CHEMICAL MEETING THE CRITERIA FOR LISTING VIA THE AUTHORITATIVE BODIES MECHANISM

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Reproductive and Cancer Hazard Assessment Section
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1-Bromopropane meets the criteria for listing under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code section 25249.5 et seq.), more commonly known as Proposition 65, via the authoritative bodies listing mechanism as known to the State to cause reproductive toxicity. The regulatory requirements for listing by this mechanism are set forth in Title 22, California Code of Regulations §12306[1]. For example, the regulations include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body the Office of Environmental Health Hazard Assessment (OEHHA) uses to determine whether listing under Proposition 65 is required.

The National Toxicology Program solely as to final reports of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) is one of five institutions which have been identified as authoritative bodies for identification of chemicals as causing reproductive toxicity for the purposes of Proposition 65 (§12306(1)(3)). NTP-CERHR has identified 1-bromopropane as causing reproductive toxicity. OEHHA has found that this chemical has been "formally identified" as causing reproductive toxicity as required by §12306(d). 1-Bromopropane was the subject of a report published by NTP-CERHR which concludes that this chemical causes reproductive toxicity. Also, the document specifically and accurately identifies the chemical and the document meets one or more of the criteria required by §12306(d)(2).

OEHHA also finds that the criteria in regulation for "as causing reproductive toxicity" (§12306(g)) have been satisfied for 1-bromopropane. In making this evaluation, OEHHA relied upon the discussion of data by NTP-CERHR in making its finding that the specified chemical causes reproductive toxicity. A brief discussion of the relevant reproductive and developmental toxicity studies providing evidence for the finding is presented below. Much of the discussion is taken verbatim from the NTP-CERHR report. The statements in bold reflect data and conclusions that satisfy the criteria for the sufficiency of evidence for reproductive toxicity (§12306(g)). The full citation for the NTP-CERHR document is given in this report.

[1] All further references are to Title 22 of the California Code of Regulations unless otherwise indicated.

Chemical Meeting the Criteria for Listing as Known to the State to Cause Reproductive Toxicity

Chemical	CAS No.	Toxicological Endpoints	Chemical Use	Reference
1-Bromopropane (1-BP)	106-94-5	developmental toxicity male reproductive toxicity female reproductive toxicity	Mostly used as a solvent for fats, waxes, or resins. Some is used to synthesize pharmaceuticals, insecticides, flavors and fragrances. 1-BP is also used in some spray adhesives and in cleaning metal and electronic components.	NTP-CERHR (2003)

1-Bromopropane (1-BP) (CAS No. 106-94-5).

1-BP caused developmental toxicity in rats in the form of decreased fetal weight and increased incidence of skeletal variations.

1-BP caused numerous adverse reproductive effects in male and female rats including decreased fertility, decreased numbers of implantation sites and litter size, increased precoital interval; decreased prostate weight, decreased sperm mobility and normal sperm count in males, and increased ovarian follicular cysts and estrous cycle length in females.

The NTP-CERHR has concluded that there is clear evidence of adverse effects for developmental and reproductive toxicity (males and females) in laboratory animals (NTP-CERHR, 2003). Further, NTP-CERHR states that "Although there is no direct evidence that exposure of people to 1-BP adversely affects reproduction or development, studies reviewed by the expert panel and more recent studies in rats show that exposure to 1-BP can adversely affect reproduction and development." "[T]he NTP judges the scientific evidence of effects in laboratory animals sufficient to conclude that 1-BP may adversely affect human development and reproduction if exposures are sufficiently high." (NTP-CERHR, 2003, p. 2).

The *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of 1-Bromopropane* by the NTP-CERHR Bromopropanes Expert Panel is incorporated into NTP-CERHR (2003) as Appendix II. The Expert Panel reviewed numerous studies of 1-BP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003, Appendix II), is summarized below.

DEVELOPMENTAL TOXICITY
Experimental Animal Data

A standard developmental toxicity study was conducted using Crl: CD (SD) IGS BR Sprague-Dawley rats (Huntingdon Life Sciences, 2001). Pregnant animals (25/group) were exposed to actual vapor concentrations of 0, 520, 2,530, or 5,060 mg/m^3 [0, 103, 503, or 1,005 ppm] 1-BP (99.9% purity) for 6 hours/day from gd 6–19 (day of conception = day 0). Exposures were conducted in whole-body chambers under dynamic conditions. Concentrations were monitored by infrared (IR) spectrometry. Nominal chamber concentrations were selected to produce a gradation of effects from the lowest to the highest dose. Pregnancy was terminated on gd 20 and the fetuses removed by cesarean section.

Lacrimation and salivation were observed in animals exposed to 1,005 ppm (5,060 mg/m^3). Mean maternal bodyweight and bodyweight corrected for gravid uterus weight in the 503 ppm (2,530 mg/m^3) and 1,005 ppm (5,060 mg/m^3) groups were significantly reduced compared to the concurrent controls; maternal bodyweight gain and food consumption were also significantly reduced in these two groups. Fetal bodyweight was significantly reduced in all treated groups. A significant treatment-related increase in the litter incidence of bent ribs was seen in the 1,005 ppm (5,060 mg/m^3) group, but the authors considered this condition reversible and frequently observed in untreated rats. A slight (but not significant) increase in the incidence of wavy ribs was observed in litters exposed to 503 ppm (2,530 mg/m^3) 1-BP. No fetuses with wavy ribs were found in either control litters or in those recovered from dams inhaling 103 ppm (520 mg/m^3). Reduced skull ossification was observed at 503 and 1,005 ppm (2,530 and 5,060 mg/m^3), and was associated with maternal toxicity and reduced fetal bodyweight by authors. (Huntingdon Life Sciences, 2001).

Limited information about developmental toxicity associated with exposure to 1-BP is also available from a two-generation reproductive toxicity study in which Crl:CD(SD) IGS BR rats were exposed to 0, 100, 250, 500, or 750 ppm [0, 503, 1,257, 2,514, 3,771 mg/m^3] 1-BP vapors (99.8% purity) during premating, mating, gestation, and lactation (pnd 5-21) (WIL Research Laboratories, 2001). Exposure concentrations within each chamber were measured 9–10 times during each exposure period by a validated GC method. Exposure of F0 rats commenced at 7 weeks of age and F1 rats began direct exposure at weaning. Exposures were conducted for at least 70 days prior to mating. Females were not exposed on pnd 0–4 and only they, not their litters, were exposed during pnd 5–21. Therefore, offspring (litters) were indirectly exposed to the test chemical *in utero* and through nursing. In addition, the F1 pups selected randomly for propagation of F2 litters were directly exposed from postnatal day 22 forward. Results in treated animals were compared to both air control and historical control data from WIL Research Laboratories.

Statistically significant reductions in live litter size were observed in F_0 and F_1 females exposed to 500 ppm and no offspring were observed in F_0 females exposed to 750 ppm.

Given that there were proportionate decreases in implantation sites in the F_0 dams, the Panel noted the plausible explanation of an effect on fertility rather than adverse effects on development. Significant reductions in neonatal weight gain during nursing occurred in F_1 males and F_2 males and females of the 500 ppm group.

REPRODUCTIVE TOXICITY

Experimental Animal Toxicity

In the two-generation reproductive toxicity study described above (WIL Research Laboratories, 2001) the potential adverse effects of 1-BP whole-body inhalation exposure in F0 and F1 parental rats were evaluated; reproductive capabilities were examined in the F0 and F1 generations.

Prior to mating, the F0 female rats exhibited increased estrous cycle length. While this effect appeared to be dose-related, statistical analysis of the data was not conducted, in part because several animals in each of the high dose groups did not cycle at all. However, the study authors considered values for the 500 and 750 ppm groups to be test agent related since they exceed the range of their historic control data for this end point (4.1–5.1 days). Reproductive performance was impaired in the higher dosage F0 groups as evidenced by significant decreases in male/female mating index in the 750 ppm group, and in the male/female fertility index in the 500 and 750 ppm groups. An increased time to coitus in the F0 500 and 750 ppm groups was not statistically significant but was considered test agent related since it exceeded historical control values. None of the females in the F0 750 ppm group became pregnant. The numbers of implantation sites and pups born to F0 females were significantly reduced in the 500 ppm group.

At necropsy, significant reductions in F0 absolute reproductive organ weights were observed for ovary (750 ppm), cauda epididymis (500 and 750 ppm), prostate (≥250 ppm, but did not decrease with increasing dose), seminal vesicles (750 ppm), and pituitary (750 ppm). Significant decreases in relative weights of these organs were only observed in the 750 ppm group for caudae epididymides and ovaries. Ovarian histologic analysis in F0 rats in the 750 ppm group revealed a significant increase in the incidence of ovaries with reduced numbers of corpora lutea and with follicular luteinized cysts. In males, a slightly increased incidence of seminiferous tubule degeneration was not considered treatment related since lesions in 4 of 6 affected rats were of minimal severity. Also, testicular sperm counts (absolute or per gram testis) were not significantly altered by treatment. An analysis of cauda epididymal spermatozoa from F0 rats revealed significant reductions in morphologically normal sperm at ≥250 ppm. However the decrease from 99.7% normal sperm in controls to 99.3% at 250 ppm was not considered to be treatment related because this value is above historical control value of 99.0%. Cauda epididymal sperm numbers were significantly reduced at 750 ppm and the percentage of motile sperm was significantly reduced at 500 and 750 ppm.

1-BP exposure in the F1 animals was initiated on pnd 22. Twenty-five rats/sex/group in control and 100–500 ppm treatment groups were selected for mating. The mating experiment was conducted as described for the F0 rats. Increased estrous cycle lengths in the 250 and 500 ppm F1 groups (4.9 and 5.1 days) were within ranges of historical controls (4.1–5.1) but were nevertheless attributed by the authors to be related to 1-BP treatment. This judgement was based on the fact that 3 and 4 animals, in the 250 and 500 ppm groups, respectively, had no complete estrous cycles (versus only 1 each in the control and 100 ppm groups). Again, no statistical analysis was performed for this endpoint. No significant effects were noted for F1 fertility or mating indices, days to mating, gestation length, or birthing complications. However, authors noted that non-significant and non-dose-related reductions in fertility indices in the F1 100, 250, and 500 ppm groups (68, 64, 72%, respectively) were below fertility indices of historical controls (~90%). Mean numbers of implantation sites were reduced in the F1 dams in the 250 and 500 ppm groups with statistical significance achieved at the higher dose level. Live litter size was significantly decreased at 500 ppm. Apparent increases in the incidence of ovarian follicular cysts and interstitial cell hyperplasia (mild) in F1 females in the 500 ppm group were not statistically significant. Absolute (but not relative) epididymis and pituitary weights were significantly reduced in the F1 500 ppm males. Lesions observed in testes were considered minimal and their incidence was not altered significantly by treatment, although there appeared to be a trend. Other male reproductive organs were histologically normal. The percentage of motile sperm was slightly, but significantly, reduced in the F1 males (from 89% in controls to 85%) at 250 ppm. The study authors did not consider this treatment-related since this value exceeds that of historic controls. However, the percentage of motile sperm was further (and significantly) reduced to 74% in the 500 ppm group. The percentages of morphologically normal sperm were significantly reduced at 500 ppm. A slight but statistically significant reduction from 99.5% normal sperm in controls to 98.9% in the 100 ppm group was not considered to be test article related because the difference was very small, and no significant changes were seen in the 250 ppm group

A study by Ichihara et al. (Ichihara et al., 2000) examined the dose response of 1-BP-induced testicular toxicity including sperm measures (motility/morphology) and detailed testicular histology (testes fixed in Bouin's and stained with period acid Schiff 's reagent). In the examination of testicular histology, subtle changes in seminiferous tubule cell associations were evaluated. These included enumeration of spermatogenic cells in stage VII tubules and elongated spermatids retained in stage IX−XI tubules (normally released at stage VIII). Eight-to-nine, 10-week-old male Wistar rats were exposed to air or 200, 400, or 800 ppm [1,006, 2,012, or 4,025 mg/m^3] 1-BP vapors (99.81% purity) for 8 hours/day for 12 weeks. Chamber concentrations of 1-BP were measured by GC and reported. At the end of the exposure period the rats were sacrificed and necropsied. Data were evaluated by one-way ANOVA followed by Dunnett's method. Significant reductions in absolute organ weights were observed for seminal vesicles (≥200 ppm), epididymides and pituitary (≥400 ppm), and

prostate (800 ppm). Significant reductions in relative organ weights were noted in seminal vesicles (≥200 ppm) and epididymides (800 ppm). Bodyweight gain was reduced in the 400 and 800 ppm groups. Histopathological changes were observed in the epididymides, prostate, and seminal vesicles of the 800 ppm group. Epididymides had reduced duct cavity diameter, wider interstitial space, increased epithelial cell height and contained neutrophils or degenerated epithelial cells. Prostate and seminal vesicles had reduced alveoli size and degenerated cells were observed in the seminal vesicle cavity. Histological evaluation of testes revealed vacuolated seminiferous epithelium in 2 of 9 rats of the 800 ppm group. The numbers of retained elongated spermatids in stages IX, X, and XI were significantly increased in 400 and 800 ppm groups and a significant increase in degenerating spermatocytes in stage VII was seen in the 800 ppm group. Sperm quality was also affected as observed by significant reductions in sperm count and motility and increases in tailless sperm at ≥400 ppm. At 800 ppm a significant increase in sperm with abnormal heads (banana-like or straight) was observed. Plasma testosterone level was significantly reduced in the 800 ppm group, but there were no changes in follicle stimulating hormone (FSH) or luteinizing hormone (LH) levels.

Saito-Suzuki et al. (1982) conducted dominant lethal studies in rats to determine the structure-activity relationships of 5 halogenated propanes, including 1-BP (>98% purity). Eleven-week-old male Crl: Sprague Dawley rats (n=15/group) were gavaged with 10% of the acute lethal dose of each compound in olive oil for 5 days. 1-BP was administered at a dose of 400 mg/kg bw. Olive oil was the negative control and 1,2-dibromo-3-chloropropane was the positive control (n=15/group). At 1–8 weeks after treatment, the males were mated weekly with untreated females. Data were analyzed using Fisher's Exact Method and the Mann-Whitney U test. 1-BP treatment had no effect on male fertility. Females (n=15/time period) were sacrificed 13–14 days after mating and examined for corpora lutea, implants, live embryos, and early and late embryonic deaths. 1-BP treatment increased the number of mean dead implants at the 8-week mating but had no effect on the dominant lethal mutation index (live embryos per test female/live embryos per control females).

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