Cytogenetic Studies in Humans After Short-Term Exposure to Ethylene Dibromide

Kyle Steenland, M.S.; Anthony Carrano, Ph.D.; David Clapp, Ph.D.; Jennifer Ratcliffe, Ph.D.; Linda Ashworth, B.S.; and Ted Meinhardt, Ph.D.

Ethylene dibromide (EDB) has been shown to increase sister chromatid exchange in animal cells in vitro, but its cytogenetic effects in humans have not been previously studied. A solution containing EDB is used in the summer months in Colorado to spray felled pine trees to kill pine beetles. We have assessed the frequencies of sister chromatid exchanges and chromosomal aberrations in the peripheral blood lymphocytes of 14 sprayers both before and after exposure. Six nonexposed individuals also were tested. Full-shift personal breathing-zone air samples indicated that the sprayers were exposed to an average of 60 ppb of EDB, as an eight-hour time weighted average. The range of exposure was from 5 to 281 ppb. Workers sprayed EDB for only five to 26 days during the summer, with an average of 14 days. After adjusting for smoking and the use of prescription medicine, there was no statistically significant difference between the frequencies of either sister chromatid exchange or chromosomal aberrations before and after spraying.

Ethylene dibromide (EDB) has been used in the United States as a lead scavenger in gasoline and as an active component of some 100 pesticides used as soil, fruit, and grain fumigants.[1,2] EDB has been shown to be strongly mutagenic and carcinogenic in a number of bacterial and mammalian tests.[1-8] A cytogenetic analysis of EDB, performed with Chinese hamster cells in culture, was positive for the induction of both sister chromatid exchanges (SCEs) and chromosomal aberrations (CA).[9] Two epidemiologic studies of humans have been inconclusive for increased cancer risk, owing to mixed exposures and small sample size.[10,11]

In 1983, the National Institute for Occupational Safety and Health (NIOSH) was asked to conduct a cytogenetic study (SCE and CA) for the state of Colorado to evaluate the use of EDB by 14 workers who sprayed felled pine trees infested with pine beatles.

The recommended NIOSH standard for EDB is 45 ppb for an eight-hour time-weighted average (TWA), with a maximum of 130 ppb during any 15-minute period.[1] The current OSHA standard for EDB is 20,000 ppb, but OSHA is currently proposing a reduction to a 100-ppb TWA and a maximum of 500 ppb in any 15-minute period.[12] The 14 sprayers in this study were exposed to airborne EDB at levels around the NIOSH recommended standard.

Methods

The design of this study was longitudinal, with each sprayer serving as his or her own control. Blood samples were drawn from 14 sprayers in May 1983 and July 1983, before and after exposure. Six nonexposed controls were also sampled at the same time points as were the sprayers. The average age of the sprayers was 25 years (range, 20 to 32 years); two of the sprayers were female.

The methods of measuring EDB exposure have been described in detail elsewhere.[13] Both full-shift and short-term personal samples were collected using charcoal tubes. To ensure that as little loss of EDB as possible occurred during storage, samples were shipped on dry ice, stored in a freezer, and analyzed within one week. Gas chromatographic analysis with electron capture detector was done according to NIOSH analytical method #1008.[14]

The procedures for the blood culture and analysis of SCEs in peripheral lymphocytes have been described.[15] The protocols for both end points are in accordance with the methods of analysis suggested for human studies in the Environmental Protection Agency Gene-Tox[16] and the March of Dimes[17] panels.

Coded blood samples were transported by courier on a commercial airline, and cultures were initiated within 24 hours of blood collection. This shipment procedure was

From the National Institute for Occupational Safety and Health, Cincinnati (Mr. Steenland and Drs. Clapp, Ratcliffe, and Meinhardt); and the Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, Calif. (Dr. Carrano and Ms. Ashworth).

Address correspondence to National Institute for Occupational Safety and Health, 4676 Columbia Pkwy., Cincinnati, OH 45226 (Mr. Steenland).

previously shown not to increase SCEs or CAs (A.C., unpublished results of studies conducted in 1980-1985.

Selection of culture medium, serum type, and concentration and duration of culture was based on historical experience that has been reported elsewhere.[18] Procedures were standardized for all cultures. A concentration of 25 μM bromodeoxyuridine was used for the aberration study to discriminate first division cells for analysis, and a 100 μM concentration was used for the SCE study. Seven cultures were established per person; four were used for the aberration study (52- and 72-hour growth), and three for the SCE analysis (72- and 96-hour growth).

At the termination of the culture, cells were harvested, fixed, and dropped on microscopic slides for scoring. Slides were then stained for the analysis of SCE and CA according to a fluorescence-plus-Giemsa method.[19,20]

For both the CA and SCE analysis, slides were coded and scored "blind" by trained individuals. For the SCE analysis, data were reported on a per chromosome basis but the SCE frequency was then normalized to 46 chromosomes per cell. Cells with less than 43 chromosomes were not scored. Eighty cells were scored per person.

For the CA analysis, cells with 46 centromeres were scored, and 200 cells were scored per person. Aberrations were scored by specific type per cell as recommended in the above reports[16,17] and by Savage[21]. Gaps (defined as discontinuities along the chromosome that are less than the width of a chromatid and are not displaced) were tabulated but not included as deletions in the analysis. A summary measure also used was the percentage of abnormal cells, i.e., the percentage of cells containing one or more aberration.

Crude data were analyzed using paired *t* tests. Under the null hypothesis, there would be no increase in either SCE or CA after exposure. To account for significant confounding variables, a standard statistical program (Biomedical Computer Programs, program 2V) was used to carry out an analysis of variance for repeated measures.[22]

Analyses were also conducted using the number of high-frequency cells as the dependent variable instead of mean SCE per chromosome.[18] Here, high-frequency cells are defined as cells in which the frequency of SCE per chromosome exceeded the 95% tolerance levels for the mean SCE per chromosome for all the cells of all nonexposed nonsmokers.

Results

Exposures — EDB represented 4% (by volume) of an emulsion that was usually sprayed onto piles of pine logs. Exposure occurred via both dermal absorption and inhalation; the authors were unable to quantify dermal absorption.

Forty-four full-shift personal samples, taken in the breathing zone, indicated an average eight-hour TWA of 60 ppb, with a range of 5 to 281 ppb.[11] Nineteen short-term samples, taken over four to 15 minutes in the breathing zone during times of peak exposures, averaged 463 ppb, with a range of 8 to 2,165 ppb.

The duration of exposure to EDB was quite short; the 14 individuals sprayed EDB for an average of only two weeks during the summer, with a range of five to 26 days.

SCE — As seen in Table 1, the average number of SCEs per chromosome for the 14 exposed sprayers was 0.197 (S.E. = 0.009) before exposure and 0.180 (S.E. = 0.008) after exposure.

A paired *t* test for the exposed individuals indicated a significant *decrease* in SCE after exposure ($p = .02$). Further analysis, stratifying on smoking, indicated that a significant decrease occurred only in the smokers.

The mean number of SCEs for the six nonexposed controls sampled before spraying was 0.185 (S.E. = 0.007), while afterward it was 0.187 (S.E. = 0.006). A paired *t* test showed no difference between samples taken at the beginning and end of summer ($p = .77$).

An analysis of variance indicated that smoking (six sprayers were smokers) and the taking of prescription medicine (two sprayers had taken antibiotics for recurrent infections) were the only potential confounders of significance. When these variables were taken into account, there was no significant difference in SCE frequency for the 14 sprayers before and after exposure ($p = .23$). None of the interaction terms was significant. Table 2 shows these results.

Duration of exposure, a continuous variable ranging from five to 26 days, had no statistically significant effect on the level of SCE. In addition, when the number of high-frequency cells was used as the dependent variable instead of the frequency of SCE, there was no change in the results.

CA — The data on CA for the exposed individuals are shown in Table 3. A paired *t* test for the 14 exposed individuals indicated no significant difference in the percentage of abnormal cells before and after exposure ($p = .49$). Similarily, a paired *t* test for the six nonexposed controls indicated no difference in the percentage of abnormal cells taken at the beginning and end of the summer ($p = .36$).

In the analysis of variance done to correct for confounding factors, no potential confounding factor (e.g., age, exposure to x-rays, flu, or drugs) was significant. There was no effect of duration of exposure on the percentage of abnormal cells. Furthermore, there was no significant difference before and after exposure when the data were analyzed according to the frequency of total aberrations per cell or according to each aberration type (Table 3).

Discussion

This study found no significant cytogenetic effects due to exposure to EDB for 14 individuals, as determined by

Table 1 — Mean Frequency of SCE per Chromosome in the Exposed Group*

Smoking Category (N)	May 1983 Before Exposure	July 1983 After Exposure
Nonsmoker (8)	0.177 (S.E. = 0.009)	0.168 (S.E. = 0.007)
Smoker (6)	0.223 (S.E. = 0.009)	0.197 (S.E. = 0.013)
Both (14)	0.197 (S.E. = 0.009)	0.185 (S.E. = 0.008)

* SCE expressed as number per chromosome, based on 80 cells scored per person; S.E.s are for unpaired data

Table 2 — Analysis of Variance for SCE, 14 Sprayers*

Effect	*df*	SS	MS	*F*	*P*
Smoking	1	0.00989	0.00989	14.22	.004
Prescription medicine	1	0.00388	0.00388	5.58	.039
Exposure (before vs post)	1	0.00033	0.00033	1.61	.233
Smoking x prescription medicine	1	0.00212	0.00212	3.04	.117
Exposure x smoking	1	0.00027	0.00027	1.32	.277
Exposure x prescription medicine	1	0.00043	0.00043	2.10	.178
Error	10	0.00207	0.00021	...	...

*Results from BMDP2V, analysis of variance for repeated measures; SS indicates sums of squares and MS indicates mean square

Table 3 — Mean and S.E.s of Chromosomal Aberrations for 14 Exposed Individuals Before/After Exposure to EDB*

	% Abnormal Cells May/July		Abberations per Cell May/July		Chromatid-type per Cell Deletions May/July		Chromatid-type per Cell Exchanges May/July		Chromosome-type per Cell Deletions May/July		Chromosome-type per Cell Exchanges May/July	
Mean	1.2	1.0	0.012	0.011	0.006	0.004	0.001	0.001	0.004	0.004	0.001	0.003
S.E.	0.05	0.05	0.001	0.001	0.0004	0.0003	0.0001	0.0001	0.0004	0.0003	0.0001	0.0004

*Under each column heading, the two values given correspond to the values before and after exposure; 200 cells were scored per person

comparing levels of SCE and CA before and after exposure.

In the analysis for SCE, a significant effect was observed for smoking and the use of prescription medicine. Smoking is known to increase the frequency of SCE.[15,23,24]

The use of six nonexposed controls was an attempt to control for laboratory variation between the first and second blood samples. The lack of significant differences in the nonexposed controls and sampled at the same two points in time as were the exposed caused the authors to be confident that experimental variation was minimal.

The authors did observe a decrease in SCE after exposure for the 14 sprayers, although that decrease was not statistically significant after taking into account smoking and the use of prescription medicine. No such decrease was observed for the six nonexposed controls. The decrease over the summer among the sprayers was seen primarily in smokers and may have been due to a decrease in smoking. Of the six sprayers here defined as smokers, one was actually an ex-smoker who had quit five months before. Five others were cigarette smokers. Of these, three indicated a decrease in cigarette consumption over the course of the summer. This is not surprising, since the work was physically demanding outside labor. All three persons who indicated a decrease in smoking, as well as the individual who had recently quit smoking, showed a decrease in SCE over the summer.

Small sample size is not likely to account for the failure to observe any increase in cytogenetic changes after exposure. First, the data showed no suggestion of any such increase. Second, using the sample size calculations published by Carrano et al,[25] for unmatched data, the present study would have had approximately 95% power to detect a 25% increase in SCE, with an alpha of 0.05.

The negative results of this study are reassuring, but obviously cannot be generalized to cohorts with higher and longer exposures.

Acknowledgment

A portion of this work was performed under NIOSH-IA-84-21 and under the auspices of the U.S. Department of Energy (contract W-7405-ENG-48) at Lawrence Livermore National Laboratory.

References

1. NIOSH, Testimony on OSHA proposed rule on occupational exposure to ethylene dibromide by Dr. J. Millar, Feb. 9, 1984, Cincinnati.

2. Ethylene dibromide (revised), in Current Intelligence Bulletin 37, publication 82-105. Cincinnati: National Institute for Occupational Safety and Health, 1982.

3. Olson WA, Habermann RT, Weisburger EK, et al: Induction of stomach cancer in rats and mice by halogenated aliphatic fumigants. *JNCI* 55:1993–1995, 1973.

4. Powers M, Voelker R, Page N, et al: Carcinogenicity of ethylene dibromide and 1,2-dibromo-3-chloropropane after oral administration in rats and mice. *Toxicol Appl Pharmacol* 33:171, 1975.

5. Van Duuren B, Goldschmidt B, Lowewengart A, et al: Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *JNCI* 63:1433–1439, 1979.

6. Carcinogenesis Bioassay of 1,2-Dibromoethane (Inhalation Study), publication (NIH) 81-1766. Bethesda, Md.: National Cancer Institute, 1981.

7. Ethylene dibromide and disulfiram toxic interaction, in Current Intelligence Bulletin No. 23, publication 78-145. Cincinnati: National Institute for Occupational Safety and Health, 1978.

8. Wang L, Winston J, Hong C, et al: Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. *Toxicol Appl Pharacol* 63:155–165, 1982.

9. Tezuka H, Ando N, Suzuki M, et al: Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. *Mutat Res* 78:177–191, 1980.

10. Ter Haar G: An investigation of possible sterility and health effects from exposure to ethylene dibromide, in Banbury Report 5 — Ethylene Dichloride: A Potential Health Risk? Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, 1980, pp 167–188.

11. Ott M, Scharnweber H, Langner R: Mortality experience of 161 employees exposed to EDB. *Br J Ind Med* 37:163–168, 1980.

12. Notice of proposed rulemaking: Occupational exposure to ethylene dibromide. *Federal Register* 48:45956–46003, Oct. 7, 1983.

13. Clapp D: Industrial Hygiene Walk-through Survey Report, Colorado Forest Service Workers. Cincinnati: Industrial Hygiene Section, Industry-Wide Studies Branch, NIOSH, March, 1984.

14. Analytical Method #1008, '1,2 Dibromoethane,' in NIOSH Manual of Analytical Methods. ed. 3. NIOSH 84-100. US Govt Printing Office, 1984, vol. 1, pp 1008-1, 1008-4.
15. Carrano A: Sister-chromatid exchange as an indicator of human exposure, in Banbury Report 13: Indicators of Genotoxic Exposure, Cold Spring Harbor, N.Y. Cold Spring Harbor Laboratory, 1982, pp 307–318.
16. Preston R, Bender M, Brewen J, et al: Mammalian in vivo and in vitro cytogenetic assays: A report of the U.S. EPA's Gene-Tox program. *Mutat Res* **87**:143–188, 1981.
17. Bloom A (Ed.), Guidelines for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards. March of Dimes, 1981.
18. Carrano A, Moore D: The rational and methodology for quantifying sister chromatid exchange in humans, in Heddle J (Ed.): Mutagenicity: New Horizons in Genetic Toxicology. New York: Academic Press Inc., 1982, pp 267–304.
19. Perry P, Wolff S: New Giemsa method for the differential staining of sister chromatids. *Nature* **251**:156–158, 1974.
20. Minkler J, Stetka D Jr, Carrano A: An ultraviolet light source for consistent differential staining of sister chromatids. *Stain Technol* **53**:359–360, 1978.
21. Savage J: Classification and relationships of induced chromosomal structural changes. *J Med Genet* **12**:102–122, 1975.
22. Biomedical Computer Programs, University of California at Los Angeles. Berkeley: University of California Press, 1979.
23. Report of the ad hoc panel on chemical carcinogenesis testing and evaluation of the National Toxicology Program Board of Scientific Counselors. Presented at National Toxicology Program, Chapel Hill, N.C.: Aug 17, 1984.
24. Lambert B, Lindblad A, Holmberg L, et al: The use of sister chromatid exchanges to monitor human populations for exposure to toxicologically harmful agents, in Wolff S (Ed.): Sister Chromatid Exchange. New York: John Wiley & Sons, Inc. 1982, pp 149–182.
25. Carrano A, Minkler J, Stetka D, et al: Variation in the baseline sister chromatid exchange frequency in human lymphocytes. *Environ Mutagen* **2**:325–337, 1980.

Challenge for Physicians

As physicians look forward and respond to the cost and organizational challenge presented, the response to these challenges must be creative and bold. Physicians must respond with the aggressive enthusiasm and effective leadership which has characterized the physician's attack of clinical problems.

If physicians do not respond in such a manner the profession faces the same fate as the master guildsman of 18th century France. In this period some of the finest furniture and decorative arts in the history of man were created. The guildsman created his work only for the pleasure of the aristocracy. It could take a decade to create a single piece of magnificent furniture. If the piece was (sic) not approved by the king it was destroyed. The guildsmen and the aristocracy neglected the needs of an impoverished population. Beauty and creative satisfaction as ends in themselves coupled with insensitivity to poverty and human needs created an explosive circumstance. In 1789 the result was the French Revolution and execution of many of the elite.

We physicians are elite. Our society has subsidized our education, provided freedom, allowed creativity, encouraged productivity, and has eulogized our efforts, to wit, the media coverage of the accomplishments in medicine. Physicians must recognize that these opportunities are exchanged for the responsibility of creating a healthier, longer living population as well as the provision of health services which the population perceives it requires at an affordable cost. Medicine must meet the needs of its fellow citizens and not pursue the personal, intellectual and economic needs of physicians as a primary goal.

— From "President's Column — Today's Physician: Pro-Active, Innovative, Bold" by D.H. Brooks, M.D., in *Bulletin of the Allegheny County Medical Society.*, May 25, 1985.