BORRISTON
LABORATORIES, INC.

FINAL REPORT

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS

Contract No. 210-81-6010
Borriston Project No. 0107-A, B, C
(Formerly 331)
January 4, 1983

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Experimental Toxicology Branch
Division of Biomedical and Behavioral Science
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Submitted by:
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5050 Beech Place
Temple Hills, MD 20748

A Subsidiary of Dynamac International, Inc.

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SUBJECT: SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS
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SUMMARY

This contract was designed to assess nine selected chemicals for their potential to cause adverse reproductive effects in the mouse, by means of a perinatal/postnatal evaluation. The experimental work was divided into three phases (designated as experimental blocks). Within each block, three chemicals were evaluated simultaneously using a shared vehicle control group in an initial maximum tolerated dose (MTD) phase and a subsequent reproductive screen. The chemicals analyzed within each block are presented below:

Experimental Block	BLI Project	Initiation	Termination	Chemicals Evaluated
I	0107-A	MTD: 10-28-81 Repro: 12-07-81	11-12-81 12-25-81	Sodium Selenite Ethylene Thiourea 2-(2-Butoxyethoxy) Ethanol
II	0107-B	MTD: 01-04-82 Repro: 06-21-82	01-19-82 07-09-82	Ethylene Glycol Diethyl Ether Diethylene Glycol Monoethyl Ether Triethylene Glycol
III	0107-C	MTD: 02-14-82 Repro: 05-17-82	03-02-82 06-06-82	Aniline p-Nitroaniline N,N-Dimethylaniline Diethylene Glycol Monoethyl Ether*

*MTD phase only - based on results of Block II MTD phase additional testing of this chemical was required and was conducted concurrently with the MTD phase for Block III; a separate control group was used for this chemical. The reproductive screen for this chemical was conducted with the second block of chemicals.

An initial MTD phase was conducted for each block of chemicals in order to establish dose levels for the subsequent reproductive screen. Each compound was administered by gavage, once daily, for eight consecutive days to separate groups of ten female CD-1 mice at five dose levels specified by the sponsor.

During each treatment period, the mice were observed twice daily for general health, mortality, and pharmacotoxic signs. Body weights were measured on Days 1, 8, 12, and 16.

Based on the results of the MTD testing, the following dose levels were selected for use in the reproductive screens:

Experimental Block	Chemical	Dose Level (mg/kg/day)
I	Sodium Selenite	3.5
	Ethylene Thiourea	100
	2-(2-Butoxyethoxy) Ethanol	500
II	Ethylene Glycol Diethyl Ether	2955
	Diethylene Glycol Monoethyl Ether	5500
	Triethylene Glycol	11270
III	Aniline	560
	p-Nitroaniline	1200
	N,N-Dimethylaniline	365

Within each block, the MTD of each chemical, suspended in the appropriate vehicle, was administered once daily by gavage to a group of 50 timed-pregnant CD-1 mice during Day 7 through Day 14 of gestation. A fourth group of 50 mice received only the vehicle and served as a control group. Following dosing, the mice were allowed to deliver their litters. Terminal sacrifice of dams and litters were performed on Day 4 postpartum. Females that did not produce litters by Day 23 of presumed gestation were sacrificed, necropsied, and non-gravid uteri treated with 10% sodium sulfide to determine the prior existence of a pregnant state.

The variables used to evaluate the reproductive hazard potential of each block of chemicals were: maternal body weights (measured upon receipt , on Days 7 and 18 of gestation and at study termination), and body weight changes (Day 18-Day 7); maternal mortality and signs of toxicity (observed twice daily); physical examinations (conducted at each body weight interval); pup

counts, litter weights, and pup weights (recorded at birth and on Day 3 postpartum); and offspring viability from birth to Day 3 postpartum .

The results of the reproductive screens are summarized as follows:

- Experimental Block I - Gestational treatment (Days 7-14) of timed-pregnant CD-1 mice with sodium selenite, ethylene thiourea, or 2-(2-butoxyethoxy) ethanol did not adversely affect the survival or gestational weight gain of the dams, or birth weight, weight gain, or viability of the F_1 generation through the first three postpartum days.

- Experimental Block II - Gestational treatment with ethylene glycol diethyl ether adversely affected reproductive outcome. Fetotoxicity was characterized by increased frequencies of dead and resorbed litters, decreased offspring viability and reduced pup weights. This chemical was also noted to be slightly toxic to maternal animals. Treatment with diethylene glycol monoethyl ether and triethylene glycol did not adversely affect reproductive outcome. Diethylene glycol monoethyl ether was considered to be slightly toxic to maternal animals based on the mortality which occurred in this group during dosing.

- Experimental Block III - Gestational treatment with p-nitroaniline adversely affected maternal survival, gestational weight gain, the ability of the dams to produce viable litters, and survival of the F_1 generation through the first three postpartum days. Treatment with aniline had no apparent effect on the dams' ability to produce live litters; however, offspring viability through the first three postpartum days was significantly lower than that of the control group. In addition, statistically significant reductions in birth weight and weight gain were seen in the aniline-treated litters. Treatment with N,N-dimethylaniline had no adverse effects on survival or weight gain of the dams, or birth weight, weight gain, or viability of the F_1 generation through the first three postpartum days.

EXPERIMENTAL METHODS - MTD PHASE
BLOCK I (BLI Project #0107-A)

Test Articles

Samples of the three chemicals to be evaluated in this study were received from the sponsor on October 20, 1981. Information pertaining to the receipt and identification of the test articles is given below:

Chemical Name	BRL No.	Physical Description	Container Description	Amount Received
Sodium selenite	430	White crystalline solid	White plastic jar	500 g
Ethylene thiourea	432	White crystalline solid	Amber bottle	500 g
2-(2-butoxyethoxy)-ethanol	431	Flammable liquid	Amber bottle	500 g

The samples were stored in the dark at room temperature in the containers in which they were received. All data which characterize the test articles with respect to identity, strength, purity, composition, and stability under conditions of use are retained by the sponsor. For dosing purposes, the purity of each compound was assumed to be 100%. The test articles were used daily from October 28 through November 4, 1981. Water was used as the vehicle. All unused compounds remaining at the end of the MTD phase were retained under appropriate storage conditions for use in the reproductive screen.

Test Animals and Husbandry

A total of 196 virgin female specific pathogen free (SPF) CD-1 albino mice were obtained from Charles River Breeding Laboratories, Inc. (Portage, Michigan) on October 21, 1981 for use in this study. The mice were 64 days of age (birth date - August 18, 1981) at the time of receipt. Upon receipt, all animals were individually examined for general physical condition and body weights were measured (range of 20.1 to 30.0 g). This strain of mouse was selected as the test system at the request of the sponsor; females were used because only females were to be exposed in the subsequent reproductive phase of the study.

The mice were housed, five per cage, in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Cages were sanitized and fresh bedding was supplied once during the study. Purina® Certified Rodent Chow® #5002 (Lot No. May 6 811H) and fresh water were available ad libitum. The mice were

maintained on a 12-hour light/dark cycle in a temperature controlled room (22±3°C)[1] with 10-15 room air changes per hour.

Prior to study initiation, the mice were quarantined for seven days in the room in which the study was to be conducted. During this period, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

Based on the observations conducted during quarantine, 160 clinically acceptable mice were randomly assigned to treatment groups, using a computer-generated random number table, as follows:

Group No.	No. of Animals	Treatment	Dose Level (mg/kg/day)	Color Code
1	10	Vehicle Control (water)	-	White
2	10	Sodium selenite (BRL 430)	2.5	Dark Blue
3	10	Sodium selenite (BRL 430)	5.0	Yellow
4	10	Sodium selenite (BRL 430)	10.0	Red
5	10	Sodium selenite (BRL 430)	20.0	Orange
6	10	Sodium selenite (BRL 430)	40.0	Light Green
7	10	Ethylene thiourea (BRL 432)	75.0	Grey
8	10	Ethylene thiourea (BRL 432)	150.0	Light Blue
9	10	Ethylene thiourea (BRL 432)	300.0	Gold
10	10	Ethylene thiourea (BRL 432)	600.0	Ivory
11	10	Ethylene thiourea (BRL 432)	1200.0	Pink
12	10	2-(2-butoxyethoxy) ethanol (BRL 431)	125.0	Green
13	10	2-(2-butoxyethoxy) ethanol (BRL 431)	250.0	Copper
14	10	2-(2-butoxyethoxy) ethanol (BRL 431)	500.0	Blue w/white stripe
15	10	2-(2-butoxyethoxy) ethanol (BRL 431)	1000.0	Red w/white stripe
16	10	2-(2-butoxyethoxy) ethanol (BRL 431)	2000.0	Green w/white stripe

Each animal received a unique, six-digit, permanent identification number and toe clipping was performed for identification purposes. The toe clip consisted of the last three digits of the permanent animal number. The remaining digits were omitted from the toe clip, since the first two digits (81) indicated the year of study initiation, and the third digit (5) was the same for all animals on study. The toe clipping code is illustrated in Figure 1. In addition, each group of five

[1]Temperatures outside the given range were reported on various occasions throughout the study, and adjustments were made to correct the problem. A record of the frequency and duration of the temperature deviations was maintained in the project log book. This variation was not considered to have affected the outcome of the study.

mice was assigned a color-coded card which displayed the corresponding project number, individual animal numbers (six digits), treatment group, and dosage level.

Test Article Preparation and Administration

Each test article was suspended in water at a concentration which provided the proper amount of test compound to the animals at each dose level. For each test article, appropriate amounts of the compound were weighed on a Mettler H33AR® pan balance (accurate to 0.1 mg). Water was then added and the resulting test article/vehicle admixture was mixed for five minutes or until suspended on a Corning® magnetic stirrer. The dosing mixtures were prepared at study initiation and were stored refrigerated in glass beakers throughout the study, except when in use.

The test article/vehicle admixtures were administered orally by gavage, once daily, for eight consecutive days. Oral presentation via intubation was selected as the route of administration at the request of the sponsor. Each animal received the appropriate dose level of the designated compound at a constant dosing volume of 10.0 ml/kg of body weight. The dosing mixtures were thoroughly agitated just prior to and continually during dosing. Mice in Group 1 received only water at a constant volume of 10.0 ml/kg of body weight and served as the common control group for the three compounds being evaluated. All test article/vehicle admixtures remaining at the end of the treatment period were retained refrigerated at the request of the sponsor for possible future analysis.

Observations

During the dosing period (Day 1 through 8), animals were observed twice daily for signs of toxicity. The first observation was conducted approximately one hour following administration of the test article, and the second, at least five hours later. In addition, mortality checks were performed once in the morning (prior to dosing) and again in the afternoon. All animals which succumbed during the dosing phase were opened and examined in order to ascertain if death was due to the toxicity of the test chemical or due to dosing technique. The following criteria were used in determining dosing error deaths: 1) compound in the thoracic cavity; 2) compound in the lungs;

and/or 3) a hole in the esophagus. All other deaths were assumed to be treatment related. Observations for signs of toxicity and mortality were performed twice daily (morning and afternoon) on Days 9 through 15 and once (morning) on Day 16 prior to terminal sacrifice. Body weights were measured at study initiation (Day 1), Day 5[2] of dosing, on the last day of dosing (Day 8), and at termination (Day 16). Body weight changes were calculated for each animal for each interval beyond the initial (Day 1) weight; i.e., $(x_t - x_i)$ where x_i is the initial weight and x_t is the weight at time t. In addition, physical examinations were performed at each body weight interval.

Termination

All surviving mice were sacrificed by carbon dioxide asphyxiation following the collection of terminal body weights on Day 16 of the study. No necropsy examinations were performed and the carcasses were incinerated.

Statistical Analysis

For each treatment group, means and standard deviations were calculated for all body weight data (Days 1, 5, 8, 16, and weight changes for each interval beyond the Day 1 weight). Multiple t-tests[3] were performed and are provided only as a means of highlighting weight differences. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

The two factors used in estimating the MTD were compound-related mortality and body weight. The following formula was utilized for calculating the weight differential between each treated group and the control group and provided an aid for estimating the MTD:

$$0\% > \left(\frac{\text{Final T BW} - \text{Final C BW}}{\text{Final C BW}} \times 100\right) > -10\%$$

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

[2]Deviation from protocol - body weights were inadvertently measured on Day 5 instead of Day 4.

[3]Snedecor, G.W. and Cochran, W.G., Statistical Methods, Iowa State University Press, Ames, Iowa 10:258-268, 1967.

RESULTS - MTD PHASE

BLOCK I (BLI Project #0107-A)

Body weights were inadvertently not measured at the correct intervals during the study. The protocol (issued October 14, 1981) stated that weights were to be taken upon animal receipt, at study initiation (Day 1), on Day 4 of dosing, on the last day of dosing (Day 8), and at termination (Day 16). Due to an error in project scheduling, weights were taken on Day 5 of dosing rather than Day 4. Correspondence from the sponsor (dated November 16, 1981) indicated that the body weight intervals given in the protocol were incorrect, and that weights should have been measured upon animal receipt, at study initiation (Day 1), on the last day of dosing (Day 8), on the fourth day after dosing (Day 12), and at termination (Day 16). All references to body weight intervals in the text and tables of this report refer to the intervals as given in the protocol issued October 14, 1981.

The results for each chemical evaluated are discussed below.

Sodium Selenite (BRL No. 430)

Mortality - The cumulative mortality which occurred during the study in mice treated with sodium selenite is presented below. The percentage of death which was attributed to technical errors made during dosing procedures is also indicated.

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
1	0 (Control)	10	0
2	2.5	0	0
3	5.0	70	20
4	10.0	70	0
5	20.0	100	0
6	40.0	100	0

Mortality data are presented by study day in Table 1. All deaths occurred during the dosing phase. Mortality reached 100% in Groups 5 and 6 by treatment Days 4 and 2, respectively.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment (Days 1 through 16) were recorded with respect to nature, onset, and duration of each. The signs observed were those which are commonly seen prior to death. Lethargy and/or hypoactivity were the most frequently observed signs

of toxicity and were occasionally accompanied by ataxia (one Group 3 animal), gasping (one Group 4 and one Group 6 animal), tremors (one Group 5 animal), or prostration (one Group 6 animal). Disorientation (characterized by slow, wandering movements around cage with apparent loss of proper bearings) was seen only in mice from Groups 5 and 6 (incidences of 1 and 2, respectively). The number of animals per group which exhibited any one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery.

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
1	0 (Control)	1/10	0/1	1/1
2	2.5	0/10	-	-
3	5.0	1/10	1/1	0/1
4	10.0	3/10	3/3	0/3
5	20.0	2/10	2/2	0/2
6	40.0	3/10	3/3	0/3

All animals which survived the treatment period remained normal throughout the post-dosing phase of the study.

Physical examinations were conducted at each body weight interval (Days 1, 5, 8, and 16). All mice examined were normal with the exception of Animal No. 815487 (Group 2), which was noted to have a 4 mm tissue mass behind the left ear at the Day 16 exam.

Body Weights - Mean body weights (Days 1, 5, 8, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 2.

All groups (including the control) showed a net loss in body weight during the dosing interval (Days 1 through 8). No statistical differences between treated and control group values were noted. Body weight data were not available for the 20.0 and 40.0 mg/kg/day dose levels after Day 1 due to the early mortality which occurred in these groups. The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
2	2.5	0.0
3	5.0	-6.2
4	10.0	-4.0
5	20.0	-
6	40.0	-

*Relative to control (Group 1) value

MTD Determination - Based on the lethality, body weight, and pharmacotoxic sign data, it was recommended that 2.5 mg/kg/day be considered the MTD for this chemical to be used in the reproductive phase. Although this dose did not produce a significant weight loss when compared to the controls at the end of the study, 50% of the animals at the next higher dose (5.0 mg/kg) died from compound-related causes, and a dose of 10.0 mg/kg/day killed 70% of the animals. Following consultation with the sponsor, a dose level of 3.5 mg/kg/day was selected for use in the reproductive screen.

Ethylene Thiourea (BRL No. 432)

It was observed during dosing procedures that this chemical was not completely soluble in water and was difficult to keep in suspension. The test article/vehicle admixtures were vigorously stirred throughout dosing in an attempt to keep the compound in a suspended state; however, in the mixtures being administered to Groups 8 through 11, the compound immediately fell out of solution upon cessation of stirring. The two highest dose levels (600.0 and 1200.0 mg/kg) were flocculent, and difficulty was encountered in pushing the full amount of the suspension out of the syringe barrel; the animals in these two groups may not have received the full complement of the test article.

Mortality - The cumulative mortality which occurred during the study in mice treated with ethylene thiourea is presented on the following page. The percentage of death which was attributed to technical errors made during dosing procedures is also indicated.

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
1	0 (Control)	10	0
7	75.0	10	10
8	150.0	50	10
9	300.0	70	30
10	600.0	0	0
11	1200.0	0	0

Mortality data are presented by study day in Table 1. All deaths occurred during the dosing phase (Days 1 through 8). The absence of mortality in two highest dose levels (600.0 and 1200.0 mg/kg/day) supports the hypothesis that these mice were not receiving the proper dose of the compound due to the difficulty encountered in maintaining a homogeneous test article/vehicle admixture.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment (Days 1 through 16) were recorded with respect to nature, onset, and duration of each. Lethargy and/or hypoactivity were the most consistently noted signs of toxicity. All mice receiving the compound at a dose level of 300.0 mg/kg/day were noted to be hypoactive at the 5-hour post-dosing observation on Day 3. Lethargy/hypoactivity were occasionally accompanied by disorientation (one Group 7 animal), paralysis (one Group 8 animal) or hunched posture, salivation, labored breathing, and squinted eyes (all observed in a single Group 9 animal). No signs of toxicity were seen in any of the mice from Groups 10 and 11. The number of animals per group which exhibited any one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery.

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
1	0 (Control)	1/10	0/1	1/1
7	75.0	1/10	1/1[1]	0/1
8	150.0	3/10	2/3	1/3
9	300.0	9/10	6/9[2]	3/9
10	600.0	0/10	-	-
11	1200.0	0/10	-	-

[1]The cause of death was attributed to dosing error rather than toxicity.
[2]The cause of death for 3 of the 6 mice which subsequently died was attributed to dosing error rather than toxicity.

All animals which survived the treatment period remained normal throughout the post-dosing period.

No abnormalities were observed in any of the mice during the physical examinations on Days 1, 5, 8, and 16.

Body Weights - Mean body weights (Days 1, 5, 8, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 2.

Statistical comparison of each of these groups with the control animals revealed some elevated test animal weights. Interpretation of these data are complicated by mortality and the biological significance of these statistical differences is judged to be minimal.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
7	75.0	-6.2
8	150.0	-5.1
9	300.0	2.9
10	600.0	-0.4
11	1200.0	-5.5

*Relative to control (Group 1) value.

MTD Determination - Based on the results of this study, no definitive MTD was recommended. Ethylene thiourea appeared to be toxic at 150.0 mg/kg/day in that this dosage killed 50% of the animals; one, however, died due to a dosing error. Weight losses (relative to the controls) were noted at Day 16 for both the 75.0 and 150.0 mg/kg/day groups, but the next higher level (300.0 mg/kg) showed a relative increase in weight at this interval. The two highest dose levels (600.0 and 1200.0 mg/kg/day) could not be considered due to the difficulty encountered in keeping the compound in suspension. It was initially recommended that this MTD determination be repeated using a 1% methylcellulose vehicle at doses of 300.0, 150.0, 75.0, 40.0, and 20.0 mg/kg/day. If this was not possible, it was suggested that 75.0 mg/kg/day be used as the MTD in the reproductive phase. Following consultation with the sponsor, a dose level of 100.0 mg/kg/day was selected for use in the reproductive screen.

2-(2-Butoxyethoxy) Ethanol (BRL No. 431)

Mortality - The cumulative mortality observed during this study in mice treated with butoxyethoxy ethanol is shown below. The percentage of death which was attributed to technical errors made during dosing procedures is also indicated.

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
1	0 (Control)	10	0
12	125.0	30	10
13	250.0	30	20
14	500.0	30	20
15	1000.0	50	30
16	2000.0	40	10

Mortality data are presented by study day in Table 1. All deaths occurred during the dosing phase (Days 1 through 8).

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment (Days 1 through 16) were recorded with respect to nature, onset, and duration of each. Signs of toxicity were seen only in mice treated at the two highest dosage levels (Groups 15 and 16). Disorientation and lethargy were noted in all Group 15 and 16 animals immediately following test article administration on Day 1. In addition, all surviving Group 15 mice were hypoactive at the one-hour observation interval. One Group 16 animal was prostrate and gasping prior to death. The number of animals per group which exhibited any one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery.

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
1	0 (Control)	1/10	0/1	1/1
12	125.0	0/10	-	-
13	250.0	0/10	-	-
14	500.0	0/10	-	-
15	1000.0	10/10	5/10[1]	5/10
16	2000.0	10/10	4/10[2]	6/10

[1]The cause of death for 3 of the 5 mice which subsequently died was attributed to dosing error rather than toxicity.
[2]The cause of death for 1 of the 4 mice which subsequently died was attributed to dosing error rather than toxicity.

All animals which survived the treatment period remained normal throughout the post-dosing phase of the study with the exception of Animal No. 815610

(Group 15) which was observed to be thin, gasping, and unkempt in appearance on Days 14, 15, and 16. These observations were also reported for this animal upon physical examination on Day 16. No other animals exhibited abnormalities at any of the physical examination intervals.

Body Weights - Mean body weights (Days 1, 5, 8, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 2. Statistical comparison of each of these groups with control animals revealed some variation in test animal weights. Interpretation of these data are complicated by mortality and the biological significance of these statistical differences is judged to be minimal.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
12	125.0	-5.1
13	250.0	-2.6
14	500.0	-9.5
15	1000.0	-10.6
16	2000.0	-0.4

*Relative to control (Group 1) value

MTD Determination - Weight losses relative to the control group were seen in all treated groups at the end of the study. Based on the mortality data, a dose level of 500.0 mg/kg was recommended and approved as the MTD for use in the reproductive phase.

EXPERIMENTAL METHODS - REPRODUCTIVE PHASE
BLOCK I (BLI Project #0107-A)

Test Articles

The three chemicals for reproductive hazard evaluation were received from the sponsor on October 20, 1981 (prior to initiation of the MTD phase); the portion of each compound remaining at the end of the MTD phase was retained under appropriate storage conditions for use in the reproductive screen. Information pertaining to the description, receipt, and storage conditions of the test articles is given on page 4 of this report.

Test Animals and Husbandry

A total of 229 timed-pregnant female specific pathogen free (SPF), CD-1 albino mice arrived at Day 3 of gestation from Charles River Breeding Laboratories, Inc. (Portage, Michigan), on December 3, 1981. The animals were examined upon receipt for general health and physical condition, and body weights were recorded (range 19.0 to 31.3 grams). This strain and the use of timed-pregnant mice were selected as the test system at the request of the sponsor.

The mice were housed individually in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Purina® Certified Rodent Chow® #5002 (Lot No. Oct. 7 811A) and fresh water were available ad libitum. Fresh water bottles were supplied once weekly; cages were sanitized and bedding was changed once during the study (between Day 15 and Day 18 of gestation). The mice were housed in a temperature controlled room (range

18-25°C)[4] with 10-15 room air changes per hour. A 12-hour light/dark illumination cycle was maintained[5].

Prior to study initiation the mice were quarantined for four days in the room in which the study was to be conducted; this shortened quarantine period was necessitated by the use of timed-pregnant mice. During quarantine, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

On Day 7 of gestation, two hundred (200) clinically acceptable mice were assigned to treatment groups using a computer-generated randomization program. The body weights of mice assigned to the study ranged from 21.0 to 30.0 g. The allotment of animals to treatment groups was as follows:

Group No.	No. of Assumed Pregnant Females	Treatment	Dose Level* (mg/kg/day)	Color Code
1	50	Distilled water	-	White
2	50	Sodium selenite	3.5	Dk. Blue
3	50	Ethylene thiourea	100.0	Yellow
4	50	Butoxyethoxy ethanol	500.0	Red

*Dose levels were established based on the results of the MTD Phase.

Each animal received a unique, six-digit, permanent animal number and was toe clipped to reflect that number for identification purposes. The toe clip consisted of the last three digits of the permanent animal number. The remaining digits were omitted from the toe clip, since the first two digits (81) indicated the year of study initiation, and the third digit (6) was the same for all animals on study. The toe clipping code used for this study is

[4]A temperature of 18°C was reported during the morning mortality check on December 10, 1981; this was corrected and two hours later a temperature of 20°C was recorded. Also, a temperature of 18°C was reported on December 14 and was corrected that evening. These temperature deviations are not considered to have affected the outcome of the study.

[5]During the delivery phase of the study (December 18, 19, and 20, 1981), it was necessary to turn the lights on during the evening in order to weigh the litters within 12 hours of birth as specified in the protocol; a record of the duration of each nocturnal visit was maintained in the project log book.

illustrated in Figure 1[6]. The cage cards were color-coded and displayed the project number, individual animal number (six digits), treatment group and dose level. The cages for each treatment group were arranged vertically on the cage rack.

Test Article Preparation and Administration

Each test article was suspended in a distilled water vehicle at a concentration which provided the proper amount of compound for each dose level. For each test article, appropriate amounts of the compound were weighed out on a Mettler H33AR® pan balance (accurate to 0.1 mg) and placed in a 250 ml volumetric flask. Distilled water was added, q.s. (quantity sufficient) to 250 ml. The resulting test article/vehicle admixture was mixed on a Corning® magnetic stirrer for five minutes or until suspended. The dosing solutions were prepared just prior to study initiation (Day 7 of gestation) and dosages were calculated based on the body weight data recorded on that day. The solutions were used for eight consecutive days (Day 7 through Day 14 of gestation). During the study, the dosing mixtures were stored at 5°C, except when in use.

The test article admixtures were administered orally with a steel feeding needle, once daily for eight consecutive days beginning on Day 7 of gestation. Oral presentation via intubation was selected as the route of administration at the request of the sponsor. Dosing was performed at approximately the same time each day. Each animal received an appropriate dose of the designated compound at a constant dosing volume of 10.0 ml/kg of body weight based on the weights measured on Day 7 of gestation. Mice in Group 1 received distilled water at a volume of 10.0 ml/kg of body weight. This group served as the common control for the three compounds being evaluated.

Observations

All animals were observed twice daily during the study (morning and afternoon) for clinical signs of toxicity and mortality. Body weights for the

[6]Animals 816060 through 816079 and 816204 through 816219 were inadvertently toe clipped incorrectly, with the right foot representing tens and the left foot representing units; documentation was made in the daily log book in order to preclude animal identification errors. All other mice were toe clipped correctly as shown in Figure 1.

dams were recorded upon receipt (Day 3 of gestation), at study initiation (Day 7 of gestation), on Day 18 of gestation, and at termination (Day 4 postpartum). In addition, a terminal body weight was recorded on Day 23 of presumed gestation for females which did not produce litters. Body weight changes (Day 18-Day 7) were calculated for each female. Physical examinations were performed at each body weight interval. Pup counts and litter weights were recorded within 12 hours of birth and on Day 3 postpartum. A per-pup average weight (mean pup weight) was calculated for each litter at birth and Day 3 by dividing the total litter weight by the number of live pups. Litter weight and mean pup weight changes (Day 3-Birth) were also calculated, and the viability of offspring from birth to Day 3 was assessed.

Termination.

Terminal body weight measurements were taken on Day 4 postpartum for females which delivered, on Day 3 postpartum for the litters, and on Day 23 of presumed gestation for the females that did not deliver. All dams were sacrificed on Day 5 postpartum and females which failed to deliver were sacrificed on Day 24 of gestation, by asphyxiation with carbon dioxide. Pups were either decapitated or killed by an overdose of ether on Day 5 postpartum. Necropsy examinations were performed only on females which did not deliver. The nongravid uteri were treated with a 10% sodium sulfide solution to determine the prior existence of a pregnant state. All carcasses were incinerated.

Statistical Analysis

For each treatment or control group, means and standard deviations were calculated for the following parameters: maternal body weights for each interval collected; litter weights, mean pup weights, and pup counts (live and dead) for each interval collected; weight changes for dams (Day 18-Day 7 of gestation) and litters (Day 3-Birth); and offspring viability ratios from birth to Day 3 postpartum. Treatment group means were compared to the common control group by Student's t-test[7]. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

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[7]Snedecor, G.W. and Cochran, W.G., Statistical Methods, Iowa State University Press, Ames, Iowa 10:258-268, 1967.

The use of the word "significant" in this report, where groups are statistically compared, is to imply either no statistically significant difference or that a statistically significant difference is noted. The use of the word "similar" has no statistical connotation, but rather indicates that two groups have similar data sets.

RESULTS - REPRODUCTIVE PHASE
BLOCK I (BLI Project #0107-A)

Maternal Mortality and Clinical Signs

Mortality data are presented in Table 3. One control (Group 1) female (No. 816098) was found dead during the afternoon mortality check on Day 13 of gestation. Necropsy examination showed no evidence that death was due to dosing technique. No other deaths occurred during the study.

The incidence of pharmacotoxic signs observed during the study is presented in Table 4. No treatment-related signs of toxicity were observed in any of the mice during the daily observations or at the physical examination intervals. Animal Nos. 816170 (ethylene thiourea, Group 3) and 816235 (butoxyethoxy ethanol, Group 4) developed tissue masses during the study. In addition, Animal 816235 was observed to have an unkempt appearance on Day 7 and Day 16 of gestation, and Animal 816166 (ethylene thiourea) was noted to have black fluid around the vagina on Day 9 of gestation.

Maternal Body Weights

A summary of mean maternal body weights and body weight changes measured at designated intervals during the study is presented in Table 6. Mean body weights for females which did not deliver litters are presented in Table 7. The mean body weights and body weight changes of all treatment groups were similar to the control values throughout the study. The statistically increased mean body weights (Day 7 and Day 18) for ethylene thiourea-treated females which did not produce litters were judged to be incidental.

Reproductive Performance and Maternal Behavior

Litters were born on Days 18 through 21 of gestation (December 18 through December 21, 1981). The percentage of litters per group delivered on each day is summarized below:

		Litters Delivered (%)			
		Gestation Day			
Group	Treatment	18	19	20	21
1	Vehicle Control	13	70	17	0
2	Sodium Selenite	22	69	9	0
3	Ethylene Thiourea	0	76	24	0
4	Butoxyethoxy Ethanol	3	60	32	5

No delay in time to delivery was apparent in any of the treated groups as compared to the vehicle control group.

A summary of reproductive outcome is presented in Table 5. No compound-related effects on reproductive outcome were observed; the delivery index (number of live litters produced/number of mice determined to be pregnant) observed in each treated group was similar to that of the control group. Examination of sodium sulfide treated uteri indicated no prior existence of a pregnant state in any of the females which had not delivered by Day 23 of gestation. The uteri from Animals 816082 (Group 1) and 816229 (Group 4) were also examined. Animal 816082 had given birth to two pups that were found dead and partially cannibalized before any litter data could be recorded; four early resorption sites were noted upon examination of the uterus. Animal 816229 delivered a single pup which was born dead and malformed; examination of the uterus showed three early resorption sites.

Abnormal maternal behavior, which was limited to cannibalization, was observed infrequently. Evidence that the dams had cannibalized their offspring was seen in two control litters (Dams 816082 and 816092) and one Group 3 litter (Dam 816200); thus, this behavior was considered to be unrelated to treatment.

Pup Counts, Litter Weights, and Offspring Viability

Mean pup counts (live and dead), litter weights, and pup weights recorded at birth and on Day 3 postpartum, as well as mean litter weight and pup weight changes (Day 3-Birth) and offspring viability ratios (number alive at Day 3/number alive at birth), are presented in Table 6.

No significant differences between control and treated group mean values were observed for any of the litter parameters analyzed.

Conclusions

Based on the results of this study, gestational treatment (Days 7-14) of timed-pregnant CD-1 mice with sodium selenite, ethylene thiourea, or 2-(2-butoxyethoxy) ethanol did not adversely affect gestational weight gain of the dams, or birth weight, weight gain, or viability of the F_1 generation.

Raw Data and Final Report Storage

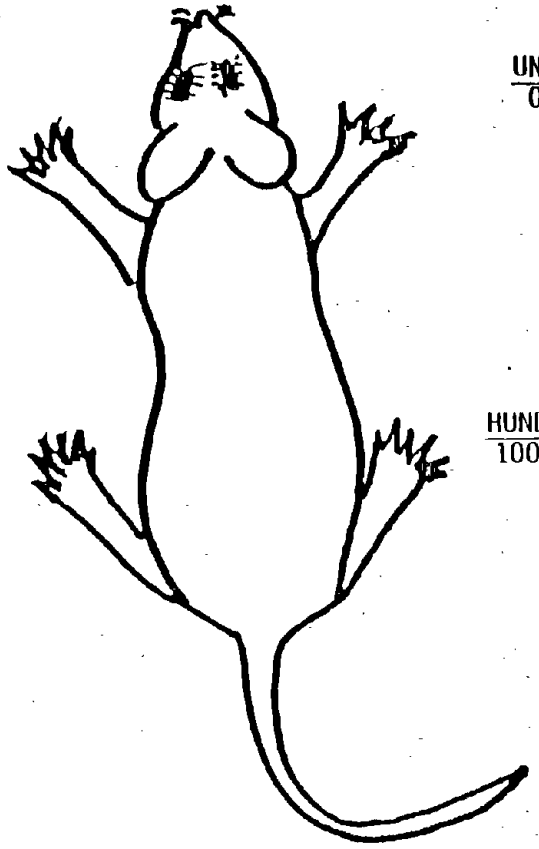
All raw data and the final report are retained in the archives at Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, Maryland, 20748.

FIGURE 2

PERMANENT NUMBER IDENTIFICATION

BY TOE CLIPPING

TENS
10-90



UNITS
0-9

HUNDREDS
100-900

0						5				
1						6				
2					7				
3					8				
4					9				

Toe-clipping code for each foot (digit 1 excluded)

TABLE 1

MORTALITY DATA FOR VEHICLE CONTROL (WATER)

SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

		Dose Level (mg/kg/day)									
		0									
Number on Test:		10									
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-								
	2	-	-								
	3	-	1								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Observation Day	1	-	-								
	2	-	-								
	3	-	-								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Cumulative Deaths*		0	1								

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 1 (Continued)

MORTALITY DATA FOR SODIUM SELENITE (BRL #430)

SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

		Dose Level (mg/kg/day)									
		2.5		5.0		10.0		20.0		40.0	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	2	-	1	-	4	-	8	-
	2	-	-	-	1	3	-	4	-	2	-
	3	-	-	2	-	2	-	1	-	-	-
	4	-	-	1	-	1	-	1	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	1	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Observation Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Cumulative Deaths*		0	0	5	2	7	0	10	0	10	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 1 (Continued)

MORTALITY DATA FOR ETHYLENE THIOUREA (BRL #432)

SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

		Dose Level (mg/kg/day)									
		75.0		150.0		300.0		600.0		1200.0	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	1	-	1	-	3	-	-	-	-
	3	-	-	3	-	3	-	-	-	-	-
	4	-	-	1	-	1	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Observation Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Cumulative Deaths*		0	1	4	1	4	3	0	0	0	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 1 (Continued)

MORTALITY DATA FOR 2-(2-BUTOXYETHOXY) ETHANOL (BRL #431)

SCREENING OF PRIORITY CHEMICALS FOR POTENTIAL REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

		Dose Level (mg/kg/day)									
		125.0		250.0		500.0		1000.0		2000.0	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	-	1	2	-
	2	-	-	1	2	-	1	-	2	-	1
	3	2	1	-	-	1	-	-	-	-	-
	4	-	-	-	-	-	-	1	-	-	-
	5	-	-	-	-	-	-	1	-	1	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	1	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Observation Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Cumulative Deaths*		2	1	1	2	1	2	2	3	3	1

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 2

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

SODIUM SELENITE (BRL #430)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 5[a]	Change (Day 5-Day 1)	Day 8	Change (Day 8-Day 1)	Day 16	Change (Day 16-Day 1)
1	MEAN	26.9	25.0	-1.8	24.8	-2.0	27.3	0.6
(0 mg/kg/day)	S.D.	1.8	2.4	1.2	2.0	1.1	2.3	0.8
	N	10	9	9	9	9	9	9
2	MEAN	27.5	26.4	-1.1	26.5	-1.0	27.3	-0.1
(2.5 mg/kg/day)	S.D.	1.7	1.9	1.5	1.8	1.3	2.0	1.2
	N	10	10	10	10	10	10	10
3	MEAN	25.6	22.7	-2.9	23.2	-2.3	25.6	0.1
(5.0 mg/kg/day)	S.D.	2.1	3.1	1.9	4.1	2.0	2.0	0.1
	N	10	4	4	3	3	3	3
4	MEAN	26.6	25.7	-1.2	26.4	-0.4	26.2	-0.7
(10.0 mg/kg/day)	S.D.	2.5	2.9	0.6	2.9	1.0	2.9	1.6
	N	10	3	3	3	3	3	3
5	MEAN	27.5	-	-	-	-	-	-
(20.0 mg/kg/day)	S.D.	1.7	-	-	-	-	-	-
	N	10	-	-	-	-	-	-
6	MEAN	25.6	-	-	-	-	-	-
(40.0 mg/kg/day)	S.D.	2.7	-	-	-	-	-	-
	N	10	-	-	-	-	-	-

[a]Deviation from protocol - body weights inadvertently measured on Day 5 instead of Day 4.

-Data not available due to death of all mice in group.

TABLE 2 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

ETHYLENE THIOUREA (BRL #432)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 5[a]	Change (Day 5-Day 1)	Day 8	Change (Day 8-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	26.9	25.0	-1.8	24.8	-2.0	27.3	0.6
	S.D.	1.8	2.4	1.2	2.0	1.1	2.3	0.8
	N	10	9	9	9	9	9	9
7 (75.0 mg/kg/day)	MEAN	24.8^{s-}	23.7	-1.2	24.6	-0.3^{s+}	25.6	0.7
	S.D.	1.4	1.9	1.3	1.5	0.6	1.5	0.7
	N	10	9	9	9	9	9	9
8 (150.0 mg/kg/day)	MEAN	25.8	23.7	-2.4	24.6	-1.6	25.9	-0.2
	S.D.	1.7	2.8	1.4	2.7	1.8	1.3	1.1
	N	10	5	5	5	5	5	5
9 (300.0 mg/kg/day)	MEAN	25.4	26.4	-0.1^{s+}	26.3	-0.2	28.1	1.6
	S.D.	2.4	3.1	0.6	2.7	1.8	2.7	1.9
	N	10	3	3	3	3	3	3
10 (600.0 mg/kg/day)	MEAN	26.9	26.5	-0.5^{s+}	26.6^{s+}	-0.3^{s+}	27.2	0.3
	S.D.	2.2	2.1	0.9	1.4	1.3	2.0	1.0
	N	10	10	10	10	10	10	10
11 (1200.0 mg/kg/day)	MEAN	26.4	25.4	-1.0	26.7^{s+}	0.4^{s+}	25.8	-0.6
	S.D.	1.5	1.9	2.6	1.9	2.1	2.3	2.3
	N	10	10	10	10	10	10	10

[a]Deviation from protocol - body weights inadvertently measured on Day 5 instead of Day 4.

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 2 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-A)

2-(2-BUTOXYETHOXY) ETHANOL (BRL #431)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 5[a]	Change (Day 5-Day 1)	Day 8	Change (Day 8-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	26.9	25.0	-1.8	24.8	-2.0	27.3	0.6
	S.D.	1.8	2.4	1.2	2.0	1.1	2.3	0.8
	N	10	9	9	9	9	9	9
12 (125.0 mg/kg/day)	MEAN	26.8	25.5	-1.0	25.2	-1.3	25.9	-0.6^{s-}
	S.D.	1.3	1.2	1.0	1.6	1.4	1.5	0.9
	N	10	7	7	7	7	7	7
13 (250.0 mg/kg/day)	MEAN	26.7	26.4	0.0^{s+}	27.1^{s+}	0.8^{s+}	26.6	0.2
	S.D.	1.7	1.9	1.4	2.0	1.8	2.7	2.1
	N	10	7	7	7	7	7	7
14 (500.0 mg/kg/day)	MEAN	24.2^{s-}	24.0	0.3^{s+}	24.7	0.6^{s+}	24.7^{s-}	0.6
	S.D.	1.6	1.7	1.1	0.8	1.2	0.6	1.2
	N	10	8	8	7	7	7	7
15 (1000.0 mg/kg/day)	MEAN	26.4	25.5	0.0^{s+}	25.1	-0.2^{s+}	24.4	-0.9
	S.D.	2.3	2.0	1.1	3.4	2.0	5.9	4.1
	N	10	6	6	5	5	5	5
16 (2000.0 mg/kg/day)	MEAN	26.3	25.9	-0.7^{s+}	26.1	-0.4^{s+}	27.2	0.7
	S.D.	2.2	1.5	0.5	1.3	0.4	1.5	0.8
	N	10	7	7	6	6	6	6

[a]Deviation from protocol - body weights inadvertently measured on Day 5 instead of Day 4.

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 3

MORTALITY DATA

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

GROUP AND TREATMENT:		1 Vehicle Control		2 Sodium Selenite 3.5 mg/kg/day		3 Ethylene Thiourea 100 mg/kg/day		4 2-(2-Butoxyethoxy) Ethanol 500 mg/kg/day	
Number on Test:		50		50		50		50	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Study Day	1[a]	-	-	-	-	-	-	-	-
	2[a]	-	-	-	-	-	-	-	-
	3[a]	-	-	-	-	-	-	-	-
	4[a]	-	-	-	-	-	-	-	-
	5[a]	-	-	-	-	-	-	-	-
	6[a]	-	-	-	-	-	-	-	-
	7[a]	-	-	-	-	-	-	-	-
	8[a]	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-
	13	-	1	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-
	19	-	-	-	-	-	-	-	-
Cumulative Deaths[b]		0	1	0	0	0	0	0	0

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.

[a]Indicates the days on which mice were dosed (Gestation Day 7 through 14).

[b]Represents total number of deaths prior to sacrifice.

TABLE 4

INCIDENCE OF PHARMACOTOXIC SIGNS

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

PHARMACOTOXIC SIGNS	GROUP AND TREATMENT:	INCIDENCE OF SIGN[a]							
		1 Vehicle Control		2 Sodium Selenite 3.5 mg/kg/day		3 Ethylene Thiourea 100 mg/kg/day		4 2-(2-Butoxyethoxy) Ethanol 500 mg/kg/day	
	INTERVAL:	AM	PM	AM	PM	AM	PM	AM	PM
Day 1									
Unkempt appearance									1/50
Day 3									
Dark fluid around vagina							1/50		
Day 9									
Subcutaneous tissue mass, throat									1/50
Day 10									
Subcutaneous tissue mass, throat								1/50	1/50
Unkept appearance								1/50	1/50
Day 11									
Subcutaneous tissue mass, throat								1/50	1/50
Day 12									
Subcutaneous tissue mass, throat								1/50	1/50
Day 15									
Subcutaneous tissue mass, throat									1/50
Day 16									
Subcutaneous tissue mass, throat									1/50
Day 17									
Tissue mass, behind left ear						1/50	1/50		

[a]Incidence (numerator) given as number per group in which each sign was observed at the specified interval; denominator indicates the number of mice which were alive at the beginning of the interval. All mice were observed to be normal on those days not shown in table.

TABLE 5

SUMMARY OF REPRODUCTIVE OUTCOME

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

	GROUP AND TREATMENT			
	1 Vehicle Control	2 Sodium Selenite 3.5 mg/kg/day	3 Ethylene Thiourea 100 mg/kg/day	4 2-(2-Butoxyethoxy) Ethanol 500 mg/kg/day
Number Treated	50	50	50	50
Number of Deaths	1	0	0	0
Number of Survivors:				
Nonpregnant	18	27	29	13
Pregnant	31	23	21	37
Fertilized without Subsequent Implantation[a]	0	0	0	0
Number of Litters:				
Live Litters	30	23	21	36
Dead Litters	1[c]	0	0	1[c]
Resorbed Litters	0	0	0	0
Delivery Index[b]:				
Ratio	30/31	23/23	21/21	36/37
Percent	97	100	100	97

[a]Determined by the presence of corpora lutea upon examination of uteri at necropsy.

[b]Delivery Index = Number of live litters produced/total number pregnant; denominator exclude mice that were determined to have been fertilized without subsequent implantation.

[c]Resorption sites were observed in the uteri of both dams which delivered dead litters.

TABLE 6

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS, AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

		GROUP AND TREATMENT			
		1 Vehicle Control	2 Sodium Selenite 3.5 mg/kg/day	3 Ethylene Thiourea 100 mg/kg/day	4 2-(2-Butoxyethoxy) Ethanol 500 mg/kg/day
MATERNAL BODY WEIGHTS (g)					
Gestation Day 3	MEAN	24.1	24.5	24.0	23.8
	S.D.	1.7	1.7	2.1	2.5
	N	31	23	21	37
Gestation Day 7	MEAN	27.2	26.3	26.6	26.6
	S.D.	1.9	1.6	1.6	1.8
	N	31	23	21	37
Gestation Day 18	MEAN	45.4	45.6	44.7	45.0
	S.D.	5.6	4.5	4.0	5.0
	N	31	22	21	37
Weight Change (Day 18 - 7)	MEAN	18.2	19.2	18.1	18.5
	S.D.	4.8	3.7	3.6	4.3
	N	31	22	21	37
Terminal (Day 4 Postpartum)	MEAN	36.4	35.1	35.3	35.4
	S.D.	4.1	2.8	3.2	3.0
	N	30	22	18	36
LITTER WEIGHTS (g)					
Birth	MEAN	15.4	14.7	14.8	15.7
	S.D.	2.9	2.5	3.1	2.9
	N	30	23	20	36
Day 3 Postpartum	MEAN	25.0	25.3	25.2	26.0
	S.D.	5.2	2.5	5.8	2.0
	N	28	23	21	35
Weight Change (Day 3 - Birth)	MEAN	9.8	10.6	10.2	10.4
	S.D.	5.2	2.5	3.4	2.0
	N	28	23	20	35

aMean maternal body weights are based on all surviving mice which produced litters; mean litter and pup weights are based on litters with live pups.

TABLE 6 (Continued)

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS, AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

		GROUP AND TREATMENT			
		1 Vehicle Control	2 Sodium Selenite 3.5 mg/kg/day	3 Ethylene Thiourea 100 mg/kg/day	4 2-(2-Butoxyethoxy) Ethanol 500 mg/kg/day
MEAN PUP WEIGHTS[b] (g)					
Birth	MEAN	1.6	1.5	1.5	1.6
	S.D.	0.3	0.2	0.2	0.2
	N	30	23	20	36
Day 3 Postpartum	MEAN	2.8	2.6	2.7	2.7
	S.D.	0.4	0.4	0.4	0.5
	N	28	23	21	35
Weight Change (Day 3 - Birth)	MEAN	1.1	1.1	1.2	1.1
	S.D.	0.5	0.3	0.3	0.3
	N	28	23	20	35
PUP COUNTS (per litter)					
Birth:					
Live	MEAN	9	10	10	10
	S.D.	3	2	2	3
	N	31	23	21	37
Dead	MEAN	0	0	0	0
	S.D.	0	0	1	0
	N	31	23	21	37
Total	MEAN	9	10	10	10
	S.D.	3	2	2	3
	N	31	23	21	37
Day 3 Postpartum:					
Live	MEAN	9	10	10	10
	S.D.	3	2	2	2
	N	30	23	21	36
Dead	MEAN	0	0	0	0
	S.D.	1	0	1	1
	N	30	23	21	36
Offspring Viability Ratio[c]	MEAN	0.97	1.00	0.96	0.99
	S.D.	0.13	0.00	0.10	0.04
	N	30	23	21	36

[a]Mean maternal body weights are base on all surviving mice which produced litters; mean litter and pup weights are based on litters with live pups.

[b]Mean Pup Weights = Litter weight/Number of live pups.

[c]Offspring Viability Ratio = Number of live pups on Day 3/Number of live pups at birth.

TABLE 7

MEAN BODY WEIGHTS - NON LITTER BEARING FEMALES[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-A)

GROUP TREATMENT		BODY WEIGHTS DAY 3 (g)	DAY 7 (g)	DAY 18 (g)	WT. CHANGE DAY (18-7) (g)	TERMINAL (g)
1	MEAN	23.9	25.2	26.3	1.3	26.8
Vehicle Control	S.D.	1.9	1.9	2.2	1.7	2.3
	N	19	19	18	18	18
2	MEAN	24.0	25.5	26.5	1.0	27.1
Sodium Selenite	S.D.	2.0	1.9	1.9	1.2	2.4
3.5 mg/kg/day	N	27	27	27	27	27
3	MEAN	24.2	26.6[s+]	27.6[s+]	1.0	27.9
Ethylene Thiourea	S.D.	2.4	1.9	2.0	1.5	2.1
100 mg/kg/day	N	29	29	29	29	29
4	MEAN	23.7	24.8	25.3	0.5	26.2
2-(2-Butoxyethoxy) Ethanol	S.D.	2.6	2.2	1.4	1.7	1.7
500 mg/kg/day	N	13	13	13	13	12

EXPERIMENTAL METHODS - MTD PHASE
BLOCK II (BLI Project #0107-B)

Test Articles

Samples of the three chemicals to be evaluated in this study were received from the sponsor on December 23, 1981. Information pertaining to the receipt and identification of the test articles is given below:

Chemical Name	BRL NO.	Physical Description	Container Description	Amount Received
Ethylene glycol diethyl ether	445	Clear liquid	Amber bottle	500 g
Diethylene glycol monoethyl ether	444	Clear liquid	Amber bottle	500 g
Triethylene glycol	443	Clear liquid	Amber bottle	500 g

The samples were stored at room temperature in the containers in which they were received. All data which characterize the test articles with respect to identity, strength, purity, composition, and stability under conditions of use are retained by the sponsor. For dosing purposes, the purity of each compound was assumed to be 100%. The test articles were used daily from January 4 through January 11, 1982. Distilled water was used as the vehicle. All unused compounds remaining at the end of the MTD phase were retained under appropriate storage conditions for use in the reproductive screen.

Test Animals and Husbandry

A shipment of 210 virgin female specific pathogen free (SPF) CD-1 albino mice were obtained from Charles River Breeding Laboratories, Inc. (Portage, Michigan) on December 30, 1981 for use in this study. The mice were 47 days of age (birth date - November 13, 1981) at the time of receipt. Upon receipt, each animal was examined for general physical condition and body weights were measured (range of 16.8 to 29.7 g). This strain of mouse was selected as the test system at the request of the sponsor; females were used because only females were to be exposed in the subsequent reproductive phase of the study.

The mice were housed, five per cage, in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Cages were sanitized and fresh bedding was supplied once during the study. Purina® Certified Rodent Chow® #5002 (Lot No. Dec 4 812) and fresh water via water bottles were available ad

libitum. The mice were maintained on a 12-hour light/dark cycle in a temperature controlled room (22±3°C)[1] with 10-15 room air changes per hour.

Prior to study initiation, the mice were quarantined for six days in the room in which the study was to be conducted. During this period, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

Based on the observations conducted during quarantine, 160 clinically acceptable mice were randomly assigned to treatment groups using a computer-generated random number table. The allotment of animals to treatment groups was as follows:

Group No.	No. of Animals	Treatment	Dose Level (mg/kg/day)	Color Code
1	10	Vehicle Control (Distilled water)	-	White
2	10	Ethylene glycol diethyl ether (BRL #445)	295	Dark Blue
3	10	Ethylene glycol diethyl ether (BRL #445)	580	Yellow
4	10	Ethylene glycol diethyl ether (BRL #445)	1180	Red
5	10	Ethylene glycol diethyl ether (BRL #445)	2365	Orange
6	10	Ethylene glycol diethyl ether (BRL #445)	4730	Light Green
7	10	Diethylene glycol monoethyl ether (BRL #444)	335	Grey
8	10	Diethylene glycol monoethyl ether (BRL #444)	670	Light Blue
9	10	Diethylene glycol monoethyl ether (BRL #444)	1340	Gold
10	10	Diethylene glycol monoethyl ether (BRL #444)	2685	Ivory
11	10	Diethylene glycol monoethyl ether (BRL #444)	5365	Pink
12	10	Triethylene glycol (BRL #443)	750	Green
13	10	Triethylene glycol (BRL #443)	1500	Copper
14	10	Triethylene glycol (BRL #443)	3005	Blue w/white stripe
15	10	Triethylene glycol (BRL #443)	6005	Red w/white stripe
16	10	Triethylene glycol (BRL #443)	12015	Green w/white stripe

Each animal received a unique six-digit permanent identification number and toe clipping was performed for identification purposes. The toe clip consisted of the last three digits of the permanent animal number. The remaining digits were omitted from the toe clip since the first two digits (82) indicated the year of study

[1]Temperatures outside the given range were reported on various occasions throughout the study, and adjustments were made to correct the problem. A record of the frequency and duration of the temperature deviations was maintained in the project log book. This variation was not considered to have affected the outcome of the study.

initiation, and the third digit (5) was the same for all animals on study. The toe clipping code used in this study is illustated in Figure 2. In addition, each group of five mice was marked with a color-coded card on which the corresponding project number, individual animal numbers (six digits), treatment group, and dosage level were printed.

Test Article Preparation and Administration

For each treatment group, a 50 ml volumetric flask was weighed on a Mettler H33AR® pan balance (accurate to 0.1 mg). The correct amount of compound appropriate for each dose level was added to the flask. Distilled water was added, quantity sufficient to 50 ml for Groups 2 through 15. The resulting test article/vehicle admixtures were transferred to 100 ml beakers and were stirred on a Corning® magnetic stirrer for 5-10 minutes. The undiluted test article was used for Group 16. The dosing mixtures were prepared just prior to study initiation and were stored in the corresponding project room in glass beakers throughout the study.

The test article/vehicle admixtures or undiluted test article were administered orally by gavage, once daily, for eight consecutive days. Oral presentation via intubation was selected as the route of administration at the request of the sponsor. Each animal in Groups 2 through 15 received the appropriate dose level of the designated compound at a constant dosing volume of 10.0 ml/kg of body weight. The dosing mixtures were thoroughly agitated just prior to and continually during dosing. The highest dose level for triethylene glycol (Group 16) required a dosing volume greater than 10 ml/kg of body weight, and it was calculated as 10.66 ml/kg based on a reported specific gravity of 1.127. Mice in Group 1 received only distilled water at a constant volume of 10.0 ml/kg of body weight and served as the common control group for the three compounds being evaluated. All test article/vehicle admixtures remaining at the end of the treatment period were retained for possible future analyses at the request of the sponsor.

Observations

During the dosing period (Day 1 through 8), animals were observed twice daily for signs of toxicity. The first observation was conducted approximately one hour following administration of the test article, and the second,

at least five hours later. In addition, mortality checks were performed once in the morning (prior to dosing) and again in the afternoon. All animals which succumbed during the dosing phase were opened and examined in order to ascertain if death was due to the toxicity of the test chemical or due to dosing technique. The following criteria were used in determining dosing error deaths: 1) compound in the thoracic cavity; 2) compound in the lungs, and/or 3) a hole in the esophagus. All other deaths were assumed to be treatment related. Observations for signs of toxicity and mortality were performed twice daily (morning and afternoon) on Days 9 through 15 and once (morning) on Day 16 prior to terminal sacrifice. Body weights were measured at study initiation (Day 1), on the last day of dosing (Day 8), on the fourth day after dosing (Day 12), and at termination (Day 16). Body weight changes were calculated for each animal for each interval beyond the initial (Day 1) weight; i.e., $(x_t - x_i)$ where x_i is the initial weight and x_t is the weight at time t. In addition, physical examinations were performed at each body weight interval.

Termination

All surviving mice were sacrificed by carbon dioxide asphyxiation following the collection of terminal body weights on Day 16 of the study. No necropsy examinations were performed and the carcasses were incinerated.

Statistical Analysis

For each treatment group, means and standard deviations were calculated for all body weight data (Days 1, 8, 12, 16, and weight changes for each interval beyond the Day 1 weight). Multiple t-tests[2] were performed and are provided only as a means of highlighting weight differences. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

The two factors used in estimating the MTD were compound-related mortality and body weight. The following formula was utilized for calculating

[2]Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods, 6th Edition Iowa State University Press.

the weight differential between each treated group and the control group and provided an aid for estimating the MTD:

$$0\% > \left(\frac{\text{Final T BW} - \text{Final C BW}}{\text{Final C BW}} \times 100\right) > -10\%$$

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

RESULTS - MTD PHASE

BLOCK II (BLI Project #0107-B)

Ethylene Glycol Diethyl Ether (BRL No. 445)

Mortality - Mortality data of mice treated with ethylene glycol diethyl ether is presented by study day in Table 8. The cumulative mortality which occurred during the study and the percentage of death attributed to technical errors made during dosing procedures is summarized below:

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
1	0(Control)	0	0
2	295	0	0
3	580	10	10
4	1180	10	0
5	2365	0	0
6	4730	100	0

The mortality in the high dose group reached 100% by Day 5, and all deaths were attributed to the toxicity of the compound. The single compound-related death which occurred in Group 4 appeared to be an anomalous result as none of the mice in the next highest dose group died.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment were recorded with respect to nature, onset, and duration of each. No signs of toxicity were observed in mice from Groups 2, 3, and 4. Lethargy was observed in all Group 5 mice at the 1-hour post-dosing observation interval on Day 1 and remained normal throughout the study. An acute toxic response was observed in mice receiving the high dose of the chemical. Death or prostration was consistently observed in all mice at the 1-hour post-dose examination interval on each day of dosing. Nine of the ten mice in this group were dead by Day 3 of dosing. The remaining mouse was found dead at the 5-hour post-dose examination on Day 5. This animal was noted to be prostrate one hour after dosing on each treatment day and exhibited tremors, disorientation (characterized by slow, wondering movements around cage with apparent loss of proper bearings), lethargy, and/or an unkempt appearance at the 5-hour post-dose examinations on Days 2, 3, and 4. The number of animals per group which exhibited any one or more of the above listed signs is shown

below, along with the number of those affected which subsequently died or showed a complete recovery.

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
1	0(Control)	0/10	-	-
2	295	0/10	-	-
3	580	0/10	-	-
4	1180	0/10	-	-
5	2365	10/10	0/10	10/10
6	4730	8/10*	8/8	0/8

*The two animals not accounted for in the number affected were found dead 1-hour post treatment on Day 1.

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1, are presented in Table 9. Body weight data for the Group 6 mice were not available after Day 1 due to the early mortality which occurred.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
2	295	-2.2
3	580	-6.4
4	1180	-1.1
5	2365	-7.1
6	4730	-

*Relative to control (Group 1) value.

A reduction in mean body weight relative to the control value was noted for all treated groups at the end of the study. Statistical evaluation of the mean body weight data showed that the weights were comparable to the respective control value at all intervals with the following exceptions: a significantly lower weight at Day 12 for Group 3 and significantly lower weights at Days 12 and 16 for Group 5. The body weight changes for all treated groups were comparable to the control values at each interval and no significant differences were noted.

MTD Determination - Based on the lethality, body weight, and pharmacotoxic sign data observed, it was recommended that 2365 mg/kg/day be considered the MTD for ethylene glycol diethyl ether. No deaths or irreversible signs of toxicity were seen in any of the mice treated at this level; however a reduction in weight relative to the control group was observed. Following consultation with the sponsor, a dose level of 2955 mg/kg/day was selected for use in the reproductive phase.

Diethylene Glycol Monoethyl Ether (BRL No. 444)

Mortality - Mortality data of mice treated with diethylene glycol monoethyl ether is presented by study day in Table 8. No compound-related deaths were seen in any of the groups. One mouse from Group 7 (low dose) died on the second day of dosing; this death was attributed to dosing error.

Pharmacotoxic Signs - No clinical signs of toxicity were observed in any of the treated mice. All mice were grossly normal upon physical examination on Days 1, 8, 12, and 16.

Body Weights - Mean body weights (Day 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1, are presented in Table 9.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
7	335	-9.0
8	670	-2.6
9	1340	0.4
10	2685	-7.9
11	5365	-5.6

*Relative to control (Group 1) value.

A reduction in body weight relative to the control value was noted for Groups 7, 8, 10, and 11; however, the mid-dose group (Group 9) showed a slight relative increase in weight as compared to the controls. Statistical evaluation of the mean body weight data revealed significantly decreased weights for Groups 7 and 10 at Days 12 and 16. The Day 12 - Day 1 and Day 16 - Day 1 weight changes for these groups were also noted to be numerically lower than the control value, although not statistically significant. The mean body

weight data for Groups 8, 9, and 11 were not significantly different than the control values at any interval. The mean weights for these three groups, as well as the mean weight changes for Groups 8 and 11, were similar to the controls. A slight numerical increase in mean body weight changes was noted at each interval for Group 9 as compared to the control values.

MTD Determination - No MTD for diethylene glycol monoethyl ether was determined due to the variability seen in the body weight data and the absence of toxic effects at all dose levels. Following consultation with sponsor it was decided that further investigation of this chemical at higher dose levels was required to establish the MTD. The testing was performed concurrently with the third experimental block of chemicals (BLI Project #0107-C), and the results are discussed in a later section of this report.

Triethylene Glycol (BRL No. 443)

Mortality - Mortality data for mice treated with triethylene glycol is presented by study day in Table 8. One mouse from Group 13 (1500 mg/kg/day) died on Day 8. Although this death was classified as compound related, it was considered to be incidental in terms of treatment, as no deaths occurred in Groups 14, 15, or 16, which received higher concentrations of the chemical.

Pharmacotoxic Signs - No signs of toxicity were observed in any of the treated mice. All animals were grossly normal upon physical examination on Days 1, 8, 12, and 16.

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 9.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential* (%)
12	750	-4.1
13	1500	-3.7
14	3005	-1.5
15	6005	-6.4
16	12015	-6.0

*Relative to control (Group 1) value.

A reduction in body weight relative to the control value was noted for all treated groups at the end of the study. Statistical evaluation revealed significantly lower than control mean body weights for Group 12 at Day 8, Group 15 at Days 8, 12, and 16, and Group 16 at Days 8 and 12. No significant differences between control and treated group mean body weight changes were noted.

MTD Determination - Based on the results of this study, a dose level of 11270 mg/kg/day was recommended and accepted as the MTD of triethylene glycol for use in the reproductive phase. This dose level was based on a dosing volume of 10 mg/kg of the undiluted material, corrected for a reported specific gravity of 1.127.

EXPERIMENTAL METHODS - REPEAT REPRODUCTIVE PHASE
BLOCK II (BLI Project #0107-B)

A study evaluating the reproductive hazard potential of ethylene glycol diethyl ether and triethylene glycol was conducted from February 8 through February 26, 1982. Diethylene glycol monoethyl ether was not evaluated at this time due to the inability to select an appropriate dose level during the MTD phase. The results of the reproductive study were inconclusive due to the low percentage of mice which was pregnant upon receipt from the supplier. A repeat reproductive evaluation was subsequently conducted at the request of the sponsor. During the interim, the MTD for diethylene glycol monoethyl ether (5500 mg/kg) was determined; therefore, the three test chemicals in the second experimental block were simultaneously assessed in the repeat reproductive evaluation. A discussion of the methods and results from the repeat reproductive study follows; all data pertaining to the first reproductive study are retained in the archives at Borriston Laboratories, Inc.

Test Articles

The three chemicals to be evaluated for reproductive hazard potential were received from the sponsor on December 23, 1981. Information pertaining to the description, receipt, and storage conditions of the test articles is given on page 37 of this report.

Test Animals and Husbandry

A total of 237 female, specific pathogen free (SPF), CD-1 albino mice, timed-pregnant to arrive on Day 2 of gestation were received from Charles River Breeding Laboratories, Inc. (Lake View, New Jersey) on June 16, 1982 (bred June 14, 1982). The animals were examined upon receipt for general health and physical condition, and body weights were recorded (range of 21.7 to 31.8 g). This strain and the use of timed-pregnant mice were selected as the test system at the request of the sponsor.

The mice were housed individually in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Purina® Certified Rodent Chow® #5002 (Lot No. Apr 15 822C) and fresh water were available ad libitum. Fresh water bottles were supplied once weekly; cages were sanitized and bedding was changed once during the study (between Day 15 and Day 18 of

gestation). The mice were housed in a temperature controlled room (22±3°C) with a ventilation cycle of 10-15 room air changes per hour. A 12-hour light/dark illumination cycle was maintained.

Prior to study initiation the mice were quarantined for five days in the room in which the study was to be conducted; this shortened quarantine period was necessitated by the use of timed-pregnant mice. During quarantine, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

On Day 7 of gestation, two hundred (200) clinically acceptable mice were assigned to treatment groups using a computer-generated randomization program.

The allotment of animals to treatment groups was as follows:

Group No.	No. of Assumed Pregnant Females	Treatment	Dose Level* (mg/kg/day)	Color Code
1	50	Distilled water	-	White
2	50	Ethylene glycol diethyl ether	2955	Dark Blue
3	50	Diethylene glycol monoethyl ether	5500	Yellow
4	50	Triethylene glycol	11270	Red

*Dose levels were established based on the results of the MTD phase; the dose level for diethylene glycol monoethyl ether was determined and reported with the MTD phase of the third block of chemicals (Project #0107-C).

Each animal received a unique, six-digit, permanent number and was toe-clipped to reflect that number for identification purposes. The toe clip consisted of the last four digits of the permanent animal number; the first two digits (82), which indicated the year of study initiation, were omitted from the toe clip. The toe-clipping code used for this study is illustrated in Figure 2. The cage cards were color-coded and displayed the project number, individual animal number (six digits), treatment group, and dose level. The cages for each treatment group were arranged vertically on the cage rack.

Test Article Preparation and Administration

Ethylene glycol diethyl ether and diethylene glycol monoethyl ether were suspended in a distilled water vehicle at a concentration which provided the

proper amount of compound for the specified dose level. The appropriate amount of each chemical was dispensed via a pipette into a 250 ml volumetric flask. Distilled water was added quantity sufficient to 250 ml. The resulting mixtures were mixed by inversion, the volume checked, and additional distilled water added (to 250 ml) if necessary. The test article/vehicle admixtures were transferred to glass beakers and were mixed for approximately five minutes on a Corning® magnetic stirrer. Triethylene glycol was used in the undiluted form. During the study, the dosing materials were stored in the corresponding project room.

The test article/vehicle admixtures or undiluted test article were administered orally with a steel feeding needle, once daily, for eight consecutive days beginning on Day 7 of gestation. Oral presentation via intubation was selected as the route of administration at the request of the sponsor. Dosing was performed at approximately the same time each day. Each animal received an appropriate dose of the designated compound at a constant dosing volume of 10.0 ml/kg of body weight based on the weights measured on Day 7 of gestation. Mice in Group 1 received distilled water at a volume of 10.0 ml/kg of body weight. This group served as the common control for the three compounds being evaluated.

Observations

All animals were observed twice daily during the study (morning and afternoon) for clinical signs of toxicity and mortality. Body weights for the dams were recorded upon receipt (Day 2 of gestation), at study initiation (Day 7 of gestation), on Day 18 of gestation, and at termination (Day 4 postpartum). In addition, a terminal body weight was recorded on Day 23 of presumed gestation for females which did not produce litters. Body weight changes (Day 18 - Day 7) were calculated for each female. Physical examinations were performed at each body weight interval. Pup counts and litter weights were recorded within 12 hours of birth and on Day 3 postpartum. A per-pup average weight (mean pup weight) was calculated for each litter at birth and Day 3 by dividing the total litter weight by the number of live pups. Litter weight and mean pup weight changes (Day 3 - Birth) were also calculated, and the viability of offspring from birth to Day 3 was assessed.

Termination

All dams were sacrificed on Day 4 postpartum and females which failed to deliver were sacrificed on Day 23 of gestation by asphyxiation with carbon dioxide. Pups were either decapitated or killed by an overdose of ether on Day 4 postpartum. Necropsy examinations were performed only on females which did not deliver. The nongravid uteri were treated with a 10% sodium sulfide solution to determine the prior existence of a pregnant state. All carcasses were incinerated.

Statistical Analysis

For each treatment or control group, means and standard deviations were calculated for the following parameters: maternal body weights for each interval collected; litter weights, mean pup weights, and pup counts (live and dead) for each interval collected; weight changes for dams (Day 18 - Day 7 of gestation) and litters (Day 3 - Birth); and offspring viability ratios from birth to Day 3 postpartum. Treatment group means were compared to the common control group by Student's t-test[6]. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

The use of the word "significant" in this report, where groups are statistically compared, is to imply either no statistically significant difference or that a statistically significant difference is noted. The use of the word "similar" has no statistical connotation, but rather indicates that two groups have similar data sets.

[6]Snedecor, G.W. and Cochran, W.G., Statistical Methods. Iowa State University Press, Ames, Iowa 10:258-268, 1967.

RESULTS - REPEAT REPRODUCTIVE PHASE
BLOCK II (BLI Project #0107-B)

Maternal Mortality and Pharmacotoxic Signs

Mortality data from all groups is presented by study day in Table 10. Four mice treated with ethylene glycol diethyl ether (Group 2) died during the dosing period; two of these deaths were attributed to dosing technique. A fifth Group 2 animal was found dead during the morning mortality check on Day 20 of gestation (Study Day 14). Seven diethylene glycol monoethyl ether-treated (Group 3) mice died from compound-related causes during the dosing phase. No compound-related deaths occurred in mice treated with triethylene glycol (Group 4); however, two animals died as a result of dosing technique. No deaths were observed in the control group.

The incidence of pharmacotoxic signs observed during the study is summarized in Table 11. Clinical signs of toxicity (prostration, lethargy, circling behavior, rapid or labored breathing, loss of righting reflex, cold to the touch) were noted on Days 1 and 2 of dosing in Groups 2 and 3 (ethylene glycol diethyl ether and diethylene glycol monoethyl ether). No pharmacotoxic signs were observed in triethylene glycol-treated (Group 4) mice (except for Animal 827122 which was noted to have a roughened haircoat from Day 4 through Day 7) or the control group.

Maternal Body Weights

A summary of mean maternal body weights and weight changes at designated intervals during the study is presented in Table 13. Mean body weight data for females which did not produce litters are presented in Table 14.

The mean maternal body weights (Day 18 and terminal) and the mean weight change (Day 18 - Day 7) for all treated groups were significantly lower than the control values. These effects on gestational weight gain are attributed to treatment with the test chemicals. The mean body weights and mean weight changes of the non-litter bearing females from the treated groups were similar to the control values at all intervals except for a significantly lower than control Day 18 mean weight in Group 4.

Reproductive Performance and Maternal Behavior

Litters were born on Days 18 through 21 of gestation (July 2 through July 5, 1982). The percentage of litters delivered on each day is summarized below:

Group	Treatment	Litter Delivered (%) Gestation Day 18	19	20	21
1	Vehicle Control	14	67	19	0
2	Ethylene Glycol Diethyl Ether	0	0	50	50
3	Diethylene Glycol Monoethyl Ether	3	69	25	3
4	Triethylene Glycol	14	66	20	0

A slight delay in time to delivery was observed on mice treated with ethylene glycol diethyl ether (Group 2). Treatment with diethylene glycol monoethyl ether and triethylene glycol had no apparent effect on time to delivery.

Reproductive outcome is summarized in Table 12. Examination of mice that had not produced litters by Day 23 of gestation revealed corpora lutea in the uteri of 12 animals (incidences of 2, 1, 5, and 4 in Groups 1, 2, 3, and 4, respectively), thus indicating that fertilization had occurred without subsequent implantation. These mice were not included in the calculation of the delivery index (number of live litters produced/number of animals determined to be pregnant) since the implantation process would have occurred prior to the initiation of treatment.

A compound-related effect on reproductive outcome was observed in mice treated with ethylene glycol diethyl ether (Group 2). Of the 45 surviving females in this group, only 12 litters were produced (4 live litters and 8 dead litters). Examination of sodium sulfide-treated uteri from those Group 2 females which had not delivered by Day 23 of presumed gestation revealed resorption sites in 23 of the 33 animals examined. Resorption sites were also observed in Group 2 animal 826983; this animal was excluded from calculations due to its death on Day 20 of gestation. The 11% delivery index observed in mice treated with ethylene glycol diethyl ether was greatly reduced as compared to the 100% delivery index observed in the vehicle control group.

Treatment with diethylene glycol monoethyl ether and triethylene glycol (Groups 3 and 4) had no apparent adverse effects on reproductive outcome. The

delivery indices were noted to be 97% and 100%, respectively, and were, therefore, similar to that of the control group (100%). Resorption sites were observed in one Group 3 animal upon examination on Day 23 of gestation.

Evidence of cannibalization was seen with similar frequency in both treated and control groups.

Pup Counts, Litter Weights, and Offspring Viability

Pup counts (live and dead) and litter weights were recorded at birth and on Day 3 postpartum. Per-pup average weights, weight changes (from birth to Day 3), and offspring viability ratios (number alive at Day 3/number alive at birth) were calculated. Mean values are summarized in Table 13.

The mean total and mean live pup counts at birth and the mean live pup count at Day 3 for the ethylene glycol diethyl ether (Group 2) litters were much lower than the control group, while the mean number of dead pups for this group was higher than the control group at both intervals. In addition, the offspring viability ratio (0.45) was greatly decreased as compared to the control value (0.99). The mean pup weights at birth and Day 3, as well as the mean pup weight change, were noted to be lower than the control group weights. These effects on pup counts, pup weights, and offspring viability through the first three postpartum days are considered to be compound related. Meaningful statistical analyses of these data could not be conducted due to the small number of ethylene glycol diethyl ether-treated females which produced litters.

The mean pup counts observed in the diethylene glycol monoethyl ether and triethylene glycol-treated groups (Groups 3 and 4, respectively) were generally comparable to the control group; the statistically lower mean live pup counts seen at Day 3 in Group 3 and at birth and Day 3 in Group 4 are considered to be incidental. The offspring viability ratios from birth through Day 3 in Groups 3 and 4 were similar to that of the control group. Mean pup weights for Groups 3 and 4 were noted to be significantly lower than the control weights at birth; however, no differences in mean pup weights were seen at Day 3, and the mean pup weight changes (Day 3 - Birth) for both of these treated groups were similar to the control values. The significantly lower than control litter weights observed in Groups 3 and 4 at Day 3 were

judged to be incidental since the mean pup weights at Day 3 were not significantly lower than the control values.

Conclusions

The results obtained from the initial reproductive hazard evaluation of ethylene glycol diethyl ether and triethylene glycol were inconclusive due to the low percentage of mice which were pregnant upon receipt from the supplier. A repeat reproductive evaluation of these two chemicals was subsequently conducted; the reproductive hazard potential of diethylene glycol monoethyl ether was also assessed at this time. Based on the results of the repeated study, ethylene glycol diethyl ether was considered to be fetotoxic. The fetotoxicity was characterized by increased frequencies of dead and resorbed litters (88.6% of total pregnancies). In addition, offspring viability and pup weights were reduced. This chemical was also considered to be slightly toxic to maternal animals. Treatment with diethylene glycol monoethyl ether and triethylene glycol had no apparent adverse effects on reproductive outcome. Diethylene glycol monoethyl ether was noted to be slightly toxic to maternal animals.

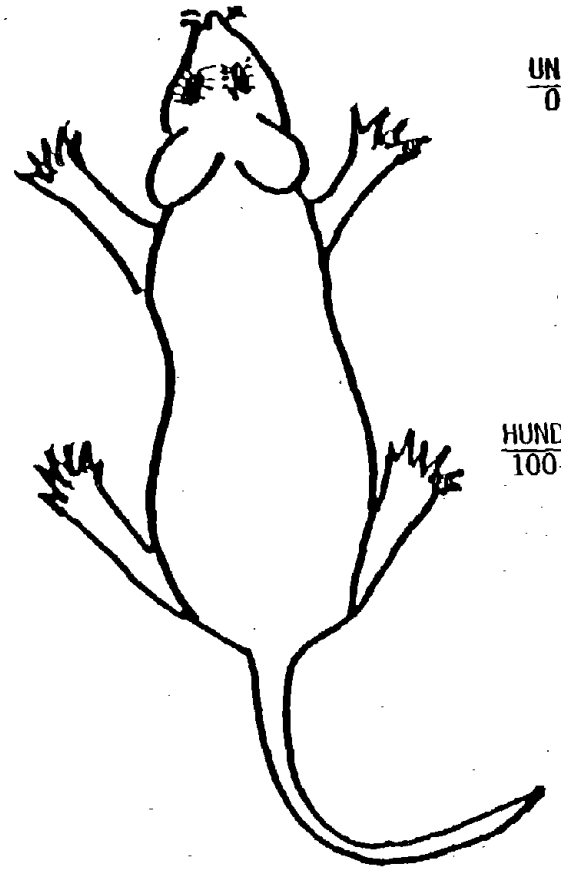
Raw Data and Final Report Storage

All raw data and the final report are retained in the archives at Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, Maryland 20748.

FIGURE 2

PERMANENT NUMBER IDENTIFICATION

BY TOE CLIPPING



0	IIII	5	ıIII
1	IIII	6	IıII
2	IıII	7	IIIı
3	IIıI	8	IıII
4	IIIı	9	IıIı

Toe-clipping code for each foot (digit 1 excluded)

TABLE 8

MORTALITY DATA FOR VEHICLE CONTROL (WATER)

SCREENING PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

		Dose Level (mg/kg/day)									
		0									
Number on Test:		10									
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-								
	2	-	-								
	3	-	-								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Observation Day	1	-	-								
	2	-	-								
	3	-	-								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Cumulative Deaths*		0	0								

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 8 (Continued)

MORTALITY DATA FOR ETHYLENE GLYCOL DIETHYL ETHER (BRL #445)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

		Dose Level (mg/kg/day)									
		295		580		1180		2365		4730	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	-	-	3	-
	2	-	-	-	1	-	-	-	-	5	-
	3	-	-	-	-	-	-	-	-	1	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	1	-	-	-	1	-
	6	-	-	-	-	-	-	-	-		
	7	-	-	-	-	-	-	-	-		
	8	-	-	-	-	-	-	-	-		
Observation Day	1	-	-	-	-	-	-	-	-		
	2	-	-	-	-	-	-	-	-		
	3	-	-	-	-	-	-	-	-		
	4	-	-	-	-	-	-	-	-		
	5	-	-	-	-	-	-	-	-		
	6	-	-	-	-	-	-	-	-		
	7	-	-	-	-	-	-	-	-		
	8	-	-	-	-	-	-	-	-		
Cumulative Deaths*		0	0	0	1	1	0	0	0	10	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 8 (Continued)

MORTALITY DATA FOR DIETHYLENE GLYCOL MONOETHYL ETHER (BRL #444)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

		Dose Level (mg/kg/day)									
		335		670		1340		2685		5365	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	1	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Observation Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Cumulative Deaths*		0	1	0	0	0	0	0	0	0	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 8 (Continued)

MORTALITY DATA FOR TRIETHYLENE GLYCOL (BRL #443)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

		Dose Level (mg/kg/day)									
		750		1500		3005		6005		12015	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	1	-	-	-	-	-	-	-
Observation Day	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-
Cumulative Deaths*		0	0	1	0	0	0	0	0	0	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 9

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

ETHYLENE GLYCOL DIETHYL ETHER (BRL #445)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	25.9	25.6	-0.4	26.3	0.4	26.7	0.8
	S.D.	2.2	1.6	3.2	1.3	3.1	1.4	3.1
	N	10	10	10	10	10	10	10
2 (295 mg/kg/day)	MEAN	24.9	24.5	-0.4	25.6	0.8	26.1	1.2
	S.D.	2.9	2.9	1.2	3.0	0.9	3.0	0.8
	N	10	10	10	10	10	10	10
3 (580 mg/kg/day	MEAN	24.0	23.8	-0.4	24.4^{s-}	0.5	25.0	0.8
	S.D.	2.4	2.3	0.6	2.3	0.5	2.7	0.8
	N	10	9	9	9	9	9	9
4 (1180 mg/kg/day)	MEAN	24.3	25.5	1.1	25.9	1.5	26.4	2.0
	S.D.	1.7	1.6	1.4	1.6	1.6	1.9	1.7
	N	10	9	9	9	9	9	9
5 (2365 mg/kg/day)	MEAN	24.2	23.8	-0.4	24.5^{s-}	0.3	24.8^{s-}	0.6
	S.D.	2.1	2.2	1.2	2.1	1.0	2.1	1.0
	N	10	10	10	10	10	10	10
6 (4730 mg/kg/day)	MEAN	25.9	-	-	-	-	-	-
	S.D.	2.0	-	-	-	-	-	-
	N	10	-	-	-	-	-	-

- Data not available due to death of all mice in group.

s- = Statistically significantly lower than control value as measured by a Student's t-test at $p<0.05$.

TABLE 9 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

DIETHYLENE GLYCOL MONOETHYL ETHER (BRL #444)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	25.9	25.6	-0.4	26.3	0.4	26.7	0.8
	S.D.	2.2	1.6	3.2	1.3	3.1	11.4	3.1
	N	10	10	10	10	10	10	10
7 (335 mg/kg/day)	MEAN	23.9	23.8	-0.3	24.0^{S-}	-0.1	24.3^{S-}	0.2
	S.D.	2.2	2.7	1.3	2.7	1.2	3.1	1.4
	N	10	9	9	9	9	9	9
8 (670 mg/kg/day)	MEAN	25.2	24.8	-0.4	25.3	0.0	26.0	0.8
	S.D.	2.3	2.4	0.7	2.2	0.9	3.0	1.3
	N	10	10	10	10	10	10	10
9 (1340 mg/kg/day)	MEAN	25.2	25.7	0.6	26.2	1.0	26.8	1.7
	S.D.	2.5	2.4	0.8	2.3	0.6	2.4	0.7
	N	10	10	10	10	10	10	10
10 (2685 mg/kg/day	MEAN	24.4	24.2	-0.2	24.3^{S-}	-0.2	24.6^{S-}	0.2
	S.D.	2.2	2.6	0.9	2.6	0.8	2.4	1.0
	N	10	10	10	10	10	10	10
11 (5365 mg/kg/day)	MEAN	24.2	24.2	0.0	24.5	0.3	25.2	1.0
	S.D.	2.3	2.6	0.6	2.5	0.6	2.6	0.7
	N	10	10	10	10	10	10	10

s- = Statistically significantly lower than control value as measured by a Student's t-test at $p<0.05$.

TABLE 9 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY COMPOUNDS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-B)

TRIETHYLENE GLYCOL (BRL #443)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	25.9	25.6	-0.4	26.3	0.4	26.7	0.8
	S.D.	2.2	1.6	3.2	1.3	3.1	1.4	3.1
	N	10	10	10	10	10	10	10
12 (750 mg/kg/day)	MEAN	24.7	23.7^{s-}	-1.0	24.8	0.1	25.6	0.9
	S.D.	2.2	2.2	0.8	2.2	0.5	2.0	0.6
	N	10	10	10	10	10	10	10
13 (1500 mg/kg/day	MEAN	25.3	24.1	-1.2	25.5	0.1	25.7	0.3
	S.D.	2.7	3.1	1.8	3.1	1.3	2.9	0.9
	N	10	10	10	9	9	9	9
14 (3005 mg/kg/day)	MEAN	25.5	24.9	-0.6	25.1	-0.3	26.3	0.8
	S.D.	1.2	1.5	1.1	1.8	1.4	2.4	2.0
	N	10	10	10	10	10	10	10
15 (6005 mg/kg/day)	MEAN	24.3	24.0^{s-}	-0.3	24.4^{s-}	0.2	25.0^{s-}	0.8
	S.D.	1.5	1.7	0.6	1.5	0.8	1.6	1.1
	N	10	10	10	10	10	10	10
16 (12015 mg/kg/day)	MEAN	24.0	23.9^{s-}	-0.1	24.7^{s-}	0.7	25.1	1.1
	S.D.	2.3	1.8	1.2	2.0	0.9	2.1	1.3
	N	10	10	10	10	10	10	10

s- = Statistically significantly lower than control value as measured by a Student's t-test at $p<0.05$.

TABLE 10

MORTALITY DATA

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE (Project #0107-B)

GROUP AND TREATMENT:		1 Vehicle Control		2 Ethylene Glycol Diethyl Ether 2955 mg/kg/day		3 Diethylene Glycol Monoethyl Ether 5500 mg/kg/day		4 Triethylene Glycol 11270 mg/kg/day	
Number on Test:		50		50		50		50	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Study Day	1[a]	-	-	-	-	3	-	-	-
	2[a]	-	-	1	-	4	-	-	-
	3[a]	-	-	-	-	-	-	-	2
	4[a]	-	-	-	-	-	-	-	-
	5[a]	-	-	-	2	-	-	-	-
	6[a]	-	-	-	-	-	-	-	-
	7[a]	-	-	1	-	-	-	-	-
	8[a]	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-
	14	-	-	1	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-
	19	-	-	-	-	-	-	-	-
Cumulative Deaths[b]		0	0	3	2	7	0	0	2

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.

[a] Indicates the days on which mice were dosed (Gestation Day 7 through 14).

[b] Represents total number of deaths prior to sacrifice.

TABLE 11

INCIDENCE OF PHARMACOTOXIC SIGNS

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE
(Project # 0107-B)

		INCIDENCE OF SIGN[a]							
	GROUP*:	1		2		3		4	
PHARMACOTOXIC SIGN	INTERVAL:	AM	PM	AM	PM	AM	PM	AM	PM
	Day 1[b]								
Lethargy					6/50		3/50		
Prostration					4/50		2/50		
Circling behavior					1/50				
Rapid breathing					4/50		2/50		
Loss of righting reflex					1/50				
	Day 2[b]								
Prostration				1/50		4/47	1/44		
Cold to the touch				1/50		3/47	1/44		
Rapid breathing				1/50					
Loss of righting reflex				1/50					
Opacity, both eyes						1/47			
	Day 3[b]								
None observed									
	Day 4[b]								
Rough hair coat, head									1/48
	Day 5[b]								
Rough hair coat, head								1/48	1/48
	Day 6[b]								
Rough hair coat, head								1/48	1/48
	Day 7[b]								
Rough hair coat, head								1/48	1/48
	Day 8[b]								
Prostration					1/46				
Labored breathing					1/46				
	Day 9[c]								
Lethargy				1/46					
Labored breathing				1/46					

[a]Incidence (numerator) given as number of mice per group in which each sign was observed at the specified interval; denominator indicates number of mice per group which were alive at the beginning of the interval.

[b]Indicates the days on which mice were dosed (Gestation Days 7 through 14).

[c]No signs were observed in any groups after Day 9.

*Group 1, Vehicle Control; Group 2, Ethylene Glycol Diethyl Ether, 2955 mg/kg/day; Group 3, Diethylene Glycol Monoethyl Ether, 5500 mg/kg/day; Triethylene Glycol, 11270 mg/kg/day.

TABLE 12

SUMMARY OF REPRODUCTIVE OUTCOME

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE (Project #0107-B)

	GROUP AND TREATMENT			
	1 Vehicle Control	2 Ethylene Glycol Diethyl Ether 2955 mg/kg/day	3 Diethylene Glycol Monoethyl Ether 5500 mg/kg/day	4 Triethylene Glycol 11270 mg/kg/day
Number Treated	50	50	50	50
Number of Deaths	0	5	7	2
Number of Survivors:				
Nonpregnant	6	9	5	8
Pregnant	42	35	33	36
Fertilized without Subsequent Implantation[a]	2	1	5	4
Number of Litters:				
Live Litters	42	4	32	36
Dead Litters	0	8	0	0
Resorbed Litters	0	23[c]	1	0
Delivery Index[b]:				
Ratio	42/42	4/35	32/33	36/36
Percent	100	11	97	100

[a]Determined by the presence of corpora lutea upon examination of uteri at necropsy.

[b]Delivery Index = Number of live litters produced/Total number pregnant; denominator excludes mice that were fertilized without subsequent implantation.

[c]Resorption sites also observed in Animal 826983 which died on Day 20 of gestation; this animal was excluded from calculations.

TABLE 13

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS, AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE (Project #0107-B)

		GROUP AND TREATMENT			
		1 Vehicle Control	2[b] Ethylene Glycol Diethyl Ether 2955 mg/kg/day	3 Diethylene Glycol Monoethyl Ether 5500 mg/kg/day	4 Triethylene Glycol 11270 mg/kg/day
Maternal Body Weights (g)					
Gestation Day 2	MEAN	26.8	26.0	26.3	26.5
	S.D.	1.7	1.7	1.7	1.6
	N	42	12	32	36
Gestation Day 7	MEAN	29.2	29.5	29.3	28.9
	S.D.	2.0	2.5	1.9	1.8
	N	42	12	32	36
Gestation Day 18	MEAN	48.6	39.5^{s-}	44.4^{s-}	45.7^{s-}
	S.D.	4.2	4.2	5.0	4.4
	N	41	12	32	35
Weight Change (Day 18-7)	MEAN	19.5	10.0^{s-}	15.1^{s-}	16.8^{s-}
	S.D.	3.1	4.4	4.4	3.7
	N	41	12	32	35
Terminal (Day 4 Postpartum)	MEAN	36.8	29.4^{s-}	35.1^{s-}	35.3^{s-}
	S.D.	3.2	2.6	2.8	2.2
	N	42	12	32	36
Litter Weights (g)					
Birth	MEAN	15.7	2.7	14.1^{s-}	13.7^{s-}
	S.D.	3.1	2.7	2.6	3.0
	N	42	4	32	35
Day 3 Postpartum	MEAN	24.6	5.2	21.9^{s-}	22.0^{s-}
	S.D.	2.7	1.3	1.8	4.0
	N	42	2	32	35
Height Change (Day 3-Birth)	MEAN	8.9	1.3.	7.8^{s-}	8.4
	S.D.	2.2	1.3	1.8	1.7
	N	42	2	32	35

[a]Mean maternal body weights are based on surviving females which delivered litters; mean litter and pup weights are based on litters with live pups.

[b]Statistical evaluation of the Group 2 litter and pup data was precluded due to the smallness of the sample size.

s- = Statistically significantly lower than control value as measured by a Student's t-test of $p<0.05$.

TABLE 13 (Continued)

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS, AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE (Project #0107-B)

		GROUP AND TREATMENT			
		1 Vehicle Control	2[b] Ethylene Glycol Diethyl Ether 2955 mg/kg/day	3 Diethylene Glycol Monoethyl Ether 5500 mg/kg/day	4 Triethylene Glycol 11270 mg/kg/day
Maternal Body Weights[c] (gm)					
Birth	MEAN	1.6	1.3	1.5[S-]	1.5[S-]
	S.D.	0.2	0.1	0.2	0.1
	N	42	4	32	35
Day 3 Postpartum	MEAN	2.5	1.9	2.4	2.4
	S.D.	0.4	0.5	0.3	0.3
	N	42	2	32	35
Weight Change	MEAN	0.9	0.6	0.9	1.0
	S.D.	0.3	0.4	0.2	0.3
	N	42	2	32	35
Pup Counts					
Birth: Live	MEAN	10	1	10	9[S-]
	S.D.	2	1	2	2
	N	42	12	32	36
Dead	MEAN	0	2	0	0
	S.D.	0	2	0	0
	N	42	12	32	36
Total	MEAN	10	3	10	10
	S.D.	2	2	2	2
	N	42	12	32	36
Day 3 Postpartum Live	MEAN	10	1	9[S-]	9[S-]
	S.D.	2	2	2	2
	N	42	4	32	36
Dead	MEAN	0	1	0	0
	S.D.	0	1	1	0
	N	42	4	32	36
Offspring Viability Ratio[d]	MEAN	0.99	0.45	0.98	0.99
	S.D.	0.02	0.53	0.06	0.03
	N	42	4	32	36

[a]Mean maternal body weights are based on surviving females which delivered litters; mean litter and pup weights are based on litters with live pups.

[b]Statistical evaluation of the Group 2 litter and pup data was precluded due to the smallness of the sample size.

TABLE 14

MEAN BODY WEIGHTS - NON LITTER BEARING FEMALES[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPEAT REPRODUCTIVE PHASE (Project #0107-B)

GROUP TREATMENT		BODY WEIGHTS DAY 2 (g)	DAY 7 (g)	DAY 18 (g)	WT. CHANGE DAY (18-7) (g)	TERMINAL (g)
1	MEAN	26.5	28.2	28.4	0.2	28.1
Vehicle Control	S.D.	2.8	1.8	1.6	0.8	1.6
	N	8	8	8	8	8
2	MEAN	26.6	29.0	29.5	0.3	27.6
Ethylene Glycol Diethyl Ether	S.D.	1.3	2.3	3.6	3.7	2.4
2955 mg/kd/day	N	38	38	34	34	33
3	MEAN	26.3	27.9	25.1	-2.3	27.2
Diethylene Glycol Monoethyl Ether	S.D.	2.1	3.0	6.9	5.9	2.4
5500 mg/kg/day	N	18	18	11	11	11
4	MEAN	25.2	27.2	26.5[S-]	-0.3	26.8
Triethylene Glycol	S.D.	2.1	2.1	1.9	1.0	2.2
11270 mg/kg/day	N	14	14	12	12	12

[a]Mean body weights are based on all surviving females which did not produce litters.

EXPERIMENTAL METHODS - MTD PHASE
BLOCK III (BLI Project #0107-C)

Test Articles

Samples of the four chemicals to be evaluated in this study were received from the sponsor; information pertaining to the receipt and identification of the test articles is given below:

Chemical Name	BRL #	Physical Description	Container Description	Date Received	Amount Received
Diethylene glycol monoethyl ether	444	Clear liquid	Glass bottle	12-23-81	500 g
Aniline	456	Clear liquid	Glass bottle	2-08-82	850 ml
p-Nitroaniline	455	Yellow crystals	Glass bottle	2-04-82	300 g
N,N-Dimethylaniline	454	Yellow crystals	Glass bottle	2-04-82	400 ml

The samples were stored at room temperature in the containers in which they were received. All data which characterize the test articles with respect to identity, strength, purity, composition, and stability under conditions of use are retained by the sponsor. For dosing purposes, the purity of each compound was assumed to be 100%. The vehicle was distilled water for diethylene glycol monoethyl ether and corn oil for aniline, p-nitroaniline, and N,N-dimethylaniline. All unused compounds remaining at the end of the MTD phase were retained under appropriate storage for use in the reproductive screen.

Test Animals and Husbandry

A total of 259 virgin female, specific pathogen free (SPF), CD-1 albino mice were obtained from Charles River Breeding Laboratories, Inc. (Lake View, New Jersey) on February 10, 1982 for use in this study. The mice were 56 days of age (birth date - December 16, 1981) at the time of receipt. Upon receipt, each animal was examined for general physical condition and body weights were measured (range of 21.8 to 29.6 g). This strain of mouse was selected as the test system at the request of the sponsor; females were used because only females were to be exposed in the subsequent reproductive phase of the study.

The mice were housed, five per cage, in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Cages were sanitized and fresh bedding was supplied once during the study. Purina® Certified Rodent Chow® #5002 (Lot No. Dec 4 812) and fresh water were available ad libitum. The mice were maintained on a 12-hour light/dark cycle in a temperature controlled room (22±3°C) with 10-15 room air changes per hour.

Prior to study initiation, the mice were quarantined for five days in the room in which the study was to be conducted. During this period, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

Based on the observations conducted during quarantine, 200 clinically acceptable mice were randomly assigned to treatment groups, using a computerized random number generator and assignment program. The allotment of animals to treatment groups was as follows:

Group Number	No. of Animals	Treatment	Dose Level (mg/kg/day)	Color Code
1	10	Vehicle Control (Water)	-	White
2	10	Vehicle Control (Corn Oil)	-	White w/red stripe
3	10	Diethylene Glycol	6000	Dark Blue
4	10	Monoethyl Ether	8000	Yellow
5	10	(BRL 444)	10270	Red
6	10	Aniline	140	Orange
7	10	(BRL 456)	280	Light Green
8	10		560	Gray
9	10		1120	Light Blue
10	10		2235	Gold
11	10	p-Nitroaniline	205	Ivory
12	10	(BRL 455)	415	Pink
13	10		830	Green
14	10		1660	Copper
15	10		3315	Dark blue w/white stripe

Group Nunber	No of Animals	Treatment	Dose Level (mg/kg/day)	Color Code
16	10	N,N-Dimethylaniline	365	Yellow w/white stripe
17	10	(BRL 454)	725	Red w/white stripe
18	10		1455	Orange w/white stripe
19	10		2910	Light green w/white stripe
20	10		5815	Gray w/white stripe

Each animal received a unique six-digit permanent identification number and toe clipping was performed for identification purposes. The toe clip consisted of the last three digits of the permanent animal number. The remaining digits were omitted from the toe clip, since the first two digits (82) indicated the year of study initiation, and the third digit (6) was the same for all animals on study. The toe clipping code used in this study is illustrated in Figure 3. In addition, each cage of five mice was marked with a color-coded card on which the corresponding project number, individual animal numbers (six digits), treatment group, and dosage level were printed.

Test Article Preparation and Administration

Diethylene Glycol Monoethyl Ether - For Group 3 and 4, appropriate amounts of the test article were weighed out into a 50 ml volumetric flask on a Mettler H33AR® pan balance (accurate to 0.1 mg). Distilled water was added, quantity sufficient, to 50 ml. The resulting test article/vehicle admixtures were mixed by repeated inversion of the capped flask and then stirred on a Corning® magnetic stirrer for 5 to 10 minutes. After mixing, the solutions were transferred to glass beakers. The undiluted test article was used for Group 5 (10270 mg/kg).

The test article/vehicle admixtures or undiluted test article were administered orally by gavage, once daily, for eight consecutive days. Each animal in Groups 3, 4, and 5 received the appropriate dose level of the compound at a constant dosing volume of 10.0 ml/kg of body weight. Mice in Group 1 received only distilled water at a constant volume of 10.0 ml/kg of body weight and served as the control group for this chemical.

Aniline, p-Nitroaniline, and N,N-Dimethylaniline - For Groups 6 through 19, the correct amount of compound appropriate for each dose level was weighed out into a volumetric flask on a Mettler H33AR® pan balance. Corn oil was added, quantity sufficient, to attain the appropriate volume. The resulting

test article/vehicle admixtures were mixed by repeated inversion, transferred to glass beakers, and stirred on a Corning® magnetic stirrer for 5 to 10 minutes. The undiluted test article was used for Group 20 (5815 mg/kg N,N-dimethylaniline).

The article/vehicle admixtures or undiluted test article were administered orally by gavage, once daily, for eight consecutive days. Each animal in Groups 6 through 20 received the appropriate dose level of the designated compound at a constant dosing volume of 5.0 ml/kg of body weight. Mice in Group 2 received only corn oil at a constant volume of 5.0 ml/kg of body weight and served as the vehicle control group for Groups 6 through 20.

The dosing mixtures for all four chemicals were prepared just prior to study initiation and were stored in the corresponding project room in glass beakers throughout the study. Those portions remaining at the end of the treatment period were retained for possible future analysis.

Oral presentation via intubation was selected as the route of administration at the request of the sponsor.

Observations

During the dosing period (Days 1 through 8), animals were observed twice daily for signs of toxicity. The first observation was conducted approximately one hour after administration of the test article, and the second, at least five hours later. In addition, mortality checks were performed once in the morning (prior to dosing) and again in the afternoon. All animals which succumbed during the dosing phase were opened and examined in order to ascertain if death was due to the toxicity of the test article or due to dosing technique. The following criteria were used in determining dosing error deaths: 1) compound in the thoracic cavity; 2) compound in the lungs; and/or 3) a hole in the esophagus. All other deaths were assumed to be compound related. Observations for signs of toxicity and mortality were performed twice daily (morning and afternoon) on Days 9 through 15 and once (morning) on Day 16 prior to terminal sacrifice. Body weights were measured at study initiation (Day 1), on the last day of dosing (Day 8), on the fourth day after dosing (Day 12), and at termination (Day 16). Body weight changes were calculated for each animal for each interval beyond the initial (Day 1)

weight; i.e., $(x_t - x_i)$ where x_i is the initial weight and x_t is the weight at time t. In addition, physical examinations were performed at each body weight interval.

Termination

All surviving mice were sacrificed by carbon dioxide asphyxiation following the collection of terminal body weights on Day 16 of the study. No necropsy examinations were performed and the carcasses were incinerated.

Statistical Analysis

For each treatment group, means and standard deviations were calculated for all body weight data (Days 1, 8, 12, 16, and weight changes for each interval beyond the Day 1 weight). Multiple t-tests[1] were performed and are provided only as a means of highlighting weight differences. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

The two factors used in estimating the MTD were compound-related mortality and body weight. The following formula was utilized for calculating the weight differential between each treated group and the control group and provided an aid for estimating the MTD:

$$0\% > \left(\frac{\text{Final T BW} - \text{Final C BW}}{\text{Final C BW}} \times 100\right) > -10\%$$

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

[1]Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods, 6th Edition Iowa State University Press, Ames, Iowa 10:258-268, 1967.

RESULTS - MTD PHASE

BLOCK III (BLI Project #0107-C)

Diethylene Glycol Monoethyl Ether (BRL No. 444)

Mortality - Mortality data of mice treated with diethylene glycol monoethyl ether are presented by study day in Table 15. The cumulative mortality which occurred during the study and the percentage of death attributed to technical errors made during dosing procedures are summarized below:

Group	Dose Level mg/kg/day	Total Mortality (%)	Dosing Error Mortality (%)
1	0 (Control)	10	10
3	6000	50	0
4	8000	100	0
5	10270	100	0

All mice at the two highest dose levels (Groups 4 and 5) were dead by Day 2 of dosing; these deaths were attributed to the toxicity of the test article.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment were recorded with respect to nature, onset, and duration of each. Lethargy, wheezing, and an unkempt appearance were the most frequently noted signs for the Group 3 mice. Prostration was observed in one animal from this group at the one-hour observation on Day 2; this animal was found dead prior to dosing on Day 3. Lethargy was the most frequently noted sign in Group 4, with eight of ten mice exhibiting this sign at the one-hour postdose observation on Day 1. Prostration, tremors, and/or ataxia were infrequently noted. All mice in this group were dead prior to dosing on Day 3. Prostration or lethargy was observed in all Group 5 mice at the one-hour postdose observation and in six of ten mice at the five-hour observation on Day 1. All mice in this group were dead prior to dosing on Day 3.

The number of animals per group exhibiting one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery:

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
1	0 (Control)	0/10	-	-
3	6000	6/10	3/6	3/6
4	8000	10/10	10/10	0/10
5	10270	10/10	10/10	0/10

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 16. Body weight data for Groups 4 and 5 (8000 and 10270 mg/kg/day) were not available after Day 1 due to the early mortality which occurred in these groups.

A net loss in body weight was noted for the Group 3 (6000 mg/kg) mice at the end of the study period. The mean weight change (Day 16-Day 1) was numerically, although not significantly, lower than the control value. The weight differential (% change in mean weight) between Group 3 and the control group at Day 16 was -6.4%.

MTD Determination - The results of this study were evaluated in conjunction with those obtained from the earlier testing performed with this chemical (Project #0107-B). High mortality rates were observed at dose levels of 6000 mg/kg and greater; therefore, a dose level of 5365 mg/kg (the high dose in the initial MTD phase) was the recommended MTD for this chemical. Following consultation with the sponsor, a dose level of 5500 mg/kg was selected for use in the reproductive screen. At this time, the decision was made to repeat the reproductive phase for the entire second block of chemicals (0107-B); therefore, this test compound was evaluated for reproductive hazard potential in the repeat study, rather than with the third block of chemicals (0107-C).

Aniline (BRL No. 456)

Mortality - Mortality data of mice treated with aniline are presented by study day in Table 15. The cumulative mortality which occurred during the

study and the percentage of death attributed to technical errors made during dosing procedures are summarized below:

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
2	0 (Control)	0	0
6	140	20	0
7	280	20	10
8	560	10	0
9	1120	100	0
10	2235	100	0

Mortality reached 100% in Groups 9 and 10 by Days 3 and 1, respectively; these deaths were attributed to the administration of the test article.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment were recorded with respect to nature, onset, and duration of each. No signs of toxicity were observed in Groups 6 and 7 (140 and 280 mg/kg, respectively) with the exception of a Group 7 animal that was paralyzed prior to death due to an injury during dosing. Lethargy was occasionally observed after dosing in mice from Group 8, and an unkempt appearance was intermittently observed in most Group 8 mice throughout the dosing phase. Tremors, prostration, squinted eyes, and a hunched and thin appearance were also noted in one animal. All mice in Group 9 exhibited convulsions within two minutes of dosing on Day 1 which persisted for approximately 15 minutes. All mice were lethargic (incidence of 9 out of 10) or prostrate (1 of 10) at the one-hour postdose interval. Lethargy was still present at the five-hour interval and was accompanied by tremors and lacrimation. Disorientation (characterized by slow, wandering movements around cage with apparent loss of bearings), unkempt appearance, prostration, tremors, and/or rapid breathing were seen in those mice which survived beyond Day 1 of dosing. All Group 9 mice were dead prior to dosing on Day 4. Mice in Group 10 exhibited convulsions within two minutes of dosing on Day 1, and death occurred in 7 of 10 animals within five minutes of dosing. Prostration was observed in the three remaining mice at the one-hour postdose interval on Day 1 and two of these three mice were found dead four hours later. The remaining Group 10 mouse exhibited prostration, tremors, lacrimation, and rapid breathing, and died prior to dosing on Day 2.

The number of animals per group exhibiting one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery:

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
2	0 (Control)	0/10	-	-
6	140	0/10	-	-
7	280	1/10	1/1	0/1
8	560	9/10[a]	0/9	9/9
9	1120	10/10	10/10	0/10
10	2235	10/10	10/10	0/10

[a]The animal not accounted for in the number affected was found dead at the one-hour postdose observation interval on Day 1.

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 16. Body weight data for Groups 9 and 10 were not available after Day 1 due to the early death of these animals.

The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential[a] (%)
6	140	1.5
7	280	-1.5
8	560	-5.7
9	1120	-
10	2235	-

[a]Relative to control (Group 2) value

A slight increase in weight relative to the control group was noted for Group 6, while Group 7 showed a slight reduction. A 5.7% reduction in weight (relative to the control group) was noted for Group 8, and the mean body weights at Days 12 and 16, as well as the mean weight changes at all intervals for this group, were significantly lower than the control values.

MTD Determination - No compound-related deaths occurred at a dose level of 140 mg/kg/day. Compound-related mortality (10%) was observed at the 280 and 560 mg/kg dose levels; therefore, a dose level of 250 mg/kg was recommended as the MTD for aniline. Following consultation with the sponsor, a dose level of 560 mg/kg was selected for use in the reproductive screen.

p-Nitroaniline (BRL No. 455)

The dosing of Groups 11 through 15 was postponed for a two-day period due to the difficulty encountered in getting the chemical into solution. Groups 1 through 14 were initiated on February 17, 1981, upon resolution of this problem. A suitable test article/vehicle admixture could not be attained at a concentration of 3315 mg/kg; therefore, Group 15 was omitted from the study at the request of the sponsor.

Mortality - Mortality data of mice treated with p-nitroaniline are presented by study day in Table 15. Deaths were observed only at the high dose, where 60% of the mice died from compound-related causes. The cumulative mortality is summarized below:

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
2	0 (Control)	0	0
11	205	0	0
12	415	0	0
13	830	0	0
14	1660	60	0

Pharmacotoxic Signs - Unkempt appearance was the only clinical sign noted for the Group 11 mice and was observed in all mice from this group at the one-hour postdose interval on Day 8. Lethargy, tremors, and an unkempt appearance were observed frequently and prostration was observed occasionally throughout the eight-day dosing period in mice from Group 12 and 13. All mice from Groups 12 and 13 recovered and remained normal throughout the eight-day post-treatment observation phase. Prostration, tremors, lethargy, and an unkempt appearance were observed frequently in all Group 14 mice during dosing. One animal from this group was also noted to have opaque eyes during the last three days of dosing and throughout the subsequent observation period. The eyes of this animal were also squinted on Days 6, and 7, and throughout the

subsequent observation period. Six of ten mice from this group died during the treatment period; those which survived continued to exhibit an unkempt appearance, lethargy, and/or opaque eyes throughout the remainder of the study.

The number of animals per group which exhibited one or more of the above-described signs is shown below, along with the number of those affected which subsequently died or showed a complete recovery:

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
2	0 (Control)	0/10	-	-
11	205	10/10	0/10	10/10
12	415	10/10	0/10	10/10
13	830	10/10	0/10	10/10
14	1660	10/10	6/10	4/10*

*One animal was observed to have opaque, squinted eyes throughout the study.

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 16. The weight differentials (% change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential[a] (%)
11	205	0.4
12	415	-0.4
13	830	-0.4
14	1660	-1.1

[a]Relative to control (Group 2) value.

The relative differences between the treated and control group weights at the end of the study were slight (less than 1% in all but Group 14), but a general dose-related trend was noted.

MTD Determination - Compound-related mortality occurred only in the high dose group. Based on the body weight data, a dose of 800 mg/kg/day was recommended as the MTD for p-nitroaniline. Following consultation with the sponsor, a dose level of 1200 mg/kg was selected for use in the reproductive screen.

N,N-Dimethylaniline (BRL No. 454)

Mortality - Mortality data of mice treated with N,N-dimethylaniline are presented by study day in Table 15. The cumulative mortality which occurred during the study and the percentage of deaths attributed to technical errors made during dosing procedures are summarized below:

Group	Dose Level (mg/kg/day)	Total Mortality (%)	Dosing Error Mortality (%)
2	0 (Control)	0	0
16	365	40	10
17	725	70	0
18	1455	100	0
19	2910	100	0
20	5815	100	0

A high mortality rate attributable to the administration of the test article was seen in all but the low-dose group. Mortality reached 100% in Groups 18, 19, and 20 by Days 4, 2, and 1, respectively.

Pharmacotoxic Signs - Pharmacotoxic signs observed during the experiment were recorded with respect to nature, onset, and duration of each. Lethargy was noted for all surviving mice from Groups 16 at the one-hour postdose observation on Day 2 of dosing and prostration or an unkempt appearance was occasionally observed in this group during the remainder of the dosing phase. Four mice died during dosing; one of these deaths resulted from dosing error rather than compound toxicity. The mice which survived the dosing phase exhibited no treatment-related clinical signs during the remainder of the study. Lethargy, tremors, and/or unkempt appearance were observed frequently in mice from Group 17 on Days 2 through 8 of dosing. Seven animals died from compound-related causes during this period. The remaining three mice were observed to be unkempt on the first day following dosing (Day 9), but showed no toxic signs during Days 10 through 16. Mice from Group 18 exhibited the first sign of toxicity (lethargy) at the five-hour postdose interval on Day 2 of dosing. Lethargy or prostration persisted until the time of death; all

mice died by Day 4 of dosing. Mice in Groups 19 and 20 exhibited prostration, lethargy, ataxia (Group 19 only), and/or tremors (observed in a single Group 20 animal) prior to death. All mice in Groups 19 and 20 were dead by Day 2 and Day 1 of dosing, respectively.

The number of animals per group exhibiting one or more of the above-described signs is shown below along with the number of those affected which subsequently died or showed a complete recovery:

Group	Dose Level (mg/kg/day)	Number Affected	Subsequent Deaths	Subsequent Recoveries
2	0 (Control)	0/10	-	-
16	365	9/10[a]	3/9[a]	6/9
17	725	9/10[b]	6/9	3/9
18	1455	10/10	10/10	0/10
19	2910	9/10[b]	9/9	0/9
20	5815	6/10[b]	6/6	0/6

[a]The animal not accounted for in the number affected was found dead at the one-hour postdose interval on Day 1 due to dosing error.

[b]The mice not accounted for in the number affected were compound-related deaths; however death was not preceded by any of the toxic signs discussed above.

Body Weights - Mean body weights (Days 1, 8, 12, and 16) and mean body weight changes for each interval beyond Day 1 are presented in Table 16. Body weight data for Groups 18, 19, and 20 were not available due to the mortality which occurred in these groups.

The weight differentials (change in mean weight) between each treated group and the control group at Day 16 are shown below:

Group	Dose Level (mg/kg/day)	Weight Differential[a] (%)
16	365	-0.4
17	725	-0.8
18	1455	-
19	2910	-
20	5815	-

[a]Relative to control (Group 2) value.

Both Groups 16 and 17 showed only minimal reductions in weight relative to the controls (less than 1%). Statistical evaluation of the mean body weight data at Days 1, 8, 12 and 16 revealed no significant differences between either treated group and the control group. The Group 17 mean weight changes were noted to be significantly lower than the control values at all intervals; however, interpretation of these data is complicated by the mortality which occurred in this group.

MTD Determination - Compound-related mortality ranging from 30% to 100% was observed in all treated groups. Since the low dose (365 mg/kg/day) resulted in three compound-related deaths, a dose slightly below this (350 mg/kg/day) was recommended as the MTD for N,N-dimethylaniline. Following consultation with the sponsor, a dose of 365 mg/kg was selected for use in the reproductive screen.

EXPERIMENTAL METHODS - REPRODUCTIVE PHASE
BLOCK III (BLI Project #0107-C)

Test Articles

The three chemicals for reproductive evaluation were received from the sponsor on February 4 and 8, 1982 (prior to initiation of the MTD phase); the portion of each compound remaining at the end of the MTD phase was retained under appropriate storage conditions for use in the reproductive screen. Information pertaining to the description, receipt, and storage conditions of the test articles is given on page 69 of this report.

Test Animals and Husbandry

A total of 259 timed-pregnant female, specific pathogen free (SPF), CD-1 albino mice arrived at Day 2 of gestation from Charles River Breeding Laboratories, Inc. (Lake View, New Jersey), on May 12, 1982. The animals were examined upon receipt for general health and physical condition, and body weights were recorded (range 22.8 to 32.5 grams). This strain and the use of timed-pregnant mice were selected as the test system at the request of the sponsor.

The mice were housed individually in suspended polycarbonate cages with San-i-cel® bedding (L.F. Klein, Baltimore, Maryland). Purina® Certified Rodent Chow® #5002 (Lot No. Jan. 7 822J) and fresh water were available ad libitum. Fresh water bottles were supplied once weekly; cages were sanitized and bedding was changed once during the study (between Day 15 and Day 18 of gestation). The mice were housed in a temperature controlled room (22±3°C)[4] with 10-15 room air changes per hour. A 12-hour light/dark illumination cycle was maintained[5].

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[4]A temperature of 27°C was reported during the morning mortality check on May 17, 1982 and was corrected. This temperature deviation is not considered to have affected the outcome of the study.

[5]During the delivery phase of the study (May 28 through June 2, 1982), it was necessary to turn the lights on during the evening in order to weigh the litters within 12 hours of birth as specified in the protocol.

Prior to study initiation the mice were quarantined for five days in the room in which the study was to be conducted; this shortened quarantine period was necessitated by the use of timed-pregnant mice. During quarantine, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups

On Day 7 of gestation, 200 clinically acceptable mice were assigned to treatment groups using a computer-generated randomization program. The body weights of mice assigned to the study ranged from 21.0 to 30.0 g. The allotment of animals to treatment groups was as follows:

Group No.	No of Assumed Pregnant Females	Treatment	Dose Level* (mg/kg/day)	Color Code
1	50	Corn Oil	-	White
2	50	Aniline	560	Dk. Blue
3	50	p-Nitroaniline	1200	Yellow
4	50	N,N-Dimethylaniline	365	Red

*Dose levels were established based on the results of the MTD Phase.

Each animal received a unique, six-digit, permanent animal number and was toe clipped to reflect that number for identification purposes. The toe clip consisted of the last three digits of the permanent animal number. The remaining digits were omitted from the toe clip, since the first two digits (82) indicated the year of study initiation, and the third digit (6) was the same for all animals on study. The toe clipping code used for this study is illustrated in Figure 3. The cage cards were color-coded and displayed the project number, individual animal number (six digits), treatment group and dose level. The cages for each treatment group were arranged vertically on the cage rack.

Test Article Preparation and Administration

Each test article was suspended in a corn oil vehicle at a concentration which provided the proper amount of compound for the selected dose level. The appropriate amount of each compound was measured out and placed in a 100 ml flask. Corn oil was added, quantity sufficient, to 100 ml. The resulting admixtures were mixed by inversion, the volumes checked and additional corn

oil added (to 100 ml), if necessary. The suspensions were then mixed on a Corning® magnetic stirrer for approximately five minutes. The dosing suspensions were prepared prior to study initiation (Day 7 of gestation) and were used for eight consecutive days (Day 7 through Day 14 of gestation). During the study, the dosing mixtures were stored at room temperature.

The test article suspensions were administered orally with a steel feeding needle, once daily for eight consecutive days beginning on Day 7 of gestation. Oral presentation via intubation was selected as the route of administration at the request of the sponsor. Dosing was performed at approximately the same time each day. Each animal received an appropriate dose of the designated compound at a constant dosing volume of 5.0 ml/kg of body weight based on the weights measured on Day 7 of gestation. Mice in Group 1 received corn oil at a volume of 5.0 mg/kg of body weight. This group served as the common control for the three compounds being evaluated.

Observations

All animals were observed twice daily during the study (morning and afternoon) for clinical signs of toxicity and mortality. Body weights for the dams were recorded upon receipt (Day 2 of gestation), at study initiation (Day 7 of gestation), on Day 18 of gestation, and at termination (Day 4 postpartum). In addition, a terminal body weight was recorded on Day 23 of presumed gestation for females which did not produce litters. Body weight changes (Day 18-Day 7) were calculated for each female. Physical examinations were performed at each body weight interval. Pup counts and litter weights were recorded within 12 hours of birth and on Day 3 postpartum. An average weight per pup (mean pup weight) was calculated for each litter at birth and Day 3 by dividing the total litter weight by the number of live pups. Litter weight and mean pup weight changes (Day 3-Birth) were also calculated, and the viability of offspring from birth to Day 3 was assessed.

Termination

All dams were sacrificed on Day 4 postpartum and females which failed to deliver were sacrificed on Day 23 of gestation by asphyxiation with carbon dioxide. Pups were either decapitated or killed by an overdose of ether on Day 4 postpartum. Necropsy examinations were performed only on females which did not deliver. The nongravid uteri were treated with a 10% sodium sulfide solution to determine the prior existence of a pregnant state. All carcasses were incinerated.

Statistical Analysis

For each treatment or control group, means and standard deviations were calculated for the following parameters: maternal body weights for each interval collected; litter weights, mean pup weights, and pup counts (live and dead) for each interval collected; weight changes for dams (Day 18-Day 7 of gestation) and litters (Day 3-Birth); and offspring viability ratios from birth to Day 3 postpartum. Treatment group means were compared to the common control group by Student's t-test[6]. A notation of s+ or s- in the tables of this report indicates that the mean value is statistically higher (s+) or lower (s-) than the respective control value at $p<0.05$.

The use of the word "significant" in this report, where groups are statistically compared, is to imply either no statistically significant difference or that a statistically significant difference is noted. The use of the word "similar" has no statistical connotation, but rather indicates that two groups have similar data sets.

[6]Snedecor, G.W. and Cochran, W.G., Statistical Methods, Iowa State University Press, Ames, Iowa 10:258-268, 1967.

RESULTS - REPRODUCTIVE PHASE
BLOCK III (BLI Project #0107-C)

Maternal Mortality and Pharmacotoxic Signs

Mortality data for all groups are presented by study day in Table 17. A total of 21 mice (42%) treated with p-nitroaniline (Group 3) died from compound-related causes. Compound-related deaths occurred in six mice from the aniline-treated group (Group 2) and two mice from the N,N-dimethylaniline-treated group (Group 4); an additional N,N-dimethylaniline-treated animal (826851) was sacrificed *in extremis* on Day 18 of gestation (Study Day 12); this animal was noted to be lethargic, prostrate, cold to the touch and had mucoid secretion from the vagina prior to sacrifice. No deaths occurred in the vehicle control group (Group 1).

The incidence of pharmacotoxic signs observed during the study is summarized in Table 18. Compound-related signs of toxicity were seen most frequently in mice treated with p-nitroaniline. Animals from this group exhibited acute toxic signs following dosing on Day 1; all mice were prostrate and approximately one-half of the mice had convulsions during this time. Prostration and/or tremors were consistently noted in the p-nitroaniline group throughout the remainder of the dosing phase (Study Days 1 through 8) with the percentage of animals affected ranging from 27% to 53%. Ataxia, difficult breathing, and yellow discoloration of the body surfaces were infrequently reported. Those animals which survived the dosing phase remained essentially normal throughout the post-treatment phase of the study. Prostration, tremors, ataxia, lethargy, piloerection, vaginal secretion, and/or anal bleeding were observed in an occasional aniline or N,N-dimethylaniline-treated animal prior to death.

Maternal Body Weights

A summary of mean maternal body weights and weight changes at designated intervals during the study is presented in Table 20. Mean body weights for females which did not produce litters are presented in Table 21.

The mean body weights (Day 18 and Terminal) and the mean weight change (Day 18-Day 7) for the p-nitroaniline-treated females which delivered litters

were significantly lower than the control values. This effect on body weight gain was attributed to treatment with p-nitroaniline.

The mean maternal body weights for the aniline-treated (Group 2) females were statistically comparable to the control values at all intervals; however, a significantly lower than control mean weight change was noted. No differences in mean maternal body weight or mean weight change were observed between the N,N-dimethylaniline-treated (Group 4) females and the control group.

The mean body weights and body weight changes for the treated females which did not produce litters were generally similar to the control values at all intervals.

Reproductive Performance and Maternal Behavior

Litters were born on Days 18 through 23 of gestation (May 28 through June 2, 1982). The percentage of litters per group delivered on each day is summarized below:

Group	Treatment	Litters Delivered (%) Gestation Day 18	19	20	21	22	23
1	Vehicle Control	17	61	17	2	2	0
2	Aniline	4	32	60	4	0	0
3	p-Nitroaniline	0	7	53	7	20	13
4	N,N-Dimethylaniline	3	67	28	3	0	0

A slight delay in time to delivery was noted for the p-nitroaniline-treated group (Group 3), in which only 60% of the litters was delivered by Day 20 of gestation as opposed to 95% in the vehicle control group. Treatment with aniline and N,N-dimethylaniline had no apparent effect on time to delivery.

Reproductive outcome is summarized in Table 19. Examination of mice that had not produced litters by Day 23 of gestation revealed corpora lutea in the uteri of 18 animals (incidences of 8, 7, and 3 in Groups 2, 3, and 4, respectively), thus indicating that fertilization had occurred without subsequent implantation. These mice were not included in the calculation of the delivery index (number of live litters produced/number of animals determined to be pregnant) since the implantation process would have occurred prior to the initiation of treatment.

A compound-related effect on reproductive outcome was observed in females treated with p-nitroaniline (Group 3), as evidenced by the reduced delivery index observed for this group (69% as opposed to 100% in the vehicle control group). Of the 29 females which survived the dosing period, only 15 litters were produced; 11 live litters and 4 dead litters. Ectromelia (all limbs) was observed in the pups delivered by Group 3 female 826788. Examination of sodium sulfide treated uteri from those Group 3 females which had not delivered litters by Day 23 of presumed gestation revealed resorption sites in one of the 14 animals examined.

Treatment with aniline and N,N-dimethylaniline (Groups 2 and 4) had no apparent adverse effects on reproductive outcome. The delivery indices were noted to be 100% and 97%, respectively, and were, therefore similar to that of the control group (100%).

Evidence of apparent cannibalization of offspring by the dams was observed more frequently in the treated groups than in the control group. The highest incidence of this behavior was observed in Group 3 (p-nitroaniline) where 47% (7/15) of the litters were cannibalized. The percentage of litters cannibalized in Group 2 and 4 was 20% (5/25) and 14% (5/36), respectively; evidence of this behavior was also seen in 5% (2/41) of the control litters.

Pup Counts, Litter Weights, and Offspring Viability

Pup counts (live and dead) and litter weights were recorded at birth and on Day 3 postpartum. Per-pup average weights, weight changes (from birth to Day 3), and offspring viability ratios (number alive at Day 3/number alive at birth) were calculated. Mean values are summarized in Table 20.

The mean total and mean live pup counts at birth and the mean live pup count at Day 3 for the p-nitroaniline-treated group (Group 3) were significantly lower than the control group while the mean number of dead pups for this group was significantly higher than the control group at both intervals. In addition, the offspring viability ratio was significantly decreased as compared to the control value. These effects on pup counts and offspring viability through the first three postpartum days are considered to be related to treatment with the test chemical. The mean litter weights, as well as the mean weight change of the Group 3 offspring, were significantly lower than the control values. This was apparently due to the lower number of pups per

litter in Group 3, as the mean pup weights and weight change (per-pup average weights) in Group 3 were statistically comparable to the control group.

The mean live and total pup counts for the aniline-treated litters (Group 2) were statistically comparable to the control group at both intervals. A significantly increased number of dead pups was noted in Group 2 at Day 3 postpartum and the offspring viability ratio through the first three postpartum days was significantly lower than that of the control group. Treatment with aniline also adversely affected birth weight and weight gain of the F_1 generation, as the mean pup weights and mean pup weight change were significantly lower than the control values.

Treatment with N,N-dimethylaniline had no adverse effects on postnatal reproductive outcome. No significant differences between the Group 4 and control group mean pup counts were noted at birth or on Day 3. The Group 4 offspring viability ratio was significantly lower than the control group; however, this difference was slight and is considered to be incidental. Statistical evaluation of mean litter and per-pup average weights revealed no noteworthy variations between the Group 4 and control group data.

Conclusions

Based on the results of this study, gestational treatment (Days 7 through 14) of timed-pregnant CD-1 mice with p-nitroaniline adversely affected maternal survival, gestational weight gain, the ability of the dams to produce viable litters, and survival of the F_1 generation through the first three postpartum days. Treatment with aniline had no apparent effect on the number of live litters produced; however, offspring viability through the first three postpartum days was significantly lower than that of the control group. In addition, reductions in birth weight and weight gain were seen in the aniline-treated litters. Treatment with N,N-dimethylaniline had no adverse effects on survivial or weight gain of the dams, or birth weight, weight gain or viability of the F_1 generation through the first three postpartum days.

Raw Data and Final Report Storage

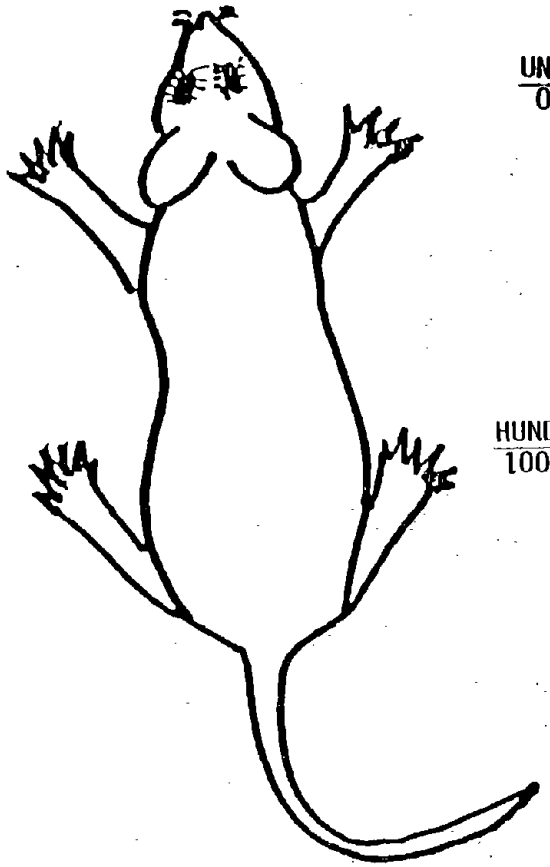
All raw data and the final report are retained in the archives at Borriston Laboratories, Inc., 5050 Beech Place, Temple Hills, Maryland 20748.

FIGURE 3

PERMANENT NUMBER IDENTIFICATION

BY TOE CLIPPING

TENS
10-90



UNITS
0-9

HUNDREDS
100-900

0	IIII	5	ıIII
1	IIII	6	IıII
2	IıII	7	IIIı
3	IIıI	8	IıIı
4	IIIı	9	IıIı

Toe-clipping code for each foot (digit 1 excluded)

TABLE 15

MORTALITY DATA FOR DIETHYLENE GLYCOL MONOETHYL ETHER (BRL #444)**

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (PROJECT #0107-C)

		Dose Level (mg/kg/day)									
		0 (Control)		6000		8000		10270			
Number on Test:		10		10		10		10			
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	1	-	7	-	8	-		
	2	-	-	1	-	3	-	2	-		
	3	-	-	-	-						
	4	-	-	-	-						
	5	-	-	-	-						
	6	-	1	-	-						
	7	-	-	-	-						
	8	-	-	-	-						
Observation Day	1	-	-	1	-						
	2	-	-	1	-						
	3	-	-	-	-						
	4	-	-	-	-						
	5	-	-	-	-						
	6	-	-	-	-						
	7	-	-	1	-						
	8	-	-	-	-						
Cumulative Deaths*		0	1	5	0	10	0				

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.

*Indicates total number of deaths prior to terminal sacrifice.

**Due to the absence of toxic effects in the initial MTD phase (Project #0107-B), these higher dose levels of diethylene glycol monoethyl ether were evaluated concurrently with the third block of chemicals (Project #0107-C); a separate vehicle control group (Group 1) was used.

TABLE 15 (Continued)

MORTALITY DATA FOR VEHICLE CONTROL (CORN OIL)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (PROJECT #0107-C)

		Dose Level (mg/kg/day)									
		0									
Number on Test:		10									
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-								
	2	-	-								
	3	-	-								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Observation Day	1	-	-								
	2	-	-								
	3	-	-								
	4	-	-								
	5	-	-								
	6	-	-								
	7	-	-								
	8	-	-								
Cumulative Deaths*		0	0								

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 15 (Continued)

MORTALITY DATA FOR ANILINE (BRL #456)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (PROJECT #0107-C)

		Dose Level (mg/kg/day)									
		140		280		560		1120		2235	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	1	-	4	-	10	-
	2	-	-	-	-	-	-	3	-		
	3	1	-	-	1	-	-	3	-		
	4	1	-	1	-	-	-				
	5	-	-	-	-	-	-				
	6	-	-	-	-	-	-				
	7	-	-	-	-	-	-				
	8	-	-	-	-	-	-				
Observation Day	1	-	-	-	-	-	-				
	2	-	-	-	-	-	-				
	3	-	-	-	-	-	-				
	4	-	-	-	-	-	-				
	5	-	-	-	-	-	-				
	6	-	-	-	-	-	-				
	7	-	-	-	-	-	-				
	8	-	-	-	-	-	-				
Cumulative Deaths*		2	0	1	1	1	0	10	0	10	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 15 (Continued)

MORTALITY DATA FOR p-NITROANILINE (BRL #455)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (PROJECT #0107-C)

		Dose Level (mg/kg/day)									
		205		415		830		1660			
Number on Test:		10		10		10		10			
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	1	-		
	2	-	-	-	-	-	-	2	-		
	3	-	-	-	-	-	-	1	-		
	4	-	-	-	-	-	-	-	-		
	5	-	-	-	-	-	-	1	-		
	6	-	-	-	-	-	-	-	-		
	7	-	-	-	-	-	-	1	-		
	8	-	-	-	-	-	-	-	-		
Observation Day	1	-	-	-	-	-	-	-	-		
	2	-	-	-	-	-	-	-	-		
	3	-	-	-	-	-	-	-	-		
	4	-	-	-	-	-	-	-	-		
	5	-	-	-	-	-	-	-	-		
	6	-	-	-	-	-	-	-	-		
	7	-	-	-	-	-	-	-	-		
	8	-	-	-	-	-	-	-	-		
Cumulative Deaths*		0	0	0	0	0	0	6	0		

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 15 (Continued)

MORTALITY DATA FOR N,N-DIMETHYLANILINE (BRL #454)

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (PROJECT #0107-C)

		Dose Level (mg/kg/day)									
		365		725		1455		2910		5815	
Number on Test:		10		10		10		10		10	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Treatment Day	1	-	-	-	-	-	-	3	-	10	-
	2	2	1	3	-	6	-	7	-		
	3	1	-	-	-	3	-				
	4	-	-	1	-	1	-				
	5	-	-	1	-						
	6	-	-	1	-						
	7	-	-	1	-						
	8	-	-	-	-						
Observation Day	1	-	-	-	-						
	2	-	-	-	-						
	3	-	-	-	-						
	4	-	-	-	-						
	5	-	-	-	-						
	6	-	-	-	-						
	7	-	-	-	-						
	8	-	-	-	-						
Cumulative Deaths*		3	1	7	0	10	0	10	0	10	0

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.

*Indicates total number of deaths prior to terminal sacrifice.

TABLE 16

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-C)

DIETHYLENE GLYCOL MONOETHYL ETHER* (BRL #444)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
1 (0 mg/kg/day)	MEAN	25.9	24.8	-1.1	25.6	-0.3	26.4	0.4
	S.D.	1.5	1.4	0.7	1.5	0.7	1.7	1.0
	N	10	9	9	9	9	9	9
3 (6000 mg/kg/day)	MEAN	26.9	25.5	-1.6	25.9	-1.4	24.7	-2.1
	S.D.	1.6	2.4	2.0	1.3	2.5	3.7	3.7
	N	10	8	8	6	6	5	5
4 (8000 mg/kg/day)	MEAN	26.2	-	-	-	-	-	-
	S.D.	1.1	-	-	-	-	-	-
	N	10	-	-	-	-	-	-
5 (10270 mg/kg/day)	MEAN	26.1	-	-	-	-	-	-
	S.D.	1.1	-	-	-	-	-	-
	N	10	-	-	-	-	-	-

*Due to the absence of toxic effects in the initial MTD phase (Project #0107-B), these higher dose levels of diethylene glycol monoethyl ether were evaluated concurrently with the third block of chemicals (Project #0107-C); a separate vehicle control group (Group 1) was used.

TABLE 16 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-C)

ANILINE (BRL #456)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
2 (0 mg/kg/day)	MEAN	25.5	24.5	-1.0	25.7	0.2	26.4	0.9
	S.D.	0.9	0.9	0.4	1.0	0.6	1.0	0.7
	N	10	10	10	10	10	10	10
6 (140 mg/kg/day)	MEAN	26.1	25.9	-0.3^{s+}	26.2	0.0	26.8	0.6
	S.D.	1.4	1.9	0.7	2.0	0.9	1.4	0.9
	N	10	8	8	8	8	8	8
7 (280 mg/kg/day)	MEAN	25.3	24.9	-0.1	25.3	0.4	26.0	1.0
	S.D.	1.6	1.4	1.4	1.3	1.5	1.2	1.6
	N	10	8	8	8	8	8	8
8 (560 mg/kg/day)	MEAN	25.9	23.3	-2.6^{s-}	23.3^{s-}	-2.7^{s-}	24.9^{s-}	-1.1^{s-}
	S.D.	1.0	2.1	2.2	3.3	3.7	1.2	1.1
	N	10	9	9	9	9	9	9
9 (1120 mg/kg/day)	MEAN	25.6	-	-	-	-	-	-
	S.D.	1.4	-	-	-	-	-	-
	N	10	-	-	-	-	-	-
10 (2235 mg/kg/day)	MEAN	26.3	-	-	-	-	-	-
	S.D.	1.3	-	-	-	-	-	-
	N	10	-	-	-	-	-	-

- Data not available due to death of all mice in group.

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 16 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-C)

p-NITROANILINE (BRL #455)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
2 (0 mg/kg/day)	MEAN	25.5	24.5	-1.0	25.7	0.2	26.4	0.9
	S.D.	0.9	0.9	0.7	1.0	0.6	1.0	0.7
	10	10	10	10	10	10	10	10
11 (205 mg/kg/day)	MEAN	26.6	25.7^{s+}	-1.0	22.9^{s-}	-3.8^{s-}	26.5	-0.2^{s-}
	S.D.	1.6	1.2	1.2	2.9	3.1	1.7	0.7
	N	10	10	10	10	10	10	10
12 (415 mg/kg/day)	MEAN	26.7	25.8^{s+}	-0.9	26.1	-0.6^{s-}	26.3	-0.4^{s-}
	S.D.	1.6	1.1	0.9	1.0	0.8	1.4	0.9
	N	10	10	10	10	10	10	10
13 (830 mg/kg/day)	MEAN	25.8	25.8^{s+}	0.0^{s+}	25.6	-0.2	26.3	0.5
	S.D.	0.6	1.5	1.3	0.7	0.5	0.9	0.6
	N	10	10	10	10	10	10	10
14 (1660 mg/kg/day)	MEAN	26.1	23.0	-3.6^{s-}	24.3	-2.5^{s-}	26.1	-1.0^{s-}
	S.D.	1.4	2.5	3.6	3.2	3.7	1.8	2.2
	N	10	4	4	4	4	4	4

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 16 (Continued)

MEAN BODY WEIGHTS WITH MEAN BODY WEIGHT CHANGES

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - MTD PHASE (Project #0107-C)

N,N-DIMETHYLANILINE (BRL #454)

Group No. (Dose Level)		Body Weight (g) Interval						
		Day 1	Day 8	Change (Day 8-Day 1)	Day 12	Change (Day 12-Day 1)	Day 16	Change (Day 16-Day 1)
2	MEAN	25.5	24.5	-1.0	25.7	0.2	26.4	0.9
(0 mg/kg/day)	S.D.	0.9	0.9	0.7	1.0	0.6	1.0	0.7
	N	10	10	10	10	10	10	10
16	MEAN	25.9	25.9	0.1^{s+}	26.2	0.4	26.3	0.5
(365 mg/kg/day)	S.D.	1.2	1.8	1.4	1.7	0.8	1.5	1.0
	N	10	6	6	6	6	6	6
17	MEAN	26.4	24.0	-3.8^{s-}	25.3	-2.5^{s-}	26.2	-1.6^{s-}
(725 mg/kg/day)	S.D.	1.4	1.4	1.0	0.7	0.5	1.0	0.7
	N	10	3	3	3	3	3	3
18	MEAN	26.8^{s+}	-	-	-	-	-	-
(1455 mg/kg/day)	S.D.	1.4	-	-	-	-	-	-
	N	10	-	-	-	-	-	-
19	MEAN	25.5	-	-	-	-	-	-
(2910 mg/kg/day)	S.D.	1.3	-	-	-	-	-	-
	N	10	-	-	-	-	-	-
20	MEAN	26.6	-	-	-	-	-	-
(5815 mg/kg/day	S.D.	1.4	-	-	-	-	-	-
	N	10	-	-	-	-	-	-

- Data not available due to death of all mice in group.

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 17

MORTALITY DATA

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

GROUP AND TREATMENT:		1 Vehicle Control		2 Aniline 560 mg/kg/day		3 p-Nitroaniline 1200 mg/kg/day		4 N,N-Dimethylaniline 365 mg/kg/day	
Number on Test:		50		50		50		50	
Cause of Death		Compound	Treatment	Compound	Treatment	Compound	Treatment	Compound	Treatment
Study Day	1[a]	-	-	-	-	4	-	-	-
	2[a]	-	-	-	-	-	-	-	-
	3[a]	-	-	-	-	-	-	-	-
	4[a]	-	-	-	-	1	-	-	-
	5[a]	-	-	-	-	5	-	-	-
	6[a]	-	-	1	-	7	-	-	-
	7[a]	-	-	-	-	1	-	-	-
	8[a]	-	-	4	-	3	-	-	-
	9	-	-	1	-	-	-	1	-
	10	-	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	1	-
	12	-	-	-	-	-	-	1[c]	-
	13	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-	-
	19	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	-
	21	-	-	-	-	-	-	-	-
Cumulative Deaths[b]		0	0	6	0	21	0	3	0

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.

[a] Indicates the days on which mice were dosed (Gestation Day 7 through 14).
[b] Represents total number of deaths prior to terminal sacrifice.
[c] Animal #826851 was sacrificed in extremis.

TABLE 18

INCIDENCE OF PHARMACOTOXIC SIGNS

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

PHARMACOTOXIC SIGNS	GROUP AND TREATMENT:	INCIDENCE OF SIGN[a]							
		1 Vehicle Control		2 Aniline 560 mg/kg/day		3 p-Nitroaniline 1200 mg/kg/day		4 N,N-Dimethylaniline 365 mg/kg/day	
	INTERVAL:	AM	PM	AM	PM	AM	PM	AM	PM
Day 1[b]									
Swelling, right forepaw		1/50	1/50						
Prostrate						c	8/50		
Convulsions						c			
Ataxia							7/50		
Yellow discoloration							13/50		
Tremors							1/50		
Difficult breathing							1/50		
Day 2[b]									
Swelling, right forepaw		1/50	1/50						
Prostrate						15/46[d]	1/46		
Tremors						11/46[d]			
Yellow discoloration							1/46		
Day 3[b]									
Swelling, right forepaw		1/50	1/50						
Prostrate						21/46[d]	1/46		
Tremors						16/46[d]			
Yellow discoloration							1/46		
Day 4[b]									
Swelling, right forepaw		1/50							
Prostrate						19/46[d]	3/46		
Tremors						14/46[d]	3/46		
Yellow discoloration							3/46		

[a]Incidence (numerator) given as number per group in which each sign was observed at the specified interval; denominator indicates the number of mice which were alive at the beginning of the interval.

[b]Indicates the days on which mice were dosed (Gestation Days 7 through 14).

[c]All Group 3 mice were prostrate and approximately one-half were convulsing following dosing on Day 1; all were normal at AM (pre-dose) observation interval on Day 1.

[d]Signs were observed following dosing; mice were normal at the AM (pre-dose) observation.

TABLE 18 (Continued)

INCIDENCE OF PHARMACOTOXIC SIGNS

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

PHARMACOTOXIC SIGNS	GROUP AND TREATMENT:	INCIDENCE OF SIGN[a]							
		1 Vehicle Control		2 Aniline 560 mg/kg/day		3 p-Nitroaniline 1200 mg/kg/day		4 N,N-Dimethylaniline 365 mg/kg/day	
	INTERVAL:	AM	PM	AM	PM	AM	PM	AM	PM
Day 5[b]									
Prostrate						24/45[d]	15/44		
Tremors						22/45[d]	14/44		
Day 6[b]									
Prostrate					1/50	20/40	8/38		
Tremors						20/40	12/38		
Difficult breathing							1/38		
Day 7[b]									
Prostrate						9/33	4/33		
Tremors						9/33	8/33		
Day 8[b]									
Prostrate				1/49	3/47	10/32	1/32		
Bleeding/bloody, anus				1/49	1/47				
Ataxia					1/47				
Lethargy					1/47				
Tremors					3/47	9/32	1/32		
Yellow discoloration						1/32			
Day 9									
Piloerection				1/45					
Thin				1/45					
Prostrate				1/45	1/45				
Tremors				1/45	1/45				
Day 10									
None observed									

[a]Incidence (numerator) given as number per group in which each sign was observed at the specified interval; denominator indicates the number of mice which were alive at the beginning of the interval.

[b]Indicates the days on which mice were dosed (Gestation Days 7 through 14).

[d]Signs were observed following dosing; mice were normal at the AM (pre-dose) observation.

TABLE 18 (Continued)

INCIDENCE OF PHARMACOTOXIC SIGNS

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

PHARMACOTOXIC SIGNS	GROUP AND TREATMENT:	INCIDENCE OF SIGN[a]							
		1 Vehicle Control		2 Aniline 560 mg/kg/day		3 p-Nitroaniline 1200 mg/kg/day		4 N,N-Dimethylaniline 365 mg/kg/day	
	INTERVAL:	AM	PM	AM	PM	AM	PM	AM	PM
Day 11									
Prostrate								1/49	
Cold to the touch								1/49	
Day 12[b]									
Prostrate								1/48[c]	
Lethargy								1/48[c]	
Cold to the touch								1/48[c]	
Mucoid secretion, vagina								1/48[c]	

[a] Incidence (numerator) given as number per group in which each sign was observed at the specified interval; denominator indicates the number of mice which were alive at the beginning of the interval.

[b] No pharmacotoxic signs were observed in any of the surviving mice after Study Day 12.

[c] Sign was observed in Animal 826851, which was sacrificed in extremis on Day 12.

TABLE 19

SUMMARY OF REPRODUCTIVE OUTCOME

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

	GROUP AND TREATMENT			
	1 Vehicle Control	2 Aniline 560 mg/kg/day	3 p-Nitroaniline 1200 mg/kg/day	4 N,N-Dimethylaniline 365 mg/kg/day
Number Treated	50	50	50	50
Number of Deaths	0	6	21	3
Number of Survivors:				
Nonpregnant	9	11	6	7
Pregnant	41	25	16	37
Fertilized without Subsequent Implantation[a]	0	8	7	3
Number of Litters:				
Live Litters	41	25	11	36
Dead Litters	0	0	4	1[c]
Resorbed Litters	0	0	1	0
Delivery Index[b]:				
Ratio	41/41	25/25	11/16	36/37
Percent	100	100	69	97

[a]Determined by the presence of corpora lutea upon examination of uteri at necropsy.

[b]Delivery Index = Number of live litters produced/Total number pregnant; denominator excludes mice that were fertilized without subsequent implantation.

[c]Litter was not delivered; 9 dead fetuses were observed in the uterus of this animal upon examination on Day 23 of gestation.

TABLE 20

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

		GROUP AND TREATMENT			
		1 Vehicle Control	2 Aniline 560 mg/kg/day	3 p-Nitroaniline 1200 mg/kg/day	4 N,N-Dimethylaniline 365 mg/kg/day
MATERNAL BODY WEIGHTS (g)					
Gestation Day 2	MEAN	26.8	26.9	26.7	26.6
	S.D.	1.6	1.4	1.6	1.6
	N	41	25	15	36
Gestation Day 7	MEAN	29.0	29.2	29.0	28.5
	S.D.	1.6	1.7	1.5	1.7
	N	41	25	15	36
Gestation Day 18	MEAN	47.7	45.0	38.8^{s-}	48.0
	S.D.	6.3	3.8	4.2	4.5
	N	41	25	15	36
Weight Change (Day 18 - 7)	MEAN	18.7	15.8^{s-}	9.8^{s-}	19.5
	S.D.	5.8	3.8	4.3	3.9
	N	41	25	15	36
Terminal (Day 4 Postpartum)	MEAN	37.5	36.3	32.0^{s-}	36.5
	S.D.	3.5	3.1	2.1	2.7
	N	41	25	15	35
LITTER WEIGHTS (g)					
Birth	MEAN	14.7	15.1	7.5^{s-}	13.9
	S.D.	4.5	3.3	4.9	4.1
	N	41	25	11	36
Day 3 Postpartum	MEAN	23.9	22.4	11.0^{s-}	22.2
	S.D.	6.5	4.4	3.3	5.9
	N	41	25	9	35
Weight Change (Day 3 - Birth)	MEAN	9.2	7.3	3.6^{s-}	8.3
	S.D.	2.7	4.4	3.3	2.4
	N	41	25	9	35

[a]Mean maternal body weights are based on all surviving mice which produced litters; mean litter and pup weights are based on litters with live pups.

s = Statistically significantly higher (s+) or lower (s-) than control value as measured by a Student's t-test at $p<0.05$.

TABLE 20 (Continued)

SUMMARY OF MEAN MATERNAL BODY WEIGHTS, LITTER WEIGHTS, PUP COUNTS AND OFFSPRING VIABILITY DATA[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

		GROUP AND TREATMENT			
		1 Vehicle Control	2 Aniline 560 mg/kg/day	3 p-Nitroaniline 1200 mg/kg/day	4 N,N-Dimethylaniline 365 mg/kg/day
MEAN PUP WEIGHTS[b] (g)					
Birth	MEAN	1.6	1.5^{S-}	1.5	1.7^{S+}
	S.D.	0.2	0.1	0.2	0.2
	N	41	25	11	36
Day 3 Postpartum	MEAN	2.7	2.3^{S-}	2.5	2.7
	S.D.	0.5	0.3	0.4	7
	N	41	25	9	35
Weight Change (Day 3 - Birth)	MEAN	1.1	0.8^{S-}	0.8	1.1
	S.D.	0.4	0.2	0.3	0.3
	N	41	25	9	35
PUP COUNTS (per litter)					
Birth					
Live	MEAN	9	10	4^{S-}	9
	S.D.	3	2	4	3
	N	41	25	15	36
Dead	MEAN	0	0	1^{S+}	0
	S.D.	1	0	1	1
	N	41	25	15	36
Total	MEAN	9	10	4^{S-}	9
	S.D.	3	2	4	3
	N	41	25	15	36
Day 3 Postpartum					
Live	MEAN	9	10	4^{S-}	8
	S.D.	3	3	4	3
	N	41	25	11	36
Dead	MEAN	0	1^{S+}	1^{S+}	0
	S.D.	0	2	2	0
	N	41	25	11	36
Offspring Viability Ratio[c]	MEAN	1.00	0.94^{S-}	0.78^{S-}	0.98^{S-}
	S.D.	0.02	0.16	0.35	0.04
	N	41	25	11	36

[a]Mean maternal body weights are based on all surviving mice which produced litters; mean litter weights are based on litters with live pups.

[b]Mean Pup Weight = Litter weight/Number of live pups.

[c]Offspring Viability Ratio = Number of live pups on Day 3/Number of live pups at Birth.

TABLE 21

MEAN BODY WEIGHTS - NON LITTER BEARING FEMALES[a]

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS - REPRODUCTIVE PHASE (Project #0107-C)

GROUP TREATMENT		BODY WEIGHTS DAY 2 (g)	DAY 7 (g)	DAY 18 (g)	WT. CHANGE DAY (18-7) (g)	TERMINAL (g)
1	MEAN	26.9	28.0	27.1	-0.9	28.1
Vehicle Control	S.D.	1.8	2.0	3.1	1.8	2.3
	N	9	9	9	9	9
2	MEAN	26.9	28.3	26.3	-1.5	28.3
Aniline	S.D.	1.6	1.8	2.5	2.8	1.7
560 mg/kg/day	N	25	25	19	19	19
3	MEAN	26.8	28.4	29.7	1.7[s+]	29.1
p-Nitroaniline	S.D.	2.0	2.4	3.4	3.1	1.8
1200 mg/kg/day	N	35	35	14	14	14
4	MEAN	26.9	28.3	29.1	0.8	29.8
N,N-Dimethylaniline	S.D.	1.5	1.9	3.0	2.6	2.4
365 mg/kg/day	N	14	14	12	12	11

[a]Mean body weights are based on all surviving females which did not produce litters.

s+ = Statistically significantly higher than control value as measured by a Student's t-test at $p<0.05$.