DETERMINATION OF THE REPRODUCTIVE EFFECTS IN MICE OF NINE SELECTED CHEMICALS

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16. Abstract (Limit: 200 words)

The reproductive effects of sodium-selenite (10102188) (SS), ethylene-thiourea (96457) (ETU), diethylene-glycol-monomethyl-ether (111773) (DEME), ethylene-glycol-monomethyl-ether (109864) (EME), ethylene-glycol-monoethyl-ether (110805) (EMEE), ethylene-glycol-monobutyl-ether (111762) (EBE), toluene (108883), 2,4-dinitrotoluene (121142) (2,4DN), and 2,4-diaminotoluene (95807) (2,4DA) were examined in mice. Female CD1-mice were given 10 milliliters per kilogram oral doses of each compound dissolved in an appropriate solvent at various concentrations to determine the maximum tolerateddose (MTD). Pregnant mice were administered the compounds orally for 8 days, the concentrations of each compound being based on the MTDs. Animals were observed for clinical symptoms and any dead mice were necropsied. Litter size, number of live pups, and litter weights were tabulated and statistically analyzed. The MTDs of SS and toluene caused no significant reproductive toxicity. ETU caused a significantly reduced number of live pups per litter. EBE and 2,4DN resulted in a large number of resorptions and maternal defects; however, the viability of the litters was not significantly affected. DEME and 2,4DA caused a large number of resorptions and maternal deaths as well as a large number of stillbirths. EME and EMEE caused extreme reproductive toxicity. No viable litters were observed.

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1. OBJECTIVE

The purpose of this study was to screen for the potential reproductive effects of nine selected NIOSH test substances. These nine chemicals were number coded during the study so that no laboratory or supervisory personnel were aware of their specific identity. Maximum tolerated dose levels were determined, and were then used in subsequent reproductive studies. The maximum tolerated dose (MTD) was initially defined as the highest dose which results in weight reduction during the study period not greater than 10% compared to the controls and which results in no animal mortality. This was changed prior to testing to include criteria of a minimum mortality to be expected in the reproductive phase. Doses were chosen by the Project Officer for the subsequent reproductive studies from the results of the MTD study, available data from the literature, clinical signs, and from other sources.

All chemicals were then assayed for their potential to produce adverse reproductive effects, by utilizing the modified postnatal mouse screening test described by Chernoff and Kavlock (Teratology, 21 (2): 33A, 1980).

2. EXPERIMENTAL DESIGN

2.1 Animals

2.1.1 Test animal

For the maximum tolerated dose studies, a total of five hundred twenty adult, virgin, non-pregnant, specific pathogen free (SPF) CD-1 female mice were used for all nine chemicals. The mice ranged in age from sixty to eighty days and ranged in weight from 25.1 to 35.8 grams, for the MTD Block I study. The mice ranged in age from six to eight weeks of age for MTD Blocks II and III. Weight ranges of mice used were 20.3 to 28.8 g for MTD II, and from 22.6 to 28.4 g for MTD Block III. They were purchased from Charles River Breeding Laboratories, either at Wilmington, MA for MTD I and MTD II (except 20), or Lakeview, NJ (LO4) for MTD III and twenty for MTD II.

For the entire reproductive phase of the study, six hundred adult female timed-pregnant 5-days specific pathogen-free CD-1 mice were used. These mice ranged in age from six to eight weeks old, and ranged in weight from 20.1 g to 36.7 g, 19.5 g to 34.0 g, and 21.5 g to 34.8 g, for Reproductive Blocks I, II, and III respectively. They were purchased from Charles River Breeding Laboratories, Wilmington, MA for Reproductive Blocks I and II, and from Lakeview, NJ for Reproductive Block III.

2.1.2 Identification and randomization procedures

Animals were identified by ear punch code. In addition, the test substance and dose level were identified on the animal cage card.

Animals were assigned to the different experimental groups by using the "Randomization of Animals to Treatment Groups" B.S.C. standard operating procedure.

2.1.3 Quarantine period and animal examination

Prior to the initiation of testing, animals were inspected for signs of debility or disease by an animal care veterinarian, and placed in quarantine in the animal room assigned for the study. Animals were observed for clinical signs daily prior to dosing by technical personnel.

2.2 Animal maintenance

2.2.1 Feed and water

Animals were supplied ad libitum with NIH-07 rat and mouse certified feed (Zeigler Brothers, Gardners, PA). Untreated tap drinking water was supplied ad libitum from an automatic watering system (Hardco, Cincinnati, Ohio).

2.2.2 Caging

Animals were randomly assigned 5 to a cage for the Maximum Tolerated Dose studies. Animals were housed individually for the Reproductive phases of the study. Polycarbonate cages containing heat treated hardwood chips (Sani-chip) as bedding and non-woven filter sheet tops, and suspended from steel racks, were used in the study. Bedding was not disturbed after day 18 of gestation in the reproductive studies. Feeders, cages, racks and filters were changed by the technical staff.

2.2.3 Environmental conditions

For the maximum tolerated dose studies, the recorded room temperature range was between 67° and 75°F except for one day when the temperature was 64°F. These temperatures were within the range stated in the protocol 86% of the readings.

For the reproductive studies, the recorded temperatures ranged from 69°F to 74°F. These temperatures were within the range stated in the protocol at all times.

The recorded range of relative humidity was between 34 and 58%, which was always within the 30 to 70% range as specified in five of the six protocols. Where a more restricting set of specifications for the humidity range was used, in the third reproductive study, the humidity measurements were 42-49% R.H., which was also within the 50±15% R.H. stated in the protocol.

Air flow was between 12 to 16 complete changes of 100% fresh air every hour. Prior to introduction into the animal room, air was filtered through roughing, Varicel and HEPA filters.

A 12-hour light/dark cycle starting at 7 A.M. using soft-type fluorescent light, was used for the duration of the study.

2.3 Study scheduling

The testing schedule for the maximum tolerated dose and reproductive phases of the experiment was arranged in a staggered fashion, so as to be more easily conducted. Dates of testing are fully outlined in Appendix 2. The nine chemicals were divided into three groups of three chemicals each. Each block of three chemicals is designated by roman numeral. The roman numerals designating each block of chemicals correspond between reproductive and MTD studies (Reproductive III examines the reproductive effects of the same chemicals tested in MTD III). Where insufficient information was obtained in an MTD block for a specific chemical, additional dose levels were subsequently run concurrently with the next MTD block. The reproductive study for that chemical was therefore run also with the following reproductive block.

2.4 Test substances formulation and disposition

Test and control substances had the following characteristics:

Chemical	Physical State	Color	Density
1. Sodium selenite	Powder	White	
2. Ethylene thiourea	Crystal	White	1.00 g/ml
3. Diethylene glycol monomethyl ether	Liquid	Colorless	0.99 g/ml
4. Ethylene glycol monomethyl ether	Liquid	Colorless	0.95 g/ml
5. Ethylene glycol monoethyl ether	Liquid	Colorless	0.91 g/ml
6. Ethylene glycol monobutyl ether	Liquid	Colorless	0.89 g/ml
7. Toluene	Liquid	Clear	0.87 g/ml
8. 2,4-dinitrotoluene	Crystal	Yellow	1.56 g/ml
9. 2,4-diaminotoluene	Crystal	Bronze	0.85 g/ml

Vehicles	Physical State	Color	Density	Purity
1. Deionized water	Liquid	Clear	1.00 g/ml	Resistance 10 mega-ohms cm
2. Corn oil	Liquid	Yellow	0.91 and 0.92 g/ml	Peroxide 10 meq/l

The contractor retains documentation of the density determinations of the MTD Block II chemicals and for all the reproductive studies. Density determinations for the formulations prepared densitometrically in MTD Blocks I and II were not retained, and cannot be specifically documented. Preparations of test substances were made in the following ways: (see key in Appendix 1)

	Volumetrically	Densitometrically
MTD I		
1. SS	X	
2. EtTu		X
3. DGME	X	
MTD II		
3. DGME (additional levels)		X
4. EGME		X
5. EGEE		X
6. EGBE		X
MTD III		
7. TOL	X	
8. DNT		X
9. DAT		X
REPRO I		
1. SS	X	
2. EtTu		X
REPRO II		
3. DGME	X	
4. EGME	X	
5. EGEE	X	
6. EGBE	X	
REPRO III		
7. TOL		X
8. DNT		X
9. DAT		X

For the first two blocks of maximum tolerated dose studies and reproductive studies, sodium selenite (SS), ethylene thiourea (EtTu), diethylene glycol monomethyl ether (DGME), ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), and ethylene glycol monobutyl ether (EGBE) were prepared, and were tested in parallel groups, using concurrent vehicle control groups as reference. Insufficient MTD data was obtained for DGME with the first five doses set in block I, so three additional dose levels were tested concurrently with MTD block II. The reproductive study on DGME was originally assigned to Reproductive Block I, but was run with Reproductive Block II because of the additional dose-setting test. In all cases for these chemicals, the vehicle was deionized water with a resistance $R > 10$ mega ohms-cm.

For the third blocks of maximum tolerated dose studies and reproductive studies, suspensions of 2,4-dinitrotoluene (DNT) and 2,4 diaminotoluene (DAT) and a solution of toluene (TOL) were prepared, and were tested in parallel groups, using a concurrent vehicle control group as reference. In all cases, the vehicle was corn oil with a peroxide content of less than 10 meq/l.

Ethylene thiourea was prepared as a suspension using a Polytron homogenizer, and maintained with constant stirring. The dose concentrations of EtTu cannot be regarded as completely reliable because of sticking to the walls of any container, and rapid settling. It was also difficult to administer due to sticking and plugging in the gavage needle. Formulations of all other chemicals were made as solutions or stirring suspensions in their vehicles and were gavaged easily.

Dose analysis, characterization, and stability for all chemicals tested in both the MTD and Reproductive phases, is the responsiblity of NIOSH. Aliquots of all doses prepared were sent by the Contractor to NIOSH for analysis.

2.5 Dose levels and dosing

For the maximum tolerated dose ten female mice were assigned to each dose level for each test compound. For the reproductive studies, fifty timed-pregnant female mice were administered the designated MTD (maximum tolerated dose) chosen from the results of the corresponding MTD study for each specific chemical. All animals were administered the formulation by gavage, using a gavage needle with a ball tip and a 0.50 ml glass syringe. All dosages were rounded to the nearest 0.05 mls. All doses were delivered at 10 ml/kg body weight.

Doses were set for the maximum tolerated dose studies as follows:

Test Substance	Dose Level
Blocks I and II: Water (vehicle control)	--
Sodium Selenite (SS)	2.5 mg/kg Body weight [a] 5.0 10.0 20.0 40.0
Ethylene thiourea (EtTu)	75 mg/kg Body weight [a] 150 300 600 1200
Diethylene glycol monomethyl ether (DGME) (Tested with Block I)	100 mg/kg Body weight [a] 250 500 1000 2000
DGME (Tested with Block II)	3000 mg/kg Body weight 6000 9000
Ethylene glycol monomethyl ether (EGME)	190 mg/kg Body weight 380 760 1520 3045
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg Body weight 450 900 1800 3605
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg Body weight 590 1180 2365 4275

[a] It was found that thirteen of the 160 Block I animals were slightly misdosed (±14.18%) due to a rounding error in the dose calculations. These deviations are, in our judgment, insignificant.

Block III: Corn Oil (vehicle control)	--
Toluene	735 mg/kg Body weight 1470 2945 5890 8700
2,4-dinitrotoluene (DNT)[b]	310 mg/kg Body weight 525 1250 2500 3500
2,4-diaminotoluene (DAT)	150 mg/kg Body weight 175 200 225 250

For the reproductive studies, doses were chosen based on the results of the MTD experiments. The dose levels chosen (see the Results section) for each chemical are as follows:

Test Substance		Dose Level
Blocks I and II:	Water (Vehicle control)	--
	SS	5 mg/kg
	EtTu	300
	DGME	4000
	EGME	1400
	EGEE	3605
	EGBE	1180
Block III:	Corn Oil (Vehicle control)	--
	TOL	2350
	DNT	390
	DAT	150

[b]The second dose level for DNT should have been 625 mg/kg BW. The actual dose prepared was thus 16% low, due to a chemistry transcription error.

Formulations of all the different compounds were administered to the animals in a volume of 10 ml/kg body weight. The dose administered was calculated from the body weight measurements for each animal on the first day of treatment.

Doses of each formulation were administered once per day, at approximately the same time of day, for eight (8) consecutive days. In timed-pregnant mice tested in the reproductive-effects studies, these doses were administered between the seventh and fourteenth gestation days, inclusive.

2.6 Procedures

2.6.1 Animal weights

The balance used to measure body weight was calibrated (using BSC Standard Operating Procedures) at the start of each day's weighing. All animals were weighed to the nearest 0.1 gram at each weighing. When litters were weighed, the balance was calibrated at the start of each day's weighing. All litters were weighed to the nearest 0.1 gram.

For the MTD (maximum tolerated dose) studies, all animals were weighed to the nearest 0.1 gram on the first and last day of dosing. The weight measured on the first day of dosing was used to calculate the dose to be given throughout the treatment period. Animals were also weighed on days four and eight after the dosing period. The final weights were obtained immediately prior to terminal sacrifice of the survivors on day eight after the dosing period.

For the reproductive studies, each female was weighed on day seven of gestation immediately prior to dosing. This body weight was used to calculate the dose volume of chemical or vehicle given throughout the treatment period.

Females were also weighed on day eighteen of gestation and on day three postpartum. Total litter weights were measured within twelve hours after birth, and again forty-eight hours later.

2.6.2 Clinical observations

For the Maximum Tolerated Dose studies, all animals were observed for clinical signs of toxicity during the first hour after dosing each day and at least one additional observation was made of each animal each day of dosing (the minimal interval between these observations was five hours). Animals were observed once daily after the dosing period until termination of the study.

The occurence of any clinical sign of toxicity during the MTD study was tabulated, showing the number of animals per dose level affected. The occurence of such signs was analyzed for each chemical independently.

For the Reproductive studies, beginning eighteen days after mating, pregnant females' cages were inspected daily for the presence of a litter or signs of cannibalism. The number of living and dead pups was counted within twelve hours of birth and again forty-eight hours later. Total litter weights were measured at these same time points.

2.6.3 Pathology

Mated females which failed to deliver a litter by the 23rd day after mating were sacrificed, necropsied, and the status of pregnancy assessed. All uteri not obviously gravid were treated with sodium sulfide to assess the prior existence of pregnancy.

All animals dying on test were necropsied as soon as possible after their discovery, to determine if the deaths were due to gavage error.

2.6.4 Data computations

The following data were recorded, computed and used in the selection of the MTD level for the reproductive study:

i. Animal body weights and clinical observations
ii. Average body weight per group per day weighed ± standard deviation compared statistically for treatment and control groups.
iii. The percent relative mean body weight change of each treated group compared to the control group for each post-treatment day that weight was measured.
iv. Mean weight change during treatment per group (difference between pre-and post-treatment weights) ± standard deviation compared statistically for treatment and control groups. The mean weight change was calculated for each treated and control group for each post-treatment day on which body weight was measured.

The following data were tabulated and used for calculations in the Reproductive study:

i. Chemical identification, dose, treatment regimen, and vehicle.
ii. Number of animals found dead in the dosing and post-dosing periods.
iii. Number of animals producing litters.
iv. Number of animals with totally resorbed litters.

v. Number of animals considered in each calculation.
vi. Mean maternal weight for each day weighed with standard deviation.
vii. Mean number of live pups found per litter within 12 hours of birth with standard deviation.
viii. Mean number of dead pups found per litter within 12 hours after birth with standard deviation.
ix. Mean number of live pups per litter on day 3 postpartum with standard deviation.
x. Mean litter weight on day 1 postpartum with standard deviation.
xi. Mean litter weight on day 3 postpartum with standard deviation.

The following calculations were made for each chemical and its concurrent control group.

i. Reproductive Index (number of females bearing viable litters divided by the number of pregnant females).
ii. Mean maternal weight change during pregnancy (weight on day eighteen of gestation minus weight on day seven of gestation) with standard deviation.
iii. Mean percent dead pups per litter with standard deviation.
iv. Mean live litter weight on day one and day three postpartum.
v. Mean percent weight change per litter from day one to day three postpartum with standard deviation.
vi. Mean percent pup viability per litter from day one to day three postpartum with standard deviation.

2.6.5 Statistical analysis

The above listed calculations (except for Reproductive Index) were analyzed by using Model I fixed treatment single classification analysis of variance (one-way ANOVA), according to the procedure described by Sokal and Rohlf (Biometry, Freeman and Co., 1969) and Snedecor and Cochran (Statistical Methods; Iowa State University Press, Iowa, 1968). Computational formulas were programmed into a TI-59 (Texas Instruments, Inc.) calculator, and tail probabilities (p) were obtained from the F-statistics using the available software.

Values of the F-test for n_1, n_2 degree of freedom at a P level of significance are given. If $F < 1$, it indicates a non-significant source of variation. In cases when $F > 1$, the value for P (level of significance) is given.

The one-way ANOVA was interpreted as follows: if $p > 0.05$, the analysis indicated no evidence for a significant variance among the different animal groups at a particular time, i.e., animals from the different groups had similar body weights. If $p < 0.05$, the one-way ANOVA indicated a significant source of variation among the animal groups, and results were interpreted as a significant effect on the body weights among

the different experimental groups. A standard Chi square analysis is used to evaluate Reproductive Indices. The procedures are described in Steel, R.G.D. and J.J. Torrie, Principles and Procedures of Statistics, McGraw Hill, New York, 1960.

Results of percentage of dead pups per litter within 12 hours postpartum were normalized using the $\sqrt{x + 0.5}$ data transformation, where x=% deaths/litter. Results of percentage of pup viability per litter between days 1 and 3, and results of percentage of body weight change per litter between days 1 and 3, were normalized using the $\sqrt{x}$ data transformation. After transformation, a Model I ANOVA was used for the analysis. These procedures are described in the reference given previously.

3. RESULTS OF THE MAXIMUM TOLERATED DOSE STUDIES

Results of the maximum tolerated dose studies are given in this section by chemical. Each test group treated with a specific chemical is part of a group of chemicals (a "block") tested with a single control group. Therefore, results are presented in order of block of testing. Each block originally contained three chemicals. Diethylene glycol monomethyl ether, originally tested for maximum tolerated dose (MTD) with Block I, had three additional dose levels tested with Block II. Therefore, additional results for it are given in section 3.2.

The mean body weights of each treated group for each weighing day are presented in tabular form. The values given are means ± standard deviations for each group, for days 1 and 8 of dosing, and for days four and eight after the dosing period. Vehicle control group weights are presented concurrently.

The percent relative body weight change of each treated group compared to the vehicle control group at the three time intervals specified is also tabulated for each chemical.

Body weight changes of female mice in each test group are also compared to the initial weight of that test group. These comparisons are tabulated for the same three time intervals specified.

Mortality and clinical signs, both during dosing, and in the post-treatment observation period are also tabulated for each chemical.

3.1 Block I MTD studies

3.1.1 Sodium selenite (SS)

Body weights of animals in the 2.5, 5.0 and 10.0 mg/kg BW were similar to the body weights of the control group. Statistical analysis of results at the end of the dosing period and during the post-dosing period (see Table 1) indicated that the different dose levels did not have a significant effect on body weights.

Body weights of animals from the 2.5, 5.0 and 10.0 mg/kg were at all times within 10% of the vehicle control group (see Table 2). All animals receiving 40.0 mg/kg BW were dead by the 3rd day of dosing, and only one animal from the 20.0 mg/kg dose group survived the dosing period.

As seen in Table 3, the animals in the lowest three dose level groups (2.5, 5.0, and 10.0 mg/kg) had an increase in mean body weights throughout the course of the study.

Statistical analyses of results at the 3 time intervals indicate that body weights among the different experimental groups were similar ($p > 0.05$).

The majority of the animals in the 20.0 and 40.0 mg/kg dose levels were dead by the end of the dosing period; all had died by the final day of study. Two natural deaths occurred in each of the 5.0 mg/kg and 10.0 mg/kg dose level groups. One single death, occurring on the 2nd day of dosing in the 2.5 mg/kg dose level, was attributed to gavage error. Mortality is summarized in Table 4. In the vehicle control group for Block I of the MTD study, one death attributed to gavage error, occurred on the first day of the post-treatment period (no control animals died in Blocks II or III).

Clinical signs of animals administered sodium selenite in the Maximum Tolerated Dose study are summarized in Tables 5 and 6.

Based on the results of the Maximum Tolerated Dose Study for Sodium selenite, a dose level of 5 mg/kg was chosen for the Reproductive study.

TABLE 1

Effect of the administration of Sodium selenite on the body weight of female mice [a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	29.6 ± 1.3 (10)	30.4 ± 1.8 [b] (5)	30.8 ± 1.4 (9)	31.8 ± 1.9 (9)
2.5	30.0 ± 2.7 (10)	30.5 ± 2.8 (9)	31.1 ± 2.8 (9)	32.2 ± 3.6 (9)
5.0	29.6 ± 2.0 (10)	30.3 ± 2.6 (8)	30.5 ± 2.9 (8)	31.0 ± 2.4 (8)
10.0	29.9 ± 1.8 (10)	30.1 ± 1.9 (8)	30.3 ± 2.1 (8)	30.7 ± 1.9 (8)
20.0	29.2 ± 1.9 (10)	30.1 (1)	--- (0)	--- (0)
40.0	29.3 ± 1.0 (10)	--- (0)	--- (0)	--- (0)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=0.259$	$F^{P}_{3,26}=0.040$	$F^{P}_{3,30}=0.166$	$F^{P}_{3,30}=0.565$
Conclusion	F <1 N.S.	F <1 N.S.	F <1 N.S.	F <1 N.S.

(a) All values are grams, mean ± S.D.; group size is indicated in parenthesis.

(b) Due to a technical problem with the watering system in one cage, only 5 animals were considered in the analysis.

(c) N.S. = Not Significant

TABLE 2

Relative effects of the administration of Sodium selenite on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day[c]	Post-dosing period 4th day	Post-dosing period 8th day
Sodium selenite	2.5 mg/kg	+ 0.3 (9)	+ 1.0 (9)	+ 1.3 (9)
	5.0 "	- 0.3 (8)	- 1.0 (8)	- 2.5 (8)
	10.0 "	- 1.0 (8)	- 1.6 (8)	- 3.5 (8)
	20.0 "	- 1.0 (1)	(0)	(0)
	40.0 "	(0)	(0)	(0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 1. Results are percent change; test group size is indicated in parenthesis.

(c) Due to a technical problem with the watering in one cage containing control animals, these values were calculated using the 5 animals from the non-affected cage.

TABLE 3

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Sodium selenite[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	+ 1.2 ± 1.2[b] (5)	+ 1.2 ± 0.6 (9)	+ 2.2 ± 1.1 (9)
2.5	+ 0.8 ± 1.1 (9)	+ 1.5 ± 0.8 (9)	+ 2.6 ± 2.0 (9)
5.0	+ 0.4 ± 1.2 (8)	+ 0.6 ± 1.2 (8)	+ 1.1 ± 0.6 (8)
10.0	+ 0.6 ± 0.7 (8)	+ 0.8 ± 0.9 (8)	+ 1.2 ± 1.4 (8)
20.0	- 2.5 (1)	(0)	(0)
40.0	(0)	(0)	(0)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{3,26}=0.648$	$F^{P}_{3,30}=1.463$	$F^{P}_{3,30}=2.107$
Conclusion[c]	F <1 N.S.	P=0.244 N.S.	P=0.120 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] Due to a technical problem with the watering in one cage, only 5 animals were considered in the analysis.

[c] N.S. = Not Significant

TABLE 4

Mortality Data for Sodium selenite

Dose Level(a)		2.5		5.0		10.0		20.0		40.0	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death(b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1									1	
	2	1						3		9	
	3							2			
	4							3			
	5					1		1			
	6					1					
	7			2							
	8										
Observation Day	1							1			
	2										
	3										
	4										
	5										
	6										
	7										
	8										
Cumulative Deaths		0	1	2		2		10		10	

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
In the vehicle control group for Block I of the MTD study, one death, attributed to gavage error, occurred on the first day of the post-treatment period.

TABLE 5

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Sodium selenite	2.5 mg/kg BW	---	a(1);g(1)	---	---
	5.0 mg/kg	---	a(1)	---	---
	10.0 mg/kg	a,b(1)	---	---	---
	20.0 mg/kg	a(10)	a,d(1);a(6);d(2)	a(5);d(2)	a(2);d(3)
	40.0 mg/kg	a,b,i(1); a(8);d(1)	a,d(3);d(6)	---	---

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	b,e,h(1)	b,e,h(1)e(3);a,e(1)
Sodium selenite	2.5 mg/kg BW	---	---	---	---
	5.0 mg/kg	---	---	d(2)	---
	10.0 mg/kg	d(1)	a,d(1)	---	---
	20.0 mg/kg	a(1);d(1)	a(1)	a,k(1)	a,k(1)
	40.0 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; f = convulsions (tremors); g = death, gavage error; h = hunched posture; i = squinted eyes; j = dehydrated; k = piloerection.

TABLE 6

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	9	g(1)	---	---	---
Sodium selenite	2.5 mg/kg BW	9	---	---	---	---
	5.0 mg/kg	8	---	---	---	---
	10.0 mg/kg	8	---	---	---	---
	20.0 mg/kg	1	d(1)	---	---	---
	40.0 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Sodium selenite	2.5 mg/kg BW	---	---	---	---
	5.0 mg/kg	---	---	---	---
	10.0 mg/kg	---	---	---	---
	20.0 mg/kg	---	---	---	---
	40.0 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; g = death, gavage error.

3.1.2 Ethylene thiourea

Body weights of animals from all dose groups were similar ($p > 0.05$) to those of the control group, both at the end of the dosing period and at the 4th day after dosing; a significant effect ($p=0.036$) on the body weights was found at the 8th day of the post-dosing period (see Table 7).

The significant effect found at the end of the post-dosing period is attributed to the differences between the weight of the animals in the 300 mg/kg dose group and the vehicle control group. The lower body weight of animals administered 300 mg/kg (27.2 ± 4.8; $n=9$) can be explained by the occurrence of a technical problem in the automatic watering system, which affected one cage of 5 animals in this group. If only those animals in the 300 mg/kg dose group not affected by the watering problem are considered, then the mean body weights (30.7 ± 4.0, $n=4$) becomes similar to those of the other dose groups, and the body weight differences become statistically non-significant.

The body weight differences for all experimental groups were within 10% of the vehicle control group values throughout the study, with the exception of the 300 mg/kg dose group at day 8 of the dosing period (-11.5%) and at day 8 of the post-dosing period (-14.5%). As explained before, a problem in the watering system was responsible for losses in body weight found at day 8 of the post-dosing period; if only those animals unaffected by this problem are taken into consideration, then the percent of body weight difference at day 8 of post-dosing becomes -3.5%.

In Table 9, at the 8th day of the dosing period, a significant effect ($p=0.028$) on the body weight changes for the different experimental groups was observed. These weight changes were not consistent with the compound dose level administered.

On day 4 of the post-dosing period, no significant differences ($p=0.064$) in weight changes were found among the various groups.

At the end of the study, there were significant differences ($p=0.010$) in body weight changes among the different groups. As previously mentioned, this statistical significance is attributed to the difference between the values of body weight change found for animals receiving 300 mg/kg, and those of the vehicle control group.

Only 3 deaths occurred in animals administered this compound. Of these 3, 1 death in the 1200 mg/kg dose level and occurring on the 4th day of dosing, was found to be related to the test compound. In the vehicle control group for Block I of the MTD study, one death attributed to gavage error, occurred on the first day of the post-treatment period (no control animals died in Blocks II or III). Mortality for this chemical is summarized in Table 10.

Clinical signs of animals administered ethylene thiourea are summarized in Tables 11 and 12.

Based on the results of the MTD study for ethylene thiourea, a dose level of 300 mg/kg was chosen for the Reproductive study.

TABLE 7

Effect of the administration of Ethylene thiourea on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days)		Post-dosing period (days)	
	1	8	4	8
Vehicle Control	29.6 ± 1.3 (10)	30.4 ± 1.8[b] (5)	30.8 ± 1.4 (9)	31.8 ± 1.9 (9)
75	29.0 ± 2.1 (10)	28.8 ± 1.6 (10)	29.2 ± 2.3 (10)	30.0 ± 2.2 (10)
150	28.9 ± 1.6 (10)	29.0 ± 1.8 (10)	29.1 ± 2.0 (10)	30.4 ± 2.0 (10)
300	29.1 ± 2.8 (10)	26.9 ± 4.4 (9)	29.0 ± 3.5 (9)	27.2 ± 4.8 (9)
600	29.2 ± 1.3 (10)	29.9 ± 1.0 (9)	29.5 ± 1.2 (9)	30.7 ± 1.2 (9)
1200	29.7 ± 0.6 (10)	29.2 ± 1.4 (9)	28.1 ± 2.6 (9)	28.7 ± 3.5 (9)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=0.296$	$F^{P}_{5,46}=1.776$	$F^{P}_{5,50}=1.202$	$F^{P}_{5,50}=2.614$
Conclusion[c]	$F<1$ N.S.	P=0.137 N.S.	P=0.322 N.S.	P=0.036

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] Due to a technical problem with the watering system in one cage, only 5 animals were considered in the analysis.

[c] N.S. = Not Significant

TABLE 8

Relative effects of the administration of Ethylene thiourea on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day[c]	Post-dosing period 4th day	Post-dosing period 8th day
Ethylene thiourea	75 mg/kg BW	- 5.3 (10)	- 5.2 (10)	- 5.7 (10)
	150 "	- 4.6 (10)	- 5.5 (10)	- 4.4 (10)
	300 "	-11.5 (9)	- 5.8 (9)	-14.5 (9)
	600 "	- 1.6 (9)	- 4.2 (9)	- 3.5 (9)
	1200 "	- 3.9 (9)	- 8.8 (9)	- 9.7 (9)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 1. Results are percent change; test group size is indicated in parenthesis.

(c) Due to a technical problem with the watering in one cage containing control animals, these values were calculated using the 5 animals from the non-affected cage.

TABLE 9

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Ethylene thiourea[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	+ 1.2 ± 1.2[b] (5)	+ 1.2 ± 0.6 (9)	+ 2.2 ± 1.1 (9)
75	- 0.2 ± 1.7 (10)	+ 0.2 ± 2.4 (10)	+ 1.0 ± 1.6 (10)
150	+ 0.1 ± 0.8 (10)	+ 0.2 ± 0.6 (10)	+ 1.5 ± 1.8 (10)
300	- 2.3 ± 3.7 (9)	- 0.3 ± 1.9 (9)	- 2.0 ± 4.1 (9)
600	+ 0.7 ± 1.3 (9)	+ 0.4 ± 1.3 (9)	+ 1.5 ± 2.4 (9)
1200	- 0.5 ± 1.4 (9)	- 1.6 ± 2.4 (9)	- 1.0 ± 3.4 (9)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{5,46}=2.790$	$F^{P}_{5,50}=2.244$	$F^{P}_{5,50}=3.397$
Conclusion[c]	P=0.028 Significant	P=0.064 N.S.	P=0.010 Significant

(a) All values are grams, mean ± S.D.; group size is indicated in parenthesis.

(b) Due to a technical problem with the watering system in one cage, only 5 animals were considered in the analysis.

(c) N.S. = Not Significant

TABLE 10

Mortality Data for Ethylene thiourea

Dose Level (a)		75	150	300	600	1200
Number on Test		(10)	(10)	(10)	(10)	(10)
Cause of Death (b)		Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage
Treatment Day	1				1	
	2					
	3					
	4					
	5					1
	6			1		
	7					
	8					
Observation Day	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
Cumulative Deaths		0	0	0 1	0 1	1

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
In the vehicle control group for Block I of the MTD study, one death, attributed to gavage error, occurred on the first day of the post-treatment period.

TABLE 11

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Ethylene thiourea	75 mg/kg BW	---	---	---	---
	150 mg/kg	---	---	---	---
	300 mg/kg	---	---	---	---
	600 mg/kg	f,b,g(1)	---	---	---
	1200 mg/kg	---	---	---	d(1)

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	b,e,h(1)	b,e,h(1)e(3);a,e(1)
Ethylene thiourea	75 mg/kg BW	---	---	---	---
	150 mg/kg	---	---	---	---
	300 mg/kg	g(1)	---	---	---
	600 mg/kg	---	---	---	---
	1200 mg/kg	---	---	a,b,e(1)	a,b,e(1)

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; f = convulsions (tremors); g = death, gavage error; h = hunched posture; i = squinted eyes; j = dehydrated; k = piloerection.

TABLE 12

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	9	g(1)	---	---	---
Ethylene thiourea	75 mg/kg BW	10	---	---	---	---
	150 mg/kg	10	---	---	---	---
	300 mg/kg	9	---	---	---	---
	600 mg/kg	9	---	---	---	---
	1200 mg/kg	9	b,a(1)	a,c(1)	a,c(1)	a,c,e(1)

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene thiourea	75 mg/kg BW	---	---	---	---
	150 mg/kg	---	---	---	---
	300 mg/kg	---	---	---	---
	600 mg/kg	---	---	---	---
	1200 mg/kg	a,c,e(1)	a,c,e(1)	a,c,e(1)	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; g = death, gavage error.

3.1.3 Diethylene glycol monomethyl ether (DGME)

For the first five dose levels tested, the body weight differences between the treated groups and the vehicle control group were well within 10% throughout the study (see Table 14).

With the exception of animals administered 0.25 g/kg BW, all other dose level groups showed body weights lower than the control group, both at the end of the dosing period, and during the post-dosing period (see Table 13).

The statistical analysis of results obtained at the 8th day of dosing indicated that body weights from all treated groups were marginally non-significant ($p=0.071$).

The marginal effect found at the 8th day of dosing became significant at the 4th day of the post-dosing period.

At the end of the study, a significant effect ($p=0.046$) on body weights among the different experimental groups was observed. The significance of this result is attributed to the difference in body weight between animals receiving 0.25 g/kg (32.7 ± 2.2; $n=10$) and those receiving 1.00 g/kg (29.9 ± 1.4; $n=9$).

However, as seen in Table 15, among these five lower dose groups, statistical analyses of results obtained at the 3 time intervals, indicate that the different dose levels had no effect on the body weight changes of the experimental groups ($p > 0.05$).

Mortality for Block I MTD testing of DGME is summarized in Table 16. One death occurred in the group receiving 1000 mg/kg at day 6 of the dosing period. This death was not due to gavage error. In the vehicle control group for Block I of the MTD study, one death attributed to gavage error, occurred on the first day of the post-treatment period (no control animals died in Blocks II or III). Clinical signs are summarized in Tables 17 and 18.

It is thought that all the anomalous effects on weights, clinical signs, and the one death are due to a watering problem in one of the cages receiving 1000 mg/kg DGME, but this cannot be specifically documented.

It was decided that three additional higher dose levels of DGME should be tested to determine the correct MTD. This was tested concurrently with Block II.

TABLE 13

Effect of the administration of Diethylene glycol monomethyl ether on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	29.6 ± 1.3 (10)	30.4 ± 1.8[b] (5)	30.8 ± 1.4 (9)	31.8 ± 1.9 (9)
100	28.7 ± 2.2 (10)	28.4 ± 2.5 (10)	28.8 ± 2.3 (10)	30.2 ± 1.9 (10)
250	30.5 ± 1.5 (10)	30.5 ± 2.1 (10)	31.6 ± 1.8 (10)	32.7 ± 2.2 (10)
500	30.4 ± 2.7 (10)	29.9 ± 1.9 (10)	30.4 ± 2.0 (10)	31.0 ± 2.4 (10)
1000	28.8 ± 0.8 (10)	28.3 ± 0.6 (9)	28.5 ± 0.9 (9)	29.9 ± 1.4 (9)
2000	29.3 ± 1.8 (10)	29.6 ± 1.4 (10)	29.1 ± 1.7 (10)	30.4 ± 2.0 (10)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=1.554$	$F^{P}_{5,48}=2.191$	$F^{P}_{5,52}=4.337$	$F^{P}_{5,52}=2.442$
Conclusion[c]	P=0.189 N.S.	P=0.071 N.S.	P=0.002	P=0.046

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] Due to a technical problem with the watering system in one cage, only 5 animals were considered in the analysis.

[c] N.S. = Not Significant

TABLE 14

Relative effects of the administration of Diethylene glycol monomethyl ether (DGME) on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day[c]	Post-dosing period 4th day	Post-dosing period 8th day
Diethylene glycol monoethyl ether (DGME)	100 mg/kg BW	- 6.6 (10)	- 6.5 (10)	- 5.0 (10)
	250 "	+ 0.3 (10)	+ 2.6 (10)	+ 2.8 (10)
	500 "	- 1.6 (10)	- 1.3 (10)	- 2.5 (10)
	1000 "	- 6.9 (9)	- 7.5 (9)	- 6.0 (9)
	2000 "	- 2.6 (10)	- 5.5 (10)	- 4.4 (10)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 1. Results are percent change; test group size is indicated in parenthesis.

(c) Due to a technical problem with the watering in one cage containing control animals, these values were calculated using the 5 animals from the non-affected cage.

TABLE 15

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Diethylene glycol monomethyl ether (DGME)[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	+ 1.2 ± 1.2[b] (5)	+ 1.2 ± 0.6 (9)	+ 2.2 ± 1.1 (9)
100	- 0.3 ± 2.4 (10)	+ 0.1 ± 0.8 (10)	+ 1.5 ± 0.7 (10)
250	0.0 ± 1.7 (10)	+ 1.1 ± 1.5 (10)	+ 2.1 ± 2.0 (10)
500	- 0.5 ± 2.3 (10)	0.0 ± 2.3 (10)	+ 0.6 ± 2.6 (10)
1000	- 0.4 ± 0.9 (9)	- 0.3 ± 1.3 (9)	+ 1.2 ± 2.0 (9)
2000	+ 0.3 ± 2.7 (10)	- 0.2 ± 2.6 (10)	+ 1.2 ± 3.3 (10)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,48}=0.572$	$F^{P}_{5,52}=1.278$	$F^{P}_{5,52}=0.798$
Conclusion[c]	F < 1 N.S.	P=0.333 N.S.	F < 1 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] Due to a technical problem with watering in one cage, only 5 animals were considered in the analysis.

[c] N.S. = Not Significant

TABLE 16

Mortality Data for Diethylene glycol monomethyl ether (DGME)

Dose Level (a)		100	250	500	1000	2000
Number on Test		(10)	(10)	(10)	(10)	(10)
Cause of Death (b)		Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage
Treatment Day	1					
	2					
	3					
	4					
	5					
	6				1	
	7					
	8					
Observation Day	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
Cumulative Deaths		0	0	0	1	0

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
In the vehicle control group for Block I of the MTD study, one death, attributed to gavage error, occurred on the first day of the post-treatment period.

TABLE 17

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	100 mg/kg BW	---	---	---	---
	250 mg/kg	---	---	---	---
	500 mg/kg	---	---	---	---
	1000 mg/kg	---	---	---	---
	2000 mg/kg	---	---	---	---

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	b,e,h(1)	b,e,h(1)e(3);a,e(1)
Diethylene glycol monomethyl ether (DGME)	100 mg/kg BW	---	---	---	---
	250 mg/kg	---	---	---	---
	500 mg/kg	---	---	---	---
	1000 mg/kg	---	h,c,j(4)d(1)	---	---
	2000 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; f = convulsions (tremors); g = death, gavage error; h = hunched posture; i = squinted eyes; j = dehydrated; k = piloerection.

TABLE 18

Clinical Observations of Animals During Post-Treatment Period(*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	9	g(1)	---	---	---
Diethylene glycol monomethyl ether (DGME)	100 mg/kg BW	10	---	---	---	---
	250 mg/kg	10	---	---	---	---
	500 mg/kg	10	---	---	---	---
	1000 mg/kg	9	---	---	---	---
	2000 mg/kg	10	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	100 mg/kg BW	---	---	---	---
	250 mg/kg	---	---	---	---
	500 mg/kg	---	---	---	---
	1000 mg/kg	---	---	---	---
	2000 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; e = weight loss; g = death, gavage error.

3.2 Block II MTD studies

3.2.1 Diethylene glycol monomethyl ether (DGME)

For the three additional dose levels tested, there is no significant statistical difference in mean group body weights among the treated groups and the control group on the first day of dosing (day 1).

At the three higher dose levels (3000, 6000, and 9000 mg/kg) statistical evaluation of the mean group body weights for treated and control grops indicated a significant difference among the groups at day 8 of the treatment period ($p=0.008$). This statistical result was attributed to the differences between the weights of the highest surviving dose group, 6000 mg/kg BW (22.5 ± 1.7 g) and the vehicle control animals (26.4 ± 2.5 g) at that period. There was no significant difference in mean group body weights among the treated groups and the control group at days 4 and 8 after dosing.

The animals in the 3000 mg/kg BW group also stayed within 10.0% of the mean body weight of the control group throughout the study. The animals surviving to the end of the dosing period in the 6000 mg/kg BW dose group lost more than 10% BW compared to control at the same time. All animals in the 9000 mg/kg BW dose group were dead by the 6th day of the study. Four animals in the 6000 mg/kg BW dose group survived to the end of the study.

Among the three higher dose levels which were tested, statistical analyses of the results obtained at day 8 of treatment indicated that a significant difference in body weight changes existed between the surviving test groups and the control group ($p=0.001$). This result is attributed to the difference in weight change between the highest surviving dose group at 6000 mg/kg BW (-2.7 ± 2.7g) and the vehicle control group (1.2 ± 1.6g). Evaluation of the results from days 4 and 8 post-treatment, indicated that the body weight changes were similar between treated and control groups for those intervals ($p > 0.05$).

Mortality for these three dose groups administered DGME is summarized in Table 22. All animals in the 9000 mg/kg BW dose group died between the second and sixth days of dosing. Four of ten animals administered 6000 mg/kg BW died by day 5 of dosing, and two more in this dose group died on the first and second days of the post-dosing period. None of these deaths can be attributed to gavage error. There were no deaths in the control group.

Clinical observations for animals administered these higher dose levels of DGME are summarized in Tables 23 and 24.

Based on the results of the MTD studies for DGME, a dose level of 4000 mg/kg was chosen for the Reproductive study.

TABLE 19

Effect of the administration of Diethylene glycol monomethyl ether (DGME) on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.2 ± 1.7 (10)	26.4 ± 2.5 (10)	27.7 ± 2.7 (10)	28.9 ± 2.8 (10)
3000	25.1 ± 1.9 (10)	25.4 ± 2.1 (10)	26.8 ± 1.7 (10)	28.2 ± 1.3 (10)
6000	24.3 ± 1.7 (10)	22.5 ± 1.7 (6)	25.8 ± 1.5 (4)	26.5 ± 1.6 (4)
9000	24.9 ± 1.8 (10)	--- (0)	--- (0)	--- (0)
Statistical Analysis				
One-Way ANOVA	$F^{P}_{3,36}=0.416$	$F^{P}_{2,23}=6.003$	$F^{P}_{2,21}=1.118$	$F^{P}_{2.21}=1.782$
Conclusion[b]	$F<1$ N.S.	P=0.008	P=0.346 N.S.	P=0.193 N.S.

(a) All values are grams, mean ± S.D.; group size is indicated in parenthesis.

(b) N.S. = Not Significant

TABLE 20

Relative effects of the administration of Diethylene glycol monomethyl ether (DGME) on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Diethylene glycol monomethyl ether (DGME)	3000 mg/kg	- 3.8 (10)	- 3.2 (10)	- 2.4 (10)
	6000 "	-14.8 (6)	- 6.9 (4)	- 8.3 (4)
	9000 "	--- (0)	--- (0)	--- (0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 19. Results are percent change; test group size is indicated in parenthesis.

TABLE 21

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Diethylene glycol monomethyl ether (DGME)[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.6 (10)	2.5 ± 1.6 (10)	3.7 ± 1.7 (10)
3000	0.3 ± 1.3 (10)	1.8 ± 1.4 (10)	3.2 ± 1.1 (10)
6000	- 2.7 ± 2.7 (6)	1.1 ± 1.0 (4)	1.8 ± 1.1 (4)
9000	--- (0)	--- (0)	--- (0)

Statistical Analysis

One-Way ANOVA	$F^{P}_{2,23}=8.838$	$F^{P}_{2,21}=1.547$	$F^{P}_{2,21}=2.766$
Conclusion	P=0.001	P=0.236 N.S.	P=0.086 N.S.

(a) All values are grams, mean ± S.D.; group size is indicated in parenthesis.

(b) N.S. = Not Significant

TABLE 22

Mortality Data for Diethylene glycol monomethyl ether (DGME)

Dose Level [a]		3000	6000	9000
Number on Test		(10)	(10)	(10)
Cause of Death [b]		Test/Gavage	Test/Gavage	Test/Gavage
Treatment Day	1			
	2			3
	3		2	3
	4			
	5		2	2
	6			2
	7			
	8			
Observation Day	1		1	
	2		1	
	3			
	4			
	5			
	6			
	7			
	8			
Cumulative Deaths		0	6	10

(a) mg/kg Body Weight

(b) Where Compound Death = a death attributable to toxicity of the test material
Where Treatment Death = a death attributable to other causes, e.g., gavage error

There were no deaths in the control group at any time.

TABLE 23

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	3000 mg/kg BW	---	---	c(1)	c(9)
	6000 mg/kg	---	a(10)c(5)b(3)i(2)j(1)	a,c(8)i(3)d(2)f(1)	c(8)a(4)h,i(3)b(1)
	9000 mg/kg	a(10)	a(8)c(5)d(3)b,i,j(1)	a,c,d(3)b,i,j(1)	c,h,i(4)b,j,p(2)e(1)

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	3000 mg/kg BW	c(10)	c(3)e(1)	a,c(10)h(2)	c,h(8)
	6000 mg/kg	c,h(6)i(5)a,d(2)	c(6)h(5)a,e,i(3)b(2)	a,c,e,h(6)i(2)	a,c,h(6)e(4)i(3)j(1)
	9000 mg/kg	a,b,c,d,h,i(2)j(1)	d(2)	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy with or without previous activity; b = dyspnea, wheezing, or decreased respiration; c = rough coat; d = death, natural; e = apparent weight loss; f = staggering and/or falling; g = death, gavage error; h = hunched; i = squinted eyes; j = prostrate or moribund; k = piloerection; l = lacrimation; m = muscle control loss; n = bleeding from urogenital region; p = decreased body temperature.

TABLE 24

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animal Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	3000 mg/kg BW	10	---	---	---	---
	6000 mg/kg	6	a(4)b,d,i,j(1)	a(4)d(1)	c(4)	---
	9000 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Diethylene glycol monomethyl ether (DGME)	3000 mg/kg BW	---	---	---	---
	6000 mg/kg	---	---	---	---
	9000 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; i = squinted eyes; j = prostrate or moribund.

3.2.2 Ethylene glycol monomethyl ether (EGME)

Mean body weights of the surviving treated groups (dose levels 190, 380, 760 and 1520 mg/kg BW) were arithmetically lower than the vehicle control group's values, from day 8 of treatment to the end of the study. Statistical analysis of the body weights measured before treatment (day 1), at the end of treatment (day 8), and post-treatment (days 4 and 8), indicated a significant difference existed among the treated group body weights prior to the initiation of dosing ($p=0.011$) and between the treated and control group body weights at the end of dosing ($p=0.035$). The significance of the day 1 values is attributed to the group mean weight differences between the 760 mg/kg BW dose group (25.9 ± 1.9g) and the 3045 mg/kg BW dose group (23.4 ± 1.6g). The significance of the treatment day 8 values is attributed to the group mean weight differences between the control group (26.4 ± 2.5 g) and the highest surviving dose group, at 1520 mg/kg BW (23.9 ± 2.2g).

There is no significant difference in mean body weights among the surviving treated and control groups at days 4 and 8 after the dosing period. Mean body weights of all animals in the 190, 380, 760 and 1520 mg/kg BW dose groups were within 10.0% of the control group mean body weights throughout the study. All animals in the 3045 mg/kg BW dose group were dead by the 7th day of the dosing period.

Effects on weight changes are seen in Table 27. On day 8 of the treatment period, a significant difference ($p=0.0057$) in body weight changes between treated groups and the control group was observed. This statistical result is attributed to the difference between the weight changes of the highest surviving dose group at 1520 mg/kg BW (-1.1 ± 1.6g) and the control group (+1.2 ± 1.6) at that time. No significant differences between treated groups and the control group in body weight changes were found for either of the post-treatment measurement intervals (days 4 and 8).

Mortality is summarized in Table 28. All animals in the highest dose group (3045 mg/kg BW) died during the dosing period, and two animals administered 1520 mg/kg BW died on the last dosing day. None of these deaths is attributable to gavage error. There were no deaths during the post-dosing period. There were no deaths in the control group.

Clinical signs of animals treated with EGME in the MTD study are summarized in Tables 29 and 30, for the treatment and post-treatment phases respectively.

A dose level of 1400 mg/kg was chosen for the Reproductive study of EGME based on the results of the Maximum Tolerated Dose study.

TABLE 25

Effect of the administration of Ethylene glycol monomethyl ether (EGME) on the body weight of female mice [a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.2 ± 1.7 (10)	26.4 ± 2.5 (10)	27.7 ± 2.7 (10)	28.9 ± 2.8 (10)
190	25.4 ± 1.1 (10)	25.2 ± 1.1 (10)	26.7 ± 1.0 (10)	28.8 ± 1.0 (10)
380	25.5 ± 1.2 (10)	26.2 ± 1.5 (10)	27.6 ± 1.2 (10)	28.8 ± 1.2 (10)
760	25.9 ± 1.9 (10)	26.2 ± 1.9 (10)	27.2 ± 1.7 (10)	28.8 ± 2.1 (10)
1520	24.8 ± 1.5 (10)	23.9 ± 2.2 (9)	26.7 ± 1.9 (8)	28.1 ± 1.9 (8)
3045	23.4 ± 1.6 (10)	--- (0)	--- (0)	--- (0)
Statistical Analysis				
One-Way ANOVA	$F^{P}_{5,54}=3.31$	$F^{P}_{4,44}=2.839$	$F^{P}_{4,43}=0.643$	$F^{P}_{4,43}=0.214$
Conclusion [b]	P=0.011	P=0.035	F< 1 N.S.	F< 1 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 26

Relative effects of the administration of
Ethylene glycol monomethyl ether (EGME)
on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Ethylene	190 mg/kg BW	- 4.5 (10)	- 3.6 (10)	- 0.3 (10)
glycol	380 "	- 0.8 (10)	- 0.4 (10)	- 0.3 (10)
monomethyl ether	760 "	- 0.8 (10)	- 1.8 (10)	- 0.3 (10)
(EGME)	1520 "	- 9.5 (9)	- 3.6 (8)	- 2.8 (8)
	3045 "	--- (0)	--- (0)	--- (0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 25. Results are percent change; test group size is indicated in parenthesis.

TABLE 27

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Ethylene glycol monomethyl ether (EGME) [a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.6 (10)	2.5 ± 1.6 (10)	3.7 ± 1.7 (10)
190	- 0.2 ± 0.9 (10)	1.4 ± 1.1 (10)	3.5 ± 1.1 (10)
380	0.4 ± 1.27 (10)	2.1 ± 1.2 (10)	3.3 ± 1.5 (10)
760	0.4 ± 1.0 (10)	1.3 ± 1.0 (10)	2.9 ± 0.8 (10)
1520	- 1.1 ± 1.6 (9)	1.5 ± 1.2 (8)	2.9 ± 0.9 (8)
3045	--- (0)	--- (0)	--- (0)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{4,44}=4.207$	$F^{P}_{4,43}=1.73$	$F^{P}_{4,43}=0.774$
Con-clusion	P=0.0057	P=0.161 N.S.	F < 1 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 28

Mortality Data for Ethylene glycol monomethyl ether (EGME)

Dose Level(a)		190		380		760		1520		3045	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death(b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1										
	2									3	
	3									4	
	4										
	5									2	
	6										
	7									1	
	8							2			
Observation Day	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
Cumulative Deaths		0		0		0		2		10	

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 29

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Ethylene glycol monomethyl ether (EGME)	190 mg/kg BW	---	c(10)l(1)	k(1)	i(2)
	380 mg/kg	---	c(3)	---	c(10)i(1)
	760 mg/kg	---	c(4)a,b(1)	c(4)k(2)	c(10)
	1520 mg/kg	---	c(5)	c(8)	c(10)
	3045 mg/kg	---	a,c(7)i(6)b(5)d(3)	a,c(5)d(4)i(3)b,h,j(2)	c(3)h,i(2)a,b,j(1)

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monomethyl ether (EGME)	190 mg/kg BW	c(10)h(2)i(2)	c(3)	c(2)	---
	380 mg/kg	c(10)	---	a(10)	c,h(3)
	760 mg/kg	c(10)h(5)i(3)	---	a(5)	h(6)a(5)c(1)
	1520 mg/kg	c,h,i(10)a(3)	c,e(10)a,i(2)h(1)	c,a(10)h(5)e(4)	c(9)h(8)a(4)d(2)i(1)
	3045 mg/kg	d(2)a,b,c,h,i,p(1)	a,c,h,i,j(1)	d(1)	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy with or without previous activity; b = dyspnea or decreased respiration; c = rough coat; d = death, natural; e = apparent weight loss; f = staggering and/or falling; g = death, gavage error; h = hunched; i = squinted eyes; j = prostrate or moribund; k = piloerection; l = lacrimation; m = muscle control loss; n = bleeding from urogenital region; p = decrease in body temperature.

TABLE 30

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
Ethylene glycol monomethyl ether (EGME)	190 mg/kg BW	10	---	---	---	---
	380 mg/kg	10	---	---	---	---
	760 mg/kg	10	h(3)	---	---	---
	1520 mg/kg	8	---	c(1)	c(2)	---
	3045 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monomethyl ether (EGME)	190 mg/kg BW	---	---	---	---
	380 mg/kg	---	---	---	---
	760 mg/kg	---	---	---	---
	1520 mg/kg	---	a,f(1)	---	---
	3045 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy; c = rough coat; f = slightly off balance; h = hunched.

3.2.3 Ethylene glycol monoethyl ether (EGEE)

Statistically, there was no significant difference among the mean body weights of any of the dose groups or control group at any of the weight measurement times ($F < 1$). The mean body weights of all the treated groups were within 10.0% of the control group mean body weights throughout the study (Tables 31 and 32).

Statistical comparison of the body weight changes of treated groups with the control group showed that these changes were similar at each of the three time intervals examined (Table 33).

Mortality for EGEE is summarized in Table 34. One animal administered 3605 mg/kg BW died on the fourth dosing day. This death was not due to gavage error or disease. No animals died during the post-dosing period of observation. There were no deaths in the control group.

Clinical signs of animals in the Maximum Tolerated Dose study of EGEE are shown, compared to controls, in Tables 35 and 36.

Based on the results of the MTD study for this chemical, a dose level of 3605 mg/kg for the Reproductive study was chosen.

TABLE 31

Effect of the administration of Ethylene glycol monoethyl ether (EGEE) on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days)		Post-dosing period (days)	
	1	8	4	8
Vehicle Control	25.2 ± 1.7 (10)	26.4 ± 2.5 (10)	27.7 ± 2.7 (10)	28.9 ± 2.8 (10)
225	24.9 ± 2.2 (10)	25.5 ± 2.5 (10)	26.7 ± 2.0 (10)	28.0 ± 2.2 (10)
450	25.2 ± 1.3 (10)	25.9 ± 1.4 (10)	27.2 ± 1.6 (10)	28.1 ± 1.4 (10)
900	25.1 ± 1.5 (10)	26.1 ± 1.4 (10)	27.3 ± 1.6 (10)	28.1 ± 1.5 (10)
1800	25.2 ± 1.4 (10)	25.7 ± 1.3 (10)	27.1 ± 1.6 (10)	28.6 ± 1.7 (10)
3605	25.2 ± 0.9 (10)	25.7 ± 0.7 (9)	26.5 ± 0.8 (9)	28.1 ± 1.2 (9)
Statistical Analysis				
One-Way ANOVA	$F^{P}_{5,54}=0.045$	$F^{P}_{5,53}=0.301$	$F^{P}_{5,53}=0.51$	$F^{P}_{5,53}=0.336$
Conclusion[b]	$F < 1$ N.S.	$F < 1$ N.S.	$F < 1$ N.S.	$F < 1$ N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 32

Relative effects of the administration of Ethylene glycol monoethyl ether (EGEE) on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg BW	- 3.4 (10)	- 3.6 (10)	- 3.1 (10)
	450 "	- 1.9 (10)	- 1.8 (10)	- 2.8 (10)
	900 "	- 1.1 (10)	- 1.4 (10)	- 2.8 (10)
	1800 "	- 2.7 (10)	- 2.2 (10)	- 1.0 (10)
	3605 "	- 2.7 (9)	- 4.3 (9)	- 2.8 (9)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 31. Results are percent change; test group size is indicated in parenthesis.

TABLE 33

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Ethylene glycol monoethyl ether (EGEE) [a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.6 (10)	2.5 ± 1.6 (10)	3.7 ± 1.7 (10)
225	0.6 ± 1.0 (10)	1.8 ± 0.5 (10)	3.1 ± 1.3 (10)
450	0.7 ± 0.7 (10)	2.0 ± 0.7 (10)	2.9 ± 1.0 (10)
900	0.9 ± 1.1 (10)	2.1 ± 1.3 (10)	3.0 ± 1.2 (10)
1800	0.6 ± 0.9 (10)	1.9 ± 1.1 (10)	3.4 ± 1.4 (10)
3605	0.5 ± 0.9 (9)	1.3 ± 1.0 (9)	2.9 ± 1.1 (9)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{5,53}=0.652$	$F^{P}_{5,53}=1.195$	$F^{P}_{5,53}=0.646$
Conclusion [b]	F < 1 N.S.	P=0.324 N.S.	F < 1 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 34

Mortality Data for Ethylene glycol monoethyl ether (EGEE)

Dose Level (a)		225		450		900		1800		3605	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death (b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1										
	2										
	3										
	4									1	
	5										
	6										
	7										
	8										
Observation Day	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
Cumulative Deaths		0		0		0		0		1	

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 35

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg BW	---	---	---	---
	450 mg/kg	---	---	---	c(10)i(1)
	900 mg/kg	---	---	c(2)	c(9)
	1800 mg/kg	---	---	c(3)	c(10)
	3605 mg/kg	a,f(10)	---	c(1)	a,c,f(9)d,i(1)

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg BW	a,c(10)	c(2)	---	c,i(1)
	450 mg/kg	a,c(10)	---	c(1)	c,h(1)
	900 mg/kg	c(10)	---	---	---
	1800 mg/kg	c(10)a(4)i(1)	c(5)a,i(1)	c(3)	c(4)h(1)
	3605 mg/kg	a,c,f(9)h(3)i(1)	a,f(9)	a,f(9)c(3)	c(3)

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy with or without previous activity; b = dyspnea or decreased respiration; c = rough coat; d = death, natural; e = apparent weight loss; f = staggering and/or falling; g = death, gavage error; h = hunched; i = squinted eyes; j = prostrate or moribund; k = piloerection; l = lacrimation; m = muscle control loss; n = bleeding from urogenital region; p = decrease in body temperature.

TABLE 36

Clinical Observations of Animals During the Post-Treatment Period (*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg BW	10	---	---	---	---
	450 mg/kg	10	---	---	---	---
	900 mg/kg	10	---	---	---	---
	1800 mg/kg	10	c(1)	---	---	---
	3605 mg/kg	9	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monoethyl ether (EGEE)	225 mg/kg BW	---	---	---	---
	450 mg/kg	---	---	---	---
	900 mg/kg	---	q(1)	q(1)	---
	1800 mg/kg	---	---	---	---
	3605 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

c = rough coat; q = left hind leg seems partially paralyzed-holds it up while walking.

3.2.4 Ethylene glycol monobutyl ether (EGBE)

Statistically, there is no significant difference in mean group body weights among the treated groups and the control group on the first day of dosing (day 1). Surviving treated groups (dose levels 295, 590, and 1180 mg/kg BW) had mean body weights that were arithmetically lower than the mean body weights of the control group from day 8 to the end of the study. Statistical analyses indicate that a significant difference exists in mean group body weights between the treated groups and the control group (Table 37) at treatment day 8 ($p=0.028$) and at day 8 post-treatment ($p=0.013$). These statistical findings are due to the differences between the weights of the highest surviving dose group at 1180 mg/kg BW (treatment day 8: 23.6 ± 2.1 g; post-treatment day 8: 25.9 ± 1.2g) and the control group weights (treatment day 8: 26.4 ± 2.5g; post-treatment day 8: 28.9 ± 2.8 g) at those periods.

The 1180 mg/kg dose group lost more than 10% body weight compared to controls at these times. The mean group body weights of animals in dose groups 295 and 590 mg/kg BW were within 10.0% of the control group values throughout the study (Table 38). All animals in the 2365 mg/kg BW and the 4725 mg/kg BW dose groups were dead by treatment day 4.

Statistical analyses of results obtained at the 3 time intervals indicated that a significant difference existed between the experimental group and the control group at all 3 periods (day 8 of dosing, $P=0.012$; day 4 post-treatment, $P=0.02$; day 8 post-treatment, $P=0.003$). These statistical results (Table 39) are attributed to the difference between the values of body weight change found for animals receiving 1180 mg/kg BW, and those of the vehicle control group.

Mortality is summarized for the MTD study of EGBE on Table 40. All animals administered 4725 mg/kg BW died by the second day of dosing. All animals administered 2365 mg/kg BW died by the fourth dosing day. One animal in the 590 mg/kg BW dose group died of gavage error on day 4 of treatment. There were no deaths in the control group.

Clinical signs for the animals administered EGBE in the MTD study are summarized for the dosing and post-dosing periods on Tables 41 and 42 respectively.

Based on the results of the MTD study for this chemical (EGBE) 1180 mg/kg was designated as the dose level to be used for the Reproductive study.

TABLE 37

Effect of the administration of Ethylene glycol monobutyl ether (EGBE) on the body weight of female mice [a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.2 ± 1.7 (10)	26.4 ± 2.5 (10)	27.7 ± 2.7 (10)	28.9 ± 2.8 (10)
295	24.7 ± 1.3 (10)	26.0 ± 1.3 (10)	26.8 ± 1.3 (10)	27.5 ± 1.2 (10)
590	25.0 ± 1.7 (10)	25.8 ± 1.7 (9)	26.6 ± 2.0 (9)	27.8 ± 1.8 (9)
1180	24.5 ± 0.8 (10)	23.6 ± 2.1 (10)	25.5 ± 0.8 (10)	25.9 ± 1.2 (10)
2365	25.3 ± 0.9 (10)	--- (0)	--- (0)	--- (0)
4725	24.9 ± 1.5 (10)	--- (0)	--- (0)	--- (0)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=0.437$	$F^{P}_{3,35}=3.412$	$F^{P}_{3,35}=2.33$	$F^{P}_{3,35}=4.135$
Conclusion [b]	$F < 1$ N.S.	P=0.028	P=0.091 N.S.	P=0.013

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 38

Relative effects of the administration of Ethylene glycol monobutyl ether (EGBE) on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg BW	- 1.5 (10)	- 3.2 (10)	- 4.8 (10)
	590 "	- 2.3 (9)	- 4.0 (9)	- 3.8 (9)
	1180 "	-10.6 (10)	- 7.9 (10)	-10.4 (10)
	2365 "	--- (0)	--- (0)	--- (0)
	4725 "	--- (0)	--- (0)	--- (0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 37. Results are percent change; test group size is indicated in parenthesis.

TABLE 39

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Ethylene glycol monobutyl ether (EGBE) [a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.6 (10)	2.5 ± 1.6 (10)	3.7 ± 1.7 (10)
295	1.3 ± 0.9 (10)	2.1 ± 1.1 (10)	2.8 ± 1.1 (10)
590	0.7 ± 1.9 (9)	1.6 ± 0.7 (9)	2.8 ± 1.1 (9)
1800	- 0.9 ± 1.8 (10)	1.0 ± 0.5 (10)	1.4 ± 1.1 (10)
2365	--- (0)	--- (0)	--- (0)
4725	--- (0)	--- (0)	--- (0)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{3,35}=4.253$	$F^{P}_{3,35}=3.74$	$F^{P}_{3,35}=5.539$
Con-clusion [b]	P=0.012	P=0.02	P=0.003

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 40

Mortality Data for Ethylene glycol monobutyl ether (EGBE)

Dose Level(a)		295		590		1180		2365		4725	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death(b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1										
	2							7		10	
	3							2			
	4			1				1			
	5										
	6										
	7										
	8										
Observation Day	1										
	2										
	3										
	4										
	5										
	6										
	7										
	8										
Cumulative Deaths		0		0	1	0		10		10	

(a)mg/kg Body Weight
(b)Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 41

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg BW	---	---	---	---
	590 mg/kg	a,h,i(10)	c(10)i(2)	c(10)g(1)	c(9)i(1)
	1180 mg/kg	c,f,i,k,m(10)j(3)	c(10)b,i(20)a(1)	c(10)i(2)	a,c,f,h,i(10)
	2365 mg/kg	b,c,f,i,k,m,(10)	d,i(7)a,c,l,n(5) j(4)b,h(2)	d(2)a,b,c, h,i,l,n(1)	d(1)
	4725 mg/kg	a,b,f,j(10)d(5)	d(5)	---	---

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg BW	i(4)c(1)	c(1)	---	---
	590 mg/kg	a,c,i(9)h(1)	c(8)	c(6)b(1)	---
	1180 mg/kg	a,c,f,h,i(10)	c(10)e(7)h(5)a(3)i(2)b(1)	a,c,e,h,i(10)m(2)	a,c,h,i(10)m(1)
	2365 mg/kg	---	---	---	---
	4725 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy with or without previous activity; b = dyspnea or decreased respiration; c = rough coat; d = death, natural; e = apparent weight loss; f = staggering and/or falling; g = death, gavage error; h = hunched; i = squinted eyes; j = prostrate or moribund; k = piloerection; l = lacrimation; m = muscle control loss; n = bleeding from urogenital region; p = decrease in body temperature.

TABLE 42

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg BW	10	---	---	---	---
	590 mg/kg	9	---	---	---	---
	1180 mg/kg	10	---	---	---	r(1)
	2365 mg/kg	0	---	---	---	---
	4725 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Ethylene glycol monobutyl ether (EGBE)	295 mg/kg BW	---	---	---	---
	590 mg/kg	---	---	---	---
	1180 mg/kg	r(1)	r(1)	r(1)	r(1)
	2365 mg/kg	---	---	---	---
	4725 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

r = end of the tail is red.

3.3 Block III MTD studies

3.3.1 Toluene (TOL)

Mean body weights (Table 43) of all test dose groups on day 1 and all surviving test groups on day 8 of dosing and at 4 days post-dosing show no statistically significant differences from the mean weights of the control group on those days.

A significant effect on body weight was shown to occur on day 8 post-dosing, which may be attributed to the decrease in the mean body weight of the highest surviving dose group, 2945 mg/kg of toluene, compared to the control group mean body weight on that day.

No surviving animal groups administered this chemical showed body weight losses greater than 10% when compared to controls on the days they were weighed (Table 44).

Table 45 summarizes the statistical evaluation of the body weight changes over the three time intervals examined. Results indicate that there are differences in body weight changes at both post-dosing intervals of test groups compared to controls. These differences may be attributed to significantly less body weight gain among animals given 1470 mg/kg and 2945 mg/kg compared to the lowest dose group or controls on the fourth day post-dosing interval, and significantly less mean body weight change in the animals of the highest surviving dose group, 2945 mg/kg, compared to the control animals on the eighth day after the dosing period.

Mortality for the MTD study of toluene is summarized in Table 46. All animals in the two highest dose groups (5890 and 8700 mg/kg BW) died by dosing day three. Two animals administered 2945 mg/kg BW were found dead on dosing day 4. None of these deaths were due to gavage error. No animals administered 735 or 1470 mg/kg BW died. There were no deaths during the post-dosing period. There were no deaths in the vehicle control group.

Clinical signs of animals administered toluene for the MTD study are summarized in Tables 47 and 48, for the dosing period and the post-treatment phase, respectively.

Based on the results of the MTD study for toluene, a dose level of 2350 mg/kg was chosen for the Reproductive study.

TABLE 43

Effect of the administration of Toluene on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.1 ± 0.9 (10)	26.2 ± 1.8 (10)	26.1 ± 1.6 (10)	27.6 ± 1.7 (10)
735	24.8 ± 1.4 (10)	26.5 ± 1.8 (10)	26.6 ± 1.7 (10)	26.2 ± 1.3 (10)
1470	25.5 ± 1.6 (10)	26.7 ± 1.5 (10)	25.2 ± 2.1 (10)	27.5 ± 1.7 (10)
2945	25.2 ± 0.9 (10)	26.8 ± 1.1 (8)	25.4 ± 1.4 (8)	25.4 ± 1.2 (8)
5890	25.3 ± 1.3 (10)	--- (0)	--- (0)	--- (0)
8700	25.7 ± 0.9 (10)	--- (0)	--- (0)	--- (0)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=0.774$	$F^{P}_{3,34}=0.244$	$F^{P}_{3,34}=1.373$	$F^{P}_{3,34}=4.771$
Conclusion[b]	F < 1 N.S.	F < 1 N.S.	P=0.268 N.S.	P=0.007 Significant

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 44

Relative effects of the administration of Toluene on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Toluene	735 mg/kg BW	+ 1.1 (10)	+ 1.9 (10)	- 5.1 (10)
	1470 "	+ 1.9 (10)	- 3.4 (10)	- 0.4 (10)
	2945 "	+ 2.3 (8)	- 2.7 (8)	- 8.0 (8)
	5890 "	--- (0)	--- (0)	--- (0)
	8700 "	--- (0)	--- (0)	--- (0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 43. Results are percent change; test group size is indicated in parenthesis.

TABLE 45

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of Toluene[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.2 (10)	1.1 ± 1.1 (10)	2.6 ± 1.1 (10)
735	1.7 ± 1.2 (10)	1.9 ± 1.0 (10)	1.5 ± 0.7 (10)
1470	1.2 ± 1.3 (10)	- 0.3 ± 2.6 (10)	2.1 ± 1.8 (10)
2945	1.7 ± 0.4 (8)	0.3 ± 0.8 (8)	0.2 ± 0.9 (8)
5890	--- (0)	--- (0)	--- (0)
8700	--- (0)	--- (0)	--- (0)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{3,34}=0.676$	$F^{P}_{3,34}=3.636$	$F^{P}_{3,34}=6.157$
Conclusion[b]	F < 1 N.S.	P=0.022 Significant	P=0.002 Significant

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 46

Mortality Data for Toluene

Dose Level(a)		735	1470	2945	5890	8700
Number on Test		(10)	(10)	(10)	(10)	(10)
Cause of Death(b)		Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage	Test/Gavage
Treatment Day	1				1	2
	2				2	7
	3				7	1
	4			2		
	5					
	6					
	7					
	8					
Observation Day	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
Cumulative Deaths		0	0	2	10	10

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 47

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
Toluene	735 mg/kg BW	---	---	---	---
	1470 mg/kg	---	a,c,i,t(1)	---	---
	2945 mg/kg	a(10)b(5)t(3)i(1)	a(9)i(5)b(2)t(2)	---	a,b,t(10)d(2)
	5890 mg/kg	a,b(10)t(4)n,p(2)d(1)	a,b,t(8)d(2)	d(7)	---
	8700 mg/kg	a(9)b,n(7)t(4)x(3)p,d(2)	d(7)a,b,t(3)h,r(1)	d(1)	---

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
Toluene	735 mg/kg BW	---	---	---	---
	1470 mg/kg	---	---	---	---
	2945 mg/kg	i,t(8)	i,t(8)b(1)	i,t(8)c(1)	a,t(8)
	5890 mg/kg	---	---	---	---
	8700 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy, slight lethargy, or weakness; b = dyspnea, wheezing or rales; c = rough coat; d = death, natural; f = soft feces; g = death, gavage error; h = hunched posture; i = squinted eyes; n = cyanosis; p = prostrate or moribund; q = head tilt; r = red crust around eyes; s = thoracic swelling; t = tremors; u = urine discolored (rusty); v = coat stained with feces; w = apparent weight loss or thinness; x = ataxia or off balance; z = decreased body temperature.

TABLE 48

Clinical Observations of Animals During the Post-Treatment Period(*)

Test Compound	Dose Level	Animal Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
Toluene	735 mg/kg BW	10	---	---	---	---
	1470 mg/kg	10	---	---	---	---
	2945 mg/kg	8	---	---	---	---
	5890 mg/kg	0	---	---	---	---
	8700 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	---	---	---	---
Toluene	735 mg/kg BW	---	---	c(1)	---
	1470 mg/kg	---	---	---	---
	2945 mg/kg	---	---	---	---
	5890 mg/kg	---	---	---	---
	8700 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; h = hunched; i = squinted eyes; j = prostrate or moribund; q = head tilt; w = apparent weight loss or thinness; x = slightly off balance; y = jaundiced.

3.3.2 2,4-dinitrotoluene (DNT)

Test and control groups started out with similar weights on dosing day one. The mean group body weight of the highest surviving dose group, 525 mg/kg was significantly different from the control group mean body weight on day 8 of dosing (Table 49).

On dosing day eight, the group administered 525 mg/kg of 2,4-dinitrotoluene lost 13.4% body weight compared to the control group. Mean group body weights of all surviving dose groups were similar to the control group mean body weights on days 4 and 8 post-dosing, and on each of these days, no animal groups given 2,4-dinitrotoluene lost more than 10% body weight compared to the control group (Table 50).

Statistical evaluation of mean body weight change of test animals compared to the control animals (Table 51) shows that there are significant differences on the eighth day of dosing and the eighth post-dosing day. These differences are attributable to weight loss among animals receiving 525 mg/kg (the highest surviving dose group) and controls on the eighth dosing day, and in animals of both surviving dose groups compared to control animals on the eighth post-dosing day.

All animals administered 1250, 2500 or 3500 mg/kg BW were dead by dosing day three. Five animals given 525 mg/kg BW were dead by dosing day seven, and one additional animal died by the first post-dosing day. None of these deaths was due to gavage error. One animal in the lowest dose group died on dosing day seven, but was found to have evidence at necropsy indicating a gavage accident. There were no deaths in the vehicle control group. Mortality is summarized for the MTD of 2,4-dinitrotoluene in Table 52.

Clinical signs for animals given this chemical for the MTD study are summarized in Tables 53 and 54.

The results of the MTD study of 2,4-dinitrotoluene were evaluated, and 390 mg/kg was chosen as the dose level of the Reproductive phase of the study.

TABLE 49

Effect of the administration of 2,4 dinitrotoluene on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.1 ± 0.9 (10)	26.2 ± 1.8 (10)	26.1 ± 1.6 (10)	27.6 ± 1.7 (10)
310	25.8 ± 1.2 (10)	26.5 ± 2.1 (9)	26.5 ± 2.2 (9)	26.5 ± 2.0 (9)
525	25.5 ± 0.6 (10)	22.7 ± 2.5 (5)	25.8 ± 1.1 (4)	26.1 ± 1.2 (4)
1250	26.1 ± 1.2 (10)	--- (0)	--- (0)	--- (0)
2500	25.3 ± 1.5 (10)	--- (0)	--- (0)	--- (0)
3500	26.0 ± 1.2 (10)	--- (0)	--- (0)	--- (0)
Statistical Analysis				
One-Way ANOVA	$F'_{5,54}=1.147$	$F'_{2,21}=6.298$	$F'_{2,20}=0.210$	$F'_{2,20}=1.465$
Conclusion[b]	P=0.347 N.S.	P=0.007 Significant	F < 1 N.S.	P=0.255 N.S.

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 50

Relative effects of the administration of 2,4 dinitrotoluene on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
2,4 dinitro-toluene (DNT)	310 mg/kg BW	+ 1.1 (9)	+ 1.5 (9)	- 4.0 (9)
	525 "	-13.4 (5)	- 1.1 (4)	- 5.4 (4)
	1250 "	--- (0)	--- (0)	--- (0)
	2500 "	--- (0)	--- (0)	--- (0)
	3500 "	--- (0)	--- (0)	--- (0)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 49. Results are percent change; test group size is indicated in parenthesis.

TABLE 51

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of 2,4 dinitrotoluene[a]

Dose level (mg/kg BW)	Dosing period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
Vehicle Control	1.2 ± 1.2 (10)	1.1 ± 1.1 (10)	2.6 ± 1.1 (10)
310	0.6 ± 2.2 (9)	0.6 ± 1.7 (9)	0.7 ± 1.3 (9)
525	- 2.5 ± 2.2 (5)	0.6 ± 1.2 (4)	0.9 ± 1.6 (4)
1250	--- (0)	--- (0)	--- (0)
2500	--- (0)	--- (0)	--- (0)
3500	--- (0)	--- (0)	--- (0)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{2,21}=7.073$	$F^{P}_{2,20}=0.331$	$F^{P}_{2,20}=5.986$
Conclusion[b]	P=0.004 Significant	F < 1 N.S.	P=0.009 Significant

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 52

Mortality Data for 2,4-dinitrotoluene

Dose Level (a)		310		525		1250		2500		3500	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death (b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1										
	2			1		9		10		10	
	3					1					
	4										
	5			2							
	6										
	7		1	2							
	8										
Observation Day	1			1							
	2										
	3										
	4										
	5										
	6										
	7										
	8										
Cumulative Deaths		0	1	6		10		10		10	

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 53

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
2,4 dinitro-toluene (DNT)	310 mg/kg BW	a(10)	a(10)h(1)	a(10)	a(10)
	525 mg/kg	a(10)	a(10)b(2)d(1)	a(9)t(1)	a,c,x(9)
	1250 mg/kg	a(10)	u(4)d(9)	d(1)	---
	2500 mg/kg	a(10)i(3)	d(10)c,r,t,u(1)	---	---
	3500 mg/kg	a,i(10)	d(10)	---	---

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
2,4 dinitro-toluene (DNT)	310 mg/kg BW	a(10)	a(10)h,w(1)	a(9)g(1)	a(9)
	525 mg/kg	a(7)x(6)c(5) d(2)b(2)w,p(1)	a,c,x(7)w(2)	a,c(7)w,(9)p(3) d,h(2)q(4)	a,c(5)h,x(2) b,s,q(1)
	1250 mg/kg	---	---	---	---
	2500 mg/kg	---	---	---	---
	3500 mg/kg	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code: a = Lethargy, slight lethargy, or weakness; b = dyspnea, wheezing or rales; c = rough coat; d = death, natural; g = death, gavage error; h = hunched posture; i = squinted eyes; p = prostrate or moribund; q = head tilt; s = thoracic swelling; t = tremors; w = apparent weight loss or thinness; x = ataxia or off balance; z = decreased body temperature.

TABLE 54

Clinical Observations of Animals During the Post-Treatment Period*

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
2,4 dinitro-toluene (DNT)	310 mg/kg BW	9	---	---	---	---
	525 mg/kg	5	x(2)d,q(1)	a,q,x(1)	---	---
	1250 mg/kg	0	---	---	---	---
	2500 mg/kg	0	---	---	---	---
	3500 mg/kg	0	---	---	---	---

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	10	---	---	---	---
2,4 dinitro-toluene (DNT)	310 mg/kg BW	9	---	---	---	---
	525 mg/kg	5	---	---	---	---
	1250 mg/kg	0	---	---	---	---
	2500 mg/kg	0	---	---	---	---
	3500 mg/kg	0	---	---	---	---

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; d = death, natural; q = head tilt; x = slightly off balance.

3.3.3 2,4-diaminotoluene (DAT)

Test and control groups started out with similar weights on dosing day one. (see Table 55). Animal groups administered 2,4-diaminotoluene showed statistically significant weight differences compared to the control group on day 8 of dosing and on days 4 and 8 post-dosing. These differences may be attributed to groups given 225 and 250 mg/kg of 2,4-diaminotoluene for the post-dosing days, and to the highest dose group, 250 mg/kg, on day eight of dosing. Animal groups administered 175 through 250 mg/kg lost more than 10% body weight compared to controls by dosing day eight. Animal groups administered 175, 225 and 250 mg/kg lost more than 10% body weight compared to controls on the fourth and eighth post-dosing days.

Mean body weight changes of animals given DAT (Table 57) are significantly different from mean body weight changes of control animals at all weighing intervals. These highly significant effects are due to losses in body weight in all groups on the eighth dosing day and the eighth post-dosing day, and in all but one group on the fourth post-dosing day. The differences of greatest significance are in the highest two dose groups compared to the control group.

Deaths in animals administered this chemical (Table 58) occurred from the fifth dosing day to the fifth post-dosing day. By the last dosing day, four, two, one, and three animals had died in groups administered 250, 225, 200 and 175 mg/kg BW, respectively. Total deaths in these groups were seven, six, six and four, respectively. There were no deaths in animals given 150 mg/kg BW. There were no gavage errors. There were no deaths in the vehicle control group.

Clinical signs for animals administered DAT in the MTD study are summarized in Tables 59 and 60 for the dosing and post-dosing periods, respectively.

The results of the Maximum Tolerated Dose study were evaluated, and a dose level of 150 mg/kg was designated for the Reproductive study.

TABLE 55

Effect of the administration of 2,4 diaminotoluene on the body weight of female mice[a]

Dose level (mg/kg BW)	Dosing period (days) 1	Dosing period (days) 8	Post-dosing period (days) 4	Post-dosing period (days) 8
Vehicle Control	25.1 ± 0.9 (10)	26.2 ± 1.8 (10)	26.1 ± 1.6 (10)	27.6 ± 1.7 (10)
150	26.1 ± 1.2 (10)	24.4 ± 2.8 (10)	25.6 ± 2.7 (10)	26.3 ± 1.5 (10)
175	25.1 ± 1.5 (10)	22.0 ± 2.0 (7)	23.0 ± 4.0 (6)	23.7 ± 3.2 (6)
200	25.1 ± 1.2 (10)	22.5 ± 4.1 (9)	27.0 ± 1.0 (4)	26.6 ± 0.5 (4)
225	25.9 ± 1.0 (10)	22.0 ± 3.1 (8)	20.9 ± 4.6 (5)	22.3 ± 5.8 (4)
250	25.6 ± 1.4 (10)	21.2 ± 3.1 (6)	20.4 ± 5.5 (4)	23.0 ± 3.1 (3)

Statistical Analysis

One-Way ANOVA	$F^{P}_{5,54}=1.333$	$F^{P}_{5,44}=3.655$	$F^{P}_{5,33}=4.028$	$F^{P}_{5,31}=3.863$
Conclusion[b]	P=0.264 N.S.	P=0.008 Significant	P=0.006 Significant	P=0.008 Significant

(a) All values are grams, mean ± S.D.; group size is indicated in parenthesis.

(b) N.S. = Not Significant

TABLE 56

Relative effects of the administration of 2,4 diaminotoluene on the body weights of female mice[a,b]

Compound	Dose Level	Dosing Period 8th day	Post-dosing period 4th day	Post-dosing period 8th day
2,4 diamino-toluene (DAT)	150 mg/kg BW	- 6.9 (10)	- 1.9 (10)	- 4.7 (10)
	175 "	-16.0 (7)	-11.9 (6)	-14.1 (6)
	200 "	-14.1 (9)	+ 3.4 (4)	- 3.6 (4)
	225 "	-16.0 (8)	-19.9 (5)	-19.2 (4)
	250 "	-19.1 (6)	-21.8 (4)	-16.7 (3)

(a) Percent of relative effect on body weights at three different time intervals after study initiation, as compared to the vehicle control group (=100%) at the same time interval.

(b) This table was prepared using the average body weights shown in Table 55. Results are percent change; test group size is indicated in parenthesis.

TABLE 57

Body weight changes of female mice as compared to the initial weight, at 3 time intervals following administration of 2,4 diaminotoluene[a]

Dose Level (mg/kg BW)	Dosing Period	Post-dosing period	
	8th day	4th day	8th day
Vehicle Control	1.2 ± 1.2 (10)	1.1 ± 1.1 (10)	2.6 ± 1.1 (10)
150	-1.7 ± 2.6 (10)	-0.5 ± 2.7 (10)	0.3 ± 1.3 (10)
175	-3.4 ± 1.6 (7)	-2.0 ± 3.2 (6)	-1.4 ± 2.5 (6)
200	-2.6 ± 3.6 (9)	1.4 ± 1.4 (4)	0.9 ± 0.9 (4)
225	-3.7 ± 2.3 (8)	-4.7 ± 3.7 (5)	-3.4 ± 5.1 (4)
250	-3.7 ± 2.4 (6)	-4.4 ± 5.0 (4)	-1.5 ± 2.1 (3)
Statistical Analysis			
One-Way ANOVA	$F^{P}_{5,44}=5.203$	$F^{P}_{5,33}=4.709$	$F^{P}_{5,31}=5.508$
Conclusion[b]	P = 0.001 Significant	P = 0.002 Significant	P = 0.001 Significant

[a] All values are grams, mean ± S.D.; group size is indicated in parenthesis.

[b] N.S. = Not Significant

TABLE 58

Mortality Data for 2,4-diaminotoluene

Dose Level (a)		150		175		200		225		250	
Number on Test		(10)		(10)		(10)		(10)		(10)	
Cause of Death (b)		Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage	Test	Gavage
Treatment Day	1										
	2										
	3										
	4										
	5									1	
	6					1					
	7							1		2	
	8			3				1		1	
Observation Day	1					2		1			
	2					2				1	
	3			1				2		1	
	4					1					
	5							1		1	
	6										
	7										
	8										
Cumulative Deaths		0		4		6		6		7	

(a) mg/kg Body Weight
(b) Where Test Death = a death attributable to toxicity of the test material
Where Gavage Death = a death attributable to gavage error
There were no deaths in the control group at any time.

TABLE 59

Clinical Observations of Animals During Dosing Period(*)

Test Compound	Dose Level	Day of Treatment 1	2	3	4
Vehicle Control	----	---	---	---	---
2,4 diamino-toluene (DAT)	150 mg/kg BW	a,i(10)	a,i(10)	a,c(8)	c(8)a(7)
	175 mg/kg	a,i(10)	a,i(10)	a,c(10)i(4)b,v(1)	a,c(9)i(1)
	200 mg/kg	a,i(10)	a,i(10)	c(10)a(8)f(1)	a,c(10)
	225 mg/kg	a,i(10)	a,i(10)	c(10)a(5)f(2)	a,c(10)h(4)
	250 mg/kg	a,i(10)	a,i(10)	c(10)a(9)	a,c(10)v(2)

Test Compound	Dose Level	Day of Treatment 5	6	7	8
Vehicle Control	----	---	---	---	---
2,4 diamino-toluene (DAT)	150 mg/kg BW	a(10)c,i(1)	a,c(10)	a,c(10)h(2)	a(8)c(7)h(1)
	175 mg/kg	a,c(10)h(6)	a,c,h(10)	a,c,h,i(10)	c(7)a(6)d(3)i(2)
	200 mg/kg	a,c(10)h(4)w(3)c(2)	a,c(10)h(6)d(1)	a,c(9)h(6)i(3)	a,c(9)h,i(5)b(4)z(1)
	225 mg/kg	a,c(10)h(4)i,w(1)	a,c(10)h(6)i(1)	a,c,h(9)i(8)d(1)	a,c(8)i,h(7)d(1)
	250 mg/kg	a,c,h(9)i(3)d,b(1)	a,c,h(9)i(5)b(2),w(1)	a,c,h(7)d,i(2)b,w(1)	c,h(6)a,i(4)b,d(1)

(*) Number of animals affected is indicated in parenthesis.

Code:
a = Lethargy, slight lethargy, or weakness; b = dyspnea, wheezing or rales; c = rough coat; d = death, natural; f = soft feces; g = death, gavage error; h = hunched posture; i = squinted eyes; n = cyanosis; p = prostrate or moribund; q = head tilt; r = red crust around eyes; s = thoracic swelling t = tremors; u = urine discolored (rusty); v = coat stained with feces; x = ataxia or off balance; z = decreased body temperature.

TABLE 60

Clinical Observations of Animals During the Post-Treatment Period*

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 1	2	3	4
Vehicle Control	----	10	---	---	---	---
2,4 diamino-toluene (DAT)	150 mg/kg BW	10	h(2)c,i(1)	c,h,i(1)	c(2)h,i(1)	c(2)h(1)
	175 mg/kg	7	c(6)h(5)a,i(3)	c,h(3)a,i(2)x(1)	c,h(2)a,d,i,y(1)	c,h(2)a,y(1)
	200 mg/kg	9	c(4)a,h,i(3)d,w(2)	d(2)a,c,h(1)	a,c,h,y(1)	c,d(1)
	225 mg/kg	8	c(7)a,h,i(6)w(4)d(1)	a,c,h,i(7)w(4)	a,c(5)h,i,w(4) y(3)d(2)	c,h(5)a,i,y(4)
	250 mg/kg	6	c(6)h(5)b,w(4)a(3)	c(3)a,b,d,h,w,i(1)	a,c(2)d,i(1)	a,c,h,i(2)b(1)

Test Compound	Dose Level	Animals Per Group	Post-treatment period (days) 5	6	7	8
Vehicle Control	----	10	---	---	---	---
2,4 diamino-toluene (DAT)	150 mg/kg BW	10	---	c(1)	---	---
	175 mg/kg	7	a(3)c,y(2)h(1)	a(4)c(3)h(2)w,y(1)	a,c,h,w,y(2)	c,h,y(2)a(1)
	200 mg/kg	9	---	---	---	---
	225 mg/kg	8	a,c(4)h,i,w,y(2)d(1)	a,c(4)h,i,y(2)w(1)	a,c,w,y(2)h,i(1)	c,w,y(2) a,h,i(1)
	250 mg/kg	6	a,c,d,h,w(1)	c,w(2)a,h(1)	c(2)a,w(1)	c(3)

(*) Number of animals affected is indicated in parenthesis.

Code:

a = Lethargy; b = dyspnea; c = rough coat; d = death, natural; h = hunched; i = squinted eyes; j = prostrate or moribund; q = head tilt; w = apparent weight loss or thinness; x = slightly off balance; y = jaundiced.

4.0 RESULTS OF THE REPRODUCTIVE STUDIES

Results of the reproductive studies and statistical analyses are summarized on Tables 61 to 78.

4.1 Sodium selenite (SS)

Maternal reproductive and litter data, and statistical analysis indicates there were no significant differences between the test and control groups for any of the measures of reproductive toxicity. The change in maternal weights from day 7 to day 18 of gestation is not significant when compared to the vehicle control. It should be noted that for this chemical, due to a technical error in the assignment of the term "day 1" of gestation, many dams gave birth just prior to their being weighed on day 18. However, the statistical analysis is conducted so that this makes no difference in the significance of the results. That is, for maternal weight gain during pregnancy, immediate post-partum maternal weights and their litter weights are added for each animal in both test and control groups, and then compared statistically. Placental weights and fluids were not included for any test or control animal or litter. All other measures are conducted exactly as specified.

4.2 Ethylene thiourea (EtTu)

Significantly fewer live pups per litter were present both 12 hours after birth and 3 days postpartum in mated females tested with this chemical. Other measures of reproductive toxicity were not significantly affected. However, the trend towards greater percentage dead pups per litter within twelve hours of birth may be noted. The same additional comments with regard to the births prior to weighing on day 18 due to inappropriate designation of "day 1" apply to this test as for the previous chemical. The statistics were handled similarly for both test and control groups.

4.3 Diethylene glycol monomethyl ether (DGME)

Five mated female mice of the fifty, which were administered this chemical at the designated tolerated dose, died during the dosing period. At necropsy, none were found to have evidence of gavage error; four were pregnant when they died. Eighteen of the remainder resorbed their litters *in utero*. Nine litters were stillborn. Only five managed to produce viable litters, out of the group mated, yielding a very low reproductive index (0.14). During the subsequent three days another litter died out entirely. The live pups uniformly did more poorly than did the live pups in the control group. Where there was sufficient data to support effective statistical analysis (all measures except number of dead pups within 12 hours), highly statistically significant differences were found between test and control groups for all other measures of reproductive toxicity.

4.4 Ethylene glycol monomethyl ether (EGME)

Seven mated female mice died, of the fifty dosed with this chemical, and only one death could be attributed to gavage error. All 7 were pregnant, and two of these pregnancies were resorbed. Every one of the remaining (30) pregnant mice resorbed their litters in utero. There were no viable births, and no litters even carried to term. No statistical manipulations were necessary, therefore, to conclude that this material is highly toxic to the normal reproductive process at all stages except the earliest ones of fertilization and implantation. The reproductive index was 0.00, due to uniform failure of gestational completion, and the deaths of all fetuses in utero.

4.5 Ethylene glycol monoethyl ether (EGEE)

Five mated female mice died in the test group (two during the dosing period and three after the dosing period was completed), and three of these five were pregnant. No deaths could be attributed to gavage error. All of the (32) remaining pregnant mice resorbed their litters in utero. No viable births occurred, and the reproductive index was 0.00. Due to the highly feto-toxic nature of these effects, to all reproductive phases subsequent to implantation, no statistical analysis is required for its demonstration. The toxic effects of this and the previous chemical on the reproductive process appear similar, by the measures tested in these experiments.

4.6 Ethylene glycol monobutyl ether (EGBE)

Eleven mated female mice dosed with this chemical died; four during the dosing period, and seven after dosing was completed. None of the deaths were due to gavage error. Five of these eleven were pregnant. One of these pregnant mice gave birth and died subsequently. The difference between the test and control groups in the number of viable litters produced is a reflection of both the direct toxicity of the chemical, and also its reproductive effects, i.e., that of causing in utero deaths. The difference in the reproductive index is markedly affected by the seven resorbed litters in the test group. No other statistical measures of reproductive toxicity were significantly different between test and control groups. However, the trend toward lower maternal body weight gains during pregnancy and lower maternal body weights at term, may be noted.

4.7 Toluene (TOL)

Only one test animal died during the experiment and there were no statistically significant differences between test and control groups in any of the categories for evaluation of reproductive toxicity. A nonsignificant trend towards lower weight gain in pregnancy may be noted.

4.8 2,4-dinitrotoluene (DNT)

Ten of the mated females dosed with this chemical, of which four were pregnant, died during the dosing period; none of these deaths could be attributed to gavage error when examined at necropsy. An additional five animals were found dead in the post-dosing observation period of which two were pregnant. Five additional litters were found to have been resorbed, and this also made a substantial contribution to the low (0.68) reproductive index. Other measures of reproductive toxicity were not statistically significantly different between test and control, but there is a trend toward lower maternal weight gains during pregnancy.

4.9 2,4-diaminotoluene (DAT)

Highly significant differences are found between test and control groups for most measures of reproductive toxicity. There were four litters found to have been stillborn, which caused highly significant effects on maternal weights and weight gains. (In addition, there was marked mortality of the dosed animals. Four died during dosing and thirteen in the post-dosing period. Sixteen of these seventeen animals were pregnant. This fact might lead one to suppose that pregnancy itself might increase susceptibility to the toxic effects of this chemical). An additional eleven litters were resorbed. Litter weight data was based on such a small number of litters and pups (left alive), and the dispersion of this data was great enough that three measures of litter weight data on day three postpartum were statistically not significant. However, the reason for this is that there was so much previously expressed toxic effect and mortality.

TABLE 61. Sodium selenite: Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 5.0 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test	0	0	
No. mice producing viable litters	31	30	
Stillborn litters	0	0	
No. mated mice with totally resorbed litters[a]	3	4	
Total Non-Pregnant	16	16	
Reproductive Index[b]	0.91	0.88	N.S.
Mated animals: Initial body weight (day 7)	26.6 ± 2.5 (50)	26.1 ± 3.1 (50)	N.S
Maternal plus litter weight of animals delivering on day 18	48.3 ± 4.7 (31)	47.0 ± 5.6 (22)	N.S
Change in weight (day 7-18)	21.0 ± 4.0 (31)	19.3 ± 3.9 (22)	N.S.
Maternal BW 3 days postpartum (those with viable litters)	35.2 ± 3.3 (31)	34.3 ± 3.3 (30)[d]	N.S

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) Eight mice gave birth after day 18.

Table 62. Sodium selenite: Litter Data

	Vehicle Control deionized H20	Test dose 5.0 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	31	30	
Stillborn litters	0	0	
Live pups per litter within 12 h. postpartum	10.2 ± 1.8	9.1 ± 2.9	N.S.
Dead pups per litter within 12 h. postpartum	0.2 ± 0.4	0.2 ± 0.4	N.S.
% dead pups/litter within 12 h. postpartum[a]	[1.1 ± 1.0]	[1.2 ± 1.1]	[N.S.]
Live pups per litter on day 3 postpartum	10.1 ± 1.9	9.1 ± 2.9	N.S.
% pup viability per litter (days 1-3)[b]	[9.9 ± 0.3]	[10.0 ± 0.1]	[N.S.]
Litter wt. on day 1 postpartum (g)	15.7 ± 2.8	14.4 ± 3.9	N.S.
Litter wt. on day 3 postpartum (g)	20.0 ± 3.5	19.0 ± 4.9	N.S.
Change in litter weight between days 1-3 (g)	4.3 ± 1.5	4.6 ± 1.4	N.S.
% Change in litter weight between days 1-3[d]	[5.1 ± 1.7]	[5.4 ± 1.9]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation , where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability=(No. live pups at day 3/No. live pups at day 1)x100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight=(Wt.(day 3)-Wt.(day 1)/Wt.(day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 63. Ethylene thiourea: Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 300 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test	0	0	
No. mice producing viable litters	31	29	
Stillborn litters	0	0	
No. mated mice with totally resorbed litters[a]	3	2	
Total Non-Pregnant	16	19	
Reproductive Index[b]	0.91	0.94	N.S.
Mated animals: Initial body weight (day 7)	26.6 ± 2.5 (50)	25.3 ± 3.0 (50)	N.S.
Maternal plus litter weight of animals delivering on day 18	48.3 ± 4.7 (31)	48.6 ± 5.8 (18)	N.S.
Change in BW (day 7-18)	21.0 ± 4.0 (31)	21.8 ± 5.2 (18)	N.S.
Maternal BW 3 days postpartum (those with viable litters)	35.2 ± 3.3 (31)	34.4 ± 3.4 (29)[d]	N.S.

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) Eleven mice gave birth after day 18.

Table 64. Ethylene thiourea: Litter Data

	Vehicle Control deionized H20	Test dose 300 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	31	29	
Stillborn litters	0	0	
Live pups per litter within 12 h. postpartum	10.2 ± 1.8	8.8 ± 2.6	$\alpha = 0.02$
Dead pups per litter within 12 h. postpartum	0.2 ± 0.4	0.3 ± 0.7	N.S.
% dead pups/litter within 12 h. postpartum[a]	[1.1 ± 1.0]	[1.6 ± 2.0]	[N.S.]
Live pups per litter on day 3 postpartum	10.1 ± 1.9	8.7 ± 2.8	$\alpha = 0.03$
% pup viability per litter (days 1-3)[b]	[9.9 ± 0.3]	[9.8 ± 0.8]	[N.S.]
Litter wt. on day 1 postpartum (g)	15.7 ± 2.8	14.8 ± 4.1	N.S.
Litter wt. on day 3 postpartum (g)	20.0 ± 3.5	19.2 ± 5.7	N.S.
Change in litter weight between days 1-3 (g)	4.3 ± 1.5	4.4 ± 2.3	N.S.
% Change in litter weight between days 1-3[d]	[5.1 ± 1.7]	[5.1 ± 2.6]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation, where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability = (No. live pups at day 3/No. live pups at day 1) x100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight = (Wt. (day 3) - Wt. (day 1) / Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 65. Diethylene glycol monomethyl ether (DGME): Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 4000 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	5	
Post-dosing	0	0	
Non-pregnant of these	0	1	
No. mice producing viable litters	31	5	
Stillborn litters	0	9	
No. mated mice with totally resorbed litters[a]	1	18	
Total Non-Pregnant	17[d]	14	
Reproductive Index[b]	0.97	0.14	$\alpha <$ 0.01
Mated animals: Initial body weight (day 7)	28.3 ± 2.5 (50)	28.1 ± 3.1 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	48.6 ± 4.6 (31)	39.4 ± 4.1 (14)	$\alpha <$ 0.01
Change in BW (day 7-18)	19.7 ± 3.7 (31)	10.2 ± 3.5 (14)	$\alpha <$ 0.01
Maternal BW 3 days postpartum (those with viable litters)	33.9 ± 3.9 (31)	28.2 ± 1.5 (4)[e]	$\alpha <$ 0.01

(a) As determined by the sulfide nidation site test.
(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.
(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).
(d) One animal in the vehicle control group was not accounted for after day 18.
(e) Maternal body weight 3 days post-partum for one animal was not taken.

Table 66. Diethylene glycol monomethyl ether (DGME): Litter Data

	Vehicle Control deionized H20	Test dose 4000 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	31	5	
No. stillborn litters	0	9	
Live pups per litter within 12 h. postpartum	10.1 ± 2.8	3.2 ± 2.2 (5)	$\alpha < 0.01$
Dead pups per litter within 12 h. postpartum	0.1 ± 0.4	0.6 ± 0.5 (5)	N.S.
% dead pups/litter within 12 h postpartum[a]	[0.9 ± 0.9]	[3.4 ± 2.7] (5)	[$\alpha < 0.01$]
Live pups per litter on day 3 postpartum	10.1 ± 2.8	1.3 ± 1.3 (4)[e]	$\alpha < 0.01$
% pup viability per litter (days 1-3)[b]	[10.0 ± 0.1]	[4.5 ± 3.6] (4)[e]	[$\alpha < 0.01$]
Litter wt. on day 1 postpartum (g)	16.1 ± 3.7	4.6 ± 2.9 (5)	$\alpha < 0.01$
Litter wt. on day 3 postpartum (g)	20.3 ± 4.3	2.5 ± 1.7 (3)[e]	$\alpha < 0.01$
Change in litter weight between days 1-3 (g)	4.2 ± 1.1	-4.2 ± 2.6 (3)[e]	$\alpha < 0.01$
% Change in litter weight between days 1-3[d]	[5.1 ± 0.7]	[-7.6 ± 2.1] (3)[e]	[$\alpha < 0.01$]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation, where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability = (No. live pups at day 3/No. live pups at day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight = (Wt. (day 3) - Wt. (day 1) / Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

(e) Day 3 viability and weight not recorded for one litter.

TABLE 67. Ethylene glycol monomethyl ether (EGME): Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 1400 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	4	
Post-dosing	0	3	
Non-pregnant of these	0	0	
No. mice producing viable litters	31	0	
Stillborn litters	0	0	
No. mated mice with totally resorbed litters[a]	1	32[e]	
Total Non-Pregnant	17[d]	13	
Reproductive Index[b]	0.97	0.00	
Mated animals: Initial body weight (day 7)	28.3 ± 2.5 (50)	28.5 ± 2.5 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	48.6 ± 4.6 (31)	---	----
Change in BW (day 7-18)	19.7 ± 3.7 (31)	---	----
Maternal BW 3 days postpartum (those with viable litters)	33.9 ± 3.9 (31)	---	----

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) One animal in the vehicle control group was unaccounted for after day 18.

(e) Two of these animals died early on test.

Table 68. Ethylene glycol monomethyl ether (EGME): Litter Data

	Vehicle Control deionized H2O	Test dose 1400 mg/kg
No. viable litters (n)	31	0
No. stillborn litters	0	0
Live pups per litter within 12 h. postpartum	10.1 ± 2.8	-
Dead pups per litter within 12 h. postpartum	0.1 ± 0.4	-
% dead pups/litter within 12 h. postpartum[a]	[0.9 ± 0.9]	-
Live pups per litter on day 3 postpartum	10.1 ± 2.8	-
% pup viability per litter (days 1-3)[b]	[10.0 ± 0.1]	-
Litter wt. on day 1 postpartum (g)	16.1 ± 3.7	-
Litter wt. on day 3 postpartum (g)	20.3 ± 4.3	-
Change in litter weight between days 1-3 (g)	4.2 ± 1.1	-
% Change in litter weight between days 1-3[c]	[5.1 ± 0.7]	-

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation, where r = % deaths/litter. [ ] indicates transformed data.

(b) % change in litter weight=(Wt. (day 3)-Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

(c) % change in litter weight=(Wt. (day 3)-Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 69. Ethylene glycol monoethyl ether (EGEE): Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 3605 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	2	
Post-dosing	0	3	
Non-pregnant of these	0	2	
No. mice producing viable litters	31	0	
Stillborn litters	0	0	
No. mated mice with totally resorbed litters[a]	1	32	
Total Non-Pregnant	17[d]	15	
Reproductive Index[b]	0.97	0.00	---
Mated animals: Initial body weight (day 7)	28.3 ± 2.5 (50)	28.5 ± 2.5 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	48.6 ± 4.6 (31)	---	---
Change in BW (day 7-18)	19.7 ± 3.7 (31)	---	---
Maternal BW 3 days postpartum (those with viable litters)	33.9 ± 3.9 (31)	---	---

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) One animal in the vehicle control group was unaccounted for after day 18.

Table 70. Ethylene glycol monoethyl ether (EGEE): Litter Data

	Vehicle Control deionized H2O	Test dose 3605 mg/kg
No. viable litters (n)	31	0
No. stillborn litters	0	0
Live pups per litter within 12 h. postpartum	10.1 ± 2.8	-
Dead pups per litter within 12 h. postpartum	0.1 ± 0.4	-
% dead pups/litter within 12 h. postpartum[a]	[0.9 ± 0.9]	-
Live pups per litter on day 3 postpartum	10.1 ± 2.8	-
% pup viability per litter (days 1-3)[b]	[10.1 ± 0.1]	-
Litter wt. on day 1 postpartum (g)	16.1 ± 3.7	-
Litter wt. on day 3 postpartum (g)	20.3 ± 4.3	-
Change in litter weight between days 1-3 (g)	4.2 ± 1.1	-
% Change in litter weight between days 1-3[c]	[5.1 ± 0.7]	-

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation, where r = % deaths/litter. [ ] indicates transformed data.

(b) % change in litter weight=(Wt.(day 3)-Wt.(day 1)/Wt.(day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

(c) % change in litter weight=(Wt.(day 3)-Wt.(day 1)/Wt.(day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 71. Ethylene glycol monobutyl ether (EGBE): Maternal and Reproductive Data

	Vehicle Control deionized H20	Test dose 1180 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	4	
Post-dosing	0	7[f]	
Non-pregnant of these	0	6	
No. mice producing viable litters	31	24	
Stillborn litters	0	0	
No. mated mice with totally resorbed litters[a]	1	7	
Total Non-Pregnant	17[d]	15	
Reproductive Index[b]	0.97	0.69	$\alpha < 0.01$
Mated animals:			
Initial body weight (day 7)	28.3 ± 2.5 (50)	28.0 ± 2.1 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	48.6 ± 4.6 (31)	44.2 ± 5.1 (24)	N.S.
Change in BW (day 7-18)	19.7 ± 3.7 (31)	15.3 ± 3.9 (24)	N.S.
Maternal BW 3 days postpartum (those with viable litters)	33.9 ± 3.9 (31)	31.0 ± 4.1 (22)[e]	N.S.

(a) As determined by the sulfide nidation site test.
(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.
(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).
(d) One animal in the vehicle control group was unaccounted for after day 18.
(e) One mother died 2 days after delivering; one maternal weight 3 days post-partum was not taken.
(f) One mother died 2 days after delivering viable pups.

Table 72. Ethylene glycol monobutyl ether (EGBE): Litter Data

	Vehicle Control deionized H20	Test dose 1180 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	31	24	
No. stillborn litters	0	0	
Live pups per litter within 12 h. postpartum	10.1 ± 2.8	9.8 ± 2.7 (24)	N.S.
Dead pups per litter within 12 h. postpartum	0.1 ± 0.4	0.2 ± 0.5 (24)	N.S.
% dead pups/litter within 12 h postpartum[a]	[0.9 ± 0.9]	[1.1 ± 1.1] (24)	[N.S.]
Live pups per litter on day 3 postpartum	10.1 ± 2.8	9.7 ± 2.8 (23)[e]	N.S.
% pup viability per litter (days 1-3)[b]	[10.0 ± 0.1]	9.9 ± 0.3 (23)[e]	[N.S.]
Litter wt. on day 1 postpartum (g)	16.1 ± 3.7	15.0 ± 3.7 (24)	N.S.
Litter wt. on day 3 postpartum (g)	20.3 ± 4.3	18.8 ± 5.1 (23)[e]	N.S.
Change in litter weight between days 1-3 (g)	4.2 ± 1.1	3.7 ± 1.7 (23)[e]	N.S.
% Change in litter weight between days 1-3[d]	[5.1 ± 0.7]	[4.8 ± 1.2] (23)[e]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation , where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability=(No. live pups at day 3/No. live pups at day 1)x100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight=(Wt. (day 3)-Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

(e) Day three postpartum viability and weight not taken for one litter.

TABLE 73. Toluene: Maternal and Reproductive Data

	Vehicle Control Corn Oil	Test dose 2350 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	1	
Post-dosing	0	0	
Non-pregnant of these	0	1	
No. mice producing viable litters	30	28	
Stillborn litters	1	2	
No. mated mice with totally resorbed litters[a]	2	0	
Total Non-Pregnant	16[d]	19[e]	
Reproductive Index[b]	0.91	0.93	N.S.
Mated animals: Initial body weight (day 7)	28.2 ± 1.7 (50)	28.4 ± 2.3 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	47.9 ± 4.9 (31)	44.8 ± 5.0 (30)	N.S.
Change in BW (day 7-18)	19.4 ± 4.1 (31)	15.9 ± 4.5 (30)	N.S.
Maternal BW 3 days postpartum (those with viable litters)	34.8 ± 3.2 (30)	35.0 ± 3.1 (28)	N.S.

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) One animal in the vehicle control group was unaccounted for after day 18.

(e) One animal's numerical identification in this group was incorrectly read at necropsy, and one animal was unaccounted for after day 18.

Table 74. Toluene: Litter Data

	Vehicle Control Corn Oil	Test dose 2350 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	30	28	
No. Stillborn litters	1	2	
Live pups per litter within 12 h. postpartum	10.6 ± 2.4	9.6 ± 3.4	N.S.
Dead pups per litter within 12 h. postpartum	0.03 ± 0.18	0.11 ± 0.31	N.S.
% dead pups/litter within 12 h. postpartum[a]	[0.77 ± 0.40]	[1.02 ± 0.96]	[N.S.]
Live pups per litter on day 3 postpartum	10.0 ± 2.8	9.5 ± 3.3	N.S.
% pup viability per litter (days 1-3)[b]	[9.68 ± 0.91]	[9.97 ± 0.10]	[N.S.]
Litter wt. on day 1 postpartum (g)	16.8 ± 3.1	15.0 ± 4.4	N.S.
Litter wt. on day 3 postpartum (g)	21.7 ± 5.9	20.6 ± 5.7	N.S.
Change in litter weight between days 1-3 (g)	5.0 ± 4.5	5.7 ± 1.7	N.S.
% Change in litter weight between days 1-3[d]	[4.9 ± 3.9]	[6.2 ± 0.7]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation , where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability=(No. live pups at day 3/No. live pups at day 1)x100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight=(Wt. (day 3)-Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 75. 2,4-dinitrotoluene (DNT): Maternal and Reproductive Data

	Vehicle Control Corn Oil	Test dose 390 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	10	
Post-dosing	0	5	
Non-pregnant of these	0	9	
No. mice producing viable litters	30	23	
No. Stillborn litters	1	0	
No. mated mice with totally resorbed litters[a]	2	5	
Total Non-Pregnant	16[d]	16	
Reproductive Index[b]	0.91	0.68	α =0.01
Mated animals: Initial body weight (day 7)	28.2 ± 1.7 (50)	27.6 ± 2.9 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	47.9 ± 4.9 (31)	45.8 ± 5.0 (23)	N.S.
Change in BW (day 7-18)	19.4 ± 4.1 (31)	16.9 ± 4.9 (23)	N.S.
Maternal BW 3 days postpartum (those with viable litters)	34.8 ± 3.2 (30)	35.0 ± 2.8 (23)	N.S.

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) One animal in the vehicle control group was unaccounted for after day 18.

Table 76. 2,4-dinitrotoluene (DNT): Litter Data

	Vehicle Control Corn Oil	Test dose 390 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	(30)	(23)	
No. Stillborn litters	1	0	
Live pups per litter within 12 h. postpartum	10.6 ± 2.4	10.3 ± 3.2	N.S.
Dead pups per litter within 12 h. postpartum	0.03 ± 0.18	0.09 ± 0.29	N.S.
% dead pups/litter within 12 h. postpartum[a]	[0.77 ± 0.40]	[0.98 ± 0.94]	[N.S.]
Live pups per litter on day 3 postpartum	10.0 ± 2.8	10.2 ± 3.3	N.S.
% pup viability per litter (days 1-3)[b]	[9.68 ± 0.91]	[9.88 ± 0.48]	[N.S.]
Litter wt. on day 1 postpartum (g)	16.8 ± 3.1	16.4 ± 4.0	N.S.
Litter wt. on day 3 postpartum (g)	21.7 ± 5.9	21.8 ± 5.6	N.S.
Change in litter weight between days 1-3 (g)	5.0 ± 4.5	5.4 ± 2.2	N.S.
% Change in litter weight between days 1-3[d]	[4.9 ± 3.9]	[5.5 ± 2.3]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation, where r = % deaths/litter. [ ] indicates transformed data.
(b) % pup viability = (No. live pups at day 3/No. live pups at day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.
(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).
(d) % change in litter weight = (Wt. (day 3) - Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

TABLE 77. 2,4-diaminotoluene (DAT): Maternal and Reproductive Data

	Vehicle Control Corn Oil	Test dose 150 mg/kg	Statistical Analysis[c]: Model I ANOVA or chi-square
No. mated mice initially on study	50	50	
No. died on test			
Dosing period	0	4	
Post-dosing	0	13	
Non-pregnant of these	0	1	
No. mice producing viable litters	30	5	
No. Stillborn litters	1	4	
No. mated mice with totally resorbed litters[a]	2	24[e]	
Total Non-Pregnant	16[d]	14	
Reproductive Index[b]	0.91	0.14	$\alpha <$ 0.01
Mated animals: Initial body weight (day 7)	28.2 ± 1.7 (50)	28.2 ± 2.4 (50)	N.S.
Maternal BW of animals prior to delivering on day 18	47.9 ± 4.9 (31)	41.7 ± 4.7 (9)	$\alpha <$ 0.01
Change in BW (day 7-18)	19.4 ± 4.1 (31)	13.3 ± 4.2 (9)	$\alpha <$ 0.01
Maternal BW 3 days postpartum (those with viable litters)	34.8 ± 3.2 (30)	30.0 ± 2.0 (5)	$\alpha <$ 0.01

(a) As determined by the sulfide nidation site test.

(b) Defined as the ratio of the number of animals producing viable litters divided by the number of mice ever pregnant.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) One animal in the vehicle control group was unaccounted for after day 18.

(e) Thirteen of these mice died early, on test.

Table 78. 2,4-diaminotoluene (DAT): Litter Data

	Vehicle Control Corn Oil	Test dose 150 mg/kg	Statistical Analysis[c]: Model I ANOVA
No. viable litters (n)	30	5	
No. Stillborn litters	1	4	
Live pups per litter within 12 h. postpartum	10.6 ± 2.4	5.4 ± 2.7	$\alpha < 0.01$
Dead pups per litter within 12 h. postpartum	0.03 ± 0.18	0.80 ± 1.10	$\alpha < 0.01$
% dead pups/litter within 12 h postpartum[a]	[0.77 ± 0.40]	[2.46 ± 2.42]	[$\alpha < 0.01$]
Live pups per litter on day 3 postpartum	10.0 ± 2.8	4.4 ± 2.9	$\alpha < 0.01$
% pup viability per litter (days 1-3)[b]	[9.68 ± 0.91]	[9.06 ± 2.10]	[N.S.]
Litter wt. on day 1 postpartum (g)	16.8 ± 3.1	7.6 ± 4.0	$\alpha < 0.01$
Litter wt. on day 3 postpartum (g)	21.7 ± 5.9	9.3 ± 6.3	$\alpha < 0.01$
Change in litter weight between days 1-3 (g)	5.0 ± 4.5	1.7 ± 3.6	N.S.
% Change in litter weight between days 1-3[d]	[4.9 ± 3.9]	[3.8 ± 6.7]	[N.S.]

(a) $\bar{x}$, SD, and ANOVA after $\sqrt{r + 0.5}$ data transformation , where r = % deaths/litter. [ ] indicates transformed data.

(b) % pup viability=(No. live pups at day 3/No. live pups at day 1)x100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % pup viability. [ ] indicates transformed data.

(c) Numbers are mean ± S.D.; Group size is indicated in parenthesis; Significance is given as alpha level, or as not significant (N.S.).

(d) % change in litter weight=(Wt. (day 3)-Wt. (day 1)/Wt. (day 1) x 100. $\bar{x}$, SD, and ANOVA, after $\sqrt{r}$ data transformation, where r = % change in litter weight. [ ] indicates transformed data.

5.0 DISCUSSION AND CONCLUSIONS

Nine NIOSH compounds were tested in the mouse modified postnatal screening assay for reproductive toxicity. This test (Chernoff and Kavlock, Teratology 21 (2):33A, 1980) is used to identify and prioritize chemicals in need of further reproductive toxicology testing. This method screens chemicals for embryonic, fetal and neonatal toxic responses, using daily treatment of pregnant mice during the period of major embryonic organogenesis with a previously determined maximum tolerated dose level.

Sodium selenite and toluene cause no significant reproductive toxicity at their designated maximum tolerated doses by these tests.

Ethylene thiourea caused significantly reduced numbers of live pups per litter within 12 hours post-partum and at 3 days post-partum. Whether fewer live births actually occurred, or there was cannibalization of live pups by the mother, this is still a significant effect.

Ethylene glycol monobutyl ether and 2,4-dinitrotoluene cause substantial numbers of resorptions and maternal deaths, and therefore reduce the reproductive index markedly. Weights of mothers and offspring, and the viability of litters which are carried to term, however are not significantly affected.

Diethylene glycol monomethyl ether and 2,4-diaminotoluene also cause substantial numbers of resorptions and maternal deaths, but also substantial numbers of stillbirths, and a greatly reduced reproductive index. Statistical analyses of data for reproductive toxicity in mothers and litters were highly significant.

Ethylene glycol monomethyl ether and ethylene glycol monoethyl ether caused extreme reproductive toxicity. No viable litters were born to mated females dosed with the designated maximum tolerated dose of these chemicals at all. All pregnancies of surviving mothers were resorbed *in utero*.

APPENDIX 1

The following are the code numbers used during the conduct of all phases of the test, with their corresponding true chemical names and abbreviations used in this report:

CODE	NAME	ABBREVIATION
08141	Sodium selenite	SS
1426LE	Ethylene thiourea	EtTu
5120TD	Diethylene glycol monomethyl ether	DGME
3623ME	Ethylene glycol monomethyl ether	EGME
0914EH	Ethylene glycol monoethyl ether	EGEE
0614PE	Ethylene glycol monobutyl ether	EGBE
478186	Toluene	TOL
055099	2,4-dinitrotoluene	DNT
850550	2,4-diaminotoluene	DAT

APPENDIX 2

Study Schedule

The following is the study schedule for all phases of these experiments:

DATES	BLOCK
11/19/81 - 12/4/81	Maximum Tolerated Dose Block I
1/8/82 - 1/23/82	Maximum Tolerated Dose Block II
1/22/82 - 2/8/82	Reproductive Block I
3/4/82 - 3/19/82	Maximum Tolerated Dose Block III
3/18/82 - 4/3/82	Reproductive Block II
4/29/82 - 5/15/82	Reproductive Block III

These dates are from the first date of dosing until the necropsy of the last surviving animals for each block.

APPENDIX 3

Supervisory Personnel

Supervisory personnel for this study were:

1. Study Director: Kirby N. Smith, DVM
2. Chemistry Manager: Theodore Olsson, III, B.S.
3. Technical Supervisor: Matthew King, B.S.

APPENDIX 4

Protocol Deviations

Balances for weighing were calibrated at the beginning of each day's weighing. Calibrations were not made at the completion of all animals receiving the same chemical or after every twentieth litter.

APPENDIX 5

Storage Location

Original raw data for this report, and this Final Report are stored in the Archives at the Contractor's laboratory, and microfilm raw data and this Final Report are stored by the Sponsor.

Quality Assurance Report

SPONSOR: NIOSH

PROJECT NO.: 10867

FINDINGS: The final report for Project 10867 has been reviewed by BSC Quality Assurance Personnel. All reported results have been found to accurately reflect the original data.

QA Inspections	Date	Findings Reported to Study Director	Findings Reported to BSC President
Protocol Review (MTD I)	11/10/81	11/10/81	12/10/81
Randomization (MTD I)	11/13/81	11/13/81	12/10/81
Animal Care (MTD I)	11/13/81	11/13/81	12/10/81
Dose Formulation (MTD I)	11/13/81	11/13/81	12/10/81
Final Report Review (MTD I)	1/5 - 1/7/82	1/6/82	----
Final Report Review (MTD II)	2/18 - 3/9/82	2/22/82 3/5/82 3/8/82 3/9/82	----
Final Report Review (MTD III)	5/7 - 6/22/82	5/18/82 6/21/82	----
Reproductive III			
Protocol Review	4/22/82	----	----
Animal Receipt	4/27/82	5/10/82	5/6/82
Randomization	4/27/82	4/27/82	5/6/82
Verification of Randomization	4/28/82	4/29/82	5/6/82
Animal Health Assessment	4/28/82	4/29/82	5/6/82
Dose Formulation	4/28/82	4/29/82	5/6/82

QA Inspections	Date	Findings Reported to Study Director	Findings Reported to BSC President
Body Weight Determinations	4/29/82	4/29/82	5/6/82
Test and Control Article Administration	5/5/82	5/10/82	5/6/82
Clinical Observations	5/5/82	5/10/82	5/6/82
Animal Care	5/5/82	5/10/82	5/6/82
Body Weight Determination	5/10/82	5/10/82	6/17/82
Litter Count and Body Weights	5/13/82	5/13/82	6/17/82
Resorption Site Detection	5/15/82	5/17/82	6/17/82
Final Report Review MTD I,II,III & Reproductive I,II,III	12/6/82 - 1/7/83	12/16/82 12/28/82 1/6/83	1/7/83

QUALITY ASSURANCE OFFICER: Peter J. Moore

DATE: 1/7/83

DISCLAIMER

The results and conclusions of this study are based upon tests conducted in the laboratories of Bioassay Systems Corporation. All tests were conducted in accordance with generally accepted analytical techniques and current procedures for biological testing. Bioassay Systems disclaims, however, any responsibility for the ultimate selection of said tests. No attempt has been made to either compare the results of these tests to the results obtained from other analytical techniques or to independently evaluate the efficacy of the laboratory procedures utilized. Consequently, no representations, either expressed or implied, are made or are intended to be made regarding the selection of these tests.