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Ethylene Dichloride: The Influence of Disulfiram or Ethanol on Oncogenicity, Metabolism, and DNA Covalent Binding in Rats[1]

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Ethylene Dichloride: The Influence of Disulfiram or Ethanol on Oncogenicity, Metabolism, and DNA Covalent Binding in Rats. CHEEVER, K. L., CHOLAKIS, J. M., EL-HAWARI, A. M., KOVATCH, R. M., AND WEISBURGER, E. K. (1990) *Fundam. Appl. Toxicol.* **14**, 243–261. Male and female Sprague–Dawley rats were exposed to 50 ppm ethylene dichloride (EDC) for 7 hr/day, 5 days/week, for 2 years by inhalation. Additional rats were exposed to 50 ppm EDC either with 0.05% disulfiram in the diet or with 5% ethanol in the drinking water. Histopathologic lesions related to the combination of inhaled EDC and dietary disulfiram were observed in the liver, mammary, and testicular tissues of rats. This combined exposure resulted in a significant increase in the incidence of intrahepatic bile duct cholangiomas in both male and female rats. Male rats exposed to both EDC and disulfiram also had an increased incidence of subcutaneous fibromas, neoplastic nodules, and interstitial cell tumors in the testes. The female rats exposed to EDC and disulfiram also had a higher incidence of mammary adenocarcinomas. No significant increase in the number of any tumor type was observed in rats exposed to only EDC, disulfiram, or ethanol. Similarly, no significant increase in the number of tumors was observed in rats exposed to inhaled EDC and ethanol in water. At the end of the 2-year period animals from each group were evaluated for EDC metabolism and DNA binding. Blood levels of EDC at the end of a 7-hr exposure period were significantly higher for rats exposed to both EDC and disulfiram than for rats exposed to EDC alone. In addition, the elimination of a single oral dose of radiolabeled EDC was affected. The urinary excretion of ^{14}C from control rats was 47 to 55% of the administered dose with 28 to 30% detected as unchanged EDC in the breath. In disulfiram-treated rats, only 35 to 36% of the administered ^{14}C was eliminated in the urine with 41 to 55% as unchanged EDC in the breath. The urinary metabolite HPLC profile was qualitatively unchanged by long-term EDC, disulfiram, or ethanol treatment, either alone or in combination, and consisted primarily of thiodiglycolic acid, thiodiglycolic acid sulfoxide, and chloroacetic acid.

Ethylene dichloride [EDC, 1,2-dichloroethane, CAS Registry No. 107-06-2 (1977)] is widely used in a variety of industrial and commercial applications. EDC is used primarily in chemical synthesis, as a fuel additive, and as an industrial solvent (Drury and Hammons, 1979; NIOSH, 1978a; IARC,

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1979; Gold, 1980; Davidson *et al.*, 1982). The production of EDC in the United States is estimated to range from 3.6 to 4.3 million tons annually (USITC, 1976–1984) and as many as 200,000 workers may be exposed to EDC in the workplace (NIOSH, 1978a,b). EDC has been shown to be absorbed by the inhalation, oral, and dermal routes (Reitz *et al.*, 1980, 1982; Spreafico *et al.*, 1980).

Previous studies in the rat have produced inconsistent results on the carcinogenic effect of EDC. In one study using Osborne-Mendel rats the daily administration of 47 or 95 mg/kg body wt of EDC by gavage for 78 weeks resulted in increased squamous-cell carcinomas of the stomach, subcutaneous fibromas, and hemangiosarcomas in male and mammary adenocarcinomas and fibroadenomas in female rats (Weisburger, 1977; NCI, 1978a). These effects were similar, albeit of lower toxicity, to those reported for ethylene dibromide (EDB) when that analog was administered by the same route (Weisburger, 1977; NCI, 1978b). However, the EDC exposure of Sprague–Dawley rats at concentrations of 150 to 250 ppm by inhalation for 78 weeks resulted in no noticeable increase in the incidence of tumors (Maltoni *et al.*, 1980). Such differences in toxicity could be related to possible alteration of EDC metabolism or rate of elimination in the strain and route of administration studied.

The metabolism of EDC has been reported to include *in vivo* and *in vitro* formation of 2-chloroethanol, chloroacetic acid, and thiodiglycolic acid as well as the cysteine and glutathione (GSH) conjugates (Yllner, 1971; Anders and Livesey, 1980; Guengerich *et al.*, 1980; Reitz *et al.*, 1980, 1982; Sawada *et al.*, 1987). One possible reactive intermediate, 2-chloroacetaldehyde, was mutagenic in the TA 100 bacterial system (McCann *et al.*, 1975), and the mutagenic effects of EDC in the TA 1535 system were increased by addition of rat liver cytosol fraction (Guengerich *et al.*, 1980, 1981; Reitz *et al.*, 1980; Rannug, 1980a,b). Other possible reactive intermediates, *S*-(2-chloroethyl)-L-cysteine and *S*-(2-chloroethyl)-GSH, have been shown to form covalent nucleotide adducts *in vitro* (Guengerich *et al.*, 1980; Reed and Foureman, 1986). The alteration of the metabolism *in vivo* of certain related halogenated hydrocarbons, EDB and vinyl chloride, has been shown to result in significantly increased tumor formation in rats. The carcinogenicity of EDB at 20 ppm, using Sprague–Dawley rats, was potentiated by coadministration of 0.05% disulfiram (DS) in the diet (Plotnick *et al.*, 1979, 1980; Wong *et al.*, 1982). An increase in tumor formation was reported between ethanol (ET) and vinyl chloride (Radike *et al.*, 1977), and similar increases in the toxicity of EDC have been demonstrated (Francovitch *et al.*, 1986; Igwe *et al.*, 1986). One possible explanation for this increased toxicity is that inhibition of the microsomal aldehyde dehydrogenase system, either directly with DS or competitively with ET, would allow either a buildup of the reactive haloacetaldehyde or increased production of *S*-(2-chloroethyl)-L-cysteine and *S*-(2-chloroethyl)-GSH (Hill *et al.*, 1978). The reported similarities in the metabolism of EDB and vinyl chloride (e.g., formation of the mutagenic haloacetaldehyde intermediate) suggested that the ingestion of DS or ET, drugs commonly encountered in the workplace, would influence the metabolism and induction of tumors by EDC. Such chemical interaction is a concept that is too often neglected in the estimation of chemical or industrial causes of cancer (Infante and Pohl, 1988). Thus, the objective of this study was to evaluate in the rat the oncogenicity, metabolism, and DNA covalent binding of EDC at 50 ppm, the current U.S. occupational standard, with or without either DS in the diet or ET in the drinking water.

METHODS

Chemicals. The test compound, EDC (>99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI)[2]

[2] Mention of company or product names is not to be considered an endorsement by the National Institute for Occupational Safety and Health.

and [1,2-U-^{14}C]EDC was synthesized by New England Nuclear (Boston, MA) with a specific activity of 50 mCi/mmol and a radiochemical purity > 99% by gas–liquid chromatography (GC). The radiochemical purity of this radioisotope was verified by high-performance liquid chromatography (HPLC) with radioactivity detection. Commercially available reference compounds of the highest purity corresponding to possible metabolites were obtained. Chloroacetic acid (98%) was purchased from Fisher Scientific Co. (Fairlawn, NJ). Thiodiglycolic acid (97%) and disulfiram (98%) were purchased from Sigma Chemical Co. (St. Louis, MO) and ethanol (95%) was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Thiodiglycolic acid sulfoxide was prepared by reaction of thiodiglycolic acid with 5% hydrogen peroxide in accordance with the method of Reitz *et al.* (1982). Purity and stability studies were conducted on EDC, DS in diet, and ET in water prior to the initiation of the study, and reanalysis of EDC and DS was conducted periodically throughout the study.

Animals. Sprague–Dawley [Crl:CD(SD)BR outbred], cesarean-derived rats, weighing 51 to 75 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Immediately upon receipt, these animals were placed in quarantine and maintained on powdered Purina 5002 rodent chow (Ralston Purina Co., St. Louis, MO) and distilled water *ad libitum*. The animals were housed individually and were maintained in an AALAC-accredited facility throughout the course of the study. The male and female rats utilized for the chronic study were observed for 12 days and were 5.5 to 6 weeks old at the initiation of the study. Additional animals, 4- to 5-month-old male (502 to 522 g) and female (267 to 322) rats from the same source, were used for preliminary studies on EDC blood levels, metabolism, excretion, and covalent binding.

Experimental design. At completion of the quarantine period, six groups of 50 male and 50 female rats were exposed to either 50 ppm EDC or filtered air for 7 hr/day, 5 days/week (except holidays) for 24 months. These groups were housed separately in Hazleton Model 1000 stainless-steel exposure chambers (Hazleton Systems, Inc., Aberdeen, MD) and were maintained on powdered diet and distilled water or were given either DS in the diet or ET in the drinking water at levels previously shown to affect the carcinogenicity of related halogenated compounds (Plotnick *et al.*, 1979, 1980; Radike *et al.*, 1977). The group designations are shown below:

Group	Air	Diet	Water
Control	Filtered	Standard	Distilled
DS	Filtered	0.05% DS	Distilled
ET	Filtered	Standard	5% ET
EDC	50 ppm EDC	Standard	Distilled
EDC/DS	50 ppm EDC	0.05% DS	Distilled
EDC/ET	50 ppm EDC	Standard	5% ET

Water or 5% ET was supplied to the chambers using an automatic watering system obtained from Edstrom Industries (Waterford, WI), except during periods of water consumption determination. The powdered diet was removed during the daily exposure periods, and the 24-hr food and water consumption was determined for each rat periodically throughout the course of the study. Environmental conditions in the chambers were maintained at 23.3 ± 1.5°C with a relative humidity of 50 ± 15% and a 12-hr light–dark cycle was established using fluorescent light. At the end of the 24-month exposure period, subgroups of five male and five female rats were used for the determination of either EDC elimination from the blood, EDC metabolism, or DNA covalent binding. Radiolabeled EDC for use in the metabolism and DNA covalent binding studies was administered by gavage at a dose of 150 mg/kg body wt. The dosing solutions were prepared by mixing appropriate amounts of the radiolabeled EDC with EDC in corn oil to give a concentration of 75 mg/ml. For the metabolism studies, each rat received 115 to 140 μCi/kg body wt, and for the study of DNA covalent binding the administered radioactivity was 241 to 260 μCi/kg body wt.

Animal observation. All groups of rats were examined twice daily for signs of toxicity. Examinations of all rats for palpable masses were conducted prior to the initial exposure to EDC, and at weekly intervals after 4 months. All rats were weighed weekly for the first 8 weeks of the study and monthly thereafter.

Vapor generation, food and water preparation, and analysis. The rats were exposed to EDC in 2.2-m^3-volume stainless-steel and glass chambers. The chambers were operated under dynamic airflow and were maintained at a slight negative pressure relative to the surrounding area. Air supplied to the chambers was filtered through activated charcoal and a HEPA filter prior to use. The 50 ppm EDC test atmospheres were generated by passing compressed air through undiluted EDC at room temperature. Vapors from the flask were diluted to the desired concentration with filtered air, mixed, and distributed to the chambers through Teflon lines. Chambers receiving only filtered air were housed in a separate room. Total chamber airflow was maintained at approximately 400 liters/min (12 air changes/hr). The distribution of EDC within each chamber was found to vary by less than 6%. The nominal concentration of EDC (ratio of the amount of EDC vaporized to the total airflow through the chamber during the exposure period) was determined on a daily basis for each chamber.

The concentration of EDC in each chamber was determined on an hourly basis by GC using an automated sampling system, consisting of computer programmed solenoid actuated sampling loops, in conjunction with a Model 2400 GC (Varian Associates, Palo Alto, CA), fitted with a 1.8-m-long × 2-mm-i.d. nickel column packed with 10% Carbowax 20M-TPA on 80/100-mesh Chromosorb W AW (Supelco, Inc., Bellefonte, PA). The oven temperature was maintained at 70°C and a nitrogen carrier gas flow rate was 30 ml/min. Peaks were detected by flame ionization detector and peak areas were measured

using a Varian Model CDS 111 integrator. The analytical system was checked prior to each exposure with at least one standard of known concentration as well as quality control samples, and the chamber concentrations were adjusted hourly as needed to maintain uniform exposure conditions.

Diet and water preparation and analysis. The preparation of 0.05% DS diet was done by weighing out 30 kg of powdered Purina 5002 diet and transferring this diet to the stainless-steel bowl of a PK twin-shell blender. A portion of this diet was mixed with 15 g of DS in a mortar and the resulting mixture was slowly added to the remaining diet during a 35-min mixing period. After the diet was mixed, random samples were taken for analysis and the remainder was stored for up to 1 month at −32°C until use. Diet samples weighing 10 g were shaken for 30 min with 100 ml of acetonitrile to extract DS (mean recovery 99%). Prior to analysis the extracts were clarified by centrifugation, and a 10-ml aliquot was mixed with 3 ml of an internal standard solution containing 0.75 mg/ml of butyrophenone (Aldrich). Samples were passed through a 0.45-μm-pore-size Acrodisc filter (Gelman Sciences, Ann Arbor, MI). Duplicate aliquots of each sample were analyzed by HPLC using a Model ALC/GPC liquid chromatograph equipped with a Model 6000A pump (Waters Associates, Milford, MA). Samples were introduced by a Waters 710B automatic sample injector module, and eluted DS was detected by a Waters 440 spectrophotometric detector set at 280 nm. Chromatographic separations were carried out using a Waters Z-Module fitted with a 115-mm-long × 8-mm-i.d. radial compression column packed with 10-μm-particle-size Bondapak C18. An isocratic solvent system consisting of methanol:water (75:25) was used and the flow rate was maintained at 3 ml/min. Chromatographic data were stored and processed with a Model 4400 Nelson data system (Nelson Analytical, Inc., Cupertino, CA).

Drinking water containing 5% ET was prepared by mixing 95% ET with distilled water. After the water was mixed, the ET concentrations were determined using a Varian Model 920 GC fitted with a 3.0-m-long × 2-mm-i.d. nickel column packed with 100/120-mesh Chromosorb 105 (Supelco, Inc.). The oven temperature was maintained at 170°C and a helium carrier gas flow rate was 30 ml/min. Peaks were detected using a thermal conductivity detector with the filament current set at 150 mA in accordance with the method of Hillbrom (1971), and chromatographic data were stored and processed with a Model 4400 Nelson data system.

Blood analysis. The clearance of blood EDC was determined for rats from the EDC, EDC/DS, and EDC/ET groups following termination of a typical 7-hr exposure period. Blood samples were taken from the orbital sinus of five male and five female rats from each exposure group and corresponding numbers of control animals at 0.25 and 2 hr after cessation of EDC generation during Week 103 of exposure. Immediately after collection 1-ml blood samples were injected into a 10-ml septum vial containing 3 ml of a 0.2 μg/ml trichloroethylene internal standard solution with 10% citric acid as a preservative. After blood samples were mixed, each was allowed to equilibrate at 40°C for 30 min prior to analysis of 0.1-ml headspace aliquots by GC. The headspace analysis was performed using a Varian Model 3700 GC fitted with a 1.8-m-long × 4-mm-i.d. glass column packed with 20% SP-2100/0.1% Carbowax 20M on 100/120-mesh Supelcoport (Supelco, Inc.). The oven temperature was maintained at 65°C, with a nitrogen carrier gas flow of 70 ml/min. Peaks eluted from the column were detected using an electron capture detector as described by Zuccato *et al.* (1980), and EDC levels were calculated using the EDC:trichloroethylene area ratio linear response curve.

Metabolism. The metabolism of EDC was evaluated for 2-year-old rats from the Control, DS, ET, EDC, EDC/DS, and EDC/ET groups prior to termination of the animals 1 week after cessation of 103 weeks of EDC exposure. DS or ET exposure was continued for DS, ET, EDC/DS, and EDC/ET groups during the 1-week interval prior to administration of radiolabeled EDC. Radiolabeled EDC was administered to three male and three female rats from each group by gavage at a dose of 150 mg/kg body wt. Immediately following the administration of the individual doses, the rats were placed in separate Roth-type glass metabolism cages for collection of exhaled compounds, urine, and feces. The airflow, consisting of dried laboratory air scrubbed to remove carbon dioxide and organic vapors, was maintained through the cages at a rate of 200 ml/min. The emergent air was passed first through activated charcoal to trap unchanged EDC in the exhaled breath (Albrecht *et al.*, 1986) and subsequently through an absorption tower filled with 500 ml of 5 M ethanolamine in 2-methoxyethanol to trap expired $^{14}CO_2$. The charcoal and ethanolamine solutions were changed at 1, 3, 6, 12, and 24 hr. Urine was collected at 6, 12, and 24 hr and feces were taken. The urine and feces samples were frozen at −20°C until analysis. Charcoal samples weighing 1 g were desorbed for 24 hr with 15 ml of Bio-Solv (Beckman Instruments, Fullerton, CA), a toluene-based scintillation fluid. The ^{14}C elimination profile was determined by liquid scintillation spectrometry (LSC) by counting appropriate amounts of charcoal desorbate, urine, or ethanolamine in 10 ml of PGS scintillation cocktail (Amersham). Feces were burned in a sample oxidizer (Tri-Carb Model B306, Packard Instrument Co., Fullerton, CA). The $^{14}CO_2$ from the combustion was absorbed in 6 ml of Carbo-Sorb (Packard) and analyzed in 12 ml Permafluor V (Packard).

The radioactivity in the samples was measured in a refrigerated Model 3255 Tri-Carb liquid scintillation spectrometer (Packard). Counting efficiencies were determined by the external standard method of Horrocks (1974).

Separation and quantification of urinary metabolites. Prior to analyzing the urine samples by HPLC, suitable conditions for the separation of compounds representing reported EDC metabolites were established. Samples

were chromatographed at 0.5 ml/min using the Waters HPLC system equipped with a Bio-Rad 300-mm-long × 4-mm-i.d. stainless-steel column packed with 10-μm-particle-size Aminex HPLX-87H ion exchange resin and were detected by a Waters 440 spectrophotometric detector set at 214 nm. Baseline separation of EDC metabolites was accomplished using an isocratic solvent system consisting of 0.005 N sulfuric acid as described by Reitz *et al.* (1982). Radioactive components from urinary samples were detected and quantified using a Flo-One Model HP radioactivity monitor (Radiomatic Instruments and Chemical Co., Tampa, FL) after the column eluent was mixed 1:3 with Scintiverse LC scintillation fluid (Fisher Chemical Co.).

DNA covalent binding. The hepatic DNA covalent binding in 2-year-old rats was evaluated for Control, DS, ET, EDC, EDC/DS, and EDC/ET groups. Radiolabeled EDC was administered to three male and three female rats from each group by gavage at a dose of 150 mg/kg body wt 10 to 14 days after cessation of 103 weeks of EDC exposure. The animals were anesthetized with ether 6 hr after dosing and euthanized by exsanguination. The livers were immediately removed, rinsed with 0.1 M Tris/0.01 M EDTA, weighed, frozen in liquid nitrogen, and stored at −70°C until isolation of the DNA. For the isolation of hepatic DNA, livers were homogenized in 2 vol of 0.1 M Tris/0.01 M EDTA (pH 7.7) and adjusted to 1.5% with 0.1 vol of 15% SDS. Samples were homogenized at full speed for 15 sec using a Polytron Model PCU-1 homogenizer equipped with a PC-10 probe (Kinematica GmbH, Luzern, Switzerland) prior to incubation for 30 min at 37°C with 100 μg/ml Pronase B (Calbiochem, La Jolla, CA). The samples were extracted successively with 2 vol of water-saturated Kirby's phenol (phenol:*m*-cresol:8-hydroxyquinoline:water, 500:70:0.5:55) and chloroform:isoamyl alcohol (24:1) by shaking for 10 min at 37°C followed by centrifugation at 8500*g* for 30 min. Following this, 5 ml of 5 M NaCl was added to the aqueous layer and DNA was precipitated by the addition of 1.5 vol of cold ethanol. The crude DNA was allowed to stand at −20°C overnight, washed twice with 50 ml of 70% ethanol, and dried under vacuum. The DNA was subsequently redissolved at 2 mg/ml in 0.015 M NaCl:0.0015 M trisodium citrate:0.01 M EDTA buffer. Following the addition of 100 μl of 1 M Tris-HCl (pH 7.4) and 100 μg/ml RNase XII-A (Sigma), which was heated at 90°C for 10 min, the DNA was incubated for 1 hr at 37°C. After incubation the DNA was extracted with an equal volume of Kirby's phenol for removal of any residual protein. DNA was subsequently precipitated by the addition of 1.5 vol of cold ethanol and washed successively with 20-ml volumes of ethanol, ethanol:acetone (1:1), and acetone. The DNA was dried under vacuum and stored at −20°C until analysis. For analysis, hepatic DNA and appropriate calf thymus DNA standards (Sigma) were dissolved in 0.1 M Tris–HCl (pH 7.7). Samples were diluted 1:10 with 1 *N* NH_4OH, and 10-μl aliquots were dried at 60°C and dissolved in 200 μl of a 300 mg/ml 3,5-diaminobenzoic acid solution. The samples were incubated for 30 min at 60°C with 2 ml of 0.6 N perchloric acid. DNA was quantified by fluorescence spectrometry using an Aminco-Bowman Model SPF 500C spectrofluorometer (SLM/Aminco Instruments, Inc., Urbana, IL) set at an excitation wavelength of 420 nm and an emission wavelength of 520 nm as described by Setaro and Morley (1976). Protein levels (<1%) were analyzed spectrophotometrically by reaction with Coomassie brilliant blue G-250 by the method of Bradford (1976). For determination of DNA radioactivity, a 0.5-ml aliquot of each sample was digested by incubation for 30 min at 80°C with 0.2 ml of 35% perchloric acid. The samples were counted in 10 ml of PCS cocktail by liquid scintillation spectrometry.

Gross pathology. Except for rats designated for preliminary studies, all rats were subjected to a complete gross necropsy examination by a veterinary pathologist. At termination rats were anesthetized with carbon dioxide and euthanized by exsanguination. The terminal body weights and tissue weights were recorded for all animals. Representative sections of all major organs and tissues listed below were preserved in neutral, phosphate-buffered 10% formalin fixative. Tissues routinely examined included accessory sex organs, adipose tissue, adrenal glands, aorta, brain (cerebrum, cerebellum, and brain stem), esophagus, eyes, heart, kidneys, large intestine (cecum and colon), larnyx and pharynx, liver, lungs, lymph nodes (thoracic and mesenteric), mammary tissue, nasal cavity and turbinates, ovaries, pancreas, parathyroid, pituitary, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, skull, small intestine (duodenum, ileum, and jejunum), spinal cord, spleen, sternum, vertebral bone and bone marrow, stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus, and any other grossly recognized lesion.

Rats which died or were found in a moribund condition were also subjected to a complete gross examination. Animals found dead outside the normal observation hours were refrigerated until necropsy. As with the animals euthanized at termination, representative sections of all major organ were collected from each animal and preserved in neutral phosphate-buffered formalin.

Histopathology. An extensive set of tissues was processed by rinsing with ethanol and embedded in paraffin. The tissues were trimmed, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin for general morphological examination by light microscopy. The tissues examined included adrenal gland, bone, bone marrow, brain, colon, esophagus, heart, kidney, larynx, liver, lung, lymph node (thoracic and mesenteric), mammary gland, nasal cavity/mucus membrane, ovary, parathyroid, pituitary, pancreas, prostate, salivary gland, skin, small intestine, spleen, stomach (glandular and forestomach), subcutis, testes, thymus, thyroid, trachea, urinary bladder, uterus, as well as all gross lesions and tissue masses. A more limited number of tissues were examined histopathologically from animals found dead or in a

moribund condition. These included, as a minimum, brain, kidney, liver, mammary gland, pancreas, salivary gland, skin, gross lesions, and tissues masses.

Statistical analysis. Absolute and relative organ weights, body weights, food and water consumption, control and test values for EDC blood levels, metabolism, and DNA covalent binding were evaluated by analysis of variance and Dunnett's test (Liu, 1964). Gross pathologic observations were not evaluated statistically. Incidences of mortality and histopathologic observations were evaluated by Fisher's Exact Probability Test (Armitage, 1971). The level of significance chosen was $p < 0.05$.

RESULTS

Test chemical analyses. Chamber atmosphere concentrations were 50.4 ± 0.9 ppm (mean ± SD of daily time weighted average concentrations) for the target concentration of 50 ppm EDC. The concentrations of DS and ET were determined for each lot of diet or drinking water used. The dietary concentrations of DS were 0.049 ± 0.001% and the ET concentrations in drinking water were 5.07 ± 0.03% (mean ± SD). Thus the analytical concentrations for chemical exposures were within 2% of the intended target concentration for each exposure group. In addition, the analytical results for the chamber EDC concentrations were in close agreement (within 2%) of the nominal concentrations indicating minimal losses or decomposition of the test material during vaporization.

Body weights, and food and water consumption. A significant decrease in the body weights was observed for both male and female rats in the DS and EDC/DS exposure groups throughout the study. This effect, resulting from an initial depression of body weight upon exposure to the 0.05% DS diet, appeared to be a result of an initial DS avoidance, and the body weights for these groups paralleled those of the other groups throughout the study. Body weights of rats in the EDC/DS exposure groups were statistically different from their respective controls at termination of the study (Table 3). Body weights of rats in the other exposure groups were not statistically different from their respective controls.

The group mean 24-hr food consumption for male and female rats in each exposure group is shown in Table 1. On the basis of these values, the daily intake of DS was estimated to range from 10.0 to 10.6 mg/kg body wt for male and 7.0 to 7.1 mg/kg body wt for female rats. A consistent but not statistically significant decrease in food intake, ranging from −15 to −21% with respect to controls, was noted for DS- or ET-treated animals throughout the 24-month period. However, the rats exposed to ET showed no corresponding decrease in body weights, an observation which may be explained by increased caloric intake by those animals. Significant changes in the 24-hr group mean water consumption occurred during the course of the study for EDC/ET- or EDC/DS-treated male rats (Table 2). On the basis of these values, the daily intake of ET was estimated to range from 2.4 to 2.6 g/kg body wt for male and 2.3 to 2.4 g/kg body wt for female rats.

Mortality and observations. The mortality rates for the various groups of male and female rats showed no significant difference in the survival on comparison with control values, and no apparent sex-related difference in survival was noted. A relatively high percentage of the animals survived the 24-month study, with 46 to 72% of the male and 48 to 70% of the female rats euthanized at termination (Table 3). Exposure to EDC, either alone or in conjunction with DS or ET, had no discernible effect on the appearance of the animals during the course of the study. Certain tissue lesions and masses were noted at necropsy with increased frequency in the EDC-exposed groups of male and female rats on comparison with control groups. Male rats in certain groups were found to have increased liver masses (32% for the EDC/DS group vs 8% for controls) which were primarily related to bile duct cysts, kidney lesions including cronic nephropathy, calculi of the renal pelvis and associated hyperplasia of the pelvic epithelium (30% for the EDC/DS group vs 8% for controls), or testicular lesions (24% for the EDC group vs 10% for controls). For the female rats the most noteworthy observation

TABLE 1

24-hr FOOD CONSUMPTION BY MALE OR FEMALE SPRAGUE–DAWLEY RATS MEASURED DURING MONTHS 1 THROUGH 24 OF ETHYLENE DICHLORIDE EXPOSURE

	Male rats		Female rats	
Group[a]	Grams/day	% difference	Grams/day	% difference
Control	24.9 ± 1.8[b]	—	18.1 ± 5.1	—
DS	21.2 ± 5.1	−14.9	14.2 ± 4.2	−21.6
ET	20.7 ± 2.2[c]	−16.8	14.5 ± 1.5	−19.9
EDC	23.6 ± 2.3	−5.2	17.1 ± 1.9	−5.5
EDC/DS	20.0 ± 3.7	−19.7	14.4 ± 2.6	−20.4
EDC/ET	19.6 ± 1.8	−21.3	14.6 ± 1.5	−19.3

[a] Groups of 50 male and 50 female Sprague–Dawley (Crl:CD (SD)BR outbred) rats were exposed to either 50 ppm ethylene dichloride (EDC) or filtered air 7 hr/day, 5 day/week for 24 months. Certain groups were given 0.05% disulfiram (DS), by weight, in the diet or 5% ethanol (ET), by volume, in the drinking water throughout the exposure period.

[b] Calculated as the average of weekly 24-hr mean food consumption values determined throughout the 24-month study (means ± SD).

[c] Significantly greater or less than controls ($p < 0.05$).

was an increased incidence of liver masses including bile duct cysts (46% for the EDC/DS group vs 6% for controls). At termination the body weights of male and female rats exposed to EDC/DS were significantly decreased while the relative liver weights were increased in comparison with control values (Tables 3, 4).

Histopathology. Exposure-related histopathologic changes observed in rats that died spontaneously or at termination were confined to the liver, testis, skin, and mammary tissue. The overall tumor incidence for rats exposed to EDC/DS was increased when compared with control or other exposure groups (Tables 5, 6). For the liver, the inci-

TABLE 2

24-hr WATER CONSUMPTION BY MALE OR FEMALE SPRAGUE–DAWLEY RATS MEASURED DURING MONTHS 1 THROUGH 24 OF ETHYLENE DICHLORIDE EXPOSURE

	Male rats		Female rats	
Group[a]	Grams/day	% difference	Grams/day	% difference
Control	42.3 ± 18.4[b]	—	40.3 ± 8.7	—
DS	45.9 ± 7.0	8.5	40.8 ± 8.1	1.2
ET	48.2 ± 12.7	13.9	45.0 ± 7.4	11.7
EDC	48.4 ± 6.5	14.4	45.5 ± 20.4	12.9
EDC/DS	54.9 ± 18.9[c]	29.8	45.7 ± 9.4	13.4
EDC/ET	51.8 ± 10.0[c]	22.4	47.9 ± 15.0	18.9

[a] Groups of 50 male and 50 female Sprague–Dawley (Crl:CD (SD)BR outbred) rats were exposed to either 50 ppm ethylene dichloride (EDC) or filtered air 7 hr/day, 5 day/week for 24 months. Certain groups were given 0.05% disulfiram (DS), by weight, in the diet or 5% ethanol (ET), by volume, in the drinking water throughout the exposure period.

[b] Calculated as the average of weekly 24-hr mean water consumption values determined throughout the 24-month study (means ± SD).

[c] Significantly less than controls ($p < 0.05$).

TABLE 3

SURVIVAL AND FINAL BODY WEIGHTS OF MALE OR FEMALE SPRAGUE–DAWLEY RATS EXPOSED FOR 2 YEARS TO ETHYLENE DICHLORIDE

Group	2-year survival (%)[a]		Final body weight (g)	
	Male	Female	Male	Female
Control	58	54	607 ± 114[b]	418 ± 94
DS	72	70	606 ± 95	331 ± 63[c]
ET	58	56	624 ± 108	470 ± 118
EDC	60	64	672 ± 121	442 ± 88
EDC/DS	46	50	526 ± 62[c]	348 ± 77[c]
EDC/ET	54	48	657 ± 105	523 ± 155[c]

[a] Based on 50 Sprague–Dawley (Crl:CD (SD)BR outbred) rats per group.
[b] Means ± SD.
[c] Significantly less than controls ($p < 0.05$).

dence of intrahepatic bile duct cholangiomas and cysts was significantly increased for male and female rats exposed to EDC/DS. The male rats in that exposure group also had increased numbers of neoplastic nodules. The cholangiomas did not appear to be locally invasive and consisted of a proliferation of bile ducts lined by flattened epithelium. Although no metastases were observed for this lesion the pathology was reflected by an increased relative liver weight. For the mammary tissue the incidence of neoplasms was significantly increased in female EDC/DS rats. Additionally, testicular interstitial cell tumors for the EDC/DS group were significantly increased. Congestion of the mesenteric lymph node, an unusual finding, was observed for DS and EDC/DS rats of both sexes (49–61% for the DS groups and 64–71% for EDC/DS groups vs 0–4% for controls). This highly significant effect appears to be related to DS exposure. Female rats exposed to EDC showed a slight increase in the incidence of basophilic focal cellular changes in the pancreas which was not apparent in the male rat. The male and female rats in both the ET and EDC/ET groups had neoplastic and nonneoplastic lesions similar to those described for the controls (Tables 7, 8).

TABLE 4

TERMINAL ABSOLUTE AND RELATIVE LIVER WEIGHTS OF MALE OR FEMALE SPRAGUE–DAWLEY RATS EXPOSED FOR 2 YEARS TO ETHYLENE DICHLORIDE

Group	Absolute liver weight		Relative liver weight	
	Male	Female	Male	Female
Control	22.0 ± 5.0 (26)[a]	15.6 ± 3.5 (27)	3.7 ± 0.9[b]	3.8 ± 0.7
DS	23.6 ± 3.5 (33)	13.6 ± 3.0 (33)	3.9 ± 0.5	4.1 ± 0.6
ET	23.4 ± 7.5 (26)	16.8 ± 4.6 (27)	3.7 ± 1.2	3.7 ± 0.9
EDC	25.2 ± 5.3 (26)	16.5 ± 4.2 (32)	3.7 ± 0.8	3.8 ± 0.6
EDC/DS	24.3 ± 4.3 (22)	18.1 ± 5.2 (22)	4.4 ± 1.1[c]	5.3 ± 1.5[c]
EDC/ET	23.6 ± 4.6 (26)	17.4 ± 5.5 (22)	3.6 ± 0.8	3.4 ± 0.9

[a] Means ± SD (*n*).
[b] Liver weight g/100 g body wt.
[c] Significantly greater than controls ($p < 0.05$).

TABLE 5

TUMOR INCIDENCE FOR MALE SPRAGUE–DAWLEY RATS EXPOSED FOR 2 YEARS TO ETHYLENE DICHLORIDE[a]

	Control	EDC	DS	ET	EDC/DS	EDC/ET
Primary tumors	69	86	53	88	102	69
Total rats with tumors	42	45	32	42	45	42
Rats with multiple tumors	17	29	15	25	29	17
Total benign tumors	64	74	45	74	84	55
Total malignant tumors	5	12	8	14	18	14
Total malignant with metastasis	0	0	1	1	4	2

[a] Based on 50 Sprague–Dawley (Crl:CD (SD)BR outbred) rats per group.

Blood EDC levels, metabolism, and urinary excretion. The levels of unchanged EDC detected in the blood of rats after a 7-hr exposure at 50 ppm were significantly increased by DS treatment. The concentrations determined for rats exposed to EDC alone were 0.28 μg/ml for males and 0.25 μg/ml for females after exposure. Similar values were noted for the EDC/ET group animals. The EDC/DS rats, however, showed a fivefold increase in the amounts of unchanged EDC in the blood of males (1.46 μg/ml) and females (1.54 μg/ml). These values showed only slight decreases after 2 hr as shown in Table 9.

Preliminary studies using 4-month-old rats dosed at 150 mg/kg body wt with radiolabeled EDC showed that the elimination of ^{14}C was essentially complete after 24 hr. In those rats, elimination of the administered ^{14}C was primarily in the urine with 49.7 to 51.5% excreted by that route. A large portion of the administered radioactivity, 35.5 to 39.6%, was eliminated as unchanged EDC in the breath. Only small amounts of radioactivity were detected as $^{14}CO_2$ or in the feces. For the 2-year-old animals, a portion of the dose may have been sequestered in the body fat since the total recovery of administered ^{14}C at 24 hr was only 71 to 86% (Tables 10, 11). Rats exposed to [^{14}C]EDC showed similar elimination patterns. However, those patterns were altered for groups exposed to DS, EDC/DS, or EDC/ET, or female rats exposed to EDC. Significantly increased amounts of unchanged [^{14}C]EDC, 57.6 to 57.7 for EDC/DS rats, were detected in the breath. For female rats, significantly increased amounts of unchanged [^{14}C]EDC, 55.3%, were present in the breath for the DS and 40.3% was detected for the EDC rats. A corresponding significant decrease in the urinary ^{14}C was determined for those treatment groups in comparison to control rat values. The rats treated with EDC/ET, however, had increased amounts of ra-

TABLE 6

TUMOR INCIDENCE FOR FEMALE SPRAGUE–DAWLEY RATS EXPOSED FOR 2 YEARS TO ETHYLENE DICHLORIDE[a]

	Control	EDC	DS	ET	EDC/DS	EDC/ET
Primary tumors	85	87	63	72	105	80
Total rats with tumors	47	47	46	41	46	47
Rats with multiple tumors	28	26	14	20	33	22
Total benign tumors	70	72	58	61	82	73
Total malignant tumors	15	15	5	11	23	7
Total malignant with metastasis	2	1	2	2	4	1

[a] Based on 50 Sprague–Dawley (Crl:CD (SD)BR outbred) rats per group.

TABLE 7

TUMOR DATA FOR MALE RATS EXPOSED TO ETHYLENE DICHLORIDE[a]

Organ–primary tumors	Control	DS	ET	EDC	EDC/DS	EDC/ET
Liver						
Number examined	50	50	50	50	49	50
Hepatocellular carcinoma	1	0	0	1	0	0
Neoplastic nodule(s)	0	1	1	2	6[b]	4
Intrahepatic bile duct						
Number examined	50	50	50	50	49	50
Cholangioma(s)	0	0	0	0	9[b]	0
Cyst(s), number	1	0	2	0	12[b]	0
Spleen						
Number examined	50	50	50	50	50	50
Number of primary tumors	0	0	0	0	0	0
Brain						
Number examined	50	50	50	50	50	50
Astrocytoma, cerebrum	0	0	2	0	0	1
Astrocytoma, cerebellum	0	0	0	0	0	1
Pancreas						
Number examined	50	50	49	50	50	49
Adenoma(s), islets	3	0	4	3	0	2
Carcinoma, islets	0	1	3	1	1	0
Kidneys						
Number examined	50	50	50	50	50	50
Liposarcoma	0	0	0	1	0	0
Lungs						
Number examined	50	50	50	50	50	49
Adenocarcinoma	0	0	0	0	1	0
Salivary gland						
Number examined	49	50	50	50	50	50
Neurofibrosarcoma	0	1	0	0	0	0
Skin and subcutaneous						
Number examined	50	49	49	50	50	50
Fibrosarcoma	1	0	0	1	1	0
Hemangiosarcoma	0	0	1	0	0	0
Carcinosarcoma	0	0	0	0	1	0
Neurofibroma	0	0	0	0	1	0
Fibroma	2	3	1	0	10[b]	2
Mammary gland						
Number examined	44	43	43	40	38	44
Adenocarcinoma(s)	0	0	0	0	1	0
Adenoma(s)	0	1	0	0	0	1
Carcinoma	1	0	0	0	0	0
Fibroadenoma(s)	0	0	0	1	0	1
Adrenal gland						
Number examined	50	50	50	50	50	50
Adenoma, cortex	3	1	1	1	1	1
Testes						
Number examined	50	50	50	50	50	50
Interstitial cell tumor	2	3	7	3	11[b]	5
Mesothelioma	0	0	0	0	2	0
Pituitary						
Number examined	48	49	48	48	49	44
Carcinoma(s)	1	0	1	1	1	1
Adenoma(s)	30	23	30	17	30	22

TABLE 7—*Continued*

Organ–primary tumors	Control	DS	ET	EDC	EDC/DS	EDC/ET
Forestomach						
Number examined	48	50	50	49	50	50
Neoplasm(s)	0	0	0	0	0	0
Bone/marrow						
Number examined	50	50	50	50	50	50
Osteogenic sarcoma	0	1	0	0	0	1
Thyroid						
Number examined	48	50	50	50	48	47
Follicular carcinoma	0	2	1	1	0	1
Follicular adenoma	4	1	1	4	3	1
C-cell carcinoma	0	0	1	2	0	2
C-cell adenoma	6	4	8	5	5	2

[a] Based on 50 Sprague–Dawley (Crl:CD (SD)BR outbred) rats per group.
[b] Values were significantly different from control values by Fisher's Exact Probability Test, $\alpha < 0.05$.

dioactivity in the urine, 51.1 to 55.1%, and only 17.7 to 17.8% appearing unchanged in the breath.

Radioactive compounds present in each urine sample collected over the 20-hr period immediately following administration of a single dose of [1,2-U-^{14}C]EDC were separated by HPLC and quantified by LSC. An HPLC profile was constructed for each treatment group. These qualitatively identical profiles contained five compounds, which were designated by their order of elution from the HPLC column as I through V. The identities of the major metabolites, previously reported by Reitz and his co-workers (1982), were confirmed. Thiodiglycolic acid (IV), eluting at 16.5 min, amounted to 54.3 to 71% of the radioactivity present in the urine. The second most abundant metabolite, thiodiglycolic acid sulfoxide (II), eluted at 11 min and accounted for 18.0 to 32.7% of the urinary radioactivity. Chloroacetic acid (V) was eluted at 19 min in amounts ranging from 0.3 to 4.3% of the total urinary activity. Two additional metabolites, I (9 min) and III (14 min), amounting to less than 12% of the total, were unidentified. Although not statistically significant the amounts of chloroacetic acid in the urine of DS and EDC/DS rats showed an apparent increase in excretion of that metabolite (Tables 12, 13). No other quantitative group-related differences were identified.

DNA covalent binding. The radioactivity present in the blood and liver was determined, 6 hr after a single oral injection of [^{14}C]EDC, for male and female rats. The calculated amounts remaining in the blood (as parent compound) were 16.0 ± 4.8 μg/ml for males and 20.9 ± 5.7 μg/ml for females. A higher amount remained in the liver, 76.2 ± 17.7 μg/g for male and 75.4 ± 19.4 μg/g for female rats. These values which may include unbound EDC showed no significant exposure-related difference for the groups tested, and the overall blood/liver ratio was 4.8 for males and 3.6 for females. The covalent binding of EDC or EDC metabolites was evaluated after isolation and purification of the hepatic DNA for each exposure group. The presence of relatively high amounts of covalent binding, amounting to 36 to 44 micromolar equivalents per mole of DNA, was detected in unpretreated rats (Table 14). However, no significant exposure-related differences were noted.

DISCUSSION

EDC is a symmetrically substituted haloethane which is structurally similar to other compounds shown to cause cancer in laboratory animals (Weisburger, 1977), and has been reported to induce tumors in both

TABLE 8

TUMOR DATA FOR FEMALE RATS EXPOSED TO ETHYLENE DICHLORIDE[a]

Organ–primary tumors	Control	DS	ET	EDC	EDC/DS	EDC/ET
Liver						
Number examined	50	49	49	50	50	50
Hepatocellular carcinoma	0	0	0	1	1	0
Neoplastic nodule(s)	1	1	0	1	2	1
Intrahepatic bile duct						
Number examined	50	49	49	50	50	50
Cholangioma(s)	0	0	0	0	17[b]	0
Cyst(s), number	1	0	2	0	24[b]	0
Spleen						
Number examined	50	49	49	50	50	50
Number of primary tumors	0	0	0	0	0	0
Brain						
Number examined	50	49	49	50	50	50
Astrocytoma, cerebrum	1	0	1	1	0	0
Astrocytoma, cerebellum	0	0	0	0	0	1
Pancreas						
Number examined	50	49	49	50	49	49
Adenoma(s), islets	2	1	1	0	0	2
Carcinoma, islets	2	0	1	0	0	0
Kidneys						
Number examined	50	49	49	50	50	50
Number of primary tumors	0	0	0	1	0	0
Lungs						
Number examined	50	49	49	50	50	49
Number of primary tumors	0	0	0	0	0	0
Salivary gland						
Number examined	50	49	49	50	49	49
Indifferentists sarcoma	0	1	0	0	0	0
Skin and subcutaneous						
Number examined	49	49	49	50	49	50
Squamous cell carcinoma	1	0	0	0	0	0
Sarcoma, subcutis	0	0	0	1	1	0
Fibroma, subcutis	1	0	1	2	5	0
Lipoma	0	0	2	0	0	1
Mammary gland						
Number examined	50	46	45	50	48	47
Adenocarcinoma(s)	4	2	6	5	12[b]	2
Adenoma(s)	2	2	2	4	3	4
Fibroadenoma(s)	15	5	12	21	19	15
Adrenal gland						
Number examined	50	50	50	50	50	50
Adenoma, cortex	2	1	1	2	0	2
Ovary						
Number examined	48	47	49	48	47	41
Sarcoma(s)	0	0	0	0	1	0
Stromal tumor	0	1	0	0	0	0
Granulosa theca cell tumor	0	0	0	1	0	0

TABLE 8—*Continued*

Organ–primary tumors	Control	DS	ET	EDC	EDC/DS	EDC/ET
Uterus						
Number examined	49	49	49	49	50	50
Sarcoma, cervix	0	0	0	0	1	0
Stromal sarcoma, endometrium	1	0	0	0	1	1
Adenoma(s), endometrium	1	0	0	0	0	0
Leiomyoma	0	0	0	0	1	0
Adenocarcinoma	0	0	0	0	1	0
Pituitary						
Number examined	50	47	49	43	49	47
Carcinoma(s)	4	1	1	0	3	2
Adenoma(s)	37	38	33	25	35	39
Forestomach						
Number examined	48	50	50	49	50	50
Neoplasm(s)	0	0	0	0	0	0
Bone/marrow						
Number examined	50	50	50	50	50	50
Osteogenic sarcoma	1	0	0	0	0	0
Thyroid						
Number examined	49	48	48	47	49	47
Follicular carcinoma	0	0	0	0	0	0
Follicular adenoma	4	1	1	4	3	1
C-cell carcinoma	1	0	1	1	2	0
C-cell adenoma	4	0	2	2	2	0

[a] Based on 50 Sprague–Dawley (Crl:CD (SD)BR outbred) rats per group.

[b] Values were significantly different from control values by Fisher's Exact Probability Test, $\alpha < 0.05$.

B6C3F1 mice and Osborne-Mendel rats following lifetime administration by gavage (NCI, 1978a). The results of the current study indicate that the exposure of rats to EDC at 50 ppm, the current U.S. occupational standard, with coadministration of DS, resulted in an increase in the incidence of tumor formation. Although DS alone resulted in no significant tumor formation in this and other studies (NCI, 1979) at levels consistent with those used therapeutically, the combined EDC/DS treatment resulted in significant levels of intrahepatic bile duct cholangiomas and cysts in both male and female rats. The cholangiomas, an unusual lesion, consisted of a poorly circumscribed proliferation of bile ducts lined by a flattened or low cuboidal epithelium. These lesions did not appear to be locally invasive and there were no metastases. In addition, the male rats were shown to have a significantly higher incidence of interstitial cell tumors of the testes, fibroma of the subcutis, and hepatic neoplastic nodules. Similar increases in appearance of fibroma of the subcutis have been noted for male rats exposed to EDC alone (NCI, 1978a). The exposure of female rats to EDC/DS resulted in significant levels of mammary adenocarcinomas. No significant increase in tumor production, however, was observed in rats after long-term exposure either to EDC alone or to EDC in combination with ET. DS, a compound used in alcohol therapy (Pedersen, 1980), has been reported to decrease the tumor formation from certain other carcinogens through its activity as an antioxidant (Wattenberg, 1975, 1978; El-hawari *et al.*, 1989). Previous NIOSH-sponsored studies, however, have

TABLE 9

BLOOD ETHYLENE DICHLORIDE LEVELS AFTER 7-hr EXPOSURE OF MALE OR FEMALE SPRAGUE–DAWLEY RATS

Group	Sex	Time interval after exposure[a] 0.25 hr	2.25 hr
EDC	Male	0.28 ± 0.13[b]	0.22 ± 0.13
EDC/DS	Male	1.46 ± 0.48[c]	1.20 ± 0.28[c]
EDC/ET	Male	0.36 ± 0.19	0.38 ± 0.15
EDC	Female	0.26 ± 0.08	0.28 ± 0.04
EDC/DS	Female	1.54 ± 0.49[c]	1.08 ± 0.08[c]
EDC/ET	Female	0.30 ± 0.15	0.35 ± 0.13

[a] Rats exposed at 50 ppm EDC/7 hr.
[b] Blood EDC (μg/ml) (means ± SD, $n = 5$).
[c] Significantly different relative to controls ($p < 0.05$).

demonstrated that the coadministration of DS with EDC resulted in testicular atrophy and liver histopathology (Igwe *et al.*, 1986). In addition, the interaction between DS and EDB, the bromine analog of EDC, significantly increased the carcinogenicity of EDB (Plotnick, 1978; Stein *et al.*, 1978; Plotnick *et al.*, 1979; Elliot and Ashby, 1980; Wong *et al.*, 1982). Although the types of tumors reported by those investigators for EDB/DS (i.e., hemangiosarcoma and liver carcinoma) differ qualitatively from those found after exposure to EDC/DS in the present study, the results of this study show that the DS interaction observed for EDB is not limited to that particular halocarbon.

For EDC administered in combination with DS, a possible explanation of the observed carcinogenicity may be related to the presence of higher blood concentrations or changes in the disposition of the compound. After a typical exposure to EDC at 50 ppm the blood EDC levels were low in EDC/ET-exposed rats. The animals exposed to EDC/DS, however, were shown to have significantly higher blood levels of unchanged EDC than those exposed to EDC alone. After the oral administration of [1,2-U-^{14}C]EDC the EDC/DS rats eliminated a significantly larger portion of the ^{14}C unchanged in the breath and less as urinary metabolites than did the previously untreated rats. This difference appears to be due to an overall decrease in the rate of biotransformation in DS-treated rats, a finding consistent with the known enzyme-inhibiting action of DS. Inhibition of aldehyde dehydrogenase activity, and, to a lesser extent, mixed-function oxidase and alcohol dehydrogenase (Zemaitis and Greene, 1976), may contribute to this decrease. The rate of elimination of [^{14}C]EDC by 24-month-old rats was slightly lower than that previously reported for 4-week-old 150- to 250-g Osborne-Mendel rats dosed with the same 150 mg/kg body wt amount of EDC (Reitz *et al.*, 1980, 1982). Those investigators, in a series of stud-

TABLE 10

MALE RAT TOTAL 24-hr RECOVERY OF ^{14}C

Sample	Percentage of administered ethylene dichloride[a] Control	DS	ET	EDC	EDC/DS	EDC/ET
Total urinary	46.6 ± 0.7	35.2 ± 6.0[b]	45.6 ± 2.5	42.5 ± 4.4	27.6 ± 5.6[b]	51.1 ± 1.8
Volatile organic	30.5 ± 9.5	40.5 ± 6.3[b]	29.8 ± 0.3	27.3 ± 3.1	57.6 ± 8.8[b]	17.7 ± 2.0
Carbon dioxide	0.5 ± 0.1	<0.1	0.1 ± 0.1	0.1 ± 0.0	<0.1	0.2 ± 0.1
Feces	1.8 ± 0.5	1.0 ± 0.6	2.6 ± 0.5	0.9 ± 0.4	0.9 ± 0.4	1.9 ± 1.4
Total recovery	79.4 ± 9.0	76.7 ± 7.1	78.2 ± 3.2	70.8 ± 5.3	86.0 ± 5.0	71.0 ± 1.4

[a] Male Sprague–Dawley rats received 150 mg/kg body wt doses of [1,2-U-^{14}C]ethylene dichloride (means ± SE, $n = 3$).
[b] Significantly different relative to controls ($p < 0.05$).

TABLE 11

FEMALE RAT TOTAL 24-hr RECOVERY OF ^{14}C

Sample	Percentage of administered ethylene dichloride[a]					
	Control	DS[b]	ET	EDC	EDC/DS	EDC/ET
Total urinary	55.0 ± 3.6	36.4 ± 4.6[c]	41.6 ± 4.7	33.9 ± 6.1[c]	24.9 ± 3.7[c]	55.1 ± 1.3
Volatile organic	28.0 ± 4.3	55.3 ± 6.1[c]	29.8 ± 2.9	40.3 ± 4.2	57.7 ± 2.5[c]	17.8 ± 1.0
Carbon dioxide	0.7 ± 0.1	<0.1	0.2 ± 0.1	0.1 ± 0.0[c]	<0.1	0.2 ± 0.1
Feces	1.1 ± 0.2	0.2 ± 0.6	2.4 ± 0.7	0.9 ± 0.5	0.2 ± 0.1	0.9 ± 0.1
Total recovery	84.7 ± 0.5	91.9 ± 5.2	73.9 ± 3.7[c]	75.3 ± 3.1[c]	82.9 ± 4.6	73.9 ± 2.0[c]

[a] Female Sprague–Dawley rats received 150 mg/kg body wt doses of [1,2-U-^{14}C]ethylene dichloride (means ± SE, $n = 3$).

[b] Average of two rats.

[c] Significantly different relative to controls ($p < 0.05$).

ies using young adult animals, found that 85% of the radioactivity appeared in the urine, with 7 to 8% as CO_2, 4% in the carcass, and 2% in the feces at 48 hr. In a more recent study by Mitoma *et al.* (1985) 4- to 6-week-old Osborne-Mendel rats, given EDC at 100 mg/kg body wt, had 11.5, 8.2, and 7.1% of the ^{14}C appearing in the expired air, as CO_2, and in the carcass, respectively.

The chromatographic evaluation of urinary metabolites excreted by 24-month-old rats shows that the principal metabolite, thiodiglycolic acid, accounted for 54.3 to 71.0% of the radioactivity present in the urine. A glutathione conjugate, thiodiglycolic acid sulfoxide, accounted for an additional 18.7 to 32.7%, and 0.3 to 4.3% of the urinary radioactivity was present as chloroacetic acid. The urinary excretion of EDC metabolites for the Sprague–Dawley rats is consistent with values reported for young adult Osborne-Mendel rats by Reitz and co-workers (1982). Other investigators have reported the presence of *S*-carboxymethylcysteine, thiodiacetic acid, and chloroacetic acid as major urinary metabolites of 4- to 6-week-old rats (Mitoma *et al.*, 1985) and mice (Yllner, 1971; Mitoma *et al.*, 1985). Additionally, the production of the reactive intermediates, chloroacetaldehyde or *S*-(2-chloroethyl)-glutathione, has been suggested to occur (Guengerich *et al.*, 1980; Anders and Livesey, 1980; Reed and Foureman, 1986; Colacci *et al.*, 1987). Such metabolites could form covalent adducts by reaction with nucleophilic macromolecules in the cell and could be responsible

TABLE 12

MALE RAT ETHYLENE DICHLORIDE 24-hr URINARY PROFILE[a]

Compound	Control	DS	ET	EDC	EDC/DS	EDC/ET
Fraction I	1.7 ± 0.6[b]	2.3 ± 4.0	2.3 ± 1.5	2.3 ± 0.6	1.7 ± 0.6	3.7 ± 0.6
Thiodiglycolic acid sulfoxide	26.7 ± 3.1	32.7 ± 4.0	28.7 ± 2.3	28.7 ± 3.5	28.0 ± 3.5	31.3 ± 4.7
Fraction III	5.0 ± 1.0	6.3 ± 0.6	4.7 ± 0.6	7.0 ± 2.6	5.0 ± 1.0	7.3 ± 2.3
Thiodiglycolic acid	65.3 ± 4.5	57.0 ± 3.5	63.0 ± 1.0	60.0 ± 3.0	60.7 ± 2.1	54.3 ± 7.0
Chloroacetic acid	0.7 ± 4.5	1.7 ± 3.5	0.3 ± 1.0	1.7 ± 1.2	4.0 ± 2.4	2.7 ± 3.8

[a] Male Sprague–Dawley rats received 150 mg/kg body wt doses of [1,2-U-^{14}C]ethylene dichloride.

[b] Percentage of urinary radioactivity (means ± SE, $n = 3$).

TABLE 13

FEMALE RAT ETHYLENE DICHLORIDE 24-hr URINARY PROFILE[a]

Compound	Control	DS	ET	EDC	EDC/DS	EDC/ET
Fraction I	2.0 ± 0.0[b]	1.5[c]	2.0 ± 0.0	3.0 ± 1.0	1.7 ± 0.6	1.3 ± 0.6
Thiodiglycolic acid sulfoxide	23.7 ± 2.1	24.0	22.3 ± 4.0	20.0 ± 2.6	18.0 ± 1.5	28.7 ± 2.3
Fraction III	4.7 ± 0.6	5.0	6.0 ± 1.0	5.3 ± 1.2	4.3 ± 0.6	7.0 ± 2.3
Thiodiglycolic acid	68.3 ± 2.9	65.5	67.3 ± 4.9	69.3 ± 2.5	71.0 ± 1.0	60.7 ± 0.6
Chloroacetic acid	1.0 ± 0.0	3.5	1.3 ± 0.6	1.7 ± 0.6	4.3 ± 1.2	1.3 ± 0.6

[a] Female Sprague–Dawley rats received 150 mg/kg body wt doses of [1,2-U-^{14}C]ethylene dichloride.
[b] Percentage of urinary radioactivity (means ± SE, n = 3).
[c] Average of two rats.

for carcinogenic or mutagenic effects of EDC (Banerjee *et al.*, 1980; Guengerich *et al.*, 1980; Perocco and Prodi, 1981; Koga *et al.*, 1986). Reitz and his co-workers (1980) reported that *in vivo* hepatic DNA covalent binding was 13.9 to 21.3 micromolar equivalents/mole after oral administration of 150 mg EDC/kg body wt for young adult rats. The hepatic DNA covalent binding for 24-month-old rats in the current study was only slightly higher, ranging from 18.9 to 53.3 micromolar equivalents/mole of DNA. Covalent binding of DNA nucleosides by EDC has been demonstrated *in vitro* by Guengerich and his co-workers (1980). They also noted that binding activity appeared to be highest in the presence of GSH, an indication that the GSH *S*-transferase pathway is important in production of reactive EDC metabolites. Those investigators noted some binding activity in the presence of microsomal protein and NADPH. The microsomal mixed-function oxidative metabolism resulting in the formation of reactive 2-haloethylene oxides is thought to be an important mechanism in macromolecular binding by a variety of chemicals (Henschler, 1977; Guengerich *et al.*, 1980, 1981). However, *in vitro* studies conducted by Storer and Conolly (1985), which compared the role of microsomal oxidative metabolism and GSH conjugation mediated by the GSH *S*-transferases, suggest that conjugation is the primary source of EDC genotoxicity.

TABLE 14

LIVER COVALENT DNA BINDING IN MALE AND FEMALE SPRAGUE–DAWLEY RATS EXPOSED TO ETHYLENE DICHLORIDE

Group	DNA covalent binding (μmole eq/mol DNA)[a]		
	Male	Female	Combined
Control	43.5 ± 1.2[b]	36.4 ± 6.6	40.0 ± 3.4
DS	41.6 ± 8.3	29.0 ± 2.0	35.3 ± 4.8
ET	37.5 ± 3.0	26.9[c]	33.2 ± 3.8
EDC	18.9[c]	35.0 ± 3.9	28.6 ± 4.5
EDC/DS	35.6 ± 4.5	22.5 ± 4.6	29.0 ± 4.1
EDC/ET	53.3 ± 25.2	23.1 ± 7.1	38.2 ± 13.5

[a] Sprague–Dawley rats received 150 mg/kg body wt doses of [1,2-U-^{14}C]ethylene dichloride.
[b] DNA isolated by solvent extraction and bound radioactivity determined by scintillation spectrometry (means ± SE, n = 3).
[c] Average of two rats.

In summary, the results of the present study indicate that the exposure of rats to EDC at 50 ppm (7 hr/day) with DS in the diet resulted in the formation of increased numbers of tumors when compared with rats exposed to only filtered air and standard diet. The combined EDC/DS treatment resulted in a high incidence of hepatic, testicular, and mammary tumors in the rat. No increased incidence, however, was noted for the EDC, EDC/ET, DS, or ET groups. Although the levels of hepatic DNA covalent binding by orally administered [^{14}C]EDC were significant those levels did not appear to be altered

by DS treatment, and the metabolism of EDC was qualitatively the same as that of corresponding controls. However, a reduced rate of elimination for EDC, along with sustained blood levels of the unchanged compound, could be related to the carcinogenic effects observed for the combined treatment.

REFERENCES

ALBRECHT, W. N., HAGADONE, M. R., AND CHENCHIN, K. (1986). Charcoal air sampling tube storage stability and desorption efficiencies of 1,2-dibromo-3-chloropropane (DBCP) and 1,3-dichloropropene (DCP). *Bull. Environ. Contam. Toxicol.* **36,** 629–634.

ANDERS, M. W., AND LIVESEY, J. C. (1980). Metabolism of 1,2-dihaloethanes. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 331–341. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

ARMITAGE, P. (1971). *Statistical Methods in Medical Research,* pp. 362–365. Wiley, New York.

BANERJEE, S., VAN DUUREN, B. L., AND ORUAMBO, F. I. (1980). Microsome-mediated covalent binding of 1,2-dichloroethane to lung microsomal protein and salmon sperm DNA. *Cancer Res.* **40,** 2170–2173.

BRADFORD, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

CAS. (1977). *The Chemical Abstracts Service Ninth Collective Index. Volumes 76–85, 1972–1976.* Amer. Chem. Soc., Washington, DC.

COLACCI, A., GRILLI, S., LATTANZI, G., PRODI, G., TURINA, M. P., FORTI, G. C., AND MAZZULLO, M. (1987). The covalent binding of 1,1,2,2-tetrachloroethane to macromolecules of rat and mouse organs. *Teratog. Carcinog. Mutagen.* **7,** 465–474.

DAVIDSON, I. W. F., SUMNER, D. D., AND PARKER, J. C. (1982). Ethylene dichloride: A review of its metabolism, mutagenic and carcinogenic potential. *Drug Chem. Toxicol.* **5,** 319–388.

DRURY, J. S., AND HAMMONS, A. S. (1979). *Investigations of Selected Environmental Pollutants: 1,2-Dichloroethane.* EPA-560/2-78-006, PB-295 865, pp. 20–30. National Technical Information Service, Springfield, VA.

EL-HAWARI, A. M., WINSTON, J. M., SHORT, R. D., LEE, C. C., AND WOODS, J. S. (1989). The effects of disulfiram on vinyl chloride-induced toxicity and carcinogenicity. *J. Toxicol. Environ. Health,* in press.

ELLIOT, B. M., AND ASHBY, J. (1980). Ethylene dibromide and disulfiram: Studies *in vitro* and *in vivo* on the mechanism of the observed synergistic carcinogenic response. *Carcinogenesis* **1,** 1049–1057.

FRANCOVITCH, R. J., SCHOR, N. A., AND GEORGE, W. J. (1986). Effects of SKF 252A, phenobarbital, and 3-methylcholanthrene on ethylene dichloride toxicity following inhalation exposure. *J. Amer. Coll. Toxicol.* **5,** 117–126.

GOLD, L. S. (1980). Human exposure to ethylene dichloride. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 209–225. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

GUENGERICH, F. P., CRAWFORD, W. M., DOMOREDZKI, J. Y., MCDONALD, T. L., AND WATANABE, P. G. (1980). *In vitro* activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. *Toxicol. Appl. Pharmacol.* **55,** 303–317.

GUENGERICH, F. P., MASON, P. S., STOTT, W. T., FOX, T. R., AND WATANABE, P. G. (1981). Roles of 2-haloethylene oxides and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in irreversible binding to protein and DNA. *Cancer Res.* **41,** 4391–4398.

HASEMAN, J. K., AND HOEL, D. G. (1974). Tables of Gehan's generalized Wilcoxon test with fixed point sensoring. *J. Stat. Comput. Simul.* **3,** 117–135.

HENSCHLER, D. (1977). Metabolism of chlorinated alkenes and alkanes as related to toxicity. *J. Environ. Pathol. Toxicol.* **1,** 125–133.

HILL, D. L., SHIH, T.-W., JÖHNSTON, T. P., AND STRUCK, R. F. (1978). Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res.* **38,** 2438–2442.

HILLBROM, M. E. (1971). Thyroid state and voluntary alcohol consumption of albino rats. *Acta Pharmacol. Toxicol.* **29,** 95–105.

HORROCKS, D. L. (1974). *Applications of Liquid Scintillation Counting,* pp. 72–74. Academic Press, New York.

International Agency for Research on Cancer (IARC). (1979). *Monograph on the Evaluation of Carcinogenic Risk of Chemicals in Humans, Vol. 20: Ethylene Dichloride,* p. 429. IARC, Lyon, France.

IGWE, O. J., QUE HEE, S. S., AND WAGNER, W. D. (1986). Interaction between 1,2-dichloroethane and disulfiram. I. Toxicologic effects. *Fundam. Appl. Toxicol.* **6,** 733–746.

INFANTE, P. F., AND POHL, G. K. (1988). Living in a chemical world: Actions and reactions to industrial carcinogens. *Teratogen. Carcinogen. Mutagen.* **8,** 225–249.

KOGA, N., INSKEEP, P. B., HARRIS, T. M., AND GUENGERICH, F. P. (1986). *S*-[2-(N^7-Guanyl)ethyl]glutathione, the major DNA adduct formed from 1,2-dibromoethane. *Biochemistry* **25,** 2192–2198.

LIU, C. C. (1964). *Introduction to Experimental Statistics,* pp. 419–423. McGraw-Hill, New York.

MALTONI, C., VALGIMIGLI, L., AND SCARNATO, C. (1980). Long-term carcinogenic bioassays on ethylene dichloride administered by inhalation to rats and mice. In *Banbury Report 5 Ethylene Dichloride: A Po-*

tential Health Risk? (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 3–29. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

McCANN, J., SIMMON, V., STREITWIESER, D., AND AMES, B. N. (1975). Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride, and cyclophosphamide. *Proc. Natl. Acad. Sci. USA* **72,** 3190–3193.

MITOMA, C., STEEGER, T., JACKSON, S. E., WHEELER, K. P., ROGERS, J. H., AND MILMAN, H. A. (1985). Metabolic disposition study of chlorinated hydrocarbons in rats and mice. *Drug Chem. Toxicol.* **8,** 183–194.

National Cancer Institute (NCI). (1978a). *Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity.* NCI Technical Report Series No. 55, DHEW Publication No. (NIH) 78-1361. Gov. Printing Office, Washington, DC.

National Cancer Institute (NCI). (1978b). *Bioassay of 1,2-Dibromoethane for Possible Carcinogenicity.* NCI Technical Report Series No. 86, DHEW Publication No. (NIH) 78-1336. Gov. Printing Office, Washington, DC.

National Cancer Institute (NCI). (1979). *Bioassay of Tetraethylthiuram Disulfide for Possible Carcinogenicity.* NCI Technical Report Series No. 166, DHEW Publication No. (NIH) 79-1722. Gov. Printing Office, Washington, DC.

National Institute for Occupational Safety and Health (NIOSH). (1978a). *Criteria for a Recommended Standard. Occupational Exposure to Ethylene Dichloride.* DHEW Publication No. (NIOSH) 78-211. Gov. Printing Office, Washington, DC.

National Institute for Occupational Safety and Health (NIOSH). (1978b). *Current Intelligence Bulletin 25: Ethylene Dichloride.* DHEW Publication No. (NIOSH) 78-149. Gov. Printing Office, Washington, DC.

PEDERSEN, S. B. (1980). Analysis and preliminary pharmacokinetics of disulfiram. *Arch. Pharm. Chemi Sci. Ed.* **8,** 65–82.

PEROCCO, P., AND PRODI, G. (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro. *Cancer Lett.* **13,** 213–218.

PLOTNICK, H. B. (1978). Carcinogenesis in rats of combined ethylene dibromide and disulfiram. *J. Amer. Med. Assoc.* **239,** 1609.

PLOTNICK, H. B., WEIGEL, W. W., RICHARDS, D. E., AND CHEEVER, K. L. (1979). The effect of dietary disulfiram upon the tissue distribution and excretion of ^{14}C-1,2-dibromoethane in the rat. *Res. Commun. Chem. Pathol. Pharmacol.* **26,** 535–545.

PLOTNICK, H. B., WEIGEL, W. W., RICHARDS, D. E., CHEEVER, K. L., AND KOMMINENI, C. (1980). Dietary enhancement of the toxicity of ethylene dibromide. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 279–286. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

RADIKE, M. J., STEMMER, K. L., BROWN, P. G., LARSON, E., AND BINGHAM, E. (1977). Effect of ethanol and vinyl chloride on the induction of liver tumors: Preliminary report. *Environ. Health Perspect.* **21,** 153–155.

RANNUG, U. (1980a). Genotoxic effects of 1,2-dibromoethane and 1,2-dichloroethane. *Mutat. Res.* **76,** 269–275.

RANNUG, U. (1980b). The use of different metabolizing systems in the elucidation of the mutagenic effects of ethylene dichloride in Salmonella. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 83–92. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

REED, D. J., AND FOUREMAN, G. L. (1986). A comparison of the alkylating capabilities of the cysteinyl and glutathionyl conjugates of 1,2-dichloroethane. *Adv. Exp. Med.* **197,** 469–475.

REITZ, R. G., FOX, T. R., DOMORADZKI, J. Y., QUAST, J. F., LANGVARDT, P. W., AND WATANABE, P. G. (1980). Pharmacokinetics and macromolecular interactions of ethylene dichloride: Comparison of oral and inhalation exposures. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 135–144. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

REITZ, R. G., FOX, T. R., RAMSEY, J. C., QUAST, J. F., LANGVARDT, P. W., AND WATANABE, P. G. (1982). Pharmacokinetics and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. *Toxicol. Appl. Pharmacol.* **62,** 190–204.

SAWADA, M., SOFUNI, T., AND ISHIDATE, M., JR. (1987). Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo. *Mutat. Res.* **187,** 157–163.

SETARO, F., AND MORLEY, C. G. D. (1976). A modified fluorometric method for the determination of microgram quantities of DNA from cell or tissue cultures. *Anal. Biochem.* **71,** 313–317.

SPREAFICO, F., ZUCCATO, E., MARCURI, F., SIRONI, M., PAGLIALUNGA, S., MADONNA, M., AND MUSSINI, E. (1980). Pharmacokinetics of ethylene dichloride in rats treated by different routes and its long-term inhalation toxicity. In *Banbury Report 5 Ethylene Dichloride: A Potential Health Risk?* (B. Ames, P. Infante, and R. Reitz, Eds.), pp. 107–129. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

STEIN, H. P., BAHLMAN, L. J., LEIDEL, N. A., PARKER, J. C., THOMAS, A., AND MILLAR, J. D. (1978). Ethylene dibromide and disulfiram toxic interaction. *Amer. Ind. Hyg. Assoc. J.* **39,** 35–45.

STORER, R. D., AND CONOLLY, R. B. (1985). An investigation of the role of microsomal oxidative metabolism in the *in vivo* genotoxicity of 1,2-dichloroethane. *Toxicol. Appl. Pharmacol.* **77,** 36–46.

U.S. International Trade Commission (USITC). (1976–1984). *Synthetic Organic Chemicals, U.S. Production*

and Sales. USITC Publications 776, 804, 833, 920, 101, 1099, 1183, 1422, and 1588.

WATTENBERG, L. W. (1975). Inhibition of chemical carcinogenesis by antioxidants. In *The Sixth International Symposium of Princess Takamatsu Cancer Research Fund Fundamentals of Cancer Prevention,* pp. 49–55. Princess Takamatsu Cancer Research Fund, Tokyo.

WATTENBERG, L. W. (1978). Inhibitors of chemical carcinogenesis. *Adv. Cancer Res.* **26,** 197–204.

WEISBURGER, E. K. (1977). Carcinogenicity studies on halogenated hydrocarbons. *Environ. Health Perspect.* **21,** 7–23.

WONG, L. C. K., WINSTON, J. M., HONG, C. B., AND PLOTNICK, H. B. (1982). Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. *Toxicol. Appl. Pharmacol.* **63,** 155–165.

YLLNER, S. (1971). Metabolism of 1,2-dichloroethane-^{14}C in the mouse. *Acta Pharmacol. Toxicol.* **30,** 257–270.

ZEMAITIS, M. A., AND GREEN, F. E. (1976). Impairment of hepatic microsomal drug metabolism in the rat during daily disulfiram administration. *Biochem. Pharmacol.* **25,** 1355–1360.

ZUCCATO, E., MARCUCCI, F., AND MUSSININ, E. (1980). GLC determination of ethylene dichloride (EDC) in biological samples. *Anal. Lett.* **13,** 213–217.