

1,2-Dibromoethane (Ethylene Dibromide)*

Introduction

1,2-Dibromoethane (ethylene dibromide, EDB, ethylene bromide, CAS No. 106-93-4), a colorless volatile liquid, enjoys considerable use as a lead scavenger in leaded gasolines and as a soil and grain fumigant, mainly as a nematocide. The use of 1,2-dibromoethane as a fuel additive appears to be decreasing as leaded gasoline for automobiles is apparently being phased downward. Yet, more than 100 formulated pesticides contain EDB. In 1977, 120 million kilograms were produced in the United States. Lesser uses include as a dye intermediate, an industrial solvent (for resins, gums, waxes), and in some fire extinguishers.

1,2-Dibromoethane affects liver microsomes, DNA, and sperm. Human exposure to EDB can cause eye, skin and respiratory irritation as well as damage to the liver, kidney, spleen, and lungs.

Ethylene dibromide was mutagenic for *Salmonella typhimurium* G46, TA 1530 and TA 1535 without metabolic activation (1,2), for *Drosophila melanogaster* (3) and for *Tradescantia* (4).

By the gavage route of administration, 1,2-dibromoethane was carcinogenic for Osborne-Mendel rats, causing squamous cell carcinomas of the forestomach in both sexes, hepatocellular carcinomas in females, and hemangiosarcomas of the circulatory system in males. EDB was carcinogenic in B6C3F₁ mice, causing squamous cell carcinomas of the forestomach and alveolar/bronchiolar adenomas in both sexes (5-9). These lesions were seen as early as week 12 in rats and week 24 in mice.

1,2-Dibromoethane was tested again by the National Toxicology Program/National Cancer Institute (NTP/NCI) Bioassay Program, this time using inhalation exposure to determine the effects by this route because workers and the general population are exposed to airborne EDB.

Methods

Male and female inbred Fischer 344 rats and male and female hybrid B6C3F₁ mice, obtained from the Frederick Cancer Research Center, were used in this study. Control and treated groups contained 50 animals of each sex and species. All groups received Wayne Lab Blox and water *ad libitum* (except food was removed during exposure periods). Chamber control and treated groups were exposed to concentrations of 0, 10, or 40 ppm EDB for 6 hr per day, 5 days per week, for 78 to 103 weeks.

The 1,2-dibromoethane used in this inhalation study was > 99% pure. This carcinogenesis bioassay was conducted from July 1976 to July 1978 at Hazleton Laboratories, America, Inc., under a subcontract to Tracor Jitco (prime contractor for the testing program).

All animals that died during the study or that were killed at the end of the exposure period were subjected to a gross necropsy and a complete histopathological examination. Statistical analyses of survival differences among groups were done using life table methods (10,11). For tumor incidence data, pairwise comparisons were made by Fisher's exact tests, and the significance of dose-response trends was assessed by Cochran-Armitage tests (12,13). The study design conformed to the *NCI Guidelines for Carcinogen Bioassays in Small Rodents* (14).

Results

Throughout the study, mean body weights of high dose rats and high dose mice of either sex were lower than those of the corresponding untreated controls. Survival of the high dose rats of either sex and of the low and high dose female mice was significantly shorter than that in the corresponding controls.

In male rats, 38/50 (76%) of the control group and 35/50 (70%) of the low dose group lived to the end of the study at 104-106 weeks. The high dose group was killed at week 89, at which time 5/50 (10%) were still alive. In female rats, 38/50 (76%) of the control group and 39/50 (78%) of the low dose group

*Prepared by J. E. Huff. Address: National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709
This paper is a condensation of the *Carcinogenesis Bioassay Technical Report* (TR210). Single copies of the complete technical report may be obtained without cost from the Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (see reference 17).

lived to the end of the study at 104-106 weeks. The high dose female rats were terminated at week 91, at which time 8/50 (16%) were still alive.

In male mice, 13/50 (26%) of the control group, 11/50 (22%) of the low dose group, and 18/50 (36%) of the high dose group lived to the end of the study at week 79. In females, 40/50 (80%) of the control group and 19/50 (38%) of the low dose group lived to the end of the study at 104-106 weeks. The high dose female mice were killed at week 91, when 7/50 (14%) were alive.

Other than being chemically associated, the cause(s) of death in the rats and female mice was undetermined. The principal cause of early death in control and dosed male mice was ascending, suppurative urinary tract infection that resulted in necrotic, ulcerative lesions around the urethral opening, chronic or suppurative cystitis (often with urinary tract obstruction), and ascending suppurative pyelonephritis.

Exposure to 1,2-dibromoethane was also associated with hepatic necrosis and toxic nephropathy in rats or either sex, testicular degeneration in male rats, retinal degeneration in female rats, and epithelial hyperplasia of the respiratory system in mice.

Carcinomas and adenocarcinomas of the nasal cavity were observed with significantly increased incidences ($p < 0.001$) in high dose rats of either sex relative to controls. The incidences of adenocarcinomas and adenomas of the nasal cavity were also significantly increased ($p < 0.001$) in low dose rats of either sex. Adenomatous polyps of the nasal cavity showed a significantly increased incidence ($p < 0.001$) in low dose male rats. The combined incidence of alveolar/bronchiolar adenomas or carcinomas was statistically significant ($p < 0.05$) for high dose female rats.

Hemangiosarcomas of the circulatory system (mainly spleen) and mesotheliomas of the tunica vaginalis occurred in high dose male rats with significantly increased incidences ($p < 0.001$) relative to controls.

The incidence of fibroadenomas of the mammary gland was significantly elevated ($p < 0.001$) in dosed female rats compared to controls.

The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma were significantly increased ($p < 0.001$) in high dose male mice. These tumors were also increased in high-dose female mice ($p < 0.01$ for adenomas and $p < 0.001$ for carcinomas).

Hemangiosarcomas occurred in low and high dose female mice at incidences significantly greater ($p < 0.001$) than the incidence of the controls. High dose female mice also had significantly increased incidences of subcutaneous fibrosarcomas ($p < 0.001$), of nasal cavity carcinomas ($p < 0.05$), and of mammary gland adenocarcinomas ($p < 0.05$). Low dose female mice also showed a significantly increased incidence ($p < 0.001$) of mammary gland adenocarcinomas.

Table 1 catalogs those primary tumor increases that occurred in rats and Table 2 lists those in mice.

Other tumor increases, some showing a statistically significant dose-related trend (yet none of the individual dose groups were different than corresponding controls), occurred in male rats (subcutaneous fibroma: control 3/50, low dose 6/50, high dose 8/50; salivary gland sarcoma [trend statistic $p < 0.05$]: 0/49, 1/50, 3/48), in female rats (subcutaneous fibroma or fibrosarcoma [trend statistic $p < 0.01$]: 0/50, 0/50, 4/50; mammary gland adenocarcinoma [trend $p < 0.05$]: 1/50, 0/50, 4/50), in male mice (circulatory system hemangioma or hemangiosarcoma [trend $p < 0.01$]: 0/45, 0/50, 4/50), and in female mice

Table 1. Primary tumor increases in F344 rats exposed to 1,2-dibromoethane by inhalation.

Site/Tumor	Male			Female		
	Chamber control	10 ppm	40 ppm	Chamber control	10 ppm	40 ppm
Circulatory system hemangiosarcoma ^{a,b}	0/50	1/50	15/50 ^c	0/50	0/50	5/50 ^d
Lung alveolar/bronchiolar carcinoma or adenoma ^b	1/50	2/50	1/50	0/50	0/48	5/47 ^d
Mammary gland fibroadenoma ^b	0/50	0/50	0/50	4/50	29/50 ^c	24/50 ^c
Nasal cavity all tumors ^{a,e,f}	0/50	39/50 ^c	41/50 ^c	1/50	34/50 ^c	43/50 ^c
Tunica vaginalis mesothelioma ^a	0/50	7/50 ^g	25/50 ^c	-	-	-

^aDose-related trend for males ($p < 0.001$).

^bDose-related trend for females ($p < 0.005$).

^cGreater than controls ($p < 0.001$).

^dGreater than controls ($p < 0.05$).

^eDose-related trend for females ($p < 0.001$).

^fAdenoma, adenocarcinoma, adenomatous polyp, squamous cell papilloma, squamous cell carcinoma, papillary adenoma, carcinoma (individual tumor incidences are given in reference 17).

^gGreater than controls ($p < 0.01$).

Table 2. Primary tumor increases in B6C3F₁ mice exposed to 1,2-dibromoethane by inhalation.

Site/Tumor	Male			Female		
	Chamber control	10 ppm	40 ppm	Chamber control	10 ppm	40 ppm
Circulatory system hemangiosarcoma ^a	0/45	0/50	2/50	0/50	11/50 ^b	23/50 ^b
Lung alveolar/bronchiolar carcinoma or adenoma ^{a,c}	0/41	3/48	23/46 ^b	4/49	11/49 ^d	41/50 ^b
Mammary gland fibroadenoma	0/45	0/50	0/50	2/50	14/50 ^b	8/50 ^d
Nasal cavity all tumors ^{a,e}	0/45	0/50	0/50	0/50	0/50	12/50 ^b
Skin subcutaneous fibrosarcoma ^a	0/45	0/50	2/50	0/50	5/50 ^d	11/50 ^b

^aDose-related trend for females ($p < 0.001$).

^bGreater than controls ($p < 0.001$).

^cDose-related trend for males ($p < 0.001$).

^dGreater than controls ($p < 0.05$).

^eAdenoma, adenomatous polyp, carcinoma, hemangiosarcoma (individual tumor incidences are given in reference 17).

(circulatory system hemangioma [trend $p < 0.05$]: 0/50, 1/50, 4/50).

Statistically significant ($p < 0.05$) decreases in tumor incidence were found for male rats (mononuclear cell leukemia: 6/50, 7/50, 1/50; adrenal pheochromocytoma: 4/49, 5/49, 0/48; pituitary gland adenoma: 10/45, 7/48, 2/47; interstitial cell tumors of the testes: 35/50, 45/50, 10/49), in female rats (all leukemias: 6/50, 7/50, 1/50; pituitary gland adenoma: 21/50, 18/49, 4/45), and in female mice (lymphoma or leukemia: 8/50, 7/50, 1/50; pituitary gland adenoma: 8/48, 1/46, 0/40). Except for the decreases in pituitary adenoma in female mice, these decreased tumor incidences reflect a significantly lower frequency in the particular high dose group. And these lack of tumor responses are likely due to the decreases in survival observed in these high dose groups.

Discussion

A carcinogenesis bioassay of 1,2-dibromoethane, a widely used pesticide, soil and grain fumigant, and leaded gasoline additive, was conducted by exposing groups of 50 F344 rats and B6C3F₁ mice of each sex by inhalation to concentrations of 10 or 40 ppm of the 1,2-dibromoethane for 78-103 weeks. Untreated controls consisted of 50 rats and 50 mice of each sex exposed in chambers to ambient air.

Mean body weights of high dose male and female rats were lower than those of corresponding untreated controls throughout the study, and survival in the same high dose groups was significantly shorter than that of controls. Mean body weights of high dose mice were lower than those of corresponding controls throughout the study, and survival in dosed females was significantly shorter than that of controls. Control and dosed male mice had poor

survival, the principal cause of death being an ascending suppurative urinary tract infection that was unrelated to compound administration.

Among the observed compound-related nonneoplastic lesions were hepatic necrosis and toxic nephropathy in male and female rats, testicular degeneration and atrophy in males, and retinal degeneration in females. A 91-day study by Rowe et al. (15) reported compound-related nonneoplastic lesions in some of the same organs when rats were exposed to 1,2-dibromoethane in air at a concentration of 385 mg/m³ (50.2 ppm) for 7 hr per day, 5 days per week. Wong et al. (16) found testicular atrophy in 90% of male Sprague-Dawley rats exposed to a combination of EDB (20 ppm) and disulfiram (0.05%). In the NCI/NTP chronic study (17), inflammation of the nasal cavity and epithelial hyperplasia of the respiratory system were diagnosed in dosed male and female mice. Using the 13-week observations, Reznik (18) reported finding characteristics of proliferative lesions in the nasal cavities of rats and mice. Stinson et al. (19) recorded similar findings in mice using data from the two-year study (17). Other lesions observed in male mice were epithelial hyperplasia of the urinary bladder and inflammation of the prostate gland.

Nitschke et al. (20) exposed groups of ten CDF (F344) rats to 0, 3, 10, and 40 ppm EDB (five times per week) for 13 weeks; separate groups were held for another 13 weeks without additional exposure. The 3 ppm exposed group exhibited no observable lesions; the 10 ppm group showed hyperplasia of the nasal turbinates. Rats exposed to 40 ppm developed hyperplasia and nonkeratinizing squamous metaplasia of the respiratory epithelium. After the post exposure period these lesions were not observed in the rats exposed for the first 13 weeks.

Supernatant of rat liver homogenates contains an

enzyme that catalyzes the reaction between glutathione and 1,2-dibromoethane (21); Watanabe et al. (22) have calculated that 20 mg 1,2-dibromoethane (by inhalation) would deplete the rat liver of glutathione.

According to Edwards et al. (23), the small intestine, liver, kidney, and fat of male RF/Hiraki rats contained most of the radioactivity 3 hr after intraperitoneal injection of 1,2-¹⁴C-dibromoethane (40 mg/kg). When rats were given intraperitoneal injections of 1,2-¹⁴C-dibromoethane (4.2 mol) and killed after 24 hr, the largest amount of radioactivity was bound to protein, DNA, and RNA in the liver and kidney, and intermediate amounts were found in the lung, testes, stomach, and large and small intestines. Bromoacetaldehyde, an alkylating agent identified as a metabolite of 1,2-dibromoethane in rats, has been suggested to be the compound involved in the irreversible binding to protein and nucleic acid (24).

1,2-Dibromoethane has been found to affect liver microsomes, DNA, and sperm. When liver microsomes from B6C3F₁ mice and when DNA from salmon sperm were incubated with ¹⁴C-bromoacetaldehyde and ¹⁴C-bromoethanol, these metabolites of 1,2-dibromoethane were bound covalently to protein and DNA to a greater extent than was 1,2-dibromoethane (25). After Nachtomi and Sarma (21) administered 1,2-dibromoethane in single doses of 0, 5.0, 7.5, 10, 15, or 22 mg/100 g body weight in corn oil by gavage to male Wistar rats, liver DNA of dosed rats sedimented more slowly than that of untreated rats. Nachtomi and Sarma postulated that the slower sedimentation rate was caused by single strand breaks in the DNA. Dose-dependent increases in hepatic DNA single-strand breaks were shown by White et al. (26) to occur in male Swiss-Webster mice treated intraperitoneally with 25-75 mg EDB/kg body weight. More breaks were detected following preincubation in the alkaline eluting solution, suggesting (by the authors) that the DNA strand breaks result, in part, from the alkali-lability of DNA sites alkylated by EDB. No evidence was found for EDB-induced DNA-DNA or DNA-protein cross-linking (26).

In the NCI/NTP inhalation study (17), tumors of the respiratory tract and tumors of the mammary gland were found at significantly increased incidences in EDB-dosed rats. Carcinomas, adenomatous polyps, and adenocarcinomas of the nasal cavity and hemangiosarcomas of the circulatory system in high dose rats of either sex occurred at incidences higher than those in the corresponding controls. Mesotheliomas of the tunica vaginalis in high dose male rats and mammary gland fibroadenomas and the combined incidence of alveo-

lar/bronchiolar carcinomas and adenomas in high dose female rats all occurred at incidences higher than those in the corresponding controls.

In mice, as in dosed rats, tumors of the respiratory tract, (both sexes), hemangiosarcomas of the circulatory system (female), and tumors of the mammary gland (female) were found at significantly increased incidences. Alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas in high dose male and female mice, and the combined incidence of carcinomas and adenomas of the nasal cavity, fibrosarcomas of the subcutaneous tissue, and hemangiosarcomas of the circulatory system in high dose female mice, occurred at incidences significantly higher than those in the corresponding controls.

In a chronic bioassay using Sprague-Dawley rats exposed by inhalation to 20 ppm EDB alone or in combination with disulfiram (0.05% diet), Wong et al. (16) observed a considerable decrease in survival after 12-15 months, and a significant increase in tumors (EDB: mammary gland, spleen, adrenal gland, liver, kidney, and subcutaneous tissue; EDB plus disulfiram: liver, spleen, kidney, adrenal gland, thyroid gland, lung, mesentery, and mammary gland).

Data analyses from a previous gavage study (6,9,27), conducted in the same laboratory as the present study, showed increased incidences of squamous cell carcinomas of the forestomach in Osborne-Mendel rats and B6C3F₁ mice of both sexes, hepatocellular carcinomas in female rats, hemangiosarcomas (primarily of the spleen) in male rats, and alveolar/bronchiolar adenomas in male and female mice. According to Van Duuren et al. (28) long-term (62 weeks) dermal application of EDB to Ha: ICR Swiss mice caused an increased incidence of respiratory tract tumors, skin papillomas, and skin carcinomas.

In conclusion and under the conditions of this bioassay (17) 1,2-dibromoethane was carcinogenic for F344 rats, causing increased incidences of carcinomas, adenocarcinomas, adenomas of the nasal cavity, and hemangiosarcomas of the circulatory system in males and females; mesotheliomas of the tunica vaginalis and adenomatous polyps of the nasal cavity in males; and fibroadenomas of the mammary gland and alveolar/bronchiolar adenomas and carcinomas (combined) in females. 1,2-Dibromoethane was carcinogenic for B6C3F₁ mice, causing alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas in males and females; and hemangiosarcomas of the circulatory system, fibrosarcomas in the subcutaneous tissue, carcinomas of the nasal cavity, and adenocarcinomas of the mammary gland in females.

REFERENCES

1. Brem, H., Stein, A., and Rosenkranz, H. The mutagenicity and DNA-modifying effect of haloalkanes. *Cancer Res.* 34: 2576-2579 (1974).
2. Buselmaier, W., Rohrborn, G., and Propping, P. Comparative investigations on the mutagenicity of pesticides in mammalian test systems. *Mutat. Res.* 21: 25-26 (1976).
3. Vogel, E., and Chandler, J. Mutagenicity testing of cyclamate and some pesticides in *Drosophila melanogaster*. *Experientia* 30: 621-623 (1974).
4. Sparrow, A., Schairer, L., and Villalobos-Pietrini, R. Comparison of somatic mutation rates induced in *Tradescantia* by chemical and physical mutagens. *Mutat. Res.* 26: 265-274 (1974).
5. IARC. Ethylene dibromide. In: Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. International Agency for Research on Cancer, World Health Organization, Lyon, France, 1977, pp. 195-209.
6. NCI. Bioassay of 1,2-Dibromoethane for Possible Carcinogenicity. NCI-CG-TR-86. National Cancer Institute, Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, NIH Pub No. 78-1336, 1978, pp. 64.
7. Olson, W., Habermann, R., Weisburger, E., Ward, J., and Weisburger, J. Brief Communication: induction of stomach cancer in rats and mice by halogenated aliphatic fumigants. *J. Natl. Cancer Inst.* 51(6): 1933 (1973).
8. Powers, M., Voelker, R., Page, N., Weisburger, E., and Kraybill, H. Carcinogenicity of ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) after oral administration in rats and mice. *Toxicol. Appl. Pharmacol.* 33: 171-172 (1975).
9. Ward, J.M. and Habermann, R.T. Pathology of stomach cancer in rats and mice induced with the agricultural chemicals ethylene dibromide and dibromochloropropane. *Bull. Soc. Pharmacol. Environ. Pathol.* 2: 10-11 (1974).
10. Cox, D. R. Regression models and life tables. *J.R. Stat. Soc. Ser. B.* 34: 87-220 (1972).
11. Tarone, R. E. Tests for trends in life table analysis. *Biometrika* 62(3): 679-682 (1975).
12. Armitage, P. *Statistical Methods in Medical Research.* John Wiley & Sons, Inc., New York, 1971, pp. 362-365.
13. Gart, J. J. The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. *Rev. Int. Stat. Inst.* 39: 148-169 (1971).
14. Sontag, J. M., Page, N. P., and Saffiotti, U. Guidelines for carcinogen bioassay in small rodents (National Cancer Institute Carcinogenesis Technical Report Series No. 1), DHEW Publication No. 76-801, Washington, DC, 1976, 65pp.
15. Rowe, V., Spencer, H., McCollister, D., Hollingsworth, R., and Adams, E. Toxicity of ethylene dibromide determined on experimental animals. *A.M.A. Arch. Ind. Hyg. Occupational Med.* 6: 158-173 (1952).
16. Wong, L.C.K., Winston, J.M., Hong, C.B., and Plotnick, H. Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. *Toxicol. Appl. Pharmacol.* 63: 155-165 (1982).
17. NTP/NCI. Carcinogenesis Bioassay of 1,2-Dibromoethane (CAS No. 106-93-4) in F344 Rats and B6C3F₁ Mice (Inhalation Study). TR 210. National Toxicology Program, Research Triangle Park, NC, NIH Pub. No. 82-1766, 1982, 163 pp.
18. Reznik, G., Stinson, S. F., and Ward, J. M. Respiratory pathology in rats and mice after inhalation of 1,2-dibromo-3-chloropropane or 1,2-dibromoethane for 13 weeks. *Arch. Toxicol.* 46: 233-240 (1980).
19. Stinson, S. F., Reznik, G., and Ward, J. M. Characteristics of proliferative lesions in the nasal cavities of mice following chronic inhalation of 1,2-dibromoethane. *Cancer Lett.* 12(1-2): 121-129 (1981).
20. Nitschke, K. D., Kociba, R. J., Keyes, D. G., and McKenna, M. J. A thirteen week repeated inhalation study of ethylene dibromide in rats. *Fundam. Appl. Toxicol.* 1: 437-442 (1981).
21. Nachtomi, E. and Sarma, D. Repair of rat liver DNA *in vivo* damaged by ethylene dibromide. *Biochem. Pharmacol.* 26: 1941-1945 (1977).
22. Watanabe, P., Young, J., Schlachter, M., Zempel, J., and Karbowski, R. Fate of inhaled ethylene dibromide in rats. *Toxicol. Appl. Pharmacol.* 45: 224 (1978).
23. Edwards, K., Jackson, H., and Jones, A. R. Studies with alkylating esters. II. A chemical interpretation through metabolic studies of the antifertility effects of ethylene dimethanesulphonate and ethylene dibromide. *Biochem. Pharmacol.* 19: 1783-1789 (1970).
24. Hill, D., Shih, T.-W., Johnston, T., and Struck, R. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res.* 38: 2438-2442 (1978).
25. Kline, S., Banerjee, S., and Van Duuren, B. Interaction of potential activated intermediates of the carcinogen ethylene dibromide with protein and DNA *in vitro*. *Proc. Am. Assoc. Cancer Res.* 20: 86 (1979).
26. White, R. D., Sipes, I. G., Gandolfi, A. J., and Bowden, G. T. Characterization of the hepatic DNA damage caused by 1,2-dibromoethane using the alkaline elution technique. *Carcinogenesis* 2: 839-844 (1981).
27. Ward, J.M., and Habermann, R.T. Pathology of stomach cancer in rats and mice induced with the agricultural chemicals ethylene dibromide and dibromochloropropane. *Lab Invest.* 30: 392 (1974).
28. Van Duuren, B., Goldschmidt, B., Loewengart, G., Smith, A., Melchionne, S., Seidman, I., and Roth, D. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J. Natl. Cancer Inst.* 63(6): 1433-1438 (1979).