TERATOLOGIC ASSESSMENT OF ETHYLBENZENE AND 2-ETHOXYETHANOL

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ABSTRACT

These experiments were performed to evaluate developmental toxicity in rats and rabbits associated with inhalation exposure to ethylbenzene (EB) or 2-ethoxyethanol (EE). Rats were exposed for 7 hours per day, 5 days per week for 3 weeks. They were then mated and exposed daily through 19 days of gestation (d.g.). Rabbits were artifically inseminated and exposed for 7 hours daily through 18 d.g. (EE) or 24 d.g. (EB). The rats were killed and examined at 21 d.g. and the rabbits at 30 d.g. Pregnant animals were observed for evidence of toxicity including altered food consumption, body weight gains and tissue weights and for histopathologic changes. Litters were examined for the presence of external, visceral and skeletal defects as well as the incidence of growth retardation and intrauterine mortality.

Neither maternal toxicity nor embryotoxicity was observed in gravid rabbits exposed to EB at 100 or 1000 ppm, but the high level induced some indications of toxicity in pregnant rats under the conditions used. Also, a significant increase in the incidence of extra ribs was detected in rat fetuses exposed in utero to the high level. This finding was not considered to be a demonstration of true teratogenicity.

Gestational exposures to EE at 617 or 767 ppm induced significantly increased incidences of embryomortality at maternally toxic concentrations in rabbits and rats, respectively. Exposure of pregnant rabbits or rats to EE at 160 or 202 ppm, respectively, induced significantly increased incidence of terata, growth retardation and embryomortality. These lower levels also induced a significant degree of maternal toxicity in rabbits, but not in rats.

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INTRODUCTION

Changing socioeconomic and legal factors have resulted in an increase in the number of women of childbearing age employed in the working environment (Warshaw, 1977, 1978; Hunt et al., 1979). As a result, it is necessary that potential chemical teratogens be identified and the degree of their teratogenicity be quantitated to provide a basis for the assessment of exposure criteria and establishment of federal standards (Bingham 1977; Mahlum et al., 1978). Because of their widespread industrial use, ethylbenzene (EB) and 2-ethoxyethanol (EE) have been identified as agents of concern. Obtaining data on the developmental and general toxicity of these materials has become an objective of NIOSH.

ETHYLBENZENE

EB (CAS: 100-41-4) C_6H_5-C_2H_5 is a flammable colorless liquid having a boiling point of 136°C, a flash point of 20°C, and extremely low solubility in water (Stecher et al., 1968). Utilization of EB was about 2.8 million tons in the U.S. during 1976, which represented only a third of the world-wide usage that year (Kirk-Othmer, 1979). EB is an alkylated benzene intermediate used primarily for the production of styrene, but it is also found in hydrocarbon mixtures used as solvents. Based on the National Occupational Health survey conducted in 1972-1974, NIOSH estimates that approximately 57,000 people in the U.S. may be occupationally exposed to EB.

The literature on the general toxicity of EB has been reviewed and summarized by Wolf et al. (1956). At high concentrations or with prolonged exposure, it has been reported to produce irritation of the skin as well as of the eyes. When administered to rats by gavage, EB has an acute LD_{50} of about 3.5 g/kg; in 6-month feeding studies daily doses up to 136 mg/kg produced no effects while 408 and 680 mg/kg had only slight effects. Daily inhalation exposures of rats and rabbits for 7-8 hours over a 6-month period produced equivocal effects at 1250 ppm and slight to moderate effects at 2200 ppm.

The only effect on the reproductive system known to us was reported by Wolf et al. (1956). Lesions characterized as degenerative changes in the testicular germinal epithelium were found in rabbits exposed approximately six months to 600 ppm. No tests of reproductive function were reported. We are unaware of any study to evaluate the developmental toxicity of EB, other than that reported herein.

The major urinary metabolites of EB in humans exposed for 8 hr to vapors at 20-80 ppm were mandelic and phenylglyoxylic acids, representing nearly 90% of the absorbed dose (Bardodej and Bardodejova, 1970). These authors conclude that both EB and styrene are biotransformed in man by the same metabolic

pathway. The major urinary metabolite in rabbits is hippuric acid, suggesting that there may be species differences in metabolic pathways.

2-ETHOXYETHANOL

EE (CAS: 110-80-5) $HO-CH_2-CH_2-O-C_2-H_5$ (Cellosolve, ethylene glycol monoethyl ether) is a flammable colorless liquid having a boiling point of 135°C, a flash point of 41°C, and high solubility in water (Sax, 1975; Stecher et al., 1968). U.S. production of EE was approximately 100,000 tons in 1978 (Nelson et al., 1980). EE is an unsymmetrical aliphatic ether which is typical of lower-molecular weight members of the glycol ether series (Kirk-Othmer, 1979). As a group, glycol ether solvents have a rather widespread industrial use, particularly since they are soluble in water and in organic solvents (Cornish, 1980). Thus, they are useful in many oil-water combinations as solvents for resins, paints, varnishes, lacquers, dyes, soaps, and cosmetics. Based on the 1972-1974 National Occupational Health survey, NIOSH estimates that approximately 360,000 people in the U.S. were occupationally exposed to EE.

The literature on the general toxicity of EE and related mono-alkyl ethers of ethylene glycol has been reviewed and summarized repeatedly (Werner et al., 1943a,b,c; Carpenter et al., 1949, 1956; Laug et al., 1939; Smyth et al., 1941; and Patty, 1963). EE is mildly irritating to the eyes and skin and enough is readily absorbed through the skin of rabbits to induce toxicity or death (skin LD_{50} about 2 g/kg) (Patty, 1963). Ingestion of EE produced moderate irritation of the gastrointestinal tract of several species (Laug et al., 1939). The acute oral LD_{50} was 5.5 g/kg for rats and 3.1 g/kg for rabbits (Carpenter et al., 1956). Thus, rabbits were more sensitive than rats to oral administration and absorption through skin was more lethal than ingestion in rabbits. Repeated oral or subcutaneous administration to rabbits of doses up to 0.2 ml/kg/day of EE for one week was tolerated, but as few as two doses of 2.0 ml/kg caused fatal renal toxicity (Patty, 1963). Rats exposed to air containing 370 ppm of EE for 7 hr/day, 5 days/week for five weeks showed slight changes in their hemogram (Werner et al., 1943b).

Limited reproductive tract and/or developmental toxicity data are available for EE (Nelson et al., 1981; Nagano et al., 1979; Stenger et al., 1971). Various ethylene glycol mono-alkyl ethers were shown to induce dose-related testicular atrophy and leukopenia in mice gavaged 5 times/week for 5 weeks; doses of at least 1.0 g/kg/day of EE or its acetylated derivative were required to produce these effects (Nagano et al., 1979). Reproductive function was not tested.

Increased incidences of skeletal defects were induced in fetuses when pregnant rats were exposed to EE at doses of 100 µl/kg/day subcutaneously or 200-400 µl/kg/day orally throughout gestation (Stenger et al., 1971). Only low doses were used to expose pregnant mice and rabbits, so that the teratogenicity of EE in these species remained uncertain. Inhalation exposure of rats 7 hr/day on 7-13 or 14-20 days of gestation (d.g.) at 200-900 ppm EE produced dose-related increased incidences of embryomortality (Nelson et al., 1981). These authors also reported impaired performance of surviving offspring evaluated postnatally in a variety of behavioral tests. This impairment was accompanied by alterations in brain neurotransmitters as well.

Thus, there are clear indications of reproductive and developmental toxicity including prenatal mortality, growth retardation, teratogenicity and postnatal functional deficiencies.

RATIONALE

In order to set standards for protecting pregnant and potentially pregnant workers, it is necessary that putative chemical teratogens be identified and the degree of their teratogenicity be quantitated. Ethylbenzene and ethyoxyethanol have been identified as agents of concern because of their industrial use in large quantities. Their toxicity has been studied and guidelines have been established for allowable concentrations. Because of the paucity of data available on the teratogenicity of these materials, experiments were conducted on EB and EE in rats and rabbits.

MATERIALS AND METHODS

CHEMICALS

Highly purified 2-ethoxyethanol (2-EE) and ethylbenzene (EB) were obtained from Aldrich Chemical as reference materials. Several other compounds, which were possible contaminants or degradation products, were also obtained from Aldrich Chemical, including: N-propylbenzene, the three isomers of ethyltoluene, 4-phenyltoluene and cumene for EB and 2-(2-ethoxyethoxy) ethanol, bis(2-ethoxyethyl) ether and 2-methoxyethanol for 2-EE.

Pure grade EB (Lot B551, Phillips Petroleum) was used for vapor generation. The material was sampled for analyses and our gas chromatograph (GC) assays confirmed the presence of 0.1% benzene and 0.1% toluene, as reported by the manufacturer. Also, we found 0.07% cumene which had not been reported. IR and UV spectra of the material did not indicate the presence of other impurities. As a result of the impurities, animals in the high level EB chambers were exposed to 1 ppm benzene, 1 ppm toluene, and 0.7 ppm cumene.

2-EE was supplied by Dow Chemical for vapor generation and was identified by Lot #5, TK # 376. The purity of the material by GC was 99.993%; Dow specified the purity as 99.9+%. The IR spectrum showed no peaks identifiable as other organic impurities. An absorbance due to H_2O in the sample was observed; water is infinitely soluble in ethoxyethanol. The UV spectrum of a solution of 34.3 mg/ml EE in acetonitrile showed a very small absorbance at wavelengths of 225 nm (shoulder, A = 0.065) and 275 nm (A = 0.020). This absorbance was so small that it was not viewed as indicative of a purity problem and could be due to the 0.007% water observed by GC.

EXPOSURE SYSTEM

A Battelle-designed chamber (U.S. patent #4,216,741), (Moss, 1980; Brown and Moss, 1981; Moss et al., 1981), fabricated by Hazelton Systems, Inc., Aberdeen, MD, was used for whole body inhalation exposures of rats and rabbits. The total volume of the chamber was 2.3 m^3 (≅2350 ℓ) of which 1.7 m^3 was available for animals and caging. The volume occupied by animals was approximately 90 ℓ for 30 rabbits and 43 ℓ for 144 rats, or less than 5% of the total volume.* Caging consisted of three levels and each level was split into two tiers which were offset from each other and from the chamber walls (see Figure 1A). Drawer-like stainless steel cage units, composed of individual animal cages, were suspended in the space above each tier. Solid pans for the collection of urine and feces (catch pans) were suspended below each

*Assumes 1 kg body weight equals 1 liter; thus 144 x 0.3 for rats and 30 x 3.0 for rabbits.

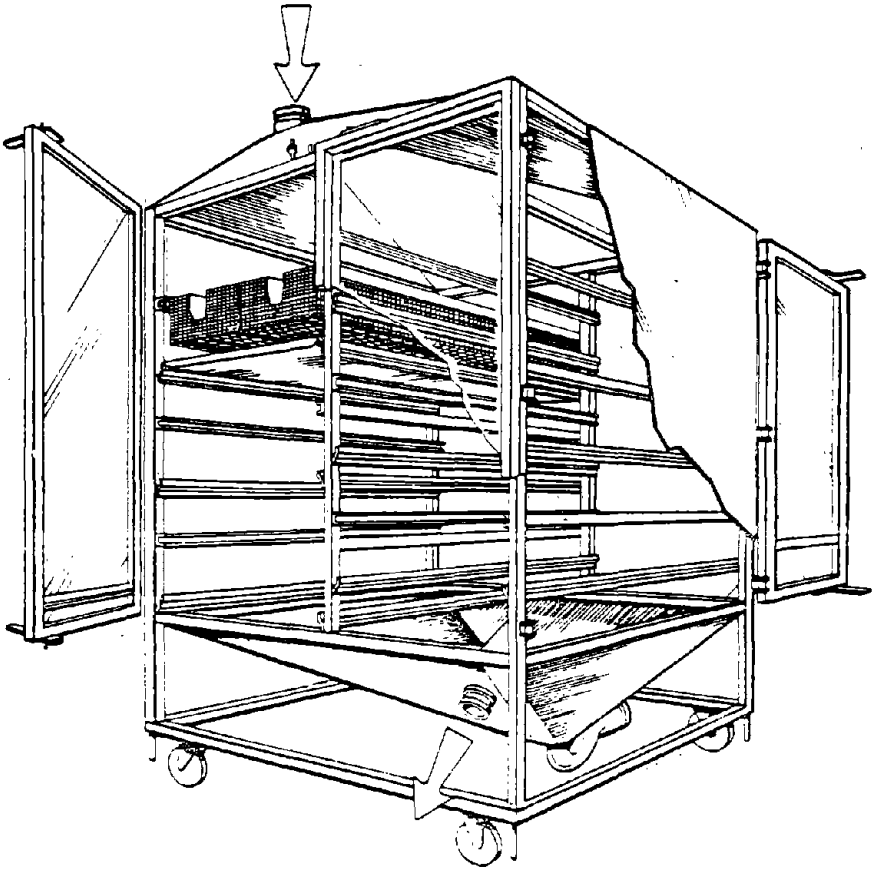


FIGURE A

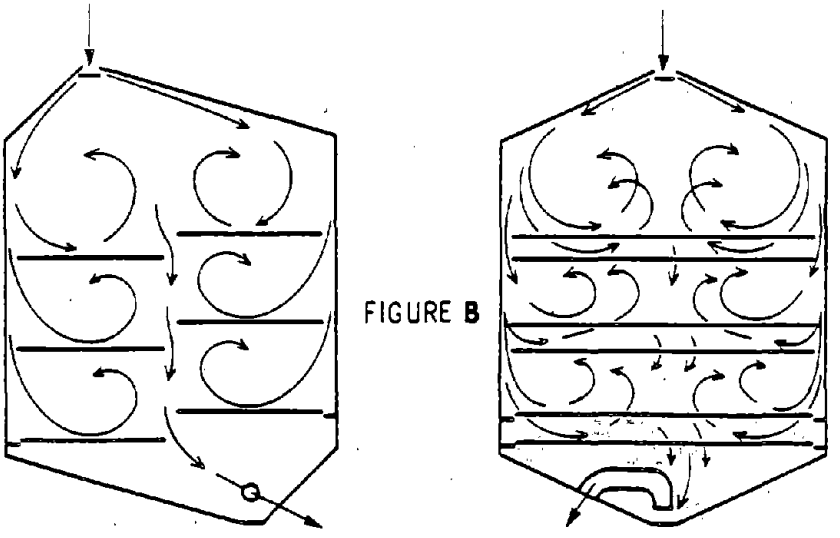


FIGURE B

FRONT VIEW

SIDE VIEW

Figure 1. Battelle exposure chamber. (A. Oblique-cut-away view of the chamber; B. Air flow patterns).

of the six cage units. All catch pans were kept in position during each exposure period.

This chamber is so designed that uniform concentrations within the chamber require that the catch pans be in position. Incoming air containing a uniform mixture of test material is diverted so that it flows as a sheet along and down the inner surfaces of the chamber. Waves are formed at each tier as the fresh exposure air flows past the edge of the catch pan (Figure 1B). Stagnant zones that might otherwise exist at the middle of each layer are cleared by flow through the space between the two tiers. Exposure air reaching the lowest level is deflected across the bottom tiers by metal strips placed in the space between the catch pan and walls.

Tests performed at Battelle Northwest show that concentrations that vary only 3 to 8% throughout the chamber can be repeatedly obtained, provided the exposure air is uniformly mixed before passing through the chamber inlet (Moss 1980; Moss et al., 1981). Independent work at the Inhalation Toxicology Research Institute of the Lovelace Foundation, Albuquerque, NM, has verified these findings (Beethe et al., 1979).

Rats and rabbits were exposed in individual stainless steel cages. The floor area of each cage was 270 cm^2 for rats (28 cm x 9.7 cm with 20 cm height) and 1300 cm^2 for rabbits (56 cm x 23 cm with 30 cm height). There were 24 rat cages or 5 rabbit cages per cage unit and six cage units per chamber. Food and water were not available during exposures.

HEPA filtered air was continually supplied to the exposure chambers and the vapors under study were introduced into the filtered air streams. Chamber total air flows were maintained at 280 ℓ/min for EB and up to 570 ℓ/min for EE. Distribution of vapors was relatively uniform with variations of about ±5% between sampling ports (one for the front and back of each of the six tiers). Exhaust was pumped from the chamber through a flow monitor and into the building exhaust system using an impulse-principle air pump with no moving parts (Vortec Corp., Model 912 transvector airflow amplifier).

At least 3 chambers were employed per species (Figure 2): one for the high-level, one for the low-level, and 1 or 2 for the filtered air. Pregestational exposures of rats to air required 2 chambers. Animals were transferred to freshly washed and sterilized chambers and cage units at least once a week.

The chamber environment was monitored with a wet/dry-bulb psychrometer to determine relative humidity (RH) and a dial thermometer to determine temperature. Both RH and temperature were recorded 2-3 times daily. Chamber temperature was controlled by the control of room temperature. Chamber environmental parameters were monitored and maintained within the following limits during the exposure periods:

Pressure:	-0.5 to -1.0 cm H_2O (relative to room)
Air Flow:	280 or 570 ℓ/min (EB or EE, respectively)
Temperature:	23 to 27°C
Relative Humidity:	35 to 65%

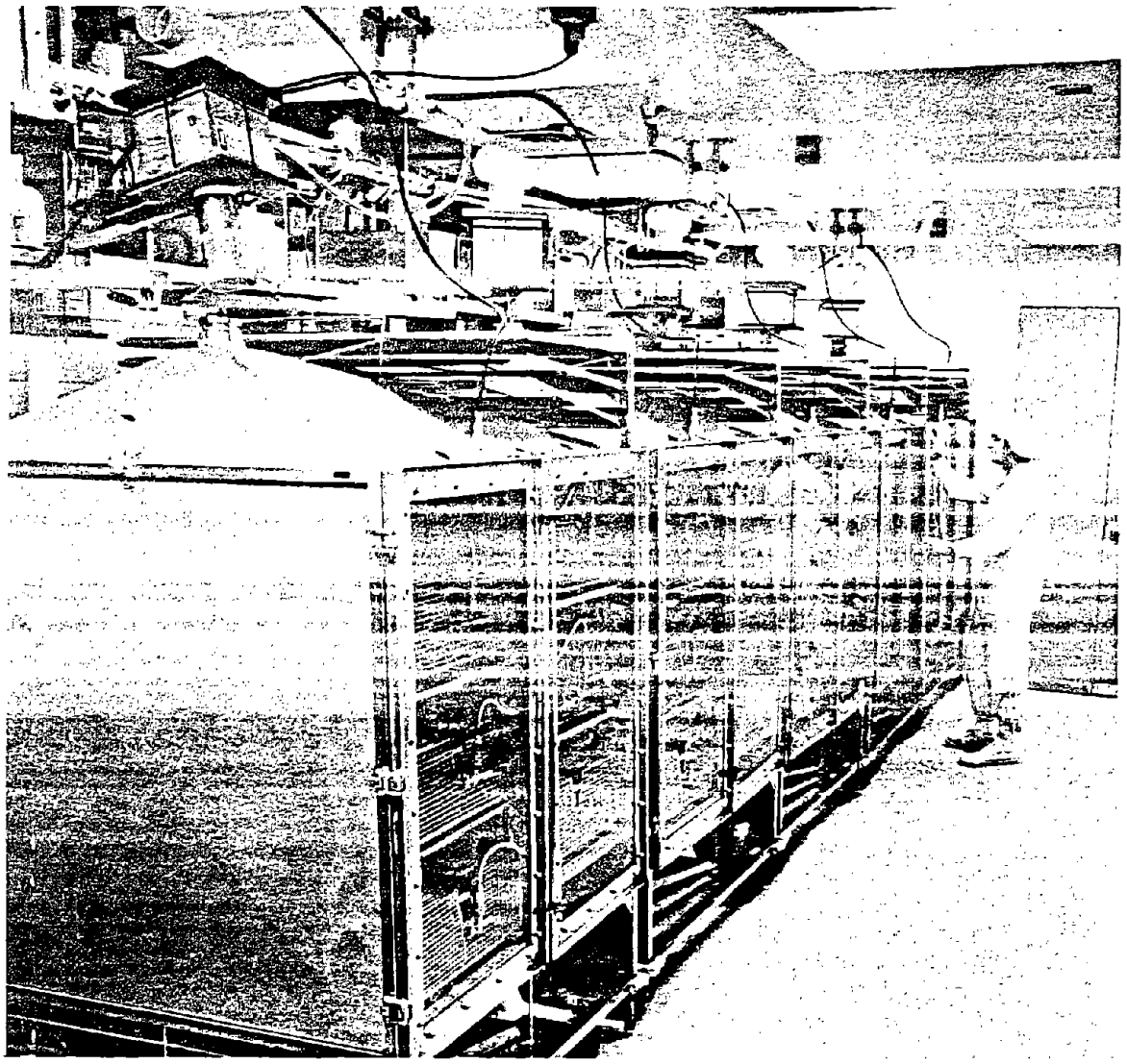


Figure 2. Typical exposure room.

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

VAPOR GENERATION

The liquid to be vaporized was pumped (Figure 3) from a 5 ℓ stainless steel reservoir by a micrometering pump (Fluid Metering, Inc., Model RH1CKC) to the surface of a cylindrical vaporizer (Figure 4) positioned in the air input duct leading directly into the exposure chamber. The reservoir was housed in a vapor hood within the exposure room. Four pump/vaporizer systems were fed from the single reservoir by incorporating a manifold liquid distribution system. Three-way valves and lines returning from the vaporizer to the reservoir facilitated filling the distribution system. This arrangement also allowed direct measurement of fluid flow on a daily basis using a graduated pipet. Pumping rates ranging from 0.14 to 1.8 ml/min were used, depending upon the material and the concentrations desired. Nitrogen was used to replace the depleted fluid in the reservoir to prevent explosions of these flammable liquids.

The vaporizer (Figure 4) consisted of a stainless steel cylinder covered with a glass fiber wick from which the liquid was vaporized. These wicks were replaced when necessitated by residue buildup. 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 71°C for EB and 81°C for EE. The cylindrical vaporizer was positioned in the fresh air duct leading directly into the exposure chamber in order to minimize material loss due to condensation on duct walls.

This generator system had several advantages over heated vat systems. The fluid was not heated until it was at the vaporizer cylinder, thus minimizing heat-induced chemical degradation. A single reservoir supplied all the exposure chambers, and the vapor was introduced directly into the air input duct to the chamber, minimizing material loss on duct walls and reducing the time for vapor build-up and decay in the chamber. Vapor concentration was relatively easy to control since it was dependent only on rate of fluid introduction to the vaporizer and on chamber dilution airflow.

The methods used for generating the desired EB and EE atmospheres were developed using a prototype generating system set up in the aerosol physics laboratory. Vapor generation and pump compatability tests were conducted with both EB and EE. In both cases, the vapor generation system was capable of generating the maximally required vapor concentration (i.e., 1,000 ppm and 750 ppm, respectively), and a linear relationship was established between the chemical detector reading and the liquid feed-rate to the vaporizor.

A pump was cycled continuously with EE for approximately 140 hours. The final pumping rate was within 1% of the initial value. Critical plastic pump parts were soaked in EB for approximately six days, after which they were compared to the new parts. No changes in dimensions occurred and no other visible effects were noted. We concluded that the vapor generator system was compatible with and adequate for EB and EE.

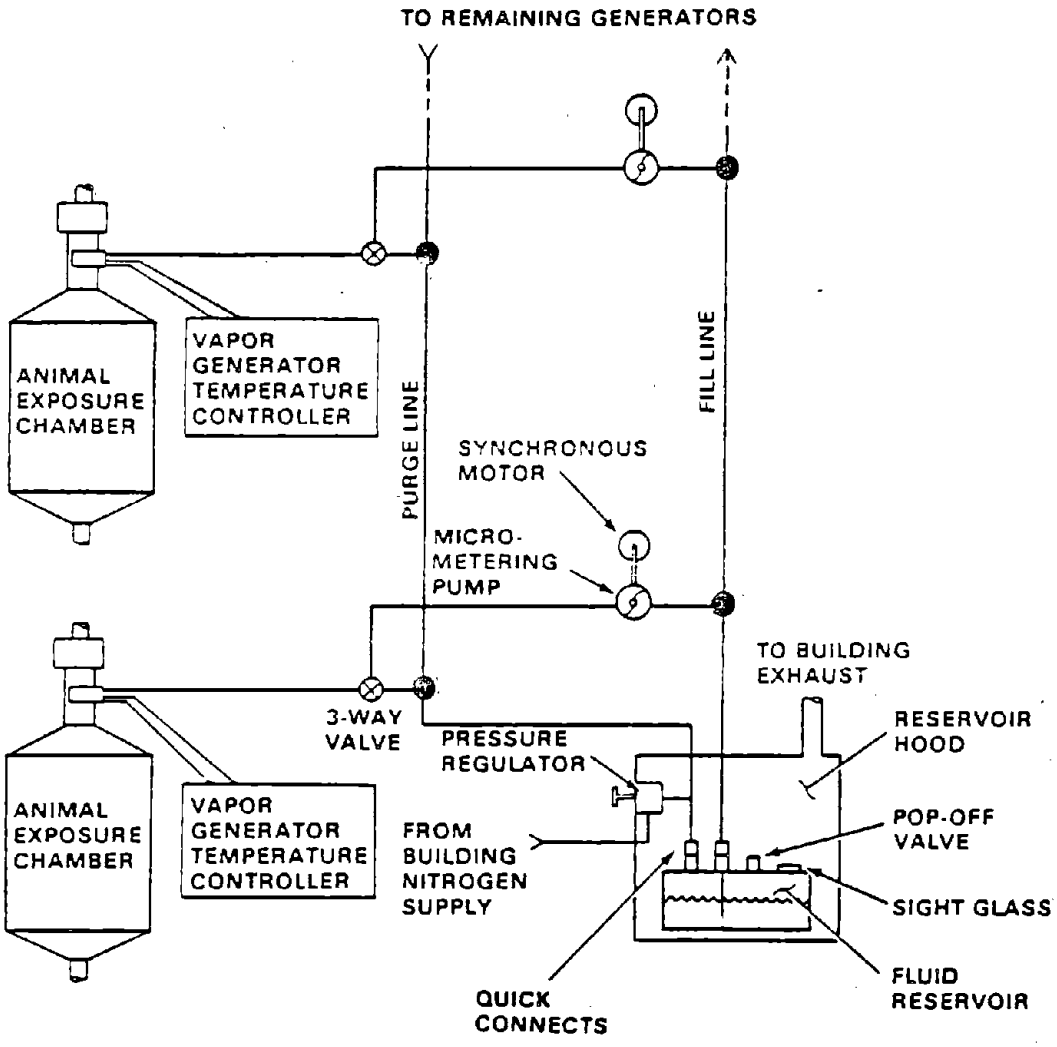


Figure 3. Vapor generation system.

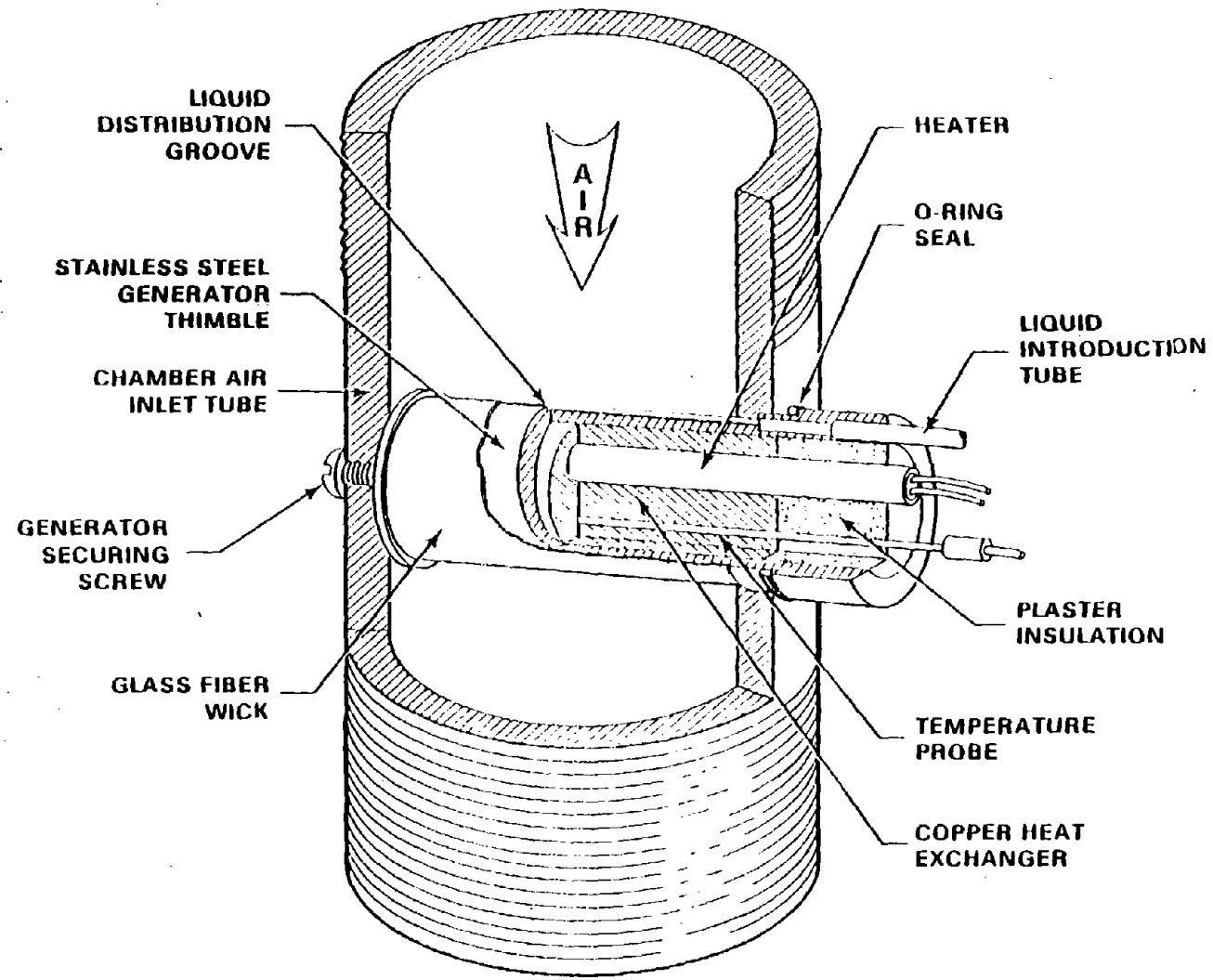


Figure 4. Vapor generation assembly .

ANALYTICAL METHODS

Our standard approach was to develop generation and analytical techniques in the aerosol physics laboratory, then to verify them (with further refinement as necessary) in the actual exposure system prior to initiation of animal experiments. This included determination of stability, accuracy and reproducibility of the generating and monitoring systems. Primary compound concentration was monitored using a gas chromatograph (GC) with a multiplexed sample valve capable of sampling from eight separate areas (Carle Instruments Inc., Model 2026).

Calibration

Calibration of the GC (Hewlett-Packard, Model 5840 A) was achieved with standard liquid dilutions of the stock exposure materials. Analysis of EB was done on a 120 cm long x 2mm I.D. glass column packed with PORAPAK-Q and operated isothermally at 215°C. Analysis of EE was done on a 120 cm long x 2mm I.D. glass column packed with PORAPAK-P and operated isothermally at 140°C. Nitrogen carrier flow was 45 ml/min for both materials.

Generation of standard curves by liquid dilutions was necessary because of the low vapor pressures (about 10 mm and 0.7 mm Hg at 25°C for EB and EE, respectively) and the tendency of these compounds to adsorb on glass surfaces. The r^2 parameter, indicating the quality of the linear fit of the data, was at least 0.999 for both materials (Figures 5 and 6). The intercept value (a; ppm) and the slope of the curve (b; area/ppm) were also calculated and are indicated on the figures. Standard curves were regenerated frequently, especially during the initial stages of exposures. Monitoring experience with flame ionization detectors (FID) showed that the curves did not shift significantly on a day-to-day basis.

Chamber concentration calibrations for both compounds were performed regularly using "grab samples" with internal standards added. The procedure was as follows: The GC monitor was set to sample one chamber repeatedly. "Grab samples" were obtained from that chamber by drawing 1-ℓ samples of the chamber atmosphere through impinger tubes containing 4-5 ml of acetonitrile. The contents of the impinger tubes were then transferred to 10-ml volumetric flasks, internal standard was added, and the flasks were filled to their calibrated capacity with acetonitrile. Toluene was used as an internal standard for EB samples and benzene was used for EE samples. The impinger samples containing internal standard were mixed and aliquots were analyzed on a separate GC. These values were compared with those from EB and EE samples prepared gravimetrically in acetonitrile containing the same internal standard solution. The calibration factor in the in-line GC monitor was then adjusted, if necessary, to give the appropriate response corresponding to the "true" chamber concentration.

Internal Standards

Analysis conditions for EE and EB were developed using a GC with a PORAPAK-P or Q column and an FID. This analysis system was compatible with the use of internal standards. The internal standard device used a diffusion tube to release a constant amount of standard into each sample before it was injected

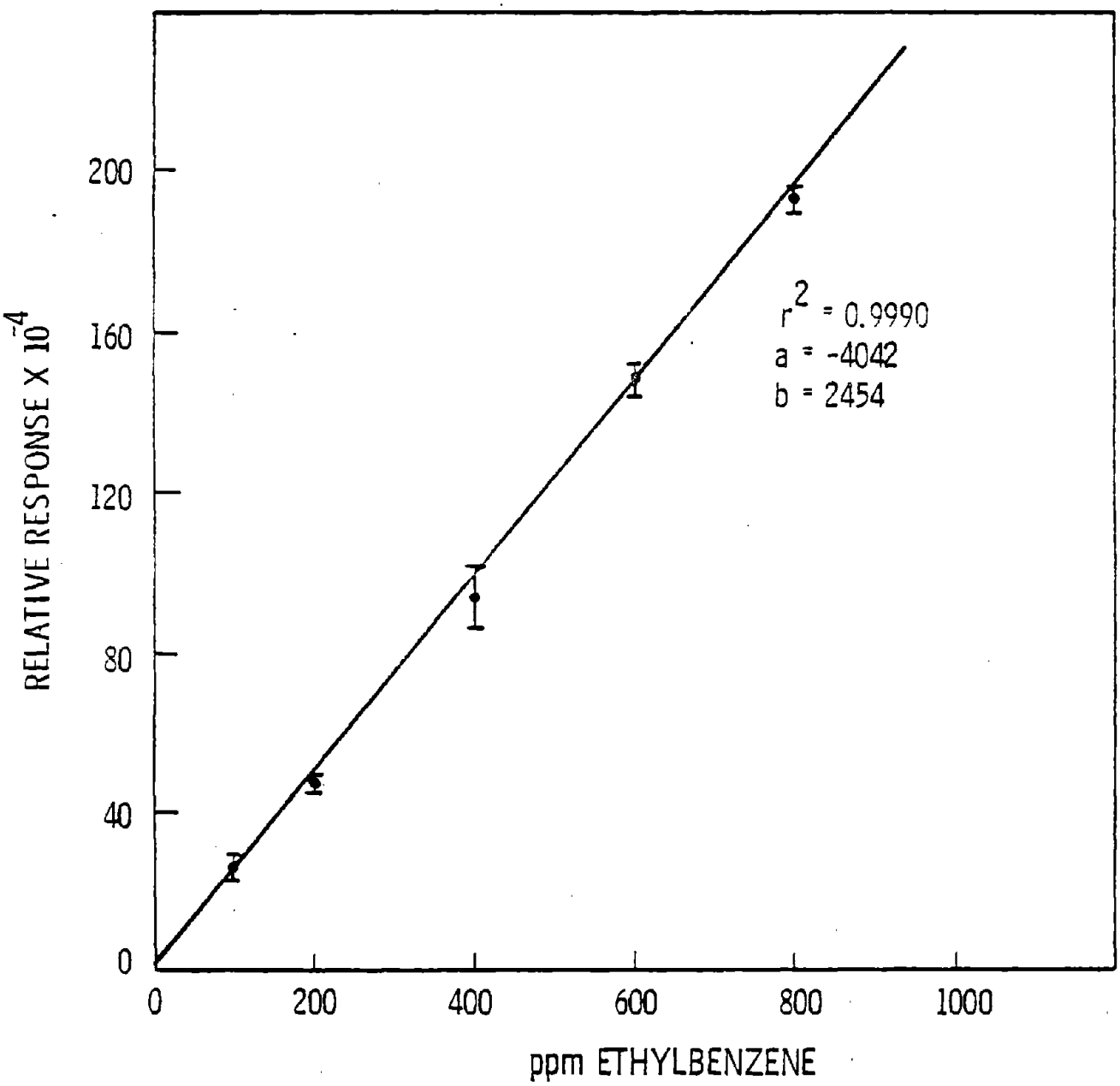


Figure 5. Typical standard curve for ethylbenzene.

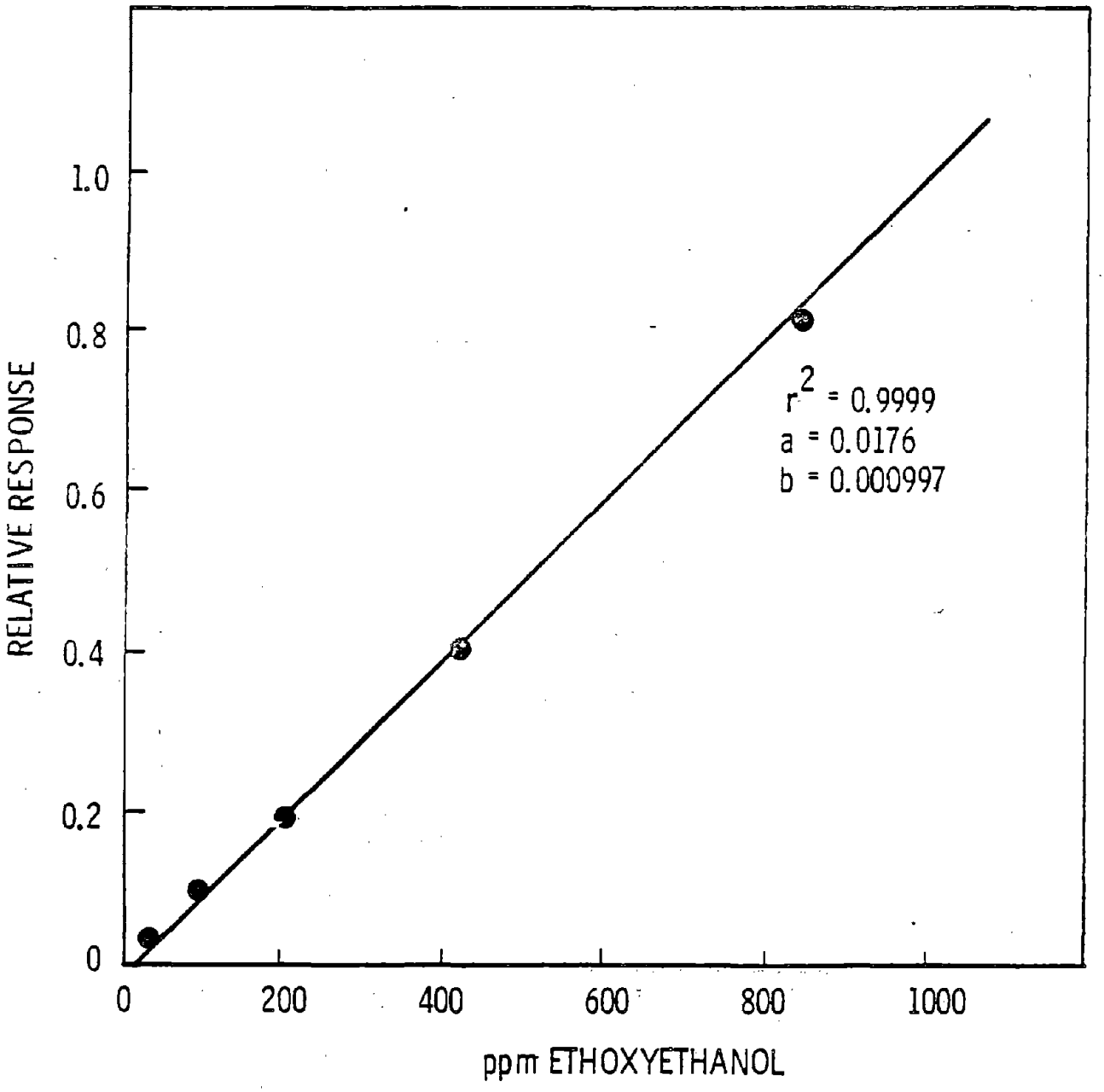


Figure 6. Typical standard curve for 2-ethoxyethanol.

into the GC (Figure 7). A regulated heat source was designed to control the temperature of the diffusion tube for the internal standards.

Under standard operating conditions, EB eluted in 3.8 minutes at a temperature of 215°C and a flow rate of 45 ml/min. Cyclohexane eluted at 1.2 min under the same conditions as EB, was well separated from EB, and was used as an internal standard for EB. Cyclohexane diffusing from a tube 5 mm I.D. x 3.3 cm long at 22.4°C produced approximately 975 ppm when introduced into air flowing at a rate of 200 ml/min.

EE eluted in 1.9 minutes at a temperature of 140°C with a flow rate of 45 ml/min. under standard operating conditions. Initially, methylene chloride was selected as an internal standard for EE. It eluted at 0.6 min and was well separated from EE at 140°C. Its diffusion at 22.4°C through a tube 4 mm I.D. x 4 cm long produced approximately 550 ppm when added to an air stream flowing at a rate of 200 ml/min. However, 2-propanol was substituted for methylene chloride on the 16th day of the ethoxyethanol exposure to reduce the frequency with which the internal standard reservoir had to be refilled. The 2-propanol peak eluted at 0.5 min at 115°C. The internal standard response remained constant during normal operations and changes in the response detected problems, such as temperature fluctuations in the GC, which could then be corrected.

Internal and external standardization methods were compared. To compare quantification with and without the internal standard, data from a typical day's exposure were evaluated using both methods. The absolute area of the internal standard showed a 0.6% coefficient of variation. This variability includes approximately 0.4% due to the GC variability and 0.5% from the delivery of the internal standard. The absolute areas of the EB response were compared to the ratios of the areas of EB and internal standard (cyclohexane). The coefficient of variation of the absolute EB areas was 7.4% and the corresponding value for the ratio of EB and internal standard was 8.1%. These results indicate that the internal standard method does not improve the precision of vapor measurement. With a precise gas sample loop injection system, the internal standard may actually decrease the precision of the measurements because it involves the comparison of two measurements.

Although the internal standard has not directly improved the quantification, we strongly believe that its use has improved the GC analysis procedure. The internal standard provides a constant (±0.5%) amount of chemical which should give a constant response on the GC. When this response changes, chamber operators look for problems with the instrumentation or gas flows. Also, changes in the internal standard alert technicians of any changes in GC calibration. Early in this study GC problems with line noise affected the microprocessor memory causing the loss of temperature set-point values. This malfunction was quickly detected by changes in the internal standard response.

Chamber Monitoring

Routine samples of the atmosphere in each chamber were constantly drawn by vacuum through teflon sample lines to a point near the input of the eight-port sample valve. This constant flow assured fresh sample at the eight-port valve. The eight-port valve, under control of selector valve multiplex

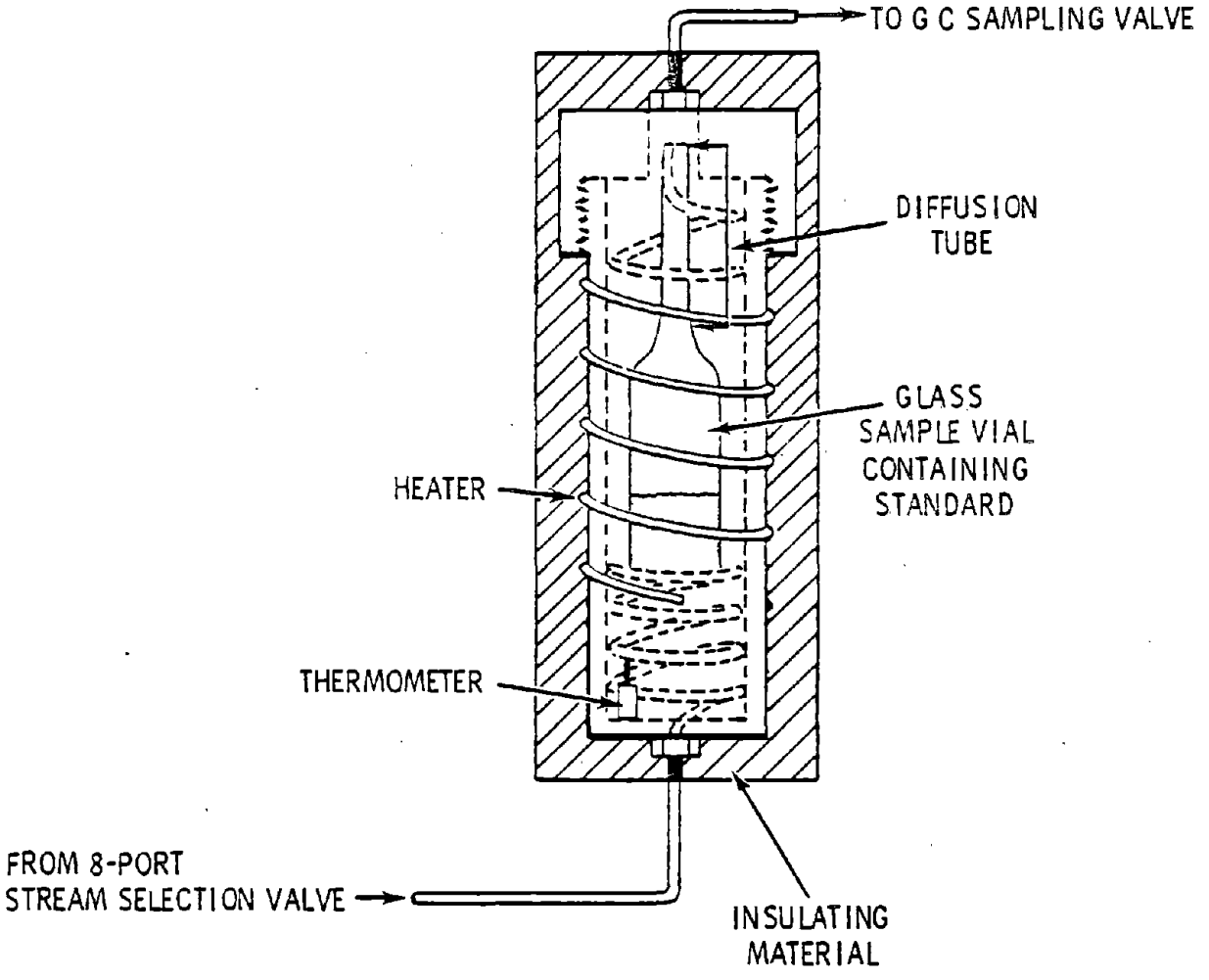


Figure 7. Internal standard device.

electronics, automatically cycled through all eight ports. The sample from each port was analyzed for chemical concentration using an appropriately calibrated GC. Samples were analyzed from each exposure chamber, the control chamber, the room and a chemical standard. Cycle time for all eight ports was approximately 32 minutes.

To monitor the exposure chamber concentrations of EB and EE, a GC was installed in the exposure suite and all sample lines were set to an initial flow rate of 150 ml/min. Chamber air samples were collected and analyzed at least four times daily. GC calibration was checked routinely and was stable throughout most of the exposure period. Changes in calibration were not usually observed except when FID detector gas bottles were changed. A calibration check was always performed following each bottle change.

Routinely, each port was sampled several times a day and the integrated response printed for inspection by the operator. The concentration of organic vapor in ppm was calculated from an area response vs. concentration curve. During the first three days of EB exposures, "grab samples" with internal standards were taken daily in addition to those obtained with the automatic sampling system. Once an internal standard was added to the automatic sampling system, "grab samples" were then taken periodically during the remainder of the EB exposures.

Daily mean chamber concentrations of EB (±SD) are plotted in Figure 8. Minor problems were encountered during the first day of pregestational rat exposures to EB. These problems were eliminated by recalibration of the GC. The exposure system performed well for the remainder of the seven-week exposure period (Figures 8-10). Average daily exposure chamber concentrations of EB (Table 1) indicate that the optimum concentrations were achieved for all of the rat and rabbit exposure groups.

The presence of bubbles of air/vapor in the liquid supply line between the reservoir and the pump assembly caused brief fluctuations in chamber concentration and resulted in the larger standard deviations (Figures 8 and 9) early in the exposure period. Variability subsequently declined (Figure 10) when the problem was corrected.

As added insurance against further bubble problems, some minor modifications were made to the generator system prior to initiating exposure with EE. The first modification was the installation of a "debubbler pump" to bypass bubbles back to the reservoir, if they should occur, rather than let them proceed on to the generator feed pumps. The second modification involved replacing a 3-way valve on the last liquid pump in the series with a simple shut-off valve. This allowed purging the system of bubbles without interrupting liquid flow to any of the generators.

Once animal exposures were initiated, we experienced difficulties in obtaining the required chamber concentrations of EE (200 and 750 ppm). The measured values with animals in the chambers were about 15-25% lower than the values obtained from empty chambers and were lower than the prescribed chamber concentrations as well. The causes were unclear and various corrective measures were taken. The differences in chamber concentrations likely represent loss of EE into or onto the animals, into moisture within the chamber,

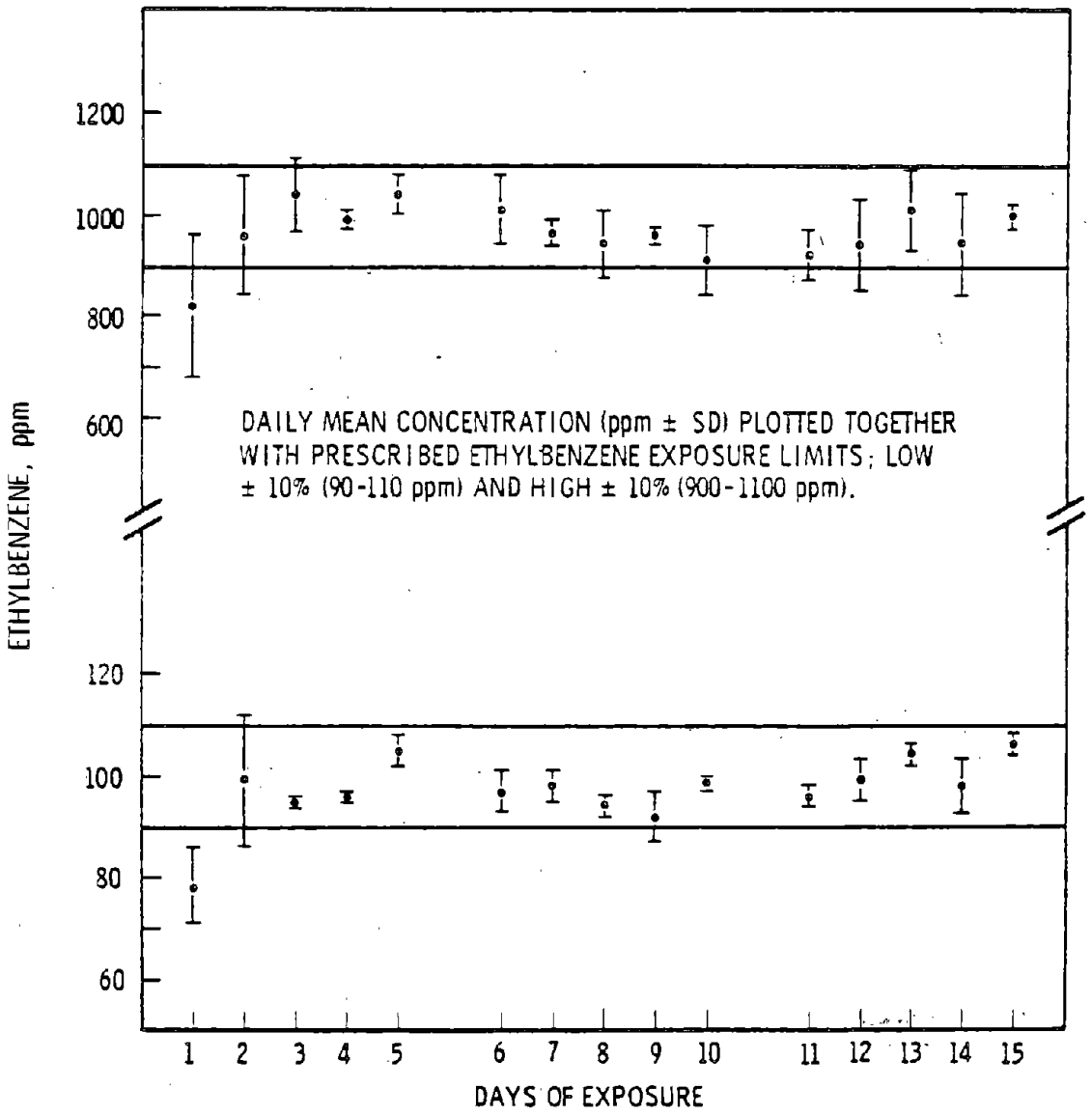


Figure 8. Chamber concentrations of ethylbenzene pregestational exposure - rat.

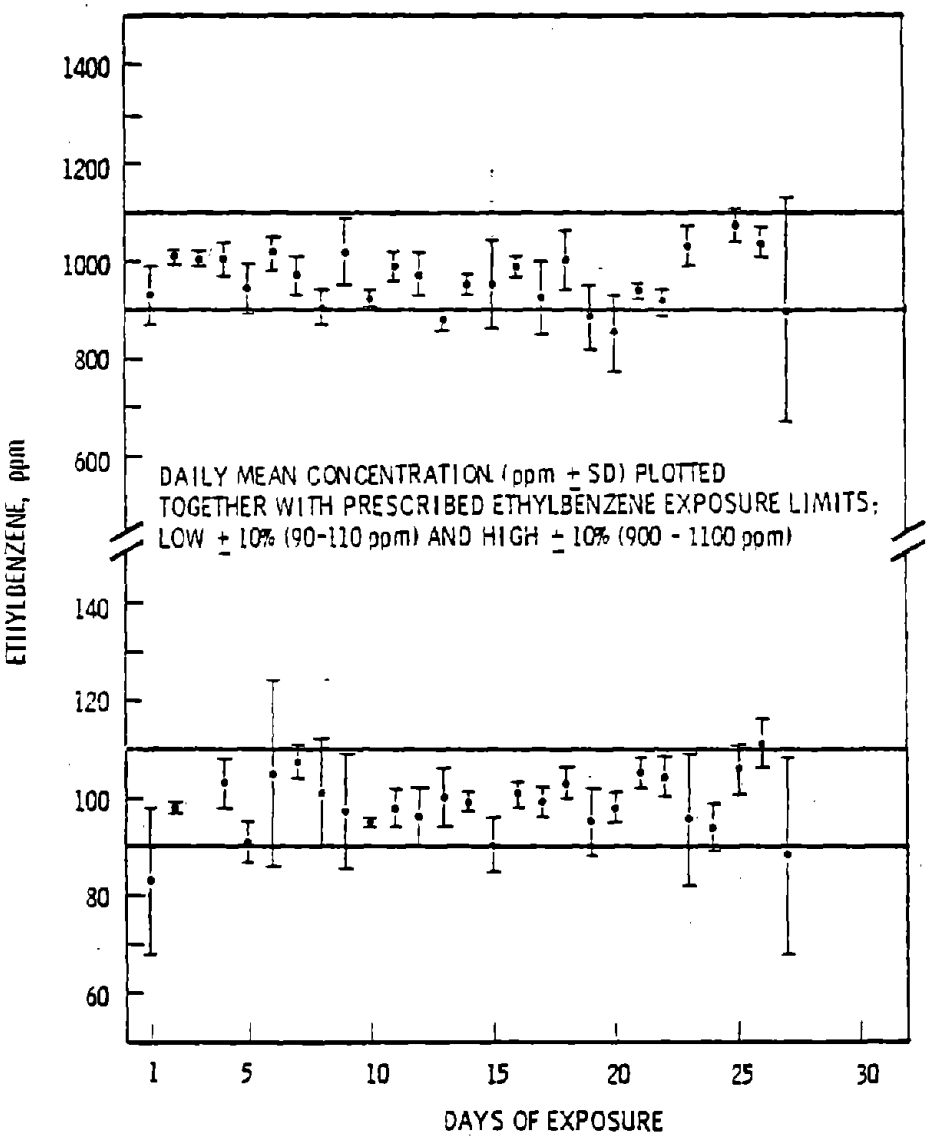


Figure 9. Chamber concentrations of ethylbenzene, gestational exposure - rabbit.

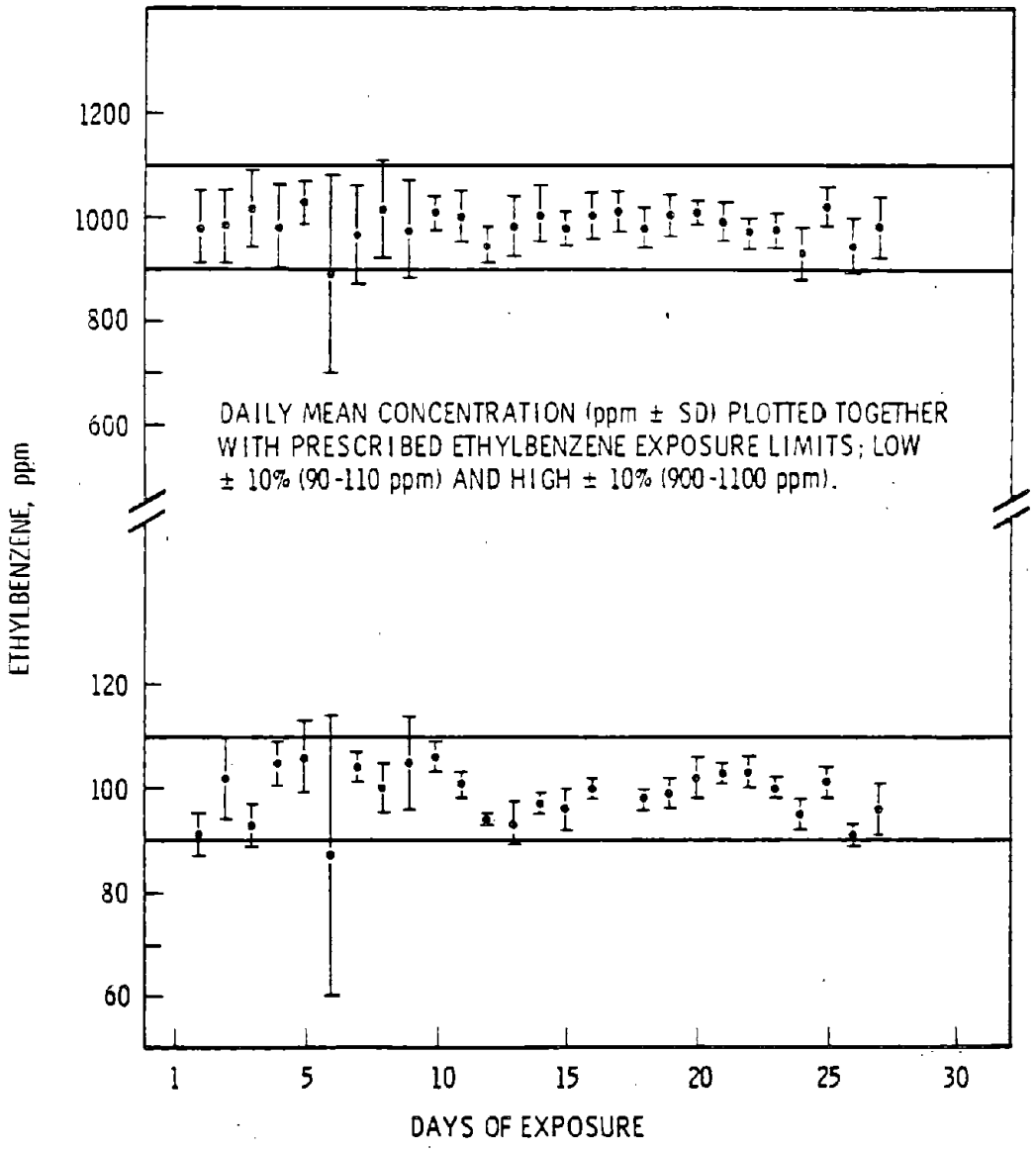


Figure 10. Chamber concentrations of ethylbenzene, gestational exposure - rat.

Table 1. Exposure chamber concentrations of ethylbenzene

Exposure Groups	Average Daily Concentration (ppm ± SD)[a]	
	"Low EB"	"High EB"
Prescribed Levels and Limits	100 ± <10	1000 ± <100
Rats - Pregestational	97 ± 6	959 ± 88
Rabbits - Gestational	99 ± 9	962 ± 76
Rats - Gestational	96 ± 7	985 ± 62

[a]The number of data contributing to each table entry ranged from 7 to 24.

and, to some extent, onto all surfaces within the chamber as reported by Werner et al. (1943c) regarding their exposure of dogs to EE. Their experience and ours suggests that under the exposure and analytical conditions used, the concentration data obtained from GC monitoring probably represents the best measure of the concentrations to which the animals were exposed.

With the agreement of the NIOSH Project Officer, the levels obtained for the rabbits over the first week were maintained (Figure 11) until early termination of their exposure (after 18 d.g.) due to evidence of maternal toxicity. It was also agreed that we would try to reach the prescribed levels for the rat exposures; this was accomplished for the gestational exposures (Figure 13), but not for the pregestational exposures (Figure 12). Average daily exposure chamber concentrations of EE obtained over the course of the various exposure periods are indicated in Table 2.

During the testing of the GC in the exposure suite for monitoring EE, carryover of samples between sample lines was detected. Line flow rates were increased to about 350 ml/min and the sample loop flows were increased to 150 ml/min. In addition, an oven was installed to heat both the stream selection and sample loop valves. This proved inadequate and the stainless steel sample lines were replaced with teflon. Periodic calibration of the GC was continued throughout the exposure period. Grab samples for calibration were taken frequently during the first two weeks until we obtained confidence in the reliability of the teflon lines. The calibration did not show the drastic shifts that had been observed with the stainless steel lines; accordingly, calibration was required less frequently. We did not observe further problems with condensation of EE in the teflon sample lines. The lines showed an acceptably short clearance time (~10 minutes).

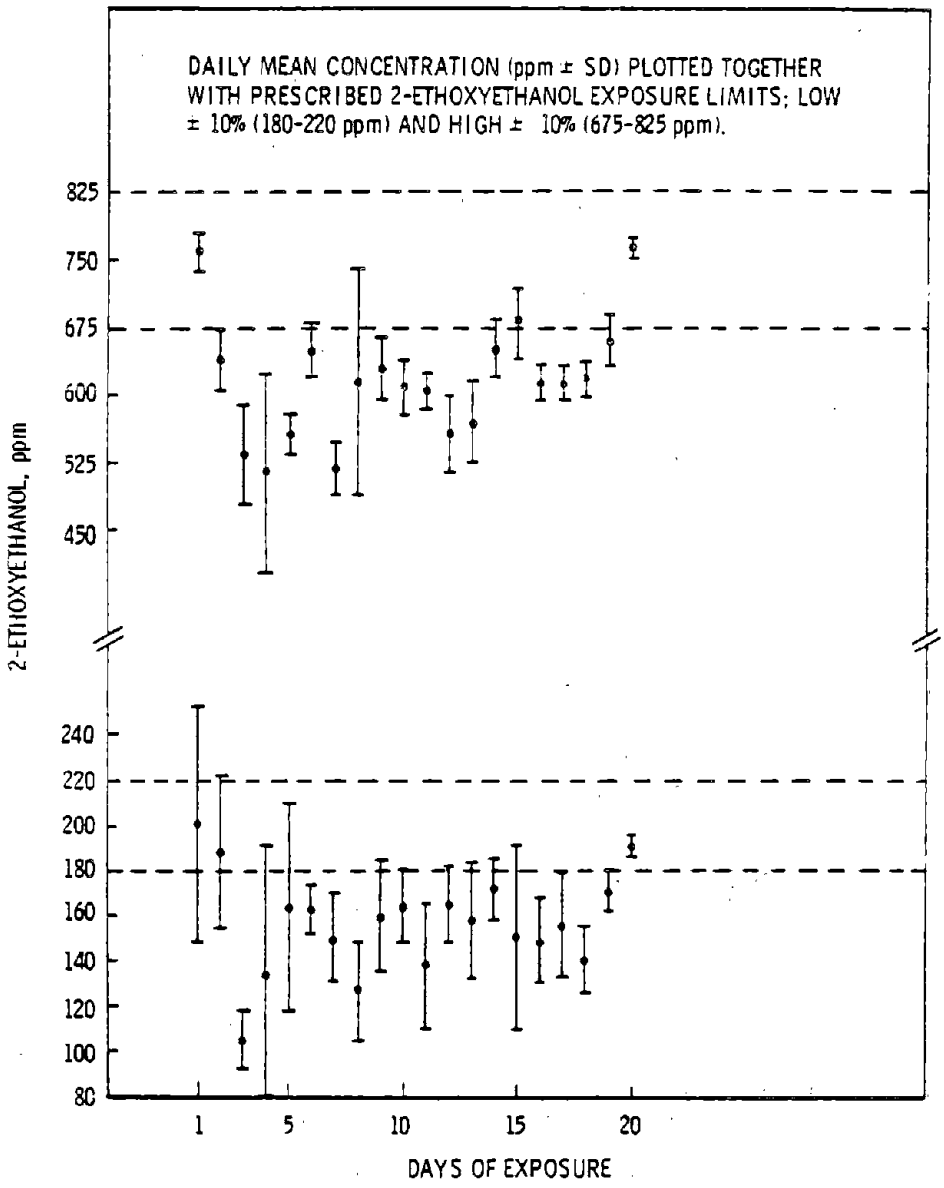


Figure 11. Chamber concentrations of ethoxyethanol, gestational exposure - rabbit.

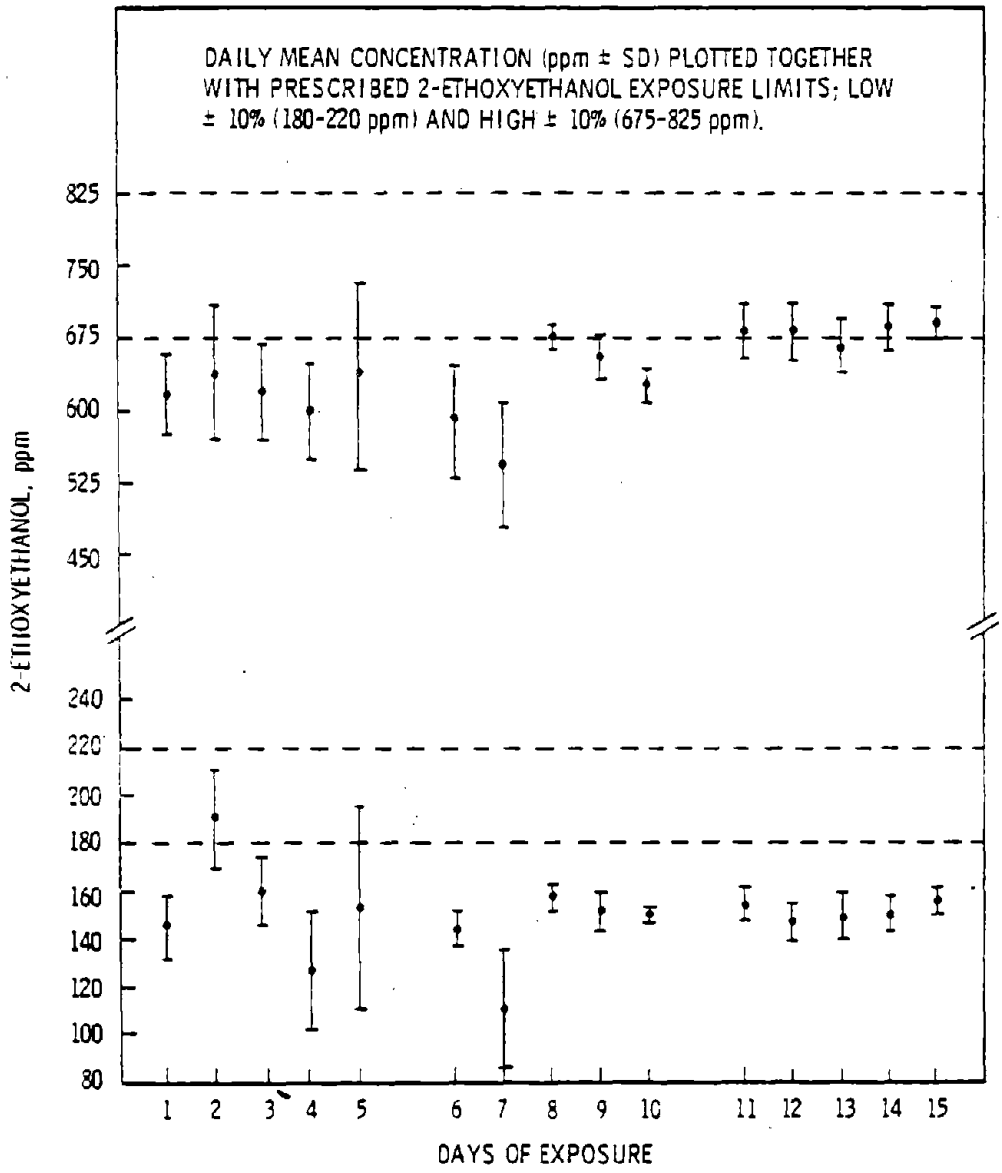


Figure 12. Chamber concentrations of ethoxyethanol, pregestational exposure - rats.

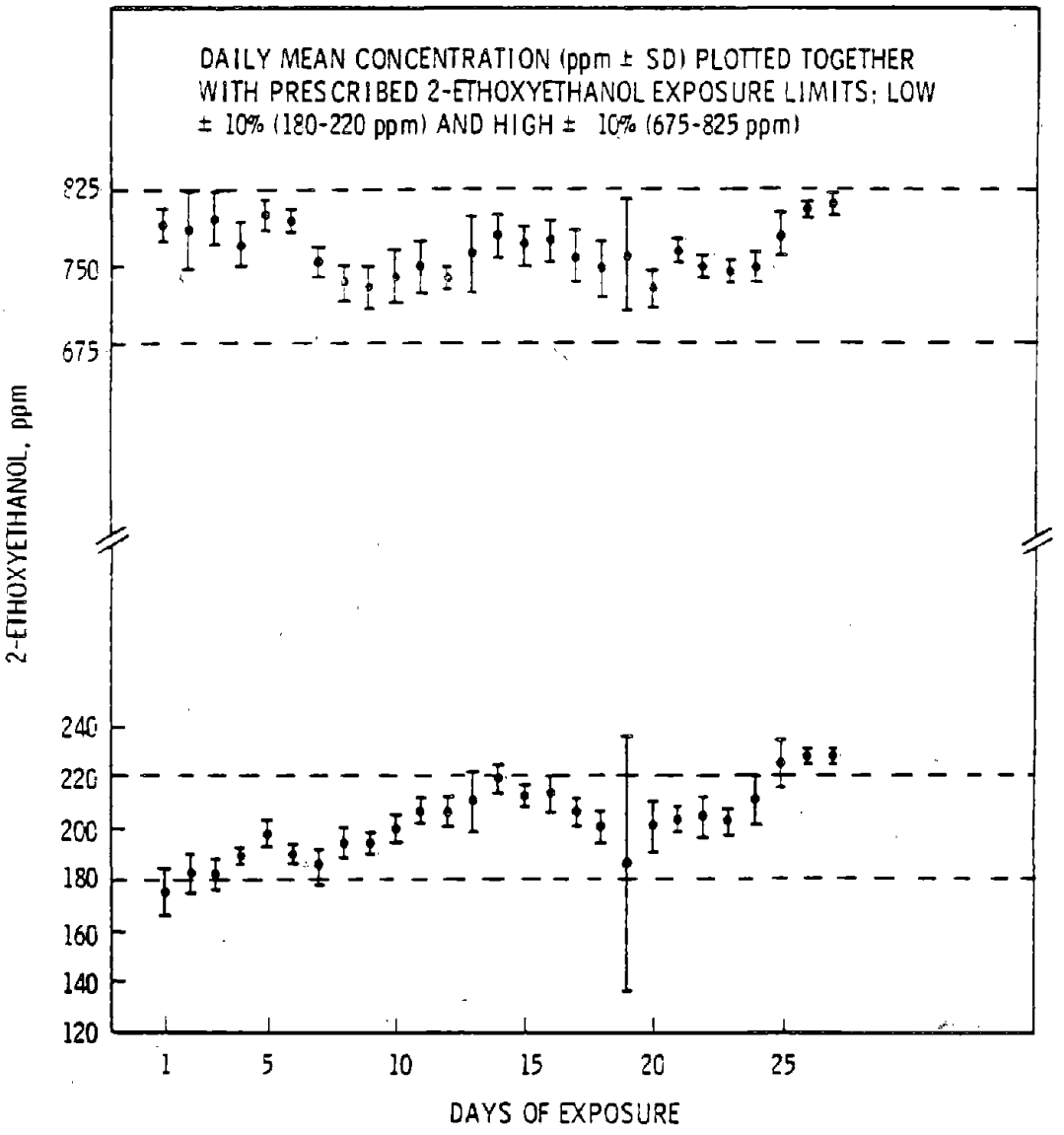


Figure 13. Chamber concentrations of ethyoxyethanol, gestational exposure - rats.

Table 2. Exposure chamber concentrations of 2-ethoxyethanol

Exposure Groups	Average Daily Concentration (ppm ± SD)[a]	
	"Low EE"	"High EE"
Prescribed Levels and Limits	200 ± <20	750 ± <75
Rats - Pregestational	150 ± 18	649 ± 50
Rabbits - Gestational	160 ± 31	617 ± 49
Rats - Gestational	202 ± 11	767 ± 22

[a]The number of data contributing to each table entry ranged from 9 to 19.

ANIMAL HUSBANDRY AND EXPOSURE PROCEDURES

Rabbits

A group of 96 sexually mature New Zealand White rabbit does (4.5-6.0 months of age and 3.0 ± 0.4 kg; mean ± SD) were obtained from White's (Kootenai, ID) for the experiments with EB. Proven breeder males of the same strain were retained from a previous study.

For the experiments with EE, a group of 86 sexually mature New Zealand White rabbit does were obtained. Neither of our usual rabbit vendors could supply all of our needs at the required date. Therefore, the required number of does was split between them: 50 does (3.4 ± 0.7 kg; 4.3 ± 0.5 mo) were purchased from White's (Kootenai, ID) and 36 does (3.5 ± 0.3 kg; 5.3 ± 0.5 mo) were purchased from Hatch's (Langley, WA). Two does, one from each supplier, died of hemorrhagic typhlitis (#9T04 and #95), and an additional doe (#32) died from a gastric hair ball which produced starvation prior to insemination. In addition, #9T03 delivered a litter of at least 8 kits shortly after receipt. Six additional does (3.3 ± 0.1 kg; 4.3 mo) were received from Hatch's to ensure adequate numbers for insemination following the loss of the 4 does during quarantine.

Ten bucks of the same stock (6.8 ± 0.1 mo) were also obtained from White's for the experiment with EE. Their training to service an artificial vagina was repeated daily for 5 trials; some mounted the teaser does, but none ejaculated. These animals were considered unsuitable and were rejected as potential semen donors. Six replacement male New Zealand White bucks (~6 mo old) were obtained from Elkhorn's (Watsonville, CA). These naive bucks were trained to serve the artificial vagina within 5 days and were used for the inseminations.

To ensure permanent and unique identification, consecutively numbered ear tags (National Band & Tag Co.) were inserted in the right ear. The females were weighed and formally randomized by a computer program (DRANDBLK) which assigned the identification numbers, randomly by animal weight, into one of three pregestational exposure groups. The rats were also identified as to pregestational exposure groups by subcutaneously injecting India ink in the forepaw: air--no mark, low--left forepaw, high--right forepaw. A similar marking of the hind paw was done after randomized assignment to gestational exposure groups.

All rats were housed in stainless steel wire cages before, during and after inhalation exposure. All caging of experimental females was individual within the exposure chamber except during the mating period, described below. At the completion of each daily exposure, the generator was disconnected, the chambers were flushed with fresh air for at least 30 min, and the chamber doors were opened. Wayne Lab-Blox was provided ad libitum, except during the daily exposure period when the food was removed. Water was supplied via an automatic watering system; this was disconnected and drained prior to initiation of each exposure. The rats were weighed at least twice weekly prior to initiating exposures during the pregestational period. During the gestational exposure period, rats were weighed prior to the daily exposures on 1, 5, 9, 13, 17 d.g. and before sacrifice at 21 d.g.

To enable a twice-weekly weighing of animals in the three pregestational exposure groups during a five day-period, it was necessary to stagger the weighing dates. For example: air, low, and high groups received their first weekly weighing (denoted as A, Tables 14 and 35) on Monday, Tuesday and Wednesday, respectively. The second weekly weight (denoted as B, Tables 14 and 35) for air, low and high groups was obtained on Wednesday, Thurday and Friday, respectively. This alternation continued on subsequent weeks.

Three days prior to initiation of pregestational exposures, the rats were placed in the exposure chamber and the food in each housing unit was weighed to allow calculation of the pre-exposure food consumption. Food consumption was recorded for a two-day period prior to inhalation exposure, thrice weekly during the pre-gestational exposure period, and at two- or three-day intervals throughout the gestational exposures. The survival, appearance, and behavior of the parental females during the experimental period was noted. Humidity was not controlled, but there were no variations in diet, light cycle, etc., or use of pesticides, medications, or other extraneous chemicals during the experimental period.

Pregestational exposures continued for three weeks (7 hours per day, 5 days per week). For breeding, the females were trarsferred to a standard rack unit and caged with males (approximately 4 females per male) three days following completion of the pregestational exposures. The following morning, after the males were returned to home cages, vaginal lavages were performed and examined for the presence of sperm. Each day, sperm-positive females from each pregestational exposure group were randomly assigned to an appropriate gestational exposure group, i.e., air-exposed animals to air, low, or high gestational groups, low-exposure animals to air or low groups, and high-exposure animals to air or high groups. Gestational exposures were started

All does were isolated for approximately 28 days, and tetracycline (oxytetracycline HCl, 600 mg/ml) was added to the drinking water as prophylaxis throughout the first three weeks of the acclimation period. Does displaying symptoms of upper respiratory infections received 300,000 units of procaine penicillin G daily for three to four days prior to insemination. All medications were discontinued at least two days prior to insemination. To ensure permanent and unique identification codes, rabbits were indelibly tattooed in the right ears.

All rabbits were housed individually in stainless steel wire cages and provided with Wayne Rabbit Diet and water ad libitum except during exposure. Following four weeks of acclimation and quarantine, the does were randomly divided, by weight (DRANDBLK), into three exposure groups; one-third of each group (also randomly selected) was artificially inseminated in the afternoons over a 3- or 4-day period. The schedule was adjusted once due to inadequate semen samples of acceptable quality; the presence of urine in the semen rendered it unacceptable.

Semen samples were collected from at least three bucks (Adams, 1961; Hafez, 1970; Tesh and Tesh, 1971; Hagen, 1974) which had been previously trained to serve an artificial vagina (Gregoire et al., 1958). Semen was collected into artificial vaginas warmed to about 45°C just prior to use. The samples were pooled and diluted with a buffered citrate-egg yolk extender to a concentration of approximately 32×10^6 motile sperm per ml. A volume of approximately 0.5 ml was used to inseminate each doe. The does were inseminated within 1-2 hours of semen collection and nearly simultaneous IV injections of pituitary luteinizing hormone (Burns Biotic, 5 mg/ml) were given at 2.5 mg/doe to induce ovulation. The morning following insemination was defined as 1 d.g.

On the morning of 1 d.g., the rabbits were placed in individual cages within the appropriate inhalation chamber. They were exposed to the test atmosphere at the high or low level or to filtered air for a 7-hour period daily throughout most of gestation. Neither food nor water was provided during exposure. The chamber was flushed with filtered air for at least 30 min following exposure. The rabbits were then removed, transferred to their individual home cages, and supplied with fresh food and water.

Food consumption was measured every two to three days during the experimental period. After termination of exposures, the animals were maintained in standard individual stainless steel caging until sacrificed. The does were weighed before their initial exposure, prior to the daily exposures on 1, 6, 10, 14, 18, and 22 d.g., on 26 d.g., and prior to sacrifice at 30 d.g.

Rats

Groups of 380 young adult female Wistar rats (150 ± 10 g, mean ± SD; ~5 weeks old) were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA) for exposure to EB and EE. They were acclimated 18 to 20 days until initiation of pregestational exposures. Groups of sixty males of the same strain (~7 weeks old) were received approximately three weeks later from the same vendor. Breeding was initiated 3 to 4 weeks after quarantine of the males.

on the day on which sperm was detected, which was designated as 1 d.g. Mating and initiation of gestational exposures continued daily until about 38 sperm-positive rats were assigned to each experimental group. Gestational exposures were performed 7 hours per day, 7 days per week, through 19 d.g.

For convenience, the pregestational exposure groups are denoted as "low level" and "high level" and the group exposed to filtered air as "air." Based upon the combination of pregestational and gestational exposures, one control and six experimental groups were formed. These groups, as follows, will be identified by the pregestational and gestational exposure in the presentation of results.

Air-Air (A-A) (Control) - 3-week pregestational exposure to filtered air followed by exposure to filtered air during days 1-19 of gestation.

Air-Low (A-L) - 3-week pregestational exposure to filtered air followed by low-level exposure during days 1-19 of gestation.

Air-High (A-H) - 3-week pregestational exposure to filtered air followed by high-level exposure during days 1-19 of gestation.

Low-Air (L-A) - 3-week pregestational low-level exposure followed by exposure to filtered air during days 1-19 of gestation.

Low-Low (L-L) - 3-week pregestational low-level exposure followed by low-level exposure during days 1-19 of gestation.

High-Air (H-A) - 3-week pregestational high-level exposure followed by exposure to filtered air during days 1-19 of gestation.

High-High (H-H) - 3-week pregestational high-level exposure followed by high-level exposure during days 1-19 of gestation.

ANIMAL SACRIFICE AND EXAMINATION PROCEDURES

The animals were sacrificed by introduction of carbon dioxide into a euthanasia chamber. The order of sacrifice was randomly determined. 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 not observed by most prosectors, so exposure group was unknown to those evaluating reproduction status and fetal measures. Pregnant rabbits were sacrificed and examined at 30 d.g. and pregnant rats at 21 d.g. The maternal necropsy procedure and evaluations of the fetuses for embryotoxicity were essentially the same for rats and rabbits. The only major difference was that all rabbit fetuses and only one-half of the rat fetuses (selected randomly) were examined for visceral abnormalities.

Necropsies were performed on all adult animals; liver, lung, spleen and kidneys were weighed. Internal abnormalities of the pregnant and nonpregnant animals were also recorded, e.g., adhesions, tumors, or evidence of infection. Samples of the ovaries, uterus, liver, lungs with trachea, and kidneys were preserved in 10% neutral buffered formalin (NBF). Histopathological examinations were performed on tissues from 25% (approximately 7 per group)

of the pregnant animals, selected at random. The residual tissues and the tissues from the remaining 75% of the animals and from the nonpregnant animals were preserved for possible future examination.

The uterus, with ovaries attached, was removed from each animal immediately upon sacrifice. The ovaries were removed and identified as to right and left, the number of corpora lutea was estimated, and the ovaries were fixed in NBF for histologic preparation and examination. The uterus was opened and counts made of living and dead fetuses and of resorptions, which were classified as to the stage of gestation at which death appeared to have occurred. The living and recently dead fetuses were removed in serial order and blotted on a moist surface. Each fetus was weighed, its crown-rump length measured, and its sex determined.

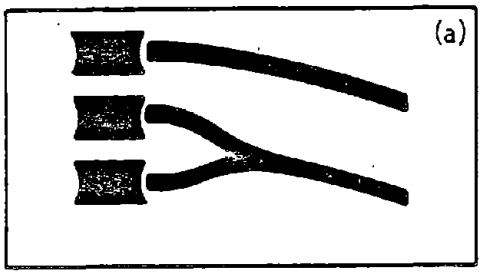
As the fetuses were removed from the surrounding membranes, the amniotic fluid was observed for any abnormalities in color or volume. Concurrently, the placentas were removed, weighed and examined; abnormal placentas, if observed, were fixed for histological preparation and examination. Each fetus was 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 sections by the methods of Wilson (1965) and vanJulsingha and Bennett (1977) for rats and rabbits, respectively. Decapitated rat fetuses and all rabbit fetuses were examined for internal abnormalities by Staples' (1974) modification of the method of Barrow and Taylor (1969). Rat fetuses not decapitated were eviscerated and their sex was verified by the presence of male or female gonads. Following evisceration, fetal rabbit carcasses were air-dried and rat fetuses were fixed in alcohol before preparation of skeletons by the Staples and Schnell (1964) modification of the method of Dawson (1926). To detect abnormalities, the cleared skeletons, stained with Alizarin Red S, were examined under low-power magnification. A defined checklist was followed to ensure inspection of all stained structures. All unusual observations were recorded. For example, observations were tabulated whenever three or more sternebrae or vertebral centra of a fetus were affected. A number of both common and unusual rib defects were noted, and some of these are illustrated in Figure 14.

Once all fetal examinations were completed, the results were tabulated according to predetermined classification of defects, namely: major malformations, minor anomalies, or common variants and/or deformations. These are working definitions to provide guidelines for a standarized classification of dysmorphology and/or dysgenesis. The general considerations for assignment to one of these categories is as follows:

Major Malformations:

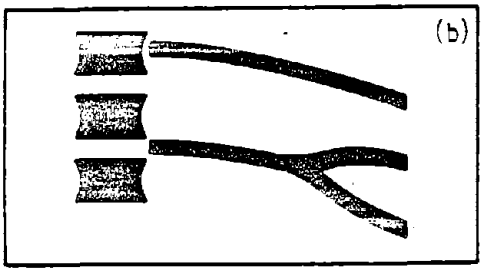
- Lethal or severely detrimental to survival
- Irreversible without intervention
- Frequently involves replication, reduction, or absence of essential structure

FUSED - lateral-ventral joining of two adjacent ribs which

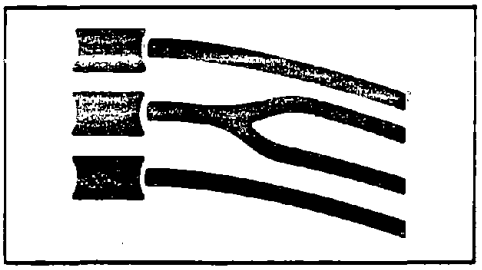


(a) clearly originate from two separate vertebrae or

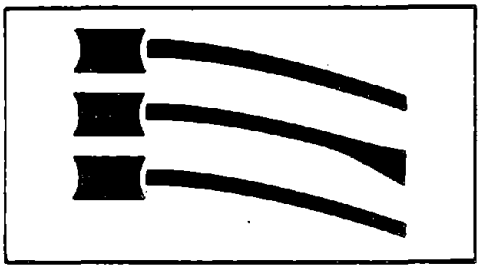
(b) appears to originate intervertebrally, with no ribs originating from the two adjacent vertebrae.



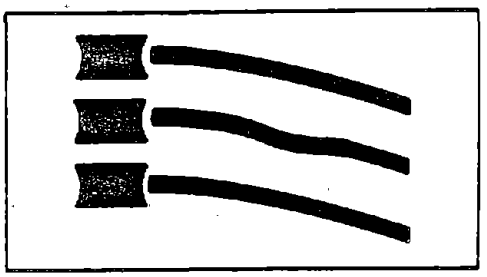
BRANCHED - lateral-ventral bifurcation of a rib which is single at its attachment to a single vertebra.



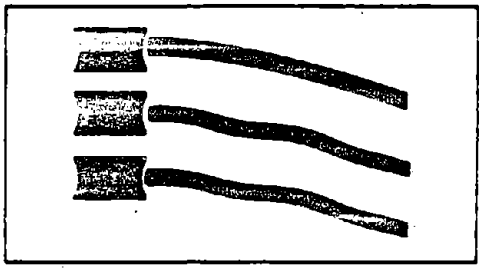
SPADE - a distinct "spade-shaped" enlargement toward the sternal end of the rib.



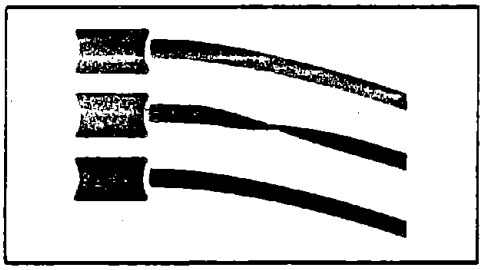
BENT - a rib with a single, distinct angulation.



WAVY - two or more undulations in a rib; usually seen in two or more adjacent ribs.



TWISTED - a distinct torsion of the rib.



KNOBBY - a distinct focal enlargement along the shaft, (often associated with bent and/or wavy ribs.)

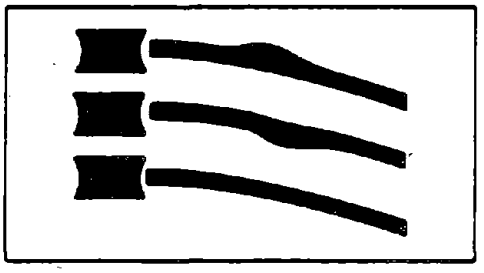


Figure 14. Rib classification criteria.

- Often results from partial or complete failure to migrate, close and/or fuse
- May include syndromes of otherwise minor anomalies

Minor Anomalies:

- Nonlethal and generally not detrimental to survival
- Generally irreversible
- Depends on degree of severity and/or frequency of occurance; may be upgraded to "major" or downgraded to "variant" accordingly.
- Frequently involves reduction or absence of nonessential structures
- Frequently involves reduction in number, size or absence of nonessential structures

Common Variants and/or Deformations:

- Nonlethal and not detrimental to survival
- Generally reversible or transitory
- Frequently associated with immaturity
- Often induced after organogenesis ("fetal pathology")
- Generally less specific than other classes of defects
- May occur with a high frequency
- Often considered to result from delay in developmental sequence

Differences in the size of specific structures are often highly subjective, so these observations are omitted from tabulations but are indicated in the text.

The rationale for categorizing abnormalities derives from the knowledge that structural changes routinely recorded in teratology studies are not of equal importance or meaning (Palmer; 1969, 1972, 1974, and 1978). Final judgments require case-by-case evaluations supported by appropriate statistical analyses of the relative incidence of defects. Thus, a dose-related increase in the incidence of any abnormality is important, regardless of classification, since the data would imply that the agent caused an effect, both qualitatively and quantitatively. In contrast, low prevalence of nonrelated defects, in the absence of an apparent dose-response relationship, should be attributed to the agent only with extreme caution. Spontaneous occurrence of defects of variable severity are inherent in all populations and represent

the background incidence (Palmer; 1968 and 1977, Perraud 1976, Kameyamsa et al. 1980, Cozens 1965, and Banerjee and Durloo 1973).

STATISTICAL EVALUATIONS

The litter was considered as the basic experimental unit for statistical analyses. The reason for using the litter as the basic unit is that statistical test procedures used in the data analysis require that the basic sampling units be independent. Fetuses are not used as experimental units because littermates may not respond independently (Haseman and Hogan 1975).

Analysis of variance (ANOVA) was the test of choice for continuous variables when several means were to be compared. Response proportions, such as number of implants per corpora lutea per dam, proportion of resorptions per litter, proportion of dead fetuses per litter, etc., were analyzed by the analysis of variance technique with an arcsin transformation (θ) of the response proportion, p. This transformation, $\theta = 2 \arcsin \sqrt{p}$, was chosen because: first, θ is more nearly normally distributed than p, and second, the standard deviation of θ is independent of long-run values of θ or p when sample size per treatment group is not too small (Eisenhart, 1947). If results of analysis of variance showed a significant treatment effect, then Duncan's multiple range test was used to make multiple comparisons among group means to investigate the possibility of a dose-response relationship. One way to express the results of Duncan's multiple range test is to divide the exposure groups into homogeneous subsets (indicated by underlining subsets) which do not differ from each other with means ordered according to increasing size, as shown in the Appendix of this report (page 96).

Comparisons of binary response variables among groups were done by chi-square tests for independence or Fisher's Exact Probability Test (Siegel, 1956).

Binary response variables include such parameters as number of pregnant females per number inseminated, number of litters with resorptions, etc.

In addition to consideration of absolute values, organ weights also were converted to percentages of total body weight (relative weight) and analyzed by the analysis of variance technique. Analysis of organ weight data examined as percentages of total body weight is as powerful and accurate in evaluating statistical significance as any other method (Weil and Gad, 1980). If the results of the analysis of variance were significant, Duncan's multiple range test was used to delineate intergroup differences.

Food consumption and body weight measurements which were repeated at certain time intervals were analyzed by the analysis of variance technique previously described. The data at each time interval were analyzed and multiple comparisons among group means were made when warranted.

The statistical computer package "Statistical Package for the Social Sciences (SPSS)"* was used for most of the analyses. Hypotheses were tested at the 0.05 level of significance.

*Supplied by Department of Political Science, National Opinion Research Center, University of Chicago.

ANIMAL OBSERVATIONS

ETHYLBENZENE EXPOSED RABBITS

Food Intake and Body Weights

Daily food consumption in all three groups ranged between 140 and 190 grams per rabbit. All groups exhibited the same degree of fluctuations in intake and there was no indication of anorexia (Table 3). Weight gain was approximately the same in all three groups throughout gestation which included the 24-day exposure period (Table 4.).

Organ Weights and Histopathology

Absolute and relative mean lung weights of the maternal rabbits were essentially the same for all groups (Table 5). The observed increased variability (standard deviation) may reflect the high weight of two lungs from animals with chronic pneumonia (40 grams for VM 56, "low-EB", and 34 grams for VM 27, "air group") or the fact that the essentially normal lungs of several animals inadvertently were not weighed. Weights of most lungs ranged from 10-18 grams. Lung weights of nonpregnant does (Table 6) were not significantly different from those of the pregnant does.

Relative liver weights of the "high-EB" group of maternal rabbits were significantly greater than those of the air and the "low-EB" groups, which were essentially the same (Table 5). Results similar to the latter two groups were obtained for the nonpregnant does (Table 6), but the differences in the high-EB group were not significant due to the smaller number of nonpregnant animals. Mean weights of kidneys and spleens of all pregnant and nonpregnant does were unremarkable (Tables 5 and 6). While the effects of the EB exposures appeared to be very similar for gravid and nongravid does (Tables 5 and 6), there were slight differences in mean absolute weights between the two female populations. This difference may be real and is suggestive of endocrine influences of pregnancy on organ weights (Souders and Morgan, 1957).

Histological examination of the lungs of the maternal rabbits showed no apparent changes related to EB exposure (Table 7). Changes present were very small foci of heterophils, lymphocytes and macrophages in alveolar areas and small increases in amounts of bronchus-associated lymphoid tissue (BALT). Animals evidencing these changes were evenly scattered through the groups. Epithelial hyperplasia of bronchi and bronchioles was uncommon and slight, except for one rabbit in the low-level group which had a moderate degree of hyperplasia associated with a moderate chronic suppurative pneumonia. One control rabbit had slight suppurative bronchitis. The other lung changes were very minor and may or may not have been related to an enzootic pathogen exposure.

Table 3. Food consumption of rabbits exposed to ethylbenzene or air by inhalation[a].

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Number of Rabbits per Group	30	29	29
Pre-exposure[b]	179 ± 61	179 ± 57	191 ± 48
1-3	183 ± 21	163 ± 54	165 ± 34
3-5	160 ± 49	176 ± 29	165 ± 34
5-7	164 ± 50	173 ± 43	171 ± 30
7-9	151 ± 31	155 ± 32	155 ± 12
9-12	173 ± 57	177 ± 36	165 ± 29
12-14	175 ± 33	181 ± 27	171 ± 34
14-16	171 ± 37	189 ± 29	177 ± 22
16-18	187 ± 43	188 ± 28	179 ± 36
18-20	187 ± 33	183 ± 31	185 ± 44
20-22	179 ± 35	173 ± 32	188 ± 29
22-24	161 ± 39	160 ± 41	173 ± 37
24-26	141 ± 40	134 ± 42	151 ± 39
26-28	171 ± 37	163 ± 46	141 ± 46

[a] Grams per rabbit per day (mean ± SD), measured every 2 or 3 days during the gestational exposures.

[b] One to three days duration immediately prior to exposure.

Table 4. Body weights of pregnant rabbits exposed to ethylbenzene or air by inhalation[a].

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Number of Rabbits per Group	24	24	23
Pre-exposure[b]	3652 ± 367	3615 ± 295	3645 ± 341
1	3765 ± 385	3692 ± 341	3767 ± 322
6	3779 ± 341	3715 ± 298	3698 ± 290
10	3837 ± 394	3761 ± 286	3587 ± 384
14	3841 ± 328	3876 ± 291	3831 ± 344
18	3941 ± 354	3902 ± 327	3858 ± 347
22	4025 ± 337	4006 ± 297	4010 ± 428
26	4100 ± 348	4100 ± 349	3997 ± 402
30	4142 ± 351	4056 ± 375	4048 ± 384

[a]Grams; mean ± SD.

[b]One to three days immediately prior to exposure.

Table 5. Organ weights of pregnant rabbits exposed to ethylbenzene or air by inhalation[a].

Observations	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Number pregnant	24	23	22
Body weight	4142 ± 351	4063 ± 382	4048 ± 384
Lung weight[b]			
absolute	14.9 ± 4.7	15.1 ± 5.8	13.1 ± 1.8
relative	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.04
Liver weight			
absolute	104.0 ± 19.2	109.1 ± 16.9	117.8 ± 22.0[c]
relative	2.5 ± 0.4	2.7 ± 0.4	2.9 ± 0.5[c]
Kidney weight			
absolute	19.3 ± 2.7	20.3 ± 2.4	19.9 ± 2.9
relative	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Spleen weight			
absolute	1.4 ± 0.37	1.3 ± 0.34	1.2 ± 0.36
relative	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

[a] Absolute weight as grams and relative weight as percent of body weight including gravid uterus; mean ± SD.

[b] Some lungs were inadvertently not weighed; group size was 23, 22, and 20 for air, low, and high, respectively.

[c] Group is significantly different by ANOVA (Duncan's). See Appendix.

Table 6. Organ weights of nonpregnant rabbits exposed to ethylbenzene or air by inhalation[a].

Observations	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Number nonpregnant	6	5	6
Body weight	3967 ± 244	4140 ± 481	3983 ± 353
Lung weight			
absolute	14.2 ± 1.2	14.6 ± 2.3	14.0 ± 1.4
relative	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.04
Liver weight			
absolute	110.2 ± 24.3	120.1 ± 13.5	132.9 ± 25.9
relative	2.8 ± 0.5	2.9 ± 0.5	3.3 ± 0.5
Kidney weight			
absolute	23.6 ± 4.6	21.2 ± 1.7	21.8 ± 3.3
relative	0.6 ± 0.1	0.5 ± 0.15	0.5 ± 0.1
Spleen weight			
absolute	1.5 ± 0.7	1.6 ± 0.4	1.6 ± 0.5
relative	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01

[a] Absolute weight as grams and relative weight as percent of body weight; mean ± SD.

Table 7. Histopathology summary of maternal rabbits exposed to ethylbenzene or air by inhalation[a].

Tissue and Observations	Exposure Levels (ppm) Air (0)	Low (99)	High (962)
Number of Animals Necropsied	30	29	29
Number of Animals Examined	6	6	6
LUNG			
Focal alveolar/perivascular inflammation	6	6	5
Increase in BALT[b]	3	4	2
Epithelial hyperplasia	1	2[c]	2
Pneumonia, suppurative	0	1[c]	0
Bronchitis, suppurative	1	0	0
KIDNEY			
Interstitial lymphoid infiltrate	5	4	5
Mineralization	2	3	3
LIVER			
Vacuolization	4[c]	4	5[c,d]
Inflammatory cells in portal area	1	1	1
Foci of heterophils	0	1	1
UTERUS AND OVARIES			
Inflammatory cells in endometrium	5	4	4
Inflammatory cells in myometrium	0	0	1

[a] Number of examined animals that had tissue changes

[b] BALT = bronchus-associated lymphoid tissue

[c] Severity of all lesions was very slight to mild, except those indicated where 1 to 2 per group were graded as moderate

[d] Fatty appearance in one sample; graded as "marked"

No apparent treatment-related cellular changes occurred in the kidneys (Table 7). One low-level and one high-level rabbit had mild interstitial lymphoid cell infiltrates, some fibrosis and tubular changes. Other minor, insignificant changes noted were rare tiny lymphoid foci scattered in interstitial areas and scattered slight tubular mineralization, both of which were evenly distributed among the groups.

The livers had variable degrees of hepatocellular vacuolation characterized by rough, nondiscrete vacuole edges, resembling glycogen deposits; the incidence of this change was evenly distributed among the experimental groups (Table 7). One high-level rabbit had marked hepatocellular vacuolation consistent with fatty change; the significance of this is questionable and is apparently not exposure-related. Slight increases in portal inflammatory cells in 3 rabbits and tiny foci of heterophils seen in 2 rabbits were represented in all groups and considered insignificant. These histological changes did not explain the significant increase in liver weight.

No significant lesions were seen in the ovaries, and the small number of inflammatory cells seen in the endometrial tissues were considered within normal limits (Table 7).

A marked fibrinonecrotic otitis externa, of unknown etiology, was present in one low dose rabbit.

Fertility and Reproductive Status

Only two does died and two others delivered prematurely out of 88 inseminated and exposed to EB or filtered air (Table 8). Thus in the absence of overt evidence of widespread maternal morbidity, mortality, or major manifestations of toxicity, the does were considered healthy and normal throughout the experimental period.

Based upon the percent of the does pregnant at sacrifice (Table 8), there was no clear evidence that daily exposure to EB from 1 through 24 d.g. altered rabbit fertility. Conception rates for the "low-EB" and "high-EB" groups were 83% and 79%, respectively, if the dead and "aborted" does are included. The distribution of nonpregnant does in each exposure group was essentially the same, and 40-67% of the nonpregnant does had corpora lutea at sacrifice.

Only one statistically significant effect was observed among the various measures of reproductive status listed in Table 9. This was the difference in mean number of live fetuses per litter in the three groups. The biological significance of the EB-related embryomortality is not understood, since there was not a concomitant change in resorbed or dead fetuses per litter. Preimplantation loss was relatively low (18-27%) based upon the ratio of mean implants per litter to mean number of corpora lutea per doe. Prenatal mortality, which was based upon the ratio of mean number of dead or resorbed fetuses per litter to mean number of implants per litter, ranged from 5-8%. Neither of these parameters indicated a "dose-related" incidence of intrauterine mortality among the three groups.

Table 8. Breeding summary of rabbits exposed to ethylbenzene or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Number Inseminated	30	29	29
Number pregnant at sacrifice	24	21	22
Number not pregnant at sacrifice	6	5	6
Number delivered prematurely	0	2[a]	0
Number died	0	1[b]	1[b]
Percent pregnant at sacrifice	80	71	78

[a] Two does, Numbers VM 23 and VM 45 delivered prematurely at 29 d.g.; no viable young.

[b] Number VM 75 (low-EB) found dead on approximately 30 d.g.; cause unknown. Number VM 85 (high-EB) found dead on approximately 11 d.g.; pneumonia presumed cause. Both were pregnant.

Table 9. Reproductive status of rabbits exposed to ethylbenzene or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Percent pregnant	80	83	79
Number of litters examined	24	21[a]	22
Number of corpora lutea per doe[b]	11 ± 2	11 ± 2	11 ± 3
Number of implants per doe[b]	8 ± 3	9 ± 3	8 ± 4
Number of implants per corpora lutea[b]	0.8 ± 0.3	0.8 ± 0.2	0.7 ± 0.3
Total fetuses (live)	182	170	158
Total fetuses (dead)	4	0	0
Number live fetuses per litter[b]	8 ± 3	7 ± 3	7 ± 3[c]
Number dead fetuses per litter[b]	0.2 ± 0.8[d]	0 ± 0	0 ± 0
Number resorptions per litter[b]	0.4 ± 0.8	0.7 ± 1.3	0.5 ± 1.1
Number of litters with resorptions	6	8	5
Percent litters with resorptions	25	35	23
Resorptions per litters with resorptions[b]	2 ± 1	2 ± 2	2 ± 2

[a] Two litters excluded from the summary; number VM 23 and VM 45 aborted.

[b] Mean ± SD.

[c] Group is significantly different by ANOVA (Duncan's). See Appendix.

[d] Litter number VM 27; all 4 implants were dead; no other litter had dead fetuses.

Fetal Measures and Teratogenicity

No significant effects of exposure were observed for fetal size (weight and length), or placental weight (Table 10). Thus, there was no evidence of intrauterine growth retardation. The sex ratios of the groups were all about the same (Table 10).

Table 10. Fetal measures from rabbit litters exposed in utero to ethylbenzene or air by inhalation[a]

Observations	Exposure Levels (ppm)		
	Air (0)	Low (99)	High (962)
Total Number of Live Fetuses	182	170	158
Body Weights (g)	43 ± 6	43 ± 8	44 ± 5
Crown-Rump Length (mm)	93 ± 11	93 ± 13	91 ± 14
Placenta Weights (g)	5.4 ± 1.2	5.2 ± 1.2	5.5 ± 1.5
Sex Ratio (% Males)	45 ± 23	45 ± 22	47 ± 27

[a]Mean ± SD of all individuals for each exposure group.

There were no significant incidences of major malformations, minor anomalies, or common variants in fetal rabbits exposed in utero to EB (Table 11). The incidences cited (Table 11) are consistent with the spontaneous incidences of these abnormalities reported for other laboratories (Cozens, 1965; Palmer, 1968). Occasionally, rudimentary ribs were incompletely ossified proximally and these observations were tabulated appropriately.

Thus, the overall impression was that daily (7 hr/day) inhalation exposure of pregnant does (1-24 d.g.) to EB at levels up to 1000 ppm did not appear to induce significant maternal toxicity, embryomortality, growth retardation, or teratogenicity in rabbits.

ETHYLBENZENE EXPOSED RATS

Food Intake and Body Weights

No significant differences among exposure groups were seen in food consumption during the pregestational or gestational exposure periods (Tables 12 and 13). Food intake was approximately 20 g/rat/day in the pregestational period and increased during late gestation to about 25 g/rat/day.

Table 11. Summary of malformations, anomalies, and variations in fetal rabbits exposed prenatally to ethylbenzene or air by inhalation[a].

Tissues and Observations	Exposure Levels (ppm) Air (0)	Low (99)	High (962)
Total No. of Litters Examined	23[d]	21	22
Total No. of Fetuses Examined	182	170	158
MAJOR MALFORMATIONS:			
Craniofacial Defects	2/2 (8.7)	0	1/1 (4.6)
Acrania	0	0	1/1 (4.6)
Cranioschisis	2/2[b] (8.7)	0	0
Cleft Lip	1/1[b] (4.2)	0	0
Cleft Palate	1/1 (4.2)	0	0
Other Defects	-	-	-
Spina Bifida	0	1/1 (4.8)	0
MINOR ANOMALIES:			
Rib Dysmorphology	0	3/3 (14.3)	0
Fused	0	2/2 (9.5)	0
Spade	0	1/1 (4.8)	0
Other Defects	-	-	-
Forelimb Flexure	1/1 (4.2)	1/1 (4.8)	0
Missing Toenail	1/1 (4.2)	0	0
Cardiac Dysmorphology	0	1/1 (4.8)	0
Thoracic Hyperchondroplasia	0	1/1 (4.8)	0
Scoliosis	0	1/1 (4.8)	0
COMMON VARIATIONS:			
Supernumerary Ribs	84/21 (91.3)	103/21 (100)	89/21 (95.5)
Extra	67/20 (87.0)	70/20 (95.2)	73/19 (86.4)
Rudimentary	42/20 (87.0)	42/20 (95.2)	26/14 (63.6)
Altered Fontanelle	2/2 (8.7)	1/1 (4.8)	1/1 (4.6)
Misshapen	0	0	1/1 (4.6)
Small	2/2 (8.7)	0	0
Large	0	1/1 (4.8)	0
Sternebral Variations	4/3 (13.0)	3/2 (9.5)	1/1 (4.6)
Misaligned	3/2 (8.7)	2/2 (9.5)	0
Fused	1/1 (4.2)	1/1 (4.8)	0
Accessory (7)	0	0	1/1 (4.6)
Ossification Defects	2/2 (8.7)	6/4 (19.0)	3/2 (9.1)
Vertebral Arch	0	1/1 (4.8)	0
Rudimentary Ribs[c]	2/2 (8.7)	5/3 (14.3)	3/2 (9.1)
Other	-	-	-
Renal Pelvic Cavitation	0	1/1 4.8	0

[a] Results are expressed as number of fetuses affected/number litters affected; % litters affected are shown below in parentheses.

[b] Same fetus; litter VM 81 fetus 3

[c] Rudimentary supernumarary ribs with proximal ossification defect (see text).

[d] Litter number VM 27: all four implants dead, so not included.

Table 12. Pregestational food consumption of rats exposed to ethylbenzene or air by inhalation[a].

Days of Exposure	Exposure Levels (ppm)		
	Air (0)	Low (97)	High (959)
Number of rats per group	159	105	105
Pre-exposure[b]	20 ± 1	21 ± 4	20 ± 4
1-2	18 ± 2	21 ± 2	14 ± 1
3-4	20 ± 2	22 ± 2	20 ± 1
5-7	21 ± 2	21 ± 0.4	22 ± 2
8-9	21 ± 1	23 ± 2	23 ± 1
10-11	21 ± 2	22 ± 2	22 ± 1
12-14	21 ± 1	21 ± 2	22 ± 2
15-16	20 ± 1	21 ± 2	20 ± 1
17-18	19 ± 1	20 ± 2	21 ± 1
19-21	21 ± 1	22 ± 1	22 ± 2

[a] Grams per rat per day; mean ± SD.

[b] Two day period immediately prior to exposure.

Table 13. Gestational food consumption of rats exposed to ethylbenzene or air by inhalation[a].

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (96)	High (985)
Number of rats per group	107	82	78
1-2	20 ± 3	20 ± 4	19 ± 4
3-4	22 ± 2	23 ± 2	22 ± 3
5-6	21 ± 3	23 ± 3	21 ± 2
7-8	20 ± 2	22 ± 3	22 ± 1
9-10	22 ± 2	24 ± 2	23 ± 2
11-12	22 ± 2	25 ± 4	24 ± 2
13-14	23 ± 2	26 ± 4	23 ± 2
15-16	26 ± 3	27 ± 4	23 ± 2

[a]Grams per rat per day; mean ± SD.

Body weights of the EB-exposed rats appeared to increase normally during the pregestational period (Table 14). Growth rates for the two EB-exposed groups were greater than that of the filtered air group during the second and third week. We cannot attribute any biological significance to this observation. The assignment of rats to the three groups was determined randomly by weight from the single shipment, so there were no known differences between groups at the outset of the pregestational exposures. The pregestational groups were randomized again after breeding, i.e., before they received their gestational exposures. There were no significant differences between air and EB-exposed rats at 1 dg (Table 15). Presumably this is because there was an interval of up to one week between the last pregestational weighing and the first gestational weighing for some animals in each group. Thus, there was time for the weights to equilibrate between the various groups. All groups gained weight during gestation (averaging approximately 90-102 g/group) and no statistically significant differences were observed (Table 15).

It should be noted that one rat (#1670) from the "high-EB" group died from unknown causes on the eighth day of pregestational exposure, but no evidence of toxicity was observed in the remaining rats.

Table 14. Pregestational body weights of rats exposed to ethylbenzene or air by inhalation[a].

Days of Pregestational Exposure	Exposure Levels (ppm)		
	Air (0)	Low (97)	High (959)
Number of rats per group	159	105	105
Pre-exposure[b]	152 ± 11	153 ± 11	153 ± 11
Week 1A	199 ± 15	201 ± 16	196 ± 19
Week 1B	202 ± 17	206 ± 17	207 ± 23
Week 2A	215 ± 18	223 ± 19[c]	225 ± 18[c]
Week 2B	219 ± 17	227 ± 20[c]	231 ± 19[c]
Week 3A	232 ± 18	238 ± 21[c]	244 ± 19[c]
Week 3B	235 ± 17	242 ± 22[c]	248 ± 19[c]

[a]Grams; mean ± SD.

[b]Two day period immediately prior to exposure.

[c]Groups significantly different by ANOVA (Duncan's). See Appendix.

Table 15. Body weights of pregnant rats exposed to ethylbenzene or air by inhalation[a].

Days of Gestation	Pregestational-Gestational Exposure Groups (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Number of rats per group	33	33	29	33	30	29	31
1	255 ± 20	252 ± 22	256 ± 21	256 ± 27	257 ± 23	264 ± 24	262 ± 22
5	272 ± 20	270 ± 22	272 ± 20	278 ± 27	274 ± 24	279 ± 26	279 ± 22
9	287 ± 20	284 ± 24	288 ± 22	288 ± 28	294 ± 23	294 ± 26	294 ± 19
13	310 ± 22	309 ± 25	311 ± 24	312 ± 29	315 ± 30	320 ± 30	315 ± 24
17	339 ± 22	336 ± 27	336 ± 32	337 ± 27	344 ± 31	341 ± 29	335 ± 26
21	379 ± 32	374 ± 34	372 ± 37	378 ± 33	381 ± 41	376 ± 35	372 ± 32

[a]Grams; mean ± SD.

Organ Weights and Histopathology

There were significant effects of EB exposure on maternal rat organ weights (Table 16). Liver, spleen, and kidney weights (absolute and relative) from the two gestationally exposed high-level groups (A-H and H-H) were significantly greater than those of all air and low-level gestationally exposed groups. This elevation in weight is not apparent in the table for the relative kidney weights (Table 16), since all values were rounded to tenths of a percent for consistency. There were no significant effects on maternal lung weights following exposure to EB. Liver weights of nonpregnant rats were also elevated for the same high-level exposure groups (Table 17).

Overall, there were similarities between rats and rabbits exposed to EB. Exposure at the high level (~1000 ppm) elevated liver weight in both species. Another similarity between the rats and rabbits exposed to EB was the differences in absolute weights, especially livers and spleens, between gravid and nongravid individuals. Again, this is suggestive that endocrine or other metabolic effects associated with pregnancy alter some organ weights.

Treatment-related changes were not evident in the histologic sections of lungs of EB-exposed rats (Table 18). Focal alveolar and perivascular infiltrates characterized by the presence of lymphocytes, plasma cells, neutrophils and/or eosinophils were seen in almost all rats of all groups and were uniformly present in a slight to mild degree. Apparent increases in bronchus-associated lymphoid tissue were slight and seen in only three rats, one in each of three different exposure groups (L-A, H-A, H-H). Bronchiolar epithelial hyperplasia was also slight and present in only one control rat and one H-A rat. The minor alveolar and perivascular infiltrates may indicate the presence of an enzootic pathogen.

The kidneys showed no histological changes related to treatment (Table 18). Considered incidental or within normal limits were: a) very tiny foci of lymphoid cells in about one-half of the rats in all groups; b) slight to mild hydronephrosis in two rats; and c) slight tubular mineralization in one rat.

Examinations of the livers provided no evidence of histological changes related to treatment (Table 18). Variable degrees of hepatocellular vacuolation resembling glycogen deposits were evenly spread among groups. Several rats in each exposure group had variable slight populations of lymphoid cells in portal areas that were within normal limits. One or two rats in each group had very small foci of necrosis and/or inflammation of unknown etiology. Three rats in the A-A and L-A groups showed evidence of extramedullary hematopoiesis.

No importance could be attributed to very small perivascular lymphoid cuffs in the ovaries of two rats, one in each of the A-L and L-L groups.

The number of inflammatory cells in the endometrium were considered within normal limits and uniformly noted in all groups. Slight numbers of inflammatory cells were seen perivascularly in the myometrium of a few rats, but it seems doubtful that this is exposure-related.

Table 16. Organ weights of pregnant rats exposed to ethylbenzene or air by inhalation[a].

Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-96	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Number pregnant	33	33	29	33	30	29	31
Body weight	378 ± 32	374 ± 34	372 ± 37	379 ± 33	381 ± 41	376 ± 35	370 ± 32
Lung weight							
absolute	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.2	1.7 ± 0.3	1.6 ± 0.2
relative	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Liver weight							
absolute	15.6 ± 1.4	15.1 ± 2.1	17.8 ± 2.1[b]	15.1 ± 1.9	15.7 ± 2.0	15.4 ± 2.1	18.5 ± 2.3[b]
relative	4.1 ± 0.2	4.0 ± 0.4	4.8 ± 0.3[b]	3.9 ± 0.8	4.1 ± 0.3	4.1 ± 0.3	5.0 ± 0.5[b]
Kidney weight							
absolute	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.3 ± 0.3[b]
relative	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1[b]	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.04	0.6 ± 0.1[b]
Spleen weight							
absolute	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2[b]	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.2[b]
relative	0.22 ± 0.03	0.21 ± 0.03	0.24 ± 0.04[b]	0.21 ± 0.05	0.22 ± 0.03	0.21 ± 0.02	0.23 ± 0.04[b]

[a] Absolute weight as grams and relative weight as percent of body weight; mean ± SD.

[b] Groups significantly different by ANOVA (Duncan's). See Appendix.

Table 17. Organ weights of nonpregnant rats exposed to ethylbenzene or air by inhalation[a].

Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Number Nonpregnant	5	1	6	8	11	10	8
Body weight	255 ± 31	280	273 ± 28	270 ± 25	266 ± 20	287 ± 28	274 ± 26
Lung weight							
absolute	1.7 ± 0.1	2.4	1.7 ± 0.3	1.6 ± 0.2	1.6 ± 0.3	1.8 ± 0.2	1.7 ± 0.2
relative	0.66 ± 0.10	0.84[b]	0.63 ± 0.06	0.59 ± 0.05	0.61 ± 0.06	0.64 ± 0.06	0.60 ± 0.06
Liver weight							
absolute	9.[illegible] ± 1.4	11.8	12.9 ± 0.9	10.7 ± 1.8	10.3 ± 1.2	11.6 ± 1.5	13.0 ± 1.6[b]
relative	3.7 ± 0.3	4.2	4.8 ± 0.7[b]	4.0 ± 0.5	3.9 ± 0.4	4.0 ± 3.4	4.8 ± 0.4[b]
Kidney weight							
absolute	1.9 ± 0.2	2.4	2.2 ± 0.1	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.2
relative	0.73 ± 0.04	0.84	0.79 ± 0.09	0.75 ± 0.05	0.79 ± 0.07	0.71 ± 0.04	0.80 ± 0.08
Spleen weight							
absolute	0.7 ± 0.1	0.7	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
relative	0.29 ± 0.08	0.25	0.27 ± 0.04	0.27 ± 0.04	0.3 ± 0.1	0.26 ± 0.05	0.25 ± 0.03

[a] Absolute weight as grams and relative weight as percent of body weight; mean ± SD.

[b] Groups significantly different by ANOVA (Duncan's). See Appendix.

Table 18. Histopathology summary of maternal rats exposed to ethylbenzene or air by inhalation[a].

Tissue and Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Number of Animals Necropsied	38	34	35	41	41	39	39
Number of Animals Examined	8	8	7	8	8	7	8
LUNG							
Focal alveolar/perivascular inflammation	8	7	6	8	8	7	7
Increase in BALT[b]	0	0	0	1	0	1	1
Epithelial hyperplasia	1	0	0	0	0	1	0
KIDNEY							
Interstitial inflammatory cells	3	4	5	3	4	4	4
Hydronephrosis	0	1	0	1	0	0	0
Mineralization	0	0	0	1	0	0	0
LIVER							
Vacuolation	4	5	5	4	4	5	5
Inflammatory cells in portal area	6	5	6	5	4	5	5
Focal necrosis/inflammation	3	1	1	3	1	2	1
Extramedullary hematopoesis	1	0	0	2	0	0	0
UTERUS AND OVARIES							
Perivascular mononuclear cells	0	1	0	0	1	0	0
Inflammatory cells in endometrium	7	8	7	8	6	7	8
Inflammatory cells in myometrium	0	2	2	0	2	2	3

[a] Number of examined animals that had tissue changes. Severity of all lesions was very slight to mild.

[b] BALT = bronchus-associated lymphoid tissue

Fertility and Reproductive Status

Rats were mated for nine consecutive nights to obtain an adequate number of females with sperm-positive vaginal lavages (Tables 19 and 20). The EB-exposed females mated well; the percent sperm positive for the nine days of breeding was 74 and 78% for the high- and low-EB groups, respectively. Only 67% of the air-exposed control females mated during the same period and this is consistent with the approximate value of 70% that we usually expect. However, the percent of sperm-positive rats that were pregnant, was significantly lower for the EB-exposed dams than the air-exposed controls (Table 19). Nevertheless, when the data on percent of sperm-positive females pregnant at 21 d.g. were rearranged for each of the gestation exposure groups and reevaluated, no statistically significant differences were observed (Table 20). The apparent discrepancy may reflect the unusually high pregnancy rate of the air-low group (97%). Generally, pregnancy is expected at 85 ± 5% of sperm-positive rats for this stock in our laboratory, but 73-81% is not unusual in unexposed groups. Thus, analysis of the data did not support a conclusion that exposure to EB reduced fertility in rats, but it does not rule out the possibility. Modification of the experimental designs would be needed to directly answer the question, "Does EB-exposure affect rat fertility?" Regardless, there were 29-33 pregnant rats in each of the gestational exposure groups at sacrifice. No statistically significant effects were observed among the various measures of reproductive status listed in Table 21.

Table 19. Breeding summary of rats exposed to ethylbenzene or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (97)	High (959)
Number of females mated	159	105	105
Number sperm positive	107	82	78
Percent sperm positive	67	78	74
Number pregnant at 21 d.g.	95	63	60
Percent pregnant at 21 d.g.	89	77[a]	77[a]

[a] $P \leq 0.05$ by Chi-Square compared to the air group.

Table 20. Pregnancy summary of rats exposed to ethylbenzene or air by inhalation.

Observations	Pregestational-Gestational Exposure Groups (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Number sperm positive	38	34	35	41	41	39	39
Number pregnant	33	33	29	33	30	29	31
Number not pregnant	5	1	6	8	11	10	8
Percent pregnant	87	97	83	81	73	74	80

Table 21. Reproductive status of rats exposed to ethylbenzene or air by inhalation.

Observations	Pregestational - Gestational Exposure Level (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Percent Pregnant	87	97	83	81	73	74	80
No. Litters Examined	33	33	29	33	30	29	31
No. of Corpora Lutea per Dam[a]	15 ± 3	16 ± 5	14 ± 3	16 ± 4	15 ± 4	17 ± 4	14 ± 2
No. of Implants per Dam[a]	12 ± 4	13 ± 3	12 ± 3	13 ± 3	11 ± 4	12 ± 4	11 ± 4
No. of Implants per Corpora Lutea[a]	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.3	0.8 ± 0.3
Total Live Fetuses	385	402	341	420	335	334	333
Total Dead Fetuses	0	0	0	0	1	1	0
No. Live Fetuses per Litter[a]	12 ± 3	12 ± 3	12 ± 4	13 ± 3	11 ± 4	12 ± 4	11 ± 4
No. Dead Fetuses per Litter[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.03± 0.18	0.04± 0.19	0.0 ± 0
No. Resorptions per Litter[a]	0.3 ± 0.5	0.5 ± 0.6	0.5 ± 1.2	0.5 ± 0.6	0.2 ± 0.5	0.5 ± 0.6	0.6 ± 0.8
No. of Litters with Resorptions	10	15	8	14	6	11	13
Percent Litters with Resorptions	30	46	28	42	20	38	42
Resorptions per Litters with Resorptions[a]	1.1 ± 0.3	1.1 ± 0.4	1.9 ± 1.7	1.4 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.5 ± 0.7

(a) Mean ± SD.

Fetal Measures and Teratogenicity

There was only one significant effect on the fetal measures (Table 22). Mean crown-rump length of the A-H group was statistically different, but since the value for the H-H group was the same as that of all other groups, the observation is evidently spurious. Otherwise body weights, placental weights and sex ratios were all within normal limits for this stock of rats.

There were no significant increases in major malformations or minor anomalies in any of the EB-exposed groups (Table 23). With the exception of supernumerary ribs, there were no significant effects of exposure on the incidence of common variations. The incidence of extra ribs was statistically increased in the A-L, A-H, and H-H groups, while rudimentary rib incidence was elevated significantly only in the A-H group. When the incidences of these two skeletal variants are combined (supernumerary ribs) that of the A-H group, but not that for the H-H group is significant. Interpretation of these results is difficult in the absence of a clear dose-response relationship. When only gestational exposure is considered for comparative purposes, then only the high-level exposed groups had an increased incidence of supernumerary ribs on the basis of percent of litters affected (69.0, versus 51.6% for A-H and H-H, respectively). The range for all of the air- and low-level exposed rats was 36.4-48.5%. Thus, there appears to be an increase in this class of abnormalities as a result of exposure to EB at the high level.

The overall impression is that daily (7 hr/day) inhalation exposure of pregnant rats (1-19 d.g.) to EB at levels up to nearly 1000 ppm had more effects than comparable exposures of pregnant rabbits. The increase of liver weight in both pregnant and nonpregnant rats, as well as in pregnant rabbits, may be an indicator of maternal toxicity or it may only reflect "detoxifying mechanisms." However, kidneys and spleens of high-level-exposed rats were also increased in weight compared to air- and low-level-exposed animals. The effect on the incidence of supernumerary ribs is noteworthy, since an increase in this skeletal variant (while not a true malformation) at approximately 1000 ppm may be an indicator of a teratogenic potential which would be fully expressed at higher levels of EB exposure (Kimmel and Wilson, 1973).

Table 22. Fetal measures from rat litters exposed in utero to ethylbenzene or air by inhalation[a].

Observations	Pregestational-Gestational Exposure Level (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Body weight (g)	3.9 ± 0.4	4.0 ± 0.7	3.8 ± 0.4	3.8 ± 0.5	4.0 ± 0.3	3.8 ± 0.3	3.8 ± 0.2
Crown-rump legnth (mm)	37 ± 2	37 ± 1	36 ± 2[b]	37 ± 2	37 ± 1	37 ± 1	37 ± 1
Placenta weight (g)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Sex ratio (% males)	50 ± 15	48 ± 15	46 ± 17	57 ± 15	51 ± 19	49 ± 19	52 ± 17

[a] Mean ± SD.

[b] Significantly different by ANOVA (Duncan's). See Appendix.

Table 23. Summary of malformations, anomalies, and variations in fetal rats exposed prenatally to ethylbenzene or air by inhalation [a].

Tissues and Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-96)	Air-High (0-985)	Low-Air (97-0)	Low-Low (97-96)	High-Air (959-0)	High-High (959-985)
Total No. of Litters Examined	33	33	29	33	30	29	31
Total No. of Fetuses Examined	385	402	341	420	335	334	333
MAJOR MALFORMATIONS							
Craniofacial Defects	0	0	1/1 (3.5)	0	1/1 (3.3)	0	0
Hydrocephaly	0	0	0	0	1/1 (3.3)	0	0
Microophthalmia	0	0	1/1 (3.5)	0	0	0	0
Ventral Wall Defects	0	0	0	0	2/2 (6.7)	0	0
Gastroschisis	0	0	0	0	1/1 (3.3)	0	0
Umbilical Hernia	0	0	0	0	1/1 (3.3)	0	0
MINOR ANOMALIES							
Rib Dysmorphology	0	0	0	0	0	0	0
Cardiovascular Defects	2/2 (6.1)	0	0	0	1/1 (3.3)	0	0
Situs Inversus Totalis	0	0	0	0	1/1 (3.3)	0	0
Transposed Pulmonary Artery	1/1 (3.0)	0	0	0	0	0	0
Retrotracheal Pulmonary Artery	1/1 (3.0)	0	0	0	0	0	0
Other Defects	-	-	-	-	-	-	-
Brachypeda	0	0	0	0	1/1 (3.3)	0	0
COMMON VARIATIONS							
Supernumerary Ribs	18/12 (36.4)	37/15 (45.5)	73/20 [b] (69.0)	37/16 (48.5)	21/11 (36.7)	22/11 (37.9)	31/16 (51.6)
Extra	1/1 (3.0)	11/6 [c] (18.2)	26/8 [c] (27.6)	10/5 (15.2)	6/3 (10.0)	6/3 (10.3)	10/7 [c] (22.6)
Rudimentary	20/13 (39.4)	33/15 (45.5)	57/20 [b] (69.0)	28/15 (45.5)	17/10 (33.3)	16/9 (31.0)	27/12 (38.7)
Reduced Ossification	18/8 (24.2)	12/10 (30.3)	9/6 (20.7)	15/11 (33.3)	9/6 (20.0)	10/9 (31.0)	11/6 (19.4)
Parietal	1/1 (3.0)	2/1 (3.0)	1/1 (3.5)	2/2 (6.1)	1/1 (3.3)	3/3 (10.3)	0
Interparietal	5/4 (12.1)	1/1 (3.0)	1/1 (3.5)	1/1 (3.0)	4/3 (10.0)	5/4 (13.8)	2/2 (6.5)
Supraoccipital	0	0	0	4/3 (10.1)	1/1 (3.3)	1/1 (3.5)	0
Sternebrae	12/5 (15.2)	4/3 (9.1)	4/2 (6.9)	4/4 (12.1)	2/2 (6.7)	15/14 (48.3)	0
Vertebral Centra	3/2 (6.1)	4/3 (9.1)	5/4 (13.8)	5/4 (12.1)	3/3 (10.0)	1/1 (3.5)	9/5 (16.1)
Caudal Vertebrae	2/2 (6.1)	0	0	0	0	0	0
Hind Limbs	0	1/1 (3.0)	0	0	0	0	0
Sternebral Variations	4/4 (12.1)	6/5 (15.2)	2/2 (6.9)	3/3 (9.1)	3/3 (10.0)	2/2 (6.9)	1/1 (3.2)
Misaligned	3/3 (9.1)	6/5 (15.2)	2/2 (6.9)	3/3 (9.1)	3/3 (10.0)	2/2 (6.9)	1/1 (3.2)
Fused	1/1 (3.0)	0	0	0	0	0	0
Other	-	-	-	-	-	-	-
Hydroureter	5/5 (15.2)	0	1/1 (3.5)	1/1 (3.0)	2/2 (6.7)	0	2/2 (6.5)

[a] Results are expressed as number of fetuses affected/number litters affected; % litters affected are show below in parentheses.

[b] Incidences of effects were significantly different from A-A controls by Chi-Square $P \leq 0.05$.

[c] Incidences of effects were significantly different from A-A controls by Fisher's Exact, $P \leq 0.05$.

ETHOXYETHANOL EXPOSED RABBITS

Food Intake, Body Weights and Mortality

Food consumption (Table 24) differed dramatically among the three exposure groups during the exposure (1-18 d.g.) and post-exposure periods (19-30 d.g.). At the beginning of the exposure period, food consumption in the high-EE group was significantly depressed to about 40 to 80% of the pre-exposure value. The subsequent gradual increase in mean food intake probably reflects the deaths of those animals that persistently ate less than about 50 g per day, but the change may have involved acclimation to the atmosphere. The rabbits of the low-EE group ate significantly less than those of the air group throughout most of the exposure period. The trend thus appears to be dose-related during exposure. Both EE-exposed groups, however, consumed more food than the controls post-exposure. The data strongly indicate that exposure of rabbits to EE by inhalation induced anorexia which persisted only during the exposure period. Water consumption was not measured.

Weight gain during exposure differed dramatically among all three animal groups (Table 25), presumably reflecting the influence of inhalation exposure, altered food intake, and pregnancy status. Net weight gain during exposure (1-18 d.g.) was about 210 and 110 g for the air- and low-EE groups, respectively, while the high-EE group lost about 250 g during this same period. Net weight gain during the post-exposure period (19-30 d.g.) was about 200, 260, and 225 g for air, low, and high groups, respectively. Thus the mean net weight gain throughout gestation was about the same for the air- and low-EE groups (375-400 g), while the high-EE group lost about 45 g. The interruption of pregnancy in the high EE group (see Table 30) may account for part of the difference in weight gain of this group compared to the other groups.

In addition to altering appetite and weight gain, there was increased mortality in the high-EE group (Table 26). Four high-level-exposed does (42, 34, 65 and 91W17) died during exposure between 10 and 13 d.g. and one ("A") was found dead at 20 d.g., two days after termination of exposure. The apparent cause of death for the two air-exposed rabbits and the one in the low-EE group was pneumonia. None of the 5 rabbits of the high-EE group had grossly apparent pneumonia. Instead, at necropsy, the stomach contents appeared to be composed of hair and recycled fecal material. This material was compacted into rather dry balls; it is doubtful they were able to pass it beyond the stomach. The intestines contained only scant ingesta. Other observations included gross evidence of fatty changes of some hearts, and all livers showed a severe degree of fatty change. There were no grossly apparent kidney changes in any rabbit. No histopathology was conducted on any of these animals.

Based on the clinical history and gross necropsy findings, the deaths in the high-EE group were presumed to have been caused primarily by the effects of the chemical on appetite and gastrointestinal function. It was not determined if this effect was direct or indirect, e.g., via the nervous system, but some indirect action seems probable since there were no consistent gross inflammatory lesions in the gastrointestinal tract.

Table 24. Food consumption of rabbits exposed to 2-ethoxyethanol or air by inhalation[a]

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Initial number of rabbits per group	29	29	29
Pre-exposure[b]	171 ± 38	151 ± 50	170 ± 37
1-2	164 ± 18	128 ± 34[c]	71 ± 41[c]
3-5	171 ± 33	138 ± 39[c]	58 ± 43[c]
6-7	182 ± 28	148 ± 38[c]	61 ± 49[c]
8-9	178 ± 26	156 ± 32[c]	75 ± 49[c]
10-11	170 ± 35	155 ± 37	82 ± 43[c]
12-13	183 ± 51	145 ± 36[c]	89 ± 43[c]
14-15	191 ± 36	156 ± 34[c]	97 ± 52[c]
16-17	182 ± 27	166 ± 50	100 ± 59[c]
18-19[d]	192 ± 25	174 ± 56	143 ± 64[c]
20-21	191 ± 37	204 ± 26	175 ± 59
22-23	191 ± 55	215 ± 50	211 ± 78
24-25	163 ± 60	190 ± 49	216 ± 70[c]
25-26	134 ± 58	178 ± 39[c]	207 ± 49[c]
27-28	135 ± 49	170 ± 48	209 ± 57[c]
28-29	132 ± 49	169 ± 66	245 ± 67[c]

[a] Grams per rabbit per day; mean ± SD.

[b] One to three days immediately prior to exposure.

[c] Groups were significantly different by ANOVA (Duncan's). See Appendix.

[d] Exposures terminated after exposure on 18 d.g. due to mortality.

Table 25. Body weights of pregnant rabbits exposed to 2-ethoxyethanol or air by inhalation[a].

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Initial number of rabbits per group	27	24	24
Pre-exposure[b]	3785 ± 414	3809 ± 479	3924 ± 394
1	3798 ± 393	3798 ± 443	3934 ± 378
6	3801 ± 395	3752 ± 434	3725 ± 370
10	3860 ± 391	3799 ± 439	3682 ± 365
14	3945 ± 392	3875 ± 427	3685 ± 347
18[c]	4001 ± 384	3941 ± 428	3661 ± 386[d]
22	4152 ± 428	4058 ± 402	3771 ± 441[d]
26	4104 ± 329	4147 ± 426	3872 ± 508
30	4189 ± 369	4202 ± 412	3879 ± 531[d]

[a]Grams; mean ± SD.

[b]One to three days immediately prior to exposure.

[c]Exposed through 18 d.g.

[d]Groups were significantly different by ANOVA (Duncan's). See Appendix.

Table 26. Mortality of rabbits exposed to 2-ethoxyethanol or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Number exposed initially	29	29	29
Number died during exposure	2	1	5
Percent mortality	7	3	17[a]

[a] $P \leq 0.05$ by Chi-Square compared to air group

Organ Weights and Histopathology

Mean maternal liver weights (absolute and relative) of both EE groups were significantly greater than those of the air group at sacrifice (Table 27). There were approximately 15 and 35% increases in absolute liver weights for the low- and high-groups, respectively. The corresponding mean kidney weights (absolute and relative) were increased about 10 or 20% above control values, but only the increase for the high-EE group was significant. Thus, there appears to be a "dose-related" increase in liver weight, but not kidney weight, following exposure to EE. There were not enough nonpregnant rabbits in any of the groups for meaningful comparisons. Mean maternal weights of lungs and spleens were unremarkable.

Convincing histological changes related to treatment were not present in the lungs (Table 28). Focal alveolar and perivascular infiltrates of lymphocytes, heterophils and macrophages were slight to mild and relatively evenly distributed among groups with perhaps a trend toward less in the high dose rabbits. There was a tendency for the exposed groups to have a small increase in bronchus-associated lymphoid tissue, based upon the fraction of samples examined histologically. Bronchiolar epithelial hyperplasia was inconsistent, but tended to be more prominent in the low level group. Slight suppurative bronchitis was present in one control rabbit, one low-level rabbit and two high-dose rabbits, and severe suppurative pleuritis was seen in one high-level rabbit.

Renal lesions showed no evidence of being treatment-related (Table 28). The high-level rabbit that had severe pleuritis also had moderate renal mineralization and moderate renal infiltrates of heterophils and lymphoid cells in the interstitial tissues. Scattered tubular mineralization was mild and evenly distributed among groups. A few other kidneys, representing all groups, showed slight lesions characterized by occasional tubular epithelial cell vacuolation and a few interstitial lymphocytes.

Table 27. Organ weights of pregnant rabbits exposed to 2-ethoxyethanol or air by inhalation[a].

Observations	Gestational Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Number pregnant	24	23	22
Body weight	4189 ± 369	4202 ± 412	3879 ± 531[b]
Lung weight			
absolute	13.2 ± 1.7	14.0 ± 2.0	15.1 ± 5.7
relative	0.3 ± 0.03	0.3 ± 0.04	0.4 ± 0.3
Liver weight			
absolute	100.0 ± 13.4	112.5 ± 20.7[b]	135.0 ± 35.6[b]
relative	2.4 ± 0.3	2.7 ± 0.4[b]	3.5 ± 0.7[b]
Kidney weight			
absolute	19.3 ± 2.8	20.9 ± 3.9	22.7 ± 3.6[b]
relative	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1[b]
Spleen weight			
absolute	1.5 ± 0.6	1.4 ± 0.9	1.6 ± 0.6
relative	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.01

[a]Mean ± SD.

[b]Groups were significantly different by ANOVA (Duncan's). See Appendix.

The livers showed no evidence of treatment-related changes (Table 28). Hepatocellular vacuolation, suggestive of glycogen deposition, was variable but evenly distributed among groups. Mononuclear inflammatory cells in portal areas were slight and evenly distributed among groups. Mild portal fibrosis was noted in one high level rabbit, as was evidenced by extramedullary hematopoiesis. Foci of necrosis were uncommon and slight except for the rabbit that had severe pleuritis, in which the liver had moderate necrosis and inflammation.

Uterine sections had slight to mild infiltration of lymphocytes and/or heterophils in the endometrium, and this tended to be more prominent in high level animals (Table 28). Four of the high-level rabbits had slight evidence of exudation of heterophils; this change was not evident in control rabbits or low-level rabbits. The finding of retained placental tissue and/or uterine involution in all six high-level does, which was not detected in control or low-level rabbits, was statistically increased. In addition, statistically significant ovarian changes were present in all of the high-level treated

Table 28. Histopathology summary of maternal rabbits exposed to 2-ethoxyethanol or air by inhalation[a].

Tissue and Observations	Exposure Levels (ppm) Air (0)	Low (160)	High (617)
Number of Animals Necropsied	27	28	24
Number of Animals Examined	6	6	6
LUNG			
Focal alveolar/perivascular inflammation	6	6[c]	4
Increase in BALT[b]	3	3[c]	5
Epithelial hyperplasia	2	3	1
Bronchitis, suppurative	1	1	2
Pleuritis, suppurative	0	0	1[d]
KIDNEY			
Interstitial lymphoid infiltrate	4	1	3[c]
Mineralization	2	3	3[c]
LIVER			
Vacuolization	5[c]	5[c]	4[c]
Inflammatory cells in portal area	4	1	5
Portal fibrosis	0	0	1
Focal necrosis/inflammation	1	0	2[c]
Extramedullary hematopoiesis	0	0	1
UTERUS AND OVARIES			
Inflammatory cells in endometrium	4	3	6
Endometritis, suppurative	0	0	4[e]
Involution/retention of placental tissue	0	0	6[e]
Corpora lutea regression	0	0	6[e]

(a) Number of examined animals that had tissue changes

(b) BALT = bronchus-associated-lymphoid tissue

(c) Severity of all lesions was very slight to mild, except those indicated where 1 to 2 per group were graded as moderate

(d) Graded as "severe"

(e) $P \leq 0.05$ by Fisher's Exact compared to air group

rabbits and none of the control or low-level rabbits (Table 28). When compared to the controls, the corpora lutea subjectively appeared smaller when viewed under a dissecting microscope. One high-level rabbit had corpora hemorrhagica. Microscopically, the cells of the corpora lutea of high-level rabbits appeared smaller and less uniform than in controls, and sterol clefts were often seen in the high-level rabbits' corpora lutea. These findings were interpreted to indicate corpora lutea regression, which was consistent with the high incidence of embryomortality of this level.

One low-level rabbit had an incidental cystic or ectatic oviduct and one high-level rabbit had a spleen with lymphoid hyperplasia as the predominate change.

Fertility and Reproductive Status

Based upon the percent of the does pregnant at sacrifice (Table 29), there was no evidence that daily exposure to EE from 1 through 18 d.g. altered rabbit fertility. If the data are included for the animals that died during exposure and for those sacrificed out of sequence, then the conception rates are 97, 86, and 93% for the air, low-EE and high-EE groups, respectively. Six of the seven nonpregnant does had corpora lutea at sacrifice; the exception was in the high-EE group, but there was no reason to believe that the finding was exposure-related.

The most striking evidence of embryotoxicity was that the uteri of all does in the high-EE group, pregnant at sacrifice, contained only early resorptions (Table 30). This 100% incidence of embryomortality was significantly different from the other groups. Therefore, the other measures of reproductive status that relate to this parameter were also significant including: a fourfold increase in number of litters with resorptions, a fourfold increase in percent of litters with resorptions and an eightfold increase in resorptions per litters with resorption. The number of implants per litter was not reduced, even though exposures were initiated at 1 d.g.

Statistically significant effects were also evident among the same measures of reproductive status for the low-EE group. The mean number of resorptions per litter was about six times that of the controls. The number of litters with resorptions and the percent of litters with resorptions was about three times that of the controls. The mean number of resorptions per litters with resorptions was about twice that of the air group. The finding of embryotoxicity in the high-EE group was not surprising in view of the marked evidence of maternal toxicity. However, since there was less severe, but still significant, embryomortality in the low-EE group, the increase was clearly dose-related. This is particularly noteworthy since there was only minor evidence of maternal toxicity (i.e., decreased body weight, reduced food consumption and increased relative liver weight) in the low-EE group.

Fetal Measures and Teratogenicity

No statistically significant effects were observed on fetal size (weight or length), placental weight, or sex ratio when the low-EE group was compared to the filtered air controls (Table 31). Thus, there was no evidence of severe intrauterine growth retardation in the low-level surviving fetuses.

Table 29. Breeding summary of rabbits exposed to 2-ethoxyethanol or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Number inseminated	29	29	29
Number pregnant at sacrifice	24	23	22
Number not pregnant at sacrifice	1	4	2
Number delivered prematurely	1	0	0
Number died prior to sacrifice[a]	2[b]	1	5
Number excluded from study[a,c]	1	1	0
Percent pregnant at sacrifice	83	79	76

[a] All pregnant

[b] Apparent cause of death was pneumonia

[c] Sacrificed on wrong days of gestation (#45 on 29 d.g. and #12 on 31 d.g.).

Table 30. Reproductive status of rabbits exposed to 2-ethoxyethanol or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)
Percent pregnant	83	79	76
Number of litters examined	24	23	22[a]
Number of corpora lutea per doe[b]	11 ± 2	11 ± 2	10 ± 2
Number of implants per doe[b]	9 ± 2	9 ± 2	8 ± 4
Number of implants per corpora lutea[b]	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.4
Total fetuses (live)	214	167[c]	0[c]
Total fetuses (dead)	0	0	0
Number live fetuses per litter[b]	9 ± 3	7 ± 2[d]	0[d]
Number dead fetuses per litter	0	0	0
Number resorptions per litter[b]	0.3 ± 0.6	2 ± 1[d]	8 ± 4[d]
Number of litters with resorptions	6	17[c]	22[c]
Percent litters with resorptions	25	74[c]	100[c]
Resorptions per litters with resorptions[b]	1 ± 1	2 ± 1[d]	8 ± 4[d]

[a] Actually only examined uterine contents for status of implants.

[b] Mean ± SD, except as noted.

[c] $P \leq 0.05$ Chi-Square compared with air group

[d] Groups were significantly different by ANOVA (Duncan's). See Appendix.

Table 31. Fetal measures from rabbit litters exposed in utero to 2-ethoxyethanol or air by inhalation[a].

Observations	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)[b]
Number of Litters	24	23	-
Body Weight (g)	47 ± 7	44 ± 11	-
Crown-Rump Length (mm)	103 ± 5	101 ± 4	-
Placenta Weight (g)	5 ± 2	6 ± 1	-
Sex Ratio (% Males)	51 ± 14	59 ± 23	-

[a]Mean ± SD.

[b]No surviving fetuses.

Significant increases in the incidence of major malformations (ventral wall defects and fusion of aorta with pulmonary artery), minor anomalies (renal changes), and common skeletal variants (supernumerary ribs with associated vertebral variations and sternebral defects) were seen in the low-EE group compared to the air controls (Table 32).

Several of the terata observed were unusual, so they are briefly described herein. There was one case of a "split peritoneum" in which the peritoneum had failed to close over two-thirds of the abdomen. The skin was intact and there was no protrusion of viscera (hernia). The cardiac dysmorphology seen was a rounded, or bulbous, heart without any other unusual characteristics. One case of "scrambled" sternebrae was seen in which most of the right and left ossified portions of the sternum were displaced, assymetrically, along the midline.

Thus, the overall conclusion is that daily (7 hr/day) inhalation exposures of pregnant does (1-18 d.g.) to EE at levels of about 620 ppm induced overt evidence of maternal toxicity and embryomortality. The low level of EE (~160 ppm) induced slight manifestations of maternal toxicity and significant incidences of embryomortality representative of a dose-response relationship. In addition, exposure to the low level induced soft tissue and skeletal abnormalities that differed both qualitatively and quantitatively from the control group.

Table 32. Summary of malformations, anomalies, and variants in fetal rabbits exposed prenatally to 2-ethoxyethanol or air by inhalation[a].

Tissues and Observations	Exposure Levels (ppm)		
	Air (0)	Low (160)	High (617)[b]
Total No. of Litters	24	23	-
Total No. of Fetuses	214	167	-
MAJOR MALFORMATIONS			
Craniofacial Defects	0	1/1 (4.4)	-
Acrania	0	1/1 (4.4)	-
Ventral Wall Defects	0	4/4[e] (17.4)	-
Omphalocoele	0	1/1[c] (4.4)	-
"Split Peritoneum"	0	1/1 (4.4)	-
Umbilical Hernia	0	2/2 (8.7)	-
Other Defects	-	-	-
Fused Aorta & Pulmonary Artery	0	5/5[e] (21.7)	-
Spina Bifida	0	1/1 (4.4)	-
Diaphragmatic Hernia	0	1/1[c] (4.4)	-
MINOR ANOMALIES			
Rib Dysmorphology	4/4 (16.7)	5/5 (21.7)	-
Knobby	2/2 (8.3)	0	-
Fused	0	1/1 (4.4)	-
Thickened Distally	2/2 (8.3)	3/3 (13.0)	-
Bent	0	1/1 (4.4)	-
Renal Changes	0	5/5[e] (21.7)	-
Cystic & Ectopic	0	3/3 (13.0)	-
Fused Kidneys	0	1/1 (4.4)	-
Unilateral Agenesis	0	1/1 (4.4)	-
Other Defects	-	-	-
Forelimb Flexure	0	3/2 (8.7)	-
Missing Toenail	0	1/1 (4.4)	-
Cardiac Dysmorphology	0	1/1 (4.4)	-
Scoliosis	0	1/1 (4.4)	-
Brachyury	0	1/1 (4.4)	-
COMMON VARIATIONS			
Supernumerary Ribs	155/23 (95.8)	164/23 (100)	-
Extra (13th pair)	129/23 (96.8)	139/23 (100)	-
Extra (13th & 14th pair)	0	21/11[e] (47.8)	-
Rudimentary (13th or 14th pair)	40/16 (66.7)	38/15 (65.2)	-

Table 32. (Cont'd)

Vertebral Variations (>12)	76/13 (54.2)	160/23[e] (100)	-
13 Thoracolumbar	74/13 (54.2)	113/20[e] (87.0)	-
14 Thoracolumbar	1/1 (4.2)	47/15[e] (65.2)	-
Double Centra	0	1/1 (4.4)	-
Extra Lumbar Arch	1/1 (4.2)	2/2 (8.7)	-
Altered Fontanelle	0	2/2 (8.7)	-
Small	0	1/1 (4.4)	-
Misshapen	0	1/1 (4.4)	-
Sternebral Variations	2/2 (8.3)	16/9[e] (39.1)	-
Misaligned	0	4/2 (8.7)	-
Scrambled	0	1/1 (4.4)	-
Bipartite	0	1/1 (4.4)	-
Fused	0	3/3 (13.0)	-
Accessory (7)	2/2 (8.3)	7/4 (17.4)	-
Ossification Defects	4/3 (12.5)	3/3 (13.0)	-
Rudimentary Ribs[d]	4/3 (12.5)	1/1 (4.4)	-
Interparietal (missing)	0	1/1 (4.4)	-
Vertebra (missing)	0	1/1 (4.4)	-

(a) Results are expressed as number of fetuses affected/number litters affected; % litters affected are shown below in parentheses.

(b) No surviving fetuses

(c) Same fetus; litter 73, fetus 3

(d) Rudimentary supernumerary ribs without ossification of proximal end

(e) Incidences of effects were significantly different from A-A by Chi-Square, $P \leq 0.05$.

ETHOXYETHANOL EXPOSED RATS

Food Intake and Body Weights

No significant differences in food consumption were seen during the pregestational and gestational periods (Tables 33 and 34), except in the high-EE group during days 1-2 of pregestational exposure. Since the observation occured only once, it is probably not meaningful. Food intake was approximately 20 g/rat/day throughout the three-week pregestational period and increased to about 25 g/rat/day during late gestation. In marked contrast to the food intake patterns of rabbits exposed to EE, there was no effect on food consumption even in the high-EE group.

Table 33. Pregestational food consumption of rats exposed to 2-ethoxyethanol or air by inhalation[a].

Days of Exposure	Exposure Levels (ppm)		
	Air (0)	Low (150)	High (648)
Number of Rats per Group	163	108	107
Pre-Exposure[b]	21 ± 1	21 ± 0.3	21 ± 1
1-2	21 ± 1	20 ± 2	18 ± 1[c]
3-4	21 ± 2	20 ± 1	19 ± 1
5-7	20 ± 1	21 ± 0.3	21 ± 1
8-10	19 ± 2	18 ± 1	17 ± 1
11-13	20 ± 2	20 ± 1	20 ± 1
14-16	20 ± 1	20 ± 1	21 ± 3
17-19	20 ± 2	19 ± 1	19 ± 2

[a] Grams per rat per day; mean ± SD.

[b] Two day period immediately prior to exposure.

[c] Groups significantly different by ANOVA (Duncan's). See Appendix.

Table 34. Gestational food consumption of rats exposed to 2-ethoxyethanol or air by inhalation[a].

Days Gestation	Exposure Levels (ppm)		
	Air (0)	Low (202)	High (767)
Number of Rats per Group	111	75	70
1 & 2	21 ± 1	20 ± 4	17 ± 8
3 & 4	23 ± 1	23 ± 4	22 ± 3
5 & 6	23 ± 2	24 ± 1	23 ± 1
7 & 8	23 ± 2	24 ± 1	24 ± 1
9 & 10	23 ± 1	24 ± 1	24 ± 3
11 & 12	24 ± 2	24 ± 1	24 ± 4
13 & 14	23 ± 2	24 ± 2	24 ± 2
15 & 16	24 ± 3	25 ± 2	25 ± 2
17 & 18	25 ± 4	24 ± 4	24 ± 4
19 & 20	25 ± 6	22 ± 4	22 ± 5

[a]Grams per rat per day; mean ± SD.

The body weights of the EE-exposed rats appeared to increase normally during the pregestational period (Table 35). Body weights of the two EE-exposed groups were significantly greater than that of the filtered air group at some weighing intervals (Weeks 1B and 2B). However, the starting and final weights of the three groups for the pregestational period were essentially the same. Thus, three weeks of exposure of nonpregnant rats to EE at up to 650 ppm did not appear to appreciably alter food consumption or growth. Gestational weight gain (1-21 d.g.) was about 100-120 g for all but two high groups (A-H and H-H) which gained only about 40 g (Table 36). As in the case of the high-EE exposed rabbits, gestational exposure of rats at the high level significantly depressed weight gain, presumably through its effect upon reproductive status (see Table 42).

Organ Weights and Histopathology

Mean liver weights of the groups exposed to the high-EE level gestationally were significantly lower than those of the groups exposed to air or low-EE (Table 37). The direction of this effect is opposite to that observed in the

Table 35. Pregestational body weights of rats exposed to 2-ethoxyethanol or air by inhalation[a].

Interval	Pregestational Exposure Levels (ppm)		
	Air (0)	Low (150)	High (649)
Number of rats per group	163	108	107
Pre-exposure[b]	163 ± 15	163 ± 14	162 ± 16
Week 1A	200 ± 18	198 ± 15	200 ± 16
Week 1B	206 ± 20	206 ± 16	213 ± 18[c]
Week 2A	213 ± 19	216 ± 19	217 ± 20
Week 2B	213 ± 17	221 ± 19[c]	225 ± 20[c]
Week 3A	227 ± 17	232 ± 20	230 ± 19
Week 3B	232 ± 19	236 ± 21	237 ± 20

[a] Grams; mean ± SD.

[b] Two day period immediately prior to exposure.

[c] Groups are significantly different by ANOVA (Duncan's). See Appendix.

Table 36. Body weights of pregnant rats exposed to 2-ethoxyethanol or air by inhalation[a].

Days of Gestation	Pregestational-Gestational Exposure Groups (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Number of rats per group	32	28	31	37	34	34	33
1	252 ± 22	254 ± 24	247 ± 23	249 ± 23	248 ± 24	248 ± 24	247 ± 21
5	276 ± 23	278 ± 25	270 ± 23	268 ± 18	271 ± 27	267 ± 26	265 ± 23
9	287 ± 24	292 ± 24	286 ± 31	280 ± 18	281 ± 28	275 ± 27	279 ± 24
13	304 ± 30	311 ± 27	302 ± 26	300 ± 20	298 ± 27	292 ± 27	294 ± 22
17	335 ± 28	336 ± 33	314 ± 25[b]	324 ± 21	326 ± 27	324 ± 29	303 ± 24[b]
21	378 ± 29	375 ± 31	294 ± 27[b]	368 ± 23	366 ± 32	369 ± 39	285 ± 27[b]

[a]Grams; mean ± SD for all nine breeding replicates.

[b]Groups are significantly different by ANOVA (Duncan's). See appendix.

Table 37. Organ weights of pregnant rats exposed to 2-ethoxyethanol or air by inhalation[a].

Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Number pregnant	32	28	31	37	34	34	33
Body weight	378 ± 29	378 ± 31	295 ± 27[b]	368 ± 23	368 ± 31	369 ± 39	285 ± 29[b]
Lung weight							
absolute	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
relative	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1[b]	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.04[b]
Liver weight							
absolute	15.5 ± 1.6	16.4 ± 2.4	11.0 ± 1.6[b]	14.8 ± 1.3	15.7 ± 1.7	14.9 ± 2.1	11.4 ± 2.0[b]
relative	4.1 ± 0.3	4.4 ± 0.5	3.7 ± 0.3[b]	4.1 ± 0.3	4.3 ± 0.4	4.0 ± 0.4	4.0 ± 0.4
Kidney weight							
absolute	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.1 ± 0.2
relative	0.5 ± 0.1	0.6 ± 0.04	0.7 ± 0.1[b]	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1[b]
Spleen weight							
absolute	0.8 ± 0.1	0.9 ± 0.2[b]	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.1[b]
relative	0.21 ± 0.03	0.23 ± 0.04	0.26 ± 0.04[b]	0.21 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.24 ± 0.02

[a] Absolute weight as grams and relative weight as percent body weight; mean ± SD.

[b] Groups are significantly different by ANOVA (Duncan's). See Appendix.

rabbits, where a dose-related increase in liver weight was seen. Mean weights of lungs and kidneys (relative) for the high level groups were significantly greater than the other groups. The absolute weights of the spleens in the A-L group were significantly different from the A-A group, but since the relative weights were not and the two gestationally exposed high-level groups were not significantly different, this observation cannot be considered biologically meaningful. The numbers of nonpregnant rats (Table 38) per group were often too small for meaningful comparisons. For example, the significant difference for the L-A lung group represents only one animal. Where group sizes are adequate, the weight of the nongravid animal tissues are similar to those of the gravid rats. However, they were generally more variable in the nongravid animals and there were few significant effects of EE exposure (e.g., in the A-H group).

Treatment-related changes were not evident in the lungs of exposed rats (Table 39). Focal alveolar and perivascular cuffs of lymphocytes, plasma cells, macrophages, neutrophils and/or some eosinophils were present in all groups to no more than a mild degree except for marked cuffing in one exposed animal that also had marked pleuritis. Slight apparent increases in bronchus-associated lymphoid tissue was seen in only 5 animals and represented 4 exposure groups (A-L, A-H, L-L and H-H). Slight bronchiolar epithelial hyperplasia was present in one exposed rat that had mild abscesses and slight pleuritis.

The kidneys had no lesions that could be related to exposure (Table 39). There were a few rats that had very slight foci of lymphoid cells in interstitial tissues, but these were considered within the range of normal. Seven rats representing the control and three exposure groups (A-L, L-A and H-H) had hydronephrosis, as well as an incidental lesion; one AH-exposed rat had mild pyelonephritis.

Liver changes could not be related to exposure (Table 39). Vacuolar changes in hepatocytes appeared to be related to glycogen deposition and rats with lesions were evenly scattered through groups. Inflammatory cells in portal areas were insignificant. Very slight foci of single cell necrosis and focal inflammation was present in rats of all groups. In the groups receiving the high dose during gestation, the necrotic foci seemed larger but that judgment was subjective and the larger foci were also seen in several lower-dose rats.

The uteri of rats in all groups had lymphocytes and neutrophils in the endometrium to a slight to mild degree (Table 39). In the A-H group, 7 of 8 had exudation of neutrophils and some epithelial necrosis and 5 of 8 in the H-H dose group had similar lesions, whereas this was not seen in any of the other groups. Evidence of significant placental retention and/or uterine involution was noted in 8 of 8 A-H dose rats and 7 of 8 H-H dose rats, whereas these changes were not evident in the rest of the groups. Incidental slight lesions of inflammatory cells in the myometrium were seen in 2 rats in low dose groups.

The ovaries of the rats that received the high doses of EE during gestation had significant changes indicative of corpora lutea regression (Table 39). The changes were relatively smaller corpora lutea size, small luteal cells, and decreased uniformity of cells in the corpora lutea. The changes did not

Table 38. Organ weights of nonpregnant rats exposed to air or 2-ethoxyethanol[a].

Observations	Pregestational-Gestational Exposure Level (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Number Not Pregnant	5	9	6	1	3	1	1
Body Weight	276 ± 22	271 ± 14	271 ± 25	288	276 ± 8	220	315
Lung Weight							
absolute	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.2	3.4	1.4 ± 0.2	1.1	1.7
relative	0.51 ± 0.12	0.50 ± 0.06	0.54 ± 0.06	1.17[b]	0.51 ± 0.06	0.50	0.54
Liver Weight							
absolute	10.4 ± 1.3	10.9 ± 1.1	10.5 ± 1.4	14.0	10.2 ± 0.1	8.8	13.1
relative	3.8 ± 0.3	4.0 ± 0.5	3.9 ± 0.4	4.9	3.7 ± 0.1	4.0	4.2
Kidney Weight							
absolute	2.1 ± 0.2	2.0 ± 0.1	1.8 ± 0.2	2.5	2.1 ± 0.05	1.8	2.3
relative	0.75 ± 0.03	0.75 ± 0.06	0.67 ± 0.04[b]	0.87	0.74 ± 0.01	0.80	0.74
Spleen Weight							
absolute	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9	0.7 ± 0.1	0.5	0.8
relative	0.29 ± 0.03	0.28 ± 0.04	0.27 ± 0.03	0.30	0.25 ± 0.04	0.21	0.26

[a]Absolute weight as grams and relative weight as percent body weight; mean ± SD.

[b]Group is significantly different by ANOVA (Duncan's). See Appendix.

Table 39. Histopathology summary of maternal rats exposed to 2-ethoxyethanol or air by inhalation[a]

Tissue and Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Number of Animals Necropsied	37	37	37	38	37	35	35
Number of Animals Examined	8	7	8	9	8	8	8
LUNG							
Focal alveolar/perivascular inflammation	7	4	5	8	7[d]	6	8
Increase in BALT[b]	0	1	1	0	2	0	1
Epithelial hyperplasia	0	0	0	0	0	1	0
Pleuritis, suppurative	0	0	0	0	1[d]	1	0
Abscess	0	0	0	0	0	1	0
KIDNEY							
Interstitial inflammatory cells	1	0	0	4[d]	2	4	2
Hydronephrosis	1	1[c]	0	2	0	0	3
Pyelonephritis	0	0	1	0	0	0	0
LIVER							
Vacuolation	7[c]	6[c]	7[c]	6	7	6[c]	7[c]
Inflammatory cells in portal area	0	0	0	0	1	0	0
Focal necrosis/inflammation	6	4	6	5	3	2	6
UTERUS AND OVARIES							
Inflammatory cells in endometrium	8	7	8	9	7	7	7
Inflammatory cells in myometrium	0	1	0	1	0	0	0
Endometritis, suppurative	0	0	7[e]	0	0	0	5[e]
Involution/retention of placental tissue	0	0	8[e]	0	0	0	7[e]
Corpora lutea regression	0	0	4[e]	0	0	0	5[e]

[a] Number of examined animals that had tissue changes

[b] BALT = bronchus-associated lymphoid tissue

[c] Severity of all lesions was very slight to mild, except those indicated where one per group was graded as "moderate".

[d] Severity of all lesions was very slight to mild, except those indicated where one per group was graded as "marked".

[e] Incidence of effects was significantly different by Fisher's Exact.

seem as pronounced as in the rabbits as only 4 of 8 in the A-H group and 5 of 8 in the H-H group seemed to have evidence of corpora lutea regression.

Fertility and Reproductive Status

Rats were mated for ten consecutive nights to obtain an adequate number of sperm-positive animals (Tables 40 and 41). The EE-exposed females mated well; the percent sperm positive for the ten days of breeding was 68, 69, and 65% for the air, low-EE, and high-EE groups, respectively. These results were in the same range as those observed for the air-exposed controls for the EB exposures. The percent of sperm-positive rats that were pregnant was exceptionally high for the EE-exposed groups (about 95%; Table 40), while only 82% of the controls were pregnant compared to an expected incidence of about 85%. In addition, when the percent of sperm-positive females pregnant at 21 d.g. was evaluated for each of the gestational exposure groups, most groups had an incidence between about 85% and 95% (Table 41). Thus, these results are similar to those obtained in rabbits; exposure to EE does not appear to alter mating behavior, breeding performance or fertility.

Table 40. Breeding summary of rats exposed to 2-Ethoxyethanol or air by inhalation.

Observations	Exposure Levels (ppm)		
	Air (0)	Low (150)	High (649)
Number of females mated	163	108	107
Number sperm positive	111	75	70
Percent sperm positive	68	69	65
Number pregnant at 21 d.g.	91	71	67
Percent pregnant at 21 d.g.	82	95	96

Significant embryolethal effects were observed associated with the high level EE exposure, induction of 100% resorptions (Table 42). These results resemble those noted above for rabbits and reported recently by Nelson et al. (1981) for rats. The mean numbers of resorptions per litter in the high-EE groups were about twenty-four-times that of the air controls, while that of the low-EE group was about twice the control value. The effects of high-EE exposure on percent of litters with resorptions was obvious. In contrast to the

Table 41. Pregnancy summary of rats exposed to 2-ethoxyethanol or air by inhalation.

Observations	Pregestational-Gestational Exposure Groups (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Number sperm positive	37	37	37	38	37	35	35
Number died	0	0	0	0	0	0	1[a]
Number pregnant	32	28	31	37	34	34	33
Number not pregnant	5	9	6	1	3	1	1
Percent pregnant	87	76	84	97	92	97	94

[a] #2265 found dead on ninth day of exposure - not pregnant.

Table 42. Reproductive status of rats exposed to 2-ethoxyethanol or air by inhalation.

Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High (649-767)
Percent pregnant	87	76	84	97	92	97	94
Number litters examined	32	28	31	37	34	34	33
Number of corpora lutea per dam[a]	17 ± 4	18 ± 5	16 ± 3	16 ± 3	16 ± 4	17 ± 4	16 ± 3
Number of implants per dam[a]	13 ± 3	13 ± 3	12 ± 4	12 ± 3	13 ± 3	13 ± 3	13 ± 3
Number of implants per corpora lutea[a]	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Total fetuses (live)	387	324	0[b]	441	402	418	0[b]
Total fetuses (dead)	0	0	0	0	0	0	0
Number live fetuses per litter[a]	12 ± 3	12 ± 3	0 ± 0[c]	12 ± 3	12 ± 3	12 ± 4	0 ± 0[c]
Number dead fetuses per litter[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Number resorptions per litter[a]	0.5 ± 0.7	1.0 ± 1.1	12.0 ± 2.9[c]	0.4 ± 0.8	0.9 ± 1.1	0.8 ± 1.4	12.5 ± 3.0[c]
Number litters with resorptions	14	18	31[b]	10	18	17	33[b]
Percent litters with resorptions	44	64	100[b]	27	53	50	100[b]
Resorptions per litters with resorptions[a]	1 ± 1	2 ± 1	12 ± 3[c]	2 ± 1	2 ± 1	2 ± 2	13 ± 3[c]

[a] Mean ± SD, except as noted.

[b] Incidences of effects were significantly different by Chi-Square.

[c] Groups are significantly different by ANOVA (Duncan's). See Appendix.

rabbits exposed to EE, the categories related to resorptions in the low-EE-exposed groups, although higher, were not significantly different from the control. The marked embryomortality induced in rats by exposure to high EE was surprising in the absence of any apparent effects on either food consumption or pregestational weight gain -- a striking contrast to the rabbits.

Fetal Measures and Teratogenicity

Body size was significantly reduced at sacrifice for the two gestationally exposed low-EE groups (Table 43). There was an approximate 25% reduction in body weight, and crown-rump length was reduced by about 10%. Thus, exposure to EE at ~200 ppm throughout gestation induced intrauterine growth retardation. There were no differences in placental weight or sex ratios among the various groups.

A significantly increased incidence of cardiovascular defects (transposed and retrotracheal pulmonary artery) was observed in the A-L, but not in the L-L group (Table 44). Although the specific defects were different, EE-exposed rabbits also had a significant incidence of cardiovascular malformations (fused aorta and pulmonary artery).

Minor skeletal defects were the predominant effects observed in the low-level EE groups (Table 44). Gestational exposure to low levels of EE incuded a significant increase of both minor skeletal anomalies and common skeletal variants in rats. The minor skeletal anomalies consisted of various categories of rib dysmorphology, especially "knobby ribs" (Table 44; Figure 14).

The increased incidence of common skeletal variants consisted of either supernumerary ribs and vertebrae, or reduced skeletal ossification.

The incidence of extra and rudimentary ribs, as well as associated thoracolumbar vertebrae, were all significantly increased in the two low-level groups compared to the three air groups, based on gestational exposures. Most of the individual skeletal variants listed in Table 44 were also significantly increased in the EE-exposed but not in the air-exposed groups. The extensive observations of reduced skeletal ossification are consistent with fetal growth retardation (Fritz and Hess, 1970; Aliverti et al., 1979; Ariyuki et al., 1980).

Thus, the overall conclusion was that daily (7 hr/day) inhalation exposure of pregnant rats (1-19 d.g.) to EE at ~750 ppm induced significantly increased incidences of maternal toxicity, as evidenced by reduced body weight gain and organ weight changes (Tables 36 and 37), and embryomortality (Table 42). Neither pregestational nor gestational exposures at ~200 ppm depressed maternal body weight (Tables 35 and 36), and exposure to this concentration did not alter maternal organ weights (Table 37). Exposure to EE at ~200 ppm during gestation significantly retarded fetal growth and induced a significant increase in the occurrence of minor anomalies and common variants in fetal skeleton (Tables 43 and 44).

Table 43. Fetal measures from rat litters exposed in utero to 2-ethoxyethanol by inhalation[a].

Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High[b] (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (649-0)	High-High[b] (649-767)
Body weight (g)	3.8 ± 0.3	3.0 ± 0.4[c]	-	3.9 ± 0.3	3.2 ± 0.3[c]	3.9 ± 0.3	-
Crown-rump length (mm)	36 ± 2	33 ± 4[c]	-	37 ± 2	34 ± 2[c]	37 ± 2	-
Placenta weight (g)	0.6 ± 0.1	0.7 ± 0.2	-	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	-
Sex ratio (% males)	47 ± 16	44 ± 14	-	54 ± 15	47 ± 17	48 ± 12	-

[a] Mean ± SD.

[b] No surviving fetuses.

[c] Groups are significantly different by ANOVA (Duncan's). See Appendix.

Table 44. Summary of malformations, anomalies, and variations in fetal rats exposed prenatally to 2-ethoxyethanol or air by inhalation[a].

Tissue and Observations	Pregestational-Gestational Exposure Levels (ppm)						
	Air-Air (0-0)	Air-Low (0-202)	Air-High (0-767)	Low-Air (150-0)	Low-Low (150-202)	High-Air (849-0)	High-High (649-767)[b]
Total No. of Litters Examined	32	29	-	37	34	33	-
Total No. of Fetuses Examined	386	324	-	441	397	420	-
MAJOR MALFORMATIONS							
Craniofacial Defects	2/2 (6.3)	0	-	0	0	0	-
Hydrocephaly	1/1 (3.1)	0	-	0	0	0	-
Anophthalmia	1/1 (3.1)	0	-	0	0	0	-
MINOR ANOMALIES							
Rib Dysmorphology	0	13/6[c] (21.4)	-	1/1 (2.7)	6/6[c] (17.6)	0	-
Wavy	0	7/4[c] (14.3)	-	0	5/5[c] (14.7)	0	-
Bent	0	5/3 (10.7)	-	0	4/3 (8.8)	0	-
Knobby	0	5/2 (7.1)	-	0	1/1 (2.9)	0	-
Fused	0	0	-	1/1 (2.7)	0	0	-
Cardiovascular Defects	0	6/4[c] (14.3)	-	0	2/2 (5.9)	0	-
Transposed Pulmonary Artery	0	0	-	0	1/1 (2.9)	0	-
Retrotracheal Pulmonary Artery	0	3/3[c] (10.3)	-	0	1/1 (2.9)	0	-
Polydactyly	0	0	-	0	1/1	0	-
COMMON VARIATIONS[e]							
Supernumerary Ribs	49/15 (46.9)	84/21[d] (75.0)	-	30/16 (43.2)	110/24[d] (70.6)	41/15 (45.5)	-
Extra (14)	5/4 (12.5)	27/13 (46.4)	-	1/1 (2.7)	25/14 (41.2)	9/5 (15.2)	-
Rudimentary	43/14 (43.8)	68/20 (71.4)	-	29/15 (40.5)	96/25 (73.5)	34/13 (39.4)	-
Extra Thoracolumbar Vertebrae	0	28/10 (35.7)	-	0	20/7 (22.6)	0	-
Reduced Ossification	32/17 (53.1)	225/26[d] (92.9)	-	17/11 (26.7)	275/33[d] (97.1)	22/13 (39.4)	-
Frontal	1/1 (3.1)	62/12 (46.1)	-	0	22/7 (20.6)	0	-
Parietal	1/1 (3.1)	22/19 (64.3)	-	2/2 (6.4)	13/12 (50.0)	0	-
Interparietal	2/2 (6.3)	64/15 (53.6)	-	0	49/17 (50.0)	4/4 (12.1)	-
Supraoccipital	1/1 (3.1)	76/21 (75.0)	-	1/1 (2.7)	75/21 (61.8)	1/1 (3.0)	-
Sternebrae	5/5 (15.6)	74/23 (82.1)	-	1/1 (2.7)	145/30 (88.2)	0	-
Vertebral Centra	0	11/7 (25.0)	-	0	13/7 (20.6)	0	-
Ribs	0	7/4 (14.3)	-	0	2/1 (2.9)	0	-
Metacarpals	1/1 (3.1)	79/17 (60.7)	-	0	39/9 (26.5)	0	-
Metatarsals	1/1 (3.1)	23/4 (14.3)	-	0	4/4 (11.8)	0	-
Sacral Vertebrae	3/3 (9.4)	104/16 (51.1)	-	0	58/12 (35.3)	0	-
Caudal Vertebrae	10/6 (18.8)	211/21 (75.0)	-	5/2 (5.4)	215/24 (70.6)	6/1 (3.0)	-
Ischium	1/1 (3.1)	0	-	0	1/1 (2.9)	0	-
Pubis	0	6/1 (3.6)	-	0	11/2 (5.9)	0	-
Sternebral Variations	0	0	-	1/1 (2.7)	1/1 (2.9)	0	-
Bipartite	0	0	-	0	1/1 (2.9)	0	-
Scrambled	0	0	-	1/1 (2.7)	0	0	-
Other Defects	-	-	-	-	-	-	-
General Edema	1/1 (3.1)	1/1 (3.6)	-	0	0	0	-

(a) Results are expressed as number of fetuses affected/number litters affected; % litters affected are shown below in parentheses.

(b) No surviving fetuses.

(c) Incidences of effects were significantly different from A-A controls by Fisher's Exact, $P \leq 0.05$.

(d) Incidences of effects were significantly different from A-A controls by Chi-Square, $P \leq 0.05$.

(e) Each category was not tested for statistical significance, since individual fetuses often had multiple effects.

CONCLUSIONS

ETHYLBENZENE

The biologically and statistically significant effects following exposure of rabbits and rats to EB are summarized in Table 45. The observed increase in maternal liver weight and reduction in mean number of live fetuses per litter were the only effects seen in rabbits which were found to be statistically significant. Liver weights of nonpregnant does at the high level were not elevated significantly compared to the air control. It is of importance that there were no appreciable differences between the mean body weights or food consumption of the three groups over the course of this experiment (Figure 15). Thus, the observed changes were not considered to be sufficient evidence to conclude that exposure to EB under this exposure regime was either maternally toxic or embryotoxic for rabbits.

The increase in pregestational weight gain of the rats may be indicative of toxicity, and the organ weight data strongly suggest that there may be maternal toxicity (Table 45). Increased size of maternal rat livers, kidneys and spleens were seen, and liver weights of nonpregnant adults were elevated also in both the A-H and H-H groups. The reduction in the percent of sperm-positive rats that were pregnant following pregestational exposures to either level suggest that EB may have adverse effects on fertility. There is some question on this point, since rearrangement of the data by gestational exposure does not reveal any significant effects. The decreased crown-rump length of the fetuses in the A-H but not in the H-H group is weak evidence of growth retardation and without accompanying depressions in mean fetal weight is probably not meaningful. The increased incidence of fetal supernumerary ribs is not considered to be a demonstration of teratogenicity, but may be an indication that exposure to higher levels might induce terata. There is not a consistent dose-reponse relationship at the levels used in this experiment. There was an elevated incidence of supernumerary ribs (extra and rudimentary) only in the A-H group, while the incidence of extra ribs alone was elevated in the A-L, A-H and H-H groups.

It is useful to consider the observed embryotoxicity on a scale relative to maternal toxicity. Thus, when a range of doses is studied and embryotoxicity is detected only at doses which are toxic to the maternal animal, one might conclude that special concern need not be directed toward the fetus. It is necessary to use caution in this approach since it is possible to overlook subtle indications of embryotoxicity in the search for statistically significant changes (Palmer, 1977). When a more limited range of doses or exposure levels are used, such as in the present studies, it becomes less likely that all concentrations will lie above the minimally toxic level for the adult. The finding of no or little embryotoxicity in the presence of maternal toxicity thus suggests, but does not prove, that an occupational

Table 45. Summary of significant effects of EB exposures on rabbits and rats.

Species	Observations	Exposure Group	Direction Of Effect	Data Table[a]
Rabbit	Maternal liver weight[b]	High	↑	5
Rabbit	No. live pups/litter	Low & High	↓	9
Rat	Pregestational body weight	Low & High	↑	14
Rat	Maternal liver weight[b]	A-H & H-H	↑	16
	Maternal kidney weight[b]	A-H & H-H	↑	16
	Maternal spleen weight[b]	A-H & H-H	↑	16
Rat	Adult liver weight[b]	A-H & H-H	↑	17
	Adult lung weight[b]	A-L only	↑	17
Rat	% pregnant post pregestational exposure	Low & High	↓	19
Rat	Pup crown-rump length	A-H only	↓	22
Rat	Supernumerary ribs	A-H only	↑	23
	Extra ribs	A-L,A-H & H-H	↑	23

[a] Table number in this report which contains numerical expression of results.

[b] Relative weight (percent of body weight).

environment which is safe for the pregnant worker will also be safe for her fetus.

Thus, the majority of the data supports the conclusion that the 1000-ppm level of EB under the conditions used for this study is toxic for pregnant rats, but probably not for pregnant rabbits. The results suggest that even higher levels administered under similar conditions might also be teratogenic in rats, but not necessarily in rabbits.

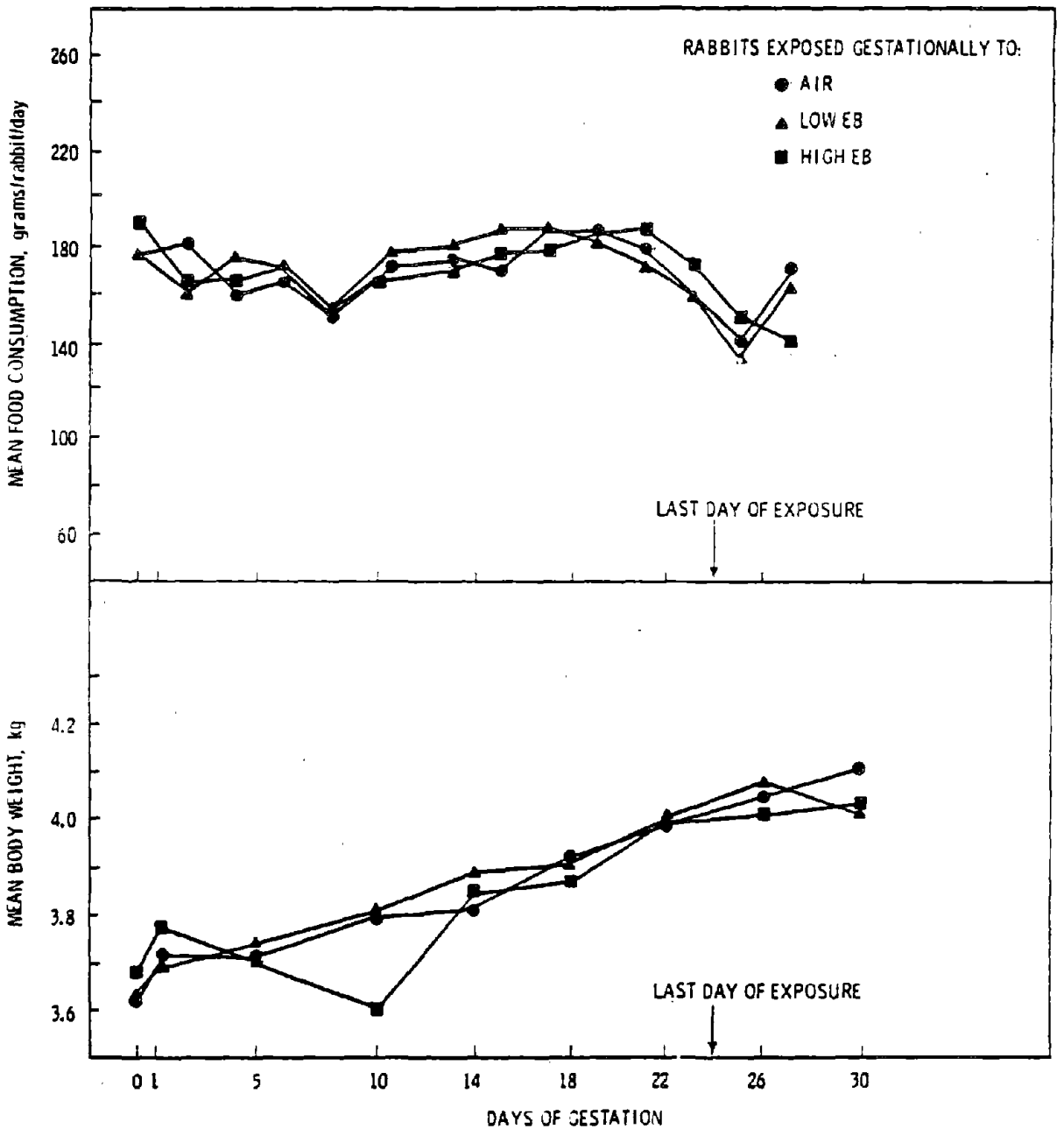


Figure 15. Summary of food consumption data and body weights for all rabbits exposed to ethylbenzene.

In contrast to our finding with EB, exposures of pregnant rabbits to EE resulted in conclusive evidence of embryotoxicity and teratogenicity in addition to maternal toxicity; the biologically and statistically significant findings are summarized in Table 46. Adverse maternal effects included dose-related depression in food consumption and consistent loss of body weight during exposure (Figure 16). These effects caused, or at least contributed to, an increased incidence of maternal mortality at the high level. Liver and kidney weights were elevated in the survivors. The most striking embryotoxic effect was the resorption incidence of 100% in all pregnant does surviving the high level. The histopathological changes in the ovaries and uteri of high-level exposed doses were consistent with the early interruption of pregnancy. A significantly increased incidence of embryomortality accompanied by a corresponding decrease in live fetuses per litter was seen even in those exposed to the low level. Suprisingly, however, growth retardation was not seen in the surviving rabbit fetuses. As was described in the "Results" section, morphologic changes detected included major malformations, minor anomalies, and variations of soft tissue and skeletons of the rabbit fetuses.

A similar spectrum of statistically significant effects was seen in pregnant rats exposed to EE. Alterations in maternal lung, liver, and kidney weights summarized in Table 46 were considered to be presumptive indications of toxicity. Food consumption was not affected, but body weight was reduced in those rats exposed gestationally to the higher level (Figure 17). However, this effect probably reflected the total incidence of interrupted pregnancy at this level and was probably not a true measure of maternal toxicity. Increased embryomortality occurred only at the high level in the rats, in contrast to the dose-related incidence in rabbits. Again, histological examination of ovaries and uteri revealed evidence of early interruption of pregnancy in those groups exposed at the higher concentration during gestation with high incidences of resorptions. There was growth retardation of the live pups from the low-level exposure group since weight, length, and skeletal ossification were reduced. Growth retardation was not seen in the surviving rabbit kits. It is particularly noteworthy that the increased incidence of terata seen in the rats occurred at the low level which did not induce maternal toxicity. The incidence of major malformations was not significantly elevated, but the incidence of minor anomalies and variations was significantly elevated. These involved both soft tissue and skeletons.

Thus, taken together these data implicate EE as being a teratogen in both rabbits and rats. Significant incidence of terata, intrauterine growth retardation, and embryomortality may be induced at levels that are below or are similar to those that induce maternal manifestations of toxicity. Based upon these bioassay data and those from other laboratories, EE has exhibited sufficient evidence of teratogenicity and embryotoxicity that it should probably be regarded as presenting similar risks to humans.

Table 46. Summary of significant effects of EE exposures on rabbits and rats.

Species	Observations	Exposure Group	Direction Of Effect	Data Table[a]
Rabbit	Maternal food consumption	Low & High	↓	24
Rabbit	Maternal body weights	High	↓	25
Rabbit	Maternal mortality	High	↑	26
Rabbit	Maternal liver weight[b]	Low & High	↑	27
	Maternal kidney weight[b]	High	↑	27
Rabbit	Histopathology of ovaries/uterus	High	↑	28
Rabbit	Incidence of resorptions/litter	Low & High	↑	30
	Incidence of live fetuses/litter	Low & High	↓	30
Rabbit	Terata	Low	↑	32
Rat	Gestational body weight	A-H & H-H	↓	36
Rat	Maternal lung weight[b]	A-H & H-H	↑	37
	Maternal liver weight[b]	A-L/ A-H	↑/↓	37
	Maternal kidney weight[b]	A-L, A-H & H-H	↑	37
	Maternal spleen weight[b]	A-L, A-H & H-H	↑	37
Rat	Histopathology of ovaries/uterus	A-H & H-H	↑	39
Rat	Incidence of resorptions/litter	A-H & H-H	↑	42
	Incidence of live fetuses/litter	A-H & H-H	↓	42
Rat	Fetal weight	A-L & L-L	↓	43
	Fetal crown-rump length	A-L & L-L	↓	43
Rat	Terata	A-L & L-L	↑	44

[a] Table number in this report which contains numerical expression of results.

[b] Relative weight, percent of body weight.

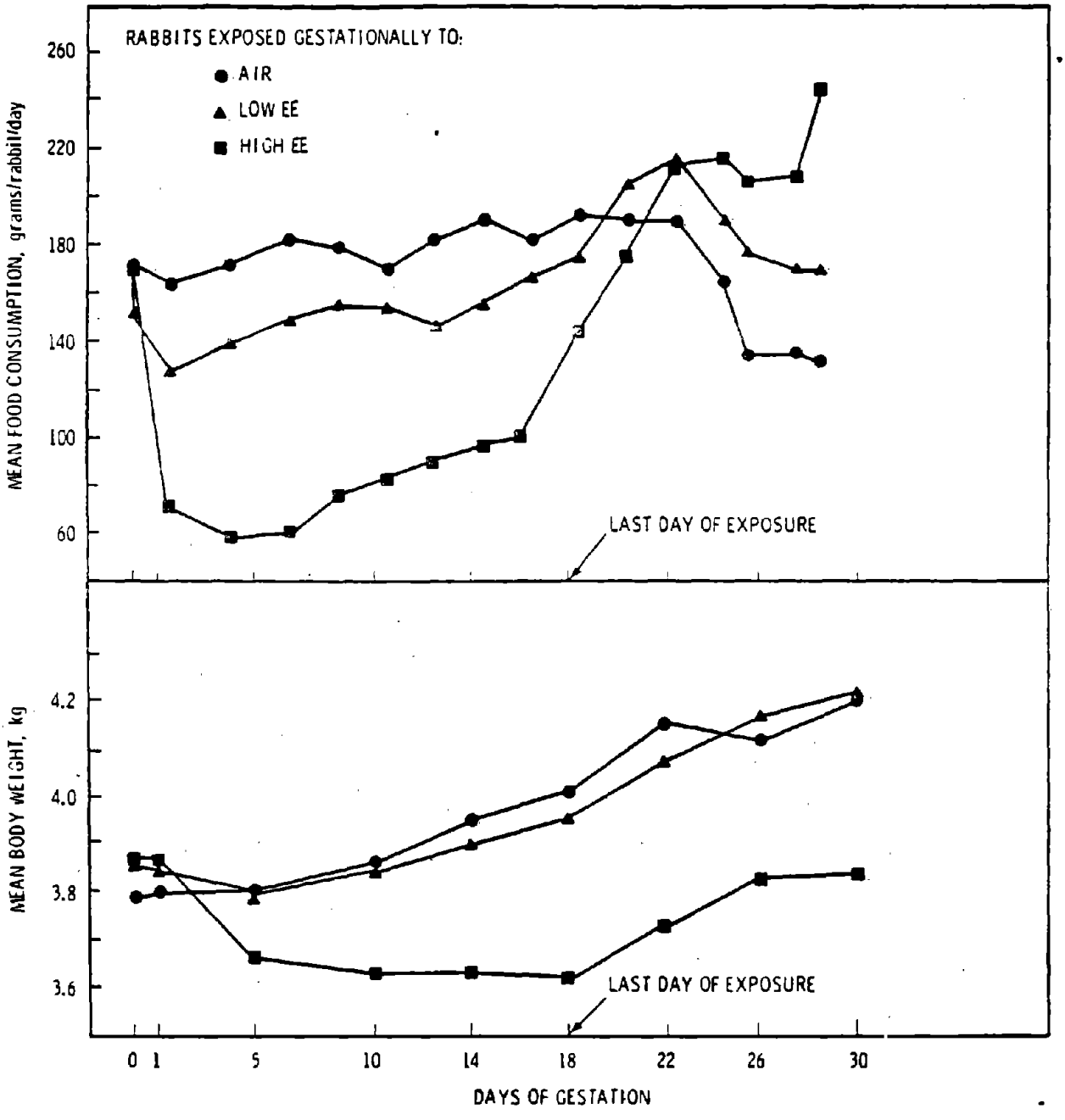


Figure 16. Summary of food consumption data and body weights for all rabbits exposed to 2-ethoxyethanol.

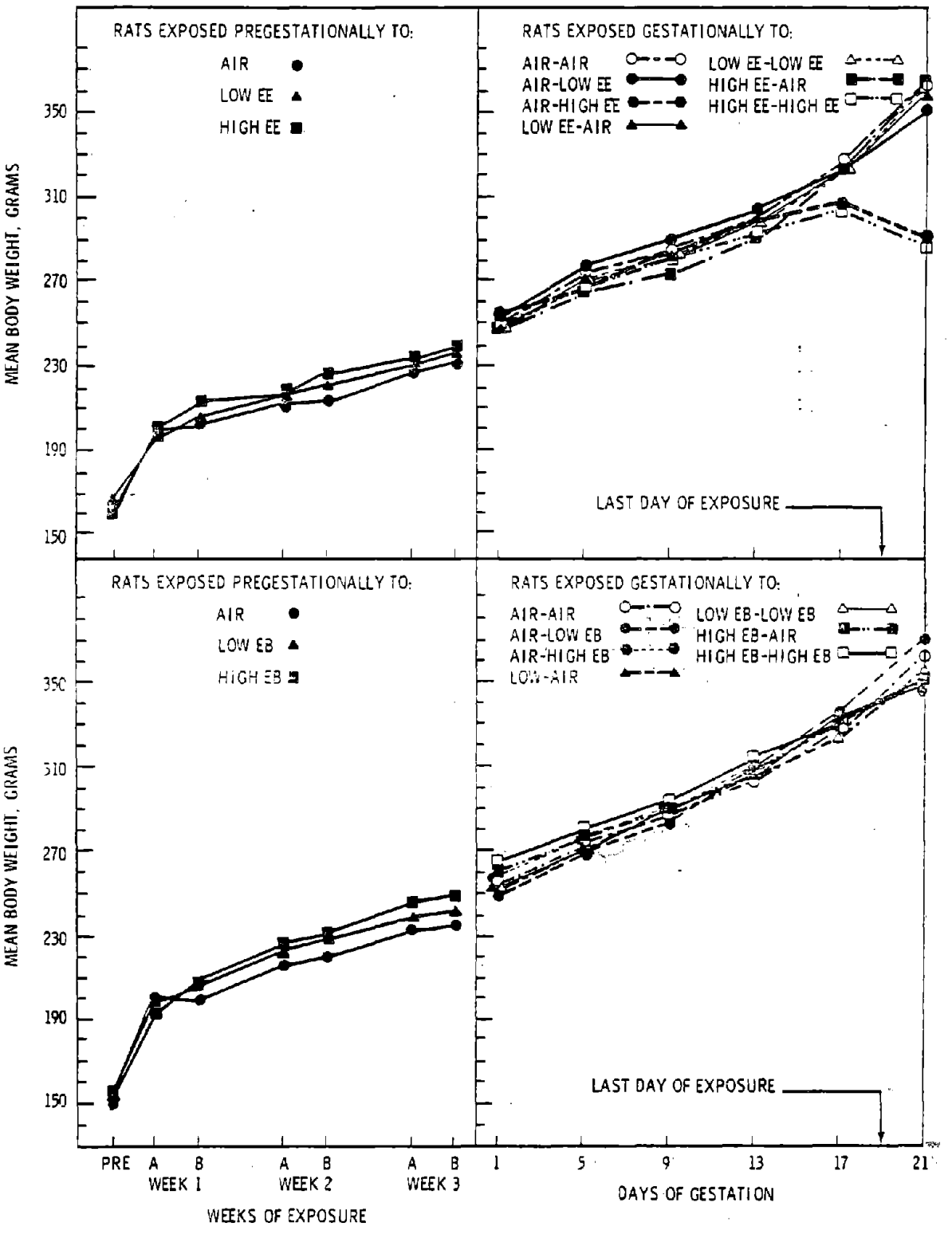


Figure 17. Summary of body weights for rats exposed to ethylbenzene or 2-ethoxyethanol.

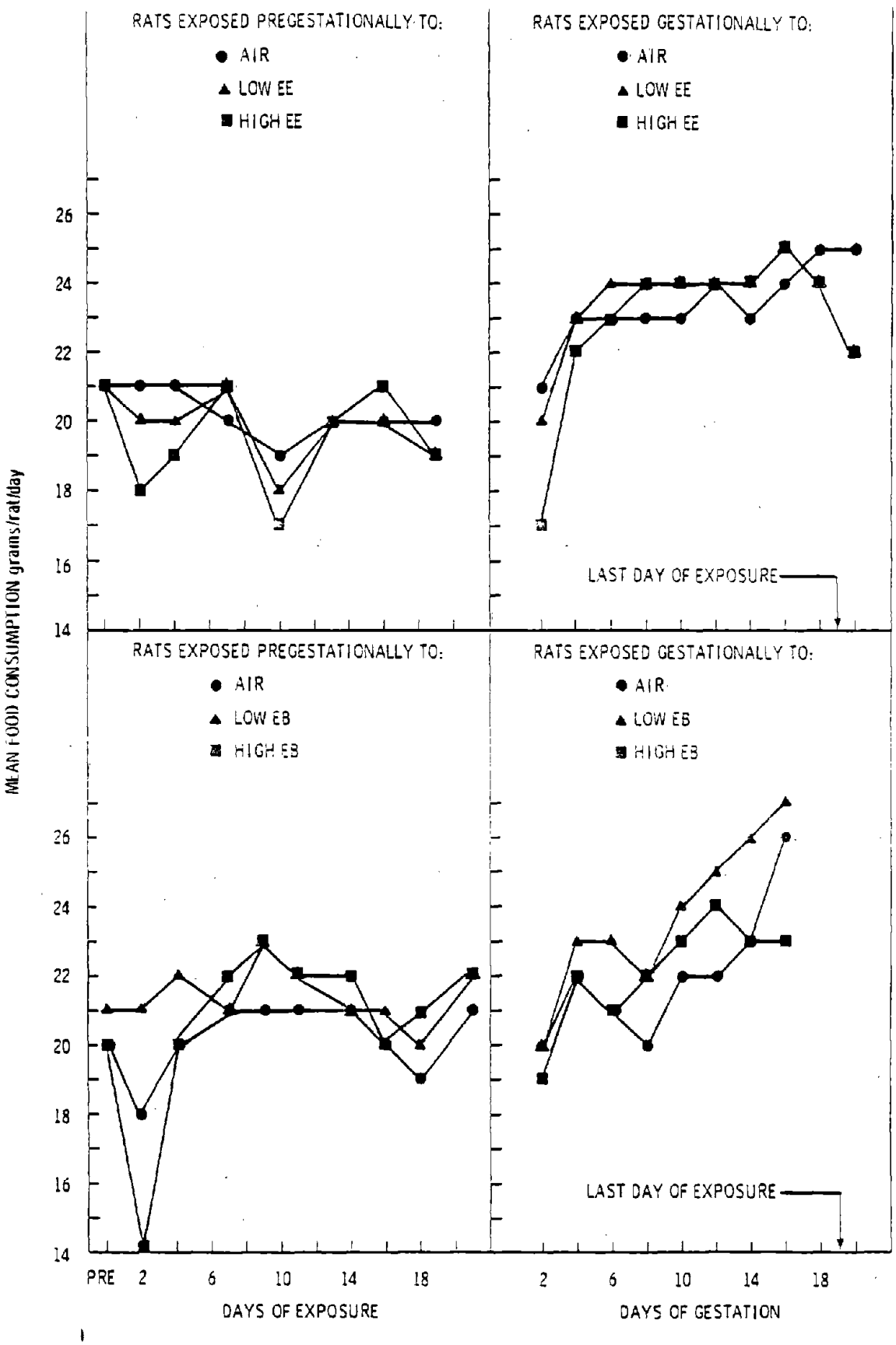


Figure 18. Summary of food consumption data for rats exposed to ethylbenzene or 2-ethoxyethanol.

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APPENDIX

Summary of Duncan's Multiple Range Analysis on EB and EE Exposed Rabbits and Rats.

The results of Duncan's multiple range test were expressed by dividing the exposure groups into homogeneous subsets which do not differ from each other. Subsets were indicated by underlining and means were ordered according to increasing size.

Data Table[a]	Compound and Species	Observation	P value	Range (listed by increasing mean value)
5	EB - Rabbit	Maternal Liver Weight	0.0045	A L H[b]
9	EB - Rabbit	No. Live/Litter	0.0450	A L H
14	EB - Rat	Pregest. body weight		
		Week 2A	0.0000	A L H
		Week 2B	0.0000	A L H
		Week 3A	0.0000	A L H
		Week 3B	0.0000	A L H
16	EB - Rat	Maternal Organ Weight		
		Liver (absol.)	0.0000	A-L L-A H-A A-A L-L A-H H-H
		Liver (rel.)	0.0000	L-A A-L H-H A-A L-L A-H H-A
		Kidney (absol.)	0.0000	L-A A-L H-A L-L A-A A-H H-H
		Kidney (rel.)	0.0000	L-A L-L A-L H-A A-A A-H H-H
		Spleen (absol.)	0.0336	A-L H-A L-A A-A L-L H-H A-H
		Spleen (rel.)	0.0007	L-A A-L H-A A-A L-L H-H A-H
17	EB - Rat	Adult Organ Weights		
		Lung (rel.)	0.0184	L-A H-H L-L A-H H-A A-A A-L
		Liver (rel.)	0.0001	A-A L-L L-A H-A A-L H-H A-H
		Kidney (rel.)	0.0478	H-A A-A L-A L-L A-H H-H A-L
22	EB-Rat	Fetal Measures		
		Crown - rump length	.0118	A-H H-H L-A H-A A-A A-L L-L
24	EE - Rabbit	Food Consumption[d]		
		1 - 2 dg	<0.05	H L A
		3 - 5 dg	<0.05	H L A
		6 - 7 dg	<0.05	H L A
		8 - 9 dg	<0.05	H L A
		10 - 11 dg	<0.05	H L A
		12 - 13 dg	<0.05	H L A
		14 - 15 dg	<0.05	H L A
		16 - 17 dg	<0.05	H L A
		18 - 19 dg	<0.05	H L A
		24 - 25 dg	<0.05	A L W
		25 - 26 dg	<0.05	A L H
		27 - 28 dg	<0.05	A L H
		28 - 29 dg	<0.05	A L H

25	EE - Rabbit	Body Weight										
		18 dg	0.0100	H	L	A						
		22 dg	0.0088	H	L	A						
		30 dg	0.0248	H	A	L						
27	EE - Rabbit	Maternal Organ Weights										
		Liver (absol.)	0.0000	A	L	H						
		Liver (rel.)	0.0000	A	L	H						
		Kidney (absol.)	0.0056	A	L	H						
		Kidney (rel.)	0.0000	A	L	H						
30	EE - Rabbit	No. Live/Litter[c]	0.0000	H	L	A						
		No. Resorp./Litters[c]	0.0000	A	L	H						
		% Litters with Resorp.	0.0000	A	L	H						
		Resorp./Litters with Resorp.	0.0000	A	L	H						
33	EE - Rat	Pregest. Food Consumption[d]										
		day 1 - 2	≤0.05	H	L	A						
35	EE - Rat	Pregest. Body Weight										
		Week 18	0.0023	L	A	H						
		Week 28	0.0000	A	L	H						
36	EE - Rat	Gest. Body Weight										
		17 dg	0.0000	H-H	A-H	L-A	H-A	L-L	A-A	A-L		
		21 dg	0.0000	H-H	A-H	L-L	L-A	H-A	A-L	A-A		
37	EE - Rat	Maternal Organ Weight										
		Lung (rel.)	0.0000	H-A	L-L	L-A	A-A	A-L	H-H	A-H		
		Liver (absol.)	0.0000	A-H	H-H	L-A	H-A	A-A	L-L	A-L		
		Liver (rel.)	0.0000	A-H	H-H	H-A	L-A	A-A	L-L	A-L		

		Kidney (absol.)	0.0123	L-A	A-A	H-A	L-L	H-H	A-H	A-L
		Kidney (rel.)	0.0000	A-A	H-A	L-L	L-A	A-L	A-H	H-H
		Spleen (absol.)	0.0001	H-H	A-H	L-A	H-A	L-L	A-A	A-L
		Spleen (rel.)	0.0000	A-A	L-A	H-A	L-L	A-L	H-H	A-H
38	EE - Rat	Adult Organ Weight								
		Lung (rel.)	0.0000	H-A	A-L	L-L	A-A	A-H	H-H	L-A
		Kidney (rel.)	0.0075	A-H	H-H	L-L	A-A	A-L	H-A	L-A
42	EE - Rat	No. Live/Litter[c]	0.0000	A-H	H-H	A-L	H-A	L-L	A-A	L-A
		No. Resorbed/Litter[c]	0.0000	L-A	A-A	L-L	H-A	A-L	A-H	H-H
		% Litters with Resorp.	0.0000	L-A	A-A	H-A	L-L	A-L	A-H	H-H
		Resorp./Litters with Resorp.	0.0000	A-A	L-A	A-L	L-L	H-A	A-H	H-H
43	EE - Rat	Fetal Body Weight	0.0000	A-L	L-L	A-A	H-A	L-A		
		Crown-Rump Length	0.0000	A-L	L-L	A-A	H-A	L-A		

(a) Refers to table in text where data is located

(b) Abbreviations of A, L and H represent air, low-level and high-level, respectively. Two letter combinations represent pregestational-gestational exposure sequence. Exposure groups are arranged by increasing order of the actual value of each group. Groups ranked as homogeneous by Duncan's are underlined.

(c) Arcsin transformation of the data preceded ANOVA and Duncan's.

(d) The P values were obtained from published tables using the calculated F value and appropriate degrees of freedom.

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16. Abstract (Limit: 200 words)

These experiments were performed to evaluate developmental toxicity in rats and rabbits associated with inhalation exposure to ethylbenzene (EB) or 2-ethoxyethanol (EE). Rats were exposed for 7 hours per day, 5 days per week for 3 weeks. They were then mated and exposed daily through 19 days of gestation (d.g.). Rabbits were artificially inseminated and exposed for 7 hours daily through 18 d.g. (EE) or 24 d.g. (EB). The rats were killed and examined at 21 d.g. and the rabbits at 30 d.g. Pregnant animals were observed for evidence of toxicity including altered food consumption, body weight gains and tissue weights and for histopathologic changes. Litters are examined for the presence of external, visceral and skeletal defects as well as the incidence of growth retardation and intrauterine mortality.

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