensitive point of departure identified in the available literature is a reduction in sperm counts and an increase in abnormal sperm in three mouse strains at 50 ppm (Liu et al, 2009). The results of the Liu et al study are seen at a lower concentration than similar effects in a different strain of mice used by the NTP, where a NOAEL of 125 ppm was identified for reductions in sperm count and motility (sperm abnormalities were not investigated). A comparison of these two mouse studies failed to identify any obvious reason for the differences in the results.

The minimum duration of exposure required to produce effects on reproductive parameters is impossible to determine with any degree of certainty. Clear effects on sperm parameters are seen in mice after 4 weeks of exposure. However, shorter duration studies (e.g. NTP 14 day) did not include any investigations of reproductive organs. A report of an abstract for an OECD 422 screening study indicates effects on reproductive parameters were observed. Such studies typically involve a two week exposure prior to mating.

In terms of studies that included an investigation of actual reproductive outcome, there is only one extensive study, in rats. BMDLs for reduced litter size were calculated to be 190 ppm for the first generation and 170 ppm for the second generation. The lowest BMDL of 150 ppm was for reduced numbers of motile sperm in the F_1 parental animals.

In a developmental toxicity study, in rats exposed by inhalation on days 6 -19 of gestation, a range of developmental effects were observed (reduced fetal weight, reduced ossification of skull bones, increased number of animals with bent / wavy ribs). There were no increases in the number of malformations. The LOAEC was 503 ppm and the NOAEC was 103 ppm; with a lowest $BMDL_{10}$ of 305 ppm for reduced fetal weight.

There is only one study which investigated the reversibility of the effects of 1-BP on sperm parameters. Banu et al, reported that after exposures to 400 ppm for 6 weeks followed by a 4 week recovery phase, the measures of sperm quality had returned to control levels. In the same study, there was no clear evidence of reversibility, even after a 14 week recovery period, in the rats exposed to 1000 ppm, a concentration producing an approximate 30% reduction in body weight and a 90% reduction in sperm count. Based on the results at 400 ppm, the contractor concludes that the effects of 1-BP on sperm in the absence of other marked toxicity are likely to be reversible.

There are isolated reports of humans exposed to 1-BP experiencing reproductive problems, but the database is limited and no firm conclusions can be drawn. Reports of historic exposure levels in industries using 1-BP have been included values in the ranges producing effects in rodents. No clear evidence of adverse effects has been identified, but no

systematic investigations of reproductive function appear to have been performed in the exposed populations. Overall, there is no reason to believe that the findings in rats and mice are not relevant to humans.

The available database on 1-BP is considered adequate as a basis for deriving reference DNELs for reproductive toxicity.

10. Critical aspects for the derivation of DNELs for reproductive toxicity of 1-bromopropane

Although there are indications of some differences in the metabolism of 1-bromopropane in humans and rodents, there is insufficient information to conclude that these differences indicate a reduced sensitivity in humans.

Data on the absorption of 1-bromopropane by are inadequate to move away from the default values for oral and inhalation exposures in ECHA guidance document R8. For dermal exposures an absorption of 10% is proposed based on *in vitro* investigations with human skin; this is considered conservative as any non-occluded dermal exposures would evaporate rapidly. The evaporation flux under laboratory conditions is approximately 500 fold greater than the dermal absorption flux and this is confirmed in the dermal absorption investigations.

As the contractor has been unable to obtain full study reports of the key studies of reproductive and developmental toxicity (WIL, Ichihara and HLS) no attempts to reanalyse the data have been made. BMDLs and NOAEC have been taken from the main review documents (TERA, 2004; CERHR, 2003).

Studies subsequent to 2004 investigating reproductive and developmental end-points have reported results broadly consistent with the studies used in the TERA review. However, the contractor has noted that some additional studies indicate sperm abnormalities, motility and count are particular sensitive end-points. The lowest LOAEC of 50 ppm (lowest concentration tested) is reported in the study of Liu et al (2009) in three strains of mice. This is below the NOAEL of 125 ppm in an NTP study for similar effects in a different strain of mouse. The report by Liu et al (2009) is considered to be reliable and there is no obvious reason to discount the results. A comparison of the Liu et al and NTP studies identified some differences in methodology but none that would explain the approximate 10 fold difference in response (taking account of dose level and magnitude of effects).

A comparison between the database on rats and mice shows that with the exception of the Liu et al data, there is little difference between the two species in terms of systemic doses. Apparent differences in terms of atmospheric concentrations of 1-BP, where the mouse appears approximately 2 fold more sensitive, can be attributed to the greater respiratory volume of mice on a body weight basis, approximately 80L/kg /h versus 40 L/kg/h in rats (Table R8-17 of the ECHA guidance document R8). The data base on rats is more extensive in that it includes full 2 generation reproduction study. The investigations in mice are primarily focussed on one or a few isolated elements of the overall reproductive process, however the findings are consistent with the effects seen in rats, including in the 2-generation study, both qualitatively and quantitatively. The Liu et al results whilst internally consistent and with no clear shortcomings are clearly different from results of a very similar study performed by the US National Toxicology Program, which measured critical parameters found to be the most sensitive by Liu et al.

Taking account of the greater sensitivity of humans, than rodents, to poor reproductive performance due to reduced semen quality / sperm counts it is considered appropriate to base the reproductive DNELs for 1-BP on reductions in sperm counts.

The most sensitive and appropriate end-point is the effects on sperm (reductions in sperm count and % motile sperm and increases in abnormal sperm) in mice exposed to 1-BP at 50 ppm (250 mg/m^3) for 4 weeks in the study of Liu et al (2009). In discussions at RAC Meetings it was concluded that even though the rat database for 1-BP was more extensive than that for mice, the data from the mouse study by Liu et al (2009) were the most appropriate as the basis for the derivation of reference, reproductive DNELs for 1-BP.

Assessment factors

The critical study for the derivation of reproductive DNELs relies on less than lifetime exposures, and are based on a LOAEC rather than a NOAEC. Consideration therefore needs to be given to the appropriate assessment factors to apply.

Duration of study

- In mice a complete spermatogenic cycle is reported to take approximately 8 weeks. In humans a complete cycle is slightly longer, reported to be approximately 11 weeks. Therefore a defined dosing duration in mice will cover more of the cycle than the equivalent duration in humans.
- 1-BP produced marked effects on sperm after 4 weeks dosing, less than a full cycle and the shortest time point investigated. There is evidence of reversibility in the only study that investigated it. These indicate the effects are on later stage sperm and that germ cells if affected, are not damaged irreparably.
- From the limited comparative data available, there is no clear indication the effects on sperm increase with increased exposure duration; the study with the lowest LOAEC having one of the shortest exposure durations.
- There were no reports of alterations in testicular histopathology (including appearance of spermatogenic tissue) in the lifetime carcinogenicity studies, but the sensitivity of the investigations (histopathology rather than specific sperm counts and observations) is uncertain.
- Kinetic data indicate 1-BP does not bioaccumulate. Therefore, provided the critical window for effects is included in the dosing period it is expected that further dosing will not produce any enhancement of effects.
- In addition, the OECD 421 reproduction screening test guideline states that 4 weeks of dosing (2 pre-mating and 2 post-mating) is sufficient to enable detection of the majority of effects on male fertility and spermatogenesis.
- The mouse 4 week study is within the OECD 421 guideline duration and therefore no additional factor for duration is considered necessary for deriving chronic DNELs from this study.

The contractor considers that the default additional assessment factors for deriving chronic DNELs from sub-acute (x6) and sub-chronic (x2) studies are not necessary for 1-BP as the critical window for effects on the most sensitive reproductive endpoint (sperm count, abnormalities and motility) has been covered adequately.

No additional factor is proposed for less than lifetime study duration.

LOAEC to NOAEC

At the lowest tested concentration in the Liu et al mouse study statistically significant increases in numbers of abnormal sperm were seen together with decreases in sperm count and in sperm motility.

- The exposures were for 8h/day every day for 4 weeks, giving limited opportunity for recovery.
- Evidence of recovery was seen in the study by Banu et al (2007).
- There is a diminution of effects with concentration of 1-BP in the Liu et al study. Some of the effects are no longer statistically significant at 50 ppm (e.g. number of motile sperm in C57 strain mice; number of abnormal sperm in DBA mice). Standard deviations are relatively large for a number of parameters (particularly sperm count) and there is overlap between control and 50 ppm results (see Table 16) .The indications are that 50 ppm is relatively close to a NOAEC.

For these reasons the contractor considers that the default assessment factor of 3 applied for deriving a DNEL from a LOAEC rather than a NOAEC can be used with no change in the case of the Liu et al data.

Default factor of 3 should be applied to move from the LOAEC to a NOAEC.

11. Derivation of reference DNELs for reproductive toxicity of 1-Bromopropane

These calculations are based on the defaults and general principles set out in ECHA guidance document R8.

The contractor proposes that the sensitive end-point of sperm effects is used to derive reference reproductive DNELs for 1-BP based on the findings in mice described in the published paper by Liu et al (2009), with a LOAEC of 50 ppm.

For workers, reference DNELs have been derived for inhalation and dermal routes. For the general population all routes (oral, inhalation and dermal) are considered potentially relevant.

Completeness of the database.

No developmental study in non-rodents (rabbits) is available and no full reproductive toxicity study is available in mice. However, the contractor considers that given the consistency of effects on rat reproduction and development, and mouse reproductive parameters there is no need to apply an additional factor for an incomplete database in the derivation of reproductive DNELs for 1-BP.

Threshold based approach

Inconsistent results for genotoxicity *in vitro* and negative results *in vivo* in a micronucleus assay in mice and in a dominant lethal test in rats indicate genotoxicity is unlikely to be a mode of action behind the reduced fertility of produced by 1-bromopropane, therefore a threshold mode of action can be assumed and an appropriate threshold based derivation can be used for the reproductive DNELs.

Reversibility

There is evidence of reversibility (Banu et al, 2007).

Absorption

No robust data on the comparative extent of inhalation and oral or dermal absorption are available. A value of 10% is proposed for dermal absorption, based on human *in vitro* data (Frasch et al, 2011) and the extensive evaporative flux of 1-BP. A default value of 100% is applied for inhalation absorption and oral absorption.

DNEL derivation

The starting point for all the proposed DNELs is the LOAEC from the Liu et al mouse study of **50 ppm = 250 mg/m^3**

The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction. 1-BP is rapidly metabolised and excreted and no extra factor for duration of exposure is considered necessary.

Workers

Inhalation

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

Inhalation absorption is considered to be the same (100%) in mice and humans. No correction is required for absorption.

= 250 mg/m^3

The mouse exposure period was 8 hours and no correction to 8 hour worker exposures is applicable.

= 250 mg/m^3

The ECHA guidance includes a factor for the increased ventilation rate of workers of 0.67.

250 x 0.67 = 167 mg/m^3

The mouse exposure was for 7 days per week, the worker exposure is assumed to be 5 days per week. A correction of 7/5 is applicable.

167 x 7/5 = 234 mg/m^3

Application of assessment factors.

The ECHA guidance proposes a default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

234 / 3 = 78 mg/m^3

For the inhalation route, the allometric factor of 7 is considered to be addressed by the increased ventilation rote of the mouse. The dynamic factor of 2.5 still applies.

78 / 2.5 = 31 mg/m^3

The application of a 5 fold intraspecies factor is recommended in the ECHA guidance for workers.

31 / 5 = 6.2 mg/m^3

Reference DNEL $_{\text{(worker, inhalation, reproduction)}}$ = 6.2 mg/m^3 (or 1.25 ppm)

Dermal

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and dermal absorption are available, but a value of 10% is proposed for dermal absorption, based on human *in vitro* data and the extensive evaporative flux of 1-BP. A default value of 100% is applied for inhalation absorption. This gives a dermal topical dose.

168 x 10 = 1680 mg/kg bw/d

The mouse exposure was for 7 days per week. Worker exposures are considered to be 5 days per week. A correction of 7 / 5 is applicable.

1680 x 7 / 5 = 2352mg/kg bw

Application of assessment factors.

For the dermal route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor = 17.5

2352 / 17.5 = 134 mg/kg bw

The application of a 5 fold intraspecies factor is recommended in the ECHA guidance for workers.

134/ 5 = 27 mg/kg bw/d

No additional factors are required for study duration or the use of a BMDL.

The ECHA guidance proposes a default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

27 / 3 = 9.0 mg/kg bw/d

Reference DNEL $_{\text{(worker, dermal, reproduction)}}$ = 9.0 mg/kg bw/d

Oral

Not required for workers.

Endpoint	Inhalation (8-hr)	Dermal
Reproductive toxicity	**6.2 mg/m^3**	**9.0 mg/kg bw**

General population

Inhalation

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

Inhalation absorption is considered to be the same (100%) in mice and humans. No correction is required for absorption.

= 250 mg/m^3

The mouse exposure period was 8 hours and a correction to 24 hour population exposures is applicable.

250 / 3 = 83 mg/m^3

The mouse exposure was for 7 days per week, the population exposure is assumed to be 7 days per week. No correction is applicable.

= 83 mg/m^3

Application of assessment factors.

The ECHA guidance proposes a default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

83 / 3 = 27.6 mg/m^3

For the inhalation route, the allometric factor of 7 is considered to be addressed by the increased ventilation rote of the mouse. The dynamic factor of 2.5 still applies.

27.6 / 2.5 = 11.0 mg/m^3

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for workers.

11.0 / 10 = 1.1 mg/m^3

Reference DNEL $_{\text{(population, inhalation, reproduction)}}$ = 1.1 mg/m^3 (or 0.22 ppm)

Dermal

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and dermal absorption are available, but a value of 10% is proposed for dermal absorption, based on human *in vitro* data and the extensive evaporative flux of 1-BP. A default value of 100% is applied for inhalation absorption. This gives a dermal topical dose.

168 x 10 = 1680 mg/kg bw/d

The mouse exposure was for 7 days per week. Population exposures are considered to be 7 days per week. No correction is applicable.

= 1680mg/kg bw

Application of assessment factors.

For the dermal route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor = 17.5

1680 / 17.5 = 96 mg/kg bw

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for the general population.

96 / 10 = 9.6 mg/kg bw/d

The ECHA guidance proposes a default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

9.6 / 3 = 3.2 mg/kg bw/d

Reference DNEL (population, dermal, reproduction) = 3.2 mg/kg bw/d

Oral

Modification of the starting point

The starting point is the inhalation LOAEC of 250 mg/m^3 for an 8 h exposure in mice. The duration of the study (8h/d; 7d/ week; 4 weeks) is considered adequate to cover the critical aspects of reproduction.

The mouse exposure period was 8 hours (480 minutes). To obtain a systemic dose, correction is required for the ventilation rate of the mouse of 1.4 L/min/kg bw.

0.25 mg/L x 1.4 x 480 = 168 mg/kg bw/d

No robust data on the comparative extent of inhalation and oral absorption are available, but default values of 100% are proposed for both routes.

= 168 mg/kg bw/d

The mouse exposure was for 7 days per week. Population exposures are considered to be 7 days per week. No correction is applicable.

= 168 mg/kg bw

Application of assessment factors.

For the orall route, the allometric scaling factor of 7 for the interspecies correction applies for mice, together with the 2.5 fold dynamic factor.

168 / 17.5 = 9.6 mg/kg bw

The application of a 10 fold intraspecies factor is recommended in the ECHA guidance for the general population.

9.6 / 10 = 0.96 mg/kg bw/d

The ECHA guidance proposes a default factor of 3 for correcting a LOAEC to a NOAEC. There is no reason to change this default value for the Liu et al data.

0.96 / 3 = 0.32 mg/kg bw/d

Reference DNEL $_{\text{(population, oral, reproduction)}}$ = 0.32 mg/kg bw/d

Endpoint	**Inhalation (24-hr)**	**Dermal**	**Oral**
Reproductive toxicity	**1.1 mg/m^3**	**3.2 mg/kg bw/d**	**0.32 mg/kg bw/d**

12 Summary

1-Bromopropane has been prioritised for Annex XIV listing due to its harmonised classification for reproductive toxicity (fertility and development) in category 1B (H360FD). The purpose of this review is to evaluate the available information relevant to deriving DNELs for the reproductive toxicity of 1-bromopropane.

The contractor has been unable to identify a document that describes the specific data driving the classification, but a number of reproductive and developmental effects have been described in the reviews and literature summarised in this report.

The contractor has identified 3 detailed reviews of the toxicity of 1-bromopropane from internet searches and requests to other regulatory agencies; these form the basis of much of this report (Table 2). The contractor has performed an extensive literature search for 1-bromopropane and identified 13 additional publications which have also been reviewed and summarised. The contractor has also reviewed registration dossiers for 1-bromopropane; some of these have provided more detailed summaries of information in the main reviews but no copies of the critical study reports. There was no new information in the registration reports that was relevant to the derivation of reproductive DNELs.

A number of studies have demonstrated that 1-BP is toxic to reproduction, affecting elements of both male and female reproductive systems. However the only detailed investigation of the entire reproductive cycle is a 2 generation inhalation study in rats (WIL, 2001). Evaluation of specific aspects of end-points related to reproductive performance has consistently shown effects on both males (particularly sperm counts, motility and abnormal sperm) and females (altered oestrous cycling and reduced maturation of ovarian follicles). The effects were seen in both rats and mice. The most sensitive end-point is a reduction in sperm counts and an increase in abnormal sperm in three mouse strains at 50 ppm (Liu et al, 2009). The results of the Liu et al study are seen at a lower concentration than similar effects in a different strain of mice used by the NTP, where a NOAEL of 125 ppm was identified for reductions in sperm count and motility (sperm abnormalities were not investigated). An investigation of the Liu et al and NTP studies identified no reasons to discount the Liu et al results.

There are isolated reports of humans exposed to 1-BP experiencing reproductive problems, but the database is limited and no firm conclusions can be drawn. However, there is no reason to believe that the findings in rats and mice are not relevant to humans.

Taking account of the greater sensitivity of humans, than rodents, to poor reproductive performance due to reduced semen quality / sperm counts it is considered appropriate to base the reproductive DNELs for 1-BP on reductions in sperm counts.

The most sensitive and appropriate end-point in the rat database is considered to be the BMDL of 150ppm for reduced sperm motility in the F_1 parents of the WIL study, as proposed in the TERA review. From the mouse database, the most appropriate point of departure is the LOAEC of 50 ppm from Liu et al for reductions in sperm motility and sperm count. Overall, it is concluded that there is no reason not to use the lower point of departure from the mouse study.

The DNELs derived for worker and the general population are shown in Table 20 below.

Table 20: Summary of reference DNELs for 1-Bromopropane

	Mouse motile sperm & sperm count LOAEC 50 ppm = 250 mg/m^3
Workers DNELs	
Inhalation	6.2 mg/m^3 (1.25 ppm)
Dermal	9.0 mg/kg bw/d
Oral	Not applicable
General Population DNELs	
Inhalation	1.1 mg/m^3 (0.22 ppm)
Dermal	3.2 mg/kg bw/d
Oral	0.32 mg/kg bw/d

ADDITIONAL INFORMATION

13 Literature search terms

The literature search for 1-BP was performed as described below:

Databases

Proquest – which included Healsafe, Medline, Toxfile and Embase

OSHline

Search criteria

No	Request
1	1-bromopropane
2	106-94-5
3	106-94-5 in CAS
4	106-94-5
5	106-94-5 in CN
6	"N-Propyl"
7	"bromide"
8	"N-Propyl bromide"
9	"Propyl"
10	"bromide"
11	"Propyl bromide"
12	bromopropane
13	#1 or #3 or #5 or #8 or #11 or #12
14	toxic*
15	developmental
16	reproducti*
17	fertility
18	fetus
19	sperm*
20	teratology
21	offspring
22	malformation
23	anti-fertility
24	mutagenic-effects
25	toxic* or developmental or reproducti* or fertility or fetus or sperm* or teratology or offspring or malformation or anti-fertility or mutagenic-effects
* 26	#13 and #25

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Annex 1

Details of the non-reproductive toxicity of 1-Bromopropane

Absorption, distribution, metabolism and excretion of 1-bromopropane.

This section is based primarily on the review by the US National Toxicology Program (NTP, 2011; 2013), supplemented by information in the registration dossiers.

Absorption

Studies in humans and laboratory animals indicate that 1-bromopropane can be absorbed following inhalation, ingestion, or dermal contact, and both inhalation and dermal exposure are likely to occur in the workplace (Cheever *et al.* 2009, Hanley *et al.* 2007). Occupational exposure studies consistently reported a correlation between ambient air levels of 1-bromopropane and levels of 1-bromopropane or metabolites in urine.

An *in vitro* study of absorption characteristics of 1-bromopropane using heat-separated human epidermal membranes demonstrated that dermal penetration of 1-bromopropane could be substantial but the actual absorption depended on the type and duration of exposure (Frasch *et al.* 2011).
Frasch et al (2011) studied the dermal absorption of 1-BP using finite and infinite dose exposures of rat and human skin samples. The human data are considered most appropriate and are summarised below. There was also an investigation of the evaporation rate of 1-BP from a petri dish to simulate evaporation following splashes on the skin. The investigations used non-radiolabelled material with analyses performed by GC-FID.

- The evaporation flux at 23°C was determined to be 470 mg/cm^2/h, with a half time for evaporation of <100 seconds. Extrapolating to 32°C to match the temperature of the *in vitro* dermal data gave a flux of approximately 600 mg/cm^2/h.

- The dermal absorption investigations are summarised in Table DA below. Human epidermis samples from female cosmetic surgery procedures were prepared by heat separation. Each element of the study used 9 replicates with samples from 3 donors. The receptor fluid in the static (Franz) cells was HEPES buffered Hanks balanced salt solution. 1-BP is moderately water soluble (*ca* 2g/L) and solubility in the receptor fluid is not seen as a rate limiting factor. Infinite dose tests had the wells covered with Para film, the infinite dose test was left unoccluded resulting in extensive evaporation. Results were presented for 3 hours even though total exposures were for 24 hours in most tests.

- For the finite dose experiments the absorption: time profile was almost linear. For the finite dose test the absorption was complete within 10 - 15 minutes, presumably due to evaporation of 1-BP. For the 10 minute exposure, absorption stopped a few minutes after the swabbing procedure.

- These data show that dermal exposures to 1-BP should result in minimal systemic exposure due to evaporation (the evaporation flux is approximately 500 times that of the dermal absorption flux). In the event that the dermal exposures result in occluded conditions (e.g. under protective gloves) or are repeated (e.g. handling wet items) a conservative dermal absorption value has been calculated based on the finite dose data. Absorption under unoccluded, finite dose conditions was 0.16% of the applied dose all within approximately 10 minutes. Extrapolating this to a 10h exposure to represent a long shift gives a value of 10%.

It is proposed to use 10% as a conservative value for the dermal absorption of 1-BP

***In vitro* dermal absorption data for 1-BP (mean +/- SD)**

Test material	Dose	Duration	Flux (μg/cm^2/h)$^{\#}$	% absorbed
Pure 1-BP	Infinite	24 hours	625 ± 176	
Saturated aqueous solution of 1-BP	Infinite	24 hours	585 ± 320	
Pure 1-BP	Finite (10ul/cm^2)	24 hours	Approximately 1	0.16
Pure 1-BP	Infinite	10 minutes	Approximately 750	
Pure Drysolv*	Infinite	24 hours	441 ± 116	
Saturated aqueous solution of Drysolv	infinite	24 hours	644 ± 255	

* Drysolv is a commercial dry-cleaning solution (95% 1-BP)
# Flux is 0-3hours except for the 10 minute exposure where it is the peak flux over 15 minutes corrected to 1 hour

The most relevant route of exposure for 1-bromopropane based on human exposures is inhalation, and metabolism studies in rats and mice show that 1-bromopropane is absorbed following inhalation (Garner *et al.* 2007, Garner *et al.* 2006, Ishidao *et al.* 2002) or oral exposure (Jones and Walsh 1979, Lee *et al.* 2010a). In male Wistar rats exposed to 1-bromopropane vapor at either 700 or 1,500 ppm, the concentration of 1-bromopropane in blood decreased linearly with time and was below the detection limit within 0.7 hours following the end of the exposure period (Ishidao *et al.* 2002). This study also reported that concentrations of bromide ion (a byproduct of 1-bromopropane metabolism) in rat blood decreased much more slowly, with a half-life of 4.7 to 15 days, depending on the exposure scenario (concentration and duration of treatment) while the half-life of bromide ion excreted in the urine was 5 to 7.5 days.

Distribution

1-Bromopropane is reported to be readily soluble in blood and fat (NTP, 2003).

No data on distribution of 1-bromopropane in humans was identified, and only one study (Garner *et al.* 2006) was found that reported limited data on distribution of radiolabeled (14C) 1-bromopropane in rats and mice after exposure by intravenous (i.v.) injection . Exhaled air, urine, and faeces were collected at various intervals up to 48 hours, and blood and tissue (reported as carcass) samples were collected 48 hours post-exposure. The total radioactivity recovered ranged from 83% to 103% with the largest percentages represented by volatile organic chemicals (VOCs) (25% to 71%), CO2 (10% to 31%) and urine (13% to 23%). Much smaller amounts were recovered from the total carcass (2% to 6%) and faeces (< 1% to 4%). Limited data were reported for radioactivity in liver, and no data were reported for recovery for other individual. The liver to blood tissue radioactivity ratios were similar (~3) regardless of dose, and dose-normalized 1-bromopropane ng equivalents/g of liver were inversely proportional to dose in both species.

Excretion

Once absorbed, the majority of 1-bromopropane is rapidly cleared from the blood by exhalation of the unchanged compound or as either CO2 or VOCs, and by urinary excretion of metabolites of 1-bromopropane or the unmetabolized molecule. Only limited information is available for the excretion of 1-bromopropane in humans, but the presence of the unmetabolized molecule in urine has been described in studies of exposed workers (Ichihara *et al.* 2004a, Kawai *et al.* 2001). Excretion of unmetabolized 1-bromopropane in urine in these studies of exposed workers was significantly correlated with exposure to 1-bromopropane in air. No studies were identified that reported urinary excretion of unmetabolized 1-bromopropane in rodents. Bromide ion is also excreted, but the specificity of this ion as a biomarker for exposure to 1-bromopropane is limited because of a relatively high background from dietary sources. Several mercapturic acid derivatives of 1-bromopropane have been identified in urine from exposed humans and experimental animals. Many more metabolites or potential metabolites have been identified from experimental animal studies using labeled 1-bromopropane.

Other studies in experimental animals have exposed rats or mice to radiolabeled (14C) 1-bromopropane by intraperitoneal (i.p.) injection (Jones and Walsh 1979) or i.v. administration through the tail vein or jugular vein (Garner *et al.* 2006). Jones and Walsh reported that 60% of a single dose of 200 mg/kg 1-bromopropane administered to rats was exhaled unchanged within 4 hours with only trace amounts detected after that time. Only 1.4% of the total dose was exhaled as CO2 and about 45% of the metabolized dose was excreted in the urine after 100 hours. A much lower recovery of 3.3% of an i.p. dose of 200 mg/kg as urinary metabolites was reported by Walsh and Jones (1977) after 100 hours.

Metabolism

The metabolites identified in humans are limited to those recovered in the urine of factory workers after exposure to 1-bromopropane. Several studies have investigated 1-bromopropane metabolism in experimental animals, and the different metabolites identified in studies by different routes of exposure indicate that the metabolism is complex.

Metabolites detected in humans

Several studies have monitored urine samples from humans occupationally exposed to 1-bromopropane in order to establish biomarkers of exposure. The predominant metabolite detected in the urine of workers is *N*-acetyl-*S*-propylcysteine (AcPrCys), and levels increased with increasing 1-bromopropane ambient exposure levels (Hanley and Dunn 2006, Hanley *et al.* 2009, 2010, Valentine *et al.* 2007). In addition to AcPrCys, several other urinary mercapturic acid conjugates were identified from 1-bromopropane-exposed workers; these included *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, and *N*-acetyl-*S*-(3-hydroxy-*n*-propyl)-L-cysteine (Cheever *et al.* 2009, Hanley *et al.* 2009). The oxidative metabolites that likely lead to the conjugates have not been reported in human studies, however no publications were identified that actually tested for them. Metabolism has been more extensively studied in experimental animals.

In vivo studies in experimental animals

Metabolism studies were conducted in rats and mice exposed by inhalation, oral, subcutaneous (s.c.), i.p., or i.v. administration and *in vitro* using rat liver microsomes (Barnsley *et al.* 1966, Garner *et al.* 2007, Garner *et al.* 2006, Jones and Walsh 1979). The four urinary mercapturic acid conjugates identified in exposed workers were also identified in experimental animals. AcPrCys was identified in the urine of rats, mice, guinea pigs, and rabbits exposed to 1-bromopropane via s.c. injection. The other metabolites were identified in the urine of rats following oral exposure. Overall, three major categories of metabolites have been identified: (1) brominated metabolites (Phase I), (2) debrominated metabolites (Phase I), and (3) glucuronide or glutathione conjugated metabolites (Phase II).

Garner *et al.* (2006) investigated the metabolism of 1-bromopropane in male F344 rats and B6C3F1 mice following inhalation or tail vein injection. These routes were selected because they do not involve first-pass metabolism and the inhalation route, specifically, is more likely to be consistent with occupational or environmental exposures compared with the oral and i.p. routes used by Jones and Walsh (1979). Much of the administered dose (40% to 70%) was exhaled unchanged. Oxidation and glutathione conjugation were the primary metabolic pathways. In both rats and mice, hydroxylation at the C2 position (forming 1-bromo-2-propanol) was the predominant pathway of oxidation. Although 1-bromo-2-propanol was not detected in the urine, resonances associated with unconjugated 1-bromo-2-propanol were detected in rat liver homogenates, and more than half of the urinary metabolites were derived from this metabolite. Although bromoacetone was not detected in the urine, its mercapturic acid conjugate, *N*-acetyl-*S*-(2-oxopropyl)cysteine, was detected in rats at levels approaching that of *N*-acetyl-*S*-(2-hydroxypropyl)cysteine, the mercapturic acid of 1-bromo-2-propanol. Another possible metabolite detected in rat liver homogenate was -bromohydrin.

Urinary metabolites in rats exposed to 1-bromopropane by i.v. injection were affected by dose (Garner *et al.* 2006). At the low dose, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) was a relatively minor component compared with earlier eluting peaks that included *N*-acetyl-*S*-(2-hydroxypropyl)cysteine. However, the relative proportion of AcPrCys increased with dose and accounted for more than 80% of the urinary

radioactivity in the high-dose group. AcPrCys is formed by direct conjugation with glutathione without oxidation. In contrast, in mice injected i.v. with 1-bromopropane, *N*-acetyl-*S*-(2-hydroxypropyl)cysteine was the single predominant metabolite at all dose levels.

A pathway overlapping in part with that described by Garner *et al.* (2006) was reported by Jones and Walsh (1979), who investigated the metabolism of 1-bromopropane in male Sprague-Dawley rats following five consecutive daily oral doses. Four possible metabolic pathways were identified (Figure 2-2). The first pathway involved direct conjugation with glutathione to produce the urinary metabolites AcPrCys and *N*-acetyl-*S*-propylcysteine-*S*-oxide. The second pathway involved oxidation at C3 of 1-bromopropane to 3-bromo-1-propanol. Pathway 3 was based on oxidation of C1 of 1-bromopropane to CO2 (hydrolysis to *n*-propanol with rapid oxidation to propionic acid and decarboxylation to CO2). Pathway 4 is the proposed mechanism for forming *N*-acetyl-*S*-(2-hydroxypropyl)cysteine; however, there was no direct evidence for this pathway *in vivo*. Several additional metabolites, including 3-bromopropionic acid and *n*-propanol, were identified by Jones and Walsh that were not described by Garner *et al.* However, as suggested by Garner *et al.*, the difference in the observed metabolites might be explained by the analytical methods used by Jones and Walsh, which included concentration steps that could have amplified several minor metabolites.

Possible reactive metabolites identified in these studies of 1-bromopropane metabolism include glycidol, -bromohydrin, and propylene oxide (1,2-epoxypropane). Glycidol was identified in urine samples but not quantified by Ishidao *et al.* (2002) as a metabolite resulting from exposure of rats to 1-bromopropane by inhalation. Walsh and Jones (1977) did not detect glycidol in rats given an i.p. injection but proposed that it was a likely intermediate in formation of the urinary metabolite 2,3-dihyroxypropylmercapturic acid. Garner *et al.* (2007) identified -bromohydrin as a metabolite. Propylene oxide was proposed as a likely metabolite by Ishidao *et al.* (2002) and by Jones and Walsh (1979), but neither group detected it in their studies. The genotoxicity and potential carcinogenicity of glycidol, -bromohydrin, and propylene oxide are discussed in Section 5.

In vitro studies

Several debrominated metabolites of 1-bromopropane were identified only in studies *in vitro* using rat liver microsomes (see Table 2-1). Three metabolites of 1-bromopropane – propene, 1,2-propanediol, and propionic acid – were identified from the *in vitro* P450-catalyzed metabolism of 1-bromopropane by phenobarbital-induced rat liver microsomes; when exogenous glutathione was added to the incubation mixture, S-(1'-propyl)glutathione and S-(2'-hydroxy-1'-propyl)glutathione were detected (Tachizawa *et al.* 1982). In another *in vitro* metabolism study of 1-bromopropane by rat liver microsomes reported by Kaneko *et al.* (1997) only *n*-propyl alcohol was reported as a metabolite, but the authors noted that differences between the rate of substrate disappearance and product formation suggested that there might be other metabolic pathways.
Jones and Walsh (1979) also conducted an *in vitro* metabolism study of 1-bromopropane. Oxidation of carbons 2 and 3 (C2 and C3) of 1-bromopropane was demonstrated *in vitro*. Metabolites oxidized at C3 included 3-bromopropionate and 3-

hydroxypropionate. Evidence for C2 oxidation (i.e., formation of 1-bromo-2-propanol) was provided by the isolation of *S*-(2-hydroxypropyl)cysteine from the reaction mixture after it was reacted with cysteine in sodium hydroxide.

Studies of metabolizing enzymes

It is clear from the available studies that most of the metabolites of 1-bromopropane are formed following oxidation reactions and glutathione conjugation. The proportion of 1-bromopropane metabolized via oxidation relative to pathways dependent on direct glutathione conjugation was inversely proportional to dose in rats but independent of dose in mice (Garner *et al.* 2006). Garner *et al.* concluded that formation of *N*-acetyl-*S*-propylcysteine [AcPrCys] results from release of a bromide ion without oxidation. Barnsley *et al.* (1966) also postulated formation of *S*-*n*-propylglutathione directly from 1-bromopropane with subsequent formation of *S*-*n*-propylcysteine and AcPrCys.

The importance of the cytochromes P450 (CYP) oxidative enzymes in the metabolism of 1-bromopropane has been confirmed by the severe reduction in formation of metabolites when NADPH was eliminated from the incubation mixture with phenobarbital-induced rat liver microsomes, effectively inactivating CYP oxidation (Tachizawa *et al.* 1982). Pretreatment of rats with 1-aminobenzotriazole (ABT), a general inhibitor of CYP, significantly reduced the number of metabolites from 10 to 1 major metabolite, AcPrCys, which accounted for more than 90% of the total radioactivity (Garner *et al.* 2006). Results from a study on the induction of liver CYP isozymes in male and female Sprague-Dawley rats exposed to 1-bromopropane indicated that the expression of the CYP2E1 isozyme was enhanced while the signals for the other isozymes (CYP1A/2 and CYP2B1/2) were not, suggesting that CYP2E1 is possibly responsible for 1-bromopropane metabolism (Kim *et al.* 1999b). Further evidence for the specific contribution of CYP2E1 to metabolism of 1-bromopropane was provided by studies with Cyp2e1-/- knockout and wild-type mice (Garner *et al.* 2007). Compared with wild-type mice exposed to 1-bromopropane by inhalation for 6 hours, the elimination half-life was more than twice as long in knockout mice (3.2 vs. 1.3 hours) exposed in the same way. In addition, the ratio of glutathione conjugation to 2-hydroxylation increased 5-fold, and the urinary concentration of *N*-acetyl-*S*-(2-hydroxypropyl)cysteine was reduced by about 50%. These data indicate that CYP2E1 is responsible for much, but not all, of the oxidative metabolism of 1-bromopropane since hydroxylated metabolites were significantly decreased, but not completely eliminated, in knockout mice.

The role of glutathione conjugation was also investigated using DL-buthionine(S,R)-sulfoximine 1-aminobenzotriazole (BSO), an inhibitor of GSH synthesis (Garner *et al.* 2006). Pretreatment with BSO did not significantly alter the metabolite profile for 1-bromopropane, although there was a moderate decrease in the level of AcPrCys with a concomitant increase in other metabolites compared with rats that were exposed to 1-bromopropane alone. The authors suggested that direct conjugation of 1-bromopropane might be a relatively minor pathway compared with oxidative metabolism in mammals.

Conclusions

Studies in humans and laboratory animals indicate that 1-bromopropane can be absorbed following inhalation, ingestion, or dermal exposure. Occupational exposure occurs primarily by inhalation and dermal contact and studies of workers show a good correlation between urinary concentrations of 1-bromopropane, bromide ion, and *N*-acetyl-*S*-(*n*-propyl)-L-cysteine (AcPrCys) with their 1-bromopropane breathing zone air concentrations. Several studies have monitored urine and blood samples in workers to establish biomarkers of exposure. These studies also indicate that unmetabolized 1-bromopropane is excreted in the urine in humans but has not been reported in animal studies. The four urinary mercapturic conjugates identified from 1-bromopropane-exposed workers have also been reported as urinary metabolites from studies in rodents, including AcPrCys, *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide, *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine, and *N*-acetyl-*S*-(3-hydroxy-*n*-propyl)-L-cysteine. The oxidative metabolites that likely lead to the conjugates have not been reported in human studies; however, no publications were identified that actually tested for them.

Experimental animal studies have shown that 1-bromopropane is absorbed, rapidly distributed, and predominantly eliminated by exhalation (approximately 40% to 70%), but is also excreted in the urine and faeces. In rats and mice, most of the 1-bromopropane administered by i.v. injection was exhaled unchanged or as CO2 within 4 hours of exposure. Urinary metabolites accounted for 13% to 23% of the administered dose after 48 hours. The available studies on 1-bromopropane metabolism show that CYP catalyzed oxidation (primarily via CYP2E1) reactions and glutathione conjugation are the primary metabolic pathways. At least 16 urinary metabolites have been identified in rodent studies (either rats or mice), including several reactive intermediate metabolites (bromoacetone, glycidol, and -bromohydrin).

Although there are differences in results between rodents and humans the database is limited for humans and some differences appear to be related to the extent of investigation. The contractor considers there is no reason to assume humans are markedly different from animals in the kinetics of 1-bromopropane.

There are no data on the relative absorption via oral, dermal and inhalation routes. The contractor proposes to use the default assumptions in guidance document R8.

There are no data on the potential for 1-bromopropane or its metabolites to cross the placenta. Effects on the fetus have been reported but it is not clear if these are due to direct exposure or external factors such as reduced blood supply or nutrients.

Summary of the non-reproductive toxicity on 1-bromopropane

Acute toxicity

The acute oral and dermal LD50s for 1-bromopropane are reported in the registration dossier as >2000 mg/kg bw in rats. The acute inhalation LC50 (4h exposure) in rats is

7000 ppm (35,000 mg/m^3), with no deaths at 6000 ppm. 1-Bromopropane is classified for narcotic effects (H336).

1-Bromopropane has a harmonised classification as a skin irritant (H315), as a severe eye irritant (H317) and a respiratory irritant (H335). The skin sensitisation is equivocal, in a maximization test in guinea pigs positive reactions in above 30% of animals are reported but the interpretation in the registration dossier is that 1-bromopropane is not a skin sensitiser.

Repeat dose toxicity

A tabulated summary of the repeat dose toxicity studies for the inhalation route performed with 1-bromopropane is provided below, based on the CSR in one of the registration dossiers (ICL-IP Temeuzen).

Method	**Results**	**Remarks**	**Reference**
rat (Sprague-Dawley) male/female subacute (inhalation: aerosol) (whole body) 0 (group 1), 2.0 (group 2), 5.0 (group 3) and 8.0 (group 4) mg/L (nominal conc.) Vehicle: air Exposure: The study was a 28 day repeat dose inhalation study where animals were given full body exposure 5 days a week, for 6 hours per day. Rats were held in the exposure chamber for 375 minutes. The first 15 minutes of this was the time taken to get up to approximately 95% of the target concentration. For the final 15 minutes in the chamber the aerosol was switched off, allowing the concentration to get to approximately 5% of the target value. Based on this, the exposure duration is quoted as 6 hours. (5 days per week.)	NOAEC: < 2 mg/L air (nominal) (male/female) based on: test mat. (Lesions in the central nervous system present in some animals at low dose of 2.0 mg/L air (group 2).)	2 (reliable with restrictions) key study experimental result	Labbé. R (1997b)

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female subchronic (inhalation: aerosol) (whole body) Control (group 1), 0.5 mg/L (group 2), 1.0 mg/L (group 3), 2.0 mg/L (group 4), 3.0 mg/L (group 5). (nominal conc.) Vehicle: air Exposure: The study was a 90 day repeat dose inhalation study was conducted where animals were given full body exposure 5 days a week, for 6 hours. During daily exposure, aerosol was actively generated for 360 minutes (for the duration of the t95 and a subsequent 335 and 345 minutes at a t95 of 25 and 15 minutes, respectively. Following 360 minutes of continuous operation, aerosol generation was stopped and the chamber concentration allowed to decay for the calculated t05 (approximately 15 and/or 25 minutes), The rats were then removed from the exposure chamber and returned to their home cages.	NOEL: 1 mg/L air (nominal) (female) based on: test mat. (histopathology)	2 (reliable with restrictions) key study experimental result	Adamo-Trigiani. M (1997)
rat (Sprague-Dawley) male/female subacute (inhalation: aerosol) (whole body) 15, 20 mg/L (nominal conc.) Vehicle: air Exposure: The purpose of this study was to investigate the potential acute toxicity of a vapour formulation of 1-bromopropane following single whole-body inhalation administration to the rat and to determine a suitable high dose level for a future 28-day repeat dose study.	NOEC: < 15 mg/L air (nominal) (male/female) based on: test mat. (clinical signs; mortality; body weight; haematology; clinical chemistry; histopathology.)	2 (reliable with restrictions) supporting study experimental result	Labbé. R (1997c)

Method	Results	Remarks	Reference
Rats were held in the exposure chamber for 375 minutes. The first 15 minutes of this was the time taken to get up to approximately 95% of the target concentration. For the final 15 minutes in the chamber the aerosol was switched off, allowing the concentration to get to approximately 5% of the target value. Based on this, the exposure duration is quoted as 6 hours. (5 days per week for 2 weeks.)			
rat (Sprague-Dawley) male/female subacute (inhalation: aerosol) (whole body) 5 mg/L (nominal conc.) Vehicle: air Exposure: The purpose of this study was to investigate the potential toxicity of a vapour formulation of 1-bromopropane following a 10-day whole-body inhalation administration (5 days/week) to the rat and to determine a suitable high dose level for a future 28-day repeat dose study. Rats were held in the exposure chamber for 375 minutes. The first 15 minutes of this was the time taken to get up to approximately 95% of the target concentration. For the final 15 minutes in the chamber the aerosol was switched off, allowing the concentration to get to approximately 5% of the target value. Based on this, the exposure duration is quoted as 6 hours. (5 days per week for 2 weeks.)	NOEC: 5 mg/L air (nominal) (male/female) based on: test mat. (overall effects)	2 (reliable with restrictions) supporting study experimental result	Labbé. R (1997d)

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male/female subchronic (inhalation: vapour) (whole body) 0, 50 (group 1), 300 (group 2), 1800 (group 3) ppm (nominal conc.) Vehicle: clean air Exposure: 6 hours a day (5 days a week for 8 weeks.) equivalent or similar to OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day) (56 day)	NOAEC: 300 ppm (nominal) (male/female) based on: test mat. (organ weights)	2 (reliable with restrictions) supporting study experimental result	Moon. Y.H. et al (1998)
rat (F344/N) male/female subacute (inhalation: vapour) 125 ppm (analytical conc.) 250 ppm (analytical conc.) 500 ppm (analytical conc.) 1000 ppm (analytical conc.) 2000 ppm (analytical conc.) Vehicle: clean air Exposure: 16 days (6 hours plus T90 (12 minutes) per day, 5 days per week) equivalent or similar to OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	In the 2-week rat study, there were no treatment-related deaths and significantly lower body weights occurred only in the 1,000 and 2,000 ppm groups. Liver weights were increased in most groups of exposed rats; however, there was no histopathologic evidence of toxicity. Foci of minimal necrosis of the respiratory epithelium and minimal to mild suppurative inflammation were present in a few 1,000 and 2,000 ppm male rats.	2 (reliable with restrictions) supporting study experimental result	Morgan, D. L. et al. (2009a)
rat (F344/N) male/female subchronic (inhalation: vapour) 62.5 ppm (analytical conc.) 125 ppm (analytical conc.) 250 ppm (analytical conc.) 500 ppm (analytical conc.) 1000 ppm (analytical conc.) Vehicle: clean air Exposure: 14 weeks (6 hours plus T90 (10 minutes) per day, 5 days per week) equivalent or similar to OECD	In the 3-month study, there were no treatment-related deaths and significantly lower body weights occurred only in the 1,000 ppm male rat group. A mild cytoplasmic vacuolization was present in the liver of all 500 and 1,000 ppm rats, and in half of the 250 ppm male rats. Other	2 (reliable with restrictions) supporting study experimental result	Morgan, D. L. et al. (2009a)

Method	Results	Remarks	Reference
Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	indications of mild hepatotoxicity in exposed rats were increased liver weights and increases in serum sorbitol dehydrogenase levels. There were no exposure-related nasal lesions in rats exposed for 3 months.		
mouse (B6C3F1) male/female subacute (inhalation: vapour) 125 ppm (analytical conc.) 250 ppm (analytical conc.) 500 ppm (analytical conc.) 1000 ppm (analytical conc.) 2000 ppm (analytical conc.) Vehicle: clean air Exposure: 17 days (6 hours plus T90 (12 minutes) per day, 5 days per week) equivalent or similar to OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	Exposure to 1-bromopropane concentrations of 500 ppm or greater in the 2-week study resulted in early deaths of mice. The liver was identified as the primary target site in these mice, and severe centrilobular necrosis was likely the cause of death. In this study, moderate to marked centrilobular necrosis was present in all males and most females exposed to 500 ppm or greater. 1-Bromopropane also caused lesions in the respiratory tract of mice, although these lesions were not considered as severe as those in the liver. In the 2-week study, respiratory tract lesions were most severe in mice that died early after exposure to 500 ppm or greater. Necrosis and vacuolization of the nasal respiratory and olfactory epithelium and prominent necrosis of the bronchiolar epithelium were present only in early death mice. In	2 (reliable with restrictions) supporting study experimental result	Morgan, D. L. et al. (2009a)

Method	Results	Remarks	Reference
	survivors exposed to 500 ppm or greater, lesions consisted primarily of regeneration of the olfactory epithelium and the bronchiolar epithelium.		
mouse (B6C3F1) male/female subchronic (inhalation: vapour) 62.5 ppm (analytical conc.) 125 ppm (analytical conc.) 250 ppm (analytical conc.) 500 ppm (analytical conc.) Vehicle: clean air Exposure: 14 weeks (6 hours plus T90 (10 minutes) per day, 5 days per week) equivalent or similar to OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)	Exposure to 1-bromopropane concentrations of 500 ppm in the 3-month study resulted in early deaths of mice. The liver was identified as the primary target site in these mice, and severe centrilobular necrosis was likely the cause of death. In this study, extensive hepatic centrilobular necrosis was present only in 500 ppm mice that died early. Centrilobular chronic inflammation was present in livers of surviving 500 ppm mice, and no hepatocellular lesions were observed in mice exposed to lower concentrations except in one 250 ppm male. The centrilobular location of the lesions in the region of the liver that contains the highest levels of P450 activity suggests that the hepatotoxicity may be due to a reactive metabolite of 1-bromopropane. 1-Bromopropane also caused lesions in the respiratory tract of mice, although these lesions were not considered as severe as those in the liver. In the 3-month	2 (reliable with restrictions) supporting study experimental result	Morgan, D. L. et al. (2009a)

Method	Results	Remarks	Reference
	study, mild necrosis of the nasal respiratory and olfactory epithelium and minimal necrosis of the epithelium lining the larynx and trachea were observed primarily in 500 ppm mice that died early. Minimal regeneration of the bronchiolar epithelium was observed in the majority of 250 and 500 ppm mice that survived to the end of the study. Although the upper respiratory tract lesions in exposed mice may be due to a direct irritant effect, metabolism of 1-bromopropane to a toxic metabolite by the nasal epithelium cannot be ruled out.		

No data on repeat dose toxicity via other routes is available.

No human data on repeat dose toxicity is available.

Summary and discussion of repeated dose toxicity

The 90-day repeat dose inhalation study was selected as the key study for repeat-dose toxicity, as it represented the longest duration of repeat exposure.

Four groups of Sprague-Dawley rats, each comprising 15 males and 15 females, were subjected to 6 hour "whole-body" exposures daily (5 days/week) of a vapour formulation of 1-bromopropane for 13 weeks at target concentrations of 0.5 mg/L (Group 2), 1.0 mg/L (Group 3), 2.0 mg/L (Group 4) and 3.0 mg/L (Group 5). A similarly constituted control group (Group 1) was similarly restrained, but exposed to room air. The mean overall chamber concentration of 1 -bromopropane, determined by Miran infrared gas analyzer, was 0.5, 1.01, 2.01 and 3.0 mg/L for Groups 2, 3, 4 and 5, respectively. The mean overall analytical chamber concentration for the 4 treated groups was 0.51, 1.01, 2.00 and 2.98 mg/L for Groups 2, 3, 4 and 5, respectively. The mean concentrations obtained were within 2% of the targeted concentrations whether determined by either Miran gas analysis or by gas chromatography.

Whole-body inhalation exposure of Sprague-Dawley rats to a vapour of 1-bromopropane for 6 hours each day, 5 days each week, for a 13-week period, at chamber concentrations of 0.5 to 3.0 mg/L produced no clinical observations which

were considered to be related to treatment. Four mortalities occurred during bleeding procedures or anaesthesia but were not considered to be related to treatment. There were no obvious effects on body weight, food consumption, urinalysis, ophthalmology, functional observational battery or motor activity that could be attributed to treatment with 1-bromopropane. No treatment-related trends were evident in hematology, blood biochemistry analysis or gross pathology. There was a marginal increase in relative liver weights in Group 5 (3.0 mg/L- high dose) males. This effect was considered of questionable toxicological significance as intergroup differences were minimal and the effect was not observed in the Group 5 (3.0 mg/L- high dose) females. However, histopathological lesions were present in the liver (vacuolation of centrilobular hepatocytes) when exposed at concentrations of 2.0 mg/L (intermediate/high dose) and 3.0 mg/L (high dose). The No Observed Effect Level (NOEL) was therefore identified as 1.0 mg/L (used as a worst case NOAEC of 1000 mg/m^3).

The registrant has included repeat dose studies conducted by the National Toxicology Program, USA with 1-bromopropane. These were conducted as part of a suite of studies used to address 1-bromopropane's potential for carcinogenic effects. The NTP study data included here was obtained from a draft report published by the NTP. However, these studies are not currently citable until the final version of the report is made available. As such, the results have been included as supporting data for information purposes only.

Carcinogenicity

Two year inhalation toxicity studies in F344 rats (125, 250 or 500 ppm) and B6C3F1 mice (62.5, 125 or 250 ppm) found that 1-bromopropane caused increases in the incidence of malignant or benign tumors of the skin (keratoacanthoma; keratoacanthoma or squamous-cell carcinoma combined; and keratoacanthoma, squamous-cell carcinoma, basal-cell adenoma, or basal-cell carcinoma combined) in male rats. Increases in benign large intestine tumors (adenoma of the colon and rectum) were seen in in female and male rats, and benign or malignant lung tumors (alveolar/bronchiolar adenoma and carcinoma combined) in female mice. Increases in skin tumors in male rats, intestinal tumors in female rats, and lung tumors in female mice were statistically significant and dose related. The tumors in the large intestine of male rats, although not statistically significant, were considered to be of biological significance due to their rarity (less than 0.2% incidence in historical controls). Additionally, tumors observed that may have been related to 1-bromopropane exposure included malignant mesothelioma of the abdominal cavity and pancreatic islet tumors (adenoma) in male rats and skin tumors (keratoacanthoma, basal-cell adenoma, or basal-cell carcinoma combined) in female rats (NTP 2011; 2013).

Although clear dose response relationships were not seen for all the tumours, significant increases in one or more tumours were seen at all tested concentrations.

No studies were found evaluating modes of action for the tumor sites found in experimental animals: skin, large intestine, and lung. However, 1-bromopropane, either directly or via reactive metabolites, causes molecular alterations that are typically associated with carcinogenesis, including genotoxicity, oxidative stress, glutathione depletion, immunosuppression, and inflammation.

Cancer NOAEC is <62.5 ppm in mice.

Genotoxicity

Studies *in vivo* show that 1-bromopropane can covalently bind to protein in exposed rats and occupationally exposed workers. The available data provide some support that 1-bromopropane is genotoxic as it induced mutations in bacterial and mammalian cells and DNA damage in human cells. There is limited evidence that DNA damage was induced in leukocytes from 1-bromopropane workers. 1-Bromopropane did not induce chromosomal damage in exposed rodents (micronucleus induction assay) or gene-cell mutations (dominant lethal mutation assay).

Neurotoxicity

Neurotoxic effects of 1-bromopropane were first described in rats and were later used to identify and analyze the initial human cases (Ichihara *et al.* 2011b, Li *et al.* 2010b, Meyer-Baron *et al.* 2012). Although the molecular mechanisms of neurotoxicity are not completely understood, recent studies show that the hippocampus is especially susceptible to 1-bromopropane-induced effects and involves oxidative stress, loss of ATP production, altered GABA metabolism and reduced GABAergic feedback inhibition, inhibition of the ubiquitination-proteosome system, changes in neurotransmitter receptor expression, and modifications of intracellular signaling cascades (Fueta *et al.* 2004, Fueta *et al.* 2002b, Huang *et al.* 2011, Mohideen *et al.* 2009). Other studies indicate that the neurotoxic effects of 1-bromopropane involve glutathione depletion, protein adducts, and degeneration of noradrenergic axons (Mohideen *et al.* 2011, Valentine *et al.* 2007, Wang *et al.* 2002, Wang *et al.* 2003).

Studies in humans include more than a dozen case reports from the United States and an epidemiological study of 1-bromopropane production factory workers in China (Ichihara *et al.* 2011b, Li *et al.* 2010b). Signs and symptoms from the case reports were similar and included numbness, diminished vibration sense in the lower extremities, distal latency, and ataxia suggesting that sensory nerves were affected. Other effects included hyperreflexia, suggesting damage to the central nervous system, and neurobehavioral effects (memory disturbances and depressive or unstable mood). Li *et al.* (2010b) evaluated neurologic abnormalities in 60 women factory workers compared with age-, sex-, and region-matched controls. Significant neurological effects included dose-dependent increase in the distal latency of tibial nerves, increased threshold for vibration sense in the toes, and decreased sensory nerve conduction velocity of the sural nerve. However, the exposure assessment was based on recent exposure measurement, which may not accurately reflect past exposure.

Reported effects in rats include prolongation of motor distal latency, reduction of motor nerve conduction velocity, myelin sheath degeneration, decrease in cerebral weight, pyknotic shrinkage and degeneration of Purkinje cells in the cerebellum, ataxia, and decreased limb muscle strength (Ichihara *et al.* 2011b).

The CERHR review (NTP, 2003) concluded that the NOAEC for neurotoxicity was 200 ppm.

Immunotoxicity

1-Bromopropane has induced immunotoxic effects in mice (Lee *et al.* 2007a). T-dependent antibody response to sheep red blood cells, intracellular IL-2 production, and the absolute numbers of splenocyte subpopulations (total T-cells, CD4+ cells, CD8+ cells, macrophages, and B-cells) were all reduced in a dose-dependent manner. Thus, dose levels that resulted in decreased cellular glutathione and increased production of glutathione conjugate in spleen cells also suppressed immune function. Thus, the immunotoxicity of 1-bromopropane could be related to glutathione depletion from formation of glutathione conjugates and increased oxidative stress.

Anderson *et al.* (2010) also reported immunotoxic effects of inhaled 1-bromopropane in female B6C3F1 mice and F344/N rats. Animals (8 per group) were placed in inhalation chambers and exposed to 0, 125, 250, or 500 ppm (mice) or 0, 250, 500, or 1,000 ppm (rats) for 6 hours/day, 5 days/week, for 4 or 10 weeks. Spleen immunoglobulin (IgM) responses to sheep red blood cells (plaque-forming cell assay) were significantly decreased in mice (all exposed groups) and in rats (high-dose group only) after exposure for 10 weeks; however, the serum IgM response (ELISA assay) was not affected. Although the mechanism underlying these contradictory results is unknown, it has been observed following exposure to other chemicals (Johnson *et al.* 2000, Temple *et al.* 1993).