ENVIRONMENTAL HEALTH RESEARCH & TESTING, INC.

FINAL REPORT

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS

MONOETHANOLAMINE (CAS NO. 141-43-5)
DIETHANOLAMINE (CAS NO. 111-42-2)
TRIETHANOLAMINE (CAS NO. 102-71-6)

Contract No.: 200-84-2735
EHRT's Project No.: ETOX-85-1002

Submitted to:
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QUALITY ASSURANCE

FINAL REPORT STATEMENT

PROJECT NUMBER ETOX-85-1002 was inspected on the following dates: 3/15/85, 4/12/85, 8/2/85, 8/21/85, and 12/3-12/16/86 by the Quality Assurance Unit, according to EHRT's Standard Operating Procedures and Good Laboratory Practice Regulations. All findings were reported to the Study Director and Management on the following dates: 5/10/85, 5/10/85, 8/2/85, 8/21/85, and 12/17/86. Action has been taken in response to all items listed by Quality Assurance. It was concluded that the Final Report accurately reflects the raw data for this project. This study has been conducted in compliance with Good Laboratory Practice Regulations.

Dixie Dales 4-14-87

QUALITY ASSURANCE UNIT DATE

Q. A. INSPECTIONS

PROJECT NO. ETOX-85-1002

EVENT	DATE INSPECTED	REPORTED TO STUDY DIRECTOR	INITIALS
Dosing	3/15/85	5/10/85	WB
Sacrifice/Uterine Exam	4/12/85	5/10/85	WB
Dose Preparation	8/2/85	8/2/85	WB
Sacrifice	8/21/85	8/21/85	WB
Final Report	12/3-12/16/86	12/17/86	WB

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SPONSOR: NIOSH INITIATION DATE: March 11, 1985

MATERIALS: Priority Chemicals TERMINATION DATE: August 23, 1985

SUBJECT: Screening of Priority Chemicals for Reproductive Hazards

Contract No. 200-84-2735

EHRT Project No. ETOX-85-1002

Final Report

SUMMARY

The purpose of this contract is to screen selected chemicals, including National Toxicology Program (NTP) priority compounds, for the potential to cause adverse reproductive effects using a post-natal mouse screening test (Chernoff and Kavlock, 1982[1], 1983[2]).

The screen consists of three experimental phases. Phases I and II are range-finding studies designed as a method to identify the predicted LD_{10}, to be used in the Phase III. Phases I and II are done in experimental blocks of six (6) chemicals using nonpregnant female mice in Phase I and pregnant mice in Phase II. Phase III is done in experimental blocks of three (3) chemicals. Treatments are administered by gavage on five (5) consecutive days (Phase I) or on gestation days 6-15 (Phase II and III).

In Phase III, reproductive hazard potential is evaluated by consideration of: maternal body weights (measured at randomization, on days 6 - 15 and day 17 of gestation, and on days 0 and 3 postpartum); maternal mortality and signs of toxicity; pup counts at birth (live and dead); pup weights (recorded at birth and on Day 3 postpartum); and offspring survival from birth to Day 3 postpartum.

This report presents the results for the three phases of testing for monoethanolamine, diethanolamine, and triethanolamine.

In Phase I, monoethanolamine, diethanolamine, and triethanolamine were administered on 5 consecutive days at 10, 100, or 1000 mg/kg/day. There were three deaths produced in the 1000 mg/kg group for monoethanolamine and one death in the 100 mg/kg group for diethanolamine. Triethanolamine produced no mortality. The dose levels for Phase II were determined from Appendix 1: Dose range number 4 was selected for monoethanolamine, dose range number 2 for diethanolamine (due to the one death in the 100 mg/kg dose level), and dose range number 1 for triethanolamine. Based on the probit analysis of the mortality data from Phase II, the predicted LD_{10} was 850 mg/kg for monoethanolamine, 450 mg/kg for diethanolamine, and 1125 mg/kg for triethanolamine.

In the Phase III of the Chernoff/Kavlock post-natal mouse screening test, 450 mg/kg/day of diethanolamine affected neonatal survival and neonatal weight gain. No maternal toxicity was observed despite dosing at the predicted LD_{10}. Diethanolamine

was therefore positive in this screen. Monoethanolamine, at 850 mg/kg/day, produced 16 percent maternal mortality. The number of viable litters was significantly reduced, therefore, monoethanolamine is judged positive in this screen. Triethanolamine did not produce evidence in this test system of developmental toxicity. It is recommended that triethanolamine be retested in the Chernoff/Kavlock test at a higher dose which results in approximately 10% maternal mortality.

INTRODUCTION

Continuing concern exists for potential reproductive hazards from exposure to various chemicals in the workplace environment. The identification of these potential reproductive hazards and the use of these data to establish criteria for Federal standards are major objectives of the National Institute for Occupational Safety and Health (NIOSH). The enormous number of chemicals which are in common use, coupled with new legislation (e.g., the Toxic Substances Control Act) designed to ensure appropriate control of industrial chemicals, has severely taxed the resources available to use traditional reproductive hazard protocols in testing. The identification and prioritization of chemicals in need of more detailed, traditional reproductive hazard testing is essential to a timely study of such a large number of chemicals. The purpose of this contract is to screen selected chemicals, including National Toxicology Program (NTP) priority compounds, for the potential to cause adverse reproductive effects using a postnatal mouse screening test (Chernoff and Kavlock, 1982[1], 1983[2]).

This method screens chemicals for embryonic, fetal, and neonatal toxic responses using pregnant mice treated during major organogenesis (days 6-15 of gestation). Dosing is stopped four days prior to expected parturition, since postnatal maternal toxicity may compound neonatal results. The number of live-born pups, birth weights, and growth and survival to 3.5 days of age

are the primary response variables measured. A reduction in the average litter size as noted within 12 hours postpartum could be due to embryonic resorptions, perinatal death, and/or cannibalization of pups by the dam. All of these events can be associated with a embryotoxic, fetotoxic, or teratogenic action. Less pronounced forms of developmental toxicity may be evidenced by decreased birth weights or by a failure of pups to survive and grow at normal rates during the 72 hours after the initial observation (litters are initially observed and weighed within 12 hours of birth and then again after 72 hours--observation time periods are then approximately 12 and 84 hours post partum). Survival and growth over this period may be sensitive indicators of organ function failure, e.g., cardiac, renal, pulmonary or other systems. The postnatal mouse test may be a sensitive screen for these functional deficits, which are not evaluated in conventional teratology tests.

MATERIALS AND METHODS

The objective of this program was to assess the reproductive hazard potential of priority chemcials in the CD-1 mouse.

TEST ARTICLE

The following summary lists the three (3) test articles used in this program and the appropriate receipt and identification information. Chemicals were obtained for the NTP by the Radian Corporation and supplied to EHRT as coded chemicals for testing.

Test Article Identification Information:

CHEMICAL	SUPPLIER	CAS No.	Date Received
Monoethanolamine	Radian Corp.	141-43-5	3/6/85
Diethanolamine	Radian Corp.	111-42-2	3/6/85
Triethanolamine	Radian Corp.	102-71-6	3/6/85

Test Article Preparation and Administration - Phases I, II and III:

Each test article was admixed in distilled water at a concentration which provided the desired amount of test compound to the animals in a standard volume of 10 ml/kg body weight. For each test article, appropriate amounts of the compound were weighed on a pan balance (accurate to 0.1 mg). The vehicle was then added and the resulting test solution mixed on a magnetic stirrer until suspended. The daily dosing solutions were

prepared and color coded by the chemistry staff at study initiation and were stored refrigerated in amber glass vials to prevent photodegradation. All dosing concentrations were verified as accurate by the chemistry staff. The animal technicians dosing the animals were not aware of the test article name and were blind to doses. Each test article was identified by the number assigned by the Radian Corp. and a corresponding color. This technique allowed for nonbias clinical observations.

On the day of dosing, an aliquot was allowed to warm to room temperature prior to dosing. The test dosing solution was delivered directly into the stomach via a 20 gauge 1 1/2 inch stainless steel gavage needle attached to a 1 cc syringe. The animals were dosed once daily for five (Phase I) or ten (Phase II and III) consecutive days. Oral intubation was selected as the route of administration by the sponsor. The dosing mixtures were thoroughly vortexed just prior to and intermittently during dosing.

A vehicle control group was employed only during the Phase III. Mice in the vehicle control group received only distilled water and served as the common control group for the test compounds being evaluated with this block of chemicals.

Purity and Stability:

Test compounds used in this study were obtained from Radian Corporation, Austin, Texas. The purity and identity of the test compounds have been confirmed by the Radian Corporation. The

compilation of stability, toxicity, storage, and safe handling information was sent by Radian Corporation to EHRT.

Prior to and upon completion of the dosing interval, the EHRT Analytical Chemistry Division analyzed dosing solutions to document concentration and chemical stability. The results of the analysis are presented in Appendix 2.

Safety Precautions:

Personnel working with the test materials or in the animal rooms wore disposable gloves, masks, and other appropriate clothing such as laboratory coats and/or uniforms. All animal wastes resulting from this study were disposed of in a landfill, and carcasses in a high temperature incinerator.

Laboratory personnel practiced good sanitation and health habits. No illness or other condition of personnel that may have adversely affected the study was reported.

EXPERIMENTAL METHODS

Test Animals and Husbandry:

Virgin female (Phase I) and primigravida (Phase II and III) specific pathogen free (SPF) CD-1 albino mice, six to eight weeks of age, were obtained from Charles River Breeding Laboratories, Inc. (Kingston, New York). Upon receipt, all animals were individually examined for general physical condition. Body weights were measured within the next two days after receipt. This strain of mouse was selected by the sponsor.

The mice were individually housed in polycarbonate shoe box cages with stainless steel tops and hardwood bedding (Ab-Sorb-Dri, Lab Products, Maywood, N.J.). Cages were sanitized and fresh bedding was supplied at least once during the study. Purina Certified Rodent Chow #5002 and fresh water were available ad libitum. The mice were maintained on a 12-hour light/dark cycle in a temperature controlled room ($72^{o}\pm3^{o}F$). Due to a problem with the supplier, Ziegler Brothers NIH-07 Rodent Chow (Gardners, PA) was supplied to the Phase III animals on the day they arrived at the test facility. Purina Certified Rodent Chow #5002 was made available to the mice later the same evening. Room temperature and humidity were recorded at least once each day.

Prior to study intiation, the mice were quarantined for five days in the room in which the study was to be conducted. This shortened quarantine period was used in Phase I to parallel that used in Phases II and III with pregnant mice. During this

period, observations were performed twice daily for mortality and general physical appearance.

Assignment to Treatment Groups:

Based on the observations during quarantine, clinically acceptable mice were randomly assigned to treatment groups, using a computer-generated randomization program. Each group consisted of three (3) virgin females (Phase I), four (4) mated females (Phase II), or fifty (50) mated females (Phase III). Each animal received a unique, permanent identification number using an ear punch numbering system. In addition, each group of mice was assigned a color-coded card which displayed the corresponding project number, individual animal numbers and group number.

Clinical Observations:

During the dosing period, animals were observed twice daily for signs of toxicity. The observations were conducted once in the morning and once in the afternoon. 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 examined for evidence of dosing error. 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. Observation for signs of toxicity and mortality were also performed once on the days the animals were

not dosed (7 days post treatment for Phase I, 2 days post treatment for Phase II).

On gestation day 17, all surviving Phase II mice were sacrificed and uteri were examined. Each female was classified as pregnant or nonpregnant. Pregnant mice were noted as having live fetuses or no live fetuses.

For Phase III (reproductive phase), litter box bedding was not changed from gestation day 17 throughout the post partum observation period. Beginning on day 18 of gestation and continuing until all litters were delivered, females were observed twice daily for evidence of labor and delivery of litters. Observations were made before 9:00 a.m. and after 4:00 p.m. The day of delivery was recorded to the nearest half day.

The time, date, and gestation day were recorded when labor, fetuses, or other evidence of delivery was observed. Females were not disturbed if they were in labor or if they had not finished cleaning newborn pups. The delivery of pups was considered complete if the pups were clean and dry.

After delivery was complete, the dam was removed and weighed then placed temporarily in a holding container. The pups were removed from the cage and the nesting material was probed for dead fetuses and parts of cannibalized fetuses. The number of live and dead pups, and the weight of all live pups were recorded. The live pups of a litter were weighed together to give a litter weight, and the average litter weight was calculated. The live pups were then returned to the nesting box and the dam was returned last. Dead fetuses were discarded.

Any female that did not show signs of delivering a litter by gestation day 22 was sacrificed and the uterus examined for evidence of pregnancy. If there was no gross evidence of pregnancy, the uterus was placed in a 10 percent ammonium sulfide solution to make early implantation sites visible. Based on the presence of early implantation sites, the female was classified as having been either pregnant or never pregnant.

Following the observations made on Day 0 post partum, females and litters were not disturbed until post partum Day 3. At that time, the maternal body weight, and the number of live and dead neonatal mice, and the total litter weight of live pups were recorded. Females and litters were then sacrificed and discarded.

Body Weights:

Body weights for the Phase I animals were recorded at the time of randomization, on days 1 and 5 of treatment and on days 3 and 7 after the final dose. Phase II and III animal body weights were recorded at the time of randomization, on days 6 through 15 of gestation and on day 17 of gestation. Additional body weights measured for Phase III were recorded on post partum day 0 and post partum day 3. Body weight changes were calculated for each phase of the study.

Termination:

All surviving mice were sacrificed by carbon dioxide asphyxiation following the collection of terminal body weights. Uteri were examined for all Phase II mice, and for Phase III mice that had not delivered by gestation day 22.

STATISTICAL ANALYSES

An overall test for homogeneity of variance (Bartlett's test) and F-test were performed on the weight data of each group following randomization. Average body weight per group and average body weight change per group were calculated for treatment and control groups. Probit analysis of mortality and morbidity data generated in Phase II of the range finding study was used to determine the predicted LD_{10} for the Phase III.

An IBM PC was used to compile all the raw data. At the completion of Phase III a diskette with this information was delivered to the Project Officer. The statistics on these data were performed by Dr. Burg at the NIOSH facility in Cincinnati, Ohio.

The following statistical procedures were used for analyzing maternal data:

- Random weights (Table 21)
  analysis of variance (ANOVA) (2-tail)
  (all groups, pregnant dams)
  (all groups, viable litters only)
- Survival (Table 19) Fisher's exact test (one-tail)
  (each group vs control, all dams)
  (each group vs control, pregnant only) (Table 20B)
- Weight gains (Table 22) Mann-Whitney U-Test (2-tail)
  (each animal, day 6 to day 0 postnatal)
  (each group vs control; viable litters only)

- Proportion of viable litters Fisher's exact test (one-tail) (Table 23)
  (each group vs control, pregnant only)
- Survival of pups Chi-Square test (one-tail)
  total pups day 3/total pups day 0 Table 23

The Mann-Whitney U-test (2-tail) was used to compare each group to the concurrent control.

- Number live pups/litter (day 0, day 3) Table 23
- Length of gestation (Table 24)
- Average wt. pup (day 0, day 3) Table 25
- Average wt. gain/litter (day 3-day 0) Table 25

The p-value listed is not corrected for multiple comparisons.

RESULTS - RANGE FINDING

(PHASE I)

In the Phase I (range finding) monoethanolamine, diethanolamine and triethanolamine were administered to three (3) groups of mice, using three mice per group. Doses of 10, 100, and 1000 mg/kg/day were administered over a five-day period (Monday through Friday). Dosing was by oral intubation once per day in a standard volume of 10 ml/kg.

Body Weights:

Average body weights and average body weight changes for each dose level are presented in Table 1 for monoethanolamine, Table 3 for diethanolamine, and in Table 5 for triethanolamine.

Mortality:

Table 2, Table 4 and Table 6 present the number of deaths for monoethanolamine, diethanolamaine and triethanolamine, respectively. Within three days of the start of treatment all three mice treated with 1000 mg/kg/day monoethanolamine were dead. There were no other deaths attributed to monoethanolamine. The two deaths in the 1000 mg/kg/day diethanolamine group were caused by dosing trauma. One death in the 100 mg/kg/day diethanolamine group was attributed to its toxicity. No deaths were produced by triethanolamine.

Clinical Signs:

Monoethanolamine produced signs of systemic toxicity and death at the 1000 mg/kg of body weight/day dose level. Systemic signs included: languid behavior, labored respiration, anorexia, wheezing, hunched posture, squinted eyes, rapid breathing, soft feces, and rough haircoat. All animals in this dose level were found dead; no deaths appeared to be caused by dosing trauma. No animals in the 10 and 100 mg/kg of body weight/ day dose levels exhibited signs of systemic toxicity.

Diethanolamine produced signs of systemic toxicity and death. Two animals in the 1000 mg/kg/day group were found dead immediately after dosing and a gross necropsy was performed. Death in these two animals appeared to be caused by gavage trauma. One animal exhibited signs of systemic toxicity in the 100 mg/kg/day group. Systemic signs included hunched appearance, rough haircoat, pale eyes and extremities. The animal was found dead on post-treatment day 3. A gross necropsy was performed and the death did not appear to be caused by dosing trauma.

When triethanolamine was administered over a five day period, no signs of systemic toxicity were observed at any dose level.

Conclusion:

Based on the three deaths in the 1000 mg/kg/day monoethanolamine group, the Phase II dose levels selected from Appendix 1 were 45, 85, 160, 305, and 575 mg/kg/day.

Based on the one death attributed to diethanolamine in the 100 mg/kg/day group, the Phase II dose levels selected from Appendix 1 were 200, 380, 720, 1370, and 2605 mg/kg/day.

Triethanolamine did not produce chemical related mortality when administered at dose levels of 10, 100, and 1000 mg/kg/day. Therefore, the dose levels for the Phase II selected from Appendix 1 were 600, 1200, 2400, 4800, and 9600 mg/kg/day

PHASE 1

Test Chemical Identification: Monoethanolamine
CAS No. 141-43-5

TABLE 1

AVERAGE BODY WEIGHTS AND BODY WEIGHT CHANGES (G)
MEAN ± STANDARD DEVIATION

	DOSE LEVEL (mg/kg)		
	10	100	1000
TREATMENT DAYS			
1	23.7 ± 0.87	23.7 ± 0.98	24.4 ± 0.92
5	23.8 ± 0.38	22.7 ± 1.11	All Dead
POST TREATMENT DAYS			
3	24.6 ± 0.57	22.8 ± 1.51	All Dead
7	25.3 ± 0.21	24.6 ± 1.15	All Dead
CHANGES			
TD 5 - TD 1	+0.1	-1.0	-
PTD 7 - TD5	+1.5	+1.9	-
PTD 7 - TD1	+1.6	+0.9	-

TD = TREATMENT DAY
PTD = POST TREATMENT DAY

TABLE 2

MORTALITY (Number of Deaths)

DOSE LEVEL (mg/kg)	DAYS						
	1	2	3	4	5	6-12	TOTAL
10	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
1000	0	1	2	-	-	-	3

PHASE I

Test Chemical Identification: Diethanolamine
CAS No. 111-42-2

TABLE 3

AVERAGE BODY WEIGHTS AND BODY WEIGHT CHANGES (G)
MEAN ± STANDARD DEVIATION

	DOSE LEVEL (mg/kg) 10	100	1000
TREATMENT DAYS			
1	23.9 ± 1.10	25.0 ± 0.90	25.4 ± 0.80
5	24.3 ± 1.01	23.9 ± 3.56	27.0 ± --
POST TREATMENT DAYS			
3	25.9 ± 0.46	23.5 ± 5.02	27.5 ± --
7	25.7 ± 0.17	27.1 ± 0.40	26.6 ± --
CHANGES			
TD 5 - TD 1	+0.4	-1.1	+1.6
PTD 7 - TD 5	+1.4	+3.2	-0.4
PTD 7 - TD1	+1.8	+2.1	+1.2

TABLE 4

MORTALITY (Number of Deaths)

DOSE LEVEL (mg/kg)	DAYS 1	2	3	4	5	6-12	TOTAL
10	0	0	0	0	0	0	0
100	0	0	0	0	0	1	1
1000	0	0	1*	1*	0	0	2

* - Dosing Error

PHASE I

Test Chemical Identification: Triethanolamine
CAS No. 102-71-6

TABLE 5

AVERAGE BODY WEIGHTS AND BODY WEIGHT CHANGES (G)
MEAN ± STANDARD DEVIATION

	DOSE LEVEL (mg/kg)		
	10	100	1000
TREATMENT DAYS			
1	25.5 ± 0.82	23.8 ± 0.66	24.4 ± 1.02
5	25.0 ± 0.19	25.0 ± 0.45	25.7 ± 1.26
POST TREATMENT DAYS			
3	26.8 ± 0.56	26.3 ± 1.25	26.0 ± 0.62
7	26.9 ± 0.41	27.3 ± 1.32	26.0 ± 0.69
CHANGES			
TD 5 - TD 1	-0.5	+1.2	+1.3
PTD 7 - TD 5	+1.9	+2.3	+0.3
PTD 7 - TD1	+1.4	+3.5	+1.6

TABLE 6

MORTALITY (Number of Deaths)

DOSE LEVEL (mg/kg)	DAYS						
	1	2	3	4	5	6-12	TOTAL
10	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
1000	0	0	0	0	0	0	0

RESULTS - RANGE FINDING

(PHASE II)

Monoethanolamine, diethanolamine and triethanolamine were evaluated in Phase II using doses selected after Phase I. Due to inconclusive results from the first Phase II, a second Phase II range finding study was conducted with monoethanolamine utilizing higher dose levels. The results for each chemical evaluated are discussed below:

Body Weights:

Average body weights and average body weight changes for each test chemical can be found as follows: monoethanolamine, Tables 7, 8, 10 and 11; diethanolamine, Tables 13 and 14; and triethanolamine, Tables 16 and 17.

Mortality and Pregnancy Status:

The number of animals dead per group/number of animals per group and the pregnancy status of all animals found dead or sacrificed are presented in Tables 9 and 12 (monoethanolamine), Table 15 (diethanolamine), and Table 18 (triethanolamine).

Clinical Signs:

When monoethanolamine was administered to primigravida mice over a ten day period (gestation day 6 through gestation day 15), signs of systemic toxicity and death were produced at some dose levels. Systemic signs were as follows:

Initial Phase II

Dose Level: (mg/kg)	
45	A rough haircoat was seen in all animals. All of the animals appeared normal by treatment day 9.
85	All animals at this dose level exhibited a rough haircoat by treatment day 3. This was the only deviation from normal noted for these animals. All animals appeared normal by treatment day 10.
160	All animals at this dose level exhibited a rough haircoat by treatment day 3. On treatment day 6, one animal appeared to be underweight. All animals appeared normal by post-treatment day 2.
305	All animals at this dose level exhibited a rough haircoat by treatment day 3. Also on treatment day 3, one animal appeared languid. This animal's behavior appeared normal by treatment day 4. All animals appeared normal by treatment day 9. On treatment day 10, one animal was found dead immediately after dosing. This death was caused by dosing trauma.
575	Hunched posture, languid behavior, and a rough haircoat were exhibited by the animals at this dose level. Three animals appeared normal by post-treatment day 2, one animal still exhibited a rough haircoat.

Repeat Phase II

Since there were no treatment related deaths in the initial Phase II rangefinding study, a second Phase II rangefinding study was performed that employed higher dose levels of monoethanolamine.

Dose Level (mg/kg)	
500	One animal at this dose level exhibited languid behavior. A rough haircoat was the only other deviation from normal noted in these animals. All animals were normal by treatment day 7.
579	One animal was found dead after dosing on treatment day 2. This death was apparently caused by dosing error. A rough haircoat was the only other deviation from normal noted in these animals. All surviving animals were normal by treatment day 6.
671	A rough haircoat was noted for one animal at this dose level; this animal was normal by treatment day 7. All other animals appeared normal throughout the study.
777	A rough haircoat was noted for this dose level. One animal exhibited hyperactive behavior on treatment day 6. This behavior continued until post-treatment day 2. All other animals appeared normal by treatment day 9.
900	Signs of systemic toxicity noted at this dose level were as follows: rough haircoat, languid behavior, and hyperactivity. One animal was found dead before dosing on treatment day 6. This death did not appear to be caused by dosing trauma.

When diethanolamine was administered to primigravida mice over a ten day period (gestation day 6 through gestation day 15), signs of systemic toxicity and death were produced. Systemic signs were as follows:

Dose Level (mg/kg)	
200	A rough haircoat was seen in all animals. One animal was found dead immediately after dosing on treatment day 7; this death appeared to be caused by dosing trauma. All surviving animals were normal by treatment day 10.
380	All animals at this dose level exhibited a rough haircoat. This was the only deviation from normal noted at this dose level.
720	Signs of systemic toxicity at this dose level included: rough haircoat, languid behavior, squinted eyes, and hunched posture. One animal was found dead during the afternoon observation on treatment day 3. Two animals were found dead during the morning observation; one on treatment day 7 and one on treatment day 8. None of these deaths appeared to be caused by dosing trauma.
1370	Signs of systemic toxicity noted in this group included: rough haircoat, languid behavior, hunched posture, bloody vaginal discharge, labored breathing, squinted eyes, unsteady gait, and animals underweight. Three animals were found dead during the morning observations, two on treatment day 7 and one one treatment day 8. One animal was found dead during the afternoon observation on treatment day 9. None of these deaths appeared to be caused by dosing trauma.
2605	All animals at this dose level exhibited signs of systemic toxicity. These signs included: rough haircoat, hunched posture, prostrate, labored breathing,

Dose Level: (mg/kg)	
2605	languid behavior, bloody vaginal discharge, and pale eyes and extremities. All animals at this dose level were found dead on treatment day 5. Three animals were found dead at the morning observation and one animal was found dead at the afternoon observation. None of these deaths appeared to be caused by dosing trauma.

When triethanolamine was administered to primigravida mice over a ten day period (gestation day 6 through gestation day 15), signs of systemic toxicity and death were produced. Systemic signs were as follows.

Dose Level (mg/kg)	
600	A rough haircoat was seen in all animals. On treatment day 4, one animal was noted as having hunched posture, pale eyes and extremities. This animal was found dead during the morning observation on treatment day 5. This death did not appear to be caused by dosing trauma. Two of the surviving animals appeared normal by treatment day 9. One surviving animal exhibited a rough haircoat until post-treatment day 2.
1200	All animals at this dose level exhibited a rough haircoat by treatment day 2. This was the only deviation from normal noted for these animals. All animals appeared normal by treatment day 9.

Dose Level:
(mg/kg)

2400 All animals at this dose level exhibited a rough haircoat by treatment day 2. On post-treatment day 1, one animal appeared lanquid, exhibited hunched posture, rough haircoat, and squinted eyes. This animal was found dead during the morning observation of post-treatment day 2. This death did not appear to be caused by dosing trauma.

4800 Animals at this dose level exhibited a rough haircoat by treatment day 2. On treatment day 4, one animal was noted as not using it's left rear leg. This animal's leg appeared normal by the afternoon observation on treatment day 5. Two animals were found dead, one during the morning observation of treatment day 7 and one during the morning observation of treatment day 9. These deaths did not appear to be caused by dosing trauma.

9600 All animals in this dose level died. One animal was found dead during the afternoon observation period of treatment day 1. The remainder of the animals were found dead during the morning observation of treatment day 2. These deaths did not appear to be caused by dosing trauma.

Conclusion:

Based on the probit analysis of combined mortality data from the Phase I and II tests, the recommended doses for the Phase III reproductive study (LD_{10}) were 850 mg/kg/day for monoethanolamine, 450 mg/kg/day for diethanolamine and 1125 mg/kg/day for triethanolamine.

PHASE II

Test Chemical Identification: Monoethanolamine
CAS No. 141-43-5

TABLE 7

AVERAGE MATERNAL BODY WEIGHTS IN GRAMS
(Mean ± Standard Deviation)
(Pregnant Animals Only)

TREATMENT	DOSE LEVEL (mg/kg of body weight/day)				
DAY	45	85	160	305	575
1	28.05 ± 0.68	27.70 ± 1.39	28.20 ± 0.57	27.86 ± --	27.66 ± 0.90
n	3	2	3	1	4
2	28.84 ± 0.52	29.02 ± 2.43	28.99 ± 1.10	28.04 ± --	28.32 ± 1.33
n	3	2	3	1	4
3	28.49 ± 1.86	29.58 ± 2.74	29.55 ± 1.03	27.90 ± --	27.71 ± 3.54
n	3	2	3	1	4
4	29.26 ± 0.81	29.67 ± 2.93	30.75 ± 1.35	27.24 ± --	30.07 ± 3.41
n	3	2	3	1	4
5	31.06 ± 1.01	30.49 ± 4.14	31.73 ± 1.84	26.32 ± --	31.40 ± 5.08
n	3	2	3	1	4
6	31.87 ± 0.64	30.66 ± 6.28	33.75 ± 2.79	27.66 ± --	31.40 ± 5.08
n	3	2	3	1	4
7	34.55 ± 1.22	32.86 ± 7.33	35.93 ± 2.53	27.94 ± --	32.65 ± 5.57
n	3	2	3	1	4
8	36.55 ± 1.27	33.43 ± 8.95	37.59 ± 2.57	27.30 ± --	34.85 ± 6.60
n	3	2	3	1	4
9	37.94 ± 1.67	34.55 ± 9.89	39.99 ± 3.38	27.38 ± --	36.56 ± 7.25
n	3	2	3	1	4
10	41.06 ± 2.60	36.45 ±13.34	42.34 ± 3.71	26.62 ± --	38.58 ± 9.06
n	3	2	3	1	4
POST TREATMENT DAY					
2	46.51 ± 3.12	39.32 ±17.59	48.91 ± 4.33	28.42 ± --	43.73 ±12.32
n	3	2	3	1	4

n - number of pregnant animals in each dose level

PHASE II

Test Chemical Identification: Monoethanolamine
CAS No. 141-43-5

TABLE 8

AVERAGE MATERNAL BODY WEIGHT CHANGES IN GRAMS
(PREGNANT ANIMALS ONLY)

	Dose Level (mg/kg)				
Monoethanolamine	45	85	160	305	575
Treatment day 10 - treatment day 1	+13.01	+ 8.75	+14.14	- 1.24	+10.92
Post-treatment day 2 - treatment day 10	+ 5.45	+ 2.87	+ 6.57	+ 1.80	+ 5.15
Post-treatment day 2 - treatment day 1	+18.46	+11.62	+20.71	+ 0.56	+16.07

TABLE 9
MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg)				
	45	85	160	305	575
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	0	0	0	1[a]	0
Ammonium Sulfide Positive	-	-	-	-	-
Ammonium Sulfide Negative	-	-	-	1	-
Animals Sacrificed	4	4	4	3	4
Live Pups	3	1	3	-	3
Ammonium Sulfide Positive	-	1	-	1	1
Ammonium Sulfide Negative	1	2	1	2	-

[a] - Animal died from dosing trauma

PHASE II (Repeat)

Test Chemical Identification: Monoethanolamine
CAS No. 141-43-5

TABLE 10

AVERAGE MATERNAL BODY WEIGHTS IN GRAMS
(Mean ± Standard Deviation)
(Pregnant Animals Only)

TREATMENT	DOSE LEVEL (mg/kg of body weight/day)				
DAY	500	579	671	777	900
1	28.80 ± 0.45	28.58 ± 0.96	29.17 ± 1.17	27.57 ± 1.82	29.82 ± 0.99
n	2	4	2	2	2
2	29.60 ± 1.02	28.75 ± 1.76	29.08 ± 1.36	27.91 ± 2.22	30.00 ± 0.31
n	2	4	2	2	2
3	30.05 ± 1.32	28.91 ± 2.03	29.96 ± 1.70	28.87 ± 1.97	26.99 ± 0.61
n	2	3	2	2	2
4	30.08 ± 2.66	29.84 ± 3.00	30.06 ± 0.99	29.39 ± 1.74	27.70 ± 0.71
n	2	3	2	2	2
5	31.00 ± 3.56	30.33 ± 3.68	32.28 ± 0.99	30.20 ± 1.78	27.85 ± 0.33
n	2	3	2	2	2
6	31.71 ± 3.24	31.07 ± 3.92	33.52 ± 1.05	31.95 ± 2.53	26.39 ± 0.41
n	2	3	2	2	2
7	33.91 ± 3.13	32.23 ± 4.70	35.67 ± 2.28	34.15 ± 2.67	26.76 ± --
n	2	3	2	2	1
8	36.68 ± 2.52	33.28 ± 5.82	37.81 ± 2.67	36.26 ± 2.29	26.14 ± --
n	2	3	2	2	1
9	39.08 ± 2.80	34.88 ± 6.45	39.98 ± 2.43	38.61 ± 2.53	26.84 ± --
n	2	3	2	2	1
10	42.39 ± 2.93	37.19 ± 8.81	42.72 ± 3.93	42.01 ± 2.67	27.18 ± --
n	2	3	2	2	1
POST TREATMENT DAY					
2	49.22 ± 2.29	41.40 ±13.40	51.25 ± 4.12	49.61 ± 2.28	28.04 ± --
n	2	3	2	2	1

n - number of pregnant animals in each dose level

PHASE II (Repeat)

Test Chemical Identification: Monoethanolamine
CAS No. 141-43-5

TABLE 11

AVERAGE MATERNAL BODY WEIGHT CHANGES IN GRAMS
(PREGNANT ANIMALS ONLY)

	Dose Level (mg/kg)				
Monoethanolamine	500	579	671	777	900
Treatment day 10 - treatment day 1	+13.59	+ 8.61	+13.55	+14.44	- 2.64
Post-treatment day 2 - treatment day 10	+ 6.83	+ 4.21	+ 8.53	+ 7.60	+ 0.86
Post-treatment day 2 - treatment day 1	+20.42	+12.82	+22.08	+22.04	- 1.78

TABLE 12
MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg)				
	500	579	671	777	900
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	0	1[a]	0	0	1
Ammonium Sulfide Positive	-	1	-	-	1
Ammonium Sulfide Negative	-	-	-	-	-
Animals Sacrificed	4	3	4	4	3
Live Pups	2	2	2	2	-
Ammonium Sulfide Positive	-	1	-	-	1
Ammonium Sulfide Negative	2	-	2	2	2

[a] - Animal died from dosing trauma

PHASE II

Test Material Identification: Diethanolamine
CAS No. 111-42-2

TABLE 13

AVERAGE MATERNAL BODY WEIGHTS IN GRAMS
(Mean ± Standard Deviation)
(Pregnant Animals Only)

TREATMENT	DOSE LEVEL (mg/kg of body weight/day)				
DAY	200	380	720	1370	2605
1	27.98 ± 1.61	28.23 ± 0.58	27.71 ± 0.57	28.18 ± 0.39	28.34 ± 0.55
n	2	2	4	3	3
2	29.72 ± 0.59	29.65 ± 1.15	29.34 ± 0.80	29.86 ± 0.61	30.05 ± 1.41
n	2	2	4	3	3
3	30.89 ± 1.20	30.58 ± 1.47	30.39 ± 0.95	30.94 ± 1.43	30.96 ± 0.92
n	2	2	4	3	3
4	31.59 ± 1.37	31.54 ± 1.44	31.13 ± 0.48	31.09 ± 1.00	28.85 ± 0.92
n	2	2	3	3	3
5	32.91 ± 0.89	33.03 ± 0.81	31.47 ± 1.09	30.58 ± 1.44	27.51 ± 0.37
n	2	2	3	3	3
6	33.82 ± 0.31	34.67 ± 2.33	31.99 ± 1.97	29.50 ± 3.04	All Dead
n	2	2	3	3	0
7	36.65 ± 0.24	36.48 ± 2.12	31.63 ± 3.58	28.53 ± 3.44	All Dead
n	2	2	3	3	0
8	38.35 ± 0.44	37.80 ± 2.97	33.83 ± 5.42	30.08 ± --	All Dead
n	2	2	2	1	0
9	40.99 ± 1.54	39.57 ± 2.59	39.70 ± --	All Dead	All Dead
n	2	2	1	0	0
10	43.42 ± 2.57	42.57 ± 3.95	41.98 ± --	All Dead	All Dead
n	2	2	1	0	0
POST TREATMENT DAY					
2	49.49 ± 3.55	49.43 ± 4.79	48.40 ± --	All Dead	All Dead
n	2	2	1	0	0

n - number of pregnant animals in each dose level

PHASE II

Test Material Identification: Diethanolamine
CAS No. 111-42-2

TABLE 14

AVERAGE MATERNAL BODY WEIGHT CHANGES IN GRAMS
(PREGNANT ANIMALS ONLY)

	Dose Level (mg/kg)				
Diethanolamine	200	380	720	1370	2605
Treatment day 10 - treatment day 1	+15.44	+14.34	+14.27	0*	0*
Post-treatment day 2 - treatment day 10	+ 6.07	+ 6.86	+ 6.42	0*	0*
Post-treatment day 2 - treatment day 1	+21.51	+21.23	+20.69	0*	0*

* - all animals dead on post-treatment day 2

TABLE 15

MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg/day)				
	200	380	720	1370	2605
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	1[a]	0	3	4	4
Ammonium Sulfide Positive	-	-	3	3	3
Ammonium Sulfide Negative	1	-	-	1	1
Animals Sacrificed	3	4	1	0	0
Live Pups	2	2	1	-	-
Ammonium Sulfide Positive	-	-	-	-	-
Ammonium Sulfide Negative	1	2	-	-	-

[a] - Animal died from dosing trauma

PHASE II

Test Chemical Identification: Triethanolamine
CAS No. 102-71-6

TABLE 16

AVERAGE MATERNAL BODY WEIGHTS IN GRAMS
(Mean ± Standard Deviation)
(Pregnant Animals Only)

TREATMENT	DOSE LEVEL (mg/kg of body weight/day)				
DAY	600	1200	2400	4800	9600
1	27.92 ± 0.99	28.32 ± 0.14	26.55 ± 2.45	27.19 ± 0.84	27.53 ± 0.75
n	4	2	2	4	2
2	28.88 ± 0.76	28.68 ± 0.57	26.72 ± 3.14	27.88 ± 0.99	24.85 ± 0.78
n	4	2	2	4	2
3	29.42 ± 1.07	29.77 ± 0.69	26.88 ± 3.37	28.50 ± 0.77	All Dead
n	4	2	2	4	0
4	28.82 ± 3.81	29.71 ± 0.69	27.35 ± 4.37	29.21 ± 0.94	All Dead
n	4	2	2	4	0
5	29.59 ± 5.42	30.69 ± 1.03	28.64 ± 5.46	30.04 ± 1.64	All Dead
n	4	2	2	4	0
6	33.93 ± 1.42	32.62 ± 2.01	28.91 ± 6.15	30.63 ± 2.57	All Dead
n	3	2	2	4	0
7	35.53 ± 1.33	35.10 ± 1.92	30.04 ± 6.87	31.78 ± 3.78	All Dead
n	3	2	2	4	0
8	37.46 ± 1.13	36.79 ± 2.22	30.56 ± 8.34	34.00 ± 5.48	All Dead
n	3	2	2	3	0
9	39.02 ± 1.97	38.40 ± 3.05	32.20 ±10.21	35.85 ± 6.27	All Dead
n	3	2	2	3	0
10	41.25 ± 2.46	40.98 ± 3.25	33.10 ±11.74	42.20 ± 1.81	All Dead
n	3	2	2	2	0
POST TREATMENT DAY					
2	45.31 ± 5.33	48.25 ± 6.07	36.07 ±16.02	48.90 ± 1.10	All Dead
n	3	2	2	2	0

n - number of pregnant animals in each dose level

PHASE II

Test Chemical Identification: Triethanolamine
CAS No. 102-71-6

TABLE 17

AVERAGE MATERNAL BODY WEIGHT CHANGES (IN GRAMS)

	Dose Level (mg/kg)				
Triethanolamine	600	1200	2400	4800	9600
Treatment day 10 - treatment day 1	+13.33	+12.66	+6.55	+15.01	0*
Post-treatment day 2 - treatment day 10	+ 4.06	+ 7.27	+2.97	+ 6.70	0*
Post-treatment day 2 - treatment day 1	+17.39	+19.93	+9.52	+21.71	0*

* - all animals dead on post-treatment day 2

TABLE 18

MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg/day)				
	600	1200	2400	4800	9600
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	1	0	1	2	4
Ammonium Sulfide Positive	1	-	-	2	2
Ammonium Sulfide Negative	-	-	1	-	2
Animals Sacrificed	3	4	3	2	0
Live Pups	3	2	1	2	-
Ammonium Sulfide Positive	-	-	1	-	-
Ammonium Sulfide Negative	-	2	1	-	-

RESULTS - REPRODUCTIVE PHASE

Monoethanolamine, Diethanolamine, Triethanolamine

Based on the results of probit analysis of the combined mortality data from Phase I and Phase II, monoethanolamine was dosed at 850 mg/kg/day; diethanolamine at 450 mg/kg/day and triethanolamine at 1125 mg/kg/day. The vehicle control group received distilled water. All animals were dosed at a constant volume of 10 ml/kg/day.

Three pregnant animals were eliminated from statistical analysis, one from the control group and two from the monoethanolamine group. The animals were eliminated due to a technician error in weighing the litters.

Mortality and Survival:

Mortality table showing deaths by individual treatment days is presented in Table 19 for all the animals and in Tables 20A and B for pregnant animals. The mortality rate for all the animals treated with monoethanolamine was higher than vehicle controls (7/50 compared to 0/50, $p<0.01$). However, when considering only pregnant mice there was an indication of a higher mortality in the monoethanolamine treated mice (5/31 vs. 0/28; $p=0.034$). There were no deaths in the fifty mice of the diethanolamine, triethanolamine and control groups.

Clinical Observations:

No animals in the vehicle control group exhibited clinical signs of toxicity. Clinical signs noted in the monoethanolamine group included: languid behavior, hunched posture, labored or rapid

respiration, squinted eyes, pale extremities, wheezing and rough haircoat. Less frequent observations noted in this group were tremors, piloerection, stained fur around nose, mouth and ano-genital area, blood-like discharge from the vagina, and some animals were noted as being underweight. All surviving animals appeared normal by post-treatment day 5. Two animals were found dead on treatment day 4, on treatment day 7, and on treatment day 10. One animal was found dead on post-treatment day 4. None of these deaths appeared to be caused by dosing trauma.

No animals in the diethanolamine group showed signs of toxicity during the treatment period. However, on post-treatment day 2, one animal exhibited rapid respiration, rough haircoat, hunched posture, and was noted as being underweight. This animal appeared normal by post-treatment day 5.

In the triethanolamine group, one animal was noted as having a subcutaneous lump (treatment day 2). This did not appear to be related to the treatment. One other animal in this group exhibited signs of toxicity throughout the treatment and post-treatment period. These signs included: hunched posture, languid behavior, rough haircoat, wheezing, labored respiration, head tilt, squinted eyes, and pale extremities. All other animals in the triethanolamine group appeared normal throughout the treatment and post-treatment period.

Maternal Body Weights:

Summaries of mean maternal body weights and body weight changes measured at the designated intervals during the study are presented in Tables 21 and 22. No statistically significant group differences

were found for the randomization weights. Statistical analyses were performed to compare the maternal body weights on postpartum day 0 and 3. The the maternal weights in the monoethanolamine group was significantly decreased from the control group ($p=0.03$, using pregnant animals producing viable litters) at day 3 postpartum ($p=0.02$) but not a postpartum day 0 ($p=0.31$). The diethanolamine dams were significantly heavier than the control dams on postpartum day 0 ($p<0.001$) but were lighter than controls on postpartum day 3 ($p=0.01$). The triethanolamine group was significantly heavier from the control group at postpartum day 0 ($p=0.02$) but not at postpartum day 3 ($p=0.43$). The average weight gain from pretreatment to post partum day 0 was 3.7 grams for monoethanolamine, 6.4 grams for diethanolamine, and 5.3 grams for triethanolamine. The diethanolamine and triethanolamine dams were significantly greater (Table 22).

Reproductive Index and Litter Data:

The reproductive index, average number of live pups/litter (day 0 and day 3) and postnatal survival are presented in Table 23. With respect to monoethanolamine, the number of viable litters was significantly decreased from the controls ($p=0.002$). Reduced neonatal survival was noted in the diethanolamine group ($p<0.001$). No statistically significant effect of triethanolamine was observed upon the percent viable litters and upon pup survival.

Duration of Gestation:

Average gestation length is presented by group in Table 24. Diethanolamine produced a significant increase in the duration of gestation compared to the control group ($p\leq0.01$). No statistically

significant group differences were noted in the length of gestation for the triethanolamine group. Monoethanolamine and the control group differed at p=0.06.

Pup Weight Data:

The average weight of each live pup/litter (day 0 and day 3), average pup weight changes and average litter weights are presented in Table 25. There were no treatment related differences in average pup weights on day 0. The average pup weight on day 3 was significantly decreased from the control in the diethanolamine group ($p \leq 0.001$). Pup weight gain was significantly decreased ($p \leq 0.001$) in the diethanolamine group. No statistically significant differences were noted in the monoethanolamine and triethanolamine groups.

Conclusion:

Monoethanolamine

Monoethanolamine (850 mg/kg/day) was toxic to the pregnant animals resulting in a 16% mortality. Monoethanolamine reduced the number of viable litters but did not affect the litter size, the percent survival of the pups, the birth weight of the pups and the weight gained by the pups. Using the ranking system of Hardin (Hardin, 1987[4]) with 22 as a maximum score and for high maternal mortality the following scores should be subtracted: viable litters (0); litter size (4); percent survival of pups (5); birth weight of pups (3); and weight gain of pups (4). Therefore, the score for monoethanolamine is 6 which represents low priority for further testing. However, because of the reduced number of viable litters in the pursuit of high but not excessive maternal mortality, mono-

ethanolamine is judged positive in the Chernoff/Kavlock preliminary developmental toxicity test. It would be of interest to further test monoethanolamine in a conventional developmental toxicity test in order to evaluate whether a low priority score by a substance positive in the Chernoff/Kavlock test will predict low activity in the conventional test.

Diethanolamine

Diethanolamine (450 mg/kg/day) had no effect on maternal mortality, on litter size and on birth weight of the pups, but did decrease the number of viable litters, the percent survival of the pups and the weight gained by the pups. Using the ranking system of Hardin (Hardin, 1987[4]) with a maximum score of 22 and for low maternal mortality the following scores should be subtracted: viable litters (0); litter size (2); percent survival of the pups (0); birth weight of the pups (1); and weight gained by the pups (0). Therefore, the score for diethanolamine is 19 which represents high priority for testing in a conventional developmental toxicity test. In summary, diethanolamine is judged positive in the Chernoff/Kavlock preliminary developmental toxicity test.

Triethanolamine

Triethanolamine (1125 mg/kg/day) did not affect maternal mortality, the number of viable litters, litter size, percent survival of the pups, birth weight of the pups and weight gained by the pups. Using the ranking system of Hardin with a maximum score of 22 and for low maternal toxicity the following scores should be subtracted: viable litters (4); litter size (2); percent survival of pups (3); birth weight of pups (1); and weight gain of pups (2).

Therefore, the score for triethanolamine is 12 which represents intermediate priority for further testing. Since the dose used of triethanolamine did not result in maternal mortality and since the other two ethanolamines (mono- and di-) are positive in the Chernoff/Kavlock test, it is recommended that triethanolamine be retested in the Chernoff/Kavlock test at a higher dose which results in approximately 10% maternal mortality. Until it is retested, triethanolamine is judged to be inaccurately tested in the Chernoff/Kavlock preliminary developmental toxicity test.

REFERENCE

1 Chernoff, N. and Kavlock, R. - An In Vivo Teratology Screen Utilizing Pregnant Mice, J Toxicol Environ Health 10:541, 1982;

2 Chernoff, N., Kavlock, R.: A teratology test system which utilizes post natal growth and viability in the mouse, in Short-Term Bioassays in the Analysis of Complex Environmental Mixtures III, Plenum Publishing Co., NY, 1983 pp 417-427.

3 MacKenzie KM: Screening of Priority Chemicals for Potential Reproductive Toxicity, Final Report to NIOSH, Contract No.: 200-82-2542, Hazleton Laboratories America, Inc., Dec., 1983.

4 Hardin BD: A recommended protocol for the Chernoff/Kavlock preliminary developmental toxicity test and a proposed method for assigning priority scores based on the results of that test. Teratog Carcinog Mutagen, in press.

TABLE 19

MORTALITY
NUMBER OF ANIMALS DEAD PER GROUP

DOSE GROUP	TREATMENT DAY										
	1	2	3	4	5	6	7	8	9	10	TOTAL
Vehicle Distilled Water	0	0	0	0	0	0	0	0	0	0	0
Monoethanolamine (850 mg/kg/day)	0	0	0	2	0	0	2	0	0	2	6
Diethanolamine (450 mg/kg/day)	0	0	0	0	0	0	0	0	0	0	0
Triethanolamine (1125 mg/kg/day)	0	0	0	0	0	0	0	0	0	0	0

DOSE GROUP	POST-TREATMENT DAYS					SUMMARY OF DEATHS		
						SACRIFICE	TOXICITY	ERROR
	1	2	3	4	5			
Vehicle Distilled Water	0	0	0	0	0	50	0	0
Monoethanolamine (850 mg/kg/day)	0	0	0	1	0	43	7*	0
Diethanolamine (450 mg/kg/day)	0	0	0	0	0	50	0	0
Triethanolamine (1125 mg/kg/day)	0	0	0	0	0	50	0	0

* $p<0.01$ by the Fisher Exact Test, 1-tail.

TABLE 20A

MATERNAL STATUS

TEST CHEMICAL

	Water	Monoethanolamine	Diethanolamine	Triethanolamine
Number Evaluated	49	50	48	50
Animals Found Dead	0	7	0	0
Pregnant	0	5	0	0
Never Pregnant[a]	0	2	0	0
Animals Sacrificed[b]	49	43	48	50
Delivered Live Pups	25	13	27	31
No Live Pups[c]	3	13	7	3
Never Pregnant[a]	21	17	14	16

a- Ammonium Sulfide Negative

b- Number of animals sacrificed - includes animals sacrificed day 3 post partum and animals sacrificed on gestation day 22

c- Visually dead pups or ammonium sulfide positive

TABLE 20B

MATERNAL SURVIVAL

	PREGNANT ANIMALS		
DOSE GROUP	LIVED	DIED	PERCENT SURVIVAL
Vehicle Distilled Water	28	0	100%
Monoethanolamine (850 mg/kg/day)	26	5*	84%
Diethanolamine (450 mg/kg/day)	34	0	100%
Triethanolamine (1125 mg/kg/day)	34	0	100%

* - p=0.034 by the Fisher Exact Test, 1-tail

TABLE 21

AVERAGE BODY WEIGHTS IN GRAMS
(MEAN ± STANDARD DEVIATION)

RANDOMIZATION WEIGHTS	VEHICLE DISTILLED WATER	MONO-ETHANOLAMINE 850 mg/kg/day	DI-ETHANOLAMINE 450 mg/kg/day	TRI-ETHANOLAMINE 1125 mg/kg/day
PREGNANT FEMALES ONLY	26.1 ± 1.15	25.9 ± 0.98	25.9 ± 1.13	26.0 ± 1.11
n[a]	28	31	34	34
GESTATION DAYS				
PREGNANT FEMALES ONLY				
6	27.7 ± 1.28	27.9 ± 0.90	27.1 ± 1.74	27.9 ± 1.15
n	28	31	34	34
7	28.1 ± 1.34	27.7 ± 1.29	28.2 ± 1.72	28.3 ± 1.59
n	28	31	34	34
8	28.6 ± 1.56	26.9 ± 1.87	29.0 ± 1.71	29.2 ± 2.12
n	28	31	34	34
9	29.0 ± 1.73	26.9 ± 2.35	30.0 ± 1.98	29.7 ± 2.34
n	28	31	34	34
10	30.2 ± 2.13	27.2 ± 2.88	31.0 ± 2.15	30.7 ± 2.67
n	28	31	34	34
11	31.6 ± 2.51	28.1 ± 3.60	32.6 ± 2.79	32.1 ± 3.12
n	28	31	34	34
12	32.8 ± 3.16	28.9 ± 4.46	34.2 ± 3.29	33.6 ± 3.60
n	28	31	34	34
13	34.2 ± 3.62	30.5 ± 4.51	35.4 ± 3.96	34.9 ± 4.21
n	28	29	34	34
14	35.7 ± 4.48	30.4 ± 5.49	36.7 ± 4.65	36.5 ± 4.84
n	28	29	34	34
15	38.0 ± 5.36	31.0 ± 6.88	39.2 ± 5.80	38.7 ± 5.74
n	28	29	34	34
17	42.7 ± 7.50	33.8 ± 8.49	42.8 ± 7.58	43.3 ± 7.53
n	28	27	34	34
POSTPARTUM DAY				
0	30.3 ± 1.80[b]	29.4 ± 1.38	32.2 ± 2.10***	31.4 ± 2.14*
n	25[c]	13	27[d]	31
3	34.5 ± 2.86	31.3 ± 4.44*	32.4 ± 3.25**	34.0 ± 2.53
n	25	13	27[d]	31

[a] - n = number of animals

[b] - the postpartum body weights were analyzed statistically with the Mann- Whitney U Test with * denoting ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.0001$).

[c] - one pregnant animal eliminated from statistical analysis due to technician error in weighing the animals

[d] - two pregnant animals were eliminated from further statistical analysis due to technician error in weighing the animals

TABLE 22

AVERAGE MATERNAL BODY WEIGHT CHANGES IN GRAMS
(MEAN ± STANDARD DEVIATION)

	DISTILLED WATER	MONO-ETHANOLAMINE	DI-ETHANOLAMINE	TRI-ETHANOLAMINE
Random Weight Pregnant Only	26.1 ± 1.15[a]	25.9 ± 0.98	25.9 ± 1.13	26.0 ± 1.11
n[b]	28	31	34	28
Random Weight Viable Litters Only	26.0 ± 1.13	25.7 ± 0.96	25.9 ± 1.10	26.1 ± 1.09
n	25	13	27	31
Weight Gain Postpartum - Randomization	+4.3 ± 1.60	+3.7 ± 1.10	+6.4 ± 1.70**	+5.3 ± 1.59*
n	25	13	27	31

[a] - an analysis of variance on the results was performed, * denoting p=0.02 and ** denoting p<0.01.
[b] - n = number of animals

TABLE 23

REPRODUCTIVE AND LITTER DATA

	Reproductive Index[a]		Average Number/Litter		Postnatal Survival[b]	
	Ratio	Percent	Day 0 Live	Day 3 Live	Ratio	Percent
VEHICLE						
WATER	25/28	89.3[c]	10.2 ± 3.1	10.1 ± 3.1	242/255	94.9
n[d]			25	25		
MONOETHANOLAMINE						
850 mg/kg	13/26	50.0***	8.7 ± 3.2	8.0 ± 4.2	104/113	92.0
n			13	13		
DIETHANOLAMINE						
450 mg/kg	27/34	79.4	9.0 ± 2.7	7.0 ± 4.2***	188/243	77.4***
n			27	27		
TRIETHANOLAMINE						
1125 mg/kg	31/34	91.2	9.6 ± 2.8	9.5 ± 2.8	278/298	93.3
n			31	31		

[a]- Number of females producing viable litters/number of surviving females that were ever pregnant

[b]- Number of pups alive on day 3/number of pups alive on day 0

[c]- Reproductive index and post natal survival results were analyzed by the Chi-Square test with *** denoting ($p \leq 0.001$). The number of live pups per litter were analyzed by the Mann-Whitney U-test with *** denoting ($p \leq 0.001$). None of the other results had a p value of less than 0.05.

[d]- n= Number of litters used to calculate the mean

TABLE 24

DURATION OF GESTATION (IN DAYS)
DAMS THAT PRODUCED VIABLE LITTERS ONLY

	DOSE GROUP			
	Vehicle Water	Monoethanolamine 850 mg/kg/day	Diethanolamine 450 mg/kg/day	Triethanolamine 1125 mg/kg/day
Mean	18.2[a]	18.5	18.5***	18.3
± SD[b]	0.35	0.48	0.34	0.34
n[c]	25	13	27	31

[a] - Comparison of exposed groups to vehicle control group was evaluated by Mann-Whitney U-test with *** denoting ($p \leq 0.001$).
[b] - SD = Standard Deviation
[c] - n = number of animals that produced viable litters

TABLE 25

AVERAGE WEIGHT OF EACH LIVE PUP PER LITTER AND
DAY 3 AVERAGE LIVE PUP WEIGHTS MINUS DAY 0
AVERAGE LIVE PUP WEIGHTS (G)
MEAN ± STANDARD DEVIATION

	AVERAGE WEIGHT/PUP/LITTER (G)		CHANGE
DOSE GROUP	DAY 0	DAY 3	DAY 3 - DAY 0
Vehicle			
Water	1.5 ± 0.19[a]	2.1 ± 0.32	0.6 ± 0.18
n[b]	25	25	25
Monoethanolamine			
850 mg/kg	1.9 ± 1.54	2.0 ± 0.24	0.5 ± 0.16
n	13	11	11
Diethanolamine			
450 mg/kg	1.5 ± 0.19	1.6 ± 0.27***	0.1 ± 0.25***
n	27	23	23
Triethanolamine			
1125 mg/kg	1.6 ± 0.20	2.2 ± 0.30	0.6 ± 0.23
n	31	31	31

[a] - Results were analyzed by Mann-Whitney U-test with *** denoting ($p \leq 0.001$). There were no other statistically significant differences among the groups, ($p \leq 0.05$).
[b] - n = Number of litters with live pups

APPENDIX 1

Range-Finding Dose Sheet (RFDS)

Phase I

Number of mice dead at:			Then Phase 2
mg/kg/day 10	100	1000	Dose Ranges
-	-	0	1
-	-	1	2
-	-	2	3
-	0	3	4
-	1	3	5
-	2	3	6
0	3	3	7
1	3	3	8
2	3	3	9
3	3	3	10

Phase II

Dose Range No.	Phase II Doses (mg/kg/day)				
1	600	1200	2400	4800	9600
2	200	380	720	1370	2605
3	95	180	340	645	1225
4	45	85	160	305	575
5	20	40	75	140	270
6	10	19	35	67	127
7	5	9	17	31	60
8	2	4	8	15	28
9	1.0	1.9	3.7	6.9	13.2
10	0.5	0.9	1.7	3.3	6.2

APPENDIX 2

DOSING SOLUTIONS

1. Reagents

The monethanolamine, diethanolamine and triethanolamine used in the preparation of the dosing solutions were obtained from Radian Corporation, Austin, Texas. All chemicals were the purest grade commercially available.

2. Vehicle

The vehicle used in the preparation of the dosing solutions was distilled, deionized water.

3. Preparation

The dosing solutions were prepared in the Analytical Lab at EHRT, by the same chemist who analyzed them. The amount of chemical to be weighed for the preparation of each dosing solution was calculated by the Lab Manager. The chemicals were weighed by the chemist. The dosing solutions were shaken for 20 minutes on the automatic shaker to ensure complete solubility or homogenous distribution of the compound in the vehicle. Each chemical was color-coded and handed to the toxicology department in an amber-colored vial. A specific color tape was attached on the vial. It was labelled with the date of preparation and with a letter

referring to a certain concentration (A = lowest concentration, B = next higher concentration, etc.). For Phase III, weighing of the chemicals was witnessed by a Q.A. officer or by another chemist.

4. Storage

The dosing solutions were usually prepared the day before the beginning of the study Phase. They were stored in a refrigerator (at 4°C) for the entire length of the study Phase. The dosing solutions were vortexed just prior to gavage.

CHEMICAL ANALYSIS

Summary:

Monoethanolamine, diethanolamine and triethanolamine were analyzed by gas chromatography with a flame inoization dectector (GC/FID).

Solubility and Extraction

1. Monoethanolamine, diethanolamine and triethanolamine were found to be soluble in water and were injected on the GC directly from the aqueous solution.

Gas Chromatographic Conditions

	Monoethanolamine	Diethanolamine	Triethanolamine
Dectectors	Flame ionization	Flame ionization	Electron Capture
Dectector Temperature	300°C	300°C	250°C
Column (Glass)	Tenax 60/80 6 ft/4 mm	Tenax 60/80 6 ft/4 mm	SP 2100 6 ft/4 mm
Sample Injection (Syringe)	2-ul	2-ul	2-ul
Injection Temperature	250°C	250°C	250°C
Initial Temperature	150°C	175°C	165°C
Final Temperature	120°C	260°C	165°C (Isothermal)
Rate	12°C/min	12°C/min	-----
Flow Rate	30 ml/min Nitrogen	30 ml/min Nitrogen	30 ml/min 95% Argon 5% Methane
Concentration Range*	1mg - 100mg/ml In Water	1mg - 100mg/ml In Water	1mg/ml in Methanol (Higher Concentration, Bad Reproducibility)

* = Any concentration above the range of the gas chromatograph was diluted into the range of the gas chromatograph with the appropriate solvent.

APPENDIX 2 (CONT.)

RESULTS OF ANALYSIS - PHASE I

DOSE LEVEL:	BEFORE DOSING	AFTER DOSING
Monoethanolamine:	Expressed in mg/ml	
10 mg/kg	0.99	0.93
100 mg/kg	9.60	10.30
1000 mg/kg	101.60	107.50
Diethanolamine:	Expressed in mg/ml	
10 mg/kg	0.90	0.80
100 mg/kg	10.10	11.00
1000 mg/kg	98.70	108.00
Triethanolamine:	Expressed in mg/ml	
10 mg/kg	1.20	1.20
100 mg/kg	11.70	8.10
1000 mg/kg	98.70	108.00

RESULTS OF ANALYSIS - PHASE II

DOSE LEVEL:	BEFORE DOSING	AFTER DOSING
Monoethanolamine:	Expressed in mg/ml	
45 mg/kg	4.41	4.54
85 mg/kg	8.40	8.78
160 mg/kg	18.05	18.31
305 mg/kg	31.55	30.22
575 mg/kg	58.45	53.47
Monoethanolamine:	(Repeat) Expressed in mg/ml	
500 mg/kg	50.0	46.1
579 mg/kg	59.5	55.1
671 mg/kg	66.8	65.7
777 mg/kg	78.8	76.8
900 mg/kg	90.6	88.6
Diethanolamine:	Expressed in mg/ml	
200 mg/kg	20.37	20.80
380 mg/kg	37.62	35.57
720 mg/kg	71.86	71.36
1370 mg/kg	132.57	131.01
2605 mg/kg	269.90	262.86
Triethanolamine:	Expressed in mg/ml	
600 mg/kg	58.20	64.70
1200 mg/kg	129.04	113.07
2400 mg/kg	254.39	301.70
4800 mg/kg	477.95	459.19
9600 mg/kg	891.22	1054.00

RESULTS OF ANALYSIS - PHASE III

Animals were dosed at constant volume of 10 mg/kg.

Monoethanolamine: Expressed in mg/ml.

Dose Level: 850 mg/kg

Before Dosing	After Dosing
86.01 mg/ml	88.25 mg/ml

Diethanolamine: Expressed in mg/ml.

Dose Level: 450 mg/kg

Before Dosing	After Dosing
43.20 mg/ml	44.81 mg/ml

Triethanolamine: Expressed in mg/ml.

Dose Level: 1250 mg/kg

Before Dosing	After Dosing
113.27 mg/ml	109.43 mg/ml