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Peer reviewed

Review

# NTP-CERHR Expert Panel Report on the reproductive and developmental toxicity of 2-bromopropane<sup>☆</sup>

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## Preface

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

2-Bromopropane (2-BP) was nominated by NIOSH and selected for evaluation by the CERHR based primarily on documented evidence of worker exposures and published evidence of reproductive toxicity in both rodents and humans. In the US, 2-BP is a contaminant (<0.1%) of 1-bromopropane which is used in spray adhesives and as a precision cleaner and degreaser. 2-BP may also be used as an intermediate in the synthesis of pharmaceutical dyes and other organic chemicals. In Asia 2-BP has been used as a replacement for chlorofluorocarbons and 1,1,1-trichloroethane.

The evaluation of 2-BP was a 4-month effort by a 9-member panel of academic, private, and government scientists that culminated in a public meeting in December 2001. At that meeting, the expert panel reviewed the scientific evidence on 2-BP and reached conclusions

<sup>☆</sup> This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (<http://cerhr.niehs.nih.gov/>). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the strengths/weaknesses and utility (adequacy) of the study for a CERHR evaluation. Statements and conclusions made under strengths/weaknesses and utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the panel.

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regarding its potential effects on human reproduction. The background information on 2-BP and findings of the expert panel are contained within this report. The Expert Panel Report on 2-bromopropane is intended to (1) interpret the strength of scientific evidence that a given exposure or exposure circumstance may pose a hazard to reproduction and the health and welfare of children; (2) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects are associated with exposure to specific chemicals or classes of chemicals, including descriptions of any uncertainties that would diminish confidence in assessment of risks; and (3) identify knowledge gaps to help establish research and testing priorities. Staff scientists from the CERHR and members of the CERHR Core Committee (oversight committee to the CERHR whose members include NTP participating agencies) have reviewed the report and the CERHR will seek public review and comment through a *Federal Register* notice.

Subsequent to this comment period, the NTP will prepare the NTP-CERHR Report on 2-bromopropane that contains NTP's conclusions regarding the potential for 2-BP to adversely affect human reproduction. The NTP will base its conclusions on the Expert Panel Report on 2-bromopropane, any public comments received on that report, and any relevant information available since the expert panel meeting. The NTP-CERHR report will include the public comments and the Expert Panel Report as appendices. The NTP-CERHR Report on 2-bromopropane will be made publicly available and transmitted to health and regulatory agencies.

The NTP and the CERHR wish to thank the members of the Bromopropanes Expert Panel for their contributions to the evaluation of 2-BP. We greatly appreciate their time, effort, and objectivity during this evaluation process. We also wish to thank the contract staff for their support in convening the expert panel and preparing the Expert Panel Report.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

**Keywords:** Chlorofluorocarbons; Bromopropanes Expert Panel; Subcutaneous

**Abbreviations:** ANOVA, analysis of variance; ASTM, American Society for Testing and Materials; 1-BP, 1-bromopropane; 2-BP, 2-bromopropane; bw, bodyweight; C, celsius; CAS RN, Chemical Abstract Service Registry Number; CERHR, Center for the Evaluation of Risks to Human Reproduction; CFC, chlorofluorocarbon; CHL, Chinese hamster lung; cm<sup>2</sup>, centimeters squared; CNS, central nervous system; d, day; DL, distal latency; DMSO, dimethyl sulfoxide; EKG, electrocardiograph; USEPA, U.S. Environmental Protection Agency; F, female; fmol, femtomole; FSH, follicle stimulating hormone; g, gram; GC, gas chromatography; gd, gestation day; GST, glutathione transferase; h, hour; Hb, hemoglobin; hCG, human chorionic gonadotropin; 2,5-HD, 2,5-hexanedione; HSDB, Hazardous Substances Data Bank; Ht, hematocrit; i.p., intraperitoneal; kg, kilogram; K<sub>m</sub>, Michaelis constant; K<sub>ow</sub>, octanol-water partition coefficient; l, liter; LC<sub>50</sub>, lethal concentration, 50% mortality; LC<sub>100</sub>, lethal concentration, 100% mortality; LH, luteinizing hormone; LOAEC, lowest observed adverse effect concentration, synonymous with lowest observed adverse level (LOAEL); M, male; m<sup>3</sup>, meters cubed; MCV, motor nerve conduction velocity; mg, milligram; ML, motor latency; ml, milliliter; mmHg, millimeters mercury; mmol, millimole; mRNA, messenger RNA; MSDS, Material Safety Data Sheet; mw, molecular weight; n, number; ng, nanogram; NIEHS, National Institute of Environmental Health Sciences; NIOSH, National Institute of Occupational Safety and Health; NOAEC, no observed adverse effect concentration, synonymous with no observed adverse effect level (NOAEL); NOEC, no observed effect concentration, synonymous with no observed effect level (NOEL); NTP, National Toxicology Program; OECD, Organization for Economic Co-operation and Development; OSHA, Occupational Safety and Health Administration; PCNA, proliferating cell nuclear antigen; pg, picogram; ppm, parts per million; RBC, red blood cell; S, sulfur; s.c., subcutaneous; TUNEL, in situ analysis of DNA fragmentation; TWA, time-weighted average; U, unit; V<sub>max</sub>, maximal velocity of metabolism; WBC, white blood cell; WHO, World Health Organization; wk, week; w/v, weight per volume; YAR, yeast expressing human androgen receptor; µg, microgram; µmol, micromole

## 1. Chemistry, use, and human exposure

### 1.1. Chemistry

#### 1.1.1. Nomenclature

2-Bromopropane (2-BP): CAS RN=75-26-3

Synonym: Isopropyl bromide

#### 1.1.2. Formula and molecular mass

The molecular formula of 2-BP is C<sub>3</sub>H<sub>7</sub>Br. The structure is given in Fig. 1. The molecular mass is mw = 123.

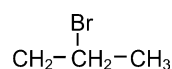


Fig. 1. 2-Bromopropane.

### 1.1.3. Chemical and physical properties

Chemical and physical properties are given in Table 1.

Conversion factors: 1 ppm  $\cong$  5.03 mg/m<sup>3</sup>;  
1 mg/m<sup>3</sup>  $\cong$  0.198 ppm.

Table 1  
Chemical and physical properties of 2-BP

Property	Value
Boiling point	59.38 °C at 760 mmHg
Melting point	-89 °C
Specific gravity	1.31 at 20 °C
Solubility in water	3180 mg/l at 20 °C
Vapor pressure	216.47 mmHg at 25 °C
Stability	Stable with normal use and storage <sup>a</sup>
reactivity	Incompatible with oxidizing agents <sup>a</sup>
Log K <sub>ow</sub>	2.14

Reviewed in HSDB [1].

<sup>a</sup> Mallinckrodt Baker [2].

#### 1.1.4. Technical products and impurities

Two studies describe the composition of 2-BP that was used at plants in Asia. In a Korean plant, the purity of 2-BP used was 97.4% and contaminants included *n*-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%) [3–5]. The reported purity of 2-BP used in a Chinese plant was 98.08% and contaminants consisted of 2-propanol (1.76%), dibromopropane (0.085%), benzene (0.055%), and trichloroethylene (0.10%) [6].

2-BP is a contaminant that may be present in 1-BP at 0.1% [7]. In the US, exposure to 2-BP has occurred through the use of 1-BP-containing spray adhesives. The composition of the spray adhesive was described as 55% 1-BP, 8% VM&P Naphtha, and 2% ethyl acetate in one case [8]; in another case the spray adhesive contained 60–70% 1-BP [9].

In the majority of animal studies described in Sections 2, 3 and 4, the purity of 2-BP was at least 99%.

### 1.2. Use and human exposure

#### 1.2.1. Production

2-BP is manufactured by heating isopropyl alcohol together with hydrogen bromide [1]. The various sources of information about US production of 2-BP are inconsistent. HSDB [1] reported that Great Lakes Chemical is a producer of 2-BP. However, an OSHA [10] report stated that 2-BP is not intentionally produced for commercial use in the US, but is a contaminant of 1-BP at concentrations of 0.1–0.2%. ASTM standards for vapor-degreasing and general grade 1-BP list 2-BP as a contaminant at a maximum of 0.1% by weight [7].

#### 1.2.2. Use

It has been reported by HSDB [1] that 2-BP is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organics. The extent of these uses and associated human exposures is unknown. In Asia, 2-BP has been used as a replacement for chlorofluorocarbons and 1,1,1-trichloroethane [3–6]. Since it is a contaminant in 1-BP, exposure to 2-BP may occur when 1-BP is used.

#### 1.2.3. Occurrence

Information on the possible exposure of the public to 2-BP through contact with air, drinking water, food, or consumer products does not exist.

Schwarzenbach et al. [11] reported on an investigation of leaks from a wastewater tank at a Swiss alkyl halide factory at which 2-BP was manufactured (>5 tons per year). After the plant ceased operation, the underlying aquifer was found to be heavily polluted. Following soil excavation and continuous groundwater pumping for 7 years, substantial concentrations of bromobenzene and chlorobenzene were found, but neither 1- nor 2-BP nor their corresponding alcohol metabolites could be detected in groundwater. In vitro studies by Schwarzenbach et al. [11] confirmed the rapid hydrolysis of 2-BP (half life of 2 days) under anaerobic conditions.

#### 1.2.4. Human exposure

In a Chinese chemical plant where 2-BP was manufactured, full workshift personal exposures to 2-BP were measured. Because sampling was only done during the winter, exposure measurements may not reflect the influence of summer temperatures on the highly volatile 2-BP. Ten men were sampled, of which one directly worked in the process (mixer) and two were plant repairmen. These workers had exposures of 0.95–5.84 ppm. The remaining men worked in the boiler area outside the plant or had office jobs (salesman, manager, engineer); only one of these seven workers had exposures that exceeded the detection limit (0.2 ppm). Among the 14 women sampled in the study, 11 had direct contact with 2-BP in the manufacturing process and their exposures ranged from 2.87 to 16.18 ppm. Of the three women who were accountants, one had an exposure of 0.88 ppm and the other two had exposures below the detection limit (0.2 ppm). Peak exposures in the manufacturing operations were measured by detector tube and reported to be 2.5–110.8 ppm [6].

In a Korean plant where 2-BP was used to clean electronic parts, the cleaning solution contained 97.4% 2-BP as well as *n*-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%) [3–5]. Since the process using that solution was no longer functioning, exposures to 2-BP were simulated to represent the seven assembly lines, each with a cleaning bath in a hood and the three or four automatic assembly lines. Area samples collected for 3 h near the cleaning baths measured 2-BP exposures of 9.2–19.6 ppm (mean 12.4 ppm). Since workers put their heads inside the hoods several times a day when cleaning parts, a 15-min sample was taken inside the hood. That measurement found a 2-BP level of 4140.7 ppm, as well as 29.8 ppm of *n*-hexane and <1 ppm of 1,1,1 trichloroethane. Unaccounted for in this exposure assessment were the two uncovered and unventilated temporary cleaning baths that were present for about 6 months, and that were believed to contribute significantly to worker exposures. No exposure information was collected for the “control” group. Also, no characterization of dermal exposure was done for this study [3–5].

In a series of investigations, NIOSH measured 2-BP levels at spray adhesive and vapor degreasing operations using 1-BP formulations that may contain 2-BP at 0.1–0.2% [7,10].

NIOSH measured 2-BP levels in the breathing zones of workers in a plant where a 1-BP-containing spray adhesive was used in the manufacture of furniture seat cushions [12]. Time-weighted average (TWA) full workshift personal exposures to 2-BP in 16 workers ranged from 0.08 to 0.68 ppm with a mean of 0.28 ppm. No local exhaust was present on the process.

NIOSH also measured 2-BP in a plant that used a 1-BP-containing spray adhesive to manufacture aircraft seat cushions [9]. The 2-BP measurements were made after the introduction of local exhaust systems for spray operations. The full workshift personal TWA 2-BP exposures in 30 workers ranged from <0.01 to 0.55 ppm with a mean of 0.14 ppm.

Short-term (15 min) exposures of 12 adhesive spray workers ranged from <0.1 to 0.4 ppm.

At a third plant where a spray adhesive containing 1-BP was used in the manufacture of furniture cushions, NIOSH measured TWA 2-BP exposures before and after improvements were made in engineering controls at spray stations. At the time of the first survey, engineering controls consisted of a slotted exhaust system at each spray table [8]. Full workshift TWA personal 2-BP exposures of the 12 sprayers ranged from 0.33 to 1.35 ppm (mean 0.66 ppm). Short term (15 min) samples of 2-BP sprayers varied from 0.3 to 1.56 ppm. 2-BP levels in adjacent area samples were 0.05–0.20 ppm. The second survey was conducted after locally-exhausted spray tables were enclosed on four sides to create spray booths [13]. The 12 spray stations were re-sampled on 3 days ( $n = 33$  samples) and personal TWA full shift exposures were reduced by about 60% (0.1–0.4 ppm, mean 0.1–0.2 ppm). Short term exposures (15 min) of sprayers varied from <0.13 to 0.4. Personal full shift TWA 2-BP exposures in non-sprayers varied from <0.01 to 0.1 ppm.

NIOSH also measured personal exposure to 2-BP in a plant where 1-BP was used as a cold vapor degreaser in the presence of a local exhaust system. Full workshift personal 2-BP exposures for 20 employees were all below the minimum detectable concentration of 0.004 ppm as were the short (13–14 min) task samples taken while an assembler used the degreaser [14].

### 1.3. Utility of data

The exposure data reviewed by the Expert Panel are of limited utility for risk assessment. There are no data available on consumer or general population exposures to 2-BP. There are limited data on current 2-BP exposures in the United States based on exposure surveys of four spray adhesive and vapor degreasing operations that used 1-BP contaminated with 2-BP. These surveys do not represent a cross-section of potential exposures since these investigations are prompted by request and require the cooperation of the companies involved. There is no information on the volume of usage or number of US workers exposed to 2-BP. None of the exposure evaluations to date have characterized the potential contribution of dermal exposures to the worker.

### 1.4. Summary of human exposure data

In Asia, 2-BP was used as a cleaning solvent in the electronics industry [3–5]. Data on 2-BP exposures in Korea and China were collected in conjunction with health effect studies. These data provide a very limited description of occupational exposures in the manufacturing of 2-BP and its use as a cleaning solvent. The Korean study relied on simulated exposures using area sampling exposure information (9.2–19.6 ppm) and did not collect any samples to represent the “control” group exposures. In addition, personal expo-

sure may have been higher than indicated by the area sampling, since two open unventilated baths were not included in the simulation. The study conducted in a Chinese 2-BP manufacturing plant reported personal exposure measures for a single winter day [6]. Only 14 of the 24 workers in the study had direct contact with 2-BP (0.95–16.18 ppm). Neither the Korean nor Chinese study characterized the dermal exposures of workers.

It has been reported by HSDB [1] that in the US 2-BP is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organics. The extent of this use is unknown. Currently in the US, 2-BP exposures most commonly occur because it is a contaminant in 1-BP at 0.1% [7]. Many of the US workplace operations involve hand contact with 1-BP, yet none of the 1-BP exposure evaluations have characterized the 2-BP exposure as a result of these dermal exposures. No information was found that documents exposure of the public to 2-BP through contact with air, drinking water, food, or consumer products.

In the US, current 2-BP levels were measured in a limited number of occupational settings where it was present in spray adhesive and vapor degreasing operations that use 1-BP in their operations. In the spray adhesive operations, NIOSH collected personal full shift TWA samples of adhesive sprayers prior to installation of or improvements to local exhaust controls that ranged from 0.08 to 1.35 ppm [8,12]. Personal full-shift TWA 2-BP samples of operations with local exhaust ranged from <0.01 to 0.55 ppm [9,13]. The 20 personal full-shift TWA samples taken from a plant where 1-BP was used in a vapor degreaser were all below the minimum detectable concentration of 0.004 ppm for 2-BP [14].

## 2. General toxicology and biological parameters

### 2.1. Toxicokinetics

#### 2.1.1. Human data

Studies providing quantitative information on absorption and distribution after 2-BP inhalation or ingestion of 2-BP were not identified. One study by Kawai et al. [15] may provide limited information about the metabolism and elimination of 2-BP in humans.

Kawai et al. [15] attempted to develop a system of biomonitoring for 2-BP based on a postulated metabolic pathway for 2-BP. They noted studies demonstrating that methyl bromide can be hydrolyzed to bromide ion and methanol *in vivo* and that a similar reaction takes place with ethylene dibromide. It was therefore postulated that 2-BP would be hydrolyzed to isopropyl alcohol, which is known to be oxidized to acetone. Urinary levels of 2-BP, bromide ion, acetone, and isopropyl alcohol were measured in five male Japanese workers exposed to 2-BP (mean area concentration of 3 mg/m<sup>3</sup> [0.6 ppm]) and the values were compared to 20 unexposed males. Only the foreman, who was thought to be exposed to the highest level of 2-BP,



had urinary levels of acetone and bromide that exceeded concentrations found in unexposed controls. 2-BP and isopropyl alcohol were not detected in the urine of workers or non-exposed controls. Kawai et al. [15] also examined the metabolism of 2-BP in rats; that portion of the study is described below under the animal data section.

**Strengths/weaknesses.** The analytical method for trapping and measuring 2-BP vapor concentrations, and for measuring 2-BP metabolite concentrations (isopropanol, acetone, bromide, parent) in urine was found to be linear over a reasonably wide dynamic range. The rat experiment used to establish the linearity of the urinary assay indicated that parent and isopropanol are not detectable in urine, suggesting that metabolism of parent is complete, as is oxidation of isopropanol to acetone. The biomonitoring of five workers exposed to a mean concentration of 3 mg/m<sup>3</sup> suggests that this concentration did not increase the urinary bromide or acetone level above background except in the one worker believed to be most highly exposed. It should be noted however, that when these metabolite concentrations were normalized to urinary creatinine, the values were within the range of unexposed controls. The strengths of the study are that the methods are reliable and the urinary monitoring permits an estimation of exposure from multiple routes.

**Utility (adequacy) for CERHR evaluation process.** The utility of this paper is that it provides confirmation of a metabolic pathway for 2-BP. Unfortunately, there are too few data to be able to link the metabolite biomarkers to exposure levels. The exposure level in the study is considerably lower than the vapor concentration associated with adverse effects in humans.

### 2.1.2. Animal data

Studies examining the quantitative absorption and distribution after 2-BP inhalation in animals were not identified.

Application of 2-BP to the intact occluded skin of Crl: SKH-hrBr nude mice found uptake at 3.1 mg/cm<sup>2</sup> h [16]. This in vivo percutaneous absorption rate was ~75% of that measured using an in vitro Frantz assay system for excised mouse skin.

**Strengths/weaknesses.** The Kim et al. [16] investigation is the only study available with which to evaluate the percutaneous absorption of 2-BP. The protocol utilized a gas chromatographic (GC) method to quantify 2-BP. Limitations are that the text of the study was in Korean, therefore a complete evaluation was not possible. Further, the data are only presented as histograms rather than specific data.

**Utility (adequacy) for CERHR evaluation process.** The utility of the paper lies in verification of the rapid dermal absorption of 2-BP. The rapid systemic absorption is important in consideration of the total absorbed dose received by workers described in the Kim et al. [3,4], Park et al. [5], Koh et al. [17], and Ichihara et al. [6] reports.

One inhalation, one parenteral, and one in vitro study provide some information on the metabolism and elimination of 2-BP.

A study by Kawai et al. [15] to develop biological monitoring for 2-BP exposure provides some information about the metabolism of 2-BP. Sixteen female Wistar rats/group [200 g, age not specified] were exposed to 0, 500, 1000, or 1500 mg/m<sup>3</sup> [99, 199, or 298 ppm] 2-BP [purity not specified] for 4 h. 2-BP concentrations in exposure chambers were monitored. Urine samples were collected during the 4 h of exposure and during the 4 h following exposure. GC was used to analyze urine for 2-BP, acetone, isopropyl alcohol, and bromide ion. Data were analyzed by Student's unpaired *t*-test. Dose-related and statistically significant increases were observed for 2-BP during exposure ( $\geq 500$  mg/m<sup>3</sup>), acetone during ( $\geq 500$  mg/m<sup>3</sup>) and after exposure ( $\geq 1000$  mg/m<sup>3</sup>), and bromide ion after exposure ( $\geq 1000$  mg/m<sup>3</sup>). The authors could not exclude the possibility that 2-BP found in urine during exposure resulted from direct contact of urine with the 2-BP vapors in air. The results of the experiment supported the theory that 2-BP is hydrolyzed to isopropyl alcohol and bromide ion, followed by oxidation of isopropyl alcohol to acetone and excretion of acetone and bromide ion through urine.

**Strengths/weaknesses.** The analytical method for trapping and measuring 2-BP vapor concentrations, and for measuring 2-BP metabolite concentrations (isopropanol, acetone, bromide, parent) in urine was found to be linear over a reasonably wide dynamic range. The rat experiment used to establish the linearity of the urinary assay indicated that 2-BP and isopropanol are not detectable in urine, suggesting that metabolism of 2-BP is complete, as is oxidation of isopropanol to acetone. The strengths of the study are that the methods are reliable and the urinary monitoring permits an estimation of exposure from multiple routes.

**Utility (adequacy) for CERHR evaluation process.** The utility of this paper is that it provides confirmation of a metabolic pathway for 2-BP. Unfortunately, there are too little data to be able to link the metabolite biomarkers to exposure levels.

Barnsley et al. [18] fed two male rats [age and strain unspecified] a diet containing <sup>35</sup>S-labelled yeast for 3 days, injected 2 of the rats subcutaneously with 0.7 ml of 40% w/v solution of 2-BP [purity not specified] in arachis oil on the fourth day, collected urine for 24 h following treatment, and measured metabolites in urine by radiochromatography. No significant levels of sulfur-containing metabolites were present in the urine at detectable levels.

**Strengths/weaknesses.** This paper may represent the state of the art in studying glutathione conjugation reactions in the early 1960s, but is of little use for risk assessment. One tentative conclusion is that no mercapturic acid or other S-containing conjugates of 2-BP were present in the urine of two rats dosed with 2-BP and fed a diet containing <sup>35</sup>S-labelled yeast.

**Utility (adequacy) for CERHR evaluation process.** This study is not of use for evaluating 2-BP. Because this study is limited due to the methodology available to the inves-

tigators, the panel does not have high confidence in its conclusions.

Kaneko et al. [19] studied the in vitro metabolism of 2-BP in hepatic microsomes of male Wistar rats by measuring the rate of substrate disappearance and rate of product (isopropyl alcohol) formation. The authors demonstrated that there were more than two sets of  $V_{\max}$  and  $K_m$  metabolic constants. According to the authors, differences in rates between substance disappearance and isopropyl alcohol formation suggested the possibility of alternate pathways besides metabolism of 2-BP to isopropyl alcohol or that isopropyl alcohol is further metabolized. The procedures and results for this experiment were reported in the form of a short communication.

**Strengths/weaknesses.** This paper provides partition coefficients that may be useful in the eventual construction of a PBPK model for 2-BP. The limited metabolism data in rat microsomes suggest multiple metabolic routes, but metabolism is not otherwise characterized.

**Utility (adequacy) for CERHR evaluation process.** This work will only have utility as the source of data for constructing PBPK models.

## 2.2. General toxicity

### 2.2.1. Human data

In 1995, the National Institute of Occupational Health, Korea Industrial Safety Corporation conducted an investigation in the tactile switch assembly section of a plant where a cluster of secondary amenorrhea was reported [3–5]. Twenty-five women and 8 men, ages 20–44 years, were employed in that part of the plant and worked 12-h shifts. The workers were involved in a process where tactile switch parts were dipped in baths of cleaning solution located within ventilation hoods. Prior to 1994, there were two temporary baths without ventilation hoods. In addition to inhalation exposures, some workers were exposed dermally when they occasionally dipped their bare hands into the cleaning solution. No personal protective equipment was used by workers. A limited number of female workers were subjected to short term exposure as they fixed problems occurring underneath the hood. Eighteen months prior to the investigation, a CFC-based cleaning solution was replaced with a solution consisting of 97.4% 2-BP with smaller percentages of *n*-heptane (0.33%), 1,2-dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%). Solvent concentrations in air were not measured during actual plant operations, so exposures were estimated by obtaining 14 area samples under a simulated manufacturing scenario. 2-BP levels outside the hood ranged from 9.2 to 19.6 ppm with a mean of 12.4 ppm. The short-term concentration of 2-BP inside the hood was measured at 4140.7 ppm and the *n*-heptane level was 29.8 ppm.

Effects in the 2-BP exposed group were compared to a control group of 65 females and 12 males who worked in another room of the same plant. Reproductive effects were a dominant finding in the 2-BP-exposed group and are dis-

cussed in Section 4. Eight women who were suffering from amenorrhea also experienced pancytopenia. Mild anemia or leukopenia were observed in the other women. A bone marrow biopsy conducted in two women with marked pancytopenia revealed hypoplastic marrow. Those two women complained that they bruised easily. Mild pancytopenia was reported in one male worker who was also azoospermic but was not reported in males with oligospermia. Blood disorders were subsequently reported to be transient [4]. Symptoms such as headache, dizziness, or weakness were reported by many workers. In males and females, clinical tests revealed no effects on blood clotting, kidney and liver function (except for one male), or thyroid function. Chest X-rays, urinalysis, and electrocardiographs (EKG) were also normal. Numbness and paralysis of hand muscles were subsequently reported by the workers [20]. Additional details are not available in a report written in English.

**Strengths/weaknesses.** These papers describe the cluster of health effects associated with 2-BP exposure. They do a good job of narrowing down the type of work associated with the adverse effect cluster and provide good circumstantial evidence of the involvement of 2-BP in the toxicity. Clinical evaluations of reproductive and hematological effects are adequate and provide some clues about mode of action. The vapor concentrations of 2-BP in the work area were simulated, but a detailed exposure scenario is not available. Only area samples were measured and the duration of short-term exposures is not known. More importantly the simulated conditions may not have replicated actual exposures that occurred when two unventilated cleaning tanks were present in the area prior to February through November 1994. Qualitative information about dermal exposure is given in that some workers occasionally (frequency and duration not specified) dipped bare hands into 97% 2-BP.

**Utility (adequacy) for CERHR evaluation process.** These papers provide a good description of the human hazard potential of dermal/inhalation exposure to relatively high levels of 2-BP in an occupational setting. There are insufficient data for dose–response assessment.

Ichihara et al. [6] examined reproductive and hematological effects in workers of a Chinese 2-BP plant in order to obtain information about dose–response relationships. Reproductive findings and complete study details are provided in Section 4. Personal air samples were collected in 14 women (age 24–54 years) and 11 males (age 31–56 years) who worked 8 h per day, 5 days per week and were employed at the plant for 5–69 months. Personal TWA exposures of 2-BP exceeded the detection limit (0.2 ppm) in 3 men and 11 women directly exposed to 2-BP; levels ranged from 0.95 to 5.84 ppm and 2.87 to 16.18 ppm in men and women, respectively. Accountants, boilers, salesmen, and the assistant manager rarely entered the factory floor and served as referents. Exposures according to job category are listed in Section 4. No information was provided about use of personal protective equipment. Five female operators, who prepare 2-BP then pour it into containers,

had slight anemia as indicated by red blood cell (RBC), hemoglobin (Hb), or hematocrit (Ht) values; exposures in those operators ranged from 5.80 to 10.74 ppm. In a comparison of accountants with normal menstrual cycles (exposure = < 0.2–0.88 ppm; age 26–34 years), operators with normal menstrual cycles (exposure = 4.09–8.60 ppm; age 25–40 years) and operators with amenorrhea or polymenorrhea (exposure = 4.14–16.18 ppm; age 39–54 years), it was found that Hb, Ht, and white blood cell (WBC) levels were lower in operators with normal cycles compared to accountants. However, levels of Hb, Ht, and WBC levels in operators with amenorrhea or polymenorrhea did not differ from those of accountants but were significantly higher than levels in female operators with normal menstrual cycles. Regression analysis revealed significant relationships between TWA exposure and RBC, Ht, and Hb levels in women. A significant inverse relationship between TWA exposure  $\times$  duration of employment and Hb and Ht levels was observed. Lower concentrations of RBCs, Hb, and Ht were observed in two males exposed to the highest concentrations of 2-BP (1.20 and 5.84 ppm). However, regression analysis revealed no significant relationship between male hematological indices and TWA or TWA  $\times$  duration of employment. The authors concluded that severe hematopoietic disorders were not observed but that a possible adverse effect on hematopoiesis following exposure to less than 10 ppm 2-BP could not be disproved. Authors stated that additional studies are needed to characterize the toxicity of 2-BP.

**Strengths/weaknesses.** Appropriate hematological and reproductive clinical measurements were made of workers in a 2-BP production plant. Vapor concentrations associated with each task in the synthesis, processing, and storage of the 2-BP were measured. The authors expressed concern that these concentrations, measured in December in a plant that did not have an air-handling system, may not have been representative of concentrations of the volatile material in warmer months. In the results section, the authors describe 2-BP concentrations for workers doing various tasks and many of the concentrations are higher than the TWA concentrations that are listed in the tables of the study. However, the authors do not describe how those values were obtained; for example, it is not known if those values represent short term measurements for a particular task. Hematological effects were correlated with higher exposures to 2-BP. There did not appear to be effects at concentrations lower than 10 ppm, but the small size of the study makes it difficult to draw definitive conclusions.

**Utility (adequacy) for CERHR evaluation process.** The report supports the Korean reports of adverse hematological effects of 2-BP. The demographics of the workforce, particularly the fact that only older women (ages 40–50) had menstrual disturbances makes this study less useful in confirming ovarian dysfunction. The airborne concentration measurements of 2-BP support an exposure–response relationship, but the data are inadequate to support the dose–response analysis phase of risk assessment. In ad-

dition, the results of regression analyses with such small number of subjects, few exposure measurements, and narrow range of mean exposure concentrations (0.9–16 ppm) is misleading. Lastly, there is no mention of short-term exposure monitoring. Quantitative data describing the frequency and duration of short-term, high exposure would have been useful for determining if adverse effects are related to peak exposures.

### 2.2.2. Animal data

Kim et al. [21] conducted an acute LC<sub>50</sub> study of 2-BP (solvent 5200; 99.01% purity) in 8–9-week-old ICR mice (from Daehan Experimental Animal Center). Three mice/sex/group inhaled 0, 26,604, 30,771, 31,864, 32,492, or 34,651 ppm 2-BP [0, 133,818, 154,778, 160,276, 163,435, or 174,295 mg/m<sup>3</sup>] (chamber concentrations monitored) for 4 h and were observed for 14 days. Male and female mice exposed to 32,492 or 34,651 ppm usually died during exposure, while mice exposed to 30,771 or 31,864 ppm lived until the end of the exposure day. No obvious lesions were observed at necropsy in the respiratory, reproductive, or hepatic organs. An LC<sub>50</sub> of 31,171 ppm was estimated using a dose–mortality curve at a 95% confidence level. The LC<sub>100</sub> was >32,905 ppm and the lowest lethal concentration was <29,528 ppm.

**Strengths/weaknesses.** The report describes an LC<sub>50</sub> determination in mice. The work was adequately done and the calculated LC<sub>50</sub> appears reliable.

**Utility (adequacy) for CERHR evaluation process.** This study provides hazard data for acute toxicity of 2-BP.

Ichihara et al. [22] conducted a study to determine the testicular and hematopoietic toxicity of 2-BP in 13-week-old Wistar rats (from Shizuoka Laboratory Animal Center). Additional details about the examination of bone marrow in this study were reported by Nakajima et al. [23,24]. This section covers systemic parameters while reproductive findings and complete study details are discussed in Section 4. The experimental protocol had nine male rats per group exposed by inhalation to 0, 300, 1000, or 3000 ppm [1509, 5031, or 15,092 mg/m<sup>3</sup>] 2-BP for 8 h per day, 7 days per week for 9 weeks. Excessive toxicity in the high-dose group led to termination of exposure after 9–11 days. Three rats in this high-dose group were sacrificed immediately after exposure; the remaining six rats were exposed to filtered air for the remainder of the 9-week study. Non-reproductive organs that were weighed and preserved in 10% formalin included the liver and kidneys. All treated rats experienced a dose-dependent reduction in bodyweight gain. Rats in the 3000 ppm group began to recover bodyweight after 2-BP exposure ended and bodyweights at the end of the study were equivalent to the 300 ppm group. Absolute kidney weight was reduced at 300 and 1000 ppm while absolute liver weight was reduced at 1000 ppm. There were no histopathological findings or effects on relative liver and kidney weights at 300 and 1000 ppm. Changes in RBC numbers were indicative of macrocytic anemia according to the study authors. Signif-



icant changes in hematological parameters included reductions in erythrocyte numbers ( $\geq 300$  ppm), Hb (1000 ppm), platelets (300 and 1000 ppm), and leukocytes (1000 ppm). Histological examinations revealed hypocellular and fatty bone marrow in rats exposed to 1000 or 3000 ppm that was characterized by dose-related increases in adipose cells and reductions in megakaryocytes [23,24]. There was only slight recovery of adipose cell and megakaryocyte numbers in the six rats of the 3000 ppm group that were exposed to air for about 7 weeks of the study; the cell numbers did not reach levels equivalent to those observed in lower dose groups. There were no changes in the ratio of granulocytes to erythrocytes at any dose.

**Strengths/weaknesses.** The study was a subchronic inhalation study in male rats, with exposures conducted for 8 h per day, 7 days per week for 9 weeks. The number of animals per group, 9, was close to the expected number of 10 for a guideline study. There were three treatment groups, but the highest concentration was excessively toxic; therefore, three of these animals were sacrificed in extremis 11 days into the dosing period and the other six were taken off treatment for the remainder of the 9 weeks. The results demonstrate the toxicity of 2-BP to hematological and male reproductive systems, without a No Observed Adverse Effect Concentration (NOAEC) identified. While this study is not a complete subchronic toxicity protocol, the examination of the male reproductive systems and hematological parameters was as or more thorough than the typical subchronic study.

**Utility (adequacy) for CERHR evaluation process.** This study helps characterize the hazard potential of 2-BP to the blood and male reproductive system. It provides support that the effects observed in the human cluster studies are indeed attributable to 2-BP exposure. The data are useful for dose–response assessment, although the lack of NOAEC should be compensated for by the calculation of a benchmark concentration.

Yu et al. [25] conducted a 2-BP toxicity study in rats to verify that adverse effects in the hematopoietic and reproductive systems of workers of a Korean electronics plant were due to 2-BP exposure [3]. Ten male Sprague–Dawley rats per group (~12 weeks old; from Daehan Animal Center) were injected intraperitoneally (i.p.) with 0, 125, 250, or 500 mg/kg bw 2-BP in olive oil, six times per week for 4 weeks. The authors acknowledged that the administration route does not pertain to occupational exposures, but stated that inhalation tests are required only if negative results are obtained with i.p. exposure. Data were evaluated by two-way ANOVA and Duncan's multiple range test. This summary describes the findings other than reproductive effects while reproductive findings and complete study details are discussed in Section 4. Clinical signs included dizziness and lethargy 15 min after dosing in all treated animals. Bodyweight gain and terminal bodyweight were significantly lower in rats exposed to 250 and 500 mg/kg bw. Significant dose-related increases in relative organ weights were observed for the adrenals of rats exposed to 250 mg/kg

bw and higher and for the lungs, spleen, liver, and brain at the highest dose (500 mg/kg bw). A histological evaluation of kidneys, liver, and pituitary revealed no distinct histopathology. Hematological evaluation demonstrated significant reductions in WBC count, lymphocyte count, Hb concentration, and mean platelet volume in the 500 mg/kg bw group. Authors also noted a dose-related trend for reductions in granulocytes and monocytes. The only significant dose-related effects observed in the blood chemistry analysis were reduced alkaline phosphatase activity and increased cholesterol in the 500 mg/kg bw per day group.

**Strengths/weaknesses.** This study was a 28-day subchronic toxicity study in male rats, with 2-BP being given i.p. 6 days per week. The analysis included bodyweights, organ weights, hematologic and reproductive parameters. The results confirm the hematological and male reproductive effects. The route of administration makes these data dubious for anything other than qualitative support of hazard. One potential concern is the high hematocrit and red cell volumes in the lowest dose 2-BP group. The values are unusually high and may signify a possible methodological problem.

**Utility (adequacy) for CERHR evaluation process.** The study is useful as part of the weight of the evidence of hazard to male reproductive and hematopoietic systems. The use of an i.p. route limits further use in risk assessment.

Because Korean workers exposed to 2-BP complained of numbness and paralysis in hand muscles, Yu et al. [20,26] studied the neurological effects of 2-BP in rats. Nine, 10-week-old male Wistar rats/group (from Shizuoka Laboratory Animal Center) were exposed to filtered air or 100 or 1000 ppm [503 or 5031 mg/m<sup>3</sup>] 2-BP vapors (99.4% purity) for 8 h per day, 7 days per week, for 12 weeks. [No rationale was provided for dose selection.] Chamber concentrations were monitored. Neurological function was tested at weeks 0, 4, 8, and 12 by measuring motor nerve conduction velocity (MCV) and distal latency (DL). Parameters evaluated at sacrifice included blood chemistry, organ weight measurement, and histopathology in an unspecified number of rats. Hematological analysis were conducted in eight rats per group and the nervous system of 1 rat per group was examined histologically. Data were analyzed by ANOVA followed by the Tukey–Kramer multiple comparison method. None of the parameters examined were affected at 100 ppm. Clinical signs of neurotoxicity, such as changes in lacrimation, salivation, pupillary response, reaction to stimuli, alertness, or pain perception, convulsions, tremors, or abnormal movements, were not observed. However, MCV was significantly reduced at week 8, and DL was significantly prolonged at weeks 8 and 12 in the 1000 ppm group. Histological examination revealed a scattered ball-like swelling in the myelin sheath of the common peroneal nerves of the tibia, but no effects were noted in spinal cord or brain. Bodyweight gain and absolute weight of brain, liver, and kidney were significantly reduced in rats of the 1000 ppm group. Histological findings for liver and kidney were not reported. Testicular histopathology is discussed in Section 4.2.2. Hematologi-

cal effects included significant reductions in erythrocytes, platelets, and leukocytes. Blood chemistry parameters (liver enzymes, glucose, protein, blood urea nitrogen, lipids, and electrolytes) were not affected. Authors concluded that long-term exposure to 1000 ppm 2-BP could lead to peripheral neuropathy.

**Strengths/weaknesses.** This study involved inhalation exposure of male rats for 8 h per day, 7 day per week, for 12 weeks to 100 or 1000 ppm 2-BP. The number of animals per group was nine, reasonable for this type of experiment, and the inhalation exposures were controlled adequately. There were progressive effects on peripheral nerve function, evaluated by repeated measures of the same animals. There was some histological evidence of myelin irregularities of peripheral nerves. The NOAEC was 100 ppm. There were no apparent central nervous system (CNS) effects. It was not clear whether the data presented in Yu et al. [26] is from the same experiment as Yu et al. [20] or is a replicate experiment. The means and standard deviations for the achieved air concentrations of 2-BP are identical for the two reports, with the exception that the earlier paper reports two decimal places, the latter, one.

**Utility (adequacy) for CERHR evaluation process.** This study identifies another potential toxic effect of 2-BP and provides data that could be used in dose–response assessment.

Zhao et al. [27] conducted a study to compare the neurotoxicity of 2-BP, 1-BP, and 2,5-hexanedione (2,5-HD). Seven to nine male Wistar rats per group (age not specified; from Seiwa Experimental Animal Institute) were injected subcutaneously (s.c.) with each chemical in olive oil 1 time per day, 5 days per week, for 4 weeks. Doses administered were 1.1, 3.7, or 11.0 mmol/kg bw 2-BP; 3.7 or 11 mmol/kg bw 1-BP; and 2.6 mmol/kg bw 2,5-HD. Purity of all chemicals was >97%. A control group of nine rats was injected with the olive oil vehicle. According to the study authors, doses of 1.1, 3.7, and 11 mmol/kg bw are equivalent to doses received by inhalation of 100, 300, and 1000 ppm BP. Bodyweights were measured weekly and maximum motor nerve conduction velocities (MCV) and motor latency (ML) were measured every 2 weeks. Data were analyzed by one-way ANOVA followed by Duncan's multiple range test. Bodyweight gain in the 11 mmol 2-BP/kg bw group was lower compared to the control group. By 2 weeks of exposure, the MCV began decreasing in treated rats and reached statistical significance in the 3.7 and 11 mmol 2-BP/kg bw groups at week 4. Dose- and time-related increases in ML occurred but were not statistically significant. All three compounds, 2-BP, 1-BP, and 2,5-hexanedione, produced qualitatively similar responses in MCV and ML. The authors concluded that 2-BP and 1-BP were equally potent and that both compounds were less potent than 2,5-hexanedione.

**Strengths/weaknesses.** This study evaluated peripheral nerve function in rats given 2-BP by s.c. injection on a 5 day per week basis for 4 weeks. It reported effects on MCV. These results appear to support those of Yu et al. [20], but

use of the s.c. route and the limited number of animals per group (seven for all but the high dose of 2-BP) limit the usefulness of this study.

**Utility (adequacy) for CERHR evaluation process.** This study confirms the hazard of 2-BP to the peripheral nervous system. The s.c. route of exposure, small sample size, and limited measurements limit its usefulness for risk assessment.

### 2.3. Genetic toxicity

Maeng and Yu [28] examined the mutagenicity and clastogenicity of 2-BP. A reverse mutation assay was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*. Five different 2-BP concentrations were tested in duplicate in a preliminary (50–5000  $\mu\text{g}$  per plate) and second (313–5000  $\mu\text{g}$  per plate) assay with and without S9 metabolic activation. Dimethyl sulfoxide (DMSO) was used as a negative control and positive controls included 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine, and 2-aminoanthracene. A dose-related increase in mutations was noted with 2-BP treatment in strain TA100 with S9 activation and in TA1535 with and without activation. Results in *E. coli* and the other *Salmonella* strains were negative. The authors stated that mutation in strains TA100 and TA1535 indicate base-pair substitutions. **[The Expert Panel notes that strains TA100 and TA1535 are known to possess nascent GST activity [29,30] which may be linked to the mutations observed.]**

For the in vitro chromosomal assay, Maeng and Yu [28] treated Chinese hamster lung (CHL) cells in duplicate for 24 h with six concentrations of 2-BP ranging from 0.077 to 2.46 mg/ml without metabolic activation and for 6 h with S9 metabolic activation. Doses were based on a preliminary study that demonstrated growth inhibition with 2-BP treatment at 4.92 mg/ml. The negative control was DMSO and positive controls were mitomycin C and cyclophosphamide. 2-BP treatment did not produce chromosomal aberrations in the presence or absence of metabolic activation.

Maeng and Yu [28] conducted a micronucleus assay by injecting 10 Sprague–Dawley rats/sex/group (from Dae-Han Experimental Animal Center) i.p. with 0, 125, 250, or 500 mg/kg bw per day 2-BP in olive oil for 28 days. On the day after treatment was completed, the rats were sacrificed and the bone marrow was stained to check for the frequency of micronucleated polychromatic erythrocytes. 2-BP treatment did not increase the frequency of micronucleated erythrocytes. Doses were sufficient to produce toxicity in rats as suggested by decreased weight gain in all treated females and males exposed to  $\geq 250$  mg/kg bw per day. According to the authors, a dose-related reduction in polychromatic erythrocytes in all treated rats may indicate 2-BP-induced hematopoietic inhibition in the bone marrow.

Ishikawa et al. [31] examined cytogenetic effects in embryos recovered from 15 Jcl:ICR strain mice/group (Clea

Japan Co.) injected i.p. with a single dose of olive oil or 300, 600, 900, or 1800 mg/kg bw 2-BP [**purity not specified**] during the early preimplantation period [**specific gestation day (gd) not specified**]. The highest dose was based upon Organization for Economic Co-operations and Development (OECD) and UK Environmental Mutagen Society guidelines and the i.p. route was selected because the authors claim it is more effective than the oral route for detecting clastogenic effects. The dams were sacrificed on gd 3 and viable embryos were examined for micronuclei frequency. The litter was considered the unit for statistical analyses which included arcsine transformation followed by one-way ANOVA and Games–Howell’s multiple comparison test. Dams experienced no changes in weight gain or other signs of toxicity. Treatment did not result in increased numbers of degenerated embryos. There was a significant, dose-related increase in the number of micronuclei/embryo and the percentages of embryos with micronuclei in the 900 and 1800 mg/kg bw dose groups. The authors also reported a 2-BP-induced reduction in cell numbers but it is not clear at which dose the effect becomes statistically significant. The authors concluded that maternal exposure to 2-BP may result in a “developmental disadvantage.”

**Strengths/weaknesses.** These studies used established in vitro methods to evaluate mutagenic potential, and in vitro and in vivo techniques to evaluate cytogenetic effects. There was a mutagenic effect in two strains of *Salmonella*, one only with metabolic activation, one with and without metabolic activation. The two strains both detect base-pair substitutions, raising the likelihood that 2-BP, at least under some circumstances, is mutagenic. The bone marrow cytogenetics assays were both negative. There was a reported increase in micronuclei in mouse embryos after maternal i.p. exposure. There is no clear explanation why the bone marrow assays were negative but the embryo assay was positive, although one could surmise that the i.p. treatment regimen exposed the embryos to much higher concentrations of 2-BP. These assays conform to accepted regulatory practices.

**Utility (adequacy) for CERHR evaluation process.** The results of these studies are directly useful in characterizing the genotoxic potential of 2-BP.

#### 2.4. Carcinogenicity

No carcinogenicity studies were located.

#### 2.5. Potentially sensitive subpopulations

There are limited data available to distinguish subpopulations that may be particularly sensitive to exposure to 2-BP. However, the epidemiological study by Ichihara et al. [6] suggests that women nearing the end of their reproductive years may be more sensitive to the follicle-depleting effects of 2-BP due to the smaller number of follicles present in their

ovaries compared to younger women. This phenomenon has been observed with other follicle-depleting toxicants such as the chemotherapeutic drug cyclophosphamide.

### 2.6. Summary of general toxicology and biological effects

#### 2.6.1. Toxicokinetics

Evidence from occupationally exposed humans and animal toxicity studies indicate that 2-BP is absorbed following inhalation exposure. There is some evidence that 2-BP is readily absorbed across the skin of nude mice [16]. Limited information is available on the metabolism of 2-BP. Measurement of urinary metabolites in humans and rats exposed to 2-BP by inhalation suggests that 2-BP is hydrolyzed to isopropyl alcohol and bromide ion, followed by oxidation of the alcohol to acetone and excretion of bromide and acetone through urine [15]. In vitro experiments with rat hepatic microsomes suggests there are multiple pathways for metabolism of 2-BP [19]. A study in rats tentatively demonstrates that 2-BP is not excreted in urine in the form of mercapturic acid or other sulfur containing conjugates [18]; however this study is limited due to the methodology available to the investigators and the panel does not have high confidence in its conclusions.

#### 2.6.2. General toxicity

Toxicity was noted in occupationally exposed humans. Blood disorders ranging from mild anemia and leukopenia to pancytopenia with hypoplastic bone marrow were observed in male and female workers exposed to 2-BP in a Korean plant at estimated exposure levels of 9.2–19.6 ppm with possible short term exposures of 4140.7 ppm [3–5]. Other symptoms reported by workers included headache, dizziness, and numbness and paralysis of hand muscles. In 11 female Chinese workers who were directly exposed to 2-BP at 2.87–16.18 ppm, there were significant inverse relationships between TWA 2-BP exposure and RBC, Ht, and Hb levels and TWA 2-BP exposure × duration of employment and Ht and Hb levels [6]. No relationship was found between hematological parameters and 2-BP exposure in three male manufacturing workers exposed to 0.95–5.84 ppm. Reproductive disorders were reported in both sexes of workers in the Korean and Chinese studies, these are discussed in Section 4. Concerns regarding exposure assessment and small study sizes limit the ability of these studies to quantify dose–response relationships for risk assessment.

An LC<sub>50</sub> of 31,171 ppm was determined for mice exposed to 2-BP by inhalation for 4 h [21]. In male rats, repeat-dose inhalation toxicity studies conducted for 9–12 weeks demonstrated that 2-BP targets the hematopoietic system at concentrations ≥300 ppm (1509 mg/m<sup>3</sup>) and the nervous system at concentrations ≥1000 ppm (5031 mg/m<sup>3</sup>) [20,22,26]. Table 2 illustrates the major general toxicity findings in the inhalation studies. Hematological effects included decreased numbers of erythrocytes, Hb, platelets, and/or leukocytes [20,22,26], while histopathological evalu-

Table 2  
Summary of general toxicity inhalation studies in male rats

Concentration in ppm (mg/m <sup>3</sup> )	Exposure regimen	Sex/species/strain	Dose: effect <sup>a</sup>	Reference
300 (1509); 1000 (5031); 15092 (15092)	8h/7d/9wk; whole body (9–11 days exposure period in high dose)	Male Wistar rat	300 ppm (1509 mg/m <sup>3</sup> ): ↓ bodyweight gain; ↓ absolute kidney weight; ↓ erythrocytes and platelets 1000 ppm (5031 mg/m <sup>3</sup> ): ↓ bodyweight gain; ↓ absolute kidney and liver weight ↓ erythrocytes, hemoglobin, platelets, hematocrit and leukocytes; hypocellular marrow 15092 ppm (15092 mg/m <sup>3</sup> ): ↓ bodyweight gain; ↓ erythrocytes; hypocellular marrow	Ichihara et al. [22]
100 (503); 1000 (5031)	8h/7d/12wk; whole body	Male Wistar rat	NOAEC = 100 ppm (503 mg/m <sup>3</sup> ) 1000 ppm (5031 mg/m <sup>3</sup> ): ↓ bodyweight; ↓ absolute brain, liver, and kidney weight; ↓ erythrocytes, platelets and leukocytes; ↓ motor nerve conduction velocity and ↑ distal latency; lesions in myelin sheath of tibial peroneal nerves	Yu et al. [20,26]

↑: increased effect; ↓: decreased effect; d: day; h: hour; wk: week.

<sup>a</sup> Reproductive effects are summarized in Section 4.

ation revealed hypocellular and fatty bone marrow [22,23]. Neurotoxicity was characterized by reduced MCV, increased distal latency, and swelling of the myelin sheath of the common peroneal nerves of the tibia [20,26]. No histological effects were noted in brain or spinal cord. There were no clinical signs of neurotoxicity such as changes in lacrimation, salivation, pupillary response, reaction to stimuli, alertness, or pain perception, convulsions, tremors, or abnormal movements. No effects on blood chemistry parameters were observed. Reproductive effects were observed and are discussed under Section 4. Two additional studies were conducted in male rats exposed i.p.. Though the route is not relevant to human exposure scenarios, the studies demonstrated adverse affects on the hematopoietic system [25] and nervous system [27] that were qualitatively consistent to those observed in the inhalation studies.

Effects on the hematopoietic and nervous system were not determined for female rats exposed through any route. One reproductive study in female rats found decreased weight gain, activity, and muscle tonus following exposure to 1000 ppm (5031 mg/m<sup>3</sup>) 2-BP vapors for 8 h per day for 9 weeks [32]. Organ weight changes included increased relative liver weight and decreased absolute spleen and absolute and relative thymus weight; no histopathological changes were noted in the organs. Reproductive effects in female rats are addressed in Section 4.

### 2.6.3. Genetic toxicity

2-BP was mutagenic in *Salmonella* strains TA100 with metabolic activation and in TA1535 with and without activation but was negative in strains TA98 and TA1537 and in *E. coli* [28]. Strains TA100 and TA1535 are known to possess nascent GST activity [29,30] which may be linked to the mutations observed.

2-BP did not induce chromosomal aberrations in an in vitro assay with Chinese hamster lung cells or bone marrow micronuclei formation in Sprague–Dawley rats [28]. There was a reported increase in micronuclei in mouse embryos after maternal i.p. exposure [31]. There is no clear explanation why the bone marrow assays were negative but the embryo assay was positive, although one could surmise that the i.p. treatment regimen exposed the embryos to much higher concentrations of 2-BP.

### 2.6.4. Carcinogenicity

No carcinogenicity data was identified.

## 3. Developmental toxicity data

### 3.1. Human data

A 26-year-old woman who suffered ovarian failure after a 16-month exposure to 2-BP in a Korean electronics plant never regained menstrual cycles but later became pregnant and gave birth to a normal full term infant [17]. A 6-month check-up revealed that the infant was healthy. Additional details of the study are included in Section 4 [3,17].

### 3.2. Experimental animal toxicity

Litter size was reduced in Sprague–Dawley rats treated i.p. with 2-BP at 300 mg/kg bw per day and higher for 14 days prior to mating and during a 7-day mating period; there were no gross abnormalities observed at doses up to 900 mg/kg bw [33]. Additional details of this study are included in Section 4.



### 3.3. Utility of data

Although the case report is interesting, it is of no real usefulness for risk assessment in that (1) it is a single instance, and it is very hard to infer anything from a single case, and (2) the exposure to 2-BP ceased prior to conception. Therefore, the embryo was not exposed to the agent of interest. In the animal study, the period of treatment does not include the period of embryogenesis, during which the organs develop and the embryo is most susceptible to agents that cause structural malformations. Therefore, the study was inadequate to assess the potential of 2-BP to cause structural abnormalities. The decreased litter size is an interesting observation, but because of the treatment schedule it is not possible to determine whether it is attributable to effects on the embryo or one or both of the parents. Finally, the i.p. route of administration renders the study of no utility for quantitative risk assessment as this is not a relevant route of exposure.

### 3.4. Summary of developmental toxicity

There are insufficient data upon which to evaluate the developmental toxicity of 2-BP in either humans or experimental animals.

In one case report, a healthy, full-term infant was born to a 26-year-old woman who suffered ovarian failure after a 16-month exposure to 2-BP prior to the pregnancy [17]. In a limited animal study, a lack of gross abnormalities was noted in the offspring of rats treated i.p. with up to 900 mg/kg bw 2-BP for 2–3 weeks prior to conception [33].

## 4. Reproductive toxicity

### 4.1. Human data

In 1995, the National Institute of Occupational Health, Korea Industrial Safety Corporation conducted an investigation in the tactile switch assembly section of a plant where a cluster of secondary amenorrhea was reported [3–5]. Twenty-five women and 8 men, ages 20–44 years, were employed in that part of the plant and worked 12 h shifts. The workers were involved in a process where tactile switch parts were dipped in baths of cleaning solution located within ventilation hoods. Prior to November 1994, two temporary baths without ventilation hoods were used in the plant. In addition to inhalation exposures, some workers were exposed dermally when they occasionally dipped their bare hands into the cleaning solution. No personal protective equipment was used. A limited number of female workers were subjected to short term exposure as they fixed problems occurring underneath the hood. Eighteen months prior to the investigation, in February 1994, a CFC-based cleaning solution was replaced with a solution consisting of 97.4% 2-BP with smaller percentages of *n*-heptane (0.33%), 1,2-

dibromopropane (0.2%), and 1,1,1-trichloroethane (0.01%). Solvent concentrations in air were not measured during actual plant operations so exposures were subsequently estimated by obtaining 14 area samples under a simulated manufacturing scenario. 2-BP levels outside the hoods ranged from 9.2 to 19.6 ppm with a mean of 12.4 ppm. The short-term concentration of 2-BP inside the hood was measured at 4140.7 ppm and the *n*-heptane level was 29.8 ppm. Effects in the 2-BP-exposed group were compared to a control group of 65 females and 12 males who worked in another room of the same plant. Medical histories revealed that 16 of the women exposed to 2-BP for 4–16 months were experiencing secondary amenorrhea. Normal menstrual cycles were reported prior to exposure. The women also had elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels, but normal prolactin levels. Blood estradiol levels were measured in three women and found to be below the detection limit of 13.6 pg/ml. Progesterone withdrawal bleedings were not observed. Ten of the women complained of hot flashes. Based on symptoms and reproductive hormone levels, the authors diagnosed the 16 women with ovarian failure. None of 66 women who worked in two other departments in the same plant had amenorrhea [5]. Semen analysis revealed azoospermia in two men and oligospermia (<20 million sperm/ml or <50% motile) in four men who were exposed for a period of 16–19 months. FSH levels were near the upper normal range and testosterone levels were in the normal range. None of the men reported a loss of libido. Based on hormonal analysis, the authors concluded that germ cells and not Leydig cells were the target tissue in the affected men. None of 12 men who worked in two other departments in the same plant developed azoospermia or oligospermia [5]. Blood disorders and other systemic effects were observed in the workers and are described in detail in Section 2. Onset of the majority of reproductive dysfunction cases in both men and women occurred during the period when two open cleaning baths without exhaust ventilation containing 2-BP were in use [5]. None of the cases had onset of reproductive dysfunction prior to the switch to the 2-BP based solvent. The authors concluded that 2-BP was the most likely cause of health problems. Previous monitoring programs ruled out involvement by other toxic agents such as ionizing radiation, lead, formaldehyde, ethylene glycol, ether and its acetates, benzene, dinitrobenzene, and dibromochloropropane. Other confounding factors that were considered included use of oral contraceptives or any other special medications, smoking, and alcohol consumption.

**Strengths/weaknesses.** A strength of this study is that reproductive problems were verified by clinical measures of semen quality and ovarian ultrasound, along with serum hormones. The discussion integrates the findings and compares them with the animal toxicology data available at the time—results are consistent across species. The higher than expected incidence of reproductive effects in this cluster



of cases, as well as the absence of reproductive abnormalities in the unexposed workers in the same plant, provides evidence that 2-BP is likely to be a human reproductive toxicant.

These three papers report on the same cluster of cases. Park et al. [5] is the most thorough of the three reports and it still suffers from lack of epidemiologic and laboratory rigor. None of the laboratory test methods are described or referenced in sufficient detail. Therefore, it is not possible to judge the quality of these data. However, methodological details for tests are not typically provided in many clinical papers when the tests are considered to be standard tests. Reporting of the test results was incomplete because the data were not presented in a table with mean values and standard deviations for measures that differed between groups. The scope of information collected by questionnaire appears to be very limited. Statistical analysis is rudimentary with no evidence that potential confounding factors such as age (especially for peri-menopausal women), sexual abstinence (for the men), medical history or lifestyle factors that may affect reproductive function and may be associated with exposure groups were appropriately controlled for. For example, it appears that no adjustments were made to control for greater numbers of smokers in the exposed versus the control group. Regression analysis could have been conducted using several different exposure (duration of employment or exposed versus unexposed, for example) and outcome measures (hormone concentrations, presence or absence of amenorrhea, azoo/oligospermia) with adjustment for age, smoking, and other potential confounders. Even more simply, bivariate analyses such as *t*-tests or chi square tests could have been used to compare the various outcomes in the exposed and unexposed groups. The study was relatively small and may have had limited power to precisely estimate the association with some outcomes. Incidence of the endpoints was considered by time of employment, but severity of effect was not related to exposure. It is noted that severity of effect appeared to be greatest in employees working during the period when unventilated tanks were present (February–November 1994). Table 4 of the Park et al. [5] study shows that 17 of 18 women who started work between February and July of 1994 developed disorders, while none of those (admittedly smaller groups) who started after August 1994 developed disorders. In this regard, it would have been most useful to use November 1994 as the cut-off date for data analysis. Incidence data expressed per 100 workers is misleading since the number of cases in each exposure group was very small. The “control” population appears to have been selected after the fact, which may have been problematic because the laboratory testing would then have been conducted at a different time than for the exposed group. On the other hand, a strength of the control groups is that they were employed in the same firm, presumably in similar jobs, except for the 2-BP exposure. There is no evidence that informed consent was obtained, or that the study protocol was reviewed by an Institutional Review Board. There is no at-

tempt to report participation rates and other factors indicative of potential selection bias. For example, it is not stated if the 33 workers comprised the entire group of workers in the tactile switch assembly section or a selected sample.

**Utility (adequacy) for CERHR evaluation process.** The findings in these papers are compelling, but the experimental design and analysis are questionable. Park et al. [5] provides the most complete details of the three reports of the same cluster, but it is still a descriptive paper. The utility of the papers for quantitative evaluation is limited because of the uncertainty in the exposure estimates, small sample size, and inadequate statistics. Because of these limitations, it is not possible to estimate human risk from a given exposure.

Koh et al. [17] conducted a pathological examination and 24-month follow-up study on the 16 Korean women who suffered 2-BP-induced ovarian failure [3]. Six of the women underwent a laparoscopic examination and ovarian biopsies were conducted in four of those women. Results of the laparoscopic examination were varied and revealed ovaries that were either atrophied, small in size, or near normal in appearance. Biopsy results were consistent with and were similar to those noted with ovarian damage from radiation or chemotherapy treatment. Findings of the biopsies included focal or diffuse fibrosis in the ovarian cortex, atrophied follicles lacking oocytes or granulosa cells, follicular developmental arrest, reduced numbers of primary follicles and corpora albicans, and hyalinization of blood vessels in the medulla. The majority of women were given estrogen–progesterone replacement therapy for 24 months. Every 6 months, the therapy was discontinued for 2–3 months to see if menstruation resumed. After 12 months of replacement therapy, consistent menstrual cycles and normal serum levels of estradiol, LH, and FSH were observed in a 24-year-old woman who was exposed to 2-BP for 5 months. One 26-year-old woman who was exposed to 2-BP for 16 months did not receive estrogen–progesterone replacement and did not resume menstruation. However, 7 months into the study she was found to be 6-weeks pregnant. Although serum estradiol levels were low for gestational stage, low serum LH and FSH levels suggested recovery of ovarian function. The woman delivered a normal full term infant and was able to breast feed. At 6 months of age, the infant was healthy and there were no problems with maternal lactation.

**Strengths/weaknesses.** This case study is a follow up on the women identified with secondary amenorrhea in Kim et al. [3,4] and Park et al. [5] studies. Ovarian biopsies from six women showed fibrosis and lack of early follicles (primary and pre-antral), a histologic picture consistent with their clinical symptoms; however, in the absence of age-matched controls, conclusions of causality cannot be drawn. Hormone data shown in Table 1 of the study report is apparently the same as is shown (without statistical analysis) in Kim et al. [3], but follow-up hormone data are reported only for two individuals who showed spontaneous recovery of ovarian function. Again, without a second hormone

assessment for the other women, lack of “recovery” of endocrine status in the other women is an assumption only.

**Utility (adequacy) for CERHR evaluation process.** The utility of this paper is that it histologically confirms the diagnosis of ovarian failure in six of the women with amenorrhea in the Kim et al. [3,4] and Park et al. [5] studies. The paper is not useful for risk assessment because no conclusions about the toxicity of 2-BP can be made due to a lack of similar measures in a comparison group. However, it would be unethical to subject healthy controls to an ovarian biopsy given the invasiveness of the procedure.

In 1996, Ichihara et al. [6] conducted a study in a 2-BP production plant in China to obtain information about dose–response for reproductive and hematopoietic effects. Personal air samples were obtained from 14 women (ages 24–54 years) and 11 men (ages 31–56 years) who worked 8 h per day, 5 days per week. Personal TWA exposures of 2-BP exceeded the detection limit of 0.2 ppm in 3 men and 11 women who were directly exposed to 2-BP and ranged from 0.95 to 5.84 ppm and 2.87 to 16.18 ppm in men and women, respectively. Those men were employed at the plant for 15–69 months, while the women were employed for 6–69 months. None of the personal air samples contained detectable levels of 2-BP impurities, including 2-propanol (1.76%), dibromopropane (0.085%), benzene (0.055%), or trichloroethylene (0.10%). Exposures according to job category are listed in Table 3. Accountants, boilers, salesmen, and the assistant manager rarely entered the factory floor and served as referents. No information was provided about use of personal protective equipment. Additional chemicals used in the manufacturing process included 2-propanol, hydrogen bromide, sulfuric acid, and sodium hydrogen-carbonate. All females were non-smokers, but 10 males smoked 3–20 cigarettes per day. None of the employees were exposed to known reproductive or hematopoietic hazards prior to working at the 2-BP factory. Interviews revealed amenorrhea in subjects ages 46, 47, and 54 years and polymenorrhea in 2 women ages 39 and 43 years. All of these women were employed as operators, who prepared and then pour 2-BP into containers, and were exposed to 2-BP at levels of 4.14–16.18 ppm. Blood levels of LH, FSH,

and estradiol were measured and they tended to be higher in females with amenorrhea or polymenorrhea, but only the LH levels reached statistical significance when compared to female accountants and operators with normal menstrual cycles and exposure to <0.2–0.88 and 4.09–8.60 ppm 2-BP, respectively. A regression analysis revealed no significant relationship between female hormonal concentrations and TWA or TWA × employment duration. A male engineer with oligoasthenozoospermia was not currently exposed to detectable 2-BP concentrations but was presumed by authors to have been exposed to high 2-BP concentrations when he set up the manufacturing process. Two males with detectable exposures to 2-BP (0.80–1.20 ppm) and two with non-detectable exposures had <50% sperm motility; one subject in each of those groups reported a shorter abstinence period compared to the other men in the study (1 day versus ≥3 days). Sperm from all workers met the World Health Organization (WHO) criteria for normal morphology and sperm count. Blood levels of LH, FSH, and testosterone were measured in men. The majority of men had LH and FSH values that were within the reference ranges and testosterone levels were within the reference range in all men. A regression analysis revealed no significant relationship between male hormonal concentrations or sperm indices and TWA or TWA × employment duration. Possible confounding factors such as past occupational exposures, oral contraception, medication history, nutritional status, and smoking were mentioned, but it is not clear if they were addressed in the analysis. Hematological parameters were also measured in the workers and are discussed in Section 2. The authors concluded that this study did not demonstrate severe reproductive toxicity from exposure to less than 10 ppm 2-BP but noted that further studies are needed.

**Strengths/weaknesses.** A strength of this occupational health study was that it was conducted in a thorough manner. Informed consent was obtained; questionnaire data on work (past exposures) and medical history, reproductive history, and menstrual status (women) was obtained, although these were not adjusted for in the analyses. Sufficient details on laboratory analyses are provided and standard WHO methods were used for semen analyses. Exposure was monitored

Table 3  
Summary of exposure information per job category in Ichihara et al. [6]

Job category	Number of workers	Direct 2-BP exposure (yes/no)	TWA exposure level (ppm)
<b>Females</b>			
Operators	9	Yes	4.1–16.18
Mixer	1	Yes	6.76
GC analyzer	1	Yes	2.87
Accountants	3	No	<0.2–0.88
<b>Males</b>			
Mixer	1	Yes	5.84
Repairmen	2	Yes	0.95
Boiler	4	No	<0.2–0.8
Engineer	1	No	<0.2
Salesmen and assistant manager	3	No	<0.2

personally and TWA exposures were calculated and used in regression analyses. Female age (>30 years) was controlled in the analysis.

The study had several weaknesses. First, the number of subjects is very small, only three male workers directly exposed to 2-BP had detectable exposures. Second, exposures were low (<10 ppm) since the study was done in winter; exposures are expected to be higher in the summer since 2-BP is volatile. Finally, there was no indication that abstinence interval was controlled/standardized in men. This could have led to misclassification of sperm parameters. Misclassification is unlikely to have been differential by exposure status, thus the effect of such misclassification would likely have biased the exposure parameters towards the null value.

**Utility (adequacy) for CERHR evaluation process.** This study is useful since it was well designed and reported, and exposure was characterized. One panel member suggested that the study may be useful in defining a NOEC for humans, but was not definitive due to small sample size and low exposures. However, a second panel member suggested that the limitations of this study make it inadequate to establish a human NOEC.

#### 4.2. Experimental animal toxicity

##### 4.2.1. Female reproductive toxicity

Kamijima et al. [32] conducted a study in rats to clarify the effects of 2-BP-induced ovarian toxicity. Seven to nine 15-week-old female slc: Wistar/ST rats/group (from Shizuoka Laboratory Animal Center) inhaled air, 100, 300, or 1000 ppm [503, 1509, 5031 mg/m<sup>3</sup>] 2-BP (99.4% purity) for 8 h per day for 9 weeks. Doses were based on a previous study in male rats that demonstrated impairment of spermatogenesis at 300 ppm and serious illness at 3000 ppm. Concentrations were monitored inside inhalation chambers. Estrous cycles were monitored for 3 weeks prior to treatment and during treatment. After exposure ended, rats with regular estrous cycles were sacrificed on the first day of diestrus. Rats experiencing prolonged estrous or diestrus stages were sacrificed following treatment [the time period between the last treatment and sacrifice is not clear]. Estrous cycle data were analyzed by the Kruskal–Wallis test and bodyweight, organ weight, and hormonal data were analyzed by analysis of variance (ANOVA); both analyses were followed by the Dunnett-type multiple comparison method. Study results are outlined in Table 4. Activity, muscle tonus, and bodyweight gain were reduced in rats exposed to 1000 ppm. Irregular estrous cycles were first observed around week 2 of treatment in rats of the 1000 ppm group. Five rats in the group were in continual diestrus with occasional estrus while the remaining four rats had continual estrus with occasional diestrus. In the 300 ppm group, a gradual prolongation of the diestrus stage was first observed at week 7 of treatment. By the end of the treatment period, all rats in the 300 ppm group had cycles consisting of continual diestrus with occasional estrus. One animal in the 100 ppm group

Table 4  
Major effects in Wistar rats in reproductive toxicity study by Kamijima et al. [32]

Number	Dose in ppm (mg/m <sup>3</sup> )	Effects
7	0	
8	100 (503)	No statistically significant effects
7	300 (1509)	↑ Irregular estrous cycles ↓ Absolute and relative uterus weight ↑ Ovarian histopathology
9	1000 (5031)	↑ Irregular estrous cycles ↓ Absolute right ovary weight and absolute and relative uterus weight ↑ Ovarian histopathology ↓ Bodyweight gain ↓ Activity and muscle tonus ↑ Relative liver weight ↓ Absolute spleen and absolute and relative thymus weight No effect on serum LH or FSH

Protocol: Female rats exposed to 2-BP vapors for 8 h per day for 9 weeks.  
Note. ↑, ↓: statistically significant increase, decrease.

became acyclic following 7–9 weeks of treatment, but statistical significance was not obtained. Changes in estrous cycles were accompanied by significantly reduced absolute right ovary weight in the 1000 ppm group. A histological evaluation was conducted in ovaries fixed in 10% formalin and stained with hematoxylin–eosin. Rats experiencing persistent estrus in the 1000 ppm group had ovaries with mostly atretic follicles, very few remaining viable oocytes, thin layers of granulosa cells in cystic follicles, and no newly formed corpora lutea. The ovaries of rats in continual diestrus in the 1000 and 300 ppm groups had reduced numbers of normal antral and growing antral follicles. Absolute and relative uterus weights were significantly decreased only in rats of the 300 and 1000 ppm group experiencing continual diestrus. Serum LH and FSH were measured, and there were no significant differences in treated rats. Organ weight effects on non-reproductive organs were only observed in the 1000 ppm group and included significantly increased relative liver weight and decreased absolute spleen and absolute and relative thymus weight with no abnormal histological findings. The authors concluded that 2-BP was the likely cause of amenorrhea in the Korean workers exposed to 2-BP.

**Strengths/weaknesses.** This study and that of Yu et al. [34], which is apparently based on the same animals, could be criticized for using a small number of rats per group (7–9); however, variability for estrous cycle length was minimized by using only those animals with a regular 4-day cycle (according to recommendations of Cooper et al. [35]). The number of cycles in each 3-week interval of treatment was analyzed with routine statistics. Since this is actually a repeated-measures design, a more appropriate statistical analysis would be that for repeated measures, using each animal as its own baseline. Hormone data did not reveal significant differences with treatment, which is surprising since LH would be expected to be high in an animal with severely

damaged ovaries. This discrepancy is not adequately explained since it could indicate a second target for 2-BP, namely the brain. It would have been helpful to have estradiol and progesterone measurements in order to better interpret the meaning of the LH and FSH concentrations. Nevertheless, the study findings are convincing. The arrest of cyclicity was both time and dose-dependent. A strength is that the study was conducted for a period of time sufficient to reveal the delayed impact at the 300 ppm dose. It would be of interest to analyze paired ovarian weights since there is biological variability between ovaries (due to different number of large follicles or corpora lutea in each). It appears that paired ovarian weight would be significantly lower in the animals exposed to 1000 ppm (Table 2 of the study). Significant effects on estrous cyclicity occurred at a dose (300 ppm) lower than that producing significant effects on bodyweight (1000 ppm). A weakness was that ovarian histology was reported only in a qualitative manner; however, the Yu et al. [34] paper dealt with quantification of variously staged follicles; together, the papers provide strong evidence for ovarian toxicity to primordial and small follicles.

**Utility (adequacy) for CERHR evaluation process.** This study is adequate for use in risk assessment. Data show clear dose- and time-dependent effects on estrous cyclicity which provides evidence of adverse effects on reproductive organ (ovarian) function, even in the absence of fertility data. Lack of effect on serum LH/FSH is inconsistent with the cyclicity effect, however.

Yu et al. [34] conducted a dose–response and a time-course experiment to identify the target cell and define the mechanism of toxicity for 2-BP-induced ovarian toxicity. Both studies used female Wistar rats (from Shizuoka Laboratory Animal Center) that were 12 weeks old at the start of exposure and monitored estrous cycles for 3 weeks prior to and during treatment. Animals were exposed in chambers to air or 2-BP (99.5%) and chamber concentrations were monitored. Exposures were based on a previous study in male rats that demonstrated impairment of spermatogenesis at 300 ppm and serious illness at 3000 ppm. At sacrifice, the right ovary was fixed in 10% neutral buffered formalin, stained with hematoxylin–eosin, and examined histologically. Follicular counts were analyzed by ANOVA followed by the Tukey–Kramer multiple comparison test. Results are outlined in Table 5. In the dose–response experiment, 7–9 rats per group were exposed to 2-BP vapors at 0, 100, 300, or 1000 ppm [503, 1509, 5031 mg/m<sup>3</sup>] for 8 h per day for 9 weeks. Following the exposure period, the rats were sacrificed on the day of diestrus I. Estrous cycles were disrupted after 7 weeks of treatment in the 300 and 1000 ppm groups, while changes in the ovary were seen at all dose levels. The numbers of primordial and growing follicles were significantly reduced in all dose groups ( $\geq 100$  ppm) and numbers of antral follicles were significantly reduced at the two highest doses (300 and 1000 ppm). Ovaries of rats from the mid- and high-dose groups were hypoplastic and contained few or no corpora lutea. For the time-course experiment, rats

Table 5  
Major effects in reproductive toxicity study in Wistar rats by Yu et al. [34]

Number	Dose in ppm (mg/m <sup>3</sup> )	Effects
7	0 <sup>a</sup>	
7	100 <sup>a</sup> (503)	↓ Primordial follicles (55% of control) ↓ Growing follicles (55% of control)
8	300 <sup>a</sup> (1509)	↑ Irregular estrous cycles ↓ Primordial follicles (55% of control) ↓ Growing follicles (50% of control) ↓ Antral follicles (50% of control)
9	1000 <sup>a</sup> (5031)	↑ Irregular estrous cycles ↓ Primordial follicles (20% of control) ↓ Growing follicles (25% of control) ↓ Antral follicles (20% of control)
7	0 <sup>b</sup>	↑ Oocyte distortions (day 5)
7/time point	3000 <sup>b</sup> (15090)	↑ Pyknotic cells and oocyte nuclei shrinkage (day 17) ↓ Primordial follicles (day 5) ↑ Apoptosis in primordial follicles (day 5) No effect on estrous cycle

Note. ↑, ↓: statistically significant increase, decrease.

<sup>a</sup> Protocol: 12-week-old female Wistar rats exposed to 2-BP vapors for 8 h per day for 9 weeks.

<sup>b</sup> Protocol: Female rats exposed to 2-BP vapors 8 h for 1 day and then sacrificed at 1, 3, 5, or 17 days following exposure.

were exposed to 0 or 3000 ppm [15,090 mg/m<sup>3</sup>] 2-BP vapors for 8 h on a single day and then sacrificed at 1, 3, 5, or 17 days following exposure. Seven rats/group were sacrificed at each time period while in estrus. Commencement of exposure was timed according to the rat's cycle to ensure that rats would be in estrus at the time of sacrifice. The right ovary was examined histologically as described above for the dose–response experiment. The left ovary was examined for apoptotic cells by labeling DNA strand breaks through incorporation of digoxigenin-conjugated deoxyuridine 5'-triphosphate (d-dUTP). In the time-course experiment, there was no effect on estrous cycles. Ovarian histopathology consisted of oocytes with distorted symmetry and nuclei on day 5 and pyknotic cells and oocyte nuclei shrinkage on day 17. Numbers of primordial follicles began decreasing on day 5 and reached statistical significance on day 17. Apoptosis was noted in oocytes and granulosa cells of primordial follicles after 5 days of exposure. The authors concluded that 2-BP induced ovarian toxicity through the destruction of primordial follicles and oocytes by apoptotic processes. They postulated that estrous cycles were subsequently disrupted when recruitment of growing and antral oocytes could no longer be supported. Therefore, follicle counts were more sensitive than monitoring of estrous cycles for detecting 2-BP-induced ovarian toxicity.

**Strengths/weaknesses.** The first experiment described in this report appears to use the same animals used by Kamijima et al. [32] to quantitatively evaluate folliculogenesis so as to identify the ovarian target(s). Differential follicle counts were made according to previously published, and



widely accepted methods. Significant decreases in primordial, growing, and antral follicles seen at the higher doses are consistent with irregular cycles and acyclicity in the Kami-jima et al. [32] study. However, differential follicle counts showed that 100 ppm was also an effective dose. The time-course study adds information about targets since it shows that primordial follicles are the first to be affected by a single (1 day) high dose. This suggests that decreases in other follicle populations are mainly the result of maturation depletion.

**Utility (adequacy) for CERHR evaluation process.** This study is adequate for use in risk assessment. Differential follicle counts establish a LOAEC of 100 ppm which is below that determined for estrous cyclicity. The time–response study provides additional evidence that 2-BP is targeting primordial follicles selectively, and provides evidence that these follicles and/or the oocytes within them die by apoptosis.

Lim et al. [33] studied 2-BP toxicity in rats to clarify effects on female reproductive function. Ten, 8-week-old female Sprague–Dawley (Crj:CD) rats/group (from Daehan Animal Center) were injected i.p. with 2-BP (99.0%) in olive oil at 0, 300, 600, or 900 mg/kg bw per day for 14 days prior to mating and during a 7-day mating period to untreated rats at a ratio of 1 male to each female. **[The rationale for dose selection was not discussed.]** Estrous cycles were monitored 2 weeks prior to and during treatment. Dams that had pups were sacrificed 1 day after giving birth and dams with no pups were sacrificed 28 days after mating. **[The evaluation of dams did not include counts of corpora lutea and implantation sites.]** Bodyweight data were analyzed by two-way ANOVA and Duncan's multiple range test; fertility data were analyzed by chi square test. Results are outlined in Table 6. Maternal weight gain was reduced in all treated groups and terminal bodyweights were significantly lower in the 600 and 900 mg/kg bw group. **[However, there were no corrections made for gravid uterine weight.]** One dam in the 600 mg/kg group died due to internal bleeding from the perforation of a blood vessel during injection. One dam in the 300 mg/kg bw group and three dams in the 900 mg/kg bw group died of unknown causes during the post-treatment period. Narcosis was reported for rats in the 600 and 900 mg/kg groups. Length of estrous cycles was increased in the 900 mg/kg bw group due to prolongation of the diestrous stage. Relative ovary weights were significantly reduced in the high dose rats but there were no effects on relative kidney, spleen, or liver weight. Histological evaluations were not conducted. Effects on reproductive function were noted but the statistical significance was not discussed. As discussed in greater detail in Table 6, treatment with 2-BP resulted in dose related decreases in fertility, number of dams giving birth, and number of pups born, but there were no abnormal pups observed. Gestation length was unaffected by 2-BP treatment. Effects on pup bodyweight could not be determined. The authors concluded that their study indicated 2-BP as the causative agent of amenorrhea in female

Table 6  
Major effects in reproductive toxicity study in Sprague–Dawley rats by Lim et al. [33]

Number <sup>a</sup>	Dose (mg/kg bw)	Effects
10/10	0	
10/9	300	↓ Weight gain Death in 1 dam ↓ Fertility (78% vs. 90%) <sup>b</sup> ↓ Dams with live pups ( <i>n</i> = 6 vs. 9) <sup>b</sup> ↓ Litter size (7.3 vs. 9.8) <sup>b</sup>
10/9	600	↑ Narcosis ↓ Weight gain Death in 1 dam ↓ Terminal bodyweight (uncorrected) ↓ Fertility (33% vs. 90%) <sup>b</sup> ↓ Dams with live pups ( <i>n</i> = 3 vs. 9) <sup>b</sup> ↓ Litter size (6.7 vs. 9.8) <sup>b</sup>
10/9	900	↑ Narcosis ↓ Weight gain ↓ Terminal bodyweight (uncorrected) Death in 3 dams ↓ Relative ovary weight ↑ Estrous cycle length from 4.6 days to 11.05 days prior to and after treatment ↓ Fertility (11% vs. 90%) <sup>b</sup> ↓ Dams with live pups ( <i>n</i> = 1 vs. 9) <sup>b</sup> ↓ Litter size (1 vs. 9.8) <sup>b</sup> No effect on gestation length and no pup abnormalities at any dose

Protocol: Female, 8-week-old rats were injected i.p. with 2-BP from 14 days prior to mating and during a 7-day mating period.

Note. ↑, ↓: statistically significant increase, decrease.

<sup>a</sup> Number of rats at beginning of study/number of rats copulating with untreated males.

<sup>b</sup> Statistical significance is not known.

workers exposed to 2-BP, but noted that additional studies including measurements of gonadotropin levels are needed. **Strengths/weaknesses.** No rationale for the dosages used was provided, nor were calculations made to permit comparisons with doses in inhalation studies. Dosages were apparently quite high as narcosis was evident in the two higher exposure groups (600 and 900 mg/kg). Importantly, narcosis can indicate sufficient neurotoxicity to impair the LH surge and this could be another mechanism by which estrous cyclicity is impaired. It is difficult to attribute decreased weight gain (Fig. 2 in study) to the exposure since it could be due to lack of pregnancy. Duration of exposure was short considering that significant effects in inhalation studies described above were not seen until 5–7 weeks of exposure. However, a non-significant disruption in estrous cyclicity has been noted following exposure to 1000 ppm 2-BP for 1–3 weeks [32]. Lack of effect on estrous cyclicity at 300 and 600 mg/kg could be due to the short exposure period. Irregular cycles seen only in the high-dose group do not explain the infertility seen in some rats at 300 and 600 mg/kg. The paper states that chi square was used to evaluate reproductive effects related to fertility indices, but Table 4 in the



Table 7  
Reproductive toxicity study in ICR mice by Sekiguchi and Honma [36]

Number	Dose (mg/kg bw)	Effects
5	0	
5	500	No effects
5	1000	↓ Number of ova ovulated (23.8 vs. 52.3)
4	2000	↓ Number of ova ovulated (6.0 vs. 52.3) ↓ Bodyweight gain Death in 1 mouse

Protocol: 51–53-day-old female ICR mice were injected i.p. with 2-BP a total of eight times over 17 days.

Note. ↑, ↓: statistically significant increase, decrease.

study does not indicate where statistically significant differences were found.

**Utility (adequacy) for CERHR evaluation process.** This study is of limited utility since route of exposure is not appropriate, dosages appear to be quite high, and duration of exposure was relatively short. Effects at high dose on estrous cyclicity are at least consistent with observations made in inhalation studies.

Sekiguchi and Honma [36] conducted a study to determine the effects of 2-BP on ovulation. Four or five, 51–53-day-old female ICR mice/group (from Charles River, Japan) were injected i.p. with 2-BP [purity not reported] in olive oil at 500, 1000, or 2000 mg/kg. [Rational for dose selection was not reported]. Mice were given eight injections every 2–3 days over a period of 17 days. Ovulation was induced by i.p. injection with pregnant mare's serum gonadotropin and human chorionic gonadotropin on the 15th and 17th day of 2-BP treatment. Mice were sacrificed and necropsied the day after the last treatment and liver, uterus and oviduct were examined. Data were analyzed by Dunnett's multiple comparison. Results are outlined in Table 7. Bodyweight gain was reduced in the 1000 and 2000 mg/kg bw groups with statistical significance achieved at 2000 mg/kg bw. However, terminal bodyweights did not differ from controls. One mouse in the 2000 mg/kg bw group died. The number of ovulated ova was significantly reduced in the 1000 and 2000 mg/kg bw groups. A non-significant reduction in absolute and relative uterus weight was also observed in the 2000 mg/kg group. There was no mention of histopathological evaluation. The authors concluded that results were consistent with humans experiencing 2-BP induced effects. It is noted that this study was published as a short communication.

**Strengths/weaknesses.** The rationale for using mice was not provided. The assay evaluates the ability of the ovary to respond to gonadotropins and is an indirect measure of the number of competent or recruitable follicles in the ovary. Results indicate that high levels of 2-BP (1000 or 2000 mg/kg) given in eight i.p. injections over 17 days, significantly reduce the number of oocytes ovulated after induction of superovulation. Other symptoms (e.g. narcosis) are not mentioned, so it is difficult to attribute effects to ovarian toxicity per se. The authors overinterpret their data, especially in relating it to the epidemiology data.

#### Utility (adequacy) for CERHR evaluation process.

There are not adequate data in this report to make it useful for risk assessment. The exposure route and systemically toxic doses also make this study of limited utility for risk assessment. The study provides indirect evidence for depletion of ovarian follicle pools in mice, as has been reported in rats.

#### 4.2.2. Male reproductive toxicity

Ichihara et al. [22] conducted a study to determine the testicular and hematopoietic toxicity of 2-BP in 13-week-old Wistar rats (from Shizuoka Laboratory Animal Center). Reproductive parameters are addressed in this section while hematological and other systemic effects are outlined in Table 8 and discussed in detail in Section 2. Nine male rats/group were exposed by inhalation to air or 300, 1000, or 3000 ppm [1509, 5031, or 15,092 mg/m<sup>3</sup>] 2-BP (99.4% purity) for 8 h per day, 7 days per week. The maximum concentration was about 10% of the LC<sub>50</sub>. Concentrations were monitored inside inhalation chambers. The controls and two lowest dose groups were exposed for 9 weeks. However, exposure in the high-dose group ended after 9–11 days due to excessive toxicity. Three rats in the high-dose group were sacrificed immediately after exposure and their testes and femurs were examined histologically. The remaining six rats were exposed to filtered air for the remainder of the exposure period and evaluated for all parameters in this study. Reproductive organs were weighed at sacrifice. The left testis and epididymis were preserved in Bouin's solution and the prostate and seminal vesicle in 10% neutral buffered formalin. Testis and epididymis were stained with periodic acid-Schiff's reagent and other tissue sections with hematoxylin–eosin. Abnormal sperm data were analyzed by Student's *t*-test and all other data by ANOVA followed by Dunnett's multiple comparison method. Significant, dose-related effects first noted at the low dose (300 ppm) included reductions in absolute and relative epididymides and testes weight, and absolute prostate and seminal vesicles weight. Significant dose-related sperm effects noted at 300 ppm and higher included reduced counts and motility and increases in tailless sperm. An increase in abnormal sperm (hooked or reflexed head) was noted at 300 ppm but could not be evaluated at higher doses because there were very few intact sperm remaining. Testicular lesions were observed at all dose levels and included atrophy of seminiferous tubules, reductions in the number of germ cells, and hyperplastic Leydig cells that increased in severity at higher doses. Vacuolation of Sertoli cells was also observed in two of the three rats sacrificed immediately after exposure to 3000 ppm for 9–11 days. Slight atrophic changes were reported for seminal vesicles and prostate of treated rats.

**Strengths/weaknesses.** Although there was a small number of male rats per group (nine), multiple outcomes of reproductive effects were obtained, including good quality histology, and sperm measures (counts, morphology, motility). Treatment was of sufficient duration (9 weeks) to detect effects on all stages of spermatogenesis. Dose range

Table 8  
Reproductive toxicity study in Wistar rats by Ichihara et al. [22]

Number	Dose in ppm (mg/m <sup>3</sup> )	Effects
9	0	
9	300 (1509)	↓ Absolute epididymides, testes, prostate, seminal vesicles, and kidney weight ↓ Relative epididymides and testes weight ↓ Sperm count (358 × 10 <sup>6</sup> /g vs. 569 × 10 <sup>6</sup> /g cauda) ↓ Sperm motility (16% vs. 86%) ↑ Tailless sperm (56% vs. 10%) ↑ Abnormal sperm (21% vs. 7%) ↑ Testicular lesions ↓ Erythrocytes and platelets ↓ Bodyweight gain
9	1000 (5031)	↓ Absolute epididymides, testes, prostate, seminal vesicles, liver, and kidney weight ↓ Relative epididymides, testes, prostate, and seminal vesicles weight ↓ Sperm count (326 × 10 <sup>6</sup> /g vs. 569 × 10 <sup>6</sup> /g cauda) ↓ Sperm motility (0% vs. 86%) ↑ Tailless sperm (98% vs. 10%) ↑ Testicular lesions ↓ Erythrocytes, hemoglobin, hematocrit, platelets, and leukocytes ↑ Bone marrow lesions ↓ Bodyweight gain
6	3000 (15092)	↓ Absolute epididymides, testes, prostate, and seminal vesicles weight ↓ Relative epididymides and testes weight ↓ Sperm count (151 × 10 <sup>6</sup> /g vs. 569 × 10 <sup>6</sup> /g cauda) ↓ Sperm motility (0% vs. 86%) ↑ Tailless sperm (99% vs. 10%) ↑ Testicular lesions ↓ Erythrocytes ↑ Bone marrow lesions ↓ Bodyweight gain

Protocol: 13-week-old male Wistar rats inhaled 2-BP vapors for 8 h per day, 7 days/week for 9 weeks in 2 lowest dose groups and 9–11 days in highest dose group.

Note. ↑, ↓: statistically significant increase, decrease.

included a toxic level (3000 ppm), but not a no-effect level. The study would have been more informative if interim observations had been made. Cessation of dosing after 9–11 days in the high dose group resulted in the demonstration that severe testicular toxicity, apparent at high doses, does not appear to be reversible, at least in the short term.

**Utility (adequacy) for CERHR evaluation process.** The study is useful for risk assessment in that it provides convincing evidence for testicular toxicity of 2-BP in a dose responsive manner. However, it does not identify a NOAEC. The study indicates lack of reversibility at the high dose within the timeframe and conditions of this study. It also suggests that effects appear to be specific for blood and testes (versus liver or kidneys), and that testes might be more sensitive than blood. Results are consistent with effects on Sertoli cells (alterations in sperm morphology and motility) and on spermatogonia (depletion of sperm numbers). Effects on accessory organs are indicative of low testosterone, although serum hormones were not measured. This could be secondary to direct effects on testis, but the design does not rule out more direct endocrine effects.

Yu et al. [26] noted atrophy of seminiferous tubules and loss of germ cells in the testes of 10-week-old Wistar rats exposed to 1000 ppm [5031 mg/m<sup>3</sup>] 2-BP vapors for

8 h per day, 7 days per week, for 12 weeks; no lesions were observed following exposure to 100 ppm [503 mg/m<sup>3</sup>] 2-BP. Additional details of this study are included in Section 2.

**Strengths/weaknesses.** This neurotoxicology study provides confirming evidence that exposure to 1000 ppm 2-BP causes testicular atrophy after subchronic (10 weeks) exposure by inhalation, and adds histologic evidence that 100 ppm may be a NOAEC for testicular toxicity. Appropriate fixation of testes appears to have been conducted for testicular evaluation, so the observations though limited in scope, appear reliable.

**Utility (adequacy) for CERHR evaluation process.** This study is adequate to suggest that 100 ppm 2-BP is a no effect level after subchronic inhalation exposure, and confirms testicular toxicity at 1000 ppm (in presence of changes in hematological indices and peripheral neuropathology). The study also provides evidence that 1-BP did not cause the same hematological changes or testicular toxicity at 1000 ppm (5–7 weeks), but was a more potent neurotoxicant than 2-BP.

Yu et al. [25] conducted a 2-BP toxicity study in rats to verify that adverse effects in the hematopoietic and reproductive systems of workers of a Korean electronics plant were

Table 9  
Major effects in reproductive toxicity study in Sprague–Dawley rats by Yu et al. [25]

Number	Dose (mg/kg bw)	Effects
10	0	
10	125	Clinical signs
10	250	Clinical signs ↓ Bodyweight gain ↓ Relative testes weight ↑ Seminiferous tube atrophy with, germ cell necrosis, Sertoli cell vacuolization, and Leydig cell hyperplasia ↑ Relative adrenal weight
10	500	Clinical signs ↓ Bodyweight gain ↓ Relative testes weight ↑ Seminiferous tube atrophy with, germ cell necrosis, Sertoli cell vacuolization, and Leydig cell hyperplasia ↑ Relative adrenal, lung, spleen, liver and brain weight ↓ White blood cell, lymphocyte, platelets, and hemoglobin ↓ Blood alkaline phosphatase activity ↑ Cholesterol

Protocol: 12-week-old male Sprague–Dawley rats injected i.p. with 2-BP on 6 days/week for 4 weeks.

Note. ↑, ↓: statistically significant increase, decrease.

due to 2-BP exposure. Ten male Sprague–Dawley rats/group (~12 weeks old; purchased from Daehan Animal Center) were injected i.p. with 0, 125, 250, or 500 mg/kg bw 2-BP (99% purity) in olive oil, 6 times/week for 4 weeks. The authors acknowledged that the administration route does not pertain to occupational exposures, but stated that inhalation tests are required only if negative results are obtained with i.p. exposure. **[The rationale for dose selection was not discussed.]** this summary describes the reproductive effects while non-reproductive findings are discussed in Section 2. Results of the study are summarized in Table 9. Rats exposed to 2-BP at 250 and 500 mg/kg bw experienced a significant, dose-related reduction in relative testicular weight. Histological examination of testes (preserved in 10% formalin and stained with hematoxylin–eosin) revealed severely atrophic tubules with necrosis of spermatogonia and spermatocytes, vacuolized Sertoli cells, and hyperplasia and hypertrophy in Leydig cells in the two highest dose groups (250 and 500 mg/kg bw). Epididymal atrophy with vacuolization of the epithelium was also observed. The dose at which this effect first occurred was not specified.

**Strengths/weaknesses.** This neurotoxicity study of 1-BP and 2-BP included reproductive outcomes. Results are limited by short exposure duration (28 days) which can miss effects on spermatogonia. However, severity of effect allowed its detection. The i.p. route of exposure is not relevant for humans.

**Utility (adequacy) for CERHR evaluation process.** This study is useful from a hazard identification standpoint be-

cause it confirms toxicity by another route. Dose–response data suggest that 125 mg/kg i.p. is the NOAEC for testicular toxicity, although other toxicities are seen at this dosage. The study also shows that testicular atrophy can be produced with shorter exposures (28 days versus 9–10 weeks) at high doses.

Wu et al. [37] conducted a reproductive toxicity study to obtain information about 2-BP toxicity in mature (9-week-old) and immature (5-week-old) male Sprague–Dawley rats (Sino-British SIPPR/BK Animal Co.). Six rats/dose/age group were injected s.c. with 0, 200, 600, or 1800 mg/kg bw 2-BP (99.6% purity), 5 days/week with treatment lasting for 5 and 7 weeks in mature and immature rats, respectively. **[There was no mention of vehicle.]** The authors stated that although exposure through the s.c. route does not occur in occupational settings, s.c. injection was chosen to ensure complete and rapid absorption. The basis for dose selection was stated to be previous data and preliminary results. After treatment, reproductive performance was assessed in the mature rats by mating them 1:2 with untreated females for 7 days. Sperm quality and testicular histology (fixed in 10% formalin and stained with hematoxylin–eosin) were examined in both age groups. Mature rats were sacrificed 4 days after mating to allow for restoration of sperm levels. Immature rats were sacrificed immediately after the treatment period. Analysis of data included ANOVA and Dunnett's test for weight effects, the Kruskal–Wallis or Mann–Whitney test for sperm, fetal, and hormonal data, and the chi square test for reproductive function data. Statistically significant results are listed in Table 10. Several effects were noted in both mature and immature rats at the lowest dose (200 mg/kg bw) and included dose-related reductions in sperm count and viability, and increases in deformed sperm and testicular lesions. Testicular lesions increased in severity with dose and included atrophied seminiferous tubules with reductions in germ cell numbers. Serum testosterone levels were first reduced at 200 and 600 mg/kg bw in mature and immature rats, respectively. A dose-related reduction in absolute and relative testicular weight was first noted in mature and immature rats at the 600 mg/kg bw dose. Bodyweight gain was significantly reduced in mature rats exposed to  $\geq 600$  mg/kg bw and immature rats at all dose levels. Additional effects seen at the highest dose level (1800 mg/kg bw) in both age groups included reductions in absolute epididymis, prostate seminal vesicle, and pituitary weight and relative epididymis weight. Dose-related adverse effects on the reproductive performance of mature rats were first noted at 600 mg/kg bw and included reduced mating and pregnancies and an increase in the number of days for pregnancy initiation to occur. Effects also noted in dams mated with the high dose group (1800 mg/kg bw per day) included a reduced number of implantation sites and increased fetal mortality. Expression of  $\beta$ -luteinizing hormone gene was measured in mature rats and was found to be increased in rats treated with 1800 mg/kg bw. Based on testicular and sperm effects, the authors estimated

Table 10  
Major effects in reproductive toxicity study in Sprague–Dawley rats by Wu et al. [37]

Number/age group	Dose (mg/kg bw/day)	Effects in mature rats	Effects in immature rats
6	0		
6	200	↓ Sperm count ( $61.6 \times 10^6/\text{ml}$ vs. $140.6 \times 10^6/\text{ml}$ ) and viability (52.3% vs. 70.8%) ↑ Deformed sperm (9.6% vs. 2.1%)  ↓ Serum testosterone (97.5 fmol/ml vs. 272 fmol/ml) ↑ Testicular lesions	↓ Bodyweight gain  ↓ Sperm count ( $61.5 \times 10^6/\text{ml}$ vs. $74.6 \times 10^6/\text{ml}$ ) and viability (51.6% vs. 64.2%) ↑ Deformed sperm (8.5% vs. 4.2%)  ↑ Testicular lesions
6	600	↓ Bodyweight gain ↓ Absolute and relative testis weight ↓ Sperm count ( $54.6 \times 10^6/\text{ml}$ vs. $140.6 \times 10^6/\text{ml}$ ) and viability (25.4% vs. 70.8%) ↑ Deformed sperm (12.6% vs. 2.1%) ↓ Serum testosterone (82.2 fmol/ml vs. 272 fmol/ml) ↑ Testicular lesions ↓ Mating (75% vs. 100%) ↓ Pregnancies (78% vs. 100%) ↑ Days to fertilization (3.9 vs. 2.8)	↓ Bodyweight gain ↓ Absolute and relative testis weight ↓ Sperm count ( $38.0 \times 10^6/\text{ml}$ vs. $74.6 \times 10^6/\text{ml}$ ) and viability (40.7% vs. 64.2%) ↑ Deformed sperm (10.8% vs. 4.2%) ↓ Serum testosterone (131 fmol/ml vs. 153 fmol/ml)  ↑ Testicular lesions
6	1800	↓ Bodyweight gain ↓ Absolute testis, epididymis, prostate, seminal vesicle, and pituitary weight ↓ Relative testis and epididymis weight ↓ Sperm count ( $11.3 \times 10^6/\text{ml}$ vs. $140.6 \times 10^6/\text{ml}$ ) and viability (0% vs. 70.8%) ↑ Deformed sperm (75.2% vs. 2.1%) ↓ Serum testosterone (80.8 fmol/ml vs. 272 fmol/ml) ↑ Testicular lesions ↓ Mating (42% vs. 100%) ↓ Pregnancies (20% vs. 100%) ↑ Days to fertilization (4.6 vs. 2.8) ↓ Implantations/litter (7.4 vs. 11.2) ↓ Viable fetuses/litter (6.0 vs. 10.2) ↑ Resorptions (5.8% vs. 1.6%) ↑ $\beta$ -LH gene expression in pituitary	↓ Bodyweight gain ↓ Absolute testis, epididymis, prostate, seminal vesicle, and pituitary weight ↓ Relative testis and epididymis weight ↓ Sperm count ( $7.8 \times 10^6/\text{ml}$ vs. $74.6 \times 10^6/\text{ml}$ ) and viability (0% vs. 64.2%) ↑ Deformed sperm (93.6% vs. 4.2%) ↓ Serum testosterone (129 fmol/ml vs. 153 fmol/ml)  ↑ Testicular lesions

Protocol: Mature (9-week-old) and immature (5-week-old) male rats were s.c. injected 5 days/week with 2-BP for 5 and 7 weeks, respectively. Mature rats were mated 1:2 with untreated females.

Note. ↑, ↓: statistically significant increase, decrease.

that the NOAEC for 2-BP was below 200 mg/kg bw per day.

**Strengths/weaknesses.** Strengths of this study include assessment of sperm counts, viability and morphology, as well as serum testosterone levels. The small number of rats per group (six) limits the power to detect effects. The design does not truly evaluate immature rats. Use of longer dosing time (7 weeks) in young rats (5 weeks of age) means that they were adult (84 days old) when evaluated (versus 98 days old for “adult” group). Therefore, they were exposed during adolescence and adulthood, and one would expect effects to be similar in both groups. The s.c. route is not directly relevant to human inhalation exposures. Another weakness is that the testosterone assay is not adequately described, and fixation of testis for histology is not optimal. Effects at two higher dosages were accompanied by

severe weight loss, so other effects are questionable as to their specificity. Fertility indices should be calculated and analyzed with the male as the unit of measure since only males were treated (see Table 5 of study).

**Utility (adequacy) for CERHR evaluation process.** This study is of limited value due to the subcutaneous route of exposure. Testicular toxicity at high doses is of questionable specificity since bodyweight was down more than 10%. The study demonstrates less severe effects at lower doses that do not affect weight, therefore it confirms inhalation studies that demonstrate that 2-BP is a testicular toxicant. Hormone (or LH mRNA) changes are probably secondary to testicular and general toxicity at the high dose and not a primary effect.

Omura et al. [38–40] used quantitative histopathology to examine mechanisms of 2-BP-induced testicular toxicity.



Table 11  
Effect on germ cells numbers in Omura et al. [38] study

Stage	Effect on germ cell numbers (% of control values)
VII	↓ Spermatogonia (45) ↓ Preleptotene spermatocytes (5)
X	↓ Spermatogonia (35) ↓ Leptotene spermatocytes (5)
XII	↓ Spermatogonia (65) ↓ Zygotene spermatocytes (5)
I	↓ Spermatogonia (20) ↓ Pachytene spermatocytes (70)
V	↓ Spermatogonia (5) ↓ Pachytene spermatocytes (50)

↓: Decreased.

They counted the numbers of different types of germ cells in seminiferous tubule cross sections selected to represent specific stages of spermatogenesis [41]. While this level of quantification is not required by OECD or EPA test guidelines, it does allow detection of subtle changes in the testis. Such information may provide insights into cellular targets and mechanism of action of a toxicant, especially after short term exposure [41], and can be used in risk assessment [42].

Omura et al. [38,39] utilized this approach to determine the type(s) of testicular cells targeted by 2-BP exposure. Four, 11-week-old Kud: Wistar rats/group were injected s.c. with saline or 1355 mg/kg bw 2-BP (>99% purity) for 5 days/week for 2 weeks. This exposure duration was selected to cover one spermatogenic cycle. According to the study authors, that dosage is equivalent to inhalation of 1000 ppm for 8 h at a respiratory minute volume of 215 ml/min/383 g bw. Following treatment, reproductive organs were weighed, testicular and epididymal sperm were counted, and sperm motility and morphology were assessed. Sperm data were evaluated by the Mann–Whitney test and other data by Student's *t*-test. Bodyweight gain was significantly reduced by 2-BP treatment (by 13%). The treated group also had significantly reduced absolute seminal vesicle weights and increased relative epididymides weights. Sperm count per testis was significantly reduced in treated rats, but 2-BP treatment had no significant effect on sperm count per gram testis weight, motility, or morphological abnormalities. Histological evaluation of the right testis (preserved in Bouin's and stained with periodic acid schiff reagent) revealed mild atrophy in only a few seminiferous tubules. The number of germ cells and Sertoli cells in seminiferous tubules at stages I, V, VII, X, and XII were determined. Treatment with 2-BP significantly reduced the numbers of spermatogonia and stage-specific spermatocytes, without affecting later cell types (spermatids) (see Table 11). Based on the changes in germ cell numbers during each stage of the cycle evaluated, the authors estimated that spermatogonia were the target of 2-BP exposure. Because spermatogonia develop into spermatocytes, the authors believed the reductions in spermatocytes to be due to depletion of spermatogonia.

Table 12  
Time-dependent reductions in spermatogonia numbers observed by Omura et al. [40]

Stage	Effect on spermatogonia numbers on each day of treatment (% of control values)				
	Day 1	Day 2	Day 3	Day 4	Day 5
VII	–	–	–	–	–
X	–	–	–	–	–
XII	–	–	↓ (80)	–	↓ (70)
I	↓ (70)	↓ (90)	↓ (60)	↓ (50)	↓ (15)
II and III	–	↓ (65)	–	↓ (70)	↓ (25)
V	–	–	–	–	↓ (60)

↓: Decreased, –: no significant effect.

However, they stated that further studies are needed to confirm these effects of 2-BP toxicity.

**Strengths/weaknesses.** Methods for fixation, histologic processing, staining, and staging of seminiferous tubules were appropriate. Timing of dosing and sacrifice are appropriate for distinguishing between spermatogonial and spermatocyte toxicity. The panel notes that the author's calculation of inhaled dose equivalents needs to be qualified. The s.c. dose may be equivalent to the delivered dose of 2-BP by inhalation over the dosing period. However, no conclusions can be made about the equivalence of the absorbed dose or the kinetics of 2-BP by the different routes. **Utility (adequacy) for CERHR evaluation process.** This study is useful for identifying a target cell (spermatogonia) following an acute exposure to a high dose of 2-BP at a dose equivalent to that causing testicular atrophy in subchronic exposure studies. If spermatogonia are also the target at lower, inhalational exposures, this would be important to human risk assessment because depletion of all testicular spermatogonia results in irreversible infertility.

Omura et al. [40] conducted a second study to confirm that spermatogonia are the target cells affected by 2-BP. Eleven-week-old Kud: Wistar rats were injected s.c. with 1355 mg/kg bw 2-BP (>99% purity) without vehicle for 1–5 days. Groups of 4 rats were sacrificed 6 h after treatment on days 1, 2, 3, 4, and 5. Five control rats were killed after 5 days of saline injection. Enumeration of spermatogenic cell types was conducted in a stage-specific manner as described above in the Omura et al. [38] study. Spermatogenic cell number data were analyzed by one-way ANOVA followed by Fisher's least significant difference procedure. As noted in Table 12, 2-BP exposure significantly reduced spermatogonia numbers in stages XII, I–III, and V. In stage I, spermatogonia numbers were reduced on each day with greater reductions occurring with increased time of treatment. A delay in the division of type B spermatogonia was also observed in rats treated for 5 days. In contrast to the Omura et al. [38] study with a 2-week exposure period, spermatocytes were generally unaffected. Statistically significant but slight reductions in pachytene spermatocyte numbers (90–95% of control values) were only observed in stage I, but the numbers did not decrease with increased time of treatment. The



authors stated that the reduction in pachytene spermatocytes had no biological significance and may have resulted from high numbers in control rats. The authors concluded that the early reduction in spermatogonia numbers demonstrated that spermatogonia are the target cells affected by 2-BP.

**Strengths/weaknesses.** This study was done according to proper methods for sample preparation and evaluation (as with their 1997 study). Shorter exposure periods help refine evaluation of sensitive cell types and increase confidence that primary effect was to the spermatogonia. The dosage used was high and, therefore, the results do not necessarily define the primary target cell at lower doses.

**Utility (adequacy) for CERHR evaluation process.** This study is adequate for defining the cellular targets of 2-BP at high doses, and results are consistent with the spectrum of effects seen after subchronic exposure. Knowledge about target cells is useful in comparing mechanisms of 1-BP and 2-BP and can be used in risk assessment.

To characterize testicular toxicity resulting from 2-BP exposure in rats, Son et al. [43] compared histological observations of spermatogenic staging to quantitative spermatogenesis measurements. Ten-week-old Sprague–Dawley rats (from Screening and Toxicology Research Center) were gavaged with 0 or 3500 mg/kg bw per day 2-BP [**purity not reported**] in corn oil for 3 days. Three control and five treated rats per day were sacrificed at 1, 3, 5, 7, 14, 28, 42, or 70 days following treatment. Histological examination of testes fixed in Bouin's solution and stained with hematoxylin–eosin or periodic acid Schiff's reagent revealed that the sequence of toxicity was damage to spermatogonia, leading to depletion of spermatocytes, spermatids, and spermatozoa that ultimately resulted in testicular atrophy (Table 13). Leydig cell hyperplasia was also observed on the last observation day. Immunohistochemical staining of Leydig cells with proliferating cell nuclear antigen (PCNA), a marker of cell proliferation, confirmed the histological observation. Evidence of partial recovery was noted by some regeneration of germ cells and spermatocytes towards the end of the observation period. Electron microscopic examination of testes from one rat/group/sacrifice

day confirmed the histological effects. Flow cytometry was used to quantify spermatogenesis based on DNA ploidy of testicular cells. The technique measured the percentages of haploid (spermatozoa, spermatids), diploid (spermatogonia, secondary spermatocytes, and Sertoli, Leydig, and connective tissue cells) and tetraploid (primary spermatocytes) cells. Data were analyzed by Dunnett's test. Results were consistent with histological findings as evidenced by time-related reductions in the percentages of diploid (days 3–28) and tetraploid cells (days 5–28). Percentages of diploid and tetraploid cells increased on day 42 and then decreased on day 70.

**Strengths/weaknesses.** This study design (acute exposure followed by serial observations) is ideal for identifying the cellular target. A specific cellular marker, PCNA, was used to confirm cell proliferation. Morphological findings were confirmed at the ultrastructural level. Flow cytometric analysis was used to confirm relative changes in cell populations according to their ploidy. A variety of histologic characteristics were monitored (in Table 1 of study) including not just depletion of specific cell types but also regeneration of germ cells, spermatid retention, Leydig cell hyperplasia, and changes in epididymal contents (all as recommended by Creasy [41], and other experts in testicular histopathology). Analysis shows progression of the pathology such that spermatogonia are first depleted, followed by spermatocytes, round spermatids and elongating spermatids. Retained spermatids are not seen until a week after treatment, about the same time that exfoliated germ cells are also seen in the epididymis. Leydig cell hyperplasia is apparent only at day 70, post-exposure. The proportion of haploid cells rises and then falls in a manner consistent with the histological findings.

**Utility (adequacy) for CERHR evaluation process** This study is adequate for tentatively identifying the initial cellular target as the spermatogonia and ruling out direct, immediate effects on testosterone production or Leydig cells. This helps interpret subchronic studies where some weight change in testosterone-dependent accessory organs were probably due to general toxicity and bodyweight loss rather than direct effects on pituitary, hypothalamus, or Leydig cells.

To obtain information about 2-BP-induced effects on spermatogenesis, Wu et al. [44] studied the effects of 2-BP on cultured rat Leydig cells. Leydig cells were obtained from 6-week-old Sprague–Dawley rats (from Sino-British SIP-PR/BK Laboratory Animal Co.). Three replicates of cells were treated with 0, 0.01, 0.1, or 1.0 mmol/l 2-BP [**1.23, 12.3, or 123 mg/l**] and 1 U human chorionic gonadotropin (hCG) hormone for 6, 12, 18, or 24 h. A significant reduction in cell viability was noted after exposure to 1.0 mmol/l 2-BP for 12 h and longer, and a non-significant reduction in viability occurred after exposure to 0.01–0.1 mmol/l 2-BP for 24 h. Testosterone production decreased significantly in cells treated with 1.0 mmol/l 2-BP for 12 h or longer. Authors concluded that 2-BP may be cytotoxic to Leydig cells in vitro. They noted that findings of this study were in con-

Table 13  
Histological analysis in Son et al. [43] study

Effect	Days adverse effect observed	Days recovery observed
Degeneration of spermatogonia in stages I–IV	1–3 and 7–70	–
Depletion of spermatogonia	3–28	42–70
Depletion of spermatocytes	5–28	42–70
Depletion of round spermatids	28–42	70
Depletion of elongate spermatids	42	70
Spermatid retention in stages IX–XII	7–28	42–70
Leydig cell hyperplasia	70	–
Oligospermia	14–70	–
Exfoliated germ cells in epididymis	7–70	–
Regeneration of germ cells	–	28–70

–: effect not observed.

trast to those of Kim et al. [3] who found no effects on testosterone levels in humans exposed to 2-BP and Ichihara et al. [22] who observed hyperplastic Leydig cells in rats exposed to 2-BP. The authors stated that differences between the results of in vivo and in vitro studies could be related to differences between the parent compound applied in vitro and metabolites of 2-BP that would result after in vivo exposure. Furthermore, decreased testosterone secretion in vivo could result in increased luteinizing hormone secretion and consequent Leydig cell hyperplasia, effects that would not occur after in vitro exposures. Differences in exposure duration and effective concentrations may also explain the different results.

**Strengths/weaknesses.** The rationale for 2-BP concentrations used was not given. The relationship between the concentrations in this study and expected levels in rats after specific in vivo exposures is unknown. No data were provided to explain how the in vitro concentrations compared to in vivo concentrations associated with toxicity. Therefore, there is no way of knowing whether the results from this study have relevance to toxicity observed in vivo. The discussion included reference to an unpublished study by the authors that is not appropriate or relevant.

**Utility (adequacy) for CERHR evaluation process.** This in vitro study confirms that 2-BP does not directly suppress hCG-induced testosterone production at concentrations that are non-toxic to the Leydig cells. As such, the study is useful but not critical for the CERHR evaluation process.

Yu et al. [45] conducted a study to determine the role of apoptosis and the possible involvement of Bcl-2 family genes and the Fas signaling system in 2-BP-induced testicular toxicity. The Bcl-2 family of genes includes both pro- and anti-apoptotic proteins; the Fas signaling system transmits apoptotic signals. Male Wistar rats (12-weeks-old) were injected percutaneously with olive oil or 1350 mg/kg 2-BP (99.5% purity) in olive oil. The dosage was similar to that used in the Omura et al. [40] study and the study authors calculated that it was equivalent to inhaling 1000 ppm for 8 h. Seven treated groups containing eight rats/evaluation period were injected daily for 1–5 days. The treated rats were euthanized at 12 h following a single treatment, 6 h following the last treatment received for 1, 2, 3, or 5 days, or at 2 or 9 days following a 5-day treatment period. A control group of eight rats was euthanized 6 h following the last day of a 5-day olive oil injection period. Six rats/group/time period were prepared for histological examination of the left testis (fixed in Bouin's) while the other two rats/group/time period were prepared for testicular examination by electron microscopy. Protein was extracted from the right testis for Western Blot analysis of BCL-2 and Fas proteins in six rats/group/time period. Data were analyzed by one-way ANOVA followed by the Tukey–Kramer multiple comparison test. Examination of stage I and stage VII seminiferous tubules revealed significant reductions in spermatogonia numbers following 3–5 days of treatment; no spermatogonia were detected at 2 and 9 days following the 5-day treatment

period. A secondary reduction in pachytene spermatocyte numbers in stage I occurred at 9 days following treatment. In situ analysis of DNA fragmentation (TUNEL) conducted in three animals/group/time period revealed that spermatogonia were undergoing apoptosis during 2-BP treatment and pachytene spermatocytes were undergoing apoptosis 9 days after the last dose was administered. DNA ladder formation in gel electrophoresis, which is a hallmark of apoptosis, was conducted in one rat/group/time period and verified the results of the TUNEL assay. Significant reductions in the expression of Bcl-2 (an anti-apoptotic protein) occurred during the first 2 days of treatment and at 9 days following treatment. Expression of Bax (a pro-apoptotic protein) was significantly increased during the first day of treatment but significantly reduced 9 days following treatment. Expression of Fas receptor was significantly upregulated during days 1, 2, and 5 days of treatment and at 2 days following the last treatment. In contrast, significant reductions in Fas ligand expression were noted on the last day of treatment and 2 days following treatment. The study authors concluded that 2-BP induces apoptosis of testicular germ cells and that Bcl-2 family genes and Fas signaling system play a role in the process.

**Strengths/weaknesses.** This is a high-dose mechanistic study that identifies apoptosis as the process of 2-BP-induced germ cell death.

**Utility (adequacy) for CERHR evaluation process.** This information is of limited use for human risk assessment.

Park et al. [46] tested 2-BP for androgenic activity in recombinant yeast expressing a human androgen receptor (YAR) linked to a  $\beta$ -galactosidase reporter gene. Testosterone was used as a positive control and DMSO was the negative control. 2-BP did not display androgenic activity.

**Strengths/weaknesses.** While the utility of this recombinant yeast assay system as a universal screen for androgens is debatable, this paper uses the method to compare a group of candidate compounds. The rationale for selecting 2-BP was apparently as an unknown that might have human exposure. The authors did not specify what solvent was used for specific treatment chemicals, other than to state that “an appropriate” solvent was used.

**Utility (adequacy) for CERHR evaluation process.** Lack of response of 2-BP in YAR (i.e. lack of androgenicity) provides indirect evidence that the mechanism of testicular toxicity of 2-BP does not involve the androgen receptor. This information is useful for risk assessment, even though none of the in vivo 2-BP studies point to any androgenic or antiandrogenic activity.

#### 4.3. Utility of data

The human epidemiological data are adequate to assess whether 2-BP is a human reproductive toxicant. Taken together, the papers by Kim et al. [3,4] and Park et al. [5] demonstrate a high prevalence of amenorrhea and azoospermia in 2-BP-exposed workers and no such abnormalities in their unexposed coworkers. However, the expo-

sure data in these and the other human studies are not adequate to permit a determination of a rigorous dose–response relationship in humans. The available data were limited by a narrow range of exposures, a small number of subjects, the absence of individual exposure data, and/or the possibility of significant dermal absorption of 2-BP that was not taken into account.

The animal data sets include studies conducted in male and female rats that are adequate to assess reproductive toxicity. For female rats, the data were sufficient to evaluate effects on estrous cycling, ovarian histology, ovarian follicle counts, and reproductive organ weights. For male rats, the data were sufficient to evaluate testicular histology, sperm measures, and accessory sex gland weights.

#### 4.4. Summary of reproductive toxicity

##### 4.4.1. Female reproductive toxicity

Adverse reproductive effects were reported in women occupationally exposed to 2-BP. Secondary amenorrhea and increased levels of FSH and LH were observed in 16 of 25 women aged 20–44 years who were exposed to 2-BP in a Korean electronics plant for 4–16 months; actual exposures were not measured but were estimated to be 9.2–19.6 ppm with potential peak exposures of up to 4141 ppm [3–5]. Statistical analyses were not performed, but it was noted that none of the 65 women who worked in two other departments in the same plant developed amenorrhea [5]. Ovarian biopsies revealed fibrosis in the ovarian cortex and atrophied follicles lacking oocytes or granulosa cells, arrest of follicular development, and reduced numbers of primary follicles and corpora albicans; however, the Expert Panel noted that a comparison was not made with ovaries from control women of similar ages [17]. One 24-year-old woman regained menstrual cycles following estrogen–progesterone replacement therapy. A 26-year woman, not on hormone therapy, never regained menstrual cycles but later became pregnant and gave birth to a healthy infant. In a study conducted at a Chinese 2-BP manufacturing plant, 11 women working in jobs with direct contact to 2-BP had personal exposure lev-

els of 2.87–16.8 ppm 2-BP [6]. Amenorrhea was noted only in three older women (46–54 years) and polymenorrhea in two women (age 39–43 years). Regression analyses found no significant relationships between concentrations of LH, FSH, or estradiol levels and 2-BP TWA or TWA × duration of exposure. These analyses were limited by the narrow range of exposures and the small number of subjects.

Reproductive effects observed in animal studies are similar to those observed in occupationally exposed women. Major effects noted in animal inhalation studies are outlined in Table 14. Nine-week inhalation studies in Wistar rats demonstrated that 2-BP targets the ovary at concentrations of  $\geq 100$  ppm ( $\geq 503$  mg/m<sup>3</sup>) and disrupts estrous cycles at concentrations  $\geq 300$  ppm ( $\geq 1509$  mg/m<sup>3</sup>) [32,34]. A NOAEC was not identified, as effects occurred at the lowest exposure levels tested. The primary estrous cycle effect was continual diestrus with occasional estrus, while a smaller number of rats experienced prolonged estrus with occasional diestrus. 2-BP exposure reduced the numbers of ovarian primordial, antral, and growing follicles. In the most severely affected rats, ovaries contained atretic follicles with few viable oocytes and thin granulosa cell layers and no corpora lutea. No changes in LH or FSH levels were observed in these rats. A mechanistic study suggested that 2-BP induces ovarian toxicity through apoptotic destruction of primordial follicles and their oocytes [34]. Two additional studies demonstrated reproductive toxicity in rodents, exposed to 2-BP i.p. [33,36], but the Expert Panel noted that the route of exposure and use of systemically toxic doses preclude their use in the evaluation process.

##### 4.4.2. Male reproductive toxicity

Two studies examined reproductive effects in men occupationally exposed to 2-BP. Azoospermia or oligospermia were observed in six of eight men exposed to 2-BP in an electronics plant in Korea for 16–19 months; exposures were estimated to be 9.2–19.6 ppm with daily short-term exposures up to 4141 ppm [3–5]. None of the 12 men employed in two other departments in the same plant developed azoo- or

Table 14  
Summary of reproductive toxicity in inhalation studies in female rats

Concentration in ppm (mg/m <sup>3</sup> )	Exposure regimen	Sex/species/strain	Dose: effect	Reference
100 (503), 300 (1509), 1000 (5031)	8h/9wk; whole body	Female Wistar rat	100 ppm (503 mg/m <sup>3</sup> ): ↓ primordial and growing follicles  300 ppm (1509 mg/m <sup>3</sup> ): disrupted estrous cycle; ↓ primordial, growing, and antral follicles; ↓ absolute and relative uterus weight  1000 ppm (5031 mg/m <sup>3</sup> ): disrupted estrous cycle; ↓ absolute ovary weight and absolute and relative uterus weight; ↓ primordial, growing, and antral follicles; ↑ atretic and cystic follicles and ↓ viable oocytes, and no corpora lutea	Kamijima et al. [32] and Yu et al. [34]

↑: Increased effect; ↓: decreased effect.

oligospermia. In a study conducted at a 2-BP plant in China, three men working in the manufacturing area for 15–69 months had personal exposure levels of 0.95–5.84 ppm 2-BP [6]. Two of the exposed men had less than 50% motile sperm, but all exposed men had normal morphology and sperm counts. Regression analyses revealed no significant relationships between sperm indices or LH, FSH, or testosterone levels and 2-BP TWA or TWA  $\times$  duration of exposure. The analyses were limited by the narrow range of exposures and the small number of subjects.

Reproductive effects in male rats exposed to 2-BP were reported in two inhalation studies; major effects in these studies are outlined in Table 15. Inhalation exposure of rats to  $\geq 300$  ppm (1509 mg/m<sup>3</sup>) for at least 9 weeks resulted in atrophy of seminiferous tubules, reductions in germ cell numbers, and hyperplastic Leydig cells [22,26] in addition to reduced sperm counts and motility with increased numbers of abnormal sperm [22]. Although inhalation is the primary route of human exposure, testicular lesions, sperm effects, and/or Leydig cell hyperplasia were also observed in animal studies with i.p., s.c., or oral exposure [25,37,43]. Although limited, a s.c. injection study in male rats treated with  $\geq 600$  mg/kg bw 2-BP for 5 weeks demonstrated reductions in mating and fertility [37]. A series of studies demonstrated that acute, high-dose, parenteral exposure to 2-BP causes apoptotic death of spermatogonia within 3 days of exposure [38,40,43,45]. 2-BP exposure also resulted in apoptosis in spermatocytes at about 9 days after the end of a 5-day treatment [45]. 2-BP did not display androgenic activity in an in vitro assay [46].

#### 4.4.3. Summary statements

There is sufficient evidence in humans that exposure to 2-BP causes reproductive toxicity in both males and females. However, the small number of exposed individuals reported and uncertainties concerning the route and dose of exposure limit the utility of this data set.

There is sufficient evidence in female rats that exposure to 2-BP causes reproductive toxicity manifested as ovarian dysfunction following inhalation at  $\geq 100$  ppm daily for 8 h per day for 9 weeks. The data are assumed relevant to consideration of human risk.

There is sufficient evidence in male rats that exposure to 2-BP causes reproductive toxicity manifested as decreased reproductive organ weights, poor sperm quality, and testicular histopathology following inhalation at  $\geq 300$  ppm daily for 8 h per day for 9 weeks. The data are assumed relevant to consideration of human risk.

## 5. Summaries, conclusions, and critical data needs

### 5.1. Summary and conclusions of reproductive and developmental hazards

Information concerning risk of developmental toxicity associated with exposure to 2-BP is lacking in humans. Data from standard animal developmental toxicity tests are lacking and ancillary data from a study designed to assess female reproductive toxicity in rats were judged to be inadequate [33].

Table 15  
Summary of reproductive toxicity in inhalation studies in male rats

Concentration in ppm (mg/m <sup>3</sup> )	Exposure regimen	Sex/species/strain	Dose: effect <sup>a</sup>	Reference
300 (1509), 1000 (5031), 3000 (15092)	8h/7d/9wk; whole body (9–11 days exposure period in high dose)	Male Wistar rat	300 ppm (1509 mg/m <sup>3</sup> ): $\downarrow$ Absolute and relative epididymides and testes weight and $\downarrow$ absolute prostate and seminal vesicles weight; $\downarrow$ sperm count and motility and $\uparrow$ abnormal sperm; $\uparrow$ seminiferous tubule atrophy and hyperplastic Leydig cells, $\downarrow$ germ cells 1000 (5031 mg/m <sup>3</sup> ): $\downarrow$ absolute and relative epididymides and testes weight and $\downarrow$ absolute prostate and seminal vesicles weight; $\downarrow$ sperm count and motility, very few intact sperm remaining; $\uparrow$ seminiferous tubule atrophy and hyperplastic Leydig cells, $\downarrow$ germ cells 3000 (15092 mg/m <sup>3</sup> ): $\downarrow$ absolute and relative epididymides and testes weight and $\downarrow$ absolute prostate and seminal vesicles weight; $\downarrow$ sperm count and motility, $\uparrow$ seminiferous tubule atrophy, hyperplastic Leydig cells and vacuolation of Leydig cells, $\downarrow$ germ cells	Ichihara et al. [22]
100 (503), 1000 (5031)	8h/7d/12wk; whole body	Male Wistar rat	Reproductive NOAEC = 100 ppm (503 mg/m <sup>3</sup> ) 1000 ppm (5031 mg/m <sup>3</sup> ): seminiferous tubule atrophy and $\downarrow$ germ cells.	Yu et al. [26]

$\uparrow$ : Increased effect;  $\downarrow$ : decreased effect, h: hour, d: days; wk: week.

<sup>a</sup> Non-reproductive effects for male rats are summarized in Section 4.



There are human and rat data for assessing reproductive toxicity. Reproductive effects observed in female rats are similar to those observed in occupationally exposed women. Specifically, inhalation exposure of female rats to 2-BP causes irregular estrous cycles, decreased ovarian and uterine weight, and ovarian histopathology, all of which become more pronounced with increasing dose [32,34]. Amenorrhea with associated ovarian pathology in selected women has been associated with occupational exposure to 2-BP [3], although the inadequate exposure assessment and small sample size limit its use in assessing human health risks. Despite these limitations, the consistency and similarity of the animal and human data support the contention that 2-BP is an ovarian toxicant in humans. Detailed evaluation of 2-BP-induced effects on rat ovarian follicles indicates that 2-BP, at concentrations as low as 100 ppm (503 mg/m<sup>3</sup>), destroys primordial and growing ovarian follicles; antral follicles were affected at the 300 ppm (1509 mg/m<sup>3</sup>) dose [34].

2-BP is also a reproductive toxicant in male rats. At concentrations as low as 300 ppm (1509 mg/m<sup>3</sup>) (by inhalation daily for 9 weeks) exposure to 2-BP resulted in decreased reproductive organ weights including the testes, epididymides, and accessory sex organs, as well as decreases in epididymal sperm counts and in the percentages of motile and morphologically normal sperm, and testicular pathology [22]. A concentration of 100 ppm (503 mg/m<sup>3</sup>) appears to be a NOAEL for testicular toxicity [26]. Evaluations conducted after i.p. [25] or s.c. [37] dosing provided evidence for altered Sertoli cell structure, Leydig cell hyperplasia, and decreased serum testosterone. Further histologic studies showed that 2-BP specifically kills spermatogonia, at least after short-term exposure to high doses [38,40]. This spectrum of effects is similar to that seen in humans occupationally exposed to 2-BP [3]. Therefore, the toxicology data from male rats, as from female rats, provide support for the contention, based on limited epidemiological data, that 2-BP is a reproductive hazard in humans.

### 5.2. Summary of human exposure

In Asia, 2-BP was used as a cleaning solvent in the electronics industry. Data on 2-BP exposures in Korea were collected in conjunction with health effects studies. These data provide a very limited description of occupational exposures during the use of 2-BP as a cleaning solvent because they were based on area samples from a simulation of the cleaning operation (9.2–19.6 ppm). Personal exposures may have been higher than indicated since two open, unventilated baths were not included in the simulation. Further, peak exposures experienced by workers as they cleaned parts inside the bath hoods were only partially simulated [3–5]. The Chinese study of 2-BP manufacturing had personal exposure measures for a single day for each worker in the study [6]. However, only 14 of the 24 workers in the

study had direct contact with 2-BP (0.95–16.18 ppm). Neither the Korean or Chinese study characterized the dermal exposures of workers.

It has been reported by HSDB [1] that in the US, 2-BP is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organic chemicals. The extent of this use is unknown. Currently in the US, 2-BP exposures most commonly occur because it is a contaminant in 1-BP at ≤0.1% [7]. No information was found that documents exposure of the public to 2-BP through contact with air, drinking water, food, or consumer products.

Current US exposure data on 2-BP are limited to workplace surveys of three spray adhesive and one cold bath degreasing operation (<0.004–1.35 ppm) [8,9,12–14]. These data cannot be considered to represent the full cross-section of potential exposures nationwide. None of the exposure evaluations to date have characterized the dermal exposures of the workers.

### 5.3. Overall conclusions

The Expert Panel judged the human and animal evidence on the developmental toxicity of 2-BP to be insufficient based upon lack of available data.

The evidence for human male and female reproductive toxicity based upon one human study in Korea was considered sufficient. The evidence was very suggestive that exposure to 2-BP was responsible for an elevated risk of adverse female reproductive endpoints such as amenorrhea and adverse male reproductive endpoints such as decreased sperm count and motility. These effects occurred in an occupational setting with potentially high short-term exposure levels. A study conducted in China, with lower estimated exposure levels, did not demonstrate a significant relationship between 2-BP exposure measures and adverse reproductive endpoints in men or women. Several methodological concerns including the small sample sizes, a failure of investigators to fully integrate the analysis of exposure and outcomes, and the very narrow range of exposures in the latter study diminish the value of these data in estimating a dose–response gradient for potential use in risk assessment.

The Expert Panel judged that sufficient evidence exists to characterize 2-BP as a reproductive toxicant in male and female rats. The rat data were assumed relevant to assessing human reproductive hazard. In female rats, inhalation of 2-BP at doses greater than or equal to 100 ppm (503 mg/m<sup>3</sup>) caused reduced follicle numbers, altered estrous cycle length, and decreased ovarian and uterine weight. No NOAEC was identified for female reproductive toxicity. In male rats, inhalation of 2-BP at levels greater than or equal to 300 ppm (1509 mg/m<sup>3</sup>) caused decreased sex accessory gland weights, decreased sperm counts and motility, increased abnormal sperm, and histological abnormalities in the testes. A NOAEC of 100 ppm (503 mg/m<sup>3</sup>) was identified for male rat reproductive toxicity.

The mechanisms by which 2-BP causes reproductive toxicity are unknown. Several studies in male rats using high, acute, parenteral doses showed that 2-BP specifically causes spermatogonia to die by apoptosis. However, the relevance of these findings to lower, non-systemically toxic doses of 2-BP is unclear. Female rat inhalation exposure to 2-BP resulted in the death of ovarian follicles of all classes. Similar to the results in the testis, apoptosis was noted in the oocytes and granulosa cells of primordial follicles following exposure to high concentrations of 2-BP.

Based upon the integration of findings from human and animal studies, the panel concluded that there is sufficient evidence that 2-BP is a reproductive hazard in men and women. Current 2-BP exposures in the United States are anticipated to be largely limited to the occupational setting by means of contamination of 1-BP. 2-BP exposure measurements evaluated by the panel ranged from <0.004 to 1.35 ppm from a few selected locations and cannot be considered to represent the full cross-section of potential exposure levels nationwide.

The panel concluded that there is minimal concern for human reproduction at the low end of this exposure range with an increase to some concern at the upper end of this range.

#### 5.4. Critical data needs

Critical data needs are defined as tests or experiments that could provide information to substantially improve an assessment of human reproductive risks. Although US exposures to 2-BP are likely to be low and largely restricted to exposures to 2-BP-contaminated 1-BP, the evidence of human reproductive toxicity underscores the need for greater confidence in the exposure estimates and toxic effects.

- **Exposure.** According to HSDB, 2-BP is used as an intermediate in chemical synthesis. In addition, 2-BP may be present as a contaminant in 1-BP at levels up to 0.1%. Limited data is available on current occupational exposures. The presence of 2-BP in the environment or consumer products is unknown. The extent of air and dermal exposure from these and other potential sources should be determined to increase confidence in the levels of exposure.
- **Effects.** Currently, there are no animal data that evaluate the developmental toxicity of 2-BP. At a minimum, a standard rodent developmental toxicity assay should be performed to improve our understanding of the potential toxic effects.

Although not considered a critical data need, the following type of study would provide information that would contribute to our understanding to the toxicity of 2-BP.

##### 5.4.1. Basis of toxicity

Because 2-BP is structurally related to a group of haloalkanes with known reproductive toxicity, mechanis-

tic studies evaluating these compounds would be useful to identify pathways of activation and targets.

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