MIDWEST RESEARCH INSTITUTE

MRI REPORT

CHRONIC INHALATION TOXICITY STUDY OF 1,2-DICHLOROETHANE IN RATS TREATED WITH DISULFIRAM OR ETHANOL

PART II: DISPOSITION, METABOLISM AND BINDING STUDIES

FINAL REPORT

July 30, 1986

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For

National Institute of Occupational Safety and Health
4676 Columbia Parkway
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Possible mechanisms of action were sought to explain the synergistic effects of simultaneously administered 1,2-dichloroethane (107062) (EDC) and disulfiram (97778) in the production of cancer in rats. In animals receiving only EDC, blood samples revealed the presence of EDC at 15 minutes and at 2 hours following the termination of the EDC exposure. Simultaneous exposure to ethanol (64175) revealed the same concentrations of EDC in the blood. In rats simultaneously exposed to EDC and disulfiram, these EDC levels were five times higher. Expired air from rats treated with EDC contained 28 to 30 percent of the unmetabolized EDC; urine contained 47 to 55 percent of the administered dose, and fecal elimination amounted to 1 to 2 percent. In rats also treated with disulfiram, expired air contained 58 percent of the administered dose. Urinary metabolic profiles for rats treated with EDC plus disulfiram were similar to those treated with EDC alone. Binding of EDC to hepatic DNA was not changed by additional exposure to disulfiram. The authors conclude that the reduced EDC elimination as a result of the simultaneous exposure to disulfiram may have been responsible for the increased incidence of hepatic, testicular, and mammary tumors found in these rats, as opposed to changes in biotransformation and binding of the offending carcinogen.

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by

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri, under Contract No. 200-82-2508 with the National Institute of Occupational Safety and Health. Mr. Kenneth Cheever was the technical monitor for the studies.

The research was conducted in the Pharmacology and Toxicology Section. The disposition and DNA binding studies were performed by Ms. M. Stoltz, Associate Biochemist, Ms. D. Czarnecki, Assistant Biochemist, Ms. K. Christianson, Assistant Biologist, Ms. J. Long, Assistant Chemist, and Ms. P. Alm, Senior Technician. The analytical studies were performed by Dr. E. Murrill, Principal Advisor for Chemistry and Biology, and Mr. F. Pallas, Associate Chemist. The study director was Dr. M. El-hawari, Head of the Pharmacology and Toxicology Section.

This report incorporates the comments provided by the sponsor on the draft final report submitted January 30, 1986.

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SUMMARY

Objectives and study design: Studies were performed to develop metabolism, disposition, and binding data that would allow an understanding of the interaction between 1,2-dichloroethane (EDC) and disulfiram (DS) or ethanol (EtOH) administered simultaneously to Sprague-Dawley (S.D.) rats. These studies were initiated following a 2-year chronic study in which six groups of male and female rats were exposed to filtered air (control), DS (0.05% in diet), EtOH (5% in water), EDC (50 ppm), EDC plus DS, or EDC plus EtOH. EDC in blood was measured in four groups of five male and five female rats exposed to EDC, EDC plus DS, and EDC plus EtOH using a gas chromatographic (GC) procedure. The elimination of radioactivity in urine, feces, and expired air was assessed following administration of an oral dose (150 mg/kg) of ^{14}C-EDC to three male and three female rats from the six treatment groups. In addition, groups of younger ($\sim$ 4 months old) male and female rats were treated with ^{14}C-EDC and recoveries of radioactivity in excreta and tissues were measured. Metabolic profiles in urine were determined using a high performance liquid chromatographic (HPLC) method. The binding of EDC metabolites to hepatic DNA was also determined in rats of the same groups following oral dosing with ^{14}C-EDC.

EDC blood levels: Blood samples were collected from male and female rats at 15 min following termination of EDC exposure and 2 hr later. The data (Table A) show that in rats exposed to EDC only, small amounts of the parent chemical were recovered in blood at 15 min. These amounts remained constant 2 hr later. Similar concentrations were recovered in blood of rats exposed to EDC plus EtOH. EDC levels in rats exposed simultaneously to EDC and DS were five times higher.

^{14}C elimination: Male and female rats were treated with ^{14}C-EDC and placed in glass metabolism cages for excreta collection. In control rats, elimination in expired air was significant (Table B); almost all of the expired ^{14}C was in the form of unmetabolized EDC (28-30%). The urine contained major amounts of the administered doses (47-55%) but fecal elimination was limited (1-2%). Only trace amounts of ^{14}C-CO_2 were recovered. Recoveries from these older rats were lower than from the 4-month-old animals. The rates of elimination in the expired air were rapid; urinary excretion rates were slower (Table C). Most of the expired ^{14}C was eliminated during the first 6 hr after dosing. In DS-treated rats, elimination in expired air increased to 41-55% and in urine decreased to 35-36%. In rats treated with EDC and DS, 58% of the dose was eliminated in expired air as EDC in both males and females. Excretion of ^{14}C in urine was reduced to 28% in males and 25% in females.

Urinary metabolic profiles: In control rats, the major urinary metabolites were thiodiglycolic acid (65-68%) and its sulfoxide (24-27%). Monochloroacetic acid (MCAA) was excreted as a minor metabolite (1%). Two other minor products (2 and 5%) were detected in urine (Table D). The distribution of these metabolites in urine was not significantly altered in EtOH- or DS-treated rats although the latter group showed increased elimination of the minor metabolite, MCAA. Urine from the female rats had slightly

higher thiodiglycolic acid and lower oxidation products. No EDC was detected in urine.

DNA binding: The binding to hepatic DNA was examined in rats treated with ^{14}C-EDC and sacrificed 6 hr later. Binding to DNA in control rats was low, only 44 μmole/mole DNA in males and 36 μmole/mole DNA in females. The binding was generally lower in the female rats and was not altered by EtOH or DS treatment (Table E).

Conclusions: The chronic toxicity studies showed that concurrent administration of EDC and DS resulted in increased hepatic, testicular, and mammary tumors compared to rats treated with EDC alone. After termination of exposure, EDC blood levels were low, but significantly higher concentrations were demonstrated in both male and female rats exposed to combined treatment of EDC and DS. The elimination of radioactivity in urine and expired air was also modified in rats receiving this combined treatment, but the urinary metabolic profiles were not significantly altered. Binding of EDC metabolites to hepatic DNA was low in all treatment groups. Differences in hepatic binding that can be implicated in the modified EDC carcinogenicity in DS-treated animals were not apparent. Therefore, reduced EDC elimination rather than changes in its biotransformation and binding may have contributed to the increased incidence of tumors demonstrated in rats exposed to EDC and DS.

TABLE A

LEVELS OF EDC IN BLOOD OF S.D. RATS FOLLOWING 2 YEARS OF INHALATION EXPOSURE TO 50 ppm OF EDC

Treatment Group	Males		Females	
	0.25 hr[a]	2.25 hr	0.25 hr	2.25 hr
	μg EDC/mL Blood			
EDC	0.28	0.22	0.26	0.28
EDC/DS	1.46	1.20	1.54	1.08
EDC/EtOH	0.36	0.38	0.30	0.35

a Sampling time after termination of exposure.

TABLE B

RECOVERY OF RADIOACTIVITY (PERCENT OF DOSE) IN EXCRETA OF S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg)

Excretum	2 Years Exposure						~ 4 Month Old
	Control	DS	EtOH	EDC	EDC/DS	EDC/EtOH	
	Male Rats						
Expired EDC	30.46	40.93	29.89	27.33	57.56	17.69	35.53
Expired CO_2	0.47	0.04	0.09	0.06	0.03	0.22	0.08
Urine	46.63	35.26	45.56	42.50	27.57	51.14	49.67
Feces	1.80	0.98	2.62	0.90	0.89	1.90	5.20
Total	79.36	77.20	78.16	70.79	86.05	70.95	92.43
	Female Rats						
Expired EDC	27.96	55.27	29.77	40.35	57.78	17.72	39.56
Expired CO_2	0.71	0.07	0.21	0.08	0.04	0.21	0.31
Urine	54.95	36.42	41.63	33.95	24.88	55.07	51.48
Feces	1.10	0.15	2.37	0.89	0.19	0.89	4.40
Total	84.72	91.92	73.98	75.26	82.90	73.88	97.13

TABLE C

RATES OF ^{14}C ELIMINATION (PERCENT OF DOSE/hr) FROM S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg)

Excretum	Time (hr)	2 Years Exposure						~ 4 Month Old Rats
		Control	DS	EtOH	EDC	EDC/DS	EDC/EtOH	
				Male Rats				
Expired EDC	1	9.071	7.062	8.336	7.971	9.260	5.945	13.932
	3	3.714	3.534	3.289	3.644	5.627	2.052	6.344
	6	1.996	2.716	1.851	2.124	3.819	1.139	2.412
	12	1.091	1.968	1.032	0.771	2.726	0.512	0.242
	24	0.106	0.570	0.270	0.089	0.490	0.096	0.018
Expired CO_2	3	0.058	0.002	0.003	0.002	0.004	0.051	0.004
	6	0.058	0.003	0.003	0.004	0.002	0.008	0.008
	12	0.008	0.001	0.002	0.004	0.002	0.004	0.005
	24	0.006	0.001	0.005	0.001	0.000	0.002	0.001
Urine	6	1.280	0.381	0.822	1.041	0.480	1.356	1.573
	12	2.669	1.256	2.142	2.316	1.494	3.140	3.681
	24	1.911	2.119	2.315	1.863	1.310	2.014	1.512
Feces	24	0.075	0.041	0.109	0.037	0.037	0.079	0.217
				Female Rats				
Expired EDC	1	9.144	11.967	8.022	8.956	10.130	6.903	18.984
	3	3.476	5.976	3.444	4.878	5.239	2.340	7.585
	6	1.910	4.419	2.017	2.895	4.364	1.024	1.511
	12	0.761	2.630	0.873	1.456	2.944	0.354	0.180
	24	0.112	0.193	0.298	0.341	0.534	0.078	0.008
Expired CO_2	3	0.087	0.005	0.007	0.003	0.004	0.007	0.009
	6	0.086	0.007	0.008	0.005	0.002	0.024	0.014
	12	0.018	0.005	0.011	0.007	0.003	0.016	0.036
	24	0.007	0.001	0.008	0.001	0.000	0.001	0.002
Urine	6	1.755	0.826	1.227	1.101	0.358	2.209	1.893
	12	3.336	2.538	2.574	2.242	1.148	3.165	4.430
	24	2.034	1.353	1.568	1.158	1.320	1.902	1.128
Feces	24	0.046	0.006	0.099	0.037	0.008	0.037	0.183

TABLE D

URINARY METABOLITES OF ^{14}C-EDC IN S.D. RATS EXPOSED FOR 2 YEARS TO OTHER TREATMENT

Treatment Group	Sex	Unknowns ~ 9 min	TDGAS[a] ~ 11 min	Unknown ~ 14 min	TDGA[a] ~ 16.5 min	MCAA[a] ~ 19 min
		Percent of Urinary Radioactivity				
Control	Male	1.7	26.7	5.0	65.3	0.7
	Female	2.0	23.7	4.7	68.3	1.0
DS	Male	2.3	32.7	6.3	57.0	1.7
	Female	1.5	24.0	5.0	65.5	3.5
EtOH	Male	2.3	28.7	4.7	63.0	0.3
	Female	2.0	22.3	6.0	67.3	1.3
EDC	Male	2.3	28.7	7.0	60.0	1.7
	Female	3.0	20.0	5.3	69.3	1.7
EDC/DS	Male	1.7	28.0	5.0	60.7	4.0
	Female	1.7	18.7	4.3	71.0	4.3
EDC/EtOH	Male	3.7	31.3	7.3	54.3	2.7
	Female	1.7	28.7	7.0	60.7	1.3

a TDGAS, thiodiglycolic acid sulfoxide; TDGA, thiodiglycolic acid; MCAA, monochloroacetic acid.

TABLE E

BINDING OF RADIOACTIVITY TO HEPATIC DNA OF S.D. RATS TREATED ORALLY WITH ^{14}C-EDC (150 mg/kg)

Treatment	Males	Females	Combined
	Micromole EDC/mole DNA		
Control	43.5	36.4	40.0
DS	41.6	29.0	35.3
EtOH	37.5	26.9	33.2
EDC	18.9	35.0	28.6
EDC/DS	35.6	22.5	29.0
EDC/EtOH	53.3	23.1	38.2

I. OBJECTIVES

The finding that disulfiram (DS), a drug used in treatment of alcoholism, potentiates the toxicity and carcinogenicity of 1,2-dibromoethane (EDB) generated considerable interest.[7,8,21,22,32] This finding was surprising in view of the earlier data that show this drug inhibits the toxicity and/or tumorigenicity of several halomethanes, halomethylenes, polycyclic hydrocarbons, nitrosamines, and hydrazines.[7a,33] To determine whether such interaction occurs with other haloalkanes, the National Institute of Occupational Safety and Health (NIOSH) supported a study to evaluate the effects of this drug on a related halocarbon, 1,2-dichloroethane (EDC). This halocarbon is produced commercially in massive amounts and is used as a solvent and industrial intermediate.[2,10,18] Many workers who handle EDC are also simultaneously exposed to DS or to related chemicals which are available commercially for use as pesticides (e.g., thiram, ziram, etc.). Some workers may also be exposed to ethanol, which is known to potentiate the toxicity of several haloalkanes.[20,31] The study, therefore, was extended to include an assessment of the interaction between ethanol (EtOH) and EDC following chronic exposure in rats.

The study design included six groups of male and female Sprague-Dawley rats which were exposed for 7 hr/day, 5 days/week, to EDC or filtered air. Some groups were fed diets containing 0.05% DS and others were exposed to 5% ethanol in the drinking water. At the end of the 2-year chronic studies, experiments to assess the metabolism, disposition, and DNA binding of ^{14}C-labeled EDC were initiated to generate data that would allow an understanding of the interaction, if any, between EDC and DS or EtOH administered simultaneously to rats over the 2-year period.

The specific objectives of the studies were:

1. To analyze EDC in blood of male and female rats exposed to 50 ppm of EDC by inhalation and rats exposed simultaneously to EDC and DS or EDC and EtOH.

2. To assess the elimination of radioactivity in urine, feces, and expired air following administration of ^{14}C-EDC to rats exposed chronically to EDC, DS, EtOH, EDC plus DS, EDC plus EtOH, and to control rats of the same age group, and to determine the metabolic profiles in urine of these animals.

3. To determine the specific binding of EDC or its metabolites to hepatic DNA following administration of ^{14}C-EDC to rats exposed for a 24-month period to EDC, DS, EDC plus DS, EDC plus EtOH, and to control rats of the same age group.

To assess the rate of elimination of ^{14}C-EDC following oral dosing, limited studies were performed in younger ($\sim$ 4 months old) male and female rats of the same strain. These rats were treated with a single oral dose of ^{14}C-EDC and recoveries of radioactivity in tissues and excreta were determined.

II. BACKGROUND

1,2-Dichloroethane (ethylene dichloride--EDC) is one of the highest volume synthetic organic chemicals manufactured in the United States. In addition to worker exposure in the chemical industry, EDC is a known contaminant of air and drinking water. The bulk of EDC produced is utilized as a chemical intermediate, but a large amount is also used for lead scavenging, grain fumigating, and as an organic solvent.[2,5,10] The current occupational exposure standard for EDC is 50 ppm[19] with a threshold limit value of 10 ppm.[1] EDC is known to produce toxic effects on the liver, kidney, heart, lungs, and nervous system. Adverse effects were reported in workers exposed to 10-50 ppm of this compound.

In a lifetime gavage study performed by the National Cancer Institute, EDC was found to elicit a carcinogenic response in rats and mice.[18,34] This response was lower than that of the bromine analog, ethylene dibromide (EDB).[17,34] Although EDB was also found to be carcinogenic to several organs after inhalation exposure,[15] EDC was reported to show no carcinogenic effect in a lifetime inhalation study.[14] These results provoked some uncertainty as to the carcinogenic potential of EDC. EDB is a more effective mutagen and possesses greater reactivity than EDC.[23] Although mammalian metabolism of both compounds appears similar, the differences in their toxic responses are presumably due to the greater ease of cleavage of the bromine-carbon bond.

EDC is rapidly and extensively absorbed through the lungs and from the gastrointestinal tract.[26,29] Inhalation is considered the primary route of exposure since EDC has a moderately high vapor pressure and a high blood/air partition coefficient. Skin absorption may be significant with direct liquid contact. Oral absorption is also important since EDC is a known water contaminant. Reitz et al.[25,26] reported that radiolabeled EDC administered orally to rats was completely absorbed.

After pulmonary or oral absorption, EDC is distributed into all body tissues. Elimination of EDC from the body is the sum of metabolism and excretion of unchanged EDC via pulmonary and other routes. Unmetabolized EDC is excreted through the lungs.[25,26] It is not ordinarily eliminated by other than the pulmonary route, but some investigators identified EDC in the urine of individuals with severe EDC poisoning. There is no definite experimental evidence concerning bioaccumulation after repeated daily exposure to EDC, although EDC has a high fat-to-blood partition coefficient. In rats, the half-life of elimination was estimated to be 25 to 57 min following single oral dosing and 13 to 35 min after inhalation exposure.[25,26,29,35]

Current knowledge of EDC metabolism is derived primarily from studies performed in recent years. Metabolites which have been identified from *in vivo* and *in vitro* studies include 2-chloroethanol, 2-chloroacetic acid, thiodiglycolic acid, and cysteine and glutathione derivatives.[3,9,36] In addition to the reactive metabolite, 2-chloroacetaldehyde, other possible reactive products formed by glutathione-dependent reactions, such as episulfonium ion, may be involved in reactions leading to tissue binding and

damage.[9] Mammalian metabolism of EDC has been studied only in the mouse and rat. In mice, Yllner recovered all administered radioactivity in urine and expired air within 24 hr.[36] Also, Reitz et al. have found that EDC is extensively metabolized in the rat.[25,26] The studies by Yllner and Reitz demonstrated that the primary route of elimination of EDC is by metabolism. Renal excretion of nonvolatile metabolites predominates.

Yllner identified three principal metabolites in urine of mice, 5-carboxymethylcysteine, thiodiglycolic acid, and chloroacetic acid.[36] In rats, Spreafico et al.[29] found the principal metabolite in rats to be thiodiglycolic acid. Chloroacetic acid was not detected, and 5-carboxymethylcysteine was detected in trace amounts. On the other hand, Reitz[25,26] detected two principal metabolites in rats, thiodiglycolic acid and its sulfoxide, although detailed identification procedures were not included in their reports.

Two principal pathways involving microsomal and cystosolic enzymes have been proposed for metabolism of EDC. Yllner originally proposed that conversion of EDC to 2-chloroacetic acid occurs through the formation of 2-chloroethanol, then 2-chloroacetaldehyde.[36] The former was identified as a minor metabolite in mice treated with EDC. Recently, Guengerich et al.[9] showed that 2-chloroethanol was a product of rat microsomal mixed-function oxidation of EDC. They proposed that part of EDC metabolism proceeded through the formation of chlorohydrin, which spontaneously dehydrohalogenates to form chloroacetaldehyde. The chloroacetaldehyde is then reduced to chloroethanol by alcohol dehydrogenase or oxidized to 2-chloroacetic acid by aldehyde dehydrogenase. The reaction proceeds in either direction depending on enzyme, substrate, and cofactor concentration. Covalent binding of EDC to microsomal protein was inhibited by the addition of alcohol or aldehyde dehydrogenase to the reaction mixture, suggesting that the aldehyde was the reactive intermediate responsible for EDC irreversible binding.[9]

The metabolism of EDC produces reactive metabolites of an electrophilic nature, including chloroacetaldehyde and the half-mustard S-(2-chloroethyl)glutathione.[3,9] These metabolites react covalently with cellular macromolecules.[4,9] Such reactions are known to be related to carcinogenic, mutagenic, and teratogenic responses. Reitz et al. determined that covalent binding to protein and DNA in rats exposed to EDC orally or by inhalation was not significantly different.[25,26] In contrast to the low covalent binding potential of EDC *in vivo*, the covalent binding of EDB was significantly higher.[7,9] Carcinogenicity of EDB was enhanced by dietary administration of DS, an inhibitor of aldehyde dehydrogenase which blocks further oxidation of bromoacetaldehyde with an increase of covalent binding.[15,22] Dietary DS increased covalent binding of EDC in the nuclei of liver cells in rats dosed with EDB.[21] In addition, orally administered DS and related compounds, e.g., diethyldithiocarbamate, caused an increase in EDB binding to DNA, RNA, and protein.[7]

III. MATERIALS AND METHODS

A. Animals

The male and female Sprague-Dawley rats used in these studies were utilized at the termination of the chronic studies. At the time these studies were initiated, the animals were 24 to 25 months old and weighed 463 to 667 g (males) and 286 to 469 g (females). For additional information on the housing and care for these animals, please see reference No. 16.

For the preliminary investigation performed to assess blood levels of EDC following oral dosing, and in studies to determine the elimination and tissue distribution of ^{14}C-EDC in younger rats, male and female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). Upon arrival, they were identified with metal eartags carrying unique identification numbers and were acclimated for a minimum of 7 days under test conditions. An attending veterinarian examined and released the animals for the study. At the initiation of the elimination studies in these animals, they were 4 to 5 months old and weighed 502 to 522 g (males) and 267 to 322 g (females).

The animals were housed in environmentally controlled rooms with 10 to 15 air changes per hour. The rooms were maintained at a temperature of 72 ± 3°F and humidity of 50 ± 15%, with a 12-hr light/dark cycle per day. During the acclimation period, the rodents were housed in polycarbonate cages on Ab-Sorb-Dri® (Ab-Sorb-Dry Company, Garfield, New Jersey) hardwood chip bedding. Throughout the study the animals were provided with certified rodent chow. Tap water was provided ad libitum. There were no known contaminants in the food or water that would interfere with the study.

Animal care and housing were in accordance with DHEW Publication No. (NIH)-78-23, 1978, "Guidelines for the Care and Use of Laboratory Animals," and the MRI Manual for Animal Care.

B. Chemicals

Nonlabeled EDC used in these studies was obtained from the same lot (No. 2401HL) utilized for the chronic inhalation experiments. Bulk chemical characterization for this lot is reported in reference No. 16. EDC and 1,1,2-trichloroethane (TCE), used as standards in the analysis for EDC in blood, were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin). The EDC was found to have a purity of greater than 99% by gas chromatography. The standards used to develop the liquid chromatographic system for the characterization of EDC metabolites in rat urine were thiodiglycolic acid (TDGA), thiodiglycolic acid sulfoxide (TDGAS), and monochloroacetic acid (MCAA). TDGA was obtained from the Sigma Chemical Company (St. Louis, Missouri). MCAA was purchased from Fisher Scientific (Fairlawn, New Jersey). TDGAS was prepared as described by Dr. R. H. Reitz (personal communication). The TDGA was reacted with 5% hydrogen peroxide for 2 hr at room temperature; then the excess water was removed under a stream of air.

The residue was a white crystalline product whose major component exhibited different ion exclusion chromatographic characteristics as compared to the reaction precursor.

Uniformly labeled ^{14}C-1,2-dichloroethane (^{14}C-EDC) used in the elimination and binding experiments was purchased from New England Nuclear (Boston, Massachusetts). The radiochemical purity was specified to be 98%. Upon receipt, the radiolabeled compound was stored frozen (-20°C) under conditions to minimize decomposition. The compound was analyzed for radiochemical purity and composition using chromatographic procedures.

C. Dosage and Treatments

^{14}C-EDC was administered orally at a dose of 150 mg/kg body weight. The EDC solutions were prepared by mixing appropriate amounts of the ^{14}C-labeled and nonlabeled compounds with Mazola® corn oil to yield 150 mg of EDC/2 mL of oil at a concentration of 75 mg/mL. For the elimination studies the specific activity ranged from 1,709 to 2,076 dpm/μg; each rat received 115 to 140 μCi/kg body weight. For the DNA binding experiments, each rat received from 241 to 260 μCi/kg body weight at a specific activity of 3,564 to 3,847 dpm/μg.

D. Experimental Design

The chronic studies were performed with the six treatment groups shown below. Rats from four groups (control, EDC, EDC/DS, and EDC/EtOH) were used for measurement of EDC levels in blood. Rats from the six dose groups were utilized in the elimination studies and in the DNA binding experiments following treatment with oral doses of ^{14}C-EDC.

Group	Air	Diet	Water
Control	Filtered	Standard	Distilled
DS	Filtered	0.05% DS	Distilled
EtOH	Filtered	Standard	5% ethanol
EDC	EDC (50 ppm)	Standard	Distilled
EDC/DS	EDC (50 ppm)	0.05% DS	Distilled
EDC/EtOH	EDC (50 ppm)	Standard	5% ethanol

1. Blood level studies: Five male and five female rats from four treatment groups (control, EDC, EDC/DS, and EDC/EtOH) were bled from the orbital sinus at 15 min following termination of the 7-hr exposure period. A second blood sample was collected 2 hr later. Blood was analyzed for EDC using a modification of the gas chromatographic procedure of Zuccato et al.[38] Detection by electron capture was used instead of the flame ionization detection employed by Zuccato.

To set up and validate the procedure before use, a few naive younger rats were treated with oral doses of EDC at 15, 50, and 150 mg/kg and were bled for analysis of EDC. The data obtained from these studies facilitated the selection of the time periods used in the sampling from animals in the chronic study.

Blood samples (1 mL) were collected and injected immediately into septum vials (10-mL capacity) containing 3 mL of 10% citric acid. The vials were stored on ice until analysis. The vials were then agitated for at least 30 min at 40°C, and a sample of the equilibrated headspace was taken for analysis.

2. Elimination studies: Three male and three female rats from each of the six dose groups were used to assess the excretion of radioactivity in the expired air (^{14}C-EDC and $^{14}CO_2$), urine, and feces following oral dosing of ^{14}C-EDC (150 mg/kg) in corn oil (2 mL/kg). Immediately following dosing, the rats were placed in individual Delmar-Roth glass metabolism cages designed for separate collection of urine, feces, and expired air. The airflow in the cages was maintained at 200 mL/min. Expired air was sampled sequentially. Nonmetabolized EDC was absorbed on activated charcoal (grade 950, Witco Chemicals, New York, New York). The charcoal (10 g) was placed into specially designed columns manufactured by Wyse Glass Specialties, Inc. (Freeland, Michigan). These columns were replaced at the times indicated below. Carbon dioxide was trapped in 5 M ethanolamine in 2-methoxyethanol during the intervals shown below.

Reitz et al.[26] presented only one value for total amounts eliminated in expired air, urine, or feces during the collection period (48 hr). In the present study, the rates of excretion were assessed by serial sampling of the expired air and urine. Expired EDC was collected at 1, 3, 6, 12, and 24 hr after dosing. $^{14}CO_2$ was sampled at 3, 6, 12, and 24 hr, and urine was collected on dry ice and sampled at 6, 12, and 24 hr. Only one fecal sample was collected at 24 hr. At the end of the 24-hr collection period the animals were kept for utilization in the binding studies.

Based on data generated earlier in our laboratories, it was expected that almost all of the ^{14}C would be eliminated by 24 hr. This was confirmed in a pilot experiment in which six younger rats (about 4 months old) were treated with oral doses of ^{14}C-EDC and were used to assess the rates of ^{14}C elimination in expired air, urine, and feces.

For analysis of EDC metabolites in urine, the urine collected on dry ice was centrifuged. Aliquots of the clear supernatant were analyzed using an HPLC procedure developed in our laboratories. Available standards were co-chromatographed with the urine.

3. Binding studies: Three male and three female rats from each of the six treatment groups were dosed orally with ^{14}C-EDC of high specific activity. The same animals utilized to assess EDC elimination were used to determine DNA binding following a recovery period ranging from 3 to 7 days. With the limited amounts of radioactivity administered in the elimination

studies and with the rapid rates of EDC elimination, only traces of radioactivity were expected to remain in the tissues of these animals. The animals were sacrificed 6 hr later. At sacrifice, the rats were anesthetized with ether and blood was withdrawn from the abdominal aorta. The livers were removed, rinsed with 0.1 M tris/0.01 M EDTA, weighed, and immediately frozen in liquid nitrogen and stored at -70°C until DNA isolation. Before freezing, ~ 0.5-g portions were removed for histopathological examination and for ^{14}C analyses.

E. Hepatic DNA Binding

The livers were processed for DNA isolation and purification according to the procedures of Joyce and Daniel.[11] DNA was quantitated using the 3,5-diaminobenzoic acid fluorescence method of Kissane and Robins.[13] The extent of binding of radioactivity to hepatic DNA was expressed as micromoles of EDC per mole of deoxyribonucleotide.

1. Isolation and purification: Hepatic DNA was isolated and purified by a modification of the method of Joyce and Daniels. The liver tissue was homogenized in two volumes of 0.1 M tris/0.01 M EDTA, pH 7.7, with a Polytron for 20 to 30 sec. A 0.5-mL sample of the liver homogenate was removed and stored at -20°C. A volume of 15% sodium dodecyl sulfate was added to give a final concentration of 1.5%. The homogenate was then treated with 100 µg/mL pronase B (Cal Biochem, La Jolla, California) at 37°C for 1 hr. Following incubation, the homogenate was extracted with an equal volume of Kirby's phenol (phenol:m-cresol:8-hydroxyquinoline:water, 500:70:0.5:55, by weight) for 30 min at room temperature with gentle shaking. After centrifugation (8,500 x g, 4°C, 30 min), the upper aqueous layer was transferred to a clean tube and extracted with an equal volume of chloroform: isoamyl alcohol (24:1, v/v) for 30 min with gentle shaking, and then centrifuged as described above. The nucleic acid solution was precipitated by addition of 1.5 volumes of cold absolute ethanol and placed at -20°C overnight.

The nucleic acids were removed from the ethanol by winding onto a glass rod and resuspended in 0.1 M tris/0.01 M EDTA, pH 7.7, at approximately 2 mg/mL. This solution was then treated with 100 µg/mL RNase A-amylase (Sigma Chemical Company, St. Louis, Missouri) for 1 hr at 37°C and then again with 100 µg/mL pronase B for 1 hr at 37°C. The solution was extracted with Kirby's phenol and chloroform:isoamyl alcohol as described above. The final aqueous extract was made 1% with respect to a saturated sodium chloride solution, and the DNA was precipitated by addition of 1.5 volumes of cold absolute ethanol. The DNA was allowed to precipitate overnight at -20°C and was then washed twice with absolute ethanol. The DNA was dried for 1 hr under a stream of nitrogen. The DNA was weighed and stored desiccated at 4°C. Additional chloroform:isoamyl alcohol extractions were sometimes required to remove residual phenol.

2. DNA analysis: The DNA was resuspended in a minimal volume of 0.1 M tris, pH 7.7, and analyzed by the fluorometric method of Kissane and Robins with modifications by Setaro and Morley.[28] A 5 mg/mL stock calf thymus DNA (Sigma Chemical Company) in 1 N NH_4OH solution was prepared.

Dilutions were made to give 0.31, 0.63, 1.25, and 2.5 mg/mL DNA standards. A 10 mg/mL standard was prepared by using twice the volume of the 5 mg/mL standard in the assay. All DNA standards and hepatic DNA samples were diluted 1 to 10 with 1 N NH_4OH. A 10-μL aliquot of each dilution was placed in a 13 x 100 test tube and then dried at 60°C for approximately 2 hr. Diaminobenzoic acid dihydrochloride (Sigma Chemical Company) was added to give a final concentration of 30 mg. The tubes were corked, vortexed, and placed in a 60°C water bath for 30 min. Before reading at 520 nm (with a 420-nm excitation wavelength) on an Aminco-Bowman spectrofluorometer, the product was diluted with 2 mL of 0.6 N perchloric acid.

3. Digestion of DNA: A 0.5-mL aliquot was removed from each rat hepatic DNA sample, and 0.2 mL of 35% perchloric acid was added. The solution was mixed and placed at 80°C for 30 min. The digested DNA samples were allowed to cool for 30 min at room temperature. Prior to scintillation counting, 10 mL of PCS cocktail (Amersham Corporation, Arlington Heights, Illinois) was added and the samples were counted for 20 min each.

4. Data analysis: Radioactivity bound to hepatic DNA is presented in terms of micromoles of ^{14}C-EDC per mole of DNA. Calculations were performed using 339.00 as the molecular weight for deoxyribonucleotides and 187.88 as the molecular weight for EDC (Merck Index). Calculations for each analysis were performed as follows:

a. The specific activity of ^{14}C-EDC (3,564 or 3,847 dpm/μg) was converted to dpm/μM by multiplying by the molecular weight of EDC (187.88).

b. The micromoles of EDC per 0.5-mL sample of DNA were determined by dividing the dpm per DNA sample by the specific activity in terms of dpm/μM.

c. The moles of DNA per 0.5-mL sample were calculated by dividing the sample weight (grams DNA per 0.5-mL sample) by the molecular weight of deoxyribonucleotides (339.00).

d. The micromoles of ^{14}C-EDC per 0.5-mL sample were then divided by the moles of DNA per 0.5-mL sample to determine the micromoles of ^{14}C-EDC bound per mole DNA.

F. Analysis of EDC in Blood

1. Gas chromatography (GC): The analysis of EDC in rat blood was performed using a Varian 3700 GC equipped with an electron capture detector. The chromatographic separation was obtained using a 6 ft x 4 mm ID glass column packed with 20% SP-2100/0.1% Carbowax 1500 on Supelcoport 100/120. Nitrogen was used as the carrier gas at a flow rate of 70 cc/min. The injector was maintained at 200°C and the detector at 250°C. The separation was performed isothermally at 65°C; then the column temperature was briefly raised to 85°C between sample analyses to remove other retained materials. Trichloroethane (TCE) was used as an internal standard for the analysis.

EDC and TCE eluted from this chromatographic system at approximately 3 and 6 min, respectively. Gas chromatograms of headspace samples of rat blood spiked with multiple levels of EDC and containing a constant amount of the internal standard (0.86 μg/mL blood) are illustrated in Figure 1.

2. Sample preparation: The internal standard, TCE, was prepared at a concentration of approximately 0.2 μg/mL in 10% citric acid. An aliquot (3.0 mL) of the citric acid solution containing the internal standard (TCE) was pipetted into 10-mL glass septum vials, and the vials were then sealed with Teflon®-faced silicone septa. The vials were refrigerated prior to and after the addition of animal blood until the equilibration period before the analysis. An aliquot of blood (1.0 mL) was carefully measured and then injected by syringe into the septum vial. Each vial was equilibrated for at least 30 min in a 40°C shaking water bath. Aliquots (0.1 cc) of the vials' headspace were chromatographed.

The standard curve was generated from control blood samples spiked with aqueous solutions of EDC.

3. Method of validation: For the analysis of rat blood following the chronic inhalation studies, an EDC standard response curve was established. EDC concentrations (ranging from 0.1 to 2.5 μg/mL EDC) versus the EDC/TCE chromatographic area ratio provided a linear regression line with a correlation of greater than 0.99. The average deviation of EDC/TCE area ratios obtained in duplicate injections for five levels of EDC spiked into blood was found to be 3.8%.

G. Determination of Urinary Profiles

1. High performance liquid chromatography (HPLC): The urinary metabolic profiles were obtained using a Waters M6000A solvent pump fitted with a Bio-Rad Aminex HPLX-87H column (300 x 7.8 mm ID). The mobile phase consisted of 0.005 N sulfuric acid at a flow rate of 0.5 mL/min. The retention indices of standards were determined using a Waters Model 440 absorbance detector with an extended wavelength module at 214 nm. For radioactive detection the eluate was mixed 1:3 with Fisher Scintiverse LC and monitored using a 500-μL flow cell with a Radiomatic Flo-One Model HP. The retentions of TDGA, TDGAS, and MCAA obtained by ultraviolet detection were 10, 15, and 18 min, respectively (see Figure 2). There is a delay of approximately 0.5 mL (1 min) between the detection on the UV and that on the radioactive monitor. A typical radiochromatograph of a urine sample is illustrated in Figure 3.

2. Sample preparation: Rat urine samples were frozen upon collection. They were thawed briefly for filtration through a 0.45-μm Acrodisc-CR (Gelman), and then refrozen until assay. Direct injection (100 μL) of the filtered urine was performed with no additional cleanup.

3. Method validation: The precision of the HPLC system for EDC urinary metabolite profiling was determined by analyzing a single urine

sample in triplicate. The urine was collected (0-24 hr) from a rat dosed orally with ^{14}C-EDC. The percentages of the three largest metabolites (TDGA, TDGAS, and the unknown at retention time 14.4 min) were found to be 69.7 ± 1.2%, 20.3 ± 1.0%, and 5.5 ± 0.1%, with RSDs of 1.7, 4.9, and 1.8%, respectively. The recovery of radioactivity from the ion exclusion column was conducted simultaneously with the precision study. The radioactivity in the chromatographic eluate was compared to the amount loaded into the injection valve. The results from triplicate analysis showed that 97 ± 2% of the radioactivity applied to the column was recovered in the eluate.

H. Radioactivity Analyses

1. Sample preparation: The charcoal was desorbed by shaking ∿ 1-g quantities in 15 mL of toluene-based scintillation fluid.* Radioactivity was determined using liquid scintillation counting. Aliquots (50 µL to 2 mL) of urine, cage rinse, CO_2 traps, and fecal homogenate were counted after addition of scintillation cocktail (PCS, Amersham Corporation, Arlington Heights, Illinois).

For studies in the younger rats, samples (50 µL to 0.5 mL) of whole blood, plasma, and RBC's were analyzed for total radioactivity. Livers, kidneys, lungs, GI tracts, and samples of muscle were weighed and homogenized in four volumes of 10% ethanol, and 0.5-mL aliquots (equivalent to 100 mg tissue) were measured for ^{14}C analysis. Samples (∿ 100 mg) of fat and skin were weighed and assayed directly. All organs and tissues were analyzed in duplicate and were combusted in a Packard Tricarb Sample Oxidizer (Model C306). Permafluor V® in combination with Carbo-Sorb® (Packard Instrument Company) was used as the scintillation cocktail for the oxidized samples.

2. Radioactivity measurement: Vials were cooled for a minimum of 24 hr before counting in a liquid scintillation counter (Packard Tricarb Model 3255). Correction for background was carried out automatically by the counter. Background determinations were obtained from the average of natural counts of several tissue homogenates from nontreated animals. The counting efficiency was determined using the automatic external standard (AES) method. An AES versus efficiency curve was prepared by processing a quench curve set through the counter under the conditions used throughout the experiment. Assays not within ± 10% of the mean of the duplicates were reassayed in duplicate except when the sample was no longer available or when radioactivity counts were low, i.e., less than two times the background. For these studies, background counts ranged from 25.0 to 35.3 counts per minute.

* For every 3 L of toluene, the fluid contains:
14.2 g of PPO: 2,5-diphenyl-1,3-oxazole
0.8 g of POPOP: 1,4-bis-[2-(4-methyl-5-phenyl-1,3-oxazolyl)]-benzene
300 mL of BBS-3: Bio-Solv™, Beckman Instruments, Fullerton, CA.

3. Data processing and analysis: Carbon-14 contents in blood and tissues are tabulated and presented in terms of microgram equivalents per milliliter (blood) or gram (tissue) and percentage of the administered dose. The percent of dose excreted in expired air, urine, and feces is also presented in tables as well as graphically.

Individual calculations for each sample were performed with an Apple II Plus computer as follows:

a. Cpm (counts per minute) for each sample was converted to dpm (disintegrations per minute).

$$\frac{\text{cpm}}{\text{efficiency}} = \text{dpm/sample}$$

b. Dpm per g or mL was calculated.

$$\frac{\text{dpm/sample}}{\text{sample weight or volume (g or mL)}} = \text{dpm/g or mL}$$

c. Dpm per g or mL was divided by the specific activity of the compound (dpm/μg) to obtain the μg equivalents/g or mL.

$$\frac{\text{dpm/g or mL}}{\text{specific activity}} = \mu\text{g/g or mL}$$

d. This was multiplied by the total weight or volume of the organ or excreta in order to obtain the total amounts in the organ or excreta.

$$\mu\text{g/g or mL} \times \text{total weight or volume} = \mu\text{g/organ or excreta}$$

e. The μg/organ or excreta was divided by the total dose administered in order to obtain the percentage of the administered dose.

$$\frac{\mu\text{g/organ or excreta} \times 100}{\text{total dose (in } \mu\text{g)}} = \% \text{ of administered dose}$$

The percent of administered dose recovered in blood, muscle, and skin was calculated based on 7, 40, and 16%, respectively, of body weight. Percent recovery in plasma and RBC's was calculated based on 60 and 40%, respectively, of the total blood volume. These estimates were based on data from the published literature.

IV. RESULTS

The studies presented below were performed with male and female Sprague-Dawley rats that were exposed during a 2-year period to EDC (50 ppm by inhalation), EDC plus DS (0.05% in feed), EDC plus EtOH (5% in drinking water), and the respective controls that were exposed to filtered air, DS, or EtOH. Groups of the male and female rats that were exposed to EDC were utilized to measure blood levels of EDC at the end of the 7-hr daily inhalation exposure. In addition, rats from all the dose groups were treated with oral doses of ^{14}C-labeled EDC (150 mg/kg) and utilized to assess the elimination of EDC and metabolites in urine, feces, and expired air. The same animals were later treated with another dose of ^{14}C-EDC and sacrificed at 6 hr to determine the hepatic DNA binding. Limited studies were performed to assess the elimination and tissue distribution of radioactivity in younger ($\sim$ 4 months old) rats treated with the same dose of ^{14}C-EDC.

A. EDC Blood Levels

Blood levels of EDC were measured using a modification of the chromatographic procedure of Zaccato et al. Blood was sampled at 0.25 and 2.25 hr after termination of inhalation exposure. Preliminary studies in rats treated with oral doses (15, 50, and 150 mg/kg) of EDC showed fast disappearance of EDC from blood.

The control rats (exposed to filtered air) showed no EDC in blood. Data from the other three groups studied (EDC, EDC plus DS, and EDC plus EtOH) are shown in Table 1 (individual animals) and in Table 2 (summary). In rats exposed to EDC only, small amounts of the parent chemical were recovered in blood at 15 min following termination of exposure (an average of 0.28 μg/mL in males and 0.25 μg/mL in females). These amounts remained relatively constant 2 hr later. Similar concentrations were recovered in blood of rats exposed to EDC plus EtOH. On the other hand, EDC levels in animals exposed simultaneously to EDC and DS were five times higher (1.5 μg/mL at 15 min). The EDC levels in these animals decreased slightly at 2 hr later, but the levels remained significantly higher than in animals exposed to EDC or to EDC plus EtOH.

B. ^{14}C Elimination

Data from earlier studies performed in our laboratory showed that ^{14}C elimination in rats treated with oral doses of ^{14}C-EDC was essentially complete within 24 hr after dosing. These findings were confirmed in the studies (presented below) in which younger ($\sim$ 4 months old) rats were treated with oral doses of ^{14}C-EDC (150 mg/kg). The studies with animals exposed chronically to EDC and other treatments were, therefore, terminated at 24 hr following administration of oral doses of ^{14}C-EDC. This allowed treatment of all six groups within 10 days following termination of the chronic studies. During this 10-day period the animals receiving DS in diet or EtOH in the drinking water continued to receive these treatments until the metabolism

and binding studies were completed. The rats used in these studies were not sacrificed at the end of the collection periods but were allowed to recover for use in the DNA binding studies. To allow complete recovery of the expired radioactivity, several traps were used to collect the parent compound (in charcoal) and any oxidation metabolites, e.g., $^{14}CO_2$ (in ethanolamine/methoxyethanol). Methanol traps surrounded with dry ice were placed between the charcoal and alkaline traps to prevent any ^{14}C carryover between these traps.

The data from individual animals are shown in Tables 3 through 8 and are summarized in Tables 9 and 10 for males and females, respectively. Also, cumulative excretion of radioactivity in urine, feces, and expired air is presented graphically in Figures 4 and 5. In control rats, 30% (males) and 28% (females) of the administered doses were excreted as non-metabolized EDC during 24 hr after dosing. Most of the expired EDC was eliminated during the first 6 hr after treatment. Expired $^{14}CO_2$ was very limited (< 1% of the administered doses). Excretion of ^{14}C in urine was slower, but the total amount eliminated in 24 hr was higher than in expired air (47% in males and 55% in females). Only 1 to 2% of the dose was recovered in feces during the 24-hr period.

Male rats exposed to EDC during the 24-month period showed an elimination pattern similar to the controls, while the females showed slightly higher recovery in expired air, primarily as EDC. Rats receiving EtOH in drinking water demonstrated similar elimination as in controls. On the other hand, a different elimination pattern was demonstrated in rats exposed to EDC and EtOH. While EDC elimination in expired air decreased, the excretion of ^{14}C in urine showed some increases.

In both the DS and EDC plus DS-treated groups, the elimination of radioactivity in expired air and urine was significantly different from the control rats or rats treated with EDC, EtOH, or EDC plus EtOH. In the DS-treated groups, EDC elimination increased to 41% (males) and 55% (females) while ^{14}C urinary excretion decreased to 35% (males) and 36% (females). In rats treated with EDC and DS, 58% of the dose was eliminated in expired air as EDC in both the males and females. Excretion of ^{14}C in urine was reduced to 28% in males and 25% in females.

To allow comparison of these data to those obtained from younger ($\sim$ 4 months old) animals, male and female Sprague-Dawley rats were treated with the same dose (150 mg/kg) of ^{14}C-EDC and placed in glass metabolism cages for excreta collection during the same time periods. At the end of 24 hr, these animals were sacrificed for tissue sampling to account for total ^{14}C recovery. The excretion data from these animals are shown in Table 11, while tissue distribution data are presented in Table 12. Figure 6 shows the cumulative elimination of radioactivity in expired air, urine, and feces.

In the male rats, excretion of ^{14}C in expired air and urine totaled 36 and 40%, respectively, in 24 hr. Almost all the ^{14}C excreted in the expired air was in the form of unmetabolized EDC. Also, significant amounts of the administered doses (5%) were recovered in feces. The female rats

showed similar excretion patterns. The rates of ^{14}C elimination (percent of dose per hour) were faster than in older rats and the total recovery in 24 hr was higher.

Radioactivity remaining in tissue 24 hr following treatment of the younger rats with ^{14}C-EDC is shown in Table 12. In male rats, the highest concentrations of ^{14}C were found in liver, kidneys, and GI tracts. The blood radioactivity was evenly distributed between plasm and red cells. Female rats demonstrated similar distribution of ^{14}C in tissue and blood as compared to males.

C. Urinary Metabolic Profiles

Urine collected from all groups of rats used in the elimination studies was used to determine the profiles of EDC metabolites. A high performance liquid chromatography (HPLC) procedure was developed and used to determine the metabolic profiles in urine of rats treated with ^{14}C-EDC. Standard metabolites obtained commercially (TDGA and MCAA) or synthesized in our laboratory (TDGAS) were used to assist in metabolite identification. The urinary metabolites were tentatively identified as TDGA and TDGAS (major) and MCAA (minor). Two other unidentified minor metabolites eluting after 9 and 14 min were present. No attempt was made to isolate and identify these metabolites.

Prior to determination of the metabolic profiles in urine of different groups, three samples from selected animals were analyzed. These samples were collected between 0-6 hr, 6-12 hr, and 12-24 hr. Since the data (Table 13) indicated no significant difference between these samples, one sample of the 24-hr pooled urine was generated from each animal and used for analysis.

The data obtained from HPLC analyses of urine from individual animals are shown in Tables 13 and 14 and summarized in Table 15. In control rats, most of the urinary radioactivity was recovered as TDGA (65-68%) and as TDGAS (24-27%). Only 1% of the urinary ^{14}C was identified as MCAA. The two unknown minor metabolites constituted about 2 and 5% of the radioactivity. No EDC was detected. Urine from all treatment groups showed similar metabolic profiles although urine of DS-treated rats showed higher elimination of MCAA. The urine from the female animals in all treatment groups had slightly higher TDGA and lower oxidation products (TDGAS) compared to the males.

D. DNA Binding

Rats from all exposure groups were treated with oral doses of ^{14}C-EDC and livers were removed at 6 hr for assessment of the binding to hepatic DNA. The data from these studies are presented in Tables 19 (individual animals) and 20 (summary). These data showed that the binding of EDC metabolites to hepatic DNA was limited (36-44 μmol/mole DNA). The binding was similar in all dose groups. EDC binding was generally lower in the female rats except in the EDC treatment group.

V. DISCUSSION

Concurrent administration of EDC and DS resulted in an increase in hepatic, testicular, and mammary tumors compared to rats treated with EDC alone.[16] A stronger carcinogenic combination was reported for EDB and DS.[15] In addition to enhancing EDB carcinogenicity, DS also increased organ toxicity and lethality due to EDB. The enhancement of EDC and EDB toxic effects by DS contrasts with the known effects of this antioxidant which inhibits the toxicity and carcinogenicity of several compounds.[7a,33] Studies on the mechanisms involved in this toxic combination are, therefore, essential to safety assessment.

Blood levels of EDC were very low following exposure to EDC by inhalation. EDC disappearance from blood following termination of exposure was slow; the levels remained the same during the first 2 hr after exposure. In animals treated with ethanol, EDC levels were similar to the control animals, but in rats treated with DS, EDC levels were significantly higher. Whether differences in DS-treated animals are due to slower biotransformation of EDC or simply due to slower EDC elimination is not certain. However, DS-treated rats eliminated more of the nonmetabolized EDC in the expired air, which is probably due to a slower rate of EDC biotransformation in the presence of DS. This drug is a known enzyme inhibitor which, in addition to inhibiting the mixed-function oxidase activity, also inhibits aldehyde dehydrogenase and, to a lesser extent, alcohol dehydrogenase activities.[37]

In the present study, the disposition data obtained from older rats were similar to those obtained from younger animals. However, the data also showed that the older rats had slower elimination and lower recovery in excreta than the younger animals. The higher adipose tissue in older rats may explain the lower ^{14}C recovery during the first 24 hr after dosing. However, slower fecal excretion resulting from slower GI emptying or slower rates of biliary elimination may also explain these findings. The extent of biliary excretion of EDC metabolites is not known, but glutathione reaction products are known to be excreted to a significant extent in bile.

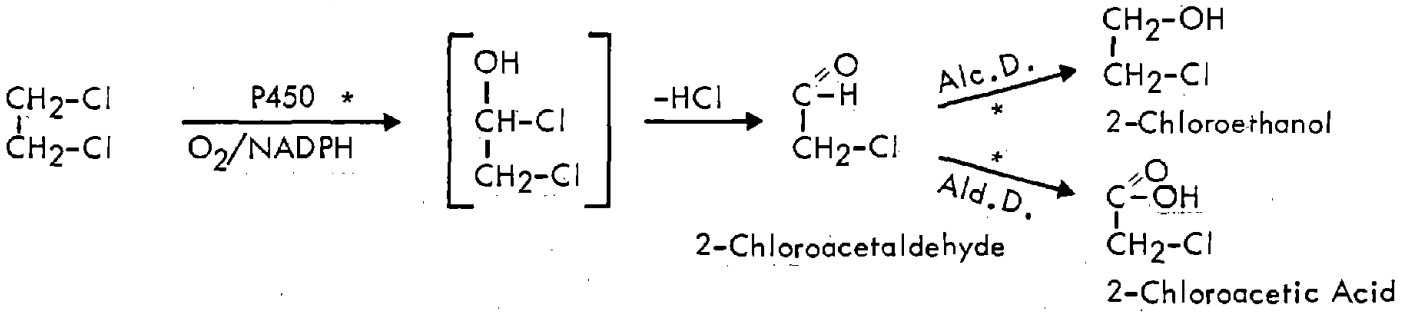
Although the disposition in older rats was similar to that observed in younger animals, a significant difference from reported studies[25,26] was the very limited excretion of $^{14}CO_2$ in expired air. The lower excretion of $^{14}CO_2$ also contrasts with our earlier findings which demonstrated higher $^{14}CO_2$ elimination in the expired air. The conditions used in the present study were similar to those reported by Reitz et al.[25,26] Although the limited recovery of expired $^{14}CO_2$ may have been due to trapping with "alkaline" charcoal, it might have also been due to differences in the rat strains used by Reitz (Osborne-Mendel) or in our earlier studies (Fischer 344).

The binding of EDC or its metabolites to hepatic DNA was limited. This is in contrast to the finding with EDB which is known to have significant DNA binding. Both DS and/or EtOH had no effect on the extent of binding. This also contrasts with earlier findings which showed increased EDB binding in the presence of DS.[7,21] Although the effect of DS on EDB binding was not examined in rats treated chronically, studies in younger rats showed

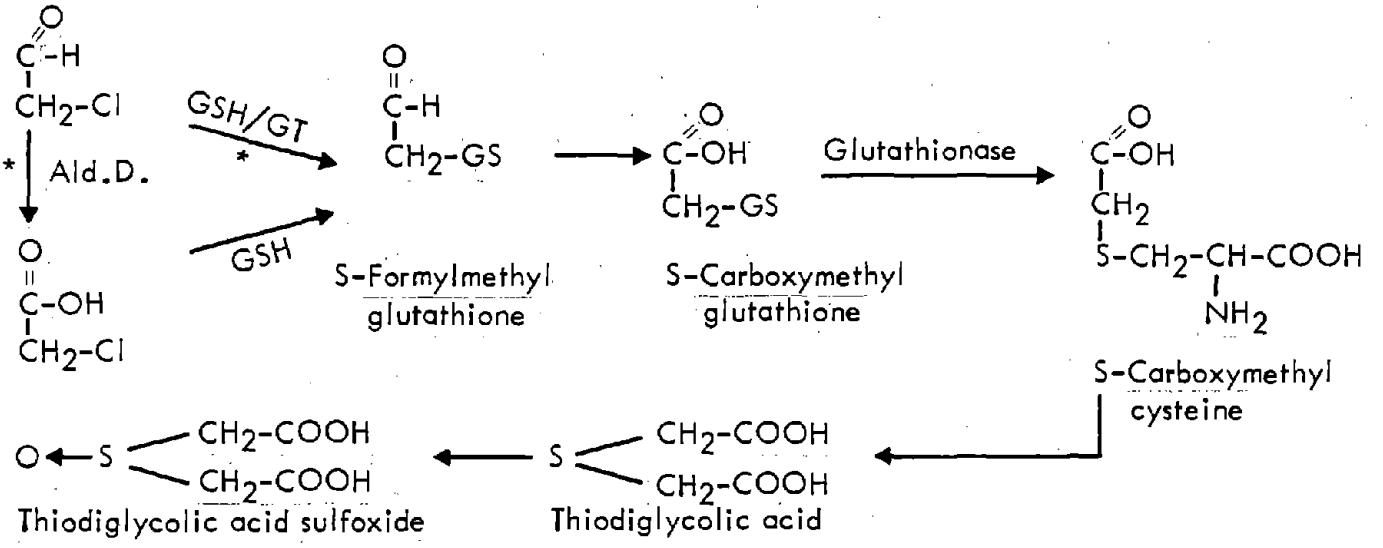
that short-term administration of DS in the diet or single dosing of DS by gavage increased EDB binding to hepatic macromolecules.[7] Plotnick et al.[21] showed that feeding DS in the diet for 10 days increased the binding of EDB metabolites to liver cell nuclei. In our laboratory, administration of a single dose of DS, its metabolite, diethyldithiocarbamate, or related prodcuts, e.g., ziram, increased the binding of EDB to DNA, RNA, and protein.[7]

Urinary metabolites separated by HPLC showed two major products, thiodiglycolic acid and its oxide, and three minor products (monochloroacetic acid and two unidentified metabolites). These findings are similar to those reported by Reitz et al. in which the two major metabolites were detected in rat urine.[25,26] However, our data and those of Reitz contrast with those of Spréafico[29] in which only the thiodiglycolic acid was detected in rat urine. The metabolic data were similar for male and female rats, although the sulfoxide derivative was produced to a smaller extent in the females. Urinary metabolites were not significantly altered in rats treated with DS, ethanol, or EDC or with a combination of EDC and DS or EDC and EtOH although in the DS-treated rats an increase in a minor metabolite, MCAA, was noted.

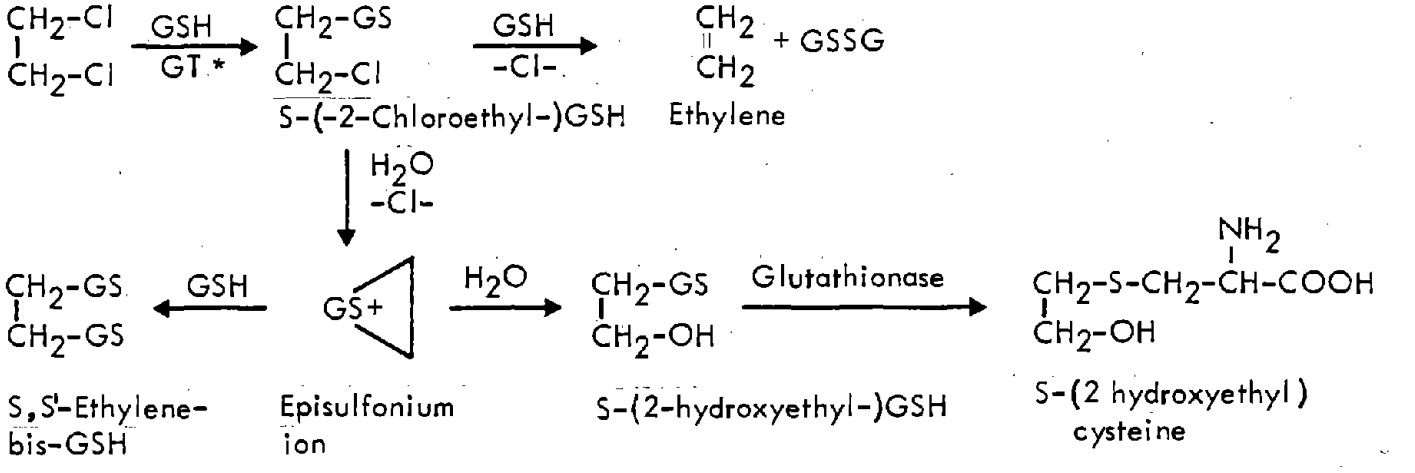
The metabolism of EDC occurs through multiple microsomal and cytosolic pathways (Chart I). Theoretically, DS can alter EDC metabolism and, accordingly, its elimination and binding, at several of these pathways. Alteration of aldehyde dehydrogenase activity by DS was expected to result in increased accumulation of the toxic metabolite, chloroacetaldehyde. However, the increased elimination of MCAA in urine suggest that inhibition of the activity of this enzyme did not occur. DS effects on the biotransformation of EDC in the present study appeared limited in causing a decrease in the total metabolism of this compound. Therefore, the reduced EDC elimination rather than changes in its biotransform and binding may have contributed to the increased incidence of tumors demonstrated in rats exposed to EDC and DS.



(1) Microsomal Metabolism of 1,2-Dichloroethane



(2) Further Microsomal Metabolism of 2-Chloroacetaldehyde



(3) Cytosolic Metabolism of 1,2-Dichloroethane

* Possible disulfiram effects

Chart I: Metabolic Pathways of 1,2-Dichloroethane

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TABLE 1

LEVELS OF EDC IN BLOOD OF INDIVIDUAL S.D. RATS FOLLOWING 2 YEARS OF INHALATION EXPOSURE TO 50 ppm OF EDC[a]

	μg EDC/mL Blood				
Male Rat No.	Sampling Time (hr)[b] 0.25	2.25	Female Rat No.	Sampling Time (hr)[b] 0.25	2.25
			EDC Group		
AX010	0.2	0.3	AX360	0.2	0.2
AX013	0.3	0.3	AX387	0.3	0.3
AX268	0.5	0.2	AX420	0.3	0.3
AX303/679	0.2	0.1	AX773	0.2	0.3
AX318	0.2	0.2	AX932	0.3	0.3
Mean ± S.E.	0.28 ± 0.13	0.22 ± 0.08		0.26 ± 0.05	0.28 ± 0.04
			EDC/DS Group		
AX048	2.0	1.5	AX397	2.4	1.1
AX060	0.9	1.1	AX405	1.2	1.0
AX065	1.9	1.5	AX466	1.4	1.0
AX242	1.4	0.9	AX467	1.4	1.2
AX256	1.1	1.0	AX877	1.3	1.1
Mean ± S.E.	1.46 ± 0.48	1.2 ± 0.28		1.54 ± 0.49	1.08 ± 0.08
			EDC/EtOH Group		
AX206	0.3	0.4	AX388	0.4	0.5
AX298	0.3	0.4	AX409	0.3	0.3
AX304	0.2	0.2	AX669	0.3	0.4
AX728	0.7	0.6	AX818	0.3	-[a]
AX841	0.3	0.3	AX869	0.2	0.2
Mean ± S.E.	0.36 ± 0.19	0.38 ± 0.15		0.30 ± 0.07	0.35 ± 0.13

a Blood from control groups (5 males and 5 females) showed no detectable EDC. Detection limit was 0.1 μg EDC/mL blood.

b After termination of exposure.

TABLE 2

LEVELS OF EDC IN BLOOD OF MALE AND FEMALE S.D. RATS FOLLOWING 2 YEARS OF INHALATION EXPOSURE TO 50 ppm OF EDC

Treatment Group	Males[a]		Females[a]	
	0.25 hr[b]	2.25 hr	0.25 hr	2.25 hr
	µg EDC/mL Blood			
EDC	0.28 ± 0.13	0.22 ± 0.08	0.26 ± 0.05	0.28 ± 0.04
EDC/DS	1.46 ± 0.48	1.20 ± 0.28	1.54 ± 0.49	1.08 ± 0.08
EDC/EtOH	0.36 ± 0.19	0.38 ± 0.15	0.30 ± 0.07	0.35 ± 0.13

a Mean ± S.E. of five determinations.
b Sampling time after termination of exposure.

TABLE 3

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO FILTERED AIR (CONTROL GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX046	AX162	AX171	Mean ± S.E.	AX356	AX361	AX416	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	5.485	11.373	10.354	9.071 ± 1.817	13.277	6.449	7.705	9.144 ± 2.098
	3	2.966	10.226	9.094	7.429 ± 2.255	10.212	5.669	4.975	6.952 ± 1.642
	6	2.019	8.236	8.044	6.100 ± 2.041	6.494	4.261	6.904	5.886 ± 0.821
	12	0.694	11.860	7.167	6.574 ± 3.237	4.719	3.261	5.821	4.600 ± 0.741
	24	0.788	1.991	1.095	1.291 ± 0.361	1.153	1.526	1.453	1.377 ± 0.114
Expired CO_2	3	0.169	0.180	0.170	0.173 ± 0.004	0.286	0.238	0.259	0.261 ± 0.014
	6	0.194	0.165	0.163	0.174 ± 0.010	0.261	0.246	0.264	0.257 ± 0.006
	12	0.051	0.036	0.048	0.045 ± 0.005	0.099	0.096	0.120	0.105 ± 0.008
	24	0.055	0.049	0.126	0.077 ± 0.025	0.073	0.103	0.076	0.084 ± 0.010
Urine	6	8.977	7.043	7.028	7.683 ± 0.647	10.838	9.149	11.607	10.531 ± 0.726
	12	12.865	19.689	15.490	16.015 ± 1.987	19.737	19.979	20.325	20.014 ± 0.171
	24	24.685	18.676	25.442	22.934 ± 2.140	17.705	31.490	24.028	24.408 ± 3.984
Feces	24	2.720	1.377	1.299	1.799 ± 0.461	0.705	1.462	1.146	1.104 ± 0.220
		Cumulative Percent of Dose							
Expired EDC	1	5.485	11.373	10.354	9.071 ± 1.817	13.277	6.449	7.705	9.144 ± 2.098
	3	8.451	21.599	19.448	16.499 ± 4.072	23.489	12.118	12.680	16.096 ± 3.700
	6	10.470	29.835	27.492	22.599 ± 6.102	29.983	16.379	19.584	21.982 ± 4.106
	12	11.164	41.695	34.659	29.173 ± 9.231	34.702	19.640	25.405	26.582 ± 4.388
	24	11.952	43.686	35.754	30.464 ± 9.535	35.855	21.166	26.858	27.960 ± 4.276
Expired CO_2	3	0.169	0.180	0.170	0.173 ± 0.004	0.286	0.238	0.259	0.261 ± 0.014
	6	0.363	0.345	0.332	0.347 ± 0.009	0.547	0.484	0.523	0.518 ± 0.018
	12	0.414	0.381	0.381	0.392 ± 0.011	0.646	0.580	0.643	0.623 ± 0.022
	24	0.469	0.430	0.507	0.469 ± 0.022	0.719	0.683	0.719	0.707 ± 0.012
Urine	6	8.977	7.043	7.028	7.683 ± 0.647	10.838	9.149	11.607	10.531 ± 0.726
	12	21.842	26.732	22.518	23.697 ± 1.530	30.575	29.128	31.932	30.545 ± 0.810
	24	46.527	45.408	47.960	46.632 ± 0.739	48.280	60.618	55.960	54.953 ± 3.597
Feces	24	2.720	1.377	1.299	1.799 ± 0.461	0.705	1.462	1.146	1.104 ± 0.220
Total	24	61.668	90.901	85.520	79.363 ± 8.983	85.559	83.929	84.683	84.724 ± 0.471

TABLE 4

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO DS (0.05%) IN THE DIET (DS GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX101	AX689	AX712	Mean ± S.E.	AX705[a]	AX914	AX578	Average
		Percent of Dose							
Expired EDC	1	6.277	4.787	10.122	7.062 ± 1.589		11.004	12.927	11.966
	3	4.788	4.379	12.037	7.068 ± 2.487		10.101	13.804	11.953
	6	6.036	5.793	12.611	8.147 ± 2.233		11.860	14.652	13.256
	12	8.842	12.401	14.191	11.811 ± 1.572		15.187	16.368	15.778
	24	5.028	11.896	3.586	6.837 ± 2.564		3.062	1.570	2.316
Expired CO_2	3	0.003	0.005	0.011	0.006 ± 0.002		0.014	0.016	0.015
	6	0.003	0.007	0.016	0.009 ± 0.004		0.023	0.017	0.020
	12	0.003	0.006	0.007	0.005 ± 0.001		0.025	0.033	0.029
	24	0.003	0.041	0.003	0.016 ± 0.013		0.010	0.010	0.010
Urine	6	2.326	0.866	3.663	2.285 ± 0.808		5.692	4.219	4.956
	12	8.598	3.226	10.793	7.539 ± 2.248		15.677	14.780	15.229
	24	34.398	20.284	21.617	25.433 ± 4.499		19.225	13.251	16.238
Feces	24	2.069	0.732	0.149	0.983 ± 0.568		0.008	0.299	0.154
		Cumulative Percent of Dose							
Expired EDC	1	6.277	4.787	10.122	7.062 ± 1.589		11.004	12.927	11.966
	3	11.065	9.166	22.159	14.130 ± 4.052		21.105	26.731	23.918
	6	17.101	14.959	34.770	22.277 ± 6.277		32.965	41.383	37.174
	12	25.943	27.360	48.961	34.088 ± 7.448		48.152	57.751	52.952
	24	30.971	39.256	52.547	40.925 ± 6.284		51.214	59.321	55.268
Expired CO_2	3	0.003	0.005	0.011	0.006 ± 0.002		0.014	0.016	0.015
	6	0.006	0.012	0.027	0.015 ± 0.006		0.037	0.033	0.035
	12	0.009	0.018	0.034	0.020 ± 0.007		0.062	0.066	0.064
	24	0.012	0.059	0.037	0.036 ± 0.014		0.072	0.076	0.074
Urine	6	2.326	0.866	3.663	2.285 ± 0.808		5.692	4.219	4.956
	12	10.924	4.092	14.456	9.824 ± 3.042		21.369	18.999	20.184
	24	45.322	24.376	36.073	35.257 ± 6.060		40.594	32.250	36.422
Feces	24	2.069	0.732	0.149	0.983 ± 0.568		0.008	0.299	0.154
Total	24	78.374	64.423	88.806	77.201 ± 7.063		91.888	91.946	91.917

a Found dead at ~ 9 hr after dosing.

TABLE 5

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO ETHANOL (5%) IN THE DRINKING WATER (EtOH GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX707	AX317	AX231	Mean ± S.E.	AX918	AX700	AX505	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	8.810	9.280	6.918	8.336 ± 0.722	8.303	6.566	9.198	8.022 ± 0.773
	3	8.146	5.691	5.898	6.578 ± 0.786	6.650	7.024	6.988	6.887 ± 0.119
	6	6.586	4.027	6.044	5.552 ± 0.779	4.447	7.889	5.819	6.052 ± 1.004
	12	3.900	8.069	6.597	6.189 ± 1.221	3.627	7.894	4.198	5.240 ± 1.337
	24	1.838	3.381	4.494	3.238 ± 0.770	2.712	6.120	1.879	3.570 ± 1.297
Expired CO_2	3	0.010	0.006	0.012	0.009 ± 0.002	0.023	0.007	0.034	0.021 ± 0.008
	6	0.014	0.003	0.011	0.009 ± 0.003	0.039	0.009	0.026	0.025 ± 0.009
	12	0.010	0.004	0.028	0.014 ± 0.007	0.039	0.016	0.143	0.066 ± 0.039
	24	0.013	0.038	0.134	0.062 ± 0.037	0.038	0.010	0.237	0.095 ± 0.071
Urine	6	5.207	4.225	5.366	4.933 ± 0.357	10.135	2.329	9.621	7.362 ± 2.521
	12	15.893	9.905	12.753	12.850 ± 1.729	13.908	20.985	11.434	15.442 ± 2.862
	24	19.995	35.495	27.842	27.777 ± 4.475	26.996	14.256	15.211	18.821 ± 4.097
Feces	24	1.691	2.790	3.367	2.616 ± 0.492	1.386	3.837	1.891	2.371 ± 0.747
		Cumulative Percent of Dose							
Expired EDC	1	8.810	9.280	6.918	8.336 ± 0.722	8.303	6.566	9.198	8.022 ± 0.773
	3	16.956	14.971	12.816	14.914 ± 1.195	14.953	13.590	16.186	14.910 ± 0.750
	6	23.542	18.998	18.860	20.467 ± 1.538	19.400	21.479	22.005	20.961 ± 0.795
	12	27.442	27.067	25.457	26.655 ± 0.609	23.027	29.373	26.203	26.209 ± 1.825
	24	29.280	30.448	29.951	29.893 ± 0.338	25.739	35.493	28.082	29.771 ± 2.940
Expired CO_2	3	0.010	0.006	0.012	0.009 ± 0.002	0.023	0.007	0.034	0.021 ± 0.008
	6	0.024	0.009	0.023	0.019 ± 0.005	0.062	0.016	0.060	0.046 ± 0.015
	12	0.034	0.013	0.051	0.033 ± 0.011	0.101	0.032	0.203	0.112 ± 0.050
	24	0.047	0.051	0.185	0.094 ± 0.045	0.139	0.042	0.440	0.207 ± 0.120
Urine	6	5.207	4.225	5.366	4.933 ± 0.357	10.135	2.329	9.621	7.362 ± 2.521
	12	21.100	14.130	18.119	17.783 ± 2.019	24.043	23.314	21.055	22.804 ± 0.899
	24	41.095	49.625	45.961	45.560 ± 2.471	51.039	37.570	36.266	41.625 ± 4.722
Feces	24	1.691	2.790	3.367	2.616 ± 0.492	1.386	3.837	1.891	2.371 ± 0.747
Total	24	72.113	82.914	79.464	78.164 ± 3.185	78.303	76.942	66.679	73.975 ± 3.669

TABLE 6

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO EDC (50 ppm) (EDC GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX010	AX013	AX268	Mean ± S.E.	AX360	AX387	AX420	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	8.678	8.480	6.754	7.971 ± 0.611	13.098	8.930	4.840	8.956 ± 2.384
	3	7.281	7.184	7.399	7.288 ± 0.621	14.421	7.893	6.951	9.755 ± 2.349
	6	7.847	5.612	5.660	6.373 ± 0.737	10.539	8.081	7.435	8.685 ± 0.946
	12	7.957	2.889	3.037	4.628 ± 1.665	4.110	13.003	9.454	8.856 ± 2.585
	24	1.866	0.820	0.522	1.069 ± 0.408	0.235	8.478	3.568	4.094 ± 2.394
Expired CO_2	3	0.005	0.004	0.009	0.006 ± 0.002	0.010	0.009	0.008	0.009 ± 0.001
	6	0.007	0.004	0.029	0.013 ± 0.008	0.010	0.020	0.015	0.015 ± 0.003
	12	0.024	0.014	0.035	0.024 ± 0.006	0.035	0.014	0.077	0.042 ± 0.019
	24	0.014	0.020	0.013	0.016 ± 0.002	0.012	0.005	0.015	0.011 ± 0.003
Urine	6	6.215	4.159	8.370	6.248 ± 1.216	9.033	4.506	6.279	6.606 ± 1.317
	12	10.714	10.027	20.954	13.898 ± 3.533	15.233	10.047	15.069	13.450 ± 1.702
	24	26.850	20.059	20.157	22.355 ± 2.248	11.709	8.022	21.941	13.891 ± 4.164
Feces	24	0.159	1.019	1.519	0.899 ± 0.397	1.897	0.516	0.268	0.894 ± 0.507
		Cumulative Percent of Dose							
Expired EDC	1	8.678	8.480	6.754	7.971 ± 0.611	13.098	8.930	4.840	8.956 ± 2.384
	3	15.959	15.664	14.153	15.259 ± 0.559	27.519	16.823	11.791	18.711 ± 4.637
	6	23.806	21.276	19.813	21.632 ± 1.166	38.058	24.904	19.226	27.396 ± 5.577
	12	31.763	24.165	22.850	26.259 ± 2.778	42.168	37.907	28.680	36.252 ± 3.981
	24	33.629	24.985	23.372	27.329 ± 3.184	42.403	46.385	32.248	40.345 ± 4.209
Expired CO_2	3	0.005	0.004	0.009	0.006 ± 0.002	0.010	0.009	0.008	0.009 ± 0.001
	6	0.012	0.008	0.038	0.019 ± 0.009	0.020	0.029	0.023	0.024 ± 0.003
	12	0.036	0.022	0.073	0.044 ± 0.015	0.055	0.043	0.100	0.066 ± 0.017
	24	0.050	0.042	0.086	0.059 ± 0.014	0.067	0.048	0.115	0.076 ± 0.020
Urine	6	6.215	4.159	8.370	6.248 ± 1.216	9.033	4.506	6.279	6.606 ± 1.317
	12	16.929	14.186	29.324	20.146 ± 4.657	24.266	14.553	21.348	20.057 ± 2.877
	24	43.779	34.245	49.481	42.502 ± 4.444	35.975	22.575	43.289	33.946 ± 6.065
Feces	24	0.159	1.019	1.519	0.899 ± 0.397	1.897	0.516	0.268	0.894 ± 0.507
Total	24	77.617	60.291	74.458	70.789 ± 5.327	80.342	69.524	75.920	75.262 ± 3.140

TABLE 7

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO EDC (50 ppm) AND DS (0.05%) IN THE DIET (EDC/DS GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX048	AX060	AX065	Mean ± S.E.	AX405	AX466	AX877	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	9.405	7.049	11.326	9.260 ± 1.237	7.661	9.963	12.767	10.130 ± 1.476
	3	11.246	8.996	13.520	11.254 ± 1.306	9.297	7.197	14.940	10.478 ± 2.312
	6	10.276	10.533	13.563	11.457 ± 1.055	11.590	12.035	15.653	13.093 ± 1.287
	12	13.265	15.559	20.269	16.364 ± 2.062	19.401	16.342	17.295	17.679 ± 0.904
	24	1.205	10.510	15.945	5.887 ± 2.686	7.446	9.606	2.154	6.402 ± 2.214
Expired CO_2	3	0.004	0.023	0.007	0.011 ± 0.006	0.006	0.017	0.010	0.011 ± 0.003
	6	0.005	0.006	0.005	0.005 ± 0.000	0.007	0.010	0.010	0.007 ± 0.001
	12	0.019	0.020	0.007	0.015 ± 0.004	0.022	0.020	0.006	0.016 ± 0.005
	24	0.002	0.001	0.002	0.002 ± 0.000	0.005	0.002	0.003	0.003 ± 0.001
Urine	6	4.103	2.228	2.310	2.880 ± 0.612	1.866	1.052	3.534	2.151 ± 0.730
	12	17.325	3.849	5.714	8.963 ± 4.216	6.670	4.931	9.065	6.889 ± 1.198
	24	16.766	19.222	11.187	15.725 ± 2.377	22.744	12.414	12.370	15.843 ± 3.451
Feces	24	1.165	0.130	1.368	0.888 ± 0.383	0.275	0.022	0.278	0.192 ± 0.085
		Cumulative Percent of Dose							
Expired EDC	1	9.405	7.049	11.326	9.260 ± 1.237	7.661	9.963	12.767	10.130 ± 1.476
	3	20.651	16.045	24.846	20.514 ± 2.542	16.958	17.160	27.707	20.608 ± 3.550
	6	30.927	26.578	38.409	31.971 ± 3.455	28.548	29.195	43.360	33.701 ± 4.833
	12	44.192	42.137	58.678	48.336 ± 5.205	47.949	45.537	60.655	51.380 ± 4.689
	24	45.397	52.647	74.623	57.556 ± 8.786	55.395	55.143	62.809	57.782 ± 2.514
Expired CO_2	3	0.004	0.023	0.007	0.011 ± 0.006	0.006	0.017	0.010	0.011 ± 0.003
	6	0.009	0.029	0.012	0.016 ± 0.006	0.013	0.027	0.020	0.020 ± 0.004
	12	0.028	0.049	0.019	0.032 ± 0.009	0.035	0.047	0.026	0.036 ± 0.006
	24	0.030	0.050	0.021	0.034 ± 0.009	0.040	0.049	0.029	0.039 ± 0.006
Urine	6	4.103	2.228	2.310	2.880 ± 0.612	1.866	1.052	3.534	2.151 ± 0.730
	12	21.428	6.077	8.024	11.843 ± 4.825	8.536	5.983	12.599	9.039 ± 1.926
	24	38.194	25.299	19.211	27.568 ± 5.596	31.280	18.397	24.969	24.882 ± 3.719
Feces	24	1.165	0.130	1.368	0.888 ± 0.383	0.275	0.022	0.278	0.192 ± 0.085
Total	24	84.786	78.126	95.223	86.045 ± 4.975	86.990	73.611	88.085	82.895 ± 4.653

TABLE 8

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO S.D. RATS EXPOSED FOR A 2 YEAR PERIOD TO EDC (50 ppm) AND ETHANOL (5%) IN THE DRINKING WATER (EDC/EtOH GROUP)

Excretum	Time After Dosing (hr)	Males				Females			
		AX206	AX298	AX304	Mean ± S.E.	AX818	AX409	AX669	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	4.617	7.467	5.750	5.945 ± 0.828	5.315	8.617	6.777	6.903 ± 0.955
	3	4.019	4.112	4.178	4.103 ± 0.046	3.814	6.003	4.227	4.681 ± 0.672
	6	5.406	2.386	2.455	3.416 ± 0.995	2.696	3.730	2.787	3.071 ± 0.331
	12	5.587	2.517	1.113	3.072 ± 1.321	2.233	2.561	1.585	2.126 ± 0.287
	24	1.194	1.707	0.554	1.152 ± 0.334	1.290	0.865	0.645	0.933 ± 0.189
Expired CO_2	3	0.008	0.435	0.020	0.154 ± 0.140	0.011	0.012	0.036	0.020 ± 0.008
	6	0.006	0.041	0.022	0.023 ± 0.010	0.027	0.021	0.172	0.073 ± 0.049
	12	0.024	0.012	0.033	0.023 ± 0.006	0.037	0.027	0.222	0.095 ± 0.063
	24	0.013	0.004	0.045	0.021 ± 0.012	0.012	0.005	0.034	0.017 ± 0.009
Urine	6	5.320	9.988	9.098	8.135 ± 1.431	12.044	15.871	11.854	13.256 ± 1.308
	12	17.889	18.959	19.679	18.842 ± 0.520	17.068	21.760	18.149	18.992 ± 1.419
	24	24.723	22.386	25.386	24.165 ± 0.910	28.523	16.697	23.240	22.820 ± 3.420
Feces	24	4.600	0.935	0.173	1.903 ± 1.366	0.817	1.128	0.737	0.894 ± 0.119
		Cumulative Percent of Dose							
Expired EDC	1	4.617	7.467	5.750	5.945 ± 0.828	5.315	8.617	6.777	6.903 ± 0.955
	3	8.636	11.579	9.928	10.048 ± 0.852	9.129	14.620	11.004	11.584 ± 1.611
	6	14.042	13.965	12.383	13.463 ± 0.541	11.825	18.350	13.791	14.655 ± 1.933
	12	19.629	16.482	13.496	16.536 ± 1.771	14.058	20.911	15.376	16.843 ± 2.160
	24	20.823	18.189	14.050	17.687 ± 1.971	15.348	21.776	16.021	17.715 ± 1.040
Expired CO_2	3	0.008	0.435	0.020	0.154 ± 0.140	0.010	0.012	0.036	0.019 ± 0.008
	6	0.014	0.476	0.042	0.177 ± 0.150	0.038	0.033	0.208	0.093 ± 0.058
	12	0.038	0.488	0.075	0.200 ± 0.144	0.075	0.060	0.430	0.188 ± 0.121
	24	0.051	0.492	0.120	0.221 ± 0.137	0.087	0.065	0.464	0.205 ± 0.129
Urine	6	5.320	9.988	9.098	8.135 ± 1.431	12.044	15.871	11.854	13.256 ± 1.308
	12	23.209	28.947	28.777	26.978 ± 1.885	29.112	37.631	30.003	32.249 ± 2.703
	24	47.932	51.333	54.163	51.143 ± 1.801	57.635	54.328	53.243	55.069 ± 1.321
Feces	24	4.600	0.935	0.173	1.903 ± 1.366	0.817	1.128	0.737	0.894 ± 0.119
Total	24	73.406	70.949	68.506	70.954 ± 1.414	73.887	77.297	70.465	73.883 ± 1.972

TABLE 9

ELIMINATION OF RADIOACTIVITY FROM MALE S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS[a]

Excretum	Time After Dosing (hr)	Control	DS	EtOH	EDC	EDC/DS	EDC/EtOH
				Percent of Dose			
Expired EDC	1	9.071 ± 1.817	7.062 ± 1.589	8.336 ± 0.722	7.971 ± 0.611	9.260 ± 1.237	5.945 ± 0.828
	3	7.429 ± 2.255	7.068 ± 2.487	6.578 ± 0.786	7.288 ± 0.621	11.254 ± 1.306	4.103 ± 0.046
	6	6.100 ± 2.041	8.147 ± 2.233	5.552 ± 0.779	6.373 ± 0.737	11.457 ± 1.055	3.416 ± 0.995
	12	6.574 ± 3.237	11.811 ± 1.572	6.189 ± 1.221	4.628 ± 1.665	16.364 ± 2.062	3.072 ± 1.321
	24	1.291 ± 0.361	6.837 ± 2.564	3.238 ± 0.770	1.069 ± 0.408	5.887 ± 2.686	1.152 ± 0.334
Expired CO_2	3	0.173 ± 0.004	0.006 ± 0.002	0.009 ± 0.002	0.006 ± 0.002	0.011 ± 0.006	0.154 ± 0.140
	6	0.174 ± 0.010	0.009 ± 0.004	0.009 ± 0.003	0.013 ± 0.008	0.005 ± 0.000	0.023 ± 0.010
	12	0.045 ± 0.005	0.005 ± 0.001	0.014 ± 0.007	0.024 ± 0.006	0.015 ± 0.004	0.023 ± 0.006
	24	0.077 ± 0.025	0.016 ± 0.013	0.062 ± 0.037	0.016 ± 0.002	0.002 ± 0.000	0.021 ± 0.012
Urine	6	7.683 ± 0.647	2.285 ± 0.808	4.933 ± 0.357	6.248 ± 1.216	2.880 ± 0.612	8.135 ± 1.431
	12	16.015 ± 1.987	7.539 ± 2.248	12.850 ± 1.729	13.898 ± 3.533	8.963 ± 4.216	18.842 ± 0.520
	24	22.934 ± 2.140	25.433 ± 4.499	27.777 ± 4.475	22.355 ± 2.248	15.725 ± 2.377	24.165 ± 0.910
Feces	24	1.799 ± 0.461	0.983 ± 0.568	2.616 ± 0.492	0.899 ± 0.397	0.888 ± 0.383	1.903 ± 1.366
				Cumulative Percent of Dose			
Expired EDC	1	9.071 ± 1.817	7.062 ± 1.589	8.336 ± 0.722	7.971 ± 0.611	9.260 ± 1.237	5.945 ± 0.828
	3	16.499 ± 4.072	14.130 ± 4.052	14.914 ± 1.195	15.259 ± 0.559	20.514 ± 2.542	10.048 ± 0.852
	6	22.599 ± 6.102	22.277 ± 6.277	20.467 ± 1.538	21.632 ± 1.166	31.971 ± 3.455	13.463 ± 0.541
	12	29.173 ± 9.231	34.088 ± 7.448	26.655 ± 0.609	26.259 ± 2.778	48.336 ± 5.205	16.536 ± 1.771
	24	30.464 ± 9.535	40.925 ± 6.284	29.893 ± 0.338	27.329 ± 3.184	57.556 ± 8.786	17.687 ± 1.971
Expired CO_2	3	0.173 ± 0.004	0.006 ± 0.002	0.009 ± 0.002	0.006 ± 0.002	0.011 ± 0.006	0.154 ± 0.140
	6	0.347 ± 0.009	0.015 ± 0.006	0.019 ± 0.005	0.019 ± 0.009	0.016 ± 0.006	0.177 ± 0.150
	12	0.392 ± 0.011	0.020 ± 0.007	0.033 ± 0.011	0.044 ± 0.015	0.032 ± 0.009	0.200 ± 0.144
	24	0.469 ± 0.022	0.036 ± 0.014	0.094 ± 0.045	0.059 ± 0.014	0.034 ± 0.009	0.221 ± 0.137
Urine	6	7.683 ± 0.647	2.285 ± 0.808	4.933 ± 0.357	6.248 ± 1.216	2.880 ± 0.612	8.135 ± 1.431
	12	23.697 ± 1.530	9.824 ± 3.042	17.783 ± 2.019	20.146 ± 4.657	11.843 ± 4.825	26.978 ± 1.885
	24	46.632 ± 0.739	35.257 ± 6.060	45.560 ± 2.471	42.502 ± 4.444	27.568 ± 5.596	51.143 ± 1.801
Feces	24	1.799 ± 0.461	0.983 ± 0.568	2.616 ± 0.492	0.899 ± 0.397	0.888 ± 0.383	1.903 ± 1.366
Recovery	24	79.363 ± 8.983	77.201 ± 7.063	78.164 ± 3.185	70.789 ± 5.327	86.045 ± 4.975	70.954 ± 1.414

a Mean ± S.E. of three rats per group.

TABLE 10

ELIMINATION OF RADIOACTIVITY FROM FEMALE S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS[a]

Excretum	Time After Dosing (hr)	Control	DS	EtOH	EDC	EDC/DS	EDC/EtOH
				Percent of Dose			
Expired EDC	1	9.144 ± 2.098	11.966[b]	8.022 ± 0.773	8.956 ± 2.384	10.130 ± 1.476	6.903 ± 0.955
	3	6.952 ± 1.642	11.953	6.887 ± 0.119	9.755 ± 2.349	10.478 ± 2.312	4.681 ± 0.672
	6	5.886 ± 0.821	13.256	6.052 ± 1.004	8.685 ± 0.946	13.093 ± 1.287	3.071 ± 0.331
	12	4.600 ± 0.741	15.778	5.240 ± 1.337	8.856 ± 2.585	17.679 ± 0.904	2.126 ± 0.287
	24	1.377 ± 0.114	2.316	3.570 ± 1.297	4.094 ± 2.394	6.402 ± 2.214	0.933 ± 0.189
Expired CO_2	3	0.261 ± 0.014	0.015	0.021 ± 0.008	0.009 ± 0.001	0.011 ± 0.003	0.020 ± 0.008
	6	0.257 ± 0.006	0.020	0.025 ± 0.009	0.015 ± 0.003	0.007 ± 0.001	0.073 ± 0.049
	12	0.105 ± 0.008	0.029	0.066 ± 0.039	0.042 ± 0.019	0.016 ± 0.005	0.095 ± 0.063
	24	0.084 ± 0.010	0.010	0.095 ± 0.071	0.011 ± 0.003	0.003 ± 0.001	0.017 ± 0.009
Urine	6	10.531 ± 0.726	4.956	7.362 ± 2.521	6.606 ± 1.317	2.151 ± 0.730	13.256 ± 1.308
	12	20.014 ± 0.171	15.229	15.442 ± 2.862	13.450 ± 1.702	6.889 ± 1.198	18.992 ± 1.419
	24	24.408 ± 3.984	16.238	18.821 ± 4.097	13.891 ± 4.164	15.843 ± 3.451	22.820 ± 3.420
Feces	24	1.104 ± 0.220	0.154	2.371 ± 0.747	0.894 ± 0.507	0.192 ± 0.085	0.894 ± 0.119
				Cumulative Percent of Dose			
Expired EDC	1	9.144 ± 2.098	11.966	8.022 ± 0.773	8.956 ± 2.384	10.130 ± 1.476	6.903 ± 0.955
	3	16.096 ± 3.700	23.918	14.910 ± 0.750	18.711 ± 4.637	20.608 ± 3.550	11.584 ± 1.611
	6	21.982 ± 4.106	37.174	20.961 ± 0.795	27.396 ± 5.577	33.701 ± 4.833	14.655 ± 1.933
	12	26.582 ± 4.388	52.952	26.209 ± 1.825	36.252 ± 3.981	51.380 ± 4.689	16.843 ± 2.160
	24	27.960 ± 4.276	55.268	29.771 ± 2.940	40.345 ± 4.209	57.782 ± 2.514	17.715 ± 1.040
Expired CO_2	3	0.261 ± 0.014	0.015	0.021 ± 0.008	0.009 ± 0.001	0.011 ± 0.003	0.019 ± 0.008
	6	0.518 ± 0.018	0.035	0.046 ± 0.015	0.024 ± 0.003	0.020 ± 0.004	0.093 ± 0.058
	12	0.623 ± 0.022	0.064	0.112 ± 0.050	0.066 ± 0.017	0.036 ± 0.006	0.188 ± 0.121
	24	0.707 ± 0.012	0.074	0.207 ± 0.120	0.076 ± 0.020	0.039 ± 0.006	0.205 ± 0.129
Urine	6	10.531 ± 0.726	4.956	7.362 ± 2.521	6.606 ± 1.317	2.151 ± 0.730	13.256 ± 1.308
	12	30.545 ± 0.810	20.184	22.804 ± 0.899	20.057 ± 2.877	9.039 ± 1.926	32.249 ± 2.703
	24	54.953 ± 3.597	36.422	41.625 ± 4.722	33.946 ± 6.065	24.882 ± 3.719	55.069 ± 1.321
Feces	24	1.104 ± 0.220	0.154	2.371 ± 0.747	0.894 ± 0.507	0.192 ± 0.085	0.894 ± 0.119
Recovery	24	84.724 ± 0.471	91.917	73.975 ± 3.669	75.262 ± 3.140	82.895 ± 4.653	73.883 ± 1.972

a Mean ± S.E. of three rats per group.
b Average of two rats.

TABLE 11

ELIMINATION OF RADIOACTIVITY FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO 4 MONTH OLD S.D. RATS

Excretum	Time After Dosing (hr)	Males				Females			
		No. 37	No. 39	No. 41	Mean ± S.E.	No. 38	No. 40	No. 42	Mean ± S.E.
		Percent of Dose							
Expired EDC	1	15.942	11.735	14.118	13.932 ± 1.218	22.445	14.838	19.668	18.984 ± 2.222
	3	14.164	11.138	12.758	12.687 ± 0.874	15.171	14.452	15.808	15.144 ± 0.392
	6	7.875	5.995	7.838	7.236 ± 0.621	1.982	5.628	5.861	4.490 ± 1.256
	12	1.120	1.386	1.855	1.454 ± 0.215	0.306	1.258	1.030	0.865 ± 0.287
	24	0.266	0.204	0.185	0.218 ± 0.024	0.052	0.085	0.081	0.073 ± 0.010
Expired CO_2	3	0.008	0.010	0.015	0.011 ± 0.002	0.037	0.021	0.019	0.026 ± 0.006
	6	0.015	0.029	0.030	0.025 ± 0.005	0.041	0.041	0.045	0.042 ± 0.001
	12	0.011	0.069	0.009	0.030 ± 0.020	0.188	0.172	0.288	0.216 ± 0.036
	24	0.007	0.025	0.015	0.016 ± 0.005	0.017	0.034	0.038	0.030 ± 0.006
Urine	6	9.814	6.974	11.528	9.439 ± 1.328	15.690	9.001	9.385	11.359 ± 2.168
	12	18.229	21.804	26.228	22.087 ± 2.313	18.798	25.063	35.878	26.580 ± 4.989
	24	13.904	22.066	18.470	18.147 ± 2.362	9.585	16.931	14.109	13.542 ± 2.139
Feces	24	10.564	2.823	2.224	5.204 ± 2.686	4.225	5.670	3.311	4.402 ± 0.687
		Cumulative Percent of Dose							
Expired EDC	1	15.942	11.735	14.118	13.932 ± 1.218	22.445	14.838	19.668	18.984 ± 2.222
	3	30.106	22.873	26.876	26.618 ± 2.092	37.616	29.290	35.476	34.127 ± 2.496
	6	37.981	28.868	34.714	33.854 ± 2.666	39.598	34.918	41.337	38.618 ± 1.917
	12	39.101	30.254	36.569	35.308 ± 2.631	39.904	36.176	42.367	39.482 ± 1.800
	24	39.367	30.458	36.754	35.526 ± 2.644	39.956	36.261	42.448	39.555 ± 1.797
Expired CO_2	3	0.008	0.010	0.015	0.011 ± 0.002	0.037	0.021	0.019	0.026 ± 0.006
	6	0.023	0.039	0.045	0.036 ± 0.007	0.078	0.062	0.064	0.068 ± 0.005
	12	0.034	0.108	0.054	0.065 ± 0.022	0.266	0.234	0.352	0.284 ± 0.035
	24	0.041	0.133	0.069	0.081 ± 0.027	0.283	0.268	0.390	0.314 ± 0.038
Urine	6	9.814	6.974	11.528	9.439 ± 1.328	15.690	9.001	9.385	11.359 ± 2.168
	12	28.043	28.778	37.756	31.526 ± 3.122	34.488	34.064	45.263	37.938 ± 3.664
	24	41.947	50.844	56.226	49.672 ± 4.163	44.073	50.995	59.372	51.480 ± 4.423
Feces	24	10.564	2.823	2.224	5.204 ± 2.686	4.225	5.670	3.311	4.402 ± 0.687

TABLE 12

RADIOACTIVITY IN BLOOD, TISSUE, AND EXCRETA AT 24 hr FOLLOWING AN ORAL DOSE OF ^{14}C-EDC (150 mg/kg) TO 4 MONTH OLD S.D. RATS

Tissue/Excretum	Males				Females			
	No. 37	No. 39	No. 41	Mean ± S.E.	No. 38	No. 40	No. 42	Mean ± S.E.
	µg Equivalents/g or mL							
Blood	5.205	8.231	5.592	6.343 ± 0.951	5.635	6.958	5.366	5.986 ± 0.492
Plasma	4.961	10.342	6.457	7.253 ± 1.604	N/A[c]	6.659	5.224	5.942 ± 0.717
RBC	4.890	7.597	4.932	5.806 ± 0.895	N/A[c]	6.357	5.225	5.791 ± 0.566
Liver	15.918	31.510	16.492	21.307 ± 5.104	12.799	15.200	15.423	14.474 ± 0.840
Kidneys	11.851	18.778	11.640	14.090 ± 2.345	5.826	9.073	6.955	7.285 ± 0.952
Lungs	3.685	8.065	2.825	4.858 ± 1.622	4.291	3.613	2.726	3.543 ± 0.453
Muscle	0.435	0.562	0.319	0.439 ± 0.070	0.336	0.493	0.273	0.367 ± 0.065
Fat[b]	0.325	1.139	0.385	0.616 ± 0.262	0.957	0.522	0.414	0.631 ± 0.166
Skin	2.456	4.447	2.668	3.190 ± 0.631	2.585	3.965	2.652	3.067 ± 0.449
GI Tract	12.871	20.507	7.144	13.507 ± 3.871	5.496	7.289	7.518	6.768 ± 0.639
	Percent of Dose							
Blood[a]	0.244	0.386	0.262	0.297 ± 0.045	0.265	0.323	0.252	0.280 ± 0.022
Plasma[a,b]	0.139	0.291	0.182	0.204 ± 0.045	N/A[c]	0.185	0.147	0.167 ± 0.019
RBC[a,b]	0.092	0.143	0.093	0.109 ± 0.017	N/A[c]	0.118	0.099	0.108 ± 0.009
Liver	0.417	0.696	0.386	0.500 ± 0.099	0.377	0.365	0.434	0.392 ± 0.021
Kidneys	0.073	0.137	0.078	0.096 ± 0.021	0.049	0.059	0.049	0.052 ± 0.003
Lungs	0.009	0.020	0.008	0.012 ± 0.004	0.017	0.014	0.011	0.014 ± 0.002
Muscle[a]	0.117	0.151	0.085	0.118 ± 0.019	0.091	0.031	0.073	0.065 ± 0.018
Skin[a]	0.263	0.476	0.286	0.342 ± 0.067	0.278	0.421	0.285	0.328 ± 0.047
GI Tract	0.534	0.915	0.288	0.579 ± 0.182	0.328	0.348	0.364	0.347 ± 0.010
Expired EDC	39.367	30.458	36.754	35.526 ± 2.644	39.956	36.261	42.448	39.555 ± 1.797
Expired CO_2	0.041	0.133	0.069	0.081 ± 0.027	0.283	0.268	0.390	0.314 ± 0.038
Urine	41.947	50.844	56.226	49.672 ± 4.163	44.073	50.995	59.372	51.480 ± 4.423
Feces	10.564	2.823	2.224	5.204 ± 2.686	4.225	5.670	3.311	4.402 ± 0.687
Recovery	93.576	87.039	96.666	92.427 ± 2.838	89.942	94.455	106.989	97.129 ± 5.099

a Based on 7, 40, and 16% of body weight for blood, muscle, and skin, respectively, and 60 and 40% of blood volume for plasma and RBCs, respectively.
b Not included in recovery estimates.
c Not available.

TABLE 13

METABOLITES IN URINE OF INDIVIDUAL S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS

Treatment Group	Sampling Period (hr)	Unknown ~ 9 min	TDGAS[a] ~ 11 min	Unknown ~ 14 min	TDGA[a] ~ 16.5 min	MCAA[a] ~ 19 min
		Percent of Urinary Radioactivity				
Control (No. AX046)	0-6	4	19	13	63	1
	6-12	2	30	7	61	0
	12-24	1	27	5	66	0
DS (No. AX101)	0-6	1	28	5	63	3
	6-12	2	27	4	65	1
	12-24	3	29	7	60	1
EtOH (No. AX707)	0-6	3	25	6	65	1
	6-12	2	29	5	61	1
	12-24	2	28	7	64	-
EDC (No. AX010)	0-6	2	25	5	66	2
	6-12	3	31	5	60	1
	12-24	2	33	5	59	0
EDC/DS (No. AX048)	0-6	3	32	6	53	6
	6-12	2	28	4	64	2
	12-24	2	35	4	58	1
EDC/EtOH (No. AX206)	0-6	2	27	3	62	3
	6-12	4	34	5	56	1
	12-24	7	23	8	61	1

a TDGAS, thiodiglycolic acid sulfoxide; TDGA, thiodiglycolic acid; MCAA, monochloroacetic acid.

TABLE 14

METABOLITES IN URINE OF INDIVIDUAL S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS

Treatment Group	Sex	Rat No.	Unknown ~ 9 min	TDGAS[a] ~ 11 min	Unknown ~ 14 min	TDGA[a] ~ 16.5 min	MCAA[a] ~ 19 min
			Percent of Urinary Radioactivity[b]				
Control	Male	AX046	2	26	6	65	0
		AX162	1	24	5	70	1
		AX171	2	30	4	61	1
		Mean ± S.E.	1.7 ± 0.6	26.7 ± 3.1	5.0 ± 1.0	65.3 ± 4.5	0.7 ± 0.6
	Female	AX356	2	22	5	70	1
		AX361	2	23	4	70	1
		AX416	2	26	5	65	1
		Mean ± S.E.	2.0 ± 0.0	23.7 ± 2.1	4.7 ± 0.6	68.3 ± 2.9	1.0 ± 0.0
DS	Male	AX101	3	28	7	61	1
		AX689	2	35	6	55	2
		AX712	2	35	6	55	2
		Mean ± S.E.	2.3 ± 0.6	32.7 ± 4.0	6.3 ± 0.6	57.0 ± 3.5	1.7 ± 0.6
	Female	AX914	2	21	5	67	4
		AX578	1	27	5	64	3
		Average	1.5	24.0	5.0	65.5	3.5
EtOH	Male	AX707	1	30	5	62	0
		AX317	2	30	4	63	0
		AX236	4	26	5	64	1
		Mean ± S.E.	2.3 ± 1.5	28.7 ± 2.3	4.7 ± 0.6	63.0 ± 1.0	0.3 ± 0.6
	Female	AX918	2	23	7	65	2
		AX700	2	26	6	64	1
		AX505	2	18	5	73	1
		Mean ± S.E.	2.0 ± 0.0	22.3 ± 4.0	6.0 ± 1.0	67.3 ± 4.9	1.3 ± 0.6
EDC	Male	AX010	2	32	5	60	1
		AX013	2	29	10	57	1
		AX268	3	25	6	63	3
		Mean ± S.E.	2.3 ± 0.6	28.7 ± 3.5	7.0 ± 2.6	60.0 ± 3.0	1.7 ± 1.2
	Female	AX360	2	22	4	69	2
		AX387	3	17	6	72	2
		AX420	4	21	6	67	1
		Mean ± S.E.	3.0 ± 1.0	20.0 ± 2.6	5.3 ± 1.2	69.3 ± 2.5	1.7 ± 0.6
EDC/DS	Male	AX048	2	32	4	59	2
		AX060	1	26	5	63	4
		AX065	2	26	6	60	6
		Mean ± S.E.	1.7 ± 0.6	28.0 ± 3.5	5.0 ± 1.0	60.7 ± 2.1	4.0 ± 2.0
	Female	AX406	2	20	4	71	3
		AX466	1	19	5	70	5
		AX877	2	17	4	72	5
		Mean ± S.E.	1.7 ± 0.6	18.7 ± 1.5	4.3 ± 0.6	71.0 ± 1.0	4.3 ± 1.2
EDC/EtOH	Male	AX206	4	26	6	61	1
		AX298	4	33	10	47	7
		AX304	3	35	6	55	0
		Mean ± S.E.	3.7 ± 0.6	31.3 ± 4.7	7.3 ± 2.3	54.3 ± 7.0	2.7 ± 3.8
	Female	AX818	2	30	6	61	1
		AX409	2	30	6	60	1
		AX669	1	26	9	61	2
		Mean ± S.E.	1.7 ± 0.6	28.7 ± 2.3	7.0 ± 1.7	60.7 ± 0.6	1.3 ± 0.6

a TDGAS, thiodiglycolic acid sulfoxide; TDGA, thiodiglycolic acid; MCAA, monochloroacetic acid.
b Pooled 0-24 hr samples.

TABLE 15

METABOLITES IN URINE OF MALE AND FEMALE S.D. RATS DOSED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS[a]

Treatment Group	Sex	Unknowns ~ 9 min	TDGAS[c] ~ 11 min	Unknown ~ 14 min	TDGA[c] ~ 16.5 min	MCAA[c] ~ 19 min
		Percent of Urinary Radioactivity[d]				
Control	Male	1.7 ± 0.6	26.7 ± 3.1	5.0 ± 1.0	65.3 ± 4.5	0.7 ± 0.6
	Female	2.0 ± 0.0	23.7 ± 2.1	4.7 ± 0.6	68.3 ± 2.9	1.0 ± 0.0
DS	Male	2.3 ± 0.6	32.7 ± 4.0	6.3 ± 0.6	57.0 ± 3.5	1.7 ± 0.6
	Female[b]	1.5	24.0	5.0	65.5	3.5
EtOH	Male	2.3 ± 1.5	28.7 ± 2.3	4.7 ± 0.6	63.0 ± 1.0	0.3 ± 0.6
	Female	2.0 ± 0.0	22.3 ± 4.0	6.0 ± 1.0	67.3 ± 4.9	1.3 ± 0.6
EDC	Male	2.3 ± 0.6	28.7 ± 3.5	7.0 ± 2.6	60.0 ± 3.0	1.7 ± 1.2
	Female	3.0 ± 1.0	20.0 ± 2.6	5.3 ± 1.2	69.3 ± 2.5	1.7 ± 0.6
EDC/DS	Male	1.7 ± 0.6	28.0 ± 3.5	5.0 ± 1.0	60.7 ± 2.1	4.0 ± 2.0
	Female	1.7 ± 0.6	18.7 ± 1.5	4.3 ± 0.6	71.0 ± 1.0	4.3 ± 1.2
EDC/EtOH	Male	3.7 ± 0.6	31.3 ± 4.7	7.3 ± 2.3	54.3 ± 7.0	2.7 ± 3.8
	Female	1.7 ± 0.6	28.7 ± 2.3	7.0 ± 1.7	60.7 ± 0.6	1.3 ± 0.6

a Mean ± S.E. of three determinations.
b Average of two determinations.
c TDGAS, thiodiglycolic acid sulfoxide; TDGA, thiodiglycolic acid; MCAA, monochloroacetic acid.
d Pooled 0-24 hr samples.

TABLE 16

BLOOD AND LIVER RADIOACTIVITY (μg/g OR mL) IN INDIVIDUAL S.D. RATS AT 6 hr AFTER ORAL DOSING WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS

Treatment Group	Blood/Tissue	Male Rats				Female Rats			
		AX046	AX162	AX171	Mean ± S.E.	AX416	AX356	AX361	Mean ± S.E.
Control	Blood	38.516	12.487	13.666	21.556 ± 8.487	28.791	24.005	16.919	23.238 ± 3.449
	Plasma	35.684	12.923	12.347	20.318 ± 7.685	30.718	26.861	16.827	24.802 ± 4.140
	RBC	28.134	8.322	13.501	16.652 ± 5.932	9.547	13.635	12.304	11.829 ± 1.204
	Liver	69.118	75.769	130.903	91.930 ± 19.581	150.097	39.053	76.105	88.418 ± 32.642
		AX101	AX689	AX712	Mean ± S.E.	AX914	AX856	AX578	Mean ± S.E.
DS	Blood	4.639	3.051	4.394	4.028 ± 0.494	57.529	4.995	5.170	22.565 ± 17.482
	Plasma	2.679	2.367	4.874	3.307 ± 0.789	60.448	5.688	4.092	23.409 ± 18.525
	RBC	6.240	3.043	3.586	4.287 ± 0.990	33.176	3.252	4.650	13.693 ± 9.750
	Liver	12.055	14.288	30.936	18.976 ± 5.841	128.063	22.126	23.930	58.340 ± 35.016
		AX231	AX317	AX707	Mean ± S.E.	AX700	AX505		Average
EtOH	Blood	30.435	12.007	19.651	20.698 ± 5.345	6.007	28.333		17.170
	Plasma	31.132	10.866	47.847	29.948 ± 10.692	5.620	30.223		17.922
	RBC	24.311	10.547	14.111	16.323 ± 4.124	4.510	16.533		10.522
	Liver	49.841	46.890	138.990	78.544 ± 30.190	41.593	75.461		58.527
		AX268	AX010		Average	AX387	AX360	AX420	Mean ± S.E.
EDC	Blood	11.153	15.365		13.259	9.412	11.667	10.657	10.579 ± 0.652
	Plasma	9.881	19.429		14.655	10.730	12.879	12.153	11.921 ± 0.631
	RBC	10.755	8.646		9.701	5.904	6.520	6.248	6.284 ± 0.192
	Liver	68.319	128.419		98.369	35.971	74.957	43.423	51.450 ± 11.949
		AX048	AX065	AX060	Mean ± S.E.	AX405	AX877	AX466	Mean ± S.E.
EDC/DS	Blood	10.689	10.352	11.675	10.905 ± 0.397	13.331	4.357	12.074	13.254 ± 0.660
	Plasma	13.156	N/A[a]	16.282	14.719	18.615	15.993	14.098	16.235 ± 1.310
	RBC	6.619	4.480	5.667	5.589 ± 0.619	10.100	7.741	5.344	7.728 ± 1.373
	Liver	48.625	69.462	85.420	67.836 ± 10.653	86.689	92.532	61.403	80.208 ± 9.553
		AX304	AX206	AX728	Mean ± S.E.	AX409	AX818	AX669	Mean ± S.E.
EDC/EtOH	Blood	43.869	19.815	12.837	25.507 ± 9.399	34.483	50.544	29.957	38.328 ± 6.246
	Plasma	50.747	21.576	13.039	28.454 ± 11.416	45.800	54.125	28.909	42.945 ± 7.418
	RBC	36.741	11.302	8.146	18.730 ± 9.052	20.166	25.936	24.215	23.439 ± 1.710
	Liver	116.327	129.635	57.910	101.291 ± 22.028	97.708	118.941	129.244	115.298 ± 9.284

a Not available.

TABLE 17

RECOVERY OF RADIOACTIVITY (% OF DOSE) IN BLOOD AND LIVER OF INDIVIDUAL S.D. RATS AT 6 hr AFTER ORAL DOSING WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS

Treatment Group	Blood/Tissue	Male Rats				Female Rats			
		AX046	AX162	AX171	Mean ± S.E.	AX416	AX356	AX361	Mean ± S.E.
Control	Blood	1.816	0.567	0.635	1.006 ± 0.405	1.344	1.118	0.792	1.085 ± 0.160
	Plasma	1.009	0.352	0.344	0.568 ± 0.220	0.860	0.750	0.473	0.694 ± 0.115
	RBC	0.531	0.152	0.251	0.311 ± 0.113	0.179	0.254	0.231	0.221 ± 0.022
	Liver	1.072	1.196	2.255	1.508 ± 0.375	2.526	0.736	1.492	1.545 ± 0.519
		AX101	AX689	AX712	Mean ± S.E.	AX914	AX856	AX578	Mean ± S.E.
DS	Blood	0.224	0.148	0.196	0.189 ± 0.022	2.702	0.235	0.243	1.060 ± 0.821
	Plasma	0.078	0.069	0.131	0.093 ± 0.019	1.704	0.161	0.116	0.660 ± 0.522
	RBC	0.121	0.059	0.064	0.081 ± 0.020	0.623	0.061	0.088	0.257 ± 0.183
	Liver	0.250	0.275	0.808	0.444 ± 0.182	2.766	0.523	0.588	1.292 ± 0.737
		AX231	AX317	AX707	Mean ± S.E.	AX700	AX505		Average
EtOH	Blood	1.421	0.555	0.904	0.960 ± 0.252	0.280	1.319		0.800
	Plasma	0.872	0.301	1.322	0.832 ± 0.295	0.157	0.844		0.501
	RBC	0.454	0.195	0.260	0.303 ± 0.078	0.084	0.308		0.196
	Liver	0.721	0.739	2.223	1.228 ± 0.498	0.561	1.122		0.842
		AX268	AX010		Average	AX387	AX360	AX420	Mean ± S.E.
EDC	Blood	0.530	0.711		0.621	0.440	0.542	0.499	0.494 ± 0.030
	Plasma	0.282	0.539		0.411	0.301	0.359	0.341	0.334 ± 0.017
	RBC	0.250	0.160		0.183	0.111	0.121	0.117	0.116 ± 0.003
	Liver	1.438	2.189		1.814	0.574	2.119	0.838	1.171 ± 0.477
		AX048	AX065	AX060	Mean ± S.E.	AX405	AX877	AX466	Mean ± S.E.
EDC/DS	Blood	0.497	0.469	0.531	0.499 ± 0.018	0.621	0.666	0.564	0.617 ± 0.030
	Plasma	0.367	N/A[a]	0.445	0.406	0.520	0.445	0.395	0.453 ± 0.036
	RBC	0.123	0.082	0.104	0.103 ± 0.012	0.188	0.144	0.100	0.144 ± 0.025
	Liver	1.201	1.976	1.833	1.670 ± 0.238	1.777	2.737	1.193	1.902 ± 0.450
		AX304	AX206	AX728	Mean ± S.E.	AX409	AX818	AX669	Mean ± S.E.
EDC/EtOH	Blood	2.003	0.915	0.603	1.174 ± 0.424	1.609	2.349	1.402	1.787 ± 0.287
	Plasma	1.390	0.598	0.368	0.785 ± 0.310	1.283	1.509	0.812	1.201 ± 0.205
	RBC	0.671	0.209	0.153	0.344 ± 0.164	0.377	0.482	0.453	0.437 ± 0.031
	Liver	1.986	2.062	1.438	0.829 ± 0.197	1.416	2.105	2.059	1.860 ± 0.222

a. Not available

TABLE 18

RADIOACTIVITY IN BLOOD AND LIVER OF S.D. RATS AT 6 hr FOLLOWING ORAL DOSING WITH ^{14}C-EDC (150 mg/kg)[a]

	Control	DS	EtOH	EDC	EDC/DS	EDC/EtOH
			Male Rats			
Blood (μg/mL)	21.6 ± 8.5	4.0 ± 0.5	20.7 ± 5.3	13.3[b]	10.9 ± 0.4	25.5 ± 9.4
Liver (μg/g)	91.9 ± 19.6	19.0 ± 5.8	78.5 ± 30.2	98.4[b]	67.8 ± 10.7	101.3 ± 22.0
Liver/Blood Ratio	4.3	4.8	3.8	7.4	6.2	4.0
			Female Rats			
Blood (μg/mL)	23.2 ± 3.4	22.6 ± 17.5	17.2[b]	10.6 ± 0.7	13.3 ± 0.6	38.3 ± 6.2
Liver (μg/g)	88.4 ± 32.8	58.3 ± 35.0	58.5[b]	51.5 ± 11.9	80.2 ± 9.6	115.3 ± 9.3
Liver/Blood Ratio	3.8	2.6	3.4	4.9	6.0	3.0

a Mean ± S.E. of three determinations.
b Average of two determinations.

TABLE 19

BINDING OF RADIOACTIVITY TO HEPATIC DNA OF INDIVIDUAL S.D. RATS TREATED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS

Treatment Group	Male Rats		Female Rats	
	Rat No.	Binding[a]	Rat No.	Binding[a]
Control	AX046	44.08	AX356	40.81
	AX162	41.28	AX361	45.06
	AX171	45.23	AX416	23.41
	Mean ± S.E.	43.53 ± 1.17	Mean ± S.E.	36.43 ± 6.62
DS	AX101	26.50	AX914	25.06
	AX689	42.86	AX578	30.12
	AX712	55.29	AX856	31.82
	Mean ± S.E.	41.55 ± 8.34	Mean ± S.E.	29.00 ± 2.03
EtOH	AX707	41.76	AX700	34.00
	AX231	38.93	AX505	19.82
	AX317	31.70		
	Mean ± S.E.	37.46 ± 3.00	Average	26.91
EDC	AX010	19.92	AX360	27.50
	AX268	17.82	AX387	40.53
			AX420	37.04
	Average	18.87	Mean ± S.E.	35.02 ± 3.89
EDC/DS	AX048	33.60	AX405	28.03
	AX060	44.17	AX466	26.11
	AX065	29.02	AX877	13.30
	Mean ± S.E.	35.60 ± 4.48	Mean ± S.E.	22.48 ± 4.62
EDC/EtOH	AX206	43.70	AX409	34.58
	AX304	100.85	AX669	10.14
	AX728	15.30	AX818	24.64
	Mean ± S.E.	53.28 ± 25.16	Mean ± S.E.	23.12 ± 7.10

a Expressed as micromole equivalents of ^{14}C-EDC bound per mole of DNA isolated from rat liver. For calculations, see methods.

TABLE 20

BINDING OF RADIOACTIVITY TO HEPATIC DNA OF MALE AND FEMALE S.D. RATS TREATED ORALLY WITH ^{14}C-EDC (150 mg/kg) FOLLOWING 2 YEARS OF EXPOSURE TO OTHER TREATMENTS[a]

Treatment Group	Males	Females	Combined
	Micromole EDC/mole DNA		
Control	43.5 ± 1.2	36.4 ± 6.6	40.0 ± 3.4
DS	41.6 ± 8.3	29.0 ± 2.0	35.3 ± 4.8
EtOH	37.5 ± 3.0	26.9	33.2 ± 3.8
EDC	18.8	35.0 ± 3.9	28.6 ± 4.5
EDC/DS	35.6 ± 4.5	22.5 ± 4.6	29.0 ± 4.1
EDC/EtOH	53.3 ± 25.2	23.1 ± 7.1	38.2 ± 13.5

a Mean ± S.E. of 3 rats or average of 2 rats.

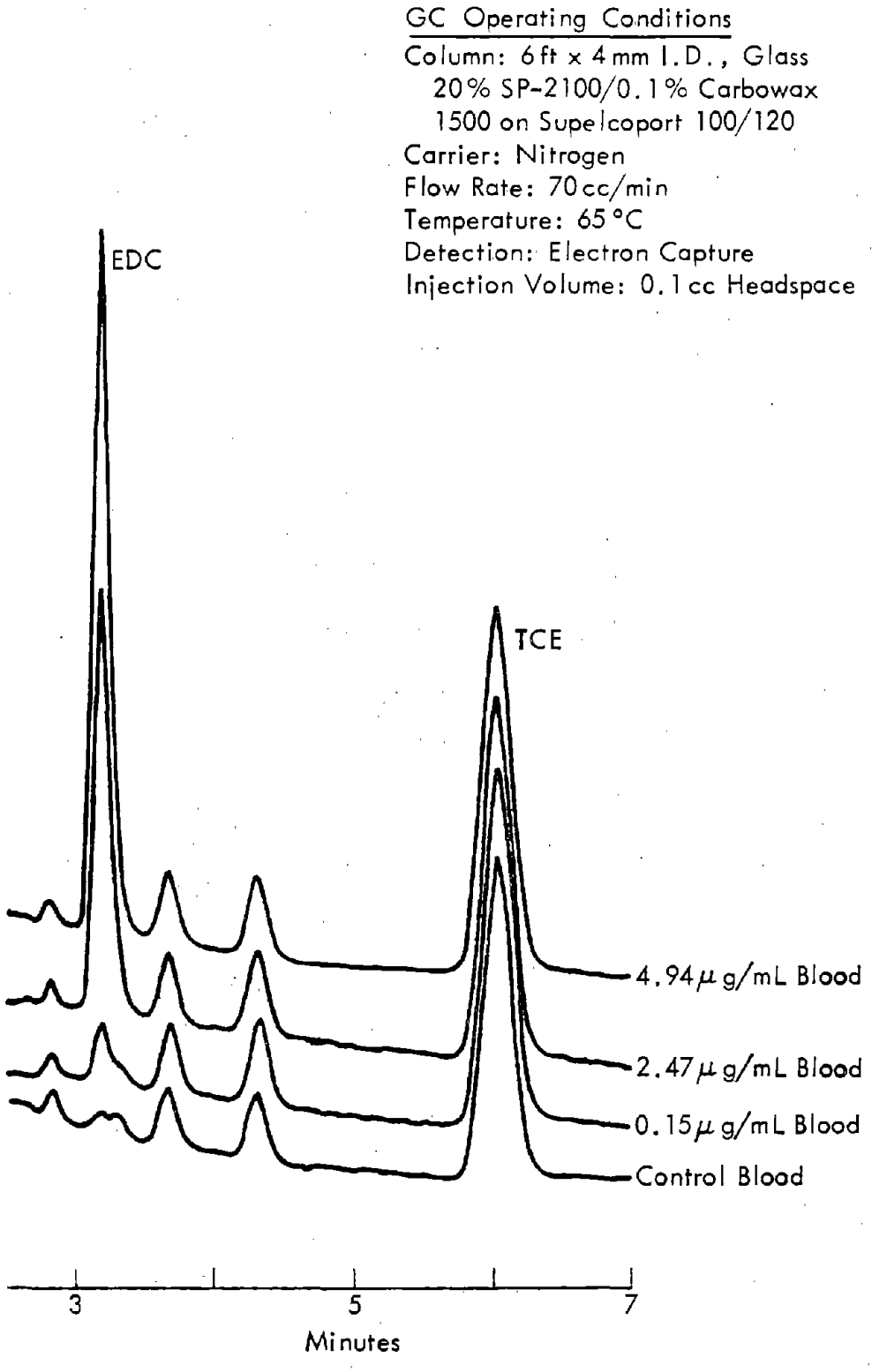

Figure 1 - Typical Gas Chromatogram of Headspaces Drawn from Rat Blood Spiked with 1,2-Dichloroethane (EDC) 1,1,2-Trichloroethane (TCE) is the Internal Standard

HPLC Operating Parameters

Column: Bio-Rad Organic Acid Analysis
Column HPX - 87 H
300 x 7.8 mm I.D.
Mobile Phase: 0.005 N H_2SO_4
Flow Rate: 0.5 mL/min
Detection: 214 nm, 1.0 AUFS

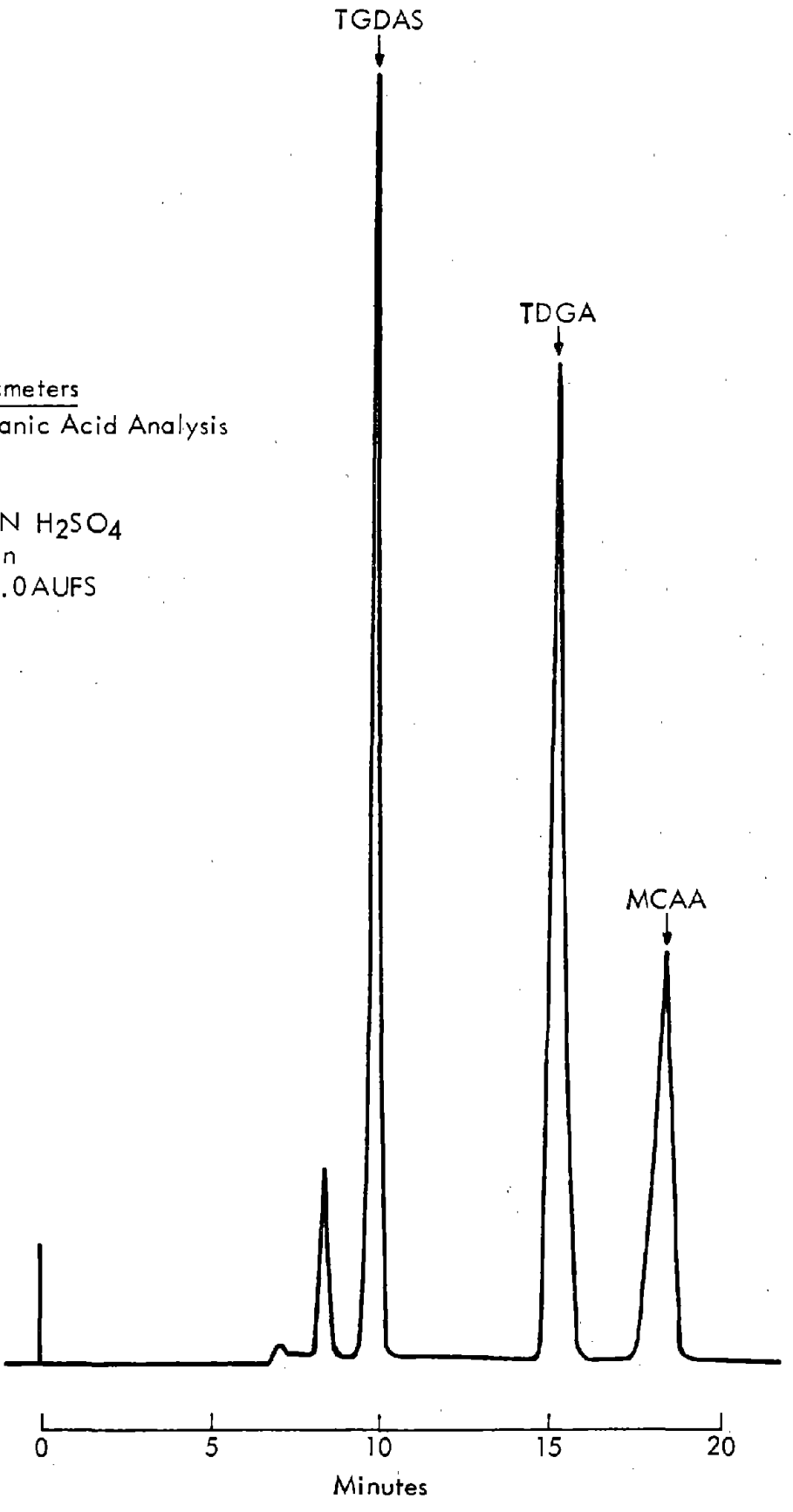

Figure 2 - High Performance Liquid Chromatograph of Thiodiglycolic Acid Sulfoxide (TGDAS), Thiodiglycolic Acid (TGDA), and Monochloroacetic Acid (MCAA)

HPLC Operating Parameters

Column: Bio-Rad Organic Acid Analysis
Column HPX - 87 H
300 x 7.8 mm I.D.
Mobile Phase: 0.005 N H_2SO_4
Flow Rate: 0.5 mL/min
Detection: Radiomatic Flo-one
Model HP
Scintillate: Fisher Scintiverse
1.5 mL/min

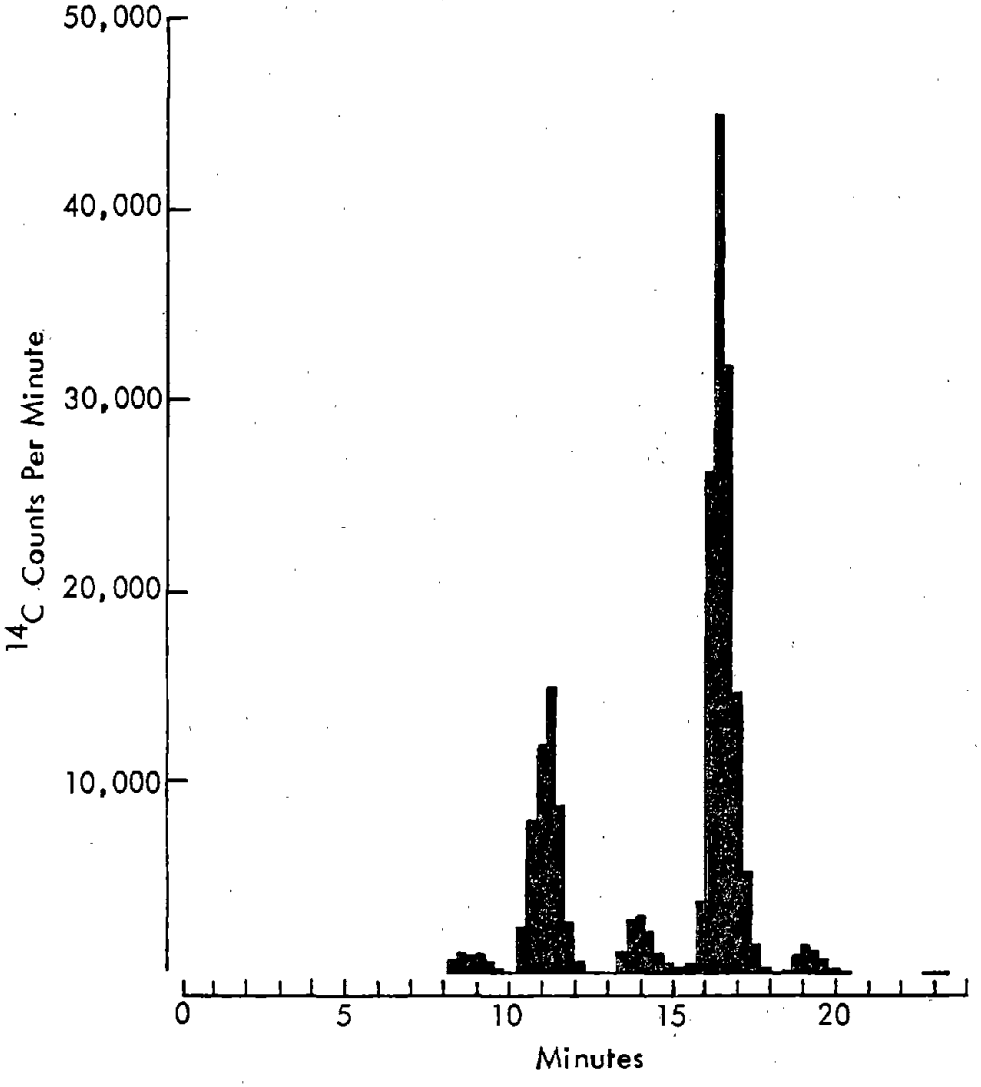

Figure 3 - Typical Radiochromatographic Profile of Urine Obtained from a Rat (No. AX268) Receiving an Oral Dose of ^{14}C-1,2-Dichloroethane (EDC) Following 2 Years of Inhalation Exposure to 50 ppm of EDC

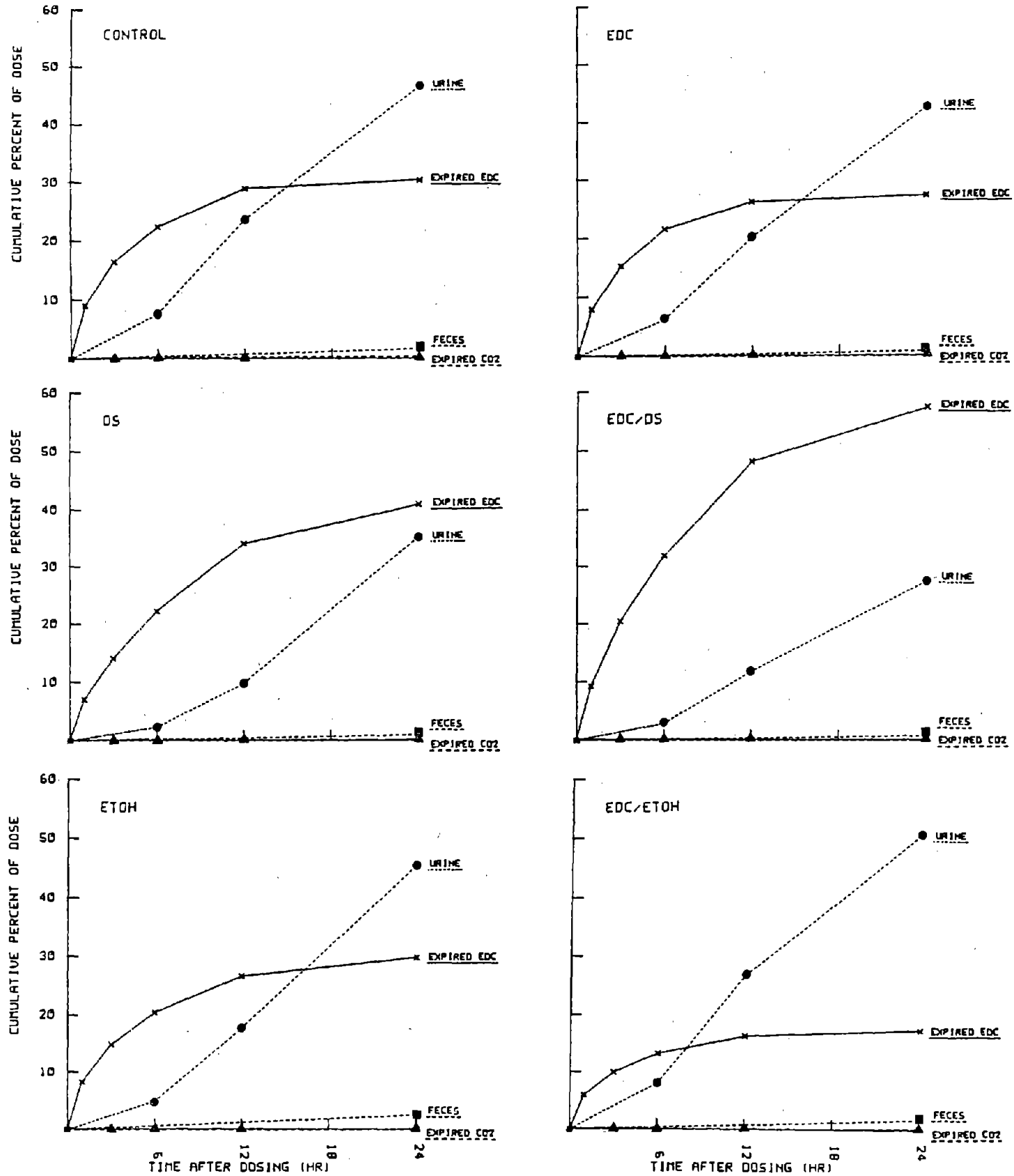

Figure 4 - Cumulative Excretion of Radioactivity from Male S.D. Rats Dosed Orally with ^{14}C-EDC (150 mg/kg) Following 2 Years of Exposure to Other Treatments

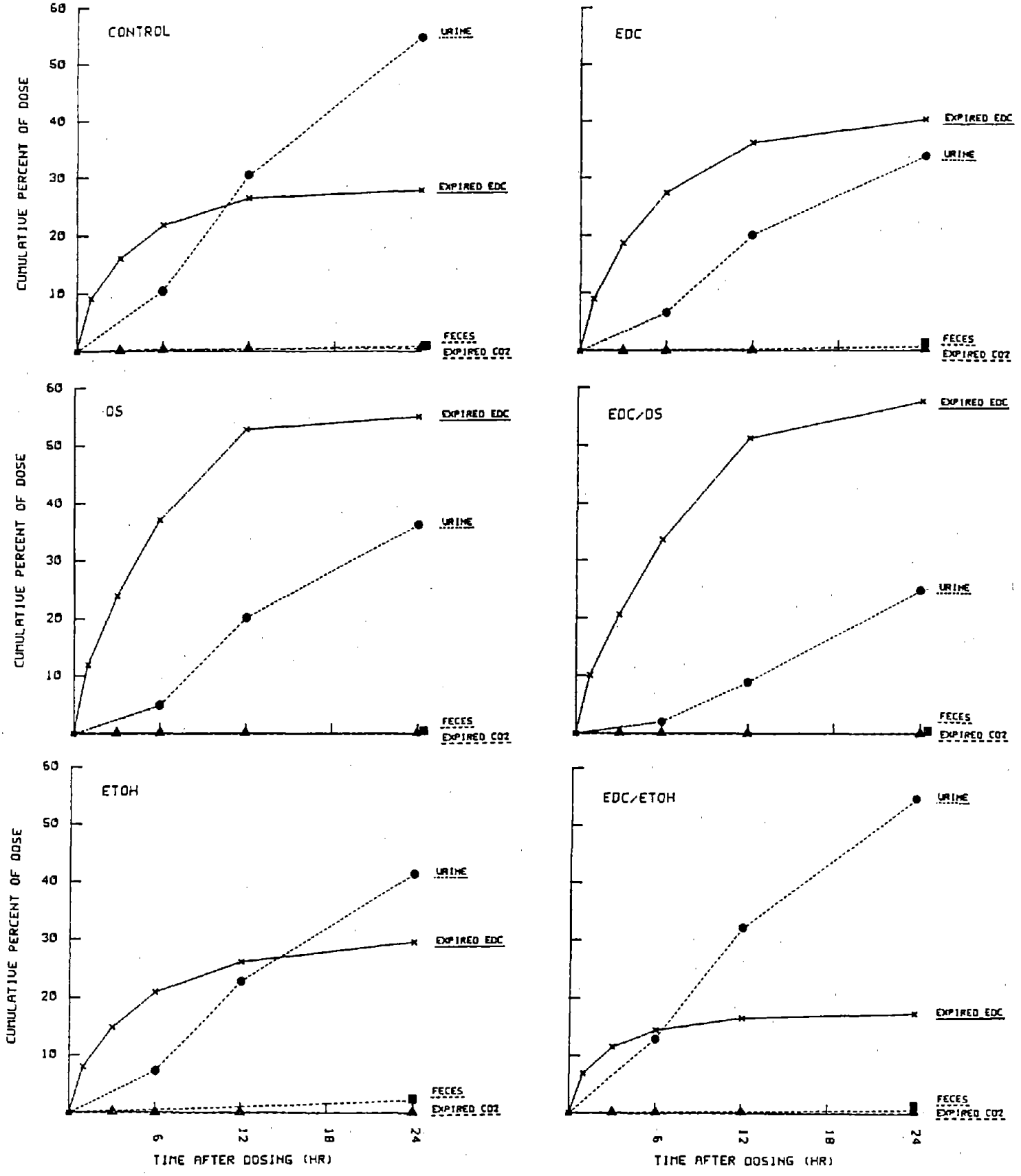

Figure 5 - Cumulative Excretion of Radioactivity from Female S.D. Rats Dosed Orally with ^{14}C-EDC (150 mg/kg) Following 2 Years of Exposure to Other Treatments

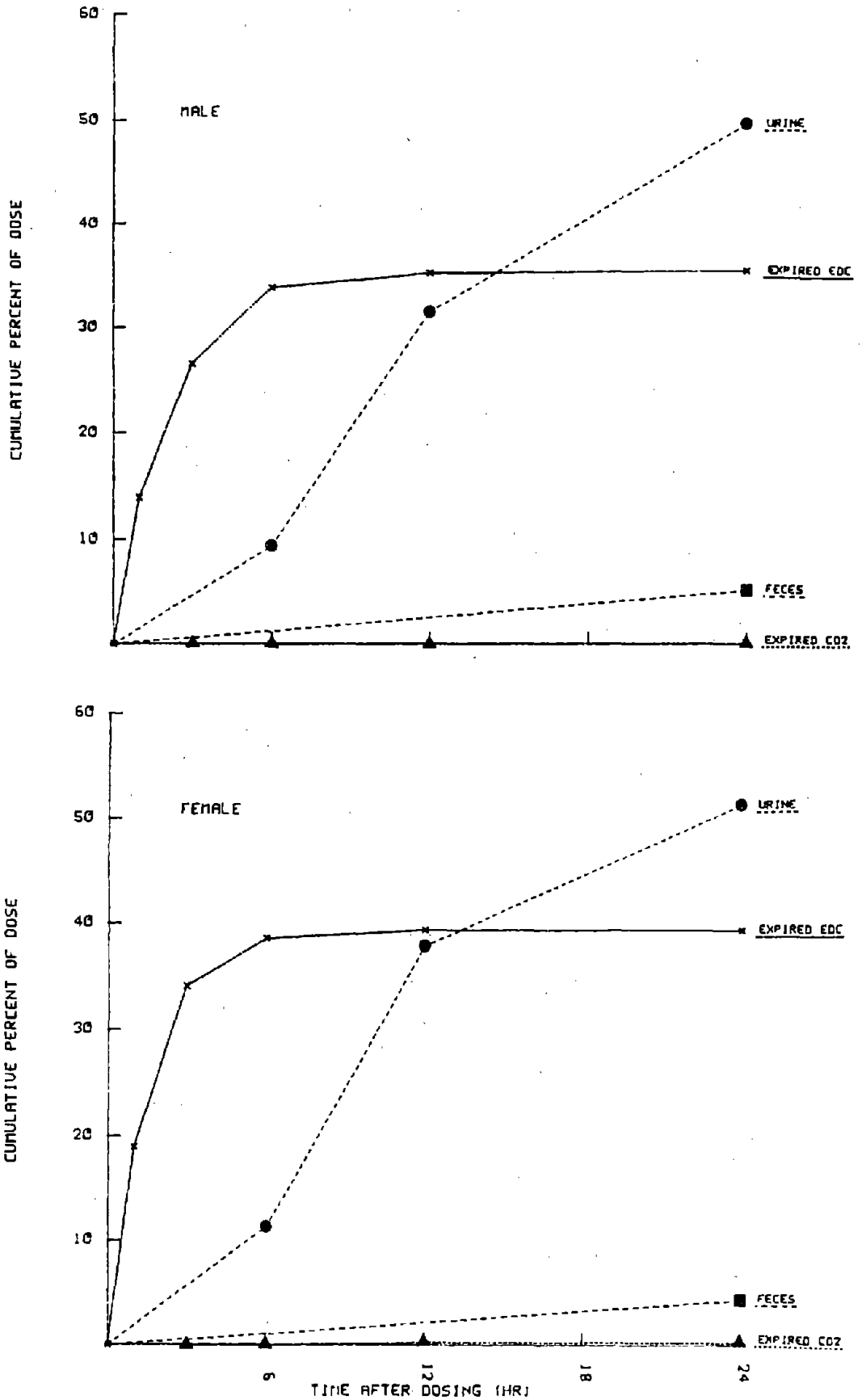

Figure 6 - Cumulative Excretion of Radioactivity Following an Oral Dose of ^{14}C-EDC (150 mg/kg) to 4 Month Old S.D. Rats