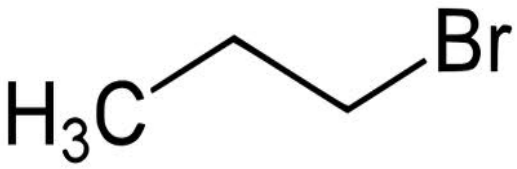
NTP
National Toxicology Program

Draft Report on Carcinogens Monograph for 1-Bromopropane

Diane L. Spencer, MS
National Institute of Environmental Health Sciences

NTP Peer Review Meeting
March 21-22, 2013

Properties and Use



1-Bromopropane (propyl bromide or 1-BP) is a volatile, brominated hydrocarbon used as a solvent in several industrial processes:

- Degreaser for electronics and metal
- Solvent vehicle for aerosolized adhesives (e.g., foam cushion manufacturing)
- Dry cleaning
- Spot remover in textile industry
- Intermediate in synthesis of pharmaceuticals, insecticides, fragrances, flavors, other chemicals

1-Bromopropane was selected as a candidate substance for the Report on Carcinogens, based upon:

- Potential for substantial human exposure
- Adequate database with which to evaluate its potential carcinogenicity

Human Exposure

Key Questions

- Is there significant exposure to 1-bromopropane for persons living in the United States?
- How are people exposed to 1-bromopropane?

U.S. Exposure to Humans

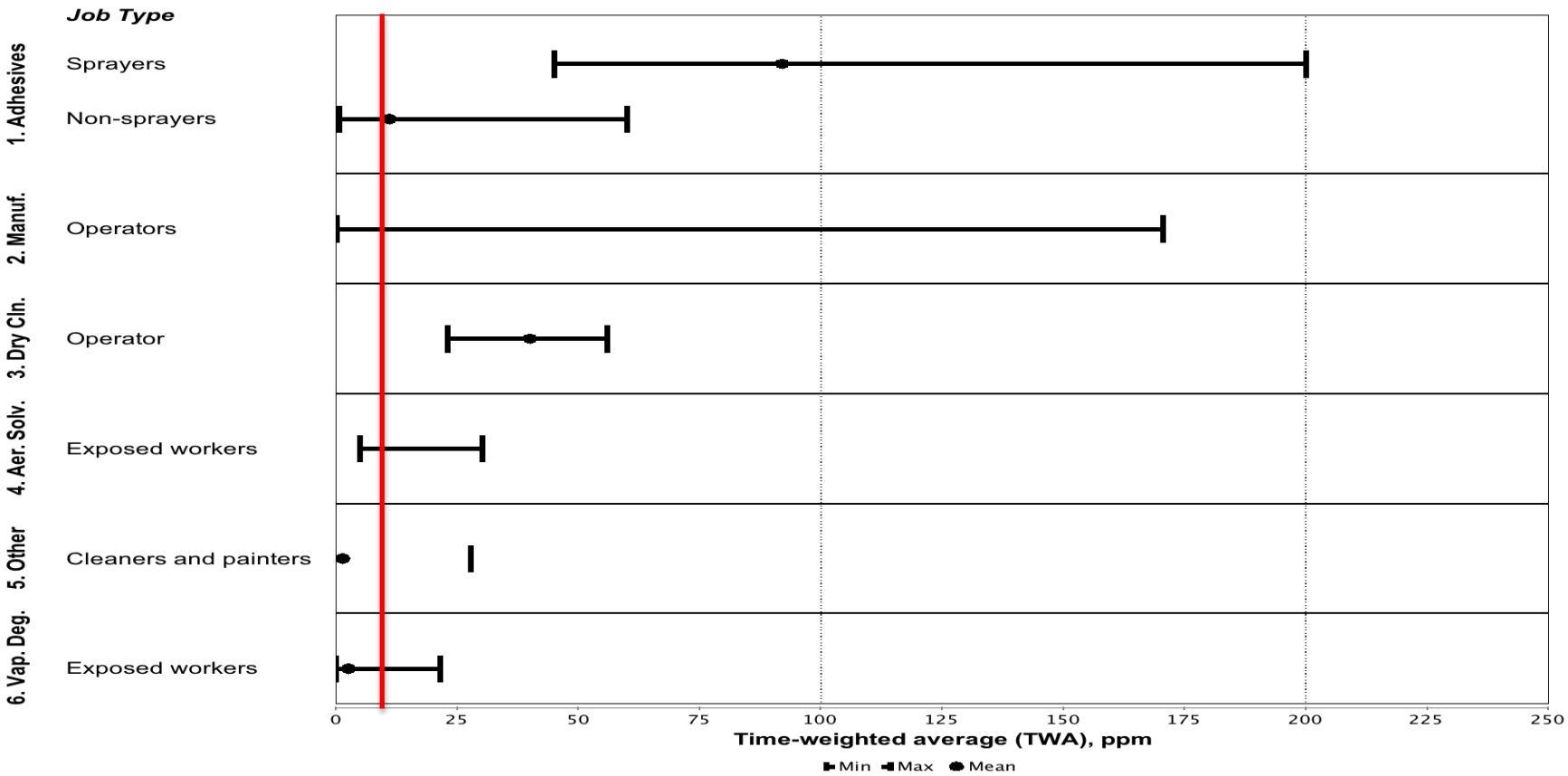
- High production volume chemical (EPA IUR/CDR)

  1 million to 15.3 million pounds per year (1998, 2002, 2006, 2012)

- Occupational exposure (industrial processes)
  - There have been recent increases in exposure to workers because of
    - Increased use in open, rather than closed, systems
    - Use as an alternative to chemicals such as trichloroethylene, PERC, methylene chloride (all classified by RoC and IARC as likely carcinogens) and two ozone-depleting chemicals, methyl chloroform, CFC-113.
  - Measured in air in occupational settings where it is used
  - Inhalation is the primary route, dermal possible
  - Metabolites of 1-bromopropane have been detected in urine of exposed workers; *N*-acetyl-*S*-propylcysteine (major metabolite) levels in urine increased with increasing levels of 1-bromopropane in ambient air (Hanley and Dunn 2006, Hanley *et al.* 2009, 2010, Valentine *et al.* 2007).

Occupational Exposure to Humans

Representative Levels (TWA) for Workers Exposed to 1-Bromopropane



American Conference of Governmental Industrial Hygienists (ACGIH)

(from Kawai *et al.* 2001, Ichihara *et al.* 2004, Hanley *et al.* 2006, 2010, Eisenberg and Ramsey 2010, Graul 2012)

Human Exposure: Summary and Conclusions

- Humans are primarily exposed to 1-bromopropane by inhalation in several different industries.
- The highest occupational exposures occur among factory workers who use spray adhesives, but many exposures in other industries are at higher levels than the recommended TWAs.
- The industrial uses of 1-bromopropane have expanded in recent years, resulting in the potential for exposure of more workers to higher levels of 1-bromopropane.
- In conclusion, there is significant exposure for persons residing in the U.S., based on occupational and production data.

Properties and Human Exposure

Questions or Clarifications?

Properties and Human Exposure: Reviewer Questions

1. Comment on whether the chemical identity and description of 1-bromopropane (Section 1: Properties and Human Exposure) are clear and technically accurate.

2. Comment on whether the information on use, production, and human exposure for 1-bromopropane (Section 1: Properties and Human Exposure and Appendix B) is clear and technically accurate.

   a. Identify any information that should be added or deleted.

3. Comment on whether adequate information is presented to document past and/or current human exposure to 1-bromopropane in the United States. Exposure can be inferred by data on usage, production, or evidence for exposure in the workplace, from the environment or consumer products, diet, or other sources due to lifestyle choices (such as tobacco smoking).

Disposition and Toxicokinetics

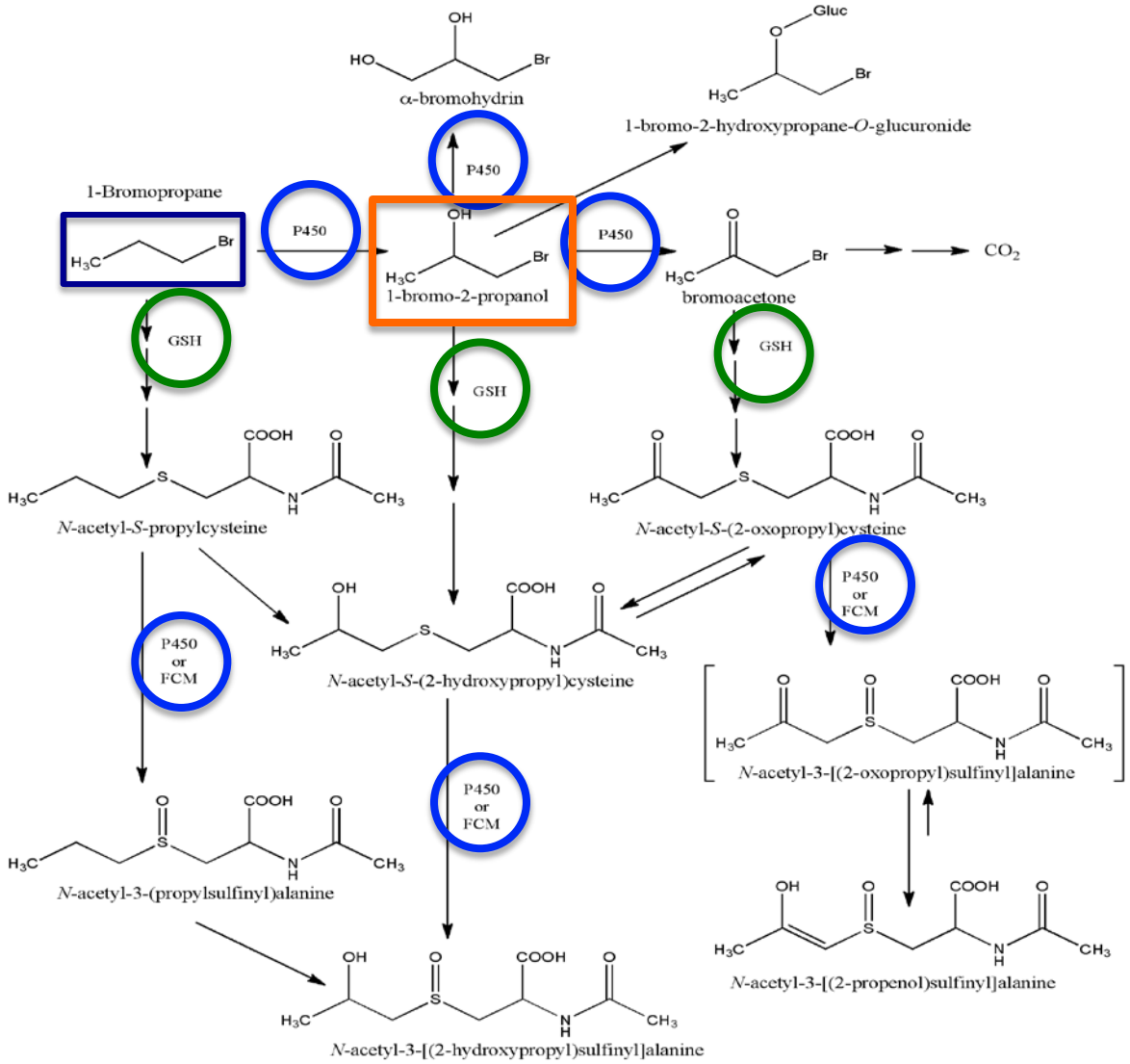
Key Questions

- What is the scope of the available database for 1-bromopropane for studies in humans and experimental animals?
- How do the results from the human studies compare with those reported for experimental animals?

Disposition and Toxicokinetics: Scope of Literature

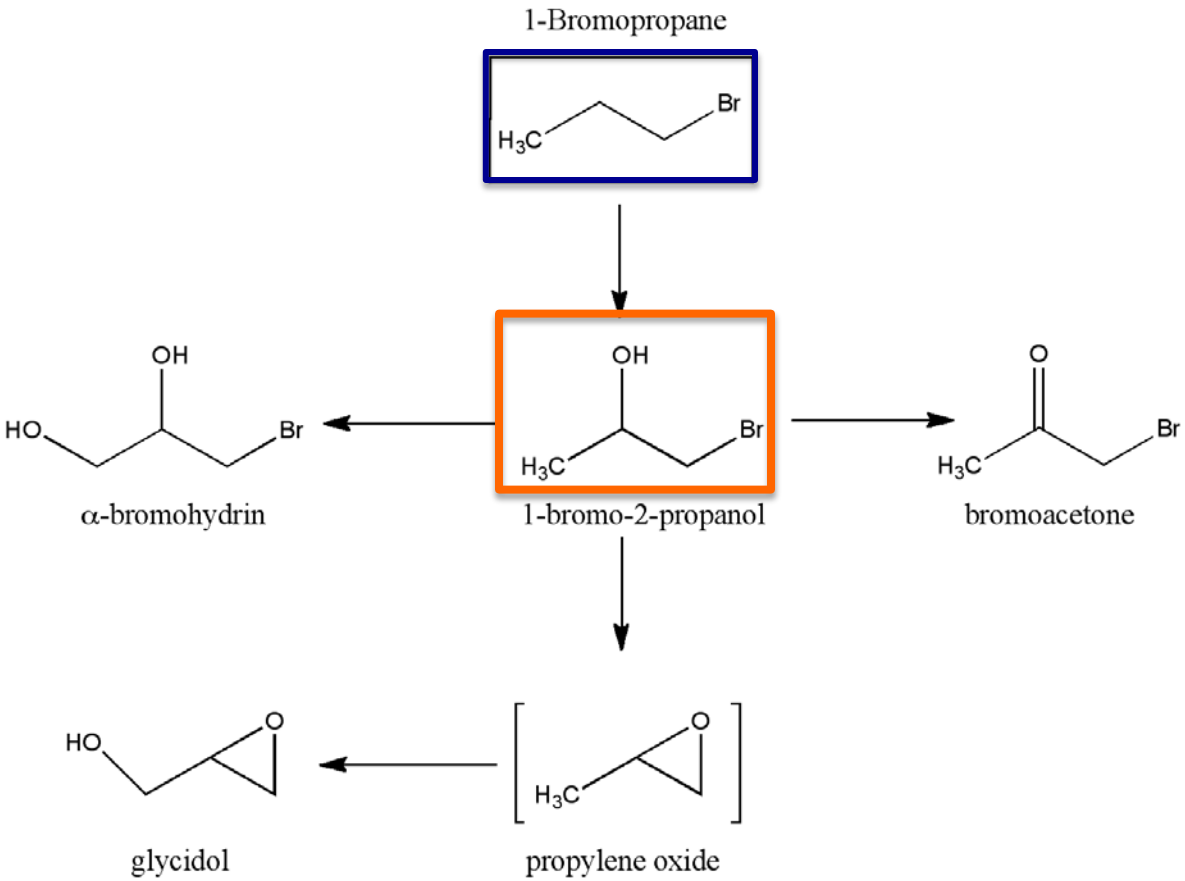
- Humans: Occupational studies
  - Studies are limited to identification of urinary biomarkers of exposure to 1-bromopropane in the workplace (i.e., unmetabolized 1-bromopropane, mercapturic acid conjugates, and bromide).
- Experimental animals
  - Urinary metabolites have been identified in studies *in vivo* in rats and mice using various methods and different routes of exposure (inhalation, oral, injection – s.c., i.p., i.v.).
  - The most informative studies were inhalation exposures in rodents.
  - Additional metabolites were identified by *in vitro* studies using rat liver microsomes.
- No toxicokinetic models of 1-bromopropane were identified for either humans or animals.

Metabolism of 1-Bromopropane



(Garner *et al.* 2006, 2007, others: urinary metabolites identified in male rats and mice)

Reactive Metabolites of 1-Bromopropane



(Garner *et al.* 2006, 2007, Ishidao *et al.* 2002)

Metabolism by Cytochromes P450

- Evidence for P450-catalyzed oxidation reactions (primarily via CYP2E1) :
  - The number of urinary metabolites in rats pretreated with a P450 inhibitor was reduced from ten to only one, *N*-acetyl-S-propylcysteine (Garner *et al.* 2006).
  - When NADPH was eliminated from an incubation mixture with phenobarbital-induced rat liver microsomes (thus inactivating P450 oxidation) there was a severe reduction in metabolite formation (Tachizawa *et al.* (1982).
  - In a study using the $Cyp2e1^{-/-}$ knockout mouse, the elimination half-life was twice as long in the knockout mice (than wild-type) after inhalation exposure (Garner *et al.* 2007).

Metabolism in Humans

- The available data in exposed humans suggest that similar metabolic pathways may occur as those observed in rodents.
- Four urinary mercapturic acid conjugates were identified from exposed workers and have also been reported as urinary metabolites from studies in experimental animals.
  - *N*-acetyl-*S*-(*n*-propyl)-L-cysteine
  - *N*-acetyl-*S*-(*n*-propyl)-L-cysteine-*S*-oxide
  - *N*-acetyl-*S*-(3-hydroxypropyl) cysteine
  - *N*-acetyl-*S*-(2-carboxyethyl) cysteine
- No studies in humans have adequately tested for oxidative metabolites or likely intermediates.
- CYP2E1, the major P450 enzyme involved in oxidative metabolism of 1-bromopropane in animal studies, is expressed in human lung and other tissues.

Disposition and Toxicokinetics: Summary

- 1-Bromopropane is absorbed in the body via different routes; inhalation is the primary route of exposure.
- The available studies on 1-bromopropane metabolism show that P450 catalyzed oxidation reactions (primarily via CYP2E1) and glutathione conjugation are the primary metabolic pathways.
- 16 urinary metabolites have been identified in rodent studies, including several reactive intermediate metabolites.
- The four urinary metabolites that have been identified in exposed workers were also found in exposed animals.

Disposition and Toxicokinetics:

Questions or Clarifications?

Disposition and Toxicokinetics: Reviewer Questions

1. Comment on whether the information on Disposition and Toxicokinetics (Section 2) is clear and technically correct, and objectively presented.
    a. Identify any information that should be added or deleted.

Human Cancer Studies

No epidemiological studies were identified that examined the relationship between human cancer and exposure specifically to 1-bromopropane.

Studies in Experimental Animals

Key Questions

- What is the level of evidence (sufficient or not sufficient) for the carcinogenicity of 1-bromopropane from studies in experimental animals?
- What are the tissue sites?

Scope of Literature

- NTP two-year carcinogenesis studies in female and male rats and mice treated with 1-bromopropane (inhalation) (NTP Technical Report # 564, 2011)
  - Chronic and associated 90-day subchronic studies; tissues were examined for neoplastic or preneoplastic endpoints, which was part of the inclusion/exclusion criteria for RoC review.

NTP animal study considered high quality

(see Draft Monograph, Appendix C for specific study quality criteria and detailed assessments)

Study Performance Elements	Assessment
Substance characterization	Purity > 99%; stability monitored
Animal husbandry	Rats (F344/N), mice ($B6C3F_1$); AAALAC facility: Adequate housing, animal care, maintenance described
Study design quality	Rats and mice, both sexes, treated 2-years inhalation, appropriate and relevant to determine exposure-related effect; historical control database route; adequate number animals/group.
Clinical observations, necropsy and pathology	Clinical observations reported; necropsies all animals; all organs, tissues examined for gross lesions; histopathology on all major organs; pathological procedures described and adequate
Data reporting and statistical methods	Data tabular; individual animal data; tumors (benign/malignant) from same organ appropriately reported as combined, cell type; historical control data for all routes
Studies informative for cancer assessment	Yes, for studies in rats and in mice. No major limitations on cancer quality study found

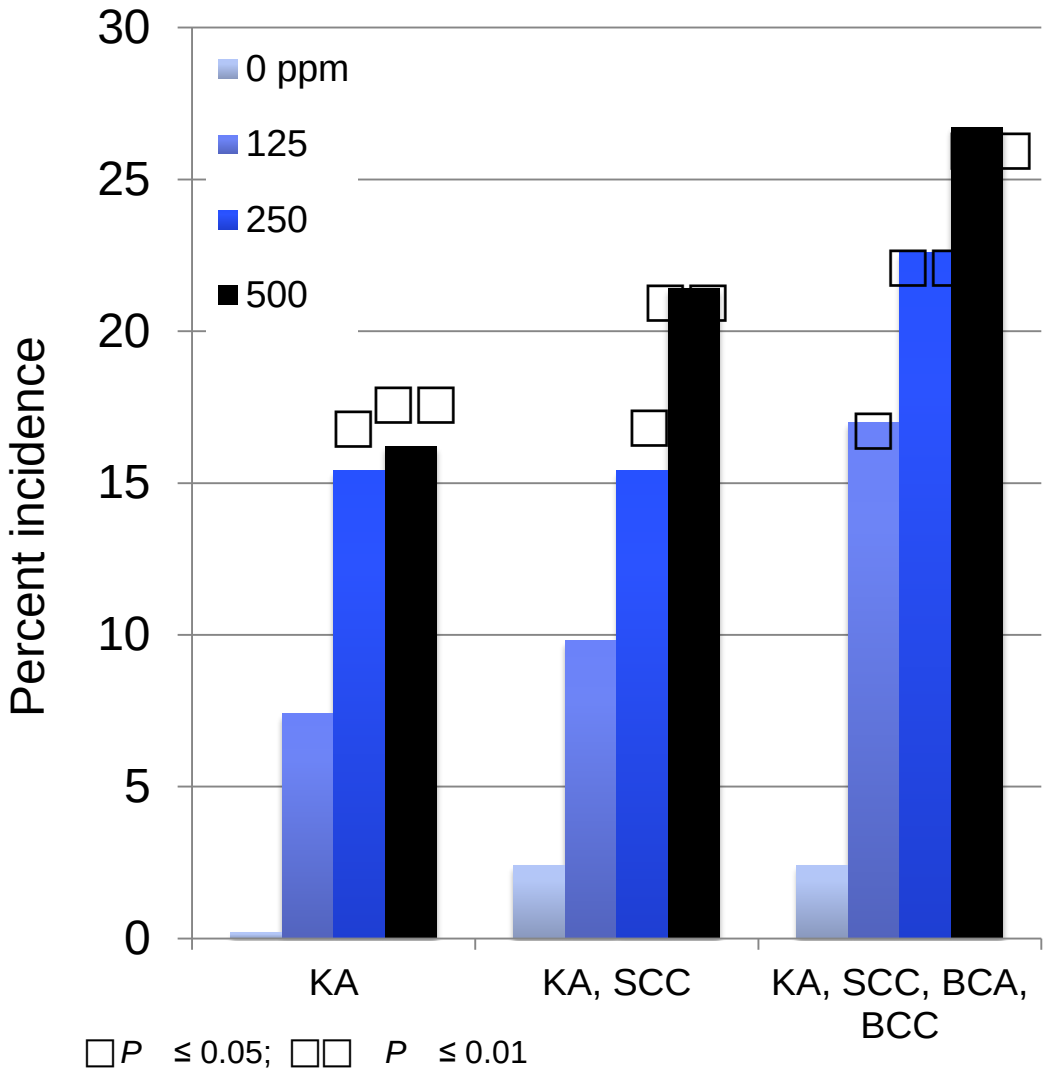
Carcinogenicity Studies in Experimental Animals

	Rat (F344/N)	Mouse ($B6C3F_1$)
Male	Skin Large intestine Mesothelium (tunica vaginalis of epididymis) Pancreatic islets	
Female	Large intestine Skin	Lung

NTP 2011

- Subchronic studies were used to determine doses used in chronic studies.
- Each exposure group included 50 males and 50 females.
- Exposed to 1-bromopropane for 6 hr/d, 5 d/wk, 105 wks in inhalation chambers
  - Rats: 0,125, 250, 500 ppm
  - Mice: 0, 62.5, 125, 250 ppm
- Increased incidence of neoplasms were effects observed at several tissue sites.

Skin Tumors in Male Rats



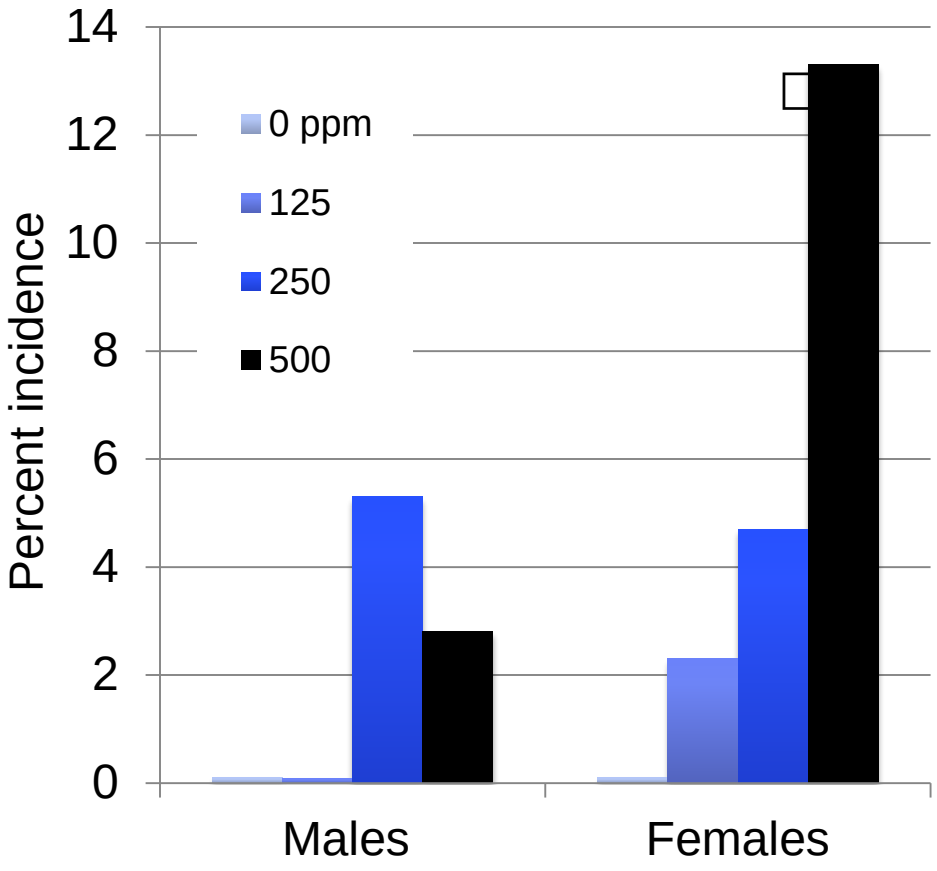
□ *P* ≤ 0.05; □□ *P* ≤ 0.01

P-trend ≤ 0.01 for KA and both combinations

- Four skin tumor types
  - Keratoacanthoma (KA)
  - Squamous cell carcinoma (SCC)
  - Basal cell adenoma (BCA)
  - Basal cell carcinoma (BCC)
- These neoplasms arise from epidermal cells or epidermal stem cells; biologically appropriate to combine for analyses.
- Keratoacanthoma can progress to squamous cell carcinoma.

(See Draft Monograph, page 30)

Large Intestine Tumors in Male and Female Rats

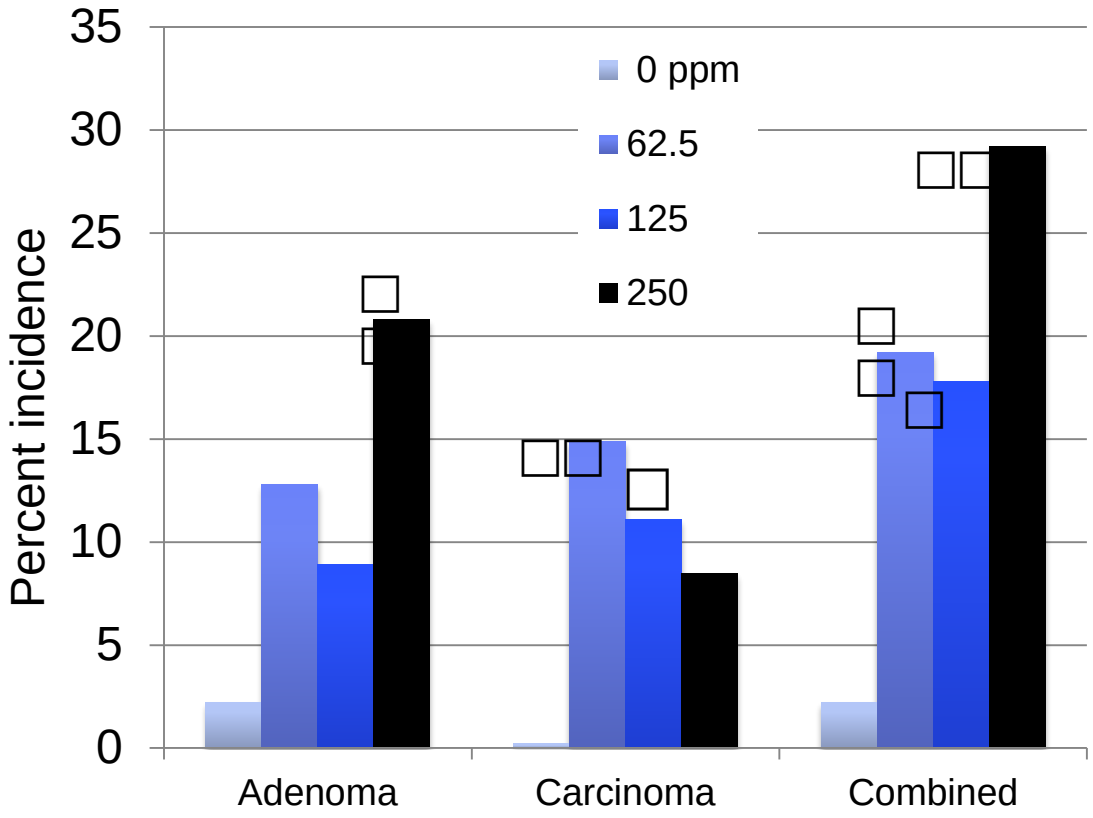


□*P* ≤ 0.05, *P*-trend = 0.004

- Statistically significant increased incidence for adenoma of large intestine (colon or rectum) in females but not males
- Very rare tumor
- Tumor incidence exceeded historical control range for male and female rats for inhalation and all routes (< 0.2%)
- Although no carcinomas were observed in this study, adenomas can progress to carcinoma
- Increased incidence of adenocarcinoma of large intestine for two brominated methanes and glycidol (metabolite of 1-bromopropane)

(See Draft Monograph, page 29)

Lung Tumors in Female Mice



□ $P \leq 0.05$; □□ $P \leq 0.01$ □□□ $P \leq 0.001$

P-trend = 0.007 (Ade); < 0.001 (Comb)

- Alveolar/bronchiolar adenomas and carcinomas
- Statistically significant increased incidence of adenoma, carcinoma, and adenoma or carcinoma combined
- Positive trend tests for adenoma and combined groups
- No exposure related response observed in male mice

(See Draft Monograph, page 32)

Other Neoplasms: May have been exposure-related

- Malignant mesothelioma – tunica vaginalis of epididymis (male rats)
  - A marginal, but statistically significant increased incidence of mesothelioma (located primarily in the epididymis of the testes) was observed in high-dose group of male rats and there was a significant dose-related trend.
- Pancreatic islet tumors (male rats)
  - Statistically significant increased incidence of benign tumors, and benign and malignant tumors combined, was observed in low and mid-dose groups
  - Findings were within the historical control range.
- Skin tumors (female rats)
  - Significant positive trend for combined tumor types, but no significant pairwise comparisons
  - No squamous cell carcinomas

(See Draft Monograph, pages 30-31)

Studies in Experimental Animals: Preliminary Recommendation

There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors in rats and mice, at multiple tissue sites, and by the occurrence of rare tumors.

- Benign and/or malignant **skin tumors** in male rats
- Benign and malignant **lung tumors** in female mice
- Benign **large intestine tumors** (rare) in male and female rats

Studies in Experimental Animals

Questions or Clarifications?

Studies in Experimental Animals: Reviewer Questions

1. Comment on whether the scientific information from cancer studies in experimental animals for 1-bromopropane (Section 4: Studies of Cancer in Experimental Animals and Appendix C) is clear, technically correct, and objectively presented.
2. Comment on whether the assessment and integration of the scientific evidence (Section 4.2: Assessment of neoplastic findings) are adequate to determine the level of evidence for carcinogenicity and to reach a listing recommendation.
    a. Provide any scientific criticisms of the NTP's interpretation and application of the evidence from the cited studies in assessing the carcinogenicity of 1-bromopropane.
    b. Identify any information that should be added or deleted.

Studies in Experimental Animals

- Panel discussion
  - RoC criteria for sufficient evidence

  There is sufficient evidence of carcinogenicity in experimental animals with an increased incidence of malignant and/or a combination of malignant and benign tumors in rats and mice or at multiple tissue sites.
- Action:
  - Vote on whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from experimental animal studies on 1-bromopropane.

Mechanistic Data and Other Relevant Effects

Key Questions

- What are potential mechanisms by which 1-bromopropane may cause cancer?
- What is the level of evidence for these mechanisms in experimental animals?
- Are there mechanistic data to suggest that the cancer findings in experimental animals are not relevant to humans?
- Could the reported alterations in immune surveillance in rodents lead to an increased incidence of tumors?

Mechanisms: Scope of Literature

- Genotoxicity: studies *in vitro*, *in vivo*, and in exposed workers; endpoints include mutations, DNA damage and micronucleus induction.
- No studies were identified that evaluated mechanisms of carcinogenicity for the tumor sites observed in experimental animals (i.e., lung, skin, large intestine).
- Therefore, we looked at mechanisms of toxicity studies and data on metabolites and analogues.

Mechanistic Data and Other Relevant Effects

- Mechanistic studies of toxicity in animals indicate 1-bromopropane (either directly or via reactive metabolites) causes molecular alterations that typically are associated with carcinogenesis:
  - Genotoxicity
  - Oxidative stress due to glutathione depletion
  - γ-Aminobutyric acid (GABA) dysfunction

Genotoxicity of 1-Bromopropane

Effect	*in vitro*	*in vivo* Rodents	Exposed workers
Mutation			
Bacteria	±		
Mammalian cells	+	–	NT
DNA damage	+	NT	+
Micronucleus induction	NT	–	NT

+ = positive, ± = both positive and negative, - = negative, NT = not tested

Key Study on 1-Bromopropane Genotoxicity

- Evidence for mutagenicity for *Salmonella typhimurium* assay
  - Negative in all strains in four studies
  - Positive in Barber *et al.* 1981
    - Used a modified assay, specifically designed for testing volatile substances: the only adequate study protocol for testing volatile substances
    - Showed 1-bromopropane induced mutations in TA100 and TA1535, both ±S9, using the modified assay, but tests were negative in standard assay
    - Authors reported four other volatile substances that were negative in the standard assay but tested positive in their modified assay

Key Study on 1-Bromopropane Genotoxicity

- Limited evidence for DNA damage (comet assay) in leukocytes from exposed workers (Toraason *et al.* 2006)
  1. Facility and job type – no clear exposure-response patterns were observed
  2. Multivariate analyses that evaluated association between DNA damage and three 1-bromopropane exposure indices (TWA levels, and serum and urinary bromide) in models controlling for potential confounders
     - Considered to be more informative because exposure was measured at the individual level, confounders were considered, wide range of exposure
     - For each of the three exposure indices, linear regression models (using log-transformed exposure indices) and exposure quartiles analyses were performed
     - All but one association between exposure and DNA were positive, although statistically significant only for TWA levels and serum quartiles analyses
  – Limitations to study: small number of exposed workers, multiple comparisons, and no unexposed controls

Genotoxicity of 1-Bromopropane Metabolites

Effect	Glycidol	[Propylene Oxide]	α-Bromohydrin	[3-Bromo-1-propanol]	1-Bromo-2-propanol
In vitro					
Mutation	+	+	+	+	
Chromosomal damage*	+	+			+
DNA damage*	+	+			
DNA adducts	+	+			
In vivo					
Mutation (germ cell)		–			
Chromosomal damage	+/–	+/–			
DNA damage*		+			
DNA adducts*		+			

*Including results in human cells

(see Draft Monograph, Table D-5)

Oxidative Stress

- Oxidative metabolites generated by P450 enzymes may exceed the glutathione-conjugating capacity or may inhibit enzymes required for glutathione synthesis.
- Reduced levels of glutathione may result in oxidative stress.
- Studies on mechanisms of toxicity have shown that 1-bromopropane causes oxidative stress in rodents which may be linked to toxic endpoints.
- Although no studies have evaluated the role of oxidative stress in 1-bromopropane–induced carcinogenicity, oxidative stress is a relevant mechanism for human carcinogenicity.

Oxidative Stress and Glutathione Depletion in Rodents

Toxicity studies (reproductive, neurological, and hepatic) in rodents have measured biomarkers of oxidative stress and glutathione depletion and assessed metabolic activation using different models.

- 1-Bromopropane causes glutathione depletion.
  - Induces dose-dependent decreases in glutathione in mice (Lee *et al.* 2007)
  - Related to Cyp2e1 expression; depletion was greater in wild type mice than in Cyp2e1$^{-/-}$ mice (Garner *et al.* 2007)
- 1-Bromopropane exposure caused oxidative stress in rodents.
  - Dose-dependent increases of oxidative stress markers (ROS, RNS) found in rat cerebellum (Subramanian *et al.* 2012)
  - Increased lipid peroxidation in male mice (Liu *et al.* 2010)
  - Altered expression of oxidative stress genes (NQO1 and HO-1) in mice (Lee *et al.* 2009, Liu *et al.* 2010)
- Several studies of 1-bromopropane suggest links between molecular alterations (i.e., oxidative metabolites, glutathione depletion and oxidative stress) and toxic endpoints.

Metabolic Activation of Halogenated Alkanes

- Several 1-bromopropane oxidative metabolites have been identified in rodents
  - Studies on five metabolites showed they are genotoxic
  - Glycidol and propylene oxide (putative metabolite) are both currently listed as *reasonably anticipated to be human carcinogens* in the RoC
- 1-Bromopropane is member of the class of halogenated alkanes
  - Halogenated alkanes readily form activated intermediates that could covalently modify biological molecules.
  - Several halogenated alkanes are listed in the RoC and/or are classified by IARC as carcinogens.

Other Potential Mechanisms

- γ-Aminobutyric acid (GABA) dysfunction
  - Evidence in rat neurotoxicity studies that inhalation exposure to 1-bromopropane causes GABA dysfunction
    - Hyperexcitability and changes in enzymatic activities in hippocampus (Fueta *et al.* 2002, 2004)
    - Degeneration of noradrenergic axons in the rat brain (Mohideen *et al.* 2009)
  - Evidence for GABA's role in carcinogenicity (in general)
    - GABA is involved in proliferation, differentiation and migration of several cell types, including cancer cells (Watanabe *et al.* 2006)
    - Altered signaling in tumor cells can lead to abnormal proliferation (Tatsuta *et al.* 1990).

Other Potential Mechanisms

One of our key questions is: Does immunomodulation play a role in 1-bromopropane carcinogenicity?

- 1-bromopropane causes immunosuppression in rats and mice
  - Spleen immunoglobulin (IgM) responses to sheep red blood cells and total T-cells were decreased (Anderson *et al.* 2010).
- 1-bromoporpane induced dose-related increases in gene expression and production of proinflammatory cytokines in mouse macrophages suggesting it can cause inflammation (Han *et al.* 2008).
- In the 2-year bioassay, there was evidence of inflammatory response in rats.
  - Exposure-related inflammatory lesions with Splendore-Hoeppli bodies were observed in rats but not mice; unclear whether these lesions may have been caused by 1-bromopropane–induced immunosuppression.
  - Lack of concordance between chronic respiratory inflammation and lung tumors in rats and mice; respiratory inflammation occurred in rats but not mice, but mice developed lung tumors.
- It is unclear whether 1-bromopropane-induced immunotoxicity plays a role in tumor development.

Synthesis

- Results of toxicity studies in experimental animals show that 1-bromopropane causes molecular alterations that are associated with carcinogenicity.
- Relevant data in humans
  - Studies of 1-bromopropane metabolism in humans have been limited to identification of potential biomarkers in urine.
  - Some evidence that humans have same metabolic pathways as animals: the same metabolites have been identified in urine of exposed workers.
  - Genotoxicity: limited evidence for DNA damage in leukocytes from exposed workers.
  - S-propylcysteine adducts in globin from 1-bromopropane-exposed workers.
- Available data support the relevance of the cancer studies in experimental animals to human carcinogenicity.

Mechanistic Data and Other Relevant Effects

Questions or Clarifications?

Mechanistic Data and Other Relevant Effects: Reviewer Questions

1. Comment on whether the genotoxicity and other mechanistic data (Section 5: Mechanistic Data and Other Relevant Effects, Appendix D, and Appendix E) presented in the cancer evaluation component for 1-bromopropane are clear, technically correct, and objectively presented.
2. Comment on whether the mechanistic data (Section 5: Mechanistic Data and Other Relevant Effects) are relevant for identifying and evaluating the potential mechanisms of action for the carcinogenic effects of 1-bromopropane.
    a. Provide any scientific criticisms of the NTP's interpretation and application of the genotoxicity data (Section 5.1: Genetic and related effects) from the cited studies for assessing effects of 1-bromopropane.
    b. Provide any scientific criticisms of the NTP's interpretation and application of the mechanistic data (Section 5.3: Mechanistic considerations and Section 5.4: Carcinogenicity of 1-bromopropane metabolites and analogues) from the cited studies for assessing effects of 1-bromopropane.
    c. Identify any information that should be added or deleted.

Overall Cancer Evaluation

- 1-Bromopropane is a substance to which a significant number of persons residing in the United States are exposed.
- The level of evidence of carcinogenicity from studies in experimental animals is sufficient; tumors were caused in multiple species, both rats and mice, and at multiple tissue sites, including lung, skin and large intestine.
- 1-Bromopropane causes molecular alterations in experimental systems that are relevant to possible mechanisms of human carcinogenicity and the available mechanistic data in humans are consistent with the conclusion that 1-bromopropane is *reasonably anticipated to be a human carcinogen*.

Overall Cancer Evaluation: Reviewer Questions

1. Comment on the overall cancer evaluation (Section 6: Overall Cancer Evaluation - Synthesis of Animal, Human, and Mechanistic Data) and whether the available metabolic, genotoxicity, and mechanistic data provide support for the relevance of the cancer studies in experimental animals to human carcinogenicity.
    a. Provide any scientific criticism of the NTP's overall assessment and integration of the experimental animal and mechanistic data.

Overall Cancer Evaluation: Synthesis of Animal, Human, and Mechanistic Data

- RoC criteria for *reasonably anticipated to be a human carcinogen*

  (see page iv of the Draft RoC Monograph for 1-Bromopropane)

- Action:
  - Vote on whether the scientific evidence supports the NTP's preliminary listing decision for 1-bromopropane in the RoC.

Draft Substance Profile

- Contains NTP's preliminary recommendation of the listing status of the substance.
- Summarizes the scientific information that is key to reaching a recommendation.
- Provides information on properties, use, production and exposure.
- Provides information on existing federal regulations and guidelines.

Once the listing status is approved by the Secretary, HHS, the substance profile becomes a part of the next edition of the RoC.

Draft Substance Profile: Reviewer Questions

A. Comment on whether the information on use, production, and human exposure for 1-bromopropane is clear and technically accurate.

B. Comment on whether the information presented regarding carcinogenicity and cancer studies in experimental animals is clear, technically correct, and objectively stated.

C. Comment on whether the substance profile highlights the key information from the cancer studies in experimental animal that supports the listing recommendation.

D. Comment on whether the information presented regarding studies on mechanisms of carcinogenicity and other relevant data is clear, technically correct, and objectively stated.

E. Comment on whether the substance profile highlights the studies on mechanisms of carcinogenicity and other relevant data that are key to providing support for evaluating the relevance of the cancer studies in experimental animals to human carcinogenicity.

Acknowledgements

Office of the Report on Carcinogens, DNTP

Ruth Lunn, Director

Gloria Jahnke

Contractor Support Staff

Sanford Garner, Principal Investigator

Stanley Atwood

Andrew Ewens

Ella Darden

Susan Dakin

Alton Peters

Jennifer Ratcliffe

Tracy Saunders

Acknowledgements

Technical Advisors

Kevin Hanley, MSPH, CIH

G. Scott Dotson, PhD, CIH

Office of Liaison, Policy and Review (OLPR), DNTP

Mary Wolfe, Director
Danica Andrews
Robin Guy
Denise Lasko
Lori White