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ATTN: TSCA 8(e) Coordinator

RE: Union Carbide Corporation's Submission of March 16, 1994  
Concerning Isobutanol (CASRN 78-83-1) [8EHQ-0394-12950]

Dear Sir or Madam,

As a follow-up to the above-noted submission, Union Carbide Corporation ("Union Carbide") herewith submits the following report:

"Isobutanol: Determination of the Potential for Pseudopregnancy in Female Rats following Acute Peroral Doses," Bushy Run Research Center, BRRC Report 93U1298, March 20, 1995 (120 pgs.).

In the attached report the term "Confidential" may appear. This precautionary statement was for internal use at the time of issuance of the report, and is hereby waived for the purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that publication rights to the contained information are the property of Union Carbide.



89950000169

Very truly yours,

William C. Kuryla, Ph.D.  
Associate Director  
Product Safety

Attachment

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# BUSHY RUN RESEARCH CENTER

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## STUDY TITLE

Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses

## TEST SUBSTANCE

Isobutanol

## DATA REQUIREMENTS

Not Applicable

## AUTHORS

S. M. Christopher and R. C. Myers

**Contains No CBI**

## STUDY COMPLETION DATE

March 20, 1995

## PERFORMING LABORATORY

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## LABORATORY PROJECT ID

93U1298

## SPONSOR

Solvents and Coatings Materials Division  
Union Carbide Corporation  
39 Old Ridgebury Road  
Danbury, CT 06817-0001

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**CONFIDENTIALITY STATEMENT**

This report is Union Carbide Corporation Business Confidential and is not to be released outside of the Corporation without the written consent of the Sponsor.

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS**

The portions of this study conducted at BRRC meet the requirements of Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards, 40 CFR Part 792; Organisation for Economic Co-operation and Development (OECD), C(81)30(Final) with exceptions. The exceptions are:

1. Concentrations of test mixtures used for peroral dosing were verified by gravimetric analysis.
2. Analyses for stability, homogeneity, and concentration verification of the test substance in the dosing emulsions used for peroral dosing were not conducted. All doses were given within 31 minutes after test substance preparation.

These exceptions are not expected to compromise the integrity of the results and conclusions of the study.

Study Director:

Susan M. Christopher  
Susan M. Christopher, B.S.

3-20-95

Date

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**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

SUMMARY

The potential for pseudopregnancy following single peroral doses of isobutanol (CAS No. 78-83-1) to female rats was evaluated.

In the probe study, 5 female rats received a single peroral dose of 2830 mg/kg of test substance (dosed as 28.3% w/v emulsions in 0.25% aqueous methyl cellulose solution) and were kept for a 5-day post-dose observation period, followed by sacrifice. Animals were weighed at 5 or 6 days prior to dose administration, on the day of dosing and at death or sacrifice. Vaginal smears were obtained from all probe animals each day beginning at 5 or 6 days pre-dose until death or sacrifice. Retroorbital blood samples were collected from each animal beginning at 5 or 6 days before the day of dose administration (at least 4 pre-dose samples obtained) and then daily beginning at 1 day post-dose until death or sacrifice unless animals exhibited substantial signs of toxicity at the time of collection. The uterus and ovaries were saved and evaluated microscopically for each rat; the vaginas were only saved in survivors. The ovaries were also weighed.

For the definitive study, single peroral doses of 2830 mg/kg of isobutanol, dosed as 28.3% (w/v) emulsions in aqueous methyl cellulose solution, were administered to a total of 15 female rats. A peroral dose of 10.0 ml/kg of 0.25% (w/v) aqueous methyl cellulose solution was given to each of 6 female control animals. Weights for both test and control animals were recorded at 3 or 4 days prior to dose administration, on the day of dosing and at 7 and 14 days post-dose or death. Beginning at 3 or 4 days prior to dose administration, vaginal smears were obtained daily from each study animal. Blood was collected from all test and control rats the day before and the day after dose administration. Surviving animals were then placed into 3 groups (each with 3 test and 2 control animals) which were bled on a rotating basis until death or sacrifice. All rats were bled at sacrifice. In addition, rectal body temperatures were recorded daily starting at 3 or 4 days pre-dose until sacrifice unless animals had severe signs of toxicity. Unless substantially autolyzed, the uteri, ovaries and vaginas were saved and evaluated microscopically. Ovaries were also weighed.

In the probe study, 1 of 5 rats dosed with 2830 mg/kg of isobutanol died. According to vaginal cytology results, the 4 survivors appeared to be in the proestrus stage of the estrous cycle within 1 to 3 days following dose administration. This stage was followed by an "inconclusive" estrous stage (too few cells to classify) in each survivor. Clinical pathology results showed that estradiol levels dropped at 1 to 3 days post-dose (to <1.5 pg/ml) in all survivors while progesterone levels sharply increased to levels above 75 ng/ml in 3 rats within 2 to 4 days post-dose. One probe rat did not exhibit an increase in progesterone following dose administration. Microscopic evaluation of the saved tissues indicated that all survivors were in the early to late metestrus stage when sacrificed.

In the definitive study, 6 of 15 rats dosed with 2830 mg/kg of the test substance died within 2 days following dose administration. Vaginal cytology results for the 9 survivors indicated that 8 were in proestrus for at least 8 consecutive days beginning within 4 days post-dose. One animal did not exhibit this pattern of extended proestrus. The clinical pathology results showed a decrease in estradiol levels within 1 to 2 days post-dose (not as depressed as in the probe study). However, estradiol levels did not appear to follow a normal cyclic pattern until approximately 10 days following dose administration. At 1 to 2 days after dosing, progesterone levels sharply increased to levels above 85 ng/ml in 5 rats (lesser increases were noted in 2 others). Body temperatures, when taken, were slightly depressed following dose administration but returned to normal within 2 days. Histological evaluation of the saved tissues indicated that most survivors appeared to be in estrus or metestrus stage upon sacrifice and that most had increased ovary weights. In the animals that died, most appeared to be in metestrus when examined.

Of the 6 control animals, vaginal smear examinations indicated that only 1 appeared to have an altered estrous cycle marked by the lengthening of the proestrus phase (to 5 days). The clinical measurements of both estradiol and progesterone were normal for most animals. The affected control animal (mentioned above) exhibited a decrease in estradiol and an increase in progesterone similar to animals dosed with isobutanol but not as marked. At least 1 other rat had decreased estradiol levels with no increase in progesterone. Further evaluation of the pre-dose progesterone levels for this animal indicate that progesterone was increasing at the time of dosing. Body temperatures were consistent prior to and following dose administration. Animals appeared to be in different cyclic stages when examined histologically and increased ovary weights were noted in 3. Colon adhesions (probably resulting from repeated measurement of rectal temperatures) were seen in several control animals.

Although the various tests did not give a clear or consistent indication of the extent of the effects observed or the mechanisms involved, there was an obvious disturbance of hormone production. Therefore, the results of this study indicate that the estrous cycle in the female rat is interrupted following peroral doses (at toxic levels) of isobutanol.

### OBJECTIVE

The objective of this study was to determine if hormone production in the female rat was affected following single peroral doses of isobutanol, such that evidence of pseudopregnancy would result. Basic peroral dosing procedures were conducted in accordance with EPA (TSCA) Health Effects Testing Guidelines 40 CFR Part 798 (Subpart B, Section 798.1175: acute oral toxicity and 1987 OECD Guidelines for Testing of Chemicals (Section 4: Health Effects; 401: acute oral toxicity). The probe study was conducted by standard BRRC testing methods.

### BACKGROUND INFORMATION

An acute toxicity and irritancy study was previously conducted using the test substance, BRRC Sample No. 55-270 (BRRC Report No. 92U1166). In this study, 1 female rat receiving a single peroral dose of isobutanol (2830 mg/kg) and 1 female rat subjected to static inhalation of substantially saturated vapor (6-hour exposure) exhibited pseudopregnancy. Both pseudopregnancies were characterized by the occurrence of deciduoma in the uterus which is rare for animals of the age group tested (12- to 14-weeks old).

### MATERIALS AND METHODS

The protocol, protocol amendments and protocol deviations, detailing the design and conduct of this study are included in Appendix 5.

#### Test Substance

A 1-quart sample of isobutanol, Lot No. TS3370114, CAS No. 78-83-1, was received on September 9, 1992, from UCC&P, Texas City, TX and assigned BRRC Sample No. 55-270. The test substance was a colorless, transparent, low viscosity liquid. The test substance was stored at room temperature. Additional information is included in the Test Substance Characterization Report (Appendix 1).

#### Reference (Control) Substance and Vehicle

A 100 g sample of methyl cellulose (CAS No. 9004-67-05), Lot No. 82H0722, Cat. No. M-0512, was received on August 17, 1993, from Sigma Chemical, St. Louis, MO, and assigned BRRC No. 56-338. This material was a white powder. A sufficient amount of this sample was mixed with an appropriate amount of distilled water (prepared by BRRC; CAS No. 7732-18-5) to give a 0.25% (w/v) solution. The solution was thoroughly mixed on a magnetic stirrer to ensure complete dissolution and was stored in the explosion-proof, chemical storage refrigerator in Room 109 except during periods of sample preparation. Methyl cellulose solutions are widely used in toxicology because of their relatively inert nature and good suspending properties.

#### Animals and Husbandry

For the probe study, a total of 10 female Sprague Dawley® rats arrived on August 10, 1993, from Harlan Sprague Dawley, Inc., Indianapolis, IN. A total of 25 female Sprague Dawley® rats arrived from the same supplier on August 31, 1993, to be used for the definitive test. The rats were ordered to be

approximately 12 weeks of age upon arrival (approximately 200 to 224 g according to the supplier). The birth dates for animals received for the probe and definitive studies were May 18, 1993, and June 8, 1993, respectively. The strain and species were selected because of their availability, existing historical data, and because this strain was used for previous testing with this test substance. The females were nulliparous and nonpregnant.

All animals were housed in Room 109 upon receipt where they remained until the termination of the study. Once every 3 months, 5 rats/sex received for acute testing and housed in Room 109 were subjected to a quality control evaluation, including gross pathology, parasitology and viral serology testing. Periodically, a clinical veterinarian examined the rats housed in Room 109 for any signs of health deficiencies. All animals were assigned a unique number and identified by cage cards. Animals were also identified by an ear tagging procedure during the week of receipt.

The animals were group housed (up to 3/cage) in stainless steel, wire mesh cages (approximately 23.5 x 40.0 x 18.0 cm). DACE® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) was placed under each cage and changed regularly. An automatic timer was set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). Temperature and relative humidity were recorded (Cole-Parmer Hygrothermograph® Seven-Day Continuous Recorder, Model No. 8368-00, Cole-Parmer Instrument Co., Chicago, IL). Temperature was routinely maintained at 66-77°F during the test period; relative humidity was routinely maintained at 40-70%. Any minor exceptions to these specified ranges can be found in the raw data.

Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) was available ad libitum except during dosing and was delivered by an automatic watering system with demand control valves mounted on each rack. Water analyses were provided by the supplier, Halliburton NUS Environmental Laboratories, Professional Service Industries, Inc., Lancaster Laboratories, Inc. or Chester Lab, and RJ Lee Group, Inc. at regular intervals. EPA standards for maximum levels of contaminants were not exceeded. Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) was available ad libitum. The feed was removed the day before dose administration. After dosing, feed was again made available ad libitum. Analyses for chemical composition and possible contaminants of each feed lot were performed by Agway Inc. and the results are included in BRRC files. Feed and water analysis reports were reviewed by the Study Director as they were available.

#### Animal Acclimation

The animals were acclimated for at least 5 days before dosing. Detailed clinical observations were conducted several times prior to dosing (at the time of receipt, during animal identification and during pre-dose weighing). Cage-side observations and mortality checks were conducted at least once daily. Animals considered unacceptable for the study, based on the clinical signs, were rejected for use on this study.

### Study Organization

Upon receipt, all animals were randomly assigned to cages. A total of 5 females were included for preliminary observations in the probe study. Twenty-one females were included for pre-dose observations in the definitive study. These animals were weighed and inspected for health on the scheduled pre-dose start day (5 or 6 days prior to dose administration for the probe study and 3 or 4 days prior to dosing for the definitive study). Only those exhibiting a healthy state were used. Healthy animals appeared alert, active and well groomed, with no evidence of discharge, diarrhea, breathing difficulties or locomotor abnormalities. A BRRC veterinarian was available for consultation regarding any animal health concerns. Available rats not assigned to this study were used as needed for other toxicity testing or were sacrificed.

### *Probe Study*

The body weight range on the day of dosing for probe animals was 190 to 202 grams (weighed after fasting). A total of 3 female rats were dosed on August 20, 1993. Because 1 animal died, another 2 animals were added in order to obtain at least 3 surviving females. These replacement animals were dosed on August 21, 1993. All surviving animals were sacrificed at 5 days post-dose (August 25 or 26, 1993).

### *Definitive Study*

In the definitive study, the body weight range on the day of dosing was 197 to 226 g for animals receiving isobutanol and 202 to 219 g for rats dosed with aqueous methyl cellulose solution (control animals). On September 13, 1993, a total of 9 females were dosed with the test substance and another 6 females received the control substance. However, deaths were produced in animals receiving isobutanol. Therefore, another 6 female rats were dosed with the test substance on the following day (September 14, 1993) in an attempt to obtain at least 9 survivors for subsequent evaluations. Survivors were sacrificed at 14 days after dose administration (September 27 or 28, 1993).

### Study Schedule (In-Life Phase)

Probe Study:	August 15, 1993 to August 26, 1993
Definitive Study:	September 10, 1993 to September 28, 1993

### Test Procedures/Observations

#### *Test Substance Preparation*

For both the probe and definitive studies, each dosing mixture was prepared just prior to administration by diluting the appropriate amount of isobutanol with 0.25% w/v aqueous methyl cellulose solution. All resulting emulsions were mixed for approximately 5 to 20 minutes on a magnetic stirrer.

### *Peroral Dosing*

Doses were administered by stomach intubation through a commercial 16-gauge (3-inch) ball-end stainless steel needle attached to a disposable syringe. The exact amounts of test substance and emulsion given to each rat were recorded on the raw data forms.

The rats were fasted overnight before dosing. For the probe study, a total of 5 female rats (including 2 replacement animals) were dosed with 2830 mg/kg of isobutanol in aqueous methyl cellulose solution. In the definitive study, 15 females (including 6 replacement rats) were administered 2830 mg/kg of the test substance (28.3% emulsion in aqueous methyl cellulose solution) and another 6 female rats (controls) were given 10.0 ml/kg of 0.25% (w/v) methyl cellulose solution (aqueous). For individual animals, the dosing volume was adjusted according to body weight. Dosed rats were observed frequently for signs of toxicity on the first day of the test and twice a day thereafter (except on weekends or holidays when they were examined only once) up to 5 days post-dose for probe animals and 14 days post-dose for study animals.

### *Body Weight*

In the probe study, all 5 animals were weighed at least 5 days prior to dosing (6 days for replacement animals). Each rat was also weighed on the day dosed and again at death or sacrifice.

For the definitive study, a total of 21 female rats were weighed at least 3 days prior to dosing (4 days for replacement animals). Weights were again obtained on the day of dose administration and at 7 and 14 days (or death).

All weights were recorded in the raw data.

### *Vaginal Cytology*

In both the probe and definitive studies, vaginal smears were made daily for each animal in order to determine the stage of the estrous cycle. The smears were obtained using a sterile swab which was wetted with saline solution, placed into the vagina and rolled onto a slide. The slides were then microscopically examined and the most reasonable stage (as based on predominant cell types) was recorded. A single letter representing the stage (P for proestrus, E for estrus, M for metestrus or D for diestrus) was recorded in the raw data for each animal.

Vaginal smears were made beginning 5 days prior to the first dose day in the probe study and 3 days prior to the first dose day in the definitive study. They were obtained from all animals each day until death or sacrifice. On the day of test substance administration, smears were collected after animals were dosed. The estrus stage could not be determined for several animals in the probe study because a sufficient number of cells necessary for classification could not be collected.

### *Blood Collection*

Animals to be bled were first anesthetized with methoxyflurane followed by the puncture of the retroorbital sinus with a glass capillary tube. In an attempt to reduce animal stress, bleeding was alternated between eyes for individual animals when possible.

Blood samples for rats in the probe study were collected beginning at 5 days prior to the first dose day (6 days pre-dose for replacement animals). None of the 5 probe animals were bled 2 days before the first dose day because all appeared to be pale (possibly anemic) at this time. Blood was not collected from animals on the day of dose administration. Dosed animals were bled daily beginning the day after dosing until death or sacrifice unless substantial signs of toxicity were apparent at the time of collection.

In the definitive study, all test and control animals were bled the day before and most were bled the day following dose administration. One test animal exhibited substantial signs of toxicity at 1 day and was, therefore, not bled at that time. At 2 days following dosing, all surviving animals were divided into 3 groups each consisting of 3 test and 2 control animals. Each group was then bled on a rotating schedule (with a 2-day interval between bleedings) up until sacrifice in order to eliminate animal stress caused by daily bleeding. All animals were bled on the day of sacrifice.

The following were measured or calculated:

#### Special Chemistry

estradiol  
progesterone

Details of the clinical pathology procedures are included in Appendix 2.

#### *Measurement of Rectal Temperature*

Rectal temperatures were not determined for animals in the probe study.

For animals in the definitive study, rectal temperatures (°C) were obtained using a digital thermometer. The digital thermometer was calibrated at each evaluation time by comparing the digital temperature of a beaker of water before and after use with temperatures obtained concurrently using a standard thermometer.

Rectal temperatures were recorded daily for all animals beginning at 3 days or 4 days (for replacement animals) prior to the day of dose. Temperatures were also obtained in the morning before dose administration. Rectal temperatures were taken after dosing for all animals included in the first dose group. However, because several animals receiving the test substance died, it was decided (by the Sponsor and Study Director) that the rectal temperatures of replacement animals would only be taken once on the dose day (prior to dose administration) in order to minimize stress to the animals. Body temperatures were then taken daily until death or sacrifice unless substantial signs of toxicity were apparent.

### *Necropsy and Anatomic Pathology*

At 5 days (probe study) or 14 days (definitive study) after dose administration, all survivors were anesthetized with methoxyflurane and killed by severing the brachial vessels. A complete necropsy was performed on most animals that died or were sacrificed. However, 1 animal in the probe study died on the weekend and was given an abbreviated necropsy. The following tissues, unless excessively autolyzed, were collected from each animal and retained in 10% neutral buffered formalin: uterus, vagina and ovaries. The vagina was not saved in the probe animal that died. Additional tissues were saved based on gross necropsy findings. In addition, the ovaries for each animal were weighed.

Details of the anatomic pathology procedures are included in Appendix 3.

### RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated as a result of this study will be retained in the BRRC/UCC Archives in Morrisville, VT for at least 5 years. Approximately 20 ml of the remaining sample will be retained for at least 2 years following issuance of this report (for further testing at the request of the Sponsor).

### RESULTS AND DISCUSSION

#### *Clinical Observations*

Individual clinical observations from peroral doses of isobutanol to animals in the definitive study are included in Table 1 of Appendix 4 (individual clinical observations for probe animals were not tabulated). Individual results from peroral doses to control animals are included in Table 2 of Appendix 4. Mean body weights are presented in Table 1 (probe study) and Table 2 (definitive study) of the report.

In the probe study, a total of 5 female rats were administered 2830 mg/kg of isobutanol, dosed as a 28.3% (w/v) emulsion in 0.25% aqueous methyl cellulose solution. One rat died at 2 days. Signs of toxicity for these animals included pale eyes (probably from repeated blood collection), sluggishness, an unsteady gait, prostration, lacrimation, slow breathing, piloerection, a red crust on the perinasal fur and urine stains. Survivors recovered within 2 days.

In the definitive study, a dose of 2830 mg/kg of the test substance, dosed as a 28.3% (w/v) emulsion in 0.25% aqueous methyl cellulose solution, was administered to 15 female rats and resulted in 6 deaths. Five animals died at 2.5 to 4 hours; another died within 2 days. Signs of toxicity included sluggishness, an unsteady gait, prostration, lacrimation, slow breathing, labored breathing (in 1) and piloerection. Most survivors gained weight during the 14-day post-dose observation period. However, 2 females exhibited a slight weight loss within 7 to 14 days. Survivors recovered within 1 to 2 days.

A total of 6 female control rats received a dose of 10.0 ml/kg of aqueous methyl cellulose solution (0.25% w/v in distilled water) and survived. There were no signs of toxicity apparent during the observation period. One rat lost a slight amount of weight within 7 to 14 days; all others gained weight during the 14-day observation period.

#### *Vaginal Cytology*

Summaries of vaginal cytology findings for the probe and definitive studies are presented in report Tables 1 and 2, respectively. Individual vaginal cytology results for animals in the definitive study are included in Table 5 (test animals) and Table 6 (control animals) of Appendix 4.

In the probe study, the vaginal cytology of the 4 surviving rats indicated that each was in the proestrus stage of the estrous cycle (majority of nucleated epithelial cells present) within 3 days after receiving peroral doses of the test substance (3 animals were in proestrus on the day of dosing). Furthermore, by 3 to 4 days after dose administration, the proestrus stage was followed by an "inconclusive" estrus stage where a definitive classification could not be made because of insufficient vaginal cells (a condition oftentimes observed in female rats that have recently mated).

The results of the vaginal cytology for animals receiving 2830 mg/kg of isobutanol in the definitive study are somewhat similar to those of the probe study. For all surviving animals, the onset of the proestrus stage occurred within 1 to 4 days after dose administration. Most test animals appeared to stall in the proestrus stage for at least 8 days; a total of 4 rats remained in proestrus from the time of onset (1 to 3 days) through 14 days. One female appeared to be in proestrus for only 2 days. During the 14-day observation period, there were frequent incidences of reduced or few numbers of cells and non-cellular and/or mucoid debris in vaginal specimens.

Vaginal cytology of animals dosed with the control substance suggested that these animals cycled normally during the observation period. One control female appeared to be in the proestrus stage at 3 days after dose administration and remained in this stage for 5 consecutive examination days. Overall, there was a much lower incidence of reduced numbers of cells, non-cellular debris and/or mucoid debris than observed in the test animals.

#### *Clinical Pathology*

Clinical pathology results can be found in Appendix 2.

In the probe study, estradiol levels for the 4 surviving animals dropped as low as (<)1.5 pg/ml within 1 to 3 days after the peroral administration of 2830 mg/kg of isobutanol (normal basal estradiol level = 10 pg/ml). Moreover, these levels did not increase above 15 pg/ml during the 5-day observation period (estradiol ranges from 20-30 pg/ml during estrus and 40-50 pg/ml prior to ovulation). In 3 of the 4 survivors, the progesterone levels increased to amounts higher than normal preovulatory progesterone levels (40 to 50 ng/ml). Peak values ranging from 75.4 to 108 ng/ml were determined for 3 rats within 2 to 4 days after dosing. Progesterone levels for the remaining rat were low (4.2 to 9.4 ng/ml) throughout the 5-day observation period.

In the definitive study, estradiol levels for animals receiving the test substance did not appear to follow a cyclic pattern for at least 5 to 6 days following dosing. Decreases in estradiol were noted following dose administration but were not as low as observed in the probe study (levels were within normal limits, none below 10 pg/ml, but at a prolonged incidence). Five of the 9 surviving test animals had progesterone levels above 85 ng/ml (85.2 to 120 ng/ml) within 1 to 6 days following test substance administration. More moderate increases in progesterone (highest observed values of 55.4 or 67.3 ng/ml) were also observed in another 2 test animals. The upper progesterone levels for the remaining 2 animals ranged from 10.7 to 29.6 ng/ml.

Estradiol levels for most control rats in the definitive study appeared to fall into a normal cyclic pattern. After dosing, 1 control animal exhibited a prolonged incidence of low estradiol values (not below the normal basal level) that was similar to that observed in test animals. This animal also had an increased progesterone level (up to 81 ng/ml) following dose administration. The remaining 5 control animals did not have an increase in progesterone following administration of the control substance. The highest level observed in these 5 rats was 68.0 ng/ml which was apparent in 1 at 14 days.

#### *Rectal Temperature*

Daily rectal temperatures for individual animals in the definitive study are found in Table 3 (Test Animals) and Table 4 (Control Animals) of Appendix 4.

Average rectal body temperatures ranged from 37°C to 39°C. However, the rectal body temperatures for animals dosed with isobutanol on the first dose day were depressed slightly on the day of dosing and also at 1 day following dose administration (ranging from 33°C to 35°C in some). For replacement animals, no lower temperatures were observed. However, unlike the first group of test animals, rectal temperatures were not obtained in animals exhibiting substantial signs of toxicity (typically after dosing to 1 day).

The rectal body temperature for control rats remained at 37°C to 39°C throughout the study.

#### *Necropsy and Anatomic Pathology*

Necropsy and anatomic pathology results can be found in Appendix 3.

For probe animals, notable findings at necropsy included discolored lungs and large submandibular lymph nodes in 1. Tables 1 and 2 of the Anatomic Pathology Report (Appendix 3) list a few additional findings.

The uterus, ovaries and vagina for rats in the probe study were saved and examined microscopically unless excessively autolyzed (see the detailed Anatomic Pathology Report, Appendix 3). The histologic examination of the 4 saved vaginas indicated that all 4 animals were in the metestrus stage of the estrous cycle at the time of death or sacrifice. Three of the 5 probe animals showed active corpora lutea in the ovaries. There was no apparent size change of examined ovaries.

Necropsy of animals in the definitive study (including both survivors and animals that died) revealed discolored submandibular lymph nodes, large ovaries, small ovaries (in 1) and discolored lungs. Additional findings are found in the Anatomic Pathology Report (Appendix 3). At necropsy, control animals had lesions of the colon (adhesions, hemorrhage and/or nodule), discolored or large submandibular lymph nodes, and a dilated pelvis (Table 3 of Appendix 3).

The histological evaluation of the saved uteri, ovaries and vaginas of rats in the definitive study revealed active corpora lutea in the ovaries of 12 of the 14 examined test animals (inactive corpora lutea were only observed in rats that died). See the detailed Anatomic Pathology Report (Appendix 3). The uteri of most animals also appeared to have a progestin influence. Histological vaginal and/or uterine evaluations revealed that most rats were in the metestrus or estrus stage at the time of death or sacrifice (mostly metestrus). An increase in ovarian size was noted in 6 of the 15 test animals. Although the overall histological results of the definitive study were similar to those observed in the probe study, it appeared that more variable results were evident in the test animals dosed with isobutanol indicating that the animals were probably starting to cycle by 14 days. The microscopic evaluation of the same tissues in the control animals indicated that the animals were in more varied stages of estrus when sacrificed at 14 days. Equal numbers of animals exhibited either active or inactive corpora lutea in the ovaries and an estrogenic or progestin influence in the uteri. The ovaries of 3 control animals appeared to have a size increase. Other pertinent microscopic lesions observed in control rats included adhesions and hemorrhages of the colon in 3 (possibly caused by the irritation from the thermometer used for repeated rectal temperatures).

#### CONCLUSIONS

This study was initially conducted because of the occurrence of pseudopregnancy in 2 female rats during the acute toxicity and irritancy study of isobutanol conducted at this laboratory. The animal supplier was questioned about any known incidence of pseudopregnancy among their females but no explanation was provided. It was not until other customers also expressed concern about estrous irregularities in their rats, that a more thorough investigation was conducted. As a result, the animal supplier informed BRRC that in the process of preparing timed-pregnant females for customers, a number of females may be swabbed for determination of the stage of estrous prior to mating with males. Females not in the proper stage of estrous are returned to the pool of available animals for sale to customers. Therefore, it is possible that females used in the original acute study were previously swabbed by the supplier. The process of swabbing could have induced pseudopregnancy.

High doses of isobutanol appeared to affect the normal cyclic pattern of female rats when dosed perorally, with several trends evident. The effects listed below were associated with "toxic" levels of isobutanol (clinical signs were similar to those seen in the original work). Daily vaginal cytology suggested that most animals receiving single peroral doses of the test substance remained in the proestrus stage of the estrous cycle for an abnormal length of time (up to sacrifice in some animals). In addition, some rats in the probe study had very few cells evident in the vaginal smears (a condition

often observed in recently mated female rats). The clinical pathology of test and probe animals receiving isobutanol indicated that many rats did not exhibit normal cyclic patterns of estradiol for at least 5 to 6 days following dosing. Moreover, most rats had a notable increase in progesterone production (above the typical "peak" values observed in the preovulatory phase) after receiving isobutanol. Most control animals appeared to have normal estradiol and progesterone levels. Only 1 control animal exhibited a similar trend in estradiol and progesterone levels, possibly because of vaginal stimulation from obtaining the smears. This control animal also remained in proestrus for 5 consecutive days based on vaginal cytology.

Histologic evaluation of the ovaries, uteri and vaginas suggested that most test animals receiving isobutanol appeared to be in the estrus or metestrus stage at the time of death or sacrifice. Most survivors also had active corpora lutea in the ovaries and a progestin influence in the uteri. The histopathologic results were more consistent among probe animals, probably because they were sacrificed at 5 days - a period of more pronounced effect. Animals in the definitive study were beginning to cycle normally (based on clinical pathology and vaginal cytology results) by 14 days. Microscopic examination revealed that the control animals exhibited a more normal pattern of cycling. There was no concrete evidence of pseudopregnancy (specifically deciduoma formation) found in females in this study.

In conclusion, it appears that the peroral administration of isobutanol (at toxic levels) does produce hormonal changes and an interruption of the estrous cycle in female rats. Although there may be some indication that handling by the supplier could account for some of the effects, it is apparent that treated females were more affected than controls based on cytologic, clinical pathologic and anatomic pathologic changes. However, it is not possible to differentiate whether or not single peroral doses of isobutanol had a specific effect on cycling in female rats or whether the cycling was affected by the stress impact from the toxicity of the test substance.

REVIEW AND APPROVAL

Study Director: Susan M. Christopher 3-19-95  
 Susan M. Christopher, B.S. Date

Senior Group Leader: Roy C. Myers 3-20-95  
 Roy C. Myers, B.S., DABT Date

Director: John P. Van Miller 3-21-95  
 John P. Van Miller, Ph.D., DABT Date

KEY PERSONNEL

Study Director: S. M. Christopher

Technical Coordinators: T. A. Christopher  
M. F. Kubena  
V. A. Camaione

Supervisors: L. C. Fisher  
M. A. McGee  
D. A. Neptun

Scientists: E. H. Fowler  
D. A. Neptun

Additional personnel are listed in the raw data.

TABLE 1  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

## SUMMARY OF MEAN BODY WEIGHT AND VAGINAL CYTOLOGY FOR ANIMALS IN THE PROBE STUDY

Material: Isobutanol			Sample No.: 55-270		Dose: 2830 mg/kg		
Day	No. of Animals Examined	Mean Body Weight (g) ± S.D.	Total Number of Animals in Estrous Stage				
			Proestrus	Estrus	Metestrus	Diestrus	Undefined*
Pre-Dose Determinations							
-6	2	214 ± 0.0	1	1	0	0	0
-5	3	220 ± 7.8	-	-	-	-	-
	5	---	0	3	2	0	0
-4	5	---	0	3	2	0	0
-3	5	---	0	0	3	2	0
-2	5	---	3	0	0	2	0
-1	5	---	3	1	0	1	0
Post-Dose Determinations							
0	5	194 ± 5.7	3	1	1	0	0
1	5	---	3	2	0	0	0
2	4	---	2	0	0	2	0
3	4	---	3	0	0	0	1
4	4	---	0	0	0	0	4
5	4	215 ± 8.2	1	0	0	0	3

\* For some animals, there were not enough cells present to make a definitive estrus classification.



**Isobutanol: Determination of the Potential for Pseudopregnancy in Female Rats following Acute Peroral Doses**

**QUALITY ASSURANCE UNIT INSPECTION SUMMARY**

<u>Inspection Date(s)</u>	<u>Inspection Type</u>	<u>Date QAU Report Issued To</u>	
		<u>Study Director</u>	<u>Management</u>
08-25-93 to 08-26-93	PROTOCOL	08-30-93	09-07-93
09-13-93	EVENT-DOSING	09-15-93	09-15-93
09-17-93	PROTOCOL AMENDMENT #1	09-21-93	09-21-93
10-22-93	PROTOCOL AMENDMENT #2	10-22-93	10-25-93
08-17-94 to 08-30-94	RAW DATA, REPORT	08-30-94	03-20-95
03-20-95	ARCHIVES	03-20-95	03-20-95

  
 \_\_\_\_\_  
 Susan L. Hopper, B.A.  
 Representative, Quality Assurance Unit

3-20-95  
 \_\_\_\_\_  
 Date

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**Test Substance Characterization Report**

**(17 Pages)**





**Union Carbide Corporation**

---

**STUDY TITLE**

**GLP Analysis-Final Report**

**TEST SUBSTANCE**

**Isobutanol**

**DATA REQUIREMENT**

**U.S. FDA, 21 CFR 58  
U.S. EPA TSCA, 40 CFR 792  
U.S. EPA FIFRA 40 CFR 160**

**STUDY DIRECTOR**

**Alexander E. Gabany, Jr.**

**STUDY COMPLETED ON**

**October 21, 1993**

**PERFORMING LABORATORY**

**Union Carbide Corporation  
PO Box 8361  
South Charleston, West Virginia 25303**

**UCC R/D LABORATORY PROJECT ID**

**Study # 37-AEG-65**

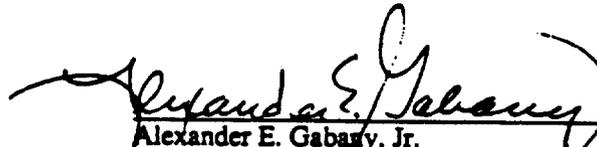
**SPONSOR COMPANY**

**Union Carbide Corporation  
Solvents and Coating Materials Division  
Danbury, Conn. 06817-0001**

**STUDY COMPLIANCE STATEMENT**

Study Compliance Statement for Union Carbide Corporation (UCC) Study # 37-AEG-65,  
isobutanol study for Bushy Run Research Center.

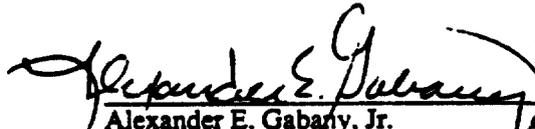
In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that the study was conducted in compliance with the U.S. EPA Section 4 Good Laboratory Practice standards; TSCA 40 CFR 792; FIFRA 40 CFR 160 and FDA, 21 CFR 58. All original raw data, records, protocols, final reports, and the sample are being retained at UCC's South Charleston, WV, Technical Center.

  
Alexander E. Gabary, Jr.      Date 10/21/83  
Study Director

**PROTOCOL DEVIATION STATEMENT**

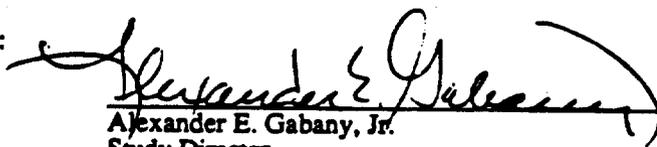
Protocol Deviation Statement for Union Carbide Corporation (UCC) Study # 37-AEG-65, isobutanol study for Bushy Run Research Center.

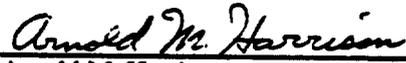
In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that there were no protocol deviations taken during the study. The study was conducted in compliance with the protocol established and signed on 5/6/93 by Alexander E. Gabany, GLP Study Director.

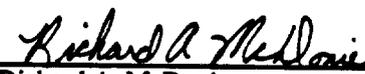
  
Alexander E. Gabany, Jr.      10/21/93  
Study Director                      date

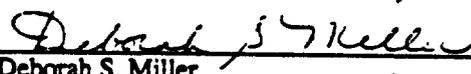
**SIGNATURE PAGE**

Submitted by: Union Carbide Corporation  
P.O. Box 8361  
South Charleston, West Virginia 25303

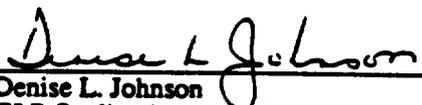
Prepared by:  10/21/93  
Alexander E. Gabany, Jr. date  
Study Director

 10/27/93  
Arnold M. Harrison date  
NMR Skill Area Specialist

 10/22/93  
Richard A. McDonie date  
GC/MS Skill Area Specialist

 10/27/93  
Deborah S. Miller date  
HS&EA Business Analytical Specialist

Quality Assurance Review by:

 11-8-93  
Denise L. Johnson date  
GLP Quality Assurance Unit  
(QAU) Representative

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## Isobutanol

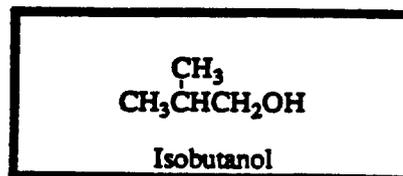
Isobutanol  
Study # 37-AEG-65

**ABSTRACT** Isobutanol was analyzed to provide analytical data as part of the toxicity study at Bushy Run Research Center. The analyses were performed in compliance with Good Laboratory Practice standards (GLP) to meet EPA Section 4 Test Rule requirements and FDA, 21 CFR 58. Gas chromatography-mass spectrometry (GC/MS) and nuclear magnetic resonance spectroscopy (NMR) techniques were independently used to confirm the sample's identity. Purity, measured by capillary GC, is 99.9%. The sample was received from Bushy Run Research Center. All raw data, documentation, records, protocols, final reports, and the sample are being retained.

**INTRODUCTION** J. J. Behen, this study's sponsor, requested that Bushy Run Research Center (BRRC) test isobutanol for toxicity. Such studies must follow GLP standards established by the EPA that require they be conducted with authentic material whose identity and purity are verified analytically.

A 30 gram sample of isobutanol (37-AEG-70) was received 5/6/93 in an amber glass bottle for analytical characterization. This sample is a subsample of a larger quantity of isobutanol (lot-#-TS3370114; BRRC # 55-270) tested at Bushy Run Research Center. A GLP protocol describing the analytical characterization of the sample was prepared (Appendix 1). The protocol called for structural identification by NMR and GC/MS and for the capillary GC measurement of any impurities identified by GC/MS.

Shown at right is the structure of Isobutanol; its Chemical Abstracts Service Registry number (CAS #) is 78-83-1.



**DISCUSSION** The data from the analyses are summarized below.

**NMR Analyses** Proton and carbon NMR data were collected in the UCC NMR Skill Center using a General Electric GN-300NB spectrometer. The acquisition parameters are shown in the figures; for the  $^1\text{H}$  NMR spectrum, the pulses used correspond to  $<3^\circ$  flip angles; the  $^{13}\text{C}$  flip angles were  $30^\circ$ ; the  $^{13}\text{C}(^1\text{H})$  (proton decoupled  $^{13}\text{C}$ ) spectrum used Waltz 16 modulation for  $^1\text{H}$  decoupling. The spectra were not acquired under quantitative conditions; the acquisition conditions were established to identify the major component and to look for any substantial impurities. The sample was dissolved in deuteriochloroform for analysis; tetramethylsilane (TMS) was added to provide an internal chemical shift reference. The TMS and deuteriochloroform were used as received.

Figure 1 shows the  $^1\text{H}$  NMR spectrum obtained from the sample 37-AEG-70. The observed chemical shifts, spin-spin coupling patterns, and relative intensities are appropriate for isobutanol: the rapidly exchanging hydroxyl proton is seen as a singlet at 4.08 ppm; the methylene protons are seen as a doublet at 3.34 ppm; the methine proton is seen as a nine-line pattern centered at 1.74 ppm; the methyl protons are seen as a doublet at 0.90 ppm.  $^{13}\text{C}$ -satellites are seen as small lines around the methyl and methylene doublets. The TMS is at 0 ppm.

Figure 2 shows the  $^{13}\text{C}(^1\text{H})$  spectrum for the same sample. No unusual or unexpected resonances are seen; the three types of carbons present in isobutanol are seen: the methylene carbon is at 69.3 ppm; the methine carbon is at 30.8 ppm; the methyl carbons are at 19.0 ppm. The 1:1:1 triplet at 77.4 ppm is from the deuteriochloroform, and TMS is at 0 ppm.

The NMR spectra are consistent with the sample being pure isobutanol.

**GC/MS Analysis** Electron ionization (EI) mass spectral data was collected in the UCC MS Skill Center using a Finnigan TSQ-70 mass spectrometer interfaced to a Hewlett-Packard (HP) 5890 gas chromatograph. The sample, 37-AEG-70, was analyzed by injecting 0.1  $\mu\text{L}$  aliquots onto a CP-Sil-5-CB capillary column held at  $30^\circ\text{C}$  for 4 minutes, and then programmed to  $250^\circ\text{C}$  at  $8^\circ/\text{minute}$ . Figure 3 shows the EI total ion current chromatogram for the sample (scanned from  $m/z$  10 to  $m/z$  310 EI mode). This chromatogram is annotated with identifications based on the components' EI spectrum.

**Capillary GC** A HP 5890 gas chromatograph equipped with a flame ionization detector was used to analyze the sample. Aliquots (1  $\mu$ L) were injected via autoinjector with a 100:1 split ratio onto a DB-1 capillary column started at 60°C and held for 4 minutes, then programmed to 250°C at 12°/minute (see Figure 4). The averages of triplicate analysis are given below (normalized chromatogram area percent).

<u>Component name</u>	<u>Retention time, min.</u>	<u>37-AEG-70</u>
n-propanol	3.5	0.02
butyraldehyde	3.9	0.01
Isobutanol	5.1	99.9
n-butanol	5.4	0.04
isobutyl formate	5.8	0.004
methyl butyrate	6.5	0.007
C <sub>8</sub> -ketone	9.4	0.005
C <sub>8</sub> -aldehyde	10.3	0.004
all others	—	0.01

**CONCLUSION** NMR spectral data and mass spectral fragmentation data from the UCC Skill Centers show that this sample is isobutanol. These independent methods satisfy the analytical requirements for structural identification, as defined in the sample protocol. Sample purity, measured by capillary GC, is = 99.9%.

**ARCHIVES** All raw data, records, protocols, samples and final reports are being retained at UCC's South Charleston, WV Technical Center as follows:

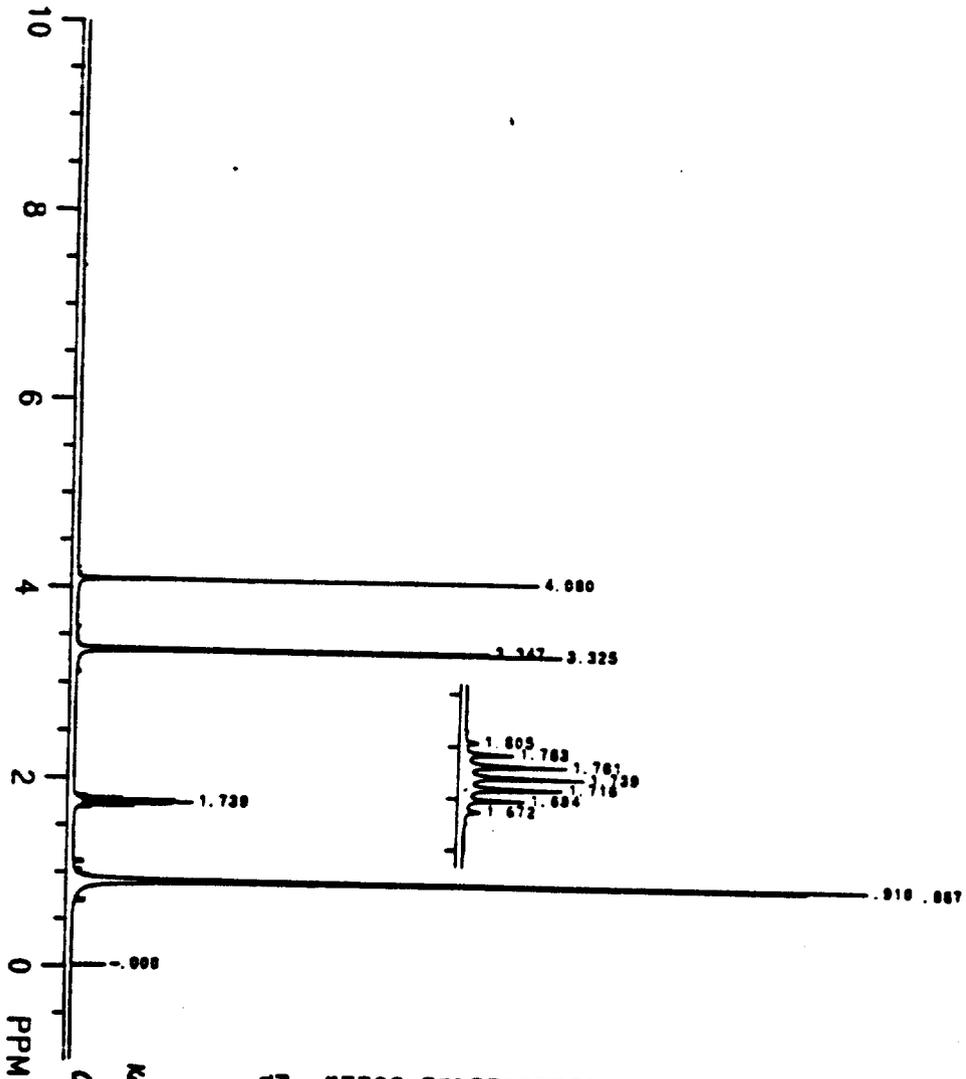
- raw data from GC, NMR, and GC/MS studies are in 770-363, 770-127 and 770-123, respectively;
- protocols, notebook and other records are to be kept in the GLP archives;
- the remainder of each sample is being kept in a locked GLP sample box in 770-363.

**ACKNOWLEDGEMENTS** We would like to thank Trudy Barker for preparing the bulk of the report, Jo Ann Coffey for sample handling and collecting the GC data, Rich McDonie for collecting the GC/MS data, and Kathy Canterbury for collecting the NMR data.

**NOTEBOOK REFERENCE:** 37-AEG-65 and related pages

**Confidentiality** No claim of confidentiality is made for any information contained in this study as it pertains to use by any government agency to which it is submitted. This document, however, is proprietary to UCC and is confidential and trade secret information in all other countries and for all purposes other than those directly related to the purposes of the reviewing agency. Information contained in these studies should not be reviewed, abstracted or used by persons other than the agency without the expressed written consent of UCC except as required to carry out statutory requirements.

Figure 1 — <sup>1</sup>H NMR Spectrum of 37-AEG-70 (Isobutanol)



TECHNICIAN  
 Nancy A. Conaway, CI/MS  
 SUPERVISOR  
 Donald R. Horvath 6/14/93

IR-79-14.858  
 CM-50  
 REFERENCED TO THE TMS

FROM 10.00  
 TO -0.00 PPM

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 DEC PPM - 0  
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 SCALE - 105.30 HZ/CM  
 - 3500 PPM/CM

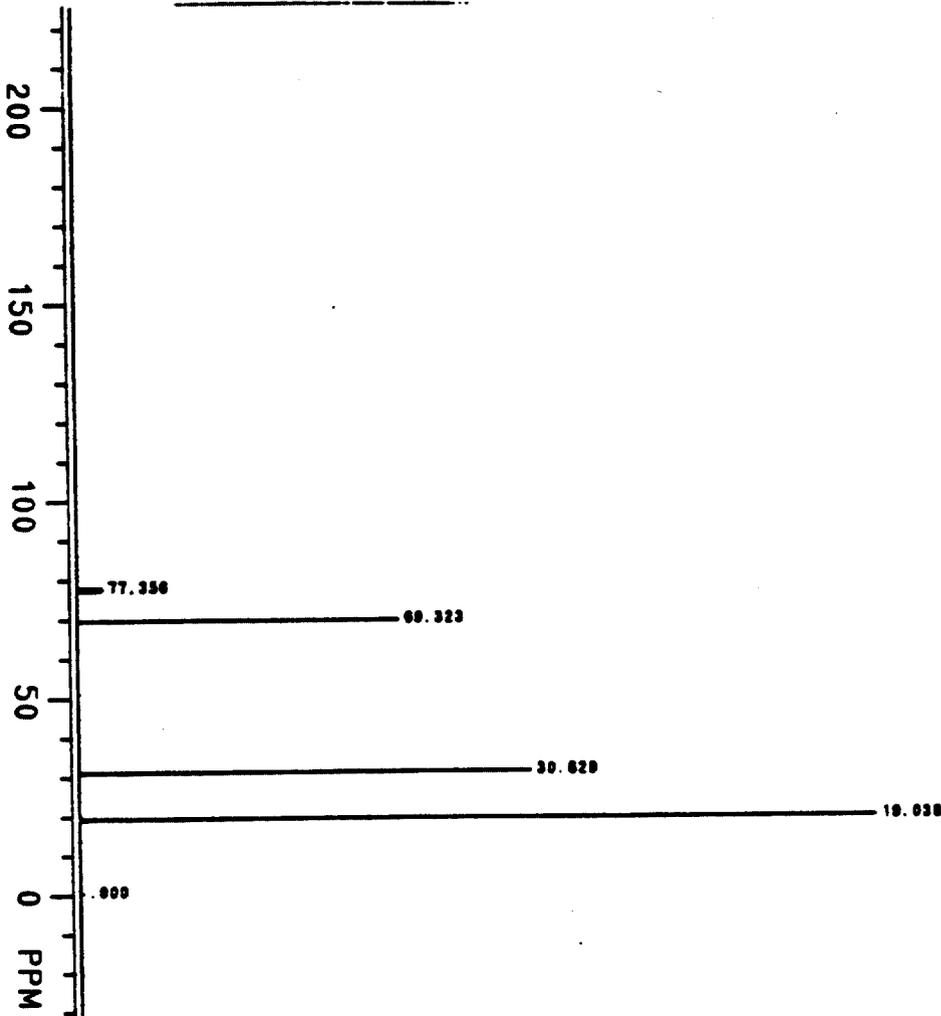
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 402484 IN SMO1

ONE PULSE SEQUENCE

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 F3 - 1.00 SEC  
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Figure 2 — <sup>13</sup>C NMR Spectrum of 37-AEG-70 (Isobutanol)



f

TECHNICIAN  
 Robert C. Strassburg 6/18/83  
 SUPERVISOR  
 David W. Johnson 6/18/83

NAME: 19.038  
 CH: 1000  
 REFERENCE TO THE TMS

ACQ: 19.038  
 CH: 1000  
 REFERENCE TO THE TMS

NAME: 30.629  
 CH: 1000  
 REFERENCE TO THE TMS

NAME: 69.323  
 CH: 1000  
 REFERENCE TO THE TMS

NAME: 77.336  
 CH: 1000  
 REFERENCE TO THE TMS

NAME: 19.038  
 CH: 1000  
 REFERENCE TO THE TMS

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 CH: 1000  
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 CH: 1000  
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Figure 3 — Capillary GC/MS RIC of 37-AEG-70 (Isobutanol)

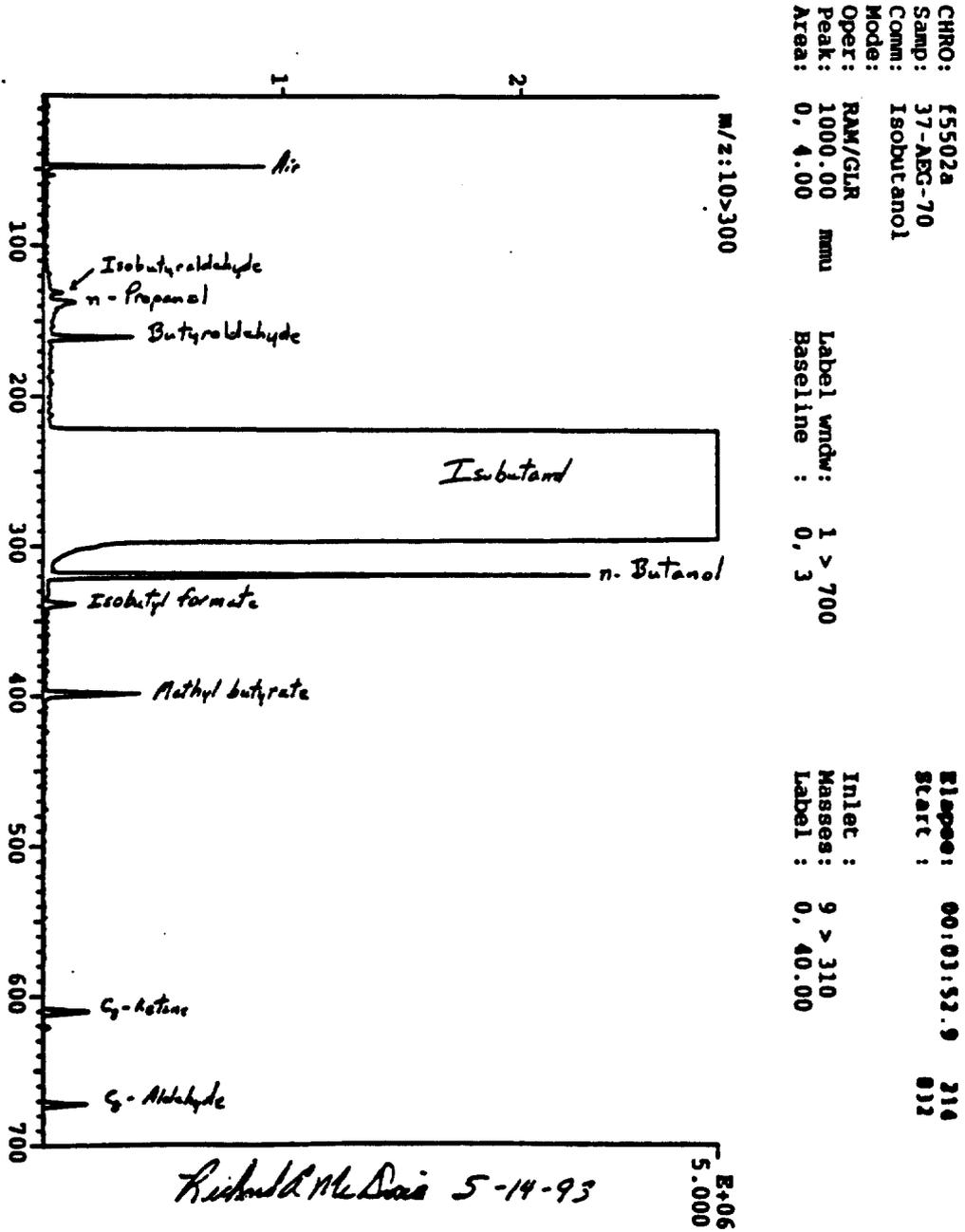
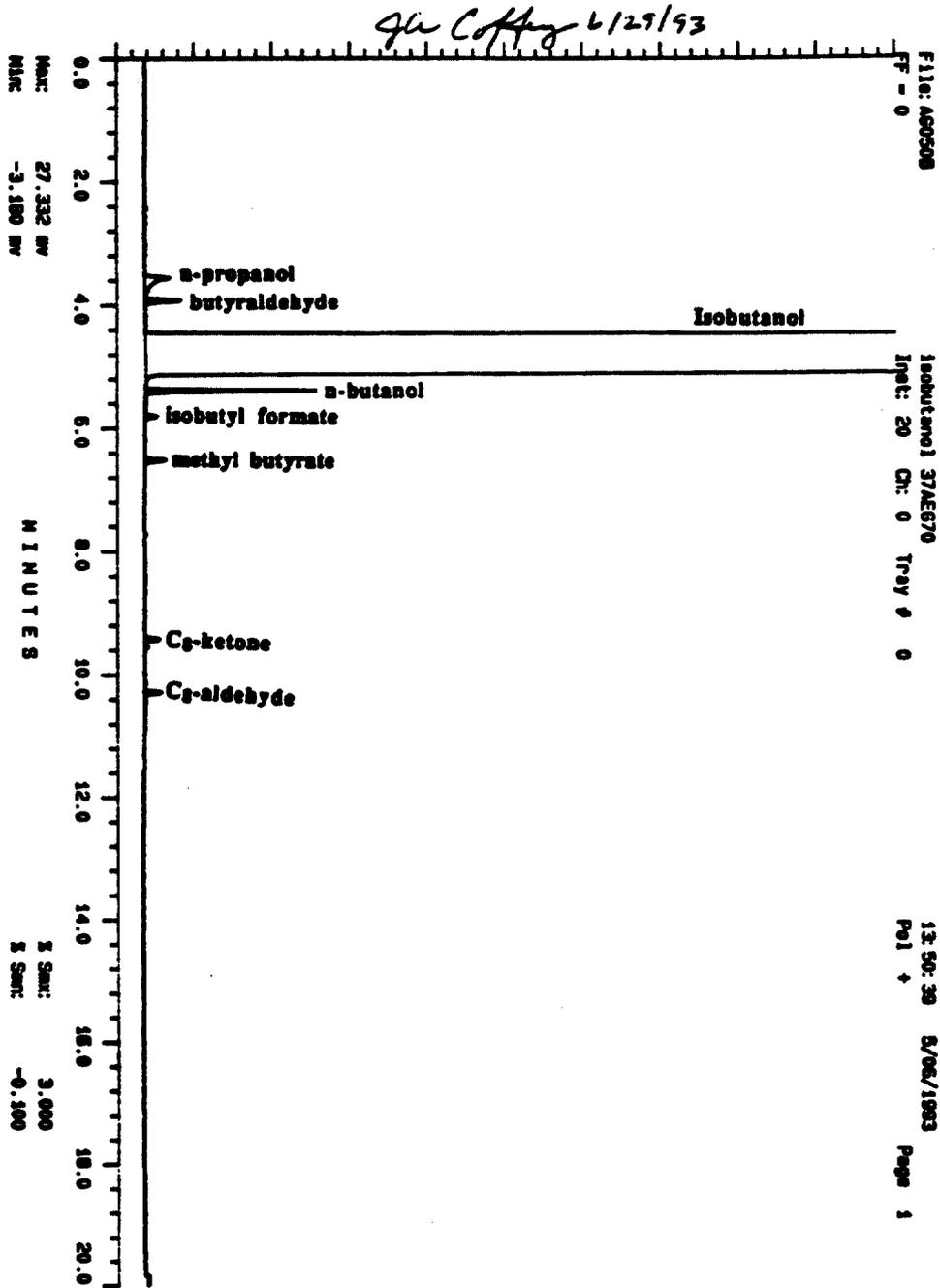


Figure 4 — Capillary Gas Chromatogram of 37-AEG-70 (Isobutanol)



APPENDIX I Protocol

# PROTOCOL

## GOOD LABORATORY PRACTICE (GLP) STUDY

**title** Isobutanol

**purpose** Analytical Characterization of Sample(s) for Toxicology Studies at Bushy Run Research Center

**study number** 37-AEG-65

**sponsor** SOLVENTS AND COATING MATERIALS DIVISION (SCMD)  
Union Carbide Corporation (UCC)  
39 Old Ridgebury Road, Danbury, Conn. 06817-0001

**testing facility** UCC Technical Center,  
South Charleston, WV 25303 (Location 511)

**Proposed Starting Date:** Thursday, May 6, 1993  
**Proposed Completion Date:** August 1, 1993  
**Estimated Date of Final Report:** September 1, 1993

**Test Substance(s) 37-AEG-70**

**Name** isobutanol; isobutyl alcohol  
**Source** lot # TS3370114; UCC, Texas City, TX  
**CAS Registry No.** 78-83-1  
**Description** colorless, non-viscous liquid  
**Purity** = 99.9%  
**Health/Safety** moderately toxic; stable. MSDS available upon request  
**Storage Conditions** room temperature

**Study Design**

- The test substance(s) will be characterized by:
- Verification of identity by proton- and carbon-NMR.
  - Verification of identity by GC/MS. An attempt will be made to identify all impurities at the concentration of  $\geq 0.1$  wt. %.
  - Quantitation of the identified impurities by capillary GC.

Reviewed and Approved by:

*Alexander E. Gablany* <sup>5/4/93</sup> *Denise L. Johnson* <sup>5/24/93</sup> *Richard C. Wise* <sup>6-1-93</sup>  
\_\_\_\_\_  
Alexander E. Gablany      date      Denise L. Johnson      date      Richard C. Wise      date  
GLP Study Director      GLP Quality Assurance Unit      Manager of Product Safety,  
(QAU) Representative      SCMD, Sponsor

This study will be performed in compliance with the following GLP standards: FDA, 21 CFR, Part 58; TSCA, 40 CFR, Part 792; and FIFRA, 40 CFR, Part 160. All changes of an approved protocol and the reasons therefor shall be documented, signed by the study director, dated, and maintained with the protocol. All raw data, reports and a sample of test substance from this study will be retained at Location 511 for at least 10 years after completion of the study. A comprehensive final report will be submitted to the Sponsor within one month after the completion of the analysis. The final report will be inspected by the QAU and will contain a signed quality assurance statement.

**Quality Assurance Unit Study Inspection Summary**

**Test Substance: ISOBUTANOL**

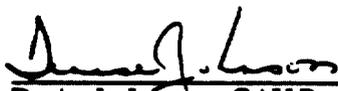
**Study No.: 37-AEG-65**

**Study Director: A.E. Gabany, B.S.**

The Quality Assurance Unit of the Union Carbide Technical Center conducted the inspections listed below and reported the results to the study director and management on the date indicated. It is the practice of this Quality Assurance Unit to report the results to both the study director and management.

<u>Date</u>	<u>Inspection</u> Type	<u>Date OAU Report Issued</u>	
		<u>To Study Director</u>	<u>To Management</u>
Feb. 10, 1992	Laboratory Compliance Review*	Feb. 10, 1992	May, 1992
May 24, 1993	Protocol Compliance Review	May 24, 1992	May 24, 1992
Nov. 8, 1993	Final Report Compliance Review	Nov. 8, 1993	Nov. 8, 1993

\*The process of doing the GLP characterization studies is audited periodically to assure these studies comply with GLP requirements. The QA unit is exempted from performing in-life study inspections for studies designed to determine physical and chemical characteristics of a test substance as described in 40 CFR 792.135.

 11-8-93  
Denise L. Johnson, QAU Representative (Date)  
Good Laboratory Practices/Quality Assurance

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**Clinical Pathology Report**

**(20 Pages)**

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### SUMMARY

Female Sprague Dawley® rats were dosed by stomach intubation with 2830 mg/kg of isobutanol in 0.25% aqueous methyl cellulose or with 0.25% aqueous methyl cellulose alone (control). Blood samples were collected for clinical pathology evaluation.

During a probe study, estradiol levels dropped and remained lower following the administration of isobutanol. Estradiol levels did not cycle for the 5 days following single administration of isobutanol. Progesterone in 3 of 4 rats increased to higher levels following isobutanol administration. The levels of progesterone following isobutanol administration were higher than normal cyclic postovulatory levels. These levels indicate a possible pseudopregnancy may have been induced.

During the definitive study, the results indicate that isobutanol prevents estradiol cycling for at least 5 - 6 days following single administration. The estradiol levels did not return to the normal cyclic pattern until 10 days after isobutanol administration. Progesterone levels in control rats remained below approximately 30 ng/ml. Progesterone levels increased to over 85 ng/ml in 5 of 9 rats following isobutanol administration. Two other rats had a similar pattern, but progesterone levels did not rise above 80 ng/ml.

### MATERIALS AND METHODS

In this study, female Sprague Dawley® rats were dosed with 2830 mg/kg of isobutanol in 0.25% aqueous methyl cellulose or 0.25% aqueous methyl cellulose (control) by stomach intubation.

Blood samples for all clinical pathology analyses were collected by retroorbital bleeding from anesthetized rats. Rats were not fasted prior to bleeding. For the probe study, blood was collected from each rat for 4 or 5 days prior to dose administration and 5 days following dose administration. No samples were taken the day of the dose administration. For the definitive study, blood was collected from rats the day before and after (with 1 exception) dose administration. The rats were divided into 3 groups for blood collection (each group consisting of control and test rats). Each group was to be bled on a rotating basis starting 2 days after dose administration until the day of sacrifice. Some modifications to the schedule were required due to deaths.

Blood was collected into blood collection tubes without anticoagulant for serum chemistry analysis. Estradiol and progesterone levels were measured using radioimmunoassays (RIA) kits from Diagnostics Products Corporation, Los Angeles, CA.

### RESULTS AND DISCUSSION

The summary of the results for the probe study are presented in Figures 1 and 2. The individual results for these animals are presented in Tables 1 and 2. The summary of the results for the definitive study are presented in Figures 3-8. The individual results for these animals are presented Tables 3-8.

Normal estradiol levels in the rat range from a basal 10 pg/ml to 20 - 30 pg/ml during estrus and 40 - 50 pg/ml during proestrus. Progesterone increases from basal levels of 1 - 5 ng/ml to 40 - 50 ng/ml following the preovulatory luteinizing hormone surge, decrease to basal levels at estrus, and increase again following diestrus.

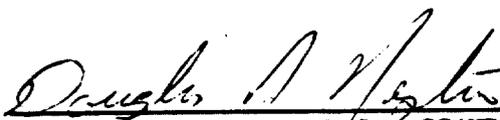
In the probe study, 1 rat died following the dosing and therefore the predosing data from this animal was not used in the figures. During the probe study, estradiol levels dropped and remained lower following the administration of isobutanol. Estradiol levels did not cycle for the 5 days following administration of isobutanol. Progesterone in 3 of 4 rats increased to higher levels following isobutanol administration. The levels of progesterone following isobutanol administration were higher than normal cyclic postovulatory levels. These levels indicate a possible pseudopregnancy may have been induced.

Several deaths occurred during the dosing of the definitive study. Dead animals were replaced in order to keep enough animals in each group. During the definitive study, the results indicate that isobutanol prevents estradiol cycling for at least 5 - 6 days following administration. The estradiol levels did return to the normal cyclic pattern 10 days following isobutanol administration. Progesterone levels in control rats remained below approximately 30 ng/ml. One control rat had high progesterone levels prior to and following dose administration. This high level may have been caused by some physical stimulation of the cervix. Progesterone levels increased to over 85 ng/ml in 5 of 9 rats following isobutanol administration. Two other rats had a similar pattern, but progesterone levels did not rise above 80 ng/ml.

#### CONCLUSION

Isobutanol caused a cessation of the cyclic pattern of estradiol circulation and an increase in progesterone. While 1 of 6 control rats exhibited a pattern of pseudopregnancy prior to dose administration, 6 of 9 rats exhibited hormone patterns of pseudopregnancy following isobutanol administration.

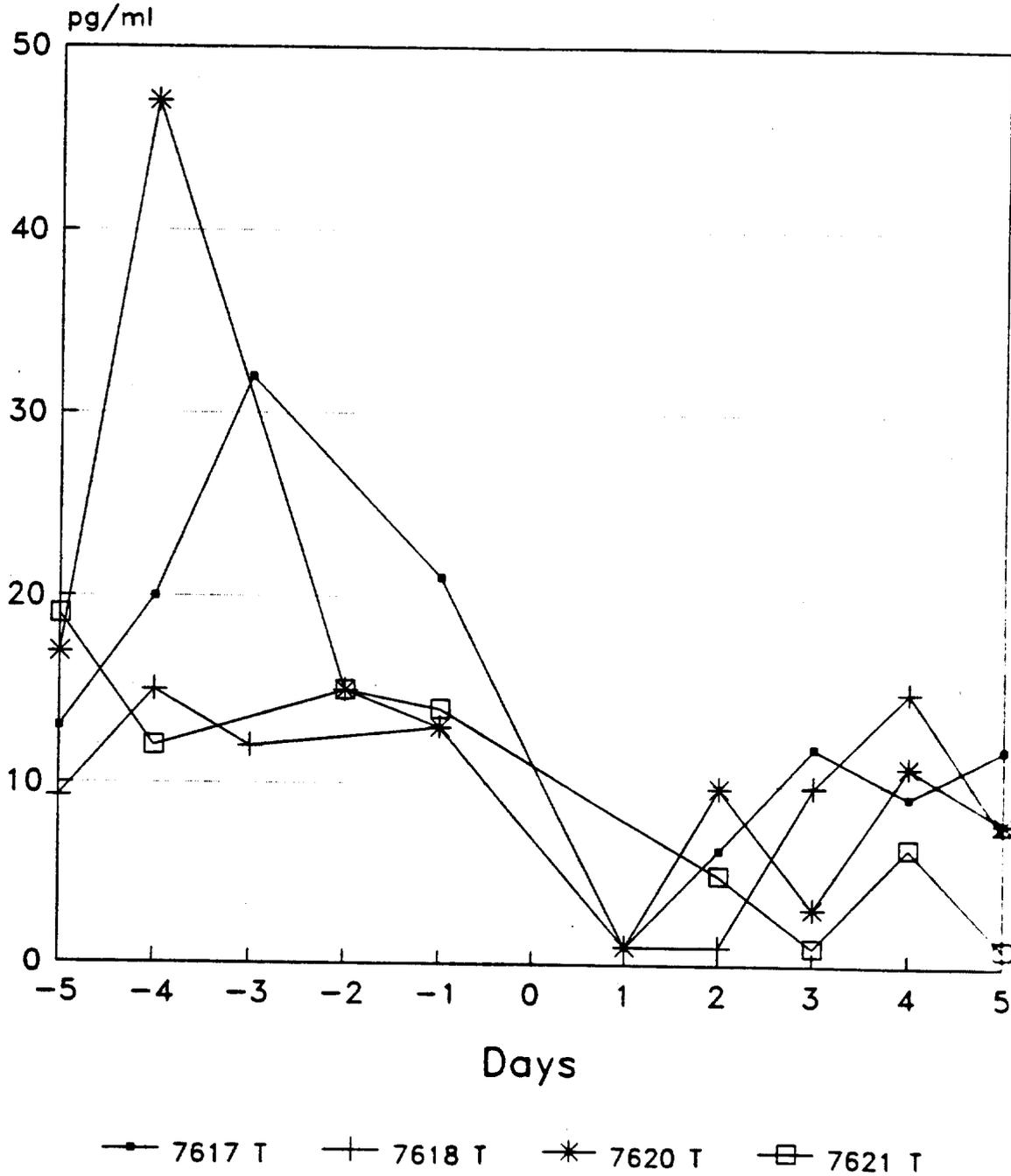
Clinical Pathologist:

  
Douglas A. Neptun, B.S., CC(NRCC), MT(ASCP)

3/19/91  
Date

FIGURE 1  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

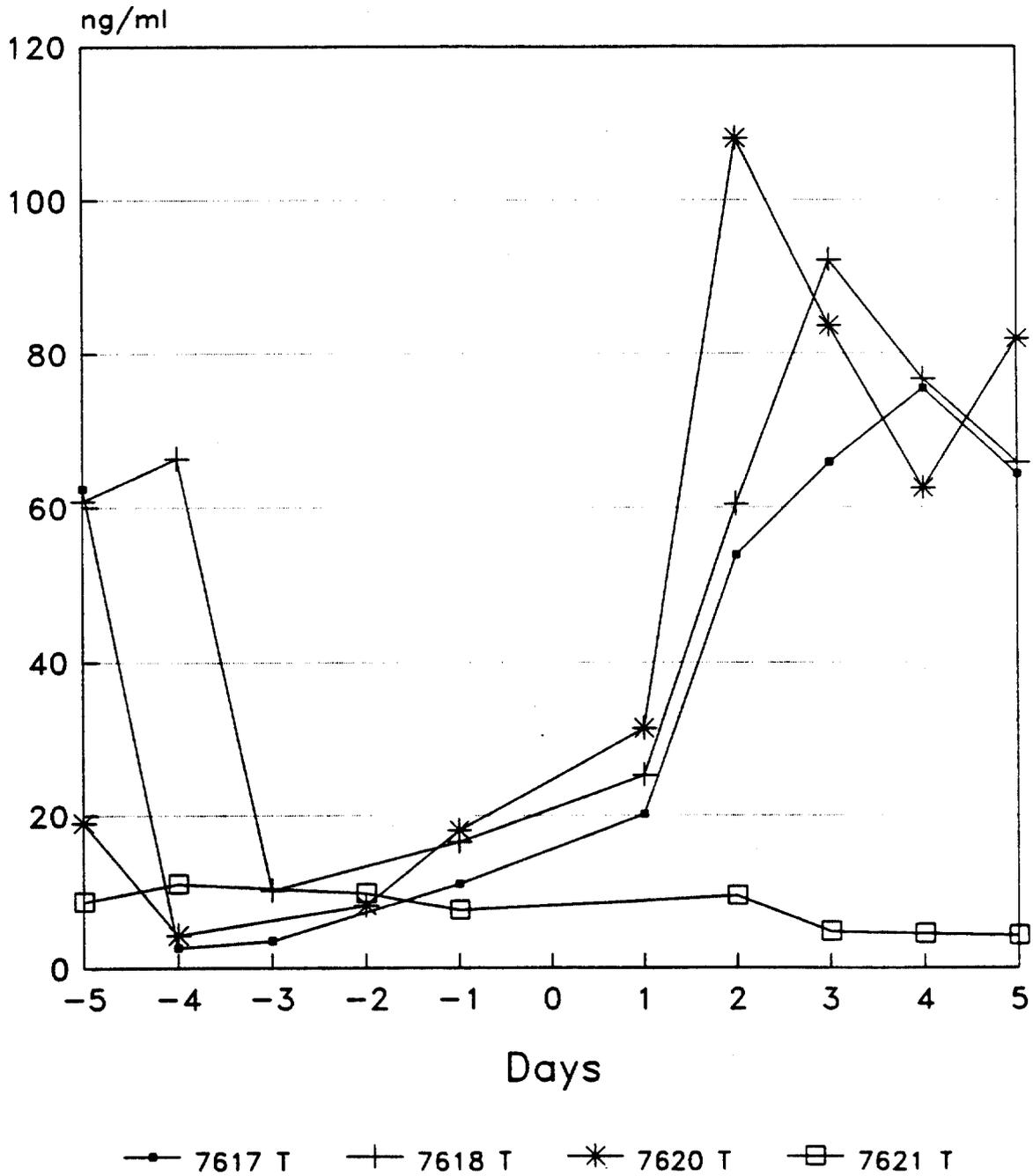
PROBE  
Estradiol



T = Isobutanol 2830 mg/kg

FIGURE 2  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

PROBE  
Progesterone



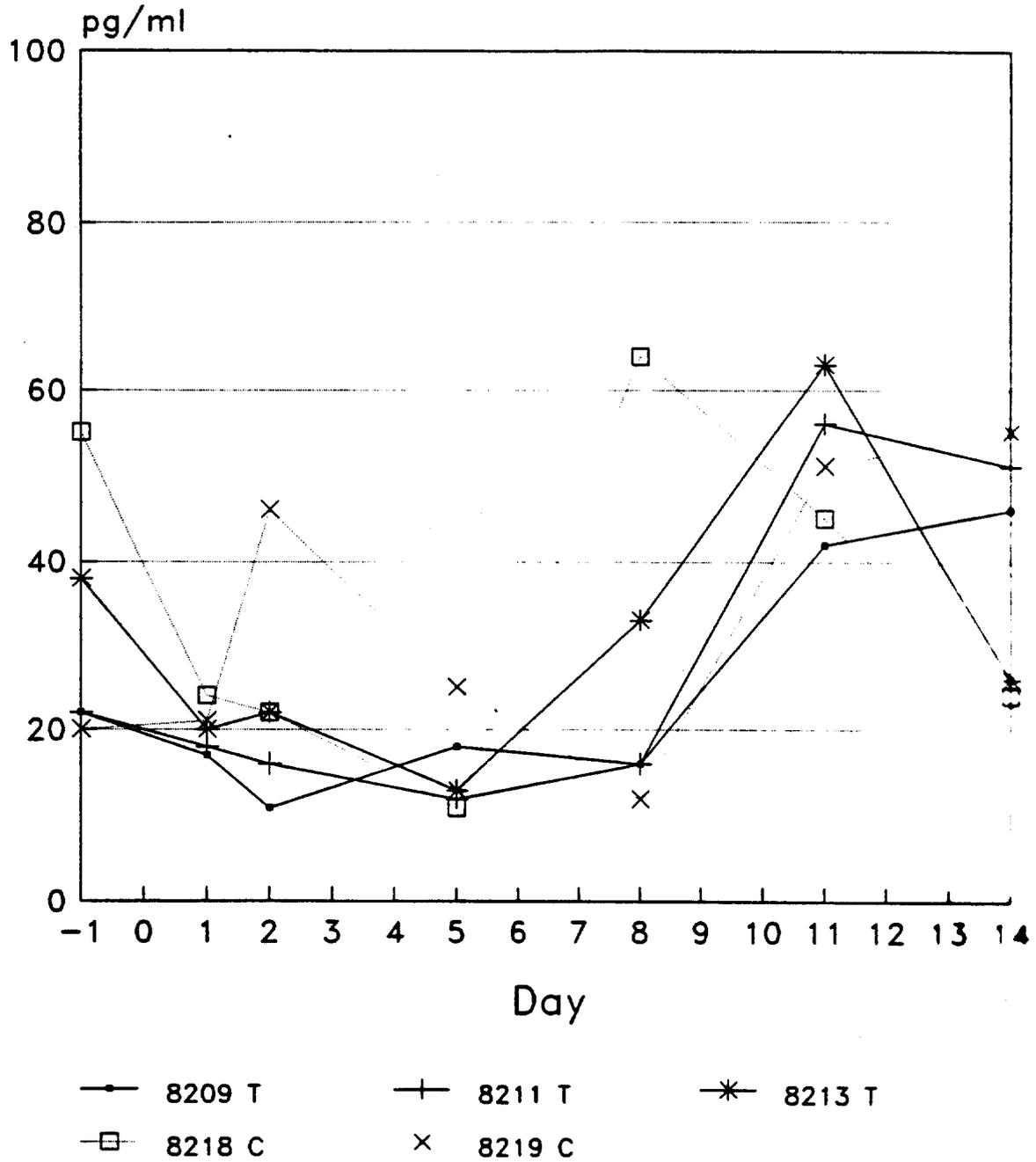
T = Isobutanol 2830 mg/kg

FIGURE 3  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE STUDY

Estradiol

Group 1

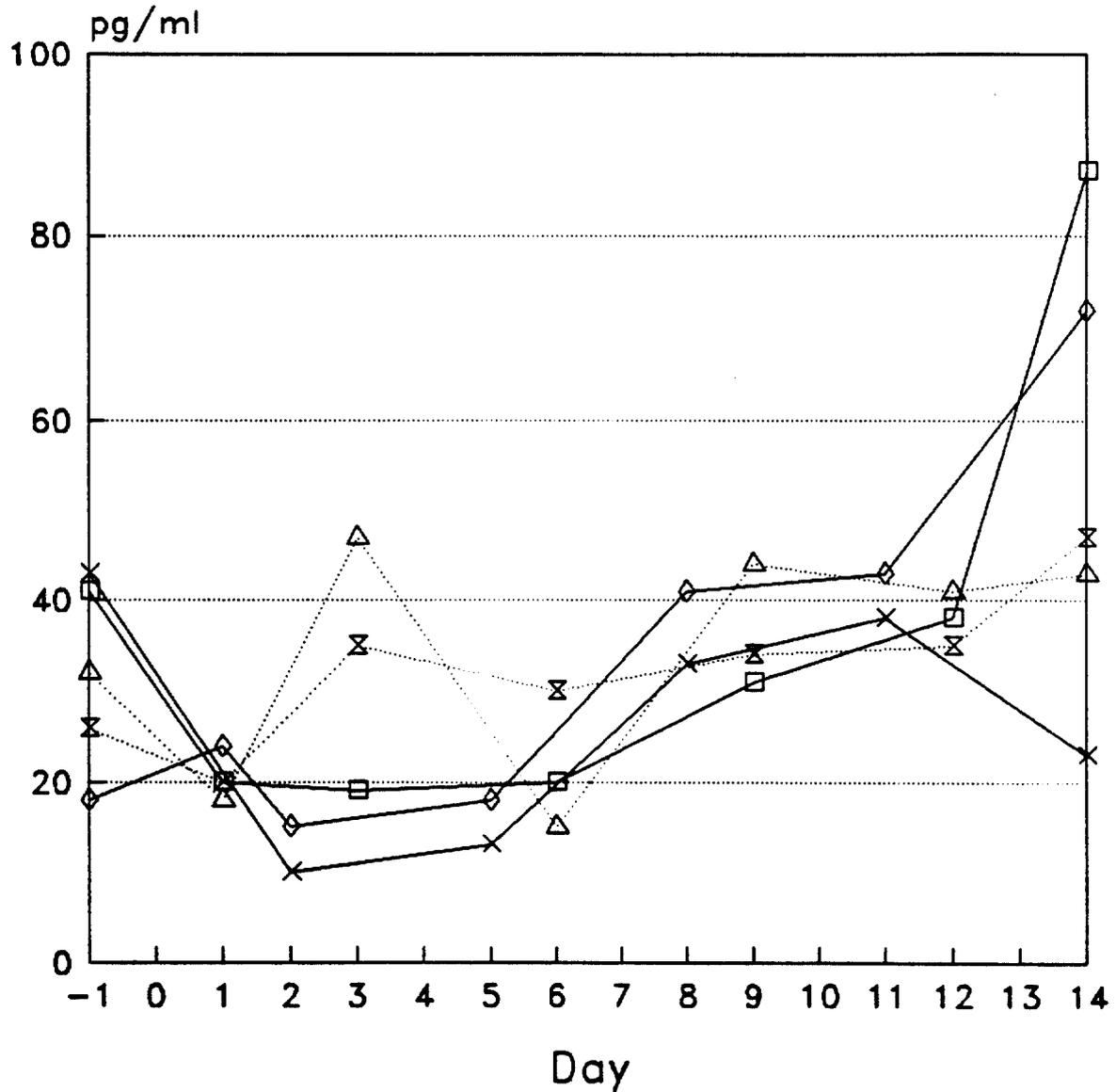


C= Control T= Isobutanol (2830 mg/kg)

FIGURE 4  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE STUDY

Estradiol  
Group 2



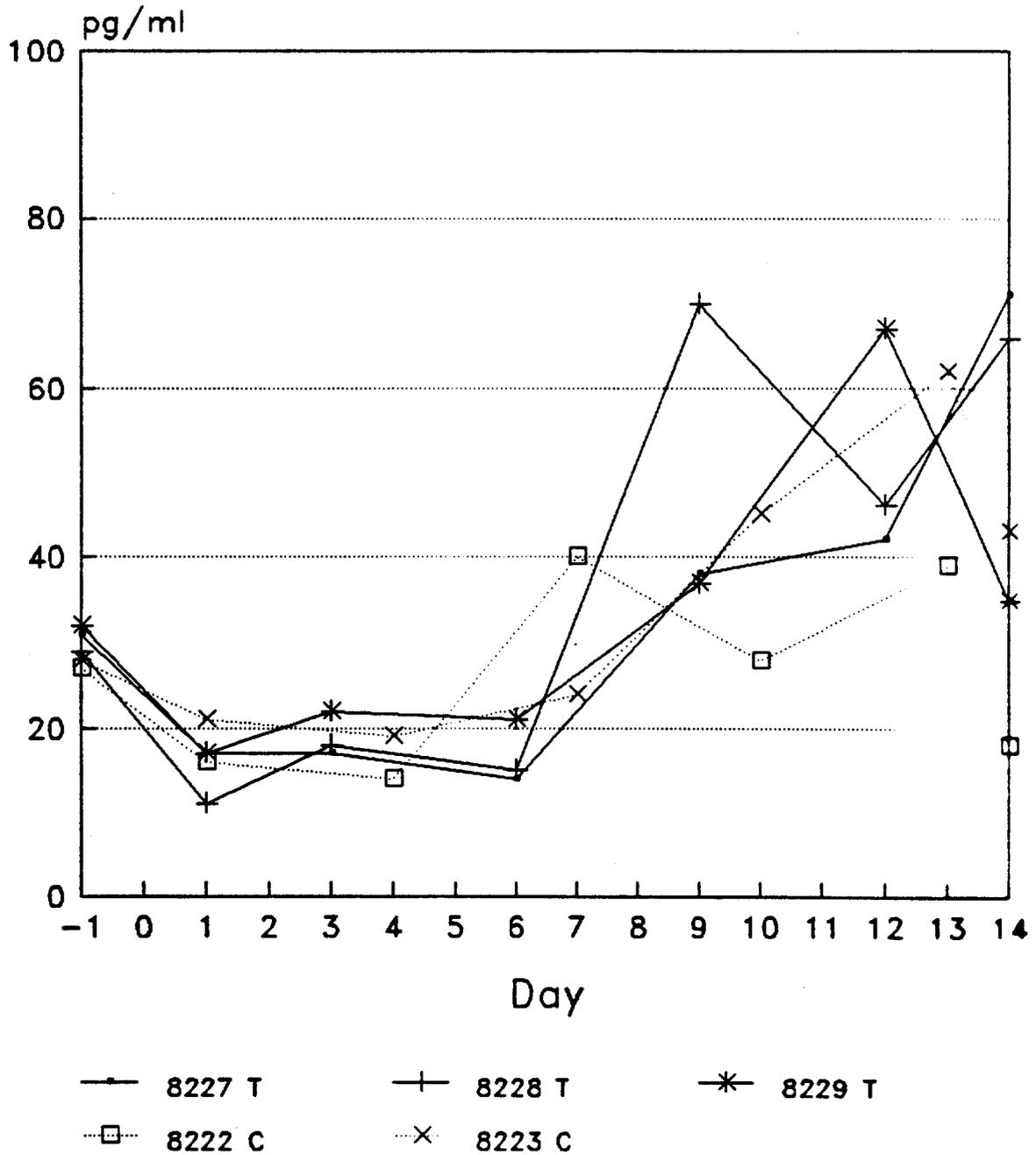
—□— 8216 T                      —×— 8224 T                      —◇— 8226 T  
- -△- - 8220 C                      - -×- - 8221 C

C= Control T= Isobutanol (2830 mg/kg)

FIGURE 5  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE STUDY

Estradiol  
Group 3

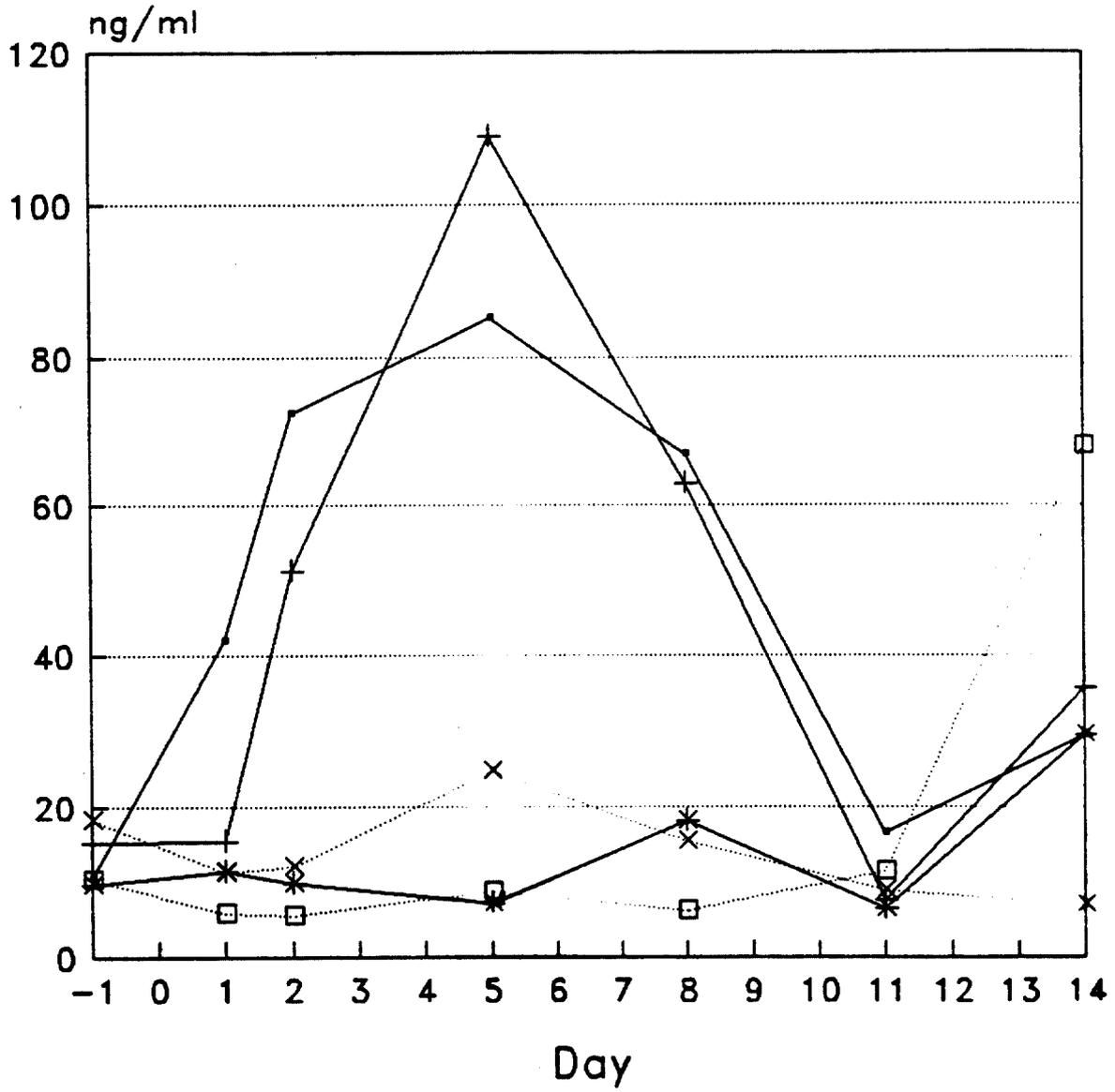


C= Control T= Isobutanol (2830 mg/kg)

**FIGURE 6**  
**ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN**  
**FEMALE RATS FOLLOWING ACUTE PERORAL DOSES**

**DEFINITIVE STUDY**

**Progesterone**  
**Group 1**



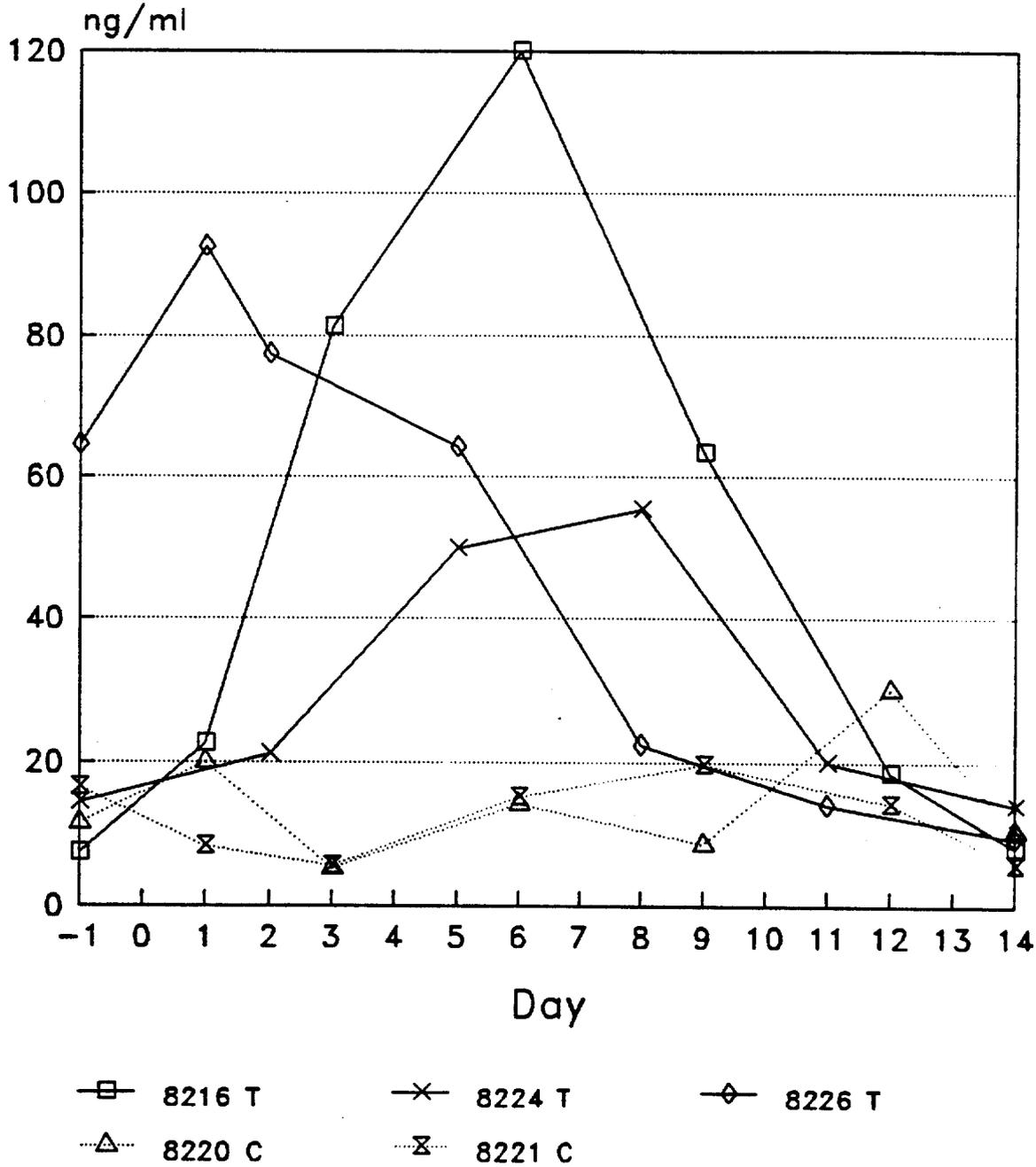
—●— 8209 T	—+— 8211 T	—*— 8213 T
—□— 8218 C	—x— 8219 C	

C= Control T= Isobutanol (2830 mg/kg)

FIGURE 7  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE STUDY

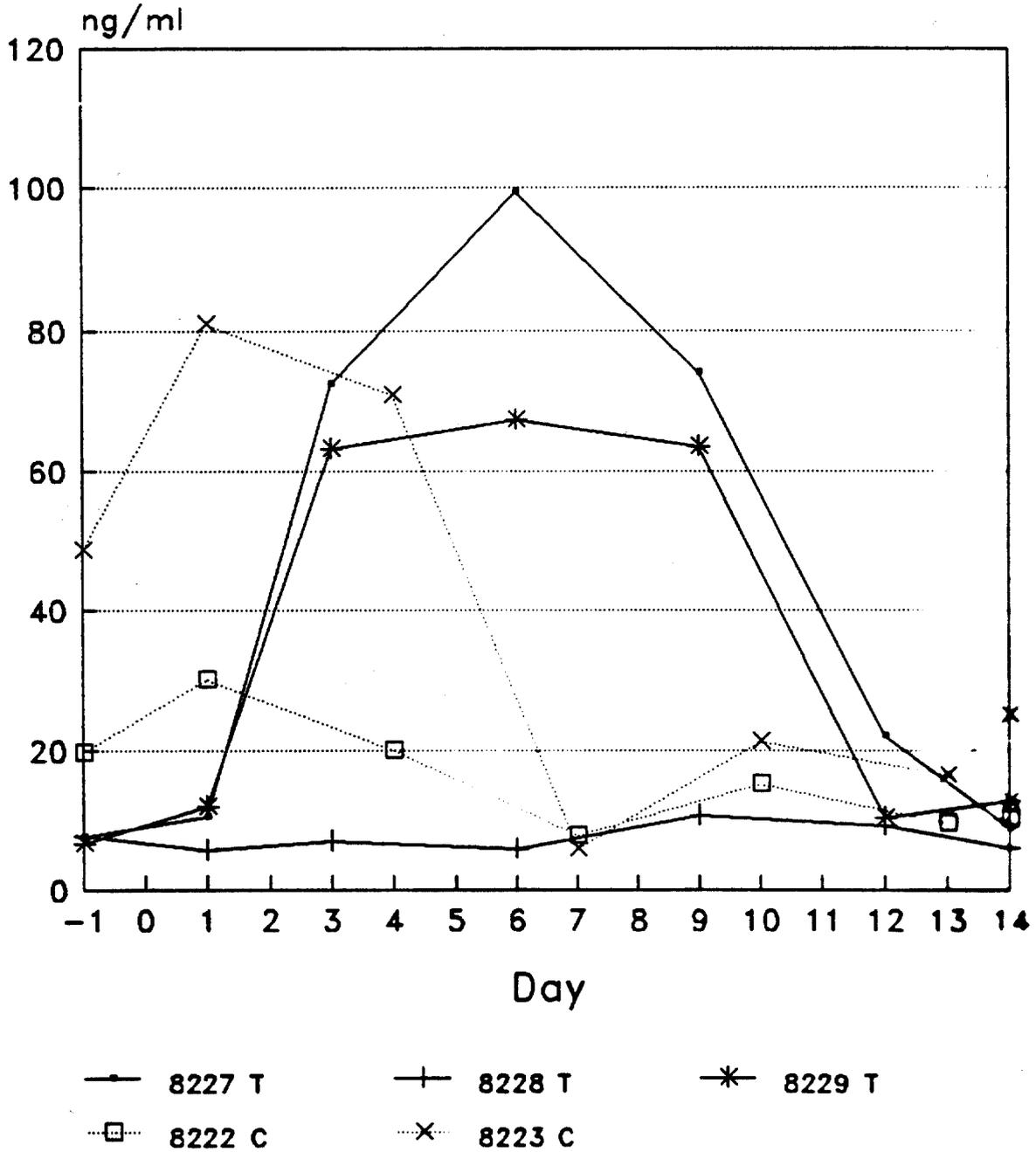
Progesterone  
Group 2



C= Control T= Isobutanol (2830 mg/kg)

FIGURE 8  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE STUDY  
Progesterone  
Group 3



C= Control T= Isobutanol (2830 mg/kg)

TABLE 1  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DAY	PROBE ESTRADIOL (pg/ml)				
	7617 T	7618 T	ANIMAL 7619 T	7620 T	7621 T
	T = ISOBUTANOL TREATED (2830 mg/kg)				
-6	NOS	NOS	NOS	15	29
-5	13	9.3	23	17	19
-4	20	15	18	47	12
-3	32	12	48	NOS	NOS
-2	NOS	NOS	NOS	15	15
-1	21	13	17	13	14
0					
1	<1.5	<1.5	NOS	<1.5	NOS
2	6.4	<1.5	NOS	9.8	5.0
3	12	9.9	NOS	3.2	<1.5
4	9.4	15	NOS	11	6.6
5	12	7.5	NOS	8.0	<1.5

NOS = NO SAMPLE

**TABLE 2**  
**ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN**  
**FEMALE RATS FOLLOWING ACUTE PERORAL DOSES**

**PROBE**  
**PROGESTERONE (ng/ml)**

DAY	7617 T	7618 T	ANIMAL 7619 T	7620 T	7621 T
	T = ISOBUTANOL TREATED (2830 mg/kg)				
-6	NOS	NOS	NOS	14.3	5.7
-5	62.4	60.8	17.0	19.0	8.6
-4	2.6	66.3	17.9	4.2	11.0
-3	3.5	10.1	5.7	NOS	NOS
-2	NOS	NOS	NOS	8.1	9.7
-1	11.0	16.5	8.4	18.0	7.5
0					
1	20.2	25.3	NOS	31.4	NOS
2	53.8	60.4	NOS	108	9.4
3	65.8	92.2	NOS	83.6	4.7
4	75.4	76.6	NOS	62.5	4.4
5	64.3	65.8	NOS	81.9	4.2

NOS = NO SAMPLE

TABLE 3  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 1  
 ESTRADIOL (pg/ml)

DAY	ANIMAL				
	8209 T	8211 T	8213 T	8218 C	8219 C
	T = ISOBUTANOL TREATED (2830 mg/kg) C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)				
-1	22	22	38	55	20
0					
1	17	18	20	24	21
2	11	16	22	22	46
3					
4					
5	18	12	13	11	25
6					
7					
8	16	16	33	64	12
9					
10					
11	42	56	63	45	51
12					
13					
14	46	51	26	24	55

TABLE 4  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 2  
 ESTRADIOL (pg/ml)

DAY	ANIMAL				
	8216 T	8224 T	8226 T	8220 C	8221 C
	T = ISOBUTANOL TREATED (2830 mg/kg) C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)				
-1	41	43	18	32	26
0					
1	20		24	18	20
2		10	15		
3	19			47	35
4					
5		13	18		
6	20			15	30
7					
8		33	41		
9	31			44	34
10					
11		38	43		
12	38			41	35
13					
14	87	23	72	43	47

TABLE 5  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 3  
 ESTRADIOL (pg/ml)

DAY	ANIMAL				
	8227 T	8228 T	8229 T	8222 C	8223 C
	T = ISOBUTANOL TREATED (2830 mg/kg) C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)				
-1	31	29	32	27	28
0					
1	17	11	17	16	21
2					
3	17	18	22		
4				14	19
5					
6	14	15	21		
7				40	24
8					
9	38	70	37		
10				28	45
11					
12	42	46	67		
13				39	62
14	71	66	35	18	43

TABLE 6  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 1  
 PROGESTERONE (ng/ml)

DAY	8209 T	8211 T	ANIMAL 8213 T	8218 C	8219 C
	T = ISOBUTANOL TREATED (2830 mg/kg) C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)				
-1	10.6	15.2	9.6	10.3	18.2
0					
1	42.0	15.4	11.3	5.7	11.2
2	72.5	51.2	9.8	5.5	12.0
3					
4					
5	85.2	109	7.1	8.6	24.7
6					
7					
8	66.9	62.9	18.1	6.0	15.4
9					
10					
11	16.4	7.5	6.4	11.4	8.7
12					
13					
14	29.6	35.8	29.6	68.0	6.8

TABLE 7  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 2  
 PROGESTERONE (ng/ml)

DAY	ANIMAL				
	8216 T	8224 T	8226 T	8220 C	8221 C
	T = ISOBUTANOL TREATED (2830 mg/kg) C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)				
-1	7.4	14.3	64.5	11.5	16.5
0					
1	22.5		92.4	19.9	8.3
2		21	77.4		
3	81.3			5.3	5.6
4					
5		49.8	64.2		
6	120			14.2	15.1
7					
8		55.4	22.4		
9	63.3			8.6	19.6
10					
11		20.0	14.1		
12	18.5			30.0	14.2
13					
14	8.1	14.0	9.4	10.5	5.8

TABLE 8  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DEFINITIVE GROUP 3  
 PROGESTERONE (ng/ml)

DAY	ANIMAL				
	8227 T	8228 T	8229 T	8222 C	8223 C
	T = ISOBUTANOL TREATED (2830 mg/kg)		C = CONTROL (10ml/kg of 0.25% METHYL CELLULOSE)		
-1	7.6	7.8	6.7	19.6	48.7
0					
1	10.5	5.8	11.9	30.2	81.0
2					
3	72.5	7.1	63.2		
4				19.9	70.9
5					
6	99.4	5.9	67.3		
7				7.8	5.9
8					
9	74.1	10.7	63.5		
10				15.2	21.2
11					
12	22.0	9.2	10.4		
13				9.6	16.5
14	8.9	6.0	12.7	10.2	25.1

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**Anatomic Pathology Report**

**(31 Pages)**

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### SUMMARY

Female Sprague Dawley® rats, purchased from Harlan Sprague Dawley Inc., Indianapolis, IN, were gavaged with 10.0 ml/kg of aqueous methyl cellulose solution (control) or 2830 mg/kg of isobutanol to determine the possibility of pseudopregnancy. Most animals received a complete necropsy (1 probe animal received an abbreviated necropsy). Selected tissues were collected from 26 female rats, preserved in fixative and microscopic examinations were performed. Four of 5 probe animals were sacrificed on Day 5 (1 died at 2 days). Nine of 15 test animals (6 died at 0.5 hrs to 2 days) and all 6 control animals were sacrificed 14 days postexposure.

It appeared from the morphology of the female genital organs that isobutanol caused the estrus cycle to be delayed longer than normal in the metestrus phase, with most of the ovaries showing active corpora lutea, and the uteri being predominantly in the progestin phase with regular appearing low columnar epithelium and a nucleus that was located part of the way between the base of the cell and the luminal surface. Many of the vaginas had some mucus present and epithelial cells that were undergoing mucification. No frank evidence of pseudopregnancy (deciduoma formation) was observed. The rats in the probe study that were sacrificed at Day 5 were more consistent in the appearance of the female genital tract than were those included in the definitive study that were sacrificed at Day 14. It appeared that the rats were starting to recycle by Day 14 with some of them showing estrogenic influence in the uteri and more active follicular development in the ovaries.

### MATERIALS AND METHODS

#### Necropsy

Rats died or were euthanized by brachial exsanguination after being anesthetized with methoxyflurane. Most animals received a complete necropsy. The uterus, vagina, and ovaries were collected from all rats and preserved in 10% neutral buffered formalin.

Ear tags were saved as animal identification.

Ovarian weights were collected from all animals at the terminal sacrifice.

#### Histopathology

Microscopic examinations were performed on the uterus, vagina, and ovaries from all rats.

All tissues to be examined were paraffin embedded, sectioned at approximately 5 microns and stained with hematoxylin and eosin. Lesions were graded, when possible, into 5 categories (minimal, mild, moderate, marked and severe).

### Statistics

No statistics were performed on the morphologic portion of this study because of the nature of the observations to be made and the small number of animals to be used.

### RESULTS AND DISCUSSION

This study was designed using the LD50 for the test material which resulted in the loss through death of some of the rats. The original design included 9 rats in the dosed group for the definitive study and only 6 in the control group. When more than 1/2 of the rats died in the definitive group, an additional group of 6 rats were dosed with the test material in order to have at least 6 rats that survived until 14 days after dosing for morphologic evaluation. These rats are tabulated separately but discussed together in the following section.

Tables 1 and 2 list the necropsy observations for the rats used in the 5 day probe study. Tables 3 and 4 list the necropsy observations for the rats originally designated for the definitive study, with Table 3 including the rats sacrificed at Day 14 and Table 4 including the rats that were found dead or sacrificed moribund. Tables 5 and 6 list the necropsy findings for the rats sacrificed at Day 14 (Table 5), or found dead or sacrificed moribund (Table 6), for the extra rats dosed for the definitive study. There were no gross lesions in any of these rats that could be attributed to gavage administration of the test material.

Tables 7-9 list the microscopic observations for the rats included in the 5 day probe study, with Table 7 including the rats that were sacrificed at Day 5, Table 8 including those rats that were found dead or sacrificed moribund, and Table 9 including all animals included in the probe study. Tables 10-12 list the microscopic observations for the rats that were originally included in the definitive study, with Table 10 including the rats that were sacrificed at Day 14, Table 11 including the rats that were found dead or sacrificed moribund, and Table 12 including all rats from this portion of the definitive study. Tables 13-15 list the microscopic observations for the rats that were dosed as "extra" rats to replace those that died or were sacrificed moribund. Table 13 includes those rats that were sacrificed at Day 14, Table 14 includes those that were found dead or were sacrificed moribund, and Table 15 includes all the rats that were used as extras.

The only microscopic findings of note in all of these rats was the interruption of the estrus cycle following administration of the isobutanol, which was seen to its greatest extent in the rats included in the probe study that were sacrificed at Day 5, but was also evident in the rats included in the definitive study. It appeared from the morphology of the female genital organs that isobutanol caused the estrus cycle to be delayed longer than normal in the metestrus phase, with most of the ovaries showing active corpora lutea, and the uteri being predominantly in the progestin phase with regular appearing low columnar epithelium and a nucleus that was located part of the way between the base of the cell and the luminal surface. Many of the vaginas had some mucus present and epithelial cells that were undergoing

mucification. No frank evidence of pseudopregnancy (deciduoma formation) was observed. The rats in the probe study that were sacrificed at Day 5 were more consistent in the appearance of the female genital tract than were those included in the definitive study that were sacrificed at Day 14. It appeared that the rats were starting to recycle by Day 14 with some of them showing estrogenic influence in the uteri and more active follicular development in the ovaries.

CONCLUSION

Rats dosed with 2830 mg/kg of isobutanol were found to have an interruption of their estrus cycles. They appeared to be delayed longer than normal in metestrus, with active corpora lutea in the ovaries, the uteri in the progestin phase, and mucification of the vaginas. The rats in the probe study that were sacrificed at Day 5 were more consistent in the appearance of the female genital tract than were those included in the definitive study that were sacrificed at Day 14. It appeared that the rats were starting to recycle by Day 14 with some of them showing estrogenic influence in the uteri and more active follicular development in the ovaries.

Pathologist:

*Edward H. Fowler*  
Edward H. Fowler, DVM, Ph.D.  
Diplomate, ACVP

*8-20-95*  
Date

TABLE 1  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF NECROPSY OBSERVATIONS

ANIMALS SACRIFICED AT DAY 5  
PROBE FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		5
NUMBER OF ANIMALS SACRIFICED		4
LYMPH ND, S-MAN SIZE INCREASE		1
EYE SIZE DECREASE		1
LUNGS COLOR CHANGE, FOCAL/MULTIFOCAL		2

GROUP LEGEND: 1 is 2830 MG/KG

TABLE 2  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF NECROPSY OBSERVATIONS

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
PROBE FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		5
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND		1
STOMACH		
COLOR CHANGE, FOCAL/MULTIFOVAL		1
LIVER		
COLOR CHANGE, FOCAL/MULTIFOVAL		1
SUBCUTIS		
COLOR CHANGE, DIFFUSE		1
LUNGS		
COLOR CHANGE, FOCAL/MULTIFOVAL		1
KIDNEYS		
COLOR CHANGE, FOCAL/MULTIFOVAL		1
GROUP LEGEND: 1 is 2830 MG/KG		

TABLE 3  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF NECROPSY OBSERVATIONS

ANIMALS SACRIFICED AT DAY 14  
 DEFINITIVE STUDY FEMALES

	GROUP:	1	2
NUMBER OF ANIMALS IN DOSE GROUP		6	9
NUMBER OF ANIMALS SACRIFICED		6	4
COLON			
ADHESION		3	0
NODULE		2	0
HEMORRHAGE		1	0
PARASITE		1	0
LYMPH ND, S-MAN			
COLOR CHANGE, DIFFUSE		2	1
COLOR CHANGE, FOCAL/MULTIFOCAL		0	1
SIZE INCREASE		2	0
THYMIC REGION			
COLOR CHANGE, FOCAL/MULTIFOCAL		1	0
OVARIES			
SIZE INCREASE		3	3
COLOR CHANGE, DIFFUSE		2	0
KIDNEYS			
DILATED PELVIS		1	0
GROUP LEGEND: 1 is 0 MG/KG, 2 is 2830 MG/KG			

TABLE 4  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF NECROPSY OBSERVATIONS

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
 DEFINITIVE STUDY FEMALES

	GROUP:	
	1	2
NUMBER OF ANIMALS IN DOSE GROUP	6	9
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND	-	5
TOTAL BODY POSTMORTEM CHANGE	-	1
STOMACH CONTENTS ABNORMAL	-	4
RECTUM CANNIBALISM	-	1
LYMPH ND, S-MAN COLOR CHANGE, DIFFUSE	-	2
THYMIC REGION COLOR CHANGE, FOCAL/MULTIFOCAI	-	1
VAGINA CANNIBALISM	-	1
LUNGS HYPERINFLATION	-	1

GROUP LEGEND: 1 is 0 MG/KG, 2 is 2830 MG/KG

TABLE 5  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF NECROPSY OBSERVATIONS

ANIMALS SACRIFICED AT DAY 14  
 ADDITIONAL FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		6
NUMBER OF ANIMALS SACRIFICED		5
COLON		
PARASITE		1
LYMPH ND, S-MAN		
COLOR CHANGE, FOCAL/MULTIFOCAL		1
SIZE INCREASE		1
OVARIES		
SIZE INCREASE		3
SIZE DECREASE		1
LUNGS		
COLOR CHANGE, FOCAL/MULTIFOCAL		1
GROUP LEGEND: 1 is 2830 MG/KG (#2)		

TABLE 6  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF NECROPSY OBSERVATIONS

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
ADDITIONAL FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		6
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND		1
STOMACH		
CONTENTS ABNORMAL		1
LUNGS		
COLOR CHANGE, DIFFUSE		1
GROUP LEGEND: 1 is 2830 MG/KG (#2)		

TABLE 7  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

ANIMALS SACRIFICED AT DAY 5  
 PROBE FEMALES

GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP	5
NUMBER OF ANIMALS SACRIFICED	4
LYMPH NO, S-MAN	
TOTAL NUMBER EXAMINED	1
PLASMACYTOSIS	1
LYMPHOID HYPERPLASIA	1
EYE	
TOTAL NUMBER EXAMINED	1
CORNEAL MINERALIZATION	1
CORNEAL ULCER	1
IRIDOCYCLITIS	1
UVEITIS	1
CATARACT	1
OVARIES	
TOTAL NUMBER EXAMINED	4
SMALL FOLLICLES	4
INACTIVE CORPORA LUTEA	2
ACTIVE CORPORA LUTEA	2
UTERUS	
TOTAL NUMBER EXAMINED	4
PROGESTIN INFLUENCE	4
VAGINA	
TOTAL NUMBER EXAMINED	4
EARLY METESTRUS	1
MUCIFICATION OF EPITHELIAL CELLS	1
MID METESTRUS	2
LATE METESTRUS	1
LUNGS	
TOTAL NUMBER EXAMINED	2
EXAMINED, UNREMARKABLE	2

GROUP LEGEND: 1 is 2830 MG/KG

TABLE 8  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF MICROSCOPIC DIAGNOSES

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
PROBE FEMALES

---

GROUP:	1
--------	---

---

NUMBER OF ANIMALS IN DOSE GROUP	5
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND	1
OVARIES	
TOTAL NUMBER EXAMINED	1
ACTIVE CORPORA LUTEA	1
UTERUS	
TOTAL NUMBER EXAMINED	1
PROGESTIN INFLUENCE	1
VAGINA	
TOTAL NUMBER EXAMINED	0
MISSING	1

---

GROUP LEGEND: 1 is 2830 MG/KG

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TABLE 9  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

DATA FOR ALL ANIMALS ON STUDY  
 PROBE FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		5
NUMBER OF ANIMALS		5
<b>LYMPH ND, S-MAN</b>		
TOTAL NUMBER EXAMINED		1
PLASMACYTOSIS		1
LYMPHOID HYPERPLASIA		1
<b>EYE</b>		
TOTAL NUMBER EXAMINED		1
CORNEAL MINERALIZATION		1
CORNEAL ULCER		1
IRIDOCYCLITIS		1
UVEITIS		1
CATARACT		1
<b>OVARIES</b>		
TOTAL NUMBER EXAMINED		5
SMALL FOLLICLES		4
INACTIVE CORPORA LUTEA		2
ACTIVE CORPORA LUTEA		3
<b>UTERUS</b>		
TOTAL NUMBER EXAMINED		5
PROGESTIN INFLUENCE		5
<b>VAGINA</b>		
TOTAL NUMBER EXAMINED		4
MISSING		1
EARLY METESTRUS		1
MUCIFICATION OF EPITHELIAL CELLS		1
MID METESTRUS		2
LATE METESTRUS		1
<b>LUNGS</b>		
TOTAL NUMBER EXAMINED		2
EXAMINED, UNREMARKABLE		2

GROUP LEGEND: 1 is 2830 MG/KG

TABLE 10  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

ANIMALS SACRIFICED AT DAY 14  
 DEFINITIVE STUDY FEMALES

	GROUP:	1	2
NUMBER OF ANIMALS IN DOSE GROUP		6	9
NUMBER OF ANIMALS SACRIFICED		6	4
<b>COLON</b>			
TOTAL NUMBER EXAMINED		2	0
NECROSIS		1	-
SEROSITIS		1	-
<b>OVARIES</b>			
TOTAL NUMBER EXAMINED		6	4
SMALL FOLLICLES		2	3
LARGE FOLLICLES		2	1
INACTIVE CORPORA LUTEA		3	0
ACTIVE CORPORA LUTEA		3	4
<b>UTERUS</b>			
TOTAL NUMBER EXAMINED		6	4
ESTROGENIC INFLUENCE		3	1
PROGESTIN INFLUENCE		3	3
<b>VAGINA</b>			
TOTAL NUMBER EXAMINED		6	4
ESTRUS		3	1
EARLY METESTRUS		1	0
MID METESTRUS		1	3
LATE METESTRUS		1	0

GROUP LEGEND: 1 is 0 MG/KG, 2 is 2830 MG/KG

TABLE 11  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
 DEFINITIVE STUDY FEMALES

	GROUP:	1	2
NUMBER OF ANIMALS IN DOSE GROUP		6	9
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND		-	5
<b>OVARIES</b>			
TOTAL NUMBER EXAMINED		0	4
TOO AUTOLYZED TO EVALUATE		-	1
SMALL FOLLICLES		-	3
LARGE FOLLICLES		-	1
INACTIVE CORPORA LUTEA		-	2
ACTIVE CORPORA LUTEA		-	2
<b>UTERUS</b>			
TOTAL NUMBER EXAMINED		0	4
TOO AUTOLYZED TO EVALUATE		-	1
ESTROGENIC INFLUENCE		-	1
PROGESTIN INFLUENCE		-	3
<b>VAGINA</b>			
TOTAL NUMBER EXAMINED		0	4
TOO AUTOLYZED TO EVALUATE		-	1
PROESTRUS		-	1
MID METESTRUS		-	2
LATE METESTRUS		-	1

GROUP LEGEND: 1 is 0 MG/KG, 2 is 2830 MG/KG

TABLE 12  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

DATA FOR ALL ANIMALS ON STUDY  
 DEFINITIVE STUDY FEMALES

	GROUP:	1	2
NUMBER OF ANIMALS IN DOSE GROUP		6	9
NUMBER OF ANIMALS		6	9
COLON			
TOTAL NUMBER EXAMINED		2	0
NECROSIS		1	-
SEROSITIS		1	-
OVARIES			
TOTAL NUMBER EXAMINED		6	8
TOO AUTOLYZED TO EVALUATE		0	1
SMALL FOLLICLES		2	6
LARGE FOLLICLES		2	2
INACTIVE CORPORA LUTEA		3	2
ACTIVE CORPORA LUTEA		3	6
UTERUS			
TOTAL NUMBER EXAMINED		6	8
TOO AUTOLYZED TO EVALUATE		0	1
ESTROGENIC INFLUENCE		3	2
PROGESTIN INFLUENCE		3	6
VAGINA			
TOTAL NUMBER EXAMINED		6	8
TOO AUTOLYZED TO EVALUATE		0	1
PROESTRUS		0	1
ESTRUS		3	1
EARLY METESTRUS		1	0
MID METESTRUS		1	5
LATE METESTRUS		1	1

GROUP LEGEND: 1 is 0 MG/KG, 2 is 2830 MG/KG

TABLE 13  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
 SUMMARY OF MICROSCOPIC DIAGNOSES

ANIMALS SACRIFICED AT DAY 14  
 ADDITIONAL FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		6
NUMBER OF ANIMALS SACRIFICED		5
<b>OVARIES</b>		
TOTAL NUMBER EXAMINED		5
CYST, LUTEAL		1
ACTIVE CORPORA LUTEA		5
MODERATELY LARGE FOLLICLES		3
<b>UTERUS</b>		
TOTAL NUMBER EXAMINED		5
ESTROGENIC INFLUENCE		4
PROGESTIN INFLUENCE		1
<b>VAGINA</b>		
TOTAL NUMBER EXAMINED		5
ESTRUS STAGE		2
MUCIFICATION OF LUMINAL EPITHELIUM		3
CORNIFICATION OF EPITHELIUM		3

GROUP LEGEND: 1 is 2830 MG/KG (#2)

TABLE 14  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF MICROSCOPIC DIAGNOSES

ALL ANIMALS FOUND DEAD/SACRIFICED MORIBUND  
ADDITIONAL FEMALES

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		6
NUMBER OF ANIMALS FOUND DEAD/SACRIFICED MORIBUND		1
OVARIES		
TOTAL NUMBER EXAMINED		1
ACTIVE CORPORA LUTEA		1
UTERUS		
TOTAL NUMBER EXAMINED		1
PROGESTIN INFLUENCE		1
VAGINA		
TOTAL NUMBER EXAMINED		1
CORNIFICATION OF EPITHELIUM		1
GROUP LEGEND: 1 is 2830 MG/KG (#2)		

TABLE 15  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES  
SUMMARY OF MICROSCOPIC DIAGNOSES

DATA FOR ALL ANIMALS ON STUDY  
ADDITIONAL FEMALES

---

	GROUP:	1
NUMBER OF ANIMALS IN DOSE GROUP		6
NUMBER OF ANIMALS		6
OVARIES		
TOTAL NUMBER EXAMINED		6
CYST, LUTEAL		1
ACTIVE CORPORA LUTEA		6
MODERATELY LARGE FOLLICLES		3
UTERUS		
TOTAL NUMBER EXAMINED		6
ESTROGENIC INFLUENCE		4
PROGESTIN INFLUENCE		2
VAGINA		
TOTAL NUMBER EXAMINED		6
ESTRUS STAGE		2
MUCIFICATION OF LUMINAL EPITHELIUM		3
CORNIFICATION OF EPITHELIUM		4

---

GROUP LEGEND: 1 is 2830 MG/KG (#2)

---

TABLE 16  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

NECROPSY PROTOCOL

FEMALES

The following tissues were examined at necropsy with no significant lesions observed unless specified on individual animal page:

TOTAL BODY	ADIPOSE TISSUE	MESENTERY/OM'TUM	PERITONEUM	PERITONEAL CAV
PLEURA	THORACIC CAV	HEART	PERICARDIAL CAV	AORTA
VASCULATURE	SALIVARY GL	ORAL/PHARYNGEAL	TONGUE	ESOPHAGUS
STOMACH	LIVER	PANCREAS	DUODENUM	JEJUNUM
ILEUM	CECUM	COLON	RECTUM	ANUS
PITUITARY	THYROID GL	PARATHYROID GL	ADRENAL GL	SKIN
SUBCUTIS	HEAD	EARS	NARES/NOSE	MAMMARY GL
PAWS/FEET	TAIL	SPLEEN	LYMPH ND, S-MAN	LYMPH ND, MED
LYMPH ND, MES	THYMIC REGION	BONE/JOINT	BONE, STERNUM	BONE, FEMUR
BONE, VERTEBRA	SKELETAL MUSCLE	DIAPHRAGM	BRAIN	SPINAL CORD
NERVE, SCIATIC	EYE	HARDERIAN GL	LACRIMAL GL	OVARIES
UTERUS	CERVIX	VAGINA	VULVA	LARYNX
TRACHEA	LUNGS	KIDNEYS	URETER	URINARY BLADDER
URETHRA	EAR TAG			

The following organs were weighed at necropsy:

OVARIES

The microscopic procedures used in this study are described in the methods section of the text.

Micro diagnosis grade codes:

1=MINIMAL, 2=MILD, 3=MODERATE, 4=MARKED, 5=SEVERE, P=PRESENT

Micro diagnosis distribution codes:

( )=FOCAL, (( ))=MULTIFOCAL, NO PARENTHESES=DIFFUSE

Micro diagnosis prefix codes:

‡ = NEOPLASM, B = BENIGN, M = MALIGNANT, §PN = PRE-NEOPLASTIC

MICRO+ indicates histologic confirmation of preceding gross diagnosis.

TABLE 17  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP:	2830 MG/KG	FEMALE	PROBE
<hr/>			
<u>ANIMAL</u>	<u>7617</u>	<u>25-AUG-93</u>	
<u>TYPE OF DEATH: SCHEDULED SACRIFICE</u>			
<u>ORGAN WEIGHT</u>	<u>ABS. (G)</u>	<u>REL.</u>	
OVARIES	0.093		GROSS: EXAMINED - NO SIGNIFICANT LESIONS
TERMINAL BODY WT.	0.0		OVARIES
			MICRO: P SMALL FOLLICLES
			3 INACTIVE CORPORA LUTEA
			UTERUS
			MICRO: 2 PROGESTIN INFLUENCE
			VAGINA
			MICRO: P MID METESTRUS
<u>ANIMAL</u>	<u>7618</u>	<u>25-AUG-93</u>	
<u>TYPE OF DEATH: SCHEDULED SACRIFICE</u>			
<u>ORGAN WEIGHT</u>	<u>ABS. (G)</u>	<u>REL.</u>	
OVARIES	0.094		LYMPH ND, S-MAN
TERMINAL BODY WT.	0.0		GROSS: SIZE INCREASE
			ONE NODE, RIGHT SIDE, 6X4X2 MM
			SAVED
			MICRO+ 4 LYMPHOID HYPERPLASIA
			MICRO: 3 PLASMACYTOSIS
			OVARIES
			MICRO: 3 ACTIVE CORPORA LUTEA
			P SMALL FOLLICLES
			UTERUS
			MICRO: 3 PROGESTIN INFLUENCE
			VAGINA
			MICRO: P MID METESTRUS
			LUNGS
			GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
			RIGHT DIAPHRAGMATIC LOBE, 2X2 MM,
			DARK RED FOCAL AREA
			SAVED
			THE FOLLOWING TISSUES WERE MICROSCOPICALLY NORMAL:
			LUNGS
<u>ANIMAL</u>	<u>7619</u>	<u>22-AUG-93</u>	
<u>TYPE OF DEATH: FOUND DEAD</u>			
			STOMACH
			GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
			GLANDULAR PORTION, DARK RED
			LIVER
			GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
			DARK MAROON MOTTLING
			SUBCUTIS
			GROSS: COLOR CHANGE, DIFFUSE
			BROWN, ENTIRE INTESTINAL TRACT
			OVARIES
			MICRO: 3 ACTIVE CORPORA LUTEA
			UTERUS
			MICRO: 3 PROGESTIN INFLUENCE
			LUNGS
			GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
			MOTTLED RED
			KIDNEYS
			GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 17  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

			PROBE		
GROUP:	2830 MG/KG	FEMALE			
-----					
<u>ANIMAL</u>	<u>7619</u>	<u>(CONTINUED)</u>			DARK BROWN AREAS, MOTTLED
					THE FOLLOWING TISSUES WERE MISSING:
					VAGINA
<u>ANIMAL</u>	<u>7620</u>	<u>26-AUG-93</u>			
TYPE OF DEATH: SCHEDULED SACRIFICE					
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	GROSS:	EXAMINED - NO SIGNIFICANT LESIONS	
OVARIES	0.117		OVARIES		
TERMINAL BODY WT.	0.0		MICRO:	3	ACTIVE CORPORA LUTEA
				P	SMALL FOLLICLES
			UTERUS		
			MICRO:	3	PROGESTIN INFLUENCE
			VAGINA		
			MICRO:	P	EARLY METESTRUS
				3	MUCIFICATION OF EPITHELIAL CELLS
<u>ANIMAL</u>	<u>7621</u>	<u>26-AUG-93</u>			
TYPE OF DEATH: SCHEDULED SACRIFICE					
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	EYE		
OVARIES	0.065		GROSS:		SIZE DECREASE
TERMINAL BODY WT.	0.0				LEFT, POST BLEEDING (AS PER EHF)
			MICRO+	4	UVEITIS
			MICRO:	3	CATARACT
				4	CORNEAL MINERALIZATION
				4	CORNEAL ULCER
				4	IRIDOCYCLITIS
			OVARIES		
			MICRO:	3	INACTIVE CORPORA LUTEA
				P	SMALL FOLLICLES
			UTERUS		
			MICRO:	1	PROGESTIN INFLUENCE
			VAGINA		
			MICRO:	P	LATE METESTRUS
			LUNGS		
			GROSS:		COLOR CHANGE, FOCAL/MULTIFOCAL
					DARK RED FOCI, AZYGOUS LOBE AND RIGHT
					DIAPHRAGMATIC LOBE
			THE FOLLOWING TISSUES WERE MICROSCOPICALLY NORMAL:		
			LUNGS		

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP:	0 MG/KG	FEMALE	DEFINITIVE STUDY	
<u>ANIMAL</u>	<u>8218</u>	<u>27-SEP-93</u>		
<u>TYPE OF DEATH: SCHEDULED SACRIFICE</u>				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.131		OVARIES	
TERMINAL BODY WT.	0.0		GROSS:	SIZE INCREASE SLIGHT, BILATERAL
			MICRO:	3 LARGE FOLLICLES
				3 INACTIVE CORPORA LUTEA
			UTERUS	
			MICRO:	3 ESTROGENIC INFLUENCE
			VAGINA	
			MICRO:	P ESTRUS
<u>ANIMAL</u>	<u>8219</u>	<u>27-SEP-93</u>		
<u>TYPE OF DEATH: SCHEDULED SACRIFICE</u>				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.098		COLON	
TERMINAL BODY WT.	0.0		GROSS:	ADHESION ADHERED TO UNDERLYING MUSCLE, VISCERAL SURFACE SAVED, INCLUDING NODULE AND HEMORRHAGE FINDINGS
			COLON	
			GROSS:	NODULE 5X2X2 MM, DARK BROWN, MULTINODULAR
			MICRO:	5 NECROSIS PERICOLONIC TISSUE MIXED WITH MUCH HEMORRHAGE IATROGENIC?
			COLON	
			GROSS:	HEMORRHAGE AREA OF ADHESION, MODERATE AMOUNT
			LYMPH ND, S-MAN	
			GROSS:	COLOR CHANGE, DIFFUSE SEVERAL NODES, DARK RED
			LYMPH ND, S-MAN	
			GROSS:	SIZE INCREASE SEVERAL NODES, 4 MM IN DIAMETER
			THYMIC REGION	
			GROSS:	COLOR CHANGE, FOCAL/MULTIFOCAL RIGHT SIDE, SCATTERED RED FOCI
			OVARIES	
			MICRO:	3 LARGE FOLLICLES
				3 INACTIVE CORPORA LUTEA
			UTERUS	
			MICRO:	3 ESTROGENIC INFLUENCE
			VAGINA	
			MICRO:	P ESTRUS
			KIDNEYS	
			GROSS:	DILATED PELVIS RIGHT, MILD
<u>ANIMAL</u>	<u>8220</u>	<u>27-SEP-93</u>		
<u>TYPE OF DEATH: SCHEDULED SACRIFICE</u>				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	LYMPH ND, S-MAN	

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP:	0 MG/KG	FEMALE	DEFINITIVE STUDY	
<hr/>				
<u>ANIMAL</u>	<u>8220</u>	<u>(CONTINUED)</u>		
OVARIES	0.129		GROSS:	SIZE INCREASE ONE NODE, 5 MM IN DIAMETER
TERMINAL BODY WT.	0.0		OVARIES GROSS:	COLOR CHANGE, DIFFUSE DARK RED, BILATERAL
			OVARIES GROSS:	SIZE INCREASE SLIGHT, BILATERAL
			MICRO+ 3	ACTIVE CORPORA LUTEA
			UTERUS MICRO: 4	ESTROGENIC INFLUENCE
			VAGINA MICRO: P	ESTRUS
<u>ANIMAL</u>	<u>8221</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	COLON	
OVARIES	0.156		GROSS:	PARASITE PARASITE, PINWORMS
TERMINAL BODY WT.	0.0		COLON GROSS:	ADHESION ADHERED TO ADIPOSE TISSUE SURROUNDING UTERUS SAVED
			COLON GROSS:	NODULE DARK RED, 3 MM IN DIAMETER SAVED
			LYMPH ND, S-MAN GROSS:	COLOR CHANGE, DIFFUSE RED, FEW NODES
			OVARIES MICRO: 3 P	INACTIVE CORPORA LUTEA SMALL FOLLICLES
			UTERUS MICRO: 1	PROGESTIN INFLUENCE
			VAGINA MICRO: P	LATE METESTRUS
<u>ANIMAL</u>	<u>8222</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	COLON	
OVARIES	0.127		GROSS:	ADHESION ADHERED TO UTERINE JUNCTION EXTENDING DOWN CERVIX SAVED
TERMINAL BODY WT.	0.0		MICRO+ 4	SEROSITIS IATROGENIC? ADHESION TO VAGINA
			OVARIES GROSS:	COLOR CHANGE, DIFFUSE RED, BILATERAL
			MICRO: 2 P	ACTIVE CORPORA LUTEA SMALL FOLLICLES

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 MG/KG FEMALE			DEFINITIVE STUDY	
<hr/>				
<u>ANIMAL</u>	<u>8222 (CONTINUED)</u>		UTERUS	
			MICRO:	3 PROGESTIN INFLUENCE
			VAGINA	
			MICRO:	P EARLY METESTRUS
<u>ANIMAL</u>	<u>8223</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>	OVARIES	
OVARIES	0.146		GROSS:	SIZE INCREASE
TERMINAL BODY WT.	0.0			SLIGHT, BILATERAL
			MICRO+	3 ACTIVE CORPORA LUTEA
			UTERUS	
			MICRO:	2 PROGESTIN INFLUENCE
			VAGINA	
			MICRO:	P MID METESTRUS

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 2830 MG/KG FEMALE			DEFINITIVE STUDY	
<hr/>				
<u>ANIMAL</u>	<u>8209</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.134			
TERMINAL BODY WT.	0.0			
			LYMPH ND, S-MAN	
			GROSS:	COLOR CHANGE, FOCAL/MULTIFOCAL RED AND TAN, ALL NODES
			OVARIES	
			GROSS:	SIZE INCREASE SLIGHT, BILATERAL
			MICRO+ 4	ACTIVE CORPORA LUTEA
			MICRO: P	SMALL FOLLICLES
			UTERUS	
			MICRO: 2	PROGESTIN INFLUENCE
			VAGINA	
			MICRO: P	MID METESTRUS
<u>ANIMAL</u>	<u>8210</u>	<u>13-SEP-93</u>		
TYPE OF DEATH: FOUND DEAD				
			STOMACH	
			GROSS:	CONTENTS ABNORMAL CLEAR LIQUID FILLED
			LYMPH ND, S-MAN	
			GROSS:	COLOR CHANGE, DIFFUSE ALL NODES, DARK RED
			OVARIES	
			MICRO: P	LARGE FOLLICLES
			3	INACTIVE CORPORA LUTEA
			UTERUS	
			MICRO: 1	PROGESTIN INFLUENCE
			VAGINA	
			MICRO: P	LATE METESTRUS
<u>ANIMAL</u>	<u>8211</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.144			
TERMINAL BODY WT.	0.0			
			OVARIES	
			GROSS:	SIZE INCREASE SLIGHT, BILATERAL
			MICRO+ 4	ACTIVE CORPORA LUTEA
			MICRO: P	SMALL FOLLICLES
			UTERUS	
			MICRO: 2	PROGESTIN INFLUENCE
			VAGINA	
			MICRO: P	MID METESTRUS ALMOST NO MATERIAL IN VAGINAL LOWER MAY HAVE BEEN WIPED OUT BY VAGINAL SMEARING
<u>ANIMAL</u>	<u>8212</u>	<u>13-SEP-93</u>		
TYPE OF DEATH: FOUND DEAD				
			STOMACH	
			GROSS:	CONTENTS ABNORMAL CLEAR LIQUID FILLED
			OVARIES	

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 2830 MG/KG FEMALE			DEFINITIVE STUDY	
<hr/>				
<u>ANIMAL</u>	<u>8212</u>	<u>(CONTINUED)</u>		
			MICRO: 3	ACTIVE CORPORA LUTEA
			P	SMALL FOLLICLES
		UTERUS		
		MICRO: 3		PROGESTIN INFLUENCE
		VAGINA		
		MICRO: P		MID METESTRUS
<u>ANIMAL</u>	<u>8213</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS. (G)</u>	<u>REL.</u>		
OVARIES	0.117		GROSS: EXAMINED	- NO SIGNIFICANT LESIONS
TERMINAL BODY WT.	0.0		OVARIES	
			MICRO: 3	ACTIVE CORPORA LUTEA
			P	SMALL FOLLICLES
		UTERUS		
		MICRO: 3		PROGESTIN INFLUENCE
		VAGINA		
		MICRO: P		MID METESTRUS
<u>ANIMAL</u>	<u>8214</u>	<u>15-SEP-93</u>		
TYPE OF DEATH: FOUND DEAD				
			TOTAL BODY	
			GROSS:	POSTMORTEM CHANGE
				ALL ORGANS, MODERATE
		RECTUM		
		GROSS:		CANNIBALISM
				ENTIRE RECTUM AND PORTION OF COLON IS GONE
		VAGINA		
		GROSS:		CANNIBALISM
				ENTIRE VAGINA AND LARGE PORTION OF CERVIX IS GONE
			MICRO: EXAMINED	- NO SIGNIFICANT LESIONS
			THE FOLLOWING TISSUES WERE TOO AUTOLYZED FOR EVALUATION:	
			OVARIES	UTERUS
				VAGINA
<u>ANIMAL</u>	<u>8215</u>	<u>13-SEP-93</u>		
TYPE OF DEATH: FOUND DEAD				
			STOMACH	
			GROSS:	CONTENTS ABNORMAL
				CLEAR LIQUID FILLED
		OVARIES		
		MICRO: 3		ACTIVE CORPORA LUTEA
		P		SMALL FOLLICLES
		UTERUS		
		MICRO: 3		PROGESTIN INFLUENCE
		VAGINA		
		MICRO: P		MID METESTRUS
		LUNGS		
		GROSS:		HYPERINFLATION
				ALL LOBES
<u>ANIMAL</u>	<u>8216</u>	<u>27-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS. (G)</u>	<u>REL.</u>		
			Lymph ND, S-MAN	

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 18  
ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 2930 MG/KG FEMALE		DEFINITIVE STUDY	
<hr/>			
<u>ANIMAL</u>	<u>8216 (CONTINUED)</u>		
OVARIES	0.137	GROSS:	COLOR CHANGE, DIFFUSE ONE NODE, RED
TERMINAL BODY WT.	0.0	OVARIES	
		GROSS:	SIZE INCREASE SLIGHT, BILATERAL
		MICRO+ 3	ACTIVE CORPORA LUTEA
		MICRO: 2	LARGE FOLLICLES
		UTERUS	
		MICRO: 3	ESTROGENIC INFLUENCE
		VAGINA	
		MICRO: P	ESTRUS
<u>ANIMAL</u>	<u>8217</u>	<u>13-SEP-93</u>	
<u>TYPE OF DEATH: FOUND DEAD</u>			
		STOMACH	
		GROSS:	CONTENTS ABNORMAL CLEAR LIQUID FILLED
		LYMPH ND, S-MAN	
		GROSS:	COLOR CHANGE, DIFFUSE ONE NODE, DARK RED
		THYMIC REGION	
		GROSS:	COLOR CHANGE, FOCAL/MULTIFOCAL SCATTERED RED FOCI
		OVARIES	
		MICRO: 3	INACTIVE CORPORA LUTEA
		P	SMALL FOLLICLES
		UTERUS	
		MICRO: 2	ESTROGENIC INFLUENCE
		VAGINA	
		MICRO: P	PROESTRUS

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 19  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 2030 MG/KG (#2) FEMALE			ADDITIONAL	
<hr/>				
<u>ANIMAL</u>	<u>8224</u>	<u>28-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.165		COLON	
TERMINAL BODY WT.	0.0		GROSS:	PARASITE PINWORMS
			OVARIES	
			GROSS:	SIZE INCREASE BILATERAL, SLIGHT
			MICRO: 3	ACTIVE CORPORA LUTEA
			(3)	CYST, LUTEAL
			((3))	MODERATELY LARGE FOLLICLES
			UTERUS	
			MICRO: 4	ESTROGENIC INFLUENCE
			VAGINA	
			MICRO: P	MUCIFICATION OF LUMINAL EPITHELIUM
			P	ESTRUS STAGE
<u>ANIMAL</u>	<u>8225</u>	<u>14-SEP-93</u>		
TYPE OF DEATH: FOUND DEAD				
			STOMACH	
			GROSS:	CONTENTS ABNORMAL CLEAR LIQUID FILLED
			OVARIES	
			MICRO: 4	ACTIVE CORPORA LUTEA EARLY FORMATION
			UTERUS	
			MICRO: 2	PROGESTIN INFLUENCE
			VAGINA	
			MICRO: 2	CORNIFICATION OF EPITHELIUM
			LUNGS	
			GROSS:	COLOR CHANGE, DIFFUSE DARK RED, ALL LOBES
<u>ANIMAL</u>	<u>8226</u>	<u>28-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.169		OVARIES	
TERMINAL BODY WT.	0.0		GROSS:	SIZE INCREASE SLIGHT, BILATERAL
			MICRO+ 3	ACTIVE CORPORA LUTEA
			UTERUS	
			MICRO: 3	PROGESTIN INFLUENCE
			VAGINA	
			MICRO: 2	MUCIFICATION OF LUMINAL EPITHELIUM
			3	CORNIFICATION OF EPITHELIUM
<u>ANIMAL</u>	<u>8227</u>	<u>28-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.129		LYMPH ND, S-MAN	
TERMINAL BODY WT.	0.0		GROSS:	COLOR CHANGE, FOCAL/MULTIFOCAL ONE NODE, RED AND TAN
			OVARIES	
			MICRO: 3	MODERATELY LARGE FOLLICLES

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 19  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

			ADDITIONAL	
GROUP: 2830 MG/KG (#2) FEMALE				
-----				
<u>ANIMAL</u>	<u>8227</u>	<u>(CONTINUED)</u>		
			UTERUS	3 ACTIVE CORPORA LUTEA
			MICRO:	4 ESTROGENIC INFLUENCE
			VAGINA	
			MICRO:	4 MUCIFICATION OF LUMINAL EPITHELIUM
				P ESTRUS STAGE
<u>ANIMAL</u>	<u>8228</u>	<u>28-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.119		OVARIES	
TERMINAL BODY WT.	0.0		MICRO:	2 MODERATELY LARGE FOLLICLES
				2 ACTIVE CORPORA LUTEA
			UTERUS	
			MICRO:	2 ESTROGENIC INFLUENCE
			VAGINA	
			MICRO:	2 CORNIFICATION OF EPITHELIUM
			LUNGS	
			GROSS:	COLOR CHANGE, FOCAL/MULTIFOCAL ONE PUNCTATE RED FOCAL AREA, EDGE OF RIGHT DIAPHRAGMATIC LOBE
<u>ANIMAL</u>	<u>8229</u>	<u>28-SEP-93</u>		
TYPE OF DEATH: SCHEDULED SACRIFICE				
<u>ORGAN WEIGHT</u>	<u>ABS.(G)</u>	<u>REL.</u>		
OVARIES	0.146		LYMPH ND, S-MAN	
TERMINAL BODY WT.	0.0		GROSS:	SIZE INCREASE ONE NODE, 5 MM IN DIAMETER
			OVARIES	
			GROSS:	SIZE DECREASE LEFT, 4 MM IN DIAMETER
			OVARIES	
			GROSS:	SIZE INCREASE RIGHT, SLIGHT
			MICRO+	3 ACTIVE CORPORA LUTEA EARLY FORMATION
			UTERUS	
			MICRO:	4 ESTROGENIC INFLUENCE LATE ESTROGENIC PHASE, ESTRUS
			VAGINA	
			MICRO:	2 CORNIFICATION OF EPITHELIUM SURFACE OF EPITHELIUM MISSING

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**In-Life Animal Data**

**(11 Pages)**

TABLE 1  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL DOSE, WEIGHT, AND CLINICAL OBSERVATION RESULTS FROM SINGLE PERORAL DOSES OF ISOBUTANOL  
 TO FEMALE RATS IN THE DEFINITIVE STUDY

Material: Isobutanol

Sample No.: 55-270

Rat Number	Dose, mg/kg b.w. <sup>a</sup>	Amt. of Sample, mg	Conc.	Dose Vol., ml	Rat Weight (g) at Day			Time to Death
					Init.	7	14	
92-8209	2830	610	28.3%	2.2	216	243	241	—
<p><u>Signs of Toxicity:</u> Sluggishness at 2 min and again at 0.5 to 4 hr (marked at 1 to 4 hr); marked unsteady gait at 2 min and again at 0.5 to 4 hr; prostration at 5 to 30 min; marked lacrimation, slow breathing at 1 to 4 hr. Recovery at 1 day.</p>								
92-8210	2830	570	28.3%	2.0	201	—	—	3-4 Hr
<p><u>Signs of Toxicity:</u> Sluggishness, unsteady gait at 5 min to 1.5 hr (marked at 1 to 1.5 hr); marked lacrimation, marked slow breathing, piloerection, prostration at 2.5 to 3 hr.</p>								
92-8211	2830	570	28.3%	2.0	200	219	226	—
<p><u>Signs of Toxicity:</u> Unsteady gait at 2 to 5 min; sluggishness at 2 to 5 min and again at 1 day; prostration at 5 min to 4 hr; slow breathing at 1 to 4 hr; piloerection at 2.5 to 4 hr; labored breathing at 1 day. Recovery at 2 days.</p>								
92-8212	2830	570	28.3%	2.0	202	—	—	2.5-3 Hr
<p><u>Signs of Toxicity:</u> Marked unsteady gait at 5 to 10 min; prostration at 10 min to 1.5 hr; marked lacrimation at 1 to 1.5 hr.</p>								
92-8213	2830	590	28.3%	2.1	209	223	232	—
<p><u>Signs of Toxicity:</u> Marked unsteady gait at 5 to 30 min; prostration at 10 min to 4 hr; lacrimation at 1 hr to 1 day (marked at 2.5 to 4 hr); sluggishness, piloerection at 1 day. Recovery at 2 days.</p>								

TABLE 1 (Continued)  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL DOSE, WEIGHT, AND CLINICAL OBSERVATION RESULTS FROM SINGLE PERORAL DOSES OF ISOBUTANOL  
 TO FEMALE RATS IN THE DEFINITIVE STUDY

Material: Isobutanol Sample No.: 55-270

Rat Number	Dose, mg/kg b.w. <sup>a</sup>	Amt. of Sample, mg	Conc.	Dose Vol., ml	Rat Weight (g) at Day			Time to Death
					Init.	7	14	

92-8214	2830	630	28.3%	2.2	223	--	--	2 Days
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Signs of Toxicity: Marked unsteady gait at 2 to 5 min; sluggishness at 2 to 5 min and again at 1 day (marked at 1 day); prostration at 5 min to 4 hr; marked lacrimation at 2.5 hr to 1 day; slow breathing, piloerection at 1 day.

92-8215	2830	610	28.3%	2.2	216	--	--	2.5-3 hr
---------	------	-----	-------	-----	-----	----	----	----------

Signs of Toxicity: Sluggishness, marked unsteady gait at 2 to 5 min; prostration at 5 min to 1.5 hr; marked lacrimation at 1 to 1.5 hr.

92-8216	2830	590	28.3%	2.1	209	233	238	--
---------	------	-----	-------	-----	-----	-----	-----	----

Signs of Toxicity: Sluggishness at 2 min to 4 hr (marked at 2.5 to 4 hr); unsteady gait at 5 min to 4 hr (marked at 5 min to 1 hr and again at 2.5 to 4 hr); lacrimation at 2.5 to 4 hr. Recovery at 1 day.

92-8217	2830	560	28.3%	2.0	197	--	--	2.5-3 Hr
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Signs of Toxicity: Sluggishness at 2 min to 1 hr; marked unsteady gait at 5 min to 1 hr; prostration, marked lacrimation at 1 to 1.5 hr.

92-8224	2830	640	28.3%	2.3	226	247	261	--
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Signs of Toxicity: Marked unsteady gait at 2 to 5 min; sluggishness at 2 to 5 min and again at 1 day (marked at 1 day); prostration at 5 min to 3 hr; lacrimation at 20 min to 1 day (marked at 2 hr to 1 day); slow breathing, piloerection at 2 hr to 1 day. Recovery at 2 days.

TABLE 1 (Continued)  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL DOSE, WEIGHT, AND CLINICAL OBSERVATION RESULTS FROM SINGLE PERORAL DOSES OF ISOBUTANOL  
 TO FEMALE RATS IN THE DEFINITIVE STUDY

Material: Isobutanol

Sample No.: 55-270

Rat Number	Dose, mg/kg b.w. <sup>a</sup>	Amt. of Sample, mg	Conc.	Dose Vol., ml	Rat Weight (g) at Day			Time to Death
					Init.	7	14	
92-8225	2830	590	28.3%	2.1	208	--	--	3 Hr
<u>Signs of Toxicity:</u> Sluggishness, marked unsteady gait at 5 to 30 min; prostration at 0.5 to 2.5 hr; lacrimation at 0.5 to 2.5 hr (marked at 2 to 2.5 hr); slow breathing, piloerection at 2 to 2.5 hr.								
92-8226	2830	610	28.3%	2.1	214	237	228	--
<u>Signs of Toxicity:</u> Marked unsteady gait at 5 to 30 min; sluggishness at 5 to 30 min and again at 1 day; marked lacrimation, slow breathing, piloerection, prostration at 2 to 3 hr. Recovery at 2 days.								
92-8227	2830	610	28.3%	2.1	214	237	238	--
<u>Signs of Toxicity:</u> Sluggishness, marked unsteady gait at 5 to 30 min; prostration at 0.5 to 3 hr; lacrimation at 0.5 to 3 hr (marked at 2 to 3 hr); slow breathing, piloerection at 2 to 3 hr. Recovery at 1 day.								
92-8228	2830	590	28.3%	2.1	209	221	224	--
<u>Signs of Toxicity:</u> Marked unsteady gait at 5 to 30 min; sluggishness at 5 min and again at 1 day; prostration at 0.5 to 3 hr; lacrimation at 0.5 hr to 1 day (marked at 2 to 3 hr); slow breathing, piloerection at 2 to 3 hr. Recovery at 2 days.								
92-8229	2830	620	28.3%	2.2	219	244	248	--
<u>Signs of Toxicity:</u> Sluggishness, marked unsteady gait at 5 to 30 min; prostration at 20 min to 3 hr; lacrimation at 20 min to 3 hr (marked at 2 to 3 hr); slow breathing, piloerection at 2 to 3 hr. Recovery at 1 day.								

<sup>a</sup> Dose given as mg of isobutanol/kg of body weight; sample dosed as 28.3% (w/v) emulsion in 0.25% (w/v) aqueous methyl cellulose solution.

TABLE 2  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

INDIVIDUAL DOSE, WEIGHT, AND CLINICAL OBSERVATION RESULTS FROM SINGLE PERORAL DOSES OF METHYL  
 CELLULOSE SOLUTION TO FEMALE RATS IN THE DEFINITIVE STUDY

Material: 0.25% Aqueous Methyl Cellulose Solution

Sample No.: 56-338

Rat Number	Dose, ml/kg b.w. <sup>a</sup>	Conc.	Dose Vol., ml	Rat Weight (g) at Day			Time to Death
				Init.	7	14	
92-8218	10.0	100%	2.0	203	224	222	--
<u>Signs of Toxicity:</u> None noted.							
92-8219	10.0	100%	2.1	209	222	229	--
<u>Signs of Toxicity:</u> None noted.							
92-8220	10.0	100%	2.0	202	224	227	--
<u>Signs of Toxicity:</u> None noted.							
92-8221	10.0	100%	2.1	211	227	238	--
<u>Signs of Toxicity:</u> None noted.							
92-8222	10.0	100%	2.1	206	222	226	--
<u>Signs of Toxicity:</u> None noted.							
92-8223	10.0	100%	2.2	219	247	245	--
<u>Signs of Toxicity:</u> None noted.							

<sup>a</sup> Dose given as ml of 0.25% (w/v) aqueous methyl cellulose solution/kg of body weight (prepared at BRRRC).

TABLE 3  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DAILY RECTAL TEMPERATURES PRIOR TO AND FOLLOWING SINGLE PERORAL DOSES OF ISOBUTANOL TO FEMALE  
 RATS IN THE DEFINITIVE STUDY

Material: Isobutanol				Sample No.: 55-270				Dose: 2830 mg/kg											
Rat Number	Pre-Dose Day				Rectal Temperature (°C)														
	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
93-8209	--	37	37	38	38 <sup>a</sup> 34	38	38	38	38	38	37	37	38	38	37	38	37	37	38
93-8210	--	38	38	39	38 <sup>a</sup> 34	Animal found dead.													
93-8211	--	38	37	38	37 <sup>a</sup> 33	35	38	38	38	38	38	38	38	38	38	38	38	37	37
93-8212	--	37	38	38	38 <sup>a</sup> 33	Animal found dead.													
93-8213	--	37	38	38	38 <sup>a</sup> 34	34	38	38	37	38	38	38	39	38	37	39	38	38	38
93-8214	--	38	38	39	39 <sup>a</sup> 34	Animal found dead.													
93-8215	--	37	38	39	39 <sup>a</sup> 33	Animal found dead.													
93-8216	--	38	38	38	38 <sup>a</sup> 34	39	38	38	38	37	37	37	38	38	37	38	38	38	38
93-8217	--	37	38	39	38 <sup>a</sup> 34	Animal found dead.													



**TABLE 4**  
**ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN**  
**FEMALE RATS FOLLOWING ACUTE PERORAL DOSES**

**DAILY RECTAL TEMPERATURES PRIOR TO AND FOLLOWING SINGLE PERORAL DOSES OF AQUEOUS METHYL CELLULOSE SOLUTION TO FEMALE RATS**

Material: 0.25% Aqueous Methyl Cellulose Solution      Sample No.: 56-338      Dose: 10.0 ml/kg

Rat Number	Rectal Temperature (°C)																	
	Pre-Dose Day			Definitive Dose Day														
	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
93-8218				38 <sup>a</sup>														
	37	38	39	38	38	37	38	38	38	38	38	38	39	38	38	38	38	38
93-8219				38 <sup>a</sup>														
	38	38	39	38	37	37	37	38	37	37	38	38	38	37	38	38	38	38
93-8220				38 <sup>a</sup>														
	38	38	39	38	37	38	38	39	38	38	38	38	39	38	38	39	38	38
93-8221				38 <sup>a</sup>														
	37	38	38	38	38	38	37	38	38	37	38	38	38	38	38	39	38	38
93-8222				38 <sup>a</sup>														
	37	38	39	38	38	38	37	38	38	38	38	39	38	37	38	38	38	38
93-8223				39 <sup>a</sup>														
	38	38	38	38	38	38	38	38	38	38	38	38	38	37	38	38	37	38

<sup>a</sup> Rectal temperature prior to dose administration.

TABLE 5  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DAILY ESTROUS CYCLE PRIOR TO AND FOLLOWING SINGLE PERORAL DOSES OF ISOBUTANOL TO FEMALE RATS IN  
 THE DEFINITIVE STUDY

Material: Isobutanol				Sample No.: 55-270										Dose: 2830 mg/kg						
Rat Number	Pre-Dose Day				Definitive Dose Day															
	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
93-8209	--	P	E	E	M	M <sup>a</sup>	M <sup>a</sup>	M	p <sup>ac</sup>	p <sup>bc</sup>	p <sup>b</sup>	p <sup>b</sup>	p <sup>b</sup>	p <sup>a</sup>	p <sup>a</sup>	p <sup>a</sup>	p <sup>b</sup>	P	E	
93-8210	--	P	E	E	M	Animal found dead.														
93-8211	--	P	P	E	M	M <sup>b</sup>	D	p <sup>bc</sup>	p <sup>bc</sup>	P	p <sup>a</sup>	p <sup>a</sup>	p <sup>a</sup>	p <sup>a</sup>	pad	pad	pad	P	P	
93-8212	--	M	M	M	M	Animal found dead.														
93-8213	--	M	M <sup>b</sup>	E	E	E	M	P	p <sup>a</sup>	p <sup>b</sup>	P	P	P	P	P	E	E	M	P	
93-8214	--	P	E	E	M	P	Animal found dead.													
93-8215	--	P	E	E	E	Animal found dead.														
93-8216	--	D	D	D	P	p <sup>b</sup>	p <sup>b</sup>	p <sup>ac</sup>	P	P	p <sup>c</sup>	p <sup>a</sup>	p <sup>b</sup>	p <sup>b</sup>	p <sup>bd</sup>	pad	pad	pad	p <sup>b</sup>	
93-8217	--	E	E	M	M	Animal found dead.														
93-8224	M	M	E	E	E	M	p <sup>bc</sup>	p <sup>a</sup>	p <sup>b</sup>	P	P	p <sup>a</sup>	pad	p <sup>bd</sup>	p <sup>a</sup>	p <sup>bd</sup>	pad	p <sup>a</sup>	E	
93-8225	M	E	E	M	M	Animal found dead.														
93-8226	P	P	P	P	P	p <sup>a</sup>	p <sup>a</sup>	p <sup>b</sup>	p <sup>a</sup>	p <sup>b</sup>	p <sup>b</sup>	p <sup>a</sup>	pad	p <sup>a</sup>	pad	P	M	M	M	

TABLE 5 (Continued)  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DAILY ESTROUS CYCLE PRIOR TO AND FOLLOWING SINGLE PERORAL DOSES OF ISOBUTANOL TO FEMALE RATS IN  
 THE DEFINITIVE STUDY

Material: Isobutanol					Sample No.: 55-270										Dose: 2830 mg/kg				
Rat Number	Pre-Dose Day				Definitive Dose Day														
	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
93-8227	M	D	P	E	E	P	P	p <sup>a</sup>	p <sup>b</sup>	p <sup>b</sup>	p <sup>b</sup>	P	p <sup>a</sup>	p <sup>a</sup>	p <sup>ad</sup>	p <sup>a</sup>	p <sup>b</sup>	p <sup>bd</sup>	P
93-8228	P	P	P	P	E	E	E	p <sup>a</sup>	p <sup>b</sup>	E	E	M	p <sup>a</sup>	E	E	E	M	M	P
93-8229	P	P	E	E	E	D	P	p <sup>a</sup>	p <sup>b</sup>	p <sup>b</sup>	P	P	p <sup>cd</sup>	p <sup>cd</sup>	p <sup>ad</sup>	p <sup>a</sup>	p <sup>c</sup>	P	P

Stage of Estrus:

- P = Proestrus
- E = Estrus
- M = Metestrus
- D = Diestrus

Other Comments:

- a = Few to very few cells present
- b = Reduced or slightly reduced number of cells present
- c = Non-cellular debris present
- d = Mucoid debris present

TABLE 6  
 ISOBUTANOL: DETERMINATION OF THE POTENTIAL FOR PSEUDOPREGNANCY IN  
 FEMALE RATS FOLLOWING ACUTE PERORAL DOSES

DAILY ESTROUS CYCLE PRIOR TO AND FOLLOWING SINGLE PERORAL DOSES OF AQUEOUS METHYL CELLULOSE  
 SOLUTION TO FEMALE RATS IN THE DEFINITIVE STUDY

Material: 0.25% Aqueous Methyl Cellulose Solution		Sample No.: 56-338		Dose: 10.0 ml/kg														
Rat Number	Pre-Dose Day			Definitive Dose Day														
	1	2	3	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
93-8218	M	M	E	E	E	E <sup>b</sup>	P	P	E	M	M <sup>a</sup>	P	E	E	M	M	E	E
93-8219	D <sup>a</sup>	D	M	M	M <sup>a</sup>	M	E	M	P	E	E	M	M	P	E	E	M	P
93-8220	M	P	E	E	E	E <sup>b</sup>	E	E	E	M	M	P	E	E	M	M	P	E
93-8221	P	P	E	M	M	P	E	E	M	P	E	E	M	P	E	E	M	D
93-8222	E	E	E	M	P	P	E	E	M	M	P	E	E	M	M	P	E	E
93-8223	P	P	M	D	D	D <sup>c</sup>	P	P <sup>c</sup>	P	P	P <sup>b</sup>	D <sup>d</sup>	E	M	P	E	E	M

Stage of Estrus:

- P = Proestrus
- E = Estrus
- M = Metestrus
- D = Diestrus

Other Comments:

- a = Few to very few cells present
- b = Reduced or slightly reduced number of cells present
- c = Non-cellular debris present
- d = Mucoid debris present

**Isobutanol: Determination of the Potential for Pseudopregnancy in  
Female Rats following Acute Peroral Doses**

**Protocol, Protocol Amendments and Protocol Deviations**

**(20 Pages)**



# BUSHY RUN RESEARCH CENTER

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## PROTOCOL

**TITLE:** Isobutanol: Determination of the Potential for Pseudopregnancy in Female Rats following Acute Peroral Doses

**BRRC PROJECT ID:** 93U1298

**SPONSOR:** Solvents and Coatings Materials Division  
Union Carbide Corporation  
39 Old Ridgebury Road  
Danbury, CT 06817-0001

**TESTING FACILITY:** Bushy Run Research Center (BRRC)  
Union Carbide Corporation  
6702 Mellon Road  
Export, PA 15632-8902

Reviewed and Approved by:

Bushy Run Research Center:

Susan M. Christopher 8-17-93  
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Tipton R. Tyler 9/30/93  
Tipton R. Tyler, Ph.D., DABT Date  
Associate Director of Applied Toxicology

Division:

Richard C. Wise 9-30-93  
Richard C. Wise Date  
Manager of Product Safety

Union Carbide Chemicals and Plastics Company Inc.  
Excellence Through Quality

EQ

OBJECTIVE

The objective of this study is to determine if hormone production in the female rat is altered following single peroral doses of the test substance such that pseudopregnancy occurs.

Design and Basis for the Study

During a recent acute toxicity study performed at BRRC (BRRC Project 92U1166), 2 female rats (1 receiving a single peroral dose and 1 subjected to inhalation of substantially saturated vapor) exhibited pseudopregnancy characterized by the occurrence of decidualoma in the uteri. Since decidualoma are rarely observed in animals of the age group dosed (12- to 14-week old female rats), it became apparent that further testing was necessary to determine if the pseudopregnancies were treatment related. This study is designed specifically for this purpose and will consist of single peroral doses of either the test or control substance to female rats along with vaginal cytology, blood analyses and histology. A probe study will first be conducted using a reduced number of animals. For the definitive study (if determined to be required), the number of test and control animals may vary, depending on the toxicity of the test substance and other factors associated with the test procedures, and, therefore, cannot be specified.

The portions of this study conducted at BRRC, except for the probe study, will be in compliance with the following guidelines and standards:

U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792.

Organisation for Economic Co-operation and Development (OECD). OECD Principles of Good Laboratory Practice, C(81)30(Final).

U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Health Effects Testing Guidelines, 40 CFR Part 798: Subpart B, Section 798.1175:acute oral toxicity.

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4: Health Effects; 401:acute oral toxicity.

PERSONNEL

All personnel who participate in the conduct of the study will be documented in the raw data.

PROJECT DATES (DEFINITIVE STUDY)

<u>Proposed Starting Date of Test Substance Administration</u>	September 13, 1993
<u>Proposed Date for Completion of In-Life Phase</u>	September 27, 1993
<u>Proposed Date for Submission of Draft Final Report</u>	November 29, 1993

METHODS

Test Substance

Product Name	Isobutanol
Chemical Name	Isobutanol
CAS Registry Number	78-83-1
Source	Union Carbide Corporation, Texas City, TX
Sponsor Identification Number	Lot No. TS3370114
BRRC Sample Number	55-270
Description	Transparent, colorless, low viscosity liquid.
Purity	Approximately 99.9% (according to the Certificate of Analysis).
Stability	The test substance is considered to be stable at room temperature for the duration of the study.
Storage Conditions	The test substance will be stored under well-ventilated conditions at ambient temperature in an appropriate storage area.
Quantity	Approximately 1 quart of the test substance was received for previous acute toxicity and primary irritancy testing. It will also be used for the proposed study. After the assigned studies have been completed, BRRC will retain the remaining test substance for at least a 3-month period (following report issuance).
Reserve Sample	A reserve sample will not be retained at BRRC. A reference sample for possible analysis or additional testing will be retained at BRRC for at least 2 years.
Safety	A Material Safety Data Sheet (MSDS) supplied by the Sponsor will be reviewed by all relevant personnel before their participation in the study. This review will be documented. Normal precautions for untested substances will be used. These procedures include the use of disposable Tyvek® or plastic coats or jumpsuits, hats, booties or shoe covers, and appropriate gloves while in the animal rooms. Eye

protection will include the use of safety glasses at all times. Disposable Tyvek® coats or smocks and appropriate gloves will be worn during administration of the test substance. In addition, monogoggles will be used when handling the test substance. Additional personal protective equipment (PPE) will be used as needed.

Test Animals

Species and Strain	Sprague Dawley® albino rats
Supplier	Harlan Sprague Dawley, Inc., Indianapolis, IN
Rationale	The Sprague Dawley® albino rat is a commonly-used species and strain for the peroral test, with which this laboratory has extensive experience.
Number and Sex	Female rats will be used as needed from the available stock of acute test animals or will be ordered as needed to complete the required tests. It is estimated that 3 female animals will be required for the probe study and 15 females will be used for definitive testing. However, these numbers may change depending upon the results obtained. The actual numbers of animals used will be documented in the raw data and the final report. It is anticipated that the total number of rats to be used will not exceed 30.
Age and Weight	The animals will be requested to be approximately 11 to 12 weeks of age and at an approximate body weight of 220-240 g on the scheduled animal receipt date.
Acclimation and Pretest Evaluations	<p>Shortly after their arrival at the laboratory, the animals will be transported to the room selected for housing. Once in the room, the animals will be removed from the shipping cartons and examined. All animals with evidence of disease or physical abnormalities will be discarded and the reason for rejection will be recorded. If an unusually large number of animals shows evidence of disease or physical abnormalities, the entire shipment of animals will be rejected for use in the study. A total of 10 rats (5/sex) will be randomly selected for a pretest health screen once every 3 months as discussed below.</p> <p>The rats will be housed 1 to 3 to a cage for an acclimation period of at least 5 days. During the acclimation period, animals will be observed for any overt clinical signs of disease or abnormality. Animals showing abnormalities deemed by the Study Director or other appropriate personnel to render the</p>

animal unacceptable for placement on study will be sacrificed and discarded and the reason for sacrifice will be recorded.

The rats will be weighed and inspected for health at 5 days (probe study) or 3 days (definitive study) prior to dose administration. Only those exhibiting a healthy state and the appropriate weight range will be used.

**Periodic Health  
Screen**

Once every 3 months, rats received in the isolation room for acute testing will be subjected to a health screen, which will consist of a serology screen, examinations for fecal parasites, and necropsy. The screen will be performed on 5 animals/sex.

The following organisms will be included in the serologic screen conducted periodically (at least once a year) throughout the facility:

Sendai virus (SEND)  
Pneumonia virus of mice (PVM)  
Rat coronavirus/Sialodacryoadenitis virus (RCV/SDA)  
Kilham rat virus (KRV)  
Toolan's H-1 virus (H-1)  
Reovirus type 3 (REO3)  
Mycoplasma pulmonis (MPUL)  
Mouse polio virus (GD-7)  
Lymphocytic choriomeningitis virus (LCMV)  
Mouse adenovirus FL/K87 (MAD)  
Minute virus of mice (MVM)  
Polyoma virus (POLY)

Fecal examination for parasites will be conducted using a cellophane tape test as a prestudy screen and by zinc sulfate flotation from cecal contents obtained at necropsy.

The purpose of this screen is to determine the suitability of the population of animals proposed for this study. Therefore, the results of this screen will be available to the Study Director soon after all tests are completed.

The rats available for acute testing will be periodically examined by a veterinarian.

**Identification**

Each animal will be assigned a unique identification number prior to the initiation of the study (shortly after receipt). Animals considered for assignment to the study will be identified by cage cards and ear tags.

Husbandry

Conditions

The rats will be housed in an appropriate animal room at BRRC from arrival until termination of the in-life phase of the study. Stainless steel cages with wire mesh floors will be used throughout all phases of the study. During acclimation and following treatment, DACB® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) will be changed at least 3 times each week.

Temperature and humidity will be recorded continuously using an automatic recorder. Temperature will be maintained at 66-77°F and relative humidity will be maintained at 40-70%. The temperature and humidity will be checked by a technician at each room check and a record will be kept indicating that it was done. Appropriate corrective action will be taken whenever readings outside the specified limits are observed.

The accuracy of the temperature and humidity recording devices will be checked periodically and calibrated when necessary. The verification and calibration data will be recorded. In the event that continuous recording cannot be maintained, the temperature and humidity will be manually recorded at each room check.

An automatic timer will be set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). In the animal room, there will be at least 10 air changes each hour.

Diet

Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) will be available ad libitum until early afternoon of the day before dosing and again within 3 to 4 hours after dosing. The analyses of chemical composition and possible contaminants of each batch of diet will be performed by Agway Inc., and the results of the analyses will be reviewed by the Study Director.

Water

Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) will be available ad libitum by an automatic watering system with demand control valves mounted on each rack. Water pressure and function of the individual cage rack systems will be checked at each room check, and a record will be kept indicating it was done. Drinking water contaminant levels will be measured at approximately 9-month intervals according to the methods specified in the EPA Safe Drinking Water Act Regulations and will comply with human drinking water requirements. The results of the analyses will be reviewed by the Study Director.

Administration of Test Substance

Route and  
Justification

The route of administration will be by gavage. The oral route is ~~considered to be a meaningful way to evaluate the toxicity of chemicals with the use pattern of the test substance and was also~~ route used in the original study where an effect was seen. ~~This route is also a potential route of human exposure.~~

T.F.C.  
9/30/19  
R.C.  
G.S.

Dose Selection

For both the probe and definitive studies, the test animals will be administered 2830 mg of the test substance/kg of body weight, the same dose level at which pseudopregnancy was observed in previous acute peroral testing. The test substance will be dosed as an emulsion (w/v) in 0.25% aqueous methyl cellulose solution. Control animals will be administered the vehicle alone.

Additional animals may possibly be added if deaths are produced and/or it is determined that additional data are needed. Other dose levels may also be required to meet goals of the study.

Vehicle and Control  
Substance

Emulsions of the test substance will be prepared as necessary using 0.25% (w/v) aqueous methyl cellulose solution (CAS No. for methyl cellulose 9004-67-5; CAS No. for water 7732-18-5). This vehicle was used in the previous acute peroral toxicity testing of the test substance and is a vehicle of low toxicity with which BRRC has extensive experience. Control animals will be dosed with aqueous methyl cellulose solution alone at volumes equivalent to those used for test animals (10 ml/kg). The identity and source of the methyl cellulose will be documented in the raw data and described in the final report.

Dose Administration

Using an appropriately-sized syringe, the required amount of test substance in the aqueous methyl cellulose solution or the aqueous methyl cellulose solution alone, will be administered by stomach intubation (to a fasted rat) through a commercial 16-gauge (3-inch) ball-end stainless steel needle (Perfektum®, Popper and Sons, Inc., New Hyde Park, NY). The exact amounts of test substance, emulsion or control substance dosed for each rat will be recorded.

The test substance will be dosed as a 28.3% (w/v) emulsion in aqueous methyl cellulose solution for the 2830 mg/kg level. For individual doses, the volume of the emulsion will be adjusted according to individual rat weights. The total volume to be administered will not exceed 2.0 ml/100 g of body weight (the limit for aqueous dilutions).

Duration of  
Treatment

Treatment will consist of a single peroral dose of the test or control substance. Treated animals will normally be observed to 5 or 7 days following dose administration (probe study), 14 days (definitive study), or to death, unless otherwise required.

Preparation of  
Dosing Solutions

Dosing emulsions will be prepared by mixing appropriate amounts of the test substance (g) and the 0.25% (w/v) aqueous methyl cellulose solution. Fresh emulsions will be prepared for each day of dosing. Mixing procedures and storage conditions will be specified in the raw data and final report.

Analysis of  
Dosing Solutions

The stability and homogeneity of the test substance in the vehicle will not be determined by BRRC. The content of the dosing mixtures will be documented by gravimetric analysis.

Study Design

Group  
Assignment

Upon receipt, animals will be randomly assigned to cages, and they will be designated for dosing according to health status, weight limits, need and availability. Animals not assigned to the study will be used for other toxicity testing, training of BRRC staff, methods development, or possible random serology testing, or they will be humanely sacrificed and discarded.

Organization

Probe Study - The probe study will consist of 3 female rats receiving 2830 mg/kg of the test substance. Additional animals may be added if deaths occur or if the results are inconclusive as determined by the Sponsor. The results will be used to determine if a full study should be conducted.

Definitive Study (may not be needed depending on the results of the probe study) - Nine female rats will be administered 2830 mg of test substance/kg of body weight. Another 6 female rats will be administered 10.0 ml/kg of 0.25% (w/v) aqueous methyl cellulose solution (control animals). All animals will be dosed on the same day. Additional animals may be included, as requested by the Sponsor and/or the Pathologist.

Experimental Evaluations

Mortality Checks  
and Clinical Signs

Dosed animals will be observed for mortality and clinical signs of toxicity frequently on the day of dosing and at least twice each scheduled work day (once in the morning and once in the afternoon) during the observation period (5 to 7 days for the probe study and 14 days for the definitive study). If signs

persist at the end of the observation period, the animals may be observed for a longer period according to the needs of the Sponsor. As appropriate (depending on the nature of observed effects), these examinations may involve animal handling. On weekends and holidays, mortality checks and observation for clinical signs of toxicity will only be conducted once a day. Detailed examinations for clinical signs of disease or abnormality, which involve animal handling, will be conducted during weight determinations.

**Sacrifice of  
Distressed Animals**

If any animal shows signs of extreme distress or is moribund, it will be sacrificed for humane reasons before the scheduled date. The Sponsor will be notified as soon as possible if it is anticipated that the sacrifice may affect the integrity of the study.

**Body Weight**

For the probe study, individual body weights will be measured 5 days prior to dose administration, just prior to dosing and at sacrifice. Individual body weights for animals included in the definitive study will be measured 3 days prior to dose administration, just prior to dosing, at 7 days and at 14 days.

**Vaginal Cytology**

In order to determine the stage of the estrous cycle, vaginal smears will be made daily for each animal. For the probe study, smears will be made beginning at 5 days prior to dose administration and ending at 5 to 7 days (assuming that female rats will normally complete 1 full estrous cycle in 5 days). In the definitive study, vaginal smears will be made daily beginning at 3 days prior to dose administration and ending at 14 days. The smears will be obtained by means of a sterile swab which will be wetted with saline solution, placed into the vagina and then rolled onto a slide. The slide may be air-dried, stained with toluidine blue O (filtered before use), and coverslipped. The cell types will then be determined by microscopic examination using first low (40-100X) and then higher (400X) magnification (lighting may be reduced as necessary). The most reasonable stage will be recorded based on the predominant cell types observed and the stages in prior smears. The single letter for the stage of the cycle (P for proestrus, E for estrus, M for metestrus or D for diestrus) will then be entered on appropriate data sheets. Whether the stage is "early" or "late" may be added to the data sheets only if it is clearly identified.

**Clinical Pathology  
Evaluations**

Clinical chemistry will be conducted on blood samples collected each day for rats in both the probe and definitive studies. For the probe study (3 animals total), blood samples will be collected daily for each

animal beginning 5 days prior to dose administration and up until the day of sacrifice. In the definitive study, the animals will be divided into 3 groups, each consisting of 3 treated and 2 control animals. Blood will be collected from 1 group of animals/day beginning 3 days prior to dose administration and up until the end of the 14-day observation period so that each group will be bled once prior to dosing and a total of 5 times after dosing. For individual animals, bleeding will be alternated between eyes in order to reduce stress on the animal. All blood samples will be obtained from methoxyflurane anesthetized animals by puncture of the retroorbital sinus. The following procedures will be determined:

Clinical Chemistry

estradiol  
progesterone

Urine will be tested for the presence of occult blood through the use of HEMASTIX® Reagent Strips (Ames).

Other clinical investigations (hematology, additional clinical chemistry, and urinalysis) may also be included, upon Sponsor request. As required, these additions will be documented by protocol amendment.

Sacrifice/Anatomic  
Pathology  
Evaluations

At the end of the observation period (approximately 5 to 7 days for the probe study and 14 days for the definitive study), all surviving rats will be anesthetized with methoxyflurane and killed by severing the brachial vessels. Animals showing signs of severe debility, particularly if death appears imminent, may be sacrificed early to prevent loss of tissues through autolysis. All animals used for probe and definitive testing, including those that die or are sacrificed during the study, will receive a complete necropsy. If necropsy is not possible soon after death, animals will be stored in a refrigerator until necropsy can be performed.

Selected tissues will be saved from each animal in both the probe and definitive tests. The saved tissues will be fixed in 10% neutral buffered formalin and evaluated microscopically. Tissues will not be saved from animals that die overnight unless it is apparent that death just occurred and tissue degradation is minimal.

The following tissues will be collected for all animals:

uterus  
vagina  
ovaries

Additional tissues as indicated by test results (signs or necropsy findings) or Sponsor instructions.

Ear tags or identifying tissues will be saved for identification purposes.

**Organ Weights**

The following organs from all surviving animals at the terminal sacrifice will be trimmed, blotted, and weighed:

ovaries

**Histopathology**

All tissues to be examined microscopically will be processed for paraffin embedding, sectioned at 5 microns, and stained with hematoxylin and eosin. Lesions will be graded as to severity, where possible, into 5 categories (minimal, mild, moderate, marked, or severe).

**Statistical Evaluations**

The data for quantitative continuous variables will be intercompared for the dose and control groups by Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests will be used following a significant ANOVA to delineate which groups differ from the control group. If Levene's test indicates homogeneous variances, the groups will be compared by an ANOVA for equal variances followed, when appropriate, by pooled variance t-tests. If Levene's test indicates heterogeneous variances, the groups will be compared by an ANOVA for unequal variances followed, when appropriate, by separate variance t-tests. For nonparametric data, the Kruskal-Wallis test followed, when appropriate, by Mann-Whitney U-tests, will be used. Incidence data will be compared using the appropriate statistical test, generally Fisher's Exact Test. Statistical analyses will be performed using either BMDP Statistical Software or other statistical programs, as deemed appropriate (Dixon, 1990; Sokal and Rohlf, 1981). The probability value of less than 0.05 (2-tailed) will be used as the critical level of significance for all tests.

**ALTERATION OF PROTOCOL**

Alterations to this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change

verbally, such change will be honored. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification. BRRC reserves the right to revise the protocol or deviate therefrom solely at the discretion of the Study Director if prior approval of the Sponsor cannot be obtained and the integrity of the study is considered in jeopardy. In this event, the Sponsor will be notified of the alteration as soon as possible, and documentation of the change will be the responsibility of the Study Director.

#### RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, specimens, and a copy of the final report generated as a result of this study will be retained in the BRRC Archives for at least 5 years.

Following the retention period specified above, the Sponsor will be contacted and given the option of taking receipt, destroying, or arranging for other storage of the data and materials. All data and materials mentioned above will remain the sole property of the Sponsor and can be removed from BRRC at the Sponsor's discretion.

#### REPORTS

##### Draft Final Report

An unaudited draft of the final report will be submitted to the Sponsor within 2 months after the completion of the terminal sacrifice. This report will be a comprehensive report which will include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results. It will include: a summary; appropriate text discussions of the experimental design, materials and methods, and results; and, as appropriate, summary mean or incidence tables of in-life and pathology data. In addition, it will contain appendices with individual animal data and other pertinent information.

##### Final Report

The draft final report will be reviewed by the Sponsor, and comments on the report will be provided to BRRC within 3 weeks from the date of submission of the draft version. BRRC will consider these comments in preparing the final report. Assuming the Sponsor's comments are received at the specified time and no major revisions are required, BRRC will submit a final report within 4 weeks of issuance of the draft report.

The final report will be audited by the Quality Assurance Unit and contain a signed quality assurance statement. It will conform to the formatting specifications of EPA PR notice 86-3.

#### ANIMAL USE POLICY

It is the goal of BRRC, through the establishment and activities of the Institutional Animal Care and Use Committee, to comply with the U.S. Animal Welfare Act and the subsequent rules promulgated by the U.S. Department of Agriculture and in effect on the date of this protocol. It has been determined that the work described herein minimizes the number of animals

used, is necessary, and uses the most appropriate species and strain in order to provide meaningful results and the most useful information for comparative purposes relative to previous studies. Furthermore, this study will be conducted humanely, and to the best of our knowledge, neither unnecessarily duplicates any previous work, nor can it be accomplished using currently available, validated nonanimal models.

GOOD LABORATORY PRACTICE COMPLIANCE

BRRC, through the administration of a quality assurance program by the Good Laboratory Practice Committee and Quality Assurance Unit, assures compliance of all phases of studies conducted at BRRC with existing regulations and generally accepted good laboratory practices.

The study will be subjected to periodic inspections and the final report will be reviewed by the BRRC Quality Assurance Unit.

REFERENCES

- Brown, M. B., and Forsythe, A. B. (1974). The Small Sample Behavior of Some Statistics Which Test the Equality of Several Means. *Technometrics* 16, 129-132.
- Dixon, W. J. (1990). *BMDP Statistical Software*. University of California Press, Berkeley, CA.
- Draper, N. R. and Smith, H. (1966). Applied Regression Analysis. John Wiley & Sons, New York, NY.
- Sokal, R. R. and Rohlf, F. J. (1981). Biometry, 2nd Edition, W. H. Freeman and Co., San Francisco, CA.



# BUSHY RUN RESEARCH CENTER

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## PROTOCOL AMENDMENT 1

**TITLE:** Isobutanol: Determination of the Potential for Pseudopregnancy in Female Rats following Acute Peroral Doses

**BRRC PROJECT ID:** 93U1298

**SPONSOR:** Solvents and Coatings Materials Division  
Union Carbide Corporation  
39 Old Ridgebury Road  
Danbury, CT 06817-0001

**TESTING FACILITY:** Bushy Run Research Center (BRRC)  
Union Carbide Corporation  
6702 Mellon Road  
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Reviewed and Approved by:

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Division:

Richard C. Wise 9-30-93  
Richard C. Wise Date  
Manager of Product Safety

Union Carbide Chemicals and Plastics Company Inc.  
Excellence Through Quality

*EQ*

The protocol is amended as follows:

Item 1

Location of  
Protocol Change

Page 9, Clinical Pathology Evaluations

Description of  
Protocol Change

Animals in the probe study were not bled daily. Bleeding was not performed on any animal at 2 days prior to dose administration, on the day of dose administration, or on postdose days if substantial signs of toxicity were apparent.

Rationale

The animals became anemic from repeated (daily) blood withdrawal. There was also concern that the added stress from this procedure either prior to or following dosing (when substantial signs of toxicity were apparent) could affect recovery and even cause deaths.

Item 2

Location of  
Protocol Change

Page 10, Clinical Pathology Evaluations

Description of  
Protocol Change

The bleeding schedule for rats included in the definitive study was changed as follows: blood will not be collected until the day before dose administration at which time all animals to be dosed will be bled; blood will not be collected from any animal on the day of dose administration; blood will be collected from all test and control animals the day after dosing (any animal exhibiting substantial signs of toxicity will not be bled); the rats will then be divided into 3 groups specifically for blood collection (each consisting of test and control animals) and each group will be bled on a rotating schedule beginning 2 days after dose administration up to the day before the first scheduled sacrifice; and, blood will be collected from all animals on the day of sacrifice. Other adjustments to the bleeding schedule may be necessary during the study. However, any change will be noted in the raw data.

Rationale

Because of the results from the probe study, it was decided that better comparisons of estradiol and progesterone levels in the blood could be made if all animals are bled the day before and the day following dose administration. Animals will not be bled on the day of dose administration or when substantial signs of toxicity are apparent because the additional stress of bleeding may affect recovery.

Item 3

Location of  
Protocol Change

Page 8, Experimental Evaluations

Description of  
Protocol Change

For all test and control animals included in the definitive test, body temperatures will be evaluated daily using simple equipment. On the day of dose administration, temperature will be evaluated for each rat prior to dose administration and possibly after dosing. If it is determined that the additional stress of this procedure may affect the recovery of animals exhibiting substantial signs of toxicity, body temperature will not be evaluated for these animals until signs subside.

Rationale

The Sponsor wanted to evaluate the potential for correlation between body temperature fluctuations and hormonal changes following peroral doses with the test substance.



# BUSHY RUN RESEARCH CENTER

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## PROTOCOL AMENDMENT 2

**TITLE:** Isobutanol: Determination of the Potential for Pseudopregnancy in Female Rats following Acute Peroral Doses

**BRRC PROJECT ID:** 93U1298

**SPONSOR:** Solvents and Coatings Materials Division  
Union Carbide Corporation  
39 Old Ridgebury Road  
Danbury, CT 06817-0001

**TESTING FACILITY:** Bushy Run Research Center (BRRC)  
Union Carbide Corporation  
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Richard C. Wise 10-29-93  
Richard C. Wise Date  
Manager of Product Safety

Union Carbide Chemicals and Plastics Company Inc.  
Excellence Through Quality



The protocol is amended as follows:

Item 1

Location of Protocol Change	Page 7, Administration of Test Substance
Description of Protocol Change	The route of administration will be by gavage. <u>The oral route was 1 route used in the original study where an effect was seen.</u>
Rationale	The Sponsor indicated that the new wording (which was hand-corrected on the original protocol by the Sponsor) more appropriately justifies the route of administration used for this study.

#### PROTOCOL DEVIATIONS

1. In the definitive study, the urine was not tested for the presence of occult blood (using HEMASTIX® Reagent Strips). This was already done in previous acute testing and was not necessary for this study.
2. The protocol states that individual body weights and vaginal smears for rats in the probe study would be measured 5 days prior to dosing. However, because 1 of the first 3 probe animals died, another 2 rats were dosed 1 day after the original dose day. Therefore, vaginal smears and weights for the additional probe animals were done at 6 days prior to dosing since pre-dose determinations for all potential probe animals were started on the same day. For the definitive study, body weights and vaginal smears were done 3 or 4 days prior to dosing instead of 3 days as stated in the protocol. As in the probe study, additional definitive study animals were dosed 1 day after the original start day to replace animals that died (as described above).

These deviations would not be expected to have any effect on the outcome of the study.