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TOXICITY STUDIES OF SELECTED CHEMICALS

TASK IV:

THE DEVELOPMENTAL TOXICITY OF ETHYLENE DIBROMIDE INHALED BY RATS AND MICE DURING ORGANOGENESIS

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FINAL REPORT

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Final Report

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PREFACE

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This work was conducted in the Biological Sciences Division under the direction of Dr. William B. House between April 1, 1977 and June 1, 1977. The experimental work was supervised directly by Dr. Cheng-Chun Lee, Assistant Director, Biological Sciences Division for Pharmacology and Toxicology; assisted by Dr. Robert D. Short, Jr. (Senior Toxicologist), Dr. Joseph M. Winston (Associate Toxicologist), Mr. Jan L. Minor (Associate Toxicologist), with the technical assistance of Mr. Brett Ferguson and Mr. Timothy Unger.

Approved for:

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SUMMARY

Ethylene dibromide (EDB) was administered at 20, 38, and 80 ppm by inhalation to pregnant Charles River CD rats and CD-1 mice for 23 hr/day. The exposures started on day 6 of gestation and lasted for a total of 10 days. Observations were made on maternal welfare and fetal development.

The results of this study indicate that (1) EDB is more toxic in adult mice than adult rats, (2) adverse effects on maternal welfare, as measured by weight gain, feed consumption, and survival were observed in both mice and rats, (3) although morphological changes were observed in fetuses from dams exposed to EDB, these effects were observed at concentrations that also affected maternal welfare, and (4) EDB was judged to be only a weak teratogen with little primary effect on development.

I. INTRODUCTION

Ethylene dibromide (1,2-dibromethane, EDB), is used as a scavenger in gasoline, a fumigant and a chemical intermediate. The estimated production of EDB was about 315 million pounds in 1972 and 331 million pounds in 1973.

The inhalation of EDB produced toxicity in experimental animals.[1] Rats exposed to a concentration of EDB in excess of 200 ppm died within 24 hr from respiratory or cardiovascular collapse. Death at concentrations less than 200 ppm were delayed and occurred sometimes as long as 12 days after treatment. During this time, rats lost weight, appeared rough and unkept, became irritable and produced a bloody nasal discharge. Chronic inhalation studies (7 hr/day for 5 days/week for 6 months) indicated that rats, guinea pigs, rabbits and monkeys generally tolerated EDB at levels of 25 ppm. The results of these studies were used to establish a threshold limit value of 20 ppm for EDB by the American Conference of Governmental Industrial Hygienists.[2]

EDB exposure also produced a more insidious type of toxicity. A carcinogenic response was demonstrated by administering EDB orally to rats (40 and 80 mg/kg/day) and mice (60 and 120 mg/kg/day) five times a week.[3] This treatment produced a high incidence of gastric squamous cell carcinomas in both species as early as 10 weeks after treatment started. The tumors, which were originally located in the forestomach, metastasized throughout the abdominal cavity. In some animals the carcinoma migrated to the lungs and other tissues.[4] EDB was also shown to be mutagenic in bacteria[5] and *Drosophila melanogaster*.[6] EDB also affected spermatogenesis and the maturation of sperm in bulls,[7] damaged spermatogenic cells in rats[8] and reduced the egg weight of laying hens.[9]

The teratogenic potential of inhaled EDB was studied in Charles River CD rats and CD-1 mice.[10] Animals were exposed to 32 ppm of EDB for 23 hr a day from days 6 through 15 of gestation. In addition to the control, a third group of animals was given a reduced diet, but no EDB exposure. In mice, it was observed that exposed animals consumed less feed and gained less weight than controls. Litter sizes were somewhat reduced as were weights, and a variety of skeletal anomalies involving incomplete ossification was noted. Because increased occurrence of similar phenomena was displayed by the reduced-diet, unexposed rats, it was determined that the defects were most likely attributable to malnourishment rather than to EDB exposure, *per se*. In rats, a similar reduction in feed consumption and weight gain was seen. Similarly, litter size was somewhat reduced, but fetal weights were near normal. As was noted in mice, many of the observed defects could well be attributable to malnourishment rather than to EDB exposure. *per se*. However, an increase in fourth-ventricular hydrocephaly, a reduction

in the occurrence of the fourteenth rib, and an increase in the frequency of wavy ribs appear to be correlated to EDB exposure in this species. The results section and tables for the above study are included in the present report as Appendix I.

The present study was undertaken to extend the teratology observations to additional concentrations of EDB. The inhalation route of exposure was selected for these studies because EDB is found in the atmosphere. The occurence of congenital defects was used as a measure of toxicity because development is a finely regulated process which is sensitive to disruption by many agents. These agents include both carcinogens and mutagens. In addition, these tests may be performed in a short period of time and are useful in identifying agents that may produce birth defects in humans.

II. METHODS

A. Animals

Charles River CD rats and CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were housed in our animal quarters for at least 7 days prior to use. The quarters were maintained at 72°C with a relative humidity of 50 $\pm$ 5% and a 7 AM to 7 PM photoperiod. Animals were given free access to powdered rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois), and tap water except where indicated in the experimental protocol. During the treatment period, feed was changed daily in order to prevent possible accumulation of EDB in the feed.

B. Animal Exposure

Rochester type stainless steel chambers with a volume of about 3.5 m^3 were used in this study. Clean air at a flow rate of 10 to 12 changes per hr entered at the top of the chamber. EDB vapor was generated by bubbling nitrogen into a glass vessel which was maintained at 30°C. EDB entered the air stream upstream from the chamber. Mixing was initiated in a plenum at the top of the chamber and completed by two squirrel cage fans and a diffusion plate.

The EDB concentration in the chamber was monitored using gas chromatography and a flame ionization detector. EDB was resolved using a stainless steel column packed with 5% didecyl phthalate on 80/100 chromosorb and nitrogen (80 ml/min) as the carrier gas. The injection, column and detector temperatures were 160°C, 145°C and 170°C, respectively. Standards were prepared by serial dilutions of an EDB stock solution prepared in carbon tetrachloride.

C. Experimental Protocol

Female rats and mice were exposed overnight to proven male breeders. Successful mating was identified the next morning (day 0 of gestation) by the presence of sperm in vaginal smears from rats and copulation plugs in mice. Mated animals were divided into control and treated groups. The control group was subdivided into animals given free access fo feed and animals whose feed was restricted. The EDB treated group was subdivided into animals exposed to 20, 38, or 80 ppm of EDB. Animals were housed in the inhalation chambers for 10 days starting on day 6 of gestation. During this time the EDB treated group was exposed to EDB for 23 hr a day. The control animals were housed under similar conditions; however, the EDB exposure was omitted.

Rats and mice were sacrificed on gestational day 20 or 18, respectively. A laparotomy was performed and the uterine horns were exposed. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed, weighed and examined for external anomalies.[11] One-half of the fetuses from each litter was fixed in Bouin's solution and examined for soft-tissue anomalies by a free-hand slicing method.[11] The remaining fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH and stained with alizarin red.[12] After differential decolorization, the skeletons were examined for anomalies.

D. Statsitical Methods

Quantitative data, reported as the mean $\pm$ standard error, were initially analyzed by Bartlett's test for homogeneity.[13] The test of significance for homogeneous data was Dunnett's procedure.[13] In contrast, heterogeneous data were analyzed by the two-sample rank test.[14] Enumeration data were analyzed with the Fisher's exact probability test.[15] For all tests the 0.05 level of significance was chosen except where indicated. The liter was considered to be the unit of observation.[16] All statistical tests, therefore, were based on the litter as the experimental unit.

III. RESULTS

A. Chamber Concentration

Rats and mice inhaled EDB 23 hr a day for 10 days starting at day 6 of gestation. During this time, the EDB concentration was measured by gas chromatography generally every 2 hr. However, as the result of a mechanical malfunction, the concentration was calculated by mass balance on one of the exposure days. The values determined during the day were averaged to yield a time weighed average concentration for that day. The average (range) of these values for each chamber during the 17 day duration of the study were 20 (15 to 22), 38 (32 to 42), and 80 (71 to 84).

B. Maternal Welfare and Reproduction

1. Rats: Adverse effects on maternal welfare and reproduction were observed in rats exposed to EDB (Table 1). Deaths occurred only at the high concentration. A weight loss was evident in rats exposed to 38 and 80 ppm of EDB. In addition, the weight gain at the end of exposure was reduced at the high concentration. Feed consumption was reduced in rats exposed to all concentrations of EDB and remained depressed in the high concentration group when the exposure was terminated. Dams exposed to 80 ppm of EDB had a reduced number of implants and evidence of embryotoxicity, as measured by increased resorptions. The body weight of fetuses from dams exposed to 38 ppm was reduced. In the feed restricted group, feed consumption and weight gain were reduced during organogenesis. When these rats were given free access to feed a compensatory weight gain occurred. Although fetal body weights were reduced, there was no evidence of embryolethality.

2. Mice: In mice, death occurred in groups exposed to 38 and 80 ppm of EDB as well as the group with restricted feed consumption (Table 2). In the last group deaths were due to canibalism. The weight change was reduced in the 20 and 38 ppm EDB exposed groups and the feed restricted group. The weight change after the exposures were terminated was normal in all groups except the one previously exposed to 38 ppm of EDB. In the group exposed to 20 ppm of EDB the percent of late resorptions was increased and fetal body weights were reduced. In the group exposed to 38 ppm of EDB, the percent of viable fetuses was reduced, the incidence of resorptions was increased, fetal body weights were reduced, and the percent of male fetuses was increased. In the feed restricted group the percent of viable fetuses was reduced and the incidence of resorptions was increased. Although only one dam produced viable fetuses, these fetuses had a reduced body weight and a reduced percent of males.

C. Anomalies

1. Rats: Hematoma were the most common external anomaly that occurred in rats. They occurred in a variety of regions and were present in all of the groups. The incidence (fetuses affected/fetuses inspected) of umbilical hernia and clubbed feet in the group exposed to 38 ppm of EDB were 1/184, and 2/184, respectively. The soft tissue anomalies observed in rats are presented in Table 3. None of these anomalies was present in any of the treated groups at an incidence that reached a level of statistical significance.

The skeletal anomalies observed in rats are reported in Table 4. A reduced percent of fetuses with normally ossified centri occurred in the group exposed to 20, but not 38 ppm of EDB. In contrast this parameter was increased in the feed restricted group. In general, EDB exposure did not dramatically alter the incidence of skeletal anomalies.

TABLE 1

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Ethylene Dibromide (ppm)				
	0[c]	20	38	80	0[d]
Number Exposed	17	15	16	16	16
Pregnant	17	11	15	16	15
Alive	17	11	15	8[e]	15
Non-pregnant	0	4	1	0	1
Alive	0	4	1	0	1
Body Weight Change[a]					
During exposure	39 ± 4	28 ± 7	-30 ± 4[f]	-77 ± 5[f]	-47 ± 4[f]
After exposure	59 ± 3	69 ± 7	67 ± 6	11 ± 6[f]	92 ± 3[f]
Feed Consumption[b]					
During exposure	19 ± 1	15 ± 2[f]	9 ± 1[f]	3 ± 0[f]	4 ± 1[f]
After exposure	22 ± 1	21 ± 1	22 ± 1	7 ± 2[f]	25 ± 1
Pregnant Survivors	17	11	15	8	15
Implants/dam	14.5 ± 0.3	13.7 ± 0.8	12.7 ± 1.0	11.3 ± 1.3[f]	13.1 ± 1.0
Viable fetuses (%)	96 ± 2	98 ± 2	98 ± 2	0 ± 0[f]	97 ± 2
Dead fetuses (%)	0 ± 0	0 ± 0	0 ± 0	13 ± 13	0 ± 0
Early resorptions (%)	4 ± 2	2 ± 2	2 ± 2	88 ± 13	3 ± 2
Late resorptions (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dams with complete resorptions	0	0	0	7[e]	0
Live Litters	17	11	15	0	15
Fetuses/dam	13.9 ± 0.3	13.4 ± 0.8	12.5 ± 1.0	--	12.5 ± 0.9
Males (%)	45 ± 3	53 ± 4	43 ± 3	--	48 ± 5
Fetal weight (gm)	4.0 ± 0.1	3.9 ± 0.0	3.6 ± 0.1[g]	--	3.4 ± 0.1[g]

a/ Gm/rat/interval for pregnant rats.
b/ Gm/rat/day for pregnant rats.
c/ Control group.
d/ Feed restricted to the amount indicated on this table.
e/ Significantly different from control (Fisher exact probability test).
f/ Significantly different from control (two sample rank test).

TABLE 2

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Ethylene Dibromide (ppm)				
	0[c]	20	38	80	0[d]
Number Exposed	18	20	20	22	18[e]
Pregnant	18	19	17	19	9
Alive	18	19	10[f]	0[f]	6[f]
Non-pregnant	0	1	3	3	4
Alive	0	1	3	0	4
Body Weight Change[a]					
During exposure	16.2 ± 0.6	7.5 ± 0.8[g]	3.8 ± 1.6	--	8.8 ± 0.7[g]
After exposure	5.6 ± 0.3	6.5 ± 0.7	0.5 ± 0.1[g]	--	4.6 ± 0.8
Feed Consumption[b]					
During exposure	6.3 ± 0.4	3.9 ± 0.3[g]	2.3 ± 0.1[g]	0.8 ± 0.1[g]	0.9 ± 0.3[g]
After exposure	6.4 ± 0.1	5.6 ± 0.2	2.9 ± 0.6[g]	--	5.8 ± 1.6
Pregnant Survivors	18	19	10		6
Implants/dam	12.3 ± 0.3	12.8 ± 0.4	12.3 ± 0.7	--	9.2 ± 1.5
Viable fetuses (%)	94 ± 2	90 ± 2	35 ± 14[g]	--	2 ± 2[g]
Dead fetuses (%)	0 ± 0	0 ± 0	1 ± 1	--	0 ± 0
Early resorptions (%)	4 ± 2	4 ± 1	51 ± 16	--	88 ± 13[g]
Late resorptions (%)	2 ± 1	6 ± 2[g]	13 ± 10	--	10 ± 10
Dams with complete resorptions	0	0	6[f]	--	5[f]
Live Litters	18	19	4	--	1
Fetuses/dam	11.5 ± 0.4	11.5 ± 0.4	4.3 ± 1.8[g]	--	0.2 ± 0.2[g]
Males (5)	53 ± 4	58 ± 3	21 ± 9[g]	--	17 ± 17[g]
Fetal weight (gm)	1.35 ± 0.03	1.09 ± 0.04[g]	0.25 ± 0.11[g]	--	0.16 ± 0.16[g]

a/ Gm/mouse/interval for pregnant mice.
b/ Gm/mouse/day for pregnant mice.
c/ Control group.
d/ Feed restricted to the amount indicated on this table.
e/ Gestational status of five dead females was not determined.
f/ Significantly different from control group (Fisher exact probability test).
g/ Significantly different from control group (two sample rank test).

TABLE 3

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN RATS

	Ethylene Dibromide (ppm)			
	0[a]	20	38	0[b]
Number of				
Litters inspected	17	11	15	15
Fetuses inspected	112	71	88	88
Soft Tissue Anomalies				
Nasal passage occluded	6.0 ± 3.0[c]	1.0 ± 1.0	6.0 ± 3.0	3.0 ± 2.0
Inferior vena cava hemorrhage	0 ± 0	0 ± 0	0 ± 0	3.2 ± 1.7
Hydronephrosis	13.0 ± 4.0	12.0 ± 4.0	14.0 ± 7.0	0 ± 0
Kidney cortex solidified	0 ± 0	1.5 ± 1.5	0 ± 0	0 ± 0
Distended urinary bladder	1.0 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Blunt snout	0 ± 0	0 ± 0	0.8 ± 0.8	0 ± 0

a/ Control group.

b/ Feed restricted group.

c/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per liter basis.

TABLE 4

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SKELETAL ANOMALIES IN RATS

	Ethylene Dibromide (ppm)			
	0[a]	20	38	0[b]
Number of				
Litters inspected	16	11	15	15
Fetuses inspected	115	75	96	97
Skeletal Anomalies				
Skull collapsed: slight	6.0 ± 2.8[c]	3.0 ± 3.0	4.5 ± 2.0	7.1 ± 3.0
marked	1.0 ± 1.0	0 ± 0	1.1 ± 1.1	1.1 ± 1.1
Occipital fontanel enlarged	0 ± 0	0 ± 0	1.9 ± 1.3	0.8 ± 0.8
Parietals incompletely ossified	0 ± 0	0 ± 0	2.2 ± 2.2	0 ± 0
Interparietals: incompletely ossified	0 ± 0	0 ± 0	3.6 ± 2.0	0 ± 0
curved medially	0 ± 0	0 ± 0	4.8 ± 3.3	0 ± 0
Supraoccipital: incompletely ossified	0 ± 0	0 ± 0	0.8 ± 0.8	0 ± 0
Squamosal: split	0 ± 0	2.5 ± 1.7	1.7 ± 1.2	0 ± 0
Hyoid bone: unossified	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0
split	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0
Sternebrae: ossified normally	56.1 ± 7.5	58.1 ± 7.4	35.2 ± 7.9	37.0 ± 9.5
unossified	11.3 ± 4.0	12.3 ± 4.6	22.8 ± 6.3	16.8 ± 4.7
incompletely ossified	30.8 ± 5.0	32.1 ± 7.4	49.2 ± 6.7	52.7 ± 8.0
split	0 ± 0	0 ± 0	0 ± 0	2.1 ± 1.4
malaligned	5.0 ± 2.7	4.1 ± 2.1	7.7 ± 2.4	4.2 ± 2.5
Centri: ossified normally	69.3 ± 5.3	47.4 ± 10.0[d]	54.8 ± 8.8	84.7 ± 4.5[d]
lobed	26.0 ± 4.7	49.7 ± 9.9	32.8 ± 7.2	14.4 ± 4.6
split	5.0 ± 1.7	8.5 ± 3.2	15.1 ± 4.6	1.9 ± 1.3
Ribs: extra	5.6 ± 2.7	0 ± 0	0 ± 0	3.6 ± 2.1
wavy	0 ± 0	0 ± 0	0.8 ± 0.8	0 ± 0
Radius absent	0 ± 0	0 ± 0	0 ± 0	1.1 ± 1.1
Paws: phalanges unossified	0 ± 0	0 ± 0	0.9 ± 0.9	0 ± 0

a/ Control group.
b/ Feed restricted group.
c/ Mean ± S.E. of the percent of fetuses with the indicated anomally calculated on a per litter basis.
d/ Significantly different from control group (two sample rank test $p < 0.10$).

2. Mice: Few external anomalies were observed in mice. Exencephally occurred in three of 218 fetuses examined from the group exposed to 20 ppm of EDB. Hematomas occurred in a few of the fetuses from groups exposed to 0 and 20 ppm of EDB.

The soft tissue anomalies observed in mice are presented in Table 5. There was a high percent of runts among the fetuses from the group exposed to 38 ppm of EDB. This may be a reflection of the reduced fetal body weight which in turn may be a reflection of the reduced feed consumption and weight gain of the dams. None of the remaining anomalies listed occurred at statistically significant rate in either of the groups exposed to EDB.

The skeletal anomalies observed in mice are reported in Table 6. EDB exposure was associated with a statistically significant increase in some of these anomalies. Of these anomalies, incomplete ossification of supraoccipitals, unossified incus, and sternebrae anomalies (unossified and incompletely ossified) were previously reported in mice exposed to 31.6 ppm of EDB[10/] (Appendix I Table 4). Unfortunately, there were no fetal mice in the present study to evaluate the effect of feed restriction on development. However, the results of the previous study suggested that the incidence of sternebrae anomalies was increased by restricting the availability of feed.

IV. DISCUSSION

Pregnant rats and mice were exposed to 0, 20, 38, or 80 ppm of EDB 23 hr a day for 10 days starting on day 6 of gestation. EDB produced effects on the dam, as measured either by weight change or feed consumption, in both species at all of the doses tested. In addition, a significant increase in adult mortality occurred in rats exposed to 80 ppm of EDB and in mice exposed to 38 and 80 ppm of EDB. Fetal mortality, as measured by the incidence of resorptions, was increased in rats exposed to 80 ppm of EDB and in mice exposed to 38 ppm of EDB. Fetal mortality and reduced fetal body weights may be due either to EDB exposure or to a decrease in feed consumption during organogenesis.

In a previous study[10/] rats similarly exposed to 31.6 ppm of EDB, had a reduced litter size as measured by implants/dam and fetuses/dam. In the present study the litter size was normal in rats exposed to 20 ppm of EDB. However, in rats exposed to 80 ppm there was a reduced number of implants/dam and none of these implants was associated with viable fetuses. Since the exposures started on gestational day 6, it is possible that the treatment may have interfered with the process of implantation. In addition, it was also reported[10/] that exposure to 31.6 ppm of EDB produced an increase in 4th ventricle hydrocephaly, reduction in the occurence of a 14th rib and an increase in the frequency of wavy ribs. These findings were not confirmed in the present study with EDB concentrations of 20, 38, and 80 ppm.

TABLE 5

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Ethylene Dibromide (ppm)		
	0[a]	20	38
Number of			
Litters inspected	18	19	4
Fetuses inspected	98	103	20
Soft Tissue Anomalies			
Cerebrum, aplasia of	0 ± 0[b]	0 ± 0	5.0 ± 5.0
Hydrocephalus: lateral ventricle	0.8 ± 0.8	2.5 ± 1.5	5.0 ± 5.0
Nasal cavity: immature	0 ± 0	0 ± 0	6.3 ± 6.3
occluded	0.8 ± 0.8	0.9 ± 0.9	0 ± 0
Nasopharyngeal canal occluded	0 ± 0	1.1 ± 1.1	0 ± 0
Small nasal sinus	0 ± 0	0 ± 0	4.2 ± 4.2
Nasal passage occluded	4.0+ 2.0	4.0 ± 2.0	18.0 ± 12.0
Nasopharypgeal canal absent	0 ± 0	1.8 ± 1.2	0 ± 0
Palate: cleft	0 ± 0	1.1 ± 1.1	0 ± 0
high	0 ± 0	1.8 ± 1.2	0 ± 0
Hydronephrosis	0.9 ± 0.9	2.2 ± 1.5	0 ± 0
Small kidney	0 ± 0	1.9 ± 1.3	6.3 ± 6.3
Kidney cortex solidified	9.0 ± 3.0	2.0 ± 1.0	0 ± 0
Exencephalus	0 ± 0	3.1 ± 1.7	0 ± 0
Runt	0 ± 0	1.0 ± 1.0	75.0 ± 25.0[c]

a/ Control group.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

c/ Significantly different from control group (two sample rank test $p < 0.05$).

TABLE 6

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Ethylene Dibromide (ppm)		
	0[a]	20	38
Number of			
Litters inspected	18	19	4
Fetuses inspected	110	115	24
Skeletal Anomalies			
Skull collapsed: slight	2.6 ± 2.0[b]	9.8 ± 4.7	5.0 ± 5.0
marked	0 ± 0	0.8 ± 0.8	0 ± 0
Nasal bones: elevated	0 ± 0	0 ± 0	11.3 ± 7.0
curved medially	1.9 ± 1.9	16.1 ± 6.4	20.8 ± 12.5
Premaxillary process incompletely ossified	0 ± 0	3.7 ± 2.8	3.6 ± 3.6
Occipital fontanel enlarged	0 ± 0	15.7 ± 6.9	88.1 ± 4.0[c]
Supraoccipital: unossified	0 ± 0	1.8 ± 1.8	41.4 ± 20.1
incompletely ossified	0 ± 0	15.7 ± 6.1[f]	54.8 ± 18.8[d]
Tympanic annulus: incompletely ossified	0 ± 0	0 ± 0	3.6 ± 3.6
Incus unossified	1.9 ± 1.3	28.5 ± 7.8[d]	95.8 ± 4.2[c]
Maxillary process: unossified	0 ± 0	0.8 ± 0.8	0 ± 0
incompletely ossified	0 ± 0	0 ± 0	4.2 ± 4.2
Maxillary: incompletely ossified	0 ± 0	0 ± 0	3.6 ± 3.6
Mandible: incompletely ossified	0 ± 0	3.7 ± 2.8	0 ± 0
Hyoid bone: unossified	0 ± 0	4.6 ± 3.6	35.5 ± 16.6[d]
incompletely ossified	0 ± 0	0.8 ± 0.8	21.7 ± 10.4[d]
Sternebrae: ossified normally	75.2 ± 5.3	64.5 ± 7.6	0 ± 0[c]
unossified	4.7 ± 2.2	7.5 ± 4.8	91.7 ± 4.8[c]
incompletely ossified	80.0 ± 3.1	19.3 ± 5.0	54.3 ± 2.5[c]
split	0 ± 0	13.0 ± 5.5	36.3 ± 17.3[d]
extra ossification between	1.9 ± 1.9	0 ± 0	0 ± 0
Centri: ossified normally	100 ± 0	100 ± 0	100 ± 0
Ribs: extra	13.9 ± 5.2	10.3 ± 3.5	12.5 ± 12.5
Pelvis: incompletely ossified	0 ± 0	2.4 ± 1.7	3.6 ± 3.6
Paws: unossified	0 ± 0	0 ± 0	25.0 ± 25.0
incompletely ossified	0 ± 0	0 ± 0	3.6 ± 3.6
phalanges unossified	0 ± 0	11.4 ± 5.8	70.8 ± 23.9[d]
Tibia: bent medially	0 ± 0	0.8 ± 0.8	0 ± 0

a/ Control group.
b/ Mean ± S.E. of the percent of fetuses with the indicated anomally calculated on a per litter basis.
c/ Significantly different from control group (two sample rank test $p < 0.01$).
d/ Significantly different from control group (two sample rank test $p < 0.05$).
e/ Significantly different from control group (two sample rank test $p < 0.10$).

Although mice similarly exposed to 31.6 ppm of EDB had a variety of skeletal anomalies, these defects were attributed to malnourishment rather than to EDB exposure, per se.[10/] Some of these previously reported anomalies (i.e., incompletely ossified supraoccipital, unossified incus, unossified sternabrae, incompletely ossified sternabrae, and split sternebrae) were observed in the present study. Since an insufficient number of litters survived in the feed restricted group, it is not possible to attribute these effects to EDB. Nevertheless the previous study suggests that malnourishment contributes to an increased incidence of these anomalies.

In summary, the results of this study indicate that (1) EDB is more toxic in pregnant mice than pregnant rats; (2) adverse effects on maternal welfare, as measured by weight gain, feed consumption and survival were observed in both rats and mice; (3) although morphological changes were observed in fetuses from dams exposed to EDB, these effects were observed at concentrations that also affected maternal welfare, and (4) EDB was judged to be only a weak teratogen with little primary effect on development.

REFERENCES

1. Rowe, V. I. et al. "Toxicity of Ethylene Dibromide Determined on Experimental Animals," AMA Arch. of Ind. Hyg. and Occup. Med., 6:158, 1952.

2. Documentation of the Threshold Limit Values, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, 1974.

3. Olson, et al. "Induction of Stomach Cancer in Rats and Mice with Halogenated Aliphatic Fumigants," J. Nat. Cancer Inst., 51(6):1993, 1973.

4. Powers, M. B., et al. "Carcinogenicity of Ethylene Dibromide (EDB) and 1,2-Dibromo-3-Chloropropane (DBCP) after Oral Administration in Rats and Mice," Toxicol. Appl. Pharmacol., 33:171, 1975.

5. Brem, H., A. B. Stein, and H. S. Rosenkranz, "The Mutagenicity and DNA-Modifying Effect of Haloalkanes," Cancer Research, 34:2576, 1974.

6. Vogel, E., and J. L. R. Chandler, "Mutagenicity Testing of Cyclamate and Some Pesticides in Drosophila Melanogaster," Experietia, 30:621, 1974.

8. Amir, D., "Sites of Spermicidal Action of Ethylene Dibromide in Bulls," J. Reprod. Fert., 35:519, 1973.

8. Edwards, K., et al. "Studies with Alkylating Agents II. A Chemical Interpretation through Metabolic Studies of the Antifertility Effects of Ethylene Dimethanesulphonate and Ethylene Dibromide," Biochem. Pharm., 19:1783, 1970.

9. Bondi, A., E. Olomucki, and M. Calderon, "Problems Connected with Ethylene Dibromide Fumigation of Cereals II. Feeding Experiments with Laying Hens," J. Sci. Food Agric., 6:600, 1955.

10. Short, R. D. et al. "Toxicity Studies of Selected Chemicals Task I: The Developmental Toxicity of Ethylene Dibromide Inhaled by Rats and Mice During Organogenesis," National Technical Information Service, Springfield, Virginia 22151, 1976.

11. Wilson, J. G., "Methods for Administering Agents and Detecting Malformations in Experimental Animals," in Teratology--Principles and Techniques, J. G. Wilson and J. Warkany (eds.), University of Chicago Press, Chicago, Illinois, pp. 262-277 (1965).

12. Staples, R. C. and V. L. Schnell, "Refinements in Rapid Clearing Techniques in the KOH-Alizarin Red S Method for Fetal Bones," Strain Technol., 39:61-63, 1964.

13. Steel, R. G. D., and J. H. Torrie, Principles and Procedures of Statistics, McGraw-Hill Book Company, New York, 1960.

14. Mann, H. B., and D. R. Whitney, "On a Test of Whether One of Two Random Variables is Stochastically Larger Than the Other," Ann. Math. Stat., 18:50-60, 1947.

15. Siegel, S., Nonparametric Statistics, McGraw-Hill, Book Company, New York, 1956.

16. Haseman, J. K., and M. D. Hogan, "Selection of the Experimental Unit in Teratology Studies," Teratology, 12:165, 1975.