Nomination of 1-Bromopropane (1-BP) and 2-Bromopropane (2-BP) for Testing by the National Toxicology Program

submitted by:

Directorate of Health Standards Programs
U.S. Occupational Safety and Health Administration
December 1999

I. Basic Chemical Information:

	1-Bromopropane	2-Bromopropane
Synonym	***n*-propyl bromide**	isopropyl bromide
CAS Registry Number	106-94-5	75-26-3
Description	flammable liquid	flammable liquid
Freezing Point	-110 ºC	N/A
Boiling Point	69 ºC	59 ºC
Vapor Pressure	143 mm Hg	N/A
Vapor Density (air=1)	4.3	N/A
Specific Gravity	1.35	1.31
Flash Point (closed cup)	21 ºC	22 ºC
Decomposition Products	hydrogen bromide	hydrogen bromide
Solubility	0.25 g/100 mL water (20ºC)	N/A

References:

Hazardous Substances Databank, National Library of Medicine
Elf Atochem. Safety Data Sheet for n-Propyl Bromide. 1997

II. Production, Use, and Exposure Information:

A. Production and Use:

Until recently, 1-BP was not produced in the U.S. in significant quantities (the major producers were located in the U.K. and Asia). Hence, 1-BP does not appear on EPA's TRI database and is not listed as a Hazardous Air Pollutant under the Clean Air Act. However, several companies have begun domestic production, anticipating major inroads into markets currently

dominated by other halogenated solvents. Two important factors have opened the door for potentially enormous increases in the domestic market for 1-BP over the next several years, perhaps even within several months: (1) although 1-BP has a small but non-zero capacity to degrade stratospheric ozone, it can substitute for potent ozone-depleters (e.g., hydrochlorofluorocarbons) in several major uses -- as EPA continues to place severe restrictions on CFCs and HCFCs, companies will look to unregulated substances such as 1-BP to replace them; and (2) in April 2000, OSHA's 1997 workplace exposure standard for methylene chloride (MC) will take full effect -- OSHA has already received numerous reports of companies switching to 1-BP rather than installing ventilation or other controls needed to reduce employee MC exposures to the requisite 25 ppm level.

Various estimates have been made of the potential market for 1-BP in the key uses to which it is likely to be put: metal cleaning and degreasing, adhesives (especially for assembling polyurethane and other foam products), and aerosol spraying. Note that all of these uses are in practice highly emissive applications, resulting in substantial releases to the ambient environment and substantial exposure to workers (unless engineering control measures are installed, which is not likely given that the major reason for switching from existing materials to 1-BP is to avoid such expenditures):

- Currently, approximately 240 million pounds of chlorinated solvents (principally trichloroethylene, perchloroethylene, and MC) are used in the U.S. each year for vapor degreasing and cold metal cleaning (1). Assuming that 1-BP remains unregulated, the combined effects of the OSHA MC rule (and anticipated OSHA perchloroethylene standard) and continued EPA regulation of the three chlorinated solvents under the NESHAP program place an upper bound on 1-BP use in these areas of 240 million pounds (IRTA notes that the actual substitution could even exceed this amount, since consumptive use of the three "traditional" solvents is currently reduced due to requirements for controlling evaporative emissions, which might not obtain for 1-BP). Similarly, virtually all of the adhesive used in foam fabrication has shifted from trichloroethane (TCA) to MC-based during the past decade following the TCA production ban; all of that market (68 million pounds annually) could potentially be replaced by 1-BP. A similarly large quantity (over 130 million pounds annually) of HCFCs are used in aerosol applications, and 1-BP could take over some or all of this market as well.
- One manufacturer (2) has estimated that an additional 2.5 million pounds of 1-BP might be used annually within the next three years as a cleaning agent in the repair and maintenance of plant machinery alone.

- According to a European research group, at a June 1999 meeting of the Montreal Protocol Open-Ended Working Group, a technical committee reported that solvent uses of 1-BP would expand to 132 million pounds within five years, consuming five percent of world bromine production (3). It is of course difficult to compare this worldwide figure with the domestic estimates above. If, however, these various estimates are even partially borne out, 1-BP could become one of the relatively few chemical substances used in the 10^7 - 10^8 million pound range with little or no adequate data available to shed light on "reasonably anticipated" reproductive toxicity and genotoxicity/carcinogenicity.

Note that 2-BP is not produced deliberately for commercial purposes in the U.S., but is at present an inevitable contaminant of 1-BP synthesis. OSHA has analyzed several samples of commercial 1-BP in the past year and found 2-BP present in each of them, in concentrations ranging from 0.1 to 0.2 percent. Therefore, if roughly 300 million pounds of 1-BP are produced in a future year, nearly 1 million pounds of 2-BP, which appears (see below) to be the more toxic isomer, will be produced concomitantly.

Note also that estimates of production assume maintenance of the status quo under EPA's Significant New Alternatives Policy (SNAP) for phasing out ozone-depleting chemicals. As a result of EPA's not yet disapproving 1-BP for any application following a petition from the Brominated Solvents Committee, it can be used for any purpose at present. EPA published an Advance Notice of Proposed Rulemaking for 1-BP in early 1999 (6a) that did not signal whether it would be approved or disapproved; EPA plans to publish a proposed rule in early 2000 which may limit 1-BP use to certain applications where substitution is likely to be of net benefit to the ozone layer (e.g., perhaps proposing not to allow substituting 1-BP for MC, as the latter chemical has a lower ozone-depleting potential).

B. Employee Exposure:

OSHA has no information at the present time on the possible number of U.S. residents who might be exposed to ambient emissions of 1-BP under various scenarios of production and use. However, even if only worker exposure is considered, and just exposures resulting from substitution away from MC are used as a lower bound, the potential breadth of 1-BP exposure elevates it beyond the exposure potential of many other substances tested by NTP in recent years. According to OSHA estimates in 1997, more than 100,000 workers are exposed to MC (4) in metal cleaning and adhesive applications alone (note: unlike comparable numbers from NIOSH's National Occupational Exposure Survey often used in NTP documents, this figure represents only

workers believed to be exposed to the substance as part of their regular duties, not total employment in establishments where the substance is used in the given application).

Limited industrial hygiene data suggest that 1-BP exposure will be quite substantial in magnitude as well as in breadth (number of workers exposed). OSHA has analyzed approximately 30 personal samples (2- to 4-hour time-weighted averages) for 1-BP (and a comparable number for 2-BP) at three facilities during the past year (5). Most 1-BP TWAs were approximately 40-80 ppm, with exposures ranging as low as 0.05 ppm and as high as 135 ppm. 2-BP exposures were generally quite consistent with the 0.1-0.2 percent contaminant levels in the primary product; TWA concentrations ranged from below the limit of detection to 0.28 ppm (0.3% of the 82 ppm exposure to 1-BP measured simultaneously for this worker).

In November 1998, NIOSH performed a detailed evaluation of 1-BP exposures at a jet aircraft seat cushion manufacturing facility in North Carolina where adhesives containing 1-BP were used (6). Sixty-nine full-shift personal samples were collected, and the average 1-BP TWA was 170 ppm, with individual worker exposures ranging between 60 and 380 ppm. Eleven area samples were also taken, with 1-BP concentrations all clustered between 107 and 161 ppm.

Therefore, depending on whether one interprets the existing animal data to support a NOAEL of 400 ppm or 200 ppm (see Section V below), it is clear that absent regulation, many workers will be exposed to concentrations of 1-BP within a factor of five of, and in some cases exceeding, the NOAEL in rodents. If the traditional 100-fold "safety factor" was applied to the NOAEL (whether to derive a dose thought likely to be below a threshold for human toxicity or a dose 100-fold lower than a "point of departure" that may represent an LED_{10} for the test animals), then most current 1-BP exposures would exceed this level by at least tenfold and by as much as 200-fold. OSHA believes a very high priority should be placed on conducting tests that would shed light on whether such large exposures pose a potential for human reproductive toxicity and in addition may pose a cancer risk, before the number of persons exposed grows from the hundreds to the tens of thousands or more.

References:

1. Comments of Institute for Research and Technical Assistance (Santa Monica, CA) to EPA "SNAP" Docket (A-91-42), 29 April 1999.

2. Comments of CRC Industries Inc. (Warminster, PA) to EPA Docket A-91-42, 23 March 1999.

3. http://www.protonique.com

4. OSHA Methylene Chloride Final Rule (62 FR 1493-1619), 10 January 1997.

5. Personal communication from OSHA Salt Lake Technical Center to Adam Finkel, October 1999.

6. Comments of NIOSH (Christopher Reh, Ph.D.) to Docket A-91-42, 1 July 1999.

6a. EPA ANPRM on n-propyl bromide, 64FR 8043-8047, 18 February 1999.

III. Toxicology:

A. Human Data

OSHA is not aware of any epidemiological studies or published case reports of toxicity except for general statements that 1-bromopropane (1-BP) is able to cause central nervous system depression, presumably at high concentrations (7). However, occupational exposure to the closely related structural analog, 2-bromopropane (2-BP), likely caused reproductive and hematologic effects reported in Korean and Chinese workers (see section IV. B below).

B. Experimental Animal Information

Single Dose Toxicity - 1-BP is not particularly toxic based on acute lethality. The four-hour LC50 in the rat is 7000 ppm (8) and the oral LD50 for the rat is greater than 2 g/kg (8). The LD50s for rat and mouse are 2.9 g/kg and 2.5 g/kg, respectively, by intraperitoneal injection (7).

Repeated Dose Toxicity - Results were reported from several repeated dose studies by inhalation in rats. ClinTrials BioResearch Laboratories of Quebec, Canada reported on a 28-day exposure study (9) and 13-week exposure study (10) with 1-BP. These were sponsored by Albemarle Corporation and submitted to EPA as part of the requirements for consideration under the SNAP program. A series of 1-BP exposures ranging from 7 days to 12 weeks have been conducted by university laboratories in Japan. The toxicity of 1-BP and 2-BP were compared for certain endpoints. The results were published in abstract form as part of 1997, 1998, and 1999 Japanese Industrial Hygiene and Occupational Health meetings.

ClinTrials Studies - In the 28 day study, groups of 10 male and 10 female Crl:CD® (SD)BR Sprague-Dawley rats were exposed to 0, 2, 5, and 8 mg/L (0, 400, 1000, or 1600 ppm) for 6 hours/day, 5 days/week. The

high dose produced significant mortality in males and females by the end of the study period. Clinical signs of neurotoxicity (convulsions, incoordination, hunched posture, etc.) were evident at the mid- and high dose. This was confirmed by impairment in a modified functional observation battery (FOB). Several organ weights (liver, kidney, brain, lung) were marginally increased; hematologic parameters (red blood cells, hemoglobin, etc.) were marginally decreased; and widespread histopathological damage was found in several tissues (testis, bone marrow, brain, spinal cord, kidney, bladder, etc.) at the 8 mg/L (1600 ppm) exposure. Where examined, many of these changes were still present to a lesser extent at the 5 mg/L (1000 ppm) exposure. While there were no apparent clinical and hematological effects at the 2 mg/L (400 ppm) exposure, mild vacuolization in the white matter of the brain was evident in almost half the animals (5/10 males; 4/10 females) examined indicating some neurological damage at this exposure level.

In the 13-week study, groups of 15 males and 15 female rats (same strain) were exposed to 0, 0.5, 1, 2, 3 mg/L (0, 100, 200, 400, and 600 ppm) for 6 hours/day, 5 days/week. The investigators found no significant treatment-related clinical, functional or hematological effects. There was a significant increase in the relative liver weights in the male rats at the two highest doses (11). This was accompanied by mild centrilobular hepatocyte vacuolation in 6/15 male rats (statistically significant elevation) at 3 mg/L (600 ppm) and 3/15 rats (non-significant elevation) at 2 mg/L (400 ppm). The combination of liver weight increases with histopathological changes indicates slight to mild liver toxicity at the 2 mg/L (400 ppm) and 3 mg/L (600 ppm) exposures. No vacuolization of brain tissue was reported at any exposure levels in this study. Based on these findings the authors reported a No Observed Adverse Effect Level (NOAEL) of 1 mg/L (200 ppm).

Japanese Studies - Investigators at Nagoya University School of Medicine exposed groups (9 to 11 rats/group) of male Wistar rats to 200 ppm, 400 ppm, 800 ppm, and 1000 ppm for 8 hours/day, 7 days/week for up to12 weeks (12, 13, 14). They primarily evaluated effects on neurological function and reproductive organs. Body weight gain and several organ weights (liver, brain, prostate, seminal vesicle, etc.) were significantly decreased at 1000 ppm (12). There was a time- and concentration-dependant decrease in grip strength and rat tail motor nerve conduction velocity (MCV) and prolonged motor nerve distal latency (12, 13). At 1000 ppm, statistically significant neurophysiological effects were evident by four weeks of exposure (12). There was evidence of myelin degeneration in the tibial nerve and neuroaxonal swelling in the medulla oblongata at the 1000 ppm and 800 ppm exposure levels (12, 13). The absolute weight of most reproductive organs and blood testosterone were significantly lower than controls at the 800 ppm level (14). The seminal

vesicle was particularly effected by 1-BP exposure with about a 50 percent reduction in weight in 800 ppm-exposed animals (14). This organ weight was still significantly reduced in the lowest exposure group (200 ppm). 1-BP also caused decreases in epididymal sperm density and motility but did not effect spermatogonia development in the testis characteristic of 2-BP exposure (13).

The Nagoya University group also investigated the effect of a seven day exposure to 1-BP on the reproductive organs in male Wistar rats (15). Animals (nine per group) were exposed to 200 ppm, 400 ppm, and 800 ppm. There was a significant decrease in body, prostate and seminal vesicle weights at 800 ppm. This exposure level also significantly reduced the epididymal motile sperm rate and the percentage of abnormal sperm without affecting sperm count. The reduction in motile sperm rate showed a concentration dependency and was significantly less than control, even at the 200 ppm level. Minor histopathological changes in the tibial nerve were found at the 800 ppm level.

Another Japanese group studied testicular toxicity in male Wistar rats exposed to 1500 ppm, 6 hours/day, 5 days/week for 3 weeks followed by a 2 week recovery period (16). Exposure to 1-BP caused a time-dependent decrease in the number of spermatogonia followed by incomplete recovery during the post-exposure period.

C. *In vitro* and Other Short-term Tests - 1-BP was reported to be mutagenic with and without metabolic activation in the Ames assay using Salmonella strains TA1535 and TA100 (17). The compound apparently has also tested negative in the reverse mutation assay using the Ames method (18). It was negative in the micronucleus test (19) and negative for dominant lethal activity in males rats given 400 mg/kg (20). Like other alkyl bromides, 1-BP is an alkylating agent and has potential to react with nucleophilic sites in cellular macromolecules.

References:

(7) Patty's Industrial Hygiene and Toxicology. Clayton G.D. and Clayton F.E. (eds). John Wiley Sons, New York, 1981-1982 pp. 3259

(8) ICF Memorandum. Acceptable Industrial Exposure Limit for N-Propyl Bromide. September, 1998

(9) ClinTrials. A 28 Day Inhalation Study of a Vapor Formulation of ALBTA1 in the Albino Rat. Report No. 91189. May, 1997

(10) ClinTrials. A 13 week Inhalation Study of a Vapor Formulation of ALBTA1 in the Albino Rat. Report No. 91190. February, 1997

(11) ICF Memorandum. Acceptable Industrial Exposure Limit for n-Propyl

Bromide. September, 1998

(12) Ichihara, Shah, Shibata, Takeuchi, and Tomoeda.. Neurotoxicity of 1-Bromopropane and 2-Bromopropane. Tokai Region Conference, 1997

(13) Ichihara, G. Reproductive and Other Disorders Due to Bromopropanes; Presented at 1999 Meeting of Japan Society for Occupational Health. ***Journal of Occupational Health*** **41:** 137, 1999

(14) Ichihara, G. et al. Histopathological Changes of Nervous System and Reproductive Organ and Blood Biochemical Findings in Rats Exposed to 1-Bromopropane; Presented at 1999 Meeting of Japan Society for Occupational Health. ***Journal of Occupational Health*** **41:** 513, 1999

(15) Wang, H. et al. Subacute Effects of 1-Bromopropane on Reproductive Organs and the Nervous System; Presented at 1999 Meeting of Japan Society for Occupational Health. ***Journal of Occupational Health*** **41:** 306, 1999

(16) Kasai, T. et al. Histopathological Study of Testicular Toxicity in Rats Exposed to 1-Bromopropane; Presented at 1999 Meeting of Japan Society for Occupational Health. ***Journal of Occupational Health*** **41:** 308, 1999

(17) Barber, E. et al. A Procedure for the Quantitative Measurement of the Mutagenicity of Volatile Liquids in the Ames Salmonella/microsome Assay. ***Mutation Research*** **90:** 31-48, 1981

(18) Elf Atochem. Ames Assay - Reverse Mutation Assay on Salmonella Typhirium. N-Propyl Bromide. HIS1005/1005A. 1994

(19) Elf Atochem. Micronucleus Test by Intraperitoneal Route in Mice. N-Propyl Bromide. Study No. 12122. September, 1995

(20) Saito-Suziki et al. Dominant Lethal Studies in Rats with 1,2-Dibromo-3-chloropropane and Its Structurally Related Compounds. ***Mutation Research*** **101:** 321-327, 1982

IV: Disposition and Structure-Activity Relationships:

A. Absorption, Distribution, Metabolism and Excretion:

OSHA is not aware of any available studies that characterize the toxicokinetics of 1-BP in experimental animals. Metabolism experiments ***in vitro*** with rat liver subcellular fractions indicate that 1-BP is conjugated with glutathione (21). It has also been shown to deplete glutathione in rat hepatocytes ***in vitro*** (22). There is some indirect qualitative evidence that oxidation to a reactive epoxide by the mixed function oxidase (MFO) system may occur ***in vitro*** (21), but its existence ***in vivo*** has yet to be established.

B. Structure-activity Correlations and Considerations:

There is both human and animal evidence that the close structural isomer of 1-BP, 2-BP, interferes with ovulation in females and sperm production in males. 2-BP causes hematopoietic toxicity as well. In a study of Korean workers (25 females and 8 males) exposed to a cleaning solvent containing >95 percent 2-BP for up to two years, it was reported that amenorrhea accompanied by high follicle stimulating hormone (FSH) levels occurred in 64 percent (16 ex. 25) of the exposed females; azoospermia (absence of sperm), oligospermia (deficiency of sperm), or reduced sperm motility occurred in 75 percent (6 ex. 8) of the exposed males; pancytopenia (reductions in erythrocytes, leukocytes, and platelets) occurred in 21 percent (7 ex. 33) of the workers (23). Reportedly, typical 2-BP concentrations in this setting averaged about 20 ppm, but this information may not be reliable.

An investigation of a Chinese chemical plant that produced 2-BP found sperm abnormalities and anemia among exposed workers (24). The Nagoya University group has shown similar reproductive effects in male (25) and female rats (26) exposed to 2-BP. Male rats exposed to 300 ppm and above had significant reductions in testis weight, sperm count, and sperm motility. Erythrocytes, leukocytes, and platelet counts in the peripheral blood were also significantly reduced at the same exposures. There were significant irregularities in the estrous cycle and decreased numbers of ovarian follicles in female rats exposed to 300 ppm and above. Another halogenated propane, 1,2-dibromo-3-chloropropane, is a well-recognized testicular toxicant.

The close structural analog, bromoethane (BE), underwent toxicity testing by the National Toxicology Program (27). In these studies, two-year inhalation exposures to female B6C3F$_1$ mice resulted in a significant increase in benign and malignant neoplasms of the uterus at the highest exposure (400 ppm). On this basis, the test report concluded that there was clear evidence of carcinogenic activity for the female mouse. There was some/equivocal evidence of carcinogenicity for other species/sex/site combinations. BE was also mutagenic in Salmonella strain TA100 and induced sister chromatid exchanges in Chinese hamster ovary cells.

References:

(21) Jones, A. and Walsh, D. The Oxidative Metabolism of 1-Bromopropane in the Rat. ***Xenobiotica* 9**: 763-772, 1979

(22) Khan, S. and O'Brien, P. 1-Bromoalkanes as New Potent Nontoxic Glutathione Depleters in Isolated Rat Hepatocytes. ***Biochem. Biophys. Res. Commun.*** 179: 436-441, 1991

(23) Kim, Y. et al. Hemopoietic and Reproductive Hazards of Korean Electronic Workers Exposed to Solvents Containing 2-Bromopropane.

***Scand J Work Environ Health* 22:** 387-391, 1996

(24) Takeuchi, Y. et al. A Review of 2-Bromopropane: Mainly on its Reproductive Toxicity. ***J Occup Health* 39:** 179-191, 1997

(25) Ichihara, G. et al. Testicular and Hemopoietic Toxicity of 2-Bromopropane, a Substitute for Ozone Layer-Depleting Chorofluorocarbons. ***J Occup Health* 39:** 57-63, 1997

(26) Kamijima, M. et al. Ovarian Toxicity of 2-Bromopropane in the Non-Pregnant Female Rat. ***J Occup Health* 39:** 144-149, 1997

(27) National Toxicology Program. Toxicology and Carcinogenesis Studies of Bromoethane in F344/N Rats and B6C3F$_1$ Mice. ***Technical Report-363***. October, 1989

V. Summary:

Two consulting firms, ICF Inc. and Environ (the latter group continuing to provide comments to EPA under the name Life Sciences Consultancy), have evaluated the toxicological data and reached slightly different conclusions. ICF concluded the NOAEL was 400 ppm based on its interpretation of the reproductive endpoints. Environ concluded that the NOAEL was 200 ppm based on the studies of Ichihara et al. (12-14) showing decreased sperm counts at 400 ppm and greater. 400 ppm is also a LOAEL for white matter vacuolization in the brain of rats. More recent work by Ichihara et al. and by Wang et al. (15) suggest that effects on the male reproductive system are visible even at 200 ppm.

OSHA suggests that there is a pressing need to pin down the “true” NOAEL—even better, to use standard study designs and statistical methods to establish a “benchmark dose” for each compound, so that regulatory agencies can rationally consider setting risk-based exposure limits. Moreover, until two-year cancer bioassays are conducted on each compound, agencies have no way to assess whether reproductive toxicity is indeed the most sensitive adverse endpoint.

VI. Ongoing Toxicological and Environmental Studies in the Government, Industry, and Academia:

OSHA is aware of two ongoing efforts to expand the toxicological data base for 1-BP. In a May, 1999 letter expressing support for the approval of 1-BP as a substitute for ozone-depleting substances in EPA’s SNAP program, the Brominated Solvents Committee (BSOC), a consortium of three 1-BP

producers, acknowledged sponsoring a two generation reproductive effects study and a developmental study in rats by inhalation (28). The study design will presumably exceed current EPA and OECD guidelines and include evaluation of sperm morphology and estrous cycle. The current status, study protocol, and timetable for completion of these studies was not stated in the submission. OSHA learned recently that these studies were halted in mid-1999 because of infertility in the control groups. BSOC has restarted the studies very recently (although reportedly using the same contractor and supplier of lab animals) and expects to provide results to EPA by July 2000. Given the preliminary data presented at the recent Annual Meeting of the Japan Society for Occupational Health, research to further understand the reproductive and neurological effects of 1-BP in experimental animals will likely be pursued by investigators at Nagoya University and elsewhere in Japan.

Reference:

(28) Brominated Solvents Committee. Letter to EPA Air Docket #A-91-42 Regarding Support for SNAP Approval of n-Propyl Bromide. May, 1999

VII. Rationale for Recommendation and Suggested Studies:

Beyond the potential for widespread occupational and possible environmental exposure, the information provided by preliminary toxicological studies on 1-BP and data from related alkyl bromides raise various concerns with regard to human health. The effects on reproductive tissue of male rats caused by short exposures to 1-BP suggest the need for longer term studies to assess reproductive function. The reproductive impairments in female and male workers exposed to its isomer, 2-BP, reinforce the need to evaluate this endpoint in both sexes along with effects on human development. The mixed results in the limited genotoxicity testing of 1-BP, its alkylating potential, and the tumorigenicity of the structurally-related BE suggest the need for additional short-term tests and a cancer bioassay. The clear neurotoxicity at high doses combined with abnormal neurophysiology at lower exposures indicate the need for more complete evaluation of neurological function. Additional toxicity testing would provide the necessary data to better identify hazard and estimate risk to workers which are essential to deriving exposure limits that protect worker health. Suggested studies are as follows. Input from the various National Toxicology Program (NTP) committees is encouraged.

OSHA believes it is essential that both reagent-grade 1-BP and 2-BP be tested separately, if at all possible. Because commercial 1-BP contains significant trace levels of 2-BP, a “negative” result from testing 1-BP alone may

not shed light on the hazard potential of the commercial product. On the other hand, a positive result if the commercial product were tested might not indicate that pure 1-BP (assuming it could be formulated in quantity) is necessarily hazardous.

OSHA recommends that NTP consider the following tests:

- Carcinogenicity Study - The full two year National Toxicology Program (NTP) bioassay protocol in both sexes of rats and mice is recommended for both compounds. The test compounds should be administered by inhalation at the maximum tolerated exposure and at least two other non-zero exposures. Specialized studies evaluating DNA binding, oncogene activation, cell proliferation, etc. should be considered as appropriate.

- Multi-generation Reproductive Study - Unless there is a successful and thorough completed study from industry sponsors, a reproductive study in exposed rats of both sexes covering at least two generations of mating is recommended. Exposure should be by inhalation and conducted by accepted NTP protocols. It is strongly recommended that spermatogenesis, estrous cycle, and hormonal endpoints be measured as well as the standard fertility and pregnancy outcomes.

- Developmental Studies - It is recommended that developmental effects be evaluated in two species. Test compounds should be administered to pregnant animals by inhalation from the period of implantation to the end of gestation. The studies should be conducted by standard protocols and the usual maternal and fetal endpoints examined.

- Subchronic Neurotoxicity Study - It is recommended that neurological endpoints be evaluated during the standard 14 week exposure study in rats. These should include a Functional Observation Battery, neurophysiological endpoints, and specialized neuropathology as appropriate.

- Genotoxicity - A battery that adequately evaluates mutagenicity and clastogenicity in mammalian systems using sensitive ***in vitro*** and ***in vivo*** test methods is recommended.

- Toxicokinetic/Mechanistic Studies - It is recommended that toxicokinetics of the test compounds be evaluated following inhalation exposure over the appropriate dose range. The extent of absorption, tissue distribution, body residence time, major metabolites and pathways of elimination should be identified. Additional studies that provide a better understanding of target organ dosimetry, mode of action or dose - response is also suggested.