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DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

Ethylene dichloride (EDC) has been reported to induce tumors in rats and mice when administered chronically by gavage. In a similar study, chronic inhalation of EDC vapor failed to induce any treatment-related tumors. To help understand the consequences of environmental exposure to EDC by either route, ^{14}C-EDC was administered to male Osborne-Mendel rats by gavage (150 mg/kg in corn oil) or inhalation (150 ppm, 6 hr). EDC was extensively metabolized following either exposure. No significant differences were observe in the route of excretion of non-volatile metabolites. In each case, ∿85% of the total metabolites appeared in the urine, with 7-8%, 4%, and 2% found in the CO_2, carcass, and feces respectively. Two major metabolites were detected in the urine after either exposure comprising ∿70% and ∿30% of total radioactivity. The major urinary metabolite was identified as thiodiacetic acid, while the other metabolite was identified as thiodiacetic acid sulfoxide, indicating the role of glutathione in metabolism of EDC. Each exposure depleted hepatic glutathione to the same extent (∿75%). No marked differences in total macromolecular binding were noted between gavage/inhalation or between "target" and "non-target" tissues. Peak blood levels of EDC were ∿5x higher after gavage versus inhalation.

Elimination of EDC from rats after inhalation exposure was adequately described by a simple two compartment pharmacokinetic model. Elimination of EDC after gavage was more complex, but could be accurately modeled by a two compartment system with saturable elimination from the central compartment.

Soluble enzymes (109,000x g supernate) prepared from the liver of phenobarbital-induced rats and incubated with EDC catalyzed the induction of mutations in S. typhimurium (TA-1535). The induction of mutation was linearly related to the covalent binding of ^{14}C-EDC to DNA purified from these bacteria. Microsomes prepared from these same rats and incubated with EDC did not produce mutations or catalyze binding of EDC to DNA.

When DNA was purified from the organs of rats exposed in vivo to ^{14}C-EDC, very little DNA alkylation was observed after either gavage (150 mg/kg) or inhalation (150 ppm, 6 hr). The degree of alkylation ranged from 2-20 alkylations/10^6 nucleotides. Only slightly highe alkyation was seen after gavage versus inhalation (2-5x), and no marked differences were noted between "target" and "non-target" organs. Therefore in vivo genotoxicity (as measured by EDC/DNA binding) does not provide a satisfactory explanation for the

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PHARMACOKINETICS AND MACROMOLECULAR INTERACTIONS OF ETHYLENE DICHLORIDE IN RATS AFTER INHALATION OR GAVAGE*

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ABSTRACT

Ethylene dichloride (EDC) has been reported to induce tumors in rats and mice when administered chronically by gavage. In a similar study, chronic inhalation of EDC vapor failed to induce any treatment-related tumors. To help understand the consequences of environmental exposure to EDC by either route, ^{14}C-EDC was administered to male Osborne-Mendel rats by gavage (150 mg/kg in corn oil) or inhalation (150 ppm, 6 hr). EDC was extensively metabolized following either exposure. No significant differences were observed in the route of excretion of non-volatile metabolites. In each case, ∿85% of the total metabolites appeared in the urine, with 7-8%, 4%, and 2% found in the CO_2, carcass, and feces respectively. Two major metabolites were detected in the urine after either exposure comprising ∿70% and ∿30% of total radioactivity. The major urinary metabolite was identified as thiodiacetic acid, while the other metabolite was identified as thiodiacetic acid sulfoxide, indicating the role of glutathione in metabolism of EDC. Each exposure depleted hepatic glutathione to the same extent (∿75%). No marked differences in total macromolecular binding were noted between gavage/inhalation or between "target" and "non-target" tissues. Peak blood levels of EDC were ∿5x higher after gavage versus inhalation.

Elimination of EDC from rats after inhalation exposure was adequately described by a simple two compartment pharmacokinetic model. Elimination of EDC after gavage was more complex, but could be accurately modeled by a two compartment system with saturable elimination from the central compartment.

Soluble enzymes (109,000x g supernate) prepared from the liver of phenobarbital-induced rats and incubated with EDC catalyzed the induction of mutations in S. typhimurium (TA-1535). The induction of mutation was linearly related to the covalent binding of ^{14}C-EDC to DNA purified

from these bacteria. Microsomes prepared from these same rats and incubated with EDC did not produce mutations or catalyze binding of EDC to DNA.

When DNA was purified from the organs of rats exposed in vivo to ^{14}C-EDC, very little DNA alkylation was observed after either gavage (150 mg/kg) or inhalation (150 ppm, 6 hr). The degree of alkylation ranged from 2-20 alkylations/10^6 nucleotides. Only slightly higher alkylation was seen after gavage versus inhalation (2-5x), and no marked differences were noted between "target" and "non-target" organs. Therefore in vivo genotoxicity (as measured by EDC/DNA binding) does not provide a satisfactory explanation for the differences noted in the two bioassays, suggesting that nongenetic factors may be important. One such factor may be the apparent saturation of EDC metabolism at the higher blood levels of EDC produced by gavage versus inhalation after nearly equivalent doses of EDC.

INTRODUCTION

Ethylene dichloride (1,2-dichloroethane, EDC, $ClCH_2$-CH_2Cl), is one of the largest volume synthetic organic chemicals manufactured in the United States, with an annual production of ~11 billion pounds. Most of this volume is used as a precursor for vinyl chloride (98%) with small amounts employed as a lead scavenger in gasoline or as a pesticide (Gold, 1980).

EDC was studied in the laboratory of Dr. Ames and reported to be "....an extremely weak mutagen..." (McCann et al., 1975). These authors further indicated that standard rat liver S-9 did not convert EDC to a more mutagenic species. However, Rannug et al. (1978) found that NADPH-independent activation of EDC to a mutagenic species occurred when bacteria were incubated with glutathione (GSH) and soluble (109,000 x g supernate) enzymes. Mutants were not produced when EDC was incubated with purified microsomes, either with or without GSH present, although in vitro binding to DNA and protein was observed under these conditions (Guengerich, et al, 1980). EDC has been reported to be mutagenic in Drosophila melanogaster by a number of authors (Fabricant, 1980).

In 1978 the National Cancer Institute reported that chronic administration of EDC by gavage induced a variety of tumors in rats and mice (NCI, 1978). Tumors were observed in male rats after administration of either the "high" dose (150 mg/kg/day) or the "low" dose (75 mg/kg/day). Almost simultaneously a report from the Bologna Tumor Centre indicated that EDC had failed to produce any treatment-related tumors in rats and mice chronically exposed to EDC vapor at concentrations up to 150 ppm for 7 hr/day (Maltoni, et al, 1980). It appears that the maximum tolerated dose was reached or exceeded in each bioassay since the top doses were reduced early in each experiment due to signs of toxicity (NCI, 1978; Maltoni, 1980). To help

understand the apparent differences between these two bioassays, and to lend perspective to risk estimations from exposure to low levels of EDC by these common routes of exposure, a thorough study of the pharmacokinetics of EDC in rats was conducted.

One of the objectives of this study was to determine the absorbed "dose" of EDC and characterize its elimination after exposure by the two routes. This was estimated by administering EDC to the animals, measuring blood levels of EDC at various times, and then constructing a pharmacokinetic model.

A second objective was to determine the major routes of elimination after exposure to ^{14}C-EDC by gavage or inhalation. This was done in order to investigate the possibility that different metabolic pathways might be involved after the two exposures. Many different pathways exist for EDC metabolism, and it is already clear that these may have different toxicological significance (Guengerich et al, 1980). Consequently, complete ^{14}C-balance studies were conducted and the major urinary metabolites of EDC were characterized.

The final objective was to examine the relative importance of the various mechanisms which might result in an increased tumor incidence such as that observed in the gavage study. Consequently, macromolecular binding (either binding to total macromolecules or specific binding to DNA) was characterized in various tissues after each type of exposure to ^{14}C-EDC *in vivo*. For comparison, DNA binding and mutagenicity were evaluated simultaneously in experiments with bacteria (*Salmonella typhimurium*). In addition, the degree of tissue damage produced by acute and subchronic exposure to EDC was evaluated using histopathology and DNA synthesis as indicators.

Male Osborne-Mendel rats were used for all studies. These animals have been reported to develop squamous cell carcinomas of the forestomach

as well as angiosarcomas of the liver and spleen (NCI, 1978). The doses administered were always 150 mg/kg (gavage) or 150 ppm/6 hr (inhalation) because these represent the highest levels of EDC administered for any significant length of time in the two bioassays.

METHODS

Animals

Male rats (Osborne-Mendel, Certified Pathogen Free) were obtained from Camm Research Lab Animals (Wayne, New Jersey 07470). The animals were 150-250 grams when received and were acclimated at least one week before use. All animals were housed in rooms designed to maintain 72°F, 50% humidity, and a 7 am - 7 pm light cycle. Food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) and water were available ad libitum except during exposures or where otherwise indicated.

Materials

Non-labeled EDC was obtained from Dow Chemical U.S.A. This material was assayed by gas chromatography at the beginning and end of the study and tested greater than 99.9% purity in each case. ^{14}C-EDC (uniformly labeled, 3.2 mCi/mM) was purchased from New England Nuclear (Lot No. 1194-143). The radiochemical purity of this material was checked by gc/ms analysis in our laboratory and was >99%.

Enzymes used in the purificiation of DNA were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade and purchased from commercial suppliers.

EDC Analysis

Samples of the test material were analyzed by gas chromatography on glass columns of 10% SP-1000 on 100/120 Chromosorb W-HP at 80° isothermal. Helium was the carrier gas at 18 ml/min. Radiochemical purity was assessed with columns of 2.5% Oronite NI-W on 60/80 Carbopack B with temperature programing from 70°C to 160°C at 10°/min. (Methane was added to the argon carrier in the transfer line to the mass spectrometer.)

EDC in blood was analyzed according to the technique of Zuccato et al, (1980). In this procedure, 0.5 ml samples of blood were collected

from an indwelling cannula and immediately injected into a 6 ml septum vial containing 1.5 ml of 10% citric acid. The vials were then agitated for at least 30 minutes at room temperature and a sample of the equilibrated head space was taken for analysis. Samples were analyzed on a Tenax G.C. (60/80 mesh) column at 120°C isothermal with 30 ml/min of nitrogen carrier gas and a flame ionization detector. A calibration curve was prepared by adding known amounts of EDC to samples of blood collected from untreated animals, and a known amount of methylene chloride was added to each vial as an internal standard.

EDC vapor concentrations in the exposure chambers were periodically determined by pumping samples from the chamber into a gas sampling loop as previously described (Reitz et al., 1980a).

Exposures

Animals were exposed to EDC at 150 mg/kg by gavage with a solution of 100 mg/ml EDC in corn oil (1.5 ml/kg body weight) or by exposure to 150 ppm EDC for 6 hr in 30 liter single pass or recirculating inhalation chambers (Reitz et al., 1980a). When a recirculating system was used, O_2 was monitored continuously with a Critikon® moisture-insensitive gaseous oxygen analyzer. CO_2 was absorbed in an Ascarite® cartridge and pure O_2 was added as necessary to maintain 20-21 volume % oxygen during exposure. Frequent small injections of ^{14}C-EDC served to keep the vapor concentration within ± 15% of the desired EDC concentration when a recirculating system was used.

All inhalation exposures were 6 hours in duration and were initiated between 7 am and 10 am. Oral dosing was also carried out between 7 am and 10 am. Animals were not fasted prior to exposures.

Procedures

^{14}C-Balance studies were carried out as described by Schumann et al., (1980). Macromolecular binding studies were performed according to the techniques of Jollow et al., (1970), as modified by Schumann et al., (1980). Protein was determined by the method of Lowry et al., (1951).

Enzymes

Microsomal and cytosolic fractions were prepared from phenobarbital-treated male Sprague-Dawley rats as described elsewhere (Guengerich, 1977a, b) and stored at -70°C. In some cases, liver enzyme preparations (S-9) were purchased from Litton Bionetics, Kensington, Maryland. The integrity of the microsomal preparations was verified by assaying the aniline hydroxylase activity according to the method of Kato and Gillette (1965).

Bacterial Mutagenicity

Mutagenicity studies were carried out in Salmonella typhimurium (TA 1535) measuring the reversion to histidine independence as described by Ames et al., (1975). This procedure was modified (Guengerich et al, 1980) to include a 30 minute preincubation period with bacteria, EDC, and enzyme preparations before adding the soft agar and plating on minimal media. Determinations were carried out in triplicate with positive and negative controls included in each experiment. Revertants were scored with an Artek® automatic colony counter after 48 hours incubation. These assays utilize a very small number of bacteria (0.1 ml of an overnight culture $\sim 10^8$ cells). In order to obtain sufficient bacteria for DNA isolation TA 1535 was grown overnight in 2 liter flasks containing 0.8% Nutrient Broth (Difco) supplemented with 0.5% NaCl and 2.0% glucose. (The glucose was autoclaved separately as a 10% solution and combined with the rest of the media after sterilization.) 750 ml portions of broth were innoculated with 1 ml overnight culture and incubated 16 hr at 30°C

with shaking. The bacteria (∿2g wet wt) were harvested by centrifugation and resuspended in either buffer (8mM $MgCl_2$, 33mM KCl in 0.1 M phosphate, pH = 7.4) or rat liver enzymes plus buffer to make a total of 6.4 ml. The protein concentrations of the enzyme preparations (before dilution) were cytosol = 22 mg/ml, microsome = 2.5 mg/ml. Incubations were carried out at 30°C in sealed scintillation vials unless otherwise noted. When indicated, other components were added at the following concentrations: glutathione (1.4 µmol/ml), glucose-6-phosphate (5 µmol/ml), NADP (4 µmol/ml), and glucose 6-phosphate dehydrogenase enzyme (Sigma, 5 Units/ml).

Characterization of Urinary Metabolites

Urine from EDC-exposed animals was collected on dry ice and kept frozen until analysis. After centrifugation to remove debris, 0.4 ml aliquots were applied to an Aminex 50W-X4 column and eluted with 0.005 N H_2SO_4: 96% of the radioactivity applied to this column was recovered in the various fractions collected and most of this was in two major peaks. The fractions were lyophilized to dryness and then derivatized with ethanolic-HCl for analysis by gas chromatography/mass spectroscopy.

Tissue Damage

The possibility that exposures to these levels of EDC might cause sufficient toxicity to stimulate cellular regeneration was investigated by microscopic examination of tissues from animals receiving either single exposures of EDC (150 mg/kg gavage; 150 ppm 6 hr inhalation) or multiple exposures by gavage. The multiple exposure gavage experiment was carried out by dosing the animals once a day for three days, allowing the animals to recover for two days, dosing for five days followed by two dosage-free days, resuming dosage for two days and then sacrificing

the animals four hr after the last dose. 3-4 Animals of the same age/weight were included in each treatment or control group. A complete microscopic examination of the single exposure animals was carried out including the following tissues:

Liver	Heart	Pancreas
Cerebral Cortex	Cerebellum	Brain Stem
Peripheral Nerve	Spinal Cord	Kidneys
Spleen	Pituitary Gland	Stomach
Cecum	Small Intestines	Mesenteric Lymph Node
Mesenteric Vessels	Mesenteric Fat	Large Intestines
Testes	Epididymis	Prostate
Urinary Bladder	Coagulating Glands	
Seminal Vesicles	Lungs	Thyroid
Parathyroid	Trachea	Esophagus
Mediastinal Fat	Aorta	Thymus
Mediastinal Lymph Node		Salivary Gland
Skeletal Muscle	Skin	Mammary Tissue
Mammary Lymph Node	Eyes	Tongue
Adrenal Glands	Cervical Lymph Node	

The following tissues were examined in the animals receiving multiple gavage doses: stomach, liver, kidneys, spleen, thymus, and mesenteric lymph node.

DNA Alkylation In Vivo

The potential of EDC to cause alkylation of DNA in vivo was estimated by determination of the specific radioactivity of DNA isolated from animals exposed to ^{14}C-EDC by gavage (150 mg/kg) or inhalation (6 hr, 150 ppm). DNA was isolated according to the procedure of Marmur (1961), with modifications to improve the purity of the final product (Reitz, et al., 1980a).

The final steps in this purification procedure involve the precipitation of isolated DNA from solution with 5% trichloroacetic acid (TCA), hydrolysis of the precipitate in a buffered solution of deoxyribonuclease, and reprecipitation by addition of more TCA to a final concentration of 5%. Next the incubation is filtered through a Millipore filter (5 micron pore size) and the filtrate is collected. This step adds significantly to the purification process, since only material susceptible to hydrolysis by the purified deoxyrbonuclease was obtained in the final filtrate.

Pharmacokinetic Modeling

A two-compartment pharmacokinetic model was constructed as a set of differential equations describing the input, transfer, and output characteristics of each compartment of the model. The time-dependent values for each variable in the model were determined by numerical integration using the Continuous System Modeling Program (IBM, 1972). Best estimates of the pharmacokinetic parameters of the model for mean blood levels were obtained by the method of least squares using a flexible polygon search routine with a convergence criterion of 10^{-3}. Areas under curves were calculated by numerical integration of the appropriate variables. Simulations were conducted by using the average parameter values of the model in the Continuous System Modeling Program. Specific details of this technique may be found in Ramsey et al., (1980).

Other Analyses

Non-protein sulfhydryl (NPSH) was determined by the method of Sedlak and Lindsay (1968). Glycogen, ribonucleic acid, and protein in the DNA preparations were determined by the methods of Shields and Burnett (1960), Brown (1946), and Bradford (1976) respectively. Normal DNA synthesis and DNA repair synthesis were evaluated as outlined elsewhere (Reitz et al, 1980a). Radioactivity was determined

by liquid scintillation counting with automatic quench correction (Beckman LS-9000). DNA concentration was determined by the procedure of Burton (1956).

RESULTS

Pharmacokinetics

Blood levels of EDC were measured at various times after a 6 hr inhalation exposure to 150 ppm EDC. The elimination of EDC appeared to be biphasic (Figure 1A) with apparent half-lives of ∿ 10 minutes (α-phase) and ∿ 28 minutes (β phase). To aid in the development of a pharmacokinetic model, blood levels of EDC were also measured two hours prior to termination of the inhalation exposure (after 4 hr of exposure). The level of EDC at this time was 8.3±1.9 μg/ml (n=4). Preliminary experiments (data not shown) indicated that the blood level of EDC had essentially reached steady state after 2.5 hours of inhalation exposure. Peak blood levels of EDC during inhalation were 8-9 μg/ml. The inhalation data could be fitted to a two-compartment pharmacokinetic model (Figure 2) similar to that proposed by Spreafico et al. (1980) for EDC elimination in rats. Data were fitted by computer optimization as outlined in Ramsey et al. (1980). The blood levels predicted by this model are shown in Figure 1A (solid line), as are the actually observed means ± standard deviations. The constants derived from computer optimization of this model are K_{12} = 0.0104 min^{-1}, K_{21} = 0.0278 min^{-1} and K_{IE} = 0.0737 min^{-1}. With an estimated input to the central compartment (K_o) of 153 μg EDC/min·kg from inhalation of 150 ppm EDC, the volume of distribution (V_D) was 265 ml/kg.

Blood levels of EDC were also measured after gavage with 150 mg/kg EDC. Absorption was very rapid following gavage. Peak blood levels were reached in less than 15 minutes, and were considerably higher (30-44 μg/ml) than observed after inhalation. The elimination of EDC after gavage was complex (Figure 1B). Although the elimination was roughly log-linear for the first part of the experiment (with an apparent half-life

of ∿ 90 min in this portion) the pattern changed considerably during the latter part of the experiment (Figure 1B). Once the blood levels of EDC fell below 5-10 µg/ml the EDC seemed to be eliminated more rapidly, with a half-life approaching that observed for the β phase after inhalation exposure (20-30 min).

The gavage data were fitted to a pharmacokinetic model similar to that employed for inhalation exposure (with K_{12} and K_{21} equal to 0.0104 min^{-1} and 0.0278 min^{-1} respectively) except that the rate of elimination was calculated as rate = $[C](\frac{V_M}{K_M+C})$ instead of rate = $[C]K_{IE}$. This substitution is employed when there is reason to believe that elimination from the central compartment may involve a saturable process or processes. In addition, absorption of the 150 mg/kg dose from the stomach was assumed to be first order with K_A = 0.400. Values for V_M, K_M, and V_D were obtained by computerized optimization routines (Ramsey et al, 1980). The values obtained in fitting the data to this model were V_M = 0.166 µg/min·ml, K_M = 1.96 µg/ml, and V_D = 3,400 ml. The computerized fit to the data is shown as the solid line in Figure 1B, with experimental data plotted as mean values ± standard deviation (n = 4 for each point except as noted).

Areas under the plot of EDC blood levels versus time (AUC) were calculated for the two sets of data. The AUC after inhalation (2,820 µg min/ml) was not greatly different than the AUC after gavage (4,560 µg min/ml).

^{14}C-Balance Studies

In the radioactive tracer experiments, ^{14}C-EDC from New England Nuclear was diluted with non-radioactive EDC to give specific activities of 1.74×10^{-2} mCi/mMole (inhalation) and 1.78×10^{-3} mCi/mMole (oral).

The results of the ^{14}C balance studies are summarized in Tables 1 and 2. In the oral balance study, 1520 µmoles [^{14}C]EDC/kg (150 mg/kg body weight) were administered. Total radioactivity recovered (body burden) was equivalent to 1539 µmole equivalents/kg (101%). It is not possible to determine percent recovery after inhalation exposure, but the body burden at the end of the 6-hour exposure was 512 µmole equivalents/kg, about one-third that seen after oral dosing.

During the first 48 hours after administration of the oral dose, 447 µmole equivalents EDC were retained in the charcoal trap designed to recover unmetabolized EDC (29% of the body burden). In contrast, only about 1.8% of the body burden was recovered as apparently unchanged EDC after inhalation exposure (9.4 µmole equivalents EDC).

Radioactivity recovered in the CO_2 traps and in the urine was composed of metabolites derived from [^{14}C]EDC (all of the urinary radioactivity was non-volatile). In addition, because of the rapid clearance of EDC from blood, it was assumed that radioactivity remaining in the body after 48 hours, or recovered in the feces or cage wash, was primarily metabolites. Thus, total metabolites after exposure by the two routes may be estimated by subtracting the amount of radioactivity recovered in the charcoal traps from the total radioactivity (Table 1). This revealed that about twice as much EDC was metabolized after oral exposure as compared with inhalation exposure (1092 vs 503 µmole equivalents). However, it must be noted that none of the urinary metabolites or ^{14}C-CO_2 excreted by the animals during the 6 hr inhalation exposure would be recovered.

In order to estimate the amount metabolized by the animals in the inhalation chamber, the total urine excreted by all four animals in the chamber was collected. The total radioactivity was then divided by the total weight of animals present in the chamber. The µmole equivalents

of EDC present in this urine were about 20% of that measured in the metabolism cages (Table 1), so it was assumed that total metabolism (including CO_2 production) was about 20% higher than measured (or approximately 600 μmoles/kg).

The primary route of elimination appears to be urinary excretion of nonvolatile metabolites (Table 1). Significant radioactivity also appeared in the CO_2 trap, with much smaller amounts detected in feces, cage wash, or carcass. After normalization for the different amounts of metabolites formed, the distribution of non-volatile radioactivity after the two routes of administration was virtually identical (Table 1, columns 2, 4).

The residual radioactivity in various tissues of the animals 48 hours after the oral or inhalation exposures was analyzed. The results are shown in Table 2. There were no striking differences noted between tissues where increased frequencies of tumors were reported in the gavage study (liver, spleen and forestomach) and tissues where increased tumor frequencies were not noted (kidney, lung, and stomach) after either route of administration. The residual radioactivity in each tissue was slightly lower after inhalation. The radioactivity in each tissue was in about the same ratio as the total metabolites after the two types of exposure (gavage:inhalation ~2:1).

Urinary Metabolites

The urinary metabolites collected after gavage (150 mg/kg) or inhalation (150 ppm, 6 hr) were analyzed by high pressure liquid chromatography. The results are shown in Figures 3A and 3B. 96% of the urinary radioactivity applied to the column was recovered in peaks A and B. In each case, peak B contained almost 70% of the radioactivity with about 26-28% recovered in peak A. The materials recovered in peaks B and A have been identified

as thiodiacetic acid and thiodiacetic acid sulfoxide respectively by gas chromatography/mass spectroscopy.

Macromolecular Binding

In addition to determining residual radioactivity in tissues 48 hr after exposure, macromolecular binding was measured shortly after exposure by the method of Jollow et al (1973). Exposure was exactly as outlined for the ^{14}C-balance studies except that animals were sacrificed 4 hr after gavage or immediately following a 6 hr inhalation exposure. The results of these experiments are summarized in Table 3. Macromolecular binding was diffuse rather than localized. That is, there were no marked differences between the group of tissues where increased incidence of tumors were observed in the gavage bioassay and those tissues where increased incidences were not observed. Except for the forestomach, slightly higher levels of macromolecular binding were observed after inhalation compared to gavage (Table 3).

DNA Alkylation and Mutagenesis in Bacteria

EDC has been demonstrated to induce mutations in the histidine reversion assay of Ames et al (1975) when incubated with soluble enzymes and GSH (Rannug, et al, 1978; Guengerich et al, 1980). Preliminary experiments indicated that the concentration of bacterial cells could be increased 500 fold before the mutation frequency (revertants/10^8 viable cells) showed any decrease (data not shown). Subsequently $\sim$ 2 g aliquots of TA-1535 were incubated with 7.06 µMoles ^{14}C-EDC/ml (specific act = 3.2 mCi/mM) and varying amounts of cytosol (109,000 xg supernate from rat liver). This treatment had no effect upon the number of viable cells (scored on nutrient agar) but produced a dose-dependent increase in both reversion frequency and incorporation of radioactivity into DNA isolated from these bacteria (Table 4). Linear regression analysis indicated a direct

relationship between the degree of alkylation (dpm/mg purified DNA) and the increase in reversion frequency (Figure 4). The correlation coefficient for linear regression was 0.9976.

A second experiment was carried out in order to compare the abilities of cytosol and purified microsomes to induce DNA alkylation and mutation upon incubation with EDC and bacteria. The results of this experiment are summarized in Table 5. In comparison with the cytosol incubation, the microsomal incubation did not increase the reversion frequency or cause alkylation of bacterial DNA by ^{14}C-EDC. Microsomal preparations have been reported to catalyze alkylation of DNA in vitro by ^{14}C-EDC (Guengerich et al, 1980). The microsome preparation used in this incubation was checked for aniline hydroxylase activity and found to be roughly equivalent to a fresh sample of S-9 from Litton Bionetics Corp. (data not shown). DNA isolated from these incubations was examined for the presence of impurities as outlined in methods. No detectable quantities of RNA or glycogen were observed (detection limit $\sim$ 0.5%) and protein concentration was 1-2% or less.

DNA Alkylation in Rats

^{14}C-EDC was diluted to a specific activity of 0.32 mCi/mM and administered to groups of 3 rats by gavage (150 mg/kg) or inhalation (150 ppm, 6 hr). Since the ^{14}C-balance studies had not indicated that appreciable quantities of volatile metabolites were formed after inhalation of EDC, no special provisions were made to remove these from the recirculating chamber. The µmole equivalents of EDC bound per mole of DNA isolated from various tissues are listed in Table 6. Two independent experiments were carried out.

In general, slightly higher levels of DNA alkylation (3-5x) were seen after gavage versus inhalation. The levels of DNA alkylation were fairly reproducible from experiment to experiment. The contamination of DNA with

other macromolecules was: RNA, <2%; Glycogen, <0.5%; and Protein, <>%. DNA alkylation levels in the kidney were roughly equivalent to those seen in the liver and stomach after gavage. DNA alkylation in the spleen was about 1/3 of that seen in the other tissues.

Tissue Damage

No treatment-related lesions were observed in any of the animals studied by gross necropsy, clinical chemistry, or microscopic examination of tissues after single gavage and inhalation exposures, or after multiple (10) gavage exposures.

A second technique was employed to examine the possible presence of toxicity sufficient to stimulate cellular regeneration. This involved a single exposure of animals to EDC by inhalation or gavage with a subsequent injection of ^{3}H-thymidine 48 hours after the EDC exposures. This technique has been used to investigate the cellular toxicity of other chemicals (Reitz et al, 1980a, 1980b; Schumann et al, 1980). The results of these experiments are listed in Table 7.

There was no increase in cellular regeneration (as indicated by increased DNA synthesis during replication) in the spleen or kidney 48 hr after exposure to EDC by either route. There was a tendency toward increased DNA synthesis in the liver after either route of exposure. However, because of the large variability and the small number of experimental animals the biological significance of these increases is unclear. (Neither increase was statistically significant.)

Glutathione Depletion

EDC was administered to groups of 6 rats by either gavage (150 mg/kg) or inhalation (150 ppm, 6 hr). Four hr after gavage or immediately following the 6 hr inhalation exposure the animals were sacrificed by decapitation and 0.5-1.0 gram samples of liver were taken for

analysis. Hepatic non-protein sulfhydryls (NPSH) were determined as outlined in methods and reported as µg equivalents of glutathione per gram of liver (wet wt.). The results of this experiment are shown in Table 8. NPSH levels following inhalation were 26% of control, while NPSH levels following gavage were 23% of control.

DISCUSSION

EDC is readily absorbed into the body following either inhalation or gavage in rats. Peak blood levels of EDC occur within 15 minutes following gavage, and are observed within an hour or two during continuous inhalation of EDC. Following exposure, EDC is rapidly eliminated from the body after either gavage or inhalation. The primary route of elimination appears to involve conjugation with glutathione to form non-volatile urinary metabolites, including the compound thiodiacetic acid reported earlier as a metabolite of EDC in mice (Yllner, 1971). The major urinary metabolites of EDC appear to be identical following either route of exposure, and are formed in the same relative amounts (Figure 3). In addition, hepatic non-protein sulfhydryl groups are depressed to about the same extent following inhalation or gavage (Table 4). Consequently the glutathione pathway(s) appears to be of about equal importance in the metabolism of EDC following either type of exposure. Smaller amounts of EDC are excreted as apparently unchanged material following gavage, or are metabolized by a pathway which releases carbon from EDC as exhaled CO_2 following both gavage and inhalation.

The rate of elimination is such that EDC cannot be detected in blood a few hours after gavage or inhalation. Furthermore, the ^{14}C balance study revealed that radioactivity from ^{14}C-EDC is almost quantitatively eliminated within 48 hours after either route of exposure. Consequently, there should be little tendency for either EDC or its metabolites to accumulate in animals following multiple exposures. These results are consistent with those reported by Spreafico et al (1980) who observed that EDC was rapidly and uniformly distributed throughout the body except for adipose tissue where a 5-10 fold higher concentration was seen. Spreafico further noted that EDC was eliminated from the individual

tissues, including adipose tissue and blood, with very similar rates (Spreafico et al, 1980).

Gavage with 150 mg/kg EDC or inhalation of 150 ppm for 6 hrs produced a similar "dose" of EDC to the test animals (estimated as the total area under the blood level/time curves), and resulted in the formation of similar amounts of EDC metabolites in each case (Table 1). However, the peak blood levels of EDC were almost 5x higher following gavage versus inhalation. This observation may be of some toxicological significance because the higher blood levels produced by gavage seem to have resulted in saturation of the normal detoxification pathways.This was first suggested by Spreafico after he noted that the apparent half life of EDC varied with dose in his gavage studies (Spreafico et al, 1980) and is suggested in these studies by the change in apparent half-life when blood levels fall below about 5-10 µg/ml of EDC after gavage (Figure 1B). Saturation of EDC metabolism could not be confirmed from the data we obtained after inhalation exposure (150 ppm), but may be inferred from Spreafico's observation that peak blood levels of EDC rose 22x (1.4 µg/ml → 31 µg/ml) when the exposure concentration was changed from 50 ppm to 250 ppm (5x)(Spreafico et al, 1980). Thus it appears that a "critical concentration" may exist in both strains of rats at about 5-10 µg EDC/ml of blood. As long as blood levels of EDC are below this "critical concentration" EDC is readily eliminated. However, once the EDC blood levels exceed the "critical concentration". elimination of EDC becomes saturated, resulting in increased half-lives and disproportionately increased AUC's. As discussed elsewhere (Ramsey & Reitz, 1980) such a situation may result in unexpected toxicity when blood levels rise above the "critical concentration".

The reports from the two bioassays are consistent with this theory. Blood levels of EDC exceeded the "critical concentration" after exposure to 250 ppm EDC but not after exposure to 50 ppm (Spreafico et al, 1980).

Exposure to 150 ppm produced blood levels in the transition region (Fig. 1A). Maltoni reported that most of the toxicity associated with exposure to 250 ppm disappeared when exposure concentrations were reduced to 150 ppm and no treatment-related effects were noted at 50 ppm or less (Maltoni et al., 1980).

In another (teratology) study, exposure of rats to 300 ppm EDC, 6 hr day, for 10 days resulted in over 50% mortality (10/16 rats died). However, exposure to 100 ppm EDC (30 rats, 10 days) produced no signs of toxicity (Rao et al., 1980).

Definite toxicity in the form of early mortality and reduced body weight was noted in the gavage bioassay at both the "high" and "low" doses of EDC (which were 150 and 75 mg/kg/day early in the NCI experiment, but were later reduced to 100 and 50 mg/kg/day). Gavage with EDC produces a sharp "spike" in the blood level curve so that the apparent "critical concentration" is exceeded after both 150 mg/kg (Figure 1B; Spreafico et al, 1980) and 50 mg/kg (Spreafico et al, 1980). The apparent "critical concentration" would not be exceeded after inhalation of less than 50 ppm EDC or after gavage with less than ∿25 mg/kg EDC (data from Spreafico et al, 1980).

The limited amount of data analyzed leaves room for some variation in the details of the pharmacokinetic models, such as the estimated values of V_m and K_m. In addition, it must be remembered that any pharmacokinetic model is only an approximation of the complex processes occurring in living organisms. Nevertheless, this pharmacokinetic analysis can give valuable guidance to the interpretation of toxicological data. For example, this analysis emphasizes the errors implicit in employing a single daily gavage to simulate exposure by inhalation or ingestion of a test material. Ingestion of EDC from environmental exposure undoubtedly occurs as a number of small "doses" spread out over the course of a day. The rapid elimination of EDC in such cases would result in much lower peak blood levels or AUC, even

when the total daily dose was equal to the amount administered by gavage.

To demonstrate this, the pharmacokinetic model used to describe the behavior of EDC after gavage was used to predict the peak blood levels and AUC after a series of 8 doses of 18.75 mg/kg administered at 3 hr intervals (total = 150 mg/kg/day). The peak blood levels and AUC's predicted for this type of exposure were much lower than those actually observed (3.96 µg/ml and 1050 µg min/ml predicted versus 36 µg/ml and 4560 µg min/ml observed). Pharmacokinetic analysis also suggests that the inhalation bioassay results (at blood levels of EDC which appear to be non-saturating) are more appropriate for evaluating the risk associated with low-level environmental exposure to EDC than the results of the gavage bioassay, where elimination of EDC was apparently saturated.

One of the most common theories of chemical carcinogenesis (the somatic mutation theory) holds that cancer results from the reaction of chemicals with DNA to induce mutations (Miller & Miller, 1966). EDC has been reported to bind to protein and DNA *in vitro* (Banerjee and Van Duuren, 1979; Guengerich et al, 1980) and has also been reported to induce mutations in bacteria (Rannug et al, 1978) and *Drosophila melanogaster* (Fabricant, 1980). Consequently the binding of EDC to macromolecules *in vivo* was investigated to determine whether this could be correlated with the tumors reported in the gavage study.

Residual radioactivity in selected tissues of rats 48 hr after exposure to ^{14}C-EDC was slightly higher after gavage versus inhalation (Table 2). In general, the levels of radioactivity reflected the relative amount of EDC metabolized by the two routes (Table 1).

As shown in Table 1, a significant amount of radioactive CO_2 was found after exposure to ^{14}C-EDC. This raised the possibility that some of the residual radioactivity observed in tissues might result from biosynthetic incorporation of C-1 fragments rather than binding of reactive EDC metabolites.

Consequently, macromolecular binding was also determined immediately following inhalation exposure or 4 hr post gavage. At this time, biosynthetic incorporation of C-1 fragments should be greatly diminished. Under these conditions, inhalation exposure produced slightly more macromolecular binding than oral exposure (Table 3). In either case, there was no major difference between gavage and inhalation data, or between sites where tumors were reported and sites where tumors were apparently absent. Thus in vivo macromolecular binding of ^{14}C-EDC was not well correlated with the production of tumors in rats.

Since Guengerich et al (1980) suggested that the multiple pathways of EDC metabolism may differ in their ability to alter DNA in vivo, binding of ^{14}C-EDC to carefully purified DNA was investigated. The first studies were carried out in Salmonella typhimurium (TA-1535). In these studies a direct correlation between binding to DNA and induction of mutations (reversion to histidine independence) was noted. Although both microsomes and soluble enzymes catalyze binding of EDC to naked DNA in vitro (Guengerich et al, 1980), only soluble enzymes (probably glutathione S-transferases) induced mutations or catalyzed DNA alkylation in intact bacteria (Table 5). The DNA alkylation and induced mutation frequency could be varied by altering the concentration of soluble enzymes in the incubation (Table 4). However, the DNA alkylation and mutation frequency always varied proportionately (Figure 4). Thus it was postulated that determination of the overall level of DNA alkylation in vivo in rats would be an indication of the extent to which a genetic (mutation) mechanism could be functioning. There are, of course, many potential sites where EDC alkylation of DNA could occur and it is unlikely that all of these sites are equivalent in their susceptibility to repair and/or ability to cause mispairing during replication. However, it seems reasonable to assume that the relative proportions of base adducts would remain fairly constant under the experimental conditions employed (i.e. one species, sex and strain of animal and only one test chemical). Because

of the low level of alkylation achieved, this could not be verified experimentally.

Two independent determinations of DNA alkylation were carried out. The results show relatively good agreement between experiments (Table 6). Gavage consistently produced higher levels of DNA alkylation than inhalation. However, it is still difficult to reconcile the results of the two contradictory bioassays with the DNA alkylation data. For example, tumors were reported in male rats after both the "high" and "low" dose levels in the gavage bioassay. Although DNA alkylation was not measured at a level equivalent to the lower gavage dose (75/50 mg/kg/day) we would expect it to be similar to that observed after the inhalation exposure which was not associated with tumor formation. Furthermore, the absolute levels of DNA alkylation observed are very low relative to those observed with several other chemicals thought to induce cancer through genetic mechanisms.

Lutz (1979) has recently reviewed the binding of chemicals to DNA in vivo and has suggested that they may be classified as having "strong", "moderate", or "weak" potential to act as genotoxic carcinogens according to whether they bind thousands, hundreds, or tens of micromoles of chemical per mole of DNA after a standard dose of 1 millimole/kg. The "Chemical Binding Indexes (CBI)" reported for several chemicals are listed in Table 9 (Lutz, 1979). The CBI calculated for EDC from these studies is such that it would be rated as having very "weak" potential to produce genotoxic lesions in vivo.

We also failed to note a relationship between DNA alkylation in the tissues studied and the reported susceptibility of these tissues to tumor formation (Table 6). DNA alkylation was about equal in the kidney and the liver and both these tissues showed more DNA alkylation than the stomach or spleen. However, tumors were not reported to occur in the kidney, while they were seen in other tissues.

Weisburger and Williams (1980) suggest that several types of mechanisms, either genetic or epigenetic, may alter the frequency of tumor expression in animal bioassays. Since the administration of high levels of halogenated hydrocarbons often produces significant tissue damage at the sites where tumors later develop (Reitz et al, 1980a, b; Schumann et al, 1980), the cellular regeneration and histopathology of selected tissues from EDC-treated animals were examined. However, no treatment-related changes were noted by microscopic examination after acute or two week repeated dosing, and a small increase in cellular regeneration in the liver of treated rats (Table 7) was not statistically significant. It is possible that prolonged administration of EDC may produce the type of tissue damage associated with tumorigenicity of materials such as chloroform, but this cannot be established from the experiments reported here.

In summary, EDC is readily metabolized and rapidly eliminated from the body after either route of exposure. However, as suggested by Spreafico et al (1980), EDC metabolism appears to be saturable, and such saturation appears more likely to occur after administration of equivalent doses by gavage than inhalation. Toxicity often results when normal detoxification is saturated. This may account for the fact that early mortality was noted in the gavage bioassay of EDC but not in the inhalation bioassay. This may also provide the most satisfactory explanation for the apparent difference in the results of the two bioassays, since total dose, GSH depletion, macromolecular binding and DNA alkylation were very similar after the two exposures and the strains of rats used in the two bioassays have each been shown to be responsive to the carcinogenicity of other halogenated hydrocarbons. Furthermore the low level of DNA alkylation suggests that the potential of EDC to produce genetic alterations in vivo is very small. Therefore in vivo genotoxicity (as measured by EDC/DNA

binding) does not provide a satisfactory explanation for the different results in the two bioassays, suggesting that non-genetic factors, such as the relatively high peak blood levels of EDC after gavage may be important.

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TITLE OF STUDY: Pharmacokinetic and Macromolecular Interactionsof Ethylene Dichloride in Rats after Inhalation or Gavage

HET-K-1985-(15)

In compliance with Good Laboratory Practice Regulations, the study phases were inspected by the Quality Assurance Unit and the results of these inspections reported to Management and the Study Director on the dates listed below. The report accurately reflects the data generated in accordance with the regulations and standard operating procedures of the laboratory. All data and the reports are located at the submitting laboratory.

Study Started:	13 June 1979	Report Issued Date:	February 3, 1981
Dates of Inspection:	9 July 1979	Date of Report:	9 July 1979
	25 October 1979		2 November 1979
	4 February 1980		5 February 1980
	3 June 1980		5 June 1980
	14 November 1980		17 November 1980

W. E. Hoover 29 January 1981

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TABLE 1

Fate of [^{14}C] EDC in Rats 48 Hours After Oral (150 mg/kg) or Inhalation (150 ppm, 6-hr) Exposure

	Oral		Inhalation	
	µmole/kg	% metabolites	µmole/kg	% metabolites
Body burden (A) (total radioactivity)	1539±391	-	512±135	-
Charcoal trap (B)	447± 60	-	9.4±0.4	-
Total metabolites (A-B)	(1092)	(100)	(503)	(100)
Urine	926±348	85.7	432±121	84.4
CO_2 trap	83.1±11.9	7.7	36.1±6.89	7.0
Total carcass (48-hr after exposure)	46.9±10.1	4.3	22.7±3.38	4.4
Feces	23.6±14.7	2.1	8.90±2.84	1.7
Cage wash	12.5± 6.37	1.1	3.34±1.34	0.7
Residue in Inhalation Chamber	-	-	98*	

Values are mean ± S.D. with n = 4 for each route of exposure, and are µmole equivalents of EDC, based on the specific activity of [^{14}C]EDC (3.2 mCi/mM).

*Total radioactivity in wash of inhalation chamber divided by total wt. of animals to give µmole equivalents/kg.

TABLE 2

Distribution of Radioactivity in Selected Tissues of Rats 48 Hours After Exposure to [^{14}C]EDC by the Oral or Inhalation Routes

	Nanomole equivalents/g tissue	
	oral (150 mg/kg)	inhalation (150 ppm, 6 hr)
Liver*	154 ± 26	75 ± 13
Kidney	120 ± 20	77 ± 8.6
Lung	51 ± 16	35 ± 5.4
Spleen*	59 ± 19	38 ± 6.0
Forestomach*	108 ± 31	37 ± 7.2
Stomach	62 ± 21	30 ± 6.4
Carcass	23 ± 4.4	13 ± 4.0

Results are reported as nanomole equivalents of EDC/g tissue ± S.D. (n = 4). Radioactivity was determined by combustion of tissue samples, trapping $^{14}CO_2$.

*Site where malignant tumors were observed in the NCI bioassay after EDC was given by gavage.

TABLE 3

Macromolecular Binding in Selected Tissues of Rats 4 Hr or 6 Hr After Exposure to [^{14}C]EDC by Oral or Inhalation Routes

	Nanomole equivalents EDC/g tissue	
	oral (150 mg/kg)	inhalation (150 ppm, 6 hr)
Liver*	175 ± 24	268 ± 45
Kidney	183 ± 25	263 ± 48
Spleen*	65 ± 21	130 ± 22
Lung	106 ± 34	147 ± 16
Forestomach*	160 ± 19	71 ± 19
Stomach	90 ± 2	156 ± 29

Results are reported as nanomole equivalents of EDC/g tissue ± S.D. (n = 4). Radioactivity was determined by scintillation counting of digests. Animals were sacrificed 4 hr after oral dosing or immediately following a 6-hr inhalation exposure.

*Site where malignant tumors were observed in the NCI study after EDC was given by gavage.

TABLE 4

Reversion Frequency and DNA Alkylation in Cultures of TA-1535 Incubated with ^{14}C-EDC and Rat Liver Enzymes plus Glutathione

	Reversion Frequency (mutants/10^8 cells)	Specific Radioactivity of DNA (dpm/mg)
Control	4.0 ± 1.5 (n = 3)	-
2.2% Cytosol*	4.6 ± 0.82 (n = 3)	8.65
7.8% Cytosol	23.5 ± 3.0 (n = 3)	27.0
27% Cytosol	80.2 ± 9.6 (n = 3)	107
71% Cytosol	111 ± 2.6 (n = 3)	137

Each incubation (9 ml vol) was carried out for 30 min at 30°C in a sealed scintillation vial and contained the following components in addition to cytosol: ^{14}C-EDC (spec act 3.2 mCi/mM), 63.5 µMoles; glutathione, 13 µ Moles; bacteria, ∿ 2 g net wt; and was buffered at pH = 7.4 with ∿ 0.1 M Phosphate.

*Cytosol is the 109,000 X g supernate from rat liver preparation (Guengerich et al, 1980). Protein concentration (before dilution) = 22.5 mg/ml.

TABLE 5

Reversion Frequency and DNA Alkyation after Incubation of Cytosol or Microsomes with ^{14}C-EDC and TA-1535

	Reversion Frequency (mutants/10^8 cells)	Specific Radioactivity of DNA (dpm/mg)
Control	5.1 ± 2.9 (n = 3)	-
Cytosol*	430 ±30 (n = 3)	111
Microsomes	6.8 ± 5.0 (n = 3)	5.7

Each 9.0 ml incubation contained ∿ 0.7g bacteria, 88.9 μ Moles ^{14}C-EDC (3.2 mCi/mM) and ∿200 μ Moles Phosphate buffer (pH = 7.4). The cytosol incubation also contained 13 μ Moles of glutathione, while the microsome incubation also contained 13 mg glucose-6-phosphate, 27.9 mg NADP, and 6.5 Units of glucose-6-phosphate dehydrogenase. The incubations were carried out at 37°C for 30 min in a sealed scintillation vial; 144 mg of cytosol protein and 16 mg of microsomal protein was used in the respective incubations.

*Cytosol is the 109,000 x g supernate from rat liver preparations (Guengerich et al, 1980).

TABLE 6

Micromole-equivalents of ^{14}C-EDC Bound/Mole of DNA
Isolated from Rats Exposed to EDC by Gavage (150 mg/kg)
or Inhalation (150 ppm, 6 hr)

	Gavage	Inhalation
Experiment #1		
Liver	21.3 ± 7.4	8.2 ± 3.3
Spleen	5.8 ± 0.7	1.8 ± 0.3
Kidney	17.4 ± 2.3	5.2 ± 3.7
Stomach	14.9*	2.8*
Experiment #2		
Liver	13.9 ± 2.1	3.3 ± 1.2
Spleen	2.5 ± 0.3	1.8 ± 0.5
Kidney	14.5 ± 6.2	2.0 ± 0.3
Stomach	6.7*	1.9*

Animals were sacrificed 4 hr post gavage or immediately following inhalation. n = 3 for all experiments. Specific radioactivity of ^{14}C-EDC was 0.32 mCi/mM.

*Tissue from all 3 animals was pooled for DNA isolation.

TABLE 7

Cellular Regeneration in Tissues of Rats Exposed to Ethylene Dichloride

Tissue	Mean Specific Radioactivity of DNA (dpm/mg) ± S.D.	
	Inhalation (150 ppm, 6 hr)	Gavage (150 mg/kg)
Liver	$\frac{13.5 \pm 7.54\ (n = 3)}{6.24 \pm 1.86\ (n = 4)}$ (2.16)	$\frac{13.2 \pm 9.76\ (n = 3)}{6.24 \pm 1.86\ (n = 4)}$ (2.11)
Kidney	$\frac{2.48 \pm 0.78\ (n = 3)}{2.86 \pm 0.71\ (n = 4)}$ (0.87)	$\frac{1.91 \pm 0.85\ (n = 3)}{2.86 \pm 0.71\ (n = 4)}$ (0.67)
Spleen	$\frac{9.29 \pm 2.51\ (n = 3)}{9.34 \pm 0.29\ (n = 4)}$ (0.99)	$\frac{8.22 \pm 1.49\ (n = 3)}{9.34 \pm 0.29\ (n = 4)}$ (0.88)

Animals were injected with tritiated thymidine 48 hr after exposure to EDC; 4 hr after the thymidine injection the animals were sacrificed and DNA was isolated from the indicated tissues. The regenerative index is calculated as the ratio of the specific radioactivity of the DNA isolated from treated animals to DNA isolated from a control group of animals receiving an identical injection with tritiated thymidine. None of the regenerative indexes were significantly different from one (Student's t-test, $p = 0.05$).

TABLE 8

Non-protein Sulfhydryl Content of Liver in Rats
Exposed to EDC by Inhalation or Gavage

	mg equivalents of glutathione/ g liver
Control (no treatment)	1.35 ± 0.31
Gavage (150 mg/kg)	0.308± 0.165*
Inhalation (150 ppm, 6 hr)	0.350± 0.086*

Samples of liver were analyzed 4 hr after gavage or immediately following inhalation. Each value report is the mean ± standard deviation for 6 rats.

*Significantly different from control ($p < 0.05$, Student's t-test).

TABLE 9

Chemical Binding Indexes (CBI) for Binding of Various Chemicals to DNA In Vivo at a Standard Dose of 1 Millimole per Kg According to Lutz (1979).

Potency Rating	Micromole Chemical/Mole DNA
Strong:	
Aflatoxin	17,000*
Dimethylnitronamine	6,000*
	(7,430)**
Moderate:	
Vinyl Chloride	525*
2-Acetylaminofluorene	560*
O-Aminoazotoluene	230*
Weak:	
Urethane	29-90*
4-Dimethylaminoazobenzene	6*
1,2-Dichloroethane (EDC)	3-12**

* Data from Lutz (1979).

** Data from Dow Laboratories.

FIGURE 1 Blood levels of EDC during or following exposure to EDC by inhalation (6 hr, 150 ppm, Figure 1A) or gavage (150 mg/kg, Figure 1B). Values predicted by the pharmacokinetic model are shown as a solid line with actual data plotted as means ± standard deviation. n = 4 unless otherwise noted.

FIGURE 2 Two compartment pharmacokinetic model used to describe the elimination of EDC in rats after inhalation or gavage.

FIGURE 3 Chromatography of urinary metabolites on an Aminex 50W-X4 column with 0.005 N H_2SO_4 eluent, collected after inhalation of ^{14}C-EDC (6 hr, 150 ppm, A) or gavage (150 mg/kg, B).

FIGURE 4 Correlation of reversion frequency in TA-1535 and DNA alkylation after incubation of bacteria with ^{14}C-EDC and various concentrations of soluble enzymes from rat liver.

FIGURE 1A

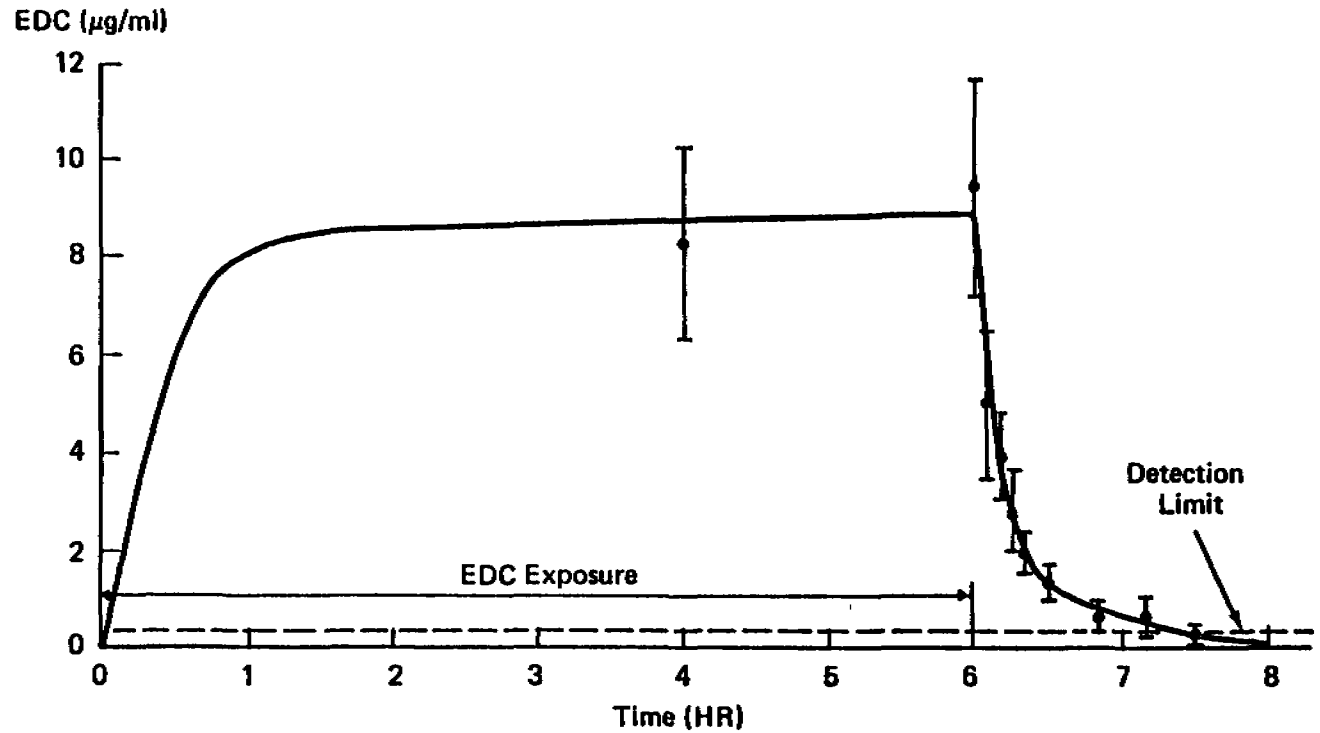


FIGURE 1B

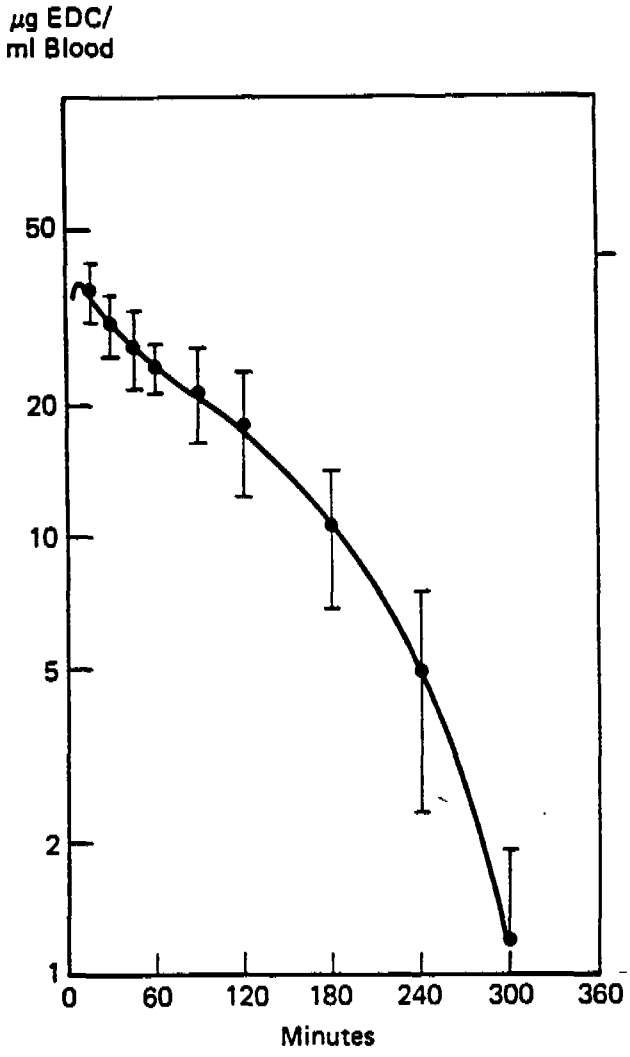


FIGURE 2

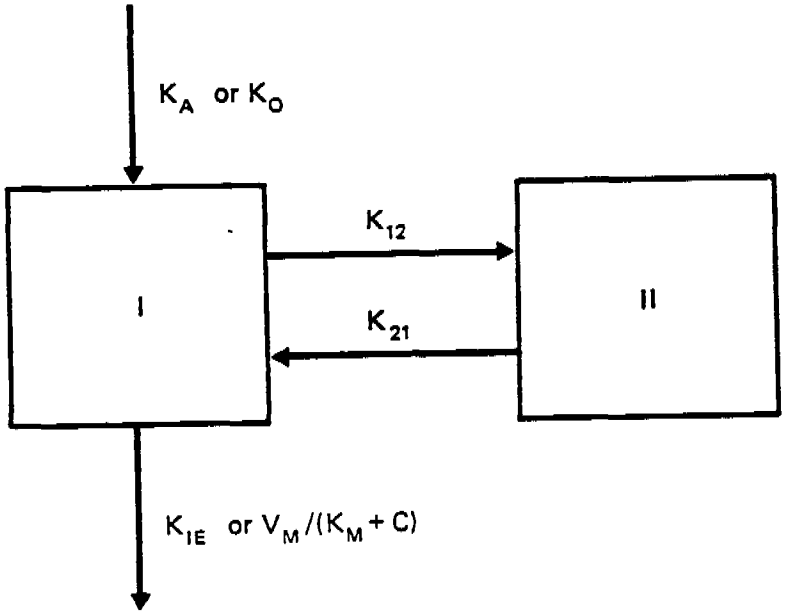


FIGURE 3A

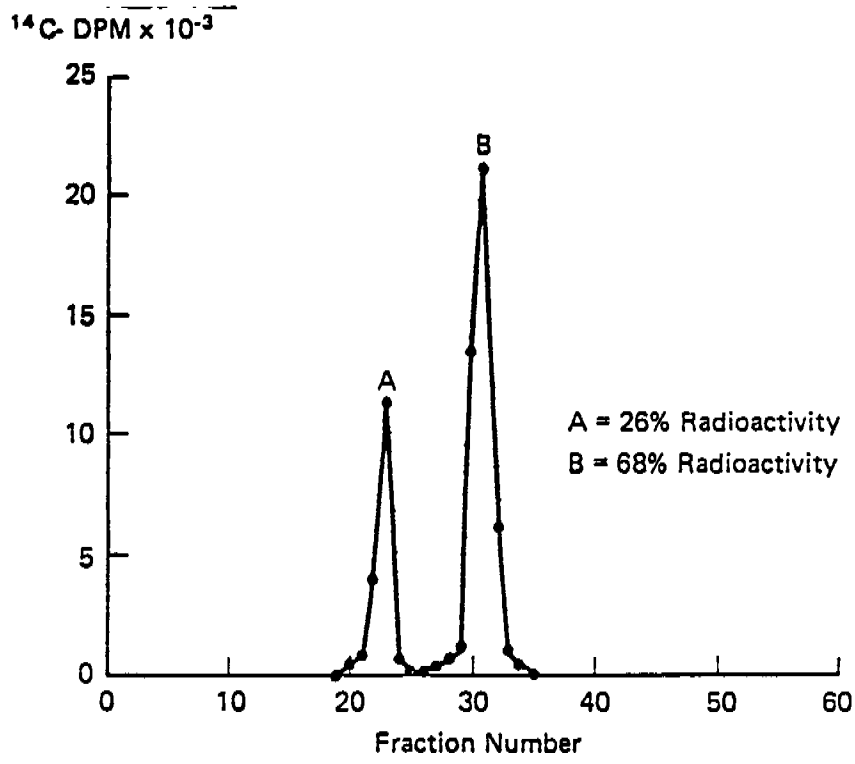

^{14}C- DPM x 10^{-3}
25
20
15
10
5
0
A
B
A = 26% Radioactivity
B = 68% Radioactivity
0
10
20
30
40
50
60
Fraction Number

FIGURE 3B

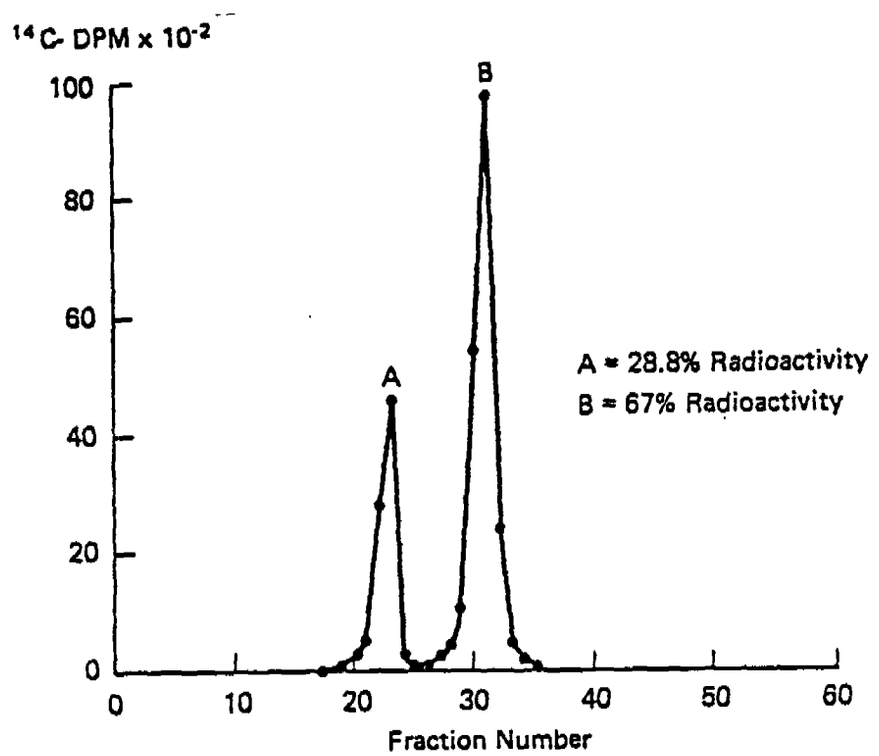
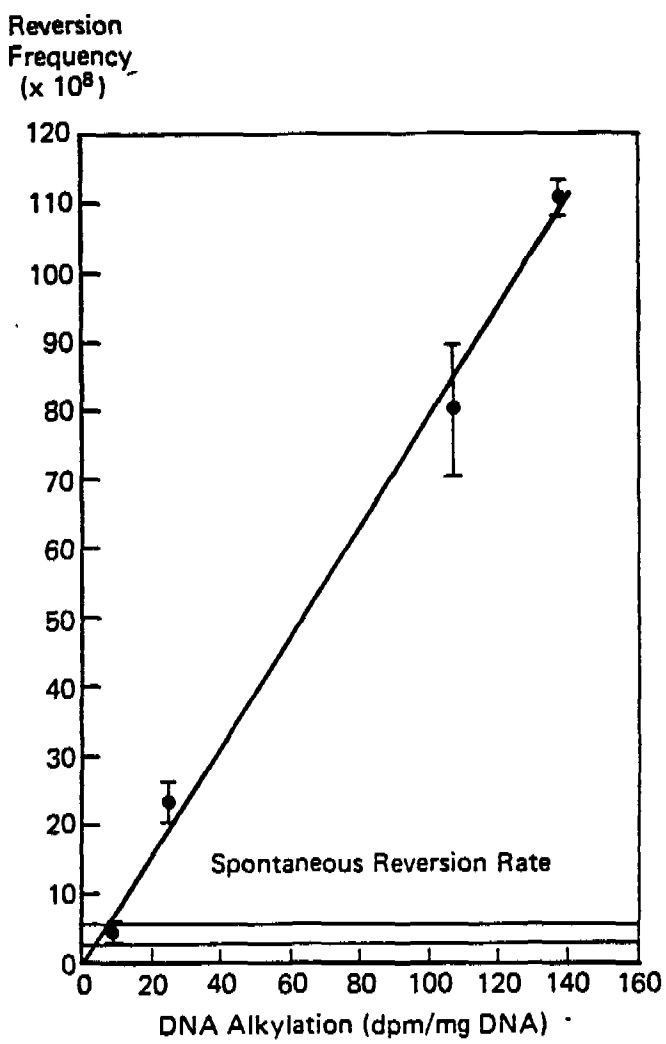


FIGURE 4



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