BROMINATED SOLVENTS COMMITTEE

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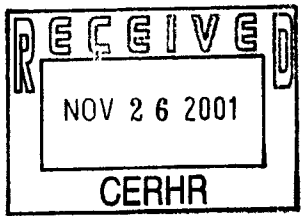

Dr. Michael D. Shelby, Ph.D.
Director, NTP Center for the Evaluation
of Risks to Human Reproduction
79 T.W. Alexander Drive, Building 4401, Room 102
P.O. Box 12233 EC-32
Research Triangle Park, NC 27709

RE: 1-bromopropane (1-BP; CASRN: 106-94-5) CERHR review

Dear Dr. Shelby,

The Bromine Solvents Consortium (BSOC) would like to submit comments to the Center for Evaluation of Risks to Human Reproduction on the draft "NTP-CERHR Expert Panel Report on Reproductive and Developmental Toxicity of 1-Bromopropane". BSOC is a consortium of three major manufacturers of 1-bromopropane - Albemarle Corporation, Great Lakes Chemical Corporation, and Dead Sea Bromine Group.

One of BSOC's major objectives was to add to the toxicity database for 1-BP such that occupational exposure limits could be set that would protect against any target organ effects. Studies were planned using standard methodologies to explore all endpoints, and conducted in independent testing laboratories under good laboratory practices. Studies used male and female animals, and were conducted by the most appropriate exposure route - inhalation. This philosophy appears to contrast with most of the more academic 1-BP work conducted in Japan, that focused on male animals and were designed for very specific endpoints of neurotoxicity or inferred effects on reproduction by examining sperm or testicular histopathology. We have provided NTP with full copies of the BSOC studies, have provided more detail in these comments, and will provide any further raw data that the scientific panel may require.

Dr. Nancy O'Malley, Albemarle Corporation, will attend the the evaluation meeting December 5-7, 2001. She can be reached at (225) 388-7611 if you have questions on the BSOC comments prior to the meeting.

Sincerely,

Gary TerHaar
Chairman, BSOC

Comments on Draft: " NTP-CERHR Expert Panel Report on Reproductive and Developmental Toxicity of 1-Bromopropane, October, 2001"

1.0 Chemistry, Usage and Exposure

1.1.3 Chemical and Physical Properties

Table 1.1 Physicochemical properties of 1-BP

Flashpoint: 25.6 C (reference cited is Aldrich, 1996)

Flammability: Flammable (reference cited is Aldrich, 1996)

Comment: Results of 11 flammability studies conclusively document that 1-BP should not be classified as flammable. They are summarized in the enclosed BSOC Letter to the Environmental Protection Agency (Fensterheim, 2000). In most cases, no flash point was determined at the temperature ranges typically used to assess flammability. When a flash point was determined, it was at a temperature greater than the level that would classify a compound as flammable under the classification systems developed by the National Fire Protection Agency, the U.S. Department of Transportation, the European Union, the International Maritime Organization, and the European Agreement Concerning the International Carriage of Dangerous Goods by Road (ADR).

To compare with other solvents, flammability limits can be expressed. The following is cited in the UNEP Technology and Economic Assessment Panel Report, April, 2001: Appendix 4: Safety Issues

"There is disagreement concerning the closed-cup flash point of NPB, but there is agreement that nPB does not have an open-cup flash point. "

Table "Comparison of the Physical Characteristics and Permitted Exposure Limits (PEL) of nPB, 1,1,1 Trichloroethane and Trichloroethylene (TCE)"

	NPB	1,1,1 TCA	TCE
Flammability Limits, %	4-7.8	7.5-12.5	8-10.5

Comment: Flammability is not indicated on the physico-chemical property section for 2-BP.

1.1.3 Table 1-1 Physicochemical properties of 1-BP
Stability Stable

Comment: The draft review of 2-BP comments under stability: "Stable with normal use and storage". This would be applicable to 1-BP also. Stability of commercial products under use conditions is enhanced with stabilizers unique for the application conditions. (see below).

1.1.4 Technical Products and Impurities

"... One case study involving a > 95% solution of 1-BP reported the contaminants of 1-BP as butyline (sic) oxide (<0.5%), 1,3 dioxalane (<2.5%) and nitromethane (<0.25%) (Sclar, 1999)"

Comment: The presence of butylene oxide, 1,3 dioxalane and nitromethane would not be contaminants of 1-BP, but additives in commerical products. These additives are present to enhance stability of the product under use conditions in vapor degreasers. Degreaser solvents can be tested periodically and further additives applied to prolong solvent acceptibility. This decreases loss of solvent, and reduces numbers of total degreaser solvent cleanout.

Table 1-2 Specifications for Vapor-Degreasing Grade and General Grade 1-BP (ASTM, 2000)
Footnote: "A tradename for 1-BP is ALBTA1 (O'Malley, 2001a)"

Comment: ALBTA1 is not a tradename. It is the name given to the 1-BP test article used in the Clintrial 13 week rat inhalation study. The ALBTA1 test article contained no stabilizers or additives as do the commercial products sold under tradenames.

1.2 Use and Human Exposure

1.2.1 Production information

"...No information is available on the quantity of 1-BP produced in the United States at this time....

"OSHA projected that up to 240 million pounds could be produced anually if it remained unregulated and was used to replace chlorinated solvents such as trichloroethylene, perchloroethylene, and methylene chloride in vapor degreasing and cold metal cleaning operations... Some of the market for HCFC's that currently have an annual consumption rate of 130 million pounds a year. In addition 2.5 million pounds of 1-BP could be used annually as a cleaning agent for machinery in manufacturing plants within 3 years."

"...Poly Systems reported that up to 80 million pounds of 1-BP could be used in metal cleaning operations, 10 million pounds in aerosols, and 55 million pounds in adhesives."

Comment: From UNEP TEAP report: "The Brominated Solvents Consortium (BSOC) estimated global sales and emissions of nPB for solvent and adhesive applications at 4839 metric tonnes in 2000, 3152 metric tonnes in 2001, and 3736 metric tonnes in 2002. Members of the BSOC are Albemarle Corporation, Great Lakes Chemical Corporation, and Bromine Compounds, Ltd. The Task Force estimates that these companies accounted for about half of nPB production in 2000. Each BSOC company separately estimated global production for each year - mindful of relevant technical, regulatory and commercial factors - and submitted these confidential estimates to BSOC. These annual company estimates were then averaged by BSOC to arrive at initial estimates that were then provided ot BSOC members for reconsideration. Each company then provided its revised estimates to BSOC and averaged to the estimates presented here."

Comment: See also Albemarle letter to M. Shelby, Sept. 26, 2001

2.0 General Toxicological and Biological Parameters

2.1 Toxicokinetics and Metabolism

Comment: The CERHR draft report cites the 1-BP blood:gas partition coefficients for humans and rats as 11.7 and 7.08; the original work (Gargas, et al. 1988) cites the values for the same coefficients for isopropyl bromide (5.95 and 2.57). The study used F-344 rats for the blood portion. Other tissue:air and liquid:air partition values calculated for the F-344 rat included muscle, liver, fat, olive oil and saline. These coefficients have shown value in PB-PK modelling. Clewell (1998) stated that the values of the coefficients for 1-BP indicated low potential for accumulation of 1-BP in the body.

Comment: The Kim et al., 1999a study is cited in this section for the work on Cytochome P-450 enzymes, and a note in the CERHR draft report indicates that method of statistical analysis was not discussed. The 1-BP testing program undertaken by H.Y. Kim and others at the Industrial Health Research Institute included acute and repeated dose inhalation toxicity studies, genetic toxicity studies (Ames and micronucleus tests), activity of Cytochrome P-450 enzymes, effects on cellular immune systems, and an assessment of olfactory behavior of rats. The statistical method section of the report on this project to the director of Korea Industrial Safety Corporation (Kim, H.Y. etal 1998) is interpreted to say that data in that portion of the study were analyzed using paired-sample t test, using SPSS. Body weight and organ weight data, as well as hematology and urinalysis data in exposed and control groups were compared with ANOVA and Duncan's multiple test method. Significance levels were ($p<0.05$) in preliminary tests, and 0.1% ($p<0.01$) in main tests.

Comment: Another in vitro liver metabolism study using Sprague Dawley rats, instead of Wistar rats as cited in the Kaneko study, was conducted by Tachizawa, et al, 1982. This study compared metabolism of 1-propyl halides (chloride, bromide, and iodide) by hepatic microsomes

from phenobarbital induced rats. Propene, 1,2 epoxypropane, 1,2 propanediol, and propionic acid were identified.

2.2 General Toxicity

2.2.1 Human Data

"Sclar (1999) published a case study of a 19 year old male who experienced weakness of the lower extremeties and the right hand, numbness, and difficulty swallowing and urinating after a 2 month ocupational exposure to a degreasing and cleaning solvent..."

Comment: Conversations between the author of the case study and the manufacturer of the 1-BP product indicated several short comings in the investigation of this case. No previous work history/previous chemical exposures were available for the patient. No list of other solvents used in the workplace, nor air monitoring in the establishment was available. A major barrier to utility of this study is loss of the patient to follow-up. It would have been valuable to see if the remarkable recovery of lower limb function before discharge continued. Initial treatment at the first hospital was for multiple sclerosis, which can have periods of reversal. On another note, skin darkening has not been associated with 1-BP exposure.

2.2.2 Animal Data

General Comment: In several places under "Utility (Adequacy) for CERHR Evaluation Process" a statement is made that the study demonstrates the toxicity of 1-BP under the conditions of exposure that are directly comparable to other substances. Does this mean the conditions of exposure are comparable, or that the results are similar to the toxicity of other substances? This is unclear.

P.9 Elf Atochem acute studies:

Comment: Replace LD50 with LC50, 4 hours. The study measured a lethal concentration, not a lethal dose.

P. 9 Kim, 1999b

"....For the acute study..."One female in the 13,000 ppm group and 4 females and 2 males in the 15000 group died within 24 hours. All rats in the 17,000 ppm group died within an unspecified time period...

Comment: According to the Report to the Industrial Health Research Institute (H.Y. Kim et al., 1998), the female rat that died in the 13,000 ppm group and the 4 female and 2 males in the 15,000 ppm group died after 12 hours had passed from start of exposure; 3 female and 4 male

rats in the 17,000 ppm group died before three hours had passed, and the rest of that group died after 12 hours had passed. All surviving animals recovered clinically within 24 hours of the end of exposure.

P. 10 Strength/Weaknesses: "..A weakness is the lack of clarity as to when animals were terminated following the last exposure, and how many days of exposure preceded necropsy"

Comment: Analytical chamber data (H.Y.Kim etal, 1998) indicates 40 exposure days, and that food was removed the last day of exposure, and necropsy occurred the next day.

P. 10 Utility...: "There is a clear dose-response for effects, and histopathologic findings can be correlated with serum biochemistry"

Comment: This statement is a bit misleading. There is not a dose response relationship for all effects. Does the statement refer only to histopathology effects? If so, the only histopath effects mentioned in the CERHR summary were hepatocytic vacuolation in all treated animals, without a dose response, and renal tubular casts in females at the high dose group. The renal tubular casts are not correlated with an increase in BUN or creatinine that would imply that the kidney pathology was reflected with changes in biochemistry representing kidney function. Similarly, liver enzymes were not increased to indicate that the vacuolation affected liver function.

Comment: The Kim study is important in assessment in that it bridges the 4 week repeated dose inhalation study and the 13 week study conducted at Clintrials. All three studies used male and female Sprague Dawley rats, and 6 hour, 5 day a week exposure protocols. These exposure protocols were designed to model workplace exposures.

P. 11 Clintrials, 1997a: 28 day inhalation study:

Comment: This study was designed as a range-finding study to choose test concentrations for a 13 week repeated dose inhalation study. Design of the 28 day study and the 13 week study would use 6 hour per day, 5 days per week exposures to mimic occupational exposures. A 13 week study in rats covers a large portion of rat lifespan, so that a no adverse effect level from such a study could be used to develop an interim workplace exposure guideline.

"An abbreviated functional observational battery revealed impaired gait (ataxia and hypotonic gait) in the 8.0 mg/L group. No neurotoxicity was seen in other groups..."

Comment: The observation of impaired gait in the high dose group doesn't necessarily indicate neurotoxic endpoints . They are signs of functional impairment, but could be just a

continuation of signs indicating moribund animals. It should be pointed that only two male animals and 7 female animals were available from the high dose group for functional observational battery at the end of the study. Onset of clinical signs indicating toxicity in these animals began on day 13 for the males, and 7 high dose males and 3 high dose females were sacrificed between day 13 to 23 for deteriorating condition. The clinical signs covered the progression of signs in moribund animals - tremors and increased activity on to weakened condition, decreased activity, lying on side, cold to the touch. The mean body weight of the two surviving male animals was 184 grams compared to 348 grams in the control animals, and many of the female animals were described as emaciated. The serum chemistry in the single high dose male from which a usable blood sample was obtained had a high blood urea nitrogen, another indicator that the animal was deteriorating.

"Microscopic evaluation indicated vacuolation of the brain for all treated groups...."

Comment: It is important not to group "vacuolation" into a single category for assessment in this study. As described in the pathologist's report, there was a distinct difference in appearance between the white matter vacuoles, and those of grey matter. There is also a difference in expectation of a background level of vacuolation in immersion fixed central nervous tissues of rats. A letter clarifying the scoring of vacuolation in this study, and in the 13 week study was written by the pathologist. (Binnington, 1997):

"...Vacuoles, which are considered to be artefactual in origin, are observed in the white matter of immersion fixed central nervous tissues of rats. The background vacuolation in the control rats was not described because it was not considered a lesion. However, during comparison evaluations, vacuolation of an obviously greater severity and extent was observed in some of the treated rats in Project 91189. The male and female control rats with the greatest degree of background white matter vacuolation were utilized for comparison of rats in all groups. Slides with brain and cervical spinal cord sections were randomly mixed and then evaluated in comparison with these two control rats. Rats which had a greater degree of vacuolation were considered to have a lesion. The severity grading of the vacuolation was based on the qualitative evaluation of the numbers of vacuoles and the extent of the vacuolation within the brain or cervical spinal cord. There were no grey matter vacuolation in the control rats of a similar nature to that identified in the dosed rats.

Comparison evaluations utilizing the same diagnostic criteria in Project Nol 91190 did not demonstrate any notable differences between control and high dose rats."

Thus, the vacuolation seen in brain white matter was seen in tissues from all animals - only in about half of the animals per group was the vacuolation increased enough to call it a lesion. In only one male and one female in the high dose group was the increase in vacuole numbers greater than a slight to mild increase.

		0 ppm	400 ppm	1,000 ppm	1,600 ppm		0 ppm	400 ppm	1,000 ppm	1,600 ppm
Total brains examined		10	10	10	10		10	10	10	10
Vacuolation, white matter	Total with lesion	0	5	6	6		0	4	5	5
	Slight	0	3	4	2		0	4	5	3
	Mild	0	2	2	3		0	0	0	1
	Moderate	0	0	0	1		0	0	0	1

		0 ppm	400 ppm	1,000 ppm	1,600 ppm		0 ppm	400 ppm	1,000 ppm	1,600 ppm
Total brains examined		10	10	10	10		10	10	10	10
Vacuolation, grey matter	Total with lesion	0	1	10	10		0	0	10	10
	Slight	0	1	9	1		0	0	10	0
	Mild	0	0	1	6		0	0	0	2
	Moderate	0	0	0	3		0	0	0	8

In the 13 week Clintrials study, the same pathologist read the brain slides, using the method described. No vacuolation of brain tissue was noted in that study where animals were exposed to levels up to 600 ppm, nor were vacuoles noted in brain tissues in the Wil 2 generation rat inhalation study at 750 ppm (Fo) or 500 ppm (F1)

"The two surviving males in the high-dose (group) had aspermatogenesis [testes fixed in Zenker's fluid].

The descriptor for testes pathology for this study was "Atrophy- Hypo/aspermatogenesis", with a grade score. The pathologist describes the testicular findings in this study as:

"Atrophic changes recorded as hypo/aspermatogenesis was identified in the testicles of 3 rats (Nos. 3004, 4004, and 4008). The testicular changes were graded as moderate in two rats (No. 3004, and No. 4004), and slight in one (No. 4008). Numerous compounds have been reported to induce atrophic changes in the testicles of rats (Heywood, R., et al., 1978). ..

P. 11 Strength/Weaknesses:

"...A weakness is the lack of clarity as to when animals were terminated following the last exposure, and how many days of exposure preceded necropsy."

Comment: The study report for this study contains a detailed calendar of events showing dates, study day, and study week. Surviving animals were necropsied on day 29 (March 18, 1996) of the study; Day 1 (February 19, 1996) was the first day of exposure. From the chamber concentration records in the report, exposure days were 1 to 5, 8 to 12, 15 to 19, and 22-26. Functional Observational Batteries were conducted on Day 17. Appendix 13 contains individual gross and histopathological findings and gives the study week and day of death. Animals that died during the course of the study were necropsied the day they were found. For example, Animal 4005 (Group 4) died on day 19, the 15th day of exposure. Exact times of death are available in raw data from this study if needed for closer analysis.

P. 11 Utility: (Adequacy)for CERHR Evaluation Process: "There is a clear dose-response for effects and histopathological findings can be correlated with serum biochemistry".

Comment: This doesn't seem to correspond to the statement previous in the text "The Expert Panel concluded that no toxicologically significant changes were seen in serum biochemistry or urinalysis."

P. 12 Clintrials, 1997b: 13 week study

Comment: The draft CERHR report indicated there was lack of detail on how many exposures occurred before necropsy. A detailed study calendar is provided in the Clintrials study report, giving study dates, study day, and event. Study day 1 was the date of first exposure. Day 89 was the date of the last exposure. Nominal and Miran Chamber concentrations were given for all the days of exposure: days 1-5, 8-12, 15-19, 22-26, 29-33, 36-40, 43-47, 50-54, 57-61, 64-68, 71-75, 78-82, 85-89. Total exposures were 65 for all animals.*** Body weights, functional observational batteries, and motor activities were conducted on animals numbered 01 to 05 in all treatment groups on day 90, and on animals numbered 06 to 10 in all treatment groups on day 91. On day 92, males numbered 01 to 08 in all treatment groups, and females numbered 01 ot 07 in all treatment groups were given detailed physical exams, sampled for hematology and biochemistry, and necropsied. The remaining animals (males numbered 09 to 15 in all treatment groups, and females numbered 08 to 15 in all treatment groups) had the same procedures on day 92.

"..... Microscopic evaluation indicated centrolobular vacuolation of the liver for the 2000 gmg/m^3 and 3000 mg/m^3 male groups."

Comment: The liver effects noted in this study were minimal in number and significance. Number and severity of the lesions are important in assessing whether these are adverse events.

The most frequent macroscopic finding at necropsy was a pale area in the liver. This incidental lesion was usually present near the fissure in the median lobe, and was associated with focal hepatocellular vacuolation. This is a common finding in rats, and was distributed across all the groups. Similarly, a periportal pattern of hepatocellular vacuolation identified in 2 control male rats and one Group 3 female rat was considered unrelated to test article administration.

A treatment-related liver effect was present in 6 of 15 high dose male rats, and 3 male (of 15) and one female (of 15) mid dose rats. This lesion was characterized by the presence of one or more prominent, clear, round, variable sized, intracytoplasmic vacuoles in the hepatocytes of the centrolobular region. All but two of the male high dose rats had a severity grade of slight; the others were graded mild. The two with mild scores had a few inflammatory foci associated with the vacuolated hepatocytes.

The presence of liver vacuolation was not correlated with any changes in liver enzymes indicating adverse function of the liver.

	Control	Group 2	Group 3	Group 4	Group 5
Liver: Diffuse vacuolation	0	0	0	3 males 1 female	6 males
Slight				4	4
Mild					2

P. 12 Yu et al, 1998

Comment: The exposures were stated to be 7 days per week under "dynamic conditions." What are "dynamic conditions?"

Strength/Weaknesses: "A strength of this study is that appropriate methods were used for histopathological evaluation of the nervous system and testes."

Comment: We agree that adequate methods were used to evaluate the testes. The length of study and high exposure concentrations should have been severe enough conditions to produce lesions if the testes were a target organ.

P 13 Ichihara (2000a) 12 week study:

Comment: No degeneration of gray or white matter of the brain in optic chiasma, caudal margin of mamillary body or caudal pole of pons was noted in the histopathologic examination. This was consistent with the lack of gray or white matter lesions in the 13 week Clintrial study.

Comment: Although there were statistical differences in grip strength if values from treated animals were compared with control values at the same time intervals, only fore limb gripstrength in 400 and 800 ppm, and hind limb grip strength in 800 ppm were decreased from the treated animals own values at time zero. Most grip strength values had increased over time zero values. The decrease in grip strength was associated with observed walking difficulties only for the 800 ppm group.

Details in this publication were much more explicit than previous publication of the same study.

P. 14 Zhao, 1999:

Comment: We agree that a major flaw in this study is the calculation of the equivalency of a subcutaneous injection of test article in oil to an inhalation dose. The electrophysiological method is not described in detail. Another study weakness is the absence of number data (only graphical representation) for the latency and velocity measurements. The exact comparisons that were made statistically are unclear also. It appears that the conduction velocity in the control groups were increasing over time. Thus, the apparent "declines' for hexanediol and 1-BP were actually failures to increase. We agree that the utility of this study is unclear.

Comment: Ohnishi, A.,et al. This study is cited in references, but not mentioned in the text of the CERHR draft report. In this study, exposure of rats to 1500 ppm for 4 weeks, 6 hours per day, 5 days per week did not show peripheral nerve toxicity (peroneal and sural peripheral nerves, spinal posterior column, and nucleus gracilis of medulla oblongata). Ataxia was thought to be from effects on cerebellum (increased degeneration of Purkinje fibers in vermis and hemisphere), although general poor condition and loss of body weight lead to the euthanasia of the animals before the 5th week of the study.

Table 2-1:

General Comment: There should be an indication of sex in Species/Strain column. The Yu and Ichihara studies (Nagoya School of Medicine) only used male Wistar rats. The Clintrials and Kim et al studies used male and female Sprague Dawley rats.

Clintrials, 1997a: "Aspermatogenesis" would be better stated "Testes atrophy" and the number of animals showing the finding. (See previous comment)

Clintrials, 1997b: Centrolobular liver vacuolation should indicate number of animals showing the lesion, and the degree of severity: 2,000 mg/m^3 - slight, 1 female, 3 males 3,000 mg/m^3 - slight 4 males, mild 2 males

2.3 Genetic Toxicity

Comment: There are other mutagenicity data for 1-BP that has not been cited. For example, Elf Atochem performed an Ames test as well as the other mutagenicity tests cited in the CERHR draft report. We have enclosed a copy of that study report available from the EPA SNAP Docket for 1-BP (" Ames test -reverse mutation assay on Salmonella typhimurium. n-Propyl Bromide. HIS10+05/1005A, Sanofi Recherche, Service de Toxicology. 1994). Kim et al., conducted Ames and micronucleus tests in conjunction with the 8 week rat inhalation study ("Toxicological Studies on Inhalation of 1-Bromopropane Using Rats, Industrial Health Research Institute", 1997). These Ames tests used standard methodology, and can be used to compare with standard Ames tests available for other chemicals. The methodology used by Barber et al for Ames tests of volatile compounds enhances delivery of volatile test articles to the test organisms by using a closed system. In acknowledgement of the "slight volatility" of 1-BP, Elf Atochem placed the plates in a closed stainless steel vessel, one vessel per test chemical.

Kim et al. conducted the 1-BP Ames test (complying with OECD and Japan Ministry of Labor requirements) using TA98, 100, 1535, 1537 and E. coli Wp2uvrA as the test organisms, and a micronucleus test using the repeated-dose inhalation exposure rats (8 weeks). The Ames test gave negative results in all species of Salmonella and E. coli tested. Micronuclei frequency in marrow of treated animals in the exposure groups of animals were not increased compared to control. Thus, the micronucleus test was negative also.

Kim's negative Ames tests and micronuclei test using 1-BP were consistent with other work (Elf Atochem's Ames and micronucleus test, Barber's previous standard Ames tests). The Elf Atochem's micronucleus test used the intraperitoneal route for exposure of Swiss mice to 1-BP. Kim's micronucleus test used inhalation exposure of Sprague Dawley rats. Thus, negative micronucleus results were found in two species, by two routes of exposure.

2.5 Summary of General Toxicology and Biological Effects

Genetic Toxicity:

Comment: The only positive bacterial genetic toxicity was in strains TA100 and TA 1535 in a closed system Ames test; standard Ames tests have been negative in all strains, with and without activation. Two micronuclei tests have been negative (mouse and rat), by both inhalation and subcutaneous routes.

3.0 DEVELOPMENTAL TOXICITY DATA

3.2 Experimental Animal Toxicity Data

P. 20 BSOC, developmental study: Bent Ribs

Comment: The CERHR draft report cites that the Expert Panel considers the high incident of bent ribs in the 996 ppm group to be a dose-related increase from the 498 ppm group and, therefore, were treatment related. The authors stated in the developmental toxicity report that they too considered the increase in bent ribs to be related to exposure, but also noted that this finding is not uncommon in untreated animals although it did not occurred in the controls of this study. Further, they cited a publication that addresses bent ribs as being a reversible finding. This would support that the occurrence of bent ribs, which is corrected within a few days post delivery, can be associated with slower development and delayed ossification due to stress on the dam. Thus, the cause of bent ribs in this study was most likely secondary due to maternal toxicity and not fetal toxicity.

P. 21 BSOC, 2 generation study:

Weakness: developmental effects may have been missed because animals were allowed to give birth.

Comment: The 2 generation study followed a standard methodology - allowing to give birth is in the standard methodology. Major developmental effects would have manifested in decreased pup survival, failure to thrive, or at necropsy of unselected pups. The Environmental Protection Agency defines developmental toxicity as an increase in the incidence of malformed offspring, decreased viability (prenatal or postnatal), altered growth, and/or functional deficits (EPA, 1986). The study design would have encompassed these endpoints.

P. 21 BSOC, Range Finding Study, 1999:

"The Expert Panel noted that exposure to 1-BP during the postweaning period reduced body weight gain and may have targeted adrenals, platelets and liver."

Comment: Although statistically significant differences were seen in offspring glucose (decreased from control in 996 females and all males), GGT (increased high does males and females), and platelets (decreased from control in high dose males and females, and mid dose females), the biological importance of these differences are not known. These changes were not to the degree that would be interpreted as clinically abnormal.

3.4 Summary of Developmental Toxicity

Evidence of developmental toxicity: < fetal weights, < ossification, >bent ribs

No teratology in teratogenicity study. < pup weights in other studies.

4.0 Reproductive Toxicity

4.1 Human data

Strength/Weaknesses: and Utility:

Comment: We agree that 3 of 42 workers reporting possible fertility problems would not be unexpected. A well designed study would also include better quantification of length of exposure as well as confounders. Matched controls would give valuable information for comparison. In this study, the low exposure (117 ppm) was probably not physiologically different than 197 ppm).

4.2 Experimental Animal Toxicity

BSOC, 2 generation study:

Table 4.1 Major effects... Wil Research 2 generation study

Comment: This study is important for several important findings/nonfindings:

1. No Brain Lesions were seen in F0 or F1 animals exposed to 750 ppm (F0) or 500 ppm (F1) of 1-BP for 7 days per week, 6 hours per day for at least 70 days prior to mating, through breeding, and pregnancy. F1 animals were also exposed in utero and through nursing. (Brain areas examined included basal ganglia, cerebellum, pons, cerebral peduncle, central gray matter, tectum, gracile nucleus, thalamus, hypothalamus, hippocampus/dentate gyrus,)

2. No deaths or neurological clinical signs from exposure were noted.

3. Exposures to 500 ppm and higher had definite effects on male and female rats, causing reduction in body weight, and affecting number of offspring. This was repeatable in the first and second generation.

4. There were no major effects in any generation at exposure to 100 ppm, for 6 hours per day, 7 days per week. Effects seen at 250 ppm are minimal, if present at all. An estrous length of 4 or 5 days is expected. The decrease in weight gain in F1 on pnd 21-28 was seen only in males at 250 ppm.

P. 27 "Sperm motility is poorly defined... Likewise, sperm count methods are poorly described.."

The computer assisted sperm analysis system (CASA) used in this study was chosen over other methods to lessen subjectivity in assessment of motility and numbers. This laboratory has extensive experience in this type of analysis, and a historical database of values from previous studies.

It is surprising that comments were not made concerning lack of detail on less sophisticated methods in other studies (ie Ichihara, 2000, "Progressive or non-progressive motile sperm were counted on an erythrocytometer (Neubauer type) under a light microscope.")

Comment: BSOC is aware that another reproductive study of 1-BP has been conducted. (Okuda, et al., 2001). The final study report is expected to be issued within a few months. We have received a translation of a brief summary of some unaudited data. A Combined Repeated Inhalation Toxicity Study with Reproduction/Developmental Toxicity Screening Test in Rats using 1-BP was conducted at Japan Bioassay Laboratories. (This laboratory conducted a similar inhalation test on 2-BP in 1997). It is our understanding that the method of the test follows OECD Guideline 422. Crj:CD(SD)IGS rats, 8 weeks of age were used for the initiation of the study. Male and female animals were exposed 6 hours per day, 7 days per week, in whole body inhalation chambers. Males were exposed for 42 days (2 weeks premating, 2 weeks mating, and 2 weeks post mating. Females were exposed for 35 to 48 days (2 weeks premating, 2 weeks mating, and for gestation (19 days). Non-pregnant females were exposed for 25 days after the end of mating. Exposure levels were 0, 94, 188, 375, 750 and 1500 ppm. Mortality was 20% for males and 50% for females in the 1500 ppm group. Clinical signs in that group included gait effects, and weakness. Sperm motility was reportedly decreased, and effects on the testes and accessory organs were noted. Ten mated pairs were used for each concentration group. Copulation was 100% except in the 1500 ppm group (0% copulation). Fertility rate was 100% through the 188 ppm group, 90% at 375, 80% at 750 ppm and 0% at 1500 ppm. For non-reproductive endpoints, No Effect Concentrations were 188 ppm in males, and 750 ppm in females. For reproductive toxicity, NOEC was 375 ppm. NOEC for FSH effects (decreased levels in males) was 94 ppm. Developmental (fertility, implants, number of newborn) NOEC was 375 ppm for mothers and 375 ppm for offspring.

We look forward to reviewing the final study.

P. 29 Ichihara et al. (2000b)

P. 31: Utility (Adequacy)

"This study is particularly useful for characterizing effects of 1-BP in males since it includes detailed histology with quantification of germ cells and serum hormones..."

Comment: Creasy (1997) notes that "detection of toxic effects" and "characterization of testicular damage" require different study designs. She states "To evaluate the potential of a chemical to produce testicular damange, the study is usually designed to maximize the exposure of the tests to the chemical using daily dosing over a prolonged (28 or 90 day) period with high maximum tolerated doses." "To identify the target cell of a toxicant, it is necessary to conduct a time course study and identify the earliest pathological changes." The length of the Ichihara study (12 weeks), exposure length (8 hours per day versus 6 hours in the 2 generation study), and concentration levels (800 ppm) guarantee prolonged levels to maximum tolerated doses. We agree that this is a useful study, detecting male target organ effects in another species of rat, but does not give as much information for risk assessment as does the 2 generation study.

Comment: The difference in exposure lengths in the Ichihara study and the 2 generation study (8 hours per day versus 6 hours per day) means that total dose to the animals for a given concentration will be higher in the Ichihara study.

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