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FINAL REPORT

ETHYLENE OXIDE
DOMINANT LETHAL MUTAGENICITY INHALATION
STUDY ON RATS

JUNE 13, 1984

PROJECT REPORT 47-87

BUSHY RUN RESEARCH CENTER

R.D. #4, MELLON ROAD

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June 13, 1984

Ethylene Oxide

Dominant Lethal Mutagenicity Inhalation Study on Rats

Sponsor: Ethylene Oxide Producers

* * * * *

ABSTRACT

Groups of sexually mature male Fischer 344 rats were exposed to ethylene oxide (EO) vapor for 6 hours per day, 5 days per week for 10 weeks. The target exposure concentrations were 100, 33, and 10 ppm of EO which are equivalent to 180, 59, and 18 mg/m³, respectively. No effects of treatment were observed from body weight determinations, clinical signs, or gross lesions at necropsy. At the end of the exposure period, the males were mated with sexually mature, unexposed, females. Low fertility rates were recorded for all treatment and air-control groups particularly, though not exclusively, during the first week of mating. Values for preimplantation loss were numerically increased in the 100 ppm exposure group in comparison to either air-control group. For the second and third weeks of mating, the number of resorptions were within the range for the two air-control groups. Although there were considerable differences between the two air-control groups for the number of resorptions, the values were increased in the 100 ppm group above the range for the controls; this was particularly noticeable during the third week. Other observations which could be indicative of a dominant lethal effect were so variable as to make it inconclusive that the resorption data were a threshold indication of a dominant lethal effect. Within the constraints of the variability within the various groups, 33 and 10 ppm are considered to be concentrations not resulting in a dominant lethal effect.

OBJECTIVE

The purpose of this study was to evaluate the potential of ethylene oxide vapor, inhaled during a period of active spermatogenesis, to produce a mutagenic effect in the germinal cell line of male rats resulting in nonviable zygotes or embryos.

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MATERIALS AND METHODS

This study was conducted according to the protocol [CHF Job #39-598 (B), dated December 1, 1976 and amended February 23 and 24, 1978, and March 29, 1978] prepared for the evaluation of possible dominant lethal mutagenicity resulting from the inhalation of ethylene oxide vapor. Any deviations to the protocol and amendments have been indicated in this report.

Test Material and Positive Control Chemical

Liquid ethylene oxide (CAS No. 75-21-8) was received in a 55-gallon drum from Union Carbide Corporation (UCC) on April 25, 1977 with the UCC identification number 7JNC45,G581. This drum was kept in a sheltered area outdoors for the duration of the study. This material was assigned the Chemical Hygiene Fellowship (CHF) Sample Number 40-163. The production site of the ethylene oxide (EO) was Union Carbide Corporation, Seadrift, TX. The tank car from which this sample was obtained was labelled Number GATX84731. Pertinent physical properties of EO are presented in Table 1. Triethylenemelamine (TEM) (CAS No. 51-18-3) which was used as the positive control chemical was received from Polysciences, Inc., Warrington, PA, on January 13 1978. This chemical was assigned the CHF Sample Number 41-29.

Liquid Ethylene Oxide Chemical Analysis

Prior to and following the termination of the inhalation exposure, EO was chemically analyzed at the Research and Development Department, UCC, South Charleston, WV to determine the purity and stability of the chemical.

Ethylene Oxide Vapor Generation

Liquid aliquots (approximately 5 pounds) from the storage drum were transferred to a stainless steel cylinder. This cylinder which was attached to the generation system was maintained at approximately 35°C by means of a constant temperature recirculating bath. The ethylene oxide vapor pressure generated at that temperature was utilized to conduct the gas through stainless steel tubing and a pressure-reducing regulator to maintain approximately three psi internal pressure. Manifolds of stainless steel tubing directed the vapor through control valves and flowmeters to the chamber air-inlet duct. Ethylene oxide vapor was diluted at the inlet duct with exposure room air and drawn into the inhalation exposure chamber.

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Inhalation Chamber Description and Operational Characteristics

All five inhalation chambers were located in Room 139. Each chamber was rectangular in shape and had a volume of approximately 4350 liters with internal dimensions of 2.1 meters long, 2.1 meters high, and 1.0 meters wide. The chambers were constructed of stainless steel and contained glass windows for animal observation. Inside each chamber, a delivery duct with holes equidistantly spaced was mounted on one of the walls and extended lengthwise along the top. An identical duct for chamber exhaust was located near the floor of the chamber, directly beneath the inlet duct. Artificial light from the room was the only source of light in the inhalation chamber. Each chamber held one carrier with animal cages.

The airflow through each chamber was approximately 2000 liters per minute, which is approximately 27 air changes per hour. During each 6-hour exposure, chamber temperature, relative humidity, and airflow rate were recorded approximately four times a day.

Ethylene Oxide Vapor Concentration Determination

All chambers, including the control chambers, were monitored for EO concentration by means of a Varian 2700 gas chromatograph (GC). Pertinent information regarding the operating conditions of the GC is presented in Table 2. An automatic sampling and recording system connected to the GC acquired the sample and recorded the data on a printer and on magnetic tape. A known standard concentration of EO was sampled several times before the start of daily chamber analysis to check the monitoring system. Furthermore, during each cycle of chamber analyses, the same standard was reanalyzed. Approximately eight samples were analyzed from each chamber each day. Conversion of parts per million, ppm (expressed as volume/volume) to mg/m^3 is $1 \text{ ppm} = 1.8 \text{ mg}/\text{m}^3$ (25°C and 760 mm Hg).

Target Chamber Concentrations and Triethylenemelamine Dosage

Target concentrations of 100, 33, and 10 ppm of EO were selected for the exposure concentrations based on the results of an eight-week inhalation study (report 45-139) on rats. In this study, young rats exposed to 100 ppm were noted to have throughout the study a lower gain in body weight than the controls, whereas only a transient change in body weight was noted for rats exposed to 50 ppm. The target exposure concentrations for the one generation reproduction (report 42-7) and teratology (report 42-13) studies were the same as the present study, since the animals from each of the three studies were exposed at the same time. There were two separate groups of animals which were exposed to room air (0 ppm of EO) and served as the negative or air-control groups (air-control I and air-control II). The purpose of having two control groups was to allow for better assessment of the variability in this study between non-chemically treated groups of animals. A sixth group of animals was treated with triethylenemelamine (0.25 mg/kg) in water by a single intraperitoneal injection 48 hours before the first day of mating. [Note: This is a deviation from the protocol which states injection will be given 24 hours before the first day of mating.]

Animals and Animal Husbandry

Thirteen-week-old virgin male Fischer 344 rats (F-344/Ma: fBR) and three separate groups of four-week, five-week, and six-week-old virgin female Fischer 344 rats were received February 14, 1978 from Microbiological Associates, Inc., Walkersville, MD. They were housed in Room 139. All animals were individually numbered by using a toe-clipping method of identification. Each animal of the same sex was given a different number. Listed on each cage card were the identification numbers for the animals within the cage.

During the non-exposure period, food (Wayne Lab Blox F-6 Laboratory Animal Diet, Allied Mills, Inc., Chicago, IL) and water (Municipal Authority of Westmoreland County, Greensburg, PA) were available ad libitum. The artificial fluorescent lighting was on a 12-hour light and 12-hour dark cycle (approximately 6:00 a.m. to 6:00 p.m.). When animals were in the room, the temperature and humidity controlling devices of the non-recirculated air supply to the room were set to maintain the environment between 66 to 77°F and 30 to 70% relative humidity.

Male rats were housed and exposed two per cage in stainless steel, wire-mesh cages (35 cm x 17 cm x 18 cm high), and females and positive control males were housed separated by sex, three per cage, in stainless steel, wire-mesh cages (23.5 cm x 20 cm x 18 cm high). Stainless steel shelf pans with a layer of Deotized Animal Cage Board® (Upjohn Co., Kalamazoo, MI) were placed under each row of cages to prevent urinary and fecal contamination of lower levels. The paperboard along with the food and water were removed during each six-hour exposure for the male rats. During the mating period, one male and one female were housed in stainless steel, wire-mesh cages (23.5 cm x 20 cm x 18 cm high).

Quality Control

Immediately upon arrival and during a two-week quarantine period, the general health status of the rats was assessed. Quality control examinations which included body weight determinations, fecal examination for intestinal parasites, blood serology, evaluation of the nasopharynx and lung for aerobic bacteriologic flora, and micropathologic examination of selected tissues were performed on a portion of the group of rats upon receipt. The results of the quality control were within normal limits for commercially available, specific pathogen-free rats; therefore, the animals were determined to be of suitable quality for the inhalation study.

Group Assignment and Number of Animals per Group

At the time of random assignment of animals into exposure groups, only the rats whose body weights were within $\pm$ two standard deviations from the mean weight of all the rats of that sex or age group were selected. Any rat having signs of poor health during the preliminary observation period was rejected. Male rats were randomly assigned to one of six groups of 10 each. The females were randomly assigned into three groups of 60 each.

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Experimental Design

A modification of the method for dominant lethal testing as presented by Green, et al. (1977) was followed in this study. The initial day of the EO exposures was on February 27, 1978. All groups of males were exposed six hours per day, five days per week for ten weeks. The last exposure day was May 5, 1978. The injection of TEM occurred on May 4, 1978. Animals were observed twice daily for possible toxic effects from ethylene oxide exposure or TEM treatment. Body weights of the males were determined prior to the initiation of the exposures, every two weeks during the exposure period, and immediately preceding the first mating period.

The first of three sequential mating weeks for all males began the day (May 6, 1978 which is one day later than that stated in the protocol) after the last exposure. At the beginning of each mating week, a virgin female, approximately 18 weeks old, was randomly assigned to a mating cage with one of the treated male rats. In anticipation of obtaining higher percentages of pregnancy, the females which had not thrown a vaginal plug within four days of mating were switched to another male within the same treatment group. The female remained with this male until a vaginal plug was noticed or until the end of the mating period for that week (i.e. 3 days). The day a vaginal plug was observed was designated as day 0 of gestation and the female was separated from the male.

Sacrifice and Necropsy

Females were sacrificed on the 13th day of gestation, or if no vaginal plug was observed, 14 days from the midweek of mating. The females were sacrificed by exsanguination through severing of the brachial vessels following the induction of anesthesia with methoxyfluorane. All gross lesions were recorded. The corpora lutea of each ovary were counted if present. The uterus of each female was examined for the number of implantation sites, and the number of early and late resorption sites. The position of implantation sites relative to the uterine horn was recorded.

The uterus, ovaries, and urinary bladder and any gross lesions were removed at necropsy and placed in 10% neutral buffered formalin. The males were sacrificed by CO₂ asphyxia. The testes and epididymides were retained in 10% neutral buffered formalin.

Experimental Data Evaluated

The appropriate records were maintained in order to determine the following:

- a) Female Fertility Index = $\frac{\text{No. of females with implantations}}{\text{No. of females mated}} \times 100$
 - b) Male Fertility Index = $\frac{\text{No. of males successfully impregnating a female}}{\text{No. of females mated}} \times 100$
 - c) Pre-implantation Loss = $\frac{\text{No. of pre-implantation loss per female}}{\text{No. of corpora lutea per female}} \times 100$
 - d) Proportions of the females with one or more or with two or more resorption sites;
 - e) Mutagenic Index (1) = $\frac{\text{No. of resorption sites per female}}{\text{No. of implantation sites per female}} \times 100$
- Mutagenic Index (2) = $\frac{\text{No. of early resorptions per female}}{\text{No. of implantation sites per female}} \times 100$

Statistical Analyses

The fiducial limit of 0.05 (two-tailed) was selected as the critical level of significance. All data of each exposure group were compared statistically to the air-control group by using the following tests: Continuous variable data (such as body weight gain) were analyzed by Bartlett's test for homogeneity of variance (Sokal and Rohlf, 1969), analysis of variance (Sokal and Rohlf, 1969), and Duncan's multiple range test (Duncan, 1955, 1957; Harter, 1960). Whenever the F value for analysis of variance was significant and Bartlett's test indicated homogeneous variance, Duncan's multiple range test was used to delineate which groups differed significantly from the control. When Bartlett's test indicated heterogeneous variance, the F-test (Sokal and Rohlf, 1969) was employed to compare each exposure group with each air-control group. The type of t-test then used was selected according to the significance of the F value. The Student's t-test (Sokal and Rohlf, 1969) was used when the F value was not significant. The Cochran t-test (Cochran and Cox, 1957) was used when the F value was significant. Contingency data were analyzed by using Fischer's Exact Test (Sokal and Rohlf, 1969).

Because there were not enough animals pregnant per group for appropriate statistical analyses, only mean or median values were reported for the following: preimplantation loss; proportions of females with one or more or with two or more resorption sites; and the two mutagenic indices.

Records

The final report, specimens, slides, and all raw data are retained in the Archives of the Bushy Run Research Center for possible further reference.

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RESULTS AND DISCUSSION

Test Material Purity and Stability

The purity of the ethylene oxide test material was stated to be greater than 99.9% ethylene oxide (P. H. Wadia letter dated October 21, 1981). Table 3 lists the results of chemical analyses which were performed prior to and following the inhalation exposure period. Based on these analyses the test material was stable through this study.

Before the initiation of the study, the chloride content of the ethylene oxide was measured by UCC at the Institute Plant Laboratory, South Charleston, WV (Table 4). The organic chloride compounds were identified by gas liquid chromatography as ethyl chloride, ethylene chlorohydrin, ethylene dichloride and vinyl chloride. The chloride content of each compound was calculated. The total of these calculated values, 10.1 ppm, agreed with the total chloride content, 10.1 ppm, as determined by a Dohrmann Chloride Analyzer.

Chamber Concentration, Temperature and Relative Humidity

The results for chamber EO concentration determinations are summarized in Table 5. The actual chamber concentrations were close to the target concentrations of 100, 33, and 10 ppm of ethylene oxide with little day-to-day variation. Mean chamber concentration values for the 100, 33, and 10 ppm ethylene oxide exposure groups were 98, 33, and 10 ppm, respectively. The respective coefficients of variation were 3, 3, and 4%.

Less than 5% of the total number of analytical samples from either of the air-control chambers were greater than the 0.05 ppm minimum detection limit. No ethylene oxide leaks from the vapor generation system were detected in the room where both the test and control chambers were located. Consequently, it is believed that these few analyses represent analytical anomalies.

Weekly means of chamber temperature and relative humidity are presented in Tables 6 and 7, respectively. The mean of the weekly means for chamber temperature ranged from 71 to 73°F and chamber relative humidity ranged from 49 to 50%. Daily mean chamber temperature values varied from 70 to 76°F and relative humidity values varied from 45 to 59%.

Observations for Toxicologic Effects

No deaths occurred in any group. No treatment-related adverse effects on appearance or demeanor were observed.

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Body Weight

The mean body weight change from Day 0 for each group during the 10 weeks of exposures are given in Table 8. There were no apparent treatment-related effects on body weights. Some statistically significant differences were noted between the EO- and air-exposed groups, but these differences either disappeared by the next weighing interval or were not representative of a dose-related response.

Fertility

Presented in Table 9 are the results of the female and male fertility index determinations. For all three weeks of mating there were no statistically significant differences between any treatment group, including the positive control group, and either air-control group.

It was noted that the fertility indices were particularly low for all groups, including the air-control groups, during the first week of mating. The results of the mating for the first week are presented in the tables, but no interpretation of the data were made because of the insufficient number of pregnant animals. Mating during the second and third weeks resulted in fertility rates of only 50 and 60% for some of the ethylene oxide and air-exposed groups. These are less than the 75% fertility rates observed in other studies with this strain of rat. Individual female and male fertility records are presented in Tables 1 and 2, respectively, of Appendix B.

Necropsy

There were no gross lesions noted at necropsy for the male rats exposed to ethylene oxide. Only a few gross findings were noted in the female rats. It is not uncommon to have low incidences of certain observations, which included mostly distention of uterine horns, in non-exposed rats. A complete listing of the gross findings for the females is presented in Table 1 of Appendix A.

Preimplantation Loss

The results for the percentages of preimplantation losses are given in Table 10. Median values for the 100 ppm and positive control groups were numerically greater in the second and third weeks in comparison with the air-controls and low and intermediate ethylene oxide groups. These results are graphically presented for individual animals in Figures 1 and 2. Given in Appendix B, Table 1, are the individual values of each pregnant animal for the number of corpora lutea and the number of implantation sites.

Resorptions and Live Implants

Results of the total number of resorptions and number of live implants per pregnant female are presented in Table 10. For the second and third weeks of mating, all values of the 33 and 10 ppm groups were within the range of values for the two air-control groups; however, the values of the 100 ppm group, particularly for the third week of mating, were beyond the range of values for the air-controls. The number of early and late resorption sites are given for each pregnant animal in Appendix B, Table 1.

The values for the proportion of pregnant females with one or more resorption sites or with two or more resorption sites (Table 10) were either high for both air-control groups or varied considerably between the two air-control groups, consequently making interpretations of the results difficult. Similar problems in variation between air-control groups are present in the interpretation of the results for the number of resorptions per implantation site and number of early resorptions per implantation sites (Table 10). The individual results of these last two observations for all animals are graphically presented in Figures 3 through 6.

Mutagenesis Data Tabulated in Reference to the Male Rat

Presented in Table 11 are the results for preimplantation losses and resorptions per implantations listed with reference to which male contributed to these results. The median values are given for mating weeks 2 and 3. For the males with two values in one week (i.e. because of two impregnations), when either the highest or lowest values are eliminated, the median results indicate the same trend as does just the median value for the group. That is, for preimplantation losses, the values of 100 ppm group were numerically greater than either the values for air-control I or II for mating weeks 2 and 3, and the values for resorptions per implantations of the 100 ppm group were numerically greater than the values for either air-control I or control II for mating week 2 but only greater than air-control I for week 3.

Discussion and Summary

Ethylene oxide exposures at concentrations as high as 100 ppm did not result in any treatment-related effects on body weight or clinical signs in sexually mature male rats exposed for 10 weeks. At necropsy, there were no gross lesions observed in these animals that could be related to treatment.

Fertility indices were low in all groups during the first week of mating. While there was improvement in fertility during the second and third weeks, in many cases the indices were less than those seen historically in animals kept in mating boxes for up to 7 days. It is possible that the shortened mating periods in this study (3 or 4 days) may have been a factor contributing to the lower fertility rates.

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For some unexplainable reason, many of the values which indicate a dominant lethal event were elevated in air-control group II during the third week of mating, which is demonstrated in Figures 4 and 6. This phenomenon, along with the low numbers of pregnant females for all groups, consequently makes interpretation of the treatment-related effects difficult. What is apparent is that there were also elevated values for the 100 ppm group, but generally, the values for the 33 and 10 ppm groups were similar to those of air-control I.

In summary, values for preimplantation loss were numerically increased in the 100 ppm exposure group in comparison to either air-control group. However, other supporting evidence of dominant lethal effects, (i.e. decrease in fertility rate or increase in resorption per implantation) were not sufficiently elevated over both controls to make a firm conclusion of the dominant lethal effects from treatment. Without the results of air-control II, 100 ppm exposure concentration would have been considered an effect level. However, with the results of air-control II considered and with the problem of low fertility rates for all groups, these results should be considered inconclusive in establishing the minimum effect level. Within the constraints of the variability of the result, it appears probable that the exposures to 33 and 10 ppm did not result in a dominant lethal effect in male Fischer 344 rats.

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Table 1

Physical Properties of Ethylene Oxide*
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Molecular Formula:	CH ₂ CH ₂ O
Molecular Weight:	44.05
CAS Registry Number	75-21-8
Boiling Point at 760 mm Hg:	10.4°C (50.7°F)
Specific Gravity at 20/20°C (H ₂ O = 1):	0.8711
Vapor Density (air = 1):	1.49
Percent Volatiles by Volume:	100
Appearance and Odor:	Colorless gas
Vapor Pressure at 20°C:	1095 mm Hg
Solubility in Water:	Complete
Evaporation Rate (butyl acetate = 1):	> 1
Flash Point (Tag open cup):	< 0°F; ASTM D 1310
Flash Point (Tag closed cup):	< 0°F; ASTM D 56
Concentration Equivalency @ 25°C and 760 mm Hg:	1 ppm=1.8 mg/m ³ 1 mg/liter = 556 ppm

* Information obtained from Material Safety Data Sheet, Union Carbide Corporation F-43130B. 4/78-4M

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Table 2

Gas Chromatographic Conditions Used for the
Exposure Chamber Analysis of Ethylene Oxide
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Column	Stainless steel tubing 1/8" o.d. x 20 ft.
Column packing	30% TERGITOL - TMN and 3% sodium methylate on Chromosorb W-NAW 60/80 mesh
Conditions	Column oven and injector at 80°C Injection loop at room temperature Interface and detector 130°C
Carrier gas	Nitrogen at 35 ml/min
Flame detector gases	Air 550 ml/min Hydrogen 42 ml/min
Sample size	1 ml
Lower limit of detection	0.05 ppm

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Table 4

Chloride Content of Liquid Ethylene Oxide
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

<u>Material</u>	<u>Organic Chloride Content¹</u> (ppm, w/v) ²	<u>Calculated</u> <u>Chloride Content</u> (ppm CL, w/v) ²
Organic Compound		
Ethyl chloride	12.2	6.7
Ethylene chlorohydrin	3	1.3
Ethylene dichloride	2	1.4
Vinyl chloride	1.3	0.73

¹ Determined by gas liquid chromatography (glc)

² To convert from w/v to v/v: multiply by reciprocal of the density of the organic chloride at 20°C, i.e.,

$$(w/v \text{ value} \times \frac{1}{\text{density at } 20^{\circ}\text{C}} = (v/v \text{ value}))$$

Table 3

Chemical Analyses and Specifications
for Drum 7JNC45, G581 of Ethylene Oxide¹
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Chemical Analysis	Analysis Date		Maximum Commercial Specifications ²
	April 23, 1977 ³	July 18, 1978	
Acidity (% by wt) ⁴	0.0016	0.0014	0.002
Aldehydes (ppm) ⁵	21	19	30
Acetylene (ppm)	Nil	Nil	Nil
Water (% by wt)	0.09	0.005	0.03
Residue (g/100 ml)	0.0012	0.0004	0.005
Color (Pt-Co) ⁶	3	1	10
Odor	Nonresidual	Nonresidual	Nonresidual
Suspended Matter	Substantially Free	Substantially Free	Substantially Free

¹ Analyses performed at the Research and Development Department,
Union Carbide Corporation, South Charleston, WV.

² Commercial specifications of Union Carbide Corporation.

³ Maximum of two analyses.

⁴ Measured as acetic acid.

⁵ Measured as acetaldehyde.

⁶ Platinum-cobalt color test.

Table 5

Inhalation Chamber Ethylene Oxide Vapor Concentration Determinations
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Ethylene Oxide Target Chamber Concentrations*			
Target concentration, ppm	100 ppm	33 ppm	10 ppm
Mean of daily means of measured concentration, ppm ¹	97.6	33.0	9.9
Coefficient of variation ² , %	3.3	2.9	3.8
Air Control Chamber Concentrations			
Air control chamber	I	II	
Target concentration, ppm	0	0	
Median of the daily medians of measured concentration, ppm ¹	ε 0.05 ³	ε 0.05 ³	
Number of individual analyses	373	367	
Number of analyses above minimum limit of detection	13	12	
Mean concentration of analyses above minimum limit of detection	0.19	0.24	
Standard deviation of analyses above minimum limit of detection	± 0.16	± 0.20	
Maximum concentration determined, ppm	0.70	0.77	
¹ Approximately 8 analyses per chamber were made each exposure day of the days that a test atmosphere was generated. ² Measurement is based on the standard deviation and the mean. It is a measure of variability of the daily means. ³ Gas chromatograph minimum detection limit.			

Table 6

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Inhalation Chamber Temperature¹

Exposure Week	Target Concentration				
	100 ppm $\bar{x} \pm SD$ ²	33 ppm $\bar{x} \pm SD$	10 ppm $\bar{x} \pm SD$	0 ppm (CI) $\bar{x} \pm SD$	0 ppm (CII) $\bar{x} \pm SD$
1	72 $\pm$ 1	72 $\pm$ 1	74 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 1
2	72 $\pm$ 1	74 $\pm$ 3	73 $\pm$ 1	72 $\pm$ 1	72 $\pm$ 1
3	71 $\pm$ 1	72 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 1
4	72 $\pm$ 1	72 $\pm$ 0	72 $\pm$ 1	72 $\pm$ 1	72 $\pm$ 1
5	70 $\pm$ 0	72 $\pm$ 1	73 $\pm$ 1	72 $\pm$ 1	72 $\pm$ 1
6	70 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 1	72 $\pm$ 0	72 $\pm$ 1
7	72 $\pm$ 1	74 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 0	72 $\pm$ 1
8	70 $\pm$ 0	74 $\pm$ 1	74 $\pm$ 1	73 $\pm$ 1	74 $\pm$ 0
9	71 $\pm$ 1	73 $\pm$ 1	72 $\pm$ 1	72 $\pm$ 1	71 $\pm$ 1
10	70 $\pm$ 0	74 $\pm$ 0	72 $\pm$ 0	72 $\pm$ 1	71 $\pm$ 1
$\bar{x} \pm SD$	71 $\pm$ 1	73 $\pm$ 1	73 $\pm$ 1	72 $\pm$ 0	72 $\pm$ 1

¹ Units = Farenheit

² $\bar{x} \pm SD$ = Mean $\pm$ standard deviation

Table 7

Ethylene Oxide Dominant Lethal Mutagenicity Inh. Test in Rats

Inhalation Chamber Relative Humidity¹

Exposure Week	Target Concentration				
	100 ppm $\bar{x} \pm SD$ ²	33 ppm $\bar{x} \pm SD$	10 ppm $\bar{x} \pm SD$	0 ppm (CI) $\bar{x} \pm SD$	0 ppm (CII) $\bar{x} \pm SD$
1	46 ± 1	46 ± 1	46 ± 1	45 ± 1	46 ± 0
2	46 ± 0	46 ± 0	46 ± 1	45 ± 0	47 ± 0
3	49 ± 3	48 ± 3	49 ± 3	48 ± 3	49 ± 3
4	49 ± 3	50 ± 4	49 ± 2	49 ± 4	50 ± 4
5	48 ± 2	48 ± 2	48 ± 2	47 ± 2	48 ± 2
6	54 ± 3	55 ± 3	54 ± 2	53 ± 2	54 ± 3
7	52 ± 4	52 ± 4	52 ± 3	51 ± 5	54 ± 3
8	52 ± 4	52 ± 4	53 ± 4	53 ± 3	53 ± 4
9	52 ± 4	51 ± 4	52 ± 4	51 ± 4	51 ± 3
10	50 ± 4	50 ± 4	49 ± 4	49 ± 4	49 ± 5
$\bar{x} \pm SD$	50 ± 3	50 ± 3	50 ± 3	49 ± 3	50 ± 3

¹ Units = % Relative humidity

² $\bar{x} \pm SD$ = Mean ± standard deviation

Table 8

Body Weight Changes of Male Fischer 344 Rats¹
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Exposure Week	Target Exposure Concentrations				
	100 ppm	33 ppm	10 ppm	Air Control I 0 ppm	Air Control II 0 ppm
	Mean $\pm$ SD ²	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Mean Body Weight, Grams, On Day 0					
	266.6 $\pm$ 18.4	273.2 $\pm$ 17.0	271.2 $\pm$ 14.3	274.4 $\pm$ 13.2	274.0 $\pm$ 16.4
Body Weight Change, Grams, From Mean Weight On Day 0					
2	13.4 $\pm$ 10.5	14.0 $\pm$ 4.6	9.8 ^{b,a} $\pm$ 5.0	16.1 $\pm$ 6.5	14.3 $\pm$ 5.4
4	20.0 ^a $\pm$ 11.6	25.1 $\pm$ 6.4	20.5 ^a $\pm$ 7.1	25.1 $\pm$ 8.2	30.0 $\pm$ 9.0
6	31.6 $\pm$ 14.1	39.3 $\pm$ 10.1	30.7 $\pm$ 8.6	32.6 $\pm$ 9.5	38.6 $\pm$ 6.5
8	46.4 $\pm$ 13.1	52.4 $\pm$ 9.5	45.2 $\pm$ 8.1	49.4 $\pm$ 10.2	52.1 $\pm$ 7.9
10	51.2 $\pm$ 13.2	57.5 $\pm$ 10.4	49.4 $\pm$ 9.0	55.7 $\pm$ 8.4	58.1 $\pm$ 8.0

a = p < 0.05

b = 0.05 > p > 0.01

¹ N = 10 rats per group

² SD = Standard deviation

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Table 9

Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Fertility Data¹

Week	Treatment					
	100 ppm	33 ppm	10 ppm	0 ppm (CI)	0 ppm (CII)	TEM (0.25 mg/kg)
	Percentage (ratio) Pregnant Females					No. of females pregnant No. mated
1	20 (2/10)	14 (1/7)	56 (5/9)	12 (1/8)	33 (3/9)	56 (5/9)
2	70 (7/10)	70 (7/10)	80 (8/10)	60 (6/10)	90 (9/10)	60 (6/10)
3	50 (5/10)	50 (5/10)	100 (10/10)	70 (7/10)	60 (6/10)	60 (6/10)
	Percentage (ratio) Males that Successfully Impregnated One or Two Females					
1	20 (2/10)	10 (1/10)	50 (5/10)	10 (1/10)	30 (3/10)	56 (5/9)
2	60 (6/10)	50 (5/10)	70 (7/10)*	60 (6/10)	70 (7/10)	60 (6/10)
3	50 (5/10)*	50 (5/10)	100 (10/10)	60 (6/10)	60 (6/10)*	50 (5/10)

¹ Number of pregnant females may be more than the number of successfully mated males because some males successfully mated with two females.

* Value may be incorrect since for one pregnant female in this group no vaginal plug was detected, consequently it is unknown which male impregnated her.

Table 10

Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Summary of Dominant Lethal Data

Week	Treatment					TEM (0.25mg/kg)
	100 ppm	33 ppm	10 ppm	0 ppm (CI)	0 ppm (CII)	
<u>Median (Quartile Deviation)*Preimplantation Loss (No. preimplantation loss/No. corpora lutea/Female X100)</u>						
1	62 (33)	-- **	40 (34)	--	30 (31)	11 (30)
2	33 (30)	9 (34)	0 (8)	0 (1)	8 (13)	30 (22)
3	42 (34)	8 (17)	8 (15)	8 (6)	14 (20)	70 (26)
<u>Total Resorptions / No. Pregnant Females</u>						
1	3.0	1.0	0.2	0.0	2.0	5.0
2	1.4	0.1	0.8	1.3	0.6	3.7
3	4.0	1.4	0.9	1.0	2.7	3.5
<u>Total Live Implants / No. Pregnant Females</u>						
1	0.5	6.0	5.6	12.0	4.7	2.0
2	5.0	7.7	9.4	9.7	8.8	2.2
3	2.6	8.6	8.4	9.3	4.5	0.3

(Continued)

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Table 1P (continued)

Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Summary of Dominant Lethal Data

Week	Treatment					TEM (0.25 mg/kg)
	100 ppm	33 ppm	10 ppm	0 ppm (CI)	0 ppm (CII)	
Proportion of Pregnant Females with One or More Resorption Sites (X100)						
1	100	--	20	--	67	100
2	71	14	50	67	44	67
3	100	60	60	86	83	100
Proportion of Pregnant Females with Two or More Resorption Sites (X100)						
1	50	--	0	--	33	100
2	43	0	12	50	11	67
3	60	40	20	14	67	83
Median (Quartile Deviation) No. Resorptions/No. Implantation Sites/Female (X100)						
1	92 (19)	- -	0 (12)	- -	9 (42)	100 (24)
2	20 (30)	0 (0)	4 (5)	13 (11)	0 (6)	76 (45)
3	44 (38)	10 (13)	9 (9)	9 (3)	42 (38)	100 (12)
Median (Quartile Deviation) No. Early Resorptions/No. Implantation Sites/Female (X100)						
1	42 (42)	- -	0 (12)	- -	0 (42)	100 (29)
2	8 (22)	0 (0)	0 (4)	4 (10)	0 (6)	60 (43)
3	14 (33)	10 (13)	0 (6)	9 (3)	34 (38)	100 (20)

* Median is the 50th percentile. Quartile deviation is (75th - 25th percentile) / 2.

**Dashes indicate that values could not be determined because only one animal was pregnant.

Table 11

Mutagenesis Data for the High Exposure and Control Groups Tabulated
According to the Male Rat Used in the Mating
Ethylene Oxide Dominant Lethal Mutagenesis Test on Rats

Treatment																				
100 ppm EO							0 ppm CI							0 ppm CII						
Mating Week							Mating Week							Mating Week						
Male	1		2		3		Male	1		2		3		Male	1		2		3	
An #	PL/CL*	R/I**	PL/CL	R/I	PL/CL	R/I	An #	PL/CL	R/I	PL/CL	R/I	PL/CL	R/I	An #	PL/CL	R/I	PL/CL	R/I	PL/CL	R/I
4786	33	83	90	100	18	44	2741	0	0	0	0	9	10	4788	62	0	31	0	36	43
4847	90	100	17	20	83	100	"					0	9	4775	0	9	10	0	55	40
"					0	100	2838			10	0	12	14	2819			80	0	10	11
2854			60	0	73	33	2799			0	22	17	0	2829			22	14		
2898			50	60	42	14	4822			0	18	8	18	"			0	17		
"			0	8			"			0	8			2862	30	83	8	0		
4774			33	0			4803			0	25			"			0	9		
2771			0	43			2903					8	9	4838			0	9	14	100
							2722					0	8	"					0	0
														2807			0	0		
														2830					nd	80
median			33	20	42	44				0	13	8	9				8	0	14	42
median ¹			25	14	30	38				0	8	8	9				0	0	23	40
median ²			42	32	58	38				0	18	8	10				10	0	25	43

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* Preimplantation loss/corpora lutea X100.

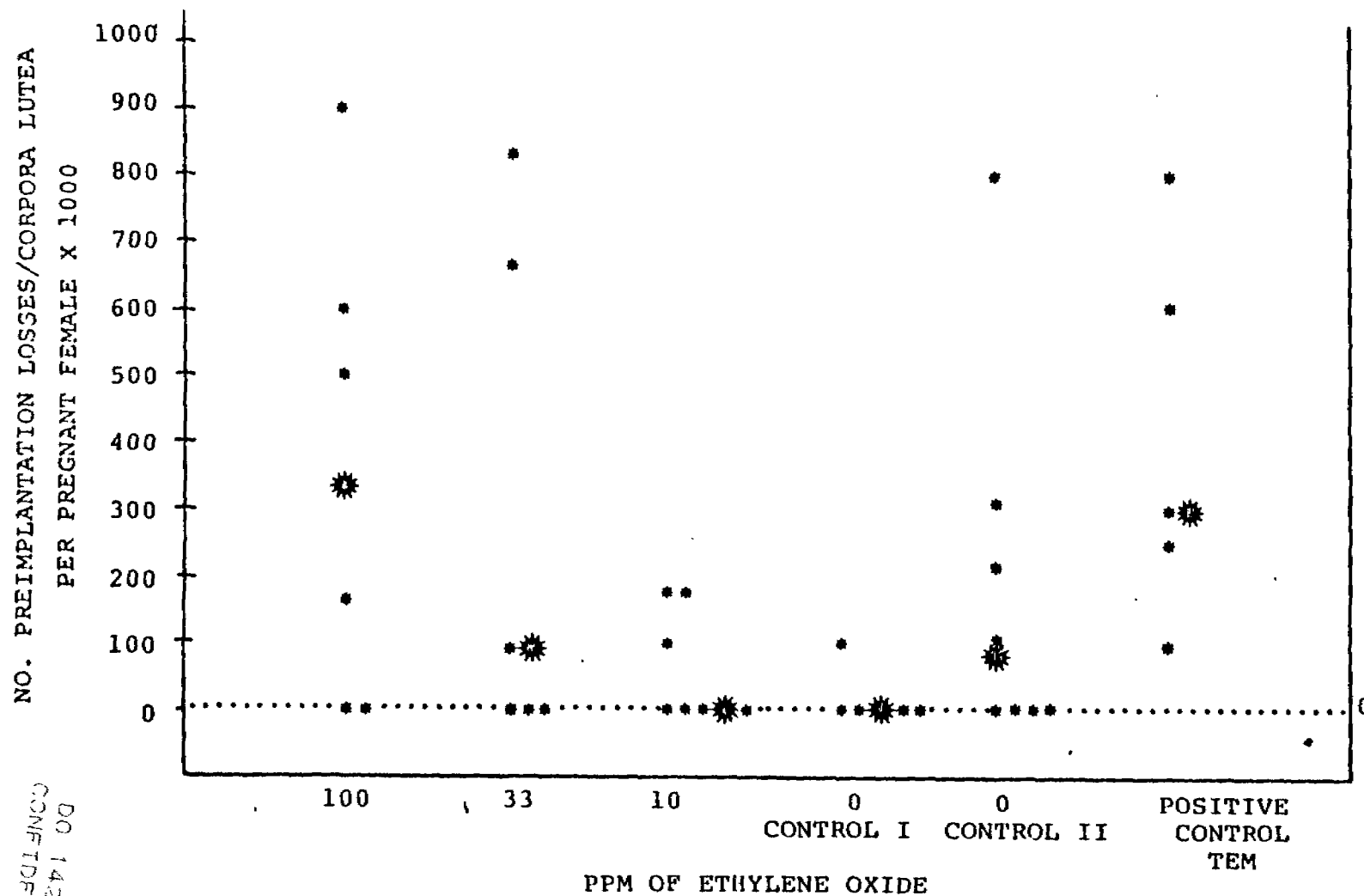
*** Early and late resorptions/implantation sites X100.

nd=not determined

1 median with the highest values of the double mating (a) eliminated.

2 median with the lowest values of the double mating (a) eliminated.

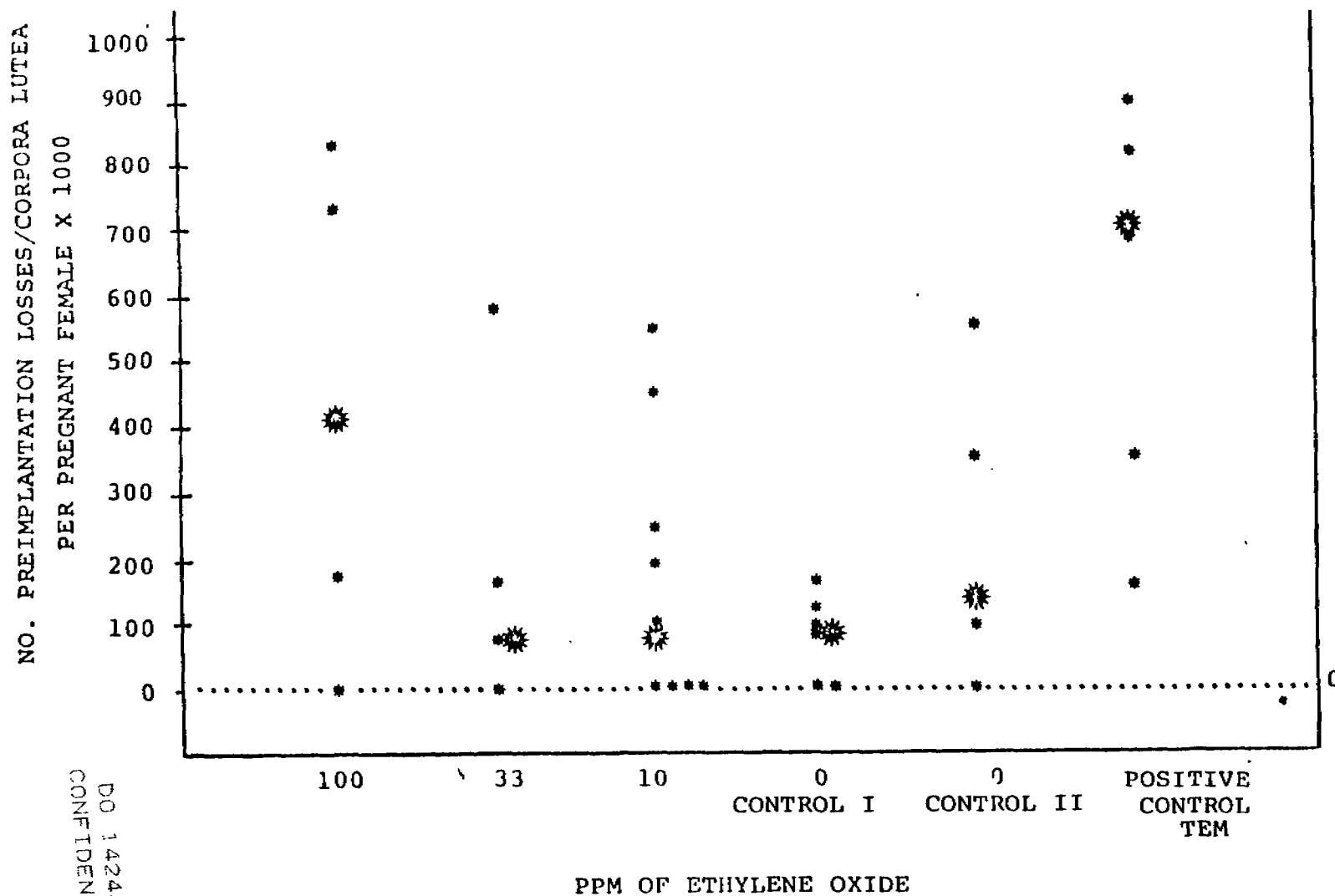
Figure 1
EO DOMINANT LETHAL MUTAGENESIS STUDY
PREIMPLANTATION LOSSES
SECOND WEEK OF MATING



* = Median and actual data point

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Figure 2
EO DOMINANT LETHAL MUTAGENESIS STUDY
PREIMPLANTATION LOSSES
THIRD WEEK OF MATING



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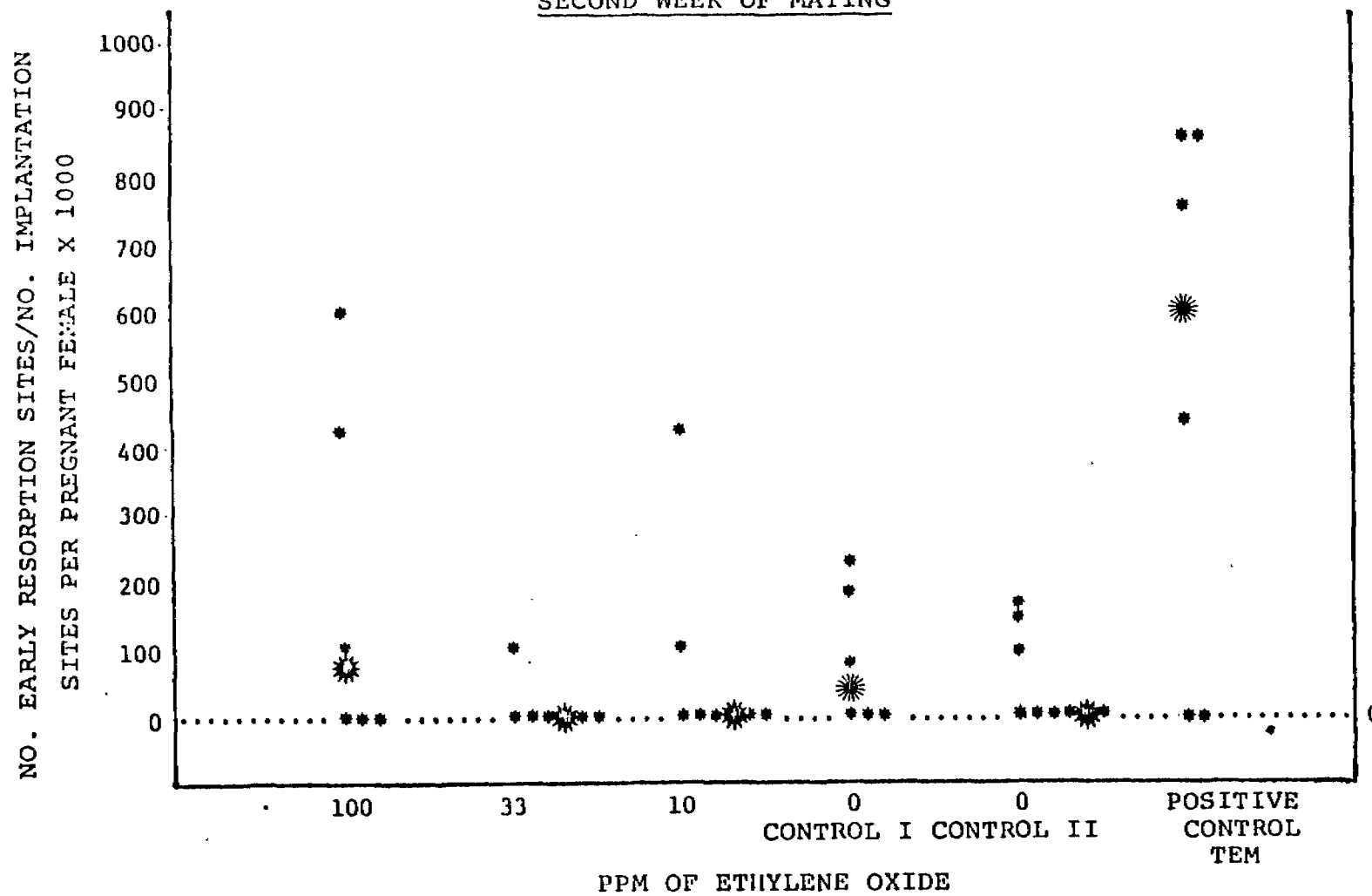
* = Median and actual data point

Figure 3

EO DOMINANT LETHAL MUTAGENESIS STUDY

EARLY RESORPTION SITES

SECOND WEEK OF MATING



☼ = Median and actual data point
☼ = Median

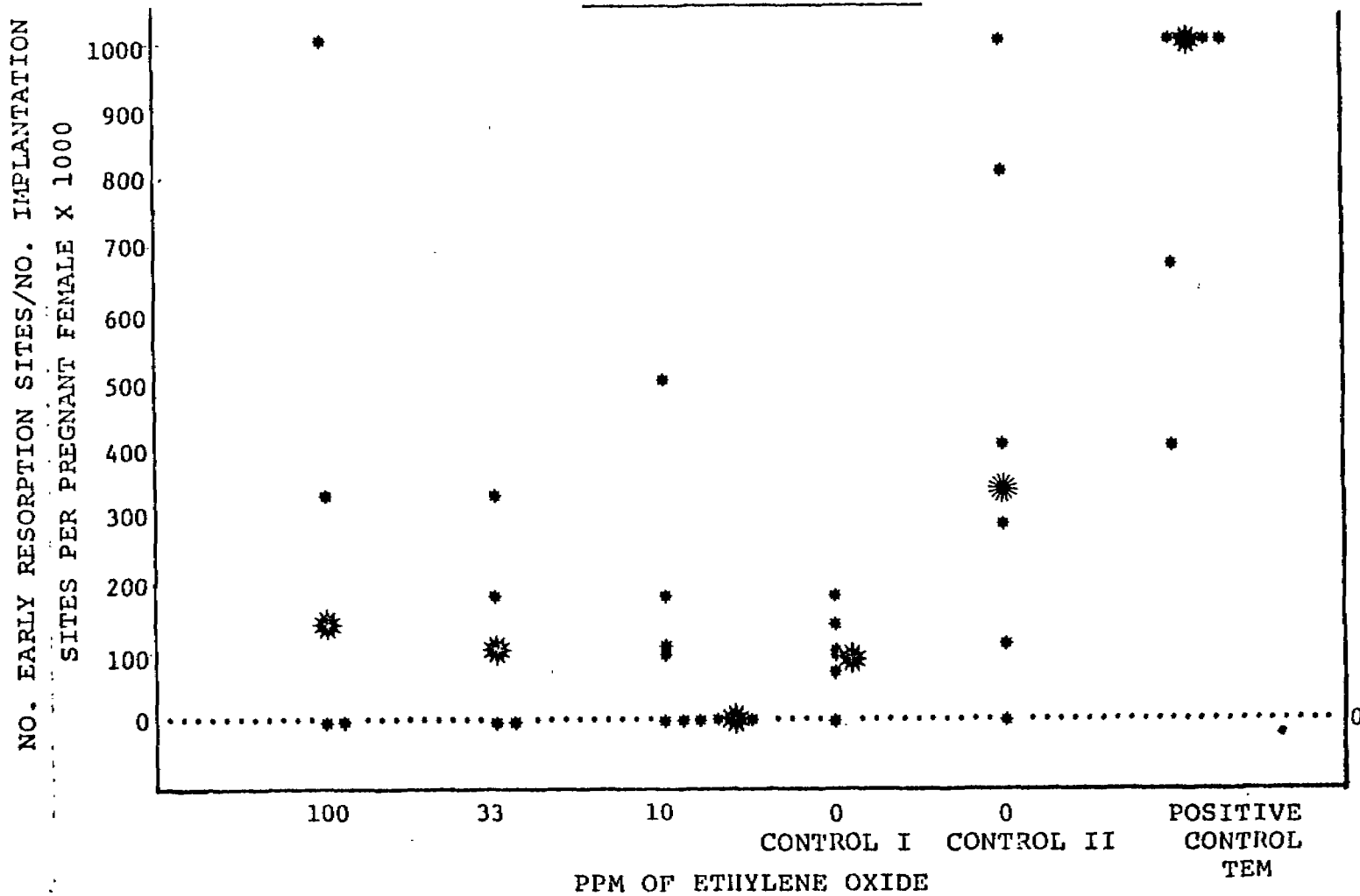
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Figure 4

EO DOMINANT LETHAL MUTAGENESIS STUDY

EARLY RESORPTION SITES

THIRD WEEK OF MATING



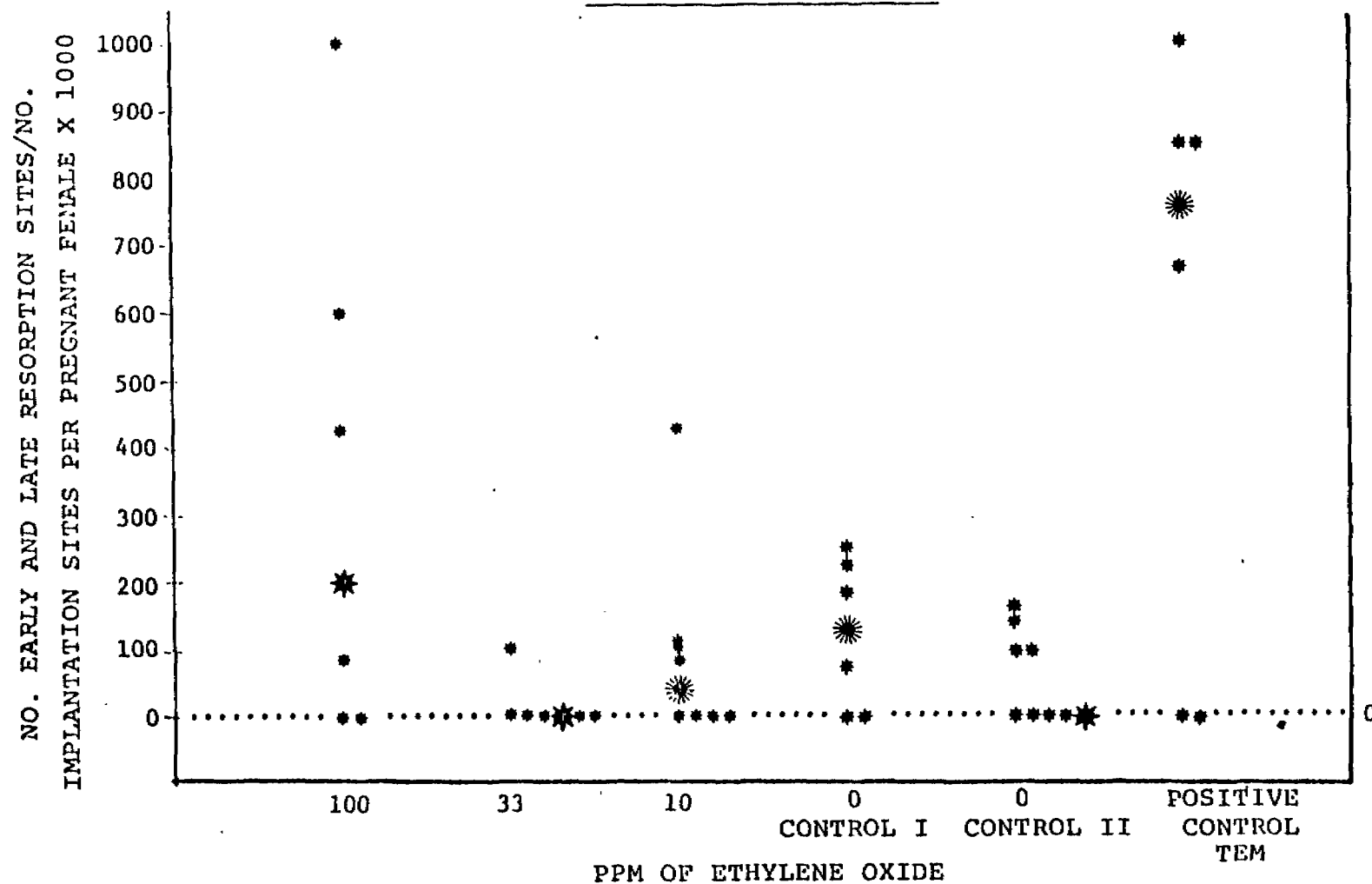
⊛ = Median and actual data point
 ⊛ = Median

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Figure 5

EO DOMINANT LETHAL MUTAGENESIS STUDY
EARLY AND LATE RESORPTION SITES
SECOND WEEK OF MATING

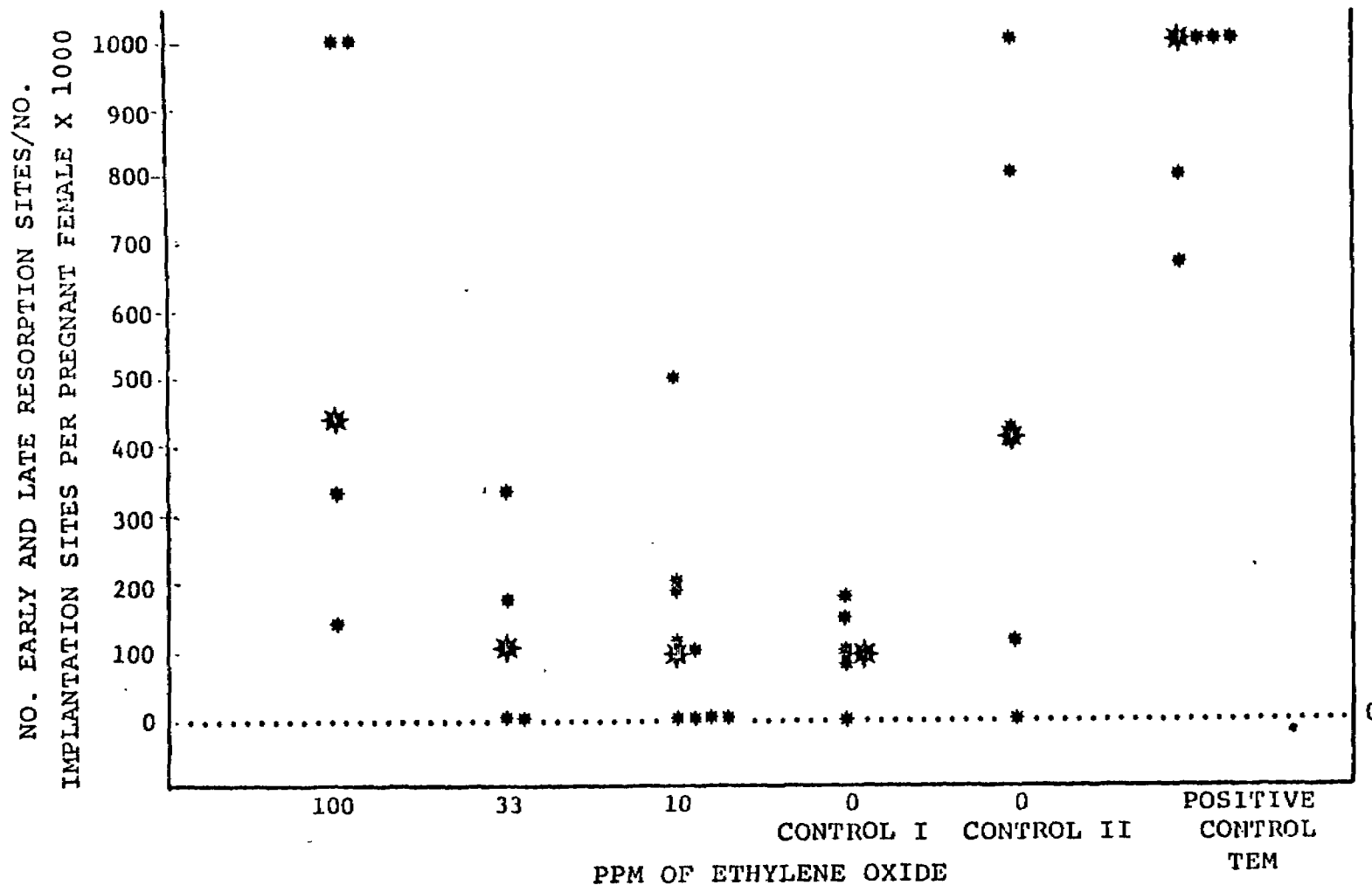


* = Median and actual data point
 ☼ = Median

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Figure 6

EO DOMINANT LETHAL MUTAGENESIS STUDY
EARLY AND LATE RESORPTION SITES
THIRD WEEK OF MATING



* = Median and actual data point

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BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632

Telephone (412) 733-5200

Quality Assurance Unit Study Inspection Summary

Test Substance: Ethylene Oxide

Study: Dominant Lethal Mutagenicity Inhalation on Rats

Study Director: William M. Snellings, Ph.D.

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

<u>Date</u>	<u>Inspection</u> <u>Type</u>	<u>Date QAU Report Issued</u>	
		<u>To Study Director</u>	<u>To Management</u>
10-22 to 10-30-79	Pathology Data	10-30-79	1-17-80
9-1-81	Event-Final Report	9-1-81	11-3-81
1-17 to 1-31-84	Raw Data, Pathology Report and Final Report	1-31-84	2-28-84

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Linda J. Calista 6/15/84
Linda J. Calista Date
Quality Assurance Unit Supervisor

APPENDIX A

Pathology Report

Ethylene Oxide Dominant Lethal Mutagenicity

Inhalation Test in Rats

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Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Pathology Results

Males. There were no gross lesions observed in treated or control rats with the exception of a Control II animal (#78-2883) which had a nodule in the peritesticular fat grossly compatible with focal fat necrosis secondary to infarction. The pathogenesis of this common lesion is believed to be initiated by torsion of tags of adipose tissue leading to localized ischemia.

Females. A few gross lesions were documented in female rats and these are summarized in Table 1. Several females had bilateral dilation of uterine horns compatible with estrus; none of these rats had implantation sites. The type and frequency of gross findings should not affect the results of this study.

R. R. Maronpot
Veterinary Pathologist

8-10-79
Date

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Table 1

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Gross Findings in Females

First Week of Mating

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
100 ppm EO	
78-3643	No gross lesions.
78-3659	No gross lesions.
78-3669	No gross lesions.
78-3670	No gross lesions.
78-3671	No gross lesions.
78-3657	No gross lesions.
78-3679	No gross lesions.
78-3683	No gross lesions.
78-3685	Distention of uterine horns compatible with estrus.
78-3686	Distention of uterine horns compatible with estrus.
78-3701	No gross lesions.
78-3724	No gross lesions.
33 ppm EO	
78-3655	No gross lesions.
78-3678	No gross lesions.
78-3690	No gross lesions.
78-3699	Polycystic right kidney and pitted surface on left kidney compatible with nephritis.
78-3702	No gross lesions.
78-3708	No gross lesions.
78-3712	No gross lesions.
78-3714	No gross lesions.
10 ppm EO	
78-3644	No gross lesions.
78-3647	No gross lesions.
78-3660	No gross lesions.
78-3662	No gross lesions.
78-3668	No gross lesions.
78-3672	No gross lesions.
78-3693	No gross lesions.
78-3694	Corneal opacity of right eye compatible with keratitis.
78-3709	No gross lesions.
78-3716	No gross lesions.
78-3720	Distention of uterine horns compatible with estrus.

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(Continued)

Table 1

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Gross Findings in Females

First Week of Mating

(Continued)

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
Control I	
78-3648	No gross lesions.
78-3656	No gross lesions.
78-3665	No gross lesions.
78-3666	No gross lesions.
78-3681	No gross lesions.
78-3689	No gross lesions.
78-3704	No gross lesions.
78-3707	No gross lesions.
78-3719	No gross lesions.
Control II	
78-3642	No gross lesions.
78-3664	No gross lesions.
78-3676	No gross lesions.
78-3688	No gross lesions.
78-3692	No gross lesions.
78-3695	Distention of uterine horns compatible with estrus.
78-3697	No gross lesions.
78-3710	No gross lesions.
78-3721	No gross lesions.
78-3722	No gross lesions.
Positive Control (0.25 mg/kg TEM)	
78-3673	No gross lesions.
78-3674	No gross lesions.
78-3675	No gross lesions.
78-3680	No gross lesions.
78-3682	No gross lesions.
78-3684	No gross lesions.
78-3700	No gross lesions.
78-3703	No gross lesions.
78-3706	No gross lesions.
78-3723	No gross lesions.

(Continued)

Table 1

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Gross Findings in Females

Second Week of Mating

(Continued)

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
100 ppm EO	
78-3565	Distention of uterine horns compatible with estrus.
78-3566	Distention of uterine horns compatible with estrus.
78-3567	No gross lesions.
78-3570	No gross lesions.
78-3572	No gross lesions.
78-3575	No gross lesions.
78-3607	Distention of uterine horns compatible with estrus.
78-3627	No gross lesions.
78-3628	No gross lesions.
78-3561	No gross lesions.
33 ppm EO	
78-3551	No gross lesions.
78-3554	No gross lesions.
78-3560	No gross lesions.
78-3581	No gross lesions.
78-3589	No gross lesions.
78-3591	No gross lesions.
78-3599	Distention of uterine horns compatible with estrus.
78-3605	Vaginal and vulval hemorrhage.
78-3618	No gross lesions.
78-3630	No gross lesions.
10 ppm EO	
78-3558	No gross lesions.
78-3559	No gross lesions.
78-3569	No gross lesions.
78-3576	No gross lesions.
78-3577	No gross lesions.
78-3578	No gross lesions.
78-3585	No gross lesions.
78-3609	No gross lesions.
78-3612	No gross lesions.
78-3621	No gross lesions.

(Continued)

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Table 1

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Gross Findings in Females

Second Week of Mating

(Continued)

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
Control I	
78-3552	No gross lesions.
78-3553	No gross lesions.
78-3573	No gross lesions.
78-3582	No gross lesions.
78-3584	No gross lesions.
78-3598	No gross lesions.
78-3613	No gross lesions.
78-3614	No gross lesions.
78-3629	No gross lesions.
78-3635	No gross lesions.
Control II	
78-3563	No gross lesions.
78-3574	No gross lesions.
78-3580	No gross lesions.
78-3588	No gross lesions.
78-3592	No gross lesions.
78-3602	No gross lesions.
78-3603	No gross lesions.
78-3606	No gross lesions.
78-3616	No gross lesions.
78-3622	No gross lesions.
Positive Control (0.25 mg/kg TEM)	
78-3549	No gross lesions.
78-3564	Periovarian cyst.
78-3586	No gross lesions.
78-3590	No gross lesions.
78-3594	No gross lesions.
78-3596	Distention of uterine compatible with e-
78-3601	No gross lesions
78-3610	No gross lesions
78-3611	No gross lesions
78-3633	No gross lesions

(Continued)

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Table 1

Ethylene Oxide Dominant Lethal Mutagenesis Test in Rats

Gross Findings in Females

Third Week of Mating

(Continued)

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
100 ppm EO	
78-3073	No gross lesions.
78-3082	No gross lesions.
78-3111	No gross lesions.
78-3123	No gross lesions.
78-3179	No gross lesions.
78-3246	No gross lesions.
78-3469	No gross lesions.
78-3480	No gross lesions.
78-3484	No gross lesions.
78-3486	No gross lesions.
33 ppm EO	
78-3064	No gross lesions.
78-3101	No gross lesions.
78-3201	No gross lesions.
78-3249	No gross lesions.
78-3467	No gross lesions.
78-3475	No gross lesions.
78-3481	Distention of uterine horns compatible with estrus.
78-3485	No gross lesions.
78-3522	No gross lesions.
78-3526	No gross lesions.
10 ppm EO	
78-3096	No gross lesions.
78-3219	No gross lesions.
78-3245	No gross lesions.
78-3464	No gross lesions.
78-3465	No gross lesions.
78-3473	No gross lesions.
78-3477	No gross lesions.
78-3487	No gross lesions.
78-3528	No gross lesions.
78-3529	No gross lesions.

(Continued)

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Table 1

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Appendix A
Page 7 of 7Ethylene Oxide Dominant Lethal Mutagenesis Test in RatsGross Findings in FemalesThird Week of Mating

(Continued)

<u>Dose and Rat Number</u>	<u>Gross Findings</u>
Control I	
78-3059	No gross lesions.
78-3074	No gross lesions.
78-3131	No gross lesions.
78-3207	No gross lesions.
78-3210	No gross lesions.
78-3462	No gross lesions.
78-3466	Distention of uterine horns compatible with estrus.
78-3476	No gross lesions.
78-3483	No gross lesions.
78-3524	No gross lesions.
Control II	
78-3063	No gross lesions.
78-3105	Distention of uterine horns compatible with estrus.
78-3110	No gross lesions.
78-3140	No gross lesions.
78-3146	No gross lesions.
78-3157	No gross lesions.
78-3206	No gross lesions.
78-3470	No gross lesions.
78-3488	No gross lesions.
78-3527	No gross lesions.
Positive Control (0.25 mg/kg TEM)	
78-3070	No gross lesions.
78-3078	Distention of uterine horns compatible with estrus.
78-3114	No gross lesions.
78-3222	No gross lesions.
78-3241	No gross lesions.
78-3250	No gross lesions.
78-3460	No gross lesions.
78-3463	No gross lesions.
78-3471	No gross lesions.
78-3482	No gross lesions.

EO = Ethylene Oxide
TEM = TriethylenemelamineDO 142477
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APPENDIX B

Individual Animal Results of Dominant Lethal Data

Ethylene Oxide Dominant Lethal

Mutagenicity Inhalation Test in Rats

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Table 1
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No.	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
		First, Second						
<u>100 ppm Ethylene Oxide</u>								
1	3643	4847	10	1	9	0	1	Y
1	3701	4774, 4786	9	6	3	5	0	Y
1	3724	4786	--	--	--	--	--	Y
1	3659	2710, 2771	--	--	--	--	--	N
1	3669*							
1	3670	2734, 2710	--	--	--	--	--	N
1	3671	2771, 2734	--	--	--	--	--	N
1	3657*							
1	3679	2842	--	--	--	--	--	Y
1	3683	2854, 2842	--	--	--	--	--	Y
1	3685	2898, 2854	--	--	--	--	--	N
1	3686	2899, 4774	--	--	--	--	--	Y
2	3628	4847, 4786	10	1	9	0	1	Y
2	3570	2854	10	4	6	0	0	Y
2	3572	2898	10	5	5	3	0	Y
2	3575	2899, 2898	8	12	0**	1	0	Y
2	3627	4786, 4774	9	6	3	0	0	Y
2	3567	2842, 2771	7	7	0	3	0	N ¹
2	3561	2710, 4847	12	10	2	1	1	Y
2	3565	2734, 2710	--	--	--	--	--	N
2	3566	2771, 2734	--	--	--	--	--	N
2	3607	4774, 2899	--	--	--	--	--	Y

(Continued)

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>100 ppm Ethylene Oxide (Continued)</u>								
3	3484	4847	12	2	10	2	0	Y
3	3073	2710, 4847	11	12	0**	0	12	H ¹
3	3179	2854	11	3	8	0	1	Y
3	3246	2898, 2854	--	--	--	--	--	H
3	3469	2899, 2898	12	7	5	1	0	Y
3	3486	4786	11	9	2	3	1	Y
3	3082	2734, 2710	--	--	--	--	--	H
3	3111	2771, 2734	--	--	--	--	--	H
3	3123	2842, 2771	--	--	--	--	--	H
3	3480	4774	--	--	--	--	--	Y
<u>33 ppm Ethylene Oxide</u>								
1	3714	4797, 4811	9	7	2	1	0	H ¹
1	3655	2715, 2726	--	--	--	--	--	H
1	3678	2779, 2747	--	--	--	--	--	H
1	3690*							
1	3699	2826, 2779	--	--	--	--	--	H
1	3702	2841, 2826	--	--	--	--	--	H
1	3708	2864, 2841	--	--	--	--	--	H
1	3712	2865, 2864	--	--	--	--	--	H

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>33 ppm Ethylene Oxide (Continued)</u>								
2	3630	4811, 4797	12	2	10	0	0	Y
2	3618	4797	10	10	0	0	0	Y
2	3581	2779, 2747	9	3	6	0	0	H ¹
2	3605	2865	8	9	0**	0	0	v
2	3551	2715	11	10	1	0	0	Y
2	3560	2747, 2726	11	10	1	0	0	Y
2	3554	2726	11	11	0	1	0	Y
2	3589	2826, 2779	--	--	--	--	--	H
2	3591	2841, 2826	--	--	--	--	--	H
2	3599	2864, 2841	--	--	--	--	--	H
3	3526	4811	12	5	7	0	0	Y
3	3522	4797	12	10	2	1	0	Y
3	3064	2715	12	11	1	2	0	Y
3	3101	2726	10	12	0**	0	0	Y
3	3467	2865	13	12	1	4	0	Y
3	3201	2747	--	--	--	--	--	Y
3	3249	2779, 2747	--	--	--	--	--	N
3	3475	2826, 2779	--	--	--	--	--	H
3	3481	2841, 2826	--	--	--	--	--	H
3	3485	2864, 2841	--	--	--	--	--	H

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>10 ppm Ethylene Oxide</u>								
1	3709	2863, 2795	12	2	10	0	0	Y
1	3693	2795, 2794	--	--	--	--	--	N
1	3660	2731, 2735	8	2	6	1	0	Y
1	3662	2735, 2740	--	--	--	--	--	N
1	3716	4810	10	6	4	0	0	Y
1	3644	4842, 4795	10	8	2	0	0	Y
1	3720	4795, 4810	--	--	--	--	--	N
1	3672	2746	10	11	0**	0	0	Y
1	3647*							
1	3668	2740, 2746	--	--	--	--	--	N
1	3694*							
2	3621	4842	7	7	0	3	0	Y
2	3558	2731, 4842	11	12	0**	0	1	Y
2	3578	2795	11	9	2	0	1	Y
2	3577	2794	11	9	2	0	0	Y
2	3612	4810, 2863	10	9	1	0	0	N
2	3585	2863	12	12	0	0	0	Y
2	3569	2740	10	10	0	1	0	Y
2	3576	2746, 2740	--	--	--	--	--	N
2	3559	2735	13	13	0	0	0	Y
2	3609	4795, 4810	--	--	--	--	--	Y

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(Continued)

Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Capital Plug Protected
<u>10 ppm Ethylene Oxide (Continued)</u>								
3	3465	4810	11	5	6	0	1	Y
3	3477	2794	11	6	5	3	0	Y
3	3487	2795, 2794	10	8	2	0	0	H
3	3528	4795	12	9	3	1	0	Y
3	3096	2731	11	10	1	0	0	Y
3	3473	2746	11	11	0	2	0	Y
3	3529	4842	11	11	0	0	0	Y
3	3245	2740	12	11	1	1	0	Y
3	3464	2863	11	11	0	0	0	Y
3	3219	2735	11	11	0	0	1	Y
<u>Air Control I (0 ppm EO)</u>								
1	3656	2741	11	12	0**	0	0	Y
1	3648	2722, 2741	--	--	--	--	--	Y
1	3665	2768, 2799	--	--	--	--	--	H
1	3666*							
1	3681	2799, 2812	--	--	--	--	--	H
1	3689	2812, 2838	--	--	--	--	--	H
1	3704	4803, 2903	--	--	--	--	--	H
1	3707	4818, 4803	--	--	--	--	--	H
1	3719	4822, 4818	--	--	--	--	--	H

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
Air Control I (0 ppm EO - Continued)								
2	3598	2838	10	9	1	0	0	Y
2	3613	2903, 2838	--	--	--	--	--	N
2	3584	2812, 2799	9	9	0	2	0	Y
2	3552	2722, 4822	10	11	0**	2	0	N ¹
2	3635	4822	13	13	0	1	0	Y
2	3629	4818, 4803	9	12	0**	0	3	N ¹
2	3573	2768, 2741	12	12	0	0	0	Y
2	3553	2741, 2722	--	--	--	--	--	N
2	3582	2799, 2768	--	--	--	--	--	N
2	3614	4803, 2903	--	--	--	--	--	N
3	3476	2838	8	7	1	1	0	Y
3	3074	2741	11	10	1	1	0	Y
3	3131	2768, 2741	11	11	0	1	0	Y
3	3207	2799	12	10	2	0	0	Y
3	3524	4822	12	11	1	2	0	Y
3	3483	2903	12	11	1	1	0	Y
3	3462	4803, 2903	--	--	--	--	--	N
3	3059	2722	11	12	0**	1	0	Y
3	3210	2812	--	--	--	--	--	N
3	3466	4818, 4803	--	--	--	--	--	N

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>Air Control II (0 ppm EO)</u>								
1	3642	4838, 4788	8	3	5	0	0	Y
1	3722	4788, 2819	--	--	--	--	--	N
1	3697	2862	10	6	3	5	0	Y
1	3710	2883, 4775	11	11	0	0	1	Y
1	3721	4775, 2807	--	--	--	--	--	N
1	3664*							
1	3676	2807, 2711	--	--	--	--	--	N
1	3688	2819, 2829	--	--	--	--	--	N
1	3692	2829, 2830	--	--	--	--	--	N
1	3695	2830	--	--	--	--	--	Y
2	3580	2819	10	2	8	0	0	Y
2	3592	2830, 2829	9	7	2	1	0	Y
2	3588	2829	12	12	0	2	0	Y
2	3616	4788	13	9	4	0	0	Y
2	3606	4775	10	9	1	0	0	Y
2	3602	2862	12	11	1	0	0	Y
2	3603	2883, 2862	9	11	0**	0	1	Y
2	3574	2807	11	12	0**	0	0	Y
2	3622	4838	11	11	0	1	0	Y
2	3563	2711, 4838	--	--	--	--	--	N

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>Air Control II (0 ppm EO - Continued)</u>								
3	3470	4775	11	5	6	2	0	Y
3	3146	2830	nd	5	nd	4	0	Y
3	3157	2862, 2830	--	--	--	--	--	N
3	3527	4838	7	6	1	6	0	Y
3	3063	2711, 4838	10	11	0**	0	0	N
3	3488	4788	11	7	4	2	1	Y
3	3110	2819	10	9	1	1	0	Y
3	3140	2829, 2819	--	--	--	--	--	N
3	3105	2807, 2711	--	--	--	--	--	N
3	3206	2883, 2862	--	--	--	--	--	N
<u>Positive Control (TEM, 0.25 mg/kg)</u>								
1	3706	2857	9	2	7	2	0	Y
1	3682	2793, 2818	8	4	4	4	0	Y
1	3684	2818, 2848	9	8	1	8	0	Y
1	3700	2848, 2857	--	--	--	--	--	N
1	3675	2775	10	10	0	4	1	Y
1	3673	2745	12	11	1	5	1	Y
1	3674	2774, 2745	--	--	--	--	--	N
1	3680	2791, 2793	--	--	--	--	--	N
1	3703*							
1	3723	4844, 2775	--	--	--	--	--	N

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Table 1 (Continued)
Ethylene Oxide Dominant Lethal Mutagenicity Inhalation Test in Rats
Individual Animal Results of Dominant Lethal Data

Mating Week Number	Female An. No.	Mated With Male An. No. First, Second	Number Corpora Lutea	Number Implantation Sites	Number Preimplantation Losses	Number Early Resorption Sites	Number Late Resorption Sites	Vaginal Plug Detected
<u>Positive Control (TEM, 0.25 mg/kg - Continued)</u>								
2	3633	4844	10	2	8	0	0	Y
2	3549	2738, 4844	10	7	3	6	0	N ¹
2	3586	2774, 2745	10	4	6	3	1	Y
2	3564	2745	--	--	--	--	--	Y
2	3611	2857, 2848	8	6	2	0	0	Y
2	3590	2775	10	7	3	6	0	Y
2	3601	2818	10	9	1	4	2	Y
2	3610	2848, 2818	--	--	--	--	--	N
2	3594	2791	--	--	--	--	--	Y
2	3596	2793, 2791	--	--	--	--	--	N
3	3241	2791	10	1	9	1	0	Y
3	3250	2793, 2791	--	--	--	--	--	N
3	3471	4844, 2857	11	2	9	2	0	Y
3	3463	2857	6	5	1	5	0	Y
3	3460	2848	10	3	7	2	0	Y
3	3222	2775	16	5	11	2	2	Y
3	3482	2818	11	7	4	7	0	Y
3	3070	2738, 4844	--	--	--	--	--	N
3	3078	2745	--	--	--	--	--	Y
3	3114	2774	--	--	--	--	--	Y

* Female was incorrectly mated with two males, each from a different treatment group, consequently, results were not included.

** More implantation sites than corpora lutea is defined as no (0) preimplantation losses.

nd = not determined; Y = yes; N = no

¹ - The number of early and late resorption sites may be incorrect since no vaginal plug was detected, and

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Table 2

Ethylene Oxide Dominant Lethal Mutogenicity Inhalation Test in Rats
Individual Fertility Results for Males

Male An. No.	Sucessfully Mated One or Two Females during Mating Weeks		
	1	2	3
<u>100 ppm Ethylene Oxide</u>			
4847	Y	Y	Y
4774	-	Y	-
4786	Y	Y	Y
2710	-	-	?
2734	-	-	-
2771	-	?*	-
2842	-	?	-
2854	-	Y	Y
2898	-	Y	Y
2899	-	-	-
<u>33 ppm Ethylene Oxide</u>			
4797	?	Y	Y
2715	-	Y	Y
2779	-	?	-
2826	-	-	-
2841	-	-	-
2864	-	-	-
2865	-	Y	Y
2726	-	Y	Y
2747	-	?	-
4811	?	-	Y

Table 2 (Continued)

Ethylene Oxide Dominant Lethal Mutogenicity Inhalation Test in Rats
Individual Fertility Results for Males

Male An. No.	Sucessfully Mated One or Two Females during Mating Weeks		
	1	2	3
<u>10 ppm Ethylene Oxide</u>			
2863	-	Y	Y
2795	Y	Y	?
2731	-	-	Y
2735	Y	Y	Y
4810	Y	?	Y
4842	-	Y	Y
4795	Y	-	Y
2746	Y	-	Y
2740	-	Y	Y
2794	-	Y	Y
<u>CI ppm</u>			
2741	Y	Y	Y
2722	-	?	Y
2768	-	-	-
2799	-	Y	Y
2812	-	-	-
4803	-	?	-
4818	-	?	-
4822	-	Y	Y
2903	-	-	Y
2838	-	Y	Y

Table 2 (Continued)
Ethylene Oxide Dominant Lethal Mutogenicity Inhalation Test in Rats
Individual Fertility Results for Males

Male An. No.	Sucessfully Mated One or Two Females during Mating Weeks		
	1	2	3
	<u>CII ppm</u>		
4838	-	Y	Y
4788	Y	Y	Y
2862	Y	Y	
2883	-	-	
4775	Y	Y	Y
2807	-	Y	
2819	-	Y	Y
2829	-	Y	
2830	-	-	Y
2711	-	-	?

Positive Control (TEM, 0.25 mg/kg)

2857	Y	-	Y
2793	-	-	-
2818	Y	Y	Y
2848	Y	Y	Y
2775	Y	Y	Y
2745	Y	Y	-
2774	-	-	-
2791	-	-	Y
4844	-	Y	-
2738	-	?	-

*Question mark indicates that male was mated with a female that also was mated with a different male. Since the female did not throw a vaginal plug (but was pregnant), it is not known which male impregnated the female.