R1751

ENVIRONMENTAL HEALTH RESEARCH & TESTING, INC.

FINAL REPORT

SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS

BENZETHONIUM CHLORIDE (CAS NO. 121-54-0)
3-ETHOXY-1-PROPANOL (CAS NO. 111-35-3)
ACETONE (CAS NO. 67-64-1)

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

Submitted to:
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16. Abstract (Limit: 200 words) Benzethonium-chloride (121540), 3-ethoxy-1-propanol (111353), and acetone (67641) were screened for their ability to cause adverse reproductive effects as evidenced through the postnatal mouse screening test. Phases one and two were intended to identify the appropriate dose levels, while phase three was intended to determine reproductive effects. Treatment was administered to female CD-1-mice by gavage on 5 consecutive days during phase one and on gestation days six through 15 in phases two and three. Benzethonium-chloride caused excessive maternal mortality and a decrease in the reproductive index, in the percent survival of the pups and in the birth weight of the pups but had no effect on litter size or weight gain. 3-Ethoxy-1-propanol caused a high maternal mortality and a decrease in the reproductive index, in the percent survival of the pups and in the birth weight of the pups. No effect was observed on litter size or weight gain. Acetone did not cause an increase in maternal mortality but did increase the weight gain by the pups and decreased the reproductive index, the percent survival of the pups and the birth weight of the pups. No effect was noted on litter size.

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SCREENING OF PRIORITY CHEMICALS FOR REPRODUCTIVE HAZARDS

Contract No.: 200-85-2735

EHRT Project No.: ETOX-85-1002

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QUALITY ASSURANCE

FINAL REPORT STATEMENT

PROJECT NUMBER ETOX-85-1002 was inspected on the following dates: 8/6/85, 8/14/85, 9/13/85, 10/22/85, and 3/3/87 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: 8/6/85, 8/14/85, 9/13/85, 10/23/85, and 3/3/87. 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.

Kelly Bailes 4-22-87

QUALITY ASSURANCE UNIT DATE

Q. A. INSPECTIONS

PROJECT NO. ETOX-85-1002

EVENT	DATE INSPECTED	REPORTED TO STUDY DIRECTOR	INITIALS
Report Review	8/6/85	8/6/85	WB
Dose Preparation	8/14/85	8/14/85	WB
Sacrifice	9/13/85	9/13/85	WB
Dosing	10/22/85	10/23/85	WB
Final Report	3/3/87	3/3/87	WB

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APPENDIX

SPONSOR: NIOSH INITIATION DATE: July 8, 1985

MATERIALS: Priority Chemicals TERMINATION DATE: October 30, 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 benzethonium chloride, 3-ethoxy-1-propanol and acetone.

In Phase I, benzethonium chloride, 3-ethoxy-1-propanol and acetone were administered on 5 consecutive days at 10, 100, or 1000 mg/kg/day. All three animals died in the 1000 mg/kg/day group for benzethonium chloride. There was no mortality in the other benzethonium chloride groups or in any of the 3-ethoxy-1-propanol or acetone groups. The dose levels for Phase II were determined from Appendix 1. Dose range number 4 was selected for benzethonium chloride. Dose range number 1 was selected for 3-ethoxy-1-propanol and for acetone. The highest dose level for acetone (9600 mg/kg) could not be achieved so that pure acetone was used for the high dose level. This calculated to 8036 mg/kg/day. Based on the probit analysis of the mortality data from Phase II, using the Statistical Analysis System (SAS) at NIOSH, the predicted LD_{10}s used for the Phase III reproduction study were as follows: 150 mg/kg/day for benzethonium chloride, 3000 mg/kg/day for 3-ethoxy-1-propanol, and 3500 mg/kg/day for acetone.

In Phase III, it is recommended that benzethonium chloride be retested at a lower dose due to excessive maternal mortality (59%). 3-Ethoxy-1-propanol is judged to have limited evidence of activity in the Chernoff/Kavlock preliminary developmental toxicity test at a dose that exhibited sufficient maternal toxicity. Acetone was judged positive in the Chernoff/Kavlock preliminary developmental toxicity test.

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 confound 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
Benzethonium Chloride	Radian Corp.	121-54-0	3/6/85
3-Ethoxy-1 Propanol	Radian Corp.	111-35-3	3/6/85
Acetone	Radian Corp.	67-64-1	3/6/85

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

Each test article was mixed 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 identity of the test article name and were blind to the dose levels. Each test article was identified by a number and a corresponding color. This technique allowed for nonbias of all 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 chemicals have been confirmed by the Radian Corporation. The compilation of stability, toxicity, storage, and safe handling

information was sent by Radian Corporation to EHRT. The vehicle was distilled water.

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 by water bottle. The mice were maintained on a 12-hour light/dark cycle in a temperature controlled room (72°± 3 F). Hepafiltered air was supplied at a rate of at least 10 room air changes per hour. 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 was 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 pups of a litter were weighed together to give a litter weight and the average individual weight of the litter 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 dams were 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.

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 18) ANOVA (2-tail)
  (all groups, pregnant dams)
  (all groups, viable litters only)
- Survival (Table 16) Fisher's Exact Test (one-tail)
  (each group vs control, all dams)
  (each group vs control, pregnant only) (Table 17B)
- Weight gains (Table 19) 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 (Table 20) (one-tail) (each group vs control, pregnant only)
- Survival of pups Chi-Square Test (one-tail) Table 20 (each group vs control)

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 20
- Length of gestation (Table 21)
- Average pup weight (day 0, day 3) Table 22
- Average wt. gain/litter (day 3 - day 0) Table 22

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

RESULTS - RANGE FINDING

(PHASE I)

In the third Phase I (range finding) experimental block benzethonium chloride, 3-ethoxy-1-propanol and acetone were evaluated. The three test chemicals were administered to three (3) groups of mice, using three mice per group. Doses of 10, 100, and 1000 mg/kg/day was 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 benzethonium chloride, in Table 3 for 3-ethoxy-1-propanol and in Table 5 for acetone.

Mortality:

Tables 2, 4 and 6 present the number of deaths for benzethonium chloride, 3-ethoxy-1-propanol and acetone, respectively. Benzethonium chloride resulted in the death of all three mice by treatment day 3 in the 1000 mg/kg of body weight/day group. These deaths did not appear to be caused by dosing trauma. There were no deaths in the lower two dose level groups of benzethonium chloride, or in any 3-ethoxy-1-propanol or acetone group.

Clinical Signs:

Benzethonium chloride 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, squinted eyes, rough haircoat, and fur stained in the vaginal area. Two animals were found dead on treatment day 2; one was found 1 hour after dosing and the other was found during the afternoon observation. One animal was found dead at the morning observation on treatment day 3. None of these deaths appeared to be caused by dosing trauma. No animals in the 10 and 100 mg/kg/day dose levels exhibited signs of systemic toxicity. No signs of systemic toxicity or death were produced at the 10, 100 or 1000 mg/kg of body weight/day dose levels of 3-ethoxy-1-propanol and acetone.

Conclusion:

Because Benzethonium chloride produced three deaths in the 1000 mg/kg/day dose level, the Phase II dose levels selected from Appendix 1 were 45, 85, 160, 305, and 575 mg/kg/day.

3-Ethoxy-1-propanol and acetone 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: Benzethonium Chloride
CAS No. 121-54-0

TABLE 1

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

	DOSE LEVEL (mg/kg)		
	10	100	1000
TREATMENT DAYS			
1	25.6 ± 0.92	24.9 ± 1.39	25.5 ± 0.53
5	26.2 ± 0.81	24.3 ± 1.52	All Dead
POST TREATMENT DAYS			
3	27.6 ± 0.88	25.5 ± 1.53	All Dead
7	28.2 ± 1.35	26.4 ± 1.54	All Dead
CHANGES			
TD 5 - TD 1	+0.6	-0.6	All Dead
PTD 7 - TD5	+2.0	+2.1	All Dead
PTD 7 - TD1	+2.6	+1.5	All Dead

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	2	1	-	-	-	3

PHASE I

Test Chemical Identification: 3-Ethoxy-1-Propanol
CAS No. 111-35-3

TABLE 3

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

	DOSE LEVEL (mg/kg)		
	10	100	1000
TREATMENT DAYS			
1	25.1 ± 0.93	25.1 ± 1.04	25.1 ± 0.65
5	24.8 ± 1.21	25.5 ± 1.05	26.1 ± 0.70
POST TREATMENT DAYS			
3	25.6 ± 1.62	25.9 ± 1.45	26.4 ± 0.08
7	26.7 ± 1.85	26.3 ± 1.56	27.4 ± 0.67
CHANGES			
TD 5 - TD 1	-.03	+0.4	+1.0
PTD 7 - TD 5	+1.9	+0.8	+1.3
PTD 7 - TD1	+1.6	+1.2	+2.3

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	0	0
1000	0	0	0	0	0	0	0

PHASE I

Test Chemical Identification: Acetone
CAS No. 67-64-1

TABLE 5

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

	DOSE LEVEL (mg/kg)		
	10	100	1000
TREATMENT DAYS			
1	25.0 ± 0.26	25.1 ± 0.81	24.9 ± 0.57
5	25.5 ± 0.43	24.7 ± 0.18	24.8 ± 1.12
POST TREATMENT DAYS			
3	26.8 ± 0.86	24.8 ± 0.63	25.8 ± 1.10
7	28.3 ± 1.49	26.3 ± 0.48	26.8 ± 1.19
CHANGES			
TD 5 - TD 1	+0.5	-0.4	-0.1
PTD 7 - TD 5	+2.8	+1.6	+2.0
PTD 7 - TD1	+3.3	+1.2	+1.9

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)

In the Phase II (range finding) experimental block benzethonium chloride, 3-ethoxy-1-propanol and acetone were evaluated. The highest dose level for 3-ethoxy-1-propanol and acetone (9600 mg/kg) could not be achieved so the pure chemicals were used for the high dose level. The density for acetone was not provided by the Radian Corporation, therefore, it was determined to be 0.7908 g/ml (Reference, Weast, R.C., Selby, S.M., and Hodgman, C.D., eds. Handbook of Chemistry and Physics, The Chemical Rubber Co., Cleveland, Ohio, 1964.). This calculated to 7908 mg/kg/day. The density for 3-ethoxy-1-propanol was provided by the Radian Corporation and was reported to be 0.904 g/ml. The high dose level was determined to be 9040 mg/kg/day. 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: benzethonium chloride, Tables 7 and 8; 3-ethoxy-1-propanol, Tables 10 and 11; and acetone, Tables 13 and 14.

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 Table 9 (benzethonium chloride), Table 12 (3-ethoxy-1-propanol), and Table 15 (acetone).

Clinical Signs:

When benzethonium chloride 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)	
45	On treatment day 4, one animal was noted as having a rough haircoat, hunched posture, and languid behavior. This was the only systemic sign noted; all other animals appeared normal.
85	On treatment day 4, one animal exhibited a rough haircoat, wheezing, and languid behavior. This was the only deviation from normal noted for these animals. All other animals appeared normal.
160	On post-treatment day 1, one animal exhibited a rough haircoat, stained fur (ano-genital region), hunched posture, and labored respiration. One animal was found dead during the afternoon observation of post-treatment day 1. This death did not appear to be caused by dosing trauma.

Dose Level (mg/kg)	
305	Animals at this dose level exhibited a rough haircoat, wheezing, hunched posture, languid behavior, rapid respiration, labored respiration, squinted eyes, and one animal was prostrate. One animal was found dead during the afternoon observation on treatment day 7. One animal was found dead during the morning observation on post-treatment day 1. These deaths did not appear to be caused by dosing trauma.
575	All animals in this dose group died. Three animals were found dead during the afternoon observation period of treatment day 3. The remaining animal was found dead during the morning observation of treatment day 4. These deaths did not appear to be caused by dosing trauma.

When 3-ethoxy-1-propanol 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	All animals in this dose group appeared normal.
1200	All animals at this dose level appeared normal.
2400	All animals in this dose group appeared normal.
4800	All animals at this dose level died. One animal was found dead during the morning observation on treatment day 2 and two animals were found dead during the

Dose Level (mg/kg)	
4800	afternoon observation of the same day. The remaining animal was found dead during the afternoon observation of treatment day 4. None of these deaths appeared to be caused by dosing trauma.
9040	All animals at this dose level were found dead during the morning observation on treatment day 2. None of these deaths appeared to be caused by dosing error.

When acetone 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	One animal at this dose level appeared to be hyperactive on treatment day 3. This was the only deviation from normal noted at this dose level.
1200	All animals at this dose level appeared normal.
2400	One animal at this dose level exhibited languid behavior and squinted eyes. This animal was found dead during the afternoon observation on treatment day 7. This death does not appear to be caused by dosing trauma.
4800	Signs of systemic toxicity noted at this dose level included: rough haircoat, squinted eyes, hunched posture, wheezing, prostrate, and death. One animal was found dead during the afternoon observation of treatment

Dose Level (mg/kg)	
4800	day 4. This death did not appear to be caused by dosing trauma. All surviving animals were normal by post-treatment day 2.
7908	All animals in this dose level died. Signs of toxicity included: rough haircoat, labored respiration, lacrimation, hunched posture, rapid respiration, wheezing, prostrate, and death. One animal was found dead during the afternoon observation of treatment day 2. The remaining animals were found dead during the afternoon observation of treatment day 4. None of these deaths appeared to be caused by dosing error.

Conclusion:

Based on probit analysis of the mortality data of Phase II, the predicted LD_{10} doses were selected for the Phase III test were benzethonium chloride, 150 mg/kg; 3-ethoxy-1-propanol, 3000 mg/kg; and acetone, 3500 mg/kg.

PHASE II

Test Chemical Identification: Benzethonium Chloride
CAS No. 121-54-0

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	29.36 ± --	28.94 ± 1.39	28.94 ± 1.84	28.51 ± 1.93	28.71 ± 0.71
n	1	3	4	4	4
2	29.24 ± --	29.05 ± 1.80	28.75 ± 1.79	27.75 ± 1.91	28.25 ± 0.90
n	1	3	4	4	4
3	30.58 ± --	29.45 ± 2.33	28.87 ± 2.27	28.50 ± 2.44	24.89 ± 0.88
n	1	3	4	4	3
4	30.08 ± --	28.41 ± 3.70	29.43 ± 2.97	27.19 ± 1.90	All Dead
n	1	3	4	4	0
5	30.82 ± --	28.46 ± 4.54	29.90 ± 2.85	25.45 ± 2.90	All Dead
n	1	3	4	4	0
6	32.14 ± --	29.55 ± 2.93	30.62 ± 3.26	25.65 ± 5.13	All Dead
n	1	3	4	4	0
7	33.18 ± --	30.11 ± 2.19	31.65 ± 3.83	26.80 ± 5.73	All Dead
n	1	3	4	4	0
8	35.40 ± --	31.29 ± 2.04	32.63 ± 4.84	28.97 ± 4.47	All Dead
n	1	3	4	3	0
9	37.10 ± --	31.35 ± 1.95	32.37 ± 5.53	28.93 ± 7.98	All Dead
n	1	3	4	3	0
10	39.52 ± --	31.73 ± 4.74	30.97 ± 3.59	28.10 ±10.32	All Dead
n	1	3	4	3	0
POST TREATMENT DAY					
2	38.74 ± --	33.20 ± 8.16	29.50 ± 4.88	35.39 ±12.46	All Dead
n	1	3	3	2	0

n - number of pregnant animals in each dose level

PHASE II

Test Chemical Identification: Benzethonium Chloride
CAS No. 121-54-0

TABLE 8

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

	Dose Level (mg/kg)				
Benzethonium Chloride	45	85	160	305	575
Treatment day 10 - treatment day 1	+10.16	+ 2.79	+2.03	-0.41	0[a]
Post-treatment day 2 - treatment day 10	- 0.78	+ 1.47	-1.47	+7.29	0[a]
Post-treatment day 2 - treatment day 1	+ 9.38	+ 4.26	+0.56	+6.88	0[a]

[a] - all animals dead by treatment day 4

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	1	2	4
Ammonium Sulfide Positive	-	-	1	2	4
Ammonium Sulfide Negative	-	-	-	-	-
Animals Sacrificed	4	4	3	2	0
Live Pups	1	2	2	1	-
Ammonium Sulfide Positive	-	1	1	1	-
Ammonium Sulfide Negative	3	1	-	-	-

PHASE II

Test Material Identification: 3-Ethoxy-1-Propanol
CAS No. 111-35-3

TABLE 10

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	9040
1	27.89 ± 1.17	27.69 ± 2.15	30.05 ± 2.33	28.43 ± 1.06	28.59 ± 0.96
n	3	3	2	3	4
2	28.73 ± 1.78	28.51 ± 2.08	29.73 ± 3.24	26.80 ± 0.79	27.79 ± 1.39
n	3	3	2	2	4
3	29.29 ± 2.72	28.86 ± 1.84	30.37 ± 4.12	30.20 ± --	All Dead
n	3	3	2	1	0
4	30.15 ± 2.97	29.43 ± 1.91	30.36 ± 4.64	30.84 ± --	All Dead
n	3	3	2	1	0
5	31.22 ± 3.05	30.94 ± 1.89	30.92 ± 5.12	All Dead	All Dead
n	3	3	2	0	0
6	32.75 ± 2.26	32.11 ± 2.01	31.23 ± 5.64	All Dead	All Dead
n	3	3	2	0	0
7	34.31 ± 2.11	34.29 ± 2.36	32.02 ± 6.05	All Dead	All Dead
n	3	3	2	0	0
8	35.69 ± 2.02	36.39 ± 3.34	32.95 ± 7.03	All Dead	All Dead
n	3	3	2	0	0
9	37.44 ± 2.40	38.06 ± 2.70	34.04 ± 8.32	All Dead	All Dead
n	3	3	2	0	0
10	39.54 ± 2.09	40.39 ± 2.75	33.49 ±10.79	All Dead	All Dead
n	3	3	2	0	0
POST TREATMENT DAY					
2	44.90 ± 2.32	45.55 ± 4.31	36.50 ±11.94	All Dead	All Dead
n	3	3	2	0	0

n - number of pregnant animals in each dose level

PHASE II

Test Material Identification: 3-Ethoxy-1-Propanol
CAS No. 111-35-3

TABLE 11

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

	Dose Level (mg/kg)				
3-Ethoxy-1-Propanol	600	1200	2400	4800	9040
Treatment day 10 - treatment day 1	+11.65	+12.70	+3.44	0[a]	0[b]
Post-treatment day 2 - treatment day 10	+ 5.36	+ 5.16	+3.01	0[a]	0[b]
Post-treatment day 2 - treatment day 1	+17.01	+17.86	+6.45	0[a]	0[b]

[a] - all animals dead by treatment day 5
[b] - all animals dead by treatment day 3

TABLE 12

MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg/day)				
	600	1200	2400	4800	9040
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	0	0	0	4	4
Ammonium Sulfide Positive	-	-	-	3	4
Ammonium Sulfide Negative	-	-	-	1	-
Animals Sacrificed	4	4	4	0	0
Live Pups	3	3	1	-	-
Ammonium Sulfide Positive	-	-	1	-	-
Ammonium Sulfide Negative	1	1	2	-	-

PHASE II

Test Chemical Identification: Acetone
CAS No. 67-64-1

TABLE 13

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	7908
1	29.36 ± 1.79	28.36 ± 0.44	27.88 ± 1.07	29.45 ± 1.45	29.85 ± 2.54
n	4	4	2	3	3
2	29.22 ± 1.51	28.21 ± 0.72	27.66 ± 1.87	28.22 ± 0.92	27.30 ± 1.65
n	4	4	2	3	3
3	29.72 ± 1.82	29.69 ± 0.99	29.66 ± 0.03	28.05 ± 2.35	24.26 ± 2.26
n	4	4	2	3	2
4	29.94 ± 2.22	29.36 ± 0.47	29.78 ± 0.20	27.19 ± 4.98	23.61 ± 2.70
n	4	4	2	3	2
5	30.91 ± 1.96	30.49 ± 0.62	31.45 ± 0.07	30.83 ± 0.16	All Dead
n	4	4	2	2	0
6	31.77 ± 2.20	31.79 ± 0.68	31.71 ± 0.58	32.92 ± 0.54	All Dead
n	4	4	2	2	0
7	33.13 ± 2.00	33.44 ± 0.45	33.10 ± 1.87	34.19 ± 0.98	All Dead
n	4	4	2	2	0
8	34.92 ± 2.38	34.76 ± 1.03	34.16 ± 1.47	35.99 ± 0.69	All Dead
n	4	4	2	2	0
9	36.53 ± 2.77	36.39 ± 0.81	35.89 ± 1.23	36.68 ± 0.06	All Dead
n	4	4	2	2	0
10	38.86 ± 3.51	38.75 ± 1.11	36.94 ± 2.57	36.33 ± 2.42	All Dead
n	4	4	2	2	0
POST TREATMENT DAY					
2	43.14 ± 3.93	44.41 ± 1.33	41.91 ± 2.62	39.99 ± 2.98	All Dead
n	4	4	2	2	0

n - number of pregnant animals in each dose level

PHASE II

Test Chemical Identification: Acetone
CAS No. 67-64-1

TABLE 14

AVERAGE MATERNAL BODY WEIGHT CHANGES (IN GRAMS)

	Dose Level (mg/kg)				
Acetone	600	1200	2400	4800	7908
Treatment day 10 - treatment day 1	+ 9.50	+10.39	+ 9.06	+ 6.88	0[a]
Post-treatment day 2 - treatment day 10	+ 4.28	+ 5.66	+ 4.97	+ 3.66	0[a]
Post-treatment day 2 - treatment day 1	+13.78	+16.05	+14.03	+10.54	0[a]

[a] - all animals dead by treatment day 5.

TABLE 15

MORTALITY AND
PREGNANCY STATUS

	DOSE LEVEL (mg/kg/day)				
	600	1200	2400	4800	7908
Number of Animals Treated	4	4	4	4	4
Animals Found Dead	0	0	1	1	4
Ammonium Sulfide Positive	-	-	-	1	3
Ammonium Sulfide Negative	-	-	1	-	1
Animals Sacrificed	4	4	3	3	0
Live Pups	4	4	2	2	-
Ammonium Sulfide Positive	-	-	-	-	-
Ammonium Sulfide Negative	-	1	1	1	-

RESULTS - REPRODUCTIVE PHASE

Benzethonium Chloride, 3-Ethoxy-1-Propanol, Acetone

Based on the probit analysis of the Phase II mortality data using the Statistical Analysis System (SAS) at NIOSH, the LD_{10} doses selected for the Phase III reproduction study were 150 mg/kg/day (benzethonium chloride), 3000 mg/kg/day (3-ethoxy-1-propanol), and 3500 mg/kg/day (acetone). The vehicle control group received distilled water. All animals were dosed at a constant volume of 10 ml/kg/day.

Mortality and Survival:

A mortality table showing all deaths by individual treatment days is presented in Table 16. Benzethonium chloride and 3-ethoxy-1-propanol caused a statistically significant ($p=<0.01$) increase in the mortality of the dams. The mortality in the acetone group did not differ from the control group.

A maternal status and survival table showing all pregnant animals are presented in Tables 17A and 17B. Benzethonium chloride and 3-ethoxy-1-propanol caused a statistically significant ($p\leq0.001$ and $p=\leq0.01$, respectively) decrease in the percent survival of the pregnant dams. Acetone did not affect the survival of the pregnant dams.

Clinical Observations:

One animal in the vehicle control group, distilled water, was found dead on post treatment day 3. On treatment day 5 the animal was noted as having hunched posture and a rough haircoat and on post

treatment day 1 the animal was prostrate. A gross necropsy was performed and the death did not appear to be caused by dosing trauma. All other control animals appeared normal throughout the study.

Benzethonium Chloride produced clinical signs of toxicity and death. Clinical signs noted in this group included: hunched posture, languid behavior, wheezing, labored respiration, rough haircoat, tremors, squinted eyes, blood-like stains (ano-genital area), prostrate, and two animals were noted as being underweight. Thirty-one animals in this group died; six animals died by dosing trauma and twenty-five animals died of toxicity.

3-Ethoxy-1-Propanol also produced clinical signs of toxicity and death. Signs of toxicity included: prostration, rapid respiration, languid behavior, rough haircoat, unsteady gait, labored respiration, and hunched posture. Thirteen animals died in this dose group; two died from apparent dosing trauma and eleven died of toxicity.

Three animals died in the acetone group. Two died of toxicity and one died from dosing trauma. Clinical signs noted in this group included: lacrimation, wheezing, rough haircoat, prostration, labored respiration, squinted eyes, and one animal was noted as being underweight. All surviving animals appeared normal throughout the study.

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 18 and 19. The randomization weights between groups were not significantly different. Benzethonium chloride caused a

significant reduction in maternal body weight at day 3 postpartum ($p \leq 0.001$) and acetone appeared to reduce slightly the maternal body weights at day 3 postpartum ($p=0.02$). There were no other differences among the groups in the postpartum maternal body weights, in the randomization body weights,or in weight changes over the selected intervals.

Reproductive Index and Litter Data:

The reproductive index, average number of live pups/litter (day 0 and day 3) and postnatal mortality are presented in Table 20. Benzethonium chloride and 3-ethoxy-1-propanol caused a significant reduction in the reproduction index ($p \leq 0.001$ and $p \leq 0.01$, respectively), while acetone only produced a suggestion of a reduced reproductive index ($p=0.05$). There were no differences among the number of live pups/litter in any of the treatment groups on day 0 or day 3. The pup survival rate (day 0 to day 3) was significantly decreased from the control for all treated groups.

Duration of Gestation:

Average gestation length is presented by group in Table 21. All three test chemicals significantly increased the duration of gestation.

Weight Data:

The average weight of each live pup/litter (day 0 and day 3), and average pup weight changes (day 3 - day 0) are presented in Table 22. Day 0 pup weights were significantly reduced in all 3 treatment

groups: benzethonium chloride (p=0.02), 3-ethoxy-1-propanol (p=0.02), and acetone (p=0.01). Pups from the acetone treatment group gained significantly more weight between day 0 and day 3.

Conclusion:

Benzethonium Chloride

Benzethonium chloride caused excessive maternal mortality and a decrease in the reproductive index, in the percent survival of the pups and in the birth weight of the pups. There was no effect upon litter size and weight gain by the pups. Using the ranking system of Hardin (Hardin, 1987[4]) with a maximum score of 22 and for a high maternal mortality the following scores are subtracted: viable litters (0); litter size (4); percent survival of the pups (0); birth weight of the pups (0); and weight gain of the pups (4). Thus, the priority score for benzethonium chloride is 14 which represent an intermediate priority. However, due to the excessive maternal mortality (59%) and the small number of surviving dams (13) and of viable litters (6), it is recommended that benzethonium chloride be retested at a lower dose. In summary, benzethonium chloride is judged provisionally positive in the Chernoff/Kavlock preliminary developmental toxicity test until retested at a lower dose level.

3-Ethoxy-1-Propanol

3-Ethoxy-1-propanol caused a high maternal mortality, and a decrease in the reproductive index, in the percent survival of the pups and in the birth weight of the pups. There was no effect upon litter size and weight gain by the pups. Using the ranking system of Hardin (Hardin, 1987[4]) with a maximum score of 22 and for high

maternal toxicity the following values should be subtracted: viable litters (0); litter size (4); percent survival of pups (0); birth weight of pups (0); and weight gain of pups (4). Thus, the score for 3-exthoxy-1-propanol is 14 which represents intermediate priority. A previous test of 3-ethoxy-1-propanol also performed at 3000 mg/kg/day, resulted in a rank score of 4 with a 26% maternal mortality (Hardin, 1987[4]). In both test a similar high maternal mortality of either 21 or 26% was obtained with a 3000 mg/kg/day dose which although higher than desired (10-20%) is not excessive. It is interesting that in our study a reduction in the reproductive index, in the percent survival of the pups and in the birth weight of the pups was observed while in the other study only a reduced litter size was observed. Therefore, the parameters affected by 3-ethoxy-1-propanol were different in the two assays indicating that the affects might not be reproducible. In summary, 3-ethoxy-1-propanol is judged to have limited evidence of activity in the Chernoff/Kavlock preliminary developmental toxicity test at a dose that exhibited sufficient maternal toxicity so that further testing is not warranted.

Acetone

Acetone did not cause an increase in maternal mortality but did increase the weight gain by the pups, decrease the reproductive index, the percent survival of the pups and the birth weight of the pups. There was no effect on litter size. Using the ranking system of Hardin (Hardin, 1987[4]) with 22 as the maximum score and for intermediate maternal mortality the following scores should be subtracted: viable litters (0); litter size (3); percent survival of

pups (0); birth weight of pups (0); and weight gain of pups (0). Therefore, the score for acetone is 19 which indicates high priority for testing in a conventional developmental toxicity test. In summary, acetone was judged to be positive 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 16

MORTALITY
NUMBER OF ANIMALS DEAD PER GROUP[a]

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
Benzethonium Chloride (150 mg/kg/day)	0	1[b]	2[c]	1	4[c]	2	2	5[b]	5	3	25
3-Ethoxy-1-Propanol (3000 mg/kg/day)	1[b]	2	0	1	2[b]	0	2	0	3	1	12
Acetone (3500 mg/kg/day)	0	0	0	0	0	1	0	0	0	0	1

DOSE GROUP	POST-TREATMENT DAYS					SUMMARY OF DEATHS		
	1	2	3	4	5	SACRIFICE	TOXICITY	TRAUMA
Vehicle Distilled Water	0	0	1	0	0	49	1	0
Benzethonium Chloride (150 mg/kg/day)	2	3	0	1	0	19	25**	6
3-Ethoxy-1-Propanol (3000 mg/kg/day)	1	0	0	0	0	37	11*	2
Acetone (3500 mg/kg/day)	0	1	0	0	1[b]	47	2	1

[a] - 50 dams per group
[b] - one death caused by dosing trauma
[c] - two deaths caused by dosing trauma
* - $p<0.01$
** - $p<0.001$

TABLE 17A

MATERNAL STATUS

	TEST CHEMICAL			
	Vehicle Water	Benzethonium Chloride	3-Ethoxy-1 Propanol	Acetone
Number Evaluated	50	44	48	49
Animals Found Dead	1	25	11	2
Pregnant	1	19	5	2
Never Pregnant[c]	0	6	6	0
Animals Delivered Live Pups	34	6	16	24
Animals Sacrificed[a]	15	13	21	23
No Live Pups[b]	2	7	7	7
Never Pregnant[c]	13	6	14	16

a - Number of animals sacrificed - no live pups delivered
b - Visually dead pups or ammonium sulfide positive
c - Ammonium Sulfide Negative

TABLE 17B

MATERNAL SURVIVAL

	PREGNANT ANIMALS		
TEST CHEMICAL	LIVED	DIED	PERCENT SURVIVAL
Vehicle Distilled Water	36	1	97%
Benzethonium Chloride (150 mg/kg/day)	13	19	41%**
3-Ethoxy-1-Propanol (3000 mg/kg/day)	23	5	82%*
Acetone (3500 mg/kg/day)	31	2	94%

* - p=0.049
** - p<0.001

TABLE 18

AVERAGE MATERNAL BODY WEIGHTS IN GRAMS
(MEAN ± STANDARD DEVIATION)

RANDOMIZATION WEIGHTS	VEHICLE WATER	BENZETHONIUM CHLORIDE 150 mg/kg/day	3-ETHOXY-1 PROPANOL 3000 mg/kg/day	ACETONE 3500 mg/kg/day
PREGNANT FEMALES ONLY	26.0 ± 0.93	26.0 ± 1.27	25.7 ± 1.01	26.0 ± 1.15
n[a]	36	13	23	31
GESTATION DAYS				
PREGNANT FEMALES ONLY				
6	28.2 ± 1.08	28.3 ± 1.52	28.3 ± 1.24	28.7 ± 1.35
n	36	13	23	31
7	29.2 ± 1.28	28.4 ± 1.73	28.4 ± 1.21	29.0 ± 1.24
n	36	13	23	31
8	30.0 ± 1.42	27.6 ± 2.65	29.3 ± 1.25	29.4 ± 1.56
n	36	13	23	31
9	30.1 ± 1.59	26.2 ± 3.72	29.0 ± 1.22	29.1 ± 1.90
n	36	13	23	31
10	30.8 ± 1.77	26.2 ± 4.38	30.2 ± 2.02	29.7 ± 2.30
n	36	13	23	31
11	32.5 ± 2.00	27.2 ± 4.32	31.3 ± 2.47	30.9 ± 2.86
n	36	13	23	31
12	34.1 ± 2.33	27.6 ± 5.01	32.4 ± 3.00	31.8 ± 3.51
n	36	13	23	31
13	35.6 ± 2.69	26.6 ± 5.56	33.3 ± 3.83	33.0 ± 4.02
n	36	13	23	31
14	37.4 ± 3.13	27.6 ± 5.79	34.6 ± 4.54	34.1 ± 4.95
n	36	13	23	31
15	39.8 ± 3.92	29.2 ± 7.10	35.8 ± 5.30	36.0 ± 5.58
n	36	13	23	31
17	45.6 ± 5.53	31.3 ± 9.61	39.4 ± 7.59	40.8 ± 7.78
n	36	13	23	31
POSTPARTUM DAY				
0	30.9 ± 1.57	30.3 ± 1.21	30.2 ± 1.47	30.3 ± 1.65
n	34	6	16	24
3	34.8 ± 2.83	30.9 ± 1.56**[b]	34.1 ± 2.39	33.0 ± 3.04*
n	34	6	16	24

[a] - n = number of animals

[b] - The postpartum body weights were analyzed statistically by the Mann-Whitney U-test with * $p \leq 0.05$; ** $p \leq 0.001$.

TABLE 19

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

	WATER	BENZETHONIUM CHLORIDE	3-ETHOXY-1 PROPANOL	ACETONE
Random Weight Pregnant Only	26.0 ± 0.93[b]	26.0 ± 1.27	25.7 ± 1.01	26.0 ± 1.15
n	36	13	23	31
Random Weight Viable Litters Only	26.0 ± 0.92	25.7 ± 1.28	25.8 ± 1.05	26.1 ± 1.22
n	34	6	16	24
Weight Gain Postpartum - Randomization	+4.9 ± 1.58	+4.6 ± 2.12	+4.4 ± 1.53	+4.2 ± 1.75
n	34	6	16	24

[a] - n = number of animals
[b] - An analysis of variance was performed on the results and no statistical significant difference was observed 1) among the weight at randomization of the groups and 2) for the weight gain postpartum - randomization.

TABLE 20

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	34/36	94.4	10.3 ± 2.78	9.9 ± 3.21	335/350	96
n[c]			34	34		
BENZETHONIUM CHLORIDE						
150 mg/kg	6/13	46.2***[d]	10.0 ± 2.28	6.7 ± 5.43	40/60	67***
n			6	6		
3-ETHOXY-1-PROPANOL						
3000 mg/kg	16/23	69.6**	10.6 ± 1.96	9.6 ± 3.22	153/170	90*
n			16	16		
ACETONE						
3500 mg/kg	24/31	77.4*	10.7 ± 1.46	9.5 ± 3.21	228/257	89**
n			24	24		

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

[d] - Reproductive Index results were analyzed by the Fisher's Exact Test with * denoting $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. The number of live pups/litter were analyzed by the Mann-Whitney U-test and none of the results were different from controls with $p \leq 0.05$.

TABLE 21
DURATION OF GESTATION (IN DAYS)
DAMS THAT PRODUCED VIABLE LITTERS ONLY

	DOSE GROUP			
	Vehicle Water	Benzethonium Chloride 150 mg/kg/day	3-Ethoxy-1-Propanol 3000 mg/kg/day	Acetone 3500 mg/kg/day
Mean	18.1	18.5*[c]	18.7**	18.5**
SD[a]	0.26	0.32	0.44	0.20
n[b]	34	6	16	24

[a] - SD = Standard Deviation
[b] - n = number of animals that produced viable litters
[c] - comparison of exposed groups to the control was evaluated by the Mann-Whitney U-test with ** $p \leq 0.01$ and *** $p \leq 0.001$

TABLE 22

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

DOSE GROUP	AVERAGE WEIGHT/PUP/LITTER (G) DAY 0	DAY 3	CHANGE DAY 3 - DAY 0
Vehicle			
Water	1.5 ± 0.19	2.0 ± 0.29	+0.5 ± 0.22
n[a]	34	33	33
Benzethonium Chloride			
150 mg/kg	1.3 ± 0.16*[b]	1.7 ± 0.32	+0.3 ± 0.16
n	6	4	4
3-Ethoxy-1-Propanol			
3000 mg/kg	1.4 ± 0.10*	1.9 ± 0.28	+0.5 ± 0.24
n	16	15	15
Acetone			
3500 mg/kg	1.4 ± 0.11**	2.1 ± 0.25	+0.7 ± 0.17***
n	24	21	21

[a] - Number of litters with live pups
[b] - Results were analyzed by the Mann-Whitney U-test with * $p \leq 0.05$; ** $p \leq 0.01$; and *** $p \leq 0.001$

APPENDIX 1

Range-Finding Dose Sheet (RFDS)

Phase I

Number of mice dead at: mg/kg/day 10	100	1000	Then Phase 2 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

CHEMICAL ANALYSIS OF DOSING SOLUTIONS

1. Reagents

The test chemicals used in the preparation of the dosing solutions were obtained from Radian Corporation, Austin, Texas.

2. Vehicle

The vehicle used in the preparation of the dosing solutions was distilled 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. The highest dose level for 3-ethoxy-1-propanol and acetone (9600 mg/kg) could not be achieved so the pure chemicals were used for the high dose level. The density for acetone was not provided by the Radian Corporation, therefore, it was determined to be 0.7908 g/ml (Reference, Weast, R.C., Selby, S.M., and Hodgman, C.D., eds. Handbook of Chemistry and Physics, The Chemical Rubber Co., Cleveland, Ohio, 1964.). This calculated to 7908 mg/kg/day. The density for 3-ethoxy-1-propanol was provided by the Radian Corporation. The density was reported to be 0.904 g/ml. The high dose level was determined to be 9040 mg/kg/day.

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

Benzethonium chloride, 3-ethoxy-1-propanol, and acetone were analyzed by gas chromatography with flame ionization detector (GC/FID).

SOLUBILITY AND EXTRACTION

Benzethonium chloride, acetone, and 3-ethyoxy-1-propanol were prepared in water. Aqueous solutions of these compounds were injected on the GC without extraction at the beginning and end of each study phase.

Gas Chromatographic Conditions

	Benzethonium Chloride	3-Ethoxy-1-Propanol	Acetone
Dectectors	Flame Ionization		
Dectector Temperature	350°C		
Column (Glass)	Tenax 60/80 6 ft/4 mm		
Sample Injection (Syringe)	2-ul		
Injection Temperature	250°C		
Initial Temperature	100°C		
Final Temperature	200°C		
Rate	9°C/min		
Flow Rate	32 ml/min Nitrogen		
Concentration Range*	1mg - 100mg/ml In Water		

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

APPENDIX 2

RESULTS OF ANALYSIS - PHASE I

DOSE LEVEL:	BEFORE DOSING	AFTER DOSING
Benzethonium Chloride: Expressed in mg/ml		
10 mg/kg	1.15	1.16
100 mg/kg	9.3	10.5
1000 mg/kg	100.8	101.1
3-Ethoxy-1-Propanol: Expressed in mg/ml		
10 mg/kg	1.03	0.99
100 mg/kg	10.04	10.16
1000 mg/kg	100.6	97.44
Acetone: Expressed in mg/ml		
10 mg/kg	1.01	0.94
100 mg/kg	9.53	9.94
1000 mg/kg	103.7	92.04

RESULTS OF ANALYSIS - PHASE II

DOSE LEVEL:	BEFORE DOSING	AFTER DOSING
Benzethonium Chloride: Expressed in mg/ml		
45 mg/kg	4.2	4.4
85 mg/kg	8.5	8.9
160 mg/kg	16.1	15.8
305 mg/kg	29.6	30.3
575 mg/kg	58.1	56.6
3-Ethoxy-1-Propanol: Expressed in mg/ml		
600 mg/kg	60.2	63.0
1200 mg/kg	155.9	130.1
2400 mg/kg	240.1	238.0
4800 mg/kg	461.5	478.2
9040 mg/kg	904.0	904.0
Acetone: Expressed in mg/ml		
600 mg/kg	59.5	62.9
1200 mg/kg	116.3	119.4
2400 mg/kg	239.9	238.0
4800 mg/kg	462.6	392.5
7908 mg/kg	790.8	790.8

APPENDIX 2 (CONT.)

RESULTS OF ANALYSIS - PHASE III

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

Benzethonium Chloride: Expressed in mg/ml

Dose Level: 150 mg/kg

Before Dosing	After Dosing
15.1 mg/ml	15.6 mg/ml

3-Ethoxy-1-Propanol: Expressed in mg/ml

Dose Level: 3000 mg/kg

Before Dosing	After Dosing
311 mg/ml	297 mg/ml

Acetone: Expressed in mg/ml

Dose Level: 3500 mg/kg

Before Dosing	After Dosing
322 mg/ml	352 mg/ml