

SCREENING OF PRIORITY CHEMICALS
FOR POTENTIAL REPRODUCTIVE HAZARD

Gary M. Booth
William S. Bradshaw
Melvin W. Carter
MESA Corporation
Orem, Utah 84057

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ABSTRACT

The objective of this work was to determine the effect of nine chemicals on selected reproductive parameters in pregnant white mice. The nine chemicals were diethylene glycol diethyl ether (diEGdiEE), ethylenethiourea (ETU), sodium selenite (SS), ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), triethylene glycol dimethyl ether (triEGdiME), p-nitrophenol (PNP), dimethyl phthalate (DMP), and naphthalene (N). The study was divided into two parts: (1) Determination of a Maximum Tolerated Dose (MTD) based on adult mortality and weight loss, and (2) A Reproductive study in which the MTD dose was administered to pregnant SPF CD-1 mice. Both the MTD and reproductive portions of the investigation were completed in blocks of three chemicals each, or a total of three blocks for the nine compounds.

The MTD values selected on the basis of the empirical results obtained in part (1) of the study were: SS, 7 mg/kg; ETU, 600 mg/kg; diEGdiEE, 3000 mg/kg, EGdiME, 2000 mg/kg; diEGdiME, 3000 mg/kg; triEGdiME, 3500 mg/kg; PNP, 400 mg/kg; DMP, 3500 mg/kg; N, 300 mg/kg. Weight loss, however, was not a sensitive indicator of toxicity in this protocol.

Analysis of the results of the Reproductive Block 1 study showed that ETU reduced the reproductive index and number of litters while diEGdiEE

decreased maternal weight, increased the number of dead pups per litter, and decreased the litter weight and weight per pup. The compound SS did not significantly affect any of the maternal or fetal measurements. Compounds triEGdiME, diEGdiME, and EGdiME from Reproductive Block 2 all caused 100% resorptions (embryo lethality) in the surviving dams and, in addition, EGdiME and diEGdiME caused 26% and 41% dam mortality, respectively. Maternal body weight gain was also reduced by all three compounds. In Reproductive Block 3, PNP decreased the number of surviving dams and significantly reduced maternal body weight. Naphthalene reduced maternal body weight, decreased the mean number of live pups per litter, decreased the litter weight, but did not affect the average weight per pup. DMP did not affect any of the parameters.

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REPORT OF THE QUALITY ASSURANCE UNIT

NIOSH PROJECT #210-81-6012

During each of the several phases of the project (NIOSH project #210-81-6012). The Quality Assurance Unit at Brigham Young University made periodic visits to the laboratories where the research was carried out to audit the research procedures for compliance with the approved protocols and standard operating procedures regarding research methods and procedures, recording and handling of data, processing data, etc. The dates of these visits, according to phase, were as follows:

MTD		
Block 1	Block 2	Block 3
10-22-81	1-25-82	5- 5-82
11-20-81	2- 1-82	5- 8-82
12- 1-81	2- 3-82	5-12-82
12- 4-81	2- 8-82	5-14-82
7- 2-82	7- 2-82	7- 2-82

REPRODUCTIVE		
Block 1	Block 2	Block 3
1-13-83	6 -1-82	7- 2-82
2- 1-83	6-11-82	7-15-82
3- 2-83	6-23-82	7-15-82
3- 7-83	8-25-82	8-30-82

The accuracy of entering data into the computer, and the accuracy of the computer analyses were also checked.

Reports were submitted to the project director regarding each phase of the study on the following dates:

MTD		
Block 1	Block 2	Block 3
12- 9-81	2-11-82	6-11-82

REPRODUCTIVE		
Block 1	Block 2	Block 3
3-24-83	4-11-82	8-10-82

The Quality Assurance Unit is satisfied that this report accurately describes the methods and procedures used in the study, and the reported results accurately reflect the raw data of the study.


Leland H. Campbell
Quality Assurance Officer

INTRODUCTION

The enormous number of chemicals which are in current use and future needs for additional compounds suggest appropriate concern for mammalian reproductive assessment. Clearly, the identification and prioritization of chemicals in need of more detailed traditional reproductive hazard testing is essential to a timely study of these compounds.

The objective of this investigation was to screen nine selected chemicals for their impact on reproductive parameters in white mice. Selection of the doses in the reproductive phase was based on a preliminary toxicity screen of the chemicals called the Maximum Tolerated Dose (MTD) study. For both studies, the nine chemicals were divided into three blocks for testing so that each block contained three treatment groups plus a control group. Specifically, the investigation assessed whether the compounds caused maternal, prenatal, and neonatal toxic responses in pregnant CD-1 mice. Chemicals were administered on days 7-14 of gestation, which circumscribed the period of major organogenesis. Among the variables that were measured were maternal mortality, number of surviving pups, birth weights, and growth and survival of the pups to 2.5 days of age. Another objective of this study was to determine if this screening test is more practical and sensitive than standard teratology tests.

MATERIALS AND METHODS

MICE

Virgin female CD-1 mice that were 61-71 days old were used in the MTD study and timed-pregnant mice of the same strain and age were used in the reproductive investigation. All mice were purchased from Charles River Associates, Lakeview New Jersey Colony, Wilmington, Massachusetts, except for those from MTD Blocks 1 and 2 which came from Portage, Michigan via Charles River Associates.

CHEMICALS

A total of nine compounds were used in this investigation. The National Institute for Occupational Safety and Health (NIOSH) supplied all compounds identified by coded names, except naphthalene (N) which was purchased commercially. However, toxicity, storage, and safe handling information was supplied. Chemical identification was supplied to the contractor after the completion of the study. The nine chemicals were diethylene glycol diethyl ether (diEGdiEE) coded as 5207BE, ethylenethiourea (ETU) coded as 1426LE, sodium selenite (SS) coded as 08141, ethylene glycol dimethyl ether (EGdiME) coded as 5311TD, diethylene glycol dimethyl ether (diEGdiME) coded as 0115LE, triethylene glycol dimethyl ether (triEGdiME) coded as 0919EH, p-nitrophenol (PNP) coded as 008853, dimethyl phthalate (DMP) coded as 720697, and N coded as 031694.

PROTOCOLS AND STANDARD OPERATIONAL PROCEDURES (SOPs)

Appendix A contains the protocols and SOPs that were followed throughout this investigation consistent with requirements of Good Laboratory Procedures (GLP).

EXPERIMENTAL DESIGN

MTD STUDIES

A total of three chemicals were tested simultaneously in each MTD study (except in MTD Block 2, where four were tested) against a vehicle control group. Thus, there were three experimental blocks (i.e. 9 chemicals) tested in this design. Block 1 included diEGdiEE, ETU, and SS. Block 2 consisted of EGdIME, diEGdIME, triEGdIME, and a repeat of diEGdiEE. Block 3 was PNP, DMP, and N. Initially, the MTD was defined as the highest dose which resulted in a weight reduction during the treatment period of 10% or less compared to the controls, and which resulted in no animal mortality. The following formula was to be utilized in determining the MTD dose level:

$$0\% < \frac{\text{Final T BW} - \text{Final C BW}}{\text{Final C BW}} \times 100 < -10\%$$

Where T = treated, C = control and BW = mean body weight

For practical purposes such a criterion for the MTD was determined to be unrealistic. All MTD levels used in each reproductive phase were based upon mortality and morbidity data. The aim was to provide a dose that would create a maximum challenge and this could best be evidenced in a dose level that produced minimum adult mortality.

Each chemical was generally tested at five concentration levels with 10 female mice per treatment level. However, N was tested at 6 concentrations

and diEGdiEE was repeated in MTD Block 2 using 3 additional treatments at 9 mice per treatment. A total of 10 mice were used as a concurrently shared vehicle control. The dose levels for each MTD Block are shown below:

<u>Group No.</u>	<u>No. of Animals (Females)</u>	<u>Dose</u>
<u>MTD Block 1</u>		
Water Control		
1	10	
Sodium Selenite		
2	10	2.5 mg/kg
3	10	5.0 mg/kg
4	10	10.0 mg/kg
5	10	20.0 mg/kg
6	10	40.0 mg/kg
Ethylene Thiourea		
7	10	75.0 mg/kg
8	10	150.0 mg/kg
9	10	300.0 mg/kg
10	10	600.0 mg/kg
11	10	1200.0 mg/kg
diEGdiEE		
12	10	125.0 mg/kg
13	10	250.0 mg/kg
14	10	500.0 mg/kg
15	10	1000.0 mg/kg
16	10	2000.0 mg/kg
<u>MTD Block 2</u>		
Water Control		
1	10	
EGdiME		
2	10	225 mg/kg
3	10	450 mg/kg
4	10	900 mg/kg
5	10	1800 mg/kg
6	10	3600 mg/kg
diEGdiME		
7	10	335 mg/kg
8	10	670 mg/kg
9	10	1340 mg/kg
10	10	2680 mg/kg
11	10	5360 mg/kg

<u>Group No.</u>	<u>No. of Animals (Females)</u>	<u>Dose</u>
<u>MTD Block 2</u>		
triEGdiME		
12	10	445 mg/kg
13	10	890 mg/kg
14	10	1780 mg/kg
15	10	3560 mg/kg
16	10	7120 mg/kg
diEGdiEE		
17	9	3000 mg/kg
18	9	6000 mg/kg
19	9	9000 mg/kg
<u>MTD Block 3</u>		
Corn Oil Control		
1	10	
p-nitrophenol		
2	10	62.5 mg/kg
3	10	125 mg/kg
4	10	250 mg/kg
5	10	500 mg/kg
6	10	1000 mg/kg
Dimethyl Phthalate		
7	10	875 mg/kg
8	10	1750 mg/kg
9	10	3500 mg/kg
10	10	7000 mg/kg
11	10	11890 mg/kg
Naphthalene		
12	10	250 mg/kg
13	10	500 mg/kg
14	10	1000 mg/kg
15	10	1200 mg/kg
16	10	2000 mg/kg
17	10	125 mg/kg

The mice were supplied by Charles River Breeding Laboratories, Inc.,
Wilmington, Massachusetts. The shipping, receiving, and age data for the
three MTD studies are shown below:

MTD Study	Date Shipped	Colony	Date Received	Birth Date	Date 1st Day Dosing	Age at Dosing (days)
1	11/10/81	Portage	11/11/81	9/9/81	11/18/81	71
2	1/13/82	Portage	1/14/82	11/21/81	1/22/82	61
3	4/19/82	Lakeview	4/20/82	2/25/82	5/1/82	65

A computer-generated random sequence was used in assigning an individual animal to its treatment group and its cage. The litter bedding (Bed-O-Cobs, which has been shown not to contain substances which induce liver enzymes) was changed weekly, except after the completion of dosing.

The mice were dosed orally by gavage once per day for eight consecutive days at the same time each day. Treatment was initiated between 7 and 10 days after shipment from the supplier. All compounds used in Blocks 1 and 2 were reported to be soluble and stable in water, while MTD Block 3 chemicals were soluble in corn oil. The stock solutions were prepared by both Drs. Booth and Bradshaw. The chemicals were administered with either distilled water or corn oil as the vehicle at a constant dose volume of 10 ml/kg body weight for both control and treated groups. The mice were given water and a standard laboratory block-feed (WAYNE LAB-BLOX) on an ad libitum basis.

All mice were weighed as part of the procedure for qualifying animals for the study and randomly assigning them to the various treatment groups. Each was also weighed individually on the date of first dosing and on the fourth and eighth days after the final dose. The empirical overall average weight per mouse, determined when assignments were made to the treatment groups, was used to calculate the dosage to be administered for all mice in the

experiment. Weighing and dosing occurred at the same time each day for each chemical. Survivors were sacrificed after the final weighing on the eighth day after dosing.

Observations of clinical signs of toxicity were conducted during the first hour after the actual dosing operation and again at 5 hours after dosing.

The mean weights and standard error of the means were calculated for each group for each time the animals were weighed.

The criterion (M) to be used originally for determining the MTD as specified by the protocol was:

$$M = \frac{\text{Final TBW} - \text{Final CBW}}{\text{Final CBW}}$$

Where TBW = treatment body weight and CBW = control body weight.

This was calculated for each weighing end point of the study. This was done to investigate the properties of the criterion for the first weighing, where no treatment effects were present, through the final weighing. Confidence intervals (95%) for M were obtained using Fieller's Theorem.

A modification of M, the criterion specified by the protocol, was also calculated as follows:

$$M^*_{gt} = \frac{(\text{BW}_{gt} - \text{BW}_{g1}) - (\text{BW}_{1t} - \text{BW}_{11})}{\text{BW}_{1t}}$$

BW_{gt} is the mean weight of the g^{th} group, where $g = 1, 2, 3$, or 4 , for the t^{th} weighing, where $t = 1, 2, 3$, or 4 . And M^*_{g4} , the modified criterion for the final weighing, is the same as specified by the protocol with the exception

that the difference in the changes in body weight for a given weighing from the initial body weight for treated and control are used in the numerator. Although the MTD criterion was changed from weight gain/loss data to mortality/morbidity data, the M and M* still have value as additional parameters of the chemical effect upon the treated mice.

REPRODUCTIVE STUDIES

All animals used in this study were obtained from Charles River Associates, Wilmington, MA, via the Lakeview New Jersey Colony. The mice were 1-2 days pregnant when shipped, and were received at our laboratory 1 day after their departure from the supplier (see below).

Reproductive Study	Date Shipped	Colony	Date Received	Date Day 1 Pregnant	Date 1st Day Dosing	Days Pregnant at Dosing (days)
1	1/5/83	Lakeview	1/7/83	1/4/83	1/10/83	7
				1/5/83	1/11/83	7
2	5/26/82	Lakeview	5/27/82	5/25/82	5/31/82	7
3	6/23/82	Lakeview	6/25/82	6/22/82	6/28/82	7

The date a vaginal plug was observed was designated day 1 of pregnancy.

A total of 50 sperm-plug positive females were assigned to each of the groups. For Reproductive Block 1, the control group was given only 10 ml/kg distilled water, the SS group was given 7 mg/kg, the ETU group was given 600 mg/kg, and the diEGdiEE group was given 3000 mg/kg. The volume of the doses was 10 ml/kg. For Reproductive Block 2, the control group was given only 10

ml/kg distilled water, the EGdiME group was given 2000 mg/kg, the diEGdiME group was given 3000 mg/kg, and the triEGdiME group was given 3500 mg/kg. For Reproductive Block 3, the control group was given 10 ml/kg table grade corn oil (Mazola[®]), the PNP group was given 400 mg/kg, the DMP group was given 3500 mg/kg, and the naphthalene group was given 300 mg/kg.

The animals were dosed on days 7-14 of gestation (at the same time each day) by oral gavage. Each of the 50 females in a particular group was housed in individual cages and randomly assigned a position on one of two cage racks. The bedding was Bed-O-Cobs, changed weekly up to day 18. The newly delivered litters were observed for three days. The number of pups and total litter weight were obtained on day 1 postpartum (within 12 hours of birth) and day 3 postpartum (48 hours after day 1 observations). Pups were weighed as a litter, not individually. In order to minimize cannibalization of the pups, the bedding was not changed from day 18 of gestation throughout the postpartum observation period.

Maternal body weights were recorded on day 7 of gestation, day 18 of gestation, and day 3 postpartum. Weight changes during treatment were calculated as the difference between the weights on day 18 and day 7 of gestation.

Gestation is usually 19 days for this strain of mice. All animals which failed to deliver by day 23 of gestation were sacrificed, necropsied and the status of pregnancy determined. All nongravid uteri were treated with a 1.0% solution of sodium sulfide to determine the existence of implantation sites.

The data for the reproductive index, number of dams surviving, and number of dams littered were analyzed using the chi-square method. The data for all the other parameters were analyzed using the general linear model to obtain the ANOVA. The comparisons for each treatment were also obtained with the same general linear model by using as dummy variables the indicator variable for the three treatments represented by the compounds under test. Thus, the t-ratios for the respective regression coefficient gave the t-test for the three treatment comparisons with the control.

Three tables for each Block were prepared:

1. A summary of the reproductive indices.
2. Averages and standard deviations for maternal weight (g) by treatment.
3. Averages and standard deviations for fetal survival, fetal mortality, fetal weight per litter, weight per pup, and percent relative gain per pup.

In addition, a summary table of the maternal survival and reproductive success for all three blocks is provided.

RESULTS AND DISCUSSION

MTD STUDIES

MTD Block 1

The mean body weights for each dose level of the various treatment groups measured during and after dosing are presented in Table 1. A comparison of the differences between these values at day 1 and day 8 of treatment shows no apparent dose-dependent effect by any of the three compounds tested. The results of a formal analysis of weight differences are shown in Table 2 (for the interval covering the 8 days of treatment) and Table 3 (for the 16-day interval covering treatment and observation).

As judged by either the original (M) or modified (M*) criteria, there was no evidence for an impact on body weight by any compound at either end-point. At each dose tested M fell within the 95% confidence limits. In addition, M* did not change toward increasingly higher negative values (as would be expected for a weight loss) as a function of increasing dose.

It is apparent that M has an upward bias due to the fact that the mean body weight of the control in this block was smaller than the corresponding means for the treated groups. As a result, none of the M values were less than 0. However, it was noted that M* had better properties in this regard with approximately equal negative and positive values. This is what one would expect if the chemicals had no effect on body weight.

The effect of treatment on adult female mortality is shown in Table 4. All ten individuals in the SS 40 mg/kg group were dead within 48 hours of the

first treatment. After five days exposure, 100% mortality was observed in the SS 20 mg/kg group. Five (one-half) of the animals in the SS 10 mg/kg group survived the eight days of treatment. There was no statistically significant effect on mortality at SS concentrations of 5 or 2.5 mg/kg.

While compounds ETU and diEGdiEE also produced no statistically significant mortality at any of the concentrations employed, the former was a special case because of its relative insolubility in water. Even at the lowest concentration of 75 mg/kg and after more than 24 hours of mechanical stirring, some undissolved particles of the solid material were evident. Dose levels of 150, 300 and 600 mg/kg were delivered as slurries. At the highest nominal level, 1200 mg/kg, the solid material was allowed to settle to the bottom of the container and the particulate-free supernatant was delivered.

It is clear from these studies that weight-loss was not an indicator of toxicity for the MTD Block 1 chemicals. In consultation with the sponsor, it was agreed that the doses for Reproductive Block 1 would be: SS = 7 mg/kg and ETU = 600 mg/kg based on their mortality effects. Compound diEGdiEE was repeated in MTD Block 2, at a concentration of 3000, 6000, and 9000 mg/kg.

MTD Block 2

Table 5 summarizes the MTD Block 2 mean body weights while the MTD criteria of M and its modification M* are presented in Tables 6-7. A summary of the deaths separated by cause for each day beginning with the first day of dosing are shown in Table 8. These data indicate that there was no evidence

of an effect of the compounds on body weight. Neither M nor M* change in a dose dependent fashion. Compound EGdiME affected the survival of the animals at the 3600 mg/kg dose, with 90% compound-related mortality. The deaths for this group occurred on the 4th and 5th treatment day. The other groups receiving this compound did not suffer deaths with the exception of one at 1800 mg/kg. Compound diEGdiME at 5360 mg/kg caused 100% compound-related mortality on day 3 of treatment. One death was observed in the 2680 mg/kg group. A 100% compound-related mortality occurred with 7120 mg/kg of triEGdiME on the 2nd and 3rd days of treatment. The group receiving 3560 mg/kg had two deaths while all other groups receiving this chemical had no deaths. Doses of 6000 and 9000 mg/kg of diEGdiEE caused 100% compound-related mortality. The deaths for these groups occurred on the 1st and 2nd days of treatment. One death was also observed at 3000 mg/kg.

These data also show that weight-loss was not an indicator of toxicity for the MTD Block 2 chemicals. The doses to be used in the reproductive study, selected on the basis of mortality effects, were EGdiME = 2000 mg/kg, diEGdiME = 3000 mg/kg, triEGdiME = 3500 mg/kg, and diEGdiEE (MTD Block 1 chemical), 3000 mg/kg.

MTD Block 3

The mean body weights for MTD Block 3 treatment groups are found in Table 9. The MTD criteria of M and M* are presented in Tables 10-11. The mortality data for each day beginning with the first day of dosing are shown in Table 12. The data from Tables 9-11 show that there was no evidence

of an effect by PNP or DMP on body weight. However, the original MTD criterion (Table 11) indicates that the 250 mg/kg level of naphthalene produced a decrease in weight when compared with the control group. This was not true, however, for the modified criterion of M*. We believe this observation at a single dose level to be of questionable biological significance.

Compound PNP affected the survival of the animals at 500 and 1000 mg/kg, with 40% (33%)* mortality at 500 mg/kg, and 80% mortality at 1000 mg/kg. The deaths for the 500 mg/kg group occurred on the 1st, 2nd, and 4th treatment days, and the 1st observation day. The deaths for the 1000 mg/kg group occurred on the 1st, 3rd, and 6th treatment days.

Compound DMP affected the survival of the animals at the 7000 mg/kg and undiluted doses, with 50% mortality and 100% mortality respectively. The deaths at a dose of 7000 mg/kg occurred on the 1st, 2nd, 3rd, and 6th treatment days. The deaths for the undiluted dose occurred on the 1st and 2nd treatment days.

Naphthalene produced 100% mortality at the 500, 1000, 1200, and 2000 mg/kg dose levels. The deaths for the 500 mg/kg group occurred on the 1st, 2nd, 3rd, 4th, and 5th days of treatment, on the 1st and 2nd days of treatment

*40% if all deaths are included; 33% if only those receiving a single dose of material are included (i.e. one animal accidentally was administered two doses and died on day 4 of treatment).

for the 1000 mg/kg group, and on the 1st day of treatment for the mice receiving 1200 and 2000 mg/kg. Naphthalene at 250 mg/kg and below was without effect.

Weight-loss also was not a sensitive indicator of toxicity for these compounds. The doses selected for Reproductive Block 3, were PNP = 400 mg/kg, DMP = 3500 mg/kg, and N = 300 mg/kg.

REPRODUCTIVE STUDIES

Reproductive Block 1

Table 13 lists the reproductive indices for each of the treatments. The reproductive index was calculated as follows: The littered survivors (LS) were divided by the pregnant survivors (PS) labelled as the LS/PS column.

Data from Table 13 clearly shows that ETU was the only treatment that significantly reduced the reproductive index when compared to the control (reproductive index = 0.743). The numbers of Na₂S positive survivors were 4, 5, 9, and 6 for distilled water, SS, ETU, and diEGdiEE, respectively. All of the dams survived in this block except for one compound-related death in the SS treatment and one dosing-error death in the diEGdiEE treatment (see Table 22 for these summaries).

The effect of treatment on maternal weight in this study is summarized in Table 14. Among dams which were pregnant (littered or Na₂S positive),

diEGdiEE was the only treatment which significantly reduced maternal body weight. In the littered group, this loss of weight in the diEGdiEE group was again observed. Some increase in weight was observed in the littered survivors for ETU on day 18 and the % Difference relative to day 7 but postpartum weight was not significantly different from the control. In the Na_2S positive group, diEGdiEE caused significantly less weight gain as shown in the % Difference column. Weights in the non-pregnant (Na_2S negative) animals were not significantly affected by any of the treatments.

Table 15 shows survival, mortality, and pup weight data for each treatment. On day +1, diEGdiEE significantly increased the number of dead pups per litter, reduced the litter weight, and reduced the weight per pup. The ETU treatment significantly reduced the number of litters. An abnormally large number of dams in this group did not produce litters and were scored as Na_2S negative (15 compared to 5 in the control group; see Table 22). Statistical analysis demonstrated that this was not a random result and we consider it more probable that a significant number of these animals carried embryos all of which died so soon after exposure to the compound that implantation sites were completely resorbed when the uteruses were treated two weeks later. ETU treatment also resulted in an unusual (but significant) increase in litter weight and weight per pup. By day +3, all of the significant differences for diEGdiEE had disappeared while the ETU treatment continued to show a significantly larger litter weight. The SS treatment apparently did not affect any of the fetal parameters on day +1 or day +3.

In summary, diEGdiEE was the only treatment which clearly adversely affected the developing fetus and also reduced maternal weight.

Reproductive Block 2

Reproductive indices for EGdiME, diEGdiME, and triEGdiME are shown in Table 16. Note that the reproductive indices for all of the compounds was 0.000 due to the fact that no litters were produced by surviving dams. Table 22 shows that the number of Na_2S positive survivors was 1, 34, 27, and 37 for distilled water, EGdiME, diEGdiME, and triEGdiME, respectively. The high number of surviving Na_2S positive dams with no litters suggests these compounds cause significant embryotoxicity.

The maternal weight data, obviously affected by the 100% prenatal mortality induced by three compounds, is shown in Table 17. Maternal weight in the pregnant mice was significantly reduced by all three compounds and these differences persisted through day 3 postpartum. In this instance, of course, this category consisted exclusively of Na_2S positive dams.

Table 18 shows a summary of the fetal parameters for control litters. The data clearly show that all three compounds caused 100% prenatal mortality in surviving pregnant dams. Examination of the uterine horns in these animals suggested that the offspring died relatively early in the embryonic phase of development. In addition, the number of compound-treated dams surviving was reduced, particularly in the EGdiME treated mice (26% dam mortality) and the diEGdiME treated mice (41% dam mortality). Group triEGdiME lost only two dams (4% dam mortality) and all of the control females survived (see Table 22).

In summary, all of the compounds caused 100% embryonic death in the surviving dams and EGdIME and diEGdIME caused 26% and 41% dam mortality respectively. Maternal weight was consequently significantly reduced by all three compounds.

Reproductive Block 3

Table 19 indicates that the reproductive index was not affected by any of the block 3 chemicals. Table 22 shows that the lack of significant differences in the LS/PS index was due to few Na_2S positive survivors compared to those that littered. This results in indices approaching or equal to unity. In other words, the pregnant dams treated with these compounds seemed to either litter or die during gestation.

Table 20 shows the effect of treatment on maternal weight. At day 7, maternal weights were about the same in all treatments. However, by day 18, maternal weights from the pregnant and littered groups in the naphthalene and PNP treatments were significantly reduced reflecting smaller litter sizes. But by day 3 postpartum, only the naphthalene treated dams had body weights that were significantly less than the controls.

The summary of all measured fetal parameters is shown in Table 21.

Naphthalene significantly decreased the number of live pups which is reflected in a decreased litter weight; weight per pup, however, was not affected. Compounds PNP and DMP did not significantly change the fetal

measurements compared to the control. The number of surviving dams was reduced by both naphthalene and PNP, but not by DMP.

In summary, PNP, DMP and naphthalene did not significantly reduced the reproductive index. However, PNP and naphthalene decreased the number of surviving dams, and significantly reduced maternal body weight. Naphthalene also significantly decreased the number of live pups and litter weight. However, weight per pup was not significantly affected by naphthalene. Compound DMP at 3500 mg/kg did not significantly affect any of the measurements during the study.

A summary of maternal survival and reproductive success for each of the nine chemicals tested is presented in Table 22.

CONCLUSIONS

Under the conditions employed in this study, body weight was not a sensitive indicator of toxicity in non-pregnant, adult female CD-1 mice, and was not useful in determining the MTD dose level for the chemicals tested. With one exception, the compounds either killed the animals, or if they survived, there was no change in body weight. Adult lethality, therefore, became the indicator parameter for toxicity, and the dose levels used in the reproductive studies were established based on a Minimum Effective Dose (MED), defined as the lowest dose which resulted in minimum mortality in a group of mice during the treatment period.

Among the dose levels used in the reproductive studies, EGdiME, diEGdiME, PNP, and naphthalene produced mortality in pregnant females (26%, 41%, 18%, and 18% respectively).

The number of live-born pups, birth weights, and growth and survival to 2.5 days of age were the major pup response variables studied in the reproductive phase. Of the nine chemicals tested, diEGdiEE was the only chemical that reduced average pup weight. For the other eight chemicals, if the pups in a litter survived the toxicity of these compounds then their body weights were not significantly decreased.

No detectable developmental toxicity was observed on surviving pups with SS or DMP. Three of the glycol derivatives (EGdiME, diEGdiME, and triEGdiME) resulted in 100% prenatal lethality, death probably occurring soon after the initiation of treatment. The toxic properties of diEGdiEE treatment were manifest directly in an elevated pup lethality and an initial decreased weight per pup. An embryo-lethal effect of ETU is inferred by the reduced number of dams which littered. Naphthalene exhibited a much lower, though significant, level of embryo-lethality based on litter size at birth and increased numbers of pregnant dams which did not survive. The data obtained for PNP for number of litters suggest the possibility of an embryotoxic effect for this compound also.

The nine chemicals tested in this study appear to fit into four categories of toxic properties. Dams exposed to either SS or DMP appear to buffer their developing offspring against toxic effects. With these compounds there was no detectable effect on prenatal toxicity at a dose level below

that which produced significant adult mortality. In contrast, EGdIME, diEGdIME, and triEGdIME constitute a second category in which the relatively high doses used produced severe prenatal lethality in addition to significant toxicity in the dams themselves. Both dam and fetus were apparently sensitive. The compound diEGdiEE and probably ETU seem to be in a third category wherein they cause significant embryoletality but without dam mortality. Naphthalene and PNP represent a fourth class of compounds in which adult toxicity is manifest before a high level of developmental toxicity occurs.

TABLE 1
 MEAN BODY WEIGHT, NON-PREGNANT ADULT FEMALES
 (GRAMS + STANDARD ERROR OF MEAN)

MTD STUDY BLOCK 1 (Contract #210-81-6012)

Chemical ¹	Dose	TREATMENT						POST TREATMENT					
		N	Day 1 of TRT	SE	N	Day 8 of TRT	SE	N	Day 4 Post TRT	SE	N	Day 8 Post TRT	SE
Water Cont.		10	23.82	.37	9	23.19	.52	9	23.62	.39	9	24.59	.33
SS	2.5 mg/kg	10	25.57	.45	9	25.31	.49	9	25.41	.45	9	26.37	.53
SS	5.0 mg/kg	10	26.13	.57	9	24.96	.52	9	25.27	.53	9	26.58	.54
SS	10.0 mg/kg	10	25.81	.50	5	25.22	.59	5	25.92	.63	5	27.48	.30
SS	20.0 mg/kg	10	24.80	.46	0	--	--	0	--	--	0	--	--
SS	40.0 mg/kg	10	25.92	.44	0	--	--	0	--	--	0	--	--
ETU	75.0 mg/kg	10	25.21	.60	10	25.10	.65	10	25.51	.62	10	26.61	.73
ETU	150.0 mg/kg	10	26.11	.63	9	25.78	.72	9	26.32	.72	9	27.81	.72
ETU	300.0 mg/kg	10	24.83	.58	10	24.55	.72	10	24.27	.66	10	25.43	.78
ETU	600.0 mg/kg	10	25.27	.68	8	25.65	.56	8	25.76	.54	8	26.52	.50
ETU	1200.0 mg/kg	10	25.65	.75	9	25.61	.63	9	25.39	.63	9	26.59	.59
diEGdiEE	125.0 mg/kg	10	25.67	.63	10	25.19	.58	10	25.23	.55	10	25.82	.66
diEGdiEE	250.0 mg/kg	10	24.98	.50	10	25.27	.32	10	25.65	.45	10	26.69	.50
diEGdiEE	500.0 mg/kg	10	25.61	.69	10	24.75	.54	10	25.00	.55	10	26.29	.67
diEGdiEE	1000.0 mg/kg	10	24.81	.63	9	25.15	.56	9	25.60	.62	9	25.55	.77
diEGdiEE	2000.0 mg/kg	10	24.48	.87	9	24.35	.77	9	24.24	.75	9	25.07	.85

¹SS = sodium selenite

ETU = ethylenethiourea

diEGdiEE = diethylene glycol diethyl ether

TABLE 2

MTD WEIGHT CRITERIA FOR LAST DAY OF TREATMENT

MTD STUDY BLOCK 1 (Contract # 210-81-6012)

CHEMICAL ¹	DOSE	ORIGINAL MTD CRITERION (M)	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)	
			LOWER	UPPER		
Water Control		0.0	-11.7	13.3		0.0
SS	2.5 mg/kg	9.2	-3.1	23.1		1.6
SS	5.0 mg/kg	7.6	-4.6	21.5		-2.3
SS	10.0 mg/kg	8.8	-5.7	24.9		0.2
SS	20.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 5 ON WEIGH DAY.				
SS	40.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 6 ON WEIGH DAY.				
ETU	75.0 mg/kg	8.2	-3.7	21.9		2.2
ETU	150.0 mg/kg	11.2	-1.2	25.3		1.3
ETU	300.0 mg/kg	5.9	-5.9	19.3		1.5
ETU	600.0 mg/kg	10.6	-2.1	25.1		4.3
ETU	1200.0 mg/kg	10.4	-1.8	24.5		2.6
diEGdiEE	125.0 mg/kg	8.6	-3.3	22.3		0.7
diEGdiEE	250.0 mg/kg	9.0	-2.9	22.7		4.0
diEGdiEE	500.0 mg/kg	6.7	-5.1	20.2		-1.0
diEGdiEE	1000.0 mg/kg	8.4	-3.7	22.4		4.2
diEGdiEE	2000.0 mg/kg	5.0	-7.0	18.7		2.2

¹SS = sodium selenite

ETU = ethylenethiourea

diEGdiEE = diethylene glycol diether ether

TABLE 3

MTD WEIGHT CRITERIA FOR 8 DAYS POST TREATMENT

MTD STUDY BLOCK 1 (Contract #210-81-6012)

CHEMICAL	DOSE	ORIGINAL MTD CRITERION (M)	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)
			LOWER	UPPER	
Water Control		0.0	-13.5	16.6	0.0
SS	2.5 mg/kg	7.2	-6.7	23.5	0.1129412
SS	5.0 mg/kg	8.1	-5.9	24.4	-1.3058977
SS	10.0 mg/kg	11.8	-5.0	30.9	3.6646957
SS	20.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 5 ON WEIGH DAY.			
SS	40.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 6 ON WEIGH DAY.			
ETU	75.0 mg/kg	8.2	-5.5	24.2	2.5666208
ETU	150.0 mg/kg	13.1	-1.2	29.8	3.7912264
ETU	300.0 mg/kg	3.4	-9.9	19.0	-0.6868318
ETU	600.0 mg/kg	7.9	-6.6	24.6	1.9769678
ETU	1200.0 mg/kg	8.1	-5.9	24.4	0.6913617
diEGdtEE	125.0 mg/kg	5.0	-8.5	20.7	-2.5169764
diEGdtEE	250.0 mg/kg	8.5	-5.2	24.3	3.8273430
diEGdtEE	500.0 mg/kg	6.9	-6.7	22.8	-0.3614741
diEGdtEE	1000.0 mg/kg	3.9	-9.8	19.9	-0.0949451
diEGdtEE	2000.0 mg/kg	2.0	-11.6	17.8	-0.6958917

1SS = sodium selenite

ETU = ethylenethiourea

diEGdtEE = diethylene glycol diethyl ether

TABLE 4
MORTALITY
MTD STUDY BLOCK 1 (Contract # 210-81-6012)

Treatment ¹	Dose (mg/kg/day)	Treatment Period (Days)								Observation Period (Days)								Cum. Deaths
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	
Water		0/0 ²	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
SS	2.5	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
SS	5.0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
SS	10.0	1/0	1/0	0/0	1/0	1/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	5/0
SS	20.0	0/0	1/0	2/0	4/0	2/0	1/0	--	--	--	--	--	--	--	--	--	--	10/0
SS	40.0	4/0	4/0	2/0	--	--	--	--	--	--	--	--	--	--	--	--	--	10/0
ETU	75.0 ³	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
ETU	150.0 ³	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
ETU	300.0 ³	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
ETU	600.0 ³	0/0	0/0	1/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
ETU	1200.0 ⁴	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
diEGdiEE	125.0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdiEE	250.0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdiEE	500.0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdiEE	1000.0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
diEGdiEE	2000.0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1

¹SS = sodium selenite

ETU = ethylenethiourea

diEGdiEE = diethylene glycol diethyl ether

²compound related deaths/deaths due to gavage-related puncture.

³delivered as insoluble slurry.

⁴delivered as particle-free supernatant.

TABLE 5

MEAN BODY WEIGHT, NON-PREGNANT ADULT FEMALES
(GRAMS $\pm$ STANDARD ERROR OR MEAN)

MTD STUDY BLOCK 2 (Contract #210-81-6012)

Chemical ¹	Dose	TREATMENT						POST TREATMENT					
		N	Day 1 of TRT	SE	N	Day 8 of TRT	SE	N	Day 4 Post TRT	SE	N	Day 8 Post TRT	SE
Water Cont.		10	24.77	.70	9	23.56	1.06	7	25.44	.82	7	25.02	1.06
EGdIME	225.0 mg/kg	10	25.33	.77	10	23.49	.85	9	24.80	.83	9	26.18	.85
EGdIME	450.0 mg/kg	10	26.25	.56	9	23.81	.96	9	24.82	.98	9	25.81	.73
EGdIME	900.0 mg/kg	10	25.74	.45	10	23.38	.78	10	24.46	.86	10	25.65	.73
EGdIME	1800.0 mg/kg	10	25.35	.56	9	25.00	.65	9	26.16	.52	9	27.08	.50
EGdIME	3600.0 mg/kg	10	25.85	.58	1	29.10	--	1	25.80	--	1	27.40	--
diEGdIME	335.0 mg/kg	10	25.29	.62	10	25.51	.64	10	25.96	.60	10	26.72	.66
diEGdIME	670.0 mg/kg	10	25.13	.53	10	25.31	.50	10	25.79	.48	10	26.59	.67
diEGdIME	1340.0 mg/kg	10	25.77	.76	10	25.68	.67	10	25.81	.68	10	26.43	.67
diEGdIME	2680.0 mg/kg	10	24.71	.42	9	23.66	.53	9	24.62	.54	9	25.53	.48
diEGdIME	5360.0 mg/kg	10	25.58	.54	--	--	--	--	--	--	--	--	--
triEGdIME	445.0 mg/kg	10	26.13	.53	10	27.29	.60	10	27.09	.67	10	28.01	.53
triEGdIME	890.0 mg/kg	10	24.51	.82	10	24.88	.69	10	25.20	.74	10	25.73	.64
triEGdIME	1780.0 mg/kg	10	25.40	.66	10	26.60	.66	10	26.58	.61	10	27.25	.63
triEGdIME	3560.0 mg/kg	10	23.47	.79	8	24.66	.95	8	25.02	.93	8	25.58	1.04
triEGdIME	7120.0 mg/kg	10	22.80	.51	--	--	--	--	--	--	--	--	--
diEGdIEE	3000.0 mg/kg	9	23.88	.98	8	25.26	.82	8	25.36	.76	8	25.95	.67
diEGdIEE	6000.0 mg/kg	9	24.44	.38	--	--	--	--	--	--	--	--	--
diEGdIEE	9000.0 mg/kg	9	23.59	.41	--	--	--	--	--	--	--	--	--

¹EGdIME = ethylene glycol dimethyl ether
diEGdIME = diethylene glycol dimethyl ether
triEGdIME = triethylene glycol dimethyl ether
diEGdIEE = diethylene glycol diethyl ether

TABLE 6
MTD WEIGHT CRITERIA FOR LAST DAY OF TREATMENT
MTD STUDY BLOCK 2 (Contract #210-81-6012)

CHEMICAL ¹	DOSE	ORIGINAL MTD CRITERION M	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)
			LOWER	UPPER	
Water Control		0.0	-18.8	23.1	0.0
EGdIME	225.0 mg/kg	-0.3	-18.5	22.2	-2.7
EGdIME	450.0 mg/kg	1.0	-17.8	24.3	-5.2
EGdIME	900.0 mg/kg	-0.8	-19.0	21.7	-4.9
EGdIME	1800.0 mg/kg	6.1	-13.2	30.0	3.6
EGdIME	3600.0 mg/kg	23.5	-21.7	74.0	18.9
diEGdIME	335.0 mg/kg	8.2	-10.7	31.9	6.0
diEGdIME	670.0 mg/kg	7.4	-11.5	30.9	5.7
diEGdIME	1340.0 mg/kg	9.0	-10.1	32.7	4.7
diEGdIME	2680.0 mg/kg	0.4	-18.4	23.5	0.6
diEGdIME	5360.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 11 ON WEIGH DAY.			
triEGdIME	445.0 mg/kg	15.8	-3.9	40.5	10.0
triEGdIME	890.0 mg/kg	5.6	-13.2	28.9	6.7
triEGdIME	1780.0 mg/kg	12.9	-6.5	37.2	10.2
triEGdIME	3560.0 mg/kg	4.6	-15.1	29.0	10.2
triEGdIME	7120.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 16 ON WEIGH DAY.			
diEGdIEE	3000.0 mg/kg	7.2	-12.8	31.8	11.0
diEGdIEE	6000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 18 ON WEIGH DAY.			
diEGdIEE	9000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 19 ON WEIGH DAY.			

¹EGdIME = ethylene glycol dimethyl ether
 diEGdIME = diethylene glycol dimethyl ether
 triEGdIME = triethylene glycol dimethyl ether
 diEGdIEE = diethylene glycol diethyl ether

TABLE 7
MTD WEIGHT CRITERIA FOR 8 DAYS POST TREATMENT
MTD STUDY BLOCK 2 (Contract #210-81-6012)

CHEMICAL ¹	DOSE	ORIGINAL MTD CRITERION (M)	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)
			LOWER	UPPER	
Water Control		0.0	-17.4	21.0	0.0
EGdIME	225.0 mg/kg	4.6	-12.1	25.1	2.4
EGdIME	450.0 mg/kg	3.1	-13.4	23.4	-2.8
EGdIME	900.0 mg/kg	2.5	-13.6	22.3	-1.4
EGdIME	1800.0 mg/kg	8.2	-8.7	29.1	5.9
EGdIME	3600.0 mg/kg	9.5	-27.2	50.2	5.2
diEGdIME	335.0 mg/kg	6.8	-9.7	27.1	4.7
diEGdIME	670.0 mg/kg	6.2	-10.2	26.5	4.8
diEGdIME	1340.0 mg/kg	5.6	-10.8	25.8	1.6
diEGdIME	2680.0 mg/kg	2.0	-14.4	22.2	2.3
diEGdIME	5360.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 11 ON WEIGH DAY 4			
triEGdIME	445.0 mg/kg	12.0	-5.0	32.9	6.5
triEGdIME	890.0 mg/kg	2.8	-13.3	22.7	3.8
triEGdIME	1780.0 mg/kg	8.9	-7.8	29.5	6.4
triEGdIME	3560.0 mg/kg	2.2	-14.7	22.9	7.4
triEGdIME	7120.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 16 ON WEIGH DAY 4			
diEGdIEE	3000.0 mg/kg	3.7	-13.4	24.5	7.2
diEGdIEE	6000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 18 ON WEIGH DAY 4			
diEGdIEE	9000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 19 ON WEIGH DAY 4			

¹EGdIME = ethylene glycol dimethyl ether
 diEGdIME = diethylene glycol dimethyl ether
 triEGdIME = triethylene glycol dimethyl ether
 diEGdIEE = diethylene glycol diethyl ether

TABLE 8

MORTALITY
MTD STUDY BLOCK 2 (Contract #210-81-6012)

Treatment ¹	Dose (mg/kg/day)	Treatment Period (Days)								No. Dead								Observation Period (Days)								Total Cum. Deaths
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8									
Water		0/0 ²	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/3
EGdIME	225	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
EGdIME	450	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
EGdIME	900	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
EGdIME	1800	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
EGdIME	3600	0/0	0/0	0/0	4/0	5/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	9/0
diEGdIME	335	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdIME	670	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdIME	1340	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
diEGdIME	2680	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
diEGdIME	5360	0/0	0/0	10/0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10/0
triEGdIME	445	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
triEGdIME	890	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
triEGdIME	1780	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
triEGdIME	3560	0/0	0/0	1/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/0
triEGdIME	7120	0/0	8/0	2/0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	10/0
diEGdIEE	3000	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
diEGdIEE	6000	8/0	1/0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	9/0
diEGdIEE	9000	3/0	6/0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	9/0

¹EGdIME = ethylene glycol dimethyl ether

diEGdIME = diethylene glycol dimethyl ether

triEGdIME = triethylene glycol dimethyl ether

diEGdIEE = diethylene glycol diethyl ether

²Compound related deaths/deaths due to gavage-related puncture.

TABLE 9

MEAN BODY WEIGHT, NON-PREGNANT ADULT FEMALES
(GRAMS $\pm$ STANDARD ERROR OF MEAN)

MTD STUDY BLOCK 3 (Contract #210-81-6012)

Chemical ¹	Dose	TREATMENT						POST TREATMENT					
		N	Day 1 of TRT	SE	N	Day 8 of TRT	SE	N	Day 4 Post TRT	SE	N	Day 8 Post TRT	SE
Corn Oil		10	27.87	.30	10	28.87	.50	10	28.72	.50	10	29.87	.56
PNP	62.5 mg/kg	10	27.06	.36	10	27.69	.47	10	28.27	.51	10	29.03	.47
PNP	125.0 mg/kg	10	27.15	.51	10	27.66	.59	10	28.02	.41	10	28.51	.36
PNP	250.0 mg/kg	10	27.25	.53	10	27.94	.44	10	28.71	.50	10	28.86	.54
PNP	500.0 mg/kg	10	27.64	.38	7	28.30	.24	6	28.55	.46	6	29.23	.56
PNP	1000.0 mg/kg	10	27.55	.62	2	30.50	.70	2	30.45	.35	2	31.00	1.40
DMP	875.0 mg/kg	10	27.37	.55	10	28.13	.79	10	28.94	.85	10	29.49	.79
DMP	1750.0 mg/kg	10	28.37	.37	10	28.45	.36	10	29.42	.32	10	30.00	.36
DMP	3500.0 mg/kg	10	27.70	.36	10	28.90	.53	9	29.33	.63	9	29.54	.52
DMP	7000.0 mg/kg	10	27.24	.48	5	28.48	.52	5	29.54	.38	5	29.94	.50
DMP	undiluted	10	27.64	.28	--	--	--	--	--	--	--	--	--
Naph	125.0 mg/kg	10	28.19	.34	10	28.13	.29	10	28.96	.31	10	29.54	.31
Naph	250.0 mg/kg	10	26.85	.53	10	27.12	.68	10	27.70	.61	10	27.57	.59
Naph	500.0 mg/kg	10	27.38	.45	--	--	--	--	--	--	--	--	--
Naph	1000.0 mg/kg	10	26.87	.49	--	--	--	--	--	--	--	--	--
Naph	1200.0 mg/kg	10	27.46	.30	--	--	--	--	--	--	--	--	--
Naph	2000.0 mg/kg	10	26.79	.35	--	--	--	--	--	--	--	--	--

¹PNP = p-nitrophenol
DMP = dimethyl phthalate
Naph = naphthalene

TABLE 10
MTD WEIGHT CRITERIA FOR LAST DAY OF TREATMENT
MTD STUDY BLOCK 3 (Contract #210-81-6012)

CHEMICAL ¹	DOSE	ORIGINAL MTD CRITERION (M)	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)
			LOWER	UPPER	
Corn Oil		0.0	-7.6	8.3	0.0
PNP	62.5 mg/kg	-4.1	-11.6	4.0	-1.3
PNP	125.0 mg/kg	-4.2	-11.7	3.9	-1.7
PNP	250.0 mg/kg	-3.2	-10.7	4.9	-1.1
PNP	500.0 mg/kg	-2.0	-10.4	7.0	-1.2
PNP	1000.0 mg/kg	5.7	-7.9	19.9	6.8
DMP	875.0 mg/kg	-2.6	-10.1	5.6	-0.8
DMP	1750.0 mg/kg	-1.5	-9.0	6.8	-3.2
DMP	3500.0 mg/kg	0.1	-7.5	8.4	0.7
DMP	7000.0 mg/kg	-1.4	-10.7	8.7	0.8
DMP	undiluted	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 11 ON WEIGH DAY 2			
Naph	125.0 mg/kg	-2.6	-10.1	5.6	-3.7
Naph	250.0 mg/kg	-6.1	-13.5	1.9	-2.5
Naph	500.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 13 ON WEIGH DAY 2			
Naph	1000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 14 ON WEIGH DAY 2			
Naph	1200.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 15 ON WEIGH DAY 2			
Naph	2000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 16 ON WEIGH DAY 2			

¹PNP = p-nitrophenol

DMP = dimethyl phthalate

Naph = naphthalene

TABLE 11
MTD WEIGHT CRITERIA FOR 8 DAYS POST TREATMENT
MTD STUDY BLOCK 3 (Contract #210-81-6012)

CHEMICAL ¹	DOSE	ORIGINAL MTD CRITERION (M)	95% CONFIDENCE LIMITS FOR M		MODIFIED MTD CRITERION (M*)
			LOWER	UPPER	
Corn Oil		0.0	-7.3	7.8	0.0
PNP	62.5 mg/kg	-2.8	-10.0	4.9	-0.1
PNP	125.0 mg/kg	-4.6	-11.7	3.1	-2.1
PNP	250.0 mg/kg	-3.4	-10.5	4.3	-1.3
PNP	500.0 mg/kg	-2.1	-10.5	6.8	-1.4
PNP	1000.0 mg/kg	3.8	-9.1	17.2	4.9
DMP	875.0 mg/kg	-1.3	-8.5	6.5	0.4
DMP	1750.0 mg/kg	0.4	-6.8	8.3	-1.2
DMP	3500.0 mg/kg	-1.1	-8.5	6.9	-0.5
DMP	7000.0 mg/kg	0.2	-8.7	9.8	2.3
DMP	undiluted	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 11 ON WEIGH DAY 4			
Naph	125.0 mg/kg	-1.1	-8.3	6.7	-2.2
Naph	250.0 mg/kg	-7.7*	-14.7	-0.2	-4.3
Naph	500.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 13 ON WEIGH DAY 4			
Naph	1000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 14 ON WEIGH DAY 4			
Naph	1200.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 15 ON WEIGH DAY 4			
Naph	2000.0 mg/kg	THERE ARE NO OBSERVATIONS IN TREATMENT GROUP 16 ON WEIGH DAY 4			

¹PNP = p-nitrophenol

DMP = dimethyl phthalate

Naph = naphthalene

* MTD Criterion significant (P < .05)

TABLE 12

MORTALITY
MTD STUDY BLOCK 3 (Contract #210-81-6012)

Treatment ¹	Dose (mg/kg/day)	Treatment Period (Days)								No. Dead								Observation Period (Days)								Total Cum. Deaths																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
Corn Oil		0/0 ²	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0

¹PNP = p-nitrophenol
DMP = dimethyl phthalate
Naph = naphthalene

²Compound related deaths/deaths due to gavage-related puncture.

*This animal accidentally received 2 doses of PNP on the day of death (day 4 of treatment).

TABLE 13
 REPRODUCTIVE INDICES AND (STANDARD ERRORS)^a
 REPRODUCTIVE BLOCK 1 (Contract #210-81-6012)

<u>CLASSIFICATION</u>	<u>SURVIVORS PREGNANT (PS)</u>	<u>SURVIVORS LITTERED (LS)</u>	<u>REPRODUCTIVE INDEX (LS/PS)</u>	
T1 Distilled H ₂ O	45	41	0.911	(.042)
T2 SS	44	39	0.886	(.048)
T3 ETU	35	26	0.743*	(.074)
T4 diEGdiEE	41	35	0.854	(.055)

* Comparison with control (T₁) significant (P< .05), Chi-Square.

^a Standard Error = $\sqrt{pq/n}$

TABLE 14

AVERAGES AND (STANDARD DEVIATIONS) FOR MATERNAL WEIGHT (g)
REPRODUCTIVE BLOCK 1 (Contract #210-81-6012)

Classification	ANIMALS ON TEST			SURVIVORS			% DIFFERENCE	Sample Size	POST PARTUM
	Sample Size	Day 7	Sample Size	Day 7	Day 18	Relative To D7			
Total on Test	200		199					199	+ 3 DAYS
T1 Distilled H ₂ O	50		50					50	
T2 Sodium Selenite (7mg/kg)	50		49					49	
T3 Ethylenethiourea (600mg/kg)	50		50					50	
T4 Diethylene glycol diethyl ether (3000mg/kg)	50		47 ¹					47	
Pregnant (Litt. or Na ₂ S Pos.) [†]	167								
T1 Distilled H ₂ O	45	27.40 (1.55)	45	27.40 (1.55)	45.88 (6.81)	67.3 (22.7)		45	32.26 (3.26)
T2 Sodium Selenite (7mg/kg)	45	27.00 (1.92)	44	27.05 (1.92)	44.97 (7.84)	66.1 (26.7)		44	31.00 (3.12)
T3 Ethylenethiourea (600mg/kg)	35	27.48 (1.59)	35	27.48 (1.59)	43.62 (10.97)	58.4 (37.6)		35	31.61 (3.84)
T4 Diethylene glycol diethyl ether (3000mg/kg)	42	27.16 (1.97)	39 ¹	27.26 (1.96)	41.12* (7.93)	50.8* (27.5)		39	29.21* (2.97)
Littered	126								
T1 Distilled H ₂ O	41	27.55 (1.52)	41	27.55 (1.52)	47.71 (3.31)	73.4 (11.1)		41	32.80 (2.83)
T2 Sodium Selenite (7mg/kg)	39	27.19 (1.84)	39	27.19 (1.84)	47.41 (3.91)	74.6 (12.5)		39	31.55 (2.79)
T3 Ethylenethiourea (600mg/kg)	26	27.67 (1.48)	26	27.67 (1.48)	49.64* (4.00)	79.4* (10.9)		26	33.27 (2.74)
T4 Diethylene glycol diethyl ether (3000mg/kg)	35	27.27 (2.07)	33 ¹	27.43 (2.02)	44.11* (3.76)	61.1* (12.8)		33	29.84* (2.73)
Na ₂ S Positive [†]	17								
T1 Distilled H ₂ O	4	25.85 (0.86)	4	25.85 (0.86)	27.13 (4.36)	4.83 (15.39)		4	26.67 (1.82)
T2 Sodium Selenite (7mg/kg)	6	25.78 (2.19)	5	25.96 (2.40)	25.94 (2.10)	0.03 (2.21)		5	26.76 (2.30)
T3 Ethylenethiourea (600 mg/kg)	9	26.93 (1.87)	9	26.93 (1.87)	26.23 (1.78)	-2.52 (3.88)		9	26.81 (2.09)
T4 Diethylene glycol diethyl ether (3000mg/kg)	7	26.60 (1.40)	6	26.35 (1.35)	24.67 (1.95)	-6.28* (7.50)		6	25.77 (1.60)
Non-pregnant (Na ₂ S Negative) [†]	56								
T1 Distilled H ₂ O	5	26.34 (1.28)	5	26.34 (1.28)	26.68 (1.29)	1.34 (3.38)		5	26.62 (1.07)
T2 Sodium Selenite (7mg/kg)	5	26.94 (0.68)	5	26.94 (0.68)	27.22 (2.33)	1.01 (7.78)		5	28.00 (2.39)
T3 Ethylenethiourea (600mg/kg)	15	26.25 (1.51)	15	26.25 (1.51)	26.39 (2.26)	0.46 (5.20)		15	26.64 (1.97)
T4 Diethylene glycol diethyl ether (3000mg/kg)	8	26.08 (2.69)	8	26.06 (2.69)	25.45 (1.65)	-1.92 (7.07)		8	26.71 (2.07)

* Comparison with Control (T₁) significant (P < .05), Chi-square.

[†] This tabulation includes both live and dead animals.

¹ Two animals with missing body weights.

TABLE 15

AVERAGES AND (STANDARD DEVIATIONS) FOR
FETAL SURVIVAL, FETAL MORTALITY, FETAL WEIGHT PER LITTER, AND WEIGHT PER PUP

REPRODUCTIVE BLOCK 1 (Contract #210-81-6012)

CLASSIFICATION	# OF DAMS SURVIVING	# OF LITTERS	# LIVE/LITTER	# DEAD/LITTER	% VIABILITY	# OF LITTERS	LITTER WEIGHT (g)	WEIGHT PER PUP (g)	% REL. GAIN ² (PER PUP)
DAY +1	198	141							
T1 Distilled H ₂ O	50	41	10.27 (1.98)	0.098 (0.30)		40 ¹	16.30 (2.99)	1.61 (0.15)	
T2 Sodium Selenite (7mg/kg)	49	39	10.44 (1.92)	0.128 (0.41)		39	16.73 (2.78)	1.61 (0.11)	
T3 Ethylenethiourea (600mg/kg)	50	26*	10.96 (1.84)	0.038 (0.20)		26	18.46* (2.89)	1.69* (0.14)	
T4 Diethylene glycol diethyl ether (3000mg/kg)	49	35	9.83 (2.33)	0.371* (1.00)		33	14.71* (3.04)	1.52* (0.18)	
DAY +3	198	141							
T1 Distilled H ₂ O	50	41	10.07 (2.13)	0.195 (0.679)	93.0 (7.1)	40	20.49 (4.15)	2.06 (0.22)	28.2 (6.29)
T2 Sodium Selenite (7mg/kg)	49	39	10.26 (2.11)	0.180 (0.683)	98.0 (7.7)	39	20.91 (3.99)	2.06 (0.25)	27.5 (10.30)
T3 Ethylenethiourea (600mg/kg)	50	26*	10.88 (1.75)	0.077 (0.27)	99.4 (2.0)	26	23.37* (3.37)	2.17 (0.22)	27.9 (7.67)
T4 Diethylene glycol diethyl ether (3000mg/kg)	49	35	9.54 (2.85)	0.286 (1.53)	96.9 (16.9)	33	18.71 (3.95)	1.95 (0.28)	28.1 (12.65)

* Comparison with Control (T₁) significant (P < .05), Chi-square.

1 One Litter weight not recorded.

2 ((Weight/Pup Day 3 - Weight Per Pup Day 1)/ Weight/Pup Day 1) x 100

TABLE 16
 REPRODUCTIVE INDICES AND (STANDARD ERRORS)^a
 REPRODUCTIVE BLOCK 2 (Contract #210-81-6012)

<u>CLASSIFICATION</u>	<u>SURVIVORS PREGNANT (PS)</u>	<u>SURVIVORS LITTERED (LS)</u>	<u>REPRODUCTIVE INDEX LS/PS</u>	
T1 Distilled H ₂ O	43	42	0.977	(.023)
T2 EGdIME	34	0	0.000*	(.000)
T3 diEGdIME	27	0	0.000*	(.000)
T4 triEGdIME	37	0	0.000*	(.000)

* Comparison with control (T₁) significant (P < .05) Chi-Square.

^a Standard Error = $\sqrt{pq/n}$

TABLE 17

AVERAGES AND (STANDARD DEVIATIONS) FOR MATERNAL WEIGHT (g)

REPRODUCTIVE BLOCK 2 (Contract #210-81-6012)

Classification	ANIMALS ON TEST		SURVIVORS		% DIFFERENCE		POST PARTUM	
	Sample Size	Day 7	Sample Size	Day 7	Day 18	Relative To D7	Sample Size	+ 3 DAYS
Total on Test	199		162				160	
T1 Distilled H ₂ O	50		48				46	
T2 Ethylene glycol	50		37				37	
dimethyl ether (2000mg/kg)								
T3 Diethylene glycol	49**		29				29	
dimethyl ether (3000mg/kg)								
T4 Triethylene glycol	50		48				48	
dimethyl ether (3500mg/kg)								
Pregnant (Littered or Na ₂ S Positive) [†]	171		138				134	
T1 Distilled H ₂ O	43	27.65 (1.81)	41 ¹	27.57 (1.81)	47.19 (4.34)	71.3 (13.3)	37 ²	33.44 (2.49)
T2 Ethylene glycol	46	27.54 (1.96)	34	27.40 (1.94)	25.42(2.54)*	-7.1 (7.3)*	34	28.04 (1.89)*
dimethyl ether (2000mg/kg)								
T3 Diethylene glycol	45	27.87 (1.54)	27	28.08 (1.48)	26.41(2.11)*	-5.8 (7.9)*	27	28.45 (1.95)*
dimethyl ether (3000mg/kg)								
T4 Triethylene glycol	38	27.97 (1.93)	37	27.92 (1.93)	28.53(3.62)*	2.4 (12.1)*	37	28.43 (2.47)*
dimethyl ether (3500mg/kg)								
Littered	42		40				36	
T1 Distilled H ₂ O	42	27.63 (1.82)	40	27.54 (1.83)	47.43 (4.10)	72.34 (11.69)	36	33.56 (2.40)
T2 Ethylene glycol	0	---	0	---	---	---	0	---
dimethyl ether (2000mg/kg)								
T3 Diethylene glycol	0	---	0	---	---	---	0	---
dimethyl ether (3000mg/kg)								
T4 Triethylene glycol	0	---	0	---	---	---	0	---
dimethyl ether (3500mg/kg)								
Na ₂ S Positive [†]	129		98				98	
T1 Distilled H ₂ O	1	28.70 (---	1	28.70 (---	37.40 (---	30.3 (---	1	28.80 (---
T2 Ethylene glycol	46	27.54 (1.96)	34	27.40 (1.94)	25.42(2.54)*	-7.2 (7.5)*	33	27.99 (1.89)
dimethyl ether (2000mg/kg)								
T3 Diethylene glycol	45	27.87 (1.54)	27	28.08 (1.48)	26.41(2.11)*	-5.8 (7.9)*	27	28.45 (1.95)
dimethyl ether (3000mg/kg)								
T4 Triethylene glycol	38	27.97 (1.93)	37	27.92 (1.93)	28.53(3.62)*	2.4 (12.1)*	37	28.43 (2.47)
dimethyl ether (3500mg/kg)								

TABLE 17 cont.
 AVERAGES AND (STANDARD DEVIATIONS) FOR MATERNAL WEIGHT (g)
 REPRODUCTIVE BLOCK 2 (Contract #210-81-6012)

Classification	ANIMAL ON TEST		SURVIVORS			% DIFFERENCE		POST PARTUM	
	Sample Size	Day 7	Sample Size	Day 7	Day 18	Relative To D7	Sample Size	+ 3 DAYS	
Non-pregnant (Na ₂ S Negative) [†]	28		24				24		
T1 Distilled H ₂ O	7	26.61 (1.81)	7	26.61 (1.81)	25.60 (1.91)	-3.8	7	27.60 (2.25)	
T2 Ethylene glycol dimethyl ether (2000mg/kg)	4	26.13 (2.17)	3	25.19 (1.40)	24.59 (0.40)	-2.2	3	27.16 (0.60)	
T3 Diethylene glycol dimethyl ether (3000mg/kg)	4	25.88 (2.53)	2	24.80 (3.82)	24.00 (1.13)	-2.4	2	26.00 (0.42)	
T4 Trimethylene glycol dimethyl ether (3500mg/kg)	12	27.31 (1.82)	11	27.17 (1.84)	26.00 (2.67)	-4.4	11	27.91 (2.49)	

* Comparison with Control(T₁) significant (P < .05), Chi-square.

** One animal was removed from the study because it was dosed with the wrong chemical.

[†] This tabulation includes both live and dead animals.

1 Two animals weight missing (Day 18).

2 Six animals with weights missing.

TABLE 18

AVERAGES AND (STANDARD DEVIATIONS) FOR
FETAL SURVIVAL, FETAL MORTALITY, FETAL WEIGHT PER LITTER, AND WEIGHT PER PUP
REPRODUCTIVE BLOCK 2 (Contract #210-81-6012)

CLASSIFICATION	# OF DMS SURVIVING	# OF LITTERS	# LIVE/LITTER	# DEAD/LITTER	% VIABILITY	LITTER WEIGHT (g)	WEIGHT PER PUP (g)	% REL. GAIN ² (PER PUP)
DAY +1	164	42						
T1 Distilled H ₂ O	50	42	9.64 (2.01)	0.31 (1.26)	15.34 (2.83)	1.60 (0.12)		
T2 Ethylene glycol dimethyl ether (200mg/kg)	37*	0*	---	---	---	---	---	---
T3 Diethylene glycol dimethyl ether (300mg/kg)	29*	0*	---	---	---	---	---	---
T4 Triethylene glycol dimethyl ether (350mg/kg)	48	0*	---	---	---	---	---	---
DAY +3	164	42						
T1 Distilled H ₂ O	50	42	9.43 (1.96)	0.21 (0.72)	98.1 (6.5)	21.23 (3.75)	2.28 (0.26)	42.2 (9.6)
T2 Ethylene glycol dimethyl ether (200mg/kg)	37*	0*	---	---	---	---	---	---
T3 Diethylene glycol dimethyl ether (300mg/kg)	29*	0*	---	---	---	---	---	---
T4 Triethylene glycol dimethyl ether (350mg/kg)	48	0*	---	---	---	---	---	---

* Comparison with Control(T₁) significant (P < .05), Chi-square.

² ((Weight/Pup Day 3 - Weight Per Pup Day 1)/Weight/Pup Day 1) x 100

TABLE 19
 REPRODUCTIVE INDICES AND (STANDARD ERRORS)^a
 REPRODUCTIVE BLOCK 3 (Contract #210-81-6012)

<u>CLASSIFICATION</u>	<u>SURVIVORS PREGNANT (PS)</u>	<u>SURVIVORS LITTERED (LS)</u>	<u>REPRODUCTIVE INDEX LS/PS</u>	
T1 Corn Oil	40	40	1.000	(.000)
T2 PNP	29	28	0.966	(.034)
T3 DMP	35	35	1.000	(.000)
T4 Naph	28	26	0.929	(.049)

* Comparison with control (T₁) significant (P < 0.5) Chi-Square.

^a Standard Error = $\sqrt{pq/n}$

TABLE 20
AVERAGES AND (STANDARD DEVIATIONS) FOR MATERNAL WEIGHT (g)
REPRODUCTIVE BLOCK 3 (Contract #210-81-6012)

Classification	ANIMALS ON TEST		SURVIVORS		% DIFFERENCE Relative To D7	Sample Size	POST PARTUM + 3 DAYS	
	Sample Size	Day 7	Sample Size	Day 18			Sample Size	
Total on Test	200		172				180	
T1 Corn Oil	50		50				50	
T2 p-nitrophenol (400mg/kg)	50		32				41	
T3 Dimethyl Phthalate (3500mg/kg)	50		49				49	
T4 Naphthalene (300mg/kg)	50		41				40	
Pregnant (Littered or Na ₂ S Positive) [†]	145		125				132	
T1 Corn Oil	40	28.33 (1.59)	40	28.33 (1.59)	81.0	40	36.61 (2.79)	
T2 p-nitrophenol (400mg/kg)	36	27.88 (2.04)	21**	27.92 (2.55)	67.0	29	35.30 (4.16)	
T3 Dimethyl Phthalate (3500mg/kg)	36	28.18 (1.81)	35	28.15 (1.83)	77.7	35	36.56 (2.76)	
T4 Naphthalene (300mg/kg)	33	28.18 (1.47)	29 ¹	28.33 (1.41)	53.5	28	32.97 (3.43)*	
Littered	129		122				129	
T1 Corn Oil	40	28.33 (1.59)	40	28.33 (1.59)	81.0	40	36.61 (2.79)	
T2 p-nitrophenol (400mg/kg)	28	28.01(2.23)	21**	27.92 (2.55)	67.0	28	35.69 (3.66)	
T3 Dimethyl Phthalate (3500mg/kg)	35	28.15 (1.83)	35	28.15 (1.83)	77.7	35	36.56 (2.76)	
T4 Naphthalene (300mg/kg)	26	28.45 (1.40)	26	28.45 (1.40)	59.8	26	33.52 (2.86)*	
Na ₂ S Positive [†]	16		3				3	
T1 Corn Oil	0	00.00 (0.00)	0	---	---	0	---	
T2 p-nitrophenol (400mg/kg)	8	27.41 (1.19)	0**	---	---	1	24.40	
T3 Dimethyl Phthalate (3500mg/kg)	1	29.00 (0.00)	0	---	---	0	---	
T4 Naphthalene (300mg/kg)	7	27.17 (1.38)	3	27.33 (1.27)	-1.30	2	25.85 (1.63)	

TABLE 20 cont.

AVERAGES AND (STANDARD DEVIATIONS) FOR MATERNAL WEIGHT (g)

REPRODUCTIVE BLOCK 3 (Contract 3210-81-6012)

Classification	ANIMALS ON TEST		SURVIVORS				
	Sample Size	Day 7	Sample Size	Day 7	Day 18	Relative to D7	Sample Size
Non-pregnant (Na ₂ S Negative) [†]	55		47				48
T1 Corn Oil	10	26.99 (2.20)	10	26.99 (2.20)	27.30 (1.55)	1.6 (8.6)	10
T2 p-nitrophenol (400mg/kg)	14	27.41 (1.76)	11**	27.49 (1.97)	27.26 (2.20)	-0.7 (5.6)	12
T3 Dimethyl Phthalate (3500mg/kg)	14	26.90 (2.27)	14	26.90 (2.27)	28.53 (5.81)	6.2 (19.5)	14
T4 Naphthalene (300mg/kg)	17	26.45 (2.47)	12	26.88 (2.56)	25.40 (2.74)	-5.5 (5.8)	12

* Comparison with Control(T₁) significant (p < .05), Chi-square.** There were 9 Dams not weighed on Day 18, they were as follows: 7 that Littered
1 that was Na₂S Negative
1 that was Na₂S Positive.

1 One animal died on Day 19.

† This tabulation includes both live and dead animals.

TABLE 21

AVERAGES AND (STANDARD DEVIATIONS) FOR
FETAL SURVIVAL, FETAL MORTALITY, FETAL WEIGHT PER LITTER, AND WEIGHT PER PUP
REPRODUCTIVE BLOCK 3 (Contract #210-81-6012)

CLASSIFICATION	# OF DWS SURVIVING	# OF LITTERS	# LIVE/LITTER	# DEAD/LITTER	% VIABILITY	SAMPLE SIZE	LITTER WEIGHT (g)	WEIGHT PER PUP (g)	SAMPLE SIZE	% REL. GAIN (PER PUP)
DAY +1	180	129				123				
T1 Corn Oil	50	40 ³	10.75 (1.81)	0.000 (0.000)		37	18.45 (2.86)	1.74 (0.15)		
T2 p-nitrophenol (400mg/kg)	41*	28 ⁴	9.79 (3.49)	0.179 (0.670)		26	17.79 (3.07)	1.71 (0.18)		
T3 Dimethyl Phthalate (3500mg/kg)	49	35 ⁵	10.54 (2.56)	0.029 (0.169)		34	18.77 (2.67)	1.74 (0.11)		
T4 Naphthalene (300mg/kg)	40*	26	8.88* (2.61)	0.077 (0.392)		26	15.11* (4.52)	1.71 (0.23)		
DAY +3	180	129				126				
T1 Corn Oil	50	40 ³	10.70 (1.80)	0.050 (0.221)	99.6 (1.9)	40 ³	26.25 (3.38)	2.50 (0.36)	37	43.5 (11.5)
T2 p-nitrophenol (400mg/kg)	41*	28 ⁴	9.71 (3.53)	0.071 (0.262)	91.9(26.2)	26	25.07 (3.87)	2.45 (0.33)	26	43.4 (11.5)
T3 Dimethyl Phthalate (3500mg/kg)	49	35 ⁵	10.46 (2.52)	0.086 (0.373)	96.4(17.0)	34	26.52 (3.28)	2.49 (0.22)	34	42.9 (11.5)
T4 Naphthalene (300mg/kg)	40*	26	8.69* (2.78)	0.192 (0.801)	97.5(11.2)	26	21.48* (6.23)	2.52 (0.55)	26	46.8 (11.5)

* Comparison with Control (T₁) significant (P < .05), Chi-square.

2 ((Weight/Pup Day 3 - Weight Per Pup Day 1)/Weight/Pup Day 1) x 100

3 Three Litters not weighed.

4 Two Litters all stillborn.

5 One litter all stillborn.

TABLE 22

A SUMMARY OF THE MATERNAL SURVIVAL AND REPRODUCTIVE SUCCESS
FOR REPRODUCTIVE BLOCKS 1, 2, AND 3.

Classification ¹	No. Treated	No. died during trt				Littered	Survivors		
		Dosing error		Cpd related			Na ₂ S+	Na ₂ S-	Total
		Na ₂ S+	Na ₂ S-	Na ₂ S+	Na ₂ S-				
<u>BLOCK 1</u>									
T1 Distilled H ₂ O	50	0	0	0	0	41	4	5	50
T2 SS	50	0	0	1	0	39	5	5	49
T3 ETU	50	0	0	0	0	26	9	15	50
T4 diEGdiEE	50	1	0	0	0	35	6	8	49
<u>BLOCK 2</u>									
T1 Distilled H ₂ O	50	0	0	0	0	42	1	7	50
T2 EGdiME	50	0	0	12	1	0	34	3	37
T3 diEGdiME	49 ²	0	0	18	2	0	27	2	29
T4 triEGdiME	50	0	0	1	1	0	37	11	48
<u>BLOCK 3</u>									
T1 Corn Oil	50	0	0	0	0	40	0	10	50
T2 PNP	50	0	0	7	2	28	1	12	41
T3 DMP	50	1	0	0	0	35	0	14	49
T4 Naph	50	0	0	5	5	26	2	12	40

¹SS = sodium selenite (7mg/kg)

ETU = ethylenethiourea (600mg/kg)

diEGdiEE = diethylene glycol diethyl ether (3000mg/kg)

EGdiME = ethylene glycol dimethyl ether (2000mg/kg)

diEGdiME = diethylene glycol dimethyl ether (3000mg/kg)

triEGdiME = triethylene glycol dimethyl ether (3500mg/kg)

PNP = p-nitrophenol (400mg/kg)

DMP = dimethyl phthalate (3500mg/kg)

Naph = naphthalene (300mg/kg)

²One animal was removed from the study because it was dosed with the wrong chemical.

APPENDIX A

STANDARD OPERATING PROCEDURES - INDEX

A. Animal Facility

- 010 Identification and Function of Individual Rooms
- 020 Physical Conditions
- 030 Maintenance and Cleaning of Permanent Fixtures
- 040 Restricted Use of Facilities

B. Animal Care

- 010 Procurement of Animals
- 020 Receipt of Animals
- 030 Cages and Bedding (including changing schedule and disposal of wastes)
- 040 Feed and Watering
- 050 Disposal of Animals

C. Chemicals

- 010 Chemical Laboratories
- 020 Receipt of Chemicals
- 030 Storage and Handling Precautions
- 040 Emergency Procedures (in the event of spills or contamination)
- 050 Preparation of Dosing Solutions
- 060 Labeling of Containers
- 070 Reference Samples for Future Analysis
- 080 Disposal of Chemicals

D. Equipment

010 Balances

020 Dosing Equipment

E. Conduct of the Experiments

011 Identification of Mice

012 Procedure for Qualifying an Individual Mouse for an Experimental Group

020 Dosing

030 Observation of effects

040 Weighing of Animals

050 Experiments with Pregnant Animals

060 Necropsy

070 Use of Na Sulfide to determine embryonic implants

F. Data Collection

010 List of Parameters for MTD Studies

020 List of Parameters for Reproductive Studies

030 General Guidelines

040 Summary Sheets

G. Records and Reports

010 Sample Records

020 Transfer of Data

030 Storage of Records

040 Microfilming

H. Statistical Analysis

010 Preparation and verification of Data Files

020 Data Analysis

I. Training of Personnel

010 General Guidelines

020 Job Descriptions

030 Tasks and Training Responsibilities

SOP #

A 010

51

Effective Date

October 1, 1981

Supercedes

original

Authorizing Signature

William J. B. Bishop

IDENTIFICATION AND FUNCTION OF INDIVIDUAL ROOMS

1. Animal quarters

- A. holding room for receipt of new shipments of mice: 950A WIDB
- B. treatment room for maintaining treated animals during the course of an MTD or Reproductive Study: 950B WIDB

2. preparation of chemicals

Chemicals will be prepared in 586 WIDB and 566 WIDB, laboratories which are connected.

3. storage of chemicals

Volatile chemicals which carry a potential for explosion will be housed in the hood, Room 742 WIDB.

Unused remnants of dosing materials will be stored in locked cabinets in 586 WIDB, and frozen aliquots of same will be stored at -90° in the freezer in the same room.

SOP #

A 020

52

Effective Date

October 1, 1981

Supersedes

original

Authorizing Signature

William J. Bradshaw

PHYSICAL CONDITIONS

1. The temperature of the individual rooms (950, 950A, 950 B WIDB) will be maintained at 72°.
2. A constant light-dark cycle (light, 6 a.m. - 6 p.m.) will be maintained in the facility.
3. Periodic inspections of the operation of exhaust fans will be requested from the physical plant personnel of the University.

SOP #

A 030 53

Effective Date

October 1, 1981

Supersedes

original

Authorizing Signature

William J. Bradshaw

MAINTENANCE AND CLEANING OF PERMANENT FIXTURES

1. Animals quarters (Rooms 950, 950A, and 950B) will be cleaned once each week. This will include removal of cage racks (which will be steam cleaned), and disinfectant mopping of the floors. Routine sweeping and mopping may be performed more often as needed.

SOP #

A 040 ⁵⁴

Effective Date

October 1, 1981

Supersedes

original

Authorizing Signature

William S. Bradshaw

RESTRICTED USE OF FACILITIES

1. Warning signs will be affixed to the doors to rooms 950 and 950B WIDB: BYU-NIOSH Study; hazardous and potentially carcinogenic chemicals in use; use of facility restricted to study personnel only.
2. Except for the unavoidable traffic through room 950, in order to gain access to the roof of the building, the rooms are relatively secluded. The staff of the animals facility have agreed to cooperate in limiting use of the rooms.
3. Persons entering room 950 B are required to wear protective lab coats and gloves, and respiratory masks if animals are being dosed.

SOP #	B 010
Effective Date	Dec. 1, 1981
Supersedes	Oct. 1, 1981
Authorizing Signature	<i>William L. Bradshaw</i>

PROCUREMENT OF ANIMALS

1. Ordering of animals will be the responsibility of Dr. Bradshaw
2. The mice must be obtained from the Lakeview, New Jersey colony of Charles River Laboratories, Inc.
3. An order will be placed, as nearly as possible, 6 weeks in advance of the expected receipt of the animals.
4. CD-1 virgin females (pathogen free-SPF), 60-80 days old will be specified for the MTD Studies.
5. Timed pregnant females of the same specifications will be obtained for the Reproductive Studies.
6. Shipping instructions will include the request that upon receipt at the Salt Lake City Airport, Heber Shelley, receiving officer at BYU be notified, and arrangements made for transport to the University in Provo (tel.: 378-4726).

SOP #	B 020 ⁵⁶
Effective Date	May 1, 1982
Supercedes	October 1, 1981
Authorizing Signature	<i>William J. Bradshaw</i>

RECEIPT OF ANIMALS

1. Newly arrived animals will be held in room 950 A WIDB.
2. Shipping cartons will be opened and the number and condition of mice determined and recorded. This will include note of the # dead or injured, or any indications of ill health
3. The identification labels (gummed tags) will be removed from each shipping carton and transferred to a notebook as part of the permanent records of the study. But see # 8 below.
4. The "breed date" on labels of pregnant females will be recorded. This is the date females were placed with males. Copulation plugs would have been found the day following, which for this study will be considered day one of pregnancy.
5. Special care will be taken to note how many different breeding groups may be part of any shipping lot.
6. The date of receiving will be designated as day 2 or day 3 for mice in an MTD Study, based on a schedule in which the shipping date from Charles River is defined as day zero. This will coincide in the Reproductive Study to the time when pregnant females were identified as sperm-positive and shipped to us.
7. All mice in one carton will be weighed as a group, and the values recorded on data sheet B020 (see attached sheet).
8. All mice from a single carton will be transferred to a fresh holding cage. The shipping label will be attached to that holding cage until the animals are removed from Room 950A and transferred to Room 950B for treatment.

BRIGHAM YOUNG UNIV	EMERY
PROVO UTAH	
HOLD AT SALT LAKE CITY 84602	
IMMED UPON ARR CONTACT	3/24/82
HEBER SHELLEY 378-4726	

FILT CRATE

OUR ORD 3376514 YOUR PO Y98664 REL-

105 FEMALE CD-1 MICE TIMPREG 1 DY:

LAKEVIEW-L04

- see # 4 above

DR WM. BRADSHAW
(BREED 3/23)

CRATE 2 OF 11

SOP #	<u>B 030</u>
Effective Date	<u>October 1, 1982</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William L. Bushman</u>

CAGES AND BEDDING

1. Three types of cages will be used:

Holding cages for newly arrived mice not yet assigned to a study, and cages for 5 animals in each MTD Study group will be the large plastic cages capable of holding 24 mice.

Mice in the Reproductive Studies will be housed individually in the metal "bread pan" cages.

2. Cages and their metal tops will be washed and autoclaved after each use (at the conclusion of a particular study).
3. Corn cob bits (Bed-0-Cobs) will be used as bedding.
4. Bedding will be changed weekly with one exception. Bedding will not be changed in the cages of mice in the Reproductive Studies after the final day of dosing (as a precaution to minimize cannibalization).

SOP #	<u>B 040</u>
Effective Date	<u>October 1, 1981</u>
Supercedes	<u>original</u>
Authorizing Signature	<u>William A. Budder</u>

FEED AND WATERING

Mice at every stage of a study from the day of receipt until the day of disposal will have free access to feed (Wayne Lab Blox) and tap water.

Water bottles and stems will be changed weekly.

Water bottles and stems will be cleaned with detergent in the 9th floor dish washer, and then sterilized in the autoclave.

SOP #	<u>B 050</u>
Effective Date	<u>October 1, 1981</u>
Supercedes	<u>original</u>
Authorizing Signature	<u>William J. Bredolinski</u>

DISPOSAL OF ANIMALS

1. Mice found dead at any stage of the project will be examined to determine the cause of death, if possible (punctured exosphagus, for example). Any anomalies in internal organs will be noted. The condition of the gravid uterus (no. implant, etc.) in mice in a Reproductive Study will also be determined.
2. Living animals at the conclusion of a study will be sacrificed by cervical dislocation.
3. Dead animals will be incinerated.

SOP #	C 010
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<i>William J. Budden</i>

CHEMICAL LABORATORIES

1. The dosing solutions will be prepared in 586 WIDB.
2. Analytical balances in rooms 586 and 566 WIDB will be used for mg level and gram level measurements, respectively.
3. Mechanical mixing of dosing solutions will be done in the hood in room 566 WIDB. Chemicals in their original containers will also be housed there during preparation of the diluted samples.

SOP #	<u>C 020</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William J. B. [Signature]</u>

RECEIPT OF CHEMICALS

1. All chemicals for the study will be shipped directly to MESA Corp., Orem, Utah. All identification of the compounds will be kept in-house at MESA so that the investigators will now know the structures of the compounds.
2. Upon receipt of the chemicals, MESA shall deliver the chemicals to the QAU who will then notify the project director that they have arrived.
3. The chemicals will be stored in locked rooms or cabinets until the stock solutions are ready to be made up (see A 010 3).

SOP #	C 030
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	

STORAGE AND HANDLING PROCEDURES

1. Storage locations (see A 010 3).
2. Frozen aliquots of original chemicals and solutions thereof will be stored in the Forma Bio-Freezer in room 466 WIDB.
3. Lab coats, gloves, and (when appropriate) respiratory masks will be worn at all times during the handling of chemicals, the preparation of dosing solutions, the dosing of animals, and the handling of treated animals.
4. Bench tops on which work with chemicals is performed will be covered with absorbent, lined paper.
5. Used implements (Kim Wipes, disposable pipettes, etc.) will be placed in separate plastic bags and incinerated.
6. All personnel will wash their hands following any contact with the dosed animals or the dosing solutions.

SOP #	C 040
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<i>William J. Boddew</i>

EMERGENCY PROCEDURES

1. A written record will be made of any spills or contamination accidents.
2. The safety officer of the University will be informed of any such event, involving the potentially hazardous chemicals used in this study.
3. Materials used in the clean-up of a chemical spill will be incinerated.
4. Personnel exposed to these chemicals as a result of a spill or accident will receive medical attention at the University Health Center.
5. The identification of unknown contaminants will be obtained from the QAU in order to assist in providing appropriate medical treatment.

SOP #	<u>C 050</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u></u>

PREPARATION OF DOSING SOLUTIONS

1. Both (and only) Drs. Booth and Bradshaw shall weigh out quantities of the test chemicals, and make up the stock solutions and dilutions.
2. Appropriate masks, gloves, and lab coats shall be worn during all of these procedures.
3. A written calculation describing the quantities of materials needed for each concentration will be produced (see attached example).

SOP #	C 060
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<i>William B. Bradshaw</i>

LABELING OF CONTAINERS

1. Different chemicals in a single study will bear a specific color and number designation.
2. Flasks or brown glass bottles containing a chemical solution will be labeled with colored tape which will bear the following information: chemical I.D. #, concentration, date of preparation, and initials of preparer.

SOP #	<u>C 070</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William J. Budden</u>

REFERENCE SAMPLES FOR FUTURE ANALYSIS

1. Appropriate amounts of the undiluted chemicals, stock solutions, and the diluted dosing solutions will be stored both at room temperature, and at -70 to -90° to be used as samples for future analysis.
2. Unused portions of solutions left after the completion of dosing will also be retained.

SOP #	<u>C 080</u>
Effective Date	<u>October 1, 1981</u>
Supercedes	<u>original</u>
Authorizing Signature	<u>William F. Bredshaw</u>

DISPOSAL OF CHEMICALS

1. All unused parent compound will be promptly returned to NIOSH at the end of the preproductive-phase of each block of chemicals. The unused dosing solutions will be incinerated.

SOP #	<u>D 010</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William E. Bunker</u>

BALANCES

1. Two balances are available for use in this study: Touch-N-Weigh, BYU # 0539, and Ohaus, Triple Beam, Physiology # 2.
2. Each balance will be calibrated every morning on which weight measurements will be made.
3. The pans and lids of the balances will be washed and dried after each day's use.
4. The same balance will be used to make all of the weight measurements on any particular day.

SOP #	<u>D 020</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u></u>

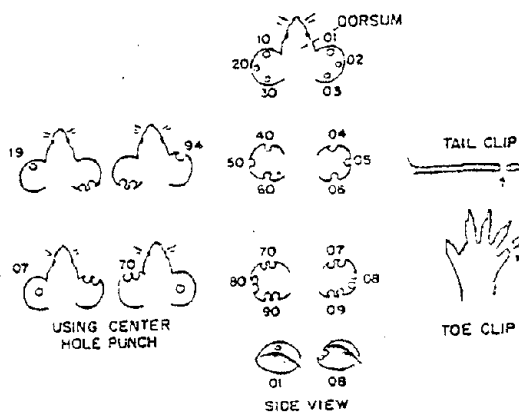
DOSING EQUIPMENT

The dosing needles used will be 1 1/4 inch, curved needles supplied by Popper and Sons. 1.0 ml syringes will be used each day for each test concentration.

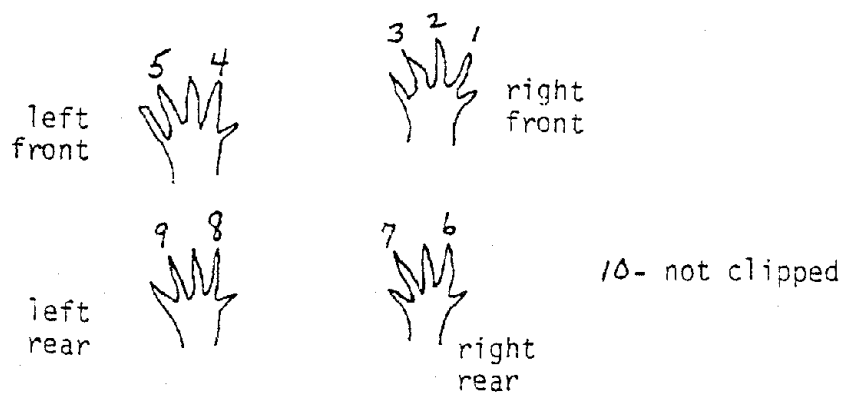
SOP #	E 011
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<u>Wm J. Bradshaw</u>

IDENTIFICATION OF MICE

- Each cage will have an identification card displayed on its front face indicating : cage #, group # (reflecting the compound and its concentration), and the individual animal numbers, 1-5 (or 6-10).
- Each animal will bear an ear punch reflecting its cage # (1-32). The numbering system to be used is illustrated below.



- Each animal will also receive a toe clip to reflect its individual animal number in the cage, 1-5 or 6-10. The numbering system to be used is illustrated below.



SOP #	<u>E 012</u>
Effective Date	<u>April 1, 1982</u>
Supersedes	<u>October 1, 1981</u>
Authorizing Signature	<u>Wm J. Bradshaw</u>

PROCEDURE FOR QUALIFYING AN INDIVIDUAL MOUSE FOR AN EXPERIMENTAL GROUP

A. MTD Study

We empirically determined the average weight of non-pregnant females in the MTD #1 batch sent from the supplier to be 25 ± 1.645 grams. It was decided that an animal would qualify if its weight were within 3 standard deviations of that mean. Therefore, a female qualifies for an MTD Study if its weight falls within the range 20.1 grams to 29.9 grams.

B. Reproductive Study

The same criterion will be applied for qualification for the reproductive study (3 standard deviations above or below the empirically determined mean for that shipment of animals). The mean for pregnant females appears to be approximately 27 grams. The acceptable weight range is therefore, 22.1 grams to 31.9 grams.

SOP #	E 020
Effective Date	December 1, 1981
Supercedes	October 1, 1981
Authorizing Signature	<i>William L. Budden</i>

DOSING

1. Mice in the Reproductive Studies will be dosed orally by gavage for 8 consecutive days during days 7-14 of gestation (day 1 being the day that a vaginal plug was discovered at Charles River Labs.).
2. Dosing will be performed beginning at 9 a.m. each day.
3. All dosing will be performed by Bill Clevenger.
4. While not in use, each dosing needle will be stored in a separate, capped vial in ethanol. Needles will be autoclaved after the conclusion of a particular study.
5. Inbetween mice in the same treatment concentration (2 cages, 10 mice for a MTD Study), the dosing needle will be rinsed in tap water and wiped dry.
6. A newly opened 1.0 mL syringe will be used each day to administer the solution to the 10 mice (2 cages) in each concentration group, and then discarded.
7. Dosing in a MTD Study will follow as nearly as possible the same schedule used for a Reproductive Study (see # 1 above).

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Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William E. Carlson</u>

OBSERVATION OF EFFECTS

1. Any immediate effects of dosing which suggest an esophageal puncture, such as blood in the mouth, will be noted.
2. At the conclusion of each morning dosing session all control and treated animals will be observed and deaths or indication of illness or abnormal behavior will be recorded.
3. At least one additional screening of each animal will be made later in the day, within 5 hours of the conclusion of dosing.
4. The date and time of discovery of any dead animal will be recorded. An internal examination will be made to determine if death was due to esophageal puncture. Anything unusual in the condition of the vital organs will be described by the examiner.

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Effective Date	October 1, 1931
Supercedes	original
Authorizing Signature	<i>William B. ...</i>

WEIGHING OF ANIMALS

1. One of the balances will be used for all weight measurements taken on a single day.
2. The balance will be calibrated before each day's weighings (see D 010).
3. Sufficient time will be allowed for each weighing so that the movement of an animal does not cause sufficient movement of the balance arm to distort the true weight value.
4. One individual among the research personnel will make all the weight measurements on a particular day.
5. Weight values will be recorded in ink on the appropriate data collection sheets immediately after the balance is read.

SOP #	<u>E 050</u>
Effective Date	<u>October 1, 1981</u>
Supercedes	<u>original</u>
Authorizing Signature	<u>William B. ...</u>

EXPERIMENTS WITH PREGNANT ANIMALS

1. With the first shipment of timed-pregnant mice received, group weight measurements will be made before the first day of dosing to determine the approximate weight per mouse of the shipment.
2. Individual weights will be determined for each mouse as a criterion for assignment to the study as part of the randomization procedure carried out one day prior to the initiation of dosing.
3. Following assignment to a particular treatment group, each mouse will be housed separately in a "bread box" type metal cage using corn cob bedding with in individual water bottle.
4. Bedding will not be changed for animals in a Reproductive Study following the last day of dosing to minimize agitation of an animal that might lead to cannibalization of pups.

SOP #	<u>E 060</u>
Effective Date	<u>October 1, 1981</u>
Supersedes	<u>original</u>
Authorizing Signature	<u>William B. Bollen</u>

NECROPSY

1. Necropsy will be performed on:

MTD Study mice which die at any stage of the experiment

Reproductive Study mice which die at any stage of the experiment

Reproductive Study mice which fail to litter

Reproductive Study mice which produce very small numbers of pups in their litters

2. Any abnormalities in the condition of the vital organs will be recorded.

3. The numbers of positions of pups in the uterine horns of pregnant mice will be recorded.

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Supercedes	<u>original</u>
Authorizing Signature	<u>William L. Boudreau</u>

USE OF SODIUM SULFIDE TO DETERMINE EMBRYONIC IMPLANTS

1. A 1% solution of Na Sulfide will be used.
2. Uterine horns will be immersed in the solutions and kept in the hoods for approximately 10 minutes and then viewed by at least 2 persons to determine and confirm the number and positions of implants.
3. A record of these observations will be made immediately on the data sheets for a Reproductive Study (see F 020).

SOP #	F 010
Effective Date	October 1, 1981
Supersedes	original
Authorizing Signature	<i>William I. Brashers</i>

LIST OF PARAMETERS FOR MTD STUDIES

1. body weight, day 1 of treatment
2. body weight, day 8 of treatment
3. body weight, 4 days post treatment
4. body weight, 8 days post treatment
5. general condition of health:
 good; listless; not moving; discharges from body orifices; any
 other states of abnormal behavior
6. date and time of the observation of a dead animal
7. a judgement as to whether death were associated with handling or
 gavage (i.e., punctured esophagus), based on internal examination
 of a dead animal
8. Measurements and observations will be recorded on the data sheet
 (F 010), "MTD Study - Weight and Toxicity Assessment" (see
 attached example).

MTD STUDY - WEIGHT AND TOXICITY ASSESSMENT

Block #	Animal #	"AGE" (DAYS)	DATE	WEIGHT (GRAMS)	CONDITION
		7			
		14			
		18			
		22			

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Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<i>William V. Darden</i>

LIST OF PARAMETERS FOR REPRODUCTIVE STUDIES.

1. maternal body weight on day 7 of gestation (before first treatment)
2. maternal body weight on day 18 of gestation
3. maternal body weight on day 3 post partum
4. number of live and dead pups observed within 12 hours of birth
5. number of live and dead pups observed on day 3 post partum
6. average litter weight of pups, day 1 post partum
7. average litter weight of pups, day 3 post partum
8. general condition of maternal health observed daily throughout the study
9. date and time of the observation of a dead animal
10. a determination of the cause of death, if due to a punctured esophogas during gavage, based on internal examination
11. a determination of the number and position of fetuses or embryos in the uterine horns of dead animals, or treatment with sodium sulfide treatment if there are no apparent offspring
12. Measurements and observations will be recorded on the data sheet (F 020), "Reproductive Study - Maternal Weight, Perinatal Lethality, Fetal Weight."

Distilled Water Control

REPRODUCTIVE STUDY - MATERNAL WEIGHT, PERINATAL LETHALITY, FETAL WEIGHT

Block # 1

Animal # 1

AGE (DAYS)	DATE	DAM WEIGHT (GRAMS)	CONDITION
7			
18			
3 post partum			

AGE (DAYS)	TOTAL BIRTHS	NO. LIVE	NO. STILLBORN	NO. DEAD	TOTAL LITTER WEIGHT (GRAMS)	AVG. WT. / PUP
day of birth						
1 post partum						
3 post partum						

SOP #	<u>F 030</u>
Effective Date	<u>March 1, 1982</u>
Supersedes	<u>October 1, 1981</u>
Authorizing Signature	<u>William V. B. Jackson</u>

GENERAL GUIDELINES

1. Whenever possible, the same individual will make all the observations at one time in a particular study (read the balance for all weight measurements on a particular day, for example).
2. All original measurements or observations (animal weights, for example) will be recorded in ink using the appropriate data sheet.
3. If an error is made in recording a measurement, a single line will be drawn through the incorrect value, and the correct value added. There will be no erasures or blotting out of incorrect values.
4. Signatures of the persons performing the various tasks in a study will be affixed to the protocol signature sheets (form F 030, see attached example) immediately following completion of each phase of the work, along with a record of the date and any other appropriate comments.

PROTOCOL - MTD STUDY, Block _____

TASK	DATE COMPLETED	COMMENTS	SIGNATURE
I. Receiving experimental animals			
A. The date and time of arrival of shipping crates at the animal quarters will be recorded.			
The shipping date from the supplier will be noted and recorded.			
The shipping label on each crate will be removed and retained for future reference.			
B. The numbers and condition of animals in each crate will be noted and recorded.			
C. Animals in each crate will be weighed as a group and transferred to a holding cage bearing the same designation as on the shipping label for that crate.			
D. Animals in holding cages will be fed and watered and checked for deaths or sickness daily until each is assigned to a treatment group.			

II. Assigning animals to a treatment group

- A. Beginning with the animals in holding cage #1 (and proceeding consecutively thereafter) mice will be: withdrawn one at a time, weighed to determine if they meet the criterion for qualifying in the study (20.1 - 29.9 grams),

assigned to a treatment group (according to the randomization sequence provided), and marked with an ear punch and toe clip to identify the correct cage (1-32) and animal (1-5, or 6-10) numbers, respectively.

The weight of each qualifying mouse will be recorded on the appropriate line of the randomization list, and the average used in the preparation of the dosing solutions.

III. Preparation of chemicals

- A. The assignment of chemicals to the four treatment groups will be made in the order (and follow the dose levels) provided by the project officer.
- B. The recommended handling precautions provided for each chemical will be followed.
- C. When possible a stock solution of the highest concentration of each chemical will be prepared, and serial dilutions made for each less concentrated solution. If a chemical is not completely soluble, separate stock solutions will be prepared for each of the suggested doses. Each of the dosing preparations will be kept in dark glass bottles, appropriately labeled.
- D. Aliquots of the original dosing preparations will be stored both at room temperature and frozen at -80°.

IV. Dosing

- A. The first day of dosing will correspond as nearly as possible to the 7th day of gestation in pregnant mice in a homologous Reproductive Study (assuming pregnant mice would be shipped one day after being designated as sperm-positive).
- B. All 50 mice receiving the same chemical (animals previously housed 5 to a cage, 2 cages to each of 5 specific dose levels) will be dosed as a block.
- C. Prior to dosing (day 1 and day 7 of the dosing period, only) each animal will be weighed and the weight recorded on the corresponding raw data sheet for that individual.
- D. Dosing will be performed orally by gavage in a volume of 0.25 ml for eight consecutive days
- E. Individual animal weights will also be measured and recorded on days 4 and 8 of the observation period following the completion of dosing (corresponding to days 18 and 22 of gestation).

1			
2			
3			
4			
5			
6			
7			
8			

F. At least twice each day during the study (prior to dosing, and 5 hours after dosing), the condition of each animal will be ascertained. Apparent illness or abnormal behavior will be noted and recorded (on the raw data sheets). The date and time of the finding of dead animals will be recorded. Dead animals will be examined internally to determine if death was gavage related (esophageal puncture).

V. Handling of data

A. After all weight measurements have been made and recorded on a particular day, the weight values will be copied from the raw data sheets onto summary sheets (one for each group - 10 mice exposed to the same concentration of a particular chemical).

B. A cumulative record of mortality in each group will also be kept on a daily basis throughout the dosing and observations periods.

C. Photocopies of the summaries will be delivered for statistical analysis after the values are verified against the raw data sheets by the QA0.

SOP #	<u>F 040</u>
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Authorizing Signature	<u>William L. Anderson</u>

SUMMARY SHEETS

1. In an MTD Study, at the conclusion of each of the 4 days on which data are to be collected, values from the raw data sheets will be transferred to summary sheet F 040 1 (see attached example):
2. Mortality data accumulated during at MTD Study will also be recorded on a running summary sheet, F 040 2 (see attached example).
3. In a Reproductive Study, at the conclusion of each of the days on which data are to be collected, values from the raw days wheets will be transferred to summary sheet F 040 3 (see attached example).

MTD STUDY - SUMMARY SHEET

Block # _____

Treatment # _____ Treatment I.D. _____

Animal #	WEIGHT BY "AGE" IN DAYS			
	7	14	18	22
1				
2				
3				
4				
5				
Cage Average:				
S.D.				

Treatment # _____ Treatment I.D. _____

6				
7				
8				
9				
10				
Cage Average:				
S.D.				
Treatment Average:				
S.D.				

[illegible]

SOP #	<u>I 010</u>
Effective Date	<u>October 1, 1981</u>
Supercedes	<u>original</u>
Authorizing Signature	<u>William F. Bradshaw</u>

GENERAL GUIDELINES

1. Laboratory technicians will be trained by Dr. Bradshaw.
2. Dr. Carter will train and supervise the work of those assisting with the statistical services required in the project.
3. Training and supervision of those functioning in quality assurance aspects of the project will be done by Dr. Campbell.
4. Each person involved in the project will read the appropriate sections of the SOP's for the work they will perform.

SOP #	I 020 1
Effective Date	October 1, 1981
Supercedes	original
Authorizing Signature	<i>William F. Bradshaw</i>

DUTIES OF TESTING FACILITY MANAGER (Bradshaw)

1. Author a detailed set of written instructions for each phase of the experimental protocol. Revise as dictated by first-hand experience with these methods. A copy of these procedures will be kept in a notebook in room 948 A of the laboratory.
2. Train test facility personnel in their duties.
3. Supervise:
 - a. maintenance of physical facilities and equipment
 - b. purchase of animals, feed, and dosing materials
 - c. feeding and care of mice
 - d. receipt of test animals
 - e. preparation of chemicals in appropriate concentrations for dosing
 - f. dosing of animals
 - g. calibration of balances
 - h. measurement and recording of animal weights, adult female toxicity and mortality, and fetolethality
 - i. transferral of raw data for individual animals to summary sheets which list together all mice in the same treatment group by cage
 - j. providing the Quality Assurance Officer with copies of the data summary sheets

SOP #	I 030
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Supercedes	original
Authorizing Signature	<i>William S. Bradshaw</i>

TASKS AND TRAINING RESPONSIBILITIES

1. Personnel Responsibilities

- a. Study Director: Dr. Gary M. Booth
- b. Supervisor of Quality Assurance Unit: Dr. L.H. Campbell
- c. Statistician: Dr. Melvin W. Carter
- d. Supervisor of Laboratory Operations: Dr. William S. Bradshaw
- e. Laboratory Technicians: William Clevenger, Michael Plasterer

2. Training Roles (see I 010).