Environmental Health Perspectives
Vol. 47, pp. 365-369, 1983

1,2-Dibromo-3-Chloropropane*

Introduction

1,2-Dibromo-3-chloropropane (DBCP, nemagon, fumazone, CAS No. 96-12-8), a contaminant (0.05%) of the flame retardant tris (2,3-dibromopropyl) phosphate, has been used primarily as a soil fumigant (30 ppm) to control nematodes. DBCP can be either injected directly into the soil or added to irrigation water. By 1972 an estimated 5.6 million kilograms were being used annually; in 1977 a total of 377,000 kilograms were used in California, mostly on grapes and tomatoes. By October 1979, DBCP could be used only on pineapple crops; in March 1981, EPA announced that manufacturers had agreed to stop making and using the compound, except for Hawaiian pineapples.

Altered testicular function (reduced spermatogenesis and infertility) has been found in employees in several plants producing or formulating DBCP (*1-6*). In 1977, low sperm counts (less than 1,000,000/mL compared with a normal value of 40,000,000/mL) were first found in workers exposed to DBCP (*7*). Some workers had no sperm. Potashnik et al. (*8*) reported a correlation between exposure time and sperm density for 23 male production line employees; exposure longer than 100 hours was associated with azoospermia and elevated follicle-stimulating hormone. Legator (*9*) has compiled a chronology of DBCP toxicity studies from 1961 to 1978.

1,2-Dibromo-3-chloropropane (technical grade) was carcinogenic by gavage to Osborne-Mendel rats and $B6C3F_1$ mice, causing squamous cell carcinomas of the forestomach in both sexes of both species and adenocarcinomas of the mammary gland in female rats (*10-12*).

1,2-Dibromo-3-chloropropane (technical grade) was retested by the National Cancer Institute/National Toxicology Program (NCI/NTP) Bioassay Program, this time using the inhalation route, since workers were being exposed to airborne DBCP and it was considered important to determine effects by this route.

Methods

Male and female inbred Fischer 344 rats and male and female hybrid $B6C3F_1$ 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, 0.6, or 3.0 ppm DBCP for 6 hr per day, 5 days per week, for 76 to 103 weeks.

The technical grade 1,2-dibromo-3-chloropropane (96% pure) used in the inhalation study contained trace amounts of epichlorohydrin and 1,2-dibromoethane.

This carcinogenesis bioassay was conducted from August 1976 to August 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 (*13,14*). 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 (*15,16*). The study design conformed to the *NCI Guidelines for Carcinogen Bioassay in Small Rodents (17)*.

Results

Accelerated mortality occurred in both species. Early deaths of high dose rats and mice were associated with respiratory tract tumors; interference with breathing and metastasis to the brain were major contributing factors in these deaths. Among male mice, survival was decreased in all

*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* (TR206). 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 *40*).

groups; urogenital infection appeared to be associated with their deaths. Surviving high dose rats were killed at week 84. The remaining high dose female mice and high and low dose male mice were killed at week 76. Low dose rats and female mice were killed at week 104. Corresponding control groups were killed at weeks 105-107, with the exception of male mice controls killed at week 80.

Increased tumor incidences were diagnosed in all DBCP-exposed groups. These neoplastic lesions were located principally within the respiratory system.

Carcinomas, squamous cell carcinomas, and adenocarcinomas of the nasal cavity and squamous cell papillomas of the tongue each occurred in high dose male rats at incidences significantly ($p < 0.05$) higher than those in the corresponding controls. Adenocarcinomas, adenomas, adenomatous polyps and squamous cell papillomas of the nasal cavity and adenomatous polyps of the nasal turbinates occurred in low dose male rats with significantly ($p < 0.01$) increased incidences relative to controls.

Carcinomas and adenocarcinomas of the nasal cavity, squamous cell papillomas of the tongue, squamous cell papillomas and carcinomas (combined) of the pharynx, and adenomas of the adrenal cortex each occurred in high dose female rats at incidences significantly ($p < 0.05$) higher than those in the corresponding controls. Also, adenomas and squamous cell papillomas of the nasal cavity, adenomas of the adrenal cortex, and fibroadenomas of the mammary gland were increased significantly ($p < 0.05$) in low dose female rats when compared with controls.

Adenocarcinomas of the nasal cavity in high dose female mice, papillary carcinomas of the lung/bronchiole in low dose female mice, carcinomas, squamous cell carcinomas of the nasal cavity, and alveolar/bronchiolar adenomas or carcinomas of the lung in high-dose male and female mice occurred at incidences significantly ($p < 0.05$) higher than those in the corresponding controls.

Table 1 lists those primary tumor increases observed in rats and Table 2 records those in mice.

Other increases though not statistically significant occurred in male rats (skin trichepithelioma: 0/50,

Table 1. Primary tumor increases in F344 rats exposed to 1,2-dibromo-3-chloropropane by inhalation.

	Male			Female		
Site/tumor	Chamber control	0.6 ppm	3.0 ppm	Chamber control	0.6 ppm	3.0 ppm
Adrenal gland cortical adenoma	1/49	6/49	3/48	0/50	7/50[a]	5/48[b]
Mammary gland fibroadenoma	0/50	0/50	0/49	4/50	13/50[b]	4/50
Nasal cavity and turbinates all tumors[c,f]	0/50	40/50[d]	39/49[d]	1/50	27/50[d]	42/50[d]
Pharynx squamous cell papilloma or carcinoma[e]	0/50	3/50	1/49	0/50	0/50	6/50[b]
Tongue squamous cell papilloma or carcinoma[c]	0/50	1/50	11/49[d]	0/50	4/50	9/50[d]

[a]Greater than controls ($p < 0.01$).
[b]Greater than controls ($p < 0.05$).
[c]Dose-related trends ($p < 0.001$).
[d]Greater than controls ($p < 0.001$).
[e]Dose-related trend, females ($p < 0.001$).
[f]Carcinoma, squamous cell papilloma or carcinoma, adenoma, adenocarcinoma, adenomatous polyp, and carcinosarcoma.

Table 2. Primary tumor increases in B6C3F$_1$ mice exposed to 1,2-dibromo-3-chloropropane by inhalation.

	Male			Female		
Site/tumor	Chamber control	0.6 ppm	3.0 ppm	Chamber control	0.6 ppm	3.0 ppm
Lung/bronchus/bronchiole all tumors[a,d]	0/41	3/40	11/45[b]	4/49	12/48[c]	18/47[b]
Nasal cavity all tumors[a,e]	0/45	1/42	19/48[b]	0/50	11/50[b]	38/50[b]

[a]Dose-related trends ($p < 0.001$).
[b]Greater than controls ($p < 0.001$).
[c]Greater than controls ($p < 0.05$).
[d]Papillary adenoma or carcinoma, squamous cell carcinoma, alveolar/bronchiolar adenoma or carcinoma.
[e]Carcinoma, squamous cell papilloma or carcinoma, adenocarcinoma, adenomatous polyp, carcinosarcoma, sarcoma, keratocanthoma, fibrosarcoma, unspecified malignant neoplasm.

1/50, 3/49 and tunica vaginalis mesothelima: 1/50, 2/50, 5/49), in male mice (circulatory system hemangiosarcoma: 0/45, 0/42, 3/48 and stomach squamous cell papilloma or carcinoma: 0/37, 0/41, 3/44), and in female mice (harderian gland adenoma: 0/50, 5/50, 1/50).

Statistically significant decreases in tumor incidence were observed for male rats (hematopoietic system leukemia or lymphoma: 6/50, 10/50, 0/49 and pituitary chromophobe adenoma: 10/45, 7/48, 0/44), for female rats (pituitary chromophobe adenoma: 20/50, 20/47, 2/46 and uterus endometrial stromal polyp: 6/50, 4/49, 0/46), and for female mice (hematopoietic system lymphoma: 8/50, 5/50, 1/50 and pituitary adenoma: 8/48, 1/44, 1/28).

Discussion

In other bioassays that employed feed, gavage, or skin application as the route of administration, DBCP was associated with increased incidences of squamous cell carcinomas or papillomas of the forestomach in Osborne-Mendel rats (*10*), Charles River albino rats (*18*), B6C3F$_1$ mice (*10*), HAM/ICR Swiss albino mice (*18*), and ICH/Ha Swiss mice (females) (*19*); adenocarcinomas of the mammary gland in Osborne-Mendel rats (females) (*10*); renal tubular tumors in Charles River albino rats (*18*); lung papillomas in ICR/Ha Swiss mice (females) (*19*); and lung tumors in Charles River rats (*18*).

In a chronic skin application study and in initiation-promotion experiments reported by Van Duuren et al. (*19*), groups of 30 female ICR/Ha Swiss mice given 11.7 or 35 mg DBCP in 0.2 mL acetone three times per week for 62 weeks had increased incidences of lung papillomas and squamous cell carcinomas or papillomas of the forestomach. In these experiments, no skin tumors were induced. However, after skin initiation with DBCP and promotion with phorbol myristate, animals had a significant increase in skin tumors.

In a chronic feeding study conducted for Dow Chemical Company in the same laboratory as the present study, groups of 60 Charles River albino rats and 50 HAM/ICR Swiss albino mice were fed diets containing DBCP for 104 or 78 weeks, respectively. DBCP in the diet was associated with statistically significant increased incidences of squamous cell carcinomas and papillomas of the forestomach in the rats and mice of either sex and of liver and renal tubular tumors in rats (*18*). In a previous NCI study conducted by the same laboratory, DBCP administered by gavage was found to be carcinogenic for Osborne-Mendel rats and B6C3F$_1$ mice, inducing squamous cell carcinomas of the forestomach in rats and mice of either sex and adenocarcinomas of the mammary gland in female rats (*10-12*).

From the results of these animal bioassays, DBCP apparently causes tumors at the direct site of exposure in the stomach and nasal cavity but not on the skin. In addition, DBCP or its metabolites cause toxic lesions and tumors at distant sites.

The contaminants of the technical grade DBCP used in the present study included several mutagens, such as epichlorohydrin (*20*), 1,2-dibromoethane (*21,22*), and 2,3-dibromo-1-propanol (*23*). The third chemical is a metabolite of tris-(2,3-dibromopropyl)-phosphate (*24,25*), the carcinogen found in the urine of some adults and children wearing sleepwear treated with this flame retardant (*23*). Epichlorohydrin (*26*) at 100 ppm and 1,2-dibromoethane (*22*) at 40 or 10 ppm induced nasal tumors when inhaled. These concentrations are considerably higher than the concentrations of the respective chemicals to which rats or mice in the current study were exposed. These rats and mice may have been exposed to a maximum level of 0.021 ppm epichloroyhydrin. In the epichlorohydrin study, only 1 of 100 Sprague-Dawley rats exposed to 30 ppm developed nasal carcinomas. It is not likely that the nasal tumors in the DBCP inhalation study were caused by exposure to these contaminants, but a cocarcinogenic effect, however remote, cannot be ruled out.

In other carcinogenesis studies on the related chemical 1,2-dibromoethane (*22,27*), results showed that this chemical was carcinogenic by two routes. Administered by gavage to Osborne-Mendel rats, 1,2-dibromoethane produced squamous cell carcinomas of the forestomach (in males and females), hepatocellular carcinomas (in females), and hemangiosarcomas (in males). Administration of 1,2-dibromoethane by the same route to B6C3F$_1$ mice produced squamous cell carcinomas of the forestomach and alveolar/bronchiolar adenomas in both sexes. When administered to F344 rats by inhalation, 1,2-dibromoethane produced a variety of nasal cavity tumors in males and females, hemangiosarcomas of the circulatory system, and mesotheliomas in the tunica vaginalis in males, and mammary gland fibroadenomas in females. Administered by inhalation to B6C3F$_1$ mice, 1,2-dibromoethane produced alveolar/bronchiolar carcinomas and adenomas in males and females and hemangiosarcomas of the circulatory system, subcutaneous fibrosarcomas, tumors of the nasal cavity, and mammary gland adenocarcinomas in females.

The direct mutagenic activity of technical grade DBCP in bacterial systems (*28*) can be attributed to contamination with epichlorohydrin (*29*), a direct acting bacterial mutagen. Several investigators,

however, have reported the mutagenic effects of DBCP in *Salmonella typhimurium* following metabolic activation with rat liver S9 [TA 1535 (*29*); TA 1530 (*28*); TA 100 (*23*)]. Because epichlorohydrin is used as a stabilizer in DBCP, investigators must specify the purity of DBCP samples studied.

The genetic effects of DBCP in mammalian germ cells have been reported for rats, mice, and humans. Lee and Suzuki (*30*) detected unscheduled DNA synthesis in the premeiotic germ cells (but not spermatozoa) of mice given a maximum tolerated dose (100 mg/kg) intraperitoneally. DBCP was also reported to induce dominant lethal mutations in rats (highest dose, 50 mg/kg) but not in mice at a high dose of 150 mg/kg (*31*). The dominant lethal effects in rats were observed in postmeiotic cells, particularly spermatids.

The available data indicate that DBCP can be metabolized by rat liver S9 to form a bacterial mutagen. DBCP or its metabolites are apparently distributed to the testes and can induce genetic damage as reflected by dominant lethal mutations and unscheduled DNA synthesis in premeiotic germ cells of rodents and perhaps by an increase in Y-chromosomal nondisjunction in humans (*32*).

The mechanism of DBCP toxicity in F344 rats is currently being investigated by the National Toxicology Program (*33-35*), with emphasis on gonadal toxicity and carcinogenicity, and on developing improved methodologies for monitoring exposed populations for deleterious effects.

From these DBCP carcinogenesis bioassay data, separate reports have been published on the carcinogenic effect of oral intubation (*10-12, 36*) on the morphology of nasal tumors induced by inhalation exposure in rats (*37*) and in mice (*38*), and on the inhalation-induced lung tumors in mice (*39*).

In conclusion and under the conditions of this bioassay (*40*), 1,2-dibromo-3-chloropropane was carcinogenic for male and female F344 rats, causing nasal cavity tumors and tumors of the tongue in both sexes, and cortical adenomas in the adrenal gland of females. 1,2-Dibromo-3-chloropropane was carcinogenic in male and female $B6C3F_1$ mice, inducing nasal cavity tumors and lung tumors.

REFERENCES

1. Biava, C., Smuckler, E., and Whorton, D. The testicular morphology of individuals exposed to dibromochloropropane. Exp. Mol. Pathol. 29: 448-458 (1978).
2. Federal Register. Labor/OSHA proposes permanent standard on occupational exposure to 1,2-dibromo-3-chloropropane. Federal Register 42(210): 57266-57267 (1977).
3. Federal Register. OSHA emergency temporary standard for exposure to 1,2-dibromo-3-chloropropane (DBCP). Federal Register 42 (175): 45535-45549 (1977).
4. Marshall, S., Whorton, D., Krauss, R., and Palmer, W. Effect of pesticides on testicular function. Urology 11(3): 257-259, 1978.
5. NIOSH. A Recommended Standard for Occupational Exposure to Dibromochloropropane. U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, 1977, pp. 13.
6. Whorton, D., Milby, T., Krauss, R., and Stubbs, H. Testicular function in DBCP exposed pesticide workers. J. Occup. Med. 21: 161-166 (1979).
7. Whorton, D., Krauss, R., Marshall, S., and Milby, T. Infertility in male pesticide workers. Lancet 2(8050): 1259-1261 (1977).
8. Potashnik, G., Yanai-Inbar, I., Sacks, M. I., and Israeli, R. Effect of dibromochloropropane on human testicular function. Israel J. Med. Sci. 15(5):438-442 (1979).
9. Legator, M.S. Chronology of studies regarding toxicity of 1,2-dibromo-3-chloropropane. Ann. N.Y. Acad. Sci. 329: 331-338 (1979).
10. NCI. Bioassay of Dibromochloropropane for Possible Carcinogenicity. NCI-CG-TR-28. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Bethesda, MD, NIH Pub. No. 78-828, 1978, p. 86.
11. Olson, W. A., Habermann, R. T., Weisburger, E. K., Ward, J. M., and Weisburger, J. H. Induction of stomach cancer in rats and mice by halogenated aliphatic fumigants. J. Natl. Cancer Inst. 51(6): 1993-1995 (1973).
12. Powers, M. B., Voelker, R. W., Page, N. P., Weisburger, E. K., and Kraybill, H. F. 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).
13. Cox, D. R. Regression models and life tables. J.R. Stat. Soc. Ser. B. 34: 87-220 (1972).
14. Tarone, R. E. Tests for trends in life table analysis. Biometrika 62(3): 679-682 (1975).
15. Armitage, P. Statistical Methods in Medical Research. John Wiley & Sons, Inc., New York, 1971, pp. 362-365.
16. 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).
17. 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/Pub/NIH-76-801, 1976, pp. 65.
18. Federal Register. Dibromochloropropane (DBCP): suspension order and notice to cancel. 44(219): 65135-65178 (1979).
19. Van Duuren, B., Goldschmidt, B., Loewengart, G., Smith, A., Melchionne, S., Seldman, I., and Roth, D. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. J. Natl. Cancer Inst. 63(6): 1433-1438 (1979).
20. IARC. Epichlorohydrin. (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 11), International Agency for Research on Cancer, World Health Organization, Lyon, 1976, p. 131.
21. 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, Vol. 15). International Agency for Research on Cancer, World Health Organization, Lyon, 1977, pp. 195-209.
22. 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, 1982, pp. 163.
23. Blum, A. and Ames, B. Flame-retardant additives as

possible cancer hazards. Science 195: 17-23, 1977.
24. IARC. Tris(2,3-dibromopropyl)phosphate. In: Some Halogenated Hydrocarbons (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 20). International Agency for Research on Cancer, World Health Organization, Lyon, 1979b, pp. 575-588.
25. Reznik, G., Reznik-Schuller, H., Rice, J. M., and Hague, B. F. Pathogenesis of toxic and neoplastic renal lesions induced by the flame retardants tris(2,3-dibromopropyl)phosphate in F344 rats, and development of colonic adenomas after prolonged oral administration. Lab. Invest. 44(1): 74-83 (1981).
26. Laskin, S., Sollakumar, A., Kuschner, M., Nelson, N., LaMendola, S., Rusch, G., Katz, G., Dulak, N., and Albert, R. Inhalation carcinogenicity of epichlorohydrin. J. Natl. Cancer Inst. 65(4): 751-755 (1980).
27. NCI. Bioassay of 1,2-Dibromoethane for Possible Carcinogenicity. NCI-CG-TR-86. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Bethesda, MD, NIH Pub No. 78-1336, 1978b, pp. 64.
28. Rosenkranz, H. Genetic activity of 1,2-dibromo-3-chloropropane, a widely-used fumigant. Bull. Environ. Contam. Toxicol. 14(1): 8-12 (1975).
29. Biles, R., Connor, T., Trieff, N. and Legator, M. The influence of contaminants on the mutagenic activity of dibromochloropropane (DBCP). J. Environ. Pathol. Toxicol. 2: 301-312 (1978).
30. Lee, I. P. and Suzuki, K. Induction of unscheduled DNA synthesis in mouse germ cells following 1,2-dibromo-3-chloropropane (DBCP) exposure. Mutat. Res. 68: 169-173 (1979).
31. Teramoto, S., Saito, R., Abyama, H., and Shirasu, Y. Dominant lethal mutation induced in male rats by 1,2-dibromo-3-chloropropane (CBCP). Mutat. Res. 77: 71-78 (1980).
32. Kapp, R., Picciano, D., and Jacobson, C. Y-Chromosomal nondisjunction in dibromochloropropane-exposed workmen. Mutat. Res. 64: 47-51 (1979).
33. Kluwe, W. M. Acute toxicity of 1,2-dibromo-3-chloropropane in the F344 male rat. I. Dose-response relationships and differences in routes of exposure. Toxicol. Appl. Pharmacol. 59: 71-83 (1981).
34. Kluwe, W. M. Acute toxicity of 1,2-dibromo-3-chloropropane in the F344 male rat. II. Development and repair of the renal, epididymal, testicular, and hepatic lesions. Toxicol. Appl. Pharmacol. 59: 84-95 (1981).
35. Kluwe, W. M., Greenwell, A., and Harrington, F. Relationship of tissue nonprotein sulfhydryls to the acute toxic effects of 1,2-dibromo-3-chloropropane. J. Pharmacol. Exptl. Therapeut. 220: 399-405 (1982).
36. IARC. 1,2-Dibromo-3-chloropropane. In: Some Halogenated Hydrocarbons (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 20). International Agency for Research Cancer, World Health Organization, Lyon, 1979a, pp. 83-96.
37. Reznik, G., Reznik-Schuller, H., Ward, J. M., and Stinson, S. F. Morphology of nasal cavity tumors in rats after chronic inhalation of 1,2-dibromo-3-chloropropane. Brit. J. Cancer 42(5): 772-781 (1980a).
38. Reznik, G., Ulland, B., Stinson, S. F., and Ward, J. M. Morphology and sex-dependent manifestations of nasal tumors in B6C3F1 mice after chronic inhalation of 1,2-dibromo-3-chloropropane J. Cancer Res. Clin. Oncol. 98: 75-83 (1980c).
39. Reznik, G., Stinson, S. F., and Ward, J. M.: Lung tumors induced by chronic inhalation of 1,2-dibromo-3-chloropropane in B6C3F1 mice. Cancer Letters 10: 339-342 (1980b).
40. NTP/NCI. Carcinogenesis Bioassay of 1,2-Dibromo-3-Chloropropane (CAS No. 96-12-8) in F344 Rats and B6C3F1 Mice (Inhalation Study). TR 206. National Toxicology Program, Research Triangle Park, NC, 1982, pp. 174.