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In vivo cytogenetic studies on mice exposed to ethylene dibromide

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Summary

The pesticide, ethylene dibromide (EDB), was evaluated with in vivo cytogenetic assays to determine its genotoxicity. CD_1 male mice were exposed to EDB through intraperitoneal injections. Bone marrow cells isolated from femora were analyzed for sister-chromatid exchange (SCE), chromosome aberration and micronucleus formation. The results showed that only certain concentrations of EDB tested caused a slight but significant increase in SCEs and chromosome aberrations. However, these increases were not dose-related. No increase in the polychromatic erythrocytes with micronuclei was observed following EDB exposure. Aslo, EDB did not cause cell-cycle delay in comparison with controls. Thus, it appears that EDB is not an effective genotoxic agent in vivo in mice.

Ethylene dibromide (EDB) is extensively used in antiknock gasoline and in fumigant mixtures. It is also used as a fire extinguisher, chemical intermediate, special solvent (for fats, oils, resins and rubber) and gage fluid. The use of EDB in fumigation mixtures may result in trace amounts of EDB or its degradation products in fumigated material such as fruits, vegetables and food grains (Rannug, 1980). Low concentrations of EDB in ambient air have been found. The main sources of these emissions are from automobiles via evaporation from fuel tanks and carburetors of cars (Fishbein, 1979).

The effects of EDB reported in humans were acute toxicity resulting from inhalation, skin absorption and ingestion. The target organs involved were liver, kidneys and testes (Olmstead, 1960). In addition, upper respiratory irritation and extensive degeneration of the heart as well as painful skin inflammation have been noted by Fanini et al. (1984). Olson et al. (1973) and Powers et al. (1975) have reported EDB-induced squamous cell carcinoma of the stomach in rats and mice. Also, increased tumors in mammary gland, spleen, adrenal gland, liver, kidney and subcutaneous tissue in rats have been shown (Kluwe et al., 1981; Wong et al., 1982).

The mutagenic potential of EDB has been well established in numerous prokaryotic and eukaryotic test systems. It has been shown to induce mutations in mammalian cell cultures (Clive, 1973), insects (Vogel and Chandler, 1974; Kale and Baum, 1981), bacteria (Tezuka et al., 1980; Ames, 1971; Brem et al., 1974), plants (Sparrow et al., 1974), fungi (de Serres and Malling, 1970; Malling, 1969) and rats (Wong et al., 1982). It is also known to

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induce a dose-related sister-chromatid exchanges (SCEs) and chromosomal aberrations in the cell cultures of Chinese hamster lung cells (Tezuka et al., 1980) and SCEs in human lymphocytes (Tucker et al., 1984). Behavioral changes in the progeny of rats treated with EDB have also been reported (Fanini et al., 1984).

Recent studies have called attention to the value of short-term in vivo cytogenetic test systems such as: SCE analysis, chromosome aberration study and micronucleus test (Hollstein et al., 1979; Kram et al., 1979). Although in vivo cytogenetic analysis may not be as amenable to rapid analysis as most of the in vitro test systems, it has an important advantage over these systems. Owing to the metabolic activation, detoxification, transportation, and distribution of chemicals that occur in the intact animal, results from the in vivo study provide more relevant information regarding the potential health hazard of chemicals to human population. The present study was designed to determine the in vivo genotoxicity of EDB, using cytogenetic tests such as SCE, chromosome aberration, and micronucleus formation in mice bone marrow cells.

Materials and methods

Chemicals and animals. Ethylene dibromide, purchased from Sigma Chemical Company, dissolved and diluted in corn oil (Mazola, commercial) was injected intraperitonealy (i.p.) into CD_1 male mice. The 8–10 weeks old mice, weighing 25–30 g, were purchased from Charles River Breeding Laboratories, Inc., Kingston, NJ, and raised under normal room temperature and relative humidity conditions for 2–4 weeks. Cyclophosphamide (CPA, Sigma Chemical Company) was used as positive control for SCE assay and triethylenemelamine (TEM, Sigma Chemical Company) was used as positive control in chromosomal aberration and micronucleus tests.

Assay systems

(1) Sister-chromatid exchange assay

The positive control chemical, CPA (10 mg/kg body weight), the negative control vehicle, corn oil, and the test compound, EDB in different dosages were injected through i.p. into 2–3 experimental animals for each treatment. The paraffin coated 5-bromo-2'-deoxyuridine (BrdU) tablets (50 mg, Boehringer Mannhein Biochemicals, Indianapolis, IN) (McFee et al., 1983) were inserted under the skin on the flank 1 h after injection of the test articles. A commercial diet (Purina Certified Laboratory Chow, rat and mouse blox) and water were given ad libitum until the animals were sacrificed. Based on previous LD_{50} studies (RTECS, 1984), 5 different concentrations ranging from 42 to 420 mg/kg were selected for the preliminary toxicity studies. 5 animals were used for each concentration tested. The 2 highest concentrations (420 and 336 mg/kg) caused 100% and 80% mortality, respectively. The remaining 3 concentrations (168, 84 and 42 mg/kg) did not show any toxicity and were thus chosen for cytogenetic studies.

Animals were sacrificed by cervical dislocation after 3 h colchicine (4 mg/kg, i.p.) treatment. For bone-marrow preparations, both femora were removed non-aseptically and the adherent muscle removed. Each femor was cleaned with pure ethyl alcohol and the head cut off with scissors. The marrow was flushed out with physiological saline into a centrifuge tube, followed by centrifugation at $285 \times g$ for 5 min. The supernatant was removed and the pellet resuspended with hypotonic (0.075 M KCl) solution for 20 min at 37°C and recentrifuged. The cells were fixed in two changes of fixative, 10 min each, in methanol–acetic acid (3 : 1). The cells were resuspended in approximately 0.5 ml of fixative dropped on to precleaned, chilled wet slides and air dried for 24 h.

A modification of the fluorescence plus Giemsa method of Perry and Wolff (1974) and Goto et al. (1978) was used for staining. Slides were stained with Hoechst 33258 (5 μg/ml) in phosphate buffer (pH 6.8) for 15 min and were then exposed with coverslips on to 'black' light (UV light, long wave, 360 nm) at 55–60°C for 7 min at a distance of 1 cm. Sorenson's phosphate buffer, pH 6.8, was used to float coverslips on the slides. The coverslips were removed and the slides rinsed in distilled water and stained with 5% Giemsa, in Sorenson's buffer, for 10–15 min. The slides were coded and 50 cells per dose were scored from each animal.

To evaluate the cellular proliferation, the frequencies of the first, second and third generation

metaphases were determined in 100 consecutive metaphase cells from each animal. Cells that had replicated their DNA exclusively before the implantation of BrdU tablet could not be distinguished from first metaphase cells, and cells that had gone through more than 3 cell cycles were counted as third mitoses. Such cells, however, cannot be expected to occur during an experimental period totalling 26 h. The proliferation rate index (*PRI*) was calculated using the following formula:

$$PRI = \frac{1\,M_1 + 2\,M_2 + 3\,M_3}{100}$$

Where M_1, M_2 and M_3 represent percentages of first, second and third or subsequent division metaphases, respectively.

The results of SCE assay, as well as the *PRI*, were compared to negative control values using Student's '*t*' test.

(2) Chromosomal aberration assay

Mice were exposed to the same concentrations of EDB as used in sister-chromatid exchange assay. 4 mice were used for each dose and animals were sacrificed by cervical dislocation at 12 h after chemical exposure. The bone marrow was flushed, centrifuged and prepared as described for SCE assay; however, the BrdU pellet was not used and the differential staining procedure was not followed in this case. The slides were coded and 75 metaphases from each animal were scored for chromosomal aberrations. The types of aberrations were classified according to the standard cytogenetic procedures (Preston et al., 1981). The number of aberrations, both including gaps and excluding gaps was compared to the negative control group using a *t*-test, after using a square-root transformation on the counts (Snedecor and Cochran, 1967).

(3) Micronucleus assay

Animals were given the test compound and the positive and negative control compounds through i.p. injection. The animals were sacrificed by cervical dislocation 24 or 48 h after chemical treatment. From the femora, bone marrow was flushed with fetal bovine serum into a 15-ml centrifuge tube and spun at $285 \times g$ for 5 min. The supernatant was removed except for a few drops and the pellet carefully mixed with the remaining serum to yield a fine suspension. One small drop of the suspension was placed on each microscope slide, spread evenly and air-dried. The slides were fixed for 5 min in absolute methanol, air-dried, and then stained with Wright–Giemsa stain according to the procedure of Schalm et al. (1975), with the following modification: phosphate buffer, pH 6.4, was used; 30 drops of stain were placed on each slide and left for 3 min; 30–40 drops of buffer were added and left for 20 min. After rinsing with distilled water and air-drying, slides were placed in xylene for 5 min and mounted with cover glasses using Permount. The incidence of micronucleated (MN) cells per 500 polychromatic erythrocytes (PCE) was determined for each animal. 5 animals were used for each test group. The ratio of normochromatic erythrocytes (NCEs) to PCEs was also calculated to determine the toxic effect of EDB to bone marrow cells. The average PCEs with MN and the average ratio of NCE to PCE from the treated were compared to those from the negative controls using a *t*-test after a square-root transformation.

Results

The data on the induction of SCE in bone marrow cells following in vivo exposure to EDB are presented in Table 1. Among the 3 doses tested, medium and high doses yielded 6.09 and 5.47 SCEs/cell, respectively. These values although not dose-related, were slightly higher than the negative control value (4.24 SCEs/cell) and statistically significant. No significant variation was observed among animals in the same group. The positive control compound, cyclophosphamide, yielded a large increase in SCE levels over those in the control animals.

The data on effect of different concentrations of EDB on cell replication in in vivo are presented in Table 2. The number of first, second, third or subsequent division cells were in close approximation in all treatments and controls. The *PRI* differences between the treated and control animals were not statistically significant.

The results on chromosome aberration assay, following in vivo exposure to EDB, show that only

TABLE 1

SISTER-CHROMATID EXCHANGE IN BONE MARROW CELLS OF MICE FOLLOWING IN VIVO EXPOSURE TO ETHYLENE DIBROMIDE

Ethylene dibromide (mg/kg)	Animal	Metaphases scored	SCEs/ metaphase [c]	Mean ± SD (per animal)	Range (per cell)
Negative control [a]	1	50	4.08	4.24 ± 0.23	0–11
	2	50	4.40		1–9
Positive control [b]	1	50	25.78	25.66 ± 0.17	11–44
	2	50	25.54		15–40
42	1	50	5.50	5.03 ± 0.66	1–12
	2	50	4.56		1–9
84	1	50	6.48	6.09 ± 0.39 [d]	1–13
	2	50	6.08		1–12
	3	50	5.70		2–13
168	1	50	5.78	5.47 ± 0.27 [e]	1–12
	2	50	5.34		1–14
	3	50	5.28		1–11

[a] Corn oil (vehicle control).
[b] Cyclophosphamide, 10 mg/kg body weight.
[c] The mice in each group did not differ significantly in their SCE level.
[d] Different from negative control ($p < 0.01$).
[e] Different from negative control ($p < 0.05$).

TABLE 2

CELL CYCLE KINETICS IN BONE MARROW CELLS OF MICE FOLLOWING IN VIVO EXPOSURE TO ETHYLENE DIBROMIDE [a]

Ethylene dibromide (mg/kg)	Animal	Percent 1st division cells [b]	Percent 2nd division cells	Percent 3rd and subsequent division cells	Proliferation rate index (*PRI*)	Mean *PRI*
Negative control [c]	1	23	60	17	1.94	1.98
	2	15	69	16	2.01	
Positive control [d]	1	26	67	7	1.81	1.88
	2	24	57	19	1.95	
42	1	20	70	10	1.90	1.94
	2	7	89	4	1.97	
84	1	25	71	4	1.79	1.90
	2	15	70	15	2.00	
	3	22	65	13	1.91	
168	1	37	53	10	1.73	1.82
	2	32	57	11	1.79	
	3	20	65	15	1.95	

[a] 100 consecutive metaphase cells were scored for the number of cell cycles traversed during BrdU-implantation period in each animal.
[b] First-division cells represent cells that have undergone no or one 'S' phase in the presence of BrdU.
[c] Corn oil (vehicle control).
[d] Cyclophosphamide, 10 mg/kg body weight.

TABLE 3

CHROMOSOME ABERRATIONS IN BONE MARROW CELLS OF MICE FOLLOWING IN VIVO EXPOSURE TO ETHYLENE DIBROMIDE

Ethylene dibromide (mg/kg) [a]	Aberrant cells		Chromosome aberrations [b]								
	− Gaps Mean ± S.D. (per animal)	+ Gaps Mean ± S.D. (per animal)	G	B	F	TD	R	TR	QR	CR	>
Negative control [c]	0.25 ± 0.50	1.00 ± 0.82	3	1							
Positive control [d]	20.25 ± 3.50	22.00 ± 4.24	27	50	36	7	16	6	5	22	43
42	1.00 ± 0.00 [e]	2.25 ± 0.50	5	1	3						
84	1.00 ± 1.15	1.75 ± 1.26	3	2	2						
168	0.75 ± 0.50	1.50 ± 0.58	3	2		1					

[a] 4 animals were used per treatment group and 75 cells were scored per animal (except positive control, wherein 114 cells were scored in total due to lack of cells).

[b] Abbreviations used: G, gaps; B, breaks; F, fragments and acentric fragments; TD, chromatid deletions; R, rings; TR, triradials; QR, quadriradials; CR, complex rearrangements; >, more than 10 aberrations.

[c] Corn oil (vehicle control).

[d] Triethylenemelamine, 2 mg/kg body weight.

[e] Different from negative control ($p < 0.05$).

the group exposed to 42 mg/kg of EDB had a slight but significant increase in aberrations (only excluding gaps) over the negative control group ($p < 0.05$, Table 3). The types of aberrations noted were gaps, breaks and fragments. The positive control compound, TEM (2 mg/kg) induced chro-

TABLE 4

MICRONUCLEI FORMATION IN BONE MARROW CELLS OF MICE FOLLOWING IN VIVO EXPOSURE TO ETHYLENE DIBROMIDE

Ethylene dibromide (mg/kg)	Harvest interval (h) [a]	PCE with MN per animal [b]		PCE with MN (%)	NCE/PCE per animal	
		Mean ± S.D.	Range		Mean ± S.D.	Range
Negative control [c]	24	1.20 ± 0.84	0–2	0.24	1.08 ± 0.34	0.73–1.54
	48	1.20 ± 0.84	0–2	0.24	0.97 ± 0.21	0.77–1.25
Positive control [d]	24	31.00 ± 4.69	24–36	6.20	2.88 ± 0.57	2.30–3.60
42	24	1.40 ± 0.55	1–2	0.28	0.79 ± 0.20	0.58–0.99
	48	1.00 ± 0.71	0–2	0.20	1.28 ± 0.43	0.65–1.86
84	24	1.60 ± 1.14	0–3	0.32	1.09 ± 0.08	1.00–1.20
	48	1.40 ± 0.55	1–2	0.28	1.62 ± 0.65	0.67–2.25
168	24	1.20 ± 0.45	1–2	0.24	1.32 ± 0.52	0.82–2.13
	48	0.75 ± 0.50	0–1	0.15	1.35 ± 0.51	0.67–1.90

Abbreviations: PCE, polychromatic erythrocytes; MN, micronuclei; NCE, normochromatic erythrocytes.

[a] 5 mice were used for each treatment group except at 168 mg/kg at 48 h interval which had only 4 due to the death of 1 mouse.

[b] 500 PCEs scored per animal.

[c] Corn oil (vehicle control).

[d] Triethylenemelamine, 2 mg/kg body weight.

mosome aberrations in approximately 75% of cells (Table 3).

The results on micronucleus assay in bone marrow cells are shown in Table 4. The number of PCE with micronuclei were not significantly different between the controls and treated cells. This was true for both 24-h and 48-h exposures. The ratio of NCE to PCE was similar between the control and treated animals. The number of PCEs with micronuclei increased from 1.2 to 31.0 following treatment with TEM.

Discussion

In the in vitro studies EDB has been reported to induce SCEs and chromosome aberrations in CHO cells (Tezuka et al., 1980) and SCEs in human peripheral lymphocytes (Tucker et al., 1984). In the present study only certain concentrations of EDB tested caused a slight but significant increase in SCEs (Table 1) and chromosome aberrations (Table 3). However, these increases were not dose-related. No increase in the number of PCEs with micronuclei was observed following EDB exposure. Although data have not yet been published, Shafik and Legator (1984) have indicated that EDB is not an effective in vivo clastogen in ICR mice.

The reason for the inconsistency in the in vivo and in vitro results is not apparent at this time. A possible explanation for the differences between in vivo and in vitro results may be the distribution of the compound. EDB is known to be absorbed and distributed into the intestine, kidney, liver and plasma at a faster rate than in the bone marrow of animals (NIOSH, 1977). Thus, there may not be enough chemical in the bone marrow to produce significant dose-related positive responses. Therefore, the mortality of the animals at higher doses, 336 and 420 mg/kg may be due to the absorption of chemical in vital organs such as liver, kidney and intestine. It is also possible that EDB is metabolized before it reaches the target cells. A few other chemical compounds such as proflavine, methylene blue and chloropromazine are known to induce SCEs in vitro; however, they do not induce significant dose-related SCEs in vivo in mammalian systems (Speit, 1982).

In addition to examining SCEs, the BrdU-differential staining technique can be utilized to assess the effects of chemicals on cell replication. Cells which have replicated once, 2 or 3 or more times in the presence of BrdU can be unequivocally identified (Schneider et al., 1976; Tice et al., 1979). If replication were inhibited, one would find a decrease in the frequency of second-replication cycle cells and an increase in the frequency of first-replication cycle cells. In the EDB-treated animals, the frequencies of the first, second, third and subsequent mitoses fail to show that the proliferation of the cells analyzed in the bone-marrow was delayed in comparison with the controls. Cyclopospham ide, a positive control, led to a pronounced increase in the SCE frequencies without affecting the proliferation of cells. In the micronucleus assay, the ratio of NCE to PCE did not differ significantly between experimentals and the controls, indicating a nontoxic effect of EDB in the bone marrow cells.

Lack of dose-response in the SCE and chromosomal aberration assays and negative results in the micronucleus test following EDB treatment indicate that EDB is not an effective genotoxic agent in vivo in mice. However, to properly evaluate its potential genetic and carcinogenic hazards to the exposed population, chronic studies may have to be carried out. Also, the genetic end-points other than those used in this study may have to be included.

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References

Ames, B.N. (1971) The detection of chemical mutagens with enteric bacteria, in: A. Hollaender (Ed.), Chemical Mutagens — Principles and Methods for Their Detection, Vol. 1, Plenum, New York, pp. 267–282.

Brem, H., A.B. Stein and H.S. Rosenkranz (1974) The mutagenicity and DNA-modifying effect of haloalkanes, Cancer Res., 34, 2576–2579.

Clive, D. (1973) Recent developments with the L5178Y TK heterozygote mutagen assay system, Environ, Health Perspect., 6, 119–125.

de Serres, F.J., and H.V. Malling (1970) Genetic analysis of *ad-3* mutants of *Neurospora crassa* induced by ethylene

dibromide — A commonly used pesticide, Newsl. Environ. Mutagen. Soc., 3, 36–37.

Fanini, D., M.S. Legator and P.M. Adams (1984) Effects of paternal ethylene dibromide exposure on F_1 generation behavior in the rat, Mutation Res., 139, 133–138.

Fishbein, L. (1979) Alkane halides, halogenated alkanols and halogenated ethers, in: L. Fishbein (Ed.), Potential Industrial Carcinogens and Mutagens, Elsevier, Amsterdam, pp. 240–265.

Goto, K., S. Maeda, Y. Kano and T. Sugiyama (1978) Factors involved in differential Giemsa-staining of sister chromatids, chromosoma, 66, 351–359.

Hollstein, M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979) Short-term tests for carcinogens and mutagens, Mutation Res., 65, 133–226.

Kale, P.G., and J.W. Baum (1981) Sensitivity of *Drosophila melanogaster* to low concentrations of gaseous mutagens: 3. Dose–rate effects, Environ. Mutagen., 3, 65–70.

Kluwe, M., R. McNish and J.B. Hook (1981) Acute nephrotoxicities and hepatotoxicities of 2-dibromo-3-chloropropane and 1,2-dibromoethane in male and female F344 rats, Toxicol. Lett., 9, 317–321.

Kram, D., E.L. Schneider, G.C. Senula and Y. Nakanishi (1979) Spontaneous and mitomycin-C induced sister-chromatid exchanges: Comparison of in vivo and in vitro systems, Mutation Res., 60, 339–347.

Malling, H.V. (1969) Ethylene dibromide: A potent pesticide with high mutagenic activity, Genetics, 61, 39.

McFee, A.F., K.W. Lowe and J.R. San Sebastian (1983) Improved sister-chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice, Mutation Res., 119, 83–88.

NIOSH (National Institute for Occupational Safety and Health), Criteria for recommended standard, occupational exposure to ethylene dibromide, DHEW (NIOSH) Publ. No. 77-221 (1977), U.S. Government Printing Office, Washington, D.C.

Olmstead, E.V. (1960) Pathological changes in ethylene dibromide poisoning, AMA Arch. Ind. Hyg. Occup. Med., 21, 45–49.

Olson, W.A., R.T. Haberman, E.K. Weisburger, J.M. Ward and J.H. Weisburger (1973) Induction of stomach cancer in rats and mice by halogenated aliphatic fumigants, J. Natl. Cancer Inst., 51, 1993–1995.

Perry, P., and S. Wolff (1974) New Giemsa method for the differential staining of sister chromatids, Nature (London), 251, 156–158.

Powers, M.B., R.W. Voelker, N.P. Page, E.K. Weisburger and H.F. Kraybill (1975) Carcinogenicity of ethylene dibromide (EDB) and 1, 2-dibromo-3-cyclopropane (DBCP) after oral administration in rats and mice, Toxicol. Appl. Pharmacol., 33, 171–172.

Preston, R.J., W. Au, M.A. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolff and J.S. Wassom (1981) Mammalian in vivo and in vitro cytogenetic assays: A report of the U.S. EPA's Gene-Tox Program, Mutation Res., 87, 143–188.

Rannug, U. (1980) Genotoxic effects of 1,2-dibromoethane and 1,2-dichloroethane, Mutation Res., 76, 269–295.

RTECS (1984) Registry of Toxic Effects of Chemical Substances (Data base), Cincinnati, OH: National Institute for Occupational Safety and Health, RTECS Citation KH9275000.

Schalm, D.W., N.C. Jain, and E.J. Carrol (1975) Veterinary Hematology, 3rd edn., Lea and Febiger, Philadelphia, p. 29.

Schneider, E.L., J.R. Chaillet and R.R. Tice (1976) In vivo BudR labelling of mammalian chromosomes, Exp. Cell Res., 100, 396–399.

Shafik, H.M., and M.S. Legator (1984) In vivo mutagenicity studies in mice following ethylene dibromide exposure, Environ. Mutagen., 6, Abstract 394–395.

Snedecor, G.W., and W.G. Cochran (1967) Statistical Methods, 6th edn., The Iowa State University Press, Ames, IO, pp. 325–327.

Sparrow, A.H., L.A. Schairer and R. Villalobos-Pietrini (1974) Comparison of somatic mutation rates induced in Tradescantia by chemical and physical mutagens, Mutation Res., 26, 265–276.

Speit, G. (1982) Intercalating substances do not induce sister-chromatid exchanges (SCEs) in vivo, Mutation Res., 104, 261–266.

Tezuka, H., N. Ando, R. Suzuki, M. Terahata, M. Moriya and Y. Shirasu (1980) Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays, Mutation Res., 78, 177–191.

Tice, R.R., E.L. Schneider and J.M. Rary (1976) The utilization of bromodeoxyuridin incorporation into DNA for the analysis of cellular kinetics, Exp. Cell Res., 102, 232–236.

Tucker, J.D., J. Xu, J. Stewart and T. Ong (1984) Detection of sister-chromatid exchanges in human peripheral lymphocytes induced by ethylene dibromide vapor, Mutation Res., 138, 93–98.

Vogel, E., and J.L.R. Chandler (1974) Mutagenicity testing of cyclamate and some pesticides in *Drosophila melanogaster*, Experientia, 30, 621–623.

Wong, L.C.K., J.M. Winston, C.B. Hong and H. Plotnick (1982) Carcinogenicity and toxicity of 1,2-dibromoethane in the rat, Toxicol. Appl. Pharmacol., 63, 155–165.