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BISPHENOL A (DGEBA):		—	

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DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA): FATE IN MALE FISCHER 344 RATS (PROBE)

By

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December 15, 1981

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ABSTRACT

Concern has been expressed that diglycidyl ether of bisphenol A (DGEBA), a major component of DER 331* epoxy resins, may represent an inhalation hazard. Due to its physical properties meaningful repeated inhalation exposure studies cannot be conducted. Thus oral toxicity studies have been proposed as an alternate means to evaluate the hazard. The purpose of this study was to obtain preliminary data on the metabolism and kinetics of DGEBA. The rationale being that oral toxicity data could be used to predict the inhalation hazard, if the fate of DGEBA were route-independent.

An atmosphere suitable for a single 6 hr inhalation exposure to ^{14}C-DGEBA could not be generated. Thus intravenous administration was substituted for the inhalation route. Route dependent differences were observed in the plasma ^{14}C-concentration time profile, tissue/ plasma ^{14}C-ratios and the urinary ^{14}C-excretion following oral and intravenous administration. These differences suggest that little of the orally administered ^{14}C-DGEBA was absorbed unchanged. Consistent with this, 4 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA. ^{14}C-DGEBA was not stable in simulated gastric fluid and degradation of ^{14}C-DGEBA in the stomach or during its initial transit through the liver could account for the route dependent differences observed in the fate of ^{14}C-DGEBA. These data indicate that oral toxicity data would be of little value in assessing the inhalation hazard. Furthermore, the inability to generate a suitable atmosphere using low airflows, high temperatures and a large surface area suggests that inhalation is an insignificant route for DGEBA exposure.

*Trademark of The Dow Chemical Company.

INTRODUCTION

The objective of these studies was to provide data on the utility of conducting oral toxicity studies to assess the possible inhalation hazard associated with the diglycidyl ether of bisphenol A (DGEBA). DGEBA, a bifunctional epoxide, is the major reactive component of DER 331* epoxy resins. Although no human exposure data are available, it has been speculated that DGEBA may represent an inhalation hazard because it contains two reactive epoxide bonds. Ideally, an inhalation study should be conducted to assess a potential inhalation hazard. However, because of the physical properties of DGEBA, it is unlikely that an atmosphere suitable for a repeated exposure inhalation study could be generated. Therefore, oral toxicity data have been proposed as a means to assess the hazard associated with inhaled DGEBA.

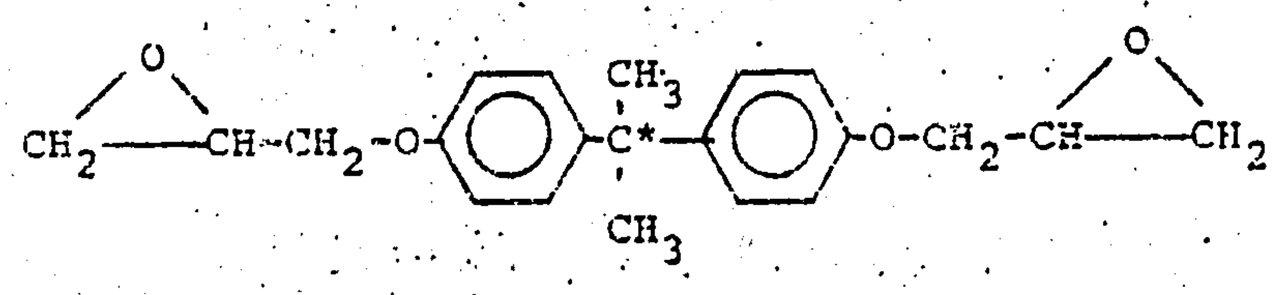
It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body, and that route dependent differences in the fate of a chemical may significantly alter its toxicity. This study was undertaken to provide preliminary data on the metabolism and kinetics of ^{14}C-DGEBA. The work described in this report was designed: (1) to determine if an atmosphere could be generated that would be suitable for a single exposure inhalation kinetic study with ^{14}C-DGEBA, (2) to compare the fate of ^{14}C-DGEBA following oral and intravenous administration, (3) to determine the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid, and (4) to compare the chromatographic profile of radioactivity in plasma following oral and intravenous administration of ^{14}C-DGEBA.

*Trademark of The Dow Chemical Company.

MATERIALS

Animals. Male Fischer 344 rats weighing 195-250 g were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. The animals were housed in rooms designed to maintain a constant temperature (72 ± 2°F), humidity (50 ± 10%), 12 hr photocycle, and 10-12 air changes per hour. Ralston Purina Rat Chow and tap water were available *ad libitum* except for an 18 hr fast prior to dosing the rats used to define the chromatographic profile of the plasma radioactivity. When required by the study objectives, juglar and biliary cannulas were implanted 48 hr prior to dosing using standard surgical procedures.

Test Material: p,p'-Diglycidyl ether of bisphenol A isopropylidine, methylene-^{14}C (^{14}C-DGEBA; Lot #ES-TDL-124) was synthetized from ^{14}C-labeled bisphenol A by T. Licky of The Dow Chemical Company.



*Location of carbon-14 label

The radiotracer had a specific activity of 7.98 μCi/mg and a radiochemical purity of 98% as determined by high pressure liquid chromatography (HPLC) with fractional collection and Carbon-14 analysis of the column effluent (T. Licky, 1979).

Reagent grade DGEBA (Lot #332R91) was used to adjust the specific activity of the ^{14}C-DGEBA in the vapor generation experiments and as an internal standard for the HPLC analysis.

EXPERIMENTAL

Vapor Generation: Reagent grade DGEBA or a mixture of reagent grade and ^{14}C-DGEBA (specific activity 1.3×10^6 dpm/g) was dissolved in methylene chloride ($MeCl_2$). An aliquot of the $MeCl_2$ solution was applied to a glass wool plug lodged in the center well of a vaporization flask (Figure 1). The $MeCl_2$ was removed by evaporation. The flask was then placed in a heating mantle and connected to the chamber as depicted in Figure 2. When connected in this manner, air passed through a Drierite® container, a flow meter, the vaporization flask, a 30 liter glass inhalation chamber, a cold finger trap containing 50 ml of $MeCl_2$ and immersed in a dry ice bath, and a second flow meter. The line connecting the vaporization flask to the chamber was constructed of 1 inch i.d. glass tubing. This line was ∿40 cm long and contained one 90° bend ∿15 cm above the glass wool plug. There were two ports in this line through which a thermocouple could be inserted, or samples of the air stream collected. One port was ∿5 cm above the glass wool plug and the other port was ∿13 cm upstream from the chamber.

During the trial vaporizations, the outer surface of the vaporization flask was heated to 410°C. Air was passed through the vaporization flask at 8 l/min. At this airflow, the temperature of the air stream 5 cm above the glass wool plug and 13 cm from the chamber was 140°C and 21-27°C, respectively. Known volumes of air (∿30 l) were drawn via the ports and passed through cold finger traps containing 50 ml of $MeCl_2$. The amount of DGEBA trapped in the cold finger traps was determined by radioactivity or HPLC analysis. The concentrations of DGEBA in the air stream at the two sample ports and exiting the chamber were calculated from the amount of DGEBA trapped and the volume of air sampled.

Fate of Orally and Intravenously Administered ^{14}C-DGEBA:

^{14}C-DGEBA was either dissolved in corn oil and administered by gavage, or suspended in fresh heparinized rat plasma[1] and administered via the jugular cannula. The oral dose was 570 µg (4.5 µCi) and the intravenous dose was 90 µg (0.70 µCi) of ^{14}C-DGEBA. Following dosing, the rat with the bile cannula was placed in a restraining cage. The other rats were placed in individual glass Roth-type metabolism cages. Plasma, urine, feces and bile samples were collected at the intervals listed below:

	Hrs After Administration of ^{14}C-DGEBA
Blood	0.5, 1.0, 1.7, 3, 4, 6, 8, 10, 12, 18 and 24
Urine	6, 12 and 24
Feces	24
Bile*	1, 2, 3, 6, 12 and 24

*Bile cannulated animal only.

The blood was centrifuged to obtain the plasma. Urine and bile were collected and stored frozen until analyzed. Aliquots of the plasma, urine and bile were added directly to ACS liquid scintillation cocktail (Mallinckrodt Chemical Company) and analyzed for radioactivity. All animals were sacrificed 24 hr after dosing. The liver, lungs, kidneys and intestines were removed from the non-bile cannulated animals. The intestine was sectioned and flushed with water to remove the intestinal contents. Aqueous homogenates of the intestine and feces were prepared. Aliquots of the tissues, feces and intestinal contents were oxidized in a biological materials oxidizer. The $^{14}CO_2$ produced during oxidation was trapped and quantitated using standard laboratory procedures.

[1]HPLC analysis demonstrated ^{14}C-DGEBA was stable in rat plasma over the interval taken to prepare and administer the dose solution.

Stability of ^{14}C-DGEBA in Simulated Gastric and Intestinal Fluid: Simulated gastric and intestinal fluid were prepared as described in the 1970 U.S. Pharmacopeia. ^{14}C-DGEBA was added to 2 ml of the simulated gastric or intestinal fluid and incubated in a 37°C water bath. Sequential aliquots of the incubation mixture were removed 5, 50, 120, and 180 (gastric only) minutes after the start of the incubation, and neutalized with 0.2 N KOH or 0.1 N HCl. Aliquots (20 µl) of the neutralized incubation mixture were analyzed by high pressure liquid chromatography (HPLC).

Chromatographic Profile of Plasma Radioactivity: The dose solutions were prepared and administered as previously described except that the amount of radioactivity administered was increased; in this segment 37.8 and 8.5 µCi of ^{14}C-DGEBA/animal were administered orally and intravenously, respectively. Administration of the oral and intravenous dosing solutions were alternated and staggered in time to control for variation due to sequence of administration, sacrifice and analysis. The animals were killed by exsanguination 4 hr after dosing. The blood was centrifuged and 20 µl of the plasma analyzed by HPLC.

High Pressure Liquid Chromatography (HPLC): The HPLC method described by Paul (1976) was used with minor modifications. The sample was applied to the column using a sample loop. Reagent grade DGEBA was co-chromatographed with those samples containing ^{14}C-DGEBA. Fractions (1-2 ml) of the column effluent were collected and analyzed for radioactivity. In addition the optical density (280 nm) of the column effluent was monitored to determine when DGEBA eluted or to quantitate DGEBA in those samples which did not contain ^{14}C-DGEBA. The HPLC conditions were as follows:

Column: 30 cm x 4 mm μPorasil (Waters® Associates)

Mobil Phase: 2 ml/min going from 7 to 100% Solvent B over 26 min using program 6 or 10 on a Waters® Associates Model 600 programmer.

Solvent A: Hexane containing 0.01% (v/v) benzene.

Solvent B: Tetrahydrofuran containing 15% (v/v) acetonitrile.

Thin Layer Chromatography: The following samples were spotted on five thin layer chromatography (TLC) plates coated with 0.1 mm of cellulose F (E. M. Reagents):

1. Stock ^{14}C-DGEBA

2. $MeCl_2$ extract from glass wool plug used in vaporization ^{14}C-DGEBA

3. Bile from bile cannulated rat

4. Urine from bile cannulated rat

5. Urine from the orally dose rat

One TLC plate was developed with each of the following solvents: dimethyl sulfoxide, pyridine, methanol, 1,4-dioxane and dimethyl foramide. The location of radioactivity on the TLC plates was determined using a beta-graph (Panax Equipment Limited).

Radioactivity Analysis: Radioactivity was quantified using a Mark II or Mark III Nuclear Chicago liquid scintillation spectrometer (Searle). Counts per min (cpm) were corrected for quench and background, and converted to μg equivalents (eq) of ^{14}C-DGEBA.

Data Analysis: Rate constants and half-lives were obtained by least squares analysis. Area under the plasma ^{14}C-concentration time curve (AUC) was calculated using the trapezodial rule. The volume of distribution (Vd_{area}) for ^{14}C-DGEBA in the rat was calculated as:

$$Vd_{area} = \frac{\text{Dose}}{\text{AUC} \times \text{Rate Constant for Elimination}}$$

RESULTS

Generation of DGEBA Vapors: The system depicted in Figure 2 was the only system found that generated measurable concentrations of DGEBA vapors at respirable temperatures. In this system DGEBA was placed on glass wool to maximize the surface area, and hot air (410°C) was used to enhance vaporization. The line connecting the vaporization flask and chamber was large, contained no restrictions, and was just long enough to permit the air stream to cool to a respirable temperature (∿22°C). However, even under these conditions, it was not possible to generate an atmosphere containing sufficient ^{14}C-DGEBA for a kinetic study.

In one trial the concentration of ^{14}C-DGEBA in the air 5 cm above the vaporization flask was 6.9 µg/l (0.5 ppm). The concentration of ^{14}C-DGEBA in air 13 cm upstream from the chamber was 0.6 µg/l (0.04 ppm), and in the air exiting the chamber was 0.05 µg/l (0.004 ppm). These data were consistent with the results of other trial vaporization; i.e., significant amounts of DGEBA could be vaporized at high temperatures but the concentration decreased rapidly when the temperature decreased or the airflow was obstructed. The observed decrease in ^{14}C-DGEBA concentrations were apparently due to condensation since the walls of the line connecting the vaporization flask to the exposure chamber were coated with a sticky film.

Higher concentrations of DGEBA vapors may have been generated in one trial vaporization in which reagent grade DGEBA and HPLC analysis were used instead of ^{14}C-DGEBA and ^{14}C-analysis. In this experiment, concentrations of DGEBA in air 5 cm above the vaporization flask, 13 cm upstream from the chamber and exiting the chamber were 51.4, 150.4, and 93.2 µg/l or 3.7, 10.8 and 6.7 ppm, respectively. However, because the lowest concentration was found just above the vaporization flask, these data are suspect. Even if these results are valid, the concentrations of DGEBA in the air were low considering the conditions used to generate the vapors, and the atmosphere was not sufficiently stable to conduct an inhalation exposure study.

<u>Fate of Orally and Intravenously Administered ^{14}C-DGEBA:</u>
Intravenous administration was substituted for the inhalation route, since it was not possible to generate an atmosphere suitable for a single inhalation exposure kinetic study. The intravenous route was selected because ^{14}C-DGEBA is

placed directly into the systemic circulation[2]. The lung is the only barrier inhaled ^{14}C-DGEBA must cross to reach the systemic circulation and once it enters the systemic circulation its fate is independent of the route of administration.

Table 1 presents the distribution of radioactivity recovered 24 hr after oral and intravenous administration of ^{14}C-DGEBA. Total recovery was low, however, the fraction of the dose recovered following oral (66%) and intravenous (52%) administration was similar in the non-bile cannulated animals. The feces was the primary route of excretion following oral (53%) and intravenous (43%) administration. The liver was the primary excretory organ for ^{14}C-DGEBA as evidenced by the fact 86% of the ^{14}C-DGEBA given intravenously was excreted <u>via</u> the bile within 24 hr. Urine was a minor route of excretion with 8 to 9% of the oral and intravenous dose excreted by this route. Another 2% of the oral and 0.5% of the intravenous dose was recovered in the intestine plus contents. The tissues, i.e. liver, kidney, lungs and intestine, contained 1 to 2% of the dose.

Differences were also observed in the distribution of radioactivity in the tissues 24 hr after oral and intravenous administration of ^{14}C-DGEBA (Table 2). The plasma contained three times more radioactivity 24 hr after the 0.43 mg/kg intravenous dose than following the 2.7 mg/kg oral dose. In contrast the tissue/plasma ^{14}C-ratios especially for the liver, intestine and lungs were higher following oral administration.

[2]Circulatory system minus the hepatic-portal system. An orally administered chemical must pass through the liver before it reaches the rest of the body and some chemicals are extensively metabolized and/or excreted during their initial transit through the liver.

The plasma ^{14}C-concentration time profiles following oral and intravenous administration of ^{14}C-DGEBA are depicted in Figures 3, 4 and 5. Pharmacokinetic parameters derived from the plasma ^{14}C-data are summarized in Table 3. The absorption of radioactivity following oral administration of ^{14}C-DGEBA was rapid (t 1/2 = 0.7 hr) and the highest plasma ^{14}C-concentration (0.97 µg eq/g) was observed 4 hr after dosing. The radioactivity was cleared from the plasma in an apparent first-order manner following both oral (Figure 3) and intravenous (Figure 4 and 5) administration. However, the radioactivity was cleared from the plasma faster following oral administration (t 1/2 = 4.8 hr) than following intravenous (t 1/2 = 7.7 hr) administration. The area under the plasma ^{14}C-concentration time curve (AUC) was slightly smaller following oral (516 µg eq min/g) than after intravenous administration (601 µg eq min/g). Assuming the radioactivity represents the same chemical entity, the relative AUC following oral and intravenous administration suggest that only 77 µg or 13% of the 570 µg of ^{14}C-DGEBA administered orally was absorbed. However, of the 77 µg absorbed 50 µg (65%) was excreted in the urine. In contrast, only 8% of the ^{14}C-DGEBA administered intravenously was excreted in the urine. The differences found in the half life for plasma radioactivity, its excretion in the urine, and the tissue/plasma ^{14}C-ratios suggest that the radioactivity in the plasma following oral and intravenous administration of ^{14}C-DGEBA represent different chemical entities.

Thin layer chromatography (TLC) of selected urine and bile samples indicated that different metabolites were excreted following oral and intravenous administration of ^{14}C-DGEBA.

Based on the TLC, no unchanged ^{14}C-DGEBA was excreted in either the urine or bile. The major metabolite found in the bile was also found in the urine following intravenous but not oral administration. Metabolites of ^{14}C-DGEBA were found in the urine following oral administration that were not found in either the bile or urine of the intravenously dosed rat.

<u>Stability of ^{14}C-DGEBA in Simulated Gastric and Intestinal Fluid</u>: The stability of epoxides are known to be pH dependent. Orally administered ^{14}C-DGEBA is subjected to pH's ranging from 1.2 in the stomach to 7.5 in the intestines. Hydrolysis of the epoxide bond(s) in the gastrointestinal tract could explain the poor absorption of orally administered ^{14}C-DGEBA and account for the observed differences in the fate of orally and intravenously administered ^{14}C-DGEBA. Therefore, the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid was investigated.

Table 4 presents the result obtained when ^{14}C-DGEBA was incubated with simulated gastric or intestinal fluid. Recovery of radioactivity applied to the HPLC column decreased from >90% for the 5 min incubations to about 70% for incubations of 50 min or more. Radioactivity in the simulated gastric fluid eluted from the HPLC column in three distinct peaks. The first peak co-eluted with reagent grade DGEBA; i.e., it had the same retention volume as the UV peak for reagent grade DGEBA. The two remaining peaks eluted after DGEBA and based on their larger retention volumes represent polar degradation products of DGEBA. The fraction

of the applied radioactivity which eluted as ^{14}C-DGEBA decreased from 77.5 to 11.0% as the incubation time was increased from 5 to 180 min. The fraction of the radioactivity in the simulated gastric fluid eluting as polar degradation products (peaks II and III) increased from 25.8 to 66.7% as the incubation time increased from 5 to 180 min. Based on these data, the half life for the degradation of ^{14}C-DGEBA in simulated gastric fluid was 70 min.

Two radioactive peaks were observed when the simulated intestinal fluid was subjected to HPLC. The first and largest peak had the same retention volume as reagent grade DGEBA. The length of the incubation had no effect on the fraction of the applied radioactivity which eluted as DGEBA or in the second peak. This suggests that ^{14}C-DGEBA is not rapidly hydrolyzed at pH's found in the small intestine.

Chromatographic Profile of Plasma Radioactivity: Based on the elimination and tissue distribution data, the radioactivity in the plasma following oral and intravenous administration appeared to represent different chemical species. In an attempt to verify this, rats were given ^{14}C-DGEBA by gavage or intravenously, sacrificed 4 hr after dosing, and their plasma collected for analysis. The mean (± SD) concentrations of radioactivity in the plasma of the orally and intravenously dosed rats were 2514 ± 514 and 559 ± 203 dpm/20 µl, respectively. Thus, the plasma from intravenously dosed rats did not contain sufficient radioactivity to permit its chromatographic profile to be resolved. However, when the plasma from the orally dosed rats was subjected to HPLC, less than 10% of the radioactivity eluted as ^{14}C-DGEBA. This suggests that only a small percentage of the orally administered ^{14}C-DGEBA reached the systemic circulation unchanged.

DISCUSSION

It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body. Observed differences in the plasma ^{14}C-concentration time profile, tissue/plasma ^{14}C-ratios and urinary excretion of radioactivity following oral and intravenous administration of ^{14}C-DGEBA suggest that little if any orally administered ^{14}C-DGEBA is absorbed unchanged, and that the chemical identity of the radioactivity in the plasma depends on the route of administration. Consistent with this, 4 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA, and the radioactivity in the urine exhibited a different chromatographic profile following oral and intravenous administration of ^{14}C-DGEBA. Thus these data indicate that oral toxicity studies would be of little value in assessing the possible hazard associated with inhalation of DGEBA.

Based on the half-life for the degradation of ^{14}C-DGEBA in simulated gastric fluid (t 1/2 = 70 min) and for the absorption of radioactivity following oral administration (t 1/2 = 42 min), over a third of the orally administered ^{14}C-DGEBA would be degraded in the gastrointestinal tract. Once absorbed, orally administered ^{14}C-DGEBA must pass through the liver to reach the rest of the body. The liver plays a major role in the excretion of ^{14}C-DGEBA as evidenced by the fact that 85% of the ^{14}C-DGEBA administered intravenously was excreted in the bile within 24 hr. Based on these observations it is not surprising that there are significant differences in the fate of orally and intravenously administered ^{14}C-DGEBA.

The problems encountered in trying to generate an atmosphere suitable for a single exposure kinetic study confirm previous

suspicions that it is unlikely a meaningful repeated inhalation exposure study could be conducted with DGEBA. Furthermore, the inability to generate a suitable atmosphere using low air flows, a large surface area, and high temperatures suggests that inhalation is an insignificant route for DGEBA exposure.

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Pharmacopeia of the United States, Eighteenth Revision, September 1, 1970.

TABLE 1: DISTRIBUTION OF RADIOACTIVITY RECOVERED 24 HOURS AFTER ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DGEBA TO RATS[a].

Treatment	Dosed Orally	Dosed Intravenously	Dosed Intravenously Bile Cannulated
Dose µg	570	90	90
mg/kg	2.7	0.43	0.43[b]
µCi	4.5	0.72	0.72
Radioactivity Recovered	µg Equivalents of ^{14}C-DGEBA (% Dose in Parenthesis)		
Feces	303 (53%)	38.7 (43%)	3 (3%)
Bile	c	c	77 (86%)
Urine	50 (9%)	6.9 (8%)	38 (42%)
Intestinal Contents	12 (2%)	0.4 (<1%)	c
Tissues[d]	13 (2%)	1.1 (1%)	c
TOTAL	378 (66%)	47.1 (52%)	118 (131%)

[a]Data are for one animal at each treatment.

[b]Body weight not available for this animal dose rate estimated assuming rat weighed 210 g.

[c]Sample not collected from this animal.

[d]Total µg eq of ^{14}C-DGEBA found in the liver, kidneys, lungs and intestines.

TABLE 2: CONCENTRATION OF RADIOACTIVITY IN THE TISSUES 24 HOURS AFTER DOSING EXPRESSED AS mg eq ^{14}C-DGEBA/g AND AS TISSUE/PLASMA RATIOS.

Tissue	Oral		Intravenous	
	µg eq/g	Tissue/Plasma	µg eq/g	Tissue/Plasma
Liver	0.64	15	0.07	0.6
Kidney	0.05	1	0.07	0.5
Lung	0.17	4	0.07	0.6
Intestine	0.55	13	0.04	0.3
Plasma	0.04		0.13	

Values based on one orally and one intravenously dosed rat.

TABLE 3: PARAMETERS DESCRIBING THE TIME COURSE OF RADIOACTIVITY IN THE RAT FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DGEBA.

	Oral	Intravenous	Bile Cannulated
Dose (µg)	570	90	90
(mg/kg)	2.7	0.43	-[a]
Maximum Plasma ^{14}C-DGEBA Conc (µg eq/g)	0.97	0.76	1.16
Time Maximum Plasma ^{14}C-DGEBA Conc Observed (hr)	4	0	0
Absorption Rate Constant (hr^{-1})	1.0	-[b]	-[b]
Half Life in hr	0.7	-[b]	-[b]
Elimination Rate Constant (hr^{-1})	0.15	0.08	0.09
Half Life in hr	4.8	8.8	7.7
Area Under ^{14}C-Plasma Conc Time Curve (µg eq . min/g)	516	601	697
Dose Adjusted Area Under ^{14}C-Plasma Conc Time Curve	0.91	6.7	7.7
Volume of Distribution (ml/Rat)	456	114	86
Clearance (ml/min/Rat)			
Urinary[c]	0.10	0.01	0.06
Biliary[c]	-[b]	-[b]	0.11
Body[d]	1.10	0.15	0.13

Parameters based on data from one animal.

[a]The body weight of this rat is not available.

[b]Calculation of this parameter is inappropriate.

[c]Calculated as amount excreted in urine or bile divided by the area under plasma ^{14}C-conc time curve.

[d]Calculated as the dose divided by the area under the plasma ^{14}C-conc time curve.

TABLE 4: ELUTION OF RADIOACTIVITY FROM THE COLUMN WHEN ^{14}C-DGEBA WAS INCUBATED WITH SIMULATED GASTRIC OR INTESTINAL FLUID, AND SUBJECTED TO HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC).

Length of Incubation (min)	Gastric Fluid				Intestinal Fluid		
	% Recovered	Peak 1	Peak 2	Peak 3	% Recovered	Peak 1	Peak 2
5	103.3	77.5	17.4	8.4	91.8	69.5	22.3
50	69.4	33.7	7.3	28.4	69.5	69.5	_[a]
120	70.1	32.9	7.4	29.4	74.2	63.2	11.0
180	77.7	11.0	12.1	54.6	_[b]	_[b]	_[b]

Values represent the percent of applied radioactivity which was recovered in the eluate, and the percent of applied radioactivity which eluted as ^{14}C-DGEBA (Peak 1) and as degradation products (Peaks 2 and 3).

[a]Only one radioactive peak was observed.

[b]A 180 min incubation was not run with simulated intestinal fluid.

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Figure 1 : Diagram of the Flask used to Vaporize ^{14}C-DGEBA. The arrows indicate the direction of airflow through the flask.

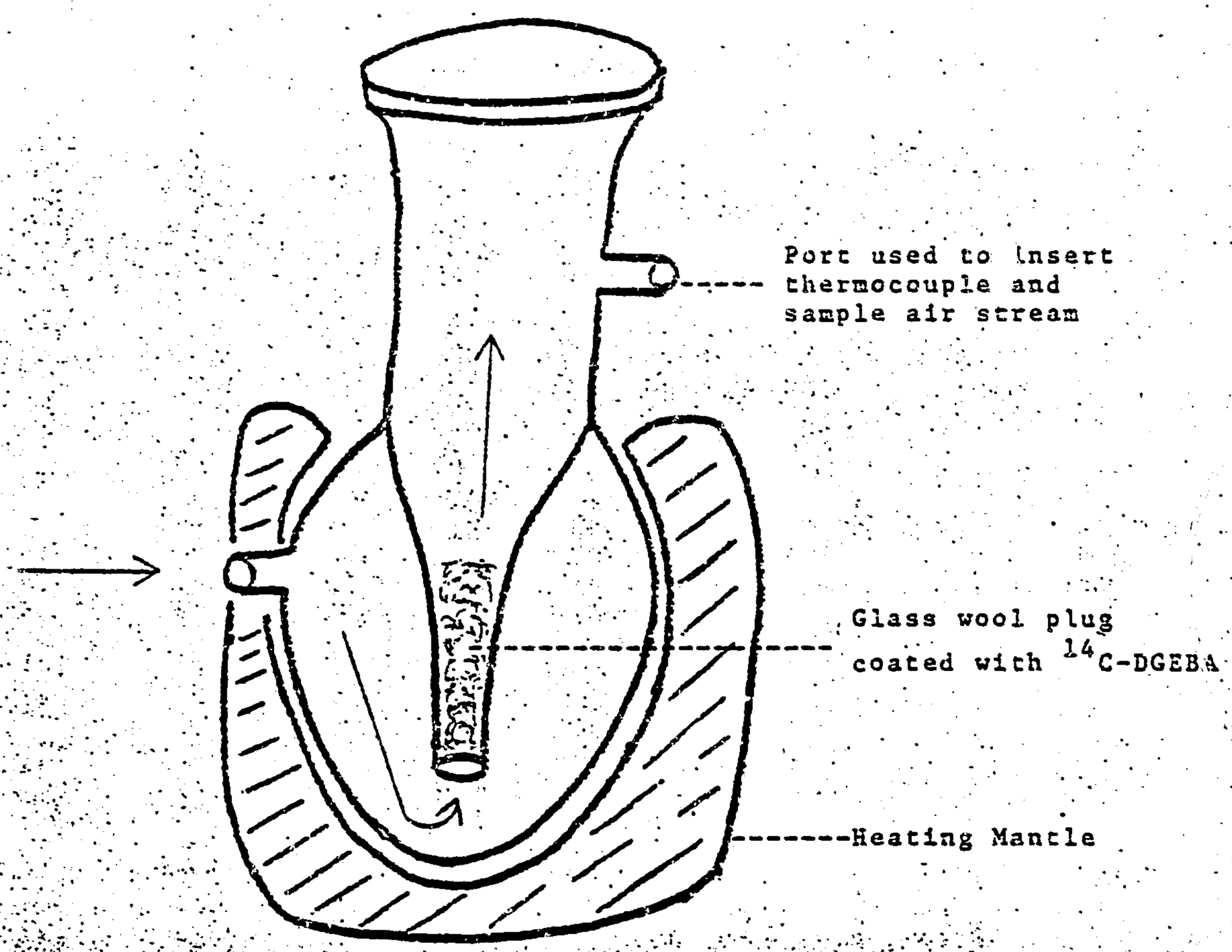

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Figure 2: Diagram of System Used to Generate ^{14}C-DSEBA Vapor. Arrows Indicate the direction of airflow through the system.

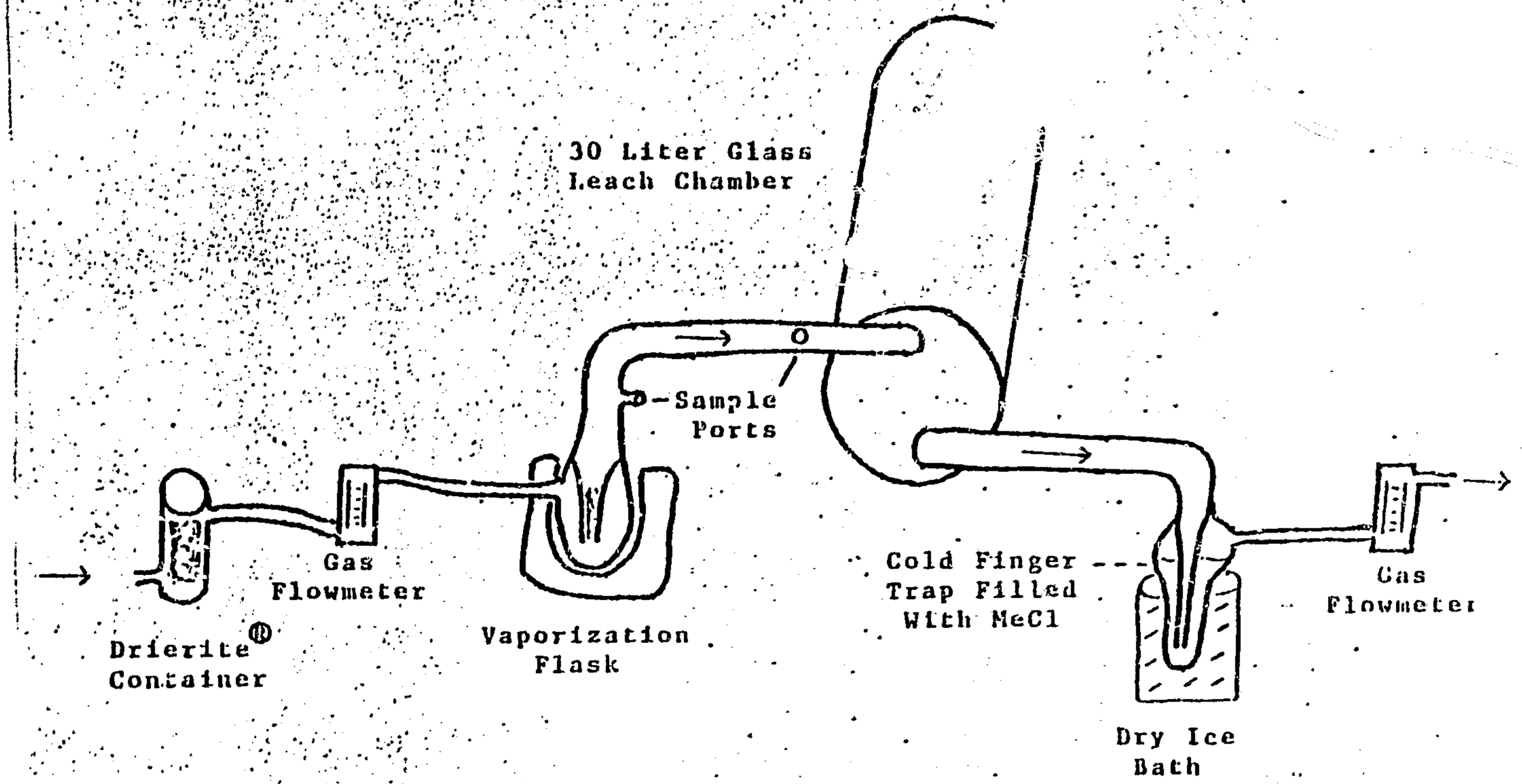

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Figure 3: Time Course of Radioactivity in Rat Plasma Following Oral administration of 2.7 mg ^{14}C-DGEBA/kg.

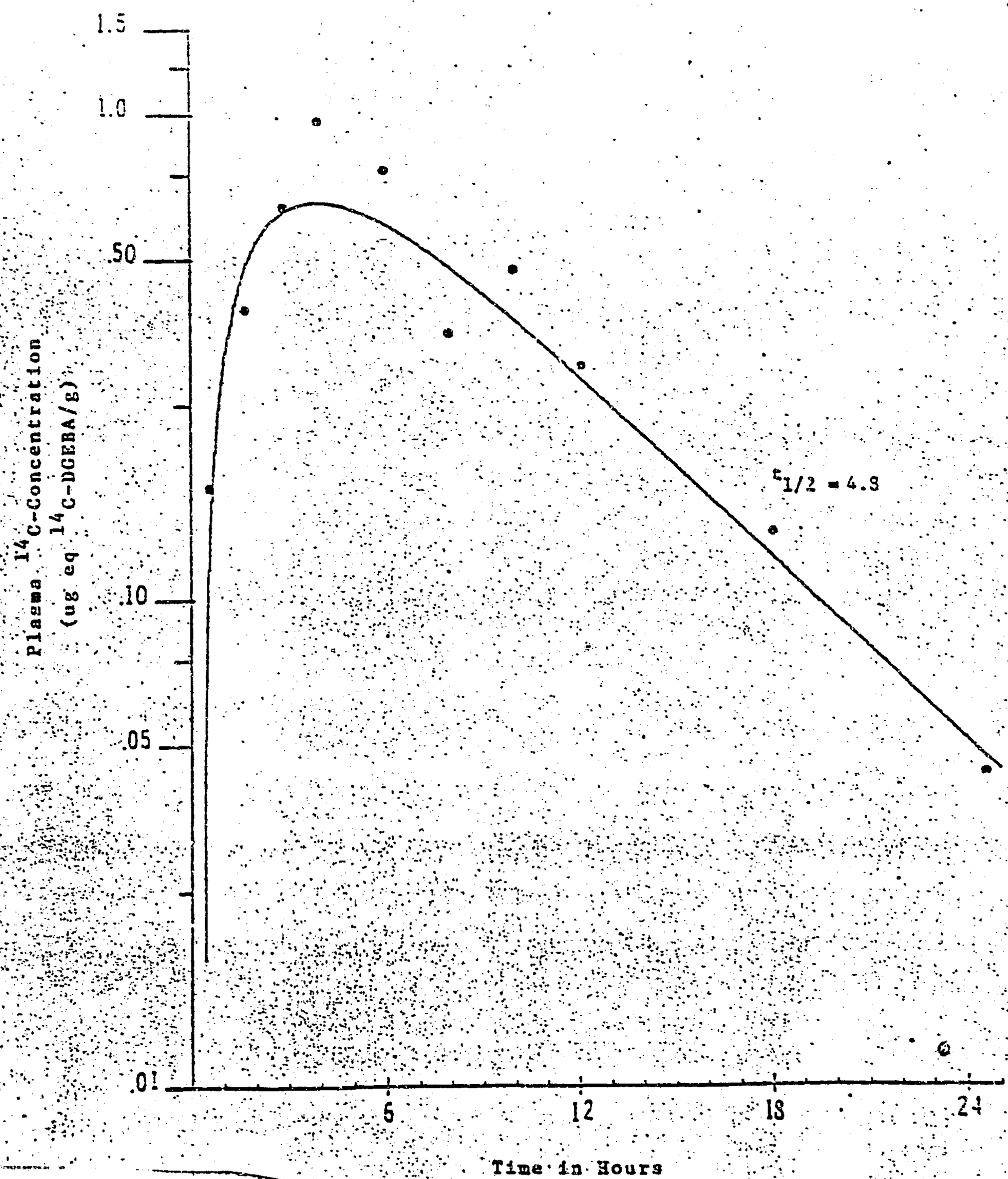


Figure 4: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.45 mg ^{14}C-DGEBA/kg.

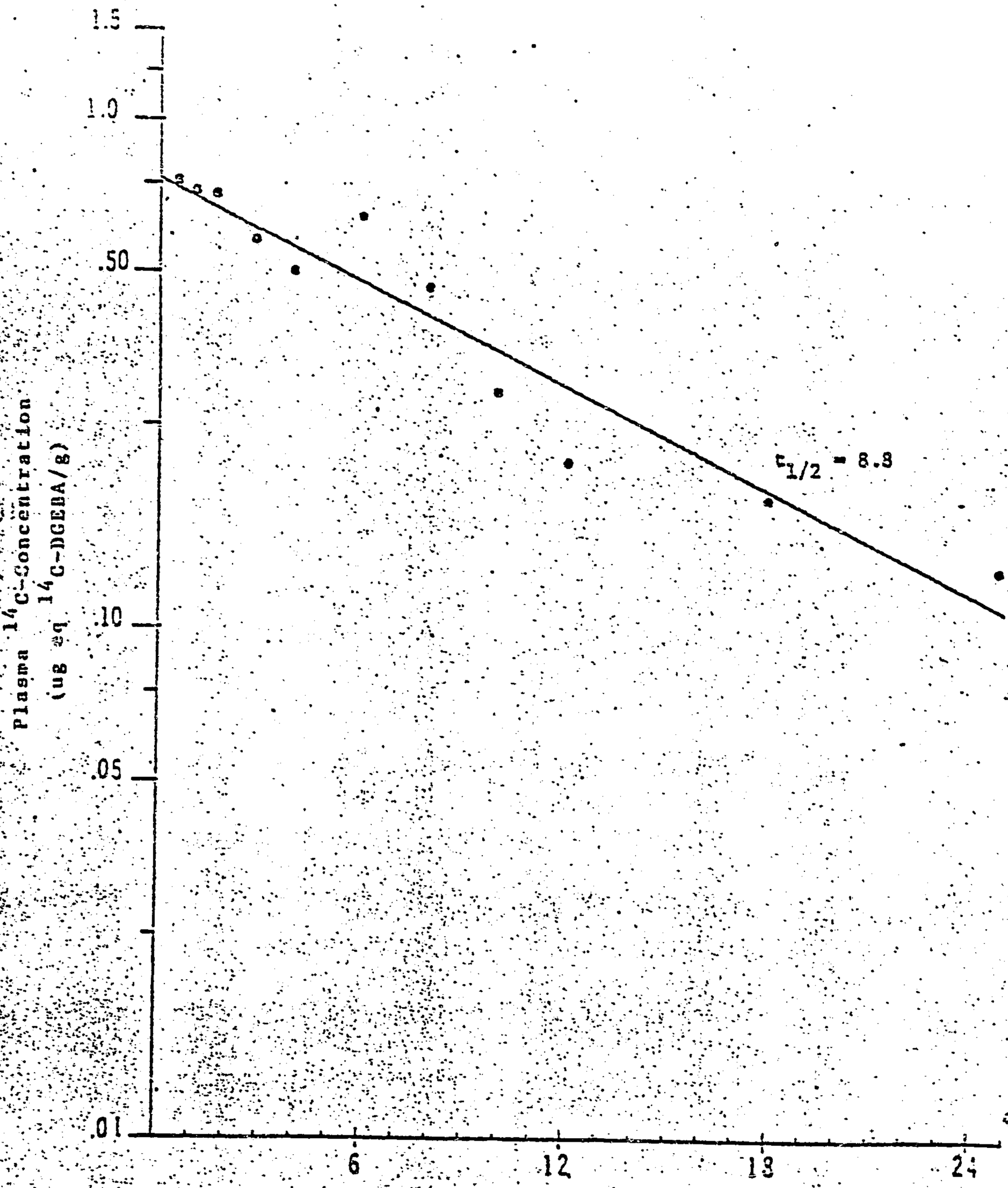
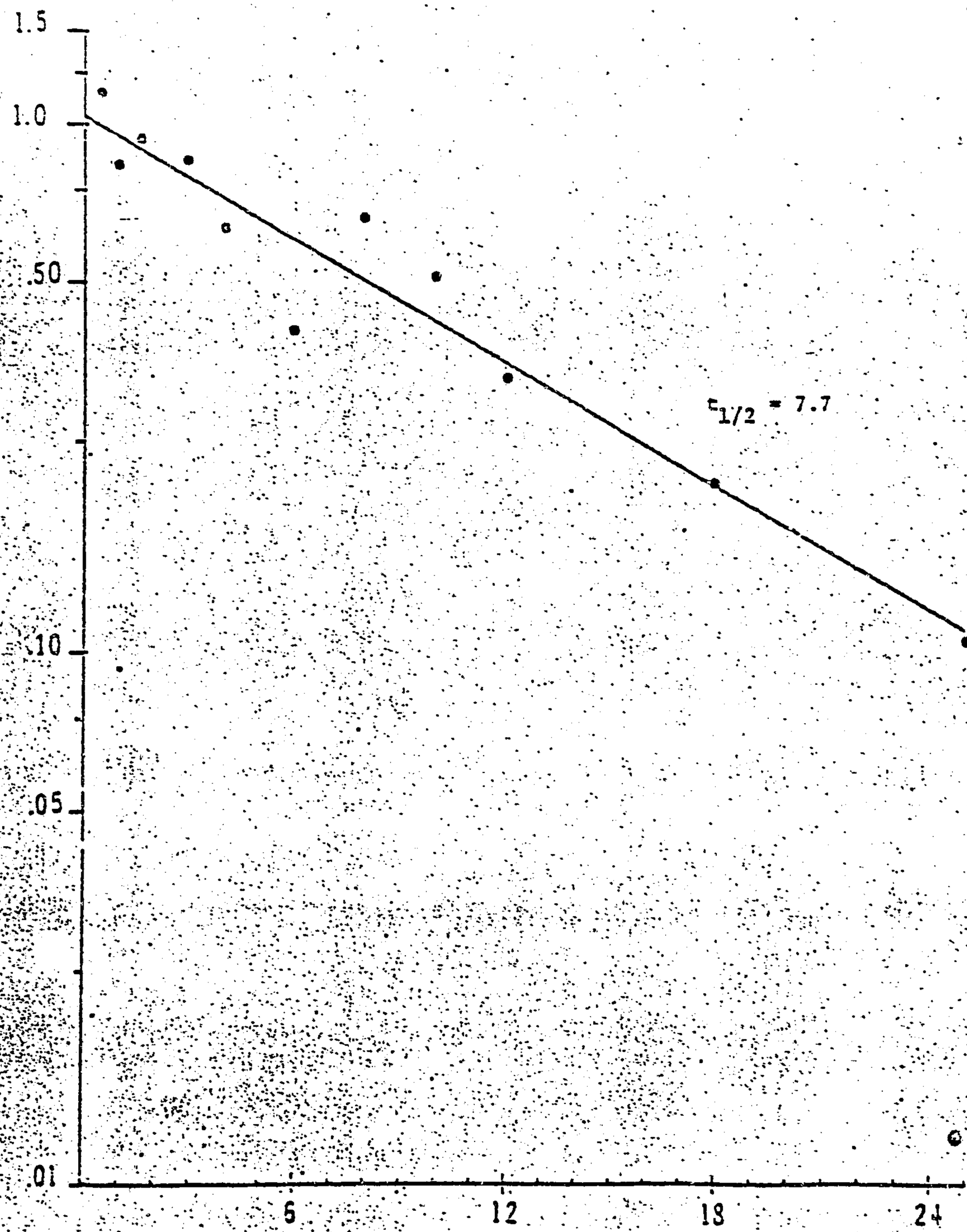


Figure 5: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.45 mg ^{14}C-DGEBA/kg to Bile Cannulated Rat.



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GLYCIDOL & ITS DERIVATIVES		999999994	
DIGLYCIDYLETHER OF BISPHENOL A		25Ø85-99-8	

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THE DOW CHEMICAL COMPANY

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Dear Sir or Madam:

We are submitting the two attached lists of Health and Safety studies as required by the referenced rule.

The first list, marked D650, is a list of studies in progress as of the date of this submission.

Please note that the study entitled "Acrylamide: A Two-Year Drinking Water Chronic Toxcitity - Onogenicity Study in CDF Fischer 344 Rats" is submitted on behalf of the following companies who are participating in the study:

Dow Chemical
American Cyanamid
Sohio
Nalco

The second list, marked D652, contains references to unpublished studies of which we are aware but do not have a copy.

Very truly yours,

R. L. Hagerman

R. L. Hagerman
Regulatory Specialist
Regulatory and Legislative Issues
Health and Environmental Sciences
1803 Building
(517)636-6855

rt

copy 7 of 8

Dow Chemical Company
D-650
Studies in Progress as of Date of Submission

1. A Mortality Study of Men Engaged in the Manufacture of Organic Dyes.
   Purpose: Examine the mortality experience of men assigned to production areas in which aromatic amines were present as raw materials or chemical intermediates.
   Data: Mortality data
   Progress: Report submitted for publication
   Date of Completion: Unknown - when accepted for publication
   CAS Nos: 62533

2. In Vitro Reactivity Study of Alkylene Oxides
   Purpose: See title
   Data: Relative kinetic data
   Progress: Started Dec. 1, 1982
   Date of Completion: Report due 1984
   CAS Nos: 106887, 75218, 75569

3. Range-Finding Study of Methylchloroform Formulation XN 50120
   Purpose: See title
   Data: Mammalian toxicity
   Progress: Report being written
   Date of Completion: Jan. '83
   CAS Nos. 71556

4. Propylene Dichloride: Acute and Two-week Inhalation Study
   Purpose: See title
   Data: Toxicity in Rats, Mice and Rabbits
   Progress: Report being written
   Date of Completion: Jan '83
   CAS Nos: 78875

5. Methyl Chloride: Inhalation Toxicity in Female C57BL6 Mice Continuously or Intermittently Exposed for 12 Days
   Purpose: See title
   Data: See title
   Progress: Report to be written
   Date of Completion: First Quarter '83
   CAS Nos. 74873

6. Methylene Chloride: A Comparison of the Pharmacokinetics and Macromolecular Interactions of Inhaled ^{14}C-Methylene Chloride in Hamsters and Rats as Related to Toxicity
Purpose: Evaluate species differences in the fate of inhaled methylene chloride
Data: See title
Progress: Pathology in progress
Date of Completion: Sept. '84
CAS Nos: 79061

7. Acrylamide: A Two Year Drinking Water Chronic Toxicity - Onogenicity Study in DCF Fischer 344 Rats
Purpose: See title
Data: See title
Progress: Pathology in progress
Date of Completion: September '84
CAS Nos: 79061

8. Acrylamide: Pharmacokinetics in the Rat
Purpose: See title
Data: See title
Progress: Report to be written
Date of Completion: First quarter '83
CAS Nos: 79061

9. Acute Range-finding - Diglycidylether of Biphenol A
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS Nos: 1675543

10. Characterization of Butylene Oxide Blood Levels, Macromolecular Binding and Hepatic Nonprotein Sulfhydryl Levels After Inhalation Exposure in Rats
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS Nos: 26249207

11. 1,1,1-Trichloroethane: Two-year Inhalation in Rats and Mice
Purpose: See title
Data:
Progress: Awaiting pathology
Date of Completion: June, '83
CAS Nos: 71556

12. Reduction Dechlorination of Chlorinated Methanes, Ethanes, & Ethylenes in Aqueous Solutions
    Purpose: Investigate non-biological, reductive dehalogenation of a series of chlorinated hydrocarbons in aqueous solution
    Data: Laboratory analytical data on concentration of chemicals in solution as a function of conditions
    Progress: Underway
    Date of Completion: Mid-1983
    CAS Nos: 75092, 71556

13. A Case-Control Study of Renal Cancer Mortality at a Texas Chemical Plant.
    Purpose: To determine if a possible etiologic agent for human renal cancer can be found related to occupation.
    Data: Mortality and past presumptive exposures to chemicals produced or used in the chemical plant.
    Progess: Preliminary report nearing completion
    Date of Completion: Early 1983

14. A Case-Control Study of Leukemia and Occupational Exposure to Ethylene Oxide.
    Purpose: To determine if past presumptive exposures to ethylene oxide are associated with leukemia.
    Data: Mortality and past presumptive exposures to ethylene oxide.
    Progress: Work histories on cases and controls being collected.
    Date of Completion: Early 1983.

15. A Cross-Sectional Study of Employees with Potential Workplace Exposure to Ethylene Oxide.
    Purpose: To determine whether those with potential exposure to ethylene oxide had a higher prevalence of abnormalities of the hematopoietic, hepatic, renal or reproductive systems than did an unexposed control group.
    Data: Medical surveillance and presumptive occupational exposure
    Progress: Preliminary report in process of review and revision
    Date of Completion: Early 1983

16. Methyl Chloride Mortality Study.
    Purpose To evaluate the mortality patterns of employees with past potential exposure to methyl chloride.
    Data: Mortality and past potential occupational exposure to methyl chloride.
    Progress: Collecting work history and exposure data.
    Date of Completion: Mid-1983

17. 1,1,1-Trichloroethane

Purpose: Define the time course of 1,1,1-trichloroethane in human volunteers following a single six-hour inhalation exposure to 350 and 35 ppm.

Data: Human pharmacokinetic study

Progress: In progress

Date of Completion: February 1983

CAS Nos: 71556

18. Acrylamide

Purpose: Evaluate vibratory sensitivity in an exposed population and controls.

Data: Field survey

Progress: Preliminary data collected.

Date of completion: Unknown; Pending development and validation of methodology.

CAS Nos: 79061

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Dow Chemical Company
D-652
References to Unpublished Studies by Others

1. Studies of the visual system in chronic acrylamide poisoned monkeys. Lapham, L.W., et. al.
   Dr. L. W. Lapham, University of Rochester Medical Center, Department of Pathology, Division of Neuropathology, Rochester, NY, 14642.

2. The integration of multiple measures in behavioral toxicology. Merison, W.H., et. al.
   Dr. W. H. Merison, University of Rochester Medical Center, Division of Toxicology, Rochester, NY, 14642

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EVALUATION OF THE GENOTOXIC POTENTIAL OF THE EPOXY RESINS XD-7342.ØØ AND XD-9Ø53.ØØ IN THE RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

Chemical Name (300 per name)	CAS No. (10)	24
GLYCIDOL & ITS DIRIVATIVES	9999999994	
HALOGENATED ALKYL EPOXIDES	9999999994	
2,2'-(((2-OXIRANYLMETHOXY)	9999999994	
PHENYL)METHYLENE)BIS-	—	

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Chemical Name (300 per name) 25	CAS No. (10) 24
(4,1-PHENYLENEOXYMETHYLENE)	9999999994
BIS-	
POLYMER (XD-9053.00)	9999999994

1B

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DOW CHEMICAL U.S.A.

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May 2, 1978

LAB. NO. PROBLEM NO.

DEPARTMENT

Toxicology Research Laboratory

TITLE

EVALUATION OF THE GENOTOXIC POTENTIAL OF THE EPOXY RESINS XD-7342.00 AND XD-9053.00 IN THE RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

13 PAGES IN FULL REPORT

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AUTHOR(S) SIGNATURE(S)

A. M. Schumann 5/3/79 S. L. Pocotte 5/3/79

REVIEWER'S SIGNATURE

P. G. Watanabe 5-7-79

P. G. Watanabe

This report is: ☐ INTERIM ☒ FINAL and mainly: ☒ NEW ☐ REVIEW

DATA REFERENCES (book and page):

[redacted]

(Refer also to earlier related reports and publications.)

PATENT STATUS: ☐ disclosure submitted ☐ case filed ☐ no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The genotoxic potential of the epoxy resins XD-7342.00 and XD-9053.00 was evaluated in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. Neither XD-7342.00 or XD-9053.00 elicited UDS at concentrations of 64, 6.4, 0.64 or 0.064 µg/ml of incubation medium. Testing higher concentrations of the epoxy resins was precluded by a lack of solubility. The lack of activity of the epoxy resins in this short-term *in vitro* assay implies a lack of genotoxic activity. However, the ability of these resins to enter the cell and interact with DNA is uncertain due to the size and reactive nature of their structure.

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INTRODUCTION

Oxirane: 2,2'-(((2-oxiranylmethoxy)phenyl)methylene), bis-(4,1-phenyleneoxymethylene) bis-, monomer (XD-7342.00) and polymer (XD-9053.00) were submitted by Western Division R&D of The Dow Chemical Company, Walnut Creek, California, for evaluation in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. This assay is currently being employed in our laboratory as a short-term *in vitro* test for assessing the genotoxic potential of chemicals. Epoxy resin XD-9053.00 is intended for use in semiconductors, transfer molding and functional power coatings.

This report is organized into sections describing (1) the background and rationale of the rat hepatocyte UDS assay, (2) the general methodology employed in conducting the assay as well as specific experimental methods utilized with XD-7342.00 and XD-9053.00, (3) the results of the present study and (4) the interpretation of the results.

BACKGROUND

Many chemicals which produce genetic toxicity (genotoxicity) react with intracellular macromolecules including DNA. Depending on whether the target cell affected is a reproductive cell (germ cell) or a somatic cell the toxic manifestation may be mutagenicity or carcinogenicity.

One of the hypotheses to date for chemical carcinogenesis is the somatic cell mutation theory (Miller & Miller, 1966). This theory postulates that chemical carcinogens interact with DNA producing alterations in the DNA molecule and the expression of the genetic code. If unrepaired, the alteration to DNA may result in cell death if critical cellular functions are impaired. However, the cell may survive and divide retaining the altered characteristics imparted to it by the genotoxic action of the chemical. One result of genotoxicity may be cellular transformation and induction of cancer. The assessment of DNA repair (unscheduled DNA synthesis: UDS) as an indicator of DNA damage has been suggested as a reliable estimate of genotoxic potential (Stich and Laishes, 1973).

The use of primary cultures of rat hepatocytes for assessing UDS has been reported by Williams (1976 and 1977). Our evaluation of the rat hepatocyte UDS assay indicated that the induction of UDS correlated well with the *in vivo* carcinogenic and *in vitro* mutagenic activity of the chemicals tested (Schumann et al., 1979). The diverse spectrum of chemicals evaluated indicate that the UDS assay may detect carcinogens with varied target organs and mutagens which produce both base-pair as well as frameshift mutations. Thus, it appears that UDS is sensitive

to a broad spectrum of carcinogenic and mutagenic chemicals. The amount of UDS elicited in this system, however, is purportedly a function of the type of DNA damage and repair invoked rather than the potency of the genotoxic agent (Williams, 1977). That this assay may not be a good predictor of in vivo potency is consistent with all other short-term in vitro carcinogenicity/mutagenicity assays.

The rat hepatocyte UDS assay involves:

1. Isolation of rat hepatocytes and establishment of primary cultures.
2. Simultaneous treatment of the cultures with the test chemical and ^{3}H-thymidine.
3. Quantitation of DNA repair (UDS) by evaluating the incorporation of the ^{3}H-thymidine into nuclear DNA by microautoradiography.

Intuitively, the use of primary cultures of rat hepatocytes is an ideal system for assessing UDS. Rat hepatocytes contain a wide spectrum of enzymes necessary to biotransform procarcinogens to their active form. Nonhepatic cultures have limited xenobiotic metabolizing capabilities and therefore require an exogenous source of enzymes in the form of hepatic microsomes. The xenobiotic metabolizing capability of long-term rat hepatocyte cultures also appears to be low (Williams, 1975). On the other hand, primary rat hepatocyte cultures retain a high degree of functional activity (Bissel et al., 1973; Bonney, 1974; Laishes and Williams, 1976; Lin and Snodgrass, 1975; Michalopoulos and Pitot, 1975). Thus, in this system a potential carcinogen/mutagen can be activated and interact within the cell in a similar manner to that which occurs in vivo.

Primary cultures of rat hepatocytes have shown the ability to activate diverse groups of procarcinogens including polycyclic aromatic hydrocarbons (epoxidation), nitrosamines (C-hydroxylation and N-dealkylation) and aromatic amines (N-hydroxylation and N-oxyesterification) (Schumann et al., 1979; Williams, 1977). Mycotoxins and azo dyes are also active in this system (Williams, 1976; 1977). In addition, this system appears to be sensitive to carcinogens with target organs other than the liver. For example, 4-nitroquinoline-1-oxide (lung), dimethylanthracene (mammary), methyl methanesulfonate (brain) and N-methyl-N-nitro-N-nitrosoguanidine and β-propiolactone (stomach) induce UDS in primary rat hepatocyte cultures.

The use of microautoradiography in assessing UDS has several advantages over liquid scintillation counting. Microautoradiography allows confirmation that the observed radioactivity is located in the nucleus and also allows the elimination of cells in S-phase (semiconservative DNA synthesis; DNA replication) due to their completely blackened nuclei. Assessing UDS by microautoradiography eliminates the need for inhibiting semiconservative DNA synthesis with hydroxyurea to "unmask" repair synthesis.

For screening test chemicals in the rat hepatocyte UDS assay, a concentration showing some degree of toxicity or limited solubility as well as fractions of that dose covering a wide spectrum of concentrations should be assessed. All tests should include a positive control and appropriate vehicle controls. Ideally, good dose-response data should be generated and used as a criterion for activity. However, in the absence of a marked dose-response relationship repeated activity in the test must be

considered a positive result. Since no single short-term *in vitro* test can adequately predict *in vivo* mutagenicity or carcinogenicity, the rat hepatocyte UDS assay should be used in conjunction with other short-term tests (e.g. bacterial mutagenicity, cell transformation) for assessing genotoxic potential and prioritizing chemicals for animal studies.

GENERAL METHODOLOGY FOR THE RAT HEPATOCYTE UDS ASSAY

Animals.

Male Sprague-Dawley rats weighing approximately 200-250 g (Spartan Research Laboratories, Haslett, MI) are acclimated to our laboratory for at least 10 days. The rats are housed in a room designed to maintain a temperature of approximately 72°F, a relative humidity of approximately 45% and a 12 hour lighting cycle. Food (Purina Rat Chow) and water are provided *ad libitum*.

Chemicals.

Thymidine-6-^{3}H (specific activity of approximately 23 Ci/mmole) is obtained from New England Nuclear (Boston, MA). Test chemicals are prepared at the desired concentration in Williams Medium E (Flow Laboratories, Rockville, MD) if water soluble or as stock solutions in dimethyl sulfoxide (DMSO, J. T. Baker Chemical Co., Phillipsburg, NJ) such that the final concentration of DMSO in the media is 0.1%. In a few instances the final concentration of DMSO in the media may be increased to 0.5 or 1.0% due to limited solubility of the test chemical.

The test chemicals are tested over a wide range of concentrations. The highest concentration levels are determined by either a lack of test chemical solubility or by toxicity to the cells. Criteria for cellular toxicity are an unflattened granular appearance, detachment of the hepatocytes from the coverslip, a decrease in nuclear labeling and/or an extremely low level of background labeling with ^{3}H-thymidine.

Preparation of Hepatocytes.

Primary cultures of rat hepatocytes are prepared by slight modification of the method described by Williams et al., (1977). All test chemicals and concentration levels assessed per assay are evaluated in hepatocytes obtained from a single rat. The rat is anesthetized with methoxyfluorane and the liver exposed by an abdominal incision. Sodium heparin (200 units) is injected into the inferior vena cava and the hepatic portal vein cannulated. The liver is then perfused for approximately 2-3 minutes with 0.5 mM ethylene glycol-bis(β-amino ethyl ether)N, N'-tetracetic acid (EGTA) in Ca^{++}, Mg^{++} free Hank's balanced salt solution (37°c) buffered with 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) to pH 7.4. The liver is subsequently perfused with 0.05-.1% collagenase (Type I, Sigma Chemical Company) in Williams Medium E (WE) (37°C) buffered with 0.05 M HEPES to pH 7.4. After approximately 12-15 min. the liver is removed from the rat and the cells dispersed in WE. The cells are sedimented by centrifugation at 50 x g for approximately 30 seconds and resuspended in fresh medium (WE) with 10% fetal bovine serum (WE + FBS). Cell viability is assessed by gently mixing one drop of the cell suspension with one drop of 0.5% trypan

blue in 0.9% saline and quantitated by the percentage of the cells excluding trypan blue. The cells in suspension are counted with a hemocytometer and diluted to a concentration of 3-5 x 10^5 cells/ml with WE + FBS.

Seeding of the Cultures.

One ml of the cell suspension (3-5 x 10^5 cells) is inoculated into a 35 x 10 mm tissue culture dish (Vangard International, Inc., Neptune, NJ) containing a 25 sq mm round Thermonox plastic coverslip (Flow Laboratories, Rockville, MD) and 2 ml of WE + FBS. The cultures are placed in a humidified (95%) 37°C incubator and maintained in an atmosphere of 95% air: 5% CO_2 for 1.5 hr. During this interval viable cells attach to the coverslip. Nonviable cells are removed by washing the cultures with 3 ml of WE at the end of the attachment interval.

Chemical Treatment.

Two ml of media containing the test chemical and ^{3}H-thymidine (5-10μCi/ml) are applied to triplicate cultures at each dose level. The cultures are then incubated for 18 hours after which they are washed for three 30 minute intervals with 1 mM nonlabeled thymidine in WE. The cells are then swelled by incubation for 10 minutes with 10% (w/v) sodium citrate and subsequently fixed with a solution of ethanol: acetic acid (3:1) for 30 minutes. The coverslips are allowed to air dry and then mounted cell side up on glass slides.

Microautoradiography.

The slides are dipped in NTB photographic emulsion (Kodak, Rochester, NY) in a dark room and stored refrigerated for 10 days. At the end of the exposure period the slides are developed with Kodak D-19 developer and stained with hemotoxylin and eosin. UDS is then quantitated by counting the number of grains over the nucleus and subtracting the mean number of

grains in 3 nuclear sized areas adjacent to the nucleus. Those cells undergoing semiconservative DNA synthesis (DNA replication) are easily visualized because of their completely blackened nuclei and not counted. Ten-twenty cells on each of two-three slides are evaluated.

Frequently the cytoplasmic background of radioactivity is slightly greater than the nuclear labeling in control or unaffected treated cultures. This results in a negative value for the net number of nuclear grains. These values will be reported as 0.0 net grains per nucleus, however, the actual values obtained are used for statistical analysis. Mean grain counts of 0 or greater will be reported as the mean ± the standard deviation. Net nuclear labeling is often observed in control cells but rarely exceeds a mean of 5 grains per nucleus. Therefore, a mean of 6 net grains per nucleus or greater (this may vary with experimental conditions) in addition to statistical significance from control is required for a positive result (Williams, 1977). In cases where nuclear labeling is very intense (approximately greater than 75 grains per nucleus) the results are reported as the best approximation of the number of grains present.

Statistics.

The net number of nuclear grains in treated cells will be compared to the appropriate control by analysis of variance and Dunnett's t-test. A mean of 6 or more net grains per nucleus (this may vary with experimental conditions) and statistical significance from control at $p \leq 0.05$ is required for a positive result.

EXPERIMENTAL METHODS FOR XD-7342.00 AND XD-9053.00

The epoxy resins were evaluated in the rat hepatocyte UDS assay as described in the general methodology section. The following describes experimental procedures specific for the present study.

XD-7342.00 and XD-9053.00 were dissolved in dimethyl sulfoxide (DMSO) and added to the medium so that the final concentration of DMSO in the incubation medium was 0.1% (v/v). Concentrations of 64, 6.4, 0.64 and 0.064 µg of XD-7342.00 or XD-9053.00 per ml of incubation medium were assessed. Higher concentrations of the epoxy resins could not be evaluated due to a lack of solubility in the medium. 2-Acetylaminofluorene (2-AAF; Aldrich Chemical Co., Milwaukee, WI) was used as a positive control at concentrations of 22.3, 2.23 and 0.223 µg per ml of incubation medium. 2-AAF was also prepared in DMSO and the final concentration of DMSO in the incubation medium was 0.1 % (v/v). Media control and 0.1% DMSO control cultures were also prepared. Ten cells on each of two slides were counted.

RESULTS

The effect of XD-7342.00 and XD-9053.00 in the rat hepatocyte UDS assay is shown in Table 1. The highest concentration tested of each epoxy resin was 64 µg/ml. Testing higher concentrations was precluded by a lack of solubility in the test system. Even at 64 µg/ml, a precipitate was observed on the slides after 18 hours of incubation. Thus, a wide spectrum of concentrations were evaluated, ranging from the solubility limit to nontoxic concentrations in which the treated and control cultures were comparable in appearance.

XD-7342.00 and XD-9053.00 failed to elicit UDS at any of the concentrations tested. Values of "0.0" net grains per nucleus reflect instances where the number of background grains in the cytoplasm exceeded the number of nuclear grains. 2-AAF, the positive control, elicited a significant degree of UDS at all three concentrations tested. At 22.3 and 2.23 µg/ml the response was too great to accurately quantitate but represented approximately 100 or more net nuclear grains. 0.22 µg/ml of 2-AAF produced 64.2 ± 22.4 (mean ±S.D.) net nuclear grains. The strong response of 2-AAF, a known animal carcinogen, indicates the responsiveness of the assay in the present study.

INTERPRETATION OF RESULTS

The lack of activity of the epoxy resins XD-7342.00 and XD-9053.00 in the rat hepatocyte UDS assay implies a lack of genotoxic activity. The various epoxide groups present in the structure of these resins, however, suggest that these should be very reactive compounds. It is hypothesized that these resins may not enter the cell due (1) to their large size or (2) to the fact that they may bind to the cell membrane. Thus, it is possible that they may never reach the target macromolecule, intranuclear DNA.

As with all short-term *in vitro* carcinogenicity/mutagenicity tests the potential genotoxic hazard for man cannot be based on any single test alone. Rather, the results of several short-term tests (e.g. bacterial mutagenicity, cell transformation, UDS) must be evaluated for assessing genotoxic potential and prioritizing chemicals for animal studies.

Written by:

A. M. Schumann 5/3/79

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S. L. Pocotte /AMS 5/3/79

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Reviewed by:

P. G. Watanabe 5-7-79

P. G. Watanabe

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TABLE 1

THE EFFECTS OF EPOXY RESINS XD-7342.00
AND XD-9053.00 IN THE RAT HEPATOCYTE UDS ASSAY

Treatment	Concentration (µg/ml)	UDS (Net Nuclear Grains)[a]
Media Control	-	0.0
0.1% DMSO Control	-	0.0
2-Acetylaminofluorene	22.3	$\geq \approx 100$
	2.23	
	0.22	64.2 ± 22.4[b]
XD-7342.00	64	0.0[c]
	6.4	0.0
	0.64	0.0
	0.064	0.0
XD-9053.00	64	0.0[c]
	6.4	0.0
	0.64	0.0
	0.064	0.0

[a]Results reported as "0.0" net nuclear grains indicates a slightly greater number of background grains in the cytoplasm compared to the nucleus.

[b]$\bar{X}$ ±S.D.; significantly greater than 0.1% DMSO control ($p \leq 0.05$).

[c]Precipitate present on slide after the 18 hour incubation.

CERTIFICATE OF AUTHENTICITY

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OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) • 206138 | No. of Pages 4

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Contract(s)

Doc Type

• P • U • M • N • S • E • A • U • N • N

Doc Title

PRELIMINARY TOXICOLOGY INFORMATION WITH COVER LETTER

Chemical Name (100 per name)	CAS No. (10)
GLYCIDOL & ITS DERIVATIVES	9999999 4
DIBROMOPHENYL GLYCIDYL ETHER DER 599	9999999 4

5/31

IB

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

December 9, 1982

RECEIVED
DEC 20 1982

Document Control Office
US Environmental Protection Agency
TS-793
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Rockville, MD 20852

OPTS-84003A

Dear Sir or Madam:

As required by 40 CFR 716, we herewith submit copies of reports which meet the requirements of the referenced rule as health and safety studies. As noted in the statement enclosed with the reports, some contain confidential business information.

The reports are separated into three categories for your convenience:

Package 1: Reports which contain no confidential business information.

Package 2: Reports which contain confidential business information.

Package 3: Reports from which confidential business information has been deleted. (Public File Copy of reports in Package 2).

Each report is marked with an identifying number at the top of the first page of the report, e.g., D-100. Use of this identification number in future correspondence concerning this submission will facilitate handling of questions.

In order to expedite the completion of our search and submission, no attempt was made to determine whether or not we manufactured or processed the chemicals which formed the subject of submitted reports. (40 CFR 716.6). Thus, submission of a report for any given material should not be construed as indicative of Dow's status as a manufacturer or processor of the material.

Many of the submitted reports contain information which is not relevant to Health or Safety Studies of listed chemicals, e.g., references to unlisted chemicals, marketing or process data, account numbers, internal document identification codes or distribution lists. Such information has been deleted from all copies submitted.

The index required by 40 CFR 716.4(b) is enclosed. It lists the new identification number and title of each report submitted in CAS number order. Please note that the index contains new Confidential Business Information.

We have also included a reprint of a recently published article dealing with methylene chloride.

Very truly yours,

Robert L. Hagerman
Regulatory Specialist
Regulatory and Legislative Issues
Health and Environmental Sciences
1001 Building
(517)636-0455

ll

D-172 87-0/0471

H

THE DOW CHEMICAL COMPANY

PRELIMINARY TOXICOLOGY [illegible]

Material: Dibromophenyl glycidyl ether
DER 599

Date: 7/25/69

Description: The test material is a light brown clear liquid with little odor. The material has a molecular weight [illegible] and was submitted by D. O. Duran, d-1217.

Structural formula:

Use: Reactive diluent for epoxy resins.

Methodical procedure: Routine skin and eye irritation studies were conducted using young, male, albino, rabbits.

Eye irritation: The test material produced no indication of pain when instilled directly into the eye of the test animal.

There was a mild transit conjunctival response in both the washed and unwashed eye. The conjunctival areas were completely clear in 24 hours.

There was no corneal response.

Skin Irritation: The covered abraded area of the abdomen showed a severe hyperemia and moderate necrosis after 24 hours. An eschar formed in this area lasting for seven days. A moderate scar was produced with loss of hair.

The covered unabraded area of the abdomen showed a severe hyperemia in 24 hours. This area began to clear in four days and was completely clear on the 10th day with only moderate loss of hair.

The uncovered area of the ear showed a slight hyperemia after one application. This area was clear in 24 hours after the application.

Preliminary Toxicology Information

July 25, 1985

Bisphenophenyl glycidyl ether DER 599.

Conclusions and Recommendations: This undiluted material is capable of causing skin burns when applied to rabbits.

We recommend that skin contact should be minimal and avoided if possible. Personnel with cuts or abrasions should handle with caution. Care should be taken not to allow material to come in contact with a covered skin area. Good personal hygiene practices should be used when handling this material.

Eye contact should afford less of a problem and minimal eye protection should be adequate when handling this material.

This is only a cursory range finding study. If this material is to reach customer potential then a more sophisticated study should be undertaken.

Terry H. Edwards
Toxicology

R. L. Schnabel, Ph.D.
Consulting Toxicologist

THE:mir

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OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

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Organ. Name: DOW CHEM CO

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MID No. (7): 0010560 | D & B No. (11): 0013818

Contract(s):

Doc Type: • R.I • G.P • H.E.A.S.D. S.U. H.S.F.N

Doc Title: SKIN SENSITIZATION POTENTIAL OF CRESYL GLYCIDYL ETHER

Chemical Name (100 per name): GLYCIDOL & ITS DERIVATIVES CRESYL GLYCIDYL ETHER

CAS No. (10): 999999994 26447-14-3

5/31

1A

410

87-8210423

BIOMEDICAL RESEARCH
Texas Division Toxicology

DOW CHEMICAL USA

INDUSTRY/FIRM	FIRM	DATE	CONTACT
		October 14, 1977	

SKIN SENSITIZING POTENTIAL OF CRESYL GLYCIDYL ETHER

REPORTED BY	CHECKED BY
M. N. Pinkerton, R. L. Schwabe	R. V. Johnston, D.V.M.

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ABOVE DATA INFORMATIVE ONLY. THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEFINED AND MAY BE CHANGED BELOW

A sample of the test material was submitted to the Comparative Toxicology Research Laboratory for evaluation of skin sensitization in guinea pigs.

A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendation of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

The skin sensitization potential of cresyl glycidyl ether was evaluated in male guinea pigs by subjecting them to a 10% (w/v) solution of the material in a 9:1 mixture of Dowanol DPM:Tween 80. Positive control animals were treated in a similar manner to a 15% solution (w/v) of DER 331 in the 9:1 mixture of Dowanol DPM:Tween 80.

Cresyl glycidyl ether sensitized ten of the ten animals, while the positive control sensitized nine of the ten animals.

Cresyl glycidyl ether did induce skin sensitization in guinea pigs under the conditions of this test procedure.

Cresyl glycidyl ether must be considered a potential skin sensitizer for humans and contact should be avoided.

RECEIVED
JAN 06 1978
CHI DAIDLAND

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Contractor:

Doc Type: • P • I • C • P • H • A • S • D 8.D. S.U M.S + N

Doc Title: ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF CRESYL GLYCIDYL ETHER

Chemical Name (IUPAC name): GLYCIDOL & ITS DERIVATIVES

CAS No. (10): 99999994

CRESYL GLYCIDYL ETHER 26447-14-3

5/6/8

1A

87-82104

D-441

BIOMEDICAL RESEARCH

June 23, 1977

ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF CRESYL GLYCIDYL ETHER

M. N. Pinkerson, R. L. Schwabel

A sample of the test material was submitted to the Biomedical Research Laboratory for evaluation of acute oral toxicity, [illegible] irritation properties, acute inhalation toxicity, and [illegible] of industrial handling hazards involving acute exposures.

A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendations of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

The acute oral lethality of the test material is low. The acute oral LD_{50} value determined in 12 albino rats was found to be greater than 2.0 g/kg of body weight. The probability that internal injury might result from acute ingestion of amounts of the test material one might encounter incidental to industrial handling is minimal.

The test material was practically non-irritating to the eye of the albino rabbit. Direct eye contact would be expected to produce no more than a very slight conjunctival inflammation. If contact occurs, contaminated eyes should be flushed with copious amounts of flowing tap water for at least 15 minutes. Safety glasses are recommended whenever the possibility of eye contact exists.

Skin irritation studies were conducted on New Zealand albino rabbits. Applications are made on open-intact, covered-intact, and covered-abraded skin areas and observations are recorded at various intervals. Applications are discontinued upon production of a substantial skin burn. The test material was practically non-irritating to the skin of an albino rabbit exposed to air. When contact was repeated 4 times, a slight chemical burn resulted. Prolonged contact (24 hours) with the skin of an albino rabbit in confinement resulted in moderate redness and a severe chemical burn. When the skin of an albino rabbit was abraded, the contact resulted in a severe chemical burn after one application.

Precautions should be taken to avoid skin contact. If contact occurs, the skin should be decontaminated with copious amounts of water from a safety shower or other suitable device and contaminated clothing thoroughly cleaned before re-use. Protective clothing, impervious to the test material should be worn as required by circumstances.

(over)

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There was no treatment-related alteration in appearance, demeanor, food consumption or survival in any of the 4 albino rats exposed in the saturated atmosphere (nominal concentration: 2.00 mg/l) for 7 hours at room temperature.

A single short-term exposure to the vapors or fumes of the tone material would not likely be harmful, however, a prolonged vapor exposure may prove injurious to health.

BIOMEDICAL AND COMPARATIVE
TOXICOLOGY RESEARCH LABORATORY

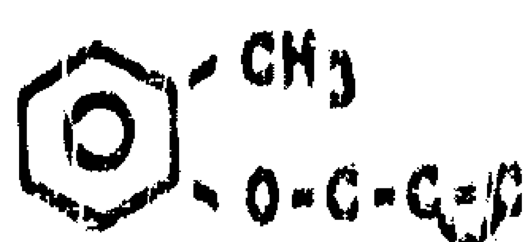
DOW CHEMICAL U.S.A.

FREEPORT, TEXAS

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA	NAME
$C_{10}O_2H_{12}$	Cresyl Glycidyl Ether
MOLECULAR WEIGHT / INDUSTRIAL HYGIENE STANDARD	SYNONYMS: CGE

STRUCTURAL FORMULA - OR COMPOSITION



BOILING POINT	EXPLOSIVE LIMITS (% VOL. IN AIR)	FLASH POINT	IGNITION TEMP.	MELTING POINT	VAPOR PRESS.
°C mmHg		> 250°F			

CORROSIVENESS (To Common Metals)	PHYSICAL STATE	COLOR
No	Liquid	Colorless

CHEMICAL REACTIVITY: Like other epoxies — Odor: Mild

STABILITY (To Heat, Change, Light)

CLASSIFICATION OF TOXIC PROPERTIES

TYPE OF CONTACT		
EYE	[X] May cause no irritation or no more than very slight to slight transitory pain ... and/or irritation of the eye. [ ] May cause sufficient injury to the eye to result in loss of time from work ...	[ ] May cause ... [ ] Vapor ... injury to the eye.
SKIN	[ ] Single prolonged exposure ... [ ] Single prolonged contact ...	[X] Single and repeated ... [ ] ...
DUST OR MIST	[ ] No systemic injury expected ... [ ] ...	[ ] ... [ ] ...
VAPOR (Vapors at room temperature)	[X] Exposure ... will cause no effects other than some very slight irritation ... [ ] Single ...	[ ] ... [ ] ... [ ] ...
INGESTION	[X] Amounts which may be swallowed incidental to handling will not cause injury ...	[ ] Amounts which may be swallowed incidental to handling ... may cause injury

COMMENTS:

000004

'6182/0474

Cresyl Glycidyl Ether

PRECAUTIONS SEE CODE BELOW

DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION

I NO CONTACT	Characterized by remote or station with equipment isolated from the work area. The persons enter as isolated areas will require the personal protection equipment for IV below.	A	A		A	A
II MINOR CONTACT	Characterized by closed systems with equipment vented outside the work area in equipment control, mechanical handling of materials in bulk. Examples are continuous reactors, mills and filters, enclosed conveyors, ventilated packaging	A	A		A	A
III DAILY CONTACT	Characterized by manual handling of materials in packaged form or bags, drums and containers. Ventilation may be provided for specific jobs. Many batch operations fall into this category.	B	D		A	A
IV CONTACT LIKELY	Characterized by hand operation. Examples are: Emergency repairs, cleaning mixing tanks, cleaning filters, taking care of spills, packaging, sampling or duty maintenance without ventilation, sampling and fire fighting.	B	D		B	A

EYE CONTACT

A No eye protection needed

B Use safety glasses without side shields.

C Use safety glasses with side shields.

D Use eye tight goggles or a full face gas mask

E Use chemical safety goggles

SKIN CONTACT**

A Both and clean clothes once per week along with the usual washing at most times should be adequate precautions.

B Greatly contaminated clothing and shoes must be removed not later than the end of the work period and must be thoroughly cleaned before reuse.

C Require shower at the end of the work period and clean cloth ing from the skin put at the start of each work day.

D Clothing should be changed and is a cause promptly upon any skin contact. Each use will require careful consideration in determining suitable protective devices and elements of personal cleanliness.

E Impervious clothing such as rubber boots, rubber aprons, and rubber gloves will be required. Specific items will be dictated or required by circumstances.

RESPIRATORY PROTECTION

DUST OR MIST

A No respiratory protection.

B No precaution required for exposure of thirty min. Duration of less to obviously dusty atmospheres. Exposure of longer duration will require the use of a dust respirator bearing the approval of the U.S. Bureau of Mines for the use with toxic dusts.

D Any exposure to obviously dusty atmospheres will require a dust respirator bearing the approval of the U.S. Bureau of Mines for use with toxic dusts.

E Any exposure to dusty atmospheres will require the use of an air line respirator, blower mask or chemical mask.

VAPOR

A No precautions necessary

B No precautions necessary for single exposures of less than ½ hour. Longer single exposure or frequently repeated exposures will require a gas mask or respirator equipped with appropriate canister

C No precautions necessary for a single exposure of no more than ten minutes. Longer exposures either single or repeated will require gas mask or respirator equipped with appropriate canister

D Gas mask with appropriate canister required at all times.

E Continuous use of air line and enter only with airline respirator, blower mask or chemical mask.

INGESTION

A No special precautions required

F Food and tobacco should not be taken in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

**GOOD PRACTICE REQUIRES THAT GROSS AMOUNTS OF ANY CHEMICAL BE KEPT OFF FROM THE SKIN AS SOON AS IS PRACTICAL.

MILITARY GAS MASK CANISTER

Organic Vapor

Author R. L. Dunn, H. H. Parker, Jr., R. V. Johnston, D. V. H.

Date December 2, 1977 Date December 2, 1977

000005

OFFICE OF TOXIC SUBSTANCES

CODING FORM FOR GLOBAL INDEXING

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Sec. Title

• R.S. • M.P. • H.P. A.S.P. S.D. S.U. U.S. I.N.

Doc. Title

ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF SANDY RESEARCH CHEMICALS

Chemical Name (100 per name)

GLYCEROL & ITS DERIVATIVES
DIGLYCERYL ESTER OF
NEOPENTYL GLYCOL

CAS No. (10)

99999994
99999994

D-408

878210469

BIOMEDICAL RESEARCH
Texas Division Toxicology

June 29, 1977

ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS
Sample Reference 79854-9-B

RECORDED BY H. Pinkerton, R. Schwebel

CHECKED BY R. V. Johnston, D.V.M.

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION MAY BE REQUIRED AS SPECIFIC USES AND MANUFACTURE AND DEVELOPMENT PROCESS CHANGE. OR REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND MANUFACTURE AND DEVELOPMENT PROCESS CHANGE.

A sample of the test material was submitted to the Comparative Toxicology Research Laboratory for evaluation of acute oral lethality, eye and skin irritation properties, acute inhalation toxicity, and for definition of industrial handling hazards involving acute exposures.

A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendation of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

The acute oral lethality of the test material is low. The acute oral LD_{50} value determined in 12 albino rats was found to be greater than 2.0 g/kg of body weight. The probability that internal injury might result from acute ingestion of amounts of the test material one might encounter incidental to industrial handling is minimal.

The test material was practically non-irritating to the eye of the albino rabbit. Direct eye contact would be expected to produce no more than a very slight conjunctival inflammation. If contact occurs, contaminated eyes should be flushed with copious amounts of flowing tap water for at least 15 minutes. Safety glasses are recommended whenever the possibility of eye contact exists.

Skin irritation studies were conducted on New Zealand albino rabbits. Applications are made on open-intact, covered-intact, and covered-abraded skin areas, and observations are recorded at various intervals. Applications are discontinued upon production of a substantial skin burn. Prolonged contact (24 hours) with the test material on the skin of an albino rabbit exposed to air resulted in moderate redness. When contact was repeated 4 times, moderate redness, moderate swelling and a slight chemical burn resulted. Prolonged contact (24 hours) with the skin of an albino rabbit in confinement resulted in moderate redness. When contact was repeated 2 times, a severe chemical burn resulted. When the skin of an albino rabbit was abraded, the contact resulted in moderate redness, severe swelling, and a slight chemical burn.

Precautions should be taken to avoid skin contact. If contact occurs, the skin should be decontaminated with copious amounts of water from a safety shower or other suitable device and contaminated clothing thoroughly cleaned

DISD

before re-use. Protective clothing, impervious to the test material should be worn as required by circumstances.

The exposure of 4 albino rats to the saturated atmosphere (nominal concentration: 0.07 mg/l) for 7 hours at room temperature resulted in 100% survival. Moderate eye and nasal irritation was observed in all animals during the exposure.

A single short-term exposure to the vapors or fumes of the test material would not likely be harmful, however, a prolonged vapor exposure may prove injurious to health.

As the diglycidyl ether of neopentyl glycol.

BIOMEDICAL AND COMPARATIVE
TOXICOLOGY RESEARCH LABORATORY

DOW CHEMICAL U.S.A.

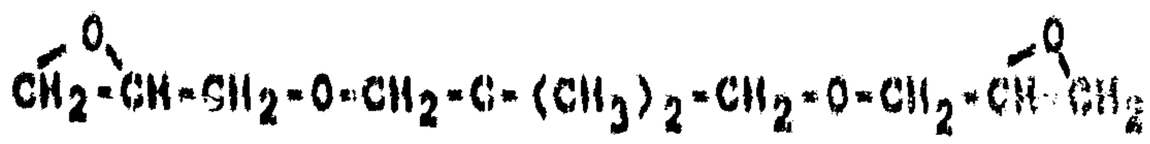
FREEPORT, TEXAS

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA | NAME

MOLECULAR WEIGHT | INDUSTRIAL HYGIENE STANDARD | SYNONYMS: Diglycidyl ether of neopentyl glycol | FILE

STRUCTURAL FORMULA OR COMPOSITION



PHYSICAL PROPERTIES	
BOILING POINT	°C mmHg
EXPLOSIVE LIMITS (% VOL. IN AIR)	
FLASH POINT	280°F °C
IGNITION TEMP	°C
MELTING POINT	°C
VAPOR PRESS mmHg 20°C	
CORROSIVENESS (To Common Metals)	
PHYSICAL STATE	Liquid
COLOR	Clear
CHEMICAL REACTIVITY	Will react with acids and amines
ODOR THRESHOLD CONCENTRATION IN AIR	Slight
STABILITY (To pH Change, Heat, Light)	Stable

TOXIC PROPERTIES — TYPE OF CONTACT	CLASSIFICATION OF TOXIC PROPERTIES	
EYE	(X) May cause no irritation or no more than very slight to slight transitory pain [redacted] and/or irritation of the eyelids	☐ May cause some permanent loss of vision (this includes damage to cornea or internal injury which is indemonstrable based on one week)
	☐ May cause sufficient injury to the eye to result in loss of time from work. (This includes damage to the cornea which heals or nearly heals in a week and/or considerable conjunctival irritation with edema.)	☐ Vapor exposure may cause severe pain, lacrymation or serious injury to the eyes
SKIN	☐ Single prolonged exposure (hours) causes no effect. Several repeated prolonged exposures may or may not cause the development of some slight irritation	(X) Single short exposure (minutes) may cause considerable irritation and/or single prolonged or frequently repeated short or intense cause a burn [redacted]
	☐ Single prolonged exposure may cause some reddening of the skin. Repeated prolonged contacts may cause considerable irritation, possibly a mild burn and/or may cause appreciable systemic injury due to absorption	☐ An exposure rapidly forms severe burns and/or serious systemic injury, even death
VAPOR OR MIST (Total of room temperature unless otherwise indicated) ☐	☐ No systemic injury expected. No irritation in nose and throat in dusty or misty atmospheres.	☐ Dusty or misty atmosphere painful in nose and throat (irritation able to must special and/or exposure may cause serious systemic injury, even death
	☐ Throat and nose irritation in a dusty or misty atmosphere is painful but not intolerable and/or prolonged or repeated exposures may cause systemic injury	☐ Short exposure (minutes) may cause death or serious systemic injury
VAPOR (Total of room temperature unless otherwise indicated)	(X) Exposures do not cause any effects other than some very slight irritation or pain in the eyes or transitory damage of the nose	☐ Exposures may cause extreme discomfort and/or serious systemic injury, and/or may cause intolerable irritation in the respiratory passages
	☐ Single exposures exceeding ½ hour, or frequently repeated exposures of shorter duration may cause slight anesthesia and/or slight systemic injury, and/or cause appreciable but not intolerable, irritation of respiratory passages.	☐ Short exposures may cause unconsciousness and/or serious systemic injury including death
		☐ Even very short exposure will cause serious systemic injury or death
INGESTION	(X) Amounts which may be swallowed incidental to industrial handling will not cause injury. However, if substantial quantities should be swallowed, more or less serious effects may occur	☐ Amounts which may be swallowed incidental to industrial handling and use may cause serious injury

COMMENTS

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87-82/0469

DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION

		PERCENTAGE OF OCCURRENCE				
		EYES	SKIN	INGESTION	INHALATION VAPOR	INHALATION DUST
I NO CONTACT	Characterized by remote operation with equipment isolated from the work area. The persons entering isolated areas will require the personal protection outlined for IV below.	A	A		A	A
II MINOR CONTACT	Characterized by closed systems with equipment vented outside the work area. In-plant equipment for mechanical handling of materials in bulk. Examples are continuous reactors, stills and filters, enclosed conveyors, ventilated packaging.	A	A		A	A
III OCCASIONAL DAILY CONTACT	Characterized by manual handling of materials in packages such as bags, drums and liners. Ventilation may be provided for specific jobs. Many batch operations fall into this category.	B	B		C	A
IV GROSS CONTACT LIKELY	Characterized by hand operation, examples are: Emergency repairs, cleaning equipment, cleaning filters, taking care of spills, packaging volatile or dusty materials without ventilation, wheeling and tray drying.	B	C		C	A

EYE CONTACT

- A No eye protection needed.
- B Use safety glasses without side shields.
- C Use safety glasses with side shields.
- D Use chemical workers goggles.
- E Use gas tight goggles or a full face gas mask.

SKIN CONTACT

- A A bath and clean clothes once per week along with the usual washing at meal times should be adequate precautions.
- B Grossly contaminated clothing and shoes must be removed not later than the end of the work period and must be thoroughly cleaned before re-use.
- C Require shower at the end of the work period and clean cloth ing from the skin out at the start of each work day.
- D Clothing should be changed and skin washed promptly upon any noticeable contact. Each use will require medical consideration to determine suitable protective devices and standards of personal cleanliness.
- E Impervious clothing such as rubber boots, rubber aprons and rubber gloves will be required. Specific items will be dictated or indicated by circumstance.

INHALATION

DUST OR MIST

- A No respiratory protection.
- B No protection required for exposure of thirty min. Duration or less in obviously dusty atmospheres. Exposures of longer duration will require the use of a dust respirator bearing the approval of the U.S. Bureau of Mines for the use with toxic dusts.
- D Any exposure to obviously dusty atmosphere will require a dust respirator bearing the approval of the U.S. Bureau of Mines for use with toxic dusts.
- E Any exposure to dusty atmosphere will require the use of an air line respirator, blower mask, or chemes mask.

VAPOR

- A No precautions necessary.
- B No precautions necessary for single exposures of less than ½ hour. Longer single exposures or frequently repeated exposures will require a gas mask or respirator equipment with appropriate canister.
- C No precautions necessary for single exposure of no more than ten minutes. Longer exposures either single or repeated, will require gas mask or respirator equipment with appropriate canister.
- D Gas mask with appropriate canister required at all times.
- E Evacuate area of odors and enter only with airline respirator, blower mask or chemes mask.

INGESTION

- A No unusual precautions required.
- E Food and tobacco should not be present in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

"GOOD PRACTICE REQUIRES THAT GROSS AMOUNTS OF ANY CHEMICAL BE REMOVED FROM THE SKIN AS SOON AS IS PRACTICAL.

"SUITABLE GAS MASK CANISTER

ORGANIC VAPOR

SIGNED M. N. Pinkerton, R. L. Daniel CHECKED R. V. Johnson, D.V.M.

DATE February 9, 1978 DATE February 9, 1978

(Confidential - For the Use of Dow Employees Only)

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OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

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Doc Type

PRELIMINARY RESULTS OF PRIMARY IRRITATION TEXTILE CHEMICAL TESTS ON GLYCEROL

Doc Title

Chemical Name (100 per name)

CAS No. (10)

Glycerol & ITS DERIVATIVES

Glycerol

999999994

556-52-5

1A

D180

Biochemical Research Laboratory

The Dow Chemical Company

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON GLYCIDOL

Signed K. J. Olson Jr. 2/4/61

Checked F. Oyen 1-26-61

File [redacted]
K. No.
Chg.
Req. by V. K. Rowe
Rept. by K. J. Olson
K. J. Olson, Feb. 3, 1961

[redacted]

PROBLEM

The carcinogenic properties of the subject material are being investigated by topical application to the mouse. A sample was also submitted for toxicological investigation and definition of industrial handling hazards.

CONCLUSIONS

Glycidol possesses a moderate to low acute oral toxicity. There should be no problem from ingestion incidental to industrial handling. It should be noted, however, that ingestion or swallowing of relatively small amounts of the material might result in some systemic injury. It is important to clearly and carefully label all containers in order that swallowing due to mistaken identity cannot occur.

The undiluted material is moderately irritating to the eye. Direct contact would likely produce rather extensive conjunctival swelling accompanied by corneal injury which might be slow to heal. It is possible that some impairment of vision might result. It is important to note that immediate and thorough washing of contaminated eyes should reduce the degree of conjunctival and corneal injury appreciably. Chemical workers goggles are recommended for handling the undiluted material whenever the likelihood of eye contact exists.

Relatively short contact with the undiluted material would likely produce a rather severe skin burn particularly in cases where it might be confined to the surface under clothing. Such reaction might be delayed for an hour or two in the cases of exposed skin as exemplified by the ungloved hands. The epoxide structure of the subject material characterizes it as being a potential sensitizing agent. Results of

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skin absorption tests conducted in the laboratory indicate that the material should present no problem from absorption through intact skin as a result of single prolonged contact. There is some indication from skin contact irritation tests that it may be absorbed through abraded skin from single contact in amounts sufficient to produce serious systemic injury. It is recommended that skin contact with the material be avoided. Protective clothing should be worn while handling whenever the likelihood of skin contact exists.

Results of acute inhalation studies indicate that there should be no problem from single exposures to the vapors of the material even when heated to temperatures of the order of 100°C. The vapors at room temperature and also at elevated temperature produced a yellow stain on the coat of the rats indicating a chemical reaction as a result of aeration.

These conclusions are based upon range finding toxicological tests and are limited to precautions for industrial handling of the material. Development of specific uses will require consideration of the health hazards and the need for further toxicological studies.

SUMMARY OF RANGE FINDING TOXICOLOGICAL DATA

Acute Oral Toxicity

Animal	Preparation Fed	Dose (g/kg)	No. Died / No. Fed	Response-Remarks
Rat	10% sol. in water	0.50	0/2	Some lung congestion liver and kidney observed at autopsy.
		1.0	2/2	Animals died overnight.

Eye Contact - Rabbit

Material	Treatment	Response-Remarks
Undiluted	Unwashed	Extensive conjunctival swelling and some necrosis accompanied by a moderate corneal injury not completely subsided in one week.
Undiluted	Washed and unwashed	Moderate conjunctival swelling and very slight corneal injury completely subsided in one week.

Skin Contact - Rabbit

Material	Condition of Skin	No. of Appl.	Site	Response-Remarks
10% aqueous solution	Intact	9	Ear	No irritation observed.
10% aqueous solution	Intact	4	Belly	Slight to moderate hyperemia and slight edema followed first and second application. Slight to moderate necrosis followed second, third and fourth application. Skin healed with slight scar in twenty-one days.
10% aqueous solution	Abraded	1	Belly	Moderate hyperemia, edema and necrosis followed first application. Skin healed with moderate scar in twenty-one days.

The animal used for the routine contact irritation test on the undiluted material died overnight following the first application. There was extensive necrosis and hyperemia as a result of this exposure.

Skin Absorption
Inhalation (Saturated Atmosphere)

Animal	Bath Temperature	Hours Exposed	No. Died No. Treated	Response-Remarks
Rat	Room	7	0/3	Animals coats turned slightly yellow in color, otherwise appeared normal during and after exposure. Some lung congestion kidney and liver injury observed at autopsy.
Rat	100°C.	7	0/3	Animals were wet and weak and stained yellow as above. Some lung congestion liver and kidney injury observed at autopsy.

Patch Test - Rabbit

Material	Duration of Exposure	Response-Remarks
Undiluted	1 hour	Slight hyperemia only
Undiluted	2 hours	Slight hyperemia and necrosis becoming obvious in twenty-four hours.
Undiluted	2 hours 40 mins.	Slight hyperemia and edema with moderate burn obvious in twenty-four hours.

Skin Absorption - Rabbit (Cuff Technique)

Material	Duration of Exposure	Dose (g/kg)	Died Treated	Response-Remarks
20% in water	24 hours	0.5	0/2	Moderate edema, hyperemia and necrosis upon removal of cuff.

FIRST AID MEASURES

Eye Contact: If the eyes are contaminated, they should be flushed immediately with copious amounts of flowing water for at least fifteen minutes.

MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY.

Skin Contact: Any injuries or irritations which may develop should receive medical attention.

All contaminated clothing, including shoes, must be removed immediately and the affected skin area flushed thoroughly with water from a safety shower, or other suitable device and cleansed with soap and plenty of water.

MEDICAL ATTENTION MUST THEN BE OBTAINED AS RAPIDLY AS POSSIBLE.

Contaminated clothing including shoes, must not be re-used until thoroughly cleaned or must be discarded.

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OFFICE OF TOXIC SUBSTANCES

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State MI

Zip 49000

Country

MRID No. (7)

000002

U.S. No. (11)

000182-01

Contractor

Doc Type

• R.I • C.P • H.E.A.S.E. 2.0, S.O.H.V.I.N

Doc Title

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON GLYCIDYL ACRYLATE AND GLYCIDYL METHACRYLATE

Chemical Name (100 per name)	CAS No. (10)
GLYCIDOL & ITS DERIVATIVES	999999994
GLYCIDYL ACRYLATE	106-90-1
GLYCIDYL METHACRYLATE	106-91-2

1s

5/31

1A

D 255

Biochemical Research Laboratory

The Dow Chemical Company

RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON GLYCIDYL ACRYLATE AND GLYCIDYL METHACRYLATE

File

K. No.

Signed K. J. Olson, Mar. 15, 1960

Chg.
Sub. By R. Brown
Ref.
Rept. By K. J. Olson

Checked R. Brown 11-12-60

PROBLEM

Limited range finding studies have been conducted on these materials and reported in and Due to the difference in toxicological properties of these two materials and also to the fact that the samples were small and the studies were incomplete, it was deemed important to repeat the range finding studies. New samples of each material were submitted to The Biochemical Research Laboratory for toxicological investigation and definition of industrial handling hazards.

CONCLUSIONS

This repeat study of the toxicological properties of these two materials bear out the conclusions presented in the first report referred to above. Range finding studies indicate that the glycidyl acrylate is approximately 10 times as toxic as glycidyl methacrylate insofar as acute oral toxicity and skin absorption are concerned. Likewise, the acrylate is much more injurious to the eyes than the methacrylate. Direct eye contact with the acrylate, undiluted or as the 10% in propylene glycol, showed in the laboratory that it would be almost certain to produce permanent impairment of vision. The methacrylate, on the other hand, would likely produce only transient corneal injury which would be expected to completely heal. Glycidyl acrylate also presents a much greater hazard and has a much higher toxicity than glycidyl methacrylate from an acute vapor inhalation standpoint.

* Ref. for glycidyl acrylate

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These conclusions are based upon range finding toxicological tests and are limited to precautions for industrial handling of the material. Development of specific uses will require consideration of the health problems presented and of the need for further toxicological studies.

ACUTE ORAL TOXICITY (TABLE I)

Glycidyl acrylate has a high acute oral toxicity. Range finding studies indicate that the LD50 is in the order of 30 mg/kg body weight in rats. Rather small amounts are capable of causing serious internal injury. These amounts can be obtained by accidental swallowing and by contamination of food and drink. Precautions should be taken to prevent the ingestion of glycidyl acrylate. The hands should be washed before smoking and eating. Food and drink should not be allowed in areas where this material is handled or used. Glycidyl methacrylate, on the other hand, possesses a moderate acute oral toxicity. Range finding tests indicate that it possesses and LD50 in the vicinity of 700 mg/kg. There should be no problem from ingestion incidental to industrial handling of the methacrylate. In either case, however, containers should be clearly labeled in order that swallowing due to mistaken identity cannot occur.

EYE CONTACT IRRITATION (TABLE II)

Undiluted glycidyl acrylate and the 10% in propylene glycol, studied in the laboratory, are capable of producing extensive damage to the conjunctival and cornea of the eye. Direct contact would be almost certain to result in some permanent impairment of vision if not complete loss of sight. Chemical worker's goggles are recommended for handling whenever the possibility of eye contact exists. It is important to note that the materials reacts very rapidly with the tissues of the eye and that if washing is to be effective, it must be done within a second after exposure. Glycidyl methacrylate, on the other hand, is only moderately irritating to the eye in the undiluted form. Permanent impairment of vision would likely not result. Immediate and thorough washing of contaminated eyes should eliminate corneal injury. Safety glasses with side shields are recommended for handling the methacrylate material. The 10% in propylene glycol is only slightly irritating to the eyes and should require minimal eye protection only for safe industrial handling.

SKIN CONTACT--IRRITATION AND ABSORPTION (TABLE III and IV)

Rabbits used for skin irritation studies on the undiluted material in the laboratory both died following the first routine application. This result indicates that both materials are rapidly absorbed through abraded skin in amounts sufficient to result in serious systemic injury, even death. It is important to avoid all contact with abraded skin or contact with skin which possesses a rash of any kind. Where intact skin is concerned the acrylate material is much more toxic

than the methacrylate. The acrylate appears to be of the order of 10 times as toxic as the methacrylate. Due to the epoxide structure of these materials, they probably possess skin sensitizing property. They are probably capable of sensitizing the skin and upon repeated contact might produce an allergic, edematous, contact-type dermatitis which might become extensive in some people. It is important to avoid skin contact with either material. Protective clothing should be worn at all times while handling, particularly in cases of cleaning up spills.

VAPOR INHALATION (TABLE IV)

Regarding acute inhalation of vapors, the glycidyl acrylate is considerably more toxic and poses a greater hazard than the methacrylate. Vapors of glycidyl acrylate present a definite problem from a single exposure at room temperature. Vapors in concentration sufficient to cause systemic injury probably are appreciably irritating and painful to the eyes and nose. They might also produce drowsiness. Concentration which might well be dangerous to life in a short time are readily attainable at room temperature and would be particularly so if the material were heated. Precautions must be taken to avoid vapor exposure. The material should be handled under a hood or in a well ventilated area. For clearning up accidental spills, it is recommended that a full-face gas mask equipped with a suitable canister be used in well ventilated areas or a self-contained breathing apparatus be used in poorly ventilated areas. The vapors of glycidyl methacrylate, on the other hand, may present a slight problem from single exposures at room temperature. Single exposure of brief duration probably would not be harmful, but prolonged vapor exposures might be injurious to health. There is indication from laboratory studies that the vapors of glycidyl methacrylate are probably also irritating to the eyes and nose. They might also produce some drowsiness. In the interest of good health, precautions should be taken to avoid breating the vapors of this material for prolonged periods. It is suggested that the material be handled under the hood or in a well ventilated area.

FIRST AID MEASURES

EYE CONTACT	[X]	If the eyes are contaminated, they should be flushed immediately with copious amounts of flowing water for at least 15 minutes.
	[X]	Medical attention should be obtained if irritation persists or develops after washing of the eyes.
	[ ]	Medical attention should be obtained.
	[(1)]	MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY.
SKIN CONTACT	[X]	Any injuries or irritations which may develop should receive medical attention.
	[ ]	Contaminated clothing and shoes should be removed and not reused until thoroughly cleaned.
	[ ]	Wash contaminated skin with soap and plenty of water.
	[X]	Contaminated clothing, including shoes, should be removed and the affected skin area should be washed thoroughly with soap and plenty of water.
	[X]	Medical attention should then be obtained.
	[ ]	Contaminated clothing and shoes should not be reused until thoroughly cleaned.
	[X]	All contaminated clothing, including shoes, must be removed immediately and the affected skin area flushed thoroughly with water from a safety shower, or other suitable device and cleaned with soap and plenty of water.
	[(1)]	MEDICAL ATTENTION MUST THEN BE OBTAINED AS RAPIDLY AS POSSIBLE.
	[(1)]	Contaminated clothing including shoes, must not be reused until thoroughly cleaned or must be discarded.
INHALATION	[X]	If a person should experience any noticeable ill effects from breathing the vapor or fumes of this material, medical attention should be obtained promptly.
	[(1)]	If a person should be overcome from breathing this material, he should be removed to fresh air at once, he must be made to rest, kept warm, and MEDICAL ATTENTION SHOULD BE OBTAINED IMMEDIATELY. If breathing stops, artificial respiration should be administered.
INGESTION	[X]	If appreciable amounts of material are swallowed, vomiting should be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Medical attention should then be obtained.
	[X]	If the material is swallowed, vomiting must be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY

COMMENTS: Note: x refers to glycidyl acrylate

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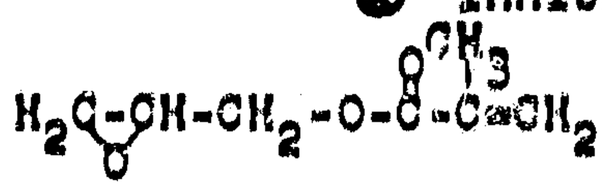
LEAD-100 PRINTED IN U.S.A. 00-07

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA: $C_7H_{10}O_3$		CHEMICAL NAME: Glycidyl methacrylate ⊗
MOLECULAR WEIGHT: 142	INDUSTRIAL HYGIENE STANDARD: CRI	SYNONYM: Methacrylic acid; 2,3-epoxypropyl ester
STRUCTURAL FORMULA - SEE BELOW		

⊗ inhibited with 20ppm MEHQ



PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT: 189 °C 760 mm Hg.	EXPLOSIVE LIMITS (% BY VOL. IN AIR)	FLASH POINT: °F.	IGNITION TEMP.: °C.	MELTING POINT: °C.	ODOR (THRESHOLD CONCENTRATION IN p/m): Pungent
SOLUBILITY: don't know				PHYSICAL STATE: liquid	COLOR: colorless
CHEMICAL REACTIVITY: see below					
STABILITY: polymerizes in acid					

TOXIC PROPERTIES

TYPE OF CONTACT	CLASSIFICATION OF TOXIC PROPERTIES	
EYE	☐ May cause no response or an over-time very slight to slight mucoidal pain and/or irritation of the eyelids.	☐ May cause some permanent loss of vision (this includes damage to cornea or internal injury which is irreversibly healed in one week.)
	☒ May cause serious injury to the eye to result in loss of time from work. (This includes damage to the cornea which heals or nearly heals in a week and/or some/possible conjunctival irritation with edema.)	☐ Vapor exposure may cause severe pain, lacrymation or serious injury to the eyes.
SKIN	☐ Single prolonged exposure (hours) causes no effect. Several repeated prolonged exposures may or may not cause the development of some slight irritation.	☐ Single short exposure (minutes) may cause considerable irritation and/or single prolonged or frequently repeated short exposures cause a burn and/or may cause systemic injury, even death.
	☒ Single prolonged exposure may cause some reddening of the skin. Repeated prolonged exposures may cause appreciable irritation, possibly a mild burn and/or may cause appreciable systemic injury due to absorption. probably a sensitizer	☐ An exposure rapidly causes severe burns and/or serious systemic injury, even death.
DUST OR MIST	☐ No systemic injury expected. No irritation to nose and throat in dusty or misty atmospheres.	☐ Dusty or misty atmosphere painful to nose and throat (intolerable to most people) and/or exposure may cause serious systemic injury.
	☐ Throat and nose irritation in a dusty or misty atmosphere is painful but not intolerable and/or prolonged or repeated exposures may cause systemic injury.	☐ Short exposure (minutes) may cause death or serious systemic injury.
VAPOR	☐ Exposures do not cause any effects other than some very slight irritation or pain to the eyes or respiratory passages at the most.	☐ Exposures may cause severe discomfort, and/or serious systemic injury, and/or may cause intolerable irritation to the respiratory passages.
	☒ Single exposures exceeding 1/1 hour, or frequently repeated exposures of shorter duration, may cause slight anesthesia and/or slight systemic injury, and/or cause appreciable, but not intolerable, irritation of respiratory passages.	☐ Short exposures may cause unconsciousness, and/or serious systemic injury, including death.
		☐ Even very short exposure will cause serious systemic injury or death.
INGESTION	☒ Amounts which may be swallowed incidental to industrial handling will not cause injury.	☐ Amounts which may be swallowed incidental to industrial handling and use may cause serious injury.

COMMENTS

000006

DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION		PRECAUTIONS (SEE CODE BELOW)				
		EYES	SKIN	INHALATION* DUST OR MIST	INHALATION* VAPOR	INGESTION
I NO CONTACT	Characterized by remote operation with equipment isolated from the work area. The personnel monitoring isolated areas will require the personal protection outlined for IV below.	A	A		A	A
II MINOR CONTACT	Characterized by closed systems with equipment located outside the work area; instrument control; mechanical handling of materials in bulk. Examples are: continuous reactors, stills and filters; sealed conveyors; ventilated packaging.	B	A		A	A
III OCCASIONAL DAILY CONTACTS	Characterized by manual handling of materials in packages such as bags, drums and fiberpaks. Ventilation may be provided for specific jobs. Many batch operations fall into this category.	C	D		B	A
IV GROSS CONTACT LIKELY	Characterized by hand operation. Examples are: Emergency repairs, cleaning equipment, cleaning filters, taking care of spills, packaging volatile or dusty materials without ventilation, shoveling and tray drying.	C	E		B	A

EYE CONTACT	A No eye protection needed. B Use safety glasses without side shields. C Use safety glasses with side shields.	D Use chemical workers goggles. E Use gas tight goggles or a full face gas mask.
SKIN** CONTACT	A A bath and clean clothes once per week along with the usual washing at mealtimes should be adequate precautions. B Generally contaminated clothing and shoes must be removed not later than the end of the work period and must be thoroughly cleaned before reuse. C Require shower at the end of the work period and clean clothing from the skin out at the start of each work day.	D Clothing should be changed and skin washed promptly upon any detectable contact. Each use will require special consideration to determine suitable protective devices and standards of personal cleanliness. E Impervious clothing such as rubber boots, rubber aprons, and rubber gloves will be required. Specific items will be dictated as required by circumstances.
INHALATION: DUST OR MIST	A No respiratory protection. B No protection required for exposure of thirty min. duration or less in obviously dusty atmosphere. Exposure of longer duration will require the use of a dust respirator bearing the approval of the U. S. Bureau of Mines for the use with toxic dusts.	D Any exposure in obviously dusty atmosphere will require a dust respirator bearing the approval of the U. S. Bureau of Mines for use with toxic dusts. E Any exposure in dusty atmosphere will require the use of an airline respirator, blower mask, or Chemox mask.
INHALATION: VAPOR	A No precautions necessary. B No precautions necessary for single exposure of less than 1/2 hour. Longer single exposure, or frequently repeated exposure will require a gas mask or respirator equipped with appropriate canister.	C No precautions necessary for single exposure of no more than ten minutes. Longer exposures either single or repeated, will require gas mask or respirator equipped with appropriate canister. D Gas mask with appropriate canister required at all times. E Evacuate area or enter and exit only with airline respirator, blower mask or Chemox mask.
INGESTION	A No unusual precautions required.	B Food and tobacco should not be present in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

**GOOD PRACTICE REQUIRES THAT GROSS AMOUNTS OF ANY CHEMICAL BE REMOVED FROM THE SKIN AS SOON AS IS PRACTICAL.

*SUITABLE GAS MASK CANISTER

organic vapor

000007

KO.

SIGNED K. J. Olson F. Oyen CHECKED H. R. Hoyle

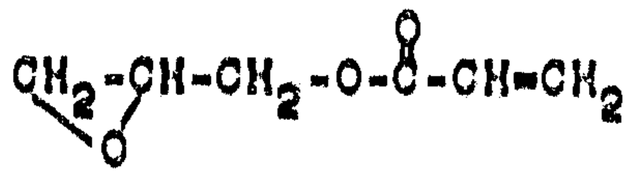
DATE 11-7-60 DATE 11-11-60

6-1960 PRINTED IN U.S.A. 50-08

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA: $C_6H_8O_3$		CHEMICAL NAME: Glycidyl Acrylate ⊗
MOLECULAR WEIGHT: 128	INDUSTRIAL HYGIENE STANDARD	SYNONYMS: CRI; Acrylic acid; 2,3-epoxy propyl ester
STRUCTURAL FORMULA OR COMPOSITION		

⊗ 0.5% hydroquinine inhibitor



PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT °C mmHg	LOWER LIMIT (% BY VOL. IN AIR)	FLASH POINT °F	IGNITION TEMP. °C	MELTING POINT °C	ODOR THRESHOLD CONCENTRATION IN AIR
					pungent

SOLUBILITY	PHYSICAL STATE	COLOR
don't know	liquid	colorless

CHEMICAL REACTIVITY: see below

STABILITY: polymerizer in acid

TOXIC PROPERTIES

TYPE OF CONTACT	CLASSIFICATION OF TOXIC PROPERTIES	
EYE	☐ May cause no response or no more than very slight to slight transitory pain and/or irritation of the eyelids.	☒ May cause some permanent loss of vision (this includes damage to cornea or internal injury which is incompletely healed in one week.)
	☐ May cause sufficient injury to the eye to result in loss of time from work. (This includes damage to the cornea which heals or nearly heals in a week and/or considerable conjunctival irritation with edema.)	☐ Vapor exposure may cause severe pain, lacrymation or serious injury to the eyes.
SKIN	☐ Single prolonged exposure (hours) causes no effect. Several repeated prolonged exposures may or may not cause the development of some slight irritation.	☐ Single short exposure (minutes) may cause considerable irritation and/or single prolonged or frequently repeated short exposures cause a burn and/or may cause systemic injury, even death.
	☐ Single prolonged exposure may cause some reddening of the skin. Repeated prolonged exposure may cause appreciable irritation, possibly a mild burn and/or may cause appreciable systemic injury due to absorption.	☒ An exposure rapidly causes severe burns and/or serious systemic injury, even death. probably a skin sensitizer
DUST OR MIST	☐ No systemic injury expected. No irritation to nose and throat in dusty or misty atmosphere.	☐ Dusty or misty atmosphere painful to nose and throat (intolerable to most people) and/or exposure may cause serious systemic injury.
	☐ Throat and nose irritation in a dusty or misty atmosphere is painful but not intolerable and/or prolonged or repeated exposures may cause systemic injury.	☐ Short exposure (minutes) may cause death or serious systemic injury.
VAPOR	☐ Exposures do not cause any effects other than some very slight irritation or pain to the eyes or respiratory passages of the nose.	☐ Exposures may cause extreme drowsiness, and/or serious systemic injury, and/or may cause intolerable irritation to the respiratory passages.
	☐ Single exposures exceeding 1/2 hour, or frequently repeated exposures of shorter duration, may cause slight anesthesia and/or slight systemic injury, and/or cause appreciable, but not intolerable, irritation of respiratory passages.	☒ Short exposures may cause unconsciousness, and/or serious systemic injury, including death.
	☐ Even very short exposure will cause serious systemic injury or death.	
INGESTION	☐ Amounts which may be swallowed incidental to industrial handling will not cause injury.	☒ Amounts which may be swallowed incidental to industrial handling and use may cause serious injury.

COMMENTS

000008

	DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION	PRECAUTIONS (SEE CODE BELOW)				
		EYES	SKIN	INHALATION* DUSTY OR MIST	INHALATION* VAPOR	INGESTION
I NO CONTACT	Characterized by remote operation with equipment isolated from the work area. The personnel entering isolated areas will require the personal protection outlined for IV below.	A	A		A	A
II MINOR CONTACT	Characterized by closed systems with equipment vented outside the work area; temporary control; mechanical handling of materials in bulk. Examples are: continuous reactors, stills and filters; enclosed conveyors; ventilated packaging.	C	A		B	A
III OCCASIONAL DAILY CONTACTS	Characterized by manual handling of materials in packages such as bags, drums and fiberpaks. Ventilation may be provided for specific jobs. Many batch operations fall into this category.	D	D		C	E
IV GROSS CONTACT LIKELY	Characterized by hand operation. Examples are: Emergency repairs, cleaning equipment, cleaning filters, taking care of spills, packaging volatile or dusty materials without ventilation, unloading and bag dying.	D	E		D	E

EYE CONTACT	A No eye protection needed. B Use safety glasses without side shields. C Use safety glasses with side shields.	D Use chemical workers goggles. E Use gas tight goggles or a full face gas mask.
SKIN** CONTACT	A A bath and clean clothes each day work along with the usual washing at mealtimes should be adequate precautions. B Grossly contaminated clothing and shoes must be removed not later than the end of the work period and must be thoroughly cleaned before re-use. C Require shower at the end of the work period and clean clothing from the skin out at the start of each work day.	D Clothing should be changed and skin washed promptly upon any detectable exposure. Each job will require special consideration to determine suitable protective devices and standards of personal cleanliness. E Impervious clothing such as rubber boots, rubber aprons, and rubber gloves will be required. Specific items will be dictated as required by circumstances.
INHALATION — DUSTY OR MIST	A No respiratory protection. B No protection required for exposure of thirty min. duration or less in obviously dusty atmosphere. Exposures of longer duration will require the use of a dust respirator bearing the approval of the U. S. Bureau of Mines for the use with toxic dusts.	D Any exposure in obviously dusty atmospheres will require a dust respirator bearing the approval of the U. S. Bureau of Mines for use with toxic dusts. E Any exposure in dusty atmospheres will require the use of an airline respirator, blower mask, or Chemox mask.
INHALATION — VAPOR	A No precautions necessary. B No precautions necessary for single exposure of less than 1/2 hour. Longer single exposures, or frequently repeated exposures will require a gas mask or respirator equipped with appropriate canister.	C No precautions necessary for single exposure of no more than ten minutes. Longer exposures either single or repeated, will require gas mask or respirator equipped with appropriate canister. D Gas mask with appropriate canister required at all times. E Evacuate area at once and enter only with airline respirator, blower mask or Chemox mask.
INGESTION	A No unusual procedures required.	B Food and tobacco should not be present in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

**GOOD PRACTICE REQUIRES THAT ANY AMOUNT OF ANY CHEMICAL IS REMOVED FROM THE SKIN AS SOON AS IS PRACTICAL.

*SUITABLE GAS MASK CANISTER

organic vapor

000009

NOTED K. J. Olson F. Oyen K.O.

DATE 11-7-60

CHECKED H. R. Hoyle

DATE 11-11-60

TABLE I

ACUTE ORAL TOXICITY IN RATS

Material	Conc. (corn oil)	Dose in g/kg body weight 0.031	0.063	0.126	0.252	0.50	1.0
Glycidyl methacrylate K-31916-3	10%	--	--	0/2 ⊗	0/2	0/2	2/2
Glycidyl acrylate K-31909-2	1.25%	0/2	0/2	--	--	--	--
	2.52%	1/2	1/2	2/2	2/2	2/2	--

⊗ Ratios refer to No. died/ No.fed.

TABLE II

EYE CONTACT IRRITATION IN RABBITS

Material	Conc.	Eye Contact Irritation Unwashed eye	Washed eye ⊗
Glycidyl methacrylate	Undiluted	Sl. to mod. conjunctivitis; sl. corneal injury cleared in one week	Sl. conjunctivitis cleared in 48 hours
Glycidyl methacrylate	10% in propylene glycol	Sl. conjunctivitis cleared in one hour	Sl. conjunctivitis cleared in 24 hours.
Glycidyl acrylate	Undiluted	Total loss of sight	Total loss of sight
Glycidyl acrylate	10% in propylene glycol	Total loss of sight	Total loss of sight

⊗ The material is applied to both eyes of the rabbit. Within 30 seconds one eye is washed with tap water for two minutes. The material is allowed to remain in the other eye.

TABLE III

SKIN CONTACT IRRITATION ON SHAVE RABBIT BELLY AND EAR

Material	Conc.	Skin Contact Irritation Ear	Intact Belly	Abraded Belly
Glycidyl methacrylate	Undiluted (1)	--	--	--
Glycidyl methacrylate	10% in DPM	Essentially no irritation	Sl. hyperemia and edema after 1st appl., mod. burn after 2nd appl. discontinued	Ess. the same as intact area. Animal lost weight.
Glycidyl acrylate	Undiluted (1)	--	--	--
Glycidyl acrylate	10% in DPM (2)	Slight hyperemia	Sl. hyperemia, edema and burn	Same as intact area.

(1) Animal died after 1st application.

(2) Animal died after 2nd application.

TABLE IV

SKIN ABSORPTION IN RABBITS (CUFF TECHNIQUE) (a)

Material	Conc.	Dose in g/kg body weight 0.015	0.031	0.063	0.126	0.252	0.50
Glycidyl methacrylate (b)					0/2	0/2	0/2
Glycidyl acrylate	3.1% soln. in DPM	0/2	1/2	1/2			

(a) Skin exposure period 24 hours.

(b) All six animals progressively lost weight during the experimental period.

TABLE V

ACUTE VAPOR INHALATION (SATURATED ATMOSPHERE)

Material	Bath Temp.	Hours Exposed	No. Died / No. Exposed	Remarks
Glycidyl methacrylate	Room	7	0/3	Eye irritation. Drowsy in ten minutes. Some lung injury at autopsy.
Glycidyl acrylate	Room	4.6	4/4	Immediate severe eye and nose irritation. Rats gasped in ten minutes.
Glycidyl acrylate	Room	2.0	3/3	One rat died over night, others died 4 to 11 days later. Kidney and G.I. irritation at autopsy.
Glycidyl acrylate	Room	1.0	0/3	Eye irritation and labored breathing in chamber. Lung, liver and kidney injury at autopsy. Saturated atmosphere, approximately 1200ppm.

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CODING FORM FOR GLOBAL INDEXING

Microfiche No. (7) • 20638 | No. of Pages

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• R. J. • U. P. • H. E. A. S. D. 8. D. S U H S P A

GUINEA PIG SENSITIZATION STUDIES CONDUCTED ON 3 METHOXY 1,2-EPOXYPROPANE (METHYL GLYCIDYL ETHER)

Chemical Name (Indexing Name) | CAS No. (10)

GLYCIDOL & ITS DERIVATIVES 3-METHOXY-1,2-EPOXYPROPANE (METHYL GLYCIDYL ETHER) | 9999999 94 430-31-0

5/31

1A

07821/376 D-160

THE DOW CHEMICAL COMPANY
BIOCHEMICAL RESEARCH LABORATORY

GUINEA PIG SENSITIZATION STUDIES CONDUCTED ON 3-METHOXY-1,2-EPOXYPROPAN. (METHYL GLYCIDYL ETHER)

TYPE
REF
SOURCE
SUB BY
REPT BY
CHECKED BY
DATE

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. INDIVIDUAL EVALUATION MUST BE MADE FOR EACH PRODUCT. EXPOSURE MAY BE REQUIRED AT SPECIFIC USES AND FORMULATIONS ARE DEVELOPED BY PRODUCT CHANGE DESIGN

A sample representative of 3-methoxy-1,2-epoxypropane was submitted to the Biochemical Research Laboratory for guinea pig skin sensitization studies.

The skin sensitizing property of 3-methoxy-1,2-epoxypropane was assessed by tests conducted on male and female guinea pigs using a test solution made up of 0.5% (w/v) of 3-methoxy-1,2-epoxypropane in Chlorothene® NU solvent. The test solution was applied to clipped and chemically depilated areas on the shoulders of the guinea pigs, twice a week for three weeks. Two weeks later the test solution and Chlorothene NU were applied to a clipped area on the animals' right and left flanks, respectively.

Guinea pigs employed as negative controls received Chlorothene NU for three weeks and were challenged, after the two week rest period, with the test solution and Chlorothene NU. Positive control animals received 15% DER® 331 epoxy resin in Dowanol DPM: Tween 80 mixture.

The sample of 3-methoxy-1,2-epoxypropane tested in this laboratory caused no skin sensitivity response in guinea pigs in skin sensitization tests. The probability of the material causing a skin sensitivity response in humans is low; however, further testing, in humans, should be considered if increase in use indicate the need.

DISTRIBUTION

000002

8782/1377

THE DOW CHEMICAL COMPANY

TEXAS DIVISION
FREEPORT TEXAS 77541

correction in original report

PRELIMINARY TOXICOLOGY INFORMATION

Material: 3 Methoxy-1, 2, Epoxy Propane **Date:** 10/24/68

Description: The material is a colorless liquid with a slight sweet odor submitted for routine skin and eye irritation studies by R. F. Mogford.

Toxicological Procedure: Routine skin and eye irritation studies were conducted using young, male, albino rabbits.

Eye Irritation: The test material produced a moderate indication of pain that persisted for less than 30 seconds.

The conjunctival response was not immediate. There was no response until one hour later when the membranes became inflamed and slightly swollen. This cleared in the washed eye after the second day and from the unwashed eye after the third day.

The cornea showed no response in either the washed or unwashed eye.

Skin Irritation: A moderate response was produced on the abraded, covered area of the abdomen after the second application. This area cleared in three days after the last application with no scarring.

No response was observed on the uncovered area of the ear with eight applications made in ten days.

Results and Recommendations: Safety glasses with side shields should give adequate eye protection.

Repeated skin contact should be avoided by workmen with cuts or abrasions.

This is only a cursory range finding study. If the product is assuming customer potential then a more sophisticated toxicological studies should be undertaken.

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CODING FORM FOR GLOBAL INDEXING

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Doc Type 22

•R.I•U.P•HEASD S.D. SYSTEM

Doc Title 23

PRELIMINARY TOXICOLOGY INFORMATION

Chemical Name (100 per name) 25 | CAS No. (10) 24

GLYCIDOL & ITS DERIVATIVES | 999999994

METHYL GLYCIDYL ETHER | 930-37-0

5/51

1A

87821378

THE DOW CHEMICAL COMPANY

TEXAS DIVISION
FREEPORT TEXAS 77541

PRELIMINARY TOXICOLOGY INFORMATION

Material: Methyl Glycidyl Ether **Date:** 12/22/69

Description: The material is a clear liquid submitted for routine skin and eye irritation studies by

Toxicological procedures: Routine skin and eye irritation studies were conducted on young, male, albino rabbits.

Eye Irritation: There were indications of severe pain produced by the material when instilled into the eyes of the rabbit.

The conjunctival membranes became inflamed and swollen immediately and were edematous within one hour. However, this condition began to subside within 24 hours.

Cloudiness over parts of both corneas was observed as the immediate response. Within one hour 50 - 100% of both corneas exhibited shallow necrosis.

No internal damage was observed.

Skin Irritation: Methyl glycidyl ether produced severe inflammation and moderate swelling on the covered abdominal area. There also was a deep scab formed on the abraded portion of this area.

There were no responses of the uncovered unabraded area of the ear to the material.

Conclusions and Recommendations: Methyl glycidyl ether can produce eye damage in rabbits.

All contact with the eyes should be avoided. If eye contact should occur, the eyes should be washed according to standard safety procedures, and immediate medical attention should be sought.

Skin burns can be produced by this material in a covered area such as under a watch band, ring, etc. This reaction is intensified if the skin is abraded. Skin contact should be avoided, particularly in a covered area.

Preliminary toxicology information - continued

Material: Methyl Glycidyl Ether Date: 12/22/69

Conclusions and Recommendations - continued

Proper eye protection should be worn at all times by potentially exposed personnel. Education as to the hazards of methyl glycidyl ether and training in decontamination and eye irrigation techniques should be mandatory for these personnel.

This cursory range finding study of this formulation of methyl glycidyl ether is one of many that have been conducted on other formulations. If this formulation is considered for customer use then further study is needed.

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CODING FORM FOR GLOBAL INDEXING

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Contract No. 21

Doc Type • R.L • U.P • H.E.A.S.D S.D. S.U H.S T.N 22

Doc Title RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON DIGLYCIDYL ETHER OF DIPHENYLOLMETHANE 23

Chemical Name (IUC per name) 25	CAS No. (10) 26
GLYCIDOL & ITS DERIVATIVES DIGLYCIDYL ETHER OF DIPHENYLOLMETHANE	999999994 999999994

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Biochemical Research Laboratory

The Dow Chemical Company

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RESULTS OF RANGE FINDING TOXICOLOGICAL TESTS ON DIGLYCIDYL ETHER OF DIPHENYLOL-METHANE

File
K No.
Chg.
Rept. By K. J. Olson

Signed Kenneth J. Olson Checked John McCollister

Date Aug. 11, 1959 Date Aug. 12, 1959

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PROBLEM

The subject material has a use in conjunction with the preparation of epoxy resins. A sample has been submitted to the Biochemical Research Laboratory for toxicological investigation and definition of industrial handling hazards.

Hemopoietic studies have been conducted on this material and appear summarized in an addendum to this report along with several other epoxides.

CONCLUSIONS

The subject material has a low acute oral toxicity and should present no problem from ingestion incidental to industrial use or general handling.

The undiluted material and the 10% in propylene glycol are essentially non-irritating to the eye. Minimal eye protection should be worn while handling when contamination is likely simply to avoid discomfort. The undiluted material is slightly to moderately irritating to intact and abraded skin. Exposure for an hour or so would probably produce no serious irritation; however, direct contact for a working day would likely result in considerable reddening and some swelling. Repeated prolonged contact for a day or two might produce a burn. The 10% solution in Dowanol DPM is essentially non-irritating to intact and abraded skin.

Experience with epoxy compounds indicates that the subject material is probably a skin sensitizing agent. Accurate evaluation of skin sensitizing properties can not be made until studies are conducted in human subjects. Due to the strong probability of this property, it is recommended that skin contact with the material be avoided. Protective clothing should be worn whenever the likelihood of gross contamination exists, particularly in cases of cleaning up spills.

(Continued)

CONCLUSIONS (CONTINUED)

Acute inhalation tests have not been conducted. Exposure to vapors at room temperature would probably pose no problem. Vapors or mist which might be evolved from the material when heated would likely produce considerable eye and nasal irritation, a condition of skin sensitization described above and possibly systemic injury. Exposure to vapors should be avoided. The material should be handled in a hood or in a well-ventilated area. If the material reaches pilot plant scale or greater it is recommended that vapor studies be initiated.

These conclusions are based upon range finding toxicological tests and are limited to precautions for industrial handling of the material. Development of specific uses will require consideration of the health problems presented and of the need for further toxicological studies.

SAMPLE INFORMATION

C.R.I. Name: Methane: bis(p-2,3-epoxypropoxyphenyl)-,

Source: [redacted]

K Number:

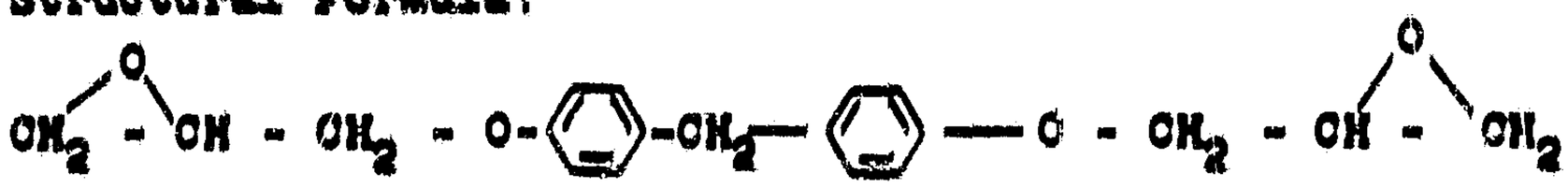
Date Request Received: 4-9-58

Date Sample Received: 4-9-58

Physical State: Liquid

Molecular Formula: $C_{19}H_{20}O_4$

Structural Formula:



EYE CONTACT	[X] If the eyes are contaminated, they should be flushed immediately with copious amounts of flowing water for at least 15 minutes. [X] Medical attention should be obtained if irritation persists or develops after washing of the eyes. [ ] Medical attention should be obtained. [ ] MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY.
SKIN CONTACT	[X] Any injuries or irritations which may develop should receive medical attention. [X] Contaminated clothing and shoes should be removed and not re-used until thoroughly cleaned. [X] Wash contaminated skin with soap and plenty of water. [ ] Contaminated clothing, including shoes should be removed and the affected skin area should be washed thoroughly with soap and plenty of water. [ ] Medical attention should then be obtained. [ ] Contaminated clothing and shoes should not be re-used until thoroughly cleaned. [ ] All contaminated clothing, including shoes, must be removed immediately and the affected skin area flushed thoroughly with water from a safety shower, or other suitable device and cleaned with soap and plenty of water. [ ] MEDICAL ATTENTION MUST THEN BE OBTAINED AS RAPIDLY AS POSSIBLE. [ ] Contaminated clothing including shoes, must not be re-used until thoroughly cleaned or must be discarded.
INHALATION	[ ] If a person should experience any noticeable ill effects from breathing the vapor or fumes of this material, medical attention should be obtained promptly. [ ] If a person should be overcome from breathing this material, he should be removed to fresh air at once, be made to rest, kept warm, and MEDICAL ATTENTION SHOULD BE OBTAINED IMMEDIATELY. If breathing stops, artificial respiration should be administered.
INGESTION	[ ] If appreciable amounts of material are swallowed, vomiting should be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Medical attention should then be obtained. [ ] If the material is swallowed, vomiting must be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY

SUMMARY OF RANGE FINDING TOXICOLOGICAL DATA

Acute Oral Toxicity

Animal	Preparation Fed	Dose (g/kg)	No.Died No.Fed	Response-Remarks
Rat	20% solution in corn oil	3.98	0/2	Some diarrhea and kidney damage observed.

Eye Contact - Rabbit

Material	Treatment	Response-Remarks
Undiluted	Washed and unwashed	No irritation observed.
10% in prop. glycol	Washed and unwashed	Slight pain only.

Skin Contact - Rabbit

Material	Condition of Skin	No. of Appl.	Site	Response-Remarks
Undiluted	Intact	4	Ear	Slight irritation followed 1st appl. and then became moderate. Slight edema and necrosis followed 3rd application - mod. necrosis followed 4th - ear healed with slight exfoliation in 21 days.
Undiluted	Intact	10	Belly	Moderate hyperemia followed 1st 4 applications, then slowly subsided in spite of further exposure. Slight edema followed 1st and 2nd applications, then subsided. Slight necrosis followed 3rd application.
Undiluted	Abraded	3	Belly	Moderate hyperemia followed 1st 3 appls. then subsided. Slight edema followed 1st 3 applications and slight necrosis followed 3rd.
10% sol. in Dowanol DPM	Intact	10	Ear	No irritation observed.
10% sol. in Dowanol DPM	Intact	10	Belly	Slight exfoliation followed 3rd and 4th appls., then became moderate.
10% sol. in Dowanol DPM	Abraded	3	Belly	Slight to moderate exfoliation between 3rd and 16th day. Skin essentially normal in 21 days.

Skin Absorption

There is no indication, from the skin irritation tests conducted, that this material is absorbed through the skin in toxic amounts.

ADDENDUM I

Preliminary Report on the Effect of Intramuscular Injection of Some Chemicals on the Hematopoietic System of Rats

The purpose of these experiments was to conduct a preliminary investigation on the possible effect of the following compounds on the hematopoietic system. All the compounds were injected intramuscularly to female rats weighing around 150 gms. The injections were made every other day for a variable period of time as described for each compound. Hematocrit hemoglobin and white cell counts were performed at intervals of time on the injected animals.

The compounds used were:

1 - Polyglycol 166 diepoxide
2 - Diethylene glycol diglycidyl
3 - Tetraethylene glycol diglycidyl ether
4 - Diglycidyl ether of diphenylol methane
5 - Diglycidyl ether of Polyglycol 166
6 - Dipropylene glycol diglycidyl ether
7 - Polyglycol P-1000 diglycidyl ether
8 - Polyglycol P-872 diglycidyl ether
9 - Polyglycol P-400 diglycidyl ether
10 - Diethylene glycol diglycidyl ether

Results

Polyglycol 166 diepoxide

This compound was injected at a dose of 400 mg./kg. every other day for a period of 30 days. Total dose per rat was 600 mg. Number of rats injected was 35.

The blood picture of 10 rats was followed up and showed no indications of anemia. The white blood cell count was elevated as a result of the abcess formation at the site of injection. The compound is an irritant on injection and causes necrosis of the tissues. Evidence of kidney irritation was also noticed as exhibited by slight bloody urine and diuresis.

Diethylene glycol diglycidyl

Rats were injected at a dose of 500 mg./kg. for the first dose; however, toxicity was apparent and more than 50% of the animals died. Animals were replaced and the dose adjusted to 250 mg./kg. The animals were injected every other day for a total number of 9 injections or 225 mg./rat. The seven following compounds were treated in the same manner as diethylene glycol diglycidyl. The injected compound caused necrosis at the site of injection and abscess formations. Blood tinged urine was also noticed indicating kidney damage.

Hematocrit varied from 40 to 24 with an average of 36.5. There was no reduction in the white blood cell count. These results indicate a slight average reduction of the hematocrit.

However, one animal out of 5 examined was definitely anemic. Whether the anemia was due to a combined effect of kidney damage and infection or a direct effect of the compound on the hematopoietic system is not definitely known at the present time. Gross appearance of the internal organs of treated animals revealed the presence of nephritis.

Tetraethylene glycol diglycidyl ether

The compound caused abscess formations at site of injection, also nephritis, hematocrit varied from 41 to 24 with an average of 30.6 or a reduction of over 20%. Again leucocytes were normal. The low hematocrit average is due to the low values obtained from one animal; however, the rest of the treated animals had low normal values.

Diglycidyl ether of diphenolyl methane

No blood changes were observed, however, kidney damage was noticed. One animal had a tumor mass involving the right kidney.

Diglycidyl ether of Polyglycol 166

No blood changes were observed. Kidney damage was noticed. No signs of irritation at the site of injection.

Dipropylene glycol diglycidyl ether

No blood changes were observed in 4 rats; however, one rat was anemic (hematocrit 28). No changes were observed in the

white cells. (One animal died on 500 mg./kg. No kidney damage.

Polyglycol P-1000 diglycidyl ether

No anemia, irritation or mortality at 500 mg./kg. No kidney damage.

Polyglycol P-872 diglycidyl ether

No anemia, no kidney damage, no irritation, no mortality at 500 mg./kg.

Polyglycol P-400 diglycidyl ether

Mild kidney damage, no anemia, no mortality at 500 mg./kg., no irritation.

Diethylene glycol diglycidyl ether

Abscesses, kidney damage. One animal had a pale bone marrow but no frank anemia. Three died on 500 mg./kg. and two more died on 250 mg./kg.

S. Sadek

S. Sadek
10-28-58

ADDENDUM II.

Report on the Effect of Intramuscular Injection of Some Epoxy Compounds on the Hematopoietic System of Rats

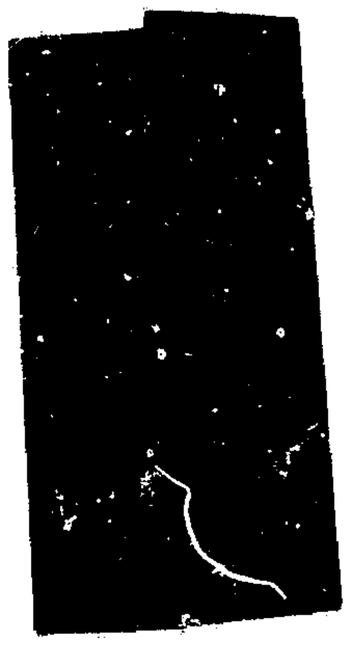
The object of this experiment was to explore the effect of prolonged administration of the following compounds on the hematopoietic system. In a previous experiment, some of the compounds used in this trial showed some pathological effects on the kidneys and depression of the hematopoietic system when injected in large doses. (See preliminary report)

The dosage level used in the present experiment was 20 mg./kg. body weight per injection.

Materials and Methods

The following compounds were tested:

1 - Diglycidyl ether
2 - Diethylene glycol diglycidyl ether
3 - Polyglycol P-400 diglycidyl ether
4 - Polyglycol P-1000 diglycidyl ether
5 - Polyglycol 166 diepoxide
6 - Tetraethylene glycol diglycidyl ether



The compounds were diluted in corn oil and injected intramuscularly to young female rats at a dose level of 20 mg./kg. body weight. The animals were injected every other day for a period of 45 days. Each animal received a total dose of 80 mg. The animals were held for a period of 30 days from the last day

of treatment to allow ample time for any effects on the hematopoietic system to develop.

At the end of the experiment, blood samples were collected from each rat for hematocrit, hemoglobin, white cell count and blood smear evaluation. The animals were weighed at the beginning of the experiment as well as at the termination. A complete autopsy was performed on each rat with special attention to the lymphatic system and bone marrow.

Results

All animals on the different treatments showed an average gain in weight of about 21 grams during the period of the experiment except the group that received diglycidyl ether. The diglycidyl ether animals showed an average weight gain of 10.1 gram and varied from 24 grams to 2 grams per animal. One animal lost 4 grams from its initial weight.

Leukocyte count, hematocrit, hemoglobin and stained blood smears showed a normal blood picture for all compounds listed above. No gross pathological changes were observed in the internal organs of any of the animals examined.

Conclusions

All compounds tested did not cause any pathological changes on the hematopoietic system as tested under the conditions of the experiment. Also, the lymph nodes and spleen of all the animals examined appeared normal in size and color.

S. Sadek

S. Sadek
6-11-59

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Contract No.

Doc Type • R.I • U.P • H.E.A.S.D S.U H.S + N

Doc Title

DNE-(O-TOLOXY)-2,3,

EPOXYPROPANE EXPERIMENT ON

GLYCIDYL (EPOXY) COMPOUNDS

Chemical Name (100 per name)	CAS No. (10)
GLYCIDOL & ITS DERIVATIVES	999999994
DNE-(O-TOLOXY-2,3, EPOXYPROPANE	999999994

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8782/1528

[redacted]

ONE-(O-TOLOXY)-2,3, EPOXYPROPANE

Since earlier work suggested (though not conclusively) that myanesin might be conjugated with glucuronic acid, it was felt that this reaction, if it occurred, would involve the hydroxy groups of the side chain. We therefore decided to investigate the epoxy analogue of myanesin to determine whether such a compound would resemble myanesin in activity, yet perhaps be longer acting and equally or more safe. Hines and Christensen found that glycidyl (epoxy) compounds were generally more potent than corresponding glycerol derivatives, and that the B- and g-carbon hydroxy groups were not necessary for relaxing activity.

This compound is a clear oily liquid at room temperature, with a sp. gr. of about 1.072, being sparingly soluble in water, but very soluble in alcohol and in propylene glycol. We investigated this compound in mice and in dogs, using a 2% solution in 85% propylene glycol, and a 10% solution in propylene glycol subcutaneously in 32 mice, and the 10% solution intravenously in 8 dogs. Doses of 75 to 500 mg./kg. were given the mice; the dogs received doses ranging from 10 to 75 mg./kg.

In the mice it was found that 75 to 200 mg./kg. caused only mild depression; 300 mg./kg. caused transient ataxia and momentary loss of the righting reflex in some animals; 500 mg./kg. caused loss of the righting reflex in 75% of the mice, but relaxation was incomplete. Moreover, all mice receiving sufficient "epoxy" to produce a measureable depression (500 mg./kg.) died within 72 hours after the injection.

In the dogs, general depression and ataxia were seen in all, lasting from 3-16 minutes. However, no specific hind-limb paralysis or abdominal relaxation was seen, and one dog died acutely following a dose of 55 mg./kg. It was concluded that this compound was an unsatisfactory substitute for myanesin.

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Doc Type • R.I. • U.P. • HEAD 8D. SUBSTANCE 21

Doc Title TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF BIS(1,2-EPOXYPROPOXY)ETHANE

Chemical Name (100 per name) 23	CAS No. (10) 24
GLYCIDOL & ITS DERIVATIVES	999999994
BIS(1,2-EPOXYPROPOXY)ETHANE	999999994

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Biochemical Research Laboratory

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TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF BIS (1,2-EPOXYPROPOXY)ETHANE

File [redacted]
K No.
Charge
Ref.
Source
Sub. By J. D. Ashley
Rept. By Carol Hendrick
K. J. Olson

Signed Carol Hendrick, Oct. 29, 1965

Signed Ken Olson, Nov. 1, 1965

Checked F. Byers 11-1-65

Carol Hendrick, Nov. 11, 1965
Ken Olson, Nov. 11, 1965

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[redacted]

PROBLEM

A sample of the subject material was submitted to the Biochemical Research Laboratory for toxicological investigation and definition of industrial handling hazards.

CONCLUSIONS

The subject material has a serious effect upon the eye. Direct eye contact is likely to result in tissue destruction possibly leading to some permanent impairment of vision. Special and particular precautions must be taken to prevent contact with the eye. Tight fitting goggles, such as chemical workers goggles, should afford suitable protection for industrial handling when eye contact is likely.

The subject material has a moderate to severe effect on intact or abraded skin. A single, prolonged exposure to abraded skin may produce marked irritation. Repeated, prolonged contact is capable of causing marked to severe damage to intact skin. Due to the

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epoxy structure the material is likely to be a skin sensitizer. Precautions must be taken to prevent skin contact with the undiluted material. Protective clothing must be worn whenever the likelihood of contact exists. A 10% Dowanol DPM solution studied in the laboratory has a moderate effect on intact or abraded skin. Repeated, prolonged contact with 10% concentration over a period of a few days may cause blistering and a superficial burn. Precautions must be taken to prevent single prolonged or repeated skin contact with such dilutions. Protective clothing, impermeable to the material should be worn as required by circumstance.

Prolonged exposure to relatively large areas of skin may result in the absorption of toxic amounts of the undiluted or the 10% dilution of the subject material.

These conclusions are based upon range finding toxicological tests and are limited to precautions for industrial handling of the material. Development of specific uses will require consideration of the health problems presented and of the need for further toxicological studies.

SUMMARY OF TOXICOLOGICAL DATA

Eye Contact - Rabbit

Material	Treatment	Response-Remarks
Undiluted	Washed	Very slight pain initially, moderate conjunctival inflammation and slight corneal injury and iritis persisting beyond one week.
Undiluted	Unwashed	Same as above.

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Skin Contact - Rabbit (Repeated Application)

Material	Condition Of Skin	No. Of Appl.	Site	Response-Remarks
Undiluted	Intact	5	Ear	Hyperemia, edema and induration of ear began after first application. A moderate burn was evident after five applications.
Undiluted	Intact	3	Belly	Slight hyperemia, moderate to extensive edema after first application. After three applications there was a moderate burn.
Undiluted	Abraded	1	Belly	Marked hyperemia, moderate to extensive edema and a moderate burn was evident after the first application. Animal was sacrificed at one week. A weight loss of 580 grams was noted during this period.
10% in Dowanol DPM	Intact	6	Ear	Slight hyperemia and edema. No further injury was apparent after the first application because it seemed the rabbit licked the compound from ear.
10% in Dowanol DPM	Intact	5	Belly	Slight hyperemia and edema, the latter of which was extensive after the first application. A moderate burn was evident after five applications.
10% in Dowanol DPM	Abraded	3	Belly	Slight hyperemia and edema after first application. A slight superficial burn was evident after the third application. Due to the poor condition of the animal the rabbit was sacrificed after nine days. During that time there was a weight loss of 200 grams.

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8788/1529

FIRST AID MEASURES

EYE CONTACT	☑	If the eyes are contaminated, they should be flushed immediately with copious amounts of flowing water for at least 15 minutes.
	☐	Medical attention should be obtained if irritation persists or develops after washing of the eyes.
	☐	Medical attention should be obtained.
	☑	MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY.
SKIN CONTACT	☑	Any injuries or irritations which may develop should receive medical attention.
	☐	Contaminated clothing and shoes should be removed and are re-used until thoroughly cleaned.
	☐	Wash contaminated skin with soap and plenty of water.
	☑	Contaminated clothing, including shoes, should be removed and the affected skin area should be washed thoroughly with soap and plenty of water.
	☐	Medical attention should then be obtained.
	☑	Contaminated clothing and shoes should not be re-used until thoroughly cleaned.
	☐	All contaminated clothing, including shoes, must be removed immediately and the affected skin area flushed thoroughly with water from a safety shower, or other suitable device and cleansed with soap and plenty of water.
	☐	MEDICAL ATTENTION MUST THEN BE OBTAINED AS RAPIDLY AS POSSIBLE.
	☐	Contaminated clothing including shoes, must not be re-used until thoroughly cleaned or must be discarded.
INHALATION	☐	If a person should experience any noticeable ill effects from breathing the vapor or fumes of this material, medical attention should be obtained promptly.
	☐	If a person should be overcome from breathing this material, he should be removed to fresh air at once, be made to rest, keep warm, and MEDICAL ATTENTION SHOULD BE OBTAINED IMMEDIATELY. If breathing stops, artificial respiration should be administered.
INGESTION	☐	If appreciable amounts of material are swallowed, vomiting should be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. Medical attention should then be obtained.
	☐	If the material is swallowed, vomiting must be induced by tickling the back of the tongue with the finger or by giving an emetic such as 2 tablespoonfuls of table salt in a glass of warm water. MEDICAL ATTENTION SHOULD THEN BE OBTAINED WITHOUT DELAY

COMMENTS:

OFFICE OF TOXIC SUBSTANCES

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Contract No.:

Doc Type: • R.I. • M.P. • HEASD P.P. SUHSTAN

Doc Title: RESULT OF TOXICOLOGICAL STUDIES ON P-BIS-(2,3-EPOXYPROPOXY)-BENZENE

Chemical Name (300 per name): GLYCIDOL & ITS DERIVATIVES; P-BIS-(2,3-EPOXYPROPOXY)BENZENE

CAS No. (10): 999999994; 999999994

3/31

1A

8782/1530

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

Subject: RESULT OF TOXICOLOGICAL STUDIES ON p-bis-(2,3-epoxypropoxy) - BENZENE

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To [redacted] Check [illegible] 3-17-48 Rept. By [illegible] 3-12-48

UNIT INDEX

Toxicological tests on animals have indicated that p-bis-(2,3-epoxypropoxy) benzene, is slightly irritating to the skin and that it is a mild skin sensitizer. We do not believe it offers any advantages from a toxicological viewpoint over 3-(2-xenoxy)-1,2-epoxy propane.

INDEX

Benzene, p-bis-(2,3-epoxypropoxy), Toxicity of.

PROBLEM

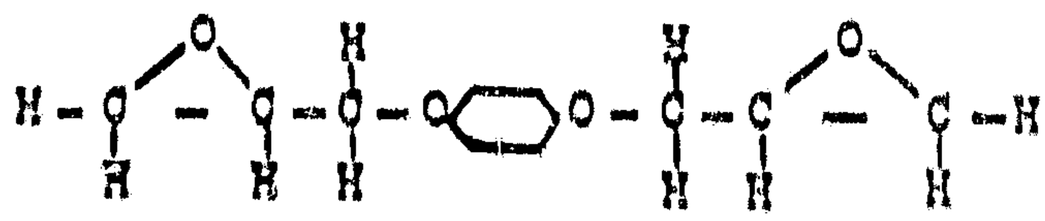
This material is being considered for use as an addition agent for Saran in place of 3-(2-xenoxy)-1,2-epoxy propane. What are its toxicological properties?

MATERIAL

Name - p-bis-(2,3-epoxypropoxy) - benzene.

Formula

Structural -



Empirical -

$C_{12}H_{14}O_4$

Source - [redacted]

Reference No - 1 - XL

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EXPERIMENTAL RESULTS

Acute Oral - The material was not sufficiently soluble in acceptable solvents for oral administration and not readily suspended in finely divided form hence could not satisfactorily be fed in single doses.

As an alternate, an attempt was made to administer the material as a part of the diet. The rats, however, won't not accept diets containing 5.0, 1.0, or 0.5% of the material.

Skin Irritation - The material as a 10% solution in butyl carbitol acetate was applied repeatedly to the ear and belly of a rabbit. A slight irritation developed on the ear and a slight to moderate irritation developed on the belly. Both seemed to heal quite rapidly when applications were terminated.

Skin Sensitization - Sensitization tests conducted on guinea pigs indicate that the test material may be a sensitizer similar to 3-(2'xenoxy)-1,2-epoxy propane. Neither of these materials are as strong a sensitizer as p-phenylenediamine. By the same technique, we succeeded in sensitizing 3 of 8 guinea pigs with 3(2X), 4 of 8 with the subject material and 7 of 7 with p-phenylenediamine.

CONCLUSIONS

p-bis(2,3-epoxypropoxy) benzene is slightly irritating to the skin and it appears to be a weak sensitizing agent.

We do not believe it offers any advantages over 3-(2-xenoxy)-1,2-epoxy propane from a toxicological viewpoint.

[redacted]

APPENDIX FOR FILE REPORT

TABULATION OF SENSITIZATION STUDIES %

Guinea Pig NO	Reaction after Last Sensitizing Dose	Reaction 24 Hours after Test Dose	Reaction [illegible]
	3(2-[illegible])-2,5-[illegible]		
211	A C	F P	[illegible]
212	A C	-	-
219	-	A C	F P
222	-	F P	-
225	-	F P	F P
231	-	F P	[illegible]
232	-	F P	-
239	-	F P	[illegible]
SCORE	0/5	1/5	[illegible]
	D-[illegible](2,3-[illegible]) [illegible]		
213	-	F P	F P
220	-	- -	-
224	A C	F P	F P
229	-	F P	-
232	-	A C	-
233	-	F P	[illegible]
236	-	F P	[illegible]
236	-	F P	[illegible]
SCORE	0/5	0/5	[illegible]

% Using Storner technique.

Note: Original data on sensitization filed T1.[illegible]-3-3

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Doc Title 23

GUINEA PIG SENSITIZATION TEST OF 1,3-DICHLOROISOPROPYL GLYCIDYL ETHER

Chemical Name (100 per name) 25 | CAS No. (10) 26

GLYCIDOL & ITS DERIVATIVES | 999999994

1,3-DICHLOROISOPROPYL GLYCIDYL ETHER | 999999994

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D-405 878211531

BIOMEDICAL RESEARCH

Dow Chemical Company

2284 March 24, 1975

GUINEA PIG SENSITIZATION TEST OF 1,3-DICHLOROISOPROPYL GLYCIDYL ETHER

REPORTED BY T. G. Pullin, R. L. Schnebel [redacted] R. V. Johnston, D.V.M.

[redacted]

A sample of the test material was submitted to the Comparative Toxicology Research Laboratory for evaluation of skin sensitization in guinea pigs.

The skin sensitization potential of the test material and butyl glycidyl ether (BGE) was evaluated in male guinea pigs by subjecting them to 0.5% (w/v) solution of the material in a 9:1 mixture of Dowanol DPM: Tween 80. Positive control animals were treated in a similar manner to 0.5% solution (w/v) of 2,4-dinitrochlorobenzene in the 9:1 mixture of Dowanol DPM: Tween 80.

The test material did not sensitize any of the animals, while the positive control sensitized all of the animals. The test material is not capable of inducing skin sensitization in guinea pigs under the conditions of this test procedure.

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OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Doc Type • R.I. • U.P. • HEASD 8D. , SUHST+N

Doc Title ACUTE TOXICOLOGICAL PROPERTIES OF SD 185

Chemical Name (100 per name)	CAS No. (10)
GLYCIDOL & ITS DERIVATIVES	999999994
DER 331	25068-38-6
BUTYL GLYCIDYL ETHER	2426-08-6

5/31

1A

87824/532

D-507

BIOMEDICAL RESEARCH
Texas Division Toxicology

DOW CHEMICAL U S A

J. Matula	7-27-73	

ACUTE TOXICOLOGICAL PROPERTIES OF SD 185

T. G. Pullin, H. N. Edwards,

REPORTED BY R. J. Scheybal CHECKED BY E. Johnston

INFORMATIVE SUMMARY FOR BUSINESS PURPOSES BASED ON THE INPUT RECEIVED. ADDITIONAL INFORMATION INCLUDING THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED FOR SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR.

A sample of SD 185 was submitted to the Texas Toxicology Laboratory for evaluation of acute lethality, eye and skin irritation properties and acute inhalation toxicity.

Eye contact with this material may result in slight, transient pain and slight conjunctival redness. Safety glasses are recommended whenever the possibility of eye contact exists.

Slight inflammation of the skin may occur with repeated, prolonged exposures. However, moderate inflammation, swelling and scaling of the skin may result from a single exposure to abraded skin.

Reasonable care and cleanliness practiced during industrial handling should avert any skin contact problems.

No special handling precautions should be necessary if vapor exposures are of short duration. If long term chronic exposures are anticipated then further studies would be necessary.

The acute oral lethality of the material is low. There is little likelihood that internal injury would result from ingestion of the small amounts one might encounter incidental to the industrial handling or use of this material.

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BIOMEDICAL RESEARCH
TEXAS DIVISION TOXICOLOGY

DOW CHEMICAL U.S.A.

FREEPORT, TEXAS

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA | NAME: BD 185

MOLECULAR WEIGHT | INDUSTRIAL HYGIENE STANDARD | SYNONYMS | FILE

STRUCTURAL FORMULA OR COMPOSITION

DER 331 with butyl glycidyl ether
96% + 4%

PHYSICAL AND CHEMICAL PROPERTIES

BOILING POINT °C mmHg	EXPLOSIVE LIMITS % VOL IN AIR	FLASH POINT °F	IGNITION TEMP °C	MELTING POINT °C	VAPOR PRESS. mmHg. 20°C
CORROSIVE NATURE (In Common Metals)			PHYSICAL STATE: Liquid resin	COLOR: straw	
THERMAL REACTIVITY				ODOR (Include Concentration in Air)	
STABILITY (In Ordinary Heat Light)					

TOXIC PROPERTIES

TYPE OF CONTACT	CLASSIFICATION	TOXIC PROPERTIES
EYE	[X] May cause permanent loss of vision (this includes damage to cornea or internal injury which is incapacitating beyond in one week.)	[ ] May cause some permanent loss of vision (this includes damage to cornea or internal injury which is incapacitating beyond in one week.)
	[ ] Vapor exposure may cause severe pain, lacrimation, if severe injury to the eyes.	[ ] Vapor exposure may cause severe pain, lacrimation, if severe injury to the eyes.
SKIN	[X] Single prolonged exposure may cause severe irritation or injury. Repeated or prolonged exposure may cause dermatitis.	[ ] Single short exposure (minutes) may cause considerable irritation and/or single prolonged or frequently repeated short exposures (minutes) cause a burn and/or may cause systemic injury, even death.
	[ ] Single prolonged exposure may cause severe burning of the skin. Repeated prolonged exposure may cause dermatitis and in sensitized persons a rash and/or may cause dermatitis and/or may cause dangerous systemic injury due to absorption.	[ ] An exposure rapidly causes severe burns and/or serious systemic injury, even death.
DUST OR MIST	[ ] Not applicable. Not applicable to mists and thick or fatty or oily atmospheres.	[ ] Thick or oily atmosphere painful to nose and throat. Exposure is most painful and/or exposure may cause serious systemic injury, even death.
	[ ] Inhalation of a heavy or oily mist may cause severe irritation of the nose and throat and/or may cause systemic injury.	[ ] Short exposure (minutes) may cause death or serious systemic injury.
VAPOR	[X] Exposure may cause irritation of the nose and throat and/or irritation of the eyes or irritation of the nose and throat.	[ ] Exposure may cause systemic illness, and/or serious systemic injury, and/or may cause intolerable irritation to the eyes, nose and throat.
Formal or some temporary or less severe injury	[ ] Single exposure may cause irritation or injury and/or prolonged or repeated exposure may cause systemic injury or death.	[ ] Short exposure may cause unconsciousness, and/or serious systemic injury including death.
		[ ] Even very short exposure will cause serious systemic injury or death.
INGESTION	[X] Amounts which may be swallowed incidental to industrial handling and use may cause serious injury.	[ ] Amounts which may be swallowed incidental to industrial handling and use may cause serious injury.

FORM VFB

DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION

		PRECAUTIONS				
		EYES	SKIN	DUST OR MIST	VAPOR	INGESTION
I NO CONTACT	Characterized by remote operations with no personal contact from the work area. The person wearing protective gear will rely to his personal protection only when required for safety.	A	A		A	A
II MINOR CONTACT	Characterized by closed systems with occasional contact outside the work area in efficient equipment. Occasional handling of materials in bulk. Examples are maintenance, lab work, pilot plant, operating supervisory, controlled packaging.	A	A		A	A
III DAILY CONTACT	Characterized by manual handling of materials in pure form such as bags, drums and containers. Ventilation may be provided for dusty jobs. Many batch operations fall into this category.	B	B		B	A
IV SHORT CONTACT LIKELY	Characterized by hand dumping, sampling and blending, cleaning equipment, cleaning filters, taking care of spills, packaging a mixture of dusty materials without ventilation, sanding and tray drying.	B	B		B	A

EYE CONTACT

- A No eye protection needed
- B Use safety glasses with side shields
- C Use safety glasses with side shields
- D Use chemical workers goggles
- E Use gas tight goggles or a full face gas mask

SKIN CONTACT

- A A bath and clean clothes each day, along with the usual washing of hands should be sufficient precautions.
- B Fully contaminated clothing and shoes must be removed not later than the end of the work period and must be thoroughly cleaned before re-use.
- C Remove shoes at the end of the work period and clean skin by rinse the work dirt at the start of each work day.
- D Clean clothes be changed and skin washed thoroughly when any dust comes contact. Each use will require special consideration in determining suitable protective devices and standards of personal cleanliness.
- E Impervious clothing such as rubber boots, rubber aprons, and rubber gloves will be required. Special items will be dictated as required by circumstances.

INHALATION

DUST OR MIST

- A No respiratory protection
- B No protection required for exposure of thirty min. Duration or less in extremely dusty atmosphere. Examples of jobs that will require the use of a dust respirator bearing the approval of the U.S. Bureau of Mines for the use with dust.
- C Any exposure to extremely dusty atmospheres will require a dust respirator bearing the approval of the U.S. Bureau of Mines for use with dust.
- D Any exposure to dusty atmospheres will require the use of an air line respirator, blower mask, or chemical mask.

VAPOR

- A Not necessary normally
- B No precautions necessary for single exposures of no more than ten minutes. Longer exposures either single or repeated will require use of respirator equipped with suitable canister.
- C Gas mask with suitable canister required at all times.
- D Use of air line with blower mask or chemical mask.

INGESTION

- A No form of protection required
- B Food and tobacco should not be present in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

*NOTE: PRACTICE MINIMIZE THAT GIVEN AMOUNTS OF ANY CHEMICAL BE REMOVED FROM THE SKIN AS SOON AS IS PRACTICAL

*SUITABLE GAS MASK CANISTER

Author: T. Pullin, R. Edwards Checked: C. Johnson, B. S. Horvath

Date: 7-27-73

BIOMEDICAL RESEARCH
Texas Toxicology Laboratory

DOW CHEMICAL U.S.A.

Freeport, Texas

T. G. Pullin, H. N. Edwards, R. L. Schwabel

ACUTE TOXICOLOGICAL PROPERTIES OF SD 185

INTRODUCTION

A sample of SD 185 was submitted to the Texas Toxicology Laboratory for evaluation of acute oral lethality, eye and skin irritation properties and acute inhalation toxicity. The material is a liquid resin.

COMPOSITION OF FORMULATION

DER 331	96%
Butyl Glycidyl Ether	4%

METHODS

Skin and eye irritation studies were conducted on New Zealand albino rabbits. Both eyes are observed for conjunctival, corneal and internal responses. One eye is washed within 30 seconds of contact while the other eye is unwashed so that the responses can be compared. Indications of pain from eye contact are also observed. Skin reactions are recorded on open-intact, covered-abraded and covered-intact areas.

Five Sprague-Dawley rats weighing from 288-364 grams with a mean of 330 grams were placed in a 56.6 liter gas environment chamber and exposed for 7 hours 45 minutes to a saturated atmosphere of the volatile components of SD 185. The saturated atmosphere was generated by passing air at 1.6 liters/minute through the bubbler containing the compound, giving an average concentration of 0.67 mg/liter. The exposure was run at room temperature.

The animals were observed periodically during and for 14 days following exposure. Weights were recorded before and periodically after exposure. All animals were necropsied 14 days post exposure.

The acute oral toxicity of the test material was assessed by administering three dose levels, 0.5, 1.0 and 2.0 gm/kg body weight by single dose gavage to each of four female Sprague-Dawley rats. The animals were fasted overnight prior to dosing, and after dosing they were allowed to eat and drink ad libitum. The rats were weighed the day following dosing and at weekly intervals for two weeks thereafter.

RESULTS

Slight, transient pain and slight conjunctival inflammation were produced by the test material when instilled into the eyes of the rabbit. Corneal and intraocular damage did not occur.

Repeated, prolonged exposures to the material on the open and covered-intact skin areas for three days produced slight inflammation.

A single, prolonged exposure on the covered abraded skin resulted in moderate inflammation, slight swelling and scaling of the skin.

All rats survived the exposure to the saturated atmosphere and exhibited no abnormal signs. Gross pathological examination of the animals at the termination of the observation period revealed no compound related changes.

There was no treatment-related alteration in appearance, demeanor, food consumption or survival in any of the animals. Gross pathological examination of the animals given the highest dosage revealed no compound related changes. The single dose oral LD_{50} for female rats is greater than 2.0 gm/kg body weight.

ANTICIPATED RESPONSES AND RECOMMENDATIONS

Eye contact with this material may result in slight, transient pain and slight conjunctival redness. Safety glasses are recommended whenever the possibility of eye contact exists.

Slight inflammation of the skin may occur with repeated, prolonged exposures. However, moderate inflammation, swelling and scaling of the skin may result from a single exposure to abraded skin.

Reasonable care and cleanliness practiced during industrial handling should avert any skin contact problems.

No special handling precautions should be necessary if vapor exposures are of short duration. If long term chronic exposures are anticipated then further studies would be necessary.

The acute oral lethality of the material is low. There is little likelihood that internal injury would result from ingestion of the small amounts one might encounter incidental to the industrial handling or use of this material.

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7-27-73

OFFICE OF TOXIC SUBSTANCES

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Doc Title ACUTE PERCUTANEOUS ABSORPTION POTENTIAL OF N-BUTYLGLYCIDYL ETHER PREPARED BY LAKE JACKSON RES CTR 23

Chemical Name (100 per name) 25	CAS No. (10) 26
GLYCIDOL & ITS DERIVATIVES	999999994
N-BUTYLGLYCIDYL ETHER	2426-08-6

5/31

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8788/1533

ACUTE DERMAL TOXICITY STUDY

OF

n-BUTYL MYCERYL LAUR

Reported By:

D. D. Lockwood and H. W. Taylor

Reviewed By:

R. V. Johnston

Health and Environmental Sciences-Texas
Lake Jackson Research Center
Acute Toxicology Laboratory
Dow Chemical U.S.A.
Freeport, Texas 77541

SUMMARY

The acute percutaneous absorption LD_{50} for male and female rabbits of n-butylglycidyl ether is 778 mg/kg (662-1012 mg/kg, 95% confidence interval), with an approximate slope of 11.5.

The acute percutaneous absorption potential was evaluated by treating 2 male and 2 female rabbits/dose level (number dead/number treated in parenthesis) with 1000 (4/4), 750 (1/4), or 500 (0/4) mg/kg of the undiluted test material. Following treatment, rabbits displayed signs of discomfort and became lethargic. Skin at the treatment site was necrotic. No internal lesions attributable to treatment were observed in the survivors upon gross pathological examination at 2 weeks.

INTRODUCTION

A sample of n-butylglycidyl ether, also known as n-BGE, was submitted to the Acute Toxicology Laboratory of Health and Environmental Sciences-Texas for evaluation of acute percutaneous absorption potential. Source of the test material, used as a diluent for epoxy resins, was Resins Technical Service and Development, Freeport, Texas.

TEST ANIMALS

The acute percutaneous absorption tests were conducted using male and female New Zealand albino rabbits (Ray Nichols Rabbitry, Lumberton,

Texas). All animals were maintained on a 12-h on light and 12-h off, in an animal care facilities accredited by the American Association for Accreditation of Laboratory Animal Care. They were supplied with commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri) and well water *ad libitum*. All animals were acclimated to the laboratory environment at least 1 week prior to testing. The rabbits were housed singly. Each animal was uniquely identified by number with an individual tattoo.

METHOD

Twenty-four hours prior to application of the test material, the entire trunk of 2 male and 2 female rabbits/dose level was clipped free of hair with electric clippers. The rabbits were treated with 1000, 750, or 500 mg/kg of the undiluted test material applied under a heavy-gauge plastic sleeve held in place with rubber bands. The plastic sleeve was covered with a cloth bandage taped securely to the marginal hair. All rabbits on test were placed in individual holding cages with free access to food and water. After 24 hours, the sleeves were removed and the skins were washed with mild soap and water, rinsed thoroughly, and dried with a soft disposable towel. The topical response at the site of application was evaluated after removal of the plastic sleeve. The animals were observed frequently during exposure and for the following 2 weeks for signs of toxicity. Body weights were recorded before and after the

8782/533

24-hour exposure period and at 1 and 2 weeks post-treatment. At 2 weeks post-treatment, all surviving rabbits were euthanized for gross pathological examination. The acute percutaneous absorption LD_{50} was calculated by the moving average method (Thompson and Weil, 1952) as implemented in a computer program.

RESULTS

The acute percutaneous absorption LD_{50} is 770 mg/kg (642-1012 mg/kg, 95% confidence internal), with an approximate slope of 11.3.

A 24-hour exposure to the test material resulted in the death of 4/4 rabbits at 1000 mg/kg, 1/4 rabbits at 750 mg/kg, and 0/4 rabbits at 500 mg/kg. Following treatment, the rabbits displayed signs of discomfort and became lethargic. The treated skin was necrotic with scabbing and scarring. Most survivors were slow to regain pretreatment weight. No internal lesions attributable to treatment were observed in the survivors upon gross pathological examination at 2 weeks.

Reported by:

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OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Doc Title: FINAL REPORT ON THE TOXICITIES EVALUATED FOR MUTAGENICITY WITH COVER LETTER

Chemical Name (100 per page): | CAS No. (10):

(2) TYCIDOL ETC. DERIVATIVES | 999999994

DCPDGE (DICYCLOPENTADIENE GLYCIDYL ETHER) | 999999994

1A

Chemical Name (100 per page)	
+ XD-7114 (DIGLYCIDYL ETHER OF NEOPENTYL GLYCOL)	9999999 4
CGE (O-CRESYL GLYCIDYL ETHER)	9999999 4
BUTYL GLYCIDYL ETHER (BGE)	9999999 4
+ EPOXIDE 3 (ALLYL GLYCIDYL ETHER)	9999999 4
* DICHLOROISOPROPYL GLYCIDYL ETHER (DCOPP)	9999999 4

D-543

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THE UNIVERSITY OF TEXAS MEDICAL BRANCH
GALVESTON, TEXAS 77550

Department of Preventive Medicine and Community Health
Division of Environmental Toxicology and Epidemiology

August 24, 1977

Christine H. Calhoun
Designee Pred-ists Dept.
Rosins TS2D, D-4503

Dear Ms. Calhoun:

We are pleased to submit a final report on the epoxides evaluated for mutagenicity.

The evidence from this study indicate that DGE is mutagenic in both *in vitro* and *in vivo* systems; XD-7114, DCOPP and CGE are less mutagenic than DGE; DER 331, DER 331 (distilled), and DCPOGE are mutagenic only in bacterial systems; while Epoxide 8 was essentially non-mutagenic.

It has been a pleasure to provide this evaluation for you, and we hope to be of service to you in the future.

Yours truly,

Marvin S. Legator, Ph.D.
Professor & Director

MSL/vc

000002

REPORT TO THE DOW CHEMICAL COMPANY

INTEGRATED MUTAGENICITY TESTING PROGRAM

SUMMARY

Several epoxides were evaluated for mutagenic activity by the following test systems: (a) microbial mutagenic assay system, (b) body fluid analysis, (c) host mediated assay, (d) induction of DNA repair, (e) micronucleus test and (f) the dominant lethal test.

DER 331, DER 331 (distilled) and DCPDGE (dicyclopentadiene glycidyl ether) were mutagenic in the microbial assay but inactive in the remainder of the test systems. In view of the inactivity in the intact animal systems, it is felt that these compounds pose the least mutagenic hazard to man of the chemicals evaluated.

XD-7114 (diglycidyl ether of neopentyl glycol) and CGE (o-cresyl glycidyl ether) were mutagenic in bacteria and induced DNA repair. In addition, mutagenicity was discovered in the urine of XD-7114 and CGE treated mice. These results plus a possibility of preimplantation loss in the dominant lethal test suggest that XD-7114 and CGE are weak mutagens.

Butyl glycidylether (BGE) was determined to be mutagenic as evidenced by an induction of increased dominant lethality, DNA repair and bacterial mutation. This data also supports the conclusion that BGE poses a hazard by skin exposure.

Epoxide 8 (allyl glycidyl ether) was only active in the microbial assay with very minimal activity. This data leads to the determination of Epoxide 8 as nonmutagenic.

Dichloroisopropyl glycidyl ether (DCOPP) was found to be mutagenic in a previous study in animal systems as seen by increased activity in the micronucleus test, cytogenetic assay and dominant lethal by gavage

It was active in this study in the host-mediated assay. This data points to DCOPP as a weak mutagen. However, the dominant lethal by topical application performed in this study was negative. This suggests that skin exposure poses less hazard.

INTRODUCTION

Exposure to toxic chemicals in our environment may be one of the major contributing factors to the mutagenic burden in man. Some man-made chemicals currently in widespread use in the population have been found to be mutagenic in sub-human experimental systems suggesting that such chemicals constitute a potential genetic hazard for man. The risk poses itself to both present and future generations: to the former, as the production of genetic lesions in somatic cells, possibly leading to cancer; while to the latter as the production of transmissible gene mutations and alterations in chromosome structure and number.

Several classes of these man-made compounds have been shown to have the ability of inducing mutations. The work of Rapoport[1] revealed that many alkylating agents are mutagenic, some of them with great potency. Haddow[2] pointed out that certain diepoxy compounds active as cross-linking agents may lead to biological alkylation. Hendy and his co-workers[3] found both radiomimetic and cytotoxic activity in their series of bisepoxides. Hine and Rowe[4] have stated that the majority of observations seem to indicate that the physiological activity of the epoxides is in three areas:

(1) central nervous system depression

(2) irritation of surface tissues and

(3) radiomimetic action.

In recognition of a potential hazard that exists concerning epoxide induced genetic damage, The Dow Chemical Company submitted two epoxy resins and six epoxy diluents to Dr. Marvin S. Legator's Laboratory, at the University of Texas Medical Branch for assessment of their mutagenic activity by means of a battery of mutagenic

tests. These test chemicals are some of the major epoxides currently used in industry and have a potential widespread exposure to the population.

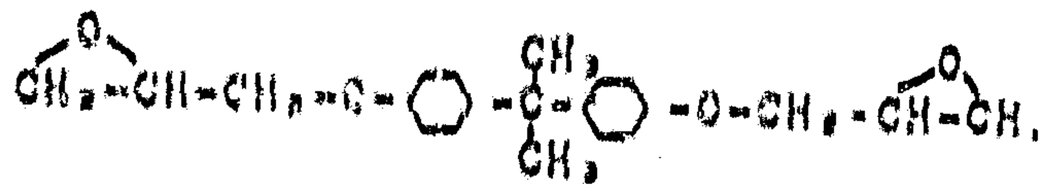
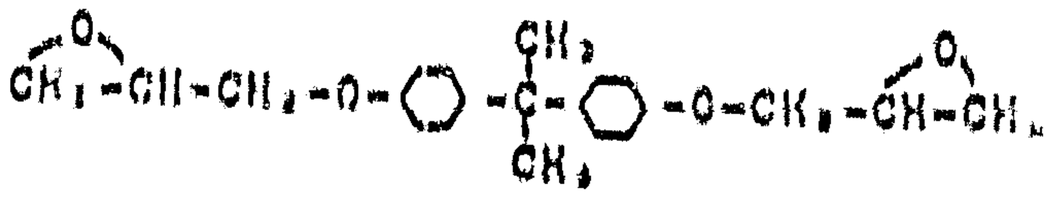
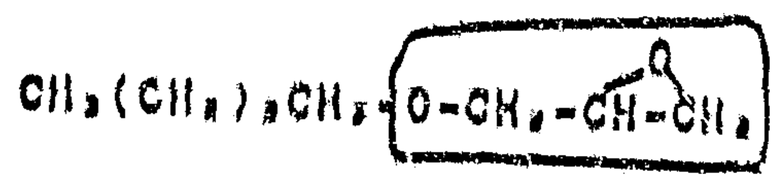
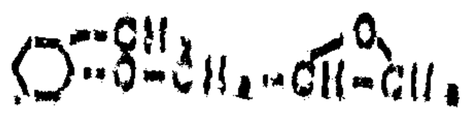
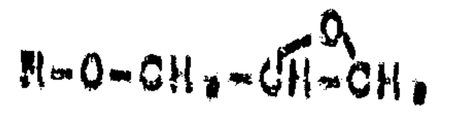
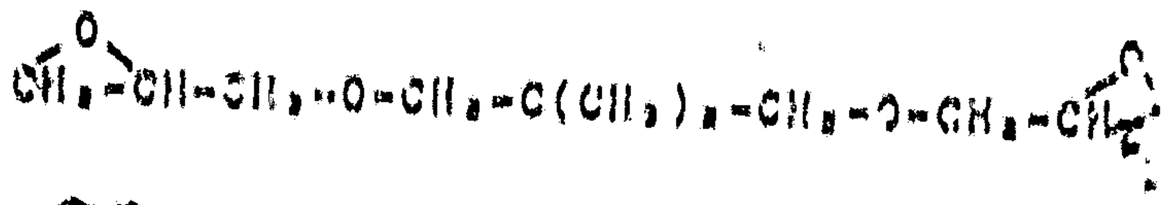
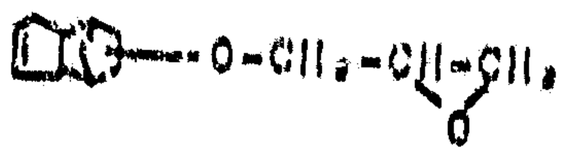
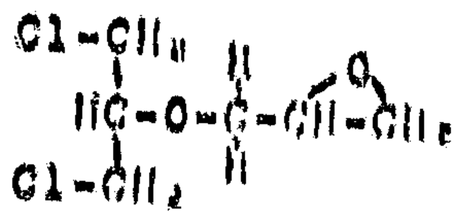
The investigation of these epoxides consisted of a comprehensive protocol involving several test systems thereby extending coverage far beyond that achieved in virtually any other test program.

MATERIALS AND METHODS

The epoxides submitted for mutagenic evaluation are shown in Table 1, and were assayed in the following test systems: (a) the microbial mutagenic test system, the well known "Ames System", (b) body fluid analysis, (c) host mediated assay, (d) induction of DNA repair, (e) the micronucleus test and (f) the dominant lethal test. The methodologies of these various test systems are shown in the following paragraphs.

TABLE 1

EPOXIDES STUDIED BY BATTERY OF MUTAGENIC TESTS

CHEMICAL	STRUCTURE
RESINS	
1. DER® 331 Diglycidyl ether of 2,2-di(p,p'-hydroxyphenyl)propane	
2. DER® 331 (Distilled) Diglycidyl ether of 2,2-di(p,p'-hydroxyphenyl)propane	
DILUENTS	
1. Butyl Glycidyl Ether (BGE)	
2. o-Cresyl Glycidyl Ether (CGE)	
3. Allyl Glycidyl Ether Epoxide 8®,	 R = C_{12}-C_{14} alkyl chain
4. Diglycidyl Ether of Neopentyl Glycol - RD-7114	
5. Dicyclopentadiene Glycidyl Ether (DCPDGE)	
6. Dichloroisopropyl Glycidyl Ether DCOIP	

®DER 331 - Registered Trademark of The Dow Chemical Company
®Epoxide 8 - Registered Trademark of Shell Oil Company

Microbial Test System. The epoxy resins and diluents were tested directly against strains TA1535 and TA92 of *Salmonella typhimurium* according to the methods of Ames *et al.*[5] "S-9" liver homogenate fraction was prepared from liver of phenobarbital-induced and Aroclor-induced rats, and a liver homogenate mix in the amount of 0.5 ml was used in the appropriate test plates as shown in Table 2. The liver homogenate mix contained S-9 fraction, 0.3 ml; $MgCl_2$ 8mM; KCl, 33mM; glucose-6-phosphate, 5mM; NADP, 4mM; and sodium phosphate (pH 7.4), 100mM.

Petri plates containing 20 ml minimal glucose agar medium (consisting of Spizizen minimal medium supplemented with 0.5 µg/ml biotin and 4 µg/ml histidine) were used for the test. Two ml of molten top agar were mixed with 0.1 ml of nutrient broth cultures of the tester strain that were grown overnight. This was then mixed with the epoxy resin or diluents. The concentration of each of these resins or diluents is shown in Table 2.

The petri plates were incubated at 37°C for 48 hours. Results were expressed as the ratio of the number of bacterial colonies (revertants to histidine prototrophy) on the plates treated with the various chemicals to the number of colonies (spontaneous revertants) on the control plates. Results are considered to be negative if the difference in the number of induced revertants compared to the number of spontaneous revertants is less than two-fold.

Body Fluid Analysis. Groups of ten $B_6D_2F_1$ female mice, 20-25 g and female ICR, 25-35 g were each given an oral dose of the resin or diluent in corn oil once a day for four days. The dosages are shown in Tables 3 and 4 for each resin or diluent. Following the treatment each group of mice was placed in a separate metabolism cage, and urine was collected in a chilled container for 24 hours. The urine was then

directly tested against TA1535 with and without the addition of β-glucuronidase (Tables 3 and 4) according to the procedure of Purslon and Ames[6].

Micronucleus Test. The micronucleus test, as described by Schmid[7] was conducted on each of the diluents or resins. In addition, triethylmelamine (TEM) was used in this test as a positive control. Each compound was administered orally once a day for five days to separate groups of ten $B_6D_2F_1$ female mice, 20-25 g, in varying dosages as seen in Table 6. TEM was injected intraperitoneally at a dosage of 0.05 mg/kg. All animals were sacrificed 4 hours after the last treatment. Extraction of bone marrow, preparation of smears, staining, and analysis of slides was then performed as described by Schmid[7]. Statistical analysis of the data from this test consisted of comparisons between the epoxide groups of mice and the control group by use of Dunnett's "t" test.

Induction of DNA Repair. The epoxides were examined for induction of excision repair measured as unscheduled DNA-synthesis in human mononucleated white cells (G-0 phase) in a short-term in vitro assay using liquid scintillation spectroscopy. Approximately 1.4 million white cells were added to freshly prepared culture medium (Eagle's minimum essential medium + 15% fetal calf serum + 2mM glutamine - Gibco) followed by either 1, 10, 100 or 500 ppm of the test compound. However, CGE was tested at 10, 100 and 1000 ppm. These concentrations ranged between 100% cell viability and the toxic dose determined on cultured cells following an in vitro exposure of 4.5 hours. The test compounds were dissolved in and diluted with dimethylsulfoxide (DMSO) prior to addition to the white cells and were added to triplicate samples of 0.1 ml quantities. Positive control samples received

4-nitroquinoline-1-oxide (4-NQO) in saline at a final concentration of 4 μM in 0.1 quantities. Immediately following the addition of each epoxide to the culture medium containing the white cells, the samples were incubated for 5 hours at 37°C.

About 30 minutes prior to harvesting the cells, the cell viability was determined by trypan blue exclusion. Following this, the cells were collected on glass fiber filters, extensively rinsed with distilled water and air-dried. Filters were subsequently placed in vials containing seven ml of toluene-PPO-POPOP mixture for liquid scintillation counts. Amount of tritiated thymidine (^{3}H-TdR) incorporation was expressed in counts per minute.

For radioautographic studies, the white cells were centrifuged and the supernatant removed following incubation. Cells were subsequently suspended in hypotonic KCl solution, exposed to methanol: glacial acetic acid (4:1) fixative, dropped on slides and allowed to air dry. The slides were then dipped in a Kodak NTB_2: distilled water emulsion, exposed at 4°C for 10 days and developed in Kodak D-19 They were then stained with Giemsa stain, and grain counts were performed over 400 nuclei per concentration. Statistical analysis of this data consisted of Duncan's Least Significant Difference Test (LSD) performed at the 0.05 level. A value less than .05 indicates a significant difference between the control cells and the chemically treated cells.

<u>Host Mediated Assay</u>. The <u>Salmonella</u> strains were maintained on Standard Methods agar slants and transferred into Nutrient broth. For enumeration of his+ revertants, Spizizen's minimal medium was used, supplemented with 0.5 μg/ml biotin and 4.0 μg histidine/ml to allow several divisions, necessary in detecting certain mutagenic agents.

The bacteria were grown to a density of approximately 2×10^7 cells/ml two hours prior to sacrifice of the animals (5th day of treatment; log phase). A 23 gauge needle was used to inoculate 2.0 ml of this cell suspension into the peritoneal cavity of female ICR albino mice (after the area of inoculation had been swabbed with ethanol). Mice weighed 25-30 g and were maintained on a diet of Purina Mouse Feed or equivalent. The experiment was allowed to run for six hours after inoculation of the bacteria suspension.

The abdominal regions of the mice were swabbed with ethanol, and 2 ml of sterile saline was injected into the peritoneal cavity of each mouse. The exudate was withdrawn using a tuberculine syringe with a 19 gauge needle. The peritoneal exudates from each mouse were then run separated.

Dilution blanks, containing 4.5 ml of sterile saline were prepared in advance. Tenfold serial dilutions were made of each peritoneal exudate (0.5 ml + 4.5 ml) yielding a concentration series from 10^0 (undiluted peritoneal exudate) through 10^{-6}.

For enumeration of the total bacterial counts, the 10^{-4} - 10^{-6} dilutions were plated on Standard-Methods agar: three plates per sample, 0.2 ml of sample per plate. Each sample was spread over the surface of the plate with a bent glass rod immersed in 95% ethanol and flamed just prior to use.

In plating for the total mutant counts on minimal agar, the 10^0 dilution was used. With the 10^0 dilution, two plates were used (0.20 ml sample/plate). All plates were incubated at 37°C. The Standard Methods agar plates were incubated for 24 hours and the minimal media for 48 hours.

Scoring in this assay was accomplished by the following parameters: No. colonies per plate x 5 = colony-forming units (CFU) per ml of sample plated. CFU/ml x 1/dilution factor (10^{-1} - 10^{-6}) = CFU/ml in diluted exudates. The mutation frequency (MF) is calculated for each sample.

$$MF = \frac{\text{His+ revertants/ml}}{\text{CFU/ml in undiluted exudate}}$$

$$MFt/MFc = \frac{\text{MF of experimental sample}}{\text{MF of control sample}}$$

MFt/MFc = 1.00 for control sample

t = treated; c = control

<u>Dominant Lethal Assay</u>. All experiments were carried out with $B_6D_2F_1$ hybrid mice; males were 8-10 weeks old at the commencement of experiments and females 8-10 weeks old when mated.

The epoxy resins and diluents were applied topically in the undiluted form. Triethylenemelamine (TEM) was obtained from the FDA and prepared freshly in 0.9% saline.

Since there was no data available regarding the percutaneous toxicity of these epoxies in mice it was necessary to conduct range-finding toxicity studies on each epoxide for 4-6 weeks to determine the dosage and surface area on the mice to be used in this study. The dosages for each epoxide determined from this range finding study were:

DER 331	~3.0 g/k
DER 331	~3.0 g/k
BGE	1.5 g/k
CGE	1.5 g/k

Epoxide 3	2.0 g/kg
XO-7114	1.5 g/kg
DCPDGE	2.0 g/kg
DCOPP	2.0 g/kg

Previous to the start of the dominant lethal assay, three untreated virgin females were randomly caged with one untreated male for a week. At the end of the week the three females were replaced by three other untreated virgin females which remained with the untreated male for the second week. The data collected from each untreated male during the two weeks of breeding prior to use in the dominant lethal established the fertility of the male and provided background data regarding litter size, spontaneous fetal deaths, etc. This data could also serve as a control for each individual male.

Following the collection of the two weeks breeding data, the dominant lethal assay was initiated. The modified procedure of Green et al[8] was used. A minimum of ten males of proven fertility were randomly selected for each epoxide. In addition, a negative control consisting of topically applied 0.9% saline and a positive control of TEM treated males was also used in this study. Males selected for the topical applications were prepared in the following manner: approximately 15-20% of the surface area of these mice were clipped by electric shears in the dorsal area; following this, the remaining hair on the clipped area was chemically depilated (Nair, Whitehall Labs, Inc.) so that no hair remained to interfere with absorption of the chemical. Chemical depilation was only used as needed following the initial removal of hair and did not exceed one dipilation per week. A minimum of 24 hours was allowed between

chemical depilation and application of the test chemical. Following this preparation, each male was treated with the dosage, as determined by the range-finding toxicity study, of the appropriate chemical three times per week for a minimum of eight weeks. Each group of males for each epoxide was weighed weekly, and the mean weight for each week was used to adjust the dosage so that the appropriate dose was maintained throughout the treatment period. Following the treatment period, three untreated virgin females were randomly caged per treated male for one week. At the end of the first week the females were replaced with three other untreated virgin females for the duration of the second week.

The positive control males were injected I.P. once with 0.2 mg/kg TEM. The week following the injection, three untreated virgin females were randomly caged with each of these males for one week. These females were then replaced with three other virgin females per male for the second week.

All females were sacrificed by cervical dislocation 13-14 days from the midweek of the caging and presumptive mating, without being checked for vaginal plugs. At autopsy, females were scored for pregnancy, total number of implants and fetal deaths.

The variables taken into account in this assay included: total number implants/pregnancy, proportion fetal deaths/pregnancy and % pregnant females. Comparisons were made between the epoxide groups of mice and control group of mice by analysis of variance.

RESULTS

The results are arranged by the individual epoxides in order to facilitate ease of comprehension of the overall mutagenicity of each epoxy. A table of results of each test for each epoxide is presented at the end of this section.

DER 331. This compound was active in the direct *in vitro* test against TA1535 at the higher dosages as seen in Table 2. There also appeared to be a possible trend of increased activity with addition of the microsomal activation system "S-9" from Aroclor liver homogenate. There was no activity shown in the direct *in vitro* test against TA98 by this chemical.

Body fluid analysis of the urine of the female BDF and ICR mice treated with DER 331 showed no activity at the dose level used in this study (Tables 3,4). The addition of β-glucuronidase, used to cleave glucuronides, did not result in a significant increase in mutagenic activity over the control.

An elevated mutation frequency was found in the host-mediated assay (Table 5). However, the growth of the microorganisms in the peritoneal cavity was low, and consequently, it appears the high mutation frequency was due to the poor growth factor rather than an effect of DER 331.

There was essentially no difference between the control animals and DER 331 treated animals in the micronucleus test as seen in Table 6. As expected, statistical analysis of the data revealed no significant difference ($p > .1$).

DER 331 produced a slight increase in tritiated thymidine (^{3}H-TdR) incorporation into nuclear DNA at 10 ppm as seen in Table 7. Figure 1 graphically illustrates this increase as well as the entire results over the various dose levels of the chemical. This increase was significantly different from the control (DMSO) at the 0.05 level. However, the results at the higher doses of 100 and 500 ppm were not significantly greater than the control, and consequently, this test was repeated.

The result from the repeat test did not exhibit reproducibility in that 10 ppm DER 331 in the repeat test did not differ significantly from the control ($p>.05$).

There was no significant increase of DER 331 over the controls in the three variables measured in the dominant lethal test (Tables 8,9). However, the proportion deaths/pregnancy for this compound was consistently and significantly ($p=.04$, Table 9) lower than the controls (Figure 2). There was also a significant effect due to time in the number implants/pregnancy and in % pregnancy.

TABLE 2

MUTAGENIC ANALYSIS OF EPOXY RESINS AND DILUENTS

USING SALMONELLA TYPHIMURIUM TA 1535

micrograms per plate	Compound	Rt/Rc Mean ± S.D. (No. of Trials) No L.H.	Phen* L.H.	Aro** L.H.
0	DER 331	34 (4)	39 (1)	30 (3)
0.5		1.97 ± 0.29	0.97	1.44 ± 0.71
1.0		2.16 ± 0.51	1.65	3.34 ± 1.84
2.0		2.58 ± 0.51	3.52	6.15 ± 4.27
0	DER 331 distilled	25 (3)	50 (1)	25 (1)
0.5		2.69 ± 0.15	1.06	2.36
1.0		3.05 ± 0.23	1.59	3.84
2.0		2.62 ± 0.17	2.42	10.04
0	XD-7114	22 (2)	39 (1)	29 (1)
0.5		2.48 ± 0.33	1.40	2.40
1.0		3.87 ± 1.25	1.88	4.47
2.0		5.49 ± 1.76	3.47	7.40
0	DGF	26 (2)	20 (1)	29 (1)
0.5		3.02 ± 1.16	1.64	1.45
1.0		7.37 ± 2.60	2.10	1.69
2.0		13.32 ± 4.60	4.20	3.76
0	DCPDGE	28 (2)	48 (1)	29 (1)
0.5		2.40 ± 0.67	1.17	2.24

umoles plate	Compound	Rt/Rc Mean ± S.E. (No. of Trials) No L.H.	Pl L.H.	Aroclor L.H.
1.0		2.40 ± 0.04	1.20	3.21
2.0		4.50 ± 1.90	1.35	5.26
0	CGE	32 (2)	40 (1)	29 (1)
0.5		23.02 ± 2.78	0.93	1.23
1.0		42.19 ± 6.26	0.85	3.09
2.0		57.49 ± 3.34	1.06	5.19
0	Epoxide B	28 (3)	41 (1)	28 (1)
0.5		1.15 ± 0.30	0.70	1.27
1.0		1.16 ± 0.11	0.72	1.29
2.0		1.29 ± 0.42	0.76	2.91

*Liver homogenate from rats pretreated with 0.1% phenobarbital drinking water for 7 days prior to sacrifice.

**Liver homogenate from rats which received 1 IP injection of Aroclor 1254 (500 mg/kg) 5 days before sacrifice.

TABLE 3

MUTAGENIC ANALYSIS OF THE URINE OF FEMALE $B_6D_2F_1$ MICE

TREATED ORALLY WITH EPOXY RESINS AND DILUENTS

Compound	Dose (mg/Kg/day x 4 days)	Survivors/10 mice	β-glucuronidase (200 U/Plate)	RT/RC*
Control (Corn Oil)	0.01 ml/g/day	9	-	-
			+	-
DER 331	1000	10	-	0.85
			+	1.00
DER 331 (distilled)	1000	9	-	1.15
			+	1.22
XD-7114 I	1000	6	-	1.27
			+	2.33
XD-7114 II	1000	9	-	0.98
			+	2.12
DCPDGE I	1000	8	-	1.08
			+	1.37
DCPDGE II	1000	10	-	1.01
			+	0.89
DCPDGE	500	10	-	1.35
			+	1.52
CGE	125	10	-	0.96
			+	1.33
Epoxide 8	1000	8	-	1.38
			+	1.11
BGE	200	9	-	1.31
			+	0.96

$$*\frac{\text{histidine revertants from urine of treated mice}}{\text{histidine revertants from urine of control mice}}$$

TABLE 4

MUTAGENIC ANALYSIS OF THE URINE OF FEMALE ICR MICE

TREATED ORALLY WITH EPOXY RESINS AND DILUENTS

Compound	Dose (mg/Kg/day X 4 days)	Survivors/10 mice	β-glucuronidase (200 U/Plate)	R_t/R_c*
Control (Corn Oil)	0.01 ml/g/day	10	-	-
			+	-
DER 331	1000	10	-	1.0
			+	1.3
DER 331 (distilled)	1000	10	-	0.74
			+	0.61
XD7114	1000	10	-	1.09
			+	2.54
DCPDGE	1000	10	-	0.97
			+	1.31
CGE	.125	10	-	1.05
			+	2.14
Epoxide 6	1000	10	-	1.21
			+	1.08
DCOPP	200	8	-	0.92
			+	1.00
BGE	200	10	-	1.25
			+	1.14
Cytoxan**	80	10	-	46.03
			+	34.79

*$\frac{\text{histidine revertants from urine of treated mice}}{\text{histidine revertants from urine of control mice}}$

**Treated one time

TABLE 5

EVALUATION OF MUTAGENICITY OF EPOXY RESINS AND DILUENTS

BY THE HOST MEDIATED ASSAY IN FEMALE ICR MICE

Compound	Dosage mg/kg/day [illegible] days	No. Mice Treated	No. Mice Died	Mutation Frequency ($\times 10^{-6}$)
Solvent (Corn Oil)	0.1 ml/g/day	30	0	4.90
DER 331	1000	10	0	27.25**
DER 331 (distilled)	1000	10	0	1.00
XD7114	1000	10	0	22.75**
DCPDGE	1000	10	0	3.60
CGE	125	10	0	0.95
Epoxide 8	1000	10	0	18.65**
DCOPP	200	10	2	8.00
BGE	200	10	0	2.15
EMS	250*	10	0	6.79
EMS	500*	5	0	46.08

*treated one time.

**elevated M.F. due to decreased growth of microorganisms in animals.

TABLE 7

INDUCTION OF DNA REPAIR AS MEASURED BY INTRANUCLEAR TRITIATED THYMIDINE INCORPORATION

COMPOUND	CONTROL		CONCENTRATION				
	SALINE	DMSO	1	10	100	500	1000 ppm
Butyl Glycidyl Ether (BGE)	* 486.45 ±38.54	544.57 ± 96.77	522.45 ± 23.69	723.90 ± 79.00	114.9 ± 170.9	93.90 ± 20.76	
O-Cresyl Glycidyl Ether (CGE)	471.05 ± 30.48	576.63 ± 67.05		802.73 ± 38.44	877.63 ± 71.87		104.07 ± 10.61
Allyl Glycidyl Ether (Epoxide 8)	468.75 ± 72.48	572.40 ± 120.46	520.53 ± 26.60	468.07 ± 46.04	148.40 ± 16.14	126.17 ± 2.25	
Dicyclopentadiene Glycidyl Ether (DGE)	493.45 ± 35.00	577.50 ± 12.73		535.30 ± 54.98	583.80 ± 14.61	64.00 ± 14.81	
Dichloroisopropyl Glycidyl Ether (DCDPE)	409.20 ± 46.81	306.13 ± 33.19	355.63 ± 30.02	457.73 ± 28.42	957.97 ± 50.89	995.20 ± 127.96	
(DER 331) Diglycidyl Ether of 2,2-di(p,p'-hydroxyphenyl) propane	45[illegible] 37.69	452.42 ± 25.20	402.00 ± 4.95	550.10 ± 16.12	369.95 ± 88.74	265.50 ± 27.58	

TABLE 7 (Cont.)

COMPOUND	CONTROL		CONCENTRATION				
	Saline	DMSO	1	10	100	500	1000 ppm
(DER 331) distilled Diglycidyl Ether of 2,2-di(p,p'-hydroxy-phenyl)propane	414.10 ± 5.80	453.73 ± 44.64	448.87 ± 35.04	532.07 ±35.04	250.37 ± 10.77	225.50 ± 37.51	
Diglycidyl Ether of Neopentyl Glycol -XD-7114	611.60 ± 88.25	545.40 ± 110.99	599.83 ± 9.07	690.03 ± 55.57	919.97 ± 61.00	750.63 ± 70.98	

* Mean count per minute ± standard deviation using liquid scintillation spectroscopy

000024

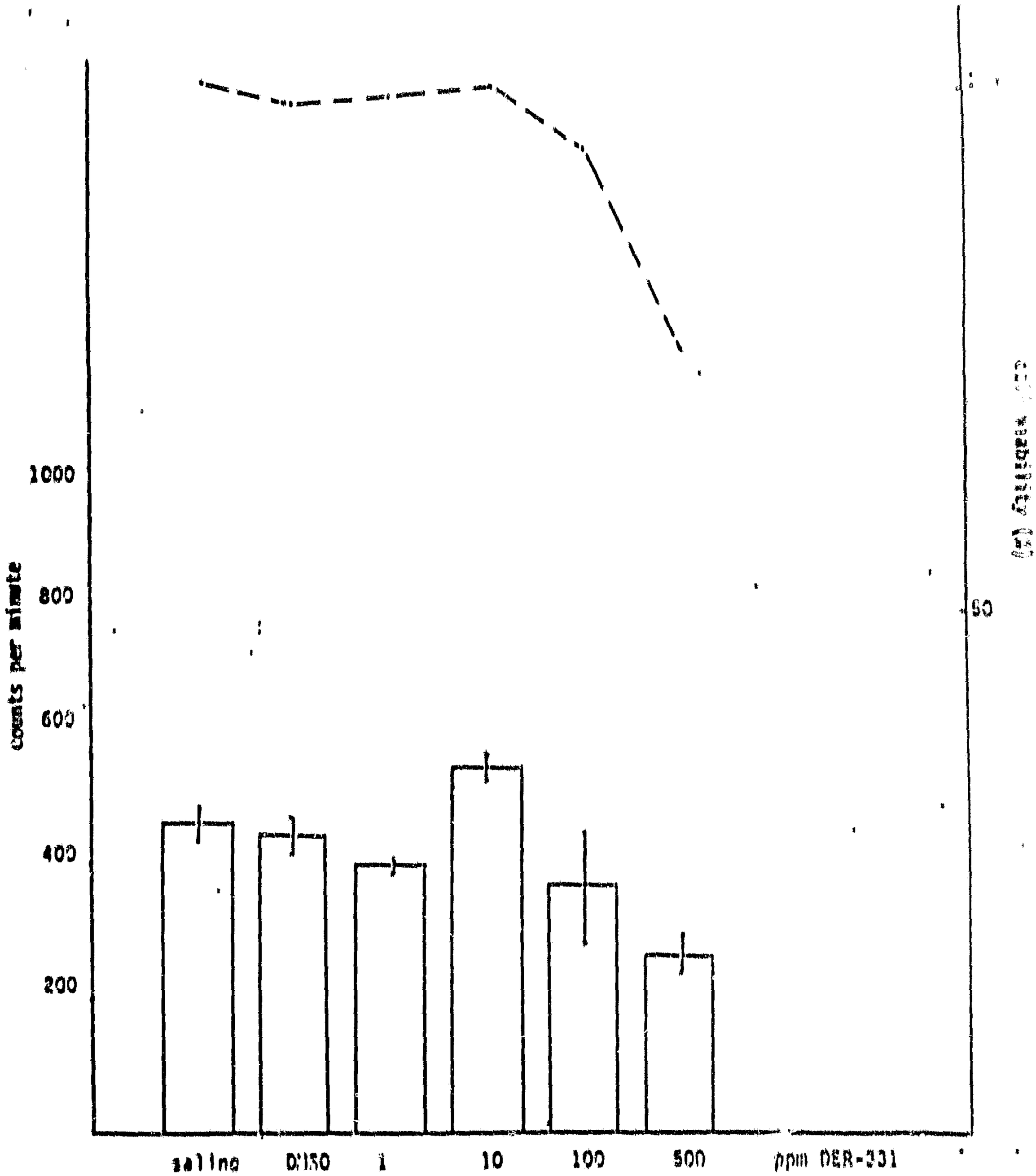


Figure 1. The effect of Diglycidyl Ether of 2,2-di(p,p'-hydroxyphenyl) propane (DER 331) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following in vitro exposure for 5 hours.

Table 8

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For DER 331 By Time

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
DER 331	Number of Implants/ Pregnancy	7.138	6.833	8.364
	Proportion Deaths/ Pregnancy	.009	.015	.007
	% Pregnancy	74.363	79.000	97.000
Control	Number of Implants/ Pregnancy	7.077	6.062	8.277
	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	73.400	83.500

Table 9

Significance Level (p)[a] of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
DER 331 vs control (Treatments)	.04	.56	.37
Time	.35	<.0001	[illegible]
DER 331 by Time	.84	.97	.36

[a] Significance level derived using two factor analysis of variance wi[illegible] factor B. Factor A = treatment. Factor B = time. (reference: [illegible] Experimental Design. B.J. Winer, 2nd Edition).

000027

DER 331 (Distilled). This chemical produced results very similar to those of DER 331 in the direct *in vitro* microbial assay. There was no activity detected in this assay when TA93 was used as the indicator organism. However, activity was found when TA1535 was used (Table 2). The phenobarbital liver homogenate appeared to slightly deactivate DER 331 (dist) since the mutagenic activity was less at all concentrations tested with the phenobarbital liver homogenate than that of the compound with no metabolic activation. However, addition of Aroclor liver homogenate activated this chemical at the highest doses.

The assay of the urine of the mice treated with DER 331 (dist.) showed no significant activity when evaluated against TA1535 (Tables 3,4). The addition of β-glucuronidase resulted in a slight insignificant increase over the control.

DER 331 (dist.) did not produce an increase in mutation frequency over the control in the host-mediated assay. In fact, the mutation frequency was considerably less than the control when 331 (dist.) was used (Table 5).

The results of the micronucleus test (Table 6) showed no significant difference ($p>.1$) between the number of micronuclei counted from the treated animals and the number from the control animals.

As found in DER 331, the distilled DER 331 also produced a significant increase ($p<.05$) in ^{3}HTdR incorporation at 10 ppm (Figure 3) but significant decreases ($p<.05$) at 100 and 500 ppm. This test was repeated to validate these results, but as found in DER 331, the results of the repeat test on DER 331 (dist.) did not differ significantly ($p>.05$) from the control.

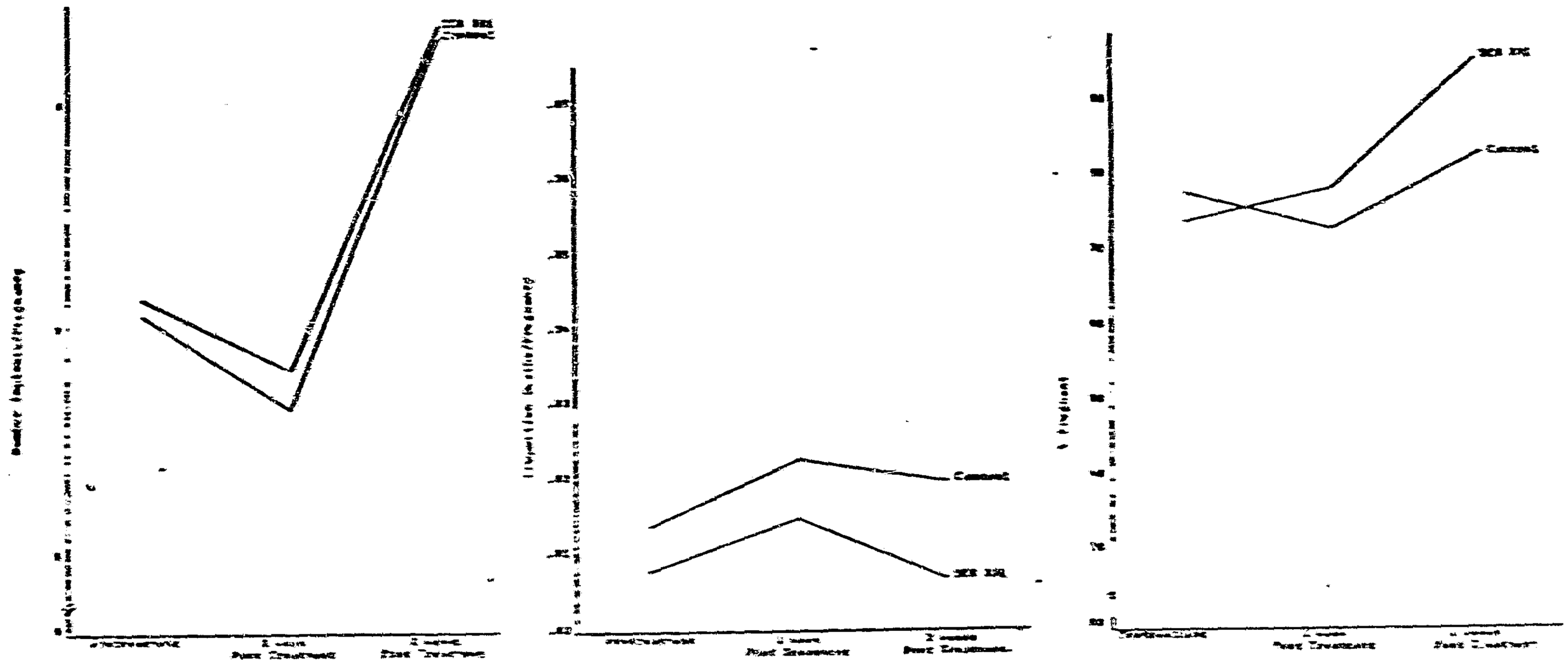


Figure 2. Comparison between DER 331 and control in the three variables measured in the dominant lethal test.

000029

In the analysis of the dominant lethal data (Table 10), DER 331 (dist.) showed a significant reduction (p=.002, Table 11) in the proportion deaths/pregnancy. This reduction as well as the other variables are illustrated in Figure 4. In addition, a significant effect due to time and time-treatment interaction was found in the number implants/pregnancy. There was no significant difference between the control and this oxalide in % pregnant.

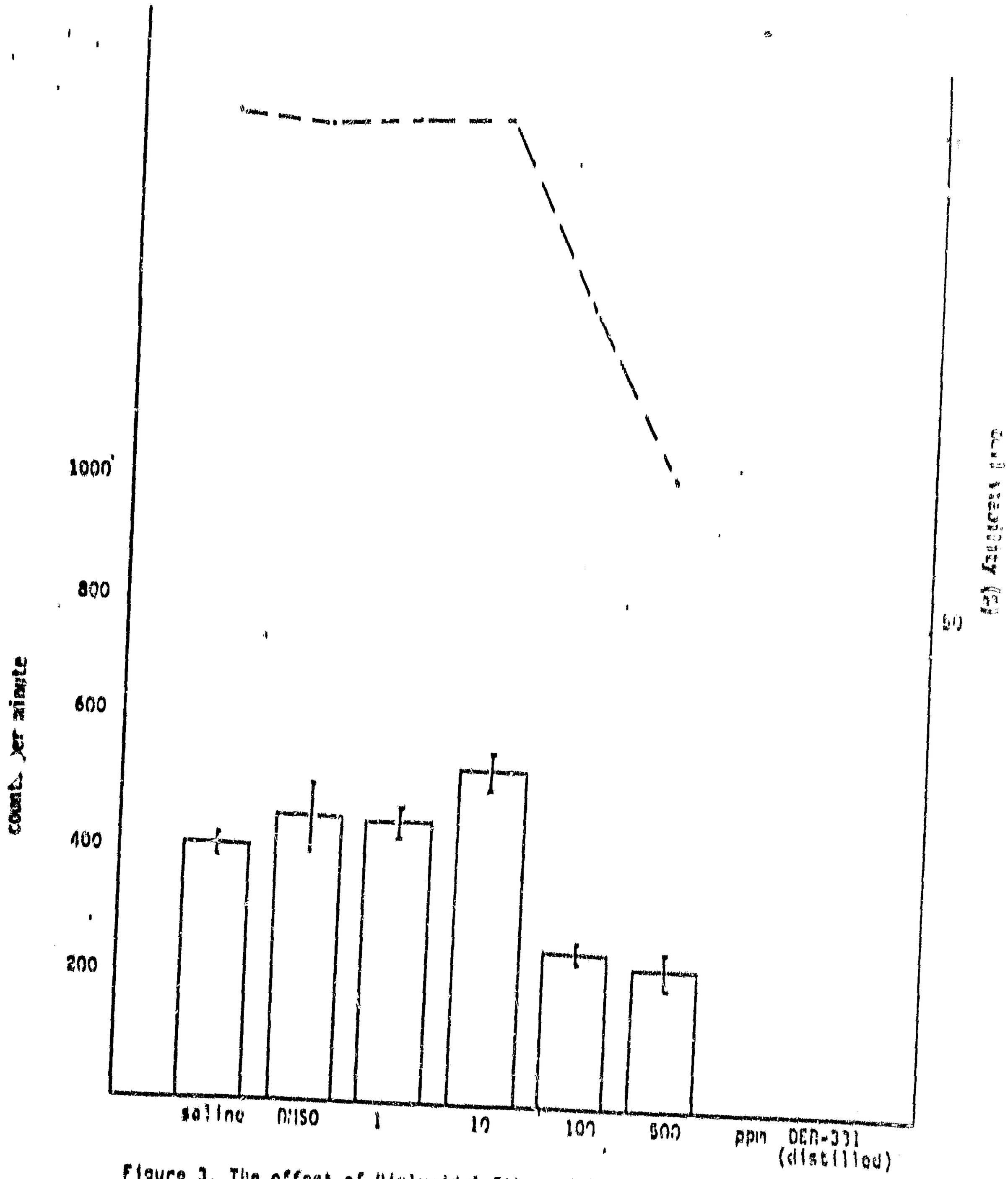


Figure 3. The effect of Diglycidyl Ether of 2,2-di(p,p'-hydroxyphenyl) propane (DER 331 Distilled) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect of cell viability (broken line) following *in vitro* exposure for 5 hours.

Table 10

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For DER 331 (Diet) By Time

		Pretreatment	1 Week Post Treatment	2 Weeks Post Treatment
	Number of Implants/ Pregnancy	7.003	7.850	7.615
DER 331 (Diet)	Proportion Deaths/ Pregnancy	.006	.003	.006
	% Pregnancy	68.181	87.909	75.818
	Number of Implants/ Pregnancy	7.077	6.662	8.277
Control	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	70.100	73.400	83.500

Table 11

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
DER 331 vs control (Dist) (Treatments)	.082	.23	.[illegible]9
Time	.8[illegible]	.00[illegible]	.[illegible]
Der 331 by Time (Dist)	.57	.005	.21

* Significance level derived using two factor analysis of variance wit[illegible] factor B. Factor A = treatment. Factor B = time. (reference: [illegible] Experimental Design. [illegible], 2nd Edition).

000033

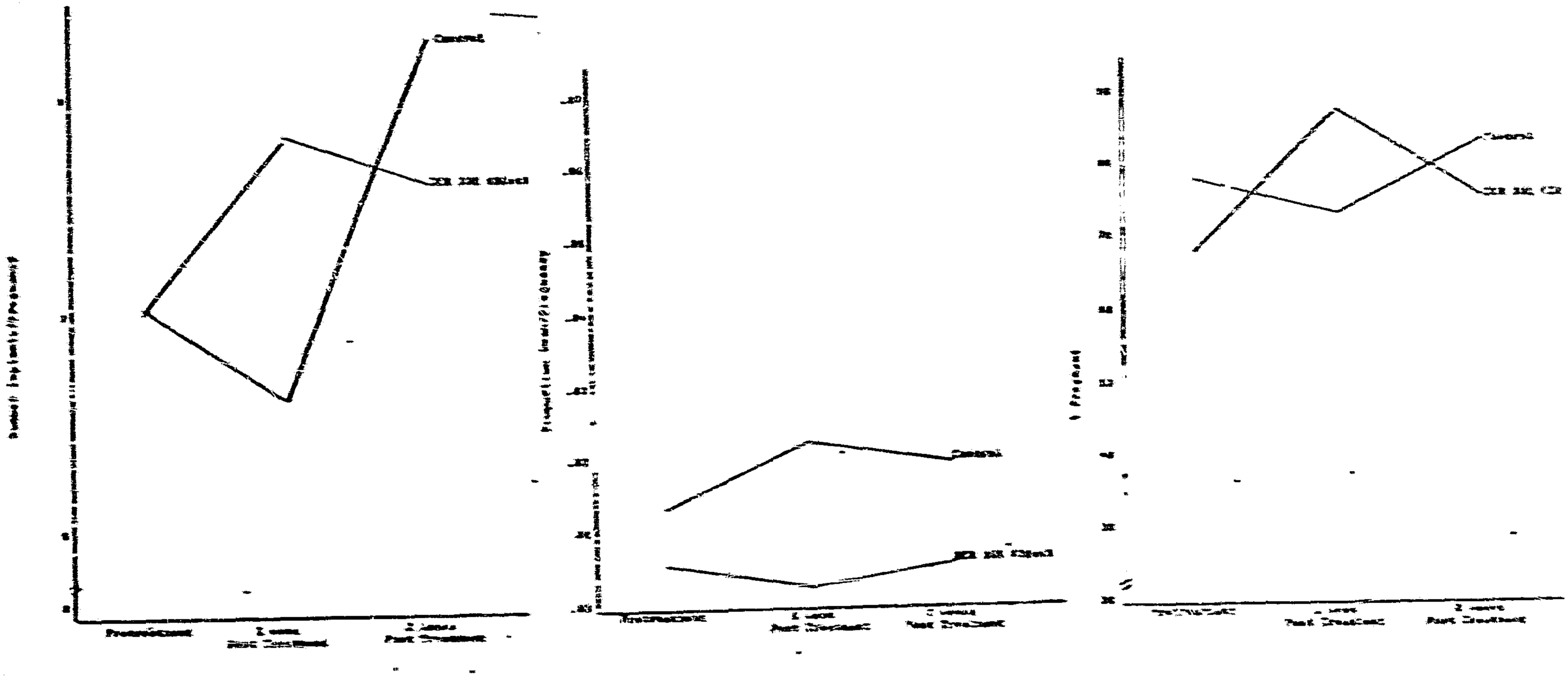


Figure 5. Comparison between DER 331 (dist.) and control in the three variables measured in dominant lethal test.

000034

XD-7114. XD-7114 was active in the microbial test system using TA1535 without microsomal activation of the liver homogenate. The results indicated that, with the exception of Aroclor liver homogenate at the two highest dosages of the test chemical, addition of liver homogenate reduced the activity of this compound. However, this test chemical was not active in the assay when TA98 was used as the indicator organism.

Mutagenic activity was detected in the urine of both species of mice that were treated with this epoxide when β-glucuronidase was added (Tables 3,4). In the initial experiment on the BDF mice, only six of ten treated mice survived, and therefore, the experiment was repeated. In the repetition, nine of ten treated mice survived, and mutagenic activity was again detected in the urine of the mice with the addition of β-glucuronidase. It should be pointed out that in all experiments activity was positive but minimal and only with the addition of β-glucuronidase.

The results in the host mediated assay were similar to those found with DER 331. There was an elevated mutation frequency apparently due to poor bacterial growth in the peritoneum rather than an effect of XD-7114.

Mutagenicity of this epoxide as evaluated by the micronucleus test was negative ($p>.1$) as evidenced by a comparison of 0.11% micronuclei counted in the XD-7114 treated mice and 0.14% in the control mice (Table 6).

There were significant increases (Duncan's LSD, $p<.05$) in unscheduled DNA synthesis induced by XD-7114 in the human mononucleated white cells at 100 and 500 ppm (Figure 5). The increase was greater

at 100 ppm, but cell viability was reduced to 70% at 500 ppm and may have affected ^{3}H-TdR incorporation. This compound was repeated in the DNA repair test using saline as the solvent and was also found to be positive.

Although there was no significant difference between XD-7114 and the control in the three variables measured in the dominant lethal test (Tables 12,13), the numbers of females in the XD-7114 group which became pregnant (% pregnant, Figure 6) was consistently lower and approached significance (p=.08). As discovered in all of the previous epoxides, the number implants/pregnancy was effected significantly by time.

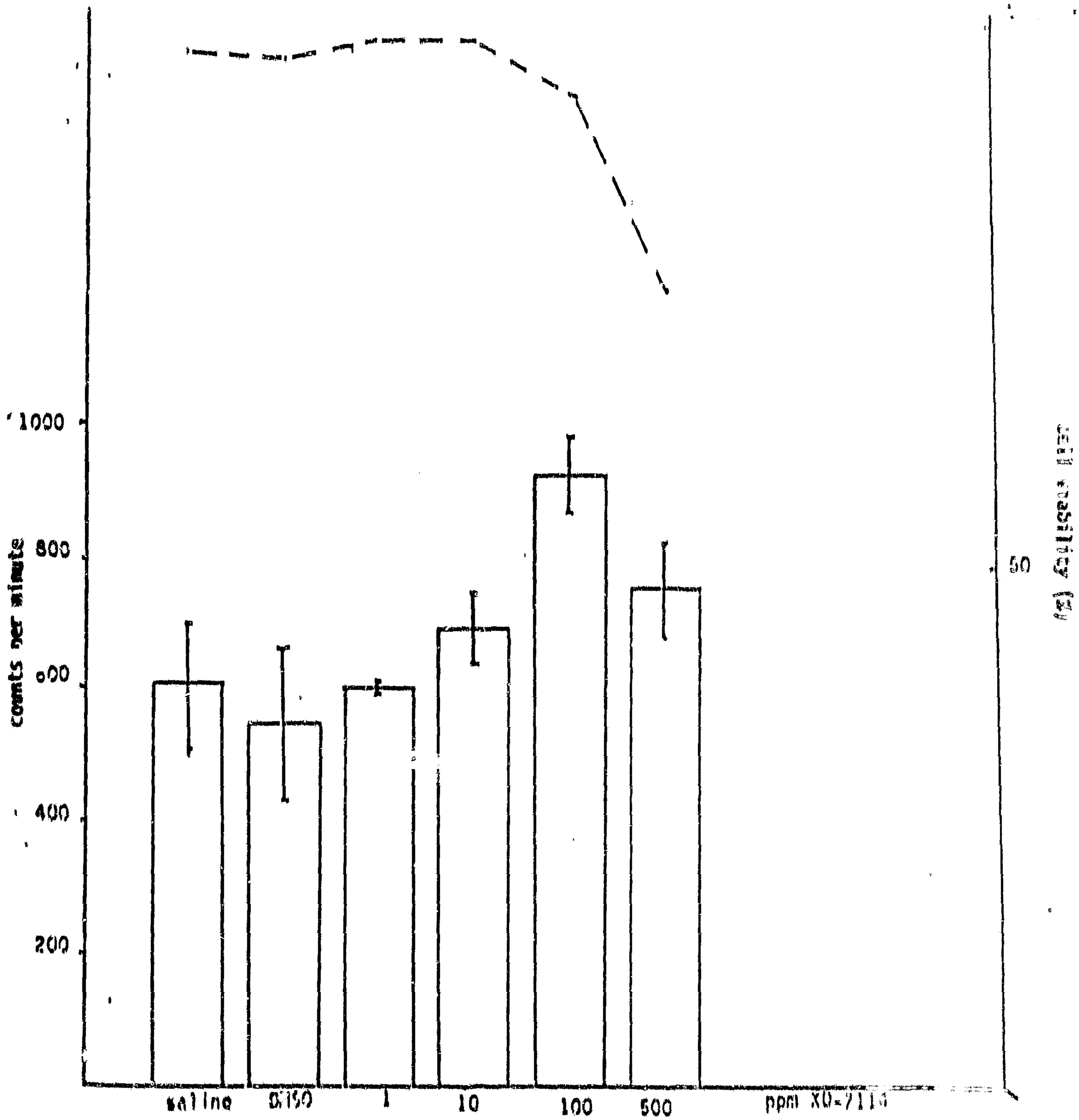


Figure 5. The effect of Diglycidyl Ether of Neopentyl Glycol (XD 7114) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

Table 12

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For XD-7114 By Time

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
XD-7114	Number of Implants/ Pregnancy	7.156	6.917	7.718
	Proportion Deaths/ Pregnancy	.011	.028	.032
	% Pregnancy	66.545	63.727	69.909
Control	Number of Implants/ Pregnancy	7.077	6.682	8.277
	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	73.400	83.500

Table 13

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
XD-7114 vs control (Treatments)	.57	.57	.08
Time	.29	<.0001	.56
XD-7114 by Time	.72	.15	.97

* Significance [illegible] using the factor analysis of variance wi[illegible] Fisher's [illegible] Test [illegible] time. (reference: [illegible] Experimental [illegible]).

0000039

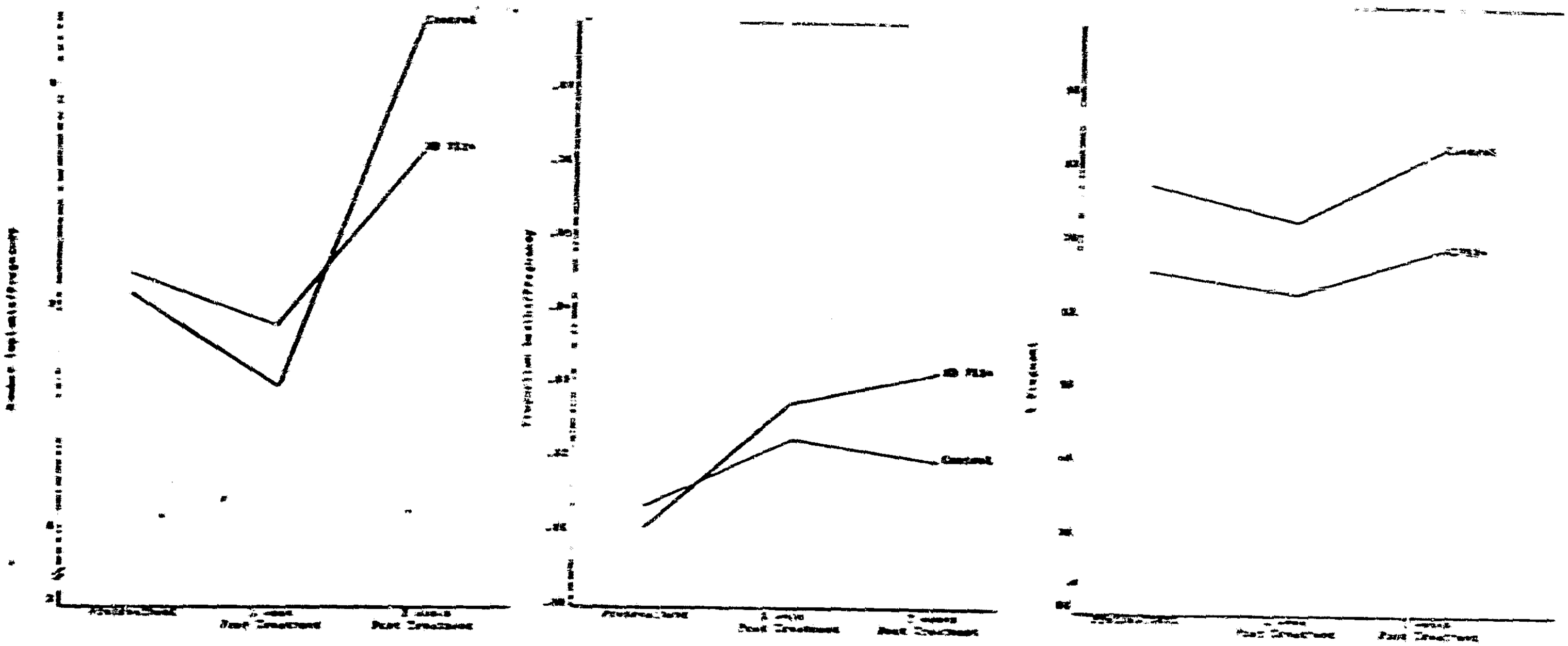


Figure 6. Comparison between XD 7114 and control in the three variables measured in dominant lethal test.

000040

BGE. As seen in Table 2, BGE was not only mutagenic when directly tested against strain TA1535 but produced a dose-response relationship. The addition of the microsomal activation system from either Aroclor or phenobarbital liver homogenate did not intensify the mutagenic activity but rather deactivated this chemical. The results with strain TA93 were not the same as those found in TA1535. This chemical was not active when TA98 was used in the direct *in vitro* assay.

There were no mutagenic substances discovered in the urine of the mice treated with 200 mg/kg BGE for four days (Tables 3,4). Addition of β-glucuronidase did not increase mutagenic activity when compared to the control.

The mutation frequency in the *Salmonella* from the BGE treated animals in the host-mediated assay was 2.15 (Table 5). This was less than the control, and it appears that BGE is not mutagenic in this test system.

The % micronuclei determined from the BGE treated animals was considerable less than the control (Table 6) although this difference was not significant ($p>.1$) as determined by Dunnett's "t" test.

A linear dose-response relationship (Figure 7) occurred in ^{3}HTdR incorporation with the dosages of 1, 10, and 100 ppm BGE. The values at 10 and 100 ppm were significantly different than the control ($p<.05$). Cell viability was dramatically reduced over the range of 100 to 500 ppm BGE, with an obvious toxic effect occurring at 500 ppm. This effect undoubtedly influenced incorporation of ^{3}HTdR at 500 ppm as seen by a significant reduction at this dosage level.

Radioautographic studies were also conducted on BGE at 100 ppm in order to confirm intranuclear incorporation of ^{3}HTdR. The results of this study (Table 14) indicated a large increase of labelled cells from the BGE treatment over the control.

In the analyses of the effects of BGE in the dominant lethal test, there was a significant increase ($p<.06$, Table 15,16) in the proportion deaths/pregnancies of the BGE treated animals over the control animals. This increase can be seen better in the graphic illustration of figure 6. In addition, there was a significant effect on this same variable due to time and a very strong time-treatment interaction, although not significant at the .05 level. Significant effects ($p<.01$) were also detected in the number implants/pregnancy due to time and time-treatment dependency. The number of pregnant females in the BGE group was consistently lower than the control group and was significant with $p<.05$.

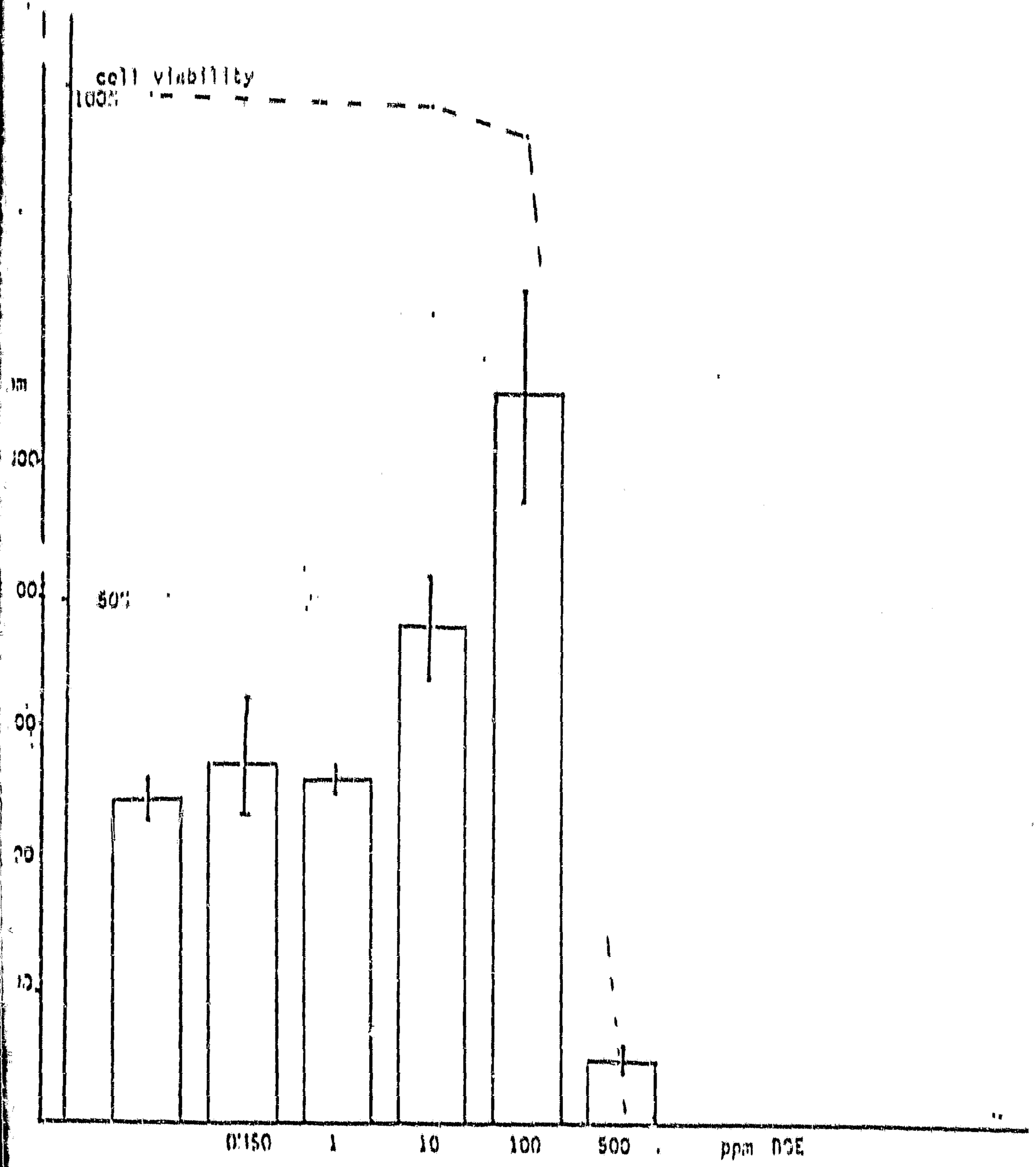


Figure 7. The effect of Butyl Glycidyl Ether (BGE) on incorporation of tritiated thymidine into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

TABLE 14

RESULTS OF RADIOAUTOGRAPHIC STUDY

(CONFIRMATION OF LIQUID SCINTILLATION STUDY)

COMPOUND	% LABELLED CELLS	MEAN GRAIN COUNT OF LABELLED CELLS
Saline-control	8.25 ± 2.6	
DMSO-control	9.5 ± 4.2	
Butyl Glycidyl Ether (BGE) (100 ppm)	52.5 ± 8.5	5.5 ± 0.21
Dichloroisopropyl Glycidyl Ether (DCOPP) diluded in saline (500 ppm)	55 ± 2.06	7.0 ± 0.46
Dichloroisopropyl Glycidyl Ether (DCOPP) diluded in, DMSO (500 ppm)	55.5 ± 9.5	7.0 ± 0.94
4-Nitroquinoline-1-oxide (4NQO)-positive control (4 μM)	97 ± 1.41	21.83 ± 1.79

Table 15

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For BGE By Time

		Pretreatment	1 week Post Treatment	2 wk Post
BGE	Number of Implants/ Pregnancy	7.286	7.184	7.[illegible]
	Proportion Deaths/ Pregnancy	.012	.076	.033
	% Pregnancy	48.454	60.545	75.818
Control	Number of Implants/ Pregnancy	7.077	6.662	8.277
	Proportion Deaths/ Pregnancy	.014	.023	.021
	% Pregnancy	78.109	73.400	83.5[illegible]

Table 16

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
BGE vs control (Treatments)	.09	.59	.05
Time	.01	.01	.10
BGE by Time	.06	.01	.34

* Significance level derived using two factor analysis of variance with repeated measures on factor.B. Factor A = treatment. Factor B = time. (reference: Statistical Principles In Experimental Design. B.J. Winer, 2nd Edition).

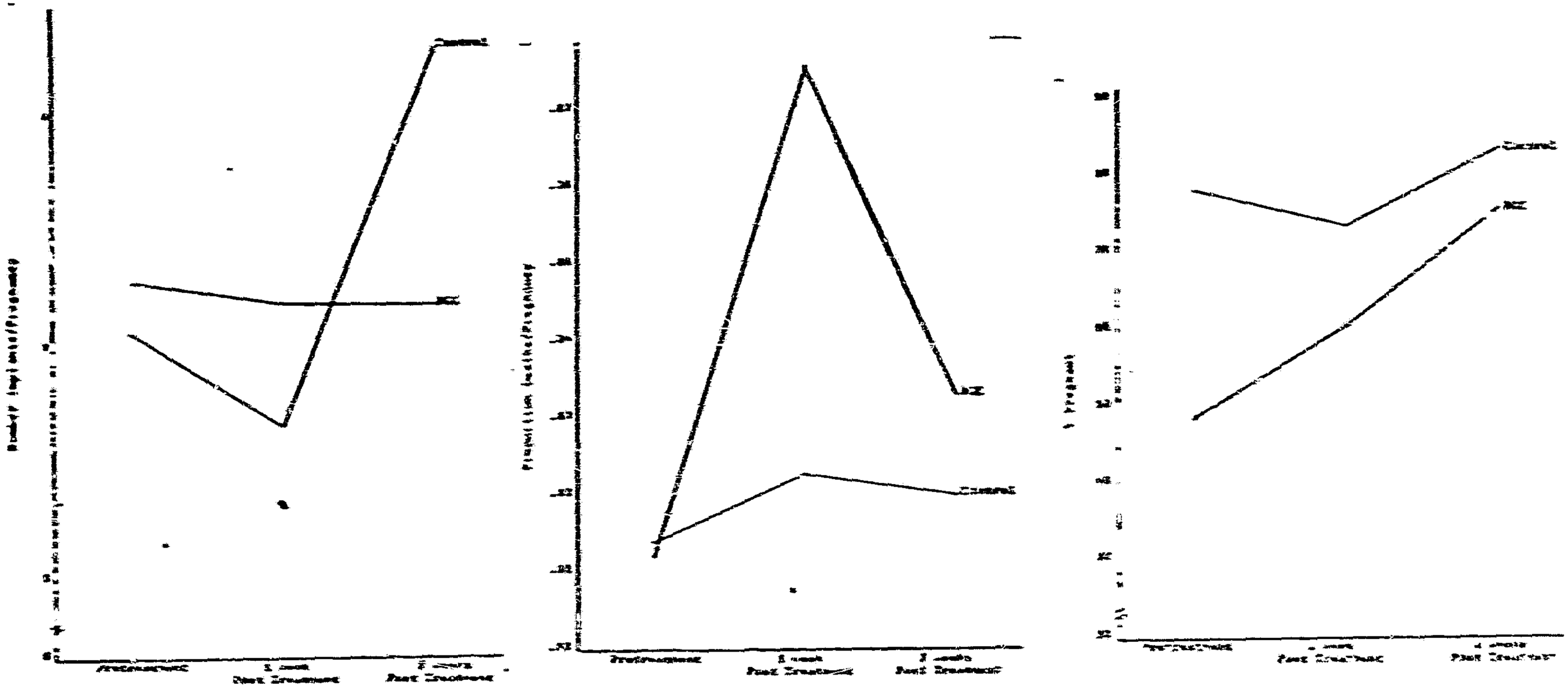


Figure 8. Comparison between BCE and control in the three variables measured in dominant lethal test.

000047

CGE. This epoxide was highly mutagenic in the direct assay against TA1534 and produced a dose-response at the three concentrations tested (Table 2). However, the mutagenic activity was significantly reduced with the addition of metabolic activation from either of the liver homogenates. As discovered in all previous epoxides, CGE was not active in the microbial assay when TA98 was used.

As shown in Table 3, evidence of significant mutagenic activity was not detected in the urine of the DDF mice treated with CGE. There was some increase in activity when β-glucuronidase was added, but this proved to be nonsignificant. However, minimal activity was discovered in the urine of the ICR mice when β-glucuronidase was added (Table 4).

CGE produced the lowest mutation frequency in the host-mediated assay (Table 5). The bacterial growth in the peritoneum was the highest in this assay, and thus it is felt that the value (0.95) is a true reflection of the effect of CGE in this assay.

This chemical exhibited no significant difference ($p>.1$) in the % micronuclei from the control (Table 6). In fact, the % micronuclei from the CGE treated animals was slightly lower than the control animals.

The increases at 10 and 100 ppm in the ^{3}HTdR incorporation were determined to be significantly different from the control by Duncan's LSD test at the .05 level. There was a marked reduction in unscheduled DNA synthesis at 1000 ppm, but cell viability was also markedly reduced from 100 ppm as seen in Figure 9, with an obvious toxic effect occurring at 1000 ppm.

The percentage pregnancy in females mated to the CGE treated males varied from 51.5 to 75.8 over the time period of the dominant lethal test (Table 17). Analysis of these values showed them to be significantly different from the control values (Table 18). There was no difference in the proportion deaths/pregnancy between CGE and the control as can

be readily seen in Figure 10. There again [illegible], as in most epoxides, a significant effect in the number [illegible]/pregnancy due to time (p=.04) and time-treated dependency (p=.0001).

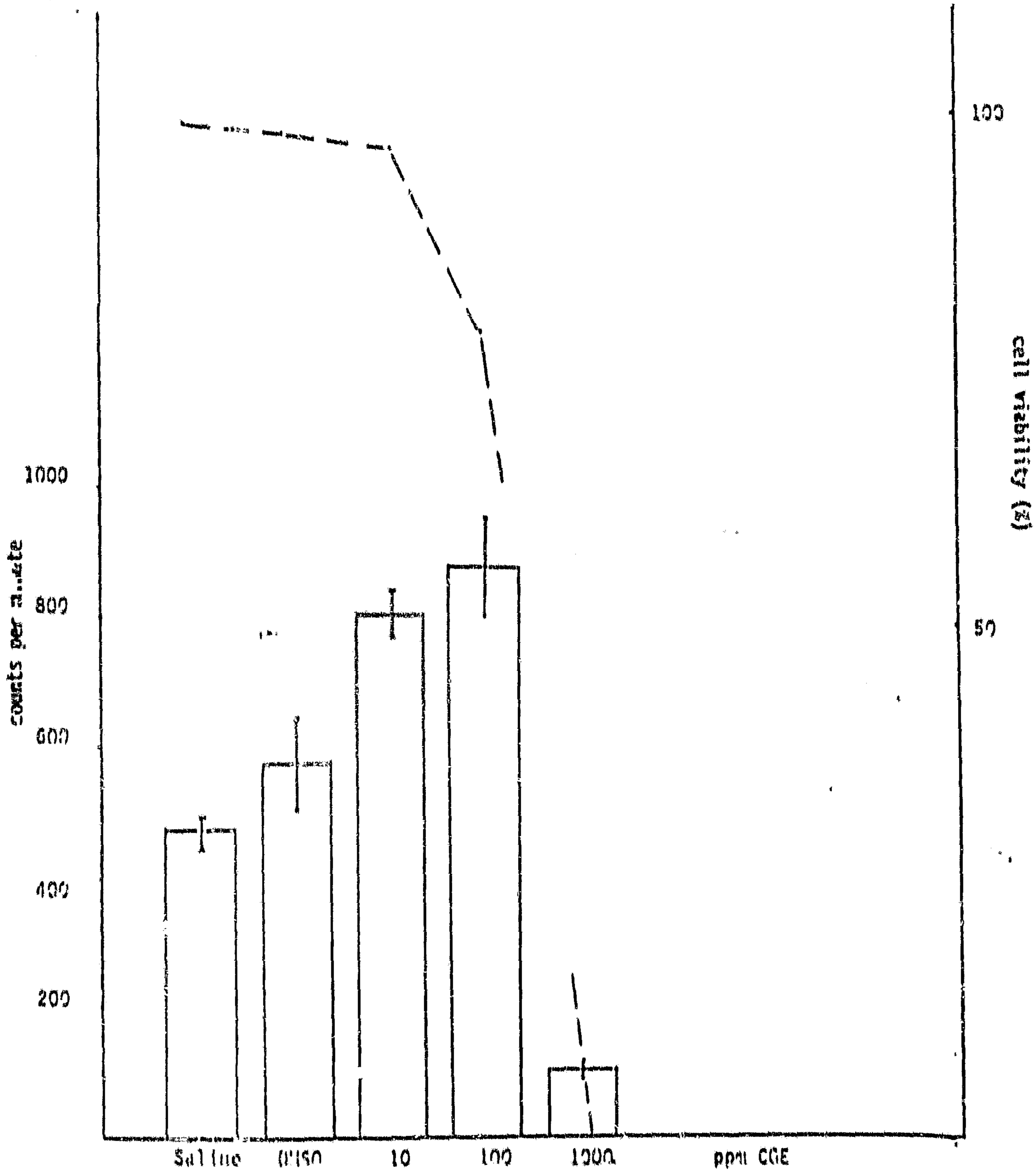


Figure 9. The effect of o-Cresyl Glycidyl Ether (CGE) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

Table 17

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For CGE By Time

		Pretreatment	1 week Post Treatment	2 [illegible] Post
CGE	Number of Implants/ Pregnancy	7.614	7.348	6.[illegible]
	Proportion Deaths/ Pregnancy	.020	.030	.020
	% Pregnancy	51.545	75.810	83.030
Control	Number of Implants/ Pregnancy	7.077	8.662	8.277
	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	73.400	83.500

Table 18

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
CGE vs control (Treatments)	.45	.86	.03
Time	.79	.04	.41
CGE by Time	.99	.0001	.18

* Significance level derived using two factor analysis of variance with repeated measures on factor B. Factor A = treatment. Factor B = time. (reference: Statistical Principles In Experimental Design. B.J. Winer, 2nd Edition).

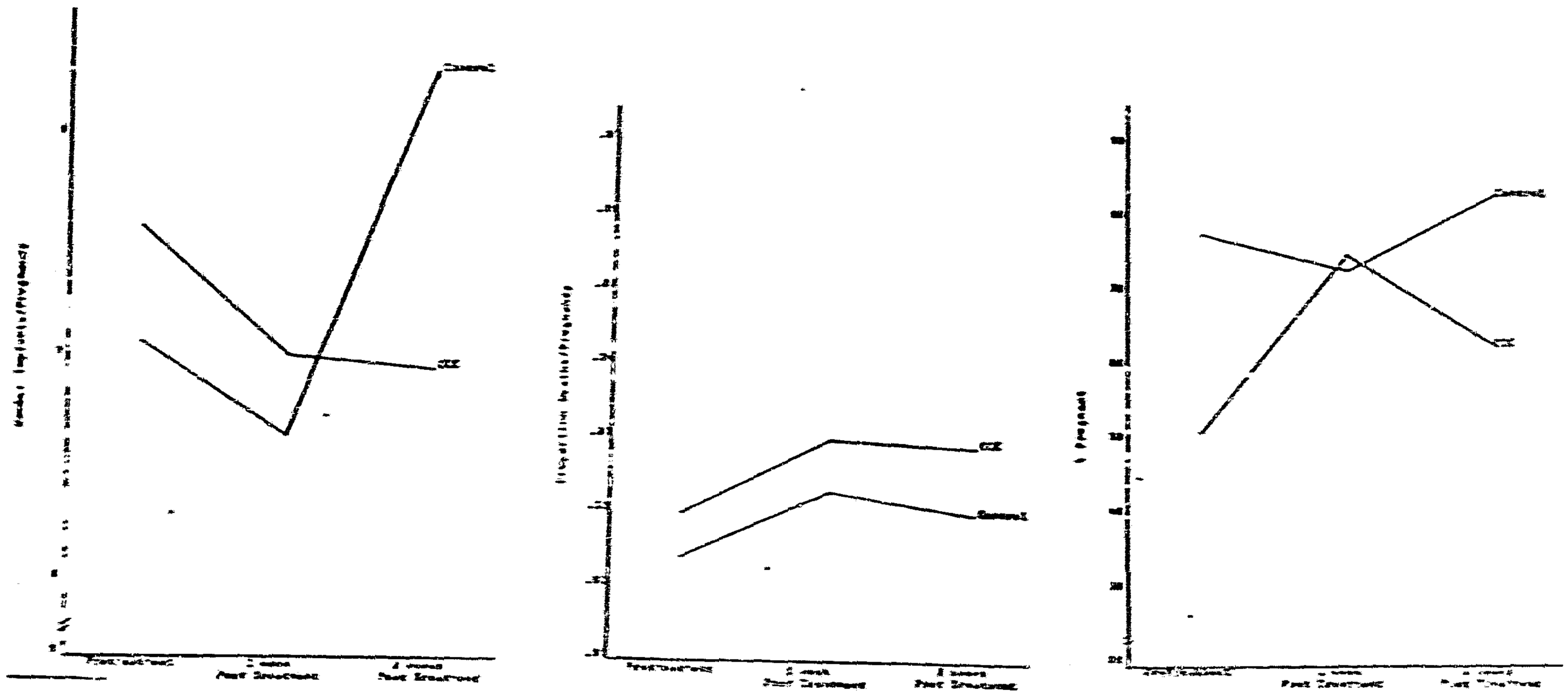


Figure 10. Comparison between CGE and control in the three variables measured in dominant lethal test.

000053

Epoxide 8. This chemical was the least active of the epoxides tested in the direct *in vitro* microbial assay. It should be noted that it only exhibited mutagenic activity at the highest dose with Aroclor metabolic activation.

The R_t/R_c values determined from the mutagenic analysis of the urine of the B6F mice treated with Epoxide 8 ranged from 1.39 without the use of [illegible] to 1.11 with the use of this enzyme (Table 3). The values with the ICR mice ranged from 1.21 to 1.03. These values were deemed insignificant in this test system.

An elevation of the mutation frequency was detected in the host-mediated assay with this epoxide. However, as found in DER 331 and XD-7114 a decreased growth of the microorganisms in the animals also occurred. Thus it is felt the elevated mutation is not an effect of Epoxide 8 but rather a reflection of the poor bacterial growth.

Epoxide 8 gave almost identical micronuclei values as the control (Table 6), and thus this chemical did not produce a mutagenic effect in terms of micronuclei at the dosage tested.

There was no increase in unscheduled DNA synthesis induced by Epoxide over the control as illustrated in Figure 11. Cell viability was extremely low at 100 ppm and nonexistant at 500 ppm. These findings were likely responsible for the significant decreases in ^{3}H-thymidine incorporation at the 100 and 500 ppm doses.

Although the proportion deaths/pregnancy for Epoxide 8 increased steadily with time, these values only ranged from 0.4% to 1.1% (Table 19) and were significantly lower ($p=.003$, Table 20) than the control values. There were no differences between the control and this epoxide in % pregnant as depicted in Figure 12. However, the ever present significant effects on the number implants/pregnancy due to time and time-treatment dependancy were present in Epoxide 8 also.

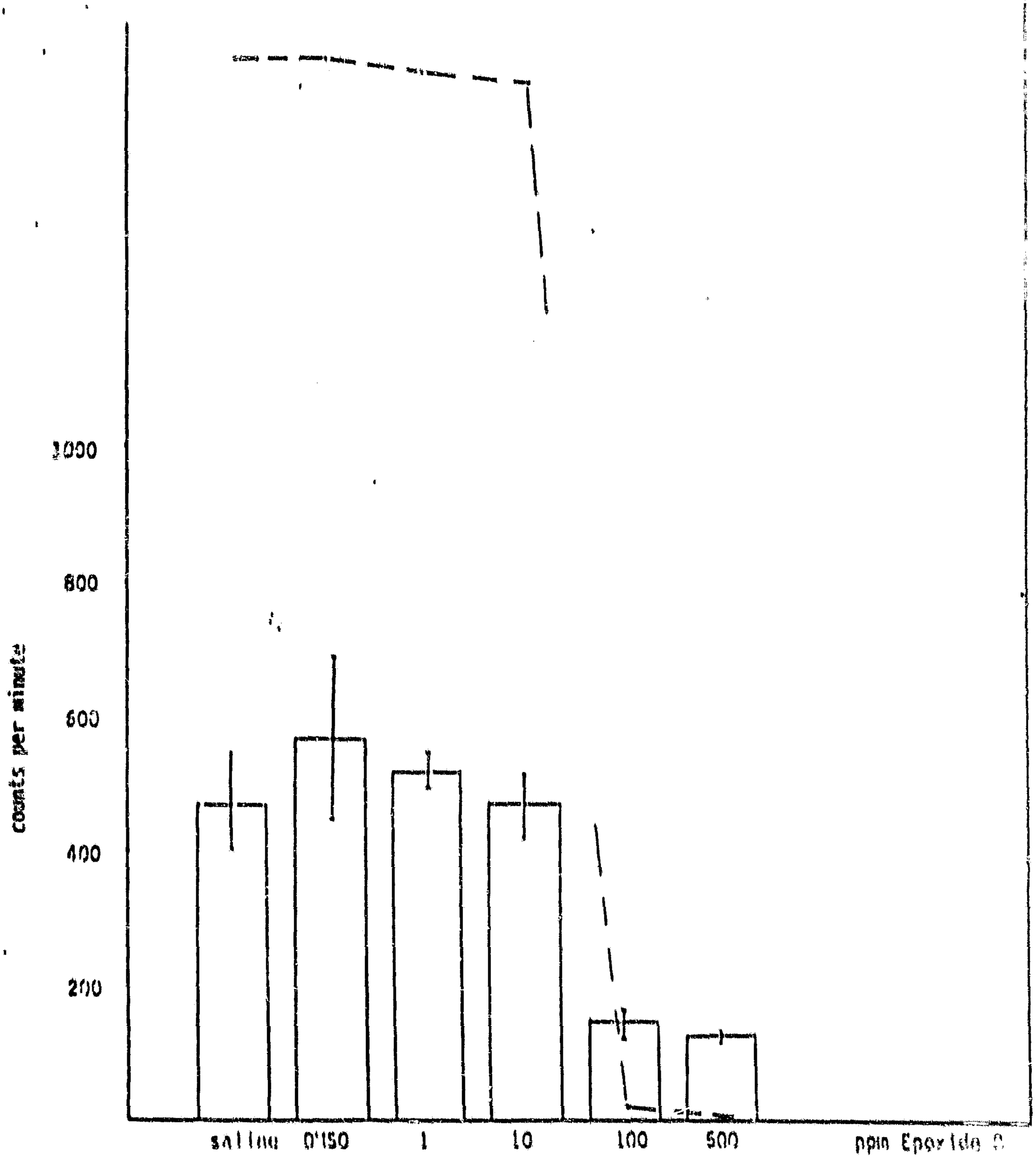


Figure 11. The effecy of Allyl Glycidyl Ether (Epoxide 8) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

Table 10

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For Epoxide 8 By Time

		Pretreatment	1 Week Post Treatment	2 Weeks Post Treatment
	Number of Implants/ Pregnancy	7.220	7.667	7.833
Epoxide 8	Proportion Deaths/ Pregnancy	.004	.006	.011
	% Pregnancy	70.000	70.100	80.000
	Number of Implants/ Pregnancy	7.077	8.862	8.277
Control	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	73.500	83.500

Table 20

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
Epoxide 8 vs control (Treatments)	.003	.40	.50
Time	.50	.0001	[illegible]
Epoxide 8 by Time	.74	.001	.93

* Significance level derived using two factor analysis of variance wit[illegible] factor B. Factor A = treatment. Factor B = time. (reference: [illegible] Experimental Design. B.J. Winer, 2nd Edition).

000057

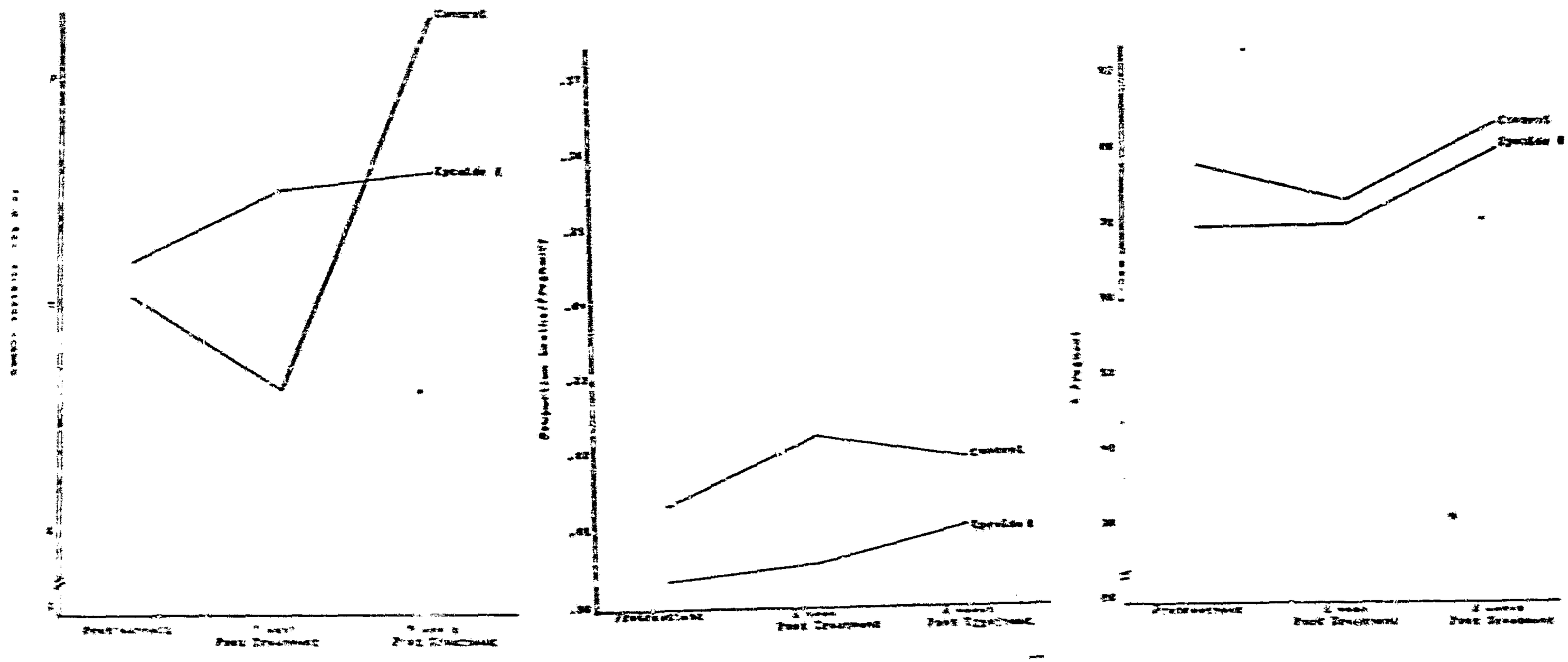


Figure 12. Comparison between Epoxide 8 and control in the three variables measured in dominant lethal test.

000058

DCPOGE. In the concentrations used in the microbial test, this epoxy diluent was mutagenic to strain TA153[illegible], but was not to strain TA[illegible]. Aroclor liver slightly activated this compound at the higher doses, but phenobarbital liver homogenate reduced the mutagenic activity at all concentrations (Table 2).

The BDF mice were dosed orally with two doses of this chemical for body fluid analysis. The first group of mice were treated with 1[illegible] (Table 3) which resulted in only five survivors of the ten mice. Significant mutagenic activity was not detected in the urine of these survivors. This dosage was repeated in another group of mice with all animals surviving although there were some toxic signs present in the animals. The results of the body fluid analysis of the urine of this group was the same as first group dosed at 1000 mg/kg. The dosage was then reduced to 500 mg/kg, and a third group of mice was treated at this level. All animals survived with no significant mutagenic constituent(s) detected in their urine. In addition there was no activity detected in the urine of the ICR mice (Table 4).

DCPOGE produced a mutation frequency in the host-mediated assay slightly lower than the control (Table 5). This nonsignificant difference from the control suggests it to be nonmutagenic in the host-mediated assay at the dose used in this study.

DCPOGE produced the highest value of micronuclei (0.23%, Table 6) of all epoxides assayed in the micronucleus test. Although it visually appeared that this might be significantly different from the control, statistical analysis by Dunnett's "t" test showed it to be nonsignificant ($p > .1$).

Unscheduled DNA synthesis was not induced by DCPMGE as seen in the illustration of ^{3}HdR incorporation in figure 13. There were no significant ($p>.05$) increases in incorporation of ^{3}H-thymidine over the control. In addition, cell viability was markedly reduced from 100 to 500 ppm of DCPMGE.

Although there was a sharp increase in the proportion deaths/pregnancy at 1 week post treatment for DCPMGE (Table 21, Figure 14), this increase was not significantly different ($p=.73$, Table 22) from the control. Examination of the raw data showed that one animal was much higher in this variable than the others and consequently, was largely responsible for this sharp increase. The only significant results discovered in the dominant lethal assay of this epoxide were significant effects in the number of implants/pregnancy and % pregnancy due to time and highly significant time-treatment dependency in the number implants/pregnancy.

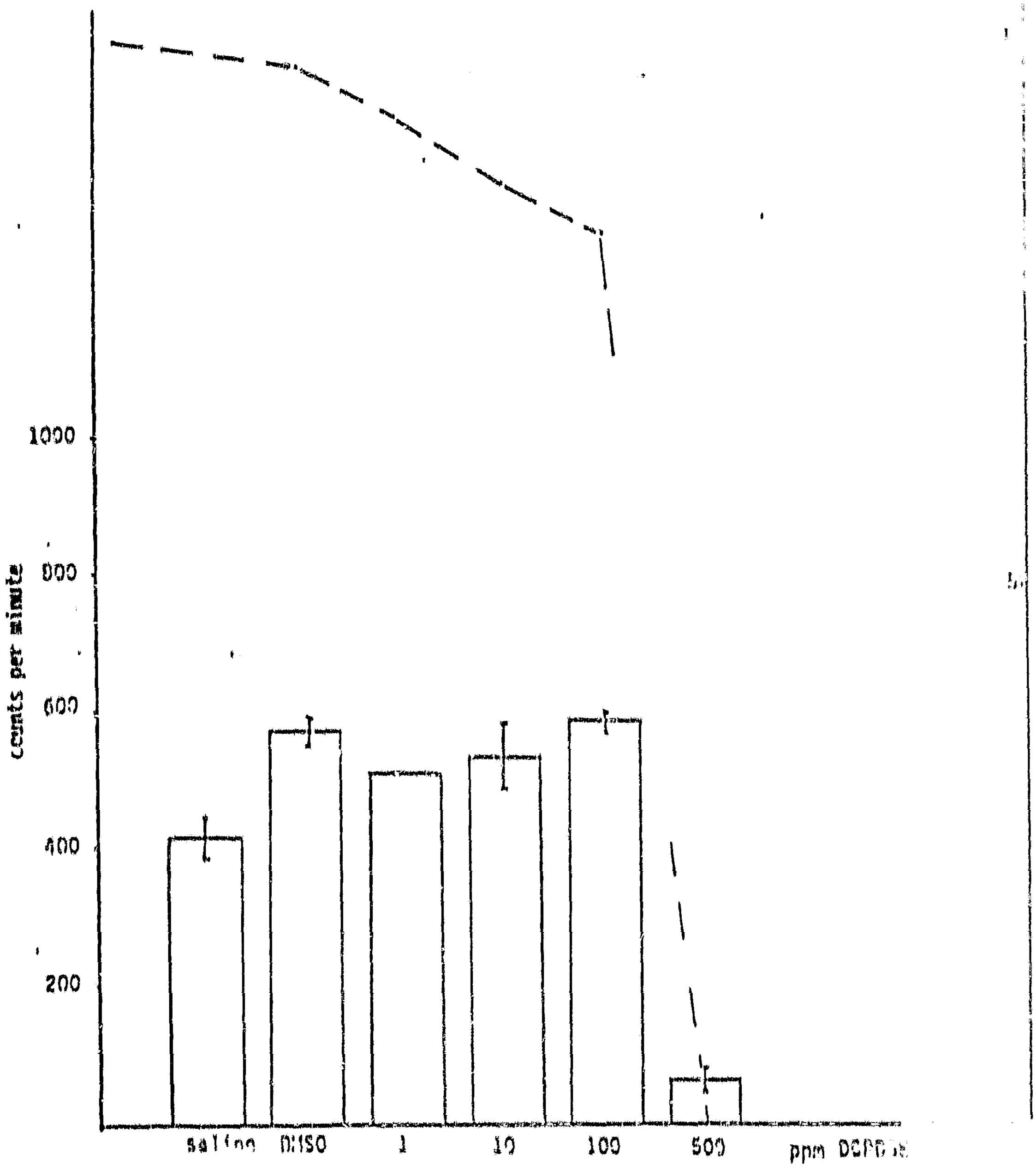


Figure 13. The effect of Dicyclopentadiene Glycidyl Ether (DCPGE) on tritiated thymidine incorporation into unstimulated, human, non-nucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

Table 21

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy For DCPDGE By Time

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
	Number of Implants/ Pregnancy	7.448	7.681	7.575
DCPDGE	Proportion Deaths/ Pregnancy	.017	.039	.012
	% Pregnancy	80.545	81.909	81.000
	Number of Implants/ Pregnancy	7.077	6.662	8.277
Control	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	73.400	83.500

Table 22

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
DCPDGE vs control (Treatments)	.73	.2[illegible]	.9[illegible]
Time	.30	.01	[illegible]
DCPDGE by Time	.58	.008	.07

* Significance level derived using two factor analysis of variance wit[illegible] factor B. Factor A = treatment. Factor B = time. [illegible] ference: St[illegible] Experimental Design. B.J. Winer, 2nd Edition).

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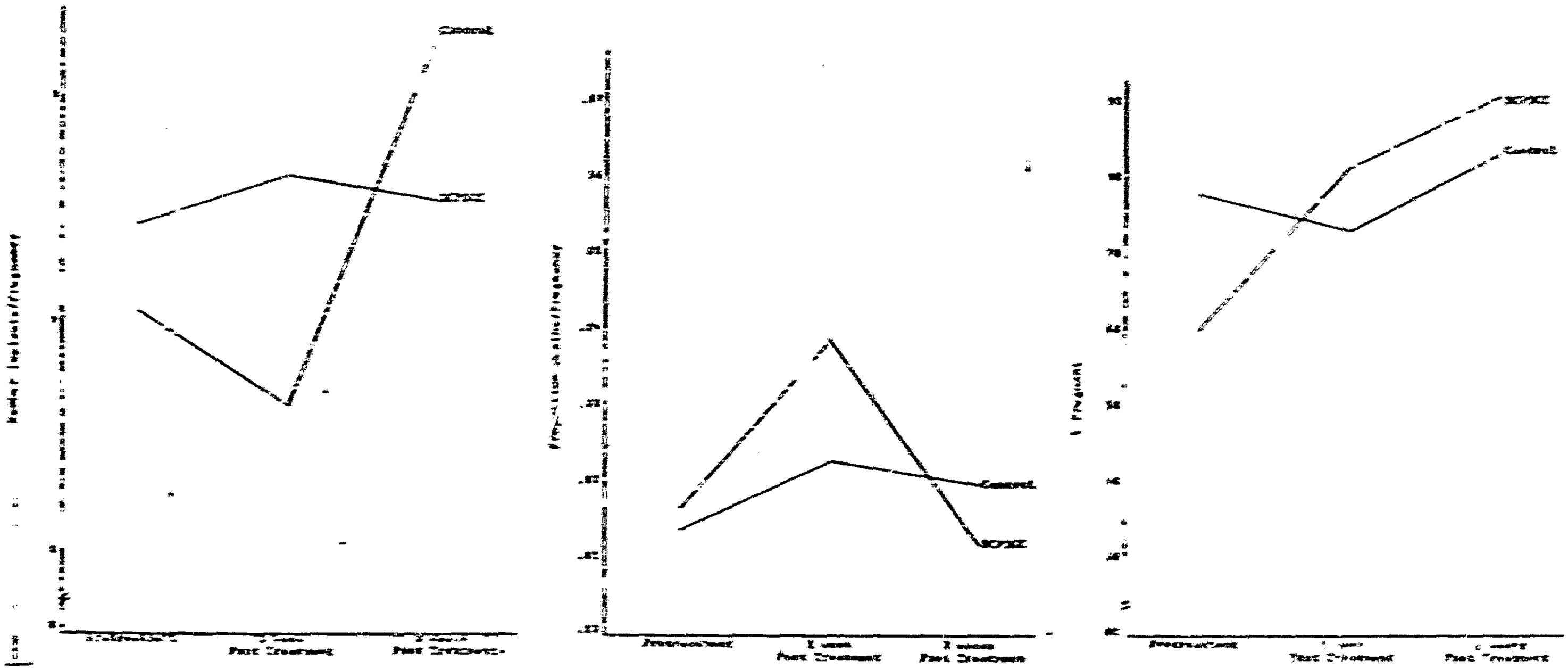

Figure 14. Comparison between DCPGE and control in the three variables measured in dominant lethal test.

000064

DCOPP. There was no mutagenicity detected in the urine of ICR mice treated with this epoxy (Table 4). However, a slight increase in mutation frequency of DCOPP over the control was found in the host-mediated assay. Since the bacterial growth was normal, it is believed this is an effect of DCOPP in the host-mediated assay.

^{3}HTdR incorporation into the human mononucleated white cells was significantly increased ($p<.05$) over the control (DMSO) when 100 and 500 ppm DCOPP was used (Figure 15). Cell viability remained high at all concentrations. This compound was repeated using saline as the solvent, and results were similar to those found with DMSO. There were significant increases ($p<.05$) in ^{3}H-thymidine insorporation at 10, 100 and 500 ppm DCOPP (Figure 16).

Radioautographic studies were conducted on this compound at 500 ppm also. The results (Table 14) were the same as found with BGE in that there was a large increase in labelled cells over the controls.

When DCOPP was compared to the control in the three variables measured in the dominant lethal test (Table 23), no significant differences (Table 24) were discovered. Although there was a sharp increase in the number of females mated to DCOPP treated males that became pregnant (Figure 17), the overall results of this variable was not significant ($p=.58$) from the control. However, there were significant effects on the number implants/pregnancy due to time and time-treatment dependency and % pregnant due to time-treatment dependency.

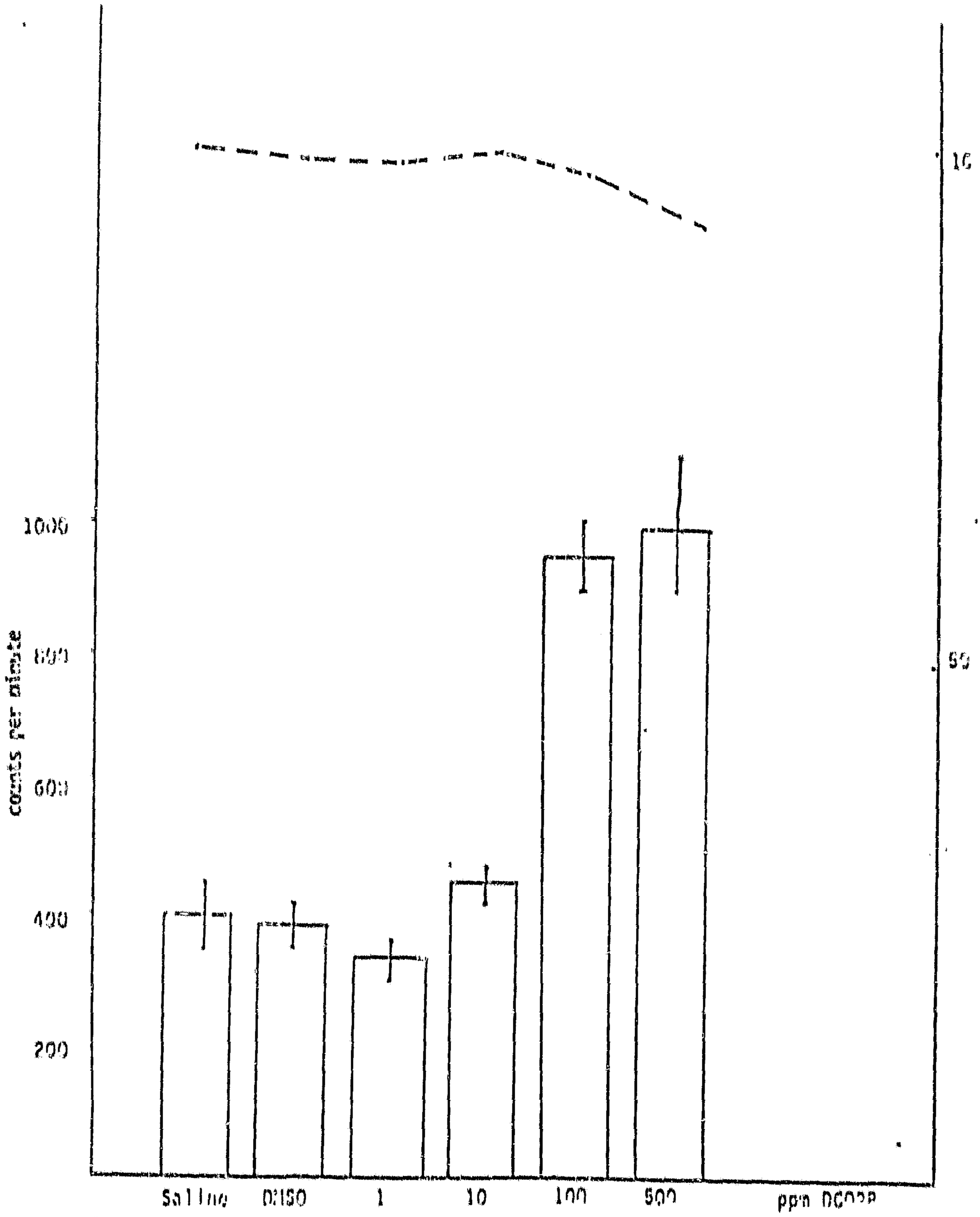

Figure 15. The effect of Dichloroisopropyl Glycidyl Ether (DCPP) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

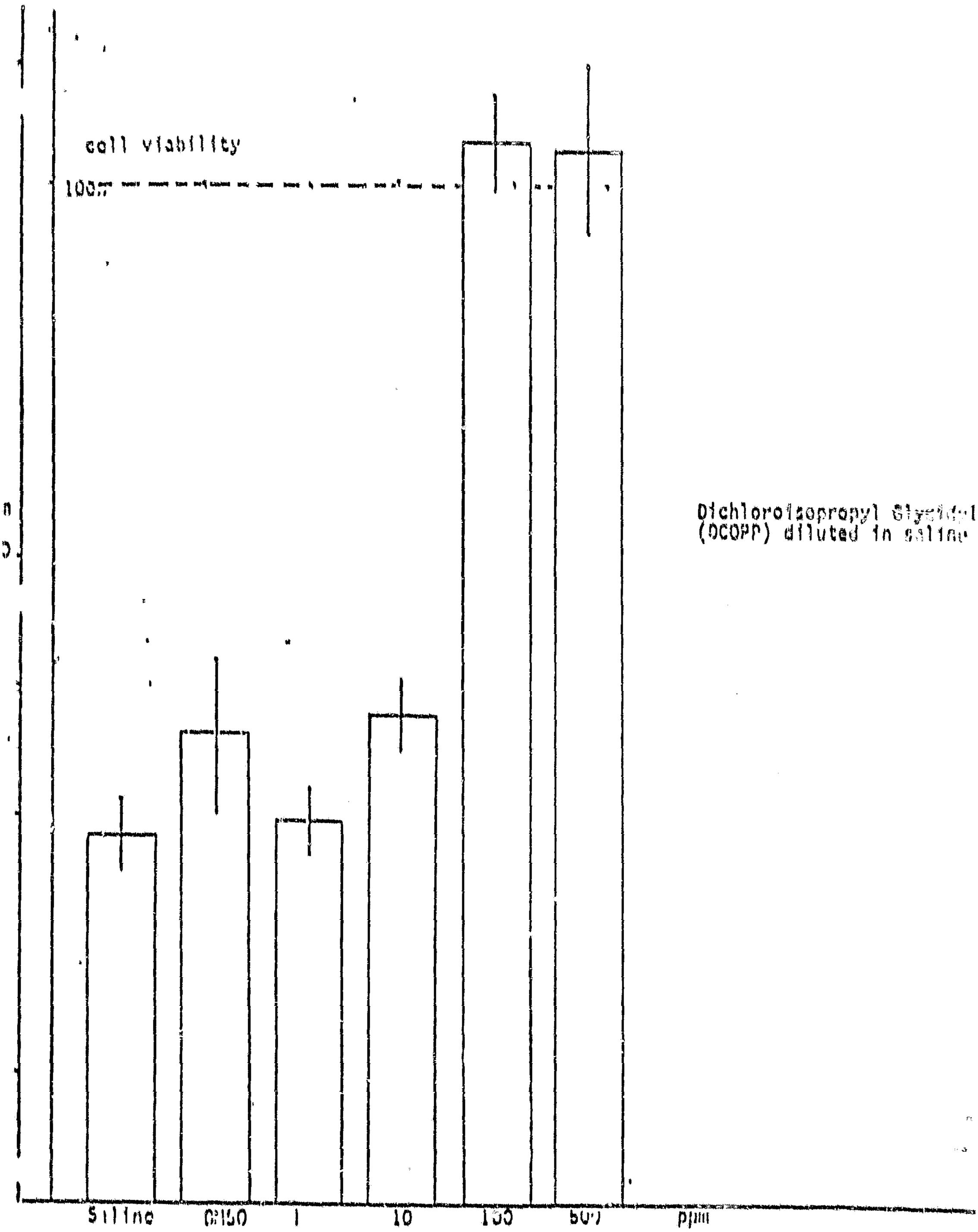

Figure 16. The effect of Dichloroisopropyl Glycidyl Ether (DCOPP) on tritiated thymidine incorporation into unstimulated, human, mononucleated white cells and the effect on cell viability (broken line) following *in vitro* exposure for 5 hours.

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Table 23

Mean Number of Implants/Pregnancy, Proportion Deaths/Pregnancy and % Pregnancy for DCOPP By Time

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
DCOPP	Number of Implants/ Pregnancy	7.449	7.567	7.551
	Proportion Deaths/ Pregnancy	.007	.014	.030
	% Pregnancy	82.000	88.000	89.818
Control	Number of Implants/ Pregnancy	7.077	6.662	8.277
	Proportion Deaths/ Pregnancy	.014	.023	.020
	% Pregnancy	78.100	93.400	83.500

000069

Table 24

Significance Level (p)* of Effects Due To Treatments, Time, and Time-Treatment Dependency

	Analysis of Variable		
	Proportion Deaths/Pregnancy	Number Implants/Pregnancy	% Pregnancy
Effect of:	p	p	p
DCOPP vs control (Treatments)	.70	.28	.56
Time	.30	.01	.20
DCOPP by Time	.51	.01	.92

* Significance level derived using two factor analysis of variance with repeated measures on factor B. Factor A = treatment. Factor B = time. (reference: Statistical Principles In Experimental Design. B.J. Winer, 2nd Edition).

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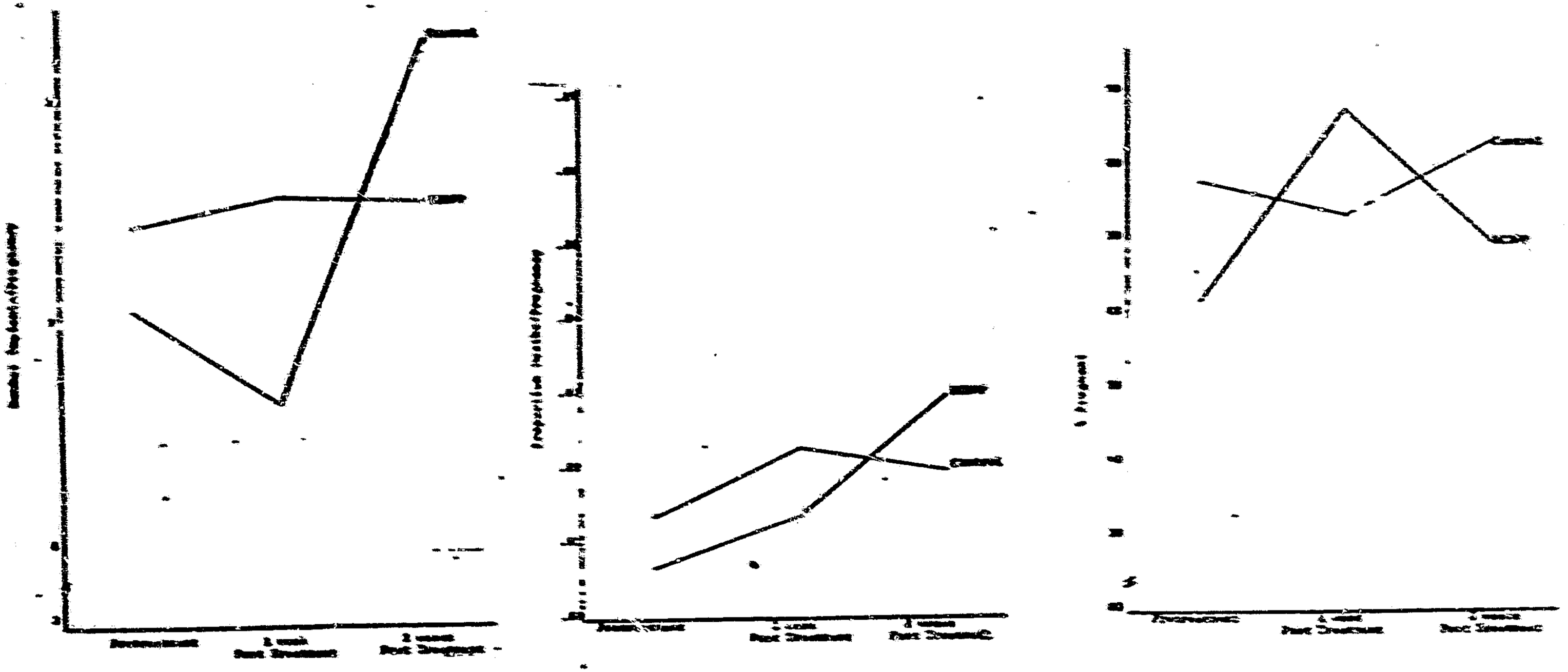

Figure 17. Comparison between DCOPP and control in the three variables measured in dominant lethal test.

000070

Positive Controls. Cyclophosphamide (Cytoxan) was used as the positive control in the body fluid analysis and produced potent mutagenicity (Table 4) in this test system. Two doses of EMS (ethylmethanesulfonate) were used as the positive control in the host mediated assay. As seen in Table, 5, there was a dose response relationship with high activity at the highest dose. TEM was highly mutagenic in the micronucleus test as depicted in Table 6. In the DNA repair study, the large increase in % labelled cells and mean grain count over labelled cells when the positive control 4NQO was used indicates high mutagenicity and that the exposed cells are capable of performing excision repair. Dominant lethality was highly evident when the positive control TEM was used (Table 25). Fetal deaths were increased at 1 and 2 week post treatment with a probable preimplantation loss occurring at 1 week post treatment also.

Table 26 presents a summary of the results of all test systems for each epoxide.

TABLE 25

MEAN NUMBER OF IMPLANTS/PREGNANCY, PROPORTION DEATHS/PREGNANCY AND % PREGNANCY FOR TEM BY TIME

		Pretreatment	1 week Post Treatment	2 weeks Post Treatment
	Number of Implants/ Pregnancy	7.70	5.63	7.99
TEM	Proportion Deaths/ Pregnancy	.013	.24	.09
	% Pregnancy	87.50	80.67	89.00

TABLE 26

RESULTS OF MUTAGENIC TESTS ON EPOXIDES

Compound	Direct In Vitro Microbial Test	Body Fluid Analysis	Mutagenic Test: Micronuclei Test	Mutagenic Test: Induction Repair	Mutagenic Test: Host-Mediated Assay	Mutagenic Test: Dominant Lethal Test
BER 331	+	−	−	−	−	−
BER 331 (Distilled)	+	−	−	−	−	−
ZB-7114	+	+	−	+	−	±
BGE	+	−	−	+	−	+
CGE	+	+[1]	−	+	−	±
Epoxide 8	+	−	−	−	−	−
BCPGE	+	−	−	−	−	−
BCPP	+[2]	−	±[2]	+	+	−[3]

+ = Positive mutagenic activity detected.
− = No mutagenic activity detected.
± = Marginal mutagenic activity detected; further experimentation needed to confirm results.

1. Negative in BDF mice.
2. Results determined in previous study.
3. Results positive in previous test by gavage.

000073

DISCUSSION

DER 331. This epoxide caused mutations of the base pair substitution type in S. typhimurium, but was not a frame shift mutagen since it showed no activity when TA98 was used. However, the results from the body fluid analysis, host mediated assay, micronucleus test, and dominant lethal assay indicate this chemical is not mutagenic in animal systems. The possibility of an active intermediate metabolite also appears remote since the use of β-glucuronidase in the body fluid analysis did not increase mutagenicity over the control. Although there was a significant increase in tritiated thymidine incorporation found at 10 ppm in the initial experiment, the results of this experiment were inconsistent. An increase at 10 ppm was not discovered in a repeat of this chemical, and the increase in the first experiment is considered an artifact. Thus this chemical is not considered to induce unscheduled DNA synthesis in human mononucleated white cells. Analysis of the number implants/pregnancy and percentage pregnancy in the dominant lethal assay showed there was an increase in these variables over time. This is not uncommon and has been found in other dominant lethal tests. It possibly could be explained by an increase in number and viability of sperm produced by the males. The significantly lower proportion deaths/pregnancy is a reflection of the pretreatment difference that was maintained throughout the study. It is unknown why there was a significant difference between the pretreatment data.

DER 331 (Distilled). The results of this compound were almost identical to the undistilled DER 331. Base pair substitution mutations were produced in S. typhimurium by DER 331 (dist.), but it appears this compound is not active in animals as evidenced by results of the body fluid analysis, micronucleus test, host mediated assay, and dominant

lethal. There was an apparent artifactual increase in ^{3}HTdR incorporation at 10 ppm in the initial DNA repair study as found in DER 331. However, after a repeat test it was not discovered, and it is felt that this epoxide, as found in DER 331, does not induce DNA repair. It is somewhat surprising that a significant reduction in the proportion deaths/pregnancy occurred with this compound as it did with DER 331. However, further analysis showed the same results as 331, that is, this difference was due to a pretreatment difference that carried throughout the experiment. Again, the increase in number of implants/pregnancy was detected with time.

XD-7114. XD-7114 was active in the direct *in vitro* microbial assay, producing substitution mutations. The body fluid analysis also indicated this chemical is active in an intact animal system. It is likely that, after further analysis of the body fluid analysis, XD-7114 is detoxified into an inactive glucuroride, but the metabolic pathway includes an active intermediate. This would explain the inactivity of this compound in the urine of the mice with no β-glucuronidase, but detection of minimal activity with the addition of the enzyme. However, mutagenicity was not detected in the micronucleus or dominant lethal tests. This could be due to the fact that the active intermediate was not present in the animal long enough to produce activity in these tests or that the mutagenic action was so minimal that it was not detected in these tests. Although there was no significant difference between XD-7114 and the control in the dominant lethal, there is some suggestion that XD-7114 produced a decrease in the number of implants/pregnancy at two weeks post treatment. This could be evidence of possible mutagenic activity, but further investigation is warranted to validate this. XD-7114 was also found to induce unscheduled DNA synthesis as evidenced by the

significant increases in ^{3}HTdR incorporation with both DMS and saline as solvents. This result in addition to the other positive effects point to the possibility of XD-7114 being a weak mutagen.

BGE. The data from the microbial assay indicates this compound is a mutagen of the base substitution type. This is rather well substantiated since the activity was high and increased in a linear fashion with increasing dosage. However, it appeared that the microsomal enzymes of the liver homogenate detoxified this chemical. Although the results were negative in the body fluid analysis, host mediated assay, and micronucleus test, mutagenic activity was detected in DNA repair study and dominant lethal test. A linear dose-response relationship was detected in the DNA repair study, while radioautographic studies confirmed the incorporation of ^{3}HTdR was intranuclear. BGE also produced an overall increase in dominant lethality as measured by a significant increase in fetal deaths (proportion deaths/pregnancy) over the control. It is apparent, particularly because of the time-treatment dependency, that the large increase occurred at 1 week post treatment with a reduction at 2 weeks post treatment. In view of the significant time-treatment interaction in the number of implants/pregnancy, differences between control and BGE were examined at 2 weeks post treatment. The results showed a significant reduction ($p=.01$) in the number of implants/pregnancy of BGE compared to the control. In view of the increased fetal deaths and no apparent toxic effect on implantation at 1 week post treatment, it is felt this is evidence of preimplantation loss at 2 weeks post treatment also. The lower percentage pregnancy females in the BGE data is due to a difference in the pretreatment data that is maintained throughout the experiment and is not a reflection of an effect due

to BGE. It is undetermined why this difference existed in the pretreatment data.

In evaluating the battery of mutagenic tests on this compound, questions arise as to why this epoxide was active in the dominant lethal and not in body fluid analysis, host-mediated assay or micronucleus test and why it appeared to be detoxified in the microbial assay and was not in the dominant lethal. One possible explanation why it was not active in the body fluid analysis, host-mediated assay or micronucleus test might be the dosage. The dose used in these three tests was low, and these tests might not be sensitive enough to detect positive activity at this level. The microsomal activation properties of liver homogenates, while incorporating an important aspect of mammalian metabolism into the microbial in vitro test, do not truly reflect the complex and dynamic metabolic processes of an intact animal. This might explain the difference between the dominant lethal and microbial assays in the detoxification of this epoxy.

CGE. This chemical is a potent mutagen against TA1535. However, it was detoxified by the liver homogenate, and this detoxification apparently occurred in the intact animal systems since no activity was detected in the host-mediated assay, micronucleus test or dominant lethal assay. This is further substantiated by the fact that mutagenic activity was detected in the urine of ICR mice only after the addition of β glucuronidase. This would indicate that CGE is detoxified in the intact animal into an inactive glucuronide. However, the activity discovered with the addition of β-glucuronidase indicates there is an active intermediate metabolite in the detoxification process. The failure to detect activity in the urine of the BDF mice might be attributable to species difference. Since the activity in the ICR mice was only minimal, the species difference of the BDF mice might account for a slight reduction in activity leading

to the failure to detect activity in the EDF species. Further analysis of the difference in the number implants/pregnancy between the control and CGE at 2 weeks post treatment showed it to be a significant reduction ($p<.05$). This could be due to preimplantation loss, but further experimentation is needed to confirm this. In addition, the positive effects in induction of excision repair cause some concern to arise about the mutagenicity of CGE.

Epoxide 8. This epoxide was essentially inactive in the entire battery of mutagenic tests. The minimal activity only at the highest dose with Aroclor metabolic activation in the microbial assay is overshadowed by the negative results in the remainder of this assay. The reduction in fetal deaths (proportion deaths/pregnancy) that occurred with Epoxide 8 is due to pretreatment differences and not a reflection of treatment effect.

DCPDGE. This epoxide, like all others, produced mutations in *S. typhimurium* of the base pair substitution class. There is some conflict in the data since Aroclor liver homogenate activated the compound but phenobarbital homogenate appeared to detoxify DCPDGE. Inactivity in the body fluid analysis, micronucleus test, host-mediated assay and dominant lethal assay indicates that this compound is detoxified in the intact animal. The sharp increase in the proportion deaths/pregnancy at 1 week post treatment was, as previously stated, largely due to one animal with a very high value. Elimination of this animal resulted in a mean value almost identical to the control at 1 week post treatment.

DCOPP. Although the microbial assay and micronucleus test were not conducted on this chemical in this study, a previous investigation was performed on DCOPP by Dr. Legator's laboratory. The results of this

investigation showed that DCOPP was positive in the microbial assay and questionable in the micronucleus test and cytogenetic studies. A dominant lethal test was also conducted in this previous investigation and found positive with oral gavage as the route of administration. The results from this investigation showed DCOPP was slightly active in the host-mediated assay and induced excision repair measured as unscheduled DNA synthesis. However, it did not exhibit any activity in the body fluid analysis on dominant lethal test. The difference in the results between the two dominant lethal studies might be attributable to the route of administration. A more pronounced effect of oral dosage over topical administration is possible. The phenomenon of the effect of route of administration on the dominant lethal test has been investigated and determined to exert a major influence. Apparently this applies to DCOPP with minimal dominant lethality occurring with oral administration but detoxification and subsequent inactivity occurring with skin application.

Positive Controls. The highly mutagenic action of all positive control chemicals in their respective test system indicates each test system was sensitive to mutagens as measured by their capability of reacting to known mutagenic compounds.

CONCLUSIONS

DER 331, DER 331 (distilled) and DCPDME were mutagenic in bacteria. However, the inactivity of these compounds in the remainder of the tests, particularly in the intact animal systems, indicates they are not mutagenic in animals. Consequently, it is felt that they pose the least mutagenic hazard to man of the chemicals evaluated.

XD-7114 and CGE were both mutagenic in the bacterial system, with CGE being a potent mutagen. DNA repair was induced by both of these chemicals, and mutagenic activity was found in the urine of mice treated with these epoxides. Although neither produced dominant lethality, an increase in micronuclei, or increased mutation frequency in the host-mediated assay, there was the possibility that they produced a preimplantation loss in the dominant lethal assay. This possibility should be investigated further to determine if it is a true effect. The evidence of the mutagenic activity expressed in an intact animal system, corresponding activity in the in vitro systems and the possibility that they produced preimplantation loss in the dominant lethal assay supports the conclusion that XD-7114 and CGE are weak mutagens.

BGE induced dominant lethality, DNA repair, and bacterial mutation. This evidence supports the conclusion that this epoxide is mutagenic and poses a hazard by skin contact.

Although positive activity was detected in bacteria treated with Epoxide B, it was very minimal and consequently, did not have a major influence in the evaluation of the mutagenicity of Epoxide B. In addition, Epoxide B did not produce any activity in the remaining tests and, based upon these results, it is believed to be nonmutagenic.

Based upon this study and the previous one conducted on MCOPP, it appears this epoxide is a weak mutagen. The increased activity in the micronucleus test, cytogenetic assay, host mediated assay and dominant lethal by gavage indicate it is mutagenic in animal systems. However, the negative findings in the dominant lethal by topical application suggest that skin exposure poses less hazard.

8782/1534

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SKIN SENSITIZATION POTENTIAL of DISTILLED DER* 331 EPOXY RESIN SAMPLE REFERENCE T8042862 1B

Chemical Name (100 per name) 25	CAS No. (10) 26
GLYCIDOL & ITS DERIVATIVES	99999994
CETYL GLYCIDYL ETHER	26447-14-3
	68988-38-6

1A

Chemical Name (300 per name)	CAS No. (10)
DOWANOL DPM	34590-94-8

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R & D REPORT

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LAB. NUMBER: (1)

LABORATORY REPORT DATE: July 30, 1979

DEPARTMENT: TEXAS TOXICOLOGY RESEARCH LABORATORY

TITLE: SKIN SENSITIZATION POTENTIAL OF DISTILLED DER® 331 EPOXY RESIN.

Sample Reference T9048608B

PAGES IN FULL REPORT

AUTHOR(S): M. N. Pinkerton

REVIEWER'S NEGATIVE: R. V. Johnston 7-30-79
R. V. Johnston, D.V.M.

This report is: ☐ INTERIM ☒ FINAL and mainly: ☐ NEW ☐ REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS: (Include in this space references to data books, and to earlier related reports, patents and publications.)

A sample of the test material was submitted to the Texas Toxicology Research Laboratory for evaluation of skin sensitization in guinea pigs.

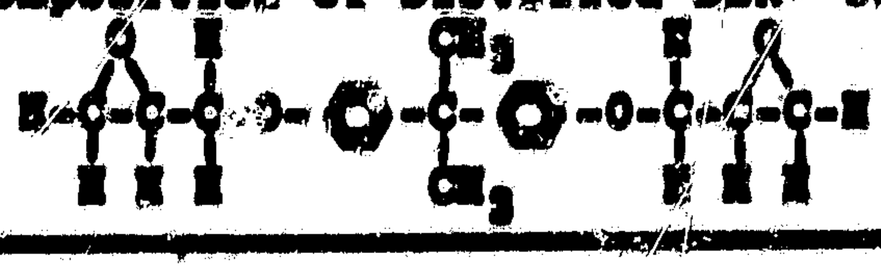
A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendation of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

The skin sensitization potential of Distilled DER® 331 epoxy resin was evaluated in male guinea pigs by subjecting them to a 10% (w/v) solution of the material in a 9:1 mixture of DOWANOL® DPM:Tween 80. Positive control animals were treated in a similar manner with a 10% (w/v) solution of Cresyl Glycidyl Ether in the 9:1 mixture of DOWANOL® DPM:Tween 80.

Distilled DER® 331 sensitized eleven of fourteen test animals, while the positive control sensitized twelve of twelve control animals. Distilled DER® 331 epoxy resin did induce skin sensitization in guinea pigs under the conditions of this test procedure and must be considered a potential skin sensitizer for humans.

Precautions should be taken to avoid skin contact. If contact occurs, the skin should be decontaminated with copious amounts of water from a safety shower or other suitable device and contaminated clothing thoroughly cleaned before re-use. Protective clothing, impervious to the test material should be worn as required by circumstances.

The composition of Distilled DER® 331 epoxy resin is:



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25068-38-6

34590-94-8

Chemical Name (300 per name) 23	CAS No. (10) 24
CRESYL GLYCIDYL ETHER	26447-14-3

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Sample Reference TR04S6821A

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AUTHOR'S SIGNATURE(S)

REVIEWER'S SIGNATURE: R. V. Johnston, D.V.M.

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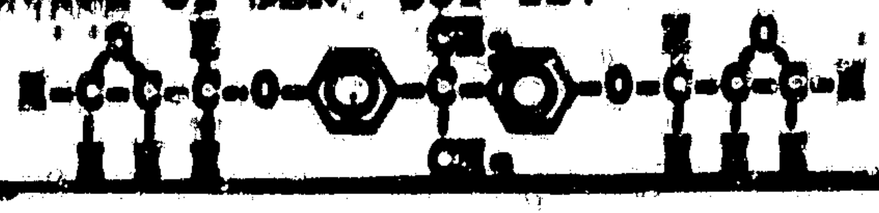
Precautions should be taken to avoid skin contact. If contact occurs, the skin should be decontaminated with copious amounts of water from a safety shower or other suitable device and contaminated clothing thoroughly cleaned before re-use. Protective clothing, impervious to the test material should be worn as required by circumstances.

The composition of DER® 331 is:



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Doc Title 23

SENSITIZATION POTENTIAL OF DOW EPOXY RESIN DER 331 IN GUINEA PIGS

Key Words (Subject) 25 Key No. (10) 26

GLYCIDOL & ITS DERIVATIVES 999999994

DOW EPOXY RESIN DER 331 25068-38-6

CENTRAAL INSTITUUT VOOR VOEDINGSONDERZOEK TNO

CENTRAL INSTITUTE FOR NUTRITION AND FOOD RESEARCH

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Title: Sensitization potential of Dow Epoxy Resin DER 331 in guinea pigs

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Investigators: Miss A.M.M. Kaiser and Dr M.P. Till

At the request of: Dow Chemical Europe, Horgen, Switzerland

Approved by: Dr A.P. de Groot

Date: May 1978

SUMMARY

1. In an experiment with guinea pigs Dow Epoxy Resin DER 331 was examined for sensitization properties.

2. A 0.1 per cent suspension in a 10 per cent aqueous solution of ethyl alcohol provoked mild to severe irritation reactions in all animals following repeated intradermal injections.

3. From the reaction to the challenge dose it was concluded that compound DER 331 provoked no sensitization as judged by the Landsteiner/Draize test.

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Sensitization potential of Dow Epoxy Resin DER 331 in guinea pigs

1. INTRODUCTION

At the request of Dow Chemical Europe, Horgen, Switzerland, Dow Epoxy Resin DER 331 was examined for possible sensitization activity in an experiment with guinea pigs.

2. MATERIAL AND METHODS

The sample was received from the principal on 7th February 1978 and was designated Dow Epoxy Resin DER 331 (Stade).

The Landsteiner/Draize method was used.

Sixteen male albino guinea pigs, weighing from 255 to 330g, were divided into 2 groups of 8 animals each; one test group and one control group.

The test substance was administered by intradermal injections. The test animals were treated repeatedly, the controls only once. Preliminary observations showed that one intradermal injection of 0.1ml of a 0.1 per cent concentration of the test compound induced moderate skin irritation. Therefore, a 0.1 per cent suspension of DER 331 in a 10 per cent aqueous solution of ethyl alcohol was used for administration. The suspension was freshly prepared once a week and stored at ambient temperature. For the challenge dose a 0.01 per cent suspension in a 10 per cent aqueous solution of ethyl alcohol was used.

The right flank of the animals was shaved with electric clippers some days before starting the treatment. Shaving was repeated before each injection and reading if necessary.

During the induction period the test animals received a total of 10 intradermal injections: 3 times weekly over a period of 22 days. The injections were given on different spots on the right flank, within an area of 3-4 square cm (see fig. 1).

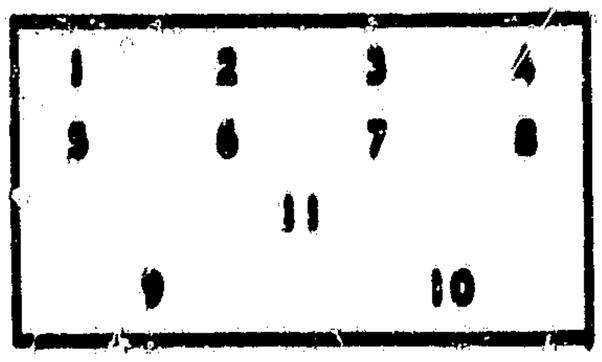

Fig. 1. Positions of the successive injections on the right flank.

As the first injection each animal received 0.05ml of the 0.1 per cent suspension; in the 2nd to the 10th injection 0.1ml was applied per animal.

The challenge dose was given two weeks after the 10th injection on spot 11 in an amount of 0.05ml of the 0.01 per cent suspension per animal. At this point the control animals were also injected with 0.05ml of 0.01 per cent suspension of the test sample. The reaction sites were examined 24 hours after the injection: diameter, colour and thickness were used as criteria for the intensity of the reaction.

3. RESULTS

The number and degree of individual positive reactions observed during the induction and challenge period with the test compound are shown in table 1.

The test compound caused mild to severe skin reactions upon repeated intradermal injections in all test animals. In most cases the middle of the reaction sites showed a yellowish spot of 2-5mm in diameter; probably a small abscess. Each reaction was visible for several days after injections afterwards a distinct scar was left.

The challenge dose provoked mild to moderate reactions in all test animals. At the same time all controls showed a positive mild to moderate reaction. There were no differences between the test and control animals with respect to the degree and incidence of erythema and edema.

From these results it may be concluded that no sensitisation occurred after treatment with the compound DER 331.

Respectfully submitted,

CENTRAL INSTITUTE FOR NUTRITION AND FOOD RESEARCH TNO

C.O.

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(Toxicologist)

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(Head of Dept Biological Toxicology)

Table 1. Individual positive reactions (marked with + signs) observed during the injection and challenge period with Rev Epoxy Resin DER 331

test animals												control animals	
no.	individual direct reaction										chal-lenge	no.	reaction to injection at the same time as the challenge
	1	2	3	4	5	6	7	8	9	10			
1948	+	+	++	++	++	++	++	++	+	++	+	1956	+
1949	+	+	+	+++	++	++	++	+	+	++	+	1957	++
1950	+	+	++	+	++	++	++	++	++	++	+	1958	+
1951	+	+	++	+	++	++	+	+	++	+++	++	1959	++
1952	+	++	+++	++	++	++	++	++	++	++	++	1960	+
1953	+	++	++	+++	++	+++	++	++	++	++	+	1961	++
1954	+	++	++	++	++	+++	+++	++	++	+++	++	1962	++
1955	+	++	+++	+++	+++	+++	+++	+++	++	++	++	1963	++
Total	8	8	8	8	8	8	8	8	8	8	8		8

Degree of reaction: + = mild

++ = moderate

+++ = severe

000005

CONTROL OF TOXIC SUBSTANCES

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DER 331 EPOXY RESIN 25068-38-6

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R & D REPORT

DOW CHEMICAL U.S.A.

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TITLE: SKIN SENSITIZATION POTENTIAL OF THREE SAMPLES OF DER* 331 EPOXY RESIN

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AUTHOR(S) SIGNATURE(S): D. D. Lockwood

SUPERVISOR'S SIGNATURE: F. J. Olson

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DATA REFERENCES (SEE ON REVERSE): None

(Refer also to earlier related reports and publications.)

PATENT STATUS: disclosure submitted / case filed / no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

Three samples of DER* 331 epoxy resin were submitted to the Toxicology Research Laboratory for comparison of skin sensitization potential. Sample 1, Lot TB0210U91, is a cloudy, off-white liquid currently used as a positive control for guinea pig skin sensitization tests. Sample 2 a current production sample, is a light yellow liquid bearing reference Lot TB0224B191. Sample 3 is a clear, light yellow highly purified sample bearing reference Lot TB0901610J. Each sample was tested on ten male Hartley albino guinea pigs as a 10% solution in DOWANOL* DPM glycol ether product, Tween 80 surfactant, 9:1. All 3 samples sensitized 10 out of 10 guinea pigs. There was essentially no difference in sensitization potential between any of the samples. Therefore all three samples must be considered potential skin sensitizers for humans.

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GLYCIDOL & ITS DERIVATIVES 999999994

DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA) 25085-99-8

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DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA): FATE IN MALE FISCHER 344 RATS (PROBE)

By

R. J. Nolan, S. Unger and L. S. Chatterton

December 15, 1981

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ABSTRACT

Concern has been expressed that diglycidyl ether of bisphenol A (DGEBA), a major component of DER 331* epoxy resins, may represent an inhalation hazard. Due to its physical properties meaningful repeated inhalation exposure studies cannot be conducted. Thus oral toxicity studies have been proposed as an alternate means to evaluate the hazard. The purpose of this study was to obtain preliminary data on the metabolism and kinetics of DGEBA. The rationale being that oral toxicity data could be used to predict the inhalation hazard, if the fate of DGEBA were route-independent.

An atmosphere suitable for a single 6 hr inhalation exposure to ^{14}C-DGEBA could not be generated. Thus intravenous administration was substituted for the inhalation route. Route dependent differences were observed in the plasma ^{14}C-concentration time profile, tissue/ plasma ^{14}C-ratios and the urinary ^{14}C-excretion following oral and intravenous administration. These differences suggest that little of the orally administered ^{14}C-DGEBA was absorbed unchanged. Consistent with this, 4 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA. ^{14}C-DGEBA was not stable in simulated gastric fluid and degradation of ^{14}C-DGEBA in the stomach or during its initial transit through the liver could account for the route dependent differences observed in the fate of ^{14}C-DGEBA. These data indicate that oral toxicity data would be of little value in assessing the inhalation hazard. Furthermore, the inability to generate a suitable atmosphere using low airflows, high temperatures and a large surface area suggests that inhalation is an insignificant route for DGEBA exposure.

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INTRODUCTION

The objective of these studies was to provide data on the utility of conducting oral toxicity studies to assess the possible inhalation hazard associated with the diglycidyl ether of bisphenol A (DGEBA). DGEBA, a bifunctional epoxide, is the major reactive component of DER 331* epoxy resins. Although no human exposure data are available, it has been speculated that DGEBA may represent an inhalation hazard because it contains two reactive epoxide bonds. Ideally, an inhalation study should be conducted to assess a potential inhalation hazard. However, because of the physical properties of DGEBA, it is unlikely that an atmosphere suitable for a repeated exposure inhalation study could be generated. Therefore, oral toxicity data have been proposed as a means to assess the hazard associated with inhaled DGEBA.

It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body, and that route dependent differences in the fate of a chemical may significantly alter its toxicity. This study was undertaken to provide preliminary data on the metabolism and kinetics of ^{14}C-DGEBA. The work described in this report was designed: (1) to determine if an atmosphere could be generated that would be suitable for a single exposure inhalation kinetic study with ^{14}C-DGEBA, (2) to compare the fate of ^{14}C-DGEBA following oral and intravenous administration, (3) to determine the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid, and (4) to compare the chromatographic profile of radioactivity in plasma following oral and intravenous administration of ^{14}C-DGEBA.

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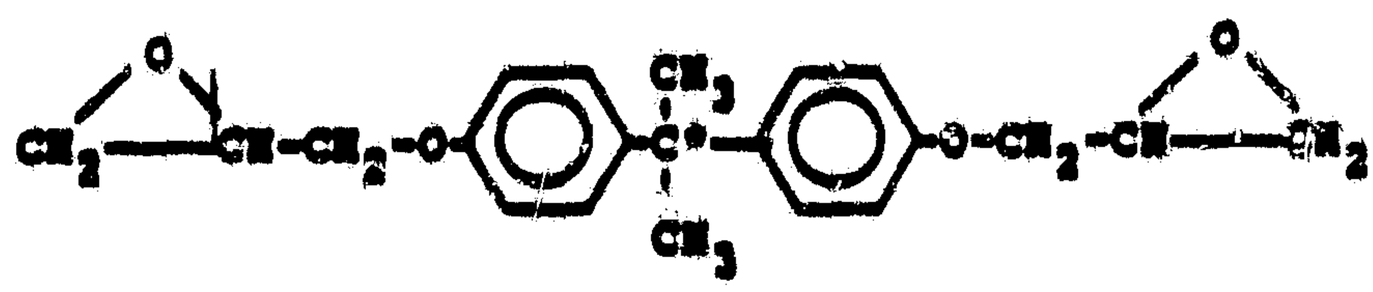
MATERIALS

Animals. Male Fischer 344 rats weighing 195-250 g were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. The animals were housed in rooms designed to maintain a constant temperature (72 ± 2°F), humidity (50 ± 10%), 12 hr photocycle, and 10-12 air changes per hour. Ralston Purina Rat Chow and tap water were available *ad libitum* except for an 18 hr fast prior to dosing the rats used to define the chromatographic profile of the plasma radioactivity. When required by the study objectives, jugular and biliary cannulas were implanted 48 hr prior to dosing using standard surgical procedures.

Test Material: p,p'-Diglycidyl ether of bisphenol A isopropylidine, methylene-^{14}C (^{14}C-DGEBA; Lot #FS-TDL-124) was synthetised from ^{14}C-labeled bisphenol A by T. Licky of The Dow Chemical Company.



*Location of carbon-14 label

The radiotracer had a specific activity of 7.98 µCi/mg and a radiochemical purity of 98% as determined by high pressure liquid chromatography (HPLC) with fractional collection and Carbon-14 analysis of the column effluent (T. Licky, 1979).

Reagent grade DGEBA (Lot #332R91) was used to adjust the specific activity of the ^{14}C-DGEBA in the vapor generation experiments and as an internal standard for the HPLC analysis.

EXPERIMENTAL

Vapor Generation: Reagent grade DGEBA or a mixture of reagent grade and ^{14}C-DGEBA (Specific activity 1.3×10^6 dpm/g) was dissolved in methylene chloride ($MeCl_2$). An aliquot of the $MeCl_2$ solution was applied to a glass wool plug lodged in the center well of a vaporization flask (Figure 1). The $MeCl_2$ was removed by evaporation. The flask was then placed in a heating mantle and connected to the chamber as depicted in Figure 2. When connected in this manner, air passed through a Drierite® container, a flow meter, the vaporization flask, a 30 liter glass inhalation chamber, a cold finger trap containing 50 ml of $MeCl_2$ and immersed in a dry ice bath, and a second flow meter. The line connecting the vaporization flask to the chamber was constructed of 1 inch i.d. glass tubing. This line was ~40 cm long and contained one 90° bend ~15 cm above the glass wool plug. There were two ports in this line through which a thermocouple could be inserted, or samples of the air stream collected. One port was ~5 cm above the glass wool plug and the other port was ~13 cm upstream from the chamber.

During the trial vaporizations, the outer surface of the vaporization flask was heated to 410°C. Air was passed through the vaporization flask at 8 l/min. At this airflow, the temperature of the air stream 5 cm above the glass wool plug and 13 cm from the chamber was 140°C and 21-27°C, respectively. Known volumes of air (~30 l) were drawn via the ports and passed through cold finger traps containing 50 ml of $MeCl_2$. The amount of DGEBA trapped in the cold finger traps was determined by radioactivity or HPLC analysis. The concentrations of DGEBA in the air stream at the two sample ports and exiting the chamber were calculated from the amount of DGEBA trapped and the volume of air sampled.

Fate of Orally and Intravenously Administered ^{14}C-DGEBA:

^{14}C-DGEBA was either dissolved in corn oil and administered by gavage, or suspended in fresh heparinized rat plasma[1] and administered via the jugular cannula. The oral dose was 570 µg (4.5 µCi) and the intravenous dose was 90 µg (0.70 µCi) of ^{14}C-DGEBA. Following dosing, the rat with the bile cannula was placed in a restraining cage. The other rats were placed in individual glass Roth-type metabolism cages. Plasma, urine, feces and bile samples were collected at the intervals listed below:

	Hrs After Administration of ^{14}C-DGEBA
Blood	0.5, 1.0, 1.7, 3, 4, 6, 8, 10, 12, 18 and 24
Urine	6, 12 and 24
Feces	24
Bile*	1, 2, 3, 6, 12 and 24

*Bile cannulated animal only.

The blood was centrifuged to obtain the plasma. Urine and bile were collected and stored frozen until analysed. Aliquots of the plasma, urine and bile were added directly to ACS liquid scintillation cocktail (Mallinckrodt Chemical Company) and analyzed for radioactivity. All animals were sacrificed 24 hr after dosing. The liver, lungs, kidneys and intestines were removed from the non-bile cannulated animals. The intestine was sectioned and flushed with water to remove the intestinal contents. Aqueous homogenates of the intestine and feces were prepared. Aliquots of the tissues, feces and intestinal contents were oxidized in a biological materials oxidizer. The $^{14}CO_2$ produced during oxidation was trapped and quantitated using standard laboratory procedures.

[1]HPLC analysis demonstrated ^{14}C-DGEBA was stable in rat plasma over the interval taken to prepare and administer the dose solution.

Stability of ^{14}C-DGEBA in Simulated Gastric and Intestinal Fluid: Simulated gastric and intestinal fluid were prepared as described in the 1970 U.S. Pharmacopeia. ^{14}C-DGEBA was added to 2 ml of the simulated gastric or intestinal fluid and incubated in a 37°C water bath. Sequential aliquots of the incubation mixture were removed 5, 30, 120, and 180 (gastric only) minutes after the start of the incubation, and neutalized with 0.2 N KOH or 0.1 N HCl. Aliquots (20 µl) of the neutralized incubation mixture were analyzed by high pressure liquid chromatography (HPLC).

Chromatographic Profile of Plasma Radioactivity: The dose solutions were prepared and administered as previously described except that the amount of radioactivity administered was increased; in this segment 37.8 and 8.5 µCi of ^{14}C-DGEBA/animal were administered orally and intravenously, respectively. Administration of the oral and intravenous dosing solutions were alternated and staggered in time to control for variation due to sequence of administration, sacrifice and analysis. The animals were killed by exsanguination 4 hr after dosing. The blood was centrifuged and 20 µl of the plasma analyzed by HPLC.

High Pressure Liquid Chromatography (HPLC): The HPLC method described by Paul (1976) was used with minor modifications. The sample was applied to the column using a sample loop. Reagent grade DGEBA was co-chromatographed with those samples containing ^{14}C-DGEBA. Fractions (1-2 ml) of the column effluent were collected and analyzed for radioactivity. In addition the optical density (280 nm) of the column effluent was monitored to determine when DGEBA eluted or to quantitate DGEBA in those samples which did not contain ^{14}C-DGEBA. The HPLC conditions were as follows:

Column: 30 cm x 4 mm μPorasil (Waters Associates)

Mobil Phase: 2 ml/min going from 7 to 100% Solvent B over 23 min using program 6 or 10 on a Waters Associates Model 600 programmer.

Solvent A: Hexane containing 0.01% (v/v) benzene.

Solvent B: Tetrahydrofuran containing 15% (v/v) acetonitrile.

Thin Layer Chromatography: The following samples were spotted on five thin layer chromatography (TLC) plates coated with 0.1 mm of cellulose F (E. M. Reagents):

1. Stock ^{14}C-DGEDA
2. $MeCl_2$ extract from glass wool plug used in vaporization ^{14}C-DGEDA
3. Bile from bile cannulated rat
4. Urine from bile cannulated rat
5. Urine from the orally dose rat

One TLC plate was developed with each of the following solvents: dimethyl sulfoxide, pyridine, methanol, 1,4-dioxane and dimethyl formamide. The location of radioactivity on the TLC plates was determined using a beta-graph (Panax Equipment Limited).

Radioactivity Analysis: Radioactivity was quantified using a Mark II or Mark III Nuclear Chicago liquid scintillation spectrometer (Searle). Counts per min (cpm) were corrected for quench and background, and converted to µg equivalents (eq) of ^{14}C-DGEBA.

Data Analysis: Rate constants and half-lives were obtained by least squares analysis. Area under the plasma ^{14}C-concentration time curve (AUC) was calculated using the trapezodial rule. The volume of distribution (Vd_{area}) for ^{14}C-DGEBA in the rat was calculated as:

$$Vd_{area} = \frac{\text{Dose}}{\text{AUC} \times \text{Rate Constant for Elimination}}$$

RESULTS

Generation of DGEBA Vapors: The system depicted in Figure 2 was the only system found that generated measurable concentrations of DGEBA vapors at respirable temperatures. In this system DGEBA was placed on glass wool to maximize the surface area, and hot air (410°C) was used to enhance vaporization. The line connecting the vaporization flask and chamber was large, contained no restrictions, and was just long enough to permit the air stream to cool to a respirable temperature (~22°C). However, even under these conditions, it was not possible to generate an atmosphere containing sufficient ^{14}C-DGEBA for a kinetic study.

In one trial the concentration of ^{14}C-DGEBA in the air 5 cm above the vaporization flask was 6.9 µg/l (0.5 ppm). The concentration of ^{14}C-DGEBA in air 13 cm upstream from the chamber was 0.6 µg/l (0.04 ppm), and in the air exiting the chamber was 0.05 µg/l (0.004 ppm). These data were consistent with the results of other trial vaporization; i.e., significant amounts of DGEBA would be vaporized at high temperatures but the concentration decreased rapidly when the temperature decreased or the airflow was obstructed. The observed decrease in ^{14}C-DGEBA concentrations were apparently due to condensation since the walls of the line connecting the vaporization flask to the exposure chamber were coated with a sticky film.

Higher concentrations of DGEBA vapors may have been generated in one trial vaporization in which reagent grade DGEBA and HPLC analysis were used instead of ^{14}C-DGEBA and ^{14}C-analysis. In this experiment, concentrations of DGEBA in air 5 cm above the vaporization flask, 13 cm upstream from the chamber and exiting the chamber were 51.4, 150.4, and 93.2 µg/l or 3.7, 10.8 and 6.7 ppm, respectively. However, because the lowest concentration was found just above the vaporization flask, these data are suspect. Even if these results are valid, the concentrations of DGEBA in the air were low considering the conditions used to generate the vapors, and the atmosphere was not sufficiently stable to conduct an inhalation exposure study.

<u>Fate of Orally and Intravenously Administered ^{14}C-DGEBA:</u>
Intravenous administration was substituted for the inhalation route, since it was not possible to generate an atmosphere suitable for a single inhalation exposure kinetic study. The intravenous route was selected because ^{14}C-DGEBA is

placed directly into the systemic circulation[2]. The lung is the only barrier inhaled ^{14}C-DGEBA must cross to reach the systemic circulation and once it enters the systemic circulation its fate is independent of the route of administration.

Table 1 presents the distribution of radioactivity recovered 24 hr after oral and intravenous administration of ^{14}C-DGEBA. Total recovery was low, however, the fraction of the dose recovered following oral (66%) and intravenous (32%) administration was similar in the non-bile cannulated animals. The feces was the primary route of excretion following oral (53%) and intravenous (43%) administration. The liver was the primary excretory organ for ^{14}C-DGEBA as evidenced by the fact 86% of the ^{14}C-DGEBA given intravenously was excreted *via* the bile within 24 hr. Urine was a minor route of excretion with 8 to 9% of the oral and intravenous dose excreted by this route. Another 2% of the oral and 0.5% of the intravenous dose was recovered in the intestine plus contents. The tissues, i.e. liver, kidney, lungs and intestine, contained 1 to 2% of the dose.

Differences were also observed in the distribution of radioactivity in the tissues 24 hr after oral and intravenous administration of ^{14}C-DGEBA (Table 2). The plasma contained three times more radioactivity 24 hr after the 0.43 mg/kg intravenous dose than following the 2.7 mg/kg oral dose. In contrast the tissue/plasma ^{14}C-ratios especially for the liver, intestine and lungs were higher following oral administration.

[2]Circulatory system minus the hepatic-portal system. An orally administered chemical must pass through the liver before it reaches the rest of the body and some chemicals are extensively metabolized and/or excreted during their initial transit through the liver.

The plasma ^{14}C-concentration time profiles following oral and intravenous administration of ^{14}C-DGEBA are depicted in Figures 3, 4 and 5. Pharmacokinetic parameters derived from the plasma ^{14}C-data are summarized in Table 3. The absorption of radioactivity following oral administration of ^{14}C-DGEBA was rapid (t 1/2 = 0.7 hr) and the highest plasma ^{14}C-concentration (0.97 µg eq/g) was observed 4 hr after dosing. The radioactivity was cleared from the plasma in an apparent first-order manner following both oral (Figure 3) and intravenous (Figure 4 and 5) administration. However, the radioactivity was cleared from the plasma faster following oral administration (t 1/2 = 4.6 hr) than following intravenous (t 1/2 = 7.7 hr) administration. The area under the plasma ^{14}C-concentration time curve (AUC) was slightly smaller following oral (316 µg eq min/g) than after intravenous administration (601 µg eq min/g). Assuming the radioactivity represents the same chemical entity, the relative AUC following oral and intravenous administration suggest that only 77 µg or 13% of the 570 µg of ^{14}C-DGEBA administered orally was absorbed. However, of the 77 µg absorbed 50 µg (65%) was excreted in the urine. In contrast, only 6% of the ^{14}C-DGEBA administered intravenously was excreted in the urine. The differences found in the half life for plasma radioactivity, its excretion in the urine, and the tissue/plasma ^{14}C-ratios suggest that the radioactivity in the plasma following oral and intravenous administration of ^{14}C-DGEBA represent different chemical entities.

Thin layer chromatography (TLC) of selected urine and bile samples indicated that different metabolites were excreted following oral and intravenous administration of ^{14}C-DGEBA.

Based on the TLC, no unchanged ^{14}C-DGEBA was excreted in either the urine or bile. The major metabolite found in the bile was also found in the urine following intravenous but not oral administration. Metabolites of ^{14}C-DGEBA were found in the urine following oral administration that were not found in either the bile or urine of the intravenously dosed rat.

<u>Stability of ^{14}C-DGEBA in Simulated Gastric and Intestinal Fluid</u>: The stability of epoxides are known to be pH dependent. Orally administered ^{14}C-DGEBA is subjected to pH's ranging from 1.2 in the stomach to 7.5 in the intestines. Hydrolysis of the epoxide bond(s) in the gastrointestinal tract could explain the poor absorption of orally administered ^{14}C-DGEBA and account for the observed differences in the fate of orally and intravenously administered ^{14}C-DGEBA. Therefore, the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid was investigated.

Table 4 presents the result obtained when ^{14}C-DGEBA was incubated with simulated gastric or intestinal fluid. Recovery of radioactivity applied to the HPLC column decreased from >90% for the 5 min incubations to about 70% for incubations of 30 min or more. Radioactivity in the simulated gastric fluid eluted from the HPLC column in three distinct peaks. The first peak co-eluted with reagent grade DGEBA; i.e., it had the same retention volume as the UV peak for reagent grade DGEBA. The two remaining peaks eluted after DGEBA and based on their larger retention volumes, represent polar degradation products of DGEBA. The fraction

of the applied radioactivity which eluted as ^{14}C-DGEBA decreased from 77.5 to 11.0% as the incubation time was increased from 5 to 180 min. The fraction of the radioactivity in the simulated gastric fluid eluting as polar degradation products (peaks II and III) increased from 25.9 to 66.7% as the incubation time increased from 5 to 180 min. Based on these data, the half life for the degradation of ^{14}C-DGEBA in simulated gastric fluid was 70 min.

Two radioactive peaks were observed when the simulated intestinal fluid was subjected to HPLC. The first and largest peak had the same retention volume as reagent grade DGEBA. The length of the incubation had no effect on the fraction of the applied radioactivity which eluted as DGEBA or in the second peak. This suggests that ^{14}C-DGEBA is not rapidly hydrolysed at pH's found in the small intestine.

<u>Chromatographic Profile of Plasma Radioactivity</u>: Based on the elimination and tissue distribution data, the radioactivity in the plasma following oral and intravenous administration appeared to represent different chemical species. In an attempt to verify this, rats were given ^{14}C-DGEBA by gavage or intravenously, sacrificed 4 hr after dosing, and their plasma collected for analysis. The mean (± SD) concentrations of radioactivity in the plasma of the orally and intravenously dosed rats were 2914 ± 514 and 559 ± 203 dpm/20 µl, respectively. Thus, the plasma from intravenously dosed rats did not contain sufficient radioactivity to permit its chromatographic profile to be resolved. However, when the plasma from the orally dosed rats was subjected to HPLC, less than 10% of the radioactivity eluted as ^{14}C-DGEBA. This suggests that only a small percentage of the orally administered ^{14}C-DGEBA reached the systemic circulation unchanged.

DISCUSSION

It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body. Observed differences in the plasma ^{14}C-concentration time profile, tissue/plasma ^{14}C-ratios and urinary excretion of radioactivity following oral and intravenous administration of ^{14}C-DGEBA suggest that little if any orally administered ^{14}C-DGEBA is absorbed unchanged, and that the chemical identity of the radioactivity in the plasma depends on the route of administration. Consistent with this, 4 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA, and the radioactivity in the urine exhibited a different chromatographic profile following oral and intravenous administration of ^{14}C-DGEBA. Thus these data indicate that oral toxicity studies would be of little value in assessing the possible hazard associated with inhalation of DGEBA.

Based on the half-life for the degradation of ^{14}C-DGEBA in simulated gastric fluid (t 1/2 = 70 min) and for the absorption of radioactivity following oral administration (t 1/2 = 42 min), over a third of the orally administered ^{14}C-DGEBA would be degraded in the gastrointestinal tract. Once absorbed, orally administered ^{14}C-DGEBA must pass through the liver to reach the rest of the body. The liver plays a major role in the excretion of ^{14}C-DGEBA as evidenced by the fact that 85% of the ^{14}C-DGEBA administered intravenously was excreted in the bile within 24 hr. Based on these observations it is not surprising that there are significant differences in the fate of orally and intravenously administered ^{14}C-DGEBA.

The problems encountered in trying to generate an atmosphere suitable for a single exposure kinetic study confirm previous

suspicions that it is unlikely a meaningful repeated inhalation exposure study could be conducted with DGEBA. Furthermore, the inability to generate a suitable atmosphere using low air flows, a large surface area, and high temperatures suggests that inhalation is an insignificant route for DGEBA exposure.

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Pharmacopeia of the United States, Eighteenth Revision, September 1, 1970.

TABLE 1: DISTRIBUTION OF RADIOACTIVITY RECOVERED 24 HOURS AFTER ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DOXEPIN TO RATS[a].

Treatment	Dosed Orally	Dosed Intravenously	Dosed Intravenously Bile Cannulated
Dose µg	570	90	90
mg/kg	2.7	0.43	0.43[b]
µCi	4.5	0.72	0.72
Radioactivity Recovered	**µg Equivalents of ^{14}C-DOXEPIN (% Dose in Parenthesis)**		
Feces	303 (53%)	38.7 (43%)	5 (5%)
Bile	c	c	77 (86%)
Urine	50 (9%)	6.9 (8%)	38 (42%)
Intestinal Contents	12 (2%)	0.4 (<1%)	c
Tissues[d]	13 (2%)	1.1 (1%)	c
TOTAL	378 (66%)	47.1 (52%)	118 (131%)

[a]Data are for one animal at each treatment.

[b]Body weight not available for this animal dose rate estimated assuming rat weighed 210 g.

[c]Sample not collected from this animal.

[d]Total µg eq of ^{14}C-DOXEPIN found in the liver, kidneys, lungs and intestines.

TABLE 2: CONCENTRATION OF RADIOACTIVITY IN THE TISSUES 24 HOURS AFTER DOSING EXPRESSED AS mg eq ^{14}C-DOEBA/g AND AS TISSUE/PLASMA RATIOS.

Tissue	Oral		Intravenous	
	mg eq/g	Tissue/Plasma	mg eq/g	Tissue/Plasma
Liver	0.64	15	0.07	0.6
Kidney	0.05	1	0.07	0.5
Lung	0.17	4	0.07	0.6
Intestine	0.55	13	0.04	0.3
Plasma	0.04		0.11	

Values based on one orally and one intravenously dosed rat.

TABLE 3: PARAMETERS DESCRIBING THE TIME COURSE OF RADIOACTIVITY IN THE RAT FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DGEBA.

	Oral	Intravenous	Bile Cannulated
Dose (µg)	570	90	90
(mg/kg)	2.7	0.43	-[a]
Maximum Plasma ^{14}C-DGEBA Conc (µg eq/g)	0.97	0.76	1.16
Time Maximum Plasma ^{14}C-DGEBA Conc Observed (hr)	4	0	0
Absorption Rate Constant (hr^{-1})	1.0	-[b]	-[b]
Half Life in hr	0.7	-[b]	-[b]
Elimination Rate Constant (hr^{-1})	0.15	0.08	0.09
Half Life in hr	4.8	8.8	7.7
Area Under ^{14}C-Plasma Conc Time Curve (µg eq . min/g)	516	601	697
Dose Adjusted Area Under ^{14}C-Plasma Conc Time Curve	0.91	6.7	7.7
Volume of Distribution (ml/Rat)	450	114	86
Clearance (ml/min/Rat)			
Urinary[c]	0.10	0.01	0.06
Biliary[c]	-	-	0.11
Body[d]	1.10	0.18	0.13

Parameters based on data from one animal.

[a]The body weight of this rat is not available.

[b]Calculation of this parameter is inappropriate.

[c]Calculated as amount excreted in urine or bile divided by the area under plasma ^{14}C-conc time curve.

[d]Calculated as the dose divided by the area under the plasma ^{14}C-conc time curve.

TABLE 4: ELUTION OF RADIOACTIVITY FROM THE COLUMN WHEN ^{14}C-DGEBA WAS INCUBATED WITH SIMULATED GASTRIC OR INTESTINAL FLUID, AND SUBJECTED TO HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC).

Length of Incubation (min)	Gastric Fluid				Intestinal Fluid		
	% Recovered	Peak 1	Peak 2	Peak 3	% Recovered	Peak 1	Peak 2
5	103.3	77.5	17.4	8.4	91.8	69.5	22.3
50	69.4	33.7	7.3	28.4	69.5	69.5	–[a]
120	70.1	32.9	7.4	29.4	74.2	63.2	11.0
180	77.7	31.0	12.1	54.6	–[b]	–[b]	–[b]

Values represent the percent of applied radioactivity which was recovered in the eluate, and the percent of applied radioactivity which eluted as ^{14}C-DGEBA (Peak 1) and as degradation products (Peaks 2 and 3).

[a]Only one radioactive peak was observed.

[b]A 180 min incubation was not run with simulated intestinal fluid.

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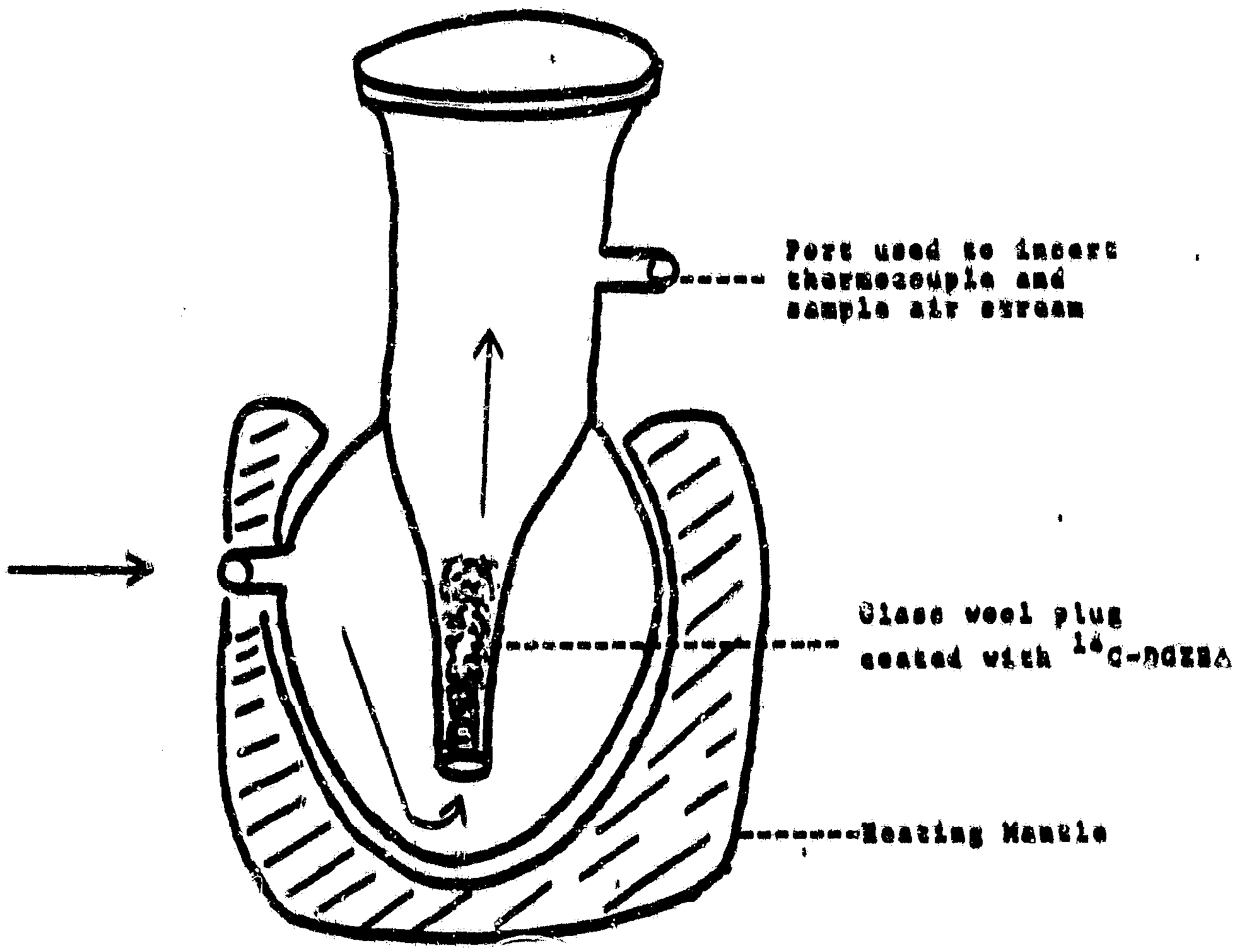
Figure 1 : Diagram of the Flask used to Vaporize ^{14}C-DGEBA. The arrows indicate the direction of airflow through the flask.



[redacted]

Figure 2: Diagram of System Used to Generate ^{14}C-DCBA Vapors. Arrows indicate the direction of airflow through the system.

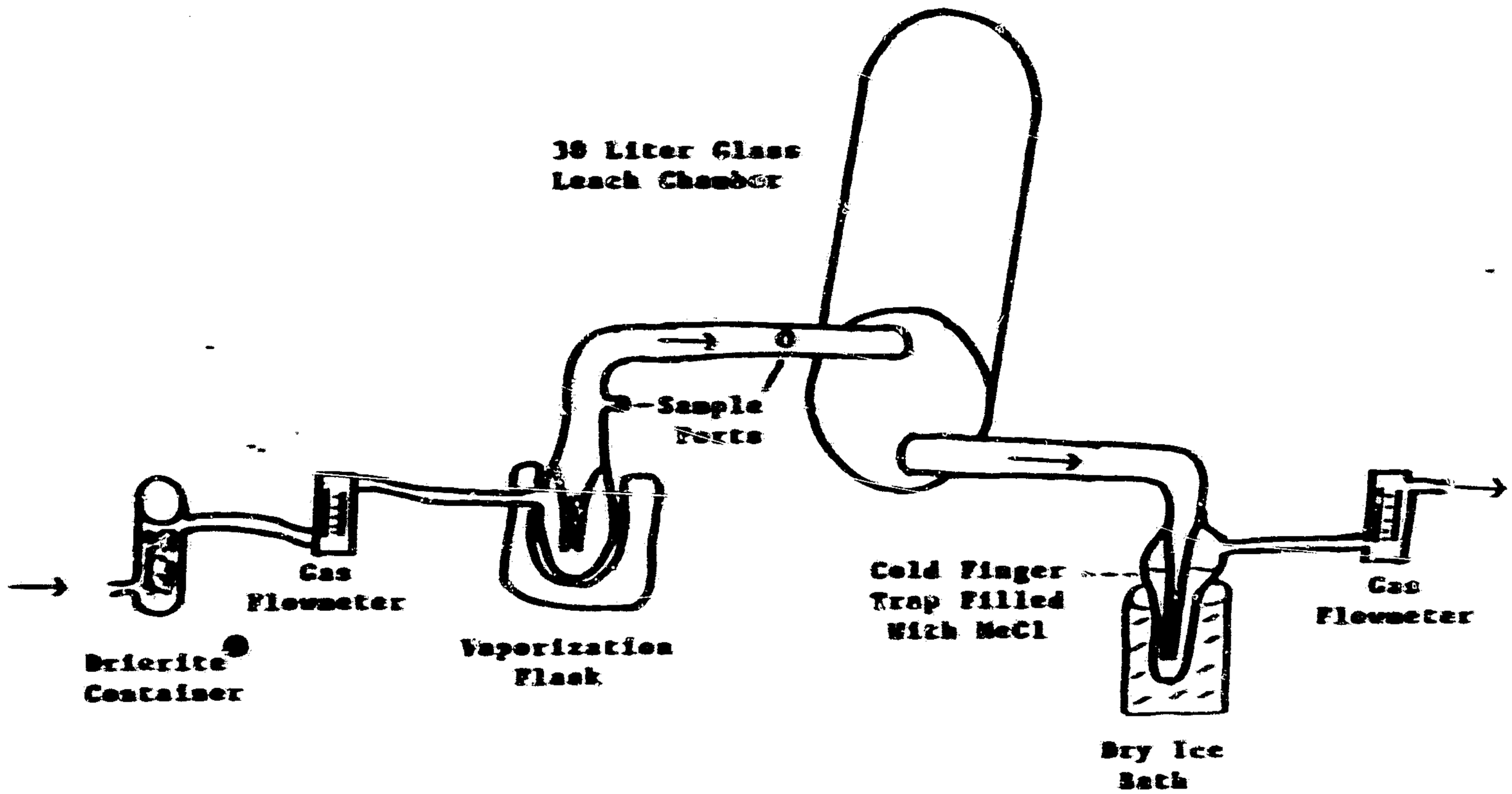


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Figure 3: Time Course of Radioactivity in Rat Plasma Following Oral administration of 2.7 mg ^{14}C-DCEBA/kg.

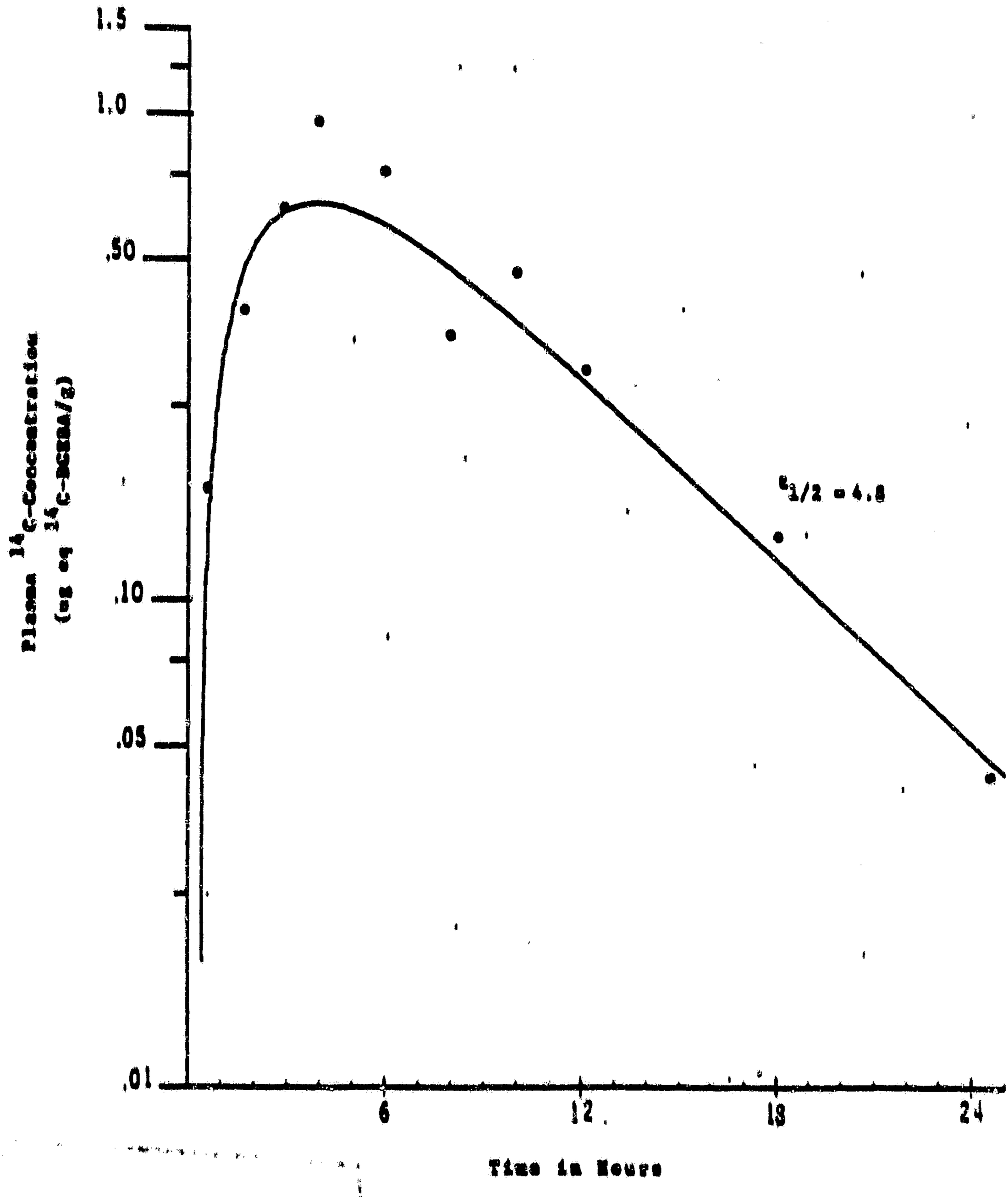


Figure 4: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.45 mg ^{14}C-DCEDA/kg.

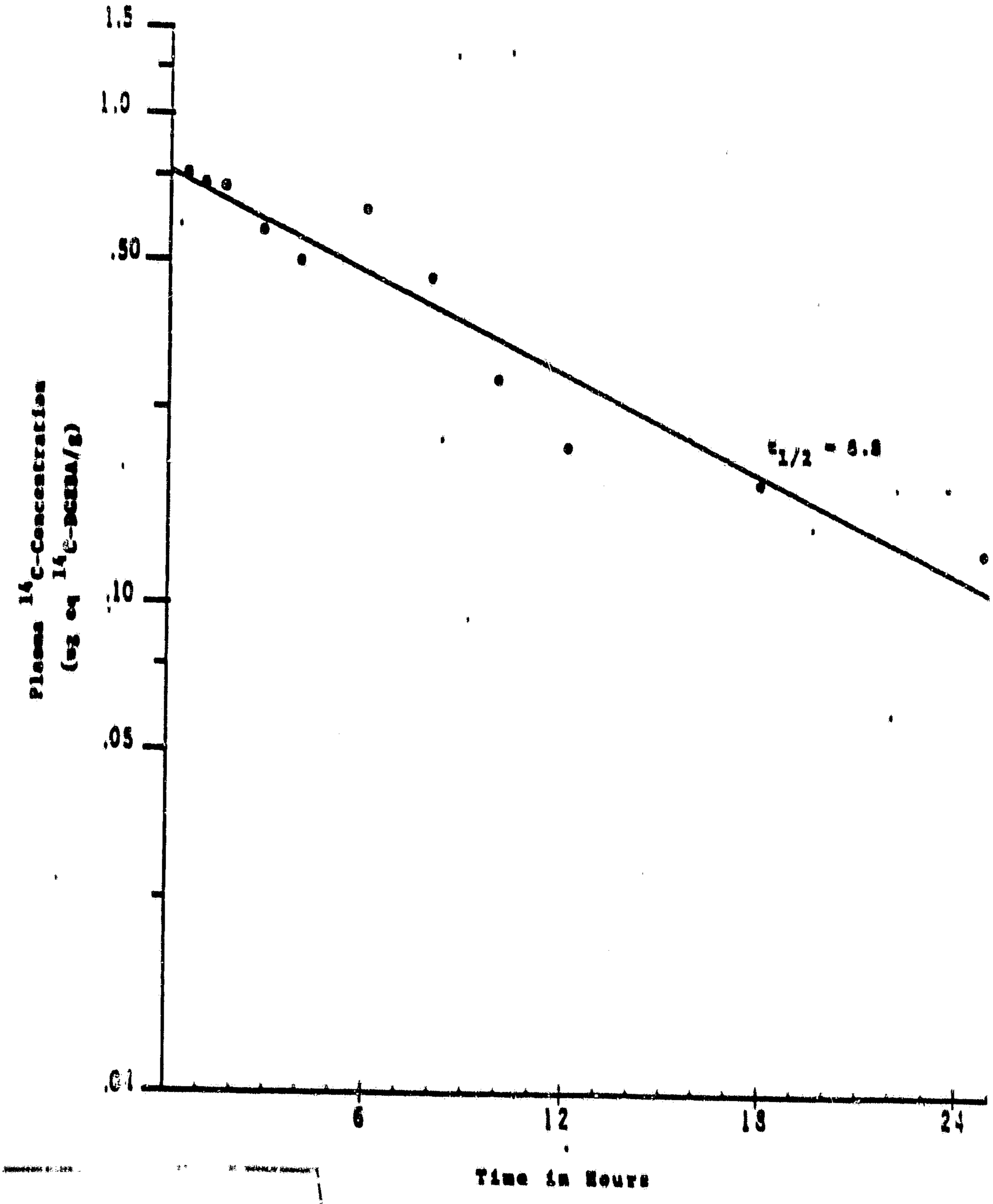
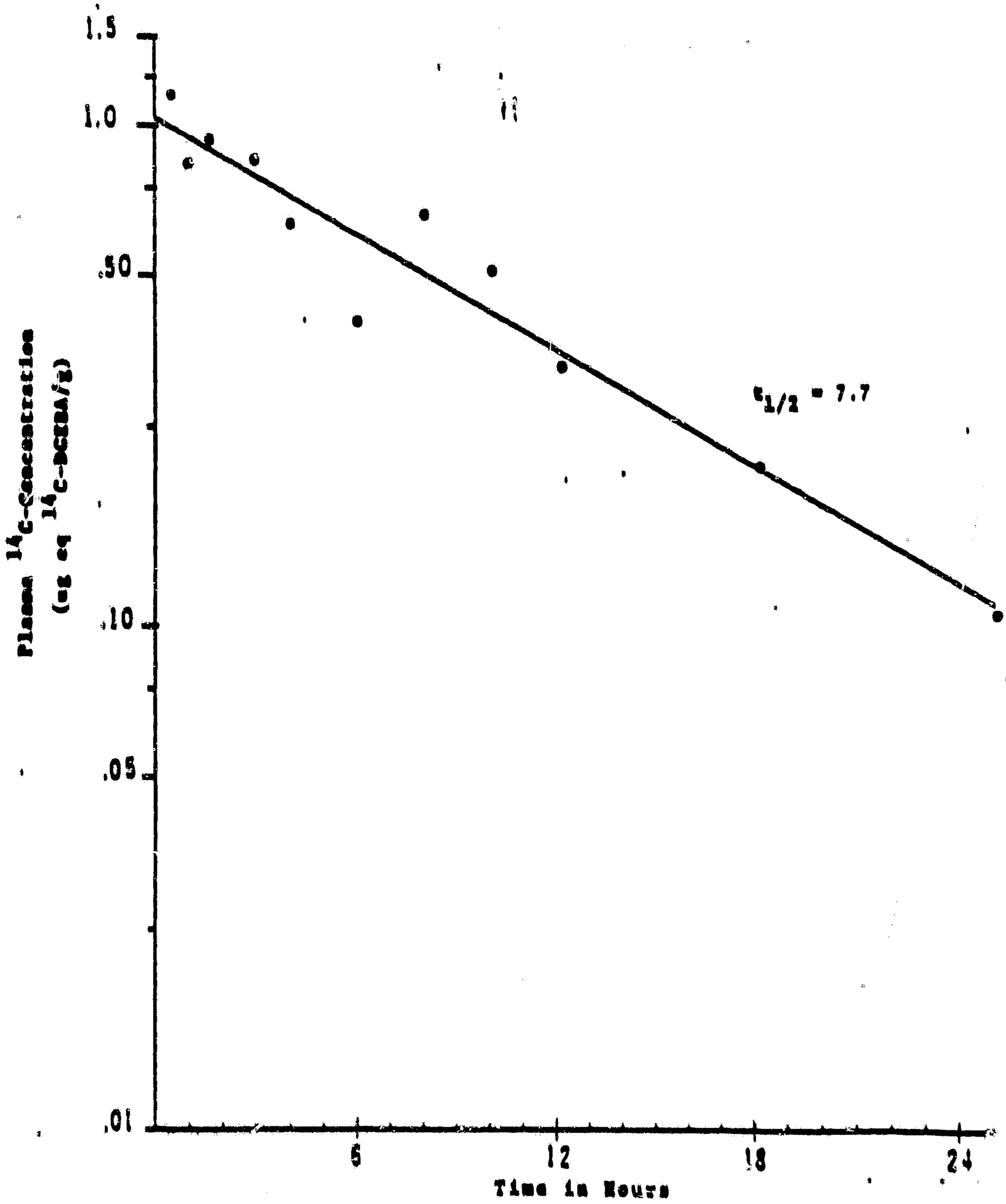


Figure 3: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.45 mg ^{14}C-DCEBA/kg to Bile Cannulated Rat.



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Doc Title: DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA) FATE IN MALE FISCHER 344 RATS (PROBE)

Chemical Name (100 char max)	CAS No. (10)
GLYCIDOL & ITS DERIVATIVES	999999994
DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA)	25085-99-8

D-406

DIGLYCIDYL ETHER OF BISPHENOL A (DGEBA): FATE IN MALE FISCHER 344 RATS (FRODA)

By

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ABSTRACT

Concern has been expressed that diglycidyl ether of bisphenol A (DGEBA), a major component of DER 331* epoxy resins, may represent an inhalation hazard. Due to its physical properties meaningful repeated inhalation exposure studies cannot be conducted. Thus oral toxicity studies have been proposed as an alternate means to evaluate the hazard. The purpose of this study was to obtain preliminary data on the metabolism and kinetics of DGEBA. The rationale being that oral toxicity data could be used to predict the inhalation hazard, if the fate of DGEBA were route-independent.

An atmosphere suitable for a single 6 hr inhalation exposure to ^{14}C-DGEBA could not be generated. Thus intravenous administration was substituted for the inhalation route. Route dependent differences were observed in the plasma ^{14}C-concentration time profile, tissue/ plasma ^{14}C-ratios and the urinary ^{14}C-excretion following oral and intravenous administration. These differences suggest that little of the orally administered ^{14}C-DGEBA was absorbed unchanged. Consistent with this, 4 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA. ^{14}C-DGEBA was not stable in simulated gastric fluid and degradation of ^{14}C-DGEBA in the stomach or during its initial transit through the liver could account for the route dependent differences observed in the fate of ^{14}C-DGEBA. These data indicate that oral toxicity data would be of little value in assessing the inhalation hazard. Furthermore, the inability to generate a suitable atmosphere using low airflows, high temperatures and a large surface area suggests that inhalation is an insignificant route for DGEBA exposure.

*Trademark of The Dow Chemical Company.

INTRODUCTION

The objective of these studies was to provide data on the utility of conducting oral toxicity studies to assess the possible inhalation hazard associated with the diol/oxidyl ether of bisphenol A (DGEBA). DGEBA, a bifunctional epoxide, is the major reactive component of DER 331* epoxy resins. Although no human exposure data are available, it has been speculated that DGEBA may represent an inhalation hazard because it contains two reactive epoxide bonds. Ideally, an inhalation study should be conducted to assess a potential inhalation hazard. However, because of the physical properties of DGEBA, it is unlikely that an atmosphere suitable for a repeated exposure inhalation study could be generated. Therefore, oral toxicity data have been proposed as a means to assess the hazard associated with inhaled DGEBA.

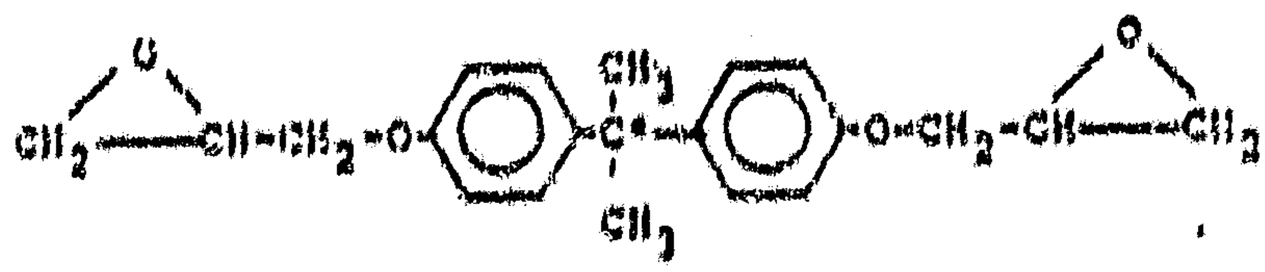
It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body, and that route dependent differences in the fate of a [illegible]ical may significantly alter its toxicity. This study was undertaken to provide preliminary data on the metabolism and kinetics of ^{14}C-DGEBA. The work described in this report was designed: (1) to determine if an atmosphere could be generated that would be suitable for a single exposure inhalation kinetic study with ^{14}C-DGEBA, (2) to compare the fate of ^{14}C-DGEBA following oral and intravenous administration, (3) to determine the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid, and (4) to compare the chromatographic profile of radioactivity in plasma following oral and intravenous administration of ^{14}C-DGEBA.

*Trademark of The Dow Chemical Company.

METHODS

Animals: Male Fischer 344 rats weighing 195-250 g were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. The animals were housed in rooms designed to maintain a constant temperature (72 ± 2°F), humidity (50 ± 10%), 12 hr photocycle, and 10-12 air changes per hour. Ralston Purina Rat Chow and tap water were available *ad libitum* except for an 18 hr fast prior to dosing the rats used to define the chromatographic profile of the plasma radioactivity. When required by the study objectives, jugular and biliary cannulas were implanted 48 hr prior to dosing using standard surgical procedures.

Test Material: p,p'-diglycidyl ether of bisphenol A isopropylidine, methylene-^{14}C (^{14}C-DGEBA; Lot #KD-TDL-124) was synthetized from ^{14}C-labeled bisphenol A by T. Licky of The Dow Chemical Company.



*Location of carbon-14 label

The radiotracer had a specific activity of 7.98 μCi/mg and a radiochemical purity of 98% as determined by high pressure liquid chromatography (HPLC) with fractional collection and Carbon-14 analysis of the column effluent (T. Licky, 1979).

Reagent grade DGEBA (Lot #152391) was used to adjust the specific activity of the ^{14}C-DGEBA in the vapor generation experiments and as an internal standard for the HPLC analysis.

EXPERIMENTAL

Vapor Generation: Reagent grade DGEBA or a mixture of reagent grade and ^{14}C-DGEBA (specific activity 1.3 x 10^6 dpm/g) was dissolved in methylene chloride ($MeCl_2$). An aliquot of the $MeCl_2$ solution was applied to a glass wool plug lodged in the center well of a vaporization flask (Figure 1). The $MeCl_2$ was removed by evaporation. The flask was then placed in a heating mantle and connected to the chamber as depicted in Figure 2. When connected in this manner, air passed through a Drierite® container, a flow meter, the vaporization flask, a 30 liter glass inhalation chamber, a cold finger trap containing 50 ml of $MeCl_2$ and immersed in a dry ice bath, and a second flow meter. The line connecting the vaporization flask to the chamber was constructed of 1 inch i.d. glass tubing. This line was ~40 cm long and contained one 90° bend ~15 cm above the glass wool plug. There were two ports in this line through which a thermocouple could be inserted, or samples of the air stream collected. One port was ~5 cm above the glass wool plug and the other port was ~13 cm upstream from the chamber.

During the trial vaporizations, the outer surface of the vaporization flask was heated to 410°C. Air was passed through the vaporization flask at 8 l/min. At this airflow, the temperature of the air stream 5 cm above the glass wool plug and 13 cm from the chamber was 140°C and 21-27°C, respectively. Known volumes of air (~30 l) were drawn *via* the ports and passed through cold finger traps containing 50 ml of $MeCl_2$. The amount of DGEBA trapped in the cold finger traps was determined by radioactivity or HPLC analysis. The concentrations of DGEBA in the air stream at the two sample ports and exiting the chamber were calculated from the amount of DGEBA trapped and the volume of air sampled.

Fate of Orally and Intravenously Administered ^{14}C-DOEBA

^{14}C-DOEBA was either dissolved in corn oil and administered by gavage, or suspended in fresh heparinized rat plasma[1] and administered via the jugular cannula. The oral dose was 5.0 mg (0.5 mCi) and the intravenous dose was 90 mg (0.10 mCi) of ^{14}C-DOEBA. Following dosing, the rat with the bile cannula was placed in a restraining cage. The other rats were placed in individual glass Roth-type metabolism cages. Plasma, urine, feces and bile samples were collected at the intervals listed below:

Hrs After Administration of ^{14}C-DOEBA

Blood	0.5, 1.0, 1.7, 3, 4, 6, 8, 10, 12, 18 and 24
Urine	6, 12 and 24
Feces	24
Bile*	1, 2, 3, 6, 12 and 24

*Bile cannulated animal only.

The blood was centrifuged to obtain the plasma. Urine and bile were collected and stored frozen until analysed. Aliquots of the plasma, urine and bile were added directly to ACS liquid scintillation cocktail (Mallinckrodt Chemical Company) and analyzed for radioactivity. All animals were sacrificed 24 hr after dosing. The liver, lungs, kidneys and intestines were removed from the non-bile cannulated animals. The intestine was sectioned and flushed with water to remove the intestinal contents. Aqueous homogenates of the intestine and feces were prepared. Aliquots of the tissues, feces and intestinal contents were oxidized in a biological materials oxidizer. The $^{14}CO_2$ produced during oxidation was trapped and quantitated using standard laboratory procedures.

[1]HPLC analysis demonstrated ^{14}C-DOEBA was stable in rat plasma over the interval taken to prepare and administer the dose solution.

Stability of ^{14}C-DOEBA in Simulated Gastric and Intestinal Fluid: Simulated gastric and intestinal fluid were prepared as described in the 1970 U.S. Pharmacopeia. ^{14}C-DOEBA was added to 2 ml of the simulated gastric or intestinal fluid and incubated in a 37°C water bath. Sequential aliquots of the incubation mixture were removed 5, 60, 120, and 180 (gastric only) minutes after the start of the incubation, and neutralized with 0.2 N KOH or 0.1 N HCl. Aliquots (20 µl) of the neutralized incubation mixture were analyzed by high pressure liquid chromatography (HPLC).

Chromatographic Profile of Plasma Radioactivity: The dose solutions were prepared and administered as previously described except that the amount of radioactivity administered was increased. In this segment 37.8 and 8.3 µCi of ^{14}C-DOEBA/animal were administered orally and intravenously, respectively. Administration of the oral and intravenous dosing solutions were alternated and staggered in time to control for variation due to sequence of administration, sacrifice and analysis. The animals were killed by exsanguination 4 hr after dosing. The blood was centrifuged and 20 µl of the plasma analyzed by HPLC.

High Pressure Liquid Chromatography (HPLC): The HPLC method described by Paul (1976) was used with minor modifications. The sample was applied to the column using a sample loop. Reagent grade DOEBA was co-chromatographed with those samples containing ^{14}C-DOEBA. Fractions (1-2 ml) of the column effluent were collected and analyzed for radioactivity. In addition the optical density (280 nm) of the column effluent was monitored to determine when DOEBA eluted or to quantitate DOEBA in those samples which did not contain ^{14}C-DOEBA. The HPLC conditions were as follows:

Column: 30 cm x 4 mm μPorasil (Waters Associates)

Mobil Phase: 2 ml/min going from 7 to 100% Solvent B over 26 min using program 6 or 10 on a Waters Associates Model 600 programmer.

Solvent A: Hexane containing 0.01% (v/v) benzene.

Solvent B: Tetrahydrofuran containing 15% (v/v) acetonitrile.

Thin Layer Chromatography: The following samples were spotted on five thin layer chromatography (TLC) plates coated with 0.1 mm of cellulose F (E. M. Reagents):

1. Stock ^{14}C-DGEBA
2. $MeCl_2$ extract from glass wool plug used in vaporization ^{14}C-DGEBA
3. Bile from bile cannulated rat
4. Urine from bile cannulated rat
5. Urine from the orally dose rat

One TLC plate was developed with each of the following solvents: dimethyl sulfoxide, pyridine, methanol, 1,4-dioxane and dimethyl foramide. The location of radioactivity on the TLC plates was determined using a beta-graph (Panax Equipment Limited).

Radioactivity Analysis: Radioactivity was quantified using a Mark II or Mark III Nuclear Chicago liquid scintillation spectrometer (Searle). Counts per min (cpm) were corrected for quench and background, and converted to mg equivalents (mg) of ^{14}C-DGEBA.

Data Analysis: Rate constants and half-lives were obtained by least squares analysis. Area under the plasma ^{14}C-concentration time curve (AUC) was calculated using the trapezodial rule. The volume of distribution (Vd_{area}) for ^{14}C-DGEBA in the rat was calculated as:

$$Vd_{area} = \frac{\text{Dose}}{\text{AUC} \times \text{Rate Constant for Elimination}}$$

RESULTS

Generation of DGEBA Vapors: The system depicted in Figure 2 was the only system found that generated measurable concentrations of DGEBA vapors at respirable temperatures. In this system DGEBA was placed on glass wool to maximize the surface area, and hot air (410°C) was used to enhance vaporization. The line connecting the vaporization flask and chamber was large, contained no restrictions, and was just long enough to permit the air stream to cool to a respirable temperature (~32°C). However, even under these conditions, it was not possible to generate an atmosphere containing sufficient ^{14}C-DGEBA for a kinetic study.

In one trial the concentration of ^{14}C-DGEBA in the air 5 cm above the vaporization flask was 5.9 µg/l (0.5 ppm). The concentration of ^{14}C-DGEBA in air 13 cm upstream from the chamber was 0.6 µg/l (0.04 ppm), and in the air exiting the chamber was 0.05 µg/l (0.004 ppm). These data were consistent with the results of other trial vaporizations; i.e., significant amounts of DGEBA could be vaporized at high temperatures but the concentration decreased rapidly when the temperature decreased or the airflow was obstructed. The observed decrease in ^{14}C-DGEBA concentrations were apparently due to condensation since the walls of the line connecting the vaporization flask to the exposure chamber were coated with a sticky film.

Higher concentrations of DGEBA vapors may have been generated in one trial vaporization in which reagent grade DGEBA and HPLC analysis were used instead of ^{14}C-DGEBA and ^{14}C-analysis. In this experiment, concentrations of DGEBA in air 5 cm above the vaporization flask, 13 cm upstream from the chamber and exiting the chamber were 51.4, 150.4, and 93.2 µg/l or 3.7, 10.8 and 6.7 ppm, respectively. However, because the lowest concentration was found just above the vaporization flask, these data are suspect. Even if these results are valid, the concentrations of DGEBA in the air were low considering the conditions used to generate the vapors, and the atmosphere was not sufficiently stable to conduct an inhalation exposure study.

<u>Fate of Orally and Intravenously Administered ^{14}C-DGEBA:</u>
Intravenous administration was substituted for the inhalation route, since it was not possible to generate an atmosphere suitable for a single inhalation exposure kinetic study. The intravenous route was selected because ^{14}C-DGEBA is

placed directly into the systemic circulation[2]. The lung is the only barrier inhaled ^{14}C-DGEBA must cross to reach the systemic circulation and once it enters the systemic circulation its fate is independant of the route of administration.

Table 1 presents the distribution of radioactivity recovered 24 hr after oral and intravenous administration of ^{14}C-DGEBA. Total recovery was low, however, the fraction of the dose recovered following oral (66%) and intravenous (52%) administration was similar in the non-bile cannulated animals. The feces was the primary route of excretion following oral (51%) and intravenous (42%) administration. The liver was the primary excretory organ for ^{14}C-DGEBA as evidenced by the fact 56% of the ^{14}C-DGEBA given intravenously was excreted <u>via</u> the bile within 24 hr. Urine was a minor route of excretion with 8 to 9% of the oral and intravenous dose excreted by this route. Another 2% of the oral and 0.5% of the intravenous dose was recovered in the intestine plus contents. The tissues, i.e. liver, kidney, lungs and intestine, contained 1 to 2% of the dose.

Differences were also observed in the distribution of radioactivity in the tissues 24 hr after oral and intravenous administration of ^{14}C-DGEBA (Table 2). The plasma contained three times more radioactivity 24 hr after the 0.43 mg/kg intravenous dose than following the 2.7 mg/kg oral dose. In contrast the tissue/plasma ^{14}C-ratios especially for the liver, intestine and lungs were higher following oral administration.

[2]Circulatory system minus the hepatic-portal system. An orally administered chemical must pass through the liver before it reaches the rest of the body and some chemicals are extensively metabolized and/or excreted during their initial transit through the liver.

The plasma ^{14}C-concentration time profiles following oral and intravenous administration of ^{14}C-DGEBA are depicted in Figures 3, 4 and 5. Pharmacokinetic parameters derived from the plasma ^{14}C-data are summarized in Table 3. The absorption of radioactivity following oral administration of ^{14}C-DGEBA was rapid (t 1/2 = 0.7 hr) and the highest plasma ^{14}C-concentration (0.97 µg eq/g) was observed 4 hr after dosing. The radioactivity was cleared from the plasma in an apparent first-order manner following both oral (Figure 3) and intravenous (Figure 4 and 5) administration. However, the radioactivity was cleared from the plasma faster following oral administration (t 1/2 = 4.8 hr) than following intravenous (t 1/2 = 7.7 hr) administration. The area under the plasma ^{14}C-concentration time curve (AUC) was slightly smaller following oral (515 µg eq min/g) than after intravenous administration (601 µg eq min/g). Assuming the radioactivity represents the same chemical entity, the relative AUC following oral and intravenous administration suggest that only 77 µg or 13% of the 570 µg of ^{14}C-DGEBA administered orally was absorbed. However, of the 77 µg absorbed 50 µg (65%) was excreted in the urine. In contrast, only 8% of the ^{14}C-DGEBA administered intravenously was excreted in the urine. The differences found in the half life for plasma radioactivity, its excretion in the urine, and the tissue/plasma ^{14}C-ratios suggest that the radioactivity in the plasma following oral and intravenous administration of ^{14}C-DGEBA represent different chemical entities.

Thin layer chromatography (TLC) of selected urine and bile samples indicated that different metabolites were excreted following oral and intravenous administration of ^{14}C-DGEBA.

Based on the TLC, no unchanged ^{14}C-DGEBA was excreted in either the urine or bile. The major metabolite found in the bile was also found in the urine following intravenous but not oral administration. Metabolites of ^{14}C-DGEBA were found in the urine following oral administration that were not found in either the bile or urine of the intravenously dosed rat.

<u>Stability of ^{14}C-DGEBA in Simulated Gastric and Intestinal Fluid</u>: The stability of epoxides are known to be pH dependent. Orally administered ^{14}C-DGEBA is subjected to pH's ranging from 1.2 in the stomach to 7.5 in the intestines. Hydrolysis of the epoxide bond(s) in the gastrointestinal tract could explain the poor absorption of orally administered ^{14}C-DGEBA and account for the observed differences in the fate of orally and intravenously administered ^{14}C-DGEBA. Therefore, the stability of ^{14}C-DGEBA in simulated gastric and intestinal fluid was investigated.

Table 4 presents the result obtained when ^{14}C-DGEBA was incubated with simulated gastric or intestinal fluid. Recovery of radioactivity applied to the HPLC column decreased from >90% for the 5 min incubations to about 70% for incubations of 30 min or more. Radioactivity in the simulated gastric fluid eluted from the HPLC column in three distinct peaks. The first peak co-eluted with reagent grade DGEBA; i.e., it had the same retention volume as the UV peak for reagent grade DGEBA. The two remaining peaks eluted after DGEBA and based on their larger retention volumes represent polar degradation products of DGEBA. The fraction

of the applied radioactivity which eluted as ^{14}C-DOEBA decreased from 77.5 to 11.0% as the incubation time was increased from 5 to 180 min. The fraction of the radioactivity in the simulated gastric fluid eluting as polar degradation products (peaks II and III) increased from 25.8 to 66.7% as the incubation time increased from 5 to 180 min. Based on these data, the half life for the degradation of ^{14}C-DOEBA in simulated gastric fluid was 70 min.

Two radioactive peaks were observed when the simulated intestinal fluid was subjected to HPLC. The first and largest peak had the same retention volume as reagent grade DOEBA. The length of the incubation had no effect on the fraction of the applied radioactivity which eluted as DOEBA or in the second peak. This suggests that ^{14}C-DOEBA is not rapidly hydrolysed at pH's found in the small intestine.

<u>Chromatographic Profile of Plasma Radioactivity</u>: Based on the elimination and tissue distribution data, the radioactivity in the plasma following oral and intravenous administration appeared to represent different chemical species. In an attempt to verify this, rats were given ^{14}C-DOEBA by gavage or intravenously, sacrificed 4 hr after dosing, and their plasma collected for analysis. The mean (± SD) concentrations of radioactivity in the plasma of the orally and intravenously dosed rats were 2514 ± 514 and 559 ± 202 dpm/20 µl, respectively. Thus, the plasma from intravenously dosed rats did not contain sufficient radioactivity to permit its chromatographic profile to be resolved. However, when the plasma from the orally dosed rats was subjected to HPLC, less than 10% of the radioactivity eluted as ^{14}C-DOEBA. This suggests that only a small percentage of the orally administered ^{14}C-DOEBA reached the systemic circulation unchanged.

DISCUSSION

It is generally recognized that toxicity is a function of the chemical species and its concentration at a critical site(s) in the body. Observed differences in the plasma ^{14}C-concentration time profile, tissue/plasma ^{14}C-ratios and urinary excretion of radioactivity following oral and intravenous administration of ^{14}C-DGEBA suggest that little if any orally administered ^{14}C-DGEBA is absorbed unchanged, and that the chemical identity of the radioactivity in the plasma depends on the route of administration. Consistent with this, 6 hr after oral administration less than 10% of the radioactivity in the plasma represented unchanged ^{14}C-DGEBA, and the radioactivity in the urine exhibited a different chromatographic profile following oral and intravenous administration of ^{14}C-DGEBA. Thus these data indicate that oral toxicity studies would be of little value in assessing the possible hazard associated with inhalation of DGEBA.

Based on the half-life for the degradation of ^{14}C-DGEBA in simulated gastric fluid (t 1/2 = 70 min) and for the absorption of radioactivity following oral administration (t 1/2 = 42 min), over a third of the orally administered ^{14}C-DGEBA would be degraded in the gastrointestinal tract. Once absorbed, orally administered ^{14}C-DGEBA must pass through the liver to reach the rest of the body. The liver plays a major role in the excretion of ^{14}C-DGEBA as evidenced by the fact that 85% of the ^{14}C-DGEBA administered intravenously was excreted in the bile within 24 hr. Based on these observations it is not surprising that there are significant differences in the fate of orally and intravenously administered ^{14}C-DGEBA.

The problems encountered in trying to generate an atmosphere suitable for a single exposure kinetic study confirm previous

suspicions that it is unlikely a meaningful repeated inhalation exposure study could be conducted with DOEDA. Furthermore, the inability to generate a suitable atmosphere using low air flows, a large surface area, and high temperatures suggests that inhalation is an insignificant route for DOEDA exposure.

Written by:

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REFERENCES

Licky, T. (1979) Preparation and analysis of p,p'-Diglycidyl Ether of Bisphenol A Isopropylidine, methylene-^{14}C. Dow Research Notebook ES-CDL-124.

Park, A. M. (1976) Liquid Chromatography of DER-331 and Other Epoxy Resins. Dow Report NVA-177.

Pharmacopeia of the United States, Eighteenth Revision, September 1, 1970.

TABLE 1: DISTRIBUTION OF RADIOACTIVITY RECOVERED 24 HOURS AFTER ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DOXBA TO RATS[a]

Treatment	Dosed Orally	Dosed Intravenously	Dosed Intravenously Bile Cannulated
Dose µg	370	90	90
mg/kg	2.7	0.43	0.43[b]
µCi	4.5	0.72	0.72

Radioactivity Recovered	µg Equivalents of ^{14}C-DOXBA (% Dose in Parenthesis)		
Feces	303 (83%)	38.7 (43%)	3 (3%)
Bile	c	c	77 (86%)
Urine	50 (9%)	6.9 (8%)	36 (42%)
Intestinal Contents	12 (2%)	0.4 (4%)	c
Tissues[d]	13 (2%)	1.1 (1%)	c
TOTAL	370 (96%)	47.1 (52%)	116 (131%)

[a]Data are for one animal at each treatment.

[b]Body weight not available for this animal dose rate estimated assuming rat weighed 210 g.

[c]Sample not collected from this animal.

[d]Total µg eq of ^{14}C-DOXBA found in the liver, kidneys, lungs and intestines.

TABLE 21 CONCENTRATION OF RADIOACTIVITY IN THE TISSUES 24 HOURS AFTER DOSING EXPRESSED AS mg eq ^{14}C-DOXRA/g AND AS TISSUE/PLASMA RATIOS.

Tissue	Oral		Intravenous	
	Mg eq/g	Tissue/Plasma	Mg eq/g	Tissue/Plasma
Liver	0.64	15	0.07	0.6
Kidney	0.05	1	0.07	0.5
Lung	0.17	4	0.07	0.6
Intestine	0.55	13	0.04	0.3
Plasma	0.04		0.13	

Values based on one orally and one intravenously dosed rat.

TABLE 3: PARAMETERS DESCRIBING THE TIME COURSE OF RADIOACTIVITY IN THE RAT FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-DOEBA.

	Oral	Intravenous	Bile Cannulated
Dose (µg)	570	90	90
(mg/kg)	2.7	0.43	-[a]
Maximum Plasma ^{14}C-DOEBA Conc (µg eq/g)	0.97	0.76	1.16
Time Maximum Plasma ^{14}C-DOEBA Conc Observed (hr)	4	0	0
Absorption Rate Constant (hr^{-1})	1.0	-[b]	-[b]
Half Life in hr	0.7	-[b]	-[b]
Elimination Rate Constant (hr^{-1})	0.15	0.08	0.09
Half Life in hr	4.8	8.8	7.7
Area Under ^{14}C-Plasma Conc Time Curve (µg eq . min/g)	526	601	697
Dose Adjusted Area Under ^{14}C-Plasma Conc Time Curve	0.21	6.7	7.7
Volume of Distribution (ml/Rat)	436	114	86
Clearance (ml/min/Rat)			
Urinary[c]	0.10	0.01	0.06
Biliary[c]	-[b]	-[b]	0.11
Body[d]	1.16	0.15	0.13

Parameters based on data from one animal.

[a]The body weight of this rat is not available.

[b]Calculation of this parameter is inappropriate.

[c]Calculated as amount excreted in urine or bile divided by the area under plasma ^{14}C-conc time curve.

[d]Calculated as the dose divided by the area under the plasma ^{14}C-conc time curve.

TABLE 4: ELUTION OF RADIOACTIVITY FROM THE COLUMN WHEN ^{14}C-DCBA WAS INCUBATED WITH SIMULATED GASTRIC OR INTESTINAL FLUID, AND SUBJECTED TO HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC).

Length of Incubation (min)	Gastric Fluid				Intestinal Fluid		
	% Recovered	Peak 1	Peak 2	Peak 3	% Recovered	Peak 1	Peak 2
5	103.3	77.5	17.4	8.4	91.8	69.5	22.3
50	69.4	33.7	7.3	28.4	69.5	69.5	-[a]
120	70.1	32.9	7.4	29.4	74.2	63.2	11.0
180	77.7	11.0	12.1	54.6	-[b]	-[b]	-[b]

Values represent the percent of applied radioactivity which was recovered in the eluate, and the percent of applied radioactivity which eluted as ^{14}C-DCBA (Peak 1) and as degradation products (Peaks 2 and 3).

[a]Only one radioactive peak was observed.

[b]A 180 min incubation was not run with simulated intestinal fluid.

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Figure 1 : Diagram of the Flask used to Vaporize ^{14}C-DGEBA. The arrows indicate the direction of airflow through the flask.

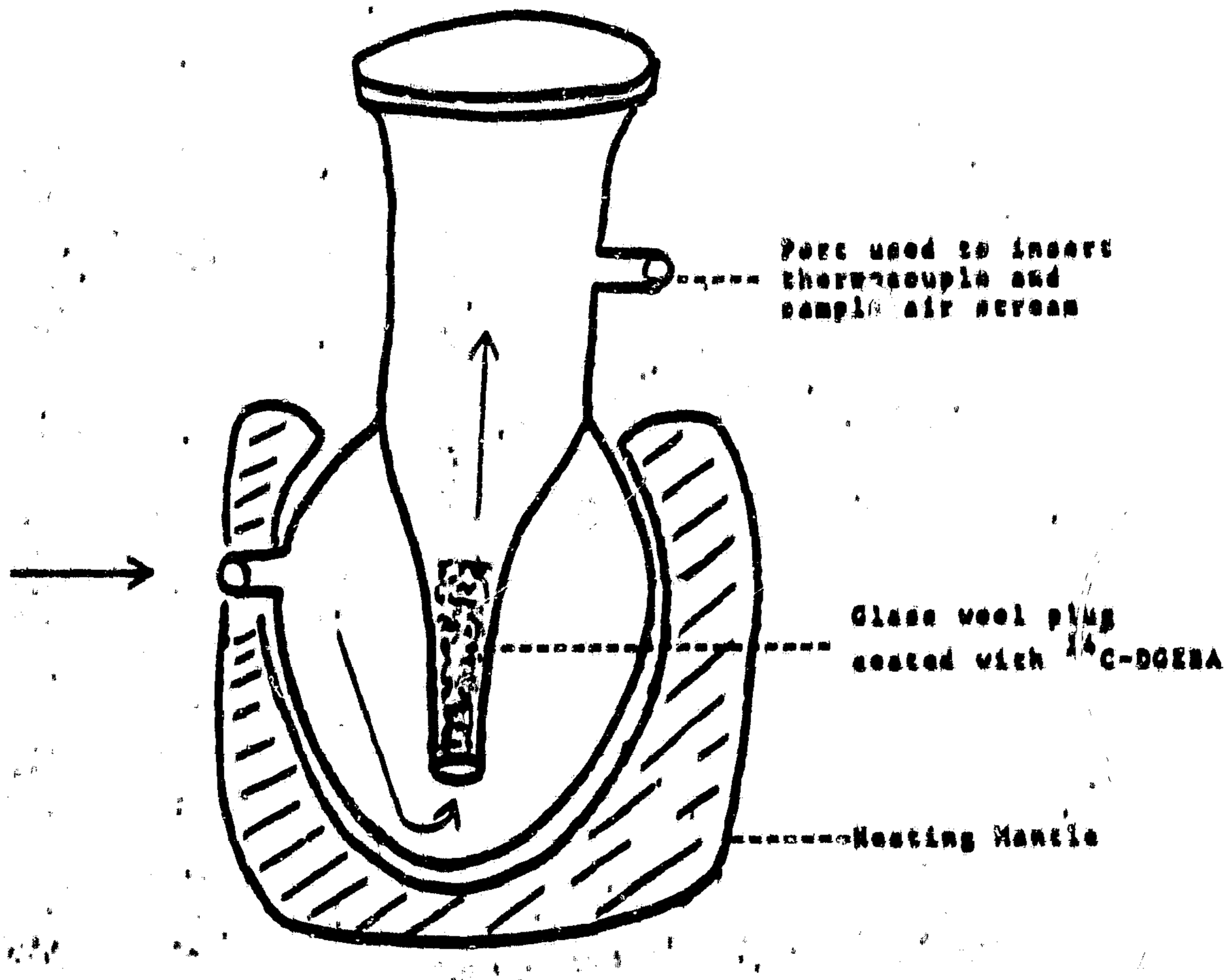

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Figure 2: Diagram of System Used to Generate ^{14}C [illegible] Arrows Indicate the direction of airflow through the [illegible]

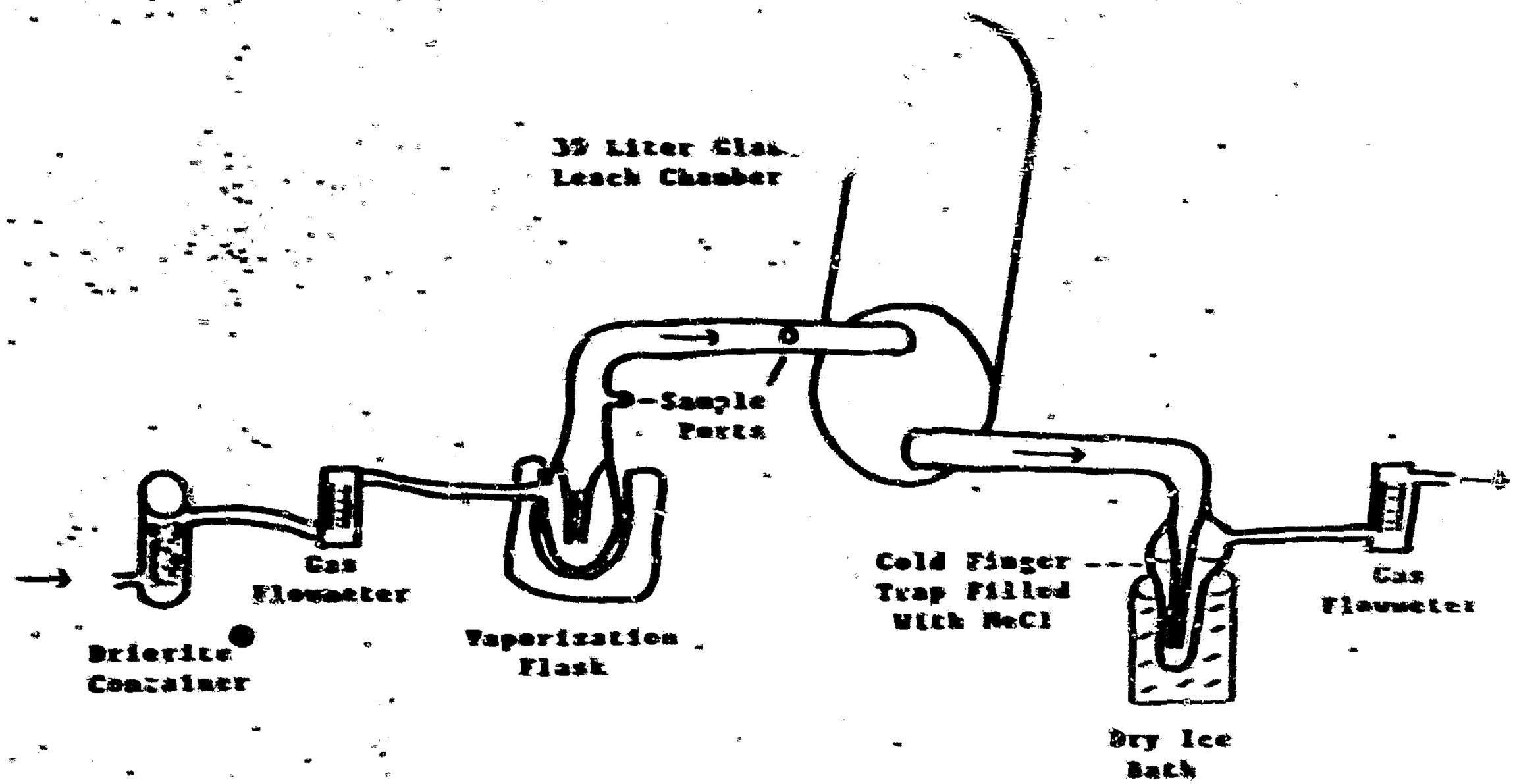

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Figure 3: Time Course of Radioactivity in Rat Plasma Following Oral administration of 2.7 mg ^{14}C-DGEBA/kg.

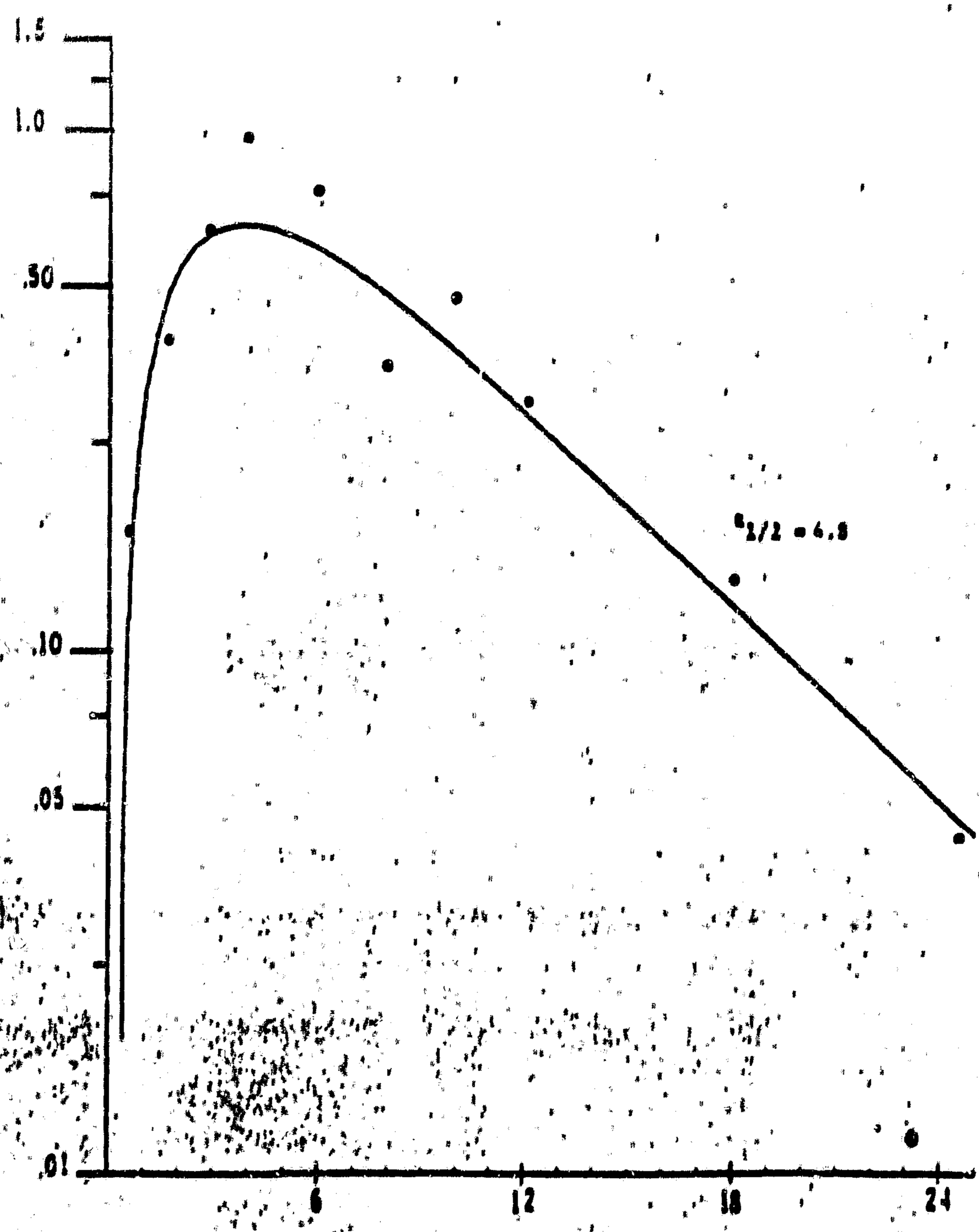

Figure 4: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.43 mg ^{14}C-DGEBA/kg.

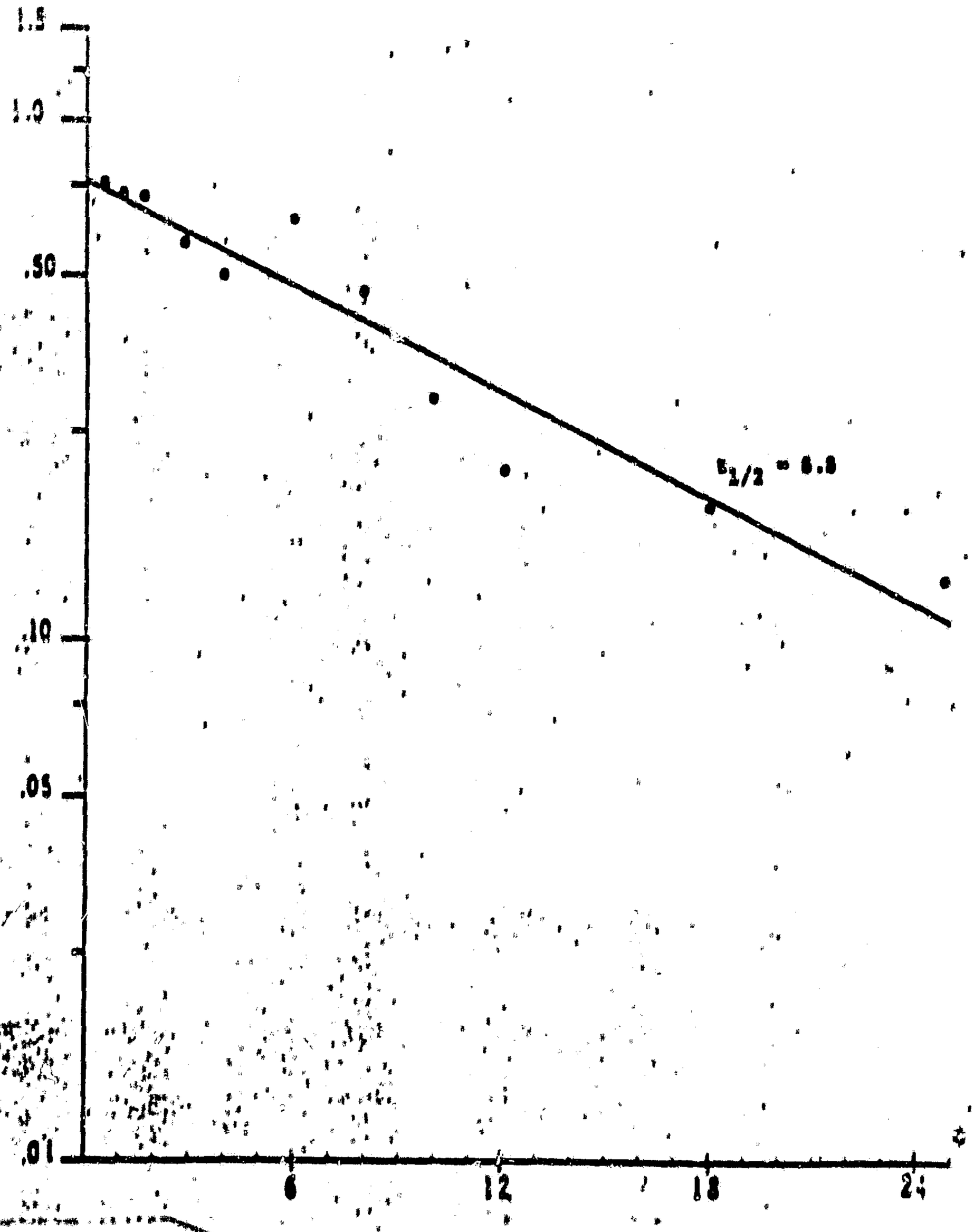

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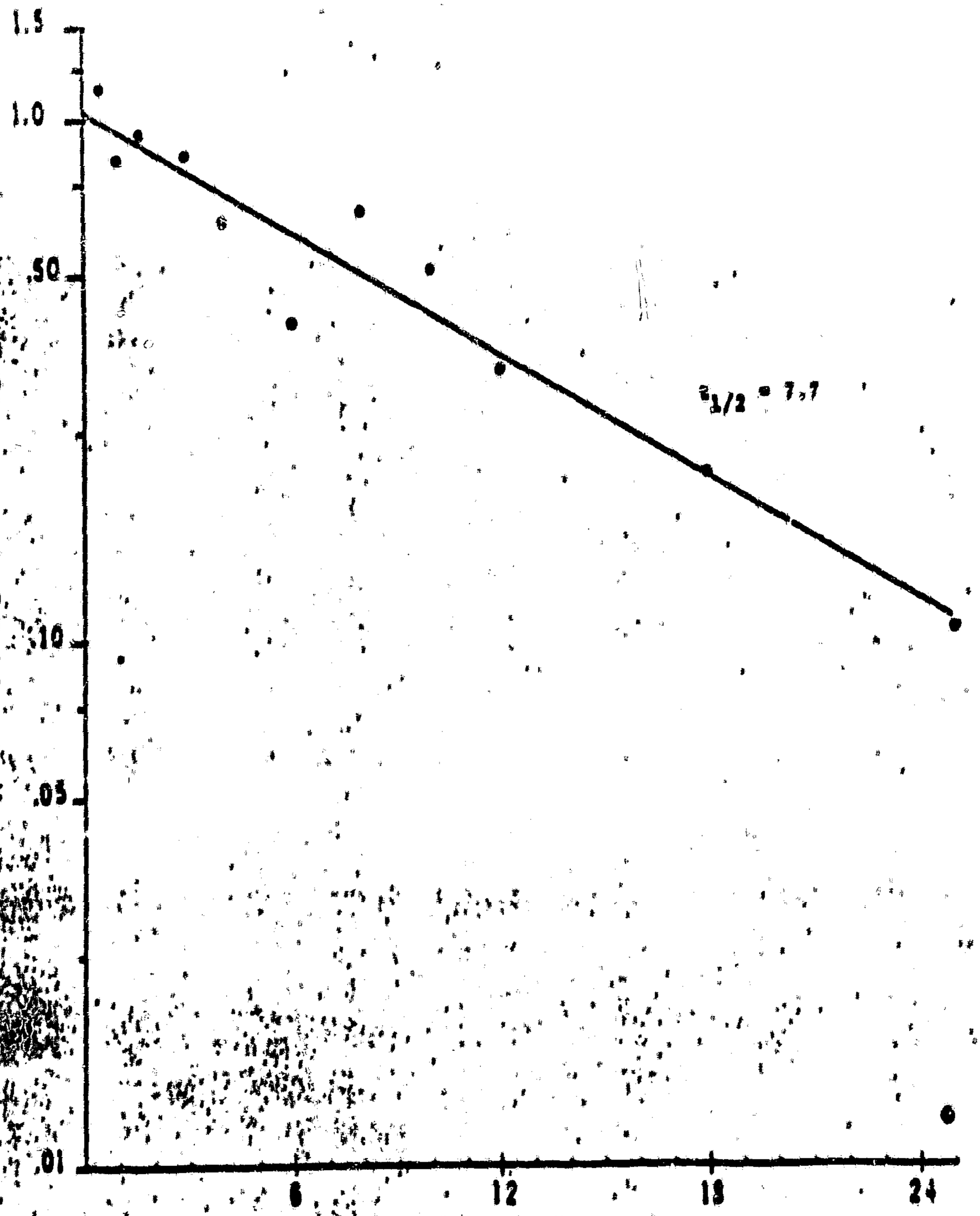
Figure 5: Time Course of Radioactivity in Rat Plasma Following Intravenous Administration of 0.45 mg ^{14}C-DCEBA/kg to Bile Cannulated Rat.



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LIST OF STUDIES IN PROGRESS

WITH COVER LETTER

Chemical Name (100 CHAR MAX) 25	CAS No. (10) 24
GLYCIDOL & ITS DERIVATIVES	999999994
DIGLYCIDYLETHER OF	25085-99-8
BISPHENOL A	

87810965

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MIDLAND, MICHIGAN 48640

December 9, 1982

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Dear Sir or Madam:

We are submitting the two attached lists of Health and Safety studies as required by the referenced rule.

The first list, marked D650, is a list of studies in progress as of the date of this submission.

Please note that the study entitled "Acrylamide: A Two-Year Drinking Water Chronic Toxicity - Onogenicity Study in CDF Fischer 344 Rats" is submitted on behalf of the following companies who are participating in the study:

Dow Chemical
American Cyanamid
Mobil
Nalco

The second list, marked D652, contains references to unpublished studies of which we are aware but do not have a copy.

Very truly yours,

R. L. Hagerman
Regulatory Specialist
Regulatory and Legislative Issues
Health and Environmental Sciences
1803 Building
(517)636-6855

rs

copy 1 of 8

000002

Dow Chemical Company
D-650
Studies in Progress as of Date of Submission

1. A Mortality Study of Men Engaged in the Manufacture of Organic Dyes.
   Purpose: Examine the mortality experience of men assigned to production areas in which aromatic amines were present as raw materials or chemical intermediates.
   Data: Mortality data
   Progress: Report submitted for publication
   Date of Completion: Unknown - when accepted for publication
   CAS Nos: 62533

2. *In Vitro* Reactivity Study of Alkylene Oxides
   Purpose: See title
   Data: Relative kinetic data
   Progress: Started Dec. 1, 1982
   Date of Completion: Report due 1984
   CAS Nos: 106887, 75218, 75569

3. Range-Finding Study of Methylchloroform Formulation XN 50120
   Purpose: See title
   Data: Mammalian toxicity
   Progress: Report being written
   Date of Completion: Jan. '83
   CAS Nos. 71556

4. Propylene Dichloride: Acute and Two-week Inhalation Study
   Purpose: See title
   Data: Toxicity in Rats, Mice and Rabbits
   Progress: Report being written
   Date of Completion: Jan '83
   CAS Nos: 78875

5. Methyl Chloride: Inhalation Toxicity in Female C57BL6 Mice Continuously or Intermittently Exposed for 12 Days
   Purpose: See title
   Data: See title
   Progress: Report to be written
   Date of Completion: First Quarter '83
   CAS Nos. 74873

6. Methylene Chloride: A Comparison of the Pharmacokinetics and Macromolecular Interactions of Inhaled ^{14}C-Methylene Chloride in Hamsters and Rats as Related to Toxicity
Purpose: Evaluate species differences in the fate of inhaled methylene chloride
Data: See title
Progress: Pathology in progress
Date of Completion: Sept. '84
CAS No: 79061

7. Acrylamide: A Two Year Drinking Water Chronic Toxicity - Onogenicity Study in DCF Fischer 344 Rats
Purpose: See title
Data: See title
Progress: Pathology in progress
Date of Completion: September '84
CAS No: 79061

8. Acrylamide: Pharmacokinetics in the Rat
Purpose: See title
Data: See title
Progress: Report to be written
Date of Completion: First quarter '83
CAS No: 79061

9. Acute Range-finding - Diglycidylether of Biphenol A
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS No: 1675543

10. Characterization of Butylene Oxide Blood Levels, Macromolecular Binding and Hepatic Nonprotein Sulfhydryl Levels After Inhalation Exposure in Rats
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS No: 26249207

11. 1,1,1-Trichloroethane: Two-year Inhalation in Rats and Mice
Purpose: See title
Data:
Progress: Awaiting pathology
Date of Completion: June, '83
CAS No: 71556

12. Reduction Dechlorination of Chlorinated Methanes, Ethanes, & Ethylenes in Aqueous Solutions
Purpose: Investigate non-biological, reductive dehalogenation of a series of chlorinated hydrocarbons in aqueous solution
Data: Laboratory analytical data on concentration of chemicals in solution as a function of conditions
Progress: Underway
Date of Completion: Mid-1983
CAS Nos: 75092, 71556

13. A Case-Control Study of Renal Cancer Mortality at a Texas Chemical Plant.
Purpose: To determine if a possible etiologic agent for human renal cancer can be found related to occupation.
Data: Mortality and past presumptive exposures to chemicals produced or used in the chemical plant.
Progess: Preliminary report nearing completion
Date of Completion: Early 1983

14. A Case-Control Study of Leukemia and Occupational Exposure to Ethylene Oxide.
Purpose: To determine if past presumptive exposures to ethylene oxide are associated with leukemia.
Data: Mortality and past presumptive exposures to ethylene oxide.
Progress: Work histories on cases and controls being collected.
Date of Completion: Early 1983.

15. A Cross-Sectional Study of Employees with Potential Workplace Exposure to Ethylene Oxide.
Purpose: To determine whether those with potential exposure to ethylene oxide had a higher prevalence of abnormalities of the hematopoietic, hepatic, renal or reproductive systems than did an unexposed control group.
Data: Medical surveillance and presumptive occupational exposure
Progress: Preliminary report in process of review and revision
Date of Completion: Early 1983

16. Methyl Chloride Mortality Study.
Purpose To evaluate the mortality patterns of employees with past potential exposure to methyl chloride.
Data: Mortality and past potential occupational exposure to methyl chloride.
Progress: Collecting work history and exposure data.
Date of Completion: Mid-1983

17. 1,1,1-Trichloroethane

Purpose: Define the time course of 1,1,1-trichloroethane in human volunteers following a single six-hour inhalation exposure to 350 and 35 ppm.

Data: Human pharmacokinetic study

Progress: In progress

Date of Completion: February 1983

CAS No: 71556

18. Acrylamide

Purpose: Evaluate vibratory sensitivity in an exposed population and controls.

Data: Field survey

Progress: Preliminary data collected.

Date of completion: Unknown; Pending development and validation of methodology.

CAS No: 79061

878210966

Dow Chemical Company
D-652
References to Unpublished Studies by Others

1. Studies of the visual system in chronic acrylamide poisoned monkeys. Lapham, L.W., et. al.
   Dr. L. W. Lapham, University of Rochester Medical Center, Department of Pathology, Division of Neuropathology, Rochester, NY, 14642.

2. The integration of multiple measures in behavioral toxicology. Merison, W.H., et. al.
   Dr. W. H. Merison, University of Rochester Medical Center, Division of Toxicology, Rochester, NY, 14642

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Doc Title EVALUATION OF THE GENOTOXIC POTENTIAL OF THE EPOXY RESINS XD-7342.00 AND XD-9053.00 IN THE RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY 23

Chemical Name (300 per name)	CAS No. (10)	
GLYCIDOL & ITS DIRIVATIVES	999999994	24
HALOGENATED ALKYL EPOXIDES	999999994	
22'-((12-OXIRANYL METHOXY)	999999994	
PHENYL)METHYLENE) BIS-		

Chemical Name (300 per name) 25	CAS No. (10) 26
2,1-PHENYLENEOXYMETHYLENE)	999999994
BIS-	
POLYMER (XD-9053.00)	999999996

878210747

R & D REPORT

DOW CHEMICAL U.S.A.

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May 3, 1978

REPORTING UNIT: Toxicology Research Laboratory

TITLE: EVALUATION OF THE GENOTOXIC POTENTIAL OF THE EPOXY RESINS XD-7342.00 AND XD-9053.00 IN THE RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

13 PAGES IN FULL REPORT

AUTHOR(S): A. M. Heimbach and S. L. Poole

AUTHOR(S) SIGNATURE(S): A. Heimbach 5/3/79 S.L. Poole 5/3/79

REVIEWER'S SIGNATURE: K. W. Wahnsiedler 5-7-79

This report is: INTERIM / FINAL and mainly: NEW / REVIEW

DATA REFERENCE (Book and page):

INVENTION DISCLOSURE (Where and publication):

PATENT STATUS: disclosure submitted / case filed / no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The genotoxic potential of the epoxy resins XD-7342.00 and XD-9053.00 was evaluated in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. Neither XD-7342.00 or XD-9053.00 elicited UDS at concentrations of 64, 6.4, 0.64 or 0.064 µg/ml of incubation medium. Testing higher concentrations of the epoxy resins was precluded by a lack of solubility. The lack of activity of the epoxy resins in this short-term *in vitro* assay implies a lack of genotoxic activity. However, the ability of these resins to enter the cell and interact with DNA is uncertain due to the size and reactive nature of their structure.

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INTRODUCTION

Oxirane: 2,2'-(((2-oxiranylmethoxy)phenyl)methylene), bis-(4,1-phenyleneoxymethylene) bis-, monomer (XD-7342.00) and polymer (XD-9053.00) were submitted by Western Division R&D of The Dow Chemical Company, Walnut Creek, California, for evaluation in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. This assay is currently being employed in our laboratory as a short-term *in vitro* test for assessing the genotoxic potential of chemicals. Epoxy resin XD-9053.00 is intended for use in semiconductors, transfer molding and functional power coatings.

This report is organized into sections describing (1) the background and rationale of the rat hepatocyte UDS assay, (2) the general methodology employed in conducting the assay as well as specific experimental methods utilized with XD-7342.00 and XD-9053.00, (3) the results of the present study and (4) the interpretation of the results.

BACKGROUND

Many chemicals which produce genetic toxicity (genotoxicity) react with intracellular macromolecules including DNA. Depending on whether the target cell affected is a reproductive cell (germ cell) or a somatic cell the toxic manifestation may be mutagenicity or carcinogenicity.

One of the hypotheses to date for chemical carcinogenesis is the somatic cell mutation theory (Miller & Miller, 1966). This theory postulates that chemical carcinogens interact with DNA producing alterations in the DNA molecule and the expression of the genetic code. If unrepaired, the alteration to DNA may result in cell death if critical cellular functions are impaired. However, the cell may survive and divide retaining the altered characteristics imparted to it by the genotoxic action of the chemical. One result of genotoxicity may be cellular transformation and induction of cancer. The assessment of DNA repair (unscheduled DNA synthesis: UDS) as an indicator of DNA damage has been suggested as a reliable estimate of genotoxic potential (Stich and Laishes, 1973).

The use of primary cultures of rat hepatocytes for assessing UDS has been reported by Williams (1976 and 1977). Our evaluation of the rat hepatocyte UDS assay indicated that the induction of UDS correlated well with the *in vivo* carcinogenic and *in vitro* mutagenic activity of the chemicals tested (Schumann et al., 1979). The diverse spectrum of chemicals evaluated indicate that the UDS assay may detect carcinogens with varied target organs and mutagens which produce both base-pair as well as frameshift mutations. Thus, it appears that UDS is sensitive

to a broad spectrum of carcinogenic and mutagenic chemicals. The amount of UDS elicited in this system, however, is purportedly a function of the type of DNA damage and repair invoked rather than the potency of the genotoxic agent (Williams, 1977). That this assay may not be a good predictor of *in vivo* potency is consistent with all other short-term *in vitro* carcinogenicity/mutagenicity assays.

The rat hepatocyte UDS assay involves:

1. Isolation of rat hepatocytes and establishment of primary cultures.
2. Simultaneous treatment of the cultures with the test chemical and ^{3}H-thymidine.
3. Quantitation of DNA repair (UDS) by evaluating the incorporation of the ^{3}H-thymidine into nuclear DNA by microautoradiography.

Intuitively, the use of primary cultures of rat hepatocytes is an ideal system for assessing UDS. Rat hepatocytes contain a wide spectrum of enzymes necessary to biotransform procarcinogens to their active form. Nonhepatic cultures have limited xenobiotic metabolizing capabilities and therefore require an exogenous source of enzymes in the form of hepatic microsomes. The xenobiotic metabolizing capability of long-term rat hepatocyte cultures also appears to be low (Williams, 1975). On the other hand, primary rat hepatocyte cultures retain a high degree of functional activity (Diesel et al., 1973; Bonney, 1974; Laishes and Williams, 1976; Lin and Snodgrass, 1975; Michalopoulos and Pitot, 1975). Thus, in this system a potential carcinogen/mutagen can be activated and interact within the cell in a similar manner to that which occurs *in vivo*.

Primary cultures of rat hepatocytes have shown the ability to activate diverse groups of procarcinogens including polycyclic aromatic hydrocarbons (epoxidation), nitrosamines (C-hydroxylation and N-dealkylation) and aromatic amines (N-hydroxylation and N-oxyesterification) (Schumann et al., 1979; Williams, 1977). Mycotoxins and azo dyes are also active in this system (Williams, 1976; 1977). In addition, this system appears to be sensitive to carcinogens with target organs other than the liver. For example, 4-nitroquinoline-1-oxide (lung), dimethylanthracene (mammary), methyl methanesulfonate (brain) and N-methyl-N'-nitro-N-nitrosoguanidine and β-propiolactone (stomach) induce UDS in primary rat hepatocyte cultures.

The use of microautoradiography in assessing UDS has several advantages over liquid scintillation counting. Microautoradiography allows confirmation that the observed radioactivity is located in the nucleus and also allows the elimination of cells in S-phase (semiconservative DNA synthesis; DNA replication) due to their completely blackened nuclei. Assessing UDS by microautoradiography eliminates the need for inhibiting semiconservative DNA synthesis with hydroxyurea to "unmask" repair synthesis.

For screening test chemicals in the rat hepatocyte UDS assay, a concentration showing some degree of toxicity or limited solubility as well as fractions of that dose covering a wide spectrum of concentrations should be assessed. All tests should include a positive control and appropriate vehicle controls. Ideally, good dose-response data should be generated and used as a criterion for activity. However, in the absence of a marked dose-response relationship repeated activity in the test must be

considered a positive result. Since no single short-term *in vitro* test can adequately predict *in vivo* mutagenicity or carcinogenicity, the rat hepatocyte UDS assay should be used in conjunction with other short-term tests (e.g. bacterial mutagenicity, cell transformation) for assessing genotoxic potential and prioritizing chemicals for animal studies.

GENERAL METHODOLOGY FOR THE RAT HEPATOCYTE UDS ASSAY

Animals.

Male Sprague-Dawley rats weighing approximately 200-250 g (Spartan Research Laboratories, Haslett, MI) are acclimated to our laboratory for at least 10 days. The rats are housed in a room designed to maintain a temperature of approximately 72°F, a relative humidity of approximately 45% and a 12 hour lighting cycle. Food (Purina Rat Chow) and water are provided *ad libitum*.

Chemicals.

Thymidine-6-^{3}H (specific activity of approximately 23 Ci/mmole) is obtained from New England Nuclear (Boston, MA). Test chemicals are prepared at the desired concentration in Williams Medium E (Flow Laboratories, Rockville, MD) if water soluble or as stock solutions in dimethyl sulfoxide (DMSO, J. T. Baker Chemical Co., Phillipsburg, NJ) such that the final concentration of DMSO in the media is 0.1%. In a few instances the final concentration of DMSO in the media may be increased to 0.5 or 1.0% due to limited solubility of the test chemical.

The test chemicals are tested over a wide range of concentrations. The highest concentration levels are determined by either a lack of test chemical solubility or by toxicity to the cells. Criteria for cellular toxicity are an unflattened granular appearance, detachment of the hepatocytes from the coverslip, a decrease in nuclear labeling and/or an extremely low level of background labeling with ^{3}H-thymidine.

Preparation of Hepatocytes.

Primary cultures of rat hepatocytes are prepared by slight modification of the method described by Williams et al., (1977). All test chemicals and concentration levels assessed per assay are evaluated in hepatocytes obtained from a single rat. The rat is anaesthetized with methoxyfluorane and the liver exposed by an abdominal incision. Sodium heparin (200 units) is injected into the inferior vena cava and the hepatic portal vein cannulated. The liver is then perfused for approximately 2-3 minutes with 0.5 mM ethylene glycol-bis(β-amino ethyl ether)N, N'-tetraacetic acid (EGTA) in Ca^{++}, Mg^{++} free Hank's balanced salt solution (37°c) buffered with 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) to pH 7.4. The liver is subsequently perfused with 0.05-.1% collagenase (Type I, Sigma Chemical Company) in Williams Medium E (WE) (37°C) buffered with 0.05 M HEPES to pH 7.4. After approximately 12-15 min. the liver is removed from the rat and the cells dispersed in WE. The cells are sedimented by centrifugation at 50 x g for approximately 30 seconds and resuspended in fresh medium (WE) with 10% fetal bovine serum (WE + FBS). Cell viability is assessed by gently mixing one drop of the cell suspension with one drop of 0.3% trypan

blue in 0.9% saline and quantitated by the percentage of the cells excluding trypan blue. The cells in suspension are counted with a hemocytometer and diluted to a concentration of 3-5 x 10^5 cells/ml with WE + FBS.

Seeding of the Cultures.

One ml of the cell suspension (3-5 x 10^5 cells) is inoculated into a 35 x 10 mm tissue culture dish (Vangard International, Inc., Neptune, NJ) containing a 25 sq mm round Thermonox plastic coverslip (Flow Laboratories, Rockville, MD) and 2 ml of WE + FBS. The cultures are placed in a humidified (95%) 37°C incubator and maintained in an atmosphere of 95% air: 5% CO_2 for 1.5 hr. During this interval viable cells attach to the coverslip. Nonviable cells are removed by washing the cultures with 3 ml of WE at the end of the attachment interval.

Chemical Treatment.

Two ml of media containing the test chemical and ^{3}H-thymidine (5-10 µCi/ml) are applied to triplicate cultures at each dose level. The cultures are then incubated for 18 hours after which they are washed for three 30 minute intervals with 1 mM nonlabeled thymidine in WE. The cells are then swelled by incubation for 10 minutes with 10% (w/v) sodium citrate and subsequently fixed with a solution of ethanol: acetic acid (3:1) for 30 minutes. The coverslips are allowed to air dry and then mounted cell side up on glass slides.

Autoradiography.

The slides are dipped in NTB photographic emulsion (Kodak, Rochester, NY) in a dark room and stored refrigerated for 10 days. At the end of the exposure period the slides are developed with Kodak D-19 developer and stained with hematoxylin and eosin. UDS is then quantitated by counting the number of grains over the nucleus and subtracting the mean number of

grains in 3 nuclear sized areas adjacent to the nucleus. These cells undergoing semiconservative DNA synthesis (DNA replication) are easily visualized because of their completely bleached nuclei and not counted. Ten-twenty cells on each of two-three slides are evaluated.

Frequently the cytoplasmic background of radioactivity is slightly greater than the nuclear labeling in control or unaffected treated cultures. This results in a negative value for the net number of nuclear grains. These values will be reported as 0.0 net grains per nucleus, however, the actual values obtained are used for statistical analysis. Mean grain counts of 0 or greater will be reported as the mean ± the standard deviation. Net nuclear labeling is often observed in control cells but rarely exceeds a mean of 5 grains per nucleus. Therefore, a mean of 6 net grains per nucleus or greater (this may vary with experimental conditions) in addition to statistical significance from control is required for a positive result (Williams, 1977). In cases where nuclear labeling is very intense (approximately greater than 75 grains per nucleus) the results are reported as the best approximation of the number of grains present.

<u>Statistics.</u>

The net number of nuclear grains in treated cells will be compared to the appropriate control by analysis of variance and Dunnett's t-test. A mean of 6 or more net grains per nucleus (this may vary with experimental conditions) and statistical significance from control at $p \leq 0.05$ is required for a positive result.

EXPERIMENTAL METHODS FOR XD-7342.00 AND XD-9033.00

The epoxy resins were evaluated in the rat hepatocyte UDS assay as described in the general methodology section. The following describes experimental procedures specific for the present study.

XD-7342.00 and XD-9033.00 were dissolved in dimethyl sulfoxide (DMSO) and added to the medium so that the final concentration of DMSO in the incubation medium was 0.1% (v/v). Concentrations of 64, 6.4, 0.64 and 0.064 µg of XD-7342.00 or XD-9033.00 per ml of incubation medium were assessed. Higher concentrations of the epoxy resins could not be evaluated due to a lack of solubility in the medium. 2-Acetylamino-fluorene (2-AAF; Aldrich Chemical Co., Milwaukee, WI) was used as a positive control at concentrations of 22.3, 2.23 and 0.223 µg per ml of incubation medium. 2-AAF was also prepared in DMSO and the final concentration of DMSO in the incubation medium was 0.1 % (v/v). Media control and 0.1% DMSO control cultures were also prepared. Ten cells on each of two slides were counted.

RESULTS

The effect of XD-7342.00 and XD-9033.00 in the rat hepatocyte UDS assay is shown in Table 1. The highest concentration tested of each epoxy resin was 64 µg/ml. Testing higher concentrations was precluded by a lack of solubility in the test system. Even at 64 µg/ml, a precipitate was observed on the slides after 18 hours of incubation. Thus, a wide spectrum of concentrations were evaluated, ranging from the solubility limit to nontoxic concentrations in which the treated and control cultures were comparable in appearance.

ED-7342.00 and ED-9053.00 failed to elicit UDS at any of the concentrations tested. Values of "0.0" net grains per nucleus reflect instances where the number of background grains in the cytoplasm exceeded the number of nuclear grains. 2-AAF, the positive control, elicited a significant degree of UDS at all three concentrations tested. At 22.3 and 2.23 µg/ml the response was too great to accurately quantitate but represented approximately 100 or more net nuclear grains. 0.22 µg/ml of 2-AAF produced 64.2 ± 22.4 (mean ± S.D.) net nuclear grains. The strong response of 2-AAF, a known animal carcinogen, indicates the responsiveness of the assay in the present study.

INTERPRETATION OF RESULTS

The lack of activity of the epoxy resins XD-7342.00 and XD-9053.00 in the rat hepatocyte UDS assay implies a lack of genotoxic activity. The various epoxide groups present in the structure of these resins, however, suggest that these should be very reactive compounds. It is hypothesized that these resins may not enter the cell due (1) to their large size or (2) to the fact that they may bind to the cell membrane. Thus, it is possible that they may never reach the target macromolecule, intranuclear DNA.

As with all short-term *in vitro* carcinogenicity/mutagenicity tests the potential genotoxic hazard for man cannot be based on any single test alone. Rather, the results of several short-term tests (e.g. bacterial mutagenicity, cell transformation, UDS) must be evaluated for assessing genotoxic potential and prioritizing chemicals for animal studies.

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TABLE 1

THE EFFECTS OF EPOXY RESINS XD-7342.00
AND XD-9053.00 IN THE RAT HEPATOCYTE UDS ASSAY

Treatment	Concentration (µg/ml)	UDS (Net Nuclear Grains)[a]
Media Control	-	0.0
0.1% DMSO Control	-	0.0
2-Acetylaminofluorene	22.3	$\geq$ 100
	2.23	
	0.22	64.2 ± 22.4[b]
XD-7342.00	64	0.0[c]
	6.4	0.0
	0.64	0.0
	0.064	0.0
XD-9053.00	64	0.0[c]
	6.4	0.0
	0.64	0.0
	0.064	0.0

[a]Results reported as "0.0" net nuclear grains indicates a slightly greater number of background grains in the cytoplasm compared to the nucleus.

[b]$\bar{X}$ ±S.D.; significantly greater than 0.1% DMSO control ($p \leq 0.05$).

[c]Precipitate present on slide after the 18 hour incubation.

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