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ABSTRACT

Maternal toxicity, reproductive performance, and developmental toxicology were evaluated in rats and rabbits following 7 hr/day inhalation exposures to 150 ppm ethylene oxide, 500 ppm propylene oxide or 1500 ppm n-butyl acetate. Rabbits were artificially inseminated and placed on one of the following exposure regimen for each chemical: 1) filtered air (control), 2) chemical exposure from 7 through 19 days of gestation (dg), and 3) chemical exposure from 1 through 19 dg. Rat-exposure regimens for each chemical were: 1) filtered air (control), 2) chemical exposure from 7 through 16 dg, 3) chemical exposure from 1 through 16 dg, and 4) chemical exposure for 5 days/wk for 3 wk prior to mating and daily from 1 through 16 dg. Unexposed males were used in mating and artificial insemination procedures.

Necropsies were performed on rats at 21 dg and on rabbits at 30 dg. Pregnant animals were examined for toxic changes, including altered food consumption, body weight, tissue weights and histopathology. Reproductive measures included the determination of numbers of corpora lutea, implantation sites, resorptions, dead fetuses and live fetuses. Live fetuses were weighed, measured, and subjected to external, visceral and skeletal examinations to detect morphologic anomalies.

No evidence of maternal toxicity, embryotoxicity, or teratogenicity was detected in rabbits exposed to 150 ppm of ethylene oxide.

Decreased food consumption and body weight and changes in relative tissue weights were observed in adult rats exposed to 150 ppm of ethylene oxide. The incidence of resorptions was increased in litters from rats exposed before breeding. Fetal size was reduced in litters from all ethylene-oxide-exposed groups of rats. Fetal morphologic changes included reduced ossification of the skull and sternebrae in all ethylene-oxide-exposed litters and an increased incidence of hydroureter in litters exposed from 7 through 16 dg. No overt teratogenicity was detected in litters of rats exposed to 150 ppm ethylene oxide.

Minimal effects on food consumption and body weight were observed in pregnant rabbits exposed to 500 ppm propylene oxide from 1 through 19 dg. The only evidence for embryotoxicity was the increased number of resorptions in litters that had resorptions. The incidence of fetal anomalies in rabbits was unaltered by propylene oxide exposure.

In all groups of rats exposed to 500 ppm propylene oxide, food consumption decreased, body weights were lower and changes in tissue weights were observed. The number of corpora lutea and implantation sites per dam and live fetuses per litter decreased in rats that received propylene oxide prior to mating. The percentage of resorbed implantation sites was highest in rats

exposed to propylene oxide from 7 through 16 dg. Fetal size was reduced, and the incidence of rib dysmorphology increased in all propylene-oxide-exposed litters. Signs of overt teratogenicity were absent.

Exposure of rabbits to 1500 ppm n-butyl acetate decreased food consumption, but no related changes in body weights were observed. Reproductive performance was unaltered by n-butyl acetate exposure. Fetal effects of n-butyl acetate exposure included increased incidences of retinal folds, misaligned sternebrae, and morphologic variations of the gallbladder in litters of rabbits exposed from 1 through 19 dg. No major malformations were observed.

Exposure of rats to 1500 ppm n-butyl acetate reduced food consumption, body weight and liver weight. Fetal size was reduced in all n-butyl-acetate-exposed litters. We observed increased incidences of fetal rib dysmorphology in rats exposed from 7 through 16 dg, and more numerous hydroureters in fetuses from rats exposed prior to mating and from 1 through 16 dg. There was no evidence of teratogenic effect following exposure of rats to 1500 ppm of n-butyl acetate.

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ACKNOWLEDGMENTS

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INTRODUCTION

Women of childbearing age may be exposed to potentially toxic or teratogenic compounds in their working environment. As a result, it is necessary to identify these compounds and to evaluate their potential maternal and fetal toxic effects. Ethylene oxide, propylene oxide, and n-butyl acetate are frequently used in industry, in hospitals, and laboratories; evaluation of their toxicity and teratogenicity in the developing animal fetus is needed to provide bases for the assessment of human exposure criteria and establishment of Federal standards.

ETHYLENE OXIDE

Ethylene oxide (CAS #75-21-8), C_2H_4O, is a flammable gas with a boiling point of 10°C, a vapor density of 1.5, and a flash point of -20°C (Patty, 1963). It is used in the production of chemicals such as ethylene glycol and its derivatives, and surfactants. Ethylene oxide is also commonly employed as a fumigant and as a cold (dry) sterilant for medical supplies. Chemically, it reacts vigorously with materials having a labile hydrogen atom. The threshold limit value (TLV) for ethylene oxide is 10 ppm, with a notice of intended change to 5 ppm; the OSHA permissible exposure limit (PEL) is 50 ppm (Lewis and Tatken, 1979).

Toxic effects of ethylene oxide include skin, eye and respiratory tract irritation, and depression of the central nervous system (Patty, 1963; Jacobson et al., 1956). Hollingsworth et al. (1956) performed repeated ethylene oxide vapor inhalation studies with rats, guinea pigs, rabbits, mice and monkeys. They found that irritation of the respiratory passages and injury to the lungs occurred following eight daily exposures, during a period of 10 days, to 204, 357, and 841 ppm of ethylene oxide. Although 122 to 157 exposures (7-hr/day) to 204 ppm of ethylene oxide over 176 to 226 days killed an appreciable number of rats and caused significant growth depression, the exposure of rabbits to similar conditions had no adverse effect on growth or viability. Data from inhalation studies with ^{14}C-ethylene oxide in mice have shown that the chemical, or a derivative, accumulates in the mucosal membranes (Appelgren et al., 1976). A significant fraction of the labeled compound was present in the liver, intestinal mucosa, epididymis, testis, cerebellum, bronchi and bone marrow.

The mutagenic potential of ethylene oxide was demonstrated by means of a dominant lethal assay in rats (Embree et al., 1977) and in mice (Generoso et al., 1981). Abrahams (1980) reported that decreased sperm counts and chromosomal aberrations were observed in hospital employees using ethylene oxide gas for sterilization of surgical instruments and dialysis equipment. Leong et al. (1974) had also observed testicular atrophy in rats following exposure to 0.5 and 1.0 ppm ethylene oxide cyclic tetramer. Thiess et al. (1981) also re-

ported that workers with industrial exposures of over 20 years to the alkylene oxides showed significantly increased rates of chromosomal aberration.

Reproduction studies were performed following inhalation exposure of male and female rats to 10, 30 or 100 ppm of ethylene oxide (Bayes, 1979). The experimental regimen included ethylene oxide exposure for 12 wk prior to cohabitation and for 2 wk during cohabitation. Females were subsequently exposed from 0 through 19 days of gestation (dg) and from 5 through 21 days postpartum. Treatment-related effects were found only in females that inhaled 100 ppm of ethylene oxide. In these females, the number of implantation sites was reduced, embryolethality increased and, at birth, there were fewer pups per litter. No adverse effects on neonatal survival or growth were observed.

LaBorde and Kimmel (1980) studied the teratogenicity of ethylene oxide in CD-1 mice following intravenous injections on 3 consecutive days at four periods during gestation. Dose levels of 75 or 150 mg/kg at gestation days 4-6 and 10-12 produced a significant increase in resorptions. An increased incidence of malformations, mostly skeletal, was observed at a dose level of 150 mg/kg/day during gestation days 6-8 and 8-10.

PROPYLENE OXIDE

Propylene oxide (CAS #75-56-9), C_3H_6O, is a highly flammable liquid with a boiling point of 34°C, a vapor density of 2.0, and a flash point of -30°C. The TLV for propylene oxide is 20 ppm (ACGIH, 1981); the PEL is 100 ppm (Lewis and Tatken, 1979). Propylene oxide is used extensively in the chemical and food manufacturing industries for the production of propylene glycol, hydroxy propyl celluloses and sugars, and surfactants. It is highly reactive chemically, but less so than ethylene oxide. Exposure to propylene oxide also causes irritation of the skin, eyes, and respiratory tract; effects on the and central nervous system are characterized by ataxia, incoordination and depression (Patty, 1963).

Repeated inhalation exposure studies with propylene oxide vapors (Rowe et al., 1956) demonstrated that rabbits and monkeys tolerated 7-hr exposures of 457 ppm for 5 days/wk over 6 to 7 mo. Under similar conditions, increased mortality was observed in rats, and weight loss and lung changes were observed in guinea pigs. Mortality data from studies with rats, mice and dogs suggest that propylene oxide vapor is one-half to one-third as toxic as ethylene oxide (Jacobson et al., 1956).

Chromosomal damage in cultured rat liver cells was observed following treatment with propylene oxide (Dean and Hodson-Walker, 1979). Mutagenicity was not observed in the in vivo studies of Bootman et al. (1979), who found that exposure of mice to two oral doses (as high as 500 mg/kg) of propylene oxide did not produce an increase in bone-marrow cell micronuclei, or evidence of mutagenicity in dominant lethal tests. Hardin et al. (1982) used three in vivo assays (rat dominant lethal, mouse sperm head morphology and Drosophila melanogaster sex-linked recessive lethal) to evaluate the mutagenic activity of propylene oxide. Results from assays in rats and mice exposed to 300 ppm of propylene oxide vapor for 7 hr/day for 5 days showed that effects were not treatment related; a significant increase in sex-linked recessive lethal mu-

tations was observed in both pre- and postmeiotic sperm cells of D. melanogaster exposed to 645 ppm of propylene oxide for 24 hr. Other recent results (Thiess et al., 1981) have demonstrated an increased chromosomal aberration rate in workers exposed to propylene oxide over a 20-yr period. No reports of assays for the teratogenicity of propylene oxide were found in the literature.

n-BUTYL ACETATE

n-Butyl acetate (CAS #123-86-4), $C_6H_{12}O_2$, is a colorless liquid with a boiling point of 126°C, a vapor density of 4.0, and a flash point of 20°C (Patty, 1963). The current TLV and PEL for n-butyl acetate are both 150 ppm (ACGIH, 1981; Lewis and Tatken, 1979).

A variety of neurophysiological measures have indicated that nerve conduction velocities are somewhat lower following long-term occupational exposure to solvent mixtures containing n-butyl acetate (Seppäläinen et al., 1978). The simple aliphatic esters such as n-butyl acetate, widely used as lacquer solvents, produce eye and respiratory tract irritation, and induce anesthesia when exposure is prolonged. These esters are relatively soluble in plasma, readily pass through the alveoli, and are believed to be rapidly hydrolyzed by liver or plasma esterases. Their rapid absorption from the airways to the blood should make these esters readily available for transport to the embryo or fetoplacental unit. No information on the developmental toxicology and teratogenicity of n-butyl acetate was found in the literature.

RATIONALE

Studies were initiated to determine the teratogenic potential of three chemicals--ethylene oxide, propylene oxide, and n-butyl acetate--using inhalation as the route of exposure to simulate exposures that may be encountered in the workplace. Inhalation exposures of rats and rabbits, representative rodent and nonrodent species, were utilized to determine the effect of these chemicals on reproductive capacity and prenatal development following exposures of the female prior to conception and/or during gestation. Of the two alkylene oxides (ethylene and propylene) under consideration, only ethylene oxide (administered intravenously in high doses to mice) has been demonstrated to be teratogenic. An increased incidence of chromosomal aberrations has been observed following long-term industrial exposures of workers to both ethylene and propylene oxide. No reports concerning the mutagenicity or teratogenicity of n-butyl acetate were found in the literature.

Since the exposure interval and the period of gestation during which the dam is exposed to the test chemicals may be significant in determining effects on reproduction and fetal development, these experiments were designed to provide exposures during critical periods of gestation and/or prior to breeding and implantation. The experimental design for rabbit exposures during gestation is shown in Table 1; that for rat exposures, which also encompass a 3-wk pregestational exposure, is shown in Table 2.

All facets of this study were conducted in compliance with the FDA's Good Laboratory Practice Regulations (FDA, 1978). See Appendix C.

Table 1. Exposure schedule for rabbit studies.

Group Designation	Days of Gestation			
	1 to 6	7 to 19	20 to 29	30
1	Filtered air	Filtered air	No exposure	Sacrifice
2	Filtered air	Test chemical	No exposure	Sacrifice
3	Test chemical	Test chemical	No exposure	Sacrifice

Table 2. Exposure schedule for rat studies.

Group Designation	3 Weeks Pregestation	Days of Gestation			
		1 to 6	7 to 16	17 to 20	21
1	Filtered air	Filtered air	Filtered air	No exposure	Sacrifice
2	Filtered air	Filtered air	Test chemical	No exposure	Sacrifice
3	Filtered air	Test chemical	Test chemical	No exposure	Sacrifice
4	Test chemical	Test chemical	Test chemical	No exposure	Sacrifice

MATERIALS AND METHODS

CHEMICALS

Ethylene Oxide

High-purity ethylene oxide (listed minimum purity, 99.7%) was purchased from Linde Division of Union Carbide (Linde Lot #01901). This material was analyzed in our laboratory before animal exposures began. The infrared (IR) spectrum of this lot of ethylene oxide (4000-600 cm^{-1}, 10-cm gas cell with NaCl windows) confirmed its identity and did not detect major impurities. Gas chromatographic (GC) analysis (2.35 m Porapak QS 80/100, 100°C isothermal, flame ionization detector [FID], 0.2 ml neat gas injected) indicated a purity of 99.83% calculated as percent of the total area of all peaks in the chromatogram. The IR and GC analyses were repeated upon completion of the exposure and showed no significant change in the purity of the ethylene oxide. The final GC analysis indicated a purity of 100.0%.

Propylene Oxide

High-purity propylene oxide (listed minimum purity, 99%) was purchased from Aldrich Chemical Company (4 x 5-gal metal pails, Lot #1230 T E). The propylene oxide was analyzed in our laboratory before animal exposures began. The IR spectrum of this material (4000-600 cm^{-1}, 0.1-mm pathlength, NaCl windows) confirmed its identity as propylene oxide and detected no major impurities. The GC analysis (2.35 m Porapak QS 80/100, 125°C isothermal, FID, 0.1 µl neat liquid injected) indicated a purity of 100.0%; no extraneous peaks were detected. The GC analysis, performed on samples taken from each of the four pails, detected no differences among samples from different containers. The IR and GC analyses were repeated after completion of the exposures and showed no change in the propylene oxide. The GC analysis again indicated a purity of 100%.

n-Butyl Acetate

n-Butyl acetate was purchased from McKesson Chemical Company (1 x 55-gal metal drum, no lot number). McKesson Chemical Company listed a minimum purity of 99% for this material but did not provide a certificate of analysis. The n-butyl acetate was analyzed in our laboratories before animal exposures began. The IR spectrum (4000-600 cm^{-1}, 0.015-mm pathlength, NaCl windows) confirmed the identity of the chemical as n-butyl acetate and did not detect major impurities. The GC analysis (1 m glass column, 20% SP2100 + 0.1% Carbowax 1500 on 100/120 Supelcoport®, 100°C isothermal) indicated a purity 99.1%. Seven impurities were detected; principally, two compounds eluting ahead of n-butyl acetate and one after it, representing 0.48%, 0.16% and 0.11% of the sample, respectively. The remaining four impurities all amounted to less than

0.07% of the sample. The IR and GC analyses were repeated after completion of the exposures and showed no significant change in purity or in the number and relative quantities of the impurities.

6-Aminonicotinamide

The 6-aminonicotinamide (6-AN), for use as a positive control teratogen, was obtained from Sigma Chemical Company (Sigma Lot #38C-0271). For comparison, this lot of 6-AN and a lot used in our previous studies (Sigma Lot #29C-0077) were analyzed together. The IR spectra (4000-600 cm^{-1}, KBR pellets) of the two lots were identical and confirmed their identities as 6-AN. No bands due to impurities were observed. The ultraviolet spectra ($\sim 4 \times 10^{-5}$ m in water) of both lots had a maximum at 266 nm with molar absorptivities of 1.45×10^4 (Lot #29C-0077) and 1.47×10^4 (Lot #38C-0271). No impurities were detected in either lot by thin layer chromatography (TLC; 0.1-mm cellulose MN 300 plates, with fluorescent indicator, developed in n-butanol:acetic acid:water, 40:10:50, and visualized under a short-wave ultraviolet lamp) or by GC examination for volatile substances (1 m 3% OV-17 on Chromosorb WHP programmed from 150-250°C). The 6-AN itself does not elute under the conditions used for this GC analysis. The results of these analyses indicated that the two lots of 6-AN were qualitatively and quantitatively similar, and both were substantially free of impurities.

EXPOSURE SYSTEM

Rats and rabbits were exposed to the test vapors within stainless steel chambers (Figure 1) designed at Battelle (U.S. patent #4,216,741; Moss, 1980; Brown and Moss, 1981; Moss et al., 1981) and fabricated by Hazelton Systems, Inc., Aberdeen, MD. Total chamber volume was 2.3 m^3 (2350 L), of which 1.7 m^3 was available for animals and caging. At the maximum capacity of 30 rabbits or 144 rats, the animals occupied less than 5% of the total chamber volume. Cages were positioned on three levels, with each level divided into two tiers which were offset from each other and from the chamber walls (Figure 1A). Drawer-like stainless steel cage units, composed of individual animal cages, were suspended in the space above each tier. Solid catch pans for the collection of animal wastes were suspended below each of the six cage units. Catch pans remained in position during each exposure period.

The chamber is designed to maintain uniform concentrations with the catch pans in place. Incoming air containing a uniform mixture of test material is diverted to flow as a sheet along and down the inner chamber surfaces. As the test-material-laden air flows past the edge of the catch pan, waves are created at each tier (Figure 1B). Stagnant zones which might otherwise exist at the middle of each layer are cleared by flow through the space between the tiers. Exposure air reaching the lowest level is deflected across the bottom tiers by metal strips placed between the catch pans and the chamber walls.

Tests performed at our laboratory have shown that concentrations can be maintained that vary by only 3 to 8% throughout the chamber, provided that the test material and exposure air are uniformly mixed before entering the exposure chamber (Moss, 1980; Moss et al., 1981). Independent testing at the Inhalation Toxicology Research Institute of the Lovelace Foundation, Albuquerque, NM has corroborated these findings (Griffis et al., 1981).

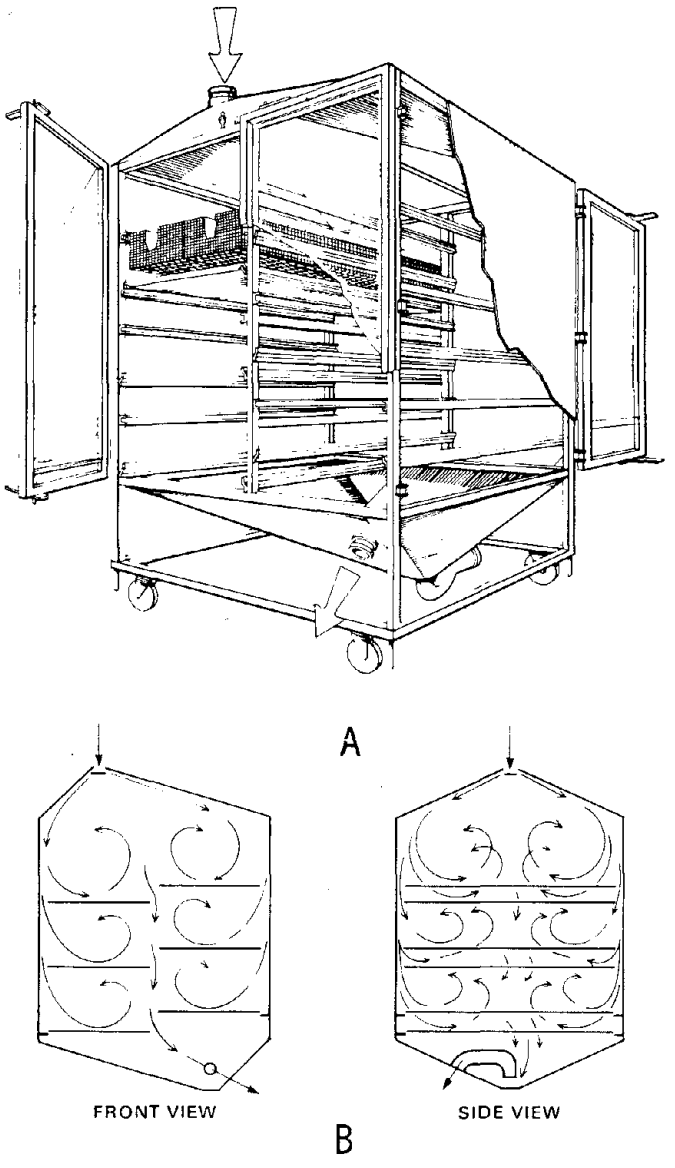

Figure 1. The inhalation exposure chamber (A. Cutaway view of chamber; B. Airflow patterns).

To achieve these conditions, HEPA-filtered air was continually supplied to the exposure chambers, and the vapors under study were introduced into the filtered air streams. Chamber air flows were maintained at 280 L/min for rats and up to 560 L/min for rabbits. Exhaust was pumped from the chamber through a flow monitor and into the building exhaust system, using an impulse-principle air pump (Vortec Corp., Model 912 transvector airflow amplifier®).

At least three chambers were available for exposure of each animal species. Rats and rabbits were exposed in individual stainless steel cages (24 rat cages or five rabbit cages/tier, six tiers/chamber). Cage floor area was 270 cm^2 for rats and 1300 cm^2 for rabbits. Rats were exposed and housed within the chamber. Rabbits were removed from the chamber and housed in other caging between exposure periods.

The environment of each chamber was monitored with a dial hygrometer to determine relative humidity (RH), and with a dial thermometer to determine temperature. Hygrometers were calibrated by wet bulb/dry bulb psychrometry; thermometers were calibrated at room temperature against a certified, traceable mercury thermometer. Both chamber RH and temperature were recorded 2-3 times daily. Chamber temperature and RH were controlled primarily by controlling room conditions. Target levels for environmental parameters during the exposure periods were:

Pressure:	-0.3 to -2.0 cm H_2O (relative to room)
Air Flow:	280 or 560 L/min (rats and rabbits, respectively)
Temperature:	23-27°C
RH:	40-60%

Chamber air flow was monitored by measuring the pressure drop of the airstream across a flow orifice incorporated in the chamber exhaust line. Chamber vacuum was monitored relative to room pressure by a vacuum gauge. Both flow and vacuum monitors had high- and low-limit alarm set points.

VAPOR GENERATION

Ethylene Oxide

Ethylene oxide vapor was supplied in the appropriate amount to each chamber by means of the generation/distribution system shown in Figure 2. Liquid ethylene oxide was forced to a boiler through an eductor tube from the ethylene oxide bottle by pressurized nitrogen (regulated at 20 psi) supplied to the bottle. Ethylene oxide was vaporized in a coiled stainless steel tube boiler maintained at about 55°C by a surrounding water bath, the temperature of which was controlled by a rod heater. Ethylene oxide vapor was conducted through a manifold to four "dual" gas-metering valves which accurately controlled the vapor flow to each chamber. Vapor from the metering valves was then routed through a three-way "purge/expose" valve and into a pipe at the chamber inlet, where the vapor was mixed with particulate- and charcoal-filtered air at a dilution of 280 L/min and 560 L/min (for rat and rabbit chambers, respectively). The purge/expose valves allowed the vapor generation and distribution system to be purged with nitrogen following an exposure period. Appropriate purge valves, the water-bath assembly, and gas-metering

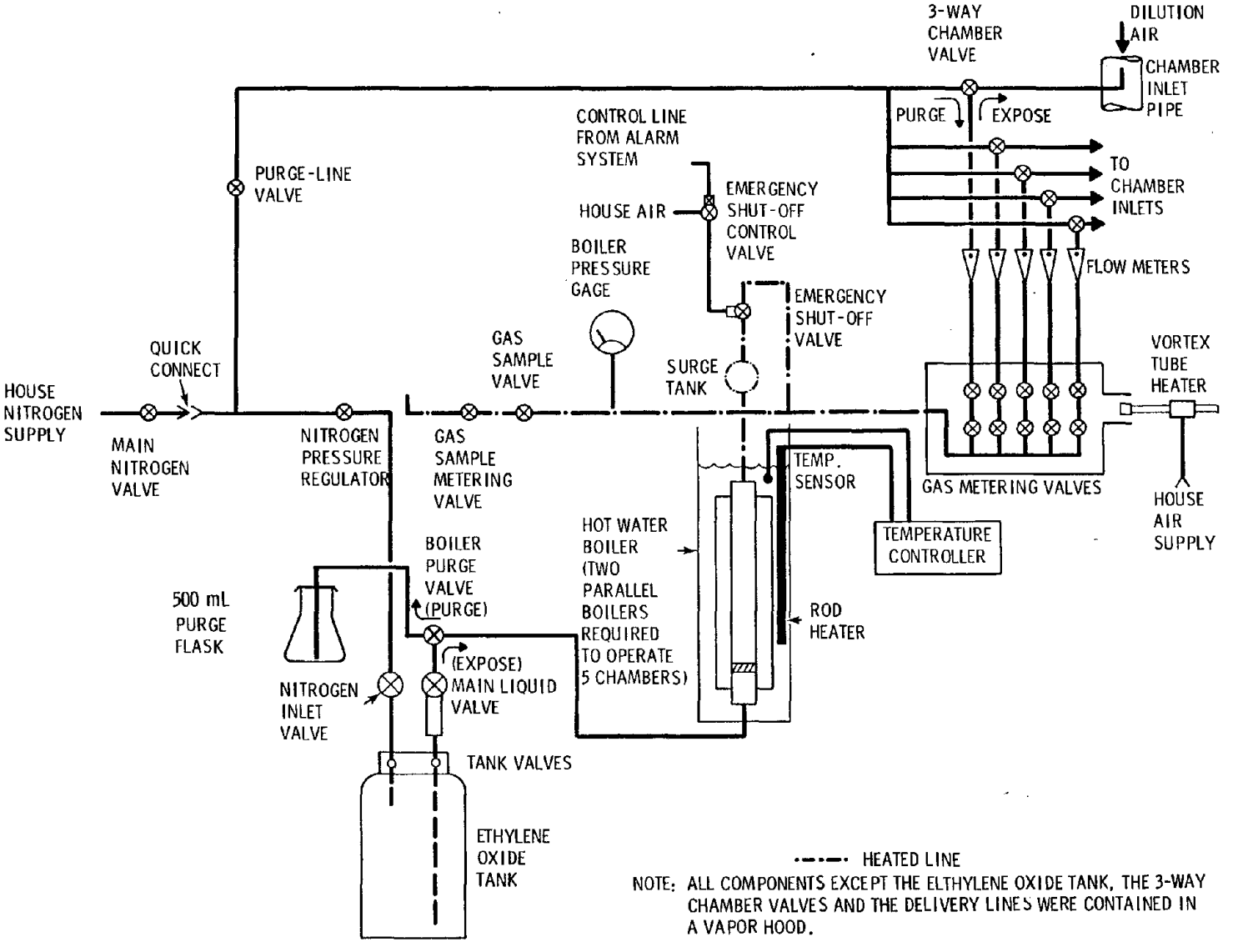

Figure 2. Ethylene oxide exposure system.

valves were all confined to a hood (vented to the building exhaust system) to minimize the hazard to animals and personnel in the event of a leak in one of these components.

An emergency shut-off valve (explosion-proof) was included in the vapor delivery line, following the water bath, to automatically shut off vapor flow in the event of an alarm condition. The gas was piped to each exposure chamber from the double-pattern metering valves. A shut-off valve located at the entrance to each chamber permitted rapid closure of gas lines. All materials in the gas distribution system were brass or stainless steel and all seals were compatible with the ethylene oxide.

Vapor concentration in the exposure chambers was monitored by GC. The detector monitored all four exposure chambers, the two control chambers, and two sites in the exposure rooms through an automatically multiplexed eight-port sample valve. The sample time per port was 5 min; thus, the concentration in each chamber was sampled once every 40 min.

Since ethylene oxide is both highly explosive and a putative carcinogen, care was taken to monitor those systems whose failure could lead to explosive gas concentrations or escape of gas into the exposure room. Each of the monitoring systems was connected in series to a general alarm that was activated if any of the monitors detected an alarm situation. Activation of the general alarm would cause both a visual and audible alarm and would close the emergency shut-off solenoid-controlled valve, thus discontinuing gas delivery to all chambers.

The monitoring system incorporated flow and vacuum monitors for each chamber, a multiplexed chemical concentration detector, a room exhaust flow monitor and the general alarm circuit. The chamber flow monitor had a low-flow alarm contact which would open if chamber flow decreased by 15%, thus preventing an increase in concentration beyond 15%. The vacuum monitor, low-vacuum alarm was designed to trip if there were a breach in the integrity of the chamber (e.g., a sample-port rubber stopper ajar) which might cause chamber vacuum to decrease below -0.3 cm H_2O relative to the room.

The multiplexed chemical concentration monitor was designed so that if any chamber were found to be 15% over the ideal concentration, or if the concentration were higher than 50 ppm in either the control chambers or the room, the detector alarm would shut off the gas flow. In order to re-establish flow, the system had to be reset manually.

The final component of the monitoring and alarm system was the room exhaust flow monitor. The air flow through the chamber was controlled by the chamber flow pump, which was independent of room exhaust. Thus, a failure of room exhaust would result in the chamber air being pumped directly into the exposure room. To minimize the escape of gas into the room, the room exhaust monitor was designed to shut off gas delivery to the chambers if room exhaust flow dropped below 50% of nominal.

All of the monitoring systems were designed to be "fail-safe" under the most common failure modes (electronic or power failure). In nearly all cases, the

failure of any part of the exposure system would be detected and/or protected by at least two remaining components of the systems in order to prevent the possibility of explosion.

Propylene Oxide and n-Butyl Acetate

The vapor generation systems for both propylene oxide and n-butyl acetate were identical except where noted below.

The detailed schematic diagram of the vapor generation system is shown in Figure 3. Propylene oxide and n-butyl acetate were contained in 1.6- and 7-L stainless steel reservoirs, respectively, which were housed in a vapor hood within the exposure room. Liquid propylene oxide was pumped from the reservoir through a manifold liquid-distribution system (¼-in. stainless steel line), by a stable micrometering pump (Fluid Metering, Inc., Model RH1CKC), at a drift-free pump rate of 10 ml/min. This pump was required to prevent bubble formation (caused by the high vapor pressure of propylene oxide at room temperature) in delivery lines between the liquid reservoir and individual metering pumps. Since the vapor pressure of n-butyl acetate is lower than that of propylene oxide, the pump was omitted from the n-butyl acetate fluid-delivery system. From this liquid distribution system, four individual pump/vaporizer systems were fed through 1/8-in. stainless steel lines. Three-way valves and return lines from the vaporizers to the manifold distribution system facilitated filling each delivery system with liquid. This arrangement allowed direct measurement of the fluid flow. Pumping rates were 0.4 and 0.8 ml/min for propylene oxide and 2.3 and 4.6 ml/min for n-butyl acetate (rat chambers and rabbit chambers, respectively). Nitrogen was used to replace the depleted liquid in the reservoir, to prevent formation of an explosive mixture.

The vaporizer (Figure 4) consisted of a stainless steel cylinder covered with a glass-fiber wick from which the liquid was vaporized. When residue accumulated, these wicks were replaced. An 80-watt heater and a temperature-sensing element were incorporated within the cylinder and connected to a remote temperature controller. Vaporizer surface temperatures were set at ~80°C. Each cylindrical vaporizer was positioned in the fresh-air duct leading directly into the exposure chamber to minimize material loss due to condensation on duct walls.

The vapor generation system was capable of vaporizing up to 7 ml/min of liquid into 280 L/min fresh air to produce vapor concentrations as high as 9000 ppm. Typically, the system maintained the required chamber concentrations of 500 ppm of propylene oxide and 1500 ppm of n-butyl acetate within ±3% of target. Concentration variations with standard deviations of less than ±3% (as determined by GC monitoring of the chamber atmosphere) were achievable for exposure periods exceeding 6 hr.

Clear Teflon® tubes of measured volume, preceded by a three-way valve, were attached just upstream from each pump to allow measurements of liquid flow rate to each vapor generator. Measurements were accomplished by momentarily switching the three-way valve from the "run" to the "test" and back to "run" position. Flow rates were determined by timing the progress of the resulting

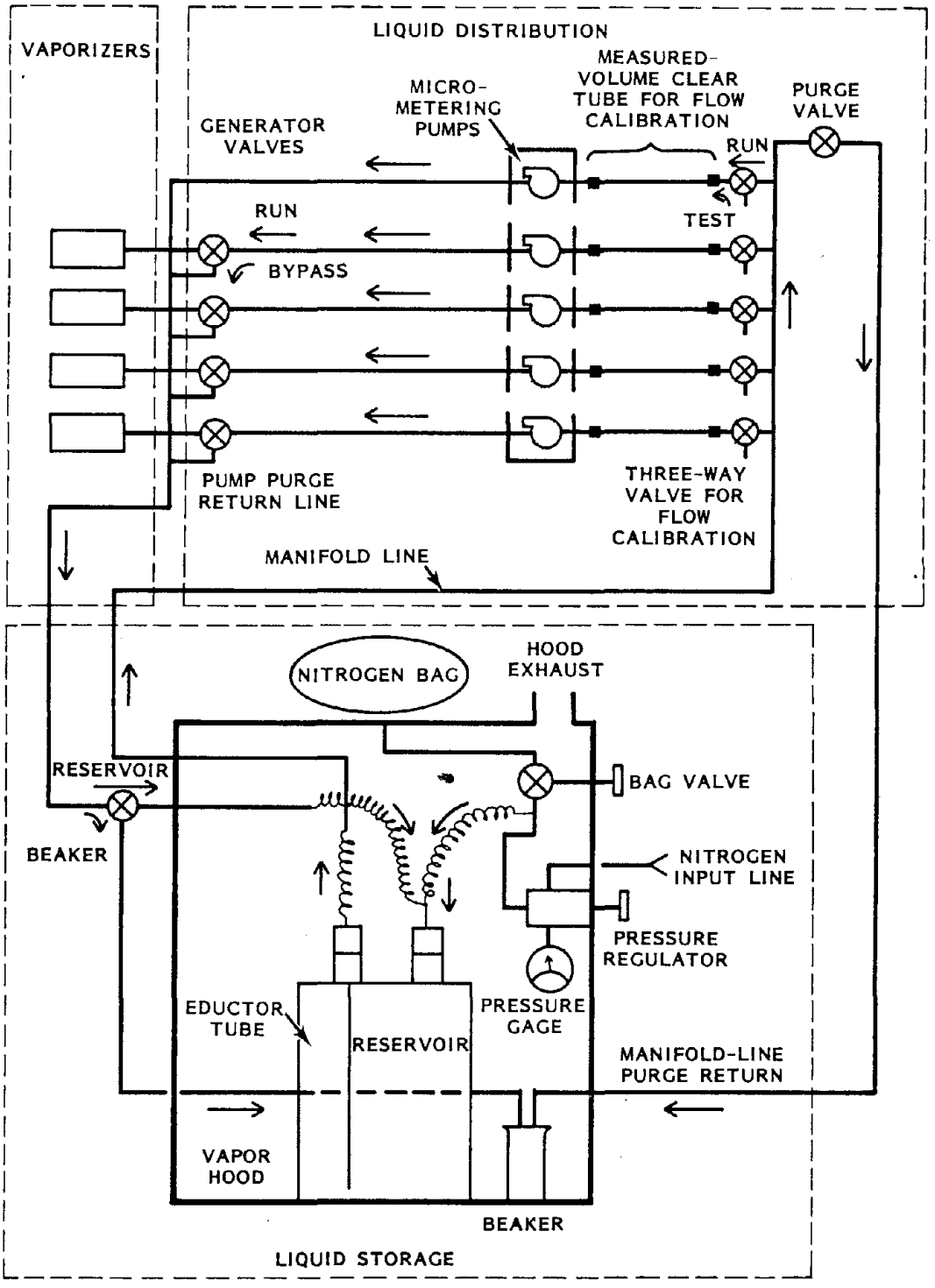

Figure 3. Schematic diagram of liquid delivery system for propylene oxide and n-butyl acetate.

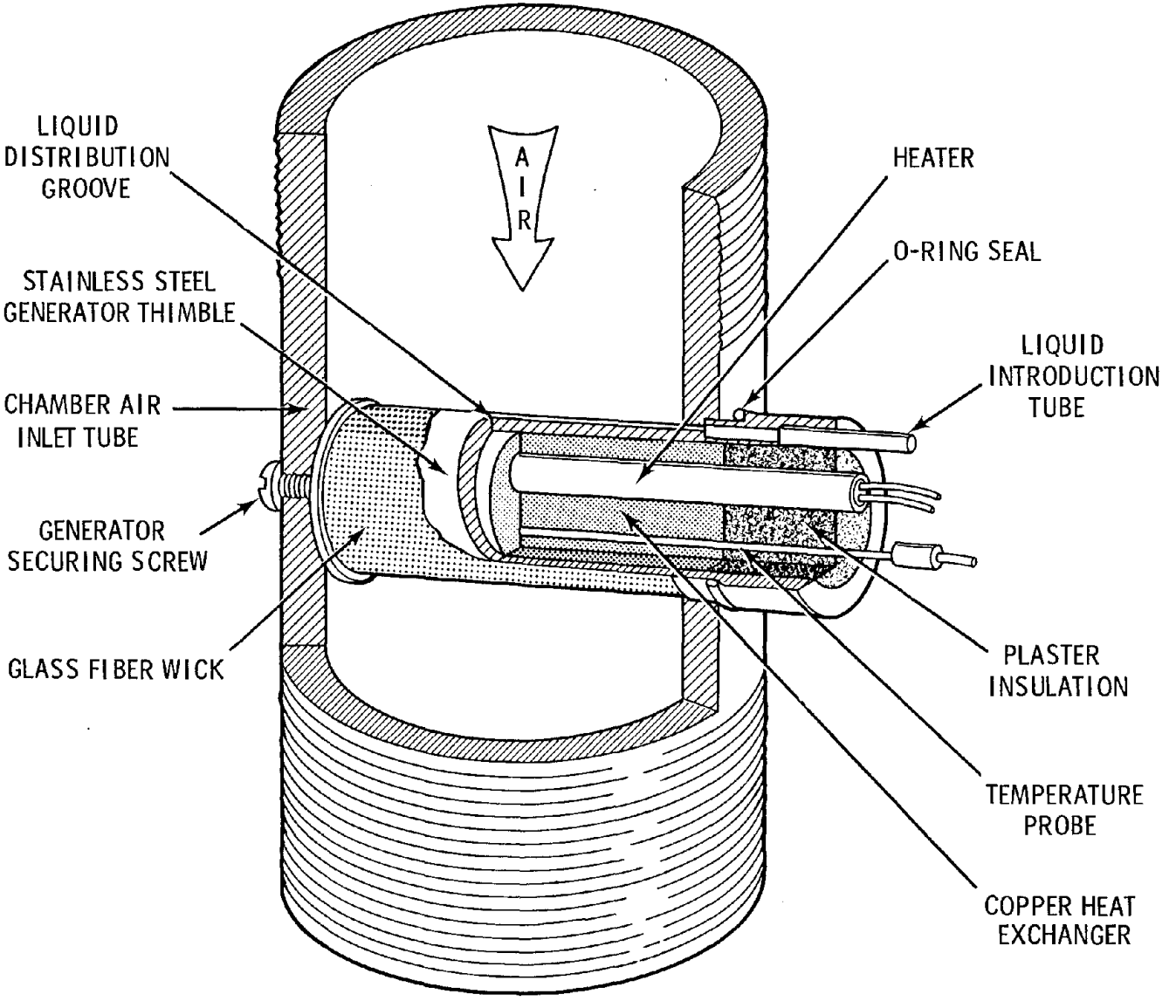

Figure 4. Vapor generation assembly for propylene oxide and n-butyl acetate.

air bubble through a clear tube of known volume. The volume of the tubes was chosen so that the error due to start- and stop-time ambiguity was less than 5%.

Exhaust and Waste Treatment

Exhaust was pulled from the exposure chamber by an impulse-principle pump (Transvector®, Vortec) which has no moving parts, thereby reducing the chance of failure. Very stable chamber air flows, ranging from 0 to 560 L/min, could be maintained by controlling the pressure of the compressed air which operated the exhaust pump. The stability of air flow through the chamber was important in maintaining the proper chemical concentration in the chamber.

The exhaust from the pump was loosely coupled to the room exhaust line to allow room air to be drawn into the exhaust around the coupling. This minimized the effect of changes in airflow to the room exhaust line on the flow of air through the chamber. The exhaust from exposure rooms was diluted with the building exhaust (75,000 cfm) prior to release from the building stack to produce environmentally acceptable stack concentrations.

CHAMBER CONCENTRATION MONITORING

Ethylene Oxide

The chamber monitoring system is shown schematically in Figure 5. The eight-port stream selection valve was mounted in close proximity to the manifold containing the sample-line flow-control valves and was connected to it using short lengths of 1/16-in. stainless steel tubing. Flows from the chambers to the manifold were maintained at 100 ml/min, thus assuring that fresh samples from the chamber were constantly available at the inlet to the eight-port valve. Sample lines to the chambers were 1/8-in. Teflon. The GC was a Hewlett-Packard 5840A, equipped with an FID, a valve driver board to control the sample loop and stream selection valves, and an auxiliary temperature control board to control the temperature of the internal standard diffusion source. The GC column was a 20 x 1/8-in. stainless steel column packed with Porapak PS and operated isothermally at 140°C with 30 ml/min of nitrogen carrier gas. The retention time of ethylene oxide was approximately 0.33 min under these conditions.

An internal standard diffusion source was placed in the sample stream between the stream selection and sample loop valves, as shown in Figure 5. Details of the construction of the source are shown in Figure 6. The reservoir was filled with approximately 0.5 ml of high-purity n-octane (99.9+%) and maintained at 35°C. With the source at 35°C, the concentration of n-octane ranges from 500 to 300 ppm with carrier gas flow rates of 30 to 50 ml/min, respectively. The retention time of n-octane was approximately 2.17 min under the conditions of this analysis.

The GC was calibrated by analyzing standards of ethylene oxide in air prepared in Teflon gas-sampling bags. The standards were prepared by flushing and filling a glass gas bulb with ethylene oxide gas drawn from the exposure cylinder, then diluting the ethylene oxide (0.5 to 3000 ml) in a Teflon gas bag

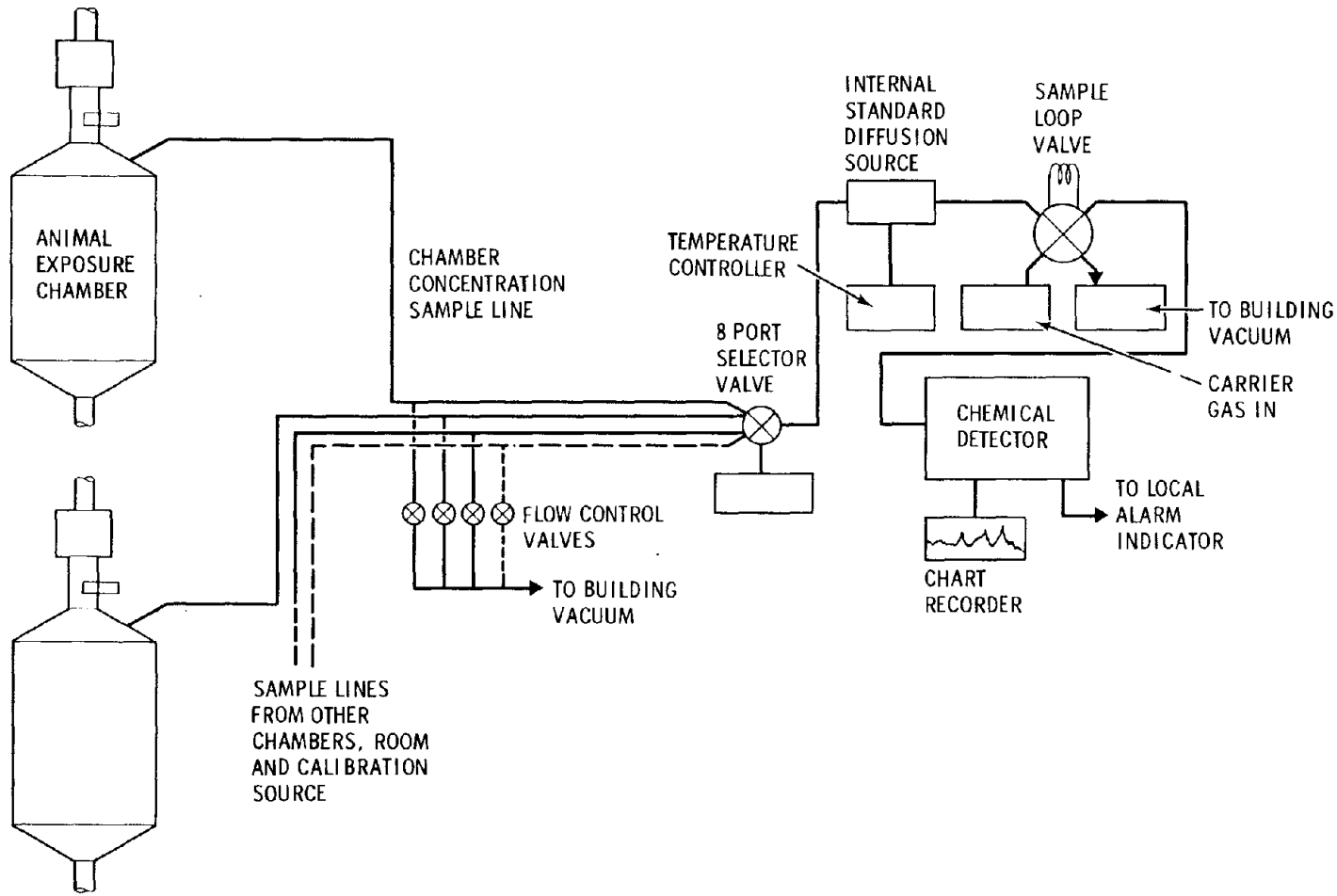

Figure 5. Test chemical concentration monitoring system.

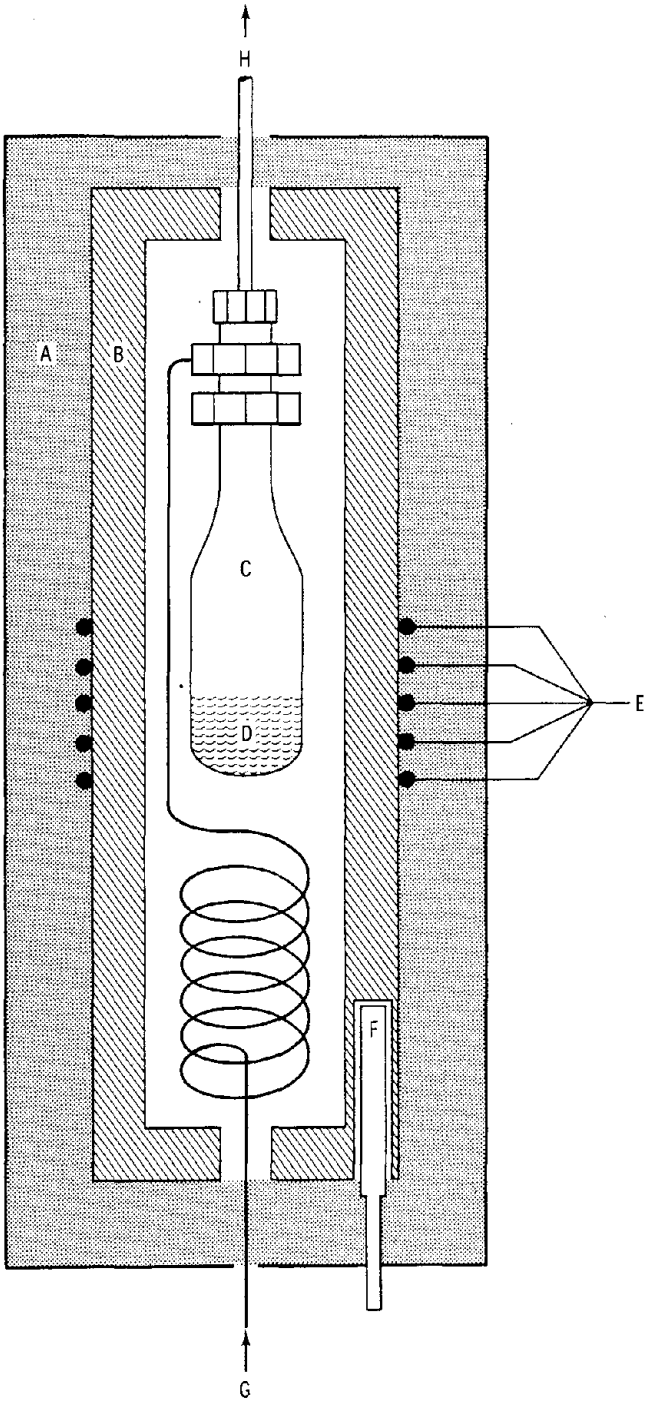

Figure 6. Construction of the internal standard diffusion source: A. thermal insulation; B. copper container; C. glass reservoir for internal standard; D. internal standard; E. heating coils; F. platinum resistance thermometer; G. sample stream inlet; H. sample stream outlet.

using gas-tight syringes (1.0 ml and 2.0 L) for the transfers. The gas bags, placed on the end of one of the room-air sampling lines, were sampled and analyzed automatically in the same manner as chamber samples. All calculations were based on peak areas, and chamber concentrations were calculated using the internal standard method. Ethylene oxide concentrations (in parts per million) were printed on the GC terminal in the exposure room as each analysis was completed.

A GC peak, subsequently identified as methane, was observed in chromatograms of both room air and chamber air during this study. Comparison of its peak height with that of ethylene oxide allowed its concentration to be estimated at 4 ppm in the rat chambers and 15 ppm in the rabbit chambers. Traces of methane have also been observed in animal rooms and chambers in other studies; the source of the methane appeared to be the waste lines.

Data for chamber concentrations obtained during animal exposures, with capacity chamber loads of rabbits and about one-half capacity loads of rats, were combined to estimate buildup and clearance times. Representative curves are shown in Appendix A, Figure A.1. Estimates of half-time for ethylene oxide buildup in both rabbit and rat chambers was 4.5 ± 0.7 min; clearance times were 3.7 ± 0.2 and 4.9 ± 0.7 min, respectively.

Mean daily concentrations for ethylene oxide in rabbit and rat exposure chambers were all within the range encompassed by ±10% of target concentration (Figure 7). Exposure group designations and mean daily concentrations for each group are listed in Appendix A (Table A.1 for rabbits and Table A.2 for rats). Days on which increased variances were detected were found to result from constricted liquid chemical delivery lines, incompatible O-rings in the micrometering valves, and GC analyzer shutdowns. Corrective measures to open constricted delivery lines included changing out the boiler, flushing all liquid delivery lines with solvent, and substituting a larger pore size filter (7 μm) for the inline filter (2 μm) used to trap polymeric solids. Since O-rings compatible with ethylene oxide were not readily available, the micrometering valves were changed and fitted with new O-rings as needed. The GC shutdowns were attributed to "noise" from the electric valving system used to switch among the various sampling locations. This problem was not resolved during the ethylene oxide exposure, but a noise-free, pneumatic system was used for subsequent exposures. When a new cylinder of ethylene oxide was introduced, most of the clogging problems diminished, suggesting that some polymer was present in the original cylinder.

Propylene Oxide

Propylene oxide concentrations in the exposure chambers were monitored using the same GC system as was described for ethylene oxide. The GC column and operating conditions were also similar, with the exception of a reduced air flow (15 ml/min) between the eight-port valve and the sample loop, utilized to increase the size of the n-octane peak relative to the propylene oxide peak. The retention times for propylene oxide and n-octane under these conditions were approximately 0.40 and 2.20 min, respectively.

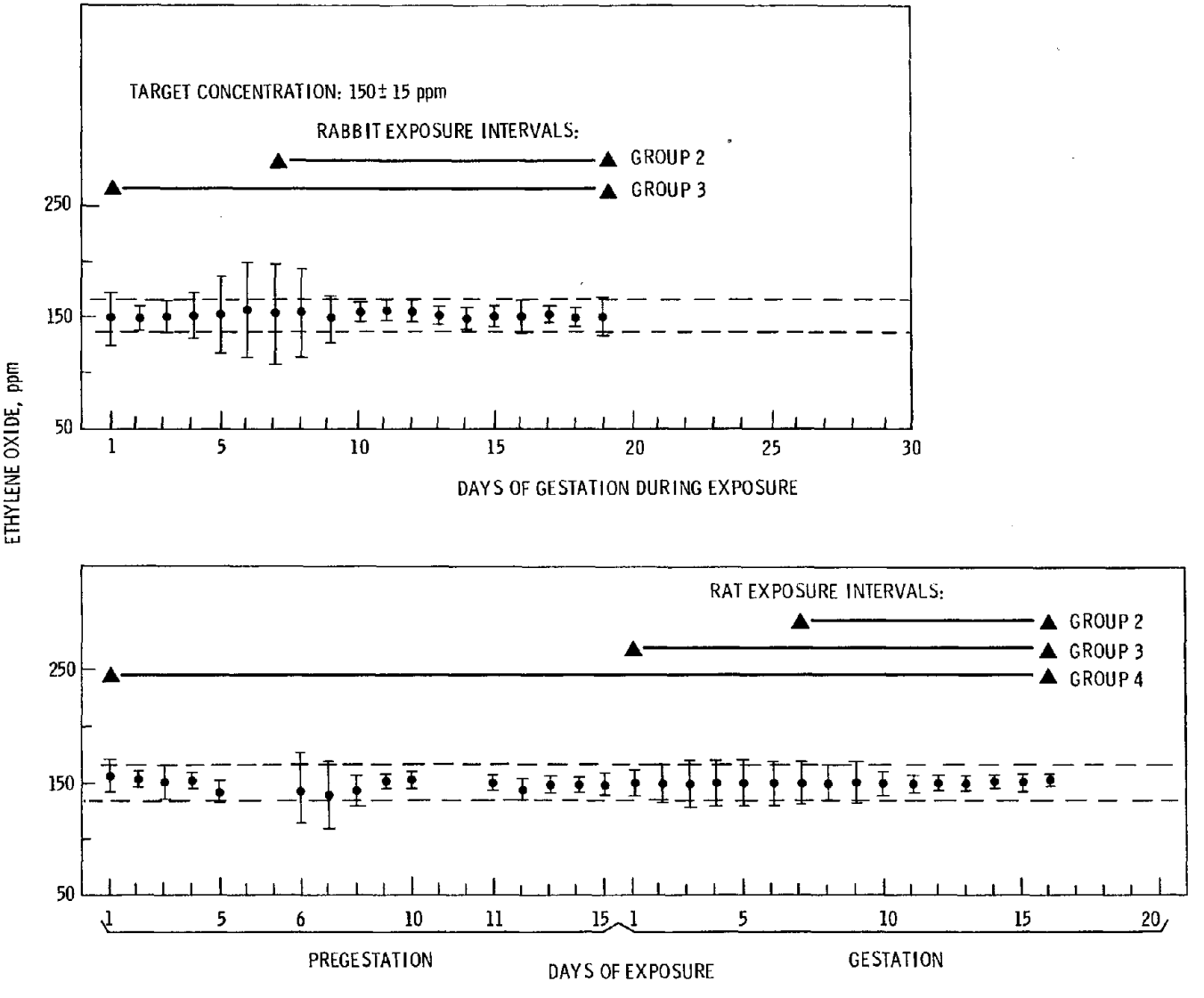

Figure 7. Mean daily concentrations for ethylene oxide in rat and rabbit exposure chambers.

The GC calibration was accomplished by analyzing standards of propylene oxide in air prepared in Teflon gas-sampling bags. The standards were prepared by injecting propylene oxide from a microsyringe into a glass tube attached to the bag filler neck. The bag was then filled through the same glass tube with a known volume of air delivered from a 1.5-L gas syringe. The quantity of propylene oxide delivered was determined by weighing the microsyringe before and after injection. The gas bags, placed on the end of one of the room-air sampling lines, were sampled and analyzed in the same manner as chamber samples. All calculations were based on peak areas, and chamber concentrations were calculated using the internal standard method.

Since the rats and rabbits were exposed serially to propylene oxide, only two exposure chambers were monitored. Therefore, two GC sampling lines were placed in each chamber, resulting in twice as many measurements per day for each chamber as were made in the ethylene oxide and n-butyl acetate exposures. Half-times for atmospheric buildup and clearance of propylene oxide in the exposure chambers were estimated using concentration data from rabbit chambers filled to capacity and rat chambers filled to one-half capacity. Representative buildup and clearance times are shown in Appendix A, Figure A.2. Half-time estimates for rabbit and rat chambers, respectively, were: for buildup, 3.6 ± 0.6 min and 6.5 ± 0.3 min; for clearance, 2.5 ± 0.1 min and 4.1 ± 0.1 min.

Mean daily concentrations of propylene oxide in rat and rabbit exposure chambers are shown in Figure 8. Mean chamber values for each exposure group are summarized in Appendix A, Table A.3 (rabbits) and Table A.4 (rats). All mean (± SD) daily concentrations fall within the range encompassed by the target concentration ±10%.

n-Butyl Acetate

The n-butyl acetate concentrations in the exposure chambers were monitored using the GC system described for ethylene oxide, except that electrically actuated (Carle) valves were replaced by pneumatically actuated (Valco) valves. The Valco valves eliminated power-line noise produced when the Carle valves were actuated, and also required less maintenance. The GC column and operating conditions for n-butyl acetate were also the same as described for ethylene oxide, except that the internal standard was changed from n-octane to n-nonane to obtain adequate separation of sample and internal standard peaks. The temperature of the diffusion source was raised from 35 to 45°C to achieve an acceptable concentration of n-nonane in the sample stream. Under these conditions, n-butyl acetate and n-nonane eluted at approximately 2.30 and 4.25 min, respectively.

The GC was calibrated using samples collected from an exposure chamber in a glass bubbler containing dimethyl formamide. The bubblers were transported to the Analytical Laboratory where an internal standard (n-dodecane) was added. The resulting solution was analyzed for n-butyl acetate on a laboratory GC calibrated against gravimetrically prepared n-butyl acetate standards in dimethyl formamide.

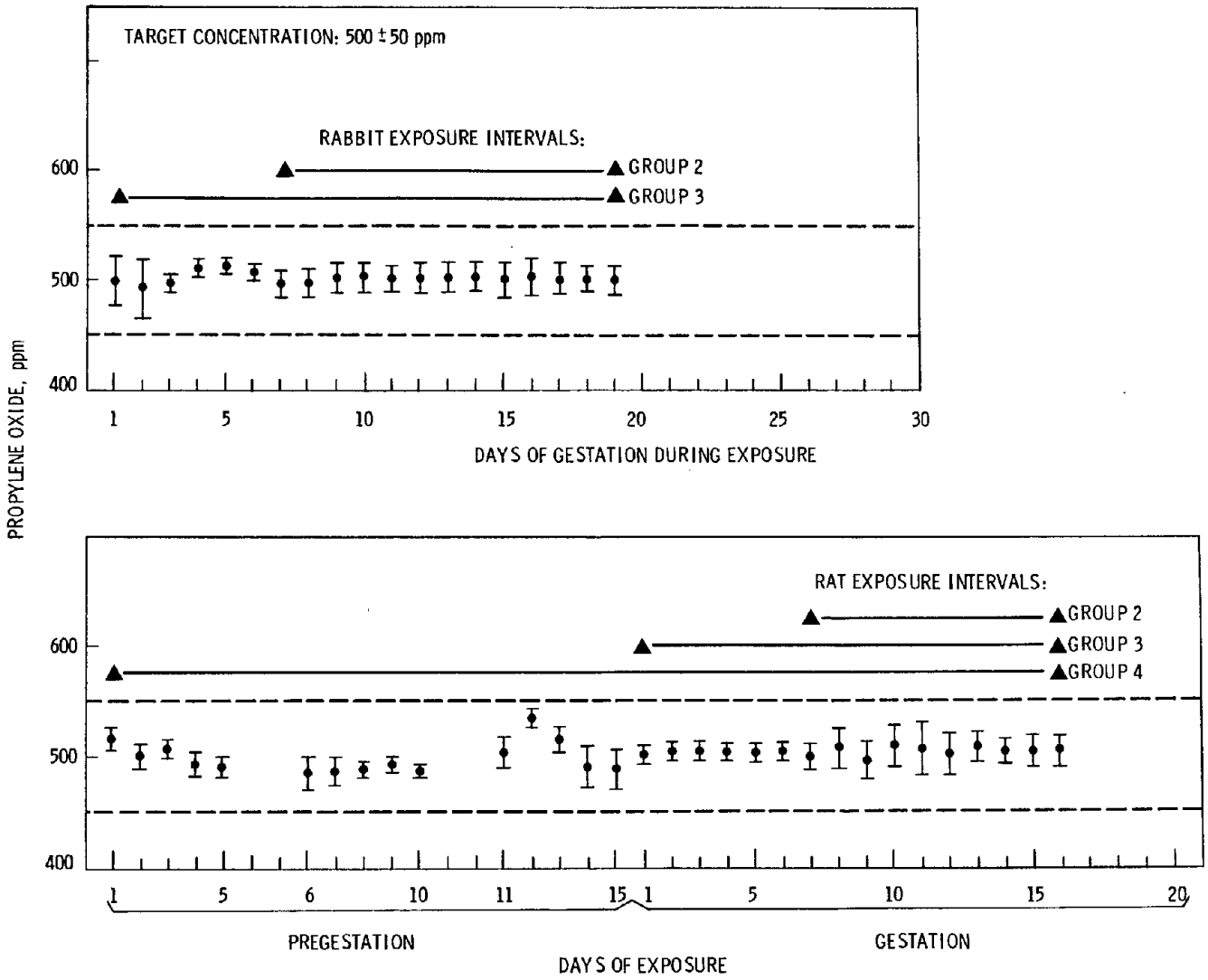

Figure 8. Mean daily concentrations for propylene oxide in rat and rabbit exposure chambers.

Knowledge of the amount of n-butyl acetate in the bubbler and of the volume of chamber air drawn through the bubbler allowed the actual concentration in the chamber to be calculated. The calculated concentration was then compared with the concentration reported by the on-line GC monitor at the time the bubbler sample was taken. If the two concentrations differed by more than 2%, a new calibration factor was entered into the monitor to make its reported values consistent with the values obtained for the bubbler samples.

Estimates of exposure chamber concentration buildup and clearance times were estimated from exposure data (Appendix A, Figure A.3). Buildup half-times for both rabbit and rat chambers were 4.7 ± 0.6 min. Clearance of n-butyl acetate differed from that of ethylene and propylene oxides in that rabbit chamber clearance times were slower than that of the rat chambers. Clearance was also affected by retention of n-butyl acetate on the animals and on chamber surfaces. Clearance to 35% of target concentration occurred by 5 and 8 min in rat and rabbit chambers, respectively; and to 10% of target concentration by 18 and 32 min.

The target mean daily concentration of n-butyl acetate in rat and rabbit exposure chambers was 1500 ± 150 ppm. Daily values within these limits were readily attained, as shown in Figure 9 and in Appendix A, Tables A.5 and A.6.

ANIMAL HUSBANDRY AND EXPOSURE PROCEDURES

Rabbits

Sexually mature, New Zealand White rabbit does (5 to 6 mo of age; body weight, about 3 kg) were obtained from R & R Rabbitry (Stanwood, WA) for experiments with the three chemicals. In addition to the 90 does required for the three experimental groups for each chemical (30 does/group), five does for replacements and two does for training bucks to the artificial vagina (AV) were obtained for each study. Eleven additional does were used as positive-control animals for the ethylene oxide study, and six does each were used as positive controls for propylene oxide and n-butyl acetate studies. Ten mature, naive bucks (6 to 7 mo old; body weight, 3 to 4 kg) of the same stock were purchased for breeding. Five of the original bucks were retained, and five were replaced for the second and for the third studies, so that a total of 10 bucks were available as semen donors for each artificial insemination period. All rabbits were identified by the supplier with a uniquely numbered, stainless steel ear tag.

To obviate the possibility of pseudopregnancy (Gibson, Staples, and Newberne, 1966), the does were isolated before the initiation of exposure (20, 18, and 24 days, respectively, for the ethylene oxide, propylene oxide, and n-butyl acetate studies). For the first 10 days of this isolation period, tetracycline (oxytetracycline, HCl, 0.63 mg/ ml) was added to the drinking water for prophylaxis. Does that displayed symptoms of upper respiratory infections during isolation received injections of 300,000 units of procaine penicillin-G daily. All medications were discontinued at least 4 days before insemination.

All rabbits were housed individually in stainless steel wire cages and provided with Wayne Rabbit Diet and water ad libitum, except during exposure.

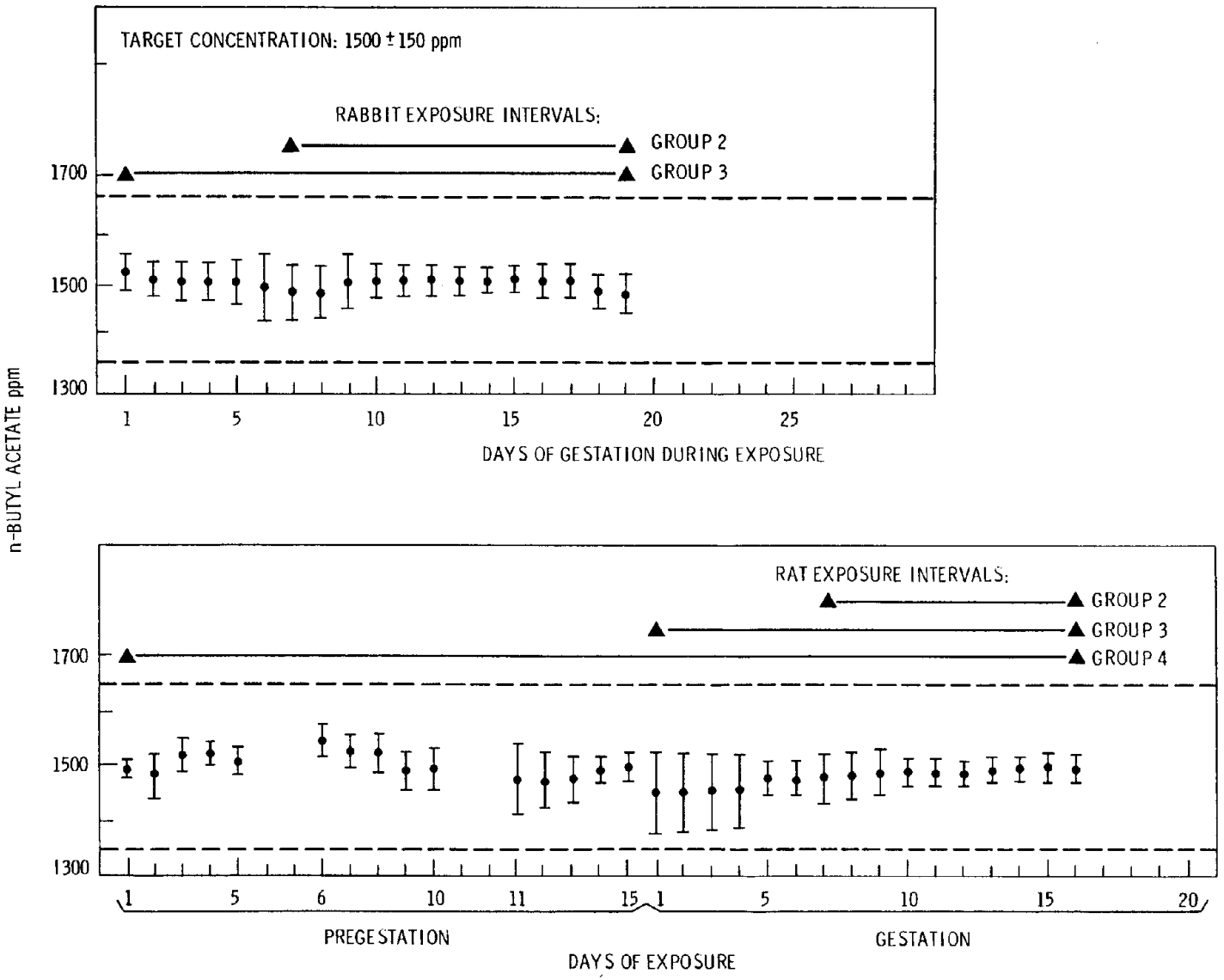

Figure 9. Mean daily concentrations for n-butyl acetate in rat and rabbit exposure chambers.

Following acclimation and quarantine, the does were divided into three exposure groups and one positive-control group by means of formal randomization (based on body weight) conducted by means of a computer program. One-third of each group (also randomly selected) was artificially inseminated in the afternoon of each day during a 3-day period.

Bucks to be used as semen donors were trained to service an AV during a period of 2 to 3 wk (Gregoire et al., 1958). On the day of artificial insemination, semen samples were collected, from at least three bucks, into an AV equipped with a reservoir warmed to about 45°C just prior to use (Adams, 1961; Hafez, 1970; Tesh and Tesh, 1971; Hagen, 1974). The samples were evaluated for motility, sperm concentration and the presence of urine, bacteria, erythrocytes and leukocytes. If the quality of the sample was satisfactory, semen samples were pooled and diluted with buffered-citrate/egg-yolk extender to a concentration of 21 to 41 million sperm/ml (Table 3). The does were inseminated with approximately 0.5 ml of the extended semen within 2 hr of semen collection. To induce ovulation, 2.5 mg of pituitary luteinizing hormone (PLH; Burns Biotec, 5 mg/ml in saline) was administered to each doe by intravenous injection immediately after insemination.

Table 3. Characteristics of extended rabbit seminal fluids used for artificial insemination.[a]

Test Chemical	Day of Insemination	No. Does Inseminated	No. Donor Bucks	Sperm (10^6/ml)	Motility[b]
Ethylene oxide	1	35	3	26	3
	2	32	3	38	4
	3	34	3	41	4
Propylene oxide	1	32	3	30	3
	2	32	5	30	3
	3	32	5	30	3
n-Butyl acetate	1	32	3	30	3
	2	32	3	40	4
	3	32	4	28	4

[a] Pooled semen samples diluted with buffered citrate/egg yolk extender
[b] Graded from 0 (no motility) to 4 (excellent motility)

The PLH, which was used for ovulation induction in ethylene and propylene oxide studies, was not commercially available for the n-butyl acetate studies.

Therefore, chorionic gonadotropin (APL; Ayerst, 500 USP units/ml in saline) was administered at a dose level of 100 USP units/doe. The morning following insemination was designated as day 1 of gestation (dg).

Exposure to the test chemical or filtered air was initiated on the morning following artificial insemination (1 dg). Rabbits were placed in individual cages within the appropriate exposure chamber for the 7-hr daily exposure period. (Table 1 shows the designated exposure regimen for each treatment group.)

Following exposure, test-chemical exposure chambers were flushed with filtered air until the chemical concentrations were at an acceptable limit for opening the chamber (5 ppm for ethylene oxide and 10 ppm for propylene oxide). Chamber concentrations of n-butyl acetate below 15 ppm (10% of the OSHA PEL of 150 ppm) were not achieved after 2 hr of filtered air flushing; therefore, fresh-air masks and protective clothing were required for technicians who transferred the rabbits to their individual home cages. The rabbits were supplied with fresh food and water at this time. Upon termination of exposures (after 19 dg), the rabbits were housed in their individual home cages until they were killed (30 dg).

Food consumption was measured for 2 wk prior to the initiation of exposure and at 5-day intervals during gestation. Body weights were measured in the morning (before exposure on 1-19 dg) on 1, 5, 10, 15, 20, 25, and 30 dg.

Rabbits assigned to the positive-control group received a single dose of 6-AN, a known teratogen (Schardein et al., 1967) at 9 dg. Freshly prepared solutions of 6-AN (10 mg/ml in an aqueous solution of 2% carboxymethyl cellulose) were administered intraperitoneally at a dose level of 3 mg/kg in all three studies, except that one-half of the rabbits in the ethylene oxide positive-control group received 2.5 mg/kg. At sacrifice, the identity of the positive-control animals was not known by those evaluating reproductive status and fetal measures. Morphologic findings in fetuses from these animals are summarized in Appendix A.

Rats

Young adult Sprague-Dawley CD female (7 to 8 wk old, 170 to 175 g) and male (7 to 8 wk old, 200 to 225 g) rats were obtained from the Charles River, Portage Facility. Groups of 235 females and 120 males purchased for the study of each material provided for 220 experimental females and 110 breeder males, plus additional animals for evaluation of health status and to compensate for any losses in shipment. Purchase requisitions specified that the rats were to be free of Mycoplasma, Sendai virus, and Corynebacterium. Upon receipt, at least five female and five male rats in each shipment were screened for these pathogens. Additional assays included culture of cecal contents for enteric pathogens, examination of animals for internal and external parasites, and histopathological examination of the lung, liver, ileum, colon, and kidney to detect diseases which might otherwise go unnoticed. Serological evaluations (Microbiological Associates) were also made for pneumonia virus of mice (PVM), Kelham rat virus (KRV), rat corona virus/sialodacryoadenitis virus (SCV/SDA), and H-1 virus (H-1). Serologic tests of the rats revealed the presence of KRV, known to be enzootic in the CD colony of the

supplier, and a few equivocal results for Mycoplasma pulmonis. Since the presence of M. pulmonis could not be substantiated by additional tests or histopathologic findings, there was little evidence to suggest infection by a primary pathogen or to question the health of the animals. Results of these tests were provided to the NIOSH Project Officer, and animals remained in quarantine until receipt of approval from NIOSH to proceed with the study.

Following approval by the Project Officer and an isolation period of 3 to 4 wk, the rats were weighed and individually identified by means of a numbered ear tag. Using formal weight randomization by means of a statistical software package, they were then assigned to two pregestational exposure groups: 1) 170 rats to filtered air or 2) 50 rats to Group 4, test chemical. The rats were then housed in individual, stainless steel cages within the exposure chambers for acclimatization.

The pregestational exposures consisted of daily 7-hr inhalation exposures, 5 days/wk for 3 wk. At the completion of each daily exposure, the test chemical exposure chambers were flushed with filtered air until an acceptable (5 ppm for ethylene oxide and 10 ppm for propylene oxide) chamber concentration was attained. Air flushing of the n-butyl acetate chambers proceeded for at least 2 hr, when the chambers were opened so that fresh food and water could be given to the animals by technicians provided with fresh-air masks and protective clothing. The chambers were then closed and supplied with filtered air during the night.

At the end of the third week of pregestational exposure, the females were mated by caging two females with one male of the same stock in a breeding cage. Copulation was determined by the presence of sperm in the vagina as determined by microscopic examination of a slide prepared from a drop of normal saline delivered into, then recovered from, the vagina with a pipette. The morning of observation of sperm was designated as 1 dg. At that time, the females which mated the previous night were weighed and randomly assigned to their first gestational exposure regimen (Table 2). Sperm-positive rats that received pregestational test chemical exposure (Group 4) continued on this regimen until 16 dg. Mated females from the combined, filtered-air exposure groups were ranked by weight and reassigned (by formal randomization) to Group 1, to be exposed to filtered air; to Group 2, to be exposed to filtered air from 1 to 6 dg and test chemical from 7 to 16 dg; or to Group 3, to be exposed to the test chemical from 1 to 16 dg.

In addition to individual ear-tag numbers, exposure groups were identified by subcutaneous injection of India ink into the left forepaw (Group 2), right forepaw (Group 3), or right hindpaw (Group 4). Group 1 rats were not marked.

Each female was placed with a male nightly until she copulated, or for 8 consecutive nights. After 8 nights of cohabitation, the population of females in which sperm was not detected were held without further mating or exposure until pregnancy could be readily detected, at which time they were necropsied (9 to 10 days).

Water was supplied by an automatic watering system, which was disconnected and drained prior to the initiation of each exposure. Wayne Lab-Blox was pro-

vided ad libitum except during the daily exposure period when the food was removed. Food consumption was monitored on a weekly basis during the 3-wk pregestational exposure and over 5-day intervals during gestation. Body weights were measured in the morning, twice per week during pregestation and on 1, 6, 11, 16, and 21 dg. The survival, appearance, and behavior of the parental females during the experimental period were noted.

From each daily mating of the filtered-air group, one or two rats were randomly selected for assignment to the positive control group which, instead of further filtered-air exposure, were housed in a separate room and given a single intraperitoneal injection of 6-AN, a known teratogen (Chamberlain and Nelson, 1963) on 12 dg. A dose of 6.5 mg 6-AN/kg was delivered from a freshly prepared 2 mg/ml solution of 6-AN in aqueous 2% carboxymethyl cellulose. The identity of the positive-control animals was not known to the prosectors at the time of sacrifice and fetal evaluation. Results from morphologic examinations of the fetuses from these rats are listed in Appendix B.

TOXICOLOGIC AND DEVELOPMENTAL EVALUATIONS

All animals were sacrificed, in a randomly determined order, by introduction of carbon dioxide into a euthanasia chamber. The animals were identified only by their unique identification number to assure that the treatment group was unknown to the prosectors. Foot markings of the rats were observed only by the prosectors performing the maternal necropsy, so the exposure group was not known to those evaluating reproductive status and fetal measures.

Female rats that were not inseminated after 8 nights were necropsied 9 to 10 days after the last night of cohabitation. Body, lung, liver, spleen, kidney, ovary, and uterus were weighed and the weights were recorded. These organs, along with any grossly abnormal tissues, were preserved in 10% neutral buffered formalin (NBF). The reproductive status of each animal was determined and the total number of implantations recorded. Uteri of all apparently nonpregnant females were stained with ammonium sulfide and examined for implantation sites (Kopf et al., 1964). Histopathologic examinations were made of all tissues collected from all animals killed in the combined filtered-air group and the test chemical group up to a maximum of five per group, or from randomized sampling of five animals if more than five were killed. In addition, all grossly abnormal tissues were examined histopathologically. All remaining tissues are being retained for possible future examinations.

Inseminated rats held until scheduled necropsy at 21 dg were examined for evidence of infections, lesions, or unusual characteristics. At least five pregnant rats from Groups 1 and 4 were screened for Mycoplasma, Corynebacterium, Sendai virus, PVM, KRV, RCV/SDA, and H-1. Results from these studies indicated the presence of KRV (which was also observed in pre-experimental screening studies). Positive titers were obtained for PVM in rats from the propylene oxide study (source of the virus is not known), and Sendai virus in animals from the n-butyl acetate study. The Sendai virus infection apparently was a result of the introduction of Osborne-Mendel rats, which were found to be infected after receipt, into another area of our animal colony. Although precautions were implemented to prevent the spread of infection, the virus was detected at necropsy of the rats exposed to n-butyl acetate. Coid and

Wardman (1971, 1972) reported that deliberate infection of pregnant rats with massive doses of live Sendai virus induced resorptions and decreased body weights but did not increase the incidence of gross fetal abnormalities. A comparison of control fetal data from all three of our studies yielded no evidence of an effect of viral infection on the incidence of resorptions or on fetal body weight.

Rabbits were killed at 30 dg. Essentially the same necropsy procedures and developmental evaluations were utilized for both rabbits and rats. Liver, lungs, spleen, kidneys, ovaries and the gravid or nongravid uterus were weighed and the weights recorded. Uteri of all apparently nonpregnant females were stained and examined for implantation sites (Kopf et al., 1964). Observations of internal abnormalities of the pregnant and nonpregnant animals were recorded (e.g., adhesions, tumors, or evidence of infection). Samples (of appropriate size for proper fixation) were taken of ovaries, uterus, liver, lungs with trachea, spleen, and kidneys of each actual or potential parental female. Any abnormal tissues were preserved in 10% NBF. A randomized sampling of tissues from 25% of the females (a maximum of eight per group) and any grossly abnormal tissues were processed by routine techniques (paraffin embedding, hematoxylin and eosin staining) and subjected to histopathological examination. The residual tissues, and the tissues from the remaining 75% of the females, were preserved for possible future examination.

The uterus, with ovaries attached, was removed from each animal. The ovaries were excised, identified as to right and left, and the number of corpora lutea estimated by counting. The excised uterus was opened, the membranes and amniotic fluid were observed for abnormalities, and living and dead fetuses, and resorptions, were counted. Mortality in utero was classified and recorded as "early" (E, placenta and conceptus indistinguishable, or metrial gland), "mid" (M, placenta distinct, embryo partially to fully formed), and "late" (L, fully formed but not viable fetus). Beginning at the right ovary, numbers were assigned, in order, to each implantation site down the right horn to the cervix. Consecutive numbers for implantation sites in the left horn proceeded from ovary to cervix.

Live and recently dead fetuses were removed in serial order, blotted on a moist surface, freed of adherent material, and weighed. A fetus was designated as stunted when its size was below the normal range of variation of its littermates, as determined by a statistical test to reject extreme observations in one direction (McLaren and Michie, 1960). The crown-rump length of each fetus was measured and recorded. Concurrently, the placentas were removed, weighed and examined; abnormal placentas, if observed, were fixed for histological preparation and examination. Each fetus was examined for gross external abnormalities under an illuminated magnifier. The fetuses of both species were randomly divided into two equal groups for more detailed teratologic examination. In one group, the heads were removed and placed in Bouin's fixative for subsequent examination of serial razor-blade-cut sections by the methods of Wilson (1965) and van Julsingha and Bennett (1977) for rats and rabbits, respectively.

All fetuses were examined for internal abnormalities using Staples' (1974) technique, which is a modification of that of Barrow and Taylor (1969), and

is similar to that described by Stertz (1977). The sex of each fetus was determined by external genitalia and visceral examination of the gonads. All fetuses were eviscerated; rat fetuses were immediately fixed in alcohol, and rabbit fetuses were skinned and air-dried prior to fixation. Following staining with alizarin red S, maceration with KOH, and clearing in glycerol (Staples and Schnell, 1964; Dawson, 1926), each skeleton was examined for abnormalities in size, shape, relative position, and degree of ossification. Results from fetal morphologic examinations were grouped into three categories (major malformations, minor anomalies, or morphologic variations) according to degree of severity, locus of fetal structural change, and incidence of these changes (Palmer, 1968, 1969, 1972, 1974, 1977, 1978; Perraud, 1976).

Data from adult animals, such as food consumption, body weight, and organ weights, are from pregnant animals only. Although formal randomization of body weights was used to select animals for the experimental groups, removal of data from nonpregnant animals from the group means tends to produce apparent deviations in initial body-weight values for some groups of animals. Results from histopathology studies are from a random sample of tissues from all females sacrificed at the termination of each study.

STATISTICAL METHODS

Binary response variables were compared among groups by chi-square tests for independence (Siegel, 1956). Pairwise comparisons for significant findings used either a two-tailed chi-square test or a Fisher's Exact Test (Siegel, 1956).

Analysis of variance (ANOVA) method was used to analyze continuous variable data. Response proportions were analyzed by ANOVA with an arcsin transformation of the response proportion. Orthogonal a priori comparisons were made among treatment group means for rabbits and rats. (See Tables 1 and 2 for details.) The orthogonal set of comparisons for rabbits was: Contrast I - Group 1 (control) versus Groups 2 and 3 (exposed to chemicals); and Contrast II - Group 2 (chemical exposure from 7 through 19 dg) versus Group 3 (chemical exposure from 1 through 19 dg). The orthogonal set of comparisons for rats was: Group 1 (control) versus Groups 2, 3, and 4 (exposed to chemicals); Groups 2 and 3 (exposed during gestation) versus 4 (exposed prior to mating and during gestation); and Contrast III - Group 2 (exposed from 7 through 16 dg) versus Group 3 (exposed from 1 through 16 dg). All orthogonal comparisons were two-tailed tests.

Absolute maternal organ weights were analyzed by analysis of covariance using the terminal body weight minus the weight of the gravid uterus (extragestational body weight) as the covariate. Relative organ weights were also analyzed as a percentage of the extragestational body weight by analysis of variance.

Body weights and crown-rump lengths for live male and female fetuses were analyzed by nested analysis of variance. The analysis takes into account the effects of treatment, litter, and sex on the body weight and crown-rump length measurements.

Repeated-measures data, such as maternal body weight, were analyzed by a multivariate repeated-measures analysis. Orthogonal polynomials were fit for each animal for which there were complete data, and a multivariate analysis of variance was performed on the coefficients to identify differences in growth patterns among exposure groups (Bock, 1975).

RESULTS

ETHYLENE OXIDE-EXPOSED RABBITS

Food Consumption and Body Weights

Although food consumption appeared to increase in all groups prior to exposure, and in Groups 2 and 3 immediately following termination of exposure on 19 dg (Figure 10), there were no significant differences among treatment groups (Table 4). Food consumption was diminished in all exposure groups during the interval from 25 to 30 dg. Comparisons of body weight values revealed no significant effect of the ethylene oxide exposures (Table 5).

Organ Weights and Histopathology

Extragestational body weights, and weights of the pregnant uterus, liver, kidneys, spleen and ovaries, were similar for all treatment groups (Table 6). Although a comparison of values for Group 1 with those of Groups 2 and 3 (Contrast I) showed no significant differences for lung weights, both absolute and relative values for Group 3 were higher than those for Group 2 (Contrast II).

There were a variety of microscopic changes in the lungs, some of which could possibly be related to Pasteurella infections. Minimal suppurative bronchitis was observed in a Group 1 rabbit; in Group 3, one doe had minimal chronic suppurative pleuritis, one had moderate diffuse subacute pneumonitis, and one had severe suppurative bronchopneumonia (Table 7). The significantly higher lung weights in Group 3 does (Table 6) were associated with the histopathologic changes: doe 1893 (lung weight, 31.8 g) had severe chronic suppurative pleuritis; doe 2358 (lung weight, 25.2 g) had severe suppurative bronchopneumonia; doe 2952 (lung weight, 21.6 g) had diffuse moderate pneumonitis. No mortality was observed in Group 3 during gestation; the deaths of two animals in Group 1 and four in Group 2 were attributed to pneumonia (Table 8).

Regressing corpora lutea were observed at necropsy in all rabbits that were not pregnant, as well as in rabbits (one from each exposure group) where pregnancy was not grossly apparent and could only be detected by staining with ammonium sulfide (Kopf et al., 1964). Corresponding uterine sections examined microscopically did not show evidence of pregnancy. In rabbits determined to be pregnant by uterine staining procedures, embryonic death presumably occurred soon after implantation, thus the endometrial changes ordinarily associated with pregnancy were not observed. One Group 1 rabbit and one Group 3 rabbit had severe suppurative metritis.

Lesions in other tissues, tabulated in Table 7, were of minimal severity. None of the observed changes in any of the tissues examined appeared to be related to ethylene oxide exposure.

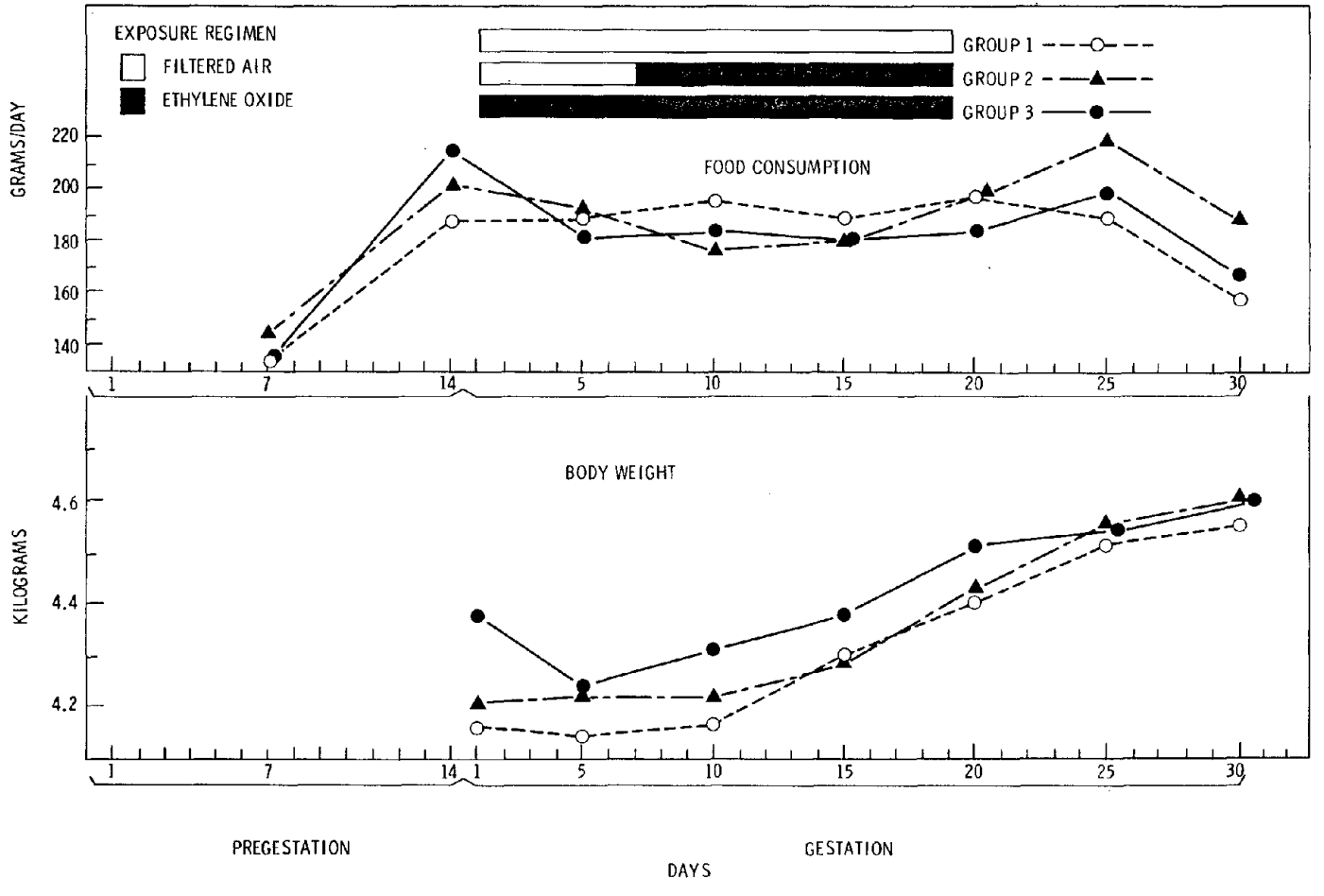

Figure 10. Food consumption and body weights of pregnant rabbits inhaling ethylene oxide or filtered air.

Table 4. Food consumption[a] of pregnant rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[b] I	Contrast[b] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	EO		
7 to 19 dg	FA	EO	EO		
NO. RABBITS:	21	20	23		
TIME OF MEASUREMENT					
Pregestation					
Week 1	133 ± 50	144 ± 44	135 ± 49	0.40	0.57
Week 2	187 ± 47	201 ± 47	214 ± 28	0.06	0.29
Gestation Days					
1 to 5	188 ± 43	190 ± 31	181 ± 24	0.80	0.41
6 to 10	195 ± 27	177 ± 37	183 ± 30	0.09	0.54
11 to 15	188 ± 22	179 ± 37	180 ± 34	0.34	0.90
16 to 20	196 ± 34	198 ± 39	183 ± 54	0.68	0.26
21 to 25	188 ± 52	219 ± 30	198 ± 46	0.09	0.12
26 to 30	157 ± 54	188 ± 39	166 ± 56	0.15	0.15

[a] Grams/rabbit/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3

Fertility and Reproductive Status

Exposure to ethylene oxide had no significant effect on the percentage of pregnant animals surviving to 30 dg. The percentage of survivors which were pregnant and the percentage calculated from data which include animals dying during exposure was not decreased by exposure to ethylene oxide (Table 8). Reproductive measures, including data for implantation sites, resorptions, and live and dead fetuses, were also unaffected by ethylene oxide exposure during pregnancy (Table 9).

Fetal Measures and Morphology

No significant effects among groups were observed for fetal body weight, crown-rump length, sex ratio, or for placenta weights (Table 10).

Table 5. Body weight (kg, mean ± SD) of pregnant rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	EO		
7 to 19 dg	FA	EO	EO		
NO. RABBITS:	21	20	23		
TIME OF MEASUREMENT					
Randomization	3.90 ± 0.35	3.99 ± 0.44	4.05 ± 0.38	0.26	0.62
Gestation day					
1	4.16 ± 0.42	4.21 ± 0.50	4.38 ± 0.38	0.24	0.20
5	4.14 ± 0.34	4.22 ± 0.40	4.24 ± 0.41	0.42	0.87
10	4.17 ± 0.30	4.22 ± 0.41	4.31 ± 0.37	0.33	0.44
15	4.30 ± 0.31	4.29 ± 0.44	4.38 ± 0.39	0.71	0.41
20	4.40 ± 0.35	4.43 ± 0.47	4.51 ± 0.43	0.52	0.54
25	4.51 ± 0.32	4.55 ± 0.47	4.54 ± 0.41	0.72	0.94
30	4.55 ± 0.33	4.60 ± 0.46	4.62 ± 0.48	0.58	0.88

[a] Probability for Contrast: I = Group 1 versus 2, 3; II = Group 2 versus 3

The incidence of morphologic alterations in the rabbit fetuses was unaffected by exposure to ethylene oxide (Table 11). One fetus in Group 3 had multiple major defects. Supernumerary ribs were present in most of the fetuses. In addition to rudimentary ribs at the lumbar I position, ossification sites were observed in some fetuses at this locus.

ETHYLENE-OXIDE-EXPOSED RATS

Food Consumption and Body Weight

During the first week of the pregestational exposure, the food intake for the ethylene-oxide-exposed rats (Group 4) was significantly higher than that of the filtered-air-exposed rats in Groups 2 and 3 (Figure 11 and Contrast II in Table 12). No other differences among groups were observed during the remaining 2 wk of the pregestational exposure. During 1 to 6 dg, food consumption of Group 1 rats (control) significantly exceeded that of all other groups

Table 6. Body and organ weights (mean ± SD) of pregnant rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	EO		
7 to 19 dg	FA	EO	EO		
NO. RABBITS:	21	20	23		
WEIGHT MEASUREMENT					
Body (g)	4548 ± 335	4603 ± 462	4622 ± 480	0.58	0.88
Pregnant uterus (g)	464 ± 233	457 ± 234	470 ± 183[c]	0.99	0.84
Extragestational (g)[b]	4083 ± 436	4146 ± 378	4151 ± 488	0.58	0.97
Lung					
Absolute[d]	14.5 ± 1.9	13.7 ± 3.8	16.0 ± 4.7	0.77	0.04*
Relative[e]	0.36 ± 0.05	0.33 ± 0.09	0.39 ± 0.13	0.95	0.05*
Liver					
Absolute	114 ± 24.3	124 ± 20.4	120 ± 24.8	0.18	0.61
Relative	2.77 ± 0.38	2.99 ± 0.48	2.89 ± 0.36	0.13	0.41
Kidneys					
Absolute	23.5 ± 3.6	24.2 ± 2.4	24.7 ± 3.7	0.32	0.62
Relative	0.58 ± 0.08	0.59 ± 0.07	0.60 ± 0.09	0.56	0.58
Spleen					
Absolute	1.67 ± 0.59	1.66 ± 0.57	1.80 ± 0.85	0.77	0.53
Relative	0.041 ± 0.013	0.040 ± 0.014	0.043 ± 0.018	0.86	0.50
Ovaries					
Absolute	0.90 ± 0.24	0.99 ± 0.24	0.89 ± 0.21	0.52	0.14
Relative	0.022 ± 0.007	0.024 ± 0.005	0.022 ± 0.005	0.81	0.16

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3. *indicates that Contrast is significant ($P \leq 0.05$)
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Missing value for weight of pregnant uterus (doe #1893)
[d] Absolute weight in grams
[e] Relative weight = (weight of organ/extragestational weight) x 100

Table 7. Histopathologic observations (number of examined animals that had tissue changes) in rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).[a]

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	EO
7 to 19 dg	FA	EO	EO
NO. RABBITS NECROPSIED:	28	25	29
NO. PREGNANT RABBITS EXAMINED MICROSCOPICALLY:	8	7	9
NO. NONPREGNANT RABBITS EXAMINED MICROSCOPICALLY:	2	3	4
OBSERVATION			
Lung			
Focal mononuclear inflammation	5[a]	9	10
Bronchial epithelial hyperplasia	2	3	4
Increased bronchus-associated lymphoid tissue	1	2	4
Suppurative lesions	1	0	3
Liver			
Portal mononuclear inflammation	6	7	9
Portal fibrosis	0	2	0
Biliary epithelial hyperplasia	1	1	0
Cholecystitis	1	0	0
Granuloma with Eimeria stiedae	1	0	0
Kidney			
Subacute/chronic nephritis[b]	9	10	12
Tubular mineralization[b]	9[c]	9[c]	10
Spleen			
Extramedullary hematopoiesis	1	0	1
Hemosiderosis	1	1	0
Ovaries			
Corpora hemorrhagica	1	1	0
Corpora lutea regression[d]	3	4	5
Uterus			
Suppurative metritis	1	0	1

[a] The severity of all lesions ranged from minimal to mild, with the exceptions noted below.
[b] Renal changes may be related to Encephalitozoon caniculi infections.
[c] One rabbit in each group had moderate tubular mineralization.
[d] Correlated with animals that were not pregnant or whose pregnancy was detectable only by uterine staining.

Table 8. Fertility of rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	EO
7 to 19 dg	FA	EO	EO
OBSERVATION			
No. inseminated	30	30	30
No. pregnant at sacrifice (30 dg)	21	20	23
No. not pregnant at sacrifice (30 dg)	7	5	6
No. aborted or delivered prematurely	0	1[a]	1[b]
No. died or euthanized	2[c]	4[d]	0
Percent pregnant at 30 dg[e]	75	80	79
Total percent pregnant[f]	77	83	80

[a] Doe 2341 aborted on 24 dg; 4 viable and 3 dead
[b] Doe 1724 delivered on 27 dg; 3 live, 1 resorption
[c] Doe 1927 died of pneumonia and septicemia on 11 dg; 14 implantation sites, all apparently viable. Doe 2462 died of pneumonia on 23 dg; 14 implantation sites, 12 apparently viable, 3 resorptions.
[d] Doe 1723 euthanized because of otitis media and pneumonia on 15 dg; 1 implantation site, resorbed in midgestation. Doe 2696 died of pneumonia on 16 dg; 10 implantation sites, all apparently viable. Doe 2326 euthanized because of pneumonia on 27 dg; 12 implantation sites, 11 viable, 1 resorption. Doe 2398 died of pneumonia on 29 dg; 8 implantation sites, 7 viable, 1 late resorption.
[e] Chi-square test (Group 1 versus 2 versus 3): $P = 0.89$.
[f] Chi-square test (Group 1 versus 2 versus 3): $P = 0.81$.

Table 9. Reproductive status of rabbits exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	EO		
7 to 19 dg	FA	EO	EO		
OBSERVATION					
Percent pregnant at 30 dg	75	80	79		
No. pregnant females examined	21	20	23		
No. litters with live fetuses	18	18	22		
No. corpora lutea/dam	9.8 ± 4.5[b]	10.8 ± 4.0	10.9 ± 3.0	0.34	0.96
No. implantations sites/dam	8.5 ± 4.1	8.4 ± 3.9	8.7 ± 3.9	0.99	0.84
No. resorptions/litter	1.10 ± 1.26	0.75 ± 0.91	1.04 ± 1.30	0.53	0.42
Early	0.62 ± 0.97	0.45 ± 0.69	0.83 ± 1.11	0.94	0.20
Mid	0.14 ± 0.48	0.10 ± 0.31	0.04 ± 0.21	0.44	0.60
Late	0.33 ± 0.58	0.20 ± 0.52	0.17 ± 0.39	0.28	0.87
Resorptions/implantation sites (%)	22.5 ± 34.1	16.9 ± 30.5	13.2 ± 21.7	0.34	0.67
No. litters with resorptions	11	10	13		
Litters with resorptions (%)[c]	52.4	50.0	56.5		
Resorptions/litters with resorptions	2.09 ± 0.94	1.50 ± 0.71	1.85 ± 1.21	0.26	0.42
No. dead fetuses/litter	0	0.053 ± 0.22	0.17 ± 0.65	0.31	0.33
No. live fetuses/litter	7.43 ± 4.25	7.60 ± 4.10	7.40 ± 3.70	0.95	0.89
Total no. live and dead fetuses	156	153	175		

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3
[b] Mean ± SD
[c] Chi-square test (Group 1 versus 2 versus 3): P = 0.91

Table 10. Fetal measures (mean ± SD) for rabbit litters exposed in utero to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	EO		
7 to 19 dg	FA	EO	EO		
OBSERVATION					
No. litters examined	18	18	22		
No. live fetuses	156	152	171		
Body weights (g)					
Female	46.1 ± 7.6	44.5 ± 8.3	43.6 ± 8.3	0.38	0.73
Male	45.9 ± 6.1	45.7 ± 5.8	45.2 ± 8.5	0.82	0.83
Crown-rump length (mm)					
Female	100.1 ± 4.4	99.9 ± 6.6	97.3 ± 7.2	0.44	0.22
Male	101.2 ± 5.4	100.8 ± 5.5	98.7 ± 7.0	0.43	0.27
Stunted[b]	(1/1) 5.5	(1/1) 5.5	0		
Placenta weight (g)	5.93 ± 1.02	5.89 ± 1.46	6.10 ± 1.28	0.86	0.45
Sex ratio (% male)	46.3 ± 19.7	55.0 ± 19.9	49.1 ± 20.4	0.32	0.36

[a] Probability for Contrast: I = Group 1 versus 2,3; II = 2 versus 3
[b] Expressed as: (number of stunted fetuses/number of litters) percentage of litters affected

Table 11. Morphologic alterations[a] in rabbit fetuses exposed in utero to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1		Group 2		Group 3	
EXPOSURE INTERVAL						
1 to 6 dg	FA		FA		EO	
7 to 19 dg	FA		EO		EO	
OBSERVATION						
No. litters with live fetuses	18		18		22	
No. fetuses examined	156		152		171	
No. heads examined	76		79		85	
MAJOR MALFORMATIONS						
Acrania	0		0		(1/1)	4.5[b]
Rachischisis	0		0		(1/1)	4.5[b]
Multiple vascular defects	0		0		(1/1)	4.5[b]
MINOR ANOMALIES						
Sternebral anomalies	(3/2)	11.1	(2/2)	11.1	0	
Misaligned	(2/1)	5.6	(2/2)	11.1	0	
Bipartite	(2/2)	11.1	(1/1)	5.6	0	
Limb anomalies	0		0		(1/1)	4.5[b]
Retinal folds	0		0		(2/2)	9.1
MORPHOLOGIC VARIATIONS						
Cardiovascular	(4/4)	22.2	(5/5)	27.8	(2/2)	9.1
Accessory vessel	(3/3)	16.7	(4/4)	22.2	(2/2)	9.1
Dilated pulmonary artery	(1/1)	5.6	(1/1)	5.6	0	
Supernumerary ribs	(104/18)	100.0	(111/17)	94.4	(132/22)	100.0
Extra	(65/16)	88.9	(87/17)	94.4	(101/22)	100.0
Rudimentary	(46/18)	100.0	(26/13)	72.2	(42/16)	72.7
Ossification at lumbar I	(10/7)	38.8	(7/5)	27.8	(9/8)	36.4
Reduced ossification	(15/5)	27.8	(4/3)	16.7	(7/4)	18.2
Skull	0		0		(1/1)	4.5
Sternebra	(14/5)	27.8	(3/2)	11.1	(7/4)	18.2
Ribs[c]	(1/1)	5.6	(1/1)	5.6	0	
Other variations						
Dental maleruption	(1/1)	5.6	0		(1/1)	4.5
Clear gallbladder	(2/1)	5.6	(2/2)	11.1	(1/1)	4.5

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected

[b] Fetus #2 in litter #2334 (heart beating at necropsy)

[c] Rudimentary supernumerary ribs with proximal ossification defect

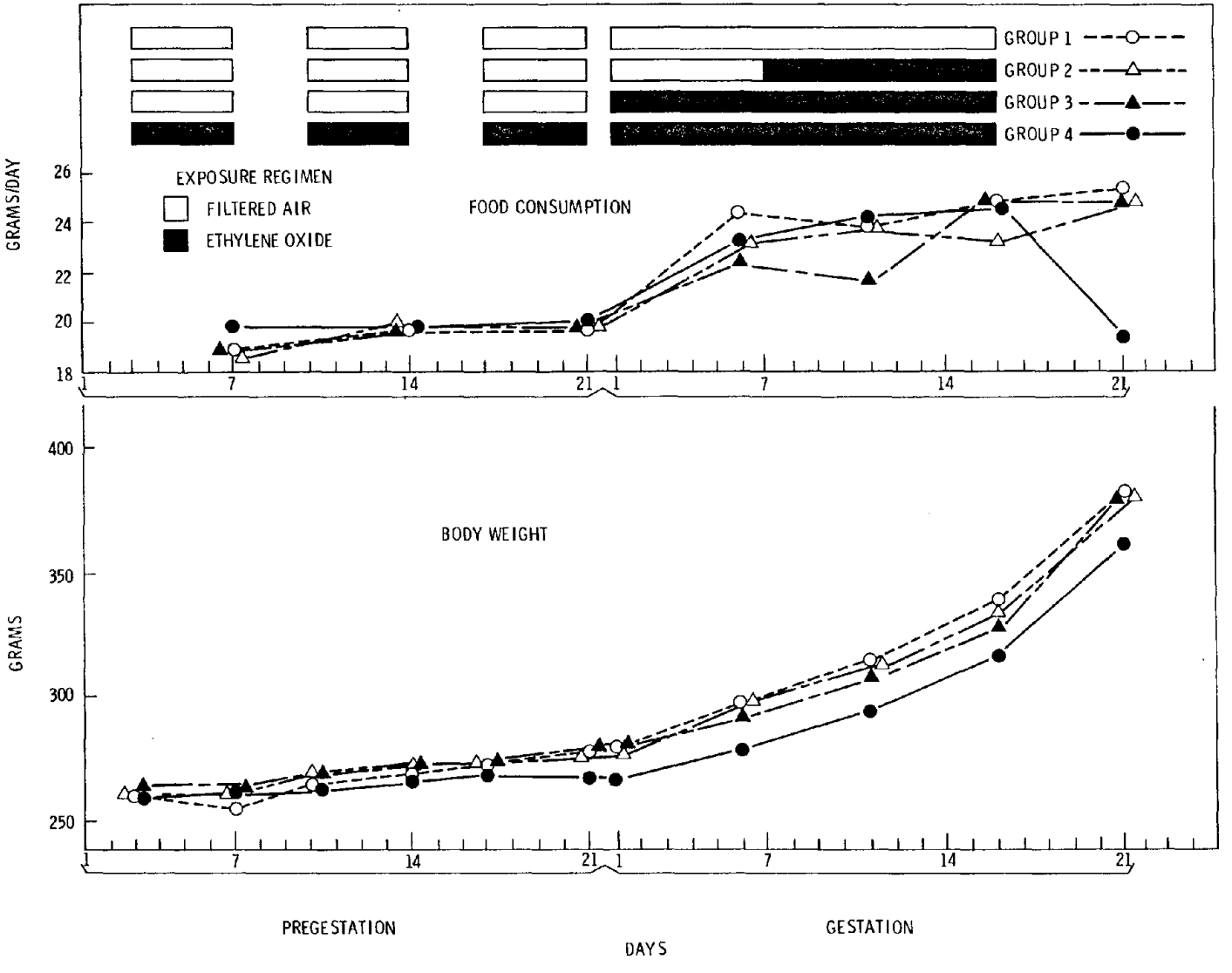

Figure 11. Food consumption and body weights of pregnant rats inhaling 150 ppm ethylene oxide or filtered air.

Table 12. Food consumption[a] of pregnant rats exposed to 150 ppm ethylene oxide (EO) or filtered air (FA)

	Group 1	Group 2	Group 3	Group 4	Contrast[b] I	Contrast[b] II	Contrast[b] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	EO			
1 to 6 dg	FA	FA	EO	EO			
7 to 16 dg	FA	EO	EO	EO			
NO. RATS:	41	41	41	39			
TIME OF MEASUREMENT							
Pregestation							
Week 1	18.9 ± 1.5	18.7 ± 1.8	18.9 ± 1.6	19.9 ± 0.3	0.25	<0.01*	0.50
Week 2	19.7 ± 0.8	19.9 ± 0.8	19.8 ± 0.8	19.8 ± 1.0	0.51	0.73	0.92
Week 3	19.7 ± 0.6	19.8 ± 0.6	19.8 ± 0.6	20.0 ± 0.6	0.27	0.19	0.90
Gestation Days							
1 to 6	24.3 ± 3.4	23.2 ± 2.3	22.4 ± 2.1	23.2 ± 3.3	0.01*	0.49	0.22
7 to 11	23.9 ± 5.4	23.7 ± 3.7	21.6 ± 2.1	24.1 ± 3.1	0.29	0.05*	0.01*
12 to 16	24.8 ± 4.6	23.2 ± 3.6	24.8 ± 4.6	24.7 ± 3.6	0.50	0.38	0.08
17 to 21	25.3 ± 6.0	24.9 ± 3.7	24.9 ± 2.7	19.3 ± 2.5	<0.01*	<0.01*	0.98

[a] Grams/rat/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3.
*indicates Contrast is significant ($P \leq 0.05$).

(Contrast I). A significant reduction in food intake was observed from 7 to 11 dg in rats of Group 3 (Contrast III), whose exposure had been initiated on 1 dg. Food consumption for Group 2 rats (exposure initiation on 7 dg) may have been ($P = 0.08$) less than that of Group 3 from 12 to 16 dg. In the interval between exposure termination and sacrifice (17 to 21 dg), food intake was significantly reduced (Contrast II) in the rats (Group 4) that inhaled ethylene oxide during the entire pregestational and gestational exposure periods.

Reductions in body weights were first observed 17 days after the initiation of pregestational exposure (Figure 11; Table 13). At that time, weights for rats exposed to ethylene oxide (Group 4) were apparently (Contrast II, $P = 0.06$) lower than those of the filtered-air-exposed rats of Groups 2 and 3. This difference was highly significant by the end of the pregestational exposure (Contrast II), and body weights of the Group 4 rats remained lower than those of Groups 2 and 3 throughout the experimental period. Body weights of the filtered-air-exposed rats (Group 1) were significantly higher than values for all other exposure groups on 6, 11 and 16 dg, but not on 21 dg (Contrast I).

Organ Weights and Histopathology

The mean weight (Table 14) for the pregnant uteri of rats exposed only to filtered air (Group 1) was significantly greater (Contrast I) than the means for the ethylene-oxide-exposed rats (Groups 2, 3 and 4). Body weights and extragestational weights for Group 4 animals were significantly lower than those of Groups 2 and 3 (Contrast II). No differences among treatments were observed for weights of lungs and ovaries, but absolute liver weights for Group 4 tended to be lower than those of Groups 2 and 3 (Contrast II). Absolute and relative weights for kidneys and spleens of ethylene-oxide-exposed groups were significantly greater than those of the filtered-air-exposed rats (Contrast I). Significant increases in relative spleen weights were correlated with the duration of exposure to ethylene oxide; i.e., the mean for Group 4, exposed for 31 days, was higher (Contrast II) than means for Groups 2 and 3 (exposed for 10 and 16 days, respectively), and Group 3 values were higher than those of Group 2 (Contrast III).

The lesions observed on histopathologic examination of the adult rats did not appear to be related to ethylene oxide exposure (Table 15) and could not be correlated with the observed differences in tissue weights. The lungs from several animals had minimal focal accumulations of mononuclear inflammatory cell in perivascular and alveolar areas; these were present in all exposure groups.

One Group 3 rat had moderate splenic extramedullary hematopoiesis, mild hepatic extramedullary hematopoiesis, minimal focal hepatic necrosis, and a neck "cyst" diagnosed as severe lymphangectasia and moderate lymphoid hyperplasia. Interstitial mononuclear inflammatory cells were minimal in kidneys of rats in all experimental groups, and mineralization of renal tubules was present in four rats, representing three groups. An observation of regression of corpora lutea in three rats, one each in Groups 1, 2 and 4, was correlated with nonpregnancy in these rats.

Table 13. Body weights (g, mean ± SD) of pregnant rats exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	Contrast[a] II	Contrast[a] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	EO			
1 to 6 dg	FA	FA	EO	EO			
7 to 16 dg	FA	EO	EO	EO			
NO. RATS:	41	41	41	39			
TIME OF MEASUREMENT							
Randomization	253 ± 15	252 ± 16	255 ± 13	253 ± 14	0.93	0.81	0.64
Pregestation day							
3[b]	260 ± 26	261 ± 20	263 ± 16	260 ± 16	0.72	0.64	0.69
7	255 ± 36	261 ± 23	263 ± 21	262 ± 17	0.14	0.87	0.74
10	264 ± 28	269 ± 19	270 ± 17	263 ± 16	0.36	0.10	0.72
14	269 ± 19	271 ± 16	273 ± 15	266 ± 18	0.73	0.11	0.58
17	272 ± 19	274 ± 17	274 ± 13	268 ± 17	0.97	0.06	0.96
21	278 ± 19	277 ± 18	280 ± 15	267 ± 17	0.37	<0.01*	0.37
Gestation day							
1	280 ± 19	279 ± 17	281 ± 15	267 ± 18	0.24	<0.01*	0.61
6	298 ± 20	298 ± 20	293 ± 17	279 ± 22	0.03*	<0.01*	0.24
11	315 ± 23	314 ± 21	308 ± 19	295 ± 25	0.03*	<0.01*	0.28
16	339 ± 24	335 ± 24	328 ± 19	317 ± 25	<0.01*	<0.01*	0.22
21	382 ± 34	381 ± 31	378 ± 27	360 ± 36	0.15	0.01*	0.71

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3.
*indicates Contrast is significant ($P \leq 0.05$)

[b] Corresponds to initiation of pregestational exposure

Table 14. Body and organ weights (mean ± SD) of pregnant rats exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	Contrast[a] II	Contrast[a] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	EO			
1 to 6 dg	FA	FA	EO	EO			
1 to 16 dg	FA	EO	EO	EO			
NO. RATS	41	41	41	39			
WEIGHT MEASUREMENT							
Body (g)	382 ± 34	381 ± 31	378 ± 27	360 ± 36	0.15	0.01*	0.71
Pregnant uterus (g)	76 ± 14	70 ± 17	71 ± 10	66 ± 20	0.02*	0.16	0.67
Extragestational (g)[b]	307 ± 23	308 ± 27	306 ± 26	294 ± 24	0.38	0.01*	0.75
Lung							
Absolute[c]	1.44 ± 0.25	1.42 ± 0.21	1.45 ± 0.22	1.44 ± 0.26	0.85	0.93	0.50
Relative[d]	0.47 ± 0.08	0.46 ± 0.07	0.48 ± 0.08	0.49 ± 0.09	0.76	0.17	0.41
Liver							
Absolute	14.1 ± 1.7	14.3 ± 2.1	14.5 ± 1.4	13.7 ± 2.6	0.96	0.07	0.72
Relative	4.60 ± 0.39	4.65 ± 0.66	4.73 ± 0.44	4.64 ± 0.79	0.46	0.61	0.54
Kidneys							
Absolute	2.07 ± 0.15	2.15 ± 0.19	2.16 ± 0.21	2.11 ± 0.17	0.03*	0.24	0.74
Relative	0.68 ± 0.05	0.70 ± 0.07	0.71 ± 0.08	0.72 ± 0.07	0.01*	0.19	0.60
Spleen							
Absolute	0.60 ± 0.10	0.61 ± 0.10	0.65 ± 0.11	0.66 ± 0.10	0.03*	0.13	0.09
Relative	0.19 ± 0.03	0.20 ± 0.03	0.21 ± 0.04	0.22 ± 0.03	<0.01*	<0.01*	0.04*
Ovaries							
Absolute	0.14 ± 0.04	0.14 ± 0.03	0.14 ± 0.03	0.13 ± 0.04	0.49	0.16	0.58
Relative	0.046 ± 0.014	0.046 ± 0.012	0.045 ± 0.012	0.044 ± 0.012	0.76	0.43	0.70

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Absolute weight in grams
[d] Relative weight = (weight of organ/extragestational weight) x 100

Table 15. Histopathologic observations in rats exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).[a]

	Group 1	Group 2	Group 3	Group 4
EXPOSURE INTERVAL				
Pregestation	FA	FA	FA	EO
1 to 6 dg	FA	FA	FA	EO
7 to 16 dg	FA	EO	EO	EO
NO. RATS NECROPSIED	44	44	45	44
NO. PREGNANT RATS EXAMINED MICROSCOPICALLY	12	11	14	12
NO. NONPREGNANT RATS EXAMINED MICROSCOPICALLY	1	1	0	1
OBSERVATION				
Lung				
Focal mononuclear inflammation	2	5	6	6
Pneumonitis	1	0	0	1
Increased bronchus-associated lymphoid tissue	1	1	2	0
Liver				
Portal mononuclear inflammation	3	2	3[b]	2
Focal necrosis	1	0	1[b]	0
Extramedullary hematopoiesis	0	1	1[b]	0
Kidney				
Focal interstitial mononuclear cells	2	3	1[c]	8
Hydronephrosis	0	1	2	0
Mineralization	1	2	0	1
Spleen				
Extramedullary hematopoiesis	0	0	1[b]	0
Ovaries				
Corpora luteal regression[d]	1	1	0	1
Uterus	0	0	0	0

[a] Number of examined animals that had tissue changes. The severity of all lesions ranged from minimal to mild, with the exceptions noted below.
[b] Lesions in rat 2309, in addition to a neck cyst classified as severe lymphangectasia with moderate lymphoid hyperplasia
[c] Hydronephrosis in rat 2463 was mild to moderate.
[d] Correlated with animals that were not pregnant

Fertility and Reproductive Status

Pregestational exposure to ethylene oxide did not have a detectable effect on the reproductive performance of these rats (Table 16); the numbers of corpora lutea and implantation sites were similar for all exposure groups (Table 17).

Table 16. Mating performance of rats[a] exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Pregestational Exposure	
	FA	EO
NO. RATS EXPOSED	169[b]	50
OBSERVATION		
No. sperm-positive rats	146	44
No. sperm-negative rats	23	6
No. sperm-negative rats pregnant at sacrifice (10 to 17 dg)	6	3
Mating failure rate (%)[c]	10	6

[a] Rats were exposed for 7 hr/day, 5 days/wk for 3 wk prior to mating.
[b] Rat 2367, which died during pregestational exposure due to starvation resulting from severe dental malocclusion, was excluded.
[c] Chi-square test: P = 0.38

The total number of resorptions per litter in Group 4 was significantly increased (Contrast II), compared with those of Groups 2 and 3 (Table 17). This increase was attributable to higher resorption incidences during early and midgestation. When the percentage of implantation sites resorbed was calculated, the value for rats receiving pregestational and gestational exposure to ethylene oxide (Group 4) was also significantly higher (Contrast II) than that for rats receiving only gestational exposure to this chemical (Groups 2 and 3). The percentage of litters with resorptions in Group 4 was not significantly higher than those of all other exposure groups, but the number of resorptions per litter with resorptions was higher in both Groups 3 and 4 animals (Contrasts II and III).

Table 17. Reproductive status of rats exposed to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	EO			
1 to 6 dg	FA	FA	EO	EO			
7 to 16 dg	FA	EO	EO	EO			
OBSERVATION							
Percent of sperm-positive females pregnant at 21 dg	93	93	91	89			
No. pregnant females examined	41	41	41	39			
No. litters with live fetuses	41	41	41	38			
No. corpora lutea/dam	15.4 ± 2.1[b]	15.2 ± 2.9	15.4 ± 2.8	14.6 ± 3.1	0.54	0.19	0.75
No. implantations sites/dam	14.7 ± 2.4	14.0 ± 3.0	14.8 ± 1.9	14.3 ± 3.3	0.57	0.90	0.24
No. resorptions/litter	0.75 ± 0.80	0.71 ± 0.87	0.92 ± 1.17	1.60 ± 1.97	0.17	0.03*	0.44
Early	0.73 ± 0.81	0.71 ± 0.87	0.85 ± 1.11	1.44 ± 1.86	0.23	0.06	0.59
Mid	0.02 ± 0.16	0	0.05 ± 0.31	0.13 ± 0.34	0.43	0.06	0.36
Late	0	0	0.02 ± 0.16	0.03 ± 0.16	0.41	0.24	0.32
Resorptions/implantation sites (%)	5.35 ± 5.65	5.39 ± 6.71	6.15 ± 7.50	13.6 ± 21.3	0.16	0.05*	0.77
No. litters with resorptions	23	20	21	27			
Litters with resorptions (%)[c]	56.1	48.8	51.2	69.2			
Resorptions/litters with resorptions	1.35 ± 0.57	1.45 ± 0.69	1.81 ± 1.03	2.30 ± 2.00	0.09	0.03*	0.03*
No. dead fetuses/litter	0	0	0	0			
No. live fetuses/litter	13.9 ± 2.49	13.3 ± 3.1	13.8 ± 1.94	12.7 ± 3.97	0.27	0.92	0.46
Total no. live and dead fetuses	570	547	567	497			

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$)

[b] Mean ± SD

[c] Chi-square test (Group 1 versus 2 versus 3 versus 4): $P = 0.26$

Fetal Measures and Morphology

Comparisons of body weights and crown-rump lengths of filtered-air-exposed fetuses (Group 1) with those of fetuses exposed to ethylene oxide during development (Groups 2, 3 and 4) indicated that ethylene oxide exposure produced significant growth reduction (Contrast I in Table 18). Body weights for both sexes of Group 4 were less than those of Groups 2 and 3 (Contrast II). Females of Group 3 tended to be lighter in weight and significantly shorter than those of Group 2 (Contrast III).

Although fetal size was reduced in Group 4, placental weights were significantly increased (Contrast II in Table 18). Placentas were also heavier for Group 3 than for Group 2 (Contrast III), but no differences were detected between means for Group 1 (control) and the groups exposed to ethylene oxide (Contrast I). Sex ratios were similar in all exposure groups.

One fetus from exposure Group 3 (Table 19) had a major malformation (diaphragmatic hernia). The incidence of hydroureter (dilatation of the ureter), which is commonly observed in rats (Perraud, 1976), tended to be higher in fetuses exposed to ethylene oxide from 7 to 16 dg (Group 2) than in Group 1 (control) fetuses. A reduction of sternebral and skull ossification, which is also common in rats (Palmer, 1972), was found in a substantial fraction of control fetuses. Significantly increased incidences of reduced ossification at these loci were evident in fetuses exposed to ethylene oxide in utero.

SUMMARY OF EFFECTS OF ETHYLENE OXIDE EXPOSURE

Significant effects (i.e., those with $P \leq 0.05$) associated with ethylene oxide exposure of pregnant rats and rabbits are summarized in Table 20. No changes in maternal, reproductive, or fetal measures could be attributed to exposure of rabbits to 150 ppm of ethylene oxide. The increased mean value for maternal lung weights for Group 3 rabbits can be attributed to the high lung weights of three rabbits and were associated with histopathologic findings of lung infections.

Several measures of maternal toxicity were evident in ethylene-oxide-exposed rats. These included sporadic decreases in food consumption as well as consistent decreases in body weight and extragestational weight in the ethylene-oxide-exposed animals, particularly those receiving both pregestational and gestational exposure to ethylene oxide. Relative renal and splenic weights were increased in ethylene-oxide-exposed rats; the increase in relative splenic weights was roughly proportional to the period of exposure.

Reproductive parameters in rats were altered by ethylene oxide exposures. Resorptions per litter and resorptions per implantation site were higher for Group 4 than for Groups 2 and 3. The early and midgestational resorptions per litter in the Group 4 rats that received the pregestational exposure tended to be higher than those of the rats exposed to ethylene oxide during gestation. No differences among treatments were observed for the percentage of litters with resorptions, but the percentage of resorptions in litters with resorptions increased as the length of the ethylene oxide exposure increased.

Table 18. Fetal measures (mean ± SD) for rat litters exposed in utero to 150 ppm ethylene oxide (EO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	EO			
1 to 6 dg	FA	FA	EO	EO			
7 to 16 dg	FA	EO	EO	EO			
OBSERVATION							
No. litters examined	41	41	41	38			
No. live fetuses	570	547	567	497			
Body weights (g)							
Female	3.56 ± 0.31	3.35 ± 0.26	3.23 ± 0.34	3.12 ± 0.31	<0.01*	0.01*	0.06
Male	3.73 ± 0.33	3.53 ± 0.29	3.47 ± 0.36	3.34 ± 0.25	<0.01*	0.02*	0.41
Crown-rump length (mm)							
Female	36.1 ± 1.2	35.3 ± 1.2	34.7 ± 1.5	34.8 ± 1.1	<0.01*	0.32	0.02*
Male	36.5 ± 1.9	36.1 ± 1.2	35.8 ± 1.4	35.6 ± 1.0	0.02*	0.17	0.35
Stunted[b]	(1/1) 2.4	(1/1) 2.4	(1/1) 2.4	0			
Placenta weight (g)	0.51 ± 0.05	0.48 ± 0.05	0.51 ± 0.07	0.58 ± 0.12	0.26	<0.01*	0.03*
Sex ratio (% male)	50.6 ± 14.4	53.1 ± 13.8	48.8 ± 11.9	52.4 ± 16.6	0.75	0.60	0.18

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).

[b] Expressed as: (number of stunted fetuses/number of litters) percentage of litters affected

Table 19. Morphologic alterations in fetal rats exposed in utero to 150 ppm ethylene oxide (EO) or filtered air (FA).[a]

	Group 1		Group 2		Group 3		Group 4	
EXPOSURE INTERVAL								
Pregestation	FA		FA		FA		EO	
1 to 6 dg	FA		FA		EO		EO	
7 to 16 dg	FA		EO		EO		EO	
OBSERVATION								
No. litters with live fetuses	41		41		41		38	
No. fetuses examined	570		547		567		497	
No. heads examined	286		276		285		245	
MAJOR MALFORMATIONS								
Diaphragmatic hernia	0		0		(1/1)	2.4	0	
MINOR ANOMALIES								
Cardiovascular anomalies	0		(1/1)	2.4	(1/1)	2.4	(1/1)	2.6
Retroesophageal subclavian	0		0		0		(1/1)	2.6
Missing innominate	0		(1/1)	2.4	(1/1)	2.4	0	
Musculoskeletal anomalies								
Brachyury	0		0		(1/1)	2.4	0	
Lordosis	0		0		(1/1)	2.4	0	
Wavy ribs	0		(3/2)	4.9	(2/1)	2.4	(1/1)	2.6
Misaligned sternebrae	(10/8)	19.5	(3/3)	7.3	(12/9)	22.0	(8/5)	13.2
Other anomalies								
Edema	0		0		(2/1)	2.4	0	
MORPHOLOGIC VARIATIONS								
Renal variations	(19/9)	22.0	(27/17)	41.5[b]	(27/9)	22.0	(18/11)	28.9
Hydroureter	(19/9)	22.0	(27/17)	41.5	(27/9)	22.0	(17/10)	26.3
Renal pelvic cavitation	0		0		0		(1/1)	2.6
Supernumerary ribs	(24/10)	24.4	(23/12)	29.3	(24/11)	26.8	(17/9)	23.7
Extra	(1/1)	2.4	(3/3)	7.3	(1/1)	2.4	0	
Rudimentary	(1/1)	2.4	0		0		0	
Ossification at lumbar I	(24/10)	24.4	(23/12)	29.3	(23/10)	24.4	(17/9)	23.7
Reduced ossification	(151/37)	90.2	(198/39)	95.1	(239/39)	95.1	(230/36)	94.7
Skull	(3/2)	4.9	(16/9)	22.0[c]	(10/9)	22.0[c]	(14/10)	26.3[c]
Vertebrae	(89/28)	68.3	(69/25)	61.0	(108/32)	78.0	(112/29)	76.3
Sternebrae	(69/23)	56.1	(145/36)	87.8[d]	(159/36)	87.8[d]	(155/33)	85.8[d]
Ribs	0		0		(1/1)	2.4	(1/1)	2.6
Pelvis	0		(3/3)	7.3	(13/5)	12.2	(8/4)	12.1
Phalanges	0		0		0		(1/1)	2.6

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected
[b] Chi-square test for renal variations: P = 0.06 for Group 1 versus 2
[c] Chi-square test for reduced ossification of the skull: P = 0.02 for Group 1 versus 2; P = 0.02 for Group 1 versus 3; P = 0.03 for Group 1 versus 4
[d] Chi-square test for reduced ossification of the sternebra: P = 0.001 for Group 1 versus 2; P = 0.001 for Group 1 versus 3; P = 0.002 for Group 1 versus 4

Table 20. Summary of significant effects ($P \leq 0.05$) of exposure to 150 ppm ethylene oxide.[a]

	RAT					RABBIT			
	Observation Period	Contrast I	Contrast II	Contrast III	Reference	Observation Period	Contrast I	Contrast II	Reference
MATERNAL OBSERVATIONS									
Food consumption									
	Pregestation								
	Week 1		4>2,3		Fig. 5,				Fig. 10
	1 to 6 dg	1>2,3,4			Table 18				Table 4
	7 to 11 dg		4>2,3	2>3					
	12 to 16 dg								
	17 to 21 dg	1>2,3,4	2,3>4						
Body weight									
	Pregestation								
	Week 3		2,3>4		Fig. 11,				Fig. 10
	1 dg		2,3>4		Table 13				Table 15
	6 dg	1>2,3,4	2,3>4						
	11 dg	1>2,3,4	2,3>4						
	16 dg	1>2,3,4	2,3>4						
	21 dg		2,3>4						
Weight of pregnant uterus	21 dg	1>2,3,4			Table 14				
Extragestational weight	21 dg		2,3>4						
Tissue weights - lung	21 dg				Table 14	30 dg		3>2[b]	Table 6
kidney		2,3,4>1[c]							
spleen		2,3,4>1[c]	4>2,3[b]	3>2[b]					
REPRODUCTIVE OBSERVATIONS	21 dg								
No. resorptions/litter			4>2,3		Table 17				Table 9
Resorptions/implants (%)			4>2,3						
Resorptions/litters with resorptions (%)			4>2,3	3>2					
FETAL OBSERVATIONS	21 dg								
Body weight									
Female		1>2,3,4	2,3>4		Table 18				Table 10
Male		1>2,3,4	2,3>4						

Crown-rump length					
Female	1>2,3,4		2>3		
Male	1>2,3,4				
Placenta weight		4>2,3	3>2		
Reduced ossification					
Skull	2,3,4>1[d]			Table 19	Table 11
Sternebra	2,3,4>1[d]				
Hydroureter	2>1[d]				

[a] Rat exposure to ethylene oxide: Group 1, none; Group 2, 7 to 16 dg; Group 3, 1 to 16 dg; Group 4, pregestation, 1 to 16 dg. Rabbit exposure to ethylene oxide: Group 1, none; Group 2, 7 to 19 dg; Group 3, 1 to 19 dg.
[b] Relative weights
[c] Absolute and relative weights
[d] Chi-square test

Fetal growth indices (body weight and crown-rump length) were reduced in the ethylene-oxide-exposed rats, particularly in those receiving both pregestational and gestational exposures. On the other hand, placental weights were higher in this group than in the rats receiving only the gestational exposures.

The incidence of reduced ossification in fetal skulls and sternebrae was higher in the ethylene-oxide-exposed rats than in the control group. This finding, together with the lower fetal body weights, suggests a pattern of retarded development. The significance of the increased incidence of the morphologic variant, dilated ureters, observed in Group 2 rats exposed from 7 through 16 dg, is not clear at this time.

There is considerable evidence of maternal toxicity in rats, particularly following a 15-day pregestational exposure prior to the 16-day gestational exposure. The subsequent effects of increased intrauterine mortality and fetal growth retardation might be attributable to maternal toxicity. Since studies of LaBorde and Kimmel (1980) demonstrated the teratogenicity of high doses of ethylene oxide administered intravenously to mice, we speculate that the embryotoxic effects we observed in rats were a result of in utero exposure to ethylene oxide, but that the mean daily concentration of 150 ppm for 7 hr/day, although maternally toxic, was less than that required to produce overt teratogenicity.

PROPYLENE-OXIDE-EXPOSED RABBITS

Food Consumption and Body Weight

Food consumption of pregnant rabbits exposed to propylene oxide (Groups 2 and 3) was less than that of the filtered-air-exposed rabbits (Group 1) during 6 to 10 ($P = 0.07$), 11 to 15 ($P < 0.01$), and 16 to 20 dg ($P = 0.01$; Figure 12, Contrast I in Table 21). Rabbits of Group 3, whose exposure was initiated on 1 dg, consumed less food than Group 2 rabbits until their exposure was initiated on 7 dg (Contrast II). From days 11 to 15, food intake for Group 3 was further depressed, and differences between the two propylene-oxide-exposed groups were again significant. Once the exposures were terminated (19 dg), food consumption was similar among all exposure groups.

Although food intake was (at times) lower in propylene-oxide-exposed rabbits, a significant depression in body weight was not observed except on 15 dg (Table 22). At that time, values for Group 3 were significantly lower than those of Group 2 (Contrast II).

Organ Weights and Histopathology

The weight of the pregnant uterus, the extragestational body weight and the weights of major organs were unaffected by exposure (Table 23). The only suggestive difference ($P = 0.07$), is a higher relative mean renal weight for rabbits of Group 3 than that for Group 2 (Contrast II).

The lesions observed in histopathologic examination of tissues from adult rabbits could not be related to propylene oxide exposure (Table 24). The most prevalent pulmonary changes were minimal to mild focal mononuclear inflammations

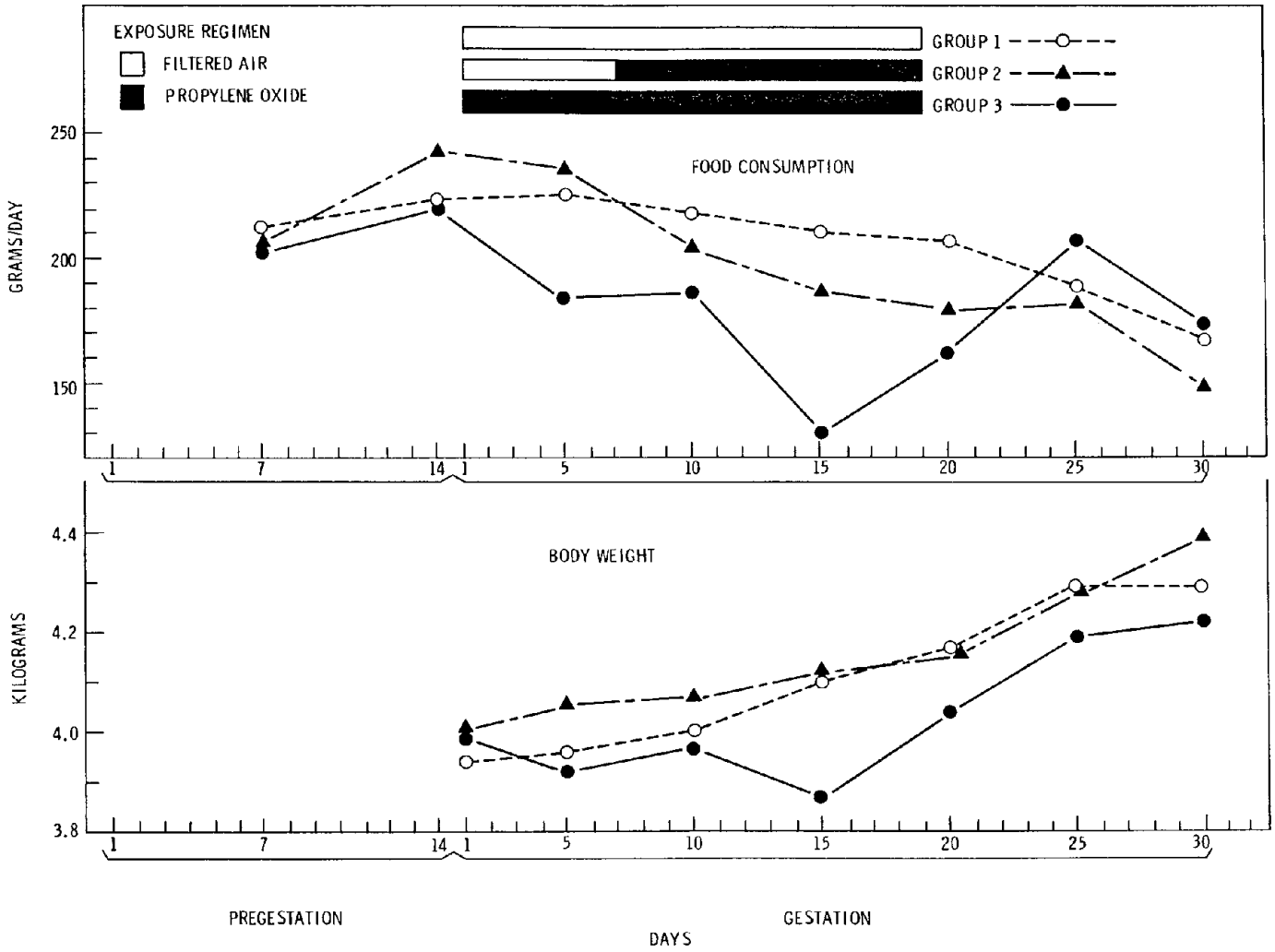

Figure 12. Food consumption and body weights of pregnant rabbits inhaling 500 ppm propylene oxide or filtered air.

Table 21. Food consumption[a] of pregnant rabbits exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[b] I	Contrast[b] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	PO		
7 to 19 dg	FA	PO	PO		
NO. RABBITS:	17	11	19		
TIME OF MEASUREMENT					
Pregestation					
Week 1	212 ± 54	206 ± 69	202 ± 56	0.66	0.85
Week 2	223 ± 40	242 ± 40	219 ± 47	0.57	0.14
Gestation Days					
1 to 5	225 ± 35	235 ± 34	183 ± 54	0.23	<0.01*
6 to 10	218 ± 35	204 ± 37	186 ± 51	0.07	0.24
11 to 15	210 ± 40	187 ± 37	130 ± 74	<0.01*	<0.01*
16 to 20	206 ± 39	179 ± 44	162 ± 45	0.01*	0.28
21 to 25	189 ± 72	182 ± 60	207 ± 63	0.80	0.34
26 to 30	167 ± 85	148 ± 90	173 ± 64	0.82	0.41

[a] Grams/rabbit/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3.
*indicates Contrast is significant ($P \leq 0.05$).

and focal aggregates of lymphocytes, macrophages and plasma cells around small vessels, small airways and/or alveoli. Although nonspecific, these changes may be related to possible Pasteurella infections inferred by the presence of suppurative bronchitis, bronchopneumonia and pleuritis in a few animals. Two Group 3 animals had minimal suppurative bronchitis, and one rabbit had severe suppurative bronchopneumonia and mild pleuritis. One Group 1 rabbit had moderate suppurative and mild pleuritis; a second rabbit in that group had mild suppurative bronchitis. Kidney changes, observed in numerous animals, consisted of minimal to mild mineralization of the proximal and distal tubules.

One Group 1 rabbit had minimal heterophil populations in the ovaries; another had a mild heterophil exudate in the uterus. Regressing corpora lutea, or ovaries without well-formed corpora lutea, correlated well with the status of

Table 22. Body weight (kg, mean ± SD) of pregnant rabbits exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	PO		
7 to 19 dg	FA	PO	PO		
NO. RABBITS:	17	11	19		
TIME OF MEASUREMENT					
Randomization	3.75 ± 0.26	3.81 ± 0.24	3.80 ± 0.24	0.46	0.96
Gestation day					
1	3.94 ± 0.30	4.01 ± 0.19	3.99 ± 0.25	0.43	0.83
5	3.96 ± 0.29	4.06 ± 0.22	3.92 ± 0.28	0.68	0.16
10	4.00 ± 0.28	4.07 ± 0.22	3.97 ± 0.31	0.75	0.30
15	4.10 ± 0.29	4.12 ± 0.24	3.87 ± 0.34	0.24	0.02*
20	4.17 ± 0.29	4.16 ± 0.26	4.04 ± 0.27	0.39	0.23
25	4.29 ± 0.33	4.28 ± 0.34	4.19 ± 0.31	0.57	0.47
30	4.29 ± 0.42	4.39 ± 0.24	4.22 ± 0.36	0.91	0.20

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3.
*indicates that Contrast is significant.

uteri that appeared to be nonpregnant at the time of necropsy. Regressing corpora lutea were also observed in two rabbits in Group 1, one in Group 2, and two in Group 3, in which implantation sites were detected by staining the uterus with ammonium sulfide (Kopf et al., 1964). Extramedullary hematopoiesis was present in liver, spleen, and kidney of one Group 1 rabbit and in the spleen of one Group 3 rabbit. These rabbits had moderate and severe bronchopneumonia, respectively.

Fertility and Reproductive Status

At sacrifice, the percentage of pregnant rabbits was low in all exposure groups, but particularly in Group 2 rabbits, which were exposed from 7 to 19 dg (Table 25). The techniques employed and the hormone (PLH) used to induce ovulation were similar to those used for artificial insemination in the ethylene oxide study. The donor bucks appeared to be healthy, and sperm quality was

Table 23. Body and organ weights (mean ± SD) of pregnant rabbits exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	PO		
7 to 19 dg	FA	PO	PO		
NO. RABBITS:	17	11	19		
WEIGHT MEASUREMENT					
Body (g)	4291 ± 419	4391 ± 241	4216 ± 358	0.91	0.21
Pregnant uterus (g)	463 ± 207	433 ± 282	374 ± 188	0.38	0.48
Extragestational (g)[b]	3826 ± 455	3958 ± 375	3842 ± 377	0.56	0.45
Lung					
Absolute[c]	16.6 ± 7.9	14.9 ± 1.3	16.5 ± 6.2	0.63	0.50
Relative[d]	0.45 ± 0.27	0.38 ± 0.03	0.43 ± 0.18	0.49	0.46
Liver					
Absolute	118 ± 30.4	126 ± 32.5	125 ± 23.4	0.38	0.95
Relative	3.04 ± 0.49	3.14 ± 0.52	3.24 ± 0.40	0.42	0.56
Kidneys					
Absolute	23.1 ± 2.9	23.5 ± 4.7	25.1 ± 3.7	0.32	0.26
Relative	0.61 ± 0.07	0.59 ± 0.10	0.66 ± 0.10	0.56	0.07
Spleen					
Absolute	1.76 ± 0.60	1.53 ± 0.54	1.65 ± 0.48	0.32	0.56
Relative	0.045 ± 0.012	0.038 ± 0.012	0.043 ± 0.012	0.24	0.34
Ovaries					
Absolute	0.88 ± 0.22	0.81 ± 0.24	0.85 ± 0.21	0.44	0.60
Relative	0.023 ± 0.006	0.021 ± 0.007	0.022 ± 0.006	0.37	0.49

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Absolute weight in grams
[d] Relative weight = (weight of organ/extragestational weight) x 100

Table 24. Histopathologic observations (number of examined animals that had tissue changes)[a] in rabbits exposed to propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	PO
7 to 19 dg	FA	PO	PO
NO. RABBITS NECROPSIED	30	26	26
NO. PREGNANT RABBITS EXAMINED MICROSCOPICALLY	7	5	9
NO. NONPREGNANT RABBITS EXAMINED MICROSCOPICALLY	4	6	3
OBSERVATION			
Lung			
Focal mononuclear inflammation	5[a]	11	10
Bronchial epithelial hyperplasia	5[b]	3	6
Increased bronchus-associated lymphoid tissue	4[c]	1	3[f]
Suppurative lesions	2	0	3
Liver			
Portal mononuclear inflammation	8	10[e]	11
Focal fibrosis	1	1	1[f]
Extramedullary hematopoiesis	1[c]	0	1
Hepatocellular vacuolation	2	1	1
Eimeria stiedae	0	2	0
Kidney			
Subacute/chronic nephritis	3	9	10
Tubular mineralization	7	6	10[f]
Extramedullary hematopoiesis	0	0	1
Spleen			
Extramedullary hematopoiesis	1[c]	0	1[f]
Ovaries			
Corpora hemorrhagica	2	1	1
Corpora lutea regression[d]	6	8	5
Suppurative inflammation	1	0	0
Uterus			
Suppurative inflammation	1	0	0

[a] The severity of all lesions ranged from minimal to mild, with the exceptions noted below.
[b] One rabbit with moderate bronchus-associated lymphoid tissue
[c] One rabbit with moderate suppurative bronchopneumonia and extramedullary hematopoiesis
[d] Correlated with animals that were not pregnant or whose pregnancy was detectably only by uterine staining
[e] Severe fibrosis and biliary hyperplasia
[f] One rabbit with severe bronchopneumonia and extramedullary hematopoiesis.

Table 25. Fertility of rabbits exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	PO
7 to 19 dg	FA	PO	PO
OBSERVATION			
No. inseminated	30	30	30
No. pregnant at sacrifice (30 dg)	17	11	19
No. not pregnant at sacrifice (30 dg)	13	15	7
No. died or euthanized	0	4[a]	4[b]
Percent pregnant at 30 dg[c]	57	42	73
Total percent pregnant[d]	57	47	67

[a] Four rabbits died from pneumonia: Doe 5651, which died on 19 dg, was not pregnant; doe 5883 died on 19 dg with 13 implants, all resorbed; doe 5875 died on 23 dg with 13 live kits and 1 resorption; doe 4077 died on 26 dg with 11 implants and 4 resorptions.

[b] Doe 4939 died on 1 dg. The probable cause of death was either an anaphylactic reaction following PLH injection or adrenocortical insufficiency. Three rabbits died from pneumonia: Doe 4934 (day 15) and doe 4929 (day 17) were not pregnant; doe 4033 died on 18 dg with one resorption.

[c] Chi-square test (Group 1 versus 2 versus 3): $P = 0.08$.

[d] Chi-square test (Group 1 versus 2 versus 3): $P = 0.30$.

good (Table 3). We speculated that the isolation period prior to insemination may not have been long enough to permit recovery from pseudopregnancy induced during shipment. In this study, 59, 31 and 74% of all inseminated does (including positive controls) were pregnant following isolation periods of 18, 19 and 20 days, respectively; 74, 72 and 91% were pregnant after 20, 21 and 22 days isolation for the ethylene oxide exposure; and, subsequently, 85, 75 and 81% were pregnant after 24, 25 and 26 days of isolation for the n-butyl acetate exposure. These results suggest that the 18-day isolation period recommended for Dutch-Belted rabbits (Gibson, Staples and Newberne, 1966) may not be adequate for other strains of rabbits such as the New Zealand whites.

Reproductive measures, such as the number of corpora lutea, implantation sites, resorptions and live and dead fetuses, were similar for all exposure groups (Table 26). The percentages of litters with resorptions were also similar,

Table 26. Reproductive status of rabbits exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	PO		
7 to 19 dg	FA	PO	PO		
OBSERVATION					
Percent pregnant at 30 dg	57	42	73		
No. pregnant females examined	17	11	19		
No. litters with live fetuses	15	9	17		
No. corpora lutea/dam	10.5 ± 4.2[b]	10.6 ± 4.6	10.1 ± 4.2	0.76	0.90
No. implantations sites/dam	8.2 ± 3.8	7.7 ± 5.0	7.8 ± 4.1	0.72	0.93
No. resorptions/litter	0.71 ± 0.92	0.73 ± 0.79	1.58 ± 2.76	0.44	0.24
Early	0.24 ± 0.56	0.36 ± 0.67	0.84 ± 2.36	0.45	0.43
Mid	0	0.09 ± 0.30	0.11 ± 0.32	0.21	0.88
Late	0.47 ± 0.87	0.27 ± 0.65	0.63 ± 1.26	0.95	0.35
Resorptions/implantation sites (%)	16.2 ± 32.2	22.8 ± 38.7	18.2 ± 32.0	0.69	0.72
No. litters with resorptions	8	6	7		
Litters with resorptions (%)[c]	47.1	54.6	36.8		
Resorptions/litters with resorptions	1.50 ± 0.76	1.33 ± 0.52	4.29 ± 3.04	0.13	0.01*
No. dead fetuses/litter	0.06 ± 0.24	0.09 ± 0.30	0	0.83	0.25
No. live fetuses/litter	7.47 ± 3.61	6.91 ± 4.72	6.21 ± 3.81	0.46	0.64
Total no. live and dead fetuses	128	77	118		

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).
[b] Mean ± SD
[c] Chi-square test (Group 1 versus 2 versus 3): P = 0.63

but Group 3 rabbits, exposed to propylene oxide from 1 to 19 dg, had a higher incidence of resorptions in litters with resorptions than did the Group 2 rabbits, which were exposed only from 7 to 19 dg (Contrast II).

Fetal Measures and Morphology

Fetal body weights, crown-rump lengths, sex ratios, and placental weights were unaffected by in utero exposure to propylene oxide (Table 27).

Table 27. Fetal measures (mean ± SD) for rabbit litters exposed in utero to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	PO		
7 to 19 dg	FA	PO	PO		
OBSERVATION					
No. litters examined	15	9	17		
No. live fetuses	127	76	118		
Body weights (g)					
Female	45.9 ± 9.9	44.6 ± 4.9	45.0 ± 10.8	0.73	0.93
Male	45.8 ± 10.4	47.5 ± 8.7	45.0 ± 9.4	0.89	0.55
Crownrump length (mm)					
Female	97.1 ± 6.8	99.1 ± 2.8	97.0 ± 9.4	0.72	0.52
Male	97.1 ± 6.1	100.9 ± 5.1	97.4 ± 7.8	0.36	0.22
Placenta weight (g)	5.9 ± 1.3	5.8 ± 1.4	6.2 ± 1.5	0.80	0.52
Sex ratio (% male)	47.0 ± 17.3	51.8 ± 25.6	57.8 ± 24.6	0.30	0.52

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3

The incidence of musculoskeletal anomalies--in particular, forelimb flexure ($P = 0.14$), and sternebral anomalies ($P = 0.06$)--was somewhat increased in fetuses of rabbits receiving the longer (1 to 19 dg) propylene oxide exposure (Table 28). According to Palmer (1972), the limb flexure often observed in rabbits appears to be due to restricted movement within the uterus and, after birth, will disappear with exercise. Minor sternebral variations, also com-

Table 28. Morphologic alterations[a] in fetal rabbits exposed in utero to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1		Group 2		Group 3	
EXPOSURE INTERVAL						
1 to 6 dg	FA		FA		PO	
7 to 19 dg	FA		PO		PO	
OBSERVATION						
No. litters with live fetuses	15		9		17	
No. fetuses examined	127		76[b]		118	
No. heads examined	63		37		61	
MINOR ANOMALIES						
Rib dysmorphology						
Knobby	0		0		(1/1)	5.9
Sternebral anomalies	(1/1)	6.7	0		(8/6)	35.3[c]
Misaligned	0		0		(5/4)	23.5
Fused	0		0		(3/3)	17.6
Bipartite	(1/1)	6.7	0		(3/3)	17.6
Limb anomalies						
Forelimb flexure	0		0		(3/3)	17.6[d]
MORPHOLOGIC VARIATIONS						
Supernumerary ribs	(74/15)	100.0	(51/9)	100.0	(78/17)	100.0
Extra	(56/15)	100.0	(40/9)	100.0	(56/17)	100.0
Rudimentary	(14/10)	66.6	(11/5)	55.5	(16/7)	41.2
Ossification at lumbar I	(4/3)	20.0	(3/2)	22.2	(6/5)	29.4
Reduced ossification	(42/12)	80.0	(16/6)	66.7	(38/12)	70.6
Sternebra	(42/12)	80.0	(16/6)	66.7	(38/12)	70.6
Pelvis	(1/1)	6.7	0		0	
Other variations						
Renal pelvic cavitation	(1/1)	6.7	(2/1)	7.2	0	

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected

[b] Dead fetus 9, litter 4035, with acrania and rachischisis, not included

[c] Fisher's Exact test for sternebral anomalies: P = 0.06 for Group 1 versus 3

[d] Fisher's Exact test for forelimb flexure: P = 0.14 for Group 1 versus 3

mon, represent a transient phase in development; however, they may provide supportive evidence of an adverse effect of an agent (Palmer, 1972).

PROPYLENE-OXIDE-EXPOSED RATS

Food Consumption and Body Weight

Food consumption in the control rats (Group 1) was higher than that of the propylene-oxide-exposed animals during the second week of the pregestational exposure and from 7 to 16 dg (Figure 13, Contrast I in Table 29). Food intake of pregnant rats of Group 4, which received both pregestational and gestational exposures of propylene oxide, was lower than that for Groups 2 and 3 (Contrast II) after the first week of pregestational exposure. Food intake in Group 4 remained lower than Groups 2 and 3 until 7 dg, when the exposure of Group 2 was initiated. During the last measurement period of the study (17 to 21 dg), values for Groups 1 and 4 were lower than those of Groups 2 and 3 (Contrasts I and II). From 7 to 11 dg, food intake of the control rats was exceptionally high and may have accentuated differences between exposed and unexposed animals at that time.

Exposure to propylene oxide prior to and following conception significantly decreased the body weight of adult rats (Figure 13, Contrast I in Table 30).

Organ Weights and Histopathology

A number of body and tissue weight changes were associated with propylene oxide exposure (Table 31). At sacrifice, body weight, weight of the pregnant uterus, and the extragestational weight of rats receiving the longest exposure to propylene oxide (Group 4) were lower (Contrast II) than those of rats exposed over shorter periods during gestation (Groups 2 and 3). Body and extragestational weights of the control rats (Group 1) exceeded those of all groups exposed to propylene oxide (Contrast I). However, the weights of the pregnant uteri of Group 2 were lower than those of Group 3 (Contrast III), despite the shorter exposure interval for Group 2 rats.

Absolute liver and spleen weights for Group 1 were higher, and relative kidney weights were lower, than values for the rats inhaling propylene oxide (Contrast I in Table 31). Relative weights of the lung and spleen were greater in Group 4 than in Groups 2 and 3 (Contrast II), and liver weights were greater in Group 3 than in Group 2 (Contrast III). These differences in relative organ weights (higher in animals subjected to longer propylene oxide exposures) may not be due to changes in a specific organ, but rather to the lower extragestational body weights (Tables 30 and 31).

Results from histopathology studies are summarized in Table 32. None of the observed lesions appeared to be associated with propylene oxide exposure. Minimal foci of mononuclear inflammatory cells in perivascular and alveolar areas of the lung were present in a few animals from all exposure groups. A mild eosinophilic perivascular reaction was observed in one Group 3 rat. A few mononuclear inflammatory cells, in foci and around the portal areas of the liver, occurred throughout the exposure groups and were considered to be within the normal range. The incidence and severity of renal changes were

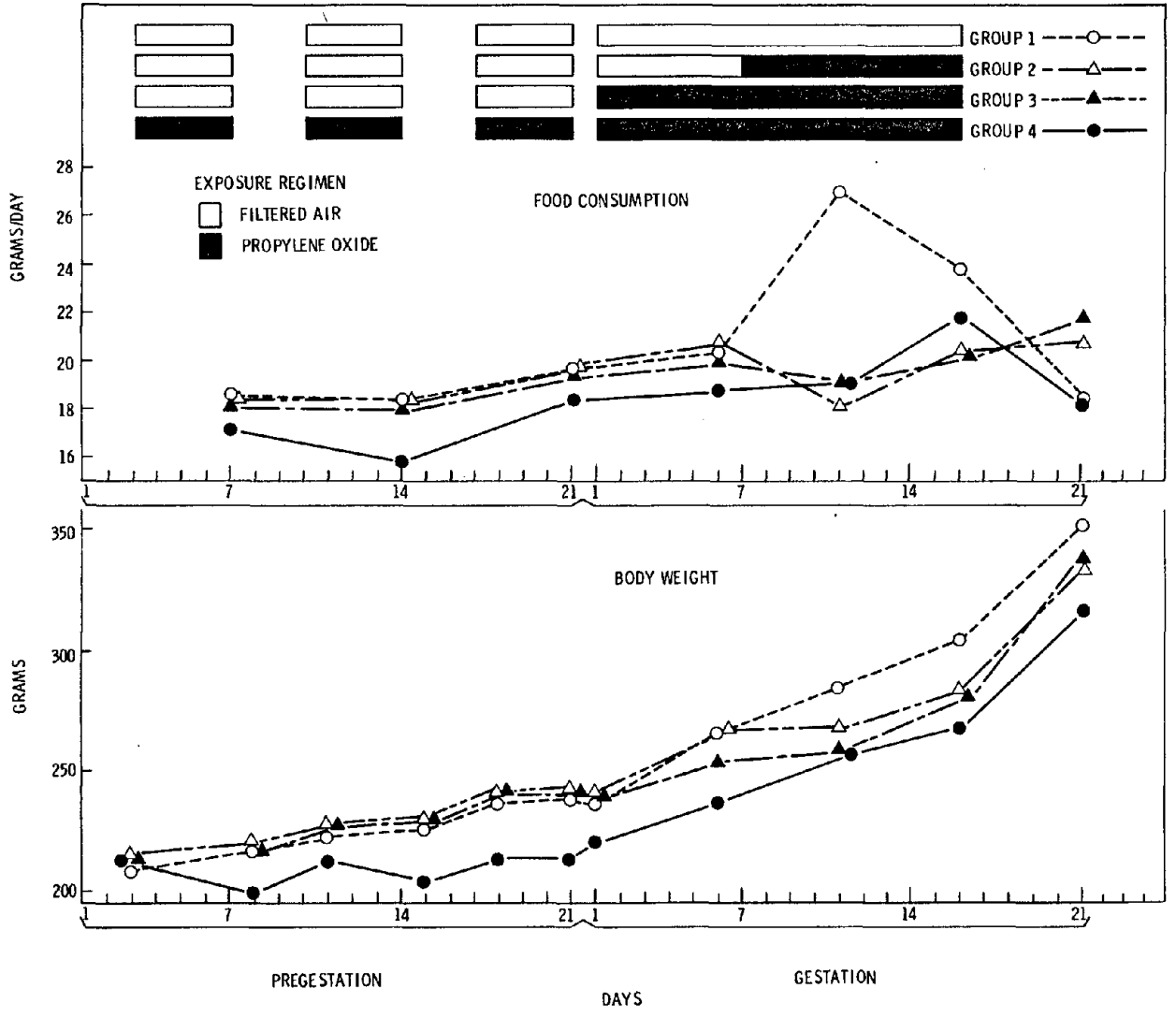

Figure 13. Food consumption and body weights of pregnant rats inhaling 500 ppm propylene oxide or filtered air.

Table 29. Food consumption[a] of pregnant rats exposed to propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[b] I	Contrast[b] II	Contrast[b] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	PO			
1 to 6 dg	FA	FA	PO	PO			
7 to 16 dg	FA	PO	PO	PO			
NO. RATS:	46	44	41	43			
TIME OF MEASUREMENT							
Pregestation							
Week 1	18.6 ± 1.7	19.1 ± 3.4	18.9 ± 1.6	17.1 ± 1.6	0.50	<0.01*	0.63
Week 2	18.4 ± 1.0	18.5 ± 1.8	18.2 ± 0.1	15.8 ± 0.9	<0.01*	<0.01*	0.28
Week 3	19.6 ± 1.6	19.7 ± 1.9	19.4 ± 1.5	18.5 ± 0.9	0.20	<0.01*	0.33
Gestation Days							
1 to 6	20.4 ± 2.6	20.8 ± 2.7	20.0 ± 3.4	18.8 ± 3.4	0.32	<0.01*	0.22
7 to 11	27.0 ± 6.1	18.1 ± 2.6	19.2 ± 5.4	19.1 ± 5.0	<0.01*	0.68	0.32
12 to 16	23.8 ± 3.5	20.5 ± 2.9	20.3 ± 1.7	21.9 ± 4.1	<0.01*	0.02*	0.79
17 to 21	18.5 ± 2.8	20.8 ± 4.1	21.8 ± 1.4	18.2 ± 3.3	<0.01*	<0.01*	0.16

[a] Grams/rat/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).

Table 30. Body weights (g, mean ± SD) of pregnant rats exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	Contrast[a] II	Contrast[a] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	PO			
1 to 6 dg	FA	FA	PO	PO			
7 to 16 dg	FA	PO	PO	PO			
NO. RATS:	46	44	41	43			
TIME OF MEASUREMENT							
Randomization	170 ± 14	173 ± 14	173 ± 14	167 ± 11	0.95	0.07	0.45
Pregestation day							
3[b]	209 ± 16	215 ± 15	213 ± 17	213 ± 13	0.11	0.62	0.68
8	216 ± 16	220 ± 14	217 ± 17	191 ± 14	0.15	<0.01*	0.36
11	222 ± 19	228 ± 19	228 ± 20	212 ± 15	0.92	<0.01*	0.89
15	226 ± 18	230 ± 18	230 ± 18	204 ± 13	0.10	<0.01*	0.95
18	236 ± 19	241 ± 17	240 ± 19	211 ± 14	0.06	<0.01*	0.87
21	238 ± 18	241 ± 16	241 ± 19	213 ± 14	0.02*	<0.01*	0.99
Gestation day							
1	237 ± 18	240 ± 17	239 ± 19	221 ± 16	0.17	<0.01*	0.96
6	266 ± 20	267 ± 20	254 ± 19	237 ± 15	<0.01*	<0.01*	<0.01*
11	285 ± 22	269 ± 21	259 ± 19	257 ± 16	<0.01*	<0.01*	0.02*
16	305 ± 29	284 ± 21	281 ± 20	268 ± 20	<0.01*	<0.01*	0.50
21	351 ± 32	333 ± 26	338 ± 25	317 ± 32	<0.01*	<0.01*	0.44

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3.
*indicates Contrast is significant ($P \leq 0.05$).
[b] Corresponds to the initiation of pregestational exposure

Table 31. Body and organ weights (mean ± SD) of pregnant rats exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	Contrast[a] II	Contrast[a] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	PO			
1 to 6 dg	FA	FA	PO	PO			
1 to 16 dg	FA	PO	PO	PO			
NO. RATS:	46	44	41	43			
WEIGHT MEASUREMENT							
Body (g)	351 ± 32	333 ± 26	338 ± 25	317 ± 32	<0.01*	<0.01*	0.44
Pregnant uterus (g)	70 ± 14	66 ± 12	72 ± 10	63 ± 17	0.12	0.02*	0.03*
Extragestational (g)[b]	280 ± 26	267 ± 21	266 ± 20	253 ± 25	<0.01*	<0.01*	0.77
Lung							
Absolute[c]	1.24 ± 0.16	1.19 ± 0.11	1.21 ± 0.16	1.19 ± 0.14	0.06	0.75	0.45
Relative[d]	0.45 ± 0.06	0.45 ± 0.04	0.46 ± 0.06	0.47 ± 0.06	0.17	0.04*	0.36
Liver							
Absolute	13.7 ± 1.40	12.8 ± 1.6	13.2 ± 1.5	12.8 ± 1.7	0.01*	0.32	0.22
Relative	4.89 ± 0.30	4.80 ± 0.45	4.99 ± 0.40	5.04 ± 0.52	0.45	0.07	0.05*
Kidneys							
Absolute	1.92 ± 0.16	1.88 ± 0.19	1.87 ± 0.19	1.83 ± 0.18	0.06	0.18	0.92
Relative	0.69 ± 0.07	0.70 ± 0.05	0.71 ± 0.05	0.73 ± 0.06	0.02*	0.07	0.86
Spleen							
Absolute	0.62 ± 0.14	0.57 ± 0.08	0.59 ± 0.09	0.61 ± 0.09	0.04*	0.20	0.29
Relative	0.22 ± 0.06	0.21 ± 0.03	0.22 ± 0.03	0.24 ± 0.04	0.86	<0.01*	0.25
Ovaries							
Absolute	0.12 ± 0.03	0.12 ± 0.03	0.12 ± 0.02	0.11 ± 0.03	0.26	0.44	0.37
Relative	0.044 ± 0.008	0.045 ± 0.011	0.044 ± 0.009	0.045 ± 0.009	0.45	0.72	0.46

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Absolute weight in grams
[d] Relative weight = (weight of organ/extragestational weight) x 100

Table 32. Histopathologic observations (number of examined animals that had tissue changes) in rats exposed to 500 ppm propylene oxide (PO) or filtered air (FA).[a]

	Group 1	Group 2	Group 3	Group 4
EXPOSURE INTERVAL				
Pregestation	FA	FA	FA	PO
1 to 6 dg	FA	FA	PO	PO
7 to 16 dg	FA	PO	PO	PO
NO. RATS NECROPSIED:	48	48	47	45
NO. PREGNANT RATS EXAMINED MICROSCOPICALLY:	15	11	12	13
NO. NONPREGNANT RATS EXAMINED MICROSCOPICALLY:	1	2	3	0
OBSERVATION				
Lung				
Focal mononuclear inflammation	2[a]	4	4	5
Eosinophilic inflammation	0	0	1	0
Liver				
Portal mononuclear inflammation	2	0	0	1
Focal mononuclear inflammation	2	3	4	3
Kidney				
Focal interstitial mononuclear inflammation	2	1	4	1
Suppurative inflammation	0	1	0	0
Hydronephrosis	1	1	2	3[b]
Mineralization	0	0	1	0
Spleen				
Chronic/active inflammation	1	0	0	0
Hemosiderosis	0	2	0	0
Ovaries				
Corpora lutea regression[c]	1	2	3	0
Uterus	0	0	0	0

[a] The severity of all lesions ranged from minimal to mild, with the exceptions noted below.
[b] Hydronephrosis in rat 959 was moderate.
[c] Correlated with animals that were not pregnant

also within the range expected. The spleen of one Group 4 rat had moderate focal necrosis with mixed cell inflammation and fibrosis; the etiology of this lesion is unknown. The degree of splenic hemosiderosis was slightly above the normal range in two Group 2 rats. Corpora luteal regression was correlated with the nonpregnant uteri except for one nonpregnant Group 4 rat that had equivocal corpora luteal involution.

Fertility and Reproductive Status

A 15-day exposure to propylene oxide prior to breeding had no effect on the mating performance of the rats (Table 33). However, the numbers of corpora lutea per dam, implantation sites per dam, and live fetuses per litter were decreased in rats receiving a pregestational exposure to propylene oxide (Contrast II in Table 34). A larger number of resorptions (occurring at early and midgestation) and a higher percentage of resorbed implantation sites were observed in Group 2 rats, exposed from 7 to 16 dg, than in animals exposed from 1 to 16 dg (Contrast III). Despite the increased resorption rate in Group 2 rats, the number of live fetuses per litter was not significantly different from that of Group 3.

Table 33. Mating performance of rats exposed to 500 ppm propylene oxide (PO) or filtered air (FA).[a]

	Pregestational Exposure	
	FA	PO
NO. EXPOSED RATS	170	50
OBSERVATION		
No. sperm-positive rats	154	45
No. sperm-negative rats	16	5
No. sperm-negative rats pregnant at sacrifice (9 to 16 dg)	1	1
Mating failure rate (%)[b]	9	8

[a] Rats were exposed for 7 hr/day, 5 days/wk for 3 wk prior to mating.
[b] Chi-square test: P = 0.86

Table 34. Reproductive status of rats exposed to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	PO			
1 to 6 dg	FA	FA	PO	PO			
7 to 16 dg	FA	PO	PO	PO			
OBSERVATION							
Percent of sperm-positive rats pregnant at 21 dg	96	92	87	96			
No. pregnant females examined	46	44	41	43			
No. litters with live fetuses	46	44	41	42			
No. corpora lutea/dam	15.4 ± 3.1[b]	15.6 ± 3.3	15.4 ± 2.2	13.8 ± 3.0	0.36	<0.01*	0.78
No. implantations sites/dam	13.9 ± 2.8	13.9 ± 2.1	14.3 ± 2.0	12.3 ± 3.2	0.44	<0.01*	0.44
No. resorptions/litter	0.87 ± 0.91	1.07 ± 1.11	0.56 ± 0.67	0.58 ± 0.70	0.38	<0.01*	0.01*
Early	0.85 ± 0.92	0.98 ± 1.11	0.54 ± 0.67	0.56 ± 0.67	0.29	0.01*	0.02*
Mid	0	0.09 ± 0.29	0	0	0.23	0.10	0.01*
Late	0.02 ± 0.15	0	0.02 ± 0.16	0.02 ± 0.15	0.80	0.65	0.39
Resorptions/implantation sites (%)	5.96 ± 6.27	7.88 ± 8.54	3.75 ± 4.43	6.61 ± 15.6	0.95	0.13	0.05*
No. litters with resorptions	27	28	19	20			
Litters with resorptions(%)[c]	58.7	63.6	46.3	46.5			
Resorptions/litters with resorptions	1.48 ± 0.70	1.68 ± 0.94	1.21 ± 0.42	1.26 ± 0.44	0.45	0.55	0.35
No. dead fetuses/litter	0	0	0	0			
No. live fetuses/litter	13.0 ± 2.7	12.8 ± 2.3	13.8 ± 1.9	11.7 ± 3.2	0.63	<0.01*	0.09
Total no. live and dead fetuses	598	565	565	505			

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant (P ≤ 0.05).
[b] Mean ± SD
[c] Chi-square test (Group 1 versus 2 versus 3 versus 4): P = 0.26

Fetal Measures and Morphology

Body weights and crown-rump lengths of both male and female fetuses exposed in utero to propylene oxide were lower than those of the control rats (Contrast I in Table 35). Body weights for Group 4 female fetuses tended to be higher than those of Groups 2 and 3 (Contrast II), and weights of male fetuses were significantly higher. Placental weights for Group 2 were lower than values for Groups 3 and 4 (Contrasts II and III).

Major morphologic malformations were observed in two fetuses in Groups 1 and 3 and one fetus in Group 2 (Table 36). The lesions described as "generalized brain dysmorphology," included massive distortion of the external and internal architecture of the brain; irregularities in the size of the olfactory lobes; and abnormalities in the shape and size of cerebral hemispheres. Hemorrhage was apparent around exterior brain surfaces. A significant increase in the incidence of fetal rib dysmorphology, particularly wavy ribs, occurred in all groups exposed to propylene oxide. Reduced ossification of vertebrae and ribs was evident in Group 3, in comparison with the fraction of affected litters in Group 1.

SUMMARY OF EFFECTS OF PROPYLENE OXIDE EXPOSURE

The effect of the exposure of rabbits to 500 ppm of propylene oxide for 7 hr/day both from 1 to 19 dg and from 7 to 19 dg was limited to periods during which food consumption and body weights of maternal animals were decreased (Table 37). These results suggest that propylene oxide was toxic to the maternal rabbit under these exposure conditions. There was no consistent evidence of embryotoxicity, although rabbits receiving propylene oxide exposure from 1 to 19 dg had a higher percentage of resorptions in litters with resorptions.

Consistent changes in indices for maternal toxicity in rats, however, were detected (Table 37). Food consumption was lower, and body weights and extragestational weights were depressed. These alterations were particularly evident in rats receiving both pregestational and gestational exposure to 500 ppm propylene oxide. Except for the spleen, changes in maternal organ weights appeared to correlate with body weight losses.

The reproductive status of the rats also was altered by exposure to propylene oxide. Although mating failure rates were unaffected by exposure to the chemical prior to breeding, the numbers of corpora lutea and implantation sites per dam and, consequently, the number of live fetuses per litter were lower in the rats receiving pregestational exposures (Group 4). Intrauterine mortality, as measured by number of resorptions and percentage of resorptions per implantation sites, particularly during early and midgestation was higher in rats exposed to propylene oxide from 7 to 16 dg (Group 2) than in those exposed from 1 to 16 dg (Group 3). The smaller litter size in Group 4 and higher resorption rate in Group 2 account for the decreased weight of the pregnant uteri in these two exposure groups.

Fetal size was reduced by exposure to propylene oxide, especially in rats receiving only the gestational exposures (Groups 2 and 3). Placental weights,

Table 35. Fetal measures (mean ± SD) for rat litters exposed in utero to 500 ppm propylene oxide (PO) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	PO			
1 to 6 dg	FA	FA	PO	PO			
7 to 16 dg	FA	PO	PO	PO			
OBSERVATION							
No. litters examined	46	44	41	43			
No. live fetuses	598	565	565	505			
Body weights (g)							
Female	3.53 ± 0.33	3.29 ± 0.27	3.41 ± 0.38	3.47 ± 0.33	0.02*	0.06	0.11
Male	3.70 ± 0.32	3.45 ± 0.30	3.52 ± 0.41	3.63 ± 0.34	<0.01*	0.03*	0.35
Crown-rump length (mm)							
Female	36.0 ± 1.7	35.1 ± 1.5	35.2 ± 2.1	35.6 ± 2.0	0.02*	0.20	0.84
Male	36.8 ± 1.6	36.0 ± 1.6	35.9 ± 1.9	36.3 ± 1.8	0.01*	0.28	0.74
Stunted[b]	(1/1) 2.2	0	(1/1) 2.4	0			
Placenta weight (g)	0.49 ± 0.06	0.47 ± 0.05	0.50 ± 0.05	0.51 ± 0.06	0.90	<0.01*	<0.01*
Sex ratio (% male)	50.8 ± 12.1	50.2 ± 12.7	45.7 ± 17.0	46.2 ± 17.4	0.18	0.52	0.17

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).

[b] Expressed as: (number of stunted fetuses/number of litters) percentage of litters affected

Table 36. Morphologic alterations in fetal rats exposed in utero to 500 ppm propylene oxide (PO) or filtered air (FA).[a]

	Group 1		Group 2		Group 3		Group 4
EXPOSURE INTERVAL							
Pregestation	FA		FA		FA		PO
1 to 6 dg	FA		FA		PO		PO
7 to 16 dg	FA		PO		PO		PO
OBSERVATION							
No. litters with live fetuses	46		44		41		42
No. fetuses examined	598		565		565		505
No. heads examined	296		284		283		251
MAJOR MALFORMATIONS							
Hydrocephaly	0		0		(1/1)	2.4	0
Meningocele	(1/1)	2.2	0		0		0
Generalized brain dysmorphology	(1/1)	2.2	0		0		0
Rachischisis	0		(1/1)	2.3[b]	0		0
Vertebral agenesis	0		0		(1/1)	2.4[c]	0
Adrenal agenesis	0		(1/1)	2.3[b]	0		0
MINOR ANOMALIES							
Visceral anomalies							
Lung hypoplasia	0		0		(1/1)	2.4[c]	0
Fused adrenals	0		(1/1)	2.3[b]	(1/1)	2.4[c]	0
Fused kidneys	0		0		(1/1)	2.4[c]	0
Ectopic gonads	0		(1/1)	2.3[b]	(1/1)	2.4[c]	0
Situs inversus totalis	0		(1/1)	2.3	0		0

Musculoskeletal anomalies								
Ectro/syndactyly	0		0		(1/1)	2.4[c]	0	
Brachyury	0		(1/1)	2.3[b]	(1/1)	2.4[c]	0	
Anury	0		0		(1/1)	2.4	0	
Rib dysmorphology	(1/1)	2.2	(12/6)	13.6[d]	(29/10)	24.4[d]	(18/9)	21.4[d]
Wavy	0		(12/6)	13.6	(28/10)	24.4	(18/9)	21.4
Bent	0		0		(1/1)	2.4	0	
Fused	(1/1)	2.2	0		0		0	
Sternebral anomalies	(2/1)	2.2	(1/1)	2.3	0		(1/1)	2.4
Misaligned	0		(1/1)	2.3	0		0	
Bipartite	(2/1)	2.2	0		0		(1/1)	2.4
MORPHOLOGIC VARIATIONS								
Renal variations	(8/5)	10.9	(6/6)	13.6	(11/6)	14.6	(16/7)	16.7
Hydroureter	(8/5)	10.9	(6/6)	13.6	(11/6)	14.6	(16/7)	16.7
Renal pelvic cavitation	(1/1)	2.2	0		0		(1/1)	2.4
Supernumerary ribs	(4/4)	8.7	(4/3)	6.8	(1/1)	2.4	(2/2)	4.8
Rudimentary	(2/2)	4.3	(1/1)	2.3	0		0	
Ossification at lumbar I	(2/2)	4.3	(3/2)	4.5	(1/1)	2.4	(2/2)	4.8
Reduced ossification	(512/46)	100.0	(530/44)	100.0	(527/41)	100.0	(446/42)	100.0
Skull	(10/6)	13.0	(10/4)	9.1	(15/8)	19.5[e]	(9/7)	16.7
Vertebra	(59/27)	58.7	(83/26)	59.0	(166/34)	82.9[e]	(98/30)	71.4
Sternebra	(506/46)	100.0	(529/44)	100.0	(523/41)	100.0[f]	(439/42)	100.0
Ribs	(1/1)	2.2	(6/4)	9.1	(11/8)	19.5[f]	(1/1)	2.4
Pelvis	(4/3)	6.5	(3/3)	6.8	(7/5)	12.2	(2/2)	4.8
Phalanges	(2/2)	4.3	(3/2)	4.5	(3/3)	7.3	(1/1)	2.4

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected
[b] Fetus 13, litter 2849
[c] Fetus 5, litter 2948
[d] Chi-square test for rib dysmorphology: P = 0.03 for Group 1 versus 2; P = 0.001 for Group 1 versus 3; P = 0.003 for Group 1 versus 4
[e] Chi-square test for reduced vertebral ossification: P = 0.01 for Group 1 versus 3
[f] Chi-square test for reduced ossification of ribs: P = 0.02 for Group 1 versus 3

Table 37. Summary of significant effects ($P \leq 0.05$) of propylene oxide exposure on pregnant rats and rabbits.[a]

	RAT					RABBIT			
	Observation Period	Contrast I	Contrast II	Contrast III	Reference	Observation Period	Contrast I	Contrast II	Reference
MATERNAL OBSERVATIONS									
Food consumption									
	Pregestation								
	Week 1		2,3>4		Fig. 13				
	Week 2	1>2,3,4	2,3>4		Table 29				
	Week 3		2,3>4						
	1 to 6 dg		2,3>4			1 to 5 dg		2>3	Fig. 12
	7 to 11 dg	1>2,3,4							Table 21
	12 to 16 dg	1>2,3,4	4>2,3			11 to 15 dg	1>2,3	2>3	
	17 to 21 dg	2,3,4>1	2,3>4			16 to 20 dg	1>2,3		
Body weight									
	Pregestation								
	Day 8		2,3>4		Fig. 13				
	11		2,3>4		Table 36				
	15		2,3>4						
	18		2,3>4						
	21	1>2,3,4	2,3>4						
	1 dg		2,3>4						
	6 dg	1>2,3,4	2,3>4	2>3					
	11 dg	1>2,3,4	2,3>4	2>3					
	16 dg	1>2,3,4	2,3>4			15 dg		2>3	Fig. 12
	21 dg	1>2,3,4	2,3>4						Table 22
Weight of pregnant uterus	21 dg		2,3>4	3>2	Table 31				Table 23
Extragestational weight		1>2,3,4	2,3>4						
Organ weights	21 dg								
Lungs - relative			4>2,3		Table 31				
Liver - absolute		1>2,3,4							
- relative				3>2					
Kidneys - relative		2,3,4>1							
Spleen - absolute		1>2,3,4							
- relative			4>2,3						
REPRODUCTIVE OBSERVATIONS	21 dg								
Corpora lutea/dam			2,3>4		Table 34				
Implantation sites/dam			2,3>4						
Resorptions - early			2,3>4	2>3					
- mid				2>3					
TOTAL			2,3>4	2>3					
Resorptions/implants (%)				2>3					
Resorptions/litters with resorptions (%)						30 dg		3>2	Table 26
Live fetuses/litter			2,3>4						
FETAL OBSERVATIONS	21 dg								
Body weight									
Female		1>2,3,4			Table 35				Table 27
Male		1>2,3,4	4>2,3						
Crown-rump length									
Female		1>2,3,4							
Male		1>2,3,4							
Placenta weight			4>2,3	3>2					
Incidence of rib dysmorphology		2,3,4>1[b]			Table 36				Table 28
Reduced ossification									
Vertebra		3>1[b]							
Rib		3>1[b]							

[a] Rat exposure to propylene oxide: Group 1, none; Group 2, 7 to 16 dg; Group 3, 1 to 16 dg; Group 4, pregestation, 1 to 16 dg. Rabbit exposure to propylene oxide: Group 1, none; Group 2, 7 to 19 dg; Group 3, 1 to 19 dg.

[b] Chi-square test

also decreased in these rats, were lowest in Group 2 animals. Major malformations were observed in two fetuses from the filtered-air-exposed group and in three fetuses from the two groups receiving gestational exposure to propylene oxide. The incidence of rib dysmorphology (primarily wavy ribs) was significantly higher in all groups of fetuses exposed in utero to propylene oxide. Reduced ossification in fetal vertebrae and ribs occurred more frequently following chemical exposure from 1 to 16 dg than after filtered-air exposure. It is not clear if these changes are due to maternal toxicity or are manifestations of developmental effects.

We may speculate that some degree of acclimation to the chemical or to exposure conditions occurred in the rats receiving the pregestational exposure since an increased resorption rate and a reduced fetal growth rate were observed in those whose propylene oxide exposure was initiated during gestation, especially at 7 dg. In the absence of an increased incidence of major malformations, the finding of a characteristic rib dysmorphology in all groups of propylene-oxide-exposed fetuses is also suggestive of an embryotoxic response under the maternally toxic conditions of the exposure.

n-BUTYL-ACETATE-EXPOSED RABBITS

Food Consumption and Body Weight

The lowest values for food consumption were consistently observed in animals from the group inhaling filtered air (Figure 14). When the two groups of n-butyl-acetate-exposed rabbits were compared (Contrast II in Table 38), there were significant differences in food consumption after the onset of exposure. From 1 to 5 dg, values for Group 2 (exposed to filtered air at this time) were higher than those of Group 3. Once the n-butyl-acetate exposure was initiated in Group 2, food consumption was lower than that of Group 3 until the exposure was terminated on 19 dg. Body weights of the rabbits (Table 39) were similar in both n-butyl-acetate-exposed groups, but the filtered-air-exposed rabbits tended to have the lowest body weights throughout the experimental period, particularly from 15 through 30 dg.

Organ Weights and Histopathology

Weights of the lungs, kidneys, and spleens of the pregnant, filtered-air-exposed rabbits were significantly lower than those of the n-butyl-acetate-exposed groups (Contrast I in Table 40). When relative organ weights (which correct for differences in body weight) were considered, these differences were not significant, although spleen weights tended to be lower in the filtered-air-exposed animals ($P < 0.06$).

Histopathologic studies performed on the rabbits necropsied at 30 dg did not relate any observed lesions to the n-butyl acetate exposures (Table 41). A variety of lung lesions were seen, some of which are probably within the range of normal; others may be related to Pasteurella infections. Small foci of mononuclear inflammatory cells were common around alveoli, small airways and small blood vessels. A few bronchi and bronchioles contained small accumulations of heterophils and minimal to mild increases in bronchus-associated lymphoid tissue (BALT). Epithelial hyperplasia of bronchi was mild in the

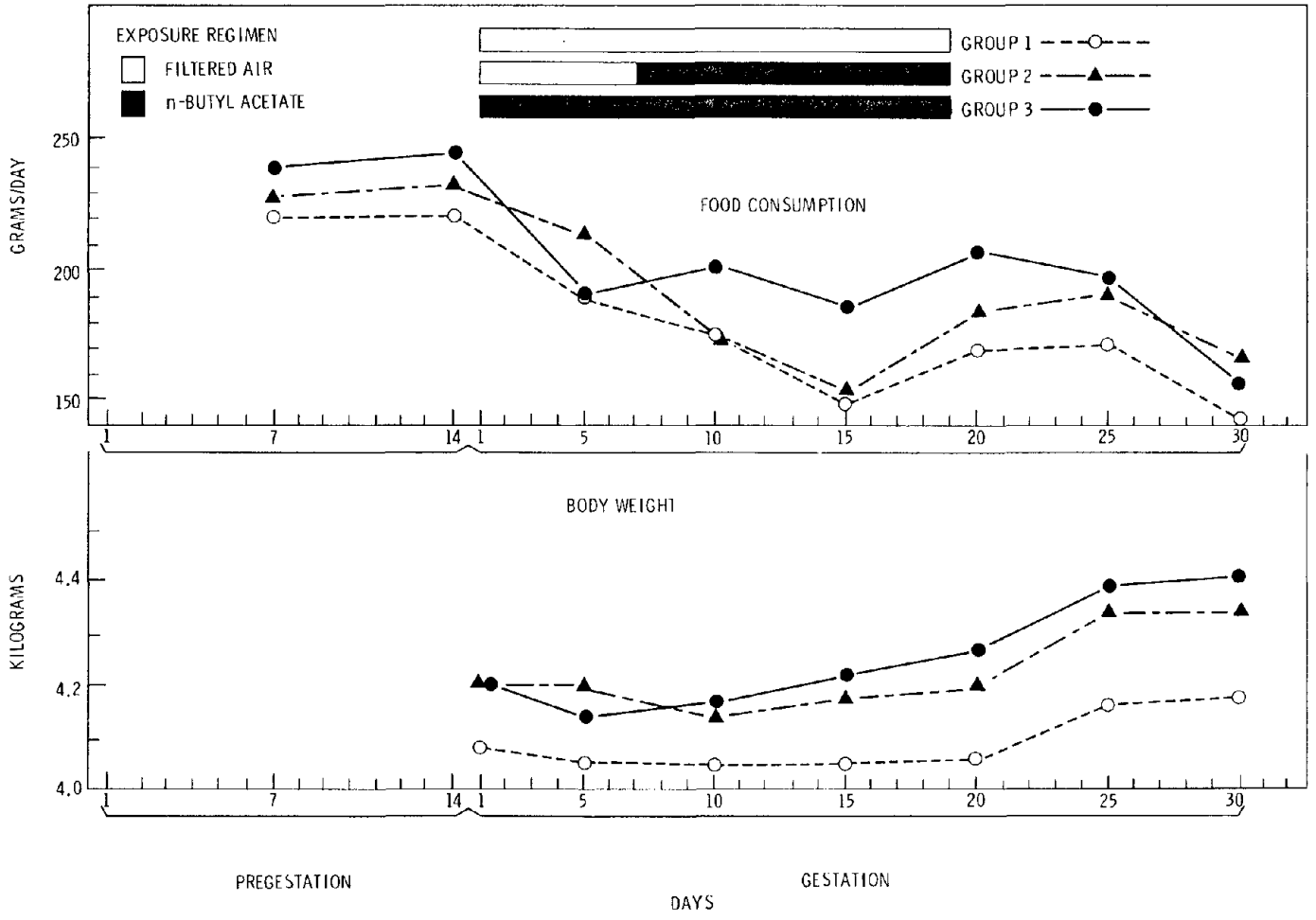

Figure 14. Food consumption and body weights of pregnant rabbits inhaling 1500 ppm n-butyl acetate or filtered air.

Table 38. Food consumption[a] of pregnant rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[b] I	Contrast[b] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	BA		
7 to 19 dg	FA	BA	BA		
NO. RABBITS:	22	21	25		
TIME OF MEASUREMENT					
Pregestation					
Week 1	220 ± 47	228 ± 40	239 ± 31	0.19	0.33
Week 2	221 ± 47	233 ± 42	245 ± 37	0.10	0.36
Gestation Days					
1 to 5	189 ± 52	215 ± 29	190 ± 35	0.22	0.04*
6 to 10	175 ± 36	173 ± 40	201 ± 35	0.22	0.01*
11 to 15	148 ± 48	154 ± 52	185 ± 51	0.10	0.04*
16 to 20	169 ± 54	173 ± 55	207 ± 74	0.21	0.07
21 to 25	171 ± 49	190 ± 57	197 ± 75	0.16	0.70
26 to 30	142 ± 56	166 ± 82	156 ± 70	0.29	0.61

[a] Grams/rabbit/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3.
*indicates Contrast is significant ($P \leq 0.05$).

Group 1 rabbits and minimal in the two n-butyl-acetate-exposed groups. The changes were evenly distributed in all groups.

Renal changes, consisting of minimal to mild tubular mineralization and subacute to chronic interstitial nephritis, were evenly distributed throughout all exposure groups. Incidental findings of extramedullary hematopoiesis in the spleen appeared minimally to mildly excessive in only four animals, one of which had peritonitis.

Regressing corpora lutea corresponded well with the status of uterine sections from nonpregnant rabbits or from animals in which pregnancy was detected by uterine staining (two rabbits in Group 1). One Group 3 rabbit had such severe suppurative metritis and peritonitis that ovarian tissue could not be identified.

Table 39. Body weight (kg, mean ± SD) of pregnant rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	BA		
7 to 19 dg	FA	BA	BA		
NO. RABBITS:	22	21	25		
TIME OF MEASUREMENT					
Randomization	4.00 ± 0.22	4.05 ± 0.35	3.96 ± 0.62	0.98	0.76
Gestation day					
1	4.09 ± 0.25	4.20 ± 0.35	4.20 ± 0.33	0.16	0.92
5	4.05 ± 0.26	4.20 ± 0.34	4.14 ± 0.32	0.17	0.53
10	4.05 ± 0.25	4.14 ± 0.36	4.17 ± 0.30	0.19	0.68
15	4.05 ± 0.23	4.18 ± 0.37	4.22 ± 0.34	0.07	0.67
20	4.06 ± 0.24	4.20 ± 0.42	4.27 ± 0.40	0.06	0.50
25	4.16 ± 0.29	4.34 ± 0.45	4.39 ± 0.47	0.06	0.66
30	4.18 ± 0.30	4.34 ± 0.38	4.41 ± 0.49	0.07	0.15

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3

Fertility and Reproductive Status

Indices for fertility and reproductive performance in rabbits were unaffected by exposure to n-butyl acetate (Tables 42 and 43). Differences among exposure groups with regard to pregnancy rates, numbers of corpora lutea, implantation sites, resorptions, and live fetuses per litter were unremarkable.

Fetal Measures and Morphology

Fetal growth measures, such as body weight and crown-rump length, were similar for n-butyl-acetate and filtered-air-exposed rabbits (Table 44). Placental weights and sex ratios were also similar.

No major malformations were observed in fetuses from any exposure group (Table 45). There was a significantly higher incidence of misaligned sternebrae and of retinal folds among fetuses from rabbits inhaling n-butyl acetate from

Table 40. Body and organ weights (mean ± SD) of pregnant rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	BA		
7 to 19 dg	FA	BA	BA		
NO. RABBITS:	22	21	25		
WEIGHT MEASUREMENT					
Body (g)	4181 ± 302	4337 ± 381	4412 ± 491	0.07	0.15
Pregnant uterus (g)	411 ± 236	402 ± 253	470 ± 233	0.68	0.58
Extragestational (g)[b]	3770 ± 263	3933 ± 367	3943 ± 443	0.08	0.22
Lung					
Absolute[c]	12.9 ± 1.5	14.1 ± 1.8	14.3 ± 2.1	0.01*	0.70
Relative[d]	0.34 ± 0.04	0.36 ± 0.04	0.37 ± 0.06	0.14	0.54
Liver					
Absolute	110.3 ± 18.6	118.0 ± 25.3	118.7 ± 25.3	0.19	0.92
Relative	2.91 ± 0.36	2.98 ± 0.51	2.99 ± 0.42	0.48	0.91
Kidneys					
Absolute	21.1 ± 2.9	22.5 ± 3.0	23.4 ± 4.0	0.04*	0.36
Relative	0.56 ± 0.07	0.57 ± 0.05	0.60 ± 0.10	0.24	0.21
Spleen					
Absolute	1.41 ± 0.46	1.69 ± 0.51	2.00 ± 1.01	0.02*	0.14
Relative	0.037 ± 0.011	0.043 ± 0.012	0.052 ± 0.029	0.06	0.13
Ovaries					
Absolute	0.87 ± 0.23	0.93 ± 0.28	0.93 ± 0.16	0.29	0.98
Relative	0.023 ± 0.006	0.024 ± 0.007	0.024 ± 0.005	0.66	0.84

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Absolute weight in grams
[d] Relative weight = (weight of organ/extragestational weight) x 100

Table 41. Histopathologic observations (number of examined animals that had tissue changes) in rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	BA
7 to 19 dg	FA	BA	BA
NO. RABBITS NECROPSIED	29	29	28
NO. PREGNANT RABBITS EXAMINED MICROSCOPICALLY	6	5	14
NO. NONPREGNANT RABBITS EXAMINED MICROSCOPICALLY	7	6	2
OBSERVATION			
Lung			
Focal mononuclear inflammation	10[a]	8	14
Bronchial epithelial hyperplasia	2	2	7
Increased bronchus-associated lymphoid tissue	1	1	2
Suppurative lesions	2	1	0
Liver			
Portal mononuclear inflammation	8	9	13
Focal fibrosis	0	0	1
Hepatocellular vacuolation	1	0	1
Eimeria stiedae	1	4	1
Kidney			
Subacute/chronic nephritis	6	7	11[c]
Tubular mineralization	9	10	10
Spleen			
Extramedullary hematopoiesis	1	1	2[c]
Ovaries			
Corpora lutea regression[b]	9	6	2
Uterus			
Suppurative inflammation	0	1	1[c]

[a] The severity of all lesions ranged from minimal to mild, with the exceptions noted below.

[b] Correlated with animals that were not pregnant or whose pregnancy was detectable only by uterine staining.

[c] Doe 4150 had moderate nephritis, severe suppurative metritis; and peritonitis; ovaries were not identifiable.

Table 42. Fertility of rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3
EXPOSURE INTERVAL			
1 to 6 dg	FA	FA	BA
7 to 19 dg	FA	BA	BA
OBSERVATION			
No. inseminated	30	30	30
No. pregnant at sacrifice (30 dg)	22	21	25
No. not pregnant at sacrifice (30 dg)	7	8	3
No. delivered prematurely	0	0	1[a]
No. died	1[b]	1[c]	1[d]
Percent pregnant at 30 dg[e]	76	72	89
Total percent pregnant[f]	77	73	90

[a] Doe 6110 delivered 8 viable kits on 29 dg.
[b] Doe 6120 died of pneumonia on 16 dg; 13 apparently viable implants.
[c] Doe 4217 died of pneumonia on 18 dg; 2 implants, both early resorptions.
[d] Doe 6152 died on 26 dg; 3 apparently viable implants. Kidney lesions were observed.
[e] Chi-square test (Group 1 versus 2 versus 3): P = 0.26.
[f] Chi-square test (Group 1 versus 2 versus 3): P = 0.23.

1 to 19 dg (Group 3) than in those exposed to filtered air (Group 1). The presence of a clear liquid in the gallbladder, rather than bile, was a more frequent variation in Group 3 fetuses than in those of Group 1.

n-BUTYL-ACETATE-EXPOSED RATS

Food Consumption and Body Weight

Food consumption was decreased in the group of rats exposed to n-butyl acetate during the first week of their pregestational exposure. These rats may have become conditioned to exposure during the second week since their food consumption for this period was higher than that of the three filtered-air-exposed groups (Figure 15, Contrasts I and II in Table 46). Food consumption during the gestational exposure (1 to 16 dg) was higher in the control animals (Group 1) than in the n-butyl-acetate-exposed rats (Contrast I). Food consumption declined significantly (Contrast III) in the period immediately following the initiation of chemical exposure (1 dg for Group 3 and 7 dg for

Table 43. Reproductive status of rabbits exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	BA		
7 to 19 dg	FA	BA	BA		
OBSERVATION					
Percent pregnant at 30 dg	76	72	89		
No. pregnant females examined	22	21	25		
No. litters with live fetuses	18	16	22		
No. corpora lutea/dam	11.7 ± 4.4[b]	12.1 ± 4.5	13.0 ± 4.1	0.44	0.53
No. implantations sites/dam	7.6 ± 3.9	7.8 ± 4.3	8.4 ± 4.0	0.64	0.63
No. resorptions/litter	1.09 ± 1.34	1.38 ± 1.40	1.12 ± 1.30	0.65	0.51
Early	0.73 ± 1.28	1.10 ± 1.30	0.72 ± 1.14	0.58	0.31
Mid	0.05 ± 0.21	0.05 ± 0.22	0.08 ± 0.28	0.77	0.65
Late	0.32 ± 0.84	0.24 ± 0.77	0.32 ± 0.56	0.84	0.70
Resorptions/implantation sites (%)	25.6 ± 38.0	33.5 ± 40.0	19.6 ± 32.5	0.93	0.20
No. litters with resorptions	11	13	13		
Litters with resorptions (%)[c]	50.0	61.9	52.0		
Resorptions/litters with resorptions	2.18 ± 1.08	2.23 ± 1.09	2.15 ± 0.99	0.98	0.85
No. dead fetuses/litter	0	0	0		
No. live fetuses/litter	6.50 ± 4.22	6.43 ± 4.53	7.24 ± 4.10	0.76	0.52
Total no. live and dead fetuses	143	135	181		

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3
[b] Mean ± SD
[c] Chi-square test (Group 1 versus 2 versus 3): P = 0.70

Table 44. Fetal measures (mean ± SD) for rabbit litters exposed in utero to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Contrast[a] I	Contrast[a] II
EXPOSURE INTERVAL					
1 to 6 dg	FA	FA	BA		
7 to 19 dg	FA	BA	BA		
NO. LITTERS EXAMINED	18	16	22		
NO. LIVE FETUSES	143	135	181		
OBSERVATION					
Body weights (g)					
Female	47.9 ± 9.0	46.9 ± 7.5	48.5 ± 7.6	0.93	0.55
Male	48.3 ± 9.9	49.3 ± 7.9	48.8 ± 7.4	0.76	0.86
Crown-rump length (mm)					
Female	101.7 ± 7.4	100.9 ± 6.4	100.3 ± 6.8	0.59	0.78
Male	101.3 ± 7.3	101.4 ± 6.8	102.0 ± 6.0	0.83	0.76
Stunted[b]	(2/2) 11.1	(1/1) 6.3	0		
Placenta weight (g)	5.8 ± 1.7	5.6 ± 0.8	6.1 ± 1.3	0.88	0.28
Sex ratio (% male)	54.5 ± 20.9	52.3 ± 18.9	58.9 ± 20.0	0.84	0.32

[a] Probability for Contrast: I = Group 1 versus 2,3; II = Group 2 versus 3
[b] Expressed as: (number of stunted fetuses/number of litters) percentage of litters affected

Table 45. Morphologic alterations[a] in fetal rabbits exposed in utero to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1		Group 2		Group 3	
EXPOSURE INTERVAL						
1 to 6 dg	FA		FA		BA	
7 to 19 dg	FA		BA		BA	
OBSERVATION						
No. litters with live fetuses	18		16		22	
No. fetuses examined	143		135		181	
No. heads examined	71		67		94	
MINOR ANOMALIES						
Rib dysmorphology	(1/1)	5.6	0		(2/2)	9.1
Branched	0		0		(1/1)	4.5
Knobby	(1/1)	5.6	0		(1/1)	4.5
Spade	0		0		(1/1)	4.5
Sternebral anomalies	(2/2)	11.1	(4/3)	18.8	(10/8)	36.4[b]
Misaligned	(1/1)	5.6	(2/2)	12.6	(9/7)	31.8[b]
Fused	0		0		(1/1)	4.5
Bipartite	(1/1)	5.6	(1/1)	6.3	0	
Extra ossification site	0		(1/1)	6.3	(1/1)	4.5
Other anomalies						
Lung hypoplasia	(1/1)	5.6[c]	0		0	
Retroesophageal aortic arch	(1/1)	5.6[c]	0		0	
Retinal folds	(5/2)	11.1	(1/1)	6.3	(14/9)	40.9[d]
MORPHOLOGIC VARIATIONS						
Supernumerary ribs	(86/18)	100.0	(99/16)	100.0	(117/22)	100.0
Extra	(62/16)	88.9	(74/14)	87.5	(83/22)	100.0
Rudimentary	(27/12)	66.7	(35/12)	75.0	(43/18)	81.8
Ossification at lumbar I	(2/2)	11.1	(3/3)	18.8	(5/5)	22.7
Reduced ossification	(52/16)	88.9	(66/16)	100.0	(81/21)	95.5
Vertebra	(1/1)	5.6	(2/2)	12.5	(1/1)	4.5
Sternebra	(52/16)	88.9	(64/16)	100.0	(80/21)	95.5
Other variations						
Dilated foramen ovale	0		0		(1/1)	4.5
Bifurcated gallbladder	0		0		(1/1)	4.5
Clear gallbladder	(9/3)	16.7	(7/4)	25.0	(22/10)	45.4[e]

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected

[b] Fisher's Exact test for misaligned sternebra: P = 0.04 for Group 1 versus 3

[c] Lung and cardiovascular anomalies in fetus 6, litter 6129, Group 1

[d] Fisher's Exact test for retinal folds: P = 0.04 for Group 1 versus 3

[e] Fisher's Exact test for clear gallbladder: P = 0.05 for Group 1 versus 3

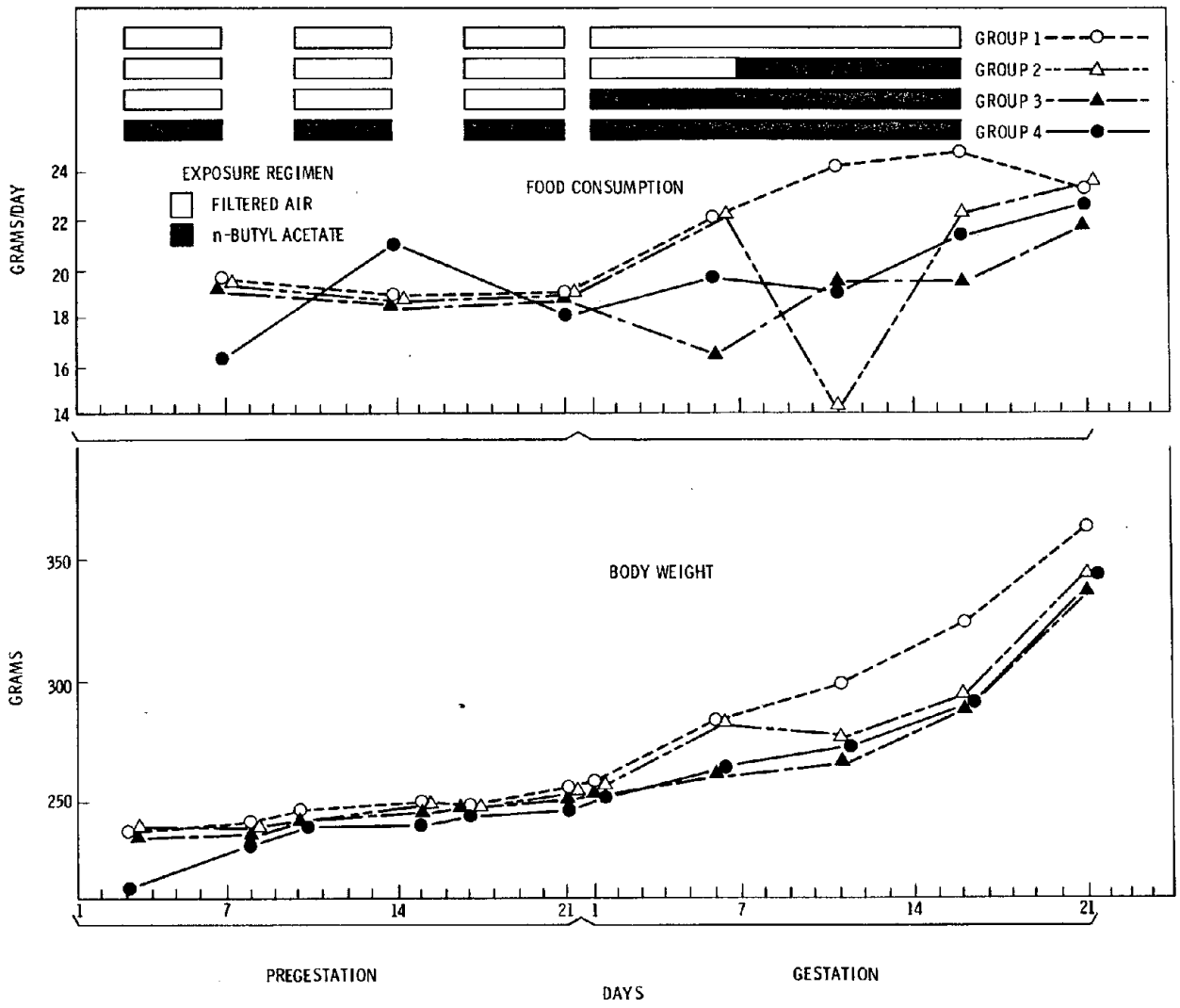

Figure 15. Food consumption and body weights of pregnant rats inhaling 1500 ppm n-butyl acetate or filtered air.

Table 46. Food consumption[a] of pregnant rats exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[b] I	Contrast[b] II	Contrast[b] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	BA			
1 to 6 dg	FA	FA	BA	BA			
7 to 16 dg	FA	BA	BA	BA			
NO. RATS:	37	42	38	43			
TIME OF MEASUREMENT							
Pregestation							
Week 1	19.6 ± 1.2	19.5 ± 1.2	19.3 ± 0.9	16.3 ± 1.1	<0.01*	<0.01*	0.45
Week 2	18.9 ± 0.8	18.8 ± 0.8	18.6 ± 0.7	21.0 ± 2.3	0.02*	<0.01*	0.59
Week 3	19.0 ± 1.6	19.0 ± 1.6	18.8 ± 1.3	18.1 ± 0.8	0.21	0.03*	0.42
Gestation Days							
1 to 6	22.1 ± 1.1	22.2 ± 1.2	16.4 ± 2.3	19.7 ± 1.7	<0.01*	<0.01*	<0.01*
7 to 11	24.2 ± 3.4	14.2 ± 4.3	19.5 ± 4.3	19.0 ± 5.4	<0.01*	<0.01*	<0.01*
12 to 16	24.8 ± 2.2	22.3 ± 1.8	19.5 ± 2.2	21.4 ± 3.1	<0.01*	<0.01*	<0.01*
17 to 21	23.4 ± 5.0	23.5 ± 2.9	21.8 ± 3.0	22.7 ± 2.1	0.24	0.06	0.03*

[a] Grams/rat/day (mean ± SD)
[b] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).

Group 2). Values for Group 2 recovered and were higher than those of Group 3, even during the period following termination of exposure.

There was a loss of body weight in the Group 4 rats during the acclimatization period (between randomization and the initiation of pregestational exposure, Table 47). This may have been a random variation since no apparent cause for this weight loss was found: food and water were readily available and there were no obvious signs of disease. By the end of the first week of exposure, body weights of these rats had recovered to the level of the other three exposure groups (Figure 15). By day 6 of the gestational exposure, body weights for Groups 3 and 4, which were inhaling n-butyl acetate, were lower than those of the filtered-air-exposed groups (Contrasts I, II and III). Body weights for the rats exposed to this chemical remained lower than those of control rats until sacrifice (Contrast I).

Organ Weights and Histopathology

At sacrifice, extragestational weight depression in n-butyl-acetate-exposed rats reflected patterns observed in body-weight comparisons (Contrast I in Table 48). Liver weights were also lower in rats inhaling n-butyl acetate (Contrast I). The relative weights of lungs and kidneys of n-butyl-acetate-exposed rats were higher than those of control animals, and these relative weights were highest in the rats exposed for 31 days (Contrasts I and II).

Tissue lesions observed during histopathologic examinations could not be related to n-butyl acetate exposure (Table 49). The only lung changes noted were small foci of mononuclear inflammatory cells in alveolar areas and around small blood vessels, and small foci of histiocytosis. These changes, observed in all groups, were considered minimal, nonspecific, and unimportant.

Liver changes, observed in all exposure groups, were apparent only as minimal mononuclear inflammatory cell populations in portal areas and minimal foci of mixed inflammatory cells scattered in the hepatic parenchyma. No changes were apparent in the spleens. Renal lesions were infrequently observed in any group; when observed, lesions were minimal to mild, except for one rat in Group 3 that had moderate hydronephrosis. Ovarian changes were limited to apparently regressing corpora lutea that correlated well with nonpregnant uteri.

Fertility and Reproductive Status

Mating performance, intrauterine mortality rate, and reproductive performance were unaffected by exposure of rats to n-butyl acetate (Tables 50 and 51).

Fetal Measures and Morphology

Body weights and crown-rump lengths of both male and female fetuses were lower in all n-butyl-acetate-exposed groups than in the filtered-air-exposed rats (Contrast I in Table 52). Neither the duration of exposure to the chemical nor the period of gestation at the initiation of exposure had a significant effect on fetal growth indices. Placental weight reductions also occurred in n-butyl-acetate-exposed rats (Contrast I). Sex ratios were unaffected.

Table 47. Body weights (g, mean ± SD) of pregnant rats exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	Contrast[a] II	Contrast[a] III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	BA			
1 to 6 dg	FA	FA	BA	BA			
7 to 16 dg	FA	BA	BA	BA			
NO. RATS:	37	42	38	43			
TIME OF MEASUREMENT							
Randomization	228 ± 21	228 ± 19	223 ± 21	231 ± 20	0.92	0.87	0.30
Pregestation day							
3[b]	239 ± 20	240 ± 21	236 ± 20	214 ± 34	0.05*	<0.01*	0.41
8	242 ± 20	241 ± 21	237 ± 21	233 ± 19	0.22	0.14	0.46
10	247 ± 22	247 ± 23	242 ± 21	241 ± 20	0.32	0.18	0.27
15	250 ± 23	249 ± 24	246 ± 21	241 ± 20	0.25	0.20	0.53
17	249 ± 22	248 ± 23	248 ± 22	245 ± 19	0.66	0.63	0.87
21	256 ± 22	255 ± 24	252 ± 22	247 ± 20	0.26	0.16	0.51
Gestation day							
1	259 ± 22	257 ± 23	254 ± 21	253 ± 22	0.27	0.41	0.60
6	283 ± 23	283 ± 24	261 ± 21	264 ± 24	<0.01*	<0.01*	<0.01*
11	299 ± 25	276 ± 23	267 ± 22	273 ± 25	<0.01*	0.18	0.09
16	324 ± 30	293 ± 24	288 ± 23	291 ± 27	<0.01*	0.49	0.39
21	363 ± 37	343 ± 30	336 ± 27	344 ± 33	<0.01*	0.61	0.33

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = 2 versus 3. * indicates Contrast is significant ($P \leq 0.05$).
[b] Corresponds to the initiation of pregestational exposure

Table 48. Body and organ weights (mean ± SD) of pregnant rats exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	BA			
1 to 6 dg	FA	FA	BA	BA			
1 to 16 dg	FA	BA	BA	BA			
NO. RATS:	37	42	38	43			
WEIGHT MEASUREMENT							
Body (g)	363 ± 37	343 ± 30	336 ± 27	344 ± 33	<0.01*	0.61	0.33
Pregnant uterus (g)	67 ± 16	64 ± 13	60 ± 15	64 ± 12	0.07	0.53	0.23
Extragestational (g)[b]	298 ± 33	280 ± 25	275 ± 25	281 ± 29	<0.01*	0.43	0.50
Lung							
Absolute[c]	1.22 ± 0.19	1.19 ± 0.11	1.19 ± 0.11	1.27 ± 0.21	0.96	0.22	0.99
Relative[d]	0.41 ± 0.06	0.42 ± 0.04	0.44 ± 0.04	0.45 ± 0.06	0.01*	0.05*	0.32
Liver							
Absolute	14.1 ± 2.1	13.2 ± 1.8	12.9 ± 1.4	13.5 ± 1.5	0.01*	0.95	0.49
Relative	4.72 ± 0.47	4.67 ± 0.45	4.71 ± 0.37	4.83 ± 0.46	0.83	0.23	0.67
Kidneys							
Absolute	2.07 ± 0.19	1.99 ± 0.22	2.01 ± 0.20	2.08 ± 0.21	0.26	0.18	0.72
Relative	0.70 ± 0.06	0.71 ± 0.06	0.73 ± 0.09	0.74 ± 0.07	0.03*	0.01*	0.07
Spleen							
Absolute	0.54 ± 0.09	0.51 ± 0.08	0.51 ± 0.07	0.52 ± 0.09	0.06	0.67	0.89
Relative	0.182 ± 0.023	0.180 ± 0.026	0.184 ± 0.025	0.187 ± 0.029	0.69	0.26	0.47
Ovaries							
Absolute	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.06	0.82	0.79
Relative	0.038 ± 0.007	0.037 ± 0.008	0.038 ± 0.007	0.038 ± 0.008	0.85	0.70	0.65

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$).
[b] Extragestational weight = (body weight) - (weight of pregnant uterus)
[c] Absolute weight in grams
[d] Relative weight = (weight of organ/extragestational weight) x 100

Table 49. Histopathologic observations (number of examined animals that had tissue changes) in rats exposed to n-butyl acetate (BA) or filtered air (FA).[a]

	Group 1	Group 2	Group 3	Group 4
EXPOSURE INTERVAL				
Pregestation	FA	FA	FA	BA
1 to 6 dg	FA	FA	BA	BA
7 to 16 dg	FA	BA	BA	BA
NO. RATS NECROPSIED:	45	45	44	46
NO. PREGNANT RATS EXAMINED MICROSCOPICALLY	14	12	13	11
NO. NONPREGNANT RATS EXAMINED MICROSCOPICALLY	2	2	1	2
OBSERVATION				
Lung				
Focal mononuclear inflammation	4	2	2	3
Alveolar histocytosis	1	1	0	2
Liver				
Portal mononuclear inflammation	3	1	3	0
Foci of mixed inflammatory cells	0	2	2	1
Hepatocellular vacuolation	1	0	0	0
Kidney				
Focal interstitial mononuclear inflammation	1	0	2	2
Hydronephrosis	1	0	2[b]	3
Mineralization	0	1	0	2
Spleen				
Ovaries				
Corpora lutea regression[c]	2	2	1	2
Uterus	0	0	0	0

[a] The severity of all lesions ranged from minimal to mild, with the exceptions noted below.
[b] Rat 3029 had moderate hydronephrosis.
[c] Correlated with animals that were not pregnant

Table 50. Mating performance of rats exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).[a]

	Pregestational Exposure	
	FA	BA
NO. EXPOSED RATS	170	50
OBSERVATION		
No. sperm-positive rats	145	46
No. sperm-negative rats	25	4
No. sperm-negative rats pregnant at sacrifice (10 to 17 dg)	6	0
Mating failure rate (%)[b]	11	8

[a] Rats were exposed for 7 hr/day, 5 days/wk for 3 wk prior to mating.
[b] Chi-square test: P = 0.52.

Two fetuses in Group 2, one in Group 3, and three in Group 4 had major malformations which included: multiple facial defects, eye defects, diaphragmatic hernias, and generalized brain dysmorphology. The lesions described as "generalized brain dysmorphology" (also observed in one fetus in the propylene oxide study), included massive distortion of the external and internal architecture of the brain; inequalities in size of the olfactory lobes, and abnormalities in shape and size of the cerebral hemispheres. Hemorrhage was apparent around exterior brain surfaces.

The incidence of rib dysmorphology was increased in fetuses of rats exposed to n-butyl acetate during gestation (Table 53). The incidence of wavy, fused and bifid ribs increased in rats exposed from 7 to 16 dg (P = 0.05), or from 1 to 16 dg (P = 0.07). Reduced pelvic ossification was also observed in fetuses of Groups 2 and 3 (P = 0.08 and P = 0.002, respectively). A larger number of fetuses with dilated ureters was noted in rats that were exposed to n-butyl acetate for 31 days than in the filtered-air-exposed animals.

SUMMARY OF EFFECTS OF n-BUTYL ACETATE EXPOSURE

Food consumption for both rats and rabbits was altered by exposure to n-butyl acetate (Table 54). Subsequent decreases in body weight, and in extragestational weight at sacrifice, occurred in rats but not in rabbits. These results indicate that maternal toxicity, as measured by body weight changes, occurred when rats were exposed to concentrations of 1500 ppm of n-butyl acetate per day.

Table 51. Reproductive status of rats exposed to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	BA			
1 to 6 dg	FA	FA	BA	BA			
7 to 16 dg	FA	BA	BA	BA			
OBSERVATION							
Percent sperm-positive females pregnant at 21 dg		82	93	89		93	
No. pregnant females examined		37	42	38[b]		43	
No. litters with live fetuses	37	42	38	43			
No. corpora lutea/dam	15.4 ± 2.7[c]	15.2 ± 2.5	15.3 ± 2.9	15.1 ± 2.5	0.69	0.93	0.93
No. implantations sites/dam	13.2 ± 2.9	13.7 ± 2.7	12.9 ± 3.1	13.5 ± 2.0	0.73	0.38	0.22
No. resorptions/litter	0.92 ± 1.16	0.88 ± 1.10	0.89 ± 1.06	1.00 ± 0.90	0.98	0.74	0.95
Early	0.81 ± 1.05	0.81 ± 1.02	0.79 ± 1.02	0.98 ± 0.89	0.80	0.70	0.93
Mid	0.11 ± 0.31	0.07 ± 0.26	0.11 ± 0.31	0	0.30	0.70	0.06
Late	0	0	0	0.02 ± 0.15			
Resorptions/implantation sites (%)	6.53 ± 7.94	6.08 ± 7.70	6.55 ± 7.62	7.73 ± 7.65	0.86	0.48	0.79
No. litters with resorptions	20	22	21	28			
Litters with resorptions (%)[b]	54.1	52.4	55.3	65.1			
Resorptions/litters with	1.70 ± 1.08	1.68 ± 0.99	1.62 ± 0.92	1.54 ± 0.64	0.64	0.70	0.65
No. dead fetuses/litter	0	0	0	0			
No. live fetuses/litter	12.3 ± 2.8	12.8 ± 2.7	12.1 ± 3.0	12.5 ± 2.3	0.74	0.31	0.21
Total no. live and dead fetuses	455	538	458	539			

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3
[b] Chi-square test (Group 1 versus 2 versus 3 versus 4): P = 0.64
[c] Mean ± SD

Table 52. Fetal measures (mean ± SD) for rat litters exposed in utero to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1	Group 2	Group 3	Group 4	Contrast[a] I	II	III
EXPOSURE INTERVAL							
Pregestation	FA	FA	FA	BA			
1 to 6 dg	FA	FA	BA	BA			
7 to 16 dg	FA	BA	BA	BA			
OBSERVATION							
No. litters examined	37	42	38	43			
No. live fetuses	455	538	458	539			
Body weights (g)							
Female	3.48 ± 0.38	3.07 ± 0.33	3.05 ± 0.42	3.20 ± 0.34	<0.01*	0.46	0.82
Male	3.63 ± 0.39	3.27 ± 0.37	3.19 ± 0.48	3.36 ± 0.34	<0.01*	0.93	0.40
Crown-rump length (mm)							
Female	36.0 ± 1.9	33.8 ± 1.9	34.2 ± 2.1	34.6 ± 1.9	<0.01*	0.11	0.32
Male	36.7 ± 1.7	34.6 ± 2.1	34.6 ± 2.1	35.2 ± 1.9	<0.01*	0.36	0.87
Stunted[b]	(3/3) 8.1	(1/1) 2.4	0	(2/2) 4.7			
Placenta weight (g)	0.48 ± 0.07	0.44 ± 0.07	0.44 ± 0.09	0.44 ± 0.05	<0.01*	0.73	0.73
Sex ratio (% male)	50.7 ± 13.8	53.8 ± 13.7	53.5 ± 12.3	48.8 ± 15.5	0.60	0.31	0.91

[a] Probability for Contrast: I = Group 1 versus 2,3,4; II = Group 4 versus 2,3; III = Group 2 versus 3. *indicates Contrast is significant ($P \leq 0.05$)

[b] Expressed as: (number of stunted fetuses/number of litters) percentage of litters affected

Table 53. Morphologic alterations[a] in fetal rats exposed in utero to 1500 ppm n-butyl acetate (BA) or filtered air (FA).

	Group 1		Group 2		Group 3		Group 4	
EXPOSURE INTERVAL								
Pregestation	FA		FA		FA		BA	
1 to 6 dg	FA		FA		BA		BA	
7 to 16 dg	FA		BA		BA		BA	
OBSERVATION								
No. litters with live fetuses	37		42		38		43	
No. fetuses examined	455		538		458		539	
No. heads examined	223		267		229		272	
MAJOR MALFORMATIONS								
Hydrocephaly (internal)	0		0		(1/1)	2.6	0	
Generalized brain dysmorphology	0		0		0		(1/1)	2.3
Cleft lip/palate	0		(1/1)	2.4[b]	0		0	
Aglossia/agnathia	0		(1/1)	2.4[b]	0		0	
Eye defects	0		0		0		(2/2)	4.6[c]
Microphthalmia	0		0		0		(2/2)	4.6[c]
Aphakia	0		0		0		(1/1)	2.3[c]
Retinal disorganization	0		0		0		(1/1)	2.3[c]
Diaphragmatic hernia	0		(1/1)	2.4	0		0	
MINOR ANOMALIES								
Visceral anomalies								
Lung lobe agenesis	(1/1)	2.7	0		0		0	
Organ agenesis (unilateral)	0		(1/1)	2.4[d]	0		0	
Asplenia	0		(1/1)	2.4	0		0	
Situs inversus totalis	0		(2/1)	2.4	0		0	
Cardiovascular anomalies	0		(1/1)	2.4[e]	(2/2)	5.3[e]	(1/1)	2.3
Retroesophageal great vessels	0		(1/1)	2.4[e]	(1/1)	2.6[e]	0	
Missing innominate	0		0		(1/1)	2.6	(1/1)	2.3
Skeletal anomalies								
Fused vertebra	0		(1/1)	2.4[f]	(1/1)	2.6[f]	0	
Rib dysmorphology	0		(6/6)	14.3[f]	(8/5)	13.1[f]	(2/2)	4.7
Wavy	0		(4/4)	9.5	(4/3)	7.9	(2/2)	4.7
Fused	0		(1/1)	2.4	(4/2)	5.3	0	
Bifid	0		(1/1)	2.4	(1/1)	2.6	0	
Sternebral anomalies	(9/7)	18.9	(1/1)	2.4	(3/2)	5.3	(9/8)	18.6
Misaligned	(6/5)	13.5	(1/1)	2.4	(2/2)	5.3	(7/7)	16.2
Scrambled	(2/1)	2.7	0		0		0	
Bipartite	(2/2)	5.4	(1/1)	2.4	0		(3/3)	7.0
Extra ossification site	0		0		(1/1)	2.6	(1/1)	2.3
Other anomalies								
Edema	0		0		0		(1/1)	2.3
MORPHOLOGIC VARIATIONS								
Renal variations	(3/3)	8.1	(5/5)	11.9	(5/3)	7.9	(22/10)	23.3[g]
Hydroureter	(3/2)	5.4	(5/5)	11.9	(5/3)	7.9	(22/10)	23.3[g]
Renal pelvic cavitation	(1/1)	2.7	(2/2)	4.8	(2/1)	2.6	(5/4)	9.3
Supernumerary ribs	(4/3)	8.1	(9/4)	9.5	(4/3)	7.9	(2/2)	4.7
Extra	0		(1/1)	2.4	0		0	
Ossification at lumbar I	(4/3)	8.1	(8/4)	9.5	(4/3)	7.9	(2/2)	4.7
Reduced ossification	(389/36)	100.0	(535/42)	100.0	(452/38)	100.0	(515/43)	100.0
Skull	(9/6)	16.2	(13/11)	26.2	(16/10)	26.3	(5/5)	11.6
Vertebra	(162/33)	89.2	(253/39)	92.9	(206/36)	94.7	(238/43)	100.0
Sternebra	(381/36)	100.0	(534/42)	100.0	(449/38)	100.0	(511/43)	100.0
Ribs	(18/11)	29.2	(27/12)	28.6[h]	(19/10)	26.3[h]	(34/15)	34.9
Pelvis	(7/2)	5.4	(18/9)	21.4[h]	(33/14)	36.8[h]	(3/3)	7.0
Limbs	0		0		0		(1/1)	2.3
Phalanges	(4/1)	2.7	(7/3)	7.1	(5/3)	7.9	(3/3)	7.0

[a] Expressed as: (number of fetuses/number of litters) percentage of litters affected
[b] Fetus 12, litter 3197
[c] Fetus 1, litter 3016
[d] Unilateral agenesis of kidney, ovary and uterus
[e] Retroesophageal aortic arch, pulmonary artery or right subclavian
[f] Chi-square test for rib dysmorphology: P = 0.05 for Group 1 versus 2; P = 0.07 for Group 1 versus 3
[g] Chi-square test for hydroureter: P = 0.05 for Group 1 versus 4
[h] Chi-square test for reduced ossification of pelvis: P = 0.08 for Group 1 versus 2; P = 0.002 for Group 1 versus 3

Table 54. Summary of significant effects ($P \leq 0.05$) of n-butyl acetate exposure on pregnant rats and rabbits.[a]

	RAT					RABBIT			
	Observation Period	Contrast I	Contrast II	Contrast III	Reference	Observation Period	Contrast I	Contrast II	Reference
MATERNAL OBSERVATIONS									
Food consumption									
	Pregestation								
	Week 1	1>2,3,4	2,3>4		Fig. 15				
	Week 2	2,3,4>1	4>2,3		Table 46				
	Week 3		2,3>4						
	1 to 6 dg	1>2,3,4	2,3>4	2>3		1 to 5 dg		2>3	Fig. 14
	7 to 11 dg	1>2,3,4	4>2,3	3>2		6 to 10 dg		3>2	Table 38
	12 to 16 dg	1>2,3,4	4>2,3	2>3		11 to 15 dg		3>2	
	17 to 21 dg			2>3					
Body weight									
	Pregestation								Fig. 14
	Day 3	1>2,3,4	2,3>4		Fig. 15				Table 39
	6 dg	1>2,3,4	2,3>4	2>3	Table 47				
	11 dg	1>2,3,4							
	16 dg	1>2,3,4							
	21 dg	1>2,3,4							
Extragestational weight	21 dg	1>2,3,4							
Organ weights									
Lungs - absolute	21 dg					30 dg	2,3>1		Table 40
- relative		2,3,4>1	4>2,3		Table 48				
Kidneys - absolute							2,3>1		
- relative		2,3,4>1	4>2,3						
Liver - absolute		1>2,3,4							
Spleen - absolute							2,3>1		
REPRODUCTIVE OBSERVATIONS					Table 51				Table 43
FETAL OBSERVATIONS	21 dg								
Body weight									
Female		1>2,3,4			Table 52				Table 44
Male		1>2,3,4							
Crown-rump length									
Female		1>2,3,4							
Male		1>2,3,4							
Placenta weight		1>2,3,4							
Incidence of rib dysmorphology		2>1[b]			Table 53				
Retinal folds							3>1[c]		
Hydroureter		4>1[b]							
Reduced pelvic ossification		2,3>1[b]							
Misaligned sternebra						30 dg	3>1[c]		Table 45
Gallbladder variations							3>1[c]		

[a] Rat exposure to ethylene oxide: Group 1, none; Group 2, 7 to 16 dg; Group 3, 1 to 16 dg; Group 4, pregestation, 1 to 16 dg. Rabbit exposure to ethylene oxide: Group 1, none; Group 2, 7 to 19 dg; Group 3, 1 to 19 dg.

[b] Chi-square test

[c] Fisher's Exact test

n-Butyl acetate exposure did not influence reproductive performance in either species. Rabbit fetuses were unaffected by chemical exposure, but fetal size was reduced in all exposed groups of rats. The observation of reduced fetal growth in rats is suggestive of embryotoxicity, but the influence of maternal toxicity cannot be ruled out. The significantly increased incidence of rib dysmorphology in rat fetuses of Group 2 and the suggestive increase in Group 3 might be considered indicators of an effect on development. We hesitate to define this as a teratogenic effect of n-butyl acetate, since a similar increase was not seen in the group of rats exposed during this period of gestation subsequent to a pregestational exposure.

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APPENDIX A

EXPOSURE CHAMBER BUILDUP AND CLEARANCE, AND MEAN DAILY CONCENTRATIONS

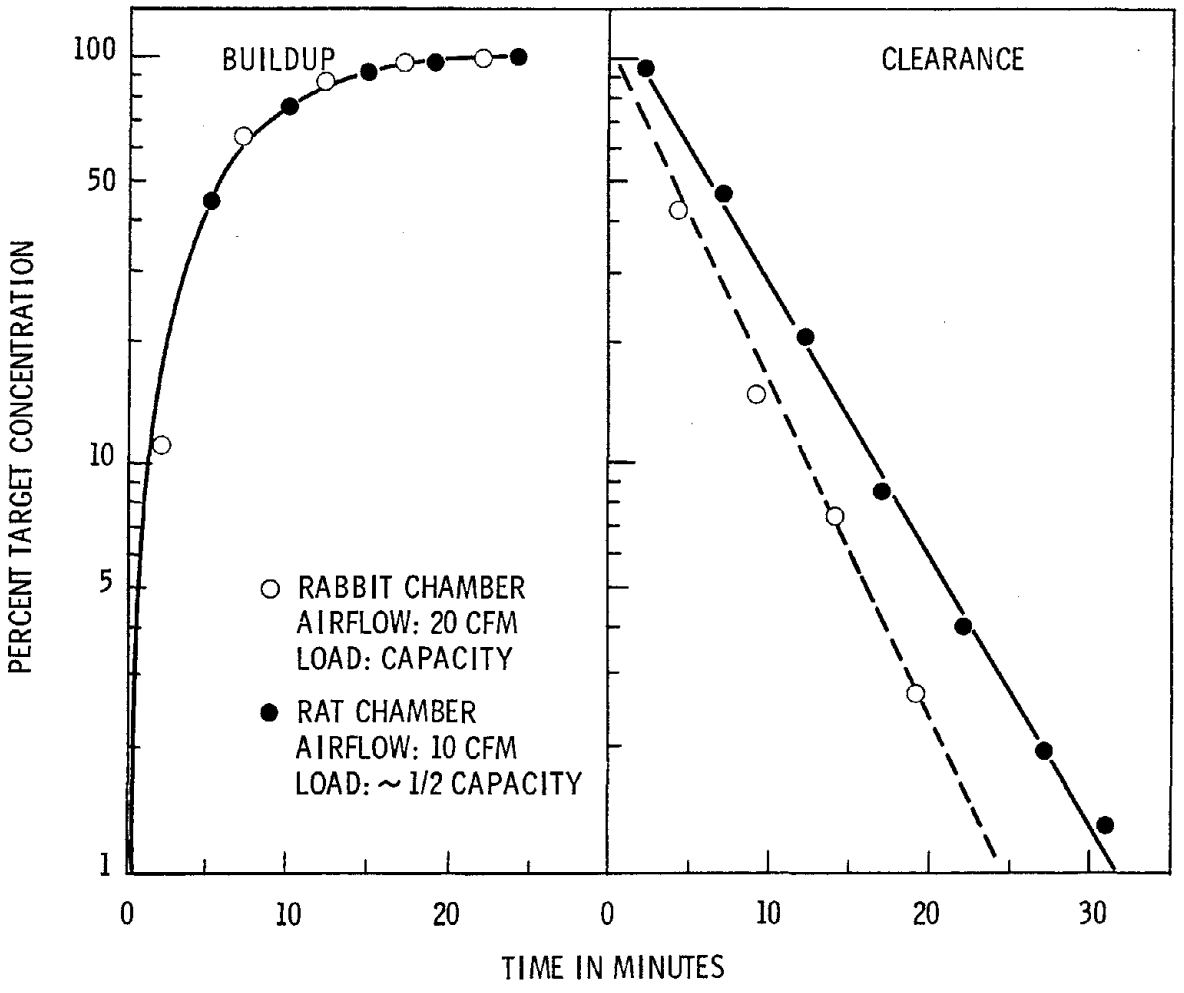

Figure A1. Buildup and clearance times for ethylene oxide during chamber charging and discharging, derived from composite exposure data.

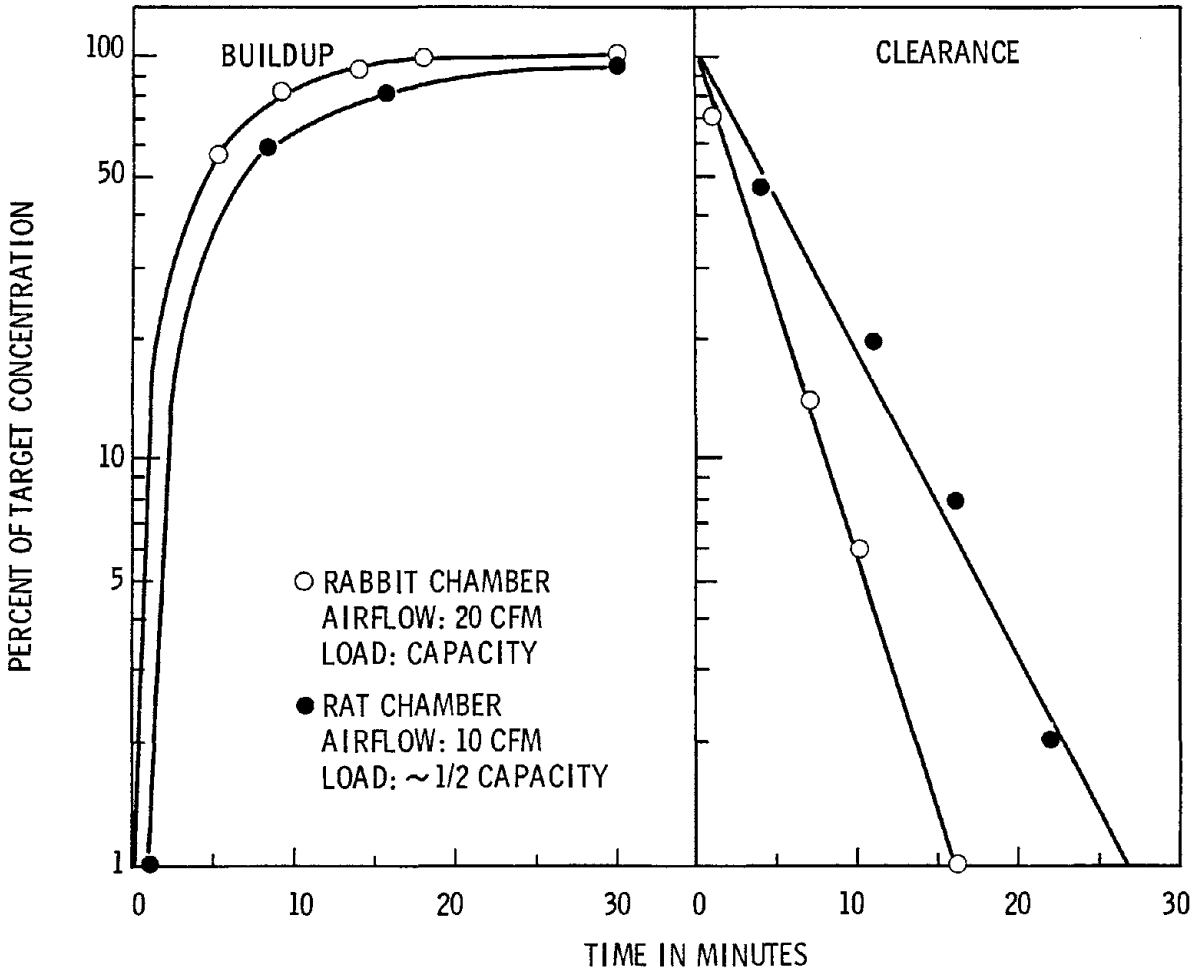

Figure A2. Buildup and clearance times for propylene oxide during chamber charging and discharging, derived from composite exposure data.

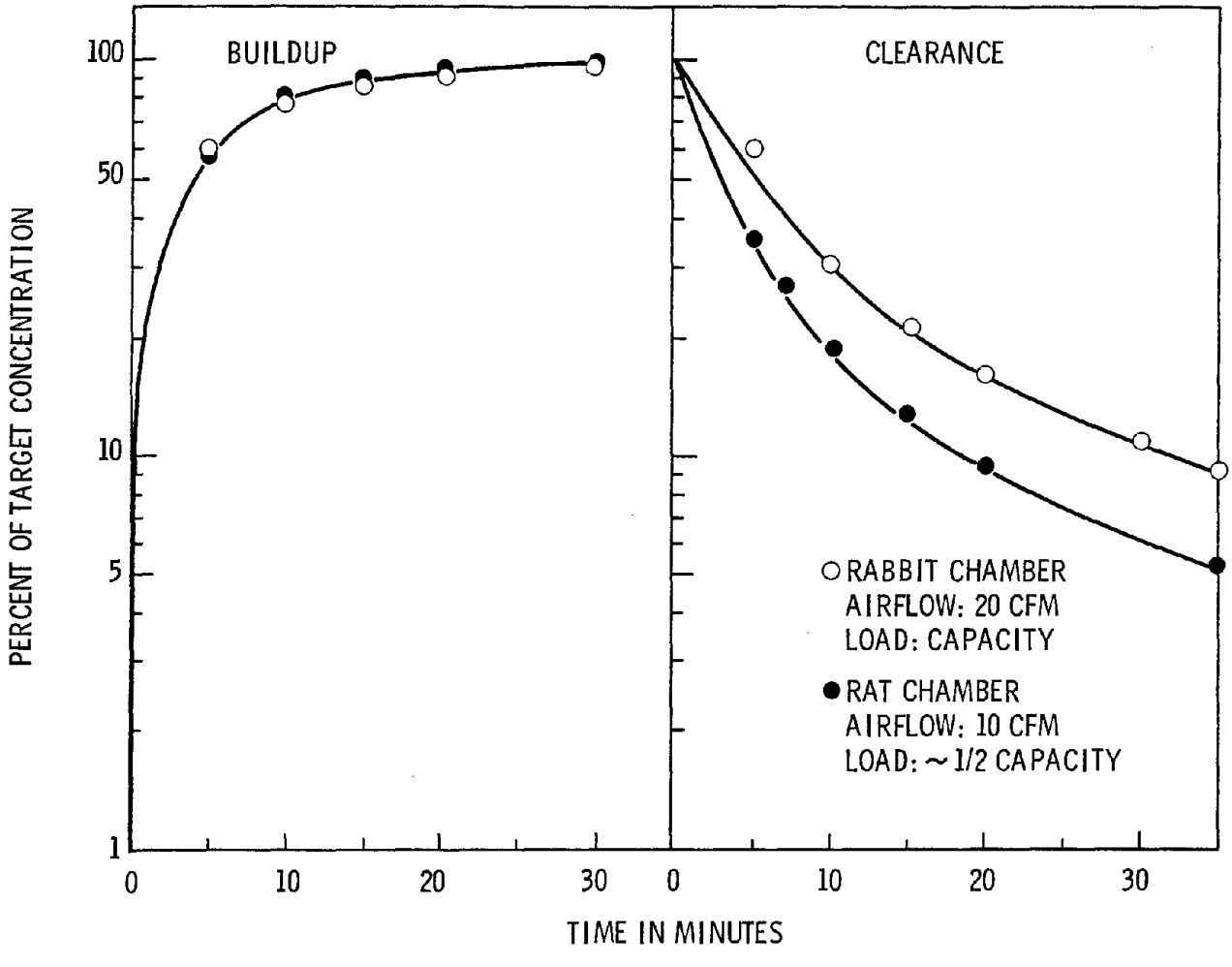

Figure A3. Buildup and clearance times for n-butyl acetate during chamber charging and discharging, derived from composite exposure data.

Table A1. Mean daily concentration of ethylene oxide[a] (ppm ± SD) in rabbit exposure chambers

Day of Gestation	Exposure Groups[b]	
	Group 2	Group 3
1		148 ± 25
2		148 ± 10
3		149 ± 14
4		150 ± 21
5		152 ± 36
6		157 ± 44
7	149 ± 49	157 ± 46
8	151 ± 44	156 ± 40
9	143 ± 24	151 ± 21
10	153 ± 5	152 ± 10
11	154 ± 6	154 ± 9
12	155 ± 6	152 ± 8
13	154 ± 6	149 ± 8
14	149 ± 7	147 ± 12
15	149 ± 6	151 ± 13
16	151 ± 6	150 ± 12
17	155 ± 3	149 ± 7
18	153 ± 4	145 ± 5
19	151 ± 20	148 ± 4

[a] Target concentration: 150 ± 15 ppm
[b] Group 1 (control) was exposed to filtered air

Table A2. Mean daily concentrations of ethylene oxide[a] (ppm ± SD) in rat exposure chambers.

Exposure	Day	Exposure Groups[b]		
		Group 2	Group 3	Group 4
Pregestational	1			157 ± 15
	2			154 ± 5
	3			152 ± 16
	4			153 ± 6
	5			142 ± 10
	6			145 ± 32
	7			141 ± 32
	8			144 ± 14
	9			153 ± 1
	10			154 ± 5
	11			149 ± 3
	12			144 ± 9
	13			149 ± 7
	14			148 ± 3
	15			148 ± 9
Gestational	1		150 ± 13	150 ± 13
	2		149 ± 17	149 ± 17
	3		150 ± 22	150 ± 22
	4		150 ± 22	150 ± 22
	5		150 ± 21	150 ± 21
	6		150 ± 21	150 ± 21
	7	151 ± 19	151 ± 19	151 ± 19
	8	149 ± 16	149 ± 16	149 ± 16
	9	150 ± 19	150 ± 19	150 ± 19
	10	149 ± 12	149 ± 12	149 ± 12
	11	149 ± 4	149 ± 4	149 ± 4
	12	148 ± 3	148 ± 3	148 ± 3
	13	149 ± 3	149 ± 3	149 ± 3
	14	150 ± 4	150 ± 4	150 ± 4
	15	150 ± 4	150 ± 4	150 ± 4
	16	151 ± 4	151 ± 4	151 ± 4

[a] Target concentration: 150 ± 15 ppm
[b] Group 1 (control) was exposed to filtered air

Table A3. Mean daily concentration of propylene oxide[a] (ppm ± SD) in rabbit exposure chambers

Day of Gestation	Exposure Groups[b]	
	Group 2	Group 3
1		499 ± 23
2		492 ± 27
3		495 ± 10
4		511 ± 9
5		513 ± 6
6		506 ± 7
7	487 ± 6	504 ± 11
8	488 ± 9	505 ± 11
9	493 ± 11	509 ± 11
10	492 ± 10	513 ± 5
11	492 ± 11	511 ± 5
12	490 ± 11	512 ± 6
13	492 ± 11	513 ± 6
14	491 ± 5	516 ± 6
15	488 ± 9	513 ± 13
16	491 ± 11	513 ± 14
17	492 ± 11	508 ± 15
18	496 ± 8	506 ± 12
19	492 ± 8	507 ± 12

[a] Target concentration: 500 ± 50 ppm
[b] Group 1 (control) was exposed to filtered air

Table A4. Mean daily concentrations of propylene oxide[a] (ppm ± SD) in rat exposure chambers.

Exposure	Day	Exposure Groups[b]		
		Group 2	Group 3	Group 4
Pregestational	1			518 ± 10
	2			501 ± 12
	3			507 ± 7
	4			493 ± 10
	5			491 ± 8
	6			484 ± 16
	7			486 ± 13
	8			488 ± 7
	9			492 ± 6
	10			485 ± 5
	11			503 ± 14
	12			534 ± 9
	13			515 ± 11
	14			491 ± 18
	15			488 ± 19
Gestational	1		501 ± 8	501 ± 8
	2		503 ± 8	503 ± 8
	3		504 ± 7	504 ± 7
	4		503 ± 7	503 ± 7
	5		502 ± 7	502 ± 7
	6		502 ± 7	502 ± 7
	7	498 ± 10	498 ± 10	498 ± 10
	8	506 ± 19	506 ± 19	506 ± 19
	9	495 ± 18	495 ± 18	495 ± 18
	10	508 ± 20	508 ± 20	508 ± 20
	11	505 ± 24	505 ± 24	505 ± 24
	12	501 ± 18	501 ± 18	501 ± 18
	13	507 ± 15	507 ± 15	507 ± 15
	14	503 ± 11	503 ± 11	503 ± 11
	15	503 ± 14	503 ± 14	503 ± 14
	16	502 ± 14	502 ± 14	502 ± 14

[a] Target concentration: 500 ± 50 ppm
[b] Group 1 (control) was exposed to filtered air

Table A5. Mean daily concentration of n-butyl acetate[a] (ppm ± SD) in rabbit exposure chambers

Day of Gestation	Exposure Groups[b]	
	Group 2	Group 3
1		1523 ± 34
2		1511 ± 32
3		1505 ± 34
4		1506 ± 34
5		1504 ± 40
6		1494 ± 59
7	1488 ± 38	1485 ± 61
8	1474 ± 39	1499 ± 55
9	1493 ± 29	1514 ± 31
10	1497 ± 29	1511 ± 26
11	1502 ± 29	1507 ± 27
12	1503 ± 29	1506 ± 24
13	1498 ± 27	1512 ± 22
14	1497 ± 26	1511 ± 13
15	1502 ± 20	1513 ± 23
16	1489 ± 31	1515 ± 24
17	1491 ± 27	1515 ± 26
18	1461 ± 40	1502 ± 21
19	1462 ± 38	1489 ± 26

[a] Target concentration: 1500 ± 150 ppm
[b] Group 1 (control) was exposed to filtered air

Table A6. Mean daily concentrations of n-butyl acetate[a] (ppm ± SD) in rat exposure chambers.

Exposure	Day	Exposure Groups[b]		
		Group 2	Group 3	Group 4
Pregestational	1			1493 ± 12
	2			1483 ± 37
	3			1520 ± 31
	4			1526 ± 19
	5			1508 ± 25
	6			1546 ± 27
	7			1527 ± 28
	8			1524 ± 35
	9			1491 ± 35
	10			1497 ± 38
	11			1476 ± 66
	12			1476 ± 50
	13			1478 ± 41
	14			1494 ± 23
	15			1499 ± 25
Gestational	1		1451 ± 73	1451 ± 73
	2		1451 ± 70	1451 ± 70
	3		1454 ± 68	1454 ± 68
	4		1456 ± 67	1456 ± 67
	5		1479 ± 29	1479 ± 29
	6		1476 ± 28	1476 ± 28
	7	1478 ± 45	1478 ± 43	1478 ± 43
	8	1495 ± 48	1485 ± 43	1485 ± 43
	9	1500 ± 46	1489 ± 42	1489 ± 42
	10	1496 ± 24	1491 ± 23	1491 ± 23
	11	1489 ± 24	1490 ± 22	1490 ± 22
	12	1490 ± 21	1491 ± 22	1491 ± 22
	13	1494 ± 20	1494 ± 21	1494 ± 21
	14	1496 ± 22	1498 ± 21	1498 ± 21
	15	1498 ± 26	1499 ± 26	1499 ± 26
	16	1500 ± 28	1497 ± 25	1497 ± 25

[a] Target concentration: 1500 ± 150 ppm
[b] Group 1 (control) was exposed to filtered air

APPENDIX B

MORPHOLOGIC ALTERATIONS IN POSITIVE CONTROL FETUSES EXPOSED IN UTERO TO 6-AMINONICOTINAMIDE

Table B1. Morphologic alterations[a] in positive control fetal rabbits exposed in utero to 6-aminonicotinamide.

	Positive Control for Study			Combined Values	
	Ethylene Oxide	Propylene Oxide	n-Butyl Acetate	Positive Control	Filtered-Air Control[b]
OBSERVATION					
No. litters with live fetuses	7	1	5	13	51
No. litters examined	45	11	35	91	426
No. heads examined	23	6	20	49	210
MAJOR MALFORMATIONS					
Craniofacial defects	30/6	6/1	20/5	(56/12) 92.3	0
Hydrocephalus	1/1	0	0	(1/1) 7.7	0
Internal hydrocephalus	0	0	1/1	(1/1) 7.7	0
Facial defects	0	1/1	5/3	(6/4) 30.8	0
Cleft palate	0	1/1	2/2	(3/3) 23.1	0
Anodontia	0	0	2/2	(2/2) 15.4	0
Anteroposterior facial dysplasia	0	0	1/1	(1/1) 7.7	0
Fused mandible/zygomatic arch	0	0	1/1	(1/1) 7.7	0
Eye defects	30/6	6/1	17/5	(53/12) 92.3	0
Anophthalmia	10/5	0	6/4	(16/9) 69.2	0
Microphthalmia	12/3	0	2/1	(14/4) 30.8	0
Aphakia	11/4	6/1	6/3	(23/8) 61.5	0
Retinal disorganization	0	5/1	10/3	(15/4) 30.8	0
Ocular dysgenesis	0	0	3/1	(3/1) 7.7	0

[a] Results are expressed as: (number fetuses/number litters/study). Percentage of litters affected is given in the summary.

[b] Summary of data from filtered-air-exposed rabbits for all three studies.

Table B1. (continued)

	Positive Control for Study			Combined Values			
	Ethylene Oxide	Propylene Oxide	n-Butyl Acetate	Positive Control		Filtered-Air Control[b]	
Cardiovascular defects	3/1	2/1	3/2	(8/4)	30.8	0	
Common truncus arteriosus	3/1	2/1	2/2	(7/4)	30.8	0	
Cardiac septal defect	0	0	1/1	(1/1)	7.7	0	
Other defects							
Vertebral dysgenesis	13/4	0	1/1	(14/5)	38.5	0	
Diaphragmatic hernia	0	0	4/2	(4/2)	15.4	0	
MINOR ANOMALIES							
Musculoskeletal anomalies							
Arthrogryposis	1/1	0	3/2	(4/3)	23.1	0	
Brachyury	11/5	1/1	1/1	(13/7)	53.8	0	
Anury	1/1	0	0	(1/1)	7.7	0	
Scoliosis	1/1	0	9/4	(10/5)	38.5	0	
Rib dysmorphology	16/7	10/1	22/4	(48/12)	92.3	(1/1)	2.0
Fused	16/7	10/1	20/4	(46/12)	92.3	0	
Branched	4/4	2/1	1/1	(7/6)	46.2	0	
Knobby	0	0	3/2	(3/2)	15.4	(1/1)	2.0
Sternebral anomalies	1/1	0	8/5	(9/6)	46.2	(6/5)	9.8
Misaligned	1/1	0	3/2	(4/3)	23.1	(3/2)	3.9
Bipartite	0	0	1/1	(1/1)	7.7	(4/4)	7.8
Extra ossification site	0	0	6/3	(6/3)	23.1	0	
Vertebral anomalies	16/6	11/1	27/4	(54/11)	84.6	0	
Misaligned	5/3	5/1	22/4	(32/8)	61.5	0	
Scrambled	0	0	1/1	(1/1)	7.7	0	
Fused	9/5	8/1	22/4	(39/10)	76.9	0	
Extra arch	8/4	6/1	14/4	(28/9)	69.2	0	
Double centra	0	1/1	4/3	(5/4)	30.8	0	

Visceral anomalies							
Renal agenesis (unilateral)	0	2/1	0	(2/1)	7.7	0	
Gallbladder agenesis	11/2	6/1	10/4	(27/7)	53.8	0	
Lung anomalies	4/3	5/1	4/1	(13/5)	38.5	(1/1)	2.0
Lobular agenesis/fusion	4/3	5/1	4/1	(13/5)	38.5	0	
Hypoplasia	0	0	0	0		(1/1)	2.0
Cardiovascular anomalies	1/1	0	0	(1/1)	7.7	(1/1)	2.0
Retroesophageal aortic arch	0	0	0	0		(1/1)	2.0
Transposed aortic arch	1/1	0	0	(1/1)	7.7	0	
Retinal folds	0	0	1/1	(1/1)	7.7	(5/2)	3.9
MORPHOLOGIC VARIATIONS							
Supernumerary ribs	11/5	8/1	25/5	(44/11)	84.6	(264/51)	100.0
Extra	3/2	6/1	17/4	(26/7)	53.8	(183/45)	82.2
Rudimentary	7/5	2/1	6/4	(15/10)	76.9	(87/40)	78.4
Ossification at lumbar I	4/3	0	5/3	(9/6)	46.2	(16/12)	23.5
Reduced ossification	12/6	8/1	32/5	(52/12)	92.3	(109/33)	64.7
Skull	1/1	0	1/1	(2/2)	15.4	0	
Vertebra	7/4	4/1	21/4	(32/9)	69.2	(1/1)	2.0
Sternebra	4/3	3/1	22/5	(29/9)	69.2	(108/33)	64.7
Limbs	1/1	0	0	(1/1)	7.7	0	
Ribs	0	0	1/1	(1/1)	7.7	(1/1)	2.0
Pelvis	0	1/1	0	(1/1)	7.7	(1/1)	2.0
Cardiovascular variations	0	0	1/1	(1/1)	7.7	(4/4)	7.8
Dilated pulmonary artery	0	0	0	0		(1/1)	2.0
Small pulmonary artery	0	0	1/1	(1/1)	7.7	0	
Accessory vessel	0	0	0	0		(3/3)	5.9
Renal variations	0	0	2/2	(2/2)	15.4	(1/1)	2.0
Renal pelvic cavitation	0	0	1/1	(1/1)	7.7	(1/1)	2.0
Misshapen	0	0	1/1	(1/1)	7.7	0	
Other variations							
Dental maleruption	1/1	0	0	(1/1)	7.7	(1/1)	2.0
Clear gallbladder	0	0	0	0		(2/1)	2.0

[a] Results are expressed as: (number fetuses/number litters/study). Percentage of litters affected is given in the summary.

[b] Summary of data from filtered-air-exposed rabbits for all three studies.

Table B2. Morphologic alterations in positive control fetal rats exposed in utero to 6-aminonicotinamide.[a]

	Positive Control for Study			Combined Values			
	Ethylene Oxide	Propylene Oxide	n-Butyl Acetate	Positive Control		Filtered-Air Control[b]	
OBSERVATION							
No. litters with live fetuses	11	10	10	31		124	
No. fetuses examined	140	130	112	382		1623	
No. heads examined	71	67	45	183		805	
MAJOR MALFORMATIONS							
Craniofacial defects	36/6	14/5	44/9	(94/20)	64.5	(2/2)	1.6
Meningocele	0	0	0	0		(1/1)	0.8
Generalized brain dysmorphology	0	0	0	0		(1/1)	0.8
Cleft lip	34/5	14/5	42/8	(90/18)	58.1	0	
Cleft palate	22/6	13/5	42/7	(77/18)	58.1	0	
Eye defects	0	0	2/1	(2/1)	3.2	0	
Microphthalmia	0	0	2/1	(2/1)	3.2	0	
Aphakia	0	0	0	0		0	
Retinal disorganization	0	0	1/1	(1/1)	3.2	0	
Skeletal defects	18/4	0	1/1	(19/5)	16.1	0	
Spina bifida	3/1	0	0	(3/1)	3.2	0	
Vertebral dysgenesis	15/3	0	1/1	(16/4)	12.9	0	
Other defects							
Common truncus arteriosus	1/1	1/1	1/1	(3/3)	9.7	0	
Diaphragmatic hernia	1/1	0	1/1	(1/1)	3.2	0	
Umbilical hernia	0	1/1	0	(1/1)	3.2	0	
Colon atresia (anterior)	0	1/1	0	(1/1)	3.2	0	

MINOR ANOMALIES							
Craniofacial anomalies	9/3	0	0	(9/3)	9.7	0	
Ankyloglossia	7/1	0	0	(7/1)	3.2	0	
Forked tongue	1/1	0	0	(1/1)	3.2	0	
Hematoma	1/1	0	0	(1/1)	3.2	0	
Visceral anomalies							
Lung lobe fusion/agenesis	3/1	0	0	(3/1)	3.2	(1/1)	0.8
Ectopic gonads	3/1	0	0	(3/1)	3.2	0	
Cardiovascular anomalies	36/6	9/5	30/6	(75/17)	54.8	0	
Retroesophageal major vessels	11/3	1/1	10/5	(22/9)	29.0	0	
Missing innominate	27/6	8/4	24/6	(59/16)	51.6	0	
Missing aortic arch	1/1	0	0	(1/1)	3.2	0	
Missing subclavian	1/1	0	0	(1/1)	3.2	0	
Musculoskeletal anomalies							
Forelimb flexure	0	46/9	26/6	(72/15)	48.4	0	
Arthrogryposis	8/2	10/4	39/8	(57/14)	45.2	0	
Ectro/syn/polydactyly	46/7	35/9	61/9	(142/25)	80.6	0	
Brachyury	0	0	1/1	(1/1)	3.2	0	
Kinky tail	14/7	10/7	18/6	(42/20)	64.5	0	
Offset tail	0	19/6	0	(19/6)	19.4	0	
Lordosis	3/2	0	0	(3/2)		0	
Rib dysmorphology							
Fused	0	0	0	0		(1/1)	0.8
Sternebral anomalies	7/6	1/1	6/3	(14/10)	32.3	(20/16)	12.9
Misaligned	1/1	0	1/1	(2/2)	6.5	(16/13)	10.5
Fused	6/5	1/1	5/3	(12/9)	29.0	0	
Bipartite	0	0	0	0		(3/3)	2.4
Scrambled	0	0	0	0		(2/1)	0.8
Vertebral anomalies	12/4	2/2	4/2	(18/8)	25.8	0	
Fused	2/1	0	0	(2/1)	3.2	0	
Misaligned	10/3	2/2	4/2	(16/7)	22.6	0	
MORPHOLOGIC VARIATIONS							
Supernumerary ribs	9/5	3/1	1/1	(13/7)	22.6	(32/17)	13.7

[a] Results are expressed as: (number fetuses/number litters/study). Percentage of litters affected is given in the summary.

[b] Summary of data from filtered-air-exposed rats for all three studies.

Table B2. (continued)

	Positive Control for Study			Combined Values			
	Ethylene Oxide	Propylene Oxide	n-Butyl Acetate	Positive Control		Filtered-Air Control[b]	
Extra	0	0	0	0		(1/1)	0.8
Rudimentary	0	0	0	0		(3/3)	2.4
Ossification at lumbar I	9/5	3/1	1/1	(13/7)	22.6	(30/15)	12.1
Reduced ossification	94/11	119/10	107/10	(320/31)	100.0	(1052/119)	96.0
Skull	23/8	11/6	27/7	(61/21)	67.7	(14/14)	11.3
Vertebra	78/10	86/9	91/10	(255/29)	93.5	(310/88)	71.0
Sternebra	45/8	119/10	107/10	(271/28)	90.3	(956/105)	84.7
Ribs	0	1/1	5/2	(6/3)	9.7	(19/12)	9.7
Pelvis	4/2	7/3	3/1	(14/6)	19.4	(11/5)	4.0
Limbs	2/2	2/1	17/4	(21/7)	22.6	0	
Phalanges	27/6	22/6	51/7	(75/19)	61.3	(6/3)	2.4
Renal variations	8/4	3/2	18/6	(29/12)	38.7	(30/17)	13.7
Hydroureter	8/4	3/2	18/6	(29/12)	38.7	(30/16)	12.9
Renal pelvic cavitation	0	1/1	1/1	(2/2)	6.5	(2/2)	1.6

[a] Results are expressed as: (number fetuses/number litters/study). Percentage of litters affected is given in the summary.

[b] Summary of data from filtered-air-exposed rats for all three studies.

APPENDIX C

GOOD LABORATORY PRACTICES

FDA's Good Laboratory Practice (Subpart J, "Records and Reports," Item 58.185)

All requirements for this regulation are contained within the Final Report with the following exceptions:

Dates of studies:

a. Ethylene oxide: 02/09/81 to 03/27/81
b. Propylene oxide: 05/31/81 to 08/20/81
c. n-Butyl acetate: 09/17/81 to 10/29/81

Reports for all studies were completed by 05/25/82.

All specimens and microfiche copies of raw data and the Final Report will be stored in a repository designated by NIOSH.

See last page.

Quality Assurance Statement

This study was conducted in accordance with the Good Laboratory Practice regulations set forth in 21 CFR 58. This report accurately reflects the raw data obtained from the study.

R A. Gilman 5-11-92
Q.A. Auditor

PERSONNEL INVOLVED

	Name	Signature	Date
Study Director	P.L. Hackett	P L Hackett	5-11-82
Aerosol Generation	M.L. Clark	M L Clark	5/12/82
	J.R. Decker	J R Decker	5/12/82
	H.S. DeFord	H S DeFord	5/12/82
Chemical Monitoring	R.E. Schirmer	Roger E Schirmer	5/12/82
	T.R. Pahl	T R Pahl	5-12-82
Animal Care and Exposure	M.G. Brown	M G Brown	5-12-82
	J.S. Hammack	J S Hammack	5/12/82
Health Evaluation	S.E. Rowe	Steve E Rowe	5/12/82
	C.A. Pierce	Cheryl A Pierce	5/12/82
Teratology	P.L. Hackett	P L Hackett	5-11-82
	R.L. Music	R L Music	5-11-82
	M.R. Sikov	M R Sikov	5/12/82
Pathology	R.A. Miller	Rodney A Miller	5-12-82
Statistics	R.L. Buschbom	R L Buschbom	5-13-82
Quality Assurance	R.A. Gelman	R A Gelman	5-13-82