RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

By:

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February 7, 1980

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LIST OF TABLES

INTRODUCTION

DBCP (1,2-dibromo-3-chloropropane) is a soil fumigant and an agricultural nematocide. It has been shown to cause testicular atrophy in laboratory animals (Torkelson, et. al., 1961), and has been associated with reduced sperm counts and sterility in exposed male workers (Whorton, et. al., 1977).

A study was designed to further assess the effects of inhaled DBCP on spermatogenesis and fertility in laboratory animals, to determine the maximum level of exposure to DBCP that does not have an effect on reproduction, and to evaluate the reversibility of these effects. Prior to starting the larger study on spermatogenesis and fertility, two probe studies were conducted. The first probe study consisted of exposing male rabbits, male dogs and male rats to 50 ppm of DBCP by inhalation. The purpose was to confirm that DBCP did have an effect on spermatogenesis, and to determine the species of choice for the larger reproduction and fertility study. The second probe study was conducted to further evaluate the appropriateness of the rabbit for the larger reproduction and fertility study. The purpose of this report is to summarize the results of the two probe studies.

Semen Evaluation. Semen evaluation was performed on dogs and rabbits. Semen collections were performed prior to placing the animals into the chambers. Semen was collected in a graduated test tube from each animal by means of an artificial vagina. After collection, the volume of semen and its color were recorded. The semen was then placed in a constant temperature bath at 37°C for 15 minutes to allow the semen to liquify.

The motility and ratio of live and dead sperm were determined after the semen had liquified. To assess motility, a large drop of semen diluted with warm saline was placed on a glass slide under a cover slip and examined at 400 X magnification. The first 100 sperm observed were ranked as follows: 1) Progressively motile (moving from place to place), 2) nonprogressively motile (twitching tails but staying in one place), and 3) non-motile (no movement). The ratio of live and dead sperm was determined by staining a drop of semen with eosin-aniline blue stain[1] and counting 100 sperm per specimen (Seager and Fletcher, 1972). The sperm count was determined using the hemocytometer method described by Miller (1955) after the method of Gordon, et. al. (1965). The actual count was determined using a Coulter Counter, Model ZBI.

Chambers. The first probe study at 50 ppm DBCP was conducted using a 1-m^3 Rochester-type exposure chamber. The main airflow in the chamber was ∿182 l/min, for an air turnover rate of ∿11 air changes/hour. The air was pulled through the chamber by an exhaust pump, such that the chamber

[1]Aniline blue, 5 g; eosin B, 0.75 g; sodium citrate, 2.9 g; water, 100 ml.

used as in the previous probe. However, certain modifications were made, in order to provide the ability to run 3 chambers from the same apparatus (see below). The air effluent direct from the vapor generator was diluted ∿3:1 with compressed air, and this mixture led to a 3-neck 1-liter plenum flask, through one neck. A second neck was capped off. The third neck joined the main chamber air intake line, but through a slight throttle, such that the pressure in the 1-liter flask was ∿1" H_2O positive with respect to ambient room air. Once target concentration (10 ppm) was reached, it was found to be a simple matter to hold the concentration to within ±3% of target by making small adjustments in airflow as required.

Design of Probe Study No. 1. In this probe study, 2 male rabbits, 5 male rats and 2 male dogs were exposed by inhalation to 50 ppm of DBCP for 6 hours/day, 5 days/week. The duration of exposure was two weeks for rabbits and rats. Exposure of dogs did not stop after two weeks, but was continued in order to maximize the potential effect in that species. A control group was not included in the probe study.

Rabbits were weighed on days 1, 6, and 14 of the study (the first day of exposure was considered to be day 1 of the study). Rats were weighed on days 1, 3, 6, 8, 10, 13, and 14. Dogs were weighed on days 1, 6, 13, and 20. On days when the animals were exposed to DBCP, body weights were recorded prior to placing the animals in the exposure chambers. Semen from dogs was collected and analyzed (See section on semen evaluation) on days -1, 6, 13, and 20 of the study. Attempts were made to collect semen from rabbits during the study, but no samples could be

technique with fluorescent light illumination immediately following the decapitation. Prior to formalin fixation, the weights of the testicles were obtained and recorded from all five rats.

The exposure of the dogs did not stop on the 14th day of the study, but was continued on to maximize the potential effect in the dogs. Both dogs died on October 29, 1977 (25th day of the study) and were presented for necropsy examination. Both dogs were examined externally and internally and any observations were recorded. Representative tissues collected during the necropsy examination were preserved in phosphate buffered 10% formalin. The routine tissues collected during the necropsy examination are listed in Table 2. Prior to the formalin fixation, the weights of the testicles were obtained and recorded from both dogs.

Representative sections from most of the tissues shown in Table 2 were processed from all animals from this probe study. Tissues were processed using conventional methods, embedded in paraffin, sectioned (5-6 μ) and stained with hematoxylin and eosin. A complete list of the organs and tissues that were examined microscopically are presented in Table 3. It should be noted that the list of tissues evaluated included animals that died as well as animals that were killed during the study. In a few cases, a tissue specimen was not obtained at necropsy or was lost during the processing. The parathyroid glands were evaluated only to the extent that they were included in the routine sections obtained from the thyroid gland.

RESULTS

Probe Study No. 1. Two male rabbits were exposed to 50 ppm of DBCP. One rabbit (77-6712) died on the 10th day of the study; the other rabbit was sacrificed on the 14th day of the study. Significant weight loss was noted in both of these rabbits (Table 4). Repeated attempts to collect semen from these rabbits on days 6 and 7 of the study were unsuccessful. Although both bucks were able to mount a teaser doe, they were unable to maintain an erection and ejaculate. No problems were encountered in collecting semen from either of these bucks one day prior to the initial exposure. Also, before being placed on this study, both of these bucks were used on numerous occasions in this laboratory as semen doners for artificial insemination; difficulties in obtaining semen did not occur during that period.

The gross and histopathologic findings and the testicular weights of the two rabbits exposed to 50 ppm DBCP are summarized in Table 5. Both rabbits had testicles that were decreased in size based on organ weight, morphological appearance or both at the time of gross necropsy. Histologically, the testicles of both rabbits had bilateral germ cell degeneration, individual cell necrosis, focal necrotic debris, apparent decrease in the number of mature spermatids and spermatocytes, and one rabbit, (77-6713), also had necrotic debris, atypical germ cells and a few multinucleated spermatids free in the tubular lumina of one epididymis. These morphological alterations in the testicles and epididymides were considered to be treatment-related alterations.

alterations that were observed in the testicles in all five rats. These changes consisted of focal or multiple tubular degenerative changes, apparent decrease in the number of germ cell layers within the tubules, occasional multinucleated spermatids, and an apparent accentuation or increased prominence of Sertoli cells in a few seminiferous tubules. These changes were rather subtle, but they appeared to be treatment-related. In addition to the testicular alterations, the kidneys from 4 of the 5 rats had epithelial cell changes at the corticomedullary junction. These changes consisted of enlarged pleomorphic epithelial cells within the tubules that were suggestive for a regenerative process. Such changes are unusual in rats of this strain and age and are, therefore, interpreted as probably treatment-related. There were a few other tissue changes observed, but these were consistent with normal or spontaneously occurring changes that are commonly seen in rats of this strain, sex and age.

Two dogs were exposed to 50 ppm of DBCP. As in the other two species, dogs exposed to DBCP had significant weight loss (Table 8). Vomiting was observed on the 3rd and 4th days of exposure. One dog was emaciated and had a dull coat and excessive dandruff; this dog died on the morning of the 25th day of the study. The other dog died during the afternoon of the 25th day; it had been unable to stand when taken out of the exposure chamber that afternoon. Evaluation of the semen of the dogs revealed no changes attributable to DBCP with respect to sperm count, volume of semen, color of semen, or motility of sperm (Table 9).

Evaluation of semen collected on the morning of the 5th day of exposure revealed no remarkable changes compared to pre-exposure values (Table 12).

Only 1 of the 4 rabbits (77-7210) exposed to 10 ppm of DBCP for 14 days had a gross lesions that consisted of a thickend, hyperemic and hemorrhagic appearance to the wall and mucosa of the urinary bladder. This finding was suggestive for cystitis, but was not considered treatment-related since no other gross lesions were observed in these 4 animals. Only the testicles and epididymides were evaluated histologically (from these rabbits). All showed slight histologic alterations within the testicles that consisted of focal individual germ cell necrosis, focal tubules with multinucleated spermatids, and focal tubules with decreased numbers of germinal cells lining the tubular lumen. These changes were considered to represent slight treatment-related effects.

QUALITY ASSURANCE STATEMENT

This report represents data generated prior to the enactment of the FDA Good Laboratory Practice Regulations. The study was conducted according to standards used in this laboratory at that time. The report accurately reflects all of the data generated. All data and reports are located at the submitting laboratory.

Study Started: Nov. 28, 1977 Report Issued: Feb. 7, 1980

Protocol Audited: 1977 Reported: 1977

Data Audited: Jan. 23, 1980 Reported: Jan. 24, 1980

Final Report Audited: Jan. 23, 1980 Reported: Jan. 24, 1980

W. E. Hoover 7 February 1980
W. E. Hoover (Date)
Quality Assurance
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TABLE 1

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

Analysis of the Test Material

	Pre-exposure	Post-exposure
	% by weight	
DBCP	97.3	96.6±0.4
Allyl Chloride	0.4	0.59±0.06
1,2,5,6-Tetrabromohexane	0.92	1.20±0.1
2-Chloropropane	0.35	0.38±0.04
1-Chloropropane	0.03	0.04±0.004
Epichlorohydrin	0.81	1.00±0.1
1-bromo-3-chloropropane	0.11	0.10±0.01

TABLE 3

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 1:

Number of Tissues and Organs Examined Histologically From Rabbits, Rats and Dogs Exposed to 50 ppm DBCP

Species	Rabbit	Rat	Dog
Number Examined	2	5	2
Liver	2	5	2
Gallbladder	1	0	2
Kidneys	2	5	2
Urinary Bladder	2	5	2
Heart	2	5	2
Anterior Mediastinal Blood Vessles (or aorta)	1	5	2
Mesenteric Blood Vessels	2	3	1
Trachea	2	5	1
Lungs	2	5	2
Tongue	0	5	2
Esophagus	2	5	0
Stomach	2	3	2
Small Intestine	2	3	2
Cecum	2	3	1
Colon	2	3	2
Salivary Glands	2	5	2
Pancreas	2	5	2
Testicles	2	5	2
Epididymis	2	4	2
Prostate	2	5	2
Seminal Vesicles	0	5	0
Coagulating Glands	0	4	0
Brain	2	5	2
Spinal Cord	1	5	0
Peripheral Nerve	2	1	1
Pituitary Gland	2	5	2
Adrenal Glands	2	5	2
Thyroid Gland	1	5	2
Parathyroid Gland(s)	1	1	1
Spleen	1	5	2
Thymus	0	5	2
Anterior Mediastinal Lymph Node(s)	0	5	1
Mesenteric Lymph Node(s)	0	4	2
Subcutaneous Lymph Node(s)	0	2	0
Skin	1	4	1
Mammary Gland	0	4	0
Skeletal Muscle	1	4	2
Eyes	2	4	2

TABLE 5

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 1:

Summary of the Gross and Histopathologic Findings in Male Rabbits Exposed to 50 ppm DBCP by Inhalation

Pathology Number	Summary of Gross and Histopathologic Observations
77-6712	Gross: Soiled hair around external nares from nasal discharge. Petechial hemorrhage and hyperemia of small intestine mucosa. Decreased ingesta in gastrointestinal tract. Purulent material in nasal turbinates. Pale liver. Debris in right outer ear canal. Testicles weighed 3.17 gms together.
	Histopathology: Testicles - bilateral multifocal germ cell degeneration, individual germ cell necrosis and increased prominence of Sertoli cells. Epididymis - unilateral decreased sperm in tubular lumina. Lungs - severe necrotizing bronchopneumonia. Trachea - mild suppurative tracheitis. Liver - slight fatty change and slight tinctorial variation to hepatocytes. Kidneys - generalized congestion and focal interstitial aggregates of mononuclear lymphoid cells.
77-6713	Gross: Hemorrhagic foci in lungs. Decreased testicular size and weighed 2.86 gms together.
	Histopathology: Testicles - bilateral multifocal germ cell degeneration, individual germ cell necrosis, focal necrotic debris, an apparent decrease in the number of spermatids and spermatocytes and occasional multinucleated spermatids. Epididymis - unilateral decreased sperm, increased necrotic cellular debris, atypical germ cells and a few multinucleated spermatids in the tubular lumina. Lung - mild acute bronchitis and bronchiolitis and peribronchial aggregates of mononuclear lymphoid cells. Heart - few arterioles with necrotic debris and mineral deposits in vessel wall.

TABLE 7

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 1:

Summary of the Gross and Histopathologic Findings in Male Rats Exposed to 50 ppm DBCP by Inhalation

Pathology Number	Summary of Gross and Histopathologic Observations
77-6714	Gross: Apparent decreased thymic size. Histopathology: Testicles - localized tubules with decreased germ cells, occasional multinucleated spermatids and increased prominence of Sertoli cells. Liver - altered tinctorial properties and vacuolation of hepatocytes. Kidneys - enlarged and pleomorphic tubular epithelial cells at the corticomedullary junction. Lungs - slight perivascular, peribronchial and interstitial aggregates of mononuclear lymphoid cells.
77-6715	Gross: No significant lesions. Histopathology: Testicles - localized tubules with decreased germ cells, occasional multinucleated spermatids and increased prominence of Sertoli cells. Liver - altered tinctorial properties and vacuolation of hepatocytes. Kidneys - enlarged and pleomorphic tubular epithelial cells at the corticomedullary junction. Lungs - slight perivascular and peribronchial aggregates of mononuclear lymphoid cells.

TABLE 8

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 1:

Body Weights (kg) of Male Dogs Exposed to 50 ppm of DBCP

	Day of Study				
Animal No.	1	6	13	20	25
NZ16	13.4	12.0	11.0	10.1	Dead
AE26	10.0	9.0	8.2	7.6	Dead

TABLE 10

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 1:

Summary of the Gross and Histopathologic Findings in Male Dogs Exposed to 50 ppm DBCP by Inhalation

Pathology Number	Summary of Gross and Histopathologic Observations
77-6889	Gross: Cachectic and dehydrated. Severe edema, hemorrhage and/or congestion of the left hind foot, subcutaneous tissue, muscles, testicles, urinary bladder, urethra, gastrointestinal tract, kidneys, anterior mediastinum, and pericardium of heart and lungs. Mottled liver. Decreased size of prostate gland. Testicles weighed 7.1 gms together and were tan in color.
	Histopathology: Testicles - degeneration, necrosis and loss of germ cells with increased prominence of Sertoli cells. Liver - slight fatty change and macrophages with brown granular cytoplasmic pigment. Lungs - suppurative pneumonia and focal nematodiasis (Filaroides hirthi). Kidneys - multifocal degeneration and necrosis of tubular epithelial cells. Multiple organs - hemorrhage, congestion and/or edema.
77-6890	Gross: Cachectic and dehydrated. Severe edema, hemorrhage and/or congestion of the tongue, subcutaneous tissue, testicles, lymph nodes, urinary bladder, prostate, gastrointestinal tract, adrenal gland, and lungs. Mottled, enlarged and friable liver. Ascites. Perforated gastric ulcer. Decreased ingesta in gastrointestinal tract. Testicles weighed 10.8 gms together. Questionable decreased size of thyroid glands. Decreased size of prostate gland.
	Histopathology: Testicles - degeneration, necrosis and loss of germ cells. Liver - slight fatty change, macrophages with brown granular cytoplasmic pigment and focal necrosis with suppurative inflammation. Kidneys - multifocal degeneration and necrosis of tubular epithelial cells. Lungs - suppurative bronchopneumonia. Colon - submucosal granulomata. Lymph nodes - lymphoid cell depletion. Multiple organs - hemorrhage, congestion and/or edema.

TABLE 12

RESULTS OF TWO INHALATION PROBE STUDIES IN RABBITS, RATS AND DOGS THAT WERE EXPOSED TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

PROBE STUDY NO. 2:

Semen Evaluation of Rabbits Exposed to 10 ppm of DBCP

Animal Number	Day of Study	Sperm Count, millions/ml	Vol. of Semen, ml	Color	Motility of Sperm (%)		
					Progressive	Non-Progressive	Non-Motile
819	-5	128	0.5	Milky	66	9	25
	5	87	1.0	Milky	79	8	13
1130	-4	58	0.5	Milky	83	6	11
	5	130	0.25	Milky	78	8	14
931	-4	51	0.5	Milky	70	13	17
	5	47	0.5	Milky	70	15	15
930	-4	48	0.4	Milky	76	7	17
	5	168	0.5	Milky	75	10	16

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HEALTH AND ENVIRONMENTAL RESEARCH
DOW CHEMICAL U.S.A.

LOCATER SHEET

FILE NO. HET-IC-1715-C(15)

DEAD STORAGE NO. 1503

TITLE OF REPORT: Fate of ethylene dibromide in rats following inhalation exposure

DATE REPORT ISSUED: 7-18-77

AUTHOR(S): R.G. Watanabe, J.D. Young, M.M. Schlachter, J.A. Zempel and R.J. Karbowski

NOTEBOOK(S) - (NUMBER AND PAGE(S):

1) NBK -13-8 4) _____

2) NBK -13-11 5) _____

3) _____ 6) _____

SPECIMENS:	PATHOLOGY NUMBERS:
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3) _____	_____
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ADDITIONAL DOCUMENTS:	LOCATION:
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TOX. NO. K-1715-(15)

FILE NO. K-1715-(15)

REQUEST FOR TOXICOLOGICAL STUDIES

1803 BLDG. HET

NAME OF MATERIAL TO BE TESTED: Ethylene Dibromide (1,2-dibromoethane)

SUBMITTED BY (Signature)	(Date)	(Building)	(Phone)
Doug Rausch (Pb. Wetmore)	9-17-76	1803	6133

SUPERVISOR (Signature)	(Date and Building)	PROJECT SAFETY COORDINATOR	(Date)

ESTIMATED COST	ACCOUNT NUMBER	LEDGER	LOCATION GROUP / FUNCTION	SECTION / SUB. FUNC	SUFFIX	PROBLEM NO. TO BE USED BY TOXICOLOGY	PROBLEM INDEX NO. LAB.	PROBLEM
25,000							51	60000128

DO NOT WRITE BELOW THIS LINE — COMPLETE ALL INFORMATION ON REVERSE

ROUTE OF ADMINISTRATION: Inhalation

LENGTH OF TREATMENT: Single Exposure

SPECIES:

RAT	☑ M ☐ F	RABBIT	☐ M ☐ F	GUINEA PIG	☐ M ☐ F
DOG	☐ M ☐ F	MONKEY	☐ M ☐ F	HAMSTER	☐ M ☐ F

OTHER

DOSES: 5, 25, 75 ppm for 6 hours

CHECK POINT	DATE
MPLE RECEIVED TOXICOLOGY	6-76
ESTIMATED STARTING TIME	10-76
ESTIMATED REPORTED DATE	2-77
REVISED ESTIMATE REPORTED	
REPORT DRAFTED	
REPORT ISSUED	

FATE OF SAMPLE	DATE
☐ RETURNED	
☐ DESTROYED	
☐ REFERENCE SAMPLE STORED	
☐ REFERENCE SAMPLE TO K STORAGE	

NOTES

DATE PROJECT COMPLETED

NOTES, CHANGES, ADVERSE EFFECTS NOTED

DO 130985
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R & D REPORT

P.G. Watanabe 9-8-77

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LABORATORY REPORT CODE: HET K-1715-(15)

DATE ISSUED: 7-18-77

DEPARTMENT: Toxicology Research Laboratory

LAB. NO. 5 1 1 6 | PROBLEM NO. 0 0 0 0 1 2 8

CRI NUMBER

TITLE: Fate of Ethylene Dibromide in Rats Following Inhalation Exposure

31 PAGES IN FULL REPORT

AUTHOR(S): P. G. Watanabe, J. D. Young, M. M. Schlachter, J. A. Zempel, R. J. Karbowski

AUTHOR(S) SIGNATURE(S): P.G. Watanabe 8-4-77; J.D. Young 8-4-77; M.M. Schlachter 8-4-77; J.A. Zempel 8-4-77; R.J. Karbowski 8-4-77

REVIEWER'S SIGNATURE: Lew Ramsey 8/4/77

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

DATA REFERENCES (book and page):

(Refer also to earlier related reports and publications.)

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

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The fate of ethylene dibromide (EDB) was studied in rats following inhalation exposure to determine if a change in the disposition of EDB in the body occurred with increasing exposure concentrations. Male rats were exposed to 7, 25, or 75 ppm ^{14}C-labeled EDB for 6 hours and the routes and rates of elimination of ^{14}C-activity were followed for 48 hours after termination of exposure. Total metabolism of EDB and hepatic glutathione (GSH) levels were also determined. The percentage of recovered radioactivity excreted by the various routes were similar for all exposure levels. The urinary excretion of radioactivity was the major route of elimination accounting for 80% of the recovered radioactivity. The rates of excretion of urinary ^{14}C-activity were similar for all exposure levels with a half-life ($t_{1/2}$) of 5.1-5.6 hours. Urinary metabolites of EDB following inhalation exposure appear to be derived from GSH conjugation. Hepatic GSH levels were 94, 77, and 59% of control following 6 hour exposures to 7, 25, and 75 ppm EDB respectively. The metabolism of EDB appears to constitute a detoxification mechanism and the data suggest that EDB is detoxified more efficiently at a low exposure (6-7 ppm) compared to higher exposures (25, 75 ppm). Thus, the fate of inhaled EDB in rats is dependent on the exposure concentration and toxicity may result in high exposures because the ability to detoxify EDB has been impaired. Furthermore, it may not be appropriate to extrapolate linearly the same toxicity incurred at levels of exposure greater than 6-7 ppm EDB to lower levels of exposure in rats.

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FATE OF ETHYLENE DIBROMIDE IN RATS FOLLOWING INHALATION EXPOSURE

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June 18, 1977

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ABSTRACT

The fate of ethylene dibromide (EDB) was studied in rats following inhalation exposure to determine if a change in the disposition of EDB in the body occurred with increasing exposure concentrations. Male rats were exposed to 7, 25, or 75 ppm ^{14}C-labeled EDB for 6 hours and the routes and rates of elimination of ^{14}C-activity were followed for 48 hours after termination of exposure. Total metabolism of EDB and hepatic glutathione (GSH) levels were also determined. The percentage of recovered radioactivity excreted by the various routes were similar for all exposure levels. The urinary excretion of radioactivity was the major route of elimination accounting for 80% of the recovered radioactivity. The rates of excretion of urinary ^{14}C-activity were similar for all exposure levels with a half-life ($t_{1/2}$) of 5.1-5.6 hours. Urinary metabolites of EDB following inhalation exposure appear to be derived from GSH conjugation. Hepatic GSH levels were 94, 77, and 59% of control following 6 hour exposures to 7, 25, and 75 ppm EDB respectively. The metabolism of EDB appears to constitute a detoxification mechanism and the data suggest that EDB is detoxified more efficiently at a low exposure (6-7 ppm) compared to higher exposures (25, 75 ppm). Thus the fate of inhaled EDB in rats is dependent on the exposure concentration and toxicity may result at high exposures

because the ability to detoxify EDB has been impaired. Furthermore, it may not be appropriate to extrapolate linearly the same toxicity incurred at levels of exposure greater than 6-7 ppm EDB to lower levels of exposure in rats.

INTRODUCTION

Ethylene dibromide (1,2-dibromoethane, EDB) is used extensively as a gasoline additive, fumigant and chemical intermediate. Exposure to EDB results primarily from industrial applications where inhaled EDB vapor constitutes the common route of entry into the body. Acute and subchronic studies on the toxicity of inhaled EDB in experimental animals (50-3000 ppm) showed that EDB caused dose-related damage to the liver, lung and kidney (Rowe, et al., 1952). Toxicity was not observed in rats exposed repeatedly to 25 ppm EDB (7 hours/day, 5 days/week) for 6 months. The current threshold limit value for industrial exposure to EDB is 20 ppm.

The metabolism of EDB has been the subject of numerous investigations. There appears to be a general consensus that the toxicity of EDB is due to the parent molecule and that the metabolism constitutes a detoxification process. Detoxification of EDB by conjugation with hepatic glutathione (GSH) mediated by GSH-S-alkyl transferase has been shown to occur in vitro (Nachtomi, 1970). Consistent with this observation was the identification of S-hydroxyethyl cysteine, its N-acetylated analogue and its respective sulfoxide as major urinary metabolites in rats following oral intubation of EDB (Nachtomi, 1966; 1970).

Of particular significance is the finding that EDB is detoxified by conjugation with GSH in vivo. This detoxification process can be saturated if doses sufficient to deplete GSH are given. This has been observed following administration of large doses of acetaminophen and bromobenzene (Gillette, 1974). If exposure to high levels of EDB similarly deplete GSH levels in the body, this may lead to increased concentrations of EDB and an increased reaction with intracellular macromolecules resulting in toxicity. In addition to morphological and functional damage to the cell, chronically induced oncogenesis may be mediated by such reactions (Miller and Miller, 1971).

Recent concern over the potential hazard of exposure to EDB was prompted by the report of induction of stomach cancer (squamous cell carcinoma) in rats and mice orally intubated with EDB at 40-200 mg/kg/day (Olson, et al., 1973). Assessing the hazard of exposure to EDB from these results is limited severely because stomach cancer was produced only at very high dose levels which caused overt toxicity and increased mortality in all the treatment groups. Furthermore, administration of a reactive chemical such as EDB in a concentrated solution via oral gavage is an unrealistic route of administration to assess the toxicity of a material such as EDB for which inhalation is the predominant route of exposure.

Studies on the fate of EDB following inhalation exposure are needed to determine if the fate of inhaled EDB changes with increasing exposure concentration. Thus the objective of this study was to characterize the fate of EDB, including reaction with intracellular macromolecules, following exposure to various atmospheric concentrations of ^{14}C-EDB in rats.

METHOD

Material.

^{14}C-labeled EDB (1,2-^{14}C-dibromoethane) was purchased from New England Nuclear (Lot #884-249, specific activity 60 mCi/mmole). Radiochemical purity was 94-95% as determined by gas-liquid chromatography on a Hewlett-Packard, Model 5750, gas chromatograph. EDB (0.2 µl) was chromatographed on a column of 10% SP-1000 on Chromosorb W (Supelco, Inc.), 100/120 mesh (6' x 1/8"). The helium carrier gas flow rate was 20 ml/min and the oven temperature was 125°C. Following passage through a thermal conductivity detector the effluent gases were trapped by bubbling directly in scintillation vials containing 20 ml of scintillant containing Concifluor (Mallinkrodt Chemical Works). Fractions were collected every 30 seconds and the radioactivity was determined by counting in a liquid scintillation spectrometer.

The non-labeled EDB used was 99% chemically pure. The non-labeled and ^{14}C-labeled EDB were mixed as vapors in a Saran bag (Anspec) to the desired specific activity and concentration.

Animals.

Male Sprague-Dawley rats (Spartan Research Laboratory) weighing 195-250 g were used in the study. All animals were housed in rooms in which a constant humidity, temperature, and 12-hour light-dark cycle (7 AM - 7 PM EST) were maintained.

Food and water were provided ad libitum except during exposure. Rats used for determination of blood EDB levels were surgically prepared by implanting a cannula in the right jugular vein (Harms and Ojeda, 1974). These rats were allowed to recover 2-3 days prior to use.

Exposure.

All rats were exposed under dynamic conditions to room air (controls) or to various concentrations of EDB. The exposures were conducted in a 1 liter plexiglass inhalation chamber which is designed to expose 4 rats simultaneously. In this apparatus the rats head protrudes into the chamber allowing exposure of the head only to the test atmosphere. The EDB atmosphere was generated by pumping an appropriate volume of concentrated EDB vapor from a Saran bag (Anspec) at a controlled rate into the chamber airflow (approximately 6 l/min). The concentrated EDB vapor was metered with a Masterflex peristaltic pump (Cole-Parmer Instrument Company) or a precision dual syringe pump. The concentration of EDB was monitored by passing the input airflow into the chamber through a gas cell of an infrared spectrophotometer (Wilks) calibrated at a wavelength of 8.5 μm. The chamber atmosphere was also analyzed at hourly intervals by gas chromatography. The column used for analysis was similar to the one described for determining the radiochemical purity of EDB. Samples for ^{14}C-activity

determinations were taken hourly by bubbling 1 ml aliquots of the chamber atmosphere into a scintillation solution containing Concifluor (Mallinckrodt Chemical). Radioactivity was determined by liquid scintillation spectrometry. The inhalation chamber was operated in a laboratory fume hood to prevent contamination of the working environment.

Fate of Radioactivity Following Exposure to Inhaled ^{14}C-EDB

Groups of 4 rats were exposed to 7, 25 or 75 ppm ^{14}C-EDB for 6 hours. The mean analytical concentrations measured by gas chromatography were 7.2±0.2 (SD), 24.8±0.4 and 74.7±1.2 ppm EDB. The respective specific activities were 582, 211 and 280 DPM/µg EDB. Control animals used for non-protein sulfhydryl determinations (4/group) were exposed concomitantly to room air.

Following the exposure one rat per exposure level was placed in a glass Roth-type metabolism cage for the collection of urine, feces and expired air. Room air was drawn through the cages at 400-500 ml/min. The air leaving the chamber was passed first through a tube containing 1 g of activated charcoal to adsorb expired EDB. Subsequent transit through a trap containing 120 ml of 5 M ethanolamine in 2-methoxy-ethanol enabled the collection of $^{14}CO_2$. The trap for $^{14}CO_2$ was maintained at room temperature.

Samples of excreta were collected for 48 hours after termination of exposure and analyzed for ^{14}C activity. Expired EDB was collected at 0.5 hr intervals for 4 hr; the CO_2 trap and urine receptable (immersed in a dry ice bath) were changed at 12 hr intervals for 48 hr; and feces were collected every 24 hr. At the termination of the study (48 hr) the animals were killed by a blow to the head.

Samples of liver, kidney, lung, fat, stomach, skin, testes, muscle and brain were collected for analysis of ^{14}C activity. The remaining carcass was homogenized (50% w/v) in distilled water and analyzed for ^{14}C activity. Samples of excreta and tissue were prepared for scintillation counting as described previously (Watanabe, et al., 1976).

Radioactivity was determined by counting in a Mark II or Mark III (Searle Analytic, Inc.) liquid scintillation spectrometer. External standard channel ratios were used to determine the counting efficiency. Counts per minute were converted to disintegrations per minute using a standard quench curve.

The remaining nine rats (3/exposure level) were killed by a blow to the head immediately following exposure. The liver was removed and a sample (1 g) was taken for determination of

non-protein sulfhydryl content (primarily GSH) by the method of Sedlak and Lindsay (1968). The remaining liver was frozen immediately on dry ice and stored at -20°C until analyzed. The carcass, liver, lung and skin were analyzed for total radioactivity as described previously. The radioactivity determined in the tissues and carcass was non-volatile, therefore this radioactivity represented the total amount of metabolized EDB. Macromolecular binding of radioactivity to liver and lung tissue was determined by the method of Jollow, et al., (1973).

Separation of Urinary ^{14}C-Activity.

Urinary ^{14}C-activity was separated by high pressure liquid chromatography. The column (stainless steel, 5 mm x 0.5 m) was packed with Zipax® SAX (duPont) ion exchange resin. Fifty µl of urine from the 0-12 hour collection interval from each rat exposed to 7, 25, or 75 ppm ^{14}C-EDB was injected on the column and eluted with a linear gradient from distilled water to 10% acetic acid, 1% pyridine over a 25 minute period (flow rate 1 ml/min). Fractions were collected at 1/2 or 1/4 minute intervals. Radioactivity in the fractions was determined by liquid scintillation spectrometry.

Blood Levels of EDB During and Following Inhalation Exposure. Two groups of 3 rats/group were exposed to 6 or 78 ppm EDB for 6 and 7 hours respectively. The mean analytical concentrations of EDB measured by gas chromatography were 5.8±0.2 (SD) and 78.2±1.1 ppm EDB. Blood samples were taken at hour intervals during the exposure, and following exposure blood samples were obtained at 15 minute intervals for 1 hour. The blood was analyzed for EDB by gas liquid chromatography on a Hewlett-Packard Model 5700 or Varian Series 2400 gas chromatographs equipped with flame ionization detectors. Whole blood (1 µl) was chromatographed on a column of 6.5% SP-1000 on Carbopack A (Supelco). The He carrier gas flow rate was 24.5 ml/min and the column temperature was 100°C.

RESULTS

The routes of excretion following inhalation exposure to various concentrations of EDB are shown in Table 1. The percentage of the recovered radioactivity excreted by the various routes was similar for all exposure levels suggesting that the routes of excretion were independent of the exposure concentration. Urinary excretion of radioactivity was the major route of excretion accounting for 80% of the total radioactivity recovered. There was significant excretion of radioactivity as expired CO_2 representing 7-8%. Only about 1% of the recovered radioactivity was eliminated by the lungs as EDB.

The rates of excretion of urinary radioactivity over a 48-hour period are shown in Figure 1. The excretion curves were fit by linear regression analysis of the logarithmically transformed data and the elimination was described by a monoexponential function. The apparent first order rate constant for urinary excretion following 7, 25, and 75 ppm exposures were 0.136±0.019 (SD), 0.127±0.016 and 0.124±0.013 hr^{-1} respectively. These corresponded to respective half-lives of 5.1, 5.5, and 5.6 hr.

The distribution of radioactivity in various tissues immediately following exposure is shown in Table 2. Highest concentrations of radioactivity were observed in the liver and kidney. All

other tissues analyzed had equivalent or lower levels of radioactivity than that in the carcass suggesting that the metabolites of EDB are generally distributed throughout all tissues with no preferential accumulation in any one tissue other than liver and kidney.

The total amount of EDB metabolized, macromolecular binding to hepatic and lung tissue and hepatic non-protein sulfhydryl levels following exposure to EDB are given in Table 3. The total amount of EDB metabolized and macromolecular binding to liver and lung tissue all increased proportionately (3-fold) to the increase in exposure concentration between 25 and 75 ppm. However, the 3.7 fold increase in exposure between 7 and 25 ppm resulted in only a 2-fold increase in total metabolism and liver binding and slightly over 1-fold increase in lung binding. Thus, the binding in the liver and lung correlated reasonably well with the total amount of EDB metabolized but rats metabolized proportionately more at 7 ppm than at 25 or 75 ppm. Hepatic non-protein sulfhydryl content (primarily glutathione, GSH) was depressed significantly from controls only following 6 hour exposures to 25 and 75 ppm. No statistically significant (Student t-test, $p < 0.05$) depression in hepatic GSH was observed following the 6-hour, 7 ppm exposure.

A typical profile of urinary radioactivity separated by high pressure liquid chromatography is shown in Figure 2. The ^{14}C-activity was separated into four radioactive peaks labeled A, B, C, and D. Peaks B and C co-eluted with authentic radiolabeled standards of N-acetyl-S-(2-hydroxyethyl) cysteine, and thiodiglycolic acid respectively. However, when the same urine samples were rechromatographed one month later, peak A was not observed and peak B was increased quantitatively equalling the sum of the two previous peaks A and B. This led to the postulate that peak A may be a cyclized form of N-acetyl-S-(2-hydroxyethyl) cysteine which hydrolyzed on storage. Thus both A and B appear to represent cysteine conjugates of EDB.

Blood levels of EDB during and following exposure to 6 and 78 ppm for exposure times of 6 and 7 hours respectively are shown in Figure 3. Upon exposure to 78 ppm EDB the blood levels approached a steady state of approximately 0.78 µg EDB/ml blood within the first hour. Following exposure the clearance of EDB over the next hour was described by a monoexponential process with an apparent first-order rate constant of 0.037±0.003 (SD) min^{-1} which corresponds to a half-life of 18.7 minutes. Upon exposure to 6 ppm the time required to reach a steady state appeared to take longer. At termination of the 6 hour exposure the concentration was 0.067 µg EDB/ml blood.

Clearance of EDB from the blood after the 6 ppm 6 hour exposure was not determined because no EDB was detected in the samples taken 15 minutes post-exposure.

If it is assumed that the pulmonary uptake of EDB is an analogous process to a constant rate intravenous infusion, then the approach to steady state blood levels can be predicted from the following relationship:

$$C_t = \frac{k_o}{V_d K} (1-e^{-Kt}) \qquad (1)$$

C_t is the concentration of EDB in the blood at a given time (t); V_d is the volume of distribution; K is the elimination rate constant from blood; and k_o is the constant rate of pulmonary uptake which is analogous to a constant infusion rate. Parameters were obtained by using the observed data for the uptake and elimination of blood EDB during exposure to 78 ppm. Subsequently those derived parameters were used to predict blood level data for both the 78 and 6 ppm exposure.

Pulmonary uptake (k_o) was calculated by dividing the total amount of EDB recovered in the body at 6 hours (determined in rats given ^{14}C-labeled EDB) by the exposure time (6 hr). The volume of distribution was then calculated at the 6 hr point by substituting the measured mean blood concentration of EDB at that time (C_t) into equation (1). Once estimates were obtained for all parameters then the blood concentration of EDB at any time (C_t) could be predicted. The uptake curve showing the predicted approach to steady state blood levels of EDB are shown along with the observed data in Figure 3. While the predicted uptake of EDB in blood closely approximated the observed data on exposure to 78 ppm, the uptake on exposure to 6 ppm was slower than that predicted during the first 2 hours of exposure. The blood concentration of EDB immediately following 6 and 78 ppm exposures increased proportionately to the increase in exposure levels.

DISCUSSION

The results indicate that rats rapidly metabolize and excrete metabolites of EDB upon inhalation exposure to atmospheric concentrations ranging from 7-75 ppm ^{14}C-EDB. The percentage of radioactivity eliminated by the various routes of excretion are similar for all exposure concentrations indicating that the routes of elimination were independent of the exposure level. Likewise, the elimination rate of ^{14}C-activity in the urine, accounting for 80% of the total amount of metabolized EDB, was independent of the exposure concentration from 7-75 ppm EDB.

The distribution of radioactivity in various tissues showed nothing remarkable. The highest concentrations of radioactivity when expressed on a per g tissue basis were found in liver and kidney which are the predominant organs involved in metabolism and excretion. It is noteworthy that the concentration in the stomach, which was the target organ following oral intubation (Olson, et al., 1973), was not greater than other non-target tissues. Furthermore, the concentration of radioactivity in the stomach did not increase disproportionately with the increase in the exposure levels. This is additional evidence that stomach cancer observed in rats following massive doses by oral gavage may be unrelated to the hazard of exposure to EDB via inhalation.

Chromatographic analyses strongly suggest that two of the major urinary metabolites of EDB are N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid. These same metabolites were reported by Nachtomi (1970) to result from oral administration of EDB to rats. It appears that following inhalation exposure, rats rapidly conjugate EDB with glutathione (GSH) and the GSH conjugates are subsequently converted to cysteine conjugates and further oxidation products of cysteine prior to urinary excretion. Only a very small quantity of EDB per se is eliminated in the expired air; thus metabolism of EDB via conjugation constitutes the primary means for detoxification.

Reaction of chemicals with intracellular macromolecules is believed to be one mechanism for toxicity including carcinogenicity. The elegant works of Mitchell, et al., (1973); Potter, et al., (1974); and Jollow, et al., (1974) showed that large doses of bromobenzene and acetaminophen overwhelm their GSH-dependent detoxification through depletion of hepatic GSH. As GSH is depleted the electrophilic intermediates formed by biotransformation of these compounds react with macromolecules rather than GSH. Toxicity is a function of the magnitude of those reactions with macromolecules. The target organs for toxicity in rats following

inhalation exposure to EDB are the liver, lung, and kidney (Rowe, et al., 1952). However, macromolecular binding of radioactivity to liver and lung tissue following exposure to EDB did not increase disproportionately with the increase in exposure concentration. Instead, the binding was directly related to the total amount of EDB metabolized. Since the metabolism of EDB is primarily a detoxification process it appears that for EDB total binding in these tissues may not correlate with toxicity. This is understandable since the methodology used measures reaction with total macromolecules, and toxicity and/or carcinogenicity is likely a function of interaction with specific macromolecular sites including nucleic acids.

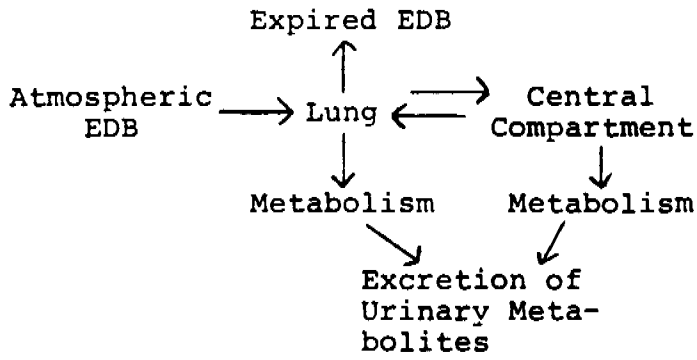
An aspect of the findings in the research reported herein worthy of discussion is that the amount of EDB biotransformed increased only 2-fold when the exposure concentration was increased from 7 to 25 ppm, a 3.4 fold increase. However, when the concentration was increased from 25 to 75 ppm, the amount of EDB biotransformed increased proportionately. The less than proportionate increase in the amount of EDB biotransformed in going from an exposure of 7 ppm to 25 ppm suggests that at the higher concentration the biotransformation process is less efficient. It is unlikely that the

difference in efficiency is related to biotransformation occurring in organs subsequent to the absorption of EDB into the blood. This is concluded because the percent of the total body burden of ^{14}C-activity expired as EDB per se subsequent to a 6 hr exposure was only 0.9 and 1.2% in rats exposed to 7 and 25 ppm EDB respectively. This small, indeed insignificant, difference is insufficient to explain why the total amount of EDB biotransformed increased only two-fold in rats exposed to a 3.4-fold higher concentration.

To explain the apparent discrepancy it is speculated that in rats EDB may be cleared from the lungs by not only blood flow but by biotransformation of EDB in pulmonary tissue. Further, this speculated pulmonary biotransformation of EDB is conceived to be saturated by exposure to high concentration of EDB and exhausted as exposure to 7 ppm EDB continues. This mechanism provides for a more efficient uptake of EDB from alveolar air when the concentration is low because pulmonary biotransformation in addition to blood flow will maintain a greater concentration gradient. At high concentrations the pulmonary biotransformation process will be overwhelmed leading to a smaller concentration gradient and consequently less efficient uptake.

Another observation supports the foregoing speculation. The time required to attain a steady state level of EDB in the blood was longer for rats exposed to 6 ppm, 5 to 6 hours, than in rats exposed to 78 ppm, 1 hr. This suggests that early in the exposure to 6 ppm EDB is efficiently taken up and biotransformed by pulmonary tissue limiting the uptake of EDB per se by blood. As exposure continues, the pulmonary biotransformation process is exhausted and consequently more of the EDB per se reaches the blood, thus accounting for the greater duration required to reach a steady state concentration in the plasma of rats exposed to 6 ppm than rats exposed to 78 ppm. In rats exposed to 78 ppm EDB pulmonary biotransformation is conceived to be quickly overwhelmed and hence steady state levels of EDB in plasma are quickly attained.

In summary, the chemical process governing the fate of inhaled EDB is conceived to occur in accordance with the following model.



In this model the central compartment is composed of blood and those tissues with a large blood flow, i.e., liver and kidney. The essential aspect of this model is that biotransformation by lung must be a saturable process. This speculation is not inconsistent with previous reports that glutathione transferases are active in the lung (Grover and Sims, 1964; Bend, et al, 1976) but that their maximum activity is considerably less than that in liver.

One of the objectives of this study was to obtain pharmacokinetic information on the fate of inhaled EDB in rats to lend perspective to results on the chronic toxicity of inhaled EDB. One such study is currently in progress (Powers, M., personal communication). Rats are being exposed daily (6 hours/day, 5 days/week) to 10 and 40 ppm EDB. Based on the evidence for increased biotransformation of EDB by the lung at exposure levels of 6-7 ppm observed in this pharmacokinetic study, proportionately more EDB may be absorbed into the blood and distributed to the tissues of rats exposed to 40 ppm than those exposed to 10 ppm. This may result in toxicity and furthermore, it may not be appropriate to extrapolate linearly the toxicity at this level to 10 ppm where pulmonary biotransformation of EDB may be more efficient.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to B. Isenbarger for analytical assistance and D. Gransden for synthesis of radiolabeled standards for metabolic identification.

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TABLE 1

Percentage ^{14}C-activity Eliminated By Male Rats During 48 Hours Following a 6 Hour Inhalation Exposure to EDB[a]

	Exposure Levels					
	7 ppm		25 ppm		75 ppm	
	%	µg equiv. EDB	%	µg equiv. EDB	%	µg equiv. EDB
Expired as:						
EDB	0.9	14	1.2	61	1.7	181
CO_2	7.3	110	8.9	294	8.1	849
Urine	81.3	1225	79.9	2633	80.4	8442
Feces	2.7	40	2.5	83	2.6	270
Tissues	7.8	117	7.5	246	7.2	760
Total		1506		3317		10502

[a]Expressed as percentage of the total radioactivity recovered, one rat per exposure level.

TABLE 2

Tissue Distribution of ^{14}C-Activity in Male Rats Following Inhalation Exposure to EDB[a] For 6 Hours

Tissue	Exposure Level		
	7 ppm	25 ppm	75 ppm
Liver	45.8±5.5	112.7±19.2	255.7±10.6
Kidney	48.2±10.9	86.6±8.1	228.8±6.9
Carcass	6.1±0.7	13.3±2.7	58.2±15.4
Lung	6.9±0.7	10.4±2.1	34.2±1.4
Fat	7.3±0.9	10.1±1.7	62.4±21.4
Stomach	8.3±1.1	9.8±1.5	38.9±13.3
Skin	3.7±0.3	8.0±1.7	19.2±2.8
Testes	4.5±0.4	7.8±2.5	24.7±3.7
Muscle	3.1±0.4	6.0±1.6	23.5±10.1
Plasma	3.3±0.8	7.7±1.5	22.2±5.1
Brain	2.7±1.4	1.3±1.3	17.7±2.2

[a]Expressed as µg equiv. EDB/g tissue; animals were killed immediately after exposure; mean ± S.D., 3 rats/exposure level.

TABLE 3

Total Metabolism, Macromolecular Binding and Hepatic Non-Protein Sulfhydryl Levels in Male Rats Following Inhalation Exposure to EDB[a]

Exposure Conc. (ppm)	µg Equivalents EDB Metabolized	Liver µg Equivalents EDB Bound per g Protein	Lung µg Equivalents EDB Bound per g Protein	Hepatic Non-Protein Sulfhydryl Content (GSH, % Control)[b]
7	1554±173[c]	7.7±0.7	2.2±1.5	94
25	3129±401	14.4±3.0	2.7±0.3	77[d]
75	11766±2218	47.2±6.4	8.2±1.6	59[d]

[a]Rats were exposed for 6 hours to EDB and killed immediately following exposure.

[b]Control rats (4) were exposed to room air and killed simultaneously with the EDB exposed group.

[c]Mean ± S. D., 3 rats/exposure level.

[d]Statistically different from controls, Student t-test, $p < 0.05$.

LEGENDS

Figure 1 Urinary excretion of ^{14}C-activity (µg equivalents EDB) versus time (hr) following inhalation exposure to 7, 25, and 75 ppm. Lines were fit by linear regression analysis and the apparent first order rate constants (k) and half-lives ($t_{1/2}$) for elimination were determined.

Figure 2 Typical separation of urinary metabolites (0-12 hour sample) by high pressure liquid chromatography (hplc) on a SAX (duPont) ion exchange column. The profile shows the separation of radioactivity representing metabolites A, B, C, and D by hplc versus time (min, flow rate = 1 ml/min).

Figure 3 Blood concentration of EDB (µg/ml) versus time (hr) during and directly following exposure to atmospheric concentrations of 6 and 78 ppm EDB (solid line). The arrow designates termination of exposure. The elimination curve of EDB following exposure to 78 ppm was fit by linear regression analysis. The dashed lines are the predicted uptake profiles using parameters derived from the 78 ppm exposure.

Figure 1

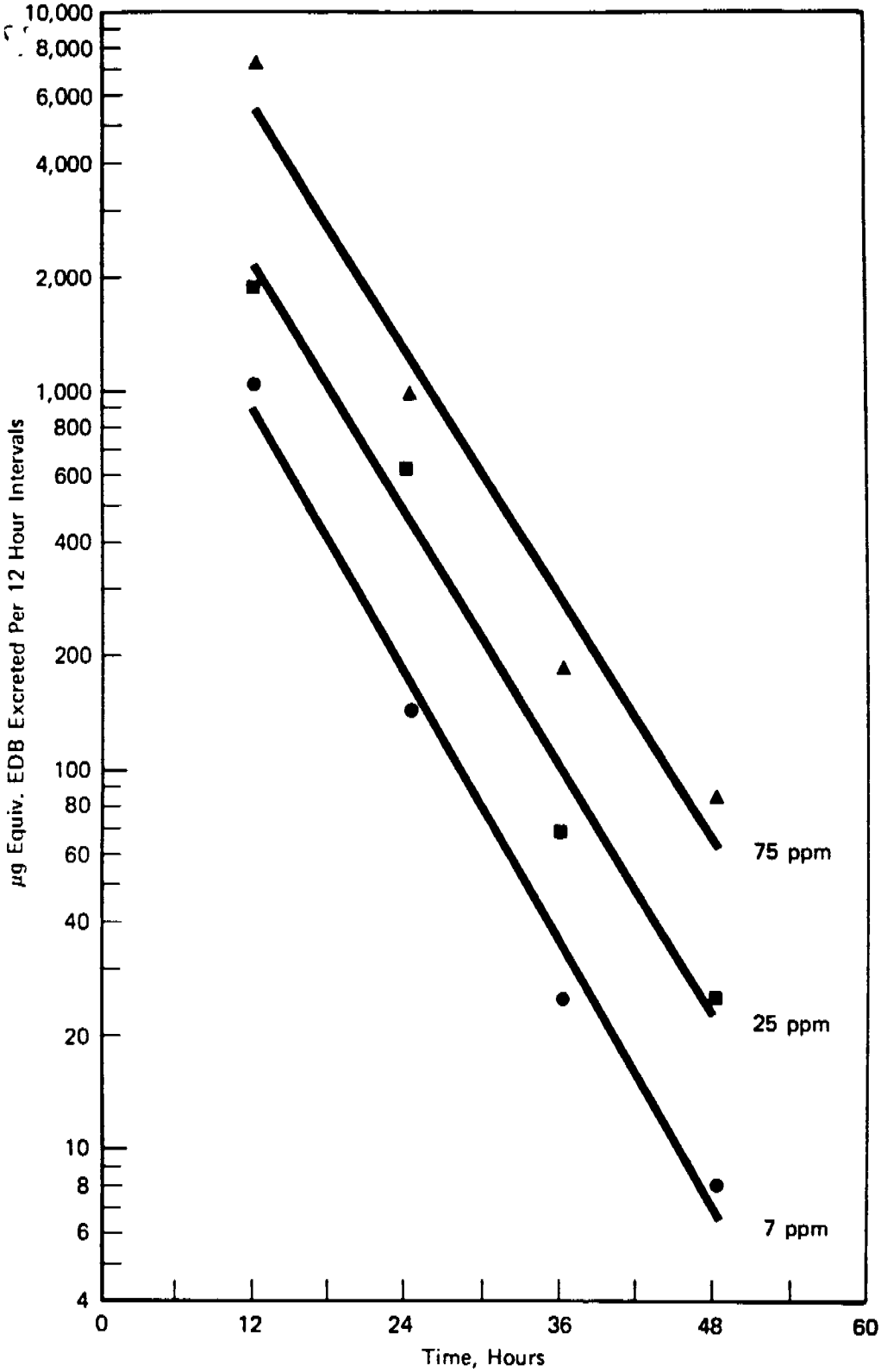

Figure 2

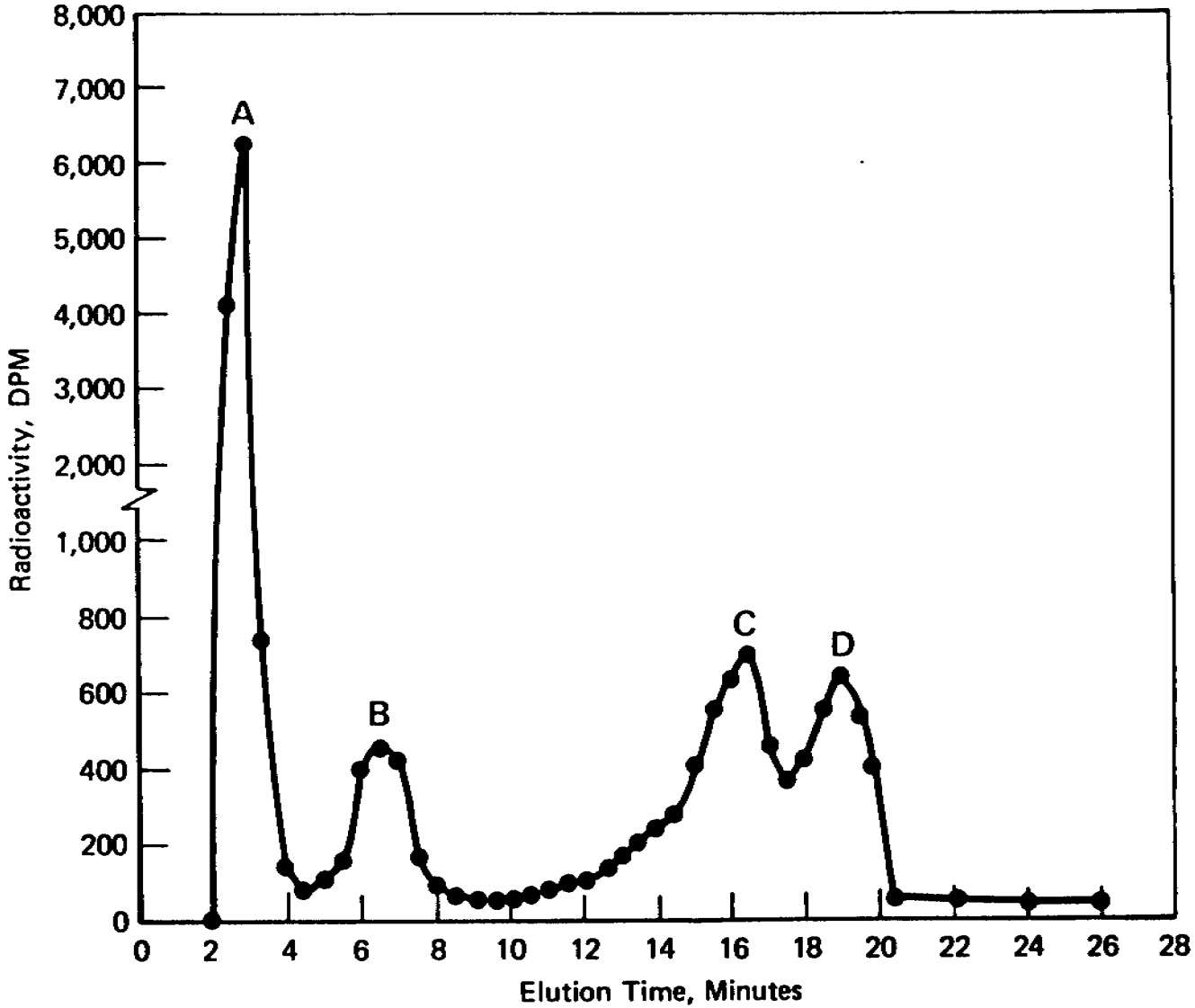

Figure 3