NIOSH

TECHNICAL REPORT

Teratologic Assessment of Butylene Oxide, Styrene Oxide and Methyl Bromide

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
National Institute for Occupational Safety and Health

TERATOLOGIC ASSESSMENT OF BUTYLENE OXIDE, STYRENE OXIDE AND METHYL BROMIDE

Melvin R. Sikov
William C. Cannon
Daniel B. Carr
Rodney A. Miller
Linda F. Montgomery
Daniel W. Phelps
Battelle, Pacific Northwest Laboratory
Richland, Washington

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NIOSH Project Officer: Richard W. Niemeier
Principal Investigator: Melvin R Sikov

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ABSTRACT

These experiments were performed to evaluate developmental toxicology in rats and rabbits associated with inhalation exposure to butylene oxide, styrene oxide, and methyl bromide. 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 d.g. 24. The rats were killed at d.g. 21 and the rabbits at d.g. 30. Pregnant animals were examined for toxic changes including altered tissue weights and histopathologic effects. Litters were evaluated using several measures of embryotoxicity and live fetuses were examined for external, visceral, and skeletal malformations.

Butylene oxide and methyl bromide produced relatively little toxic symptomology in the rats. Both concentrations of butylene oxide used (250 and 1000 ppm) produced significantly elevated mortality in the rabbits. The lower methyl bromide concentration (20 ppm) had little effect in the adult rabbit but 70 ppm produced severe neurotoxicity and 96% mortality. There were minimal embryotoxic effects attributable to butylene oxide exposure--these were seen only at maternally toxic concentrations in the rabbits. Methyl bromide was without remarkable embryotoxicity.

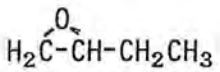
There was extensive mortality in rats that received prolonged exposure to 100 ppm styrene oxide, and 300 ppm was so rapidly lethal that exposures were terminated. Lower concentrations (15 and 50 ppm) were used for exposure of the rabbits; 50 ppm produced 79% mortality. Gestational exposure appeared to decrease fecundity by increasing loss of embryos before implantation in rats, and tended to increase the incidence of resorptions in rabbits. In both species, fetal weight and lengths were reduced by gestational exposure. The incidence of ossification defects of the sternebrae and occipital bones was increased by gestational exposure of rats to styrene oxide.

INTRODUCTION

Changing socioeconomic and legal factors have resulted in an increase in the number of women of childbearing age employed in the working environment. 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. Because of their widespread industrial use in quantity, butylene oxide, styrene oxide, and methyl bromide have been identified as agents of concern. Obtaining data on the developmental and general toxicology of these materials has become an objective of NIOSH, and of other Federal Agencies.

BUTYLENE OXIDE

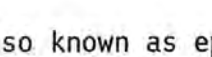
Relatively little information about butylene oxide (epoxybutane, CAS 106-88-7) is readily available beyond that presented by Hine and Rowe (1963). The most common form is 1,2-butylene oxide:



although it is frequently used as a mixture containing 10 to 20% of various 2,3-butylene oxides.

NIOSH estimates that 7 million pounds of butylene oxide are produced annually and that 200,000 workers may be exposed. Butylene oxides are used for the production of the corresponding butylene glycols and their derivatives, as well as butanol amines and surface active agents. Although butylene oxides do not appear to be absorbed through the skin, they produce irritation of the skin and eyes. Acute administration of butylene oxide to rats by gavage has an LD_{50} of about 0.5 g/kg while inhalation exposures to saturated vapor (unstated concentration) for 12 minutes were lethal and 6-minute exposures caused some delayed deaths due to secondary pneumonia. It was suggested that deaths were due to pulmonary irritation, followed by edema and pneumonitis. Repeated exposures (regimen not stated) to a vapor concentration of 400 ppm appeared to be tolerated by rats, guinea pigs, and rabbits (Hine and Rowe, 1963).

STYRENE OXIDE

Styrene oxide:  , also known as epoxyethylbenzene or epoxystyrene (CAS 96-09-3) is used as an intermediate in the production of styrene glycol and its derivatives and as a reactive diluent in the epoxy-resin industry (Hine and Rowe, 1963). It is also reported to be useful as an intermediate in the preparation of agricultural and biological chemicals, cosmetics, and surface coatings, and in the treatment of textiles and fibers. Union Carbide is the only U.S. company which produces styrene oxide. Its production is

estimated to be about 200 thousand pounds yearly (U.S. H.E.W., 1979), but NIOSH uses a production figure of 200 million pounds, with potential exposure of 90,000 workers.

Styrene oxide has been presumed to be a metabolite of styrene and has been proposed as its ultimate mutagenic metabolite (Busk, 1979). Styrene oxide has been shown to produce point mutations in microbial test systems (Salmonella and Schizosaccharomyces), Drosphila and in a hamster cell line, to affect unscheduled DNA synthesis, and to induce cytogenetic changes in mouse bone marrow cells and in microcultures of human lymphocytes (Linnainmaa et al., 1978; Loprieno et al., 1970; Busk, 1979; Donner et al., 1979).

Styrene oxide is irritating to the eyes and skin; it may also produce skin sensitization. There is some absorption through the skin; this is slow but may reach toxic levels in rabbits over a 24-hour period with an LD_{50} of 2.8 g/kg, as compared to the oral LD_{50} of 2.0 g/kg in rats and guinea pigs. Rats exposed to vapor-saturated air survived a 2-hour exposure but 3 of 6 died following a 4-hour exposure (Hine and Rowe, 1963; Lane, 1979).

Since, as indicated, styrene oxide is a metabolite of styrene, a brief consideration of the uses and toxicology of styrene and polystyrene may be appropriate. Styrene has been manufactured in the United States from natural gas and petroleum for some 40 years. One of the first uses of styrene was in the manufacture of styrene-butadiene rubber during World War II, at which time approximately 20 million pounds of styrene were manufactured per year. In 1978, 7.1 billion pounds were used and an anticipated 8.6 billion pounds will be used in 1982 (U.S. H.E.W., 1979).

"Contamination" of polystyrene with styrene monomer is very slight. Styrene is more soluble in oil or fat than in water. A Canadian study showed that the monomer could be found at concentrations of 0.25 ppb in sour cream samples taken near the surface of a polystyrene container (quoted in U. S. H.E.W., 1979). Concentrations of styrene in air are permitted in industry at levels of 100 ppm.

Teramoto (1978) has indicated in a report (available only in an abstract) that "styrene uptake" in humans exposed to its vapors (10, 50, and 150 ppm) was about 70%. After a 4 hour single exposure to rats, no significant differences in the rate constants and biological half life in tissues were noted except in the adipose tissue. After exposures at approximately 700 ppm for 4 hours/day for 5 days, the distribution and elimination of styrene in organs and tissues were similar to those observed after single exposure. No accumulation of styrene was observed. However, a trend of increasing concentration in adipose tissue was observed after i.p. administration at 350 mg/dose every 6 hours for 5 doses. Repeated 4-hour exposures (at 50 ppm or 350 ppm) for 4 months caused no significant changes in body weight or in peripheral and serum blood. An increase in serum glucose and triglyceride was observed in some animals.

Recently, a relationship between maternal occupational exposure to styrene (as well as other chemicals) and central nervous system defects in offspring was suggested anecdotally, although no definite data were presented (Holmberg, 1977).

Recent analysis and evaluation of data from bioassays of styrene led NCI scientists to conclude that the styrene test provided no evidence for carcinogenicity of the compound in either rats or mice. Styrene oxide is currently on test but no data are yet available (U.S. H.E.W., 1979).

METHYL BROMIDE

Methyl bromide (CH_3Br), also known as monobromomethane (CAS 74-83-9), is used as a fumigant, as a chemical intermediate as a methylating agent, and as a fire extinguishing material in some countries. An annual production figure of 20 million pounds and a potentially-exposed worker population of 75,000 has been estimated by NIOSH.

The toxicology and industrial uses of methyl bromide were reviewed several years ago by Irish et al. (1940) and more recently by Johnson (1978). Exposure to very high concentrations of the vapor produces rapid narcosis and death from respiratory failure. High concentrations result in lung irritation followed by congestion and edema. This may be followed by confluent bronchial pneumonia or secondary infection, leading to delayed or early deaths, respectively. Rats survived a single 8-hour exposure to 260 ppm. A series of experiments were performed involving exposures of various species to one of several different concentrations of methyl bromide for 5 day/week, 7½-8 hours/day for 6 months, or until the majority of the animals had died. Repeated exposures of rats to 100 ppm produced a response varying from essentially normal lungs to severe pneumonia, but 66 ppm appeared to be tolerated. Guinea pigs showed no significant pulmonary changes at the 100 ppm level, while a monkey developed severe convulsions. Rabbits developed pulmonary damage at this level and both rabbits and monkeys developed a characteristic paralysis. At 33 ppm, the rabbits showed both pulmonary and neurological damage although monkeys appeared normal. No effects were noted in any of these species at a concentration of 17 ppm.

Recent data from NIOSH has shown methyl bromide to be mutagenic using the Salmonella method (personal communication from Geoffrey Taylor, NIOSH).

RATIONALE

No data were found on the teratogenicity of these materials although it has been shown that styrene oxide and methyl bromide are mutagenic. In order to protect children of mothers who may be exposed to these substances during pregnancy, it is important to evaluate the potential teratogenicity of these chemicals. To detect unique species sensitivities, rats and rabbits were exposed to the chemicals by the inhalation route to evaluate their effects on reproductive capacity, including teratogenicity under conditions simulating exposure in the workplace prior to conception and/or exposure during prenatal development.

MATERIALS AND METHODS

EXPOSURE SYSTEM

Rats and rabbits were exposed in stainless steel chambers (2.3 m^3 total volume, 1.7 m^3 animal volume) built to Battelle-Northwest (BNW) specifications (Figures 1 and 2). Their operating characteristics have been described (Moss et al., 1980; Beethe et al., 1980). A polydispersed particulate aerosol (uranine dye, approximately 1 μm diameter) attained equilibrium in 15-20 min after introduction into the chamber. Concentrations measured above the six catch pans showed a 3-5% variation. The design of these chambers allows for up to 192 adult rats (depending on size) to be both exposed and housed within each chamber. Identical chambers will accommodate up to thirty rabbits in individual cages, although they must be housed in separate caging between exposure periods.

Three chambers were employed: one for the high-level, one for the low-level, and one for the pregestational filtered air exposures of rats. The same chambers were used for gestational exposures of rats. Upon completion of the rat exposures, the same chambers were used for gestational exposure of rabbits. Animals were transferred to freshly washed and sterilized chamber-caging units at least once a week.

HEPA filtered air was continually supplied to the exposure chambers and the vapors under study were introduced into the filtered air streams. Chamber air flows were maintained at 10 cfm, which provided seven air changes per hour.

ATMOSPHERE GENERATION SYSTEMS

Butylene Oxide

Methodology for generating the desired butylene oxide atmospheres was developed using an exposure chamber set up in the aerosol physics laboratory. Liquid butylene oxide was placed in a 3-necked, 500-ml distillation flask with a coarse fritted glass bubbler extending from the center neck into the liquid (Figure 3). One of the remaining necks was stoppered while the other had a tube extending into the inlet air line to the exposure chamber. Dry N_2 gas was used at 0.225-1.5 liters per minute to avoid explosive vapor concentrations in the generator. The liquid bath was heated to the temperatures necessary (14°-26°C) to generate the required amount of vapor. For the first three weeks of exposure, the butylene oxide was periodically replenished; subsequently it was replaced daily (see page 14).

The generators were located in a fume hood, and connected to the exposure chambers by 3-inch diameter flexible hose (Figure 4). The chamber exhaust was connected (through a HEPA filter) to a transvector air flow amplifier (Vortex

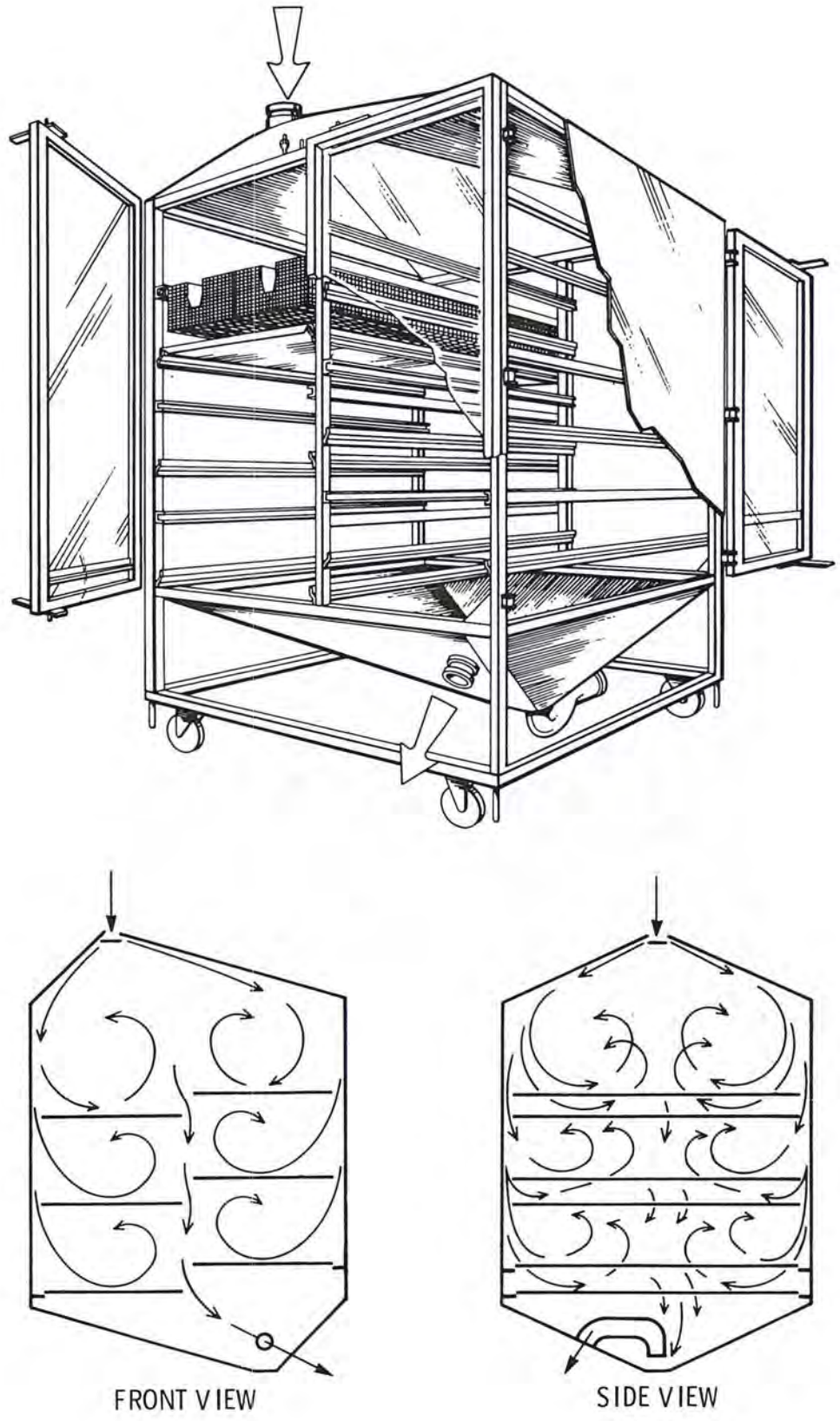


Figure 1. Battelle inhalation exposure chamber

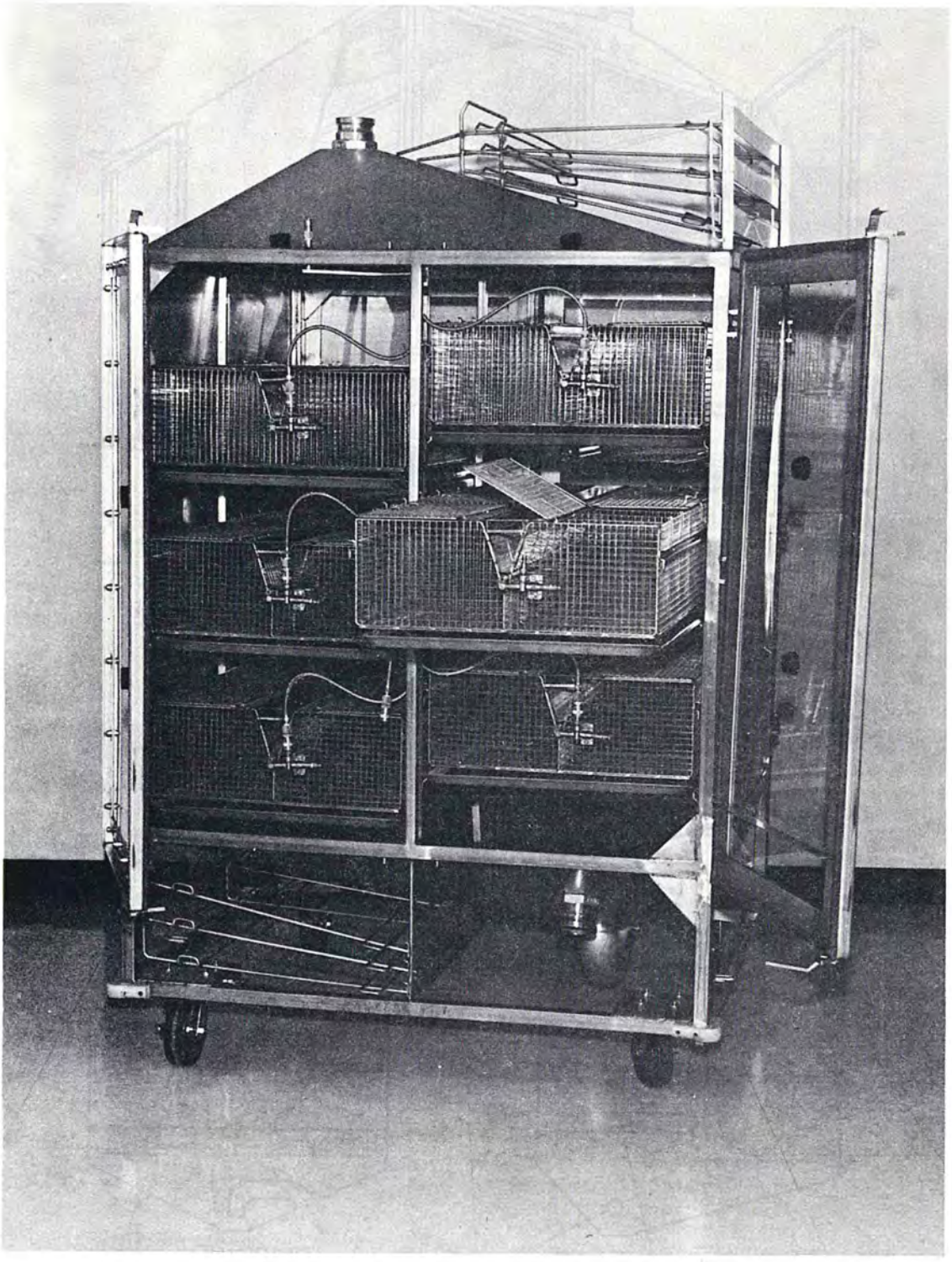


Figure 2. Photograph of Battelle inhalation exposure chamber.

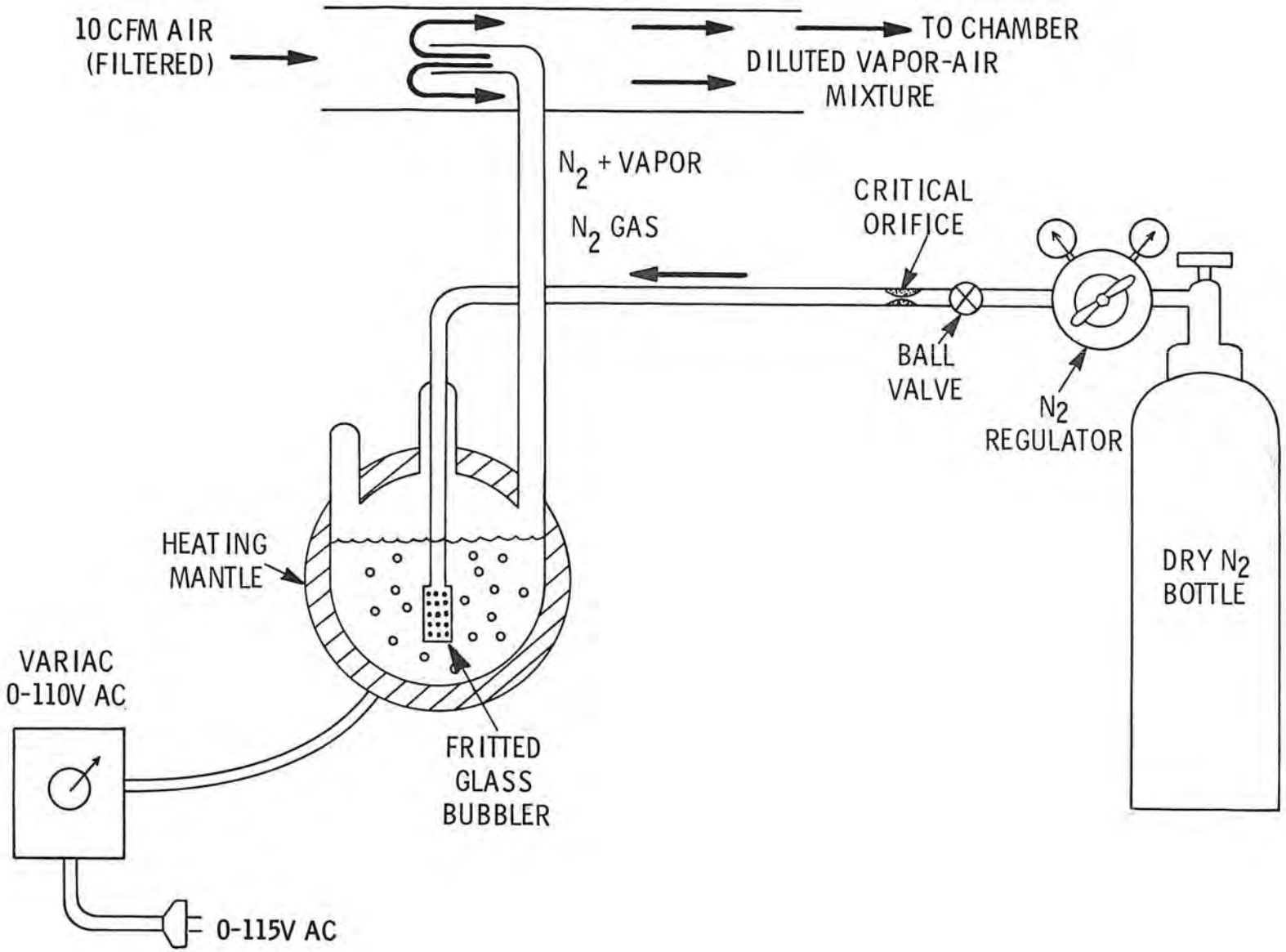


Figure 3. Details of generator for butylene oxide vapor.

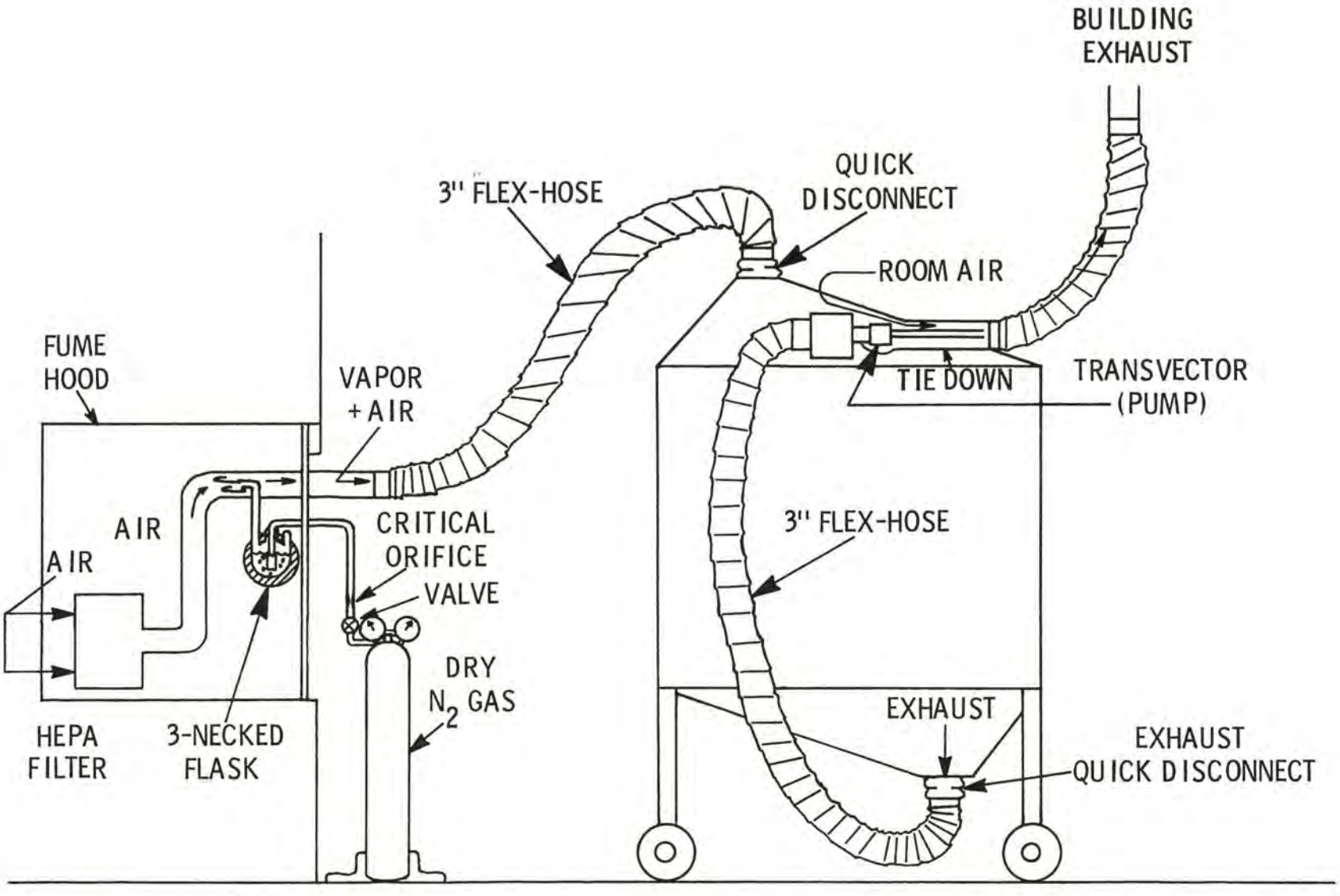


Figure 4. Relationships between principal components of animal exposure system for butylene oxide and styrene oxide.

Corp.) which introduced the exhaust into another flexible hose which was connected to a room exhaust port (Figure 5). The total flow into the room exhaust hose was approximately 125 cfm of which only 10 cfm came from the chamber. To pump 10 cfm required about 3 cfm of driving air for a total output of 13 cfm. The system was adjusted to provide vapor concentrations in the chambers of 1000 ppm and 250 ppm of butylene oxide.

Styrene Oxide

A similar generator was set up in the aerosol physics laboratory to develop appropriate procedures for styrene oxide. The low vapor pressure of this material led to a tendency toward aerosol formation. With a modified generator system, shown diagrammatically in Figure 6, the vapor still contained a small amount of aerosol (estimated to be less than 1%). In lieu of using a more elaborate generator, the vapor was introduced upstream of a HEPA filter, which, in effect, provided a vapor which was free of aerosol.

Two types of flexible hose were tested for use in the exhaust and intake of the exposure chambers. The apparatus was set up using 3-in. GlasHose® (double-ply, double-overwrap, neoprene-impregnated glass fabric manufactured by Flexhaust). Although this hose was impermeable to butylene oxide, it was permeable to styrene oxide. Styrene oxide concentrations as high as 1 ppm were measured in the room near the hose and about 25 ppm was measured adjacent to the hose when 300 ppm was in the chamber. Double-ply, 3-inch diameter flexible exhaust hose of rubber silicon-impregnated fiberglass (McMaster-Carr) also was not resistant to styrene oxide. It was necessary, therefore, to wrap the GlasHose® with aluminum-backed Mylar duct tape (Alcoa) to prevent leakage of styrene oxide and a flexible hose jacket was placed concentrically over the tubing. The outer jacket was supplied with air flow back to the generator hood to alleviate the problem of styrene oxide contamination of room air.

Methyl Bromide

Two separate systems were used to generate the exposure atmospheres of methyl bromide; each was connected to a single exposure chamber. Each system consisted of a methyl bromide gas bottle (pressure 13 psig), a manual control valve, a shut-off valve, a double pattern (Nupro #SS-2MGD) metering valve, and a flow meter for the pure gas portion of the system. The gas was mixed with two liters of air per minute and conducted to the top of the chamber through quarter-inch stainless steel tubing. This air gas mixture was introduced just downstream of the inlet HEPA filter at the top of the exposure chamber. The chambers were exhausted by means of a transvector pump (with flow and pressure monitors) into the building exhaust system through 304 stainless steel Bendway® (product of Flexhaust) flexible duct.

Initially, there were fluctuations in the methyl bromide gas flows. These were removed by heating the lines between the gas cylinders and the metering valves to avoid condensation of methyl bromide liquid and the resulting bubbling or boiling of the liquid.

Methyl bromide degraded the seals in the valves. Stainless steel solenoid valves were inserted in the gas lines (for use as fail-safe shut-off valves in the event of a loss of chamber flow or an electrical power), but failed before

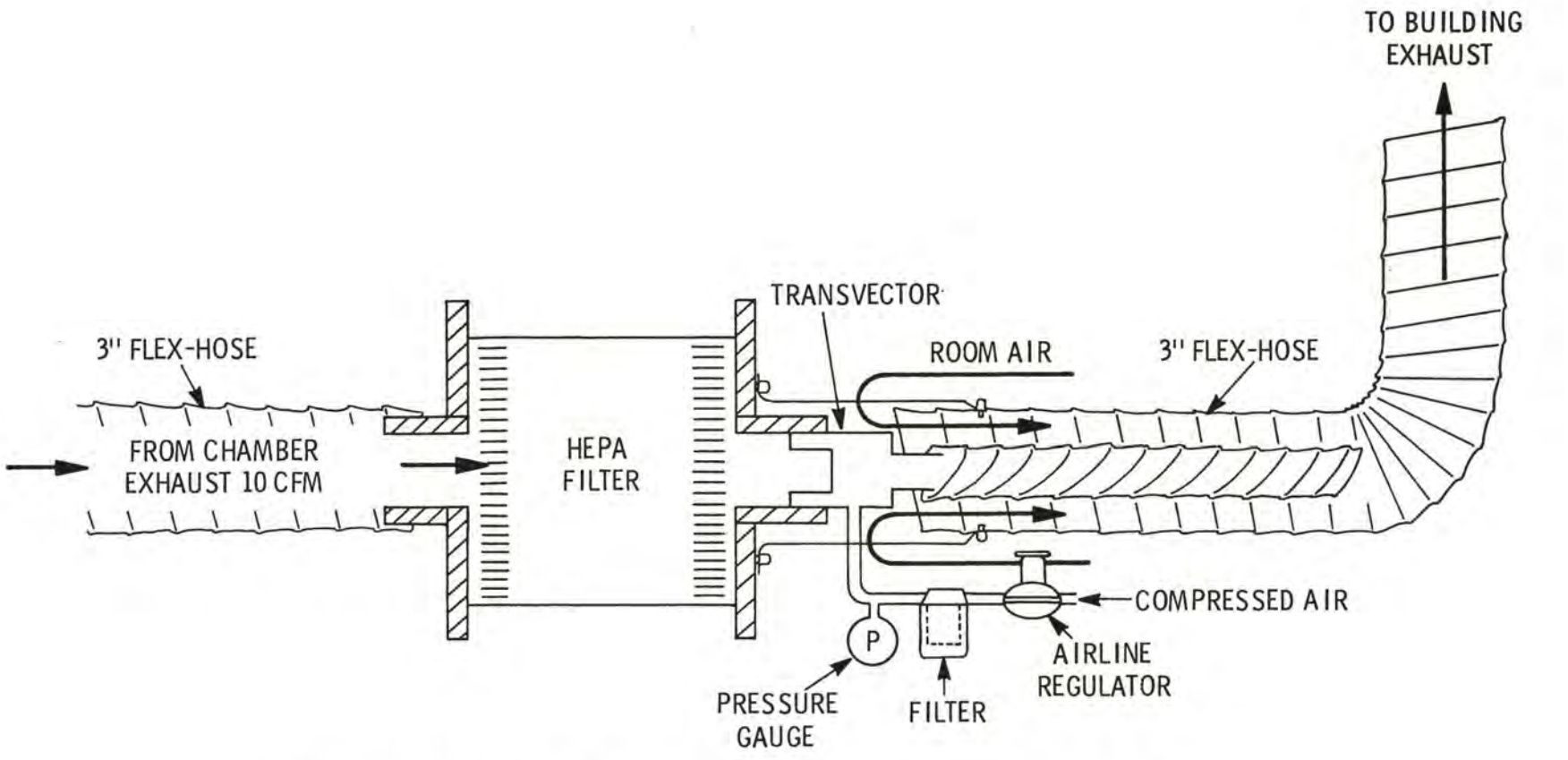


Figure 5. Details of exhaust system for animal exposure chambers.

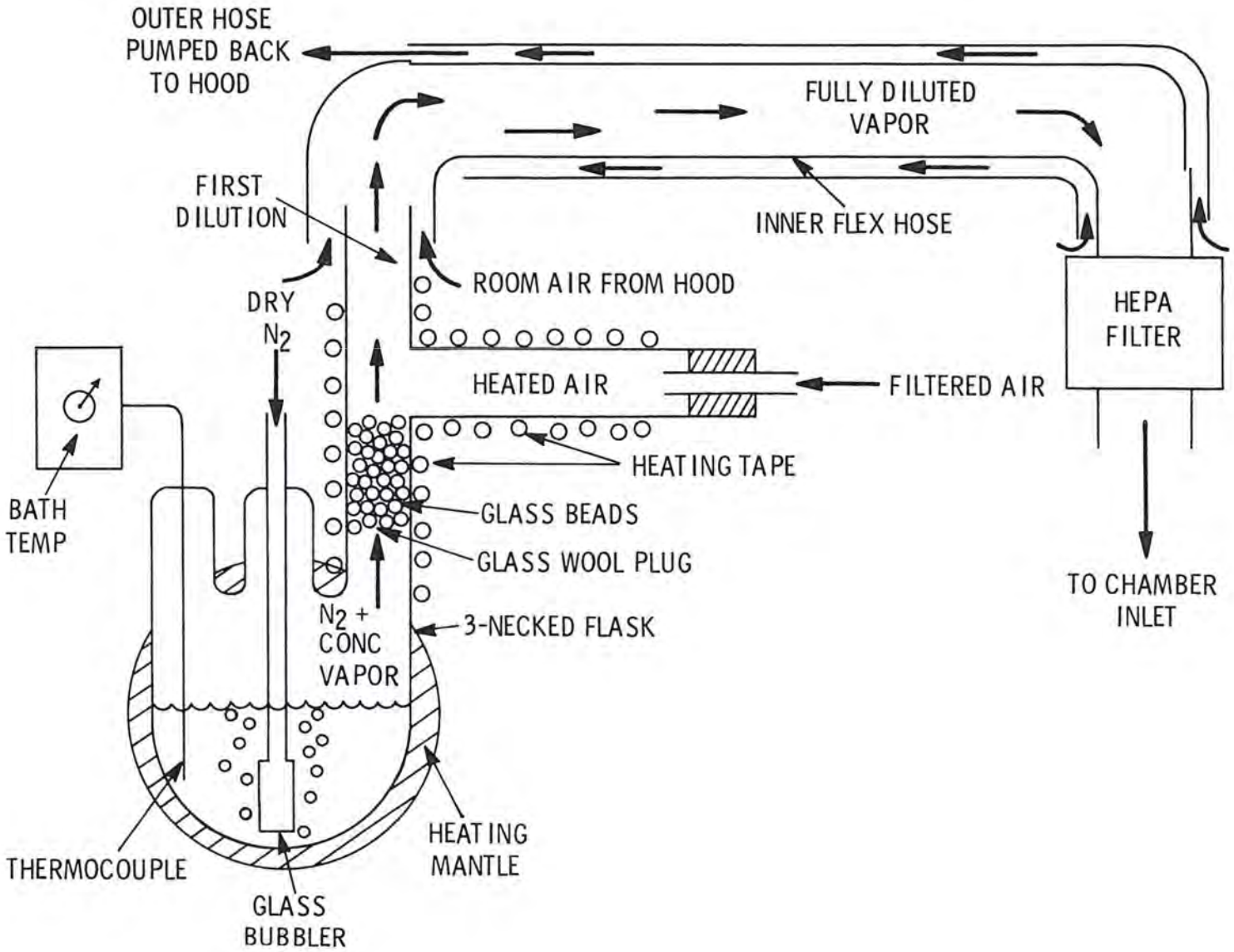


Figure 6. Details of vapor generator for styrene oxide.

the animal exposures started. We were not able to find replacement valves with Teflon or other inert type of seals before the end of the exposures. Manual shut-off valves (toggle type) in the gas lines appeared to develop leaks after slightly more than one week of use and were replaced by ball valves with Teflon seals.

ANALYTIC METHODS AND CHAMBER MONITORING

Our standard approach was to develop generation and analytical methodologies in the aerosol physics laboratory, then to verify (with further refinement as necessary) using the actual exposure system prior to initiation of animal experiments. This included determination of stability, accuracy and reproducibility.

Butylene Oxide

It was originally planned that photoionization detectors would be used for measurement of butylene oxide concentrations in the exposure chambers. Due to delays in receipt of the photoionization detectors and their inoperability after receipt, analytical procedures for butylene oxide were developed using gas chromatography with a Porapak® QS column and a thermal conductivity detector. The butylene oxide standard was purchased from Aldrich Chemical as "1,2 Epoxybutane" (Catalog No. 10997-5, p. 353 in 1977-78 catalog, 99% pure, Lot. No. PB). The exposure material (>99% pure) was purchased from Dow Chemical as "1,2 Epoxybutane," (Lot No. MM10258) which is the most common butylene oxide. Samples of the most likely isomers of butylene oxide, i.e. "trans-2,3-epoxybutane, #2316-20" and "mixture cis-2,3-epoxybutane and trans-2,3-expoxybutane," were purchased from Chemical Samples Company and were used as reference materials in the event that additional peaks appeared on the GC recordings. None of the other butylene oxides were found in the exposure material in concentrations greater than 0.1%.

Standards of butylene oxide for calibrating the gas chromatograph were prepared by dilution of a known liquid volume in a calibrated gas sampling bulb. A sample from this bulb was further diluted with air in a gas sampling bag. In cross-checking the syringes used for the gas dilutions, we obtained different results with different types of syringes. Syringes constructed of rubber and plastic delivered a lower amount of butylene oxide than a similar volume syringe constructed of glass and Teflon. The volume of each syringe was checked and found to be accurate. It is assumed that the butylene oxide was absorbed by the rubber or plastic. Since the rubber and plastic syringes were used initially to make standard dilutions, the resulting butylene oxide concentrations in the exposure chambers were approximately 15% lower than desired for the first 9 days of the pre-gestation exposure.

The data from several evaluations have shown that the sampling system and the method of standard sample preparation were reproducible. The accuracy of these procedures was checked against a known standard. A standard curve for benzene was prepared according to the procedure described for butylene oxide. A certified benzene standard of 109 ppm, purchased from Matheson (Cylinder number 22510P, Control number 12-19-78) was run and the value compared to that obtained from the standard benzene curve. The data indicated an average value of 112.8 ± 4.3 ppm when calculated from the standard curve, a deviation of

only 3.5% from the stated concentration. Matheson stated this concentration as accurate to ±2%. Also, the 109 ppm concentration was at the lower detection limits of the thermal conductivity detector and therefore, had slightly higher variability than would be expected at higher concentrations.

Routine sampling of the chambers was done during animal exposure with automatic Carle gas valves which monitored eight sampling ports throughout the chambers and room. Routinely, to verify safety as well as to establish operational stability, the points which the GC periodically monitored (every 40 min) included the supply to the high and low chambers, two points within each of the high and low chambers, one point in the control chamber and one room air sample. Occasionally, the benzene calibration standard was included in one of the eight sampling ports as a check on daily instrument calibration and operation. Each port was sampled every 40 min and the integrated response printed for inspection by the operator. The concentration of butylene oxide in ppm was calculated from an area response vs. concentration curve. A typical curve is shown in Figure 7. This standard curve was regenerated on alternate days during initial stages of exposures; it was found that this curve did not shift significantly on a day-to-day basis. Since good stability continued through the first 2 weeks of exposures, the standard curve was checked at least weekly thereafter.

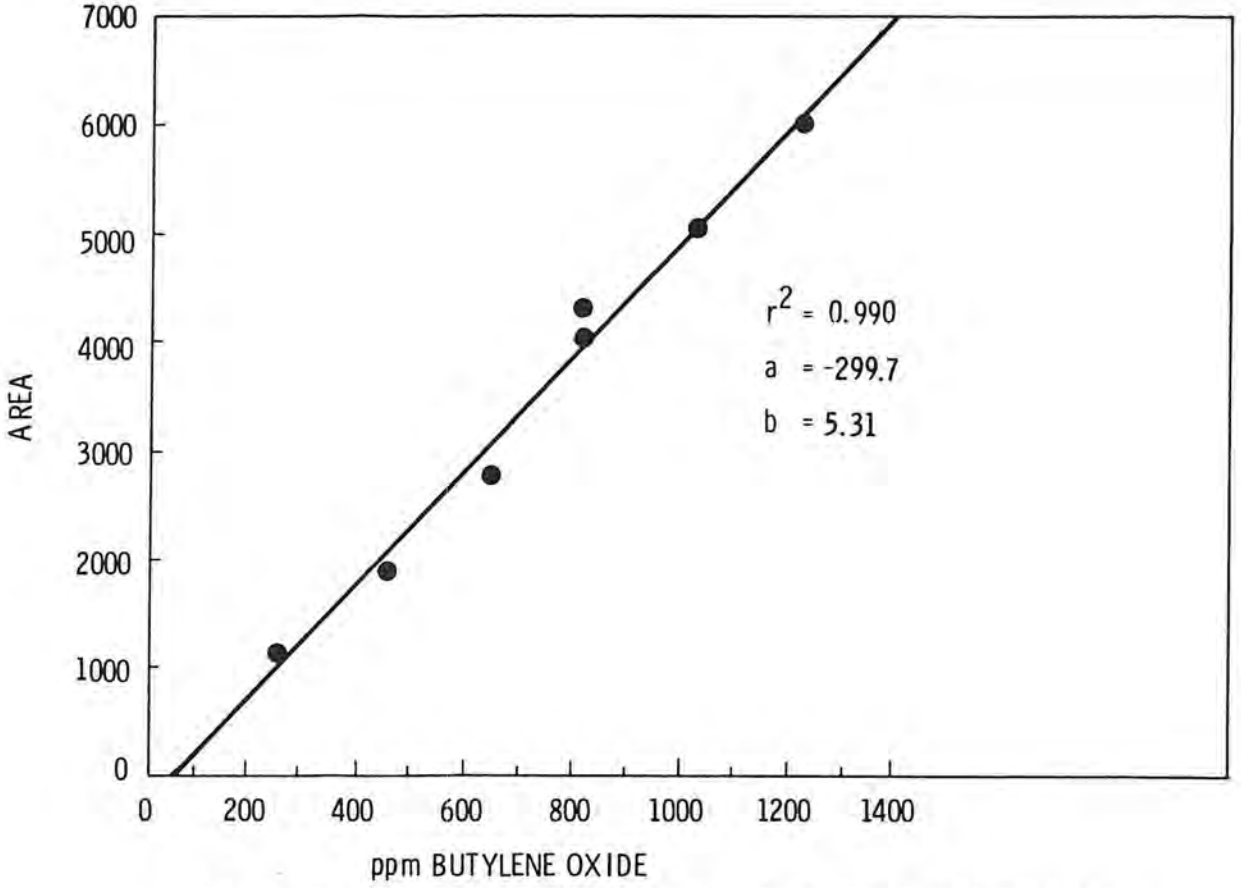


Figure 7. Typical standard calibration for butylene oxide.

Between rat and rabbit exposures, we switched from the thermal conductivity detector (TCD) to a flame ionization detector (FID). The butylene oxide curves generated using the FID had a variation of ±5.6% in the slope with a "goodness of fit" (r^2) value of about 0.99. This was a slightly larger variation (approximately ±3%) than the TCD showed earlier. The 109 ppm benzene standard continued to compare well to the benzene curve generated by our method of standards preparation. The response for the 109 ppm standard calculated at 112 ± 7 ppm. This also showed a slightly larger variation than was previously observed using the TCD but the mean remained within 3% of the standard value.

Ancillary Information--(Butylene Oxide)

A number of further calibration and verification studies were performed throughout the exposure period in addition to generation of a standard curve on a weekly basis. One of the factors included in the error of the standard curve was the reproducibility of the sampling valves. When the error in the valves was checked by letting a single sample run several times, a variation of ±2.8% was found.

Teflon sampling lines were examined for loss due to the length of sampling line. We found no loss due to the line for lengths less than about 15 feet. Lines as long as 30 feet showed less than 5% loss but took a longer time for equilibration to maximal value. Thus, there appeared to be little difference between the short sampling line to the chamber input and the longer lines to bottom chamber sampling port. Nevertheless, we used lines of equal length.

The materials in the generators originally were periodically replenished, but not replaced. After about 3 weeks of exposure, we noticed that the material in the high-level butylene oxide generator had developed a slight yellow color. To identify this material, we ran IR and UV spectra and GC analysis. The IR spectra for analytical grade butylene oxide and the yellowish butylene oxide residue were very similar. We observed no bands due to carbonyl, ether or ester functional groups which could have resulted from oxidation or polymerization of the butylene oxide. The GC traces showed no significant peaks other than those previously noted at the 0.1% level for cis- and trans-2,3-epoxybutane. The only difference seen was in the UV spectrum, which showed a very small shoulder at 200-210 nm on the major butylene oxide absorbance at 180 nm. The identity of the impurity was not examined further as the level appeared to be below 0.1% of the original exposure material. Also, if the impurity were an oxidation or polymerization product as suspected, the vapor pressure would be lower than butylene oxide which would account for the increase in concentration in the generator with time. The generation procedure was modified, however, to replace the material in the generator daily.

Chamber Concentrations--(Butylene Oxide)

Pre-exposure measurements indicated that the chamber atmospheres reached equilibrium within 20 min of the start of generation and clearance required a similar time. Concentrations within the chamber were uniform to within 10% and remained within this range over prolonged generation periods, requiring only minimal adjustment by the operator at infrequent intervals. The generator and exposure systems operated within the desired limits, as indicated

by the time averaged concentrations in the chambers throughout the pregestational and gestation exposures (Table 1).

TABLE 1. Time-Weighted Average Concentrations (Mean Daily ppm ± S.D.) of Butylene Oxide in Exposure Chambers.

Type of Exposure	Species	Location of Measurement	Exposure Level 250 ppm	1000 ppm
Pregestational	Rat	Chamber Inlet	261 ± 16	980 ± 79
		Chamber Top	244 ± 21	947 ± 83
		Chamber Bottom	219 ± 35	920 ± 90
		Chamber Mean	232 ± 29	934 ± 87
Gestational	Rat	Chamber Inlet	261 ± 23	1030 ± 107
		Chamber Top	257 ± 24	947 ± 99
		Chamber Bottom	243 ± 27	954 ± 102
		Chamber Mean	250 ± 26	956 ± 101
Gestational (Replicate I)	Rabbit	Chamber Inlet	284 ± 47	1061 ± 57
		Chamber Top	252 ± 16	971 ± 76
		Chamber Bottom	217 ± 52	920 ± 60
		Chamber Mean	234 ± 38	946 ± 67
(Replicate II)		Integrated	254 ± 6	

Styrene Oxide

The styrene oxide was purchased from Aldrich Chemical Company (Catalog No. S500-6 of 1977-78 catalog; Lot No. PC051777). This material was analyzed for impurities by IR and UV spectra and GC and appeared to be at least 99% pure.

A standard curve for the GC analysis of styrene oxide was generated by standard liquid dilutions and analysis was done on a 4-foot OV-17 column with 6% liquid phase at 175°C (Figure 8). Generation of standard curves by liquid dilutions was necessary because of the low vapor pressure of styrene oxide (approximately 400 ppm at 25°C as compared to about 186,000 ppm for butylene oxide). Since the exposure chamber sampling of vapor-phase material was to be used, it was necessary to compare standard curves generated by liquid and vapor (gas bulb) dilution. The responses were co-linear over the range 50-1000 ppm. The results using hexane as a solvent material (Figure 9),

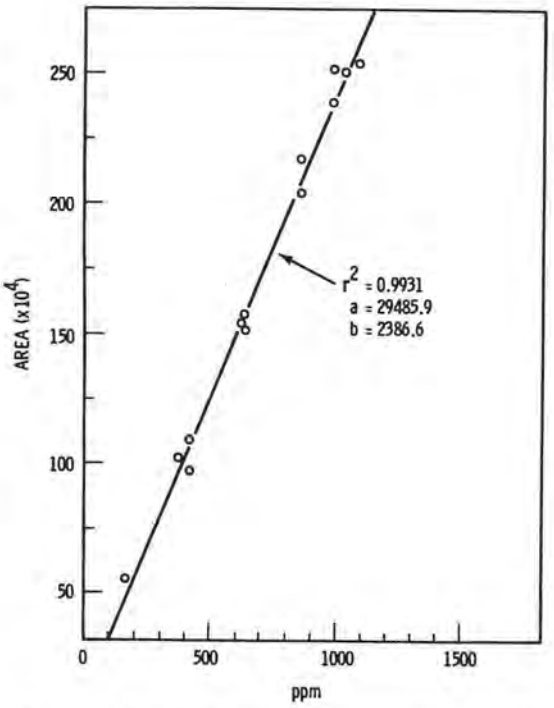


Figure 8. Typical standard curve for styrene oxide generation using liquid dilutions.

indicated that the slopes of the two lines agreed to within 2%, with high values for the goodness of fit for both lines (0.996 and 0.999). Other compounds (butylene oxide, heptane and benzene) were also used for further comparisons with favorable results, validating the use of standards prepared by liquid dilution.

Standard calibration curves for styrene oxide were generated on a weekly basis throughout the exposure periods. In an effort to further reduce drift, daily calibration checks, using a standard 80-ppm solution, were performed each morning by the exposure technician before the start of exposure and grab sampling. The slopes of the standard curves showed a coefficient of variation of 6.9% and a minimum value of 0.997 for goodness of fit over the duration of the exposures.

Since, as indicated below, on-line continuous monitoring could not be implemented, the chamber atmosphere was sampled at least four (but usually 10) times a day using a gas syringe grab-sampling technique.

The variability in syringe sampling was checked in five runs made in close sequence from the same part of the chamber. The results showed a coefficient of variation of 1.6%. Also, we developed a long probe, which could be inserted through the rabbit cages for sampling throughout the chamber. The probe consisted of a glass tube with a tee at the end; one arm of the tee was connected to a pump and the other was fitted with a rubber septum for sampling. After approximately 20 minutes at a flow of 300 ml/min through the glass tube, the chamber concentration measured with the probe differed from that measured by standard grab samples by less than 5%.

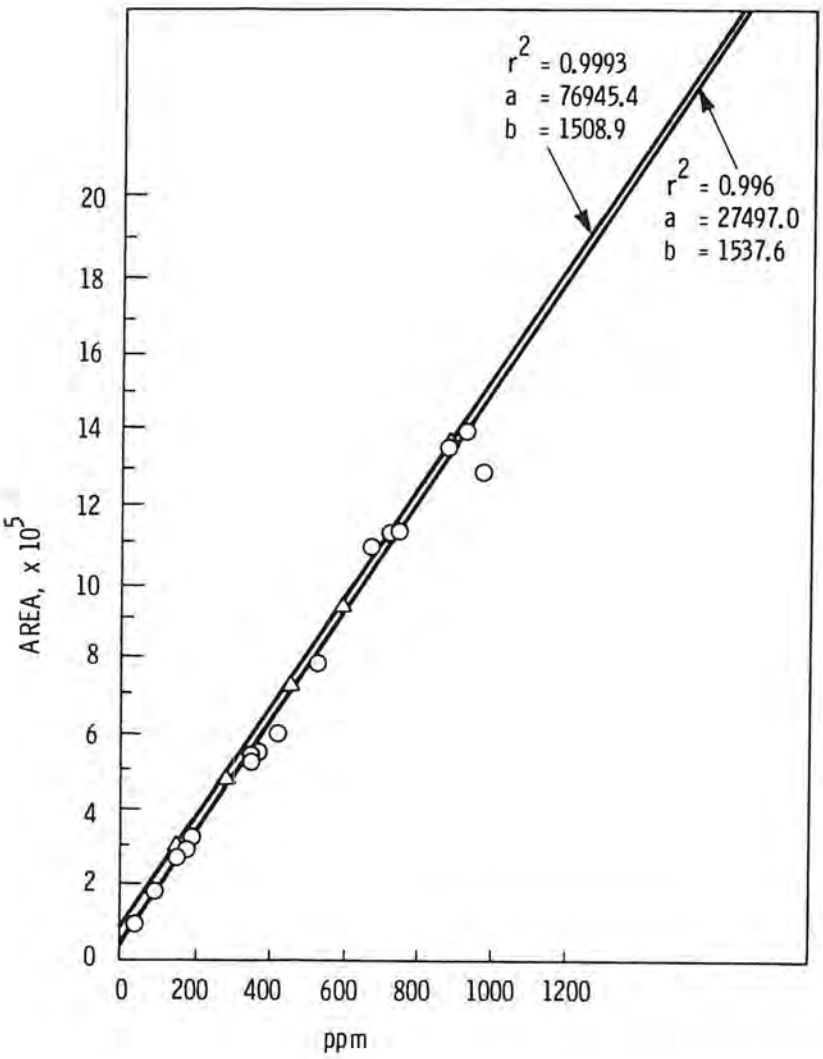


Figure 9. Comparison of standard curves for hexane generated by liquid o and vapor Δ (gas bulb) dilutions.

Ancillary Information--(Styrene Oxide)

A series of unsuccessful efforts was made to develop an on-line continuous system for direct chamber-air sampling of styrene oxide. Tests were made of variations in sample delivery through both Teflon and glass lines and on the effects of heating and sample flow on loss rates. Modifications of the GC to accommodate the sampling and switching valves inside the oven greatly decreased in-line sample losses. However, heated valves and heated glass sampling lines gave only a 50% delivery on samples drawn from the chamber-air inlet, as compared to the levels found in the chamber-air grab samples.

Further work on direct gas sampling of styrene oxide included increasing the sample flow rate, using larger glass lines, and increasing the volume of direct samples and grab samples at the 100-ppm level. Comparisons at the 15- and 50-ppm exposure levels suggested that continuous gas sampling of the atmospheres through automatic valves could be reinstituted on a routine basis for rabbit exposures. The system was tested and functioned properly for about two days before giving erroneous readings. The problem was traced to a bad valve; this was replaced, but it lasted only about a day before being fouled by the combination of heat (150°C) and styrene oxide.

The photoionization detectors (PIDs) originally planned for analysis were finally made operational during the exposure period. It was found that these units have multiple pitfalls which make them unsuitable for use with styrene oxide. Their shortcomings include sample loss by adsorption onto the system's internal surfaces, and lack of any primary calibration method. No further tests of these detectors were made with styrene oxide.

Chamber Concentrations--(Styrene Oxide)

The expression of chamber concentration was complicated by the manner of sampling. First, any deviant measurement led to a correction in concentration by the exposure technician, so that the measurement was transient. Repeated measurements were taken to achieve the desired concentration. Furthermore, there was no completely objective method for eliminating apparently erroneous measurements. Accordingly, it was decided that the most conservative approach was to indicate the mean value of the several concentration readings obtained each day. These are shown in Figure 10 for the three exposure conditions: low-level rat (100 ppm), high- and low-level rabbit (50 and 15 ppm, respectively). The optimum concentration and the ±10% concentration lines are shown.

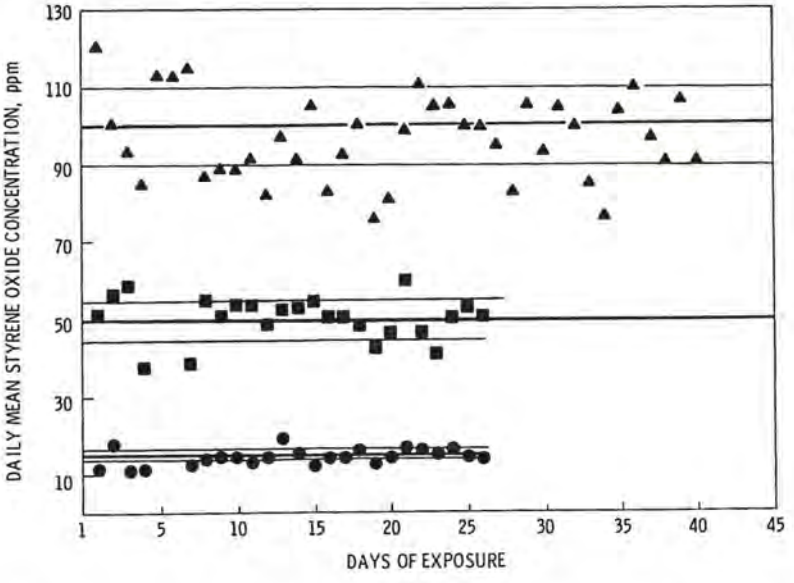


Figure 10. Daily mean concentration of styrene oxide vapor in animals exposure chambers. ▲ - low level rat; ■ - high level rabbit; ● - low level rabbit.

The superimposed individual daily mean concentrations, in most cases, fell within this range.

The mean concentration value (±SD) for the rat exposure was 97.0 ± 11.9 ppm and for the rabbit exposures were 51.0 ± 5.7 and 14.6 ± 2.1 ppm. These time-average concentrations closely approximated the desired values, with required standard deviations of about 10% of the mean.

Methyl Bromide

Methyl bromide (99.5% minimum purity) was purchased in gas cylinders from Matheson Gas Products (Cylinder Nos. B02777 and B02778). A photoionization detector (PID) equipped with an 8-port stream selection valve was set up for chamber monitoring of methyl bromide. Standard samples were made in the 10-80 ppm range by gas dilutions of the pure methyl bromide gas (Figure 11). The detector response was linear in the working range. Eight-foot Teflon lines were used to transfer the sample from the chamber to the selection valve and a different sample line was monitored every five minutes. The output from the detector was connected to a stripchart recorder and the detector gain was adjusted so that the concentration values read directly in ppm from the chart recorder.

Long term stability for the detector was not as good as was hoped, requiring periodic cleaning and recalibration of the detector. The problem seemed to be that the UV lamp progressively dropped in intensity due to a build-up of a coating. As an operational check on detector stability and response, the exposure technician ran a sample of 100 ppm benzene gas and verified the standard response for this gas against the chart recorder readings to check for detector drift.

Throughout the exposure period, the PID monitors were generally calibrated every 3 to 4 days. The goodness-of-fit value for a linear least-squares fit of the data was always equal to or greater than 0.99 and the slope averaged 0.99 with a coefficient of variation of 2.7%. The sample lines were examined for differences by running standard samples of methyl bromide and of 100 ppm benzene gas. No differences were seen between responses when the standards were introduced through the sample lines and when introduced directly into the detector.

Chamber Concentrations--(Methyl Bromide)

The exposure technician periodically checked the records from the automatic sampling system and made adjustments as needed. The values from at least two points in the chamber and the associated gas flows, were recorded at least four times per day, and usually twelve times or more. These data permitted checking of system function by the aerosol physicist.

The individual samplings of the two chambers remained quite constant and closely approximated the optimal concentrations. Almost all of the daily time-weighted average concentration values fell within ±10% of the optimum value. Calculation of the time-weighted average concentrations gave values for the pregestational rat exposures of 19.6 ± 0.9 and 68.5 ± 1.7 ppm for the low and high levels, respectively. The values during the gestational exposure

were 20.0 ± 1.5 and 68.8 ± 1.9 ppm for rats. The corresponding concentrations were 19.3 ± 0.19 and 68.7 ± 2.18 ppm for the rabbit exposures.

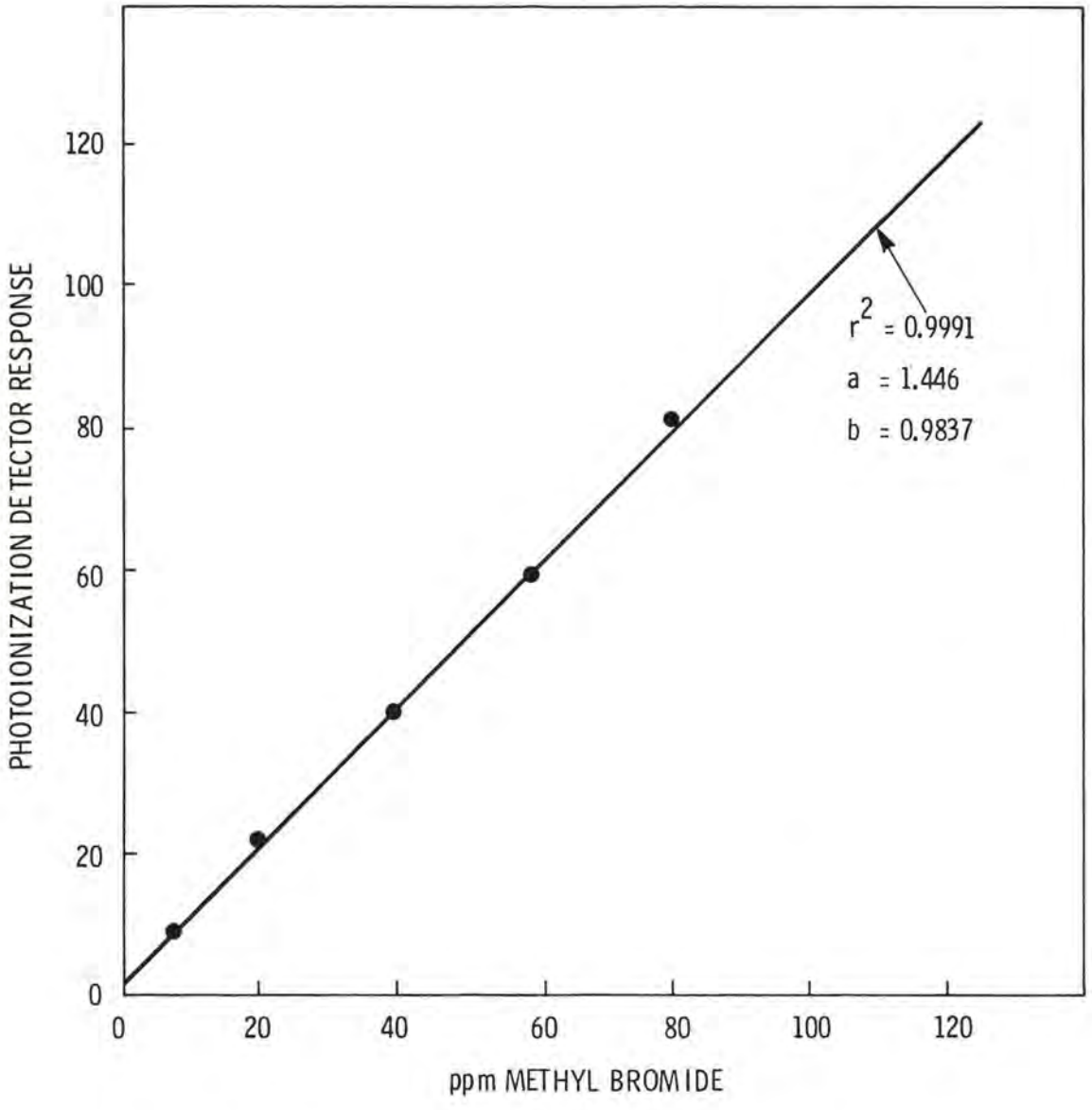


Figure 11. Typical calibration curve for photoionization detector response as a function of methyl bromide concentration.

ANIMAL PROCEDURES

Rats

A group of 380 young, adult female Wistar rats (4-5 weeks old, 100-125 g. on receipt) was purchased from Hilltop Lab Animals, Inc., Scottdale, PA, for the experiment with each agent. The rats were acclimated in our laboratory for a minimum of 10 days before initiation of experiments; in the butylene oxide experiment, they were kept for about a month before use. Males (8 weeks old, 300-324 g) of the same strain were purchased at the start of the study. Males which did not inseminate a female were culled and replaced for each subsequent experiment.

A few of the rats in the butylene oxide experiment showed marked weight loss early during the pregestational exposure period and were sacrificed. Necropsy suggested a Corynebacterium kutcheri infection, which was confirmed by cultures and histopathologic examination. One death occurred following gestational exposure. A total of five pregnant rats showed gross lesions attributable to the organism at scheduled sacrifice (d.g. 21). Since the group sizes were adequate without them, data from these animals were not included in the calculation of results. Upon receipt, eight of the rats purchased for the styrene oxide experiment were subjected to stress test by corticosteroid (dexomethosone, 2 mg) injection, killed three days later, and lung washes were obtained. Positive cultures for C. kutscheri were obtained from lung washes of three rats, but no gross lesions were detected in any animal on gross examination at the completion of the experiment. The rats for the methyl bromide experiment were not tested; they may be presumed to have had a latent infection but this did not develop into gross lesions.

The animals were housed in stainless steel cages before, during, and after inhalation exposure. Caging of experimental females was individual within the exposure chamber except during the mating period. Cage assignment was not random and the locations were not rotated. At the completion of each daily exposure, the generator was disconnected, the chambers were flushed with fresh air for at least one-half hour, 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 by an automatic watering system which was disconnected and drained prior to initiation of each exposure. Food consumption was recorded for a 2-day period prior to inhalation exposure, thrice weekly during the pre-gestational exposure period, and at 2-day intervals throughout the gestational exposures. The survival, appearance, and behavior of the parental females during the experimental period were noted. There were no variations in diet or light cycle and there was no use of pesticides, medications, or other extraneous chemicals during the experimental period. Humidity was not controlled.

Each female rat was uniquely identified by consecutively numbered ear tags (National Band and Tag Co.), which provided coding as well as identification. A computer file was created of the weights and identification numbers of the females and a randomization program was used to assign the animals randomly by weight to the three pregestational treatment groups. The rats were also coded as to pregestation exposure group by subcutaneously injecting india ink in the

forepaw: filtered air-no mark, low concentration-left, high concentration-right. A similar marking of the hind paw was done after assignment to gestational exposure groups. This procedure, which obviated errors in recording of group assignment, permitted the person who killed the rats to be aware of exposure history. Since the uterus was removed and examined by other prosectors, however, treatment group was not known during evaluations.

On the Friday prior to initiation of exposure, the animals were placed in the exposure chamber and the food in each housing unit was weighed to allow calculation of the pre-exposure food consumption. In the butylene oxide and styrene oxide experiments, the females were individually weighed again prior to placement in the exposure chambers. The female rats were weighed at least twice weekly during pregestation exposure and on days 1, 7, 14, and 21 of gestation.

Pregestational exposures ordinarily began on a Monday and were continued for 7 hours per day, 5 days a week, for 3 weeks. The females were transferred to a standard rack unit and caged with males (2:1) starting on the Monday following completion of the pregestational exposures. Vaginal lavages were performed the following mornings and examined for the presence of sperm. Sperm positive females were randomly assigned to gestational exposure groups. Gestational exposures were started on the day on which sperm were detected, which was denoted as day 1 of gestation (d.g. 1). Mating and initiation of gestational exposures continued for 7-9 days, until about 36 sperm-positive rats were assigned to each experimental group. Gestational exposures were performed 7 hours per day, 7 days per week, through d.g. 19. All exposed mated rats were sacrificed at d.g. 21. The target concentrations to which the rats were exposed are indicated below. For convenience, the exposure groups are denoted as "LOW" and "HIGH" and the group exposed to filtered air as "AIR."

	Concentration (ppm)	
	LOW	HIGH
Butylene Oxide	250	1,000
Styrene Oxide	100	300*
Methyl Bromide	20	70

*Original target high concentration (300 ppm) was lethal. Subsequently the 300 ppm (high) concentration group was omitted on instructions from the sponsor.

Based upon the combination of pregestational and gestational exposures, one control and six experimental groups were formed. These groups will be identified by the pregestational and gestational exposure in the presentation of results:

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

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

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

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

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

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

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

The animals were sacrificed by introduction of carbon dioxide into an euthanasia chamber. The order of sacrifice was random and the animals were identified only by a code number so that the treatment group was not known during teratologic examination.

Necropsies were performed on all animals; liver, lung, and kidneys were weighed. Internal abnormalities of the pregnant and nonpregnant animals, e.g., adhesions, tumors, or evidence of infection were also recorded. Samples of the ovaries, uterus, liver, lungs with trachea, and kidneys were preserved in 10% neutral buffered formalin (NBF). Histopathological examination was performed on tissues from 25% (approximately 8 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 have been preserved for possible future examination.

Teratologic Examinations

The uterus, with ovaries attached, was removed from each animal immediately upon sacrifice and the total number of implantation sites was counted. This information and the several other measures to be described were recorded on forms from which computer input was keypunched directly. The ovaries were removed and identified as right or left, the corpora lutea were counted, and the ovaries were fixed in NBF for histologic examination. The uterus was opened and counts of living and dead fetuses and of resorptions were made. The resorptions were classified as to the stage of gestation at which death appeared to have occurred.

The fetuses were removed from the surrounding membranes in serial order and the amniotic fluid was observed for any abnormalities in color. Concurrently, the placentas were removed, weighed and examined; abnormal placentas were fixed in 10% NBF for histological examination. The living and recently dead fetuses were blotted on a moist surface, weighed, the crown-rump length measured, and the sex was determined. Each fetus was examined under an illuminated magnifier for gross external abnormalities and assigned, by coin-toss, into one of two groups for more detailed teratologic examination. In one group, the heads were removed and fixed in Bouin's fluid for subsequent examination of serial thin razor blade sections by the method of Wilson (1965).

These fetuses were examined for internal abnormalities by Staples' modification of Wilson's method (Staples, 1974; Wilson, 1965). Slightly more than one-half on the fetuses were examined in this manner since we established a requirement that a minimum of 2 fetuses per horn and 4 fetuses per litter would be examined, except when precluded by the presence of fewer fetuses. The fetuses of the second group were eviscerated and together with the decapitated carcasses, were fixed in alcohol and prepared for evaluation of skeletal abnormalities by the Staples and Schnell (1964) modification of the method of Dawson (1926). The cleared Alizarin Red S stained skeletons were examined under low power magnification, following a defined checklist to ensure observation and recording of defects.

Rabbits

Groups of 72 sexually mature virgin female and adult male New Zealand White rabbits (approximately 4½-5 and 6 months of age, respectively) were obtained from Scotts Rabbitry, Vashon Island, Washington. They were fed Wayne Rabbit Diet and water ad libitum except during exposure. The rabbits were caged in individual stainless steel cages for a minimum of 18 days after receipt in our laboratory for acclimation and to insure the absence of pseudopregnancy. The females were randomly divided by weight into three groups of 24 each. The rabbits were artificially inseminated in the afternoon over the course of three successive days, using pooled semen samples (Hafez, 1970; Andrew and Staples, 1977) from bucks which had been previously trained to serve an artificial vagina (AV). Each AV was filled with water at about 45°C before use, and semen was collected into the external reservoir at room temperature. The pooled semen samples from three males were diluted with a buffered extender to a concentration of approximately 20-30 million sperm per ml. A volume of approximately 0.5 ml was used to inseminate each doe within 1-2 hours of semen collection. Ovulation was induced by nearly simultaneous i.v. injections of 2.5 mg (0.5 mℓ) of pituitary luteinizing hormone (Armour Standard, Burns Biotec). The morning following insemination was defined as d.g. 1.

On the day following insemination, 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. Neither food nor water was provided during exposure. The chamber was flushed with filtered air following exposure and the rabbits were transferred to individual home cages in separate animal quarters where they were supplied with fresh food and water.

Pregestational exposure was not included as part of the rabbit protocol. There were three groups of experimental rabbits defined as follows: LOW--low level exposure on days 1-24 of gestation; HIGH--high level exposure on days 1-24 of gestation; AIR--filtered air exposure on days 1-24 of gestation. The concentration levels employed corresponding to the above for each material are shown in the following table:

	Concentration (ppm)	
	LOW	HIGH
Butylene Oxide	250	1000
Styrene Oxide	15	50
Methyl Bromide	20	70*

*On instructions from the sponsor, exposures to methyl bromide at the 70 ppm level were terminated after exposure on d.g. 15 because of toxicity.

As indicated elsewhere in this report, pregnancy rate was low in the butylene oxide experiment and group size was considered inadequate. Accordingly, the original exposures and the resulting data were denoted as Replicate I. An additional 24 inseminated rabbits subsequently were exposed to butylene oxide at the low level (250 ppm); these animals were considered as Replicate II. The exposures of these rabbits were performed concurrently with the methyl bromide exposures and used the air group of that experiment as a control. The values for various measures obtained from the control (and from the exposed) rabbits were similar to one another in the two replicates and only occasional statistically significant differences were found. To allow better evaluation by the reader, whenever suggestive differences were obtained the data from the two replicates are presented in the tabulations of results, in addition to the pooled values. The pooled values only are presented when there were clearly no differences between replicates.

The rabbits were exposed to butylene oxide or to styrene oxide through the first 24 days of gestation. Because of the toxicity to be described in the results, rabbits were exposed to methyl bromide only through d.g. 15. The rabbits of each experiment were maintained until day 30 of gestation, at which time they were sacrificed using CO_2. The necropsy procedures and evaluations of embryotoxicity were as described for rats. The only major exception was that all fetuses were dissected and examined for visceral abnormalities, as described above.

Food consumption was measured before the exposure period began and every two days throughout exposure. Replicate I of the butylene oxide experiment was the exception, as only two food determinations were made. The adult rabbits were weighed before exposure, prior to the daily exposures on days 1, 9, 16 and 23 of gestation, and prior to sacrifice at day 30.

STATISTICAL EVALUATIONS

The question is sometimes raised as to what constitutes the basic unit of measure for teratologic data. We have usually treated the litter as the basic unit since each fetus is not an independent observation and members of a litter tend to respond similarly. In some situations, such as sex ratio, the fraction of males in each litter was calculated and used to calculate group means; differences were tested for statistical significance. The overall fraction was also calculated and these proportions were tested across groups. The litter means of quantitative measures such as crown-rump length, fetal weight, and other continuous variables were and treated as individual observations.

When there are more than two groups to compare, it is necessary to decide what comparisons are of primary interest and what multiple comparison procedure should be used. Comparisons against the control group was appropriate for the rabbits since, for all three test chemicals, mortality reduced the size of the high level group to the extent that there were effectively only two groups, providing no basis for establishing a dose response relationship. For the rats, it could be argued that some of the pregestational exposure groups could serve as "controls" for components of the gestational exposure paradigm. If provision is made for comparison involving different times and different dose levels, the power to detect differences from the unexposed control is diminished. We felt it most important to establish whether there were exposure-related differences from the unexposed controls. Less emphasis was placed on comparisons between groups, although in some instances these other comparisons have been obvious and have suggested the presence of interesting interactions.

For continuous data, an analysis of variance (ANOVA) was performed using the computer package "Statistical Package for the Social Sciences (SPSS)."* Statistically significant differences among groups were noted in the group variances for several measures and are indicated in the tables by a superscript "v." These were often seen in the absence of differences in group mean values. Such a difference in variance, indicating a change in the dispersion of the individual values about the group mean is considered to be meaningful although the cause of this variation was not clear in all instances. If the tests for equal variance among groups indicated significant differences, Dunnett's (1964) procedure for unequal variance comparisons was followed. If the variances were not significantly different, the ANOVA was used to obtain the pooled mean square error and Dunnett's procedure for equal variances was followed.

Fisher's Exact Probability Test (Siegel, 1956) was used on the maternal and fetal data for tests of differences between proportions. Bonferronni's (Graybill, 1976) method was used to adjust for the problem associated with multiple comparisons against a control group.

In analysis of repeated measures data, such as maternal weights during and after exposure, missing observations can bias the results, especially when the measurements are missing due to animal death. As this happened for several groups, the problem needed to be considered for the interpretation of the results. Essentially two analyses were done on the repeated measures data. First, t tests were used to compare groups using all available data. Second, orthogonal polynomial equations were fit for each animal for which there was complete data and a multivariate analysis of variance was performed on the coefficients in an attempt to identify differences in weight gain patterns among the exposure groups (See Bock, 1975; Cohen, 1966.) The evaluations using these two methods seemed to be complementary and clear.

Because of the uncertainties of measurement of food consumption, the means for the several measurements were treated as individual observations. Differences

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

from the control consumption values were evaluated by the "Randomization Test using t criterion" as described by Edgington (1980).

In all instances, differences from the control (air or air-air) group at probability levels of 0.05 or less were accepted as statistically significant, and are indicated in the tabulations of results by a superscript "s."

RESULTS

MORTALITY

Very few rats died in the experiments with butylene oxide or methyl bromide (Table 2). A single rat from the high group died during pregestational exposure to butylene oxide and none during gestational exposure. Two females died on the first night of breeding after exposure to methyl bromide, apparently the result of fighting in the unfamiliar multiple caging. No deaths were observed during the exposure to methyl bromide.

A substantial number of deaths occurred during the first day of pregestational exposure of rats to the 300 ppm level of styrene oxide and deaths continued to occur during the night following cessation of exposure. Exposure of this group was discontinued and the remaining rats were killed two days later. A total of 44 of the 106 animals exposed to the high level died during the 3-day period. Sixteen percent (17 of 106) of the animals exposed at 100 ppm died during pregestational exposure (Table 2). Eight of the 48 rats in the group that were initially exposed to air and then at 100 ppm of styrene oxide for gestational exposure died. All of these incidences of mortality were statistically significant compared to the air-air group during this exposure although 3/31 deaths in the low-low group was not. Deaths of mated animals exposed to the 100 ppm level were distributed through the period between 9 and 19 days of gestational exposure.

Because of the mortality noted in the rats, reduced concentrations of styrene oxide were used for exposure of rabbits: 15 and 50 ppm for the low and high concentrations, respectively. There were statistically significant increases in mortality even at these reduced concentrations: Four (17%) rabbits in the low group died--one after 2 weeks and three at the end of exposure. Nineteen (79%) of those in the high group died throughout the exposure period. Only one control animal (4%) died, the cause of death was not evident (Table 3).

Slightly over 1 week after starting methyl bromide exposures, rabbits of the high (70 ppm) concentration group began losing weight and showing generalized signs of distress. Their condition worsened to include convulsive movements and, subsequently, severe to partial (hind-limb) paresis in rabbits. After cessation of exposure* most animals maintained their condition, a few animals showed marked improvement. Deaths began to occur concurrently with the onset of symptoms and a total of 24 of the 25 rabbits in this group had died by the scheduled sacrifice at 30 d.g. (Figure 12.)

Because of low fertility in the original butylene oxide experiment with rabbits (Replicate I), the low level exposure was subsequently repeated (Replicate II). This was run concurrently with the methyl bromide experiment and

*Exposure was terminated after 15 days total.

TABLE 2. Mortality of Rats Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide Before and During Gestation. Results are expressed as number dead/number exposed and (percent)

Exposure Agent	Pregestational Exposure Level			Pregestational-Gestational Exposure Level						
	Air	Low	High	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Butylene Oxide (%)	0/159 (0)	0/106 (0)	1/106 (0.9)	0/39 (0)	0/38 (0)	0/38 (0)	0/45 (0)	0/44 (0)	0/41 (0)	1/42 (2.0)
Styrene Oxide (%)	1/159 (0.6)	17/106[s] (16.0)	44/106[s]* (41.5)	1/41 (2.0)	6/48[s] (16.7)		0/32 (0)	3/31 (9.7)		
Methyl Bromide (%)	0/159 (0)	0/106 (0)	0/104§ (0)	0/42 (0)	0/40 (0)	0/40 (0)	0/38 (0)	0/40 (0)	0/39 (0)	0/40 (0)

* Based on deaths within three days of a single 7-hour exposure; remaining rats were euthanized at that time

§ Two additional rats, which died during first night of mating, are excluded

s Indicates statistically significant ($P \leq 0.05$) difference from control value

Table 3. Mortality of Rabbits Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide Beginning on Day 1 of Gestation. Results expressed as number dead/number exposed and (percent)

Exposure Agent	Air	Low	High
Butylene Oxide			
Replicate 1*	0/24	1/23	14/24[s]
(%)	(0)	(4.3)	(58.3)
Replicate 2	0/25†	5/25[s]	
(%)	(0)	(20.0)	
Pooled	0/49	6/48[s]	14/24[s]
(%)	(0)	(12.5)	(58.3)
Styrene Oxide	1/23	4/24	19/24[s]
(%)	(4.3)	(16.7)	(79.2)
Methyl Bromide	0/25	0/25	24/25[s]
(%)	(0)	(0)	(96.0)

* See text for explanation of replicates.
† Second replicate performed concurrently with methyl bromide exposures, using same control group.
s Indicates statistically significant ($P \leq 0.05$) difference from control value.

used the filtered air group as the controls. No deaths were seen among the controls in either replicate. The mortality incidences of 1 of 23 and 5 of 25 rabbits exposed to 250 ppm were not significantly different from each other. However, the mortality in Replicate II and the pooled (6/48, 12%) mortality values were significantly higher than their respective control values. Fourteen of 24 (58%) exposed to 1000 ppm died during exposure to butylene oxide; a significant increase. Supperative pneumonia was the usual necropsy finding in rabbits which died during exposure. Positive cultures for Pasturella multocida were obtained from the lungs of many of these animals. It therefore appears that the development of overt infection was related to the butylene oxide exposure.

BODY WEIGHT

Butylene Oxide

Pregestational exposure to 1000 ppm of butylene oxide produced a slight, but statistically significant, reduction in the body weight of the rats relative to the controls at most time periods (Table 4). The differences were transient and were not statistically significant at the end of the pregestation exposure. By 7 days of gestational exposure, the rats exposed at the high levels (air-high and high-high groups) were significantly lighter than the controls, and remained lighter throughout the study (Table 5). A lesser effect on weight was seen towards the end of gestation in the low-air

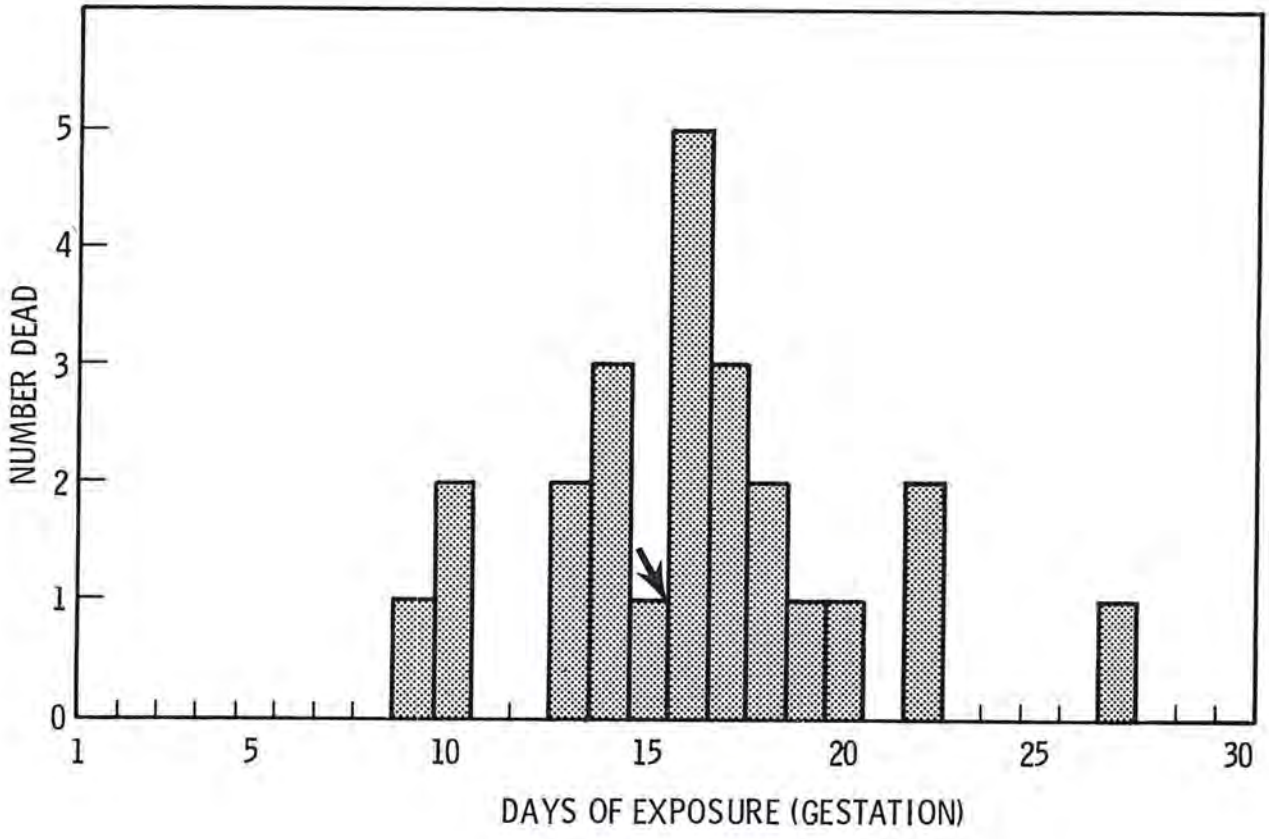


Figure 12. Pattern of mortality in rabbits receiving daily exposure to 70 ppm methyl bromide. Exposures were terminated after the fifteenth daily exposure (indicated by arrow).

and low-low groups. Similar patterns of effect were seen with analyses using orthogonal polynomials. Body weight was not significantly affected by butylene oxide exposure in the rabbit (Table 6).

Styrene Oxide

Pregestational exposure to 100 ppm of styrene oxide rapidly suppressed weight gain so that the rats of the low level group were markedly smaller than those exposed to air (Table 4). This may be a manifestation of the toxicity of this agent, in view of the rapid mortality of the animals in the 300 ppm exposure group. Although these weights are based upon all animals in the group and may thus reflect the moribund condition prior to death, the same lack of growth was seen when the data were analyzed as orthogonal polynomials, in which only survivors were considered. The rats apparently recovered from the toxicity after cessation of exposure as indicated by the subsequent increases in weight in the low-air group (Table 5). Weight gain in groups exposed to styrene oxide during gestation (air-low and low-low) was significantly reduced in comparison to the air-air group.

Rabbits were exposed to substantially lower concentrations of styrene oxide (15 and 50 ppm) than the low gestational concentration (100 ppm) used for the

TABLE 4. Body Weight (g ± S.D.) of Adult Female Rats During Daily (Pre-gestational) Inhalation Exposure to Butylene Oxide, Styrene Oxide, or Methyl Bromide.

Exposure Agent	Days of Exposure	Exposure Level		
		Air	Low	High
Butylene Oxide	Pre-exposure*	212 ± 20	212 ± 18	212 ± 18
	Pre-exposure	253 ± 20	250 ± 22	252 ± 22
	1	257 ± 22	253 ± 23	250 ± 23[s]
	3	251 ± 22	253 ± 24	248 ± 24
	8	256 ± 24	255 ± 25	248 ± 24[s]
	10	257 ± 24	256 ± 25	251 ± 22[s]
	15	254 ± 24	263 ± 28	259 ± 22[s]
	17	265 ± 25	265 ± 27	261 ± 22
Styrene Oxide	Pre-exposure*	140 ± 10	140 ± 10	139 ± 10
	Pre-exposure	173 ± 13	165 ± 20[s]	174 ± 13
	1	173 ± 13	165 ± 22	153 ± 11
	3	178 ± 14	152 ± 22[s]	
	8	196 ± 17	161 ± 23[s]	†
	10	201 ± 16	168 ± 18[s]	
	15	213 ± 16	183 ± 17[s]	
	17	219 ± 18	182 ± 16[s]	
Methyl Bromide	Pre-exposure§	157 ± 11	157 ± 11	157 ± 11
	1	171 ± 12	174 ± 16	179 ± 16[s]
	3	177 ± 14	181 ± 20	186 ± 18[s]
	8	194 ± 15	201 ± 17[s]	204 ± 16[s]
	10	200 ± 16	205 ± 18[s]	207 ± 17[s]
	15	209 ± 18	216 ± 18[s]	217 ± 18[s]
	17	220 ± 18	220 ± 19	220 ± 18

* First pre-exposure weights were taken at randomization, several days prior to exposure. Animals were weighed again 3 days before exposure.

† Exposure to high level (300 ppm) was terminated and surviving rats euthanized because of high mortality.

§ Randomized several days before initiation of exposure and weighed at that time only.

s Indicates statistically significant ($P \leq 0.05$) difference from air (control) mean.

TABLE 5. Effect of Daily Inhalation Exposure to Butylene Oxide, Styrene Oxide, or Methyl Bromide on Gestational Body Weights of Rats (g ± S.D.)

Exposure Agent	Days of Gestation	Pregestational-Gestational Exposure Level						
		Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Butylene Oxide	1	280 ± 25	277 ± 24	278 ± 25	273 ± 29	273 ± 31	271 ± 25	270 ± 25
	7	308 ± 25	304 ± 26	292 ± 24[s]	299 ± 30	301 ± 31	297 ± 26	293 ± 26[s]
	14	338 ± 28	330 ± 33	316 ± 27[s]	322 ± 37[s]	320 ± 34[s]	325 ± 26	318 ± 32[s]
	20	402 ± 36	389 ± 48	363 ± 45[s]	369 ± 58[s]	374 ± 61[s]	372 ± 51[s]	363 ± 51[s]
	21	415 ± 39	399 ± 54	375 ± 51[s]	379 ± 66[s]	386 ± 64[s]	387 ± 57[s]	373 ± 59[s]
Styrene Oxide*	1	230 ± 20	231 ± 22		212 ± 17[s]	207 ± 21[s]		
	7	256 ± 22	208 ± 19[s]		240 ± 21[s]	200 ± 19[s]		
	14	286 ± 27	217 ± 26[s]		276 ± 24	203 ± 23[s]		
	20	327 ± 46	227 ± 30[s]		335 ± 28	204 ± 35[s]		
	21	338 ± 46	232 ± 24[s]		335 ± 40	215 ± 37[s]		
Methyl Bromide	1	239 ± 19	239 ± 27	237 ± 21	247 ± 21	240 ± 17	237 ± 19	230 ± 19[s]
	7	269 ± 19	270 ± 27	266 ± 17[s]	273 ± 23	268 ± 20	258 ± 25	257 ± 20[s]
	14	303 ± 25	303 ± 31	292 ± 21[s]	303 ± 31	307 ± 23	295 ± 24	290 ± 26[s]
	21	372 ± 37	366 ± 46	359 ± 28	376 ± 38	384 ± 32	377 ± 29	368 ± 37

* The 300 ppm (high) group wasterminated during pregestational exposure; only the 100 ppm group was maintained for gestational exposures.

s Indicates statistically significant ($P \leq 0.05$ difference from air (control) mean

TABLE 6. Effect of Daily Inhalation Exposure to Butylene Oxide, Styrene Oxide, or Methyl Bromide on Gestational Body Weights of Rabbits (kg ± S.D.)

Exposure Agent	Days of Exposure	Exposure Level		
		Air	Low	High
Butylene Oxide Replicate I*	Pre-exposure	3.13 ± 0.51	3.07 ± 0.51	3.07 ± 0.49
	1	3.48 ± 0.49	3.38 ± 0.50	3.34 ± 0.49
	9	3.42 ± 0.50	3.37 ± 0.48	3.15 ± 0.54
	16	3.83 ± 0.60	3.67 ± 0.65	3.28 ± 0.55
	23	3.93 ± 0.74	3.90 ± 0.90	3.46 ± 0.52
	30	3.51 ± 0.35	3.55 ± 0.51	3.01 ± 0.44
Replicate II*	Pre-exposure	2.72 ± 0.29†	2.78 ± 0.29	
	1	3.52 ± 0.32	3.60 ± 0.30	
	9	3.62 ± 0.29	3.65 ± 0.27	
	16	3.73 ± 0.37	3.75 ± 0.31	
	23	3.94 ± 0.41	3.80 ± 0.27	
	30	3.95 ± 0.32	3.94 ± 0.28	
Styrene Oxide	Pre-exposure	2.46 ± 0.24	2.39 ± 0.29	2.43 ± 0.28
	1	2.57 ± 0.27	2.54 ± 0.33	2.62 ± 0.34
	9	2.76 ± 0.29	2.61 ± 0.36	2.44 ± 0.33[s]
	16	2.83 ± 0.30	2.75 ± 0.35	2.46 ± 0.38[s]
	23	2.90 ± 0.35	2.77 ± 0.32	2.28 ± 0.42[s]
	30	3.11 ± 0.28	2.97 ± 0.36	2.57 ± 0.18[s]
Methyl Bromide	Pre-exposure	2.72 ± 0.29	2.72 ± 0.27	2.79 ± 0.36
	1	3.52 ± 0.32	3.54 ± 0.34	3.55 ± 0.32
	9	3.62 ± 0.29	3.62 ± 0.32	3.44 ± 0.30[s]
	16	3.73 ± 0.37	3.67 ± 0.31	2.83 ± 0.23[s]
	23	3.94 ± 0.41	3.71 ± 0.29	§
	30	3.95 ± 0.32	3.77 ± 0.41	3.46

* See text for explanation of replicates.
† Replicate II exposures were concurrent with methyl bromide exposures, using same air (control) group.
§ Weight not obtained.
s Indicates statistically significant ($P \leq 0.05$) difference from air (control) mean.

rats. Nevertheless, exposure to the high level (50 ppm) resulted in statistically significant lower body weights by 9 d.g. relative to the controls; this difference persisted throughout the remainder of the study (Table 6).

Methyl Bromide

The pre-exposure weights of rats in the methyl bromide groups were identical, because of randomization by weight (Table 4). The rats assigned to the high group had gained slightly more weight by the start of exposure than had the controls, and the difference was statistically significant. The weights of these animals remained similar to or greater than the controls throughout the remainder of the pregestational exposure. Although these differences were small, they were statistically significant as were the differences in the low group at a few periods (Table 4).

Body weights of the rats in the high-high methyl bromide group were significantly less than in the air-air group at most time periods of the gestational exposure, although by term no significant differences were observed (Table 5). Transient statistically significant differences in body weight of the maternal rabbits were noted with exposure to methyl bromide (Table 6).

FOOD CONSUMPTION

Analysis of the data on food consumption is complicated by the usual uncertainties in measurement. This occasionally gave rise to mean values for a measurement period which appear to be invalid but for which there is no clear basis for their omission. As an example, the pre-exposure measurements for the rats of the methyl bromide experiment differ significantly (Table 7). Accordingly, differences in means in individual measurement periods are not indicated and no attempt was made to adjust for pre-exposure consumption values.

It should be noted that there were occasional periods for which measurement was accidentally omitted and that measurements for the rats were based on groups of animals using a common feeder. Since the rats of the three pregestational groups were redistributed for the gestational exposures, the gestational data and the analyses do not reflect the differences in prior exposure history, which makes any differences even more meaningful.

Butylene Oxide

No remarkable changes in food consumption were produced by pregestational or gestational exposure of rats to butylene oxide (Table 7). Adequate data for butylene oxide exposed rabbits are available only for the low level in Replicate II, where no effect was detected. Incomplete measurements in Replicate I over the 15-17 and 20-22 day periods confirm this lack of effect at the low dose but suggest that there may have been an effect at the high level.

Styrene Oxide

The rats exposed to the 100 ppm level of styrene oxide showed markedly reduced food consumption relative to those in the air group at most periods of

TABLE 7. Food Consumption of Adult Female Rats During Pregestational and Gestational Inhalation Exposures to Butylene Oxide, Styrene Oxide, or Methyl Bromide. Consumption was measured over indicated intervals for all rats using a common feeder and was calculated as daily consumption per animal and expressed as g ± S.D.

Measurement Period*	Agent and Exposure Level								
	Butylene Oxide			Styrene Oxide			Methyl Bromide		
	Air	Low	High	Air	Low	High	Air	Low	High
Pre-exposure	22 ± 2	22 ± 4	21 ± 4	21 ± 1	17 ± 1	21 ± 1	18 ± 4	12 ± 5	6 ± 2
Pregestation									
2 - 3	20 ± 1	20 ± 2	20 ± 3	19 ± 1	13 ± 4	†	19 ± 2	17 ± 9	25 ± 4
4 - 6	24 ± 1	24 ± 2	21 ± 4	19 ± 2	10 ± 2		23 ± 2	24 ± 1	25 ± 4
8 - 9	20 ± 1	21 ± 3	18 ± 4	21 ± 2	13 ± 1		22 ± 1	22 ± 1	22 ± 1
10 - 11	18 ± 2	20 ± 2	18 ± 2	21 ± 2	16 ± 1		23 ± 2	21 ± 1	21 ± 4
14 - 15	21 ± 1	21 ± 1	20 ± 1	20 ± 2	20 ± 5		21 ± 4	22 ± 2	22 ± 2
16 - 17	21 ± 3	21 ± 2	19 ± 1	20 ± 2	15 ± 1		22 ± 1	24 ± 2	24 ± 5
18 - 20	19 ± 1	20 ± 2	18 ± 1	19 ± 1	14 ± 1		23 ± 2	25 ± 4	22 ± 1
Mean§	20 ± 2	21 ± 1	19 ± 1	20 ± 1	14 ± 3 s		22 ± 1	22 ± 3	23 ± 2
Gestation									
1 - 3	21	21	19	27 ± 17	19 ± 15		21 ± 7	32 ± 5	25 ± 6
4 - 7	22 ± 4	24 ± 2	22 ± 6	26 ± 8	19 ± 13		23 ± 4	34 ± 8	34 ± 10
8 - 9	24 ± 3	26 ± 2	23 ± 2	24 ± 9	24 ± 15		26 ± 4	36 ± 15	35 ± 2
11 - 13	26 ± 2	26 ± 1	23 ± 1	29 ± 10	18 ± 7		26 ± 4	31 ± 8	29 ± 5
14 - 15	26 ± 2	25 ± 4	26 ± 5	20 ± 13	17 ± 6		38 ± 5	30 ± 4	36 ± 10
16 - 17	26 ± 2	27 ± 2	26 ± 2	28 ± 9	15 ± 1		32 ± 9	23 ± 8	41 ± 14
19 - 20	27 ± 3	28 ± 2	25 ± 3	25 ± 11	24 ± 9		20 ± 8	23 ± 9	20 ± 7
Mean§	25 ± 2	25 ± 2	23 ± 3	26 ± 3	19 ± 3 s		27 ± 6	30 ± 5	31 ± 7

* Periods of measurement are indicated by terminal day of measurement period, during pregestational or gestational exposure. A range of days is indicated to simplify comparisons between agents, since there were slight differences in the measurement intervals.

† Group was terminated due to high mortality.

§ Indicated means were calculated using mean values of measurement periods as observations, irrespective of variances.

s Statistically significant ($P \leq 0.05$) overall difference from air (control) group by "Randomization Test."

measurement (Table 7). This difference was statistically significant during both the pregestational and gestational periods.

As indicated, the concentrations of styrene oxide were much lower for rabbit exposures than for the rat. The rabbits in the low (15 ppm) level chamber ate markedly less than did those in the air chamber at almost all periods of food measurement; this became even more prominent subsequent to the 9 days of exposure (Table 8). The difference was significant for both periods. The animals in the high (50 ppm) dose chamber ate about half as much as did the controls, and the differences were significant throughout the study.

Methyl Bromide

Food comsumption was similar in the rats exposed to either the high or low concentration of methyl bromide as in the filtered air group. Food consumption was unaffected by exposure of rabbits to the low level (20 ppm) of methyl bromide (Table 8). The high level of methyl bromide (70 ppm), which produced severe neurological changes and deaths in the rabbits, also was found to markedly ($P \leq 0.05$) reduce food intake at times subsequent to the onset of neurological symptoms.

TISSUE WEIGHTS IN MATERNAL ANIMALS

Exposure of rats to butylene oxide or methyl bromide did not affect liver weights; however, the mean weights were significantly reduced in those groups exposed to styrene oxide during gestation (Table 9). This was primarily a reflection of the reduced body weight since the hepatic weight, expressed as a percentage of body weight (Table 10), was not affected.

Likewise in the rabbit, styrene oxide decreased mean but not relative liver weight and also affected the variances of the means (Tables 11 and 12). The liver of the only surviving rabbit in the high methyl bromide group was markedly smaller, on both an absolute and relative scale, than the mean values in the controls or low level groups.

Exposure to butylene oxide did not have a major effect on lung weight in either the rat or in the rabbit. Styrene oxide increased both the variance and mean relative weights of the lung in both species (Tables 9-12). Methyl bromide tended to increase the weight of the lung in the rabbit, although this effect was not statistically significant.

Kidney weights were affected only in the rat and only by styrene oxide. The variance and the relative kidney weights in the air-low and low-low groups were significantly increased (Table 10) although the absolute weights were not significantly different (Table 9).

No statistically significant differences in placental weight were detected.

TABLE 8. Food Consumption of Artificially Inseminated Rabbits During Daily Inhalation Exposures to Butylene Oxide, Styrene Oxide, or Methyl Bromide. Consumption was measured over indicated intervals for rabbits using individual feeders and was calculated as daily consumption per rabbit and expressed as g/day ± S.D.

Measurement Period*	Agent and Exposure Level						
	Styrene Oxide			Methyl Bromide			Butylene Oxide
	Air	Low	High	Air	Low	High	Low†
Pre-exposure	206 ± 40	233 ± 75	215 ± 46	184 ± 46	193 ± 53	182 ± 39	186 ± 41
0 - 1	199 ± 35	165 ± 38	88 ± 58				
1 - 2	194 ± 38	171 ± 30	94 ± 54	165 ± 47	177 ± 37	145 ± 36	178 ± 54
2 - 3	195 ± 35	167 ± 32	100 ± 68	166 ± 26	178 ± 28	166 ± 30	214 ± 31
3 - 4	197 ± 33	188 ± 30	112 ± 56	160 ± 37	155 ± 47	125 ± 29	147 ± 109
4 - 5	184 ± 33	183 ± 41	108 ± 60	174 ± 29	177 ± 26	94 ± 54	168 ± 37
5 - 7	189 ± 36	192 ± 43	107 ± 70	228 ± 77	222 ± 68	151 ± 75	218 ± 102
7 - 9	183 ± 24	165 ± 33	102 ± 53	265 ± 64	269 ± 36	222 ± 49	291 ± 78
9 - 11	190 ± 22	165 ± 36	85 ± 63	145 ± 56	#	#	#
11 - 13	181 ± 27	153 ± 53	86 ± 63	141 ± 53	139 ± 74	5 ± 4	145 ± 51
13 - 15	180 ± 27	148 ± 50	77 ± 47	155 ± 55	137 ± 51	30 ± 74	137 ± 63
15 - 17	177 ± 38	156 ± 46	72 ± 46	172 ± 33	154 ± 50	2 ± 2	149 ± 59
17 - 19	175 ± 46	148 ± 49	76 ± 45	215 ± 76	191 ± 70	22 ± 43	190 ± 69
19 - 21	169 ± 53	142 ± 49	72 ± 49	254 ± 89	248 ± 83	152 ± 17	242 ± 53
21 - 23	165 ± 50	137 ± 47	70 ± 43	#	286 ± 76		236 ± 18
23 - 25	160 ± 39	120 ± 55		180 ± 61	180 ± 52		#
25 - 28				160 ± 48	155 ± 67		136 ± 48
Mean to d.g.9§	192 ± 6	177 ± 12[s]	102 ± 8[s]	193 ± 43	196 ± 42	150 ± 43[s]	203 ± 51
Overall mean	183 ± 12	160 ± 20[s]	89 ± 15[s]	184 ± 40	191 ± 48	101 ± 75[s]	189 ± 49

* Days of gestation
† Replicate II, to be compared to methyl bromide air group
§ Indicated means were calculated using mean values of measurement periods as observations, irrespective of variances
\# Food weights were not recorded
s Statistically significant ($P \leq 0.05$) overall difference from air (control) group by "Randomization Test"

TABLE 9. Weights (g ± S.D.) of Selected Organs in Rats Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide Before and During Gestation

Exposure Agent	Organ	Pregestational-Gestational Exposure Level						
		Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Butylene Oxide	Liver	15.2 ± 2.0	15.4 ± 1.8	14.6 ± 1.8	14.8 ± 2.4	14.8 ± 2.4	14.6 ± 2.2	14.1 ± 1.8
	Lung	1.8 ± 0.21	1.8 ± 0.22*	1.9 ± 0.33	1.9 ± 0.25	1.9 ± 0.31	1.9 ± 0.33	1.8 ± 0.22
	Kidneys	2.1 ± 0.18	2.03 ± 0.21	2.00 ± 0.20	2.00 ± 0.25	2.0 ± 0.23	2.0 ± 0.24	2.00 ± 0.21
	Placenta[v]†	0.60 ± 0.06	0.61 ± 0.09	0.65 ± 0.15	0.61 ± 0.10	0.60 ± 0.10	0.62 ± 0.08	0.62 ± 0.07
Styrene Oxide	Liver	13.9 ± 1.7	10.3 ± 1.4[s]		14.0 ± 1.8	10.8 ± 1.2[s]		
	Lung[v]	1.5 ± 0.18	2.0 ± 0.43[s]		1.5 ± 0.19	1.6 ± 0.35		
	Kidneys	1.9 ± 0.23	1.8 ± 0.19		1.9 ± 0.23	1.7 ± 0.08		
	Placenta[v]†	0.60 ± 0.07	0.61 ± 0.16		0.58 ± 0.06	0.59 ± 0.09		
Methyl Bromide	Liver	15.6 ± 1.9	15.4 ± 1.9	15.0 ± 1.7	15.7 ± 2.0	16.2 ± 1.7	14.9 ± 1.7	15.1 ± 1.7
	Lung[v]	1.6 ± 0.28*	1.6 ± 0.25	1.5 ± 0.17	1.5 ± 0.16	1.5 ± 0.19	1.5 ± 0.19	1.5 ± 0.16
	Kidneys	2.0 ± 0.22	2.0 ± 0.22	1.9 ± 0.20	2.0 ± 0.30	2.1 ± 0.21	1.9 ± 0.20	1.9 ± 0.17
	Placenta[v]†	0.63 ± 0.16	0.57 ± 0.05	0.56 ± 0.06	0.58 ± 0.07	0.58 ± 0.12	0.57 ± 0.06	0.57 ± 0.08

* One value, clearly out of weight range, was deleted (Butylene oxide air-low, 12.38g; methyl bromide air-air, 7.74g).
† Expressed as mean of litter means.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) mean.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 10. Relative Weights (Organ Wt/Body Wt X 100) ± S.D. of Selected Organs in Rats Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide Before and During Gestation

Exposure Agent	Organ	Pregestational-Gestational Exposure Level						
		Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Butylene Oxide	Liver	3.6 ± 0.34	3.8 ± 0.35	3.7 ± 0.34	3.6 ± 0.40	3.6 ± 0.34	3.6 ± 0.43	3.5 ± 0.3
	Lung[v]	0.44 ± 0.06*	0.44 ± 0.06	0.49 ± 0.06	0.44 ± 0.07	0.46 ± 0.09	0.46 ± 0.08	0.46 ± 0.0
	Kidneys	0.50 ± 0.05	0.50 ± 0.06	0.51 ± 0.05	0.49 ± 0.06	0.50 ± 0.06	0.51 ± 0.07	0.49 ± 0.0
Styrene Oxide	Liver	3.9 ± 0.30	4.1 ± 0.41		4.0 ± 0.40	4.2 ± 0.25[s]		
	Lung[v]	0.43 ± 0.05	0.79 ± 0.16[s]		0.44 ± 0.07	0.62 ± 0.15[s]		
	Kidneys[v]	0.52 ± 0.05	0.73 ± 0.10[s]		0.53 ± 0.05	0.67 ± 0.03[s]		
Methyl Bromide	Liver	4.2 ± 0.38	4.1 ± 0.30	4.1 ± 0.30	4.1 ± 0.32	4.2 ± 0.31	3.9 ± 0.29	4.0 ± 0.2
	Lung[v]	0.43 ± 0.08*	0.42 ± 0.07	0.42 ± 0.04	0.39 ± 0.04	0.39 ± 0.04	0.40 ± 0.05	0.41 ± 0.0
	Kidneys	0.53 ± 0.05	0.53 ± 0.05	0.53 ± 0.07	0.52 ± 0.06	0.53 ± 0.04	0.51 ± 0.05	0.52 ± 0.0

* One value, clearly out of weight range, was deleted; see Table 9.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 11. Weights (g ± S.D.) of Selected Organs in Pregnant* Rabbits Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide During Gestation

Exposure Agent	Organ	Exposure Level		
		Air	Low	High
Butylene Oxide†	Number of Does	27	19	2
	Liver	105.9 ± 19.9	102.8 ± 15.1	112.7
	Lung	14.1 ± 3.7	14.5 ± 2.9	27.3
	Kidneys	21.4 ± 3.6	21.6 ± 3.3	23.1
	Placenta	5.3 ± 2.2	4.6 ± 1.3	5.6
Styrene Oxide	Number of Does	16	15	4
	Liver[v]	103.9 ± 30.7	98.4 ± 12.6	88.9 ± 17.2[s]
	Lung[v]	13.1 ± 2.4	13.6 ± 1.6	17.1 ± 5.5[s]
	Kidneys	20.1 ± 2.9	20.8 ± 2.4	18.2 ± 1.6
	Placenta	5.3 ± 1.6	4.8 ± 0.72	4.9 ± 0.83
Methyl Bromide	Number of Does	17	13	1
	Liver	98.8 ± 18.6	94.8 ± 11.4	69.3
	Lung	12.0 ± 1.3	13.3 ± 1.9	17.1
	Kidneys	19.7 ± 2.4	18.5 ± 2.3	14.7
	Placenta	4.1 ± 0.82	3.7 ± 0.66	2.7

* Data from non-pregnant animals were not included in analysis.
† Data from the two replicates did not differ significantly; therefore data were combined.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

HISTOPATHOLOGY

Rats

Lung--

A variety of pulmonary lesions, generally classified as inflammations, were frequently seen in the rats exposed to all three agents (Table 13). These differed among the three agents and reflect the prior history of the animals as well as the response to the exposure agent. As was described in Methods, this included a Corynebacterium kutcheri infection in the rats, which was most prominent in those used for the butylene oxide experiment. In 9 rats of the butylene oxide experiment, there were focal alveolar inflammations (accumulations of lymphocytes and plasma cells) which were not directly associated

TABLE 12. Relative Weights (Organ Wt/Body Wt X 100) ± S.D. of Selected Organs in Pregnant* Rabbits Exposed to Butylene Oxide, Styrene Oxide, or Methyl Bromide During Gestation

Exposure Agent	Organ	Exposure Level		
		Air	Low	High
Butylene Oxide†	Number of Does	27	19	2
	Liver	2.7 ± 0.50	2.6 ± 0.43	3.4
	Lung	0.36 ± 0.09	0.37 ± 0.08	0.82
	Kidneys	0.55 ± 0.09	0.55 ± 0.10	0.69
Styrene Oxide	Number of Does	16	15	4
	Liver[v]	3.3 ± 0.87	3.4 ± 0.42	3.4 ± 0.51[s]
	Lung[v]	0.42 ± 0.06	0.46 ± 0.07	0.67 ± 0.24[s]
	Kidneys	0.65 ± 0.10	0.71 ± 0.10	0.70 ± 0.08
Methyl Bromide	Number of Does	17	13	1
	Liver	2.5 ± 0.44	2.4 ± 0.25	2.0
	Lung	0.30 ± 0.03	0.34 ± 0.05	0.49
	Kidneys	0.49 ± 0.05	0.48 ± 0.06	0.43

* Data from non-pregnant animals were not included in analysis.
† Data from the two replicates did not differ significantly; therefore data were combined.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

with arterioles or bronchioles, and small granulomas were observed in 2 rats. Mononuclear inflammatory lesions similar to those seen in the butylene oxide experiment, were seen in 5 rats of the methyl bromide experiment, and focal granulomatous inflammation of the lungs was observed in 11 rats. This may be a response to inhaled plant material, which was noted in alveolar areas of one rat. We speculate that the granulomas seen in the other rats may reflect some unidentified exposure prior to receipt of the animals, e.g. bedding, food, or pathogens.

Suppurative bronchopneumonia and/or bronchiolitis (neutrophilic exudate in bronchioles and alveoli) were seen in the styrene oxide experiments with rats. This was considered pneumonia if there was an abundant accumulation of neutrophils, and called brochiolitis if the exudate of neutrophils in lumina was minor or within the epithelium only. Most likely the pneumonia is an extension of the bronchiolitis. As the severity of the pneumonia increased there may have been a tendency to obscure the mononuclear peribronchiolitis and perivasculitis (bronchus-associated lymphoid tissue), which were also present.

TABLE 13. Effect of Daily Pregestational and Gestational Inhalation Exposures of Rats to Butylene Oxide, Styrene Oxide, or Methyl Bromide on the Incidence and Severity* of Histopathological Lesions of the Lung

Exposure Agent	Exposure Levels†	LESIONS					
		Inflammations§	Alveolar Histiocytosis	Peribronchiolitis#	Perivasculitis#	Squamous Metaplasia	Hyperplasia/ Hypertrophy
Butylene Oxide	Air-Air	2/8 (.2)*	4/8 (.6)	8/8 (1.2)	8/8 (1.4)	0/8	0/8
	Air-Low	3/7 (.7)	3/7 (.6)	7/7 (1.3)	5/7 (1.4)	0/7	0/7
	Air-High	1/9 (.1)	4/9 (.4)	9/9 (1.2)	8/9 (1.3)	0/9	0/9
	Low-Air	1/7 (.1)	2/7 (.3)	7/7 (1.1)	7/7 (1.3)	0/7	0/7
	Low-Low	0/8	4/8 (.5)	7/8 (1.1)	8/8 (1.4)	0/8	0/8
	High-Air	3/8 (.4)	4/8 (.6)	8/8 (1.6)	8/8 (2.2)	0/8	0/8
	High-High	1/7 (.1)	4/7 (.6)	7/7 (1.7)	7/7 (1.7)	0/7	0/7
Styrene Oxide	Air-Air	0/8	1/8 (.2)	5/8 (.9)	3/8 (.6)	0/8	0/8
	Air-Low	3/5 (2.6)	0/5	2/5 (1.)	0/5	4/5 (1.8)	4/5 (1.8)
	Low-Air	0/7	3/7 (.4)	5/7 (1.)	7/7 (1.6)	0/7	0/7
	Low-Low	4/5 (1.2)	0/5	4/5 (2.)	3/5 (1.4)	5/5 (2.4)	4/5 (1.0)
Methyl Bromide	Air-Air	2/8 (.4)	0/8	7/8 (1.)	8/8 (1.3)	0/8	0/8
	Air-Low	0/8	0/8	6/8 (.8)	6/8 (.9)	0/8	0/8
	Air-High	4/8 (.5)	1/8 (.1)	7/8 (.9)	8/8 (1.4)	0/8	0/8
	Low-Air	3/8 (.4)	0/8	4/8 (.5)	5/8 (.9)	0/8	0/8
	Low-Low	2/8 (.2)	3/8 (.4)	4/8 (.5)	6/8 (.1)	0/8	0/8
	High-Air	1/8 (.2)	3/8 (.4)	6/8 (.8)	7/8 (1.1)	0/8	0/8
	High-High	4/8 (.6)	3/8 (.4)	7/8 (.9)	8/8 (1.0)	0/8	0/8

* Severity of lesions was graded as 1 - slight, 2 - mild, 3 - moderate, 4 - marked, 5 - extreme; severity is expressed as mean of all rats examined in group.

† Pregestational-gestational exposure level.

§ General category, specific diagnosis differs with exposure agent: non-specific focal mononuclear alveolar inflammation and small granulomas - butylene oxide; pneumonia/bronchiolitis - styrene oxide; focal granulomatous inflammation and non-specific focal mononuclear alveolar inflammation - methyl bromide.

\# More accurately, these categories are a measure of bronchus-associated lymphoid tissue.

These inflammations are considered to be related to exposure only in the styrene oxide study. With this agent, inflammation was not seen in the air-air or low-air groups but was seen in 3/5 and 4/5 of the rats in the air-low and low-low groups, respectively. These changes tended to be correlated with bronchiolar epithelial hypertrophy and/or hyperplasia (apparent increases in size or number of epithelial cells in bronchioles) and with squamous metaplasia. This bronchiolar epithelial squamous metaplasia was usually restricted to the largest one or two bronchioles. Often, bronchiolar epithelial hyperplasia/ hypertrophy was seen in the smaller bronchioles that did not undergo metaplastic changes.

Other changes in the lungs were seen in all experiments, but did not appear to be related to exposure. These include alveolar histiocytosis, a focal accumulation of large histiocytes in alveoli. Most rats showed slight mononuclear peribronchiolitis and perivasculitis--lymphoid nodules and plasma cells around the bronchioles and associated arterioles. These lesions are common for rats, and may reflect previous exposure to pathogenic organisms.

Kidney--

Several rats of each dose group with each agent were considered to have interstitial nephritis (Table 14). This diagnosis was used when any lymphocytes or plasma cells were seen in the interstitial tissues. These infiltrates were very minimal and considered normal. Evidence of glomerulonephrosis was not detected. A few instances of hydronephrosis were seen; this was limited to the methyl bromide experiment. Although not clearly exposure related, 4 of the 5 diagnoses of hydronephrosis were made in rats of the high level groups.

Liver--

Some prominent foci of hepatic necrosis were seen in the methyl bromide rats, but most were small (2-3 cells) and the attendant inflammatory reaction was also scant. A limited number of rats throughout the butylene oxide and styrene oxide (but not the methyl bromide) experiments were observed to have extramedullary hematopoiesis, a common observation in rats. Almost every rat had slight mononuclear pericholangitis, i.e., any infiltrate of lymphocytes and/or plasma cells around the portal triads. These were considered within the normal range.

Reproductive Tract--

Slight to mild mononuclear endometritis, i.e. any infiltrate of lymphocytes and/or plasma cells in the endometrium, was a consistent observation. These changes were, for the most part, considered within the normal range, particularly since all rats were pregnant. Apparently normal corpora lutea were noted in the sections of ovary from each rat.

Rabbits

Lung--

A variety of inflammatory lesions were also seen in the rabbits and may have been influenced by an endemic Pasteurella infection (Table 15). In the styrene

TABLE 14. Effect of Daily Pregestational and Gestational Inhalation Exposures of Rats to Butylene Oxide, Styrene Oxide, or Methyl Bromide on the Incidence and Severity* of Histopathological Changes in the Kidney, Liver, and Uterus

		SITE AND LESION						
		Kidney		Liver			Uterus	
Exposure Agent	Exposure Levels†	Interstitial Nephritis	Hydronephrosis	Necrosis and Inflammation	Extramedullary Hematopoiesis	Pericholangitis	Endometritis	Metritis
Butylene Oxide	Air-Air	1/8 (.1)*	0/8	0/8	2/8 (.2)	8/8 (1.1)	8/8 (1.3)	0/8
	Air-Low	3/7 (.4)	0/7	0/7	2/7 (.3)	7/7 (1.1)	7/7 (1.7)	0/7
	Air-High	5/9 (.6)	0/9	1/9 (.1)	2/9 (.2)	9/9 (1.4)	9/9 (1.7)	0/9
	Low-Air	4/7 (.6)	0/7	1/7 (.1)	3/7 (.4)	7/7 (1.1)	7/7 (1.7)	0/7
	Low-Low	2/8 (.2)	0/8	1/8 (.1)	0/8	8/8 (1.1)	8/8 (1.6)	0/8
	High-Air	3/8 (.6)	0/8	0/8	5/8 (.8)	8/8 (1.1)	8/8 (1.8)	0/8
	High-High	1/7 (.1)	0/7	0/7	1/7 (.1)	7/7 (1.4)	7/7 (1.7)	0/7
Styrene Oxide	Air-Air	6/7 (.1)	0/7	0/7	3/8 (.5)	7/8 (1.2)	8/8 (1.5)	0/8
	Air-Low	2/5 (.4)	0/5	0/5	3/5 (.6)	5/5 (1.)	5/5 (1.)	0/5
	Low-Air	6/7 (.9)	0/7	0/7	1/7 (.1)	7/7 (1.)	7/7 (1.6)	0/7
	Low-Low	1/5 (.2)	0/5	0/5	1/5 (.2)	5/8 (1.2)	5/5 (1.2)	0/5
Methyl Bromide	Air-Air	4/8 (.5)	0/8	7/8 (.9)	0/8	8/8 (1.)	8/8 (1.1)	1/8 (.1)
	Air-Low	4/8 (.6)	0/8	7/8 (.9)	0/8	8/8 (1.)	8/8 (1.1)	0/8
	Air-High	6/8 (.8)	2/8 (.6)	8/8 (1.)	0/8	8/8 (1.)	7/8 (.9)	0/8
	Low-Air	5/8 (.6)	1/8 (.2)	8/8 (1.)	0/8	8/8 (1.)	8/8 (1.2)	3/8 (.4)
	Low-Low	6/8 (.8)	0/8	7/8 (.9)	0/8	8/8 (1.)	8/8 (1.1)	2/8 (.2)
	High-Air	8/8 (1.)	1/8 (.1)	6/8 (.9)	0/8	8/8 (1.)	8/8 (1.)	0/8
	High-High	8/8 (1.)	1/8 (.1)	8/8 (1.)	0/8	8/8 (1.)	8/8 (1.)	1/8 (.1)

* Severity of lesions was grades as 1 - slight, 2 - mild, 3 - moderate, 4 - marked, 5 - extreme; severity is expressed as mean of all rats examined in group.

† Pregestational-gestational exposure level.

TABLE 15. Effect of Daily Inhalation Exposures of Pregnant Rabbits to Butylene Oxide, Styrene Oxide, or Methyl Bromide on the Incidence and Severity* of Histopathological Lesions of the Lung

Exposure Agent	Exposure Level	LESION						
		Inflammations†	Alveolar Histiocytosis	Peribronchitis and Bronchiolitis§	Peri-vasculitis§	Squamous Metaplasia	Micro-abscess	Hyperplasia
Styrene Oxide	Air	7/8 (.9)*	1/8 (.2)	8/8 (2.)	8/8 (1.6)	0/8	1/8 (.2)	1/8 (.1)
	Low	7/8 (1.1)	3/8 (.6)	8/8 (2.1)	8/8 (1.6)	0/8	1/8 (.1)	3/8 (.4)
	High	4/4 (2.)	2/4 (.8)	4/4 (2.)	4/4 (2.)	1/4 (2)	1/4 (.5)	4/4 (1.2)
Methyl Bromide	Air	3/5 (.6)	0/5	5/5 (1.4)	5/5 (1.2)	0/5	0/5	3/5 (.6)
	Low	4/5 (.8)	0/5	5/5 (1.4)	5/5 (1.2)	0/5	0/5	2/5 (.6)
	High	1/1 (1.)	0/1	1/1 (1.)	1/1 (1.)	0/1	0/1	1/1 (1.)
Butylene Oxide	Low#	4/5 (.8)	0/5	5/5 (1.4)	5/5 (1.2)	0/5	0/5	4/5 (.8)

* Severity of lesions was graded as 1 - slight, 2 - mild, 3 - moderate, 4 - marked, 5 - extreme; severity expressed is as mean of all rabbits examined in group.

† General category, specific diagnoses varied with exposure agent as described in text.

§ More accurately, a measure of bronchus-associated lymphoid tissue.

\# Should be compared with methyl bromide air group.

oxide experiment, the inflammation was evidenced as bronchitis, bronchiolitis, and bronchopneumonia. The bronchitis and bronchiolitis in most cases was mononuclear (lymphocytes within the epithelium) and primarily, but not necessarily, over peribronchiolar lymphoid nodules. Two of 8 controls had heterophils in bronchi and bronchioles (slight) and none had bronchopneumonia. Five of 8 low dose styrene oxide rabbits had heterophils in bronchi and bronchioles, which was severe enough to call bronchopneumonia in 1 of these 8. All of the 4 rabbits examined in the high styrene oxide group had heterophils in bronchi and bronchioles and 2 of these 4 were severe enough to call bronchopneumonia. In the methyl bromide and butylene oxide experiments, there were similar findings, as well as a few cases of focal parenchymal inflammation. This was usually a focal accumulation of macrophages with some heterophils in alveolar locations not identified with larger vessels or airways and often involved small granulomas. Suppurative lesions tended to be less common in the butylene oxide and methyl bromide experiments. One lung with a microabscess was seen in each exposure group of the styrene oxide experiment. Only a single focus of squamous metaplasia was seen and was found in the high level group; this appeared to be a response to the nearby abscess. The apparent increase in epithelial hyperplasia could easily have been a response to the inflammation and was not a dramatic change, but incidence tended to increase with dose. Differences or trends among exposure groups in the butylene oxide or methyl bromide experiments were not observed.

Other Organs--

Most findings in the kidney, liver, and uterus of rabbits (Table 16) were clearly unrelated to treatment and were similar to those noted in the rat. A slight mineralization of a few renal tubules was observed, but was considered to be within normal limits in the rabbit.

PREGNANCY RATES

Butylene Oxide

Exposure to butylene oxide led to a slightly reduced percentage of sperm-positive rats which were pregnant (Table 17) although no clear dose relationship was seen and the decrease was not statistically significant. A greater decrease was seen in the rabbits, based on the results at scheduled sacrifice (Table 17). Although this might be considered to be influenced by preimplantation mortality of does, nine of 13 does in the high group which died prior to scheduled sacrifice were pregnant. This gives an overall fraction of 11/23 or 48%, as compared to the 42% pregnant in the air group.

Styrene Oxide

Pregestational exposure to styrene oxide did not affect the percentage of the animals which were pregnant in the low-air group as compared to the air-air group. The percentage of the rats which were found to be pregnant was significantly reduced in the air-low group and was even more greatly reduced in the low-low group. The difference between these two exposed groups was not statistically significant. This effect of 100 ppm of styrene oxide thus appears to be related to exposure during early pregnancy and leads to preimplantation loss of embryos.

TABLE 16. Effect of Daily Inhalation Exposures of Pregnant Rabbits to Butylene Oxide, Styrene Oxide, or Methyl Bromide on the Incidence and Severity* of Histopathological Changes in the Kidney, Liver, and Uterus

		SITE AND LESION					
		Kidney		Liver			Uterus
Exposure Agent	Exposure Level	Tubular Mineralization	Interstitial Nephritis	Pericholangitis	Portal Fibrosis	Necrosis/ Inflammation	Endometritis/ Metritis
Styrene Oxide	Air	3/8 (.5)*	1/8 (.1)	7/8 (1.1)	0/8	0/8	1/8 (.1)
	Low	5/8 (.8)	3/8 (.4)	6/8 (1.1)	0/8	0/8	2/8 (.2)
	High	0/4	1/4 (.2)	4/4 (1.5)	0/4	0/4	1/4 (.2)
Methyl Bromide	Air	1/5 (.2)	0/5	5/5 (1.2)	1/5 (.2)	0/5	0/5
	Low	3/5 (.6)	1/5 (.4)	5/5 (1)	1/5 (.2)	2/5 (.4)	1/5 (.2)
	High	0/1	0/1	1/1 (1)	0/1	0/1	0/1
Butylene Oxide	Low	3/5 (.6)	2/5 (.4)	5/5 (1.2)	1/5 (.2)	1/5 (.2)	0/5

* Severity of lesions was graded as 1 - slight, 2 - mild, 3 - moderate, 4 - marked, 5 - extensive; severity is expressed as mean of all rabbits examined in group.

TABLE 17. Effect of Exposure to Butylene Oxide, Styrene Oxide, or Methyl Bromide on Fraction (%) of Sperm-Positive Rats or Artificially Inseminated Rabbits Which Were Pregnant at Scheduled Sacrifice*

Species	Exposure Agent	Pregestational-Gestational (rat) or Gestational (rabbit) Exposure						
		Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Rat	Butylene Oxide (%)	36/37 (97)	33/37 (89)	28/35 (80)	33/42 (79)	38/44 (86)	33/40 (83)	31/39 (80)
	Styrene Oxide (%)	34/40 (85)	12/39^{s} (31)		28/32 (88)	5/28^{s} (18)		
	Methyl Bromide (%)	40/41 (98)	34/39 (87)	39/40 (98)	35/38 (92)	40/40 (100)	37/37 (100)	38/40 (95)
		Air			Low			High
Rabbit	Butylene Oxide Replicate I† (%)	10/24 (42)			6/22 (27)			2/10 (20)
	Replicate II§ (%)	17/25 (68)			13/20 (65)			
	Pooled (%)	27/49 (55)			19/42 (45)			2/10 (20)
	Styrene Oxide (%)	16/22 (74)			15/20 (75)			4/5 (80)
	Methyl Bromide (%)	17/25 (68)			13/25 (52)			1/1 (100)

* Additional non-scheduled animals were available and were sacrificed for evaluation of reproductive success but their litters were not evaluated.
† See text for explanation of replicates.
§ Second replicate performed concurrently with methyl bromide exposures, using same control group.
s Indicates statistically significant ($P \leq 0.05$) difference from control value.

The values in Table 17 may be adjusted on the basis of examinations for pregnancy in does which died subsequent to the time of implantation. This results in overall pregnancy values of 17/23 (74%), 18/24 (75%), and 12/18 (67%) in the filtered air, 15 ppm, and 50 ppm groups, respectively. Thus, a comparable effect on implantation was not seen in the rabbits at the reduced levels of styrene oxide (Table 17) concentration used.

Methyl Bromide

No effects of exposure to methyl bromide on pregnancy rates were observed in either rats or rabbits (Table 17). Twelve of the 24 rabbits which died during the experimental period were pregnant, giving a value of 13/25 (52%) in the 70 ppm group, the same value as obtained at 20 ppm.

FETAL SIZE AND SEX RATIO

There was a marked reduction in both fetal body weight and crown-rump length in both groups of rats exposed to styrene oxide during gestation; these differences were statistically significant only in the air-low group (Table 18). These measures were unaffected in the group exposed to the low level pregestationally and then to air during gestation (low-air). A similar reduction was seen in the rabbits exposed to the high level during gestation, although the differences were not statistically significant.

Neither butylene oxide nor methyl bromide had a statistically significant effect on either the weight or length of the fetuses in rats (Table 18) or in rabbits (Table 19). In both cases, however, the fetuses of the few (2 and 1, respectively) surviving litters of high dose rabbits were markedly smaller than were those of the air or low dose groups.

Statistically significant differences in the sex ratios were not observed (Table 18).

FECUNDITY AND EMBRYOTOXICITY

Butylene Oxide

Pregestational and/or gestational exposure of rats to butylene oxide did not have statistically significant effects on any of the measures of reproductive success (Table 20). This was true also in the rabbit although in the two litters exposed to the high level (for which statistical analysis could not be performed) there was a suggestion of a decreased number of live fetuses per litter and an increase in the frequency of resorptions (Table 21).

Styrene Oxide

Exposure of rats to styrene oxide had little effect on any of these quantitative measures of reproductive success (Table 22). There was a slight decrease in the number of corpora lutea per rat in the two groups exposed to styrene oxide prior to mating (low-air and low-low), although this difference was not statistically significant. As indicated in a preceding section, the number of

TABLE 18. Weight (g ± S.D.), Length (mm ± S.D.) and Sex Ratio* of Rat Fetuses at 21 Days of Gestation After Exposure of Their Mothers Before and During Gestation to Butylene Oxide, Styrene Oxide, or Methyl Bromide. Results are calculated as mean of litter means.

Exposure Agent	Measure	Pregestational-Gestational Exposure Level						
		Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Butylene Oxide	No. Litters	35	32	28	33	36	30	30
	Weight	4.0 ± 0.37	4.0 ± 0.31	3.9 ± 0.28	4.1 ± 0.39	4.0 ± 0.46	4.2 ± 0.53	4.1 ± 0.29
	Length	37.3 ± 1.6	37.4 ± 1.3	36.8 ± 1.1	37.3 ± 1.5	37.0 ± 1.8	37.7 ± 1.6	37.3 ± 0.99
	% Males*	51.0 ± 16.3	49.8 ± 16.1	54.7 ± 15.5	49.1 ± 18.3	49.3 ± 17.5	46.2 ± 23.2	52.4 ± 14.2
		51.7	51.0	53.7	51.4	51.5	48.3	51.4
Styrene Oxide	No. Litters	34	12		28	5		
	Weight[v]	3.7 ± 0.56	2.5 ± 0.61[s]		3.7 ± 0.33	3.0 ± 0.97		
	Length	35.3 ± 1.9	31.9 ± 2.9[s]		35.2 ± 1.4	32.5 ± 3.1		
	% Males	49.7 ± 15.8	56.7 ± 26.6		50.9 ± 14.5	38.6 ± 27.3		
		51.3	52.6		50.6	46.7		
Methyl Bromide	No. Litters	37	31	36	34	38	36	36
	Weight	3.9 ± 0.32	3.9 ± 0.32	3.7 ± 0.30	3.9 ± 0.28	3.8 ± 0.25	3.9 ± 0.32	3.7 ± 0.27
	Length	37.0 ± 1.4	36.7 ± 1.6	36.2 ± 1.6	37.1 ± 1.3	36.8 ± 1.3	36.9 ± 1.2	36.2 ± 1.2
	% Males	47.4 ± 18.4	46.7 ± 17.3	49.1 ± 15.1	46.0 ± 15.2	48.0 ± 18.0	52.9 ± 10.3	49.7 ± 12.7
		46.7	47.0	48.9	45.5	48.6	52.8	49.6

* Sex ratio is expressed as mean of percent males per litter ± S.D.; percentage of all fetuses which were male is also shown.

s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.

v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 19. Weight (g ± S.D.), Length (mm ± S.D.) and Sex Ratio* of Rabbit Fetuses at 30 Days of Gestation After Exposure of Their Mothers During Gestation to Butylene Oxide, Styrene Oxide or Methyl Bromide. Results are calculated as mean of litter means.

Exposure Agent	Measure	Gestational Exposure Level		
		Air	Low	High
Butylene Oxide	No. Litters	27	19	2
	Weight	48.6 ± 7.8	46.2 ± 5.9	34.8
	Length	102.2 ± 8.2	101.9 ± 7.0	90.9
	% Males*	50.7 ± 23.7	58.9 ± 24.7	37.5
		50.6	59.7	
Styrene Oxide	No. Litters	16	15	4
	Weight	45.7 ± 9.8	42.7 ± 6.2	37.7 ± 7.6
	Length	97.8 ± 7.3	96.1 ± 6.3	92.6 ± 4.2
	% Males	58.7 ± 16.0	55.7 ± 22.6	45.0 ± 22.2
		56.7	55.2	37.5
Methyl Bromide	No. Litters	17	13	1
	Weight	46.2 ± 7.2	43.4 ± 4.7	28.2
	Length	101.2 ± 7.0	98.1 ± 5.2	86.5
	% Males	51.9 ± 15.6	48.8 ± 22.4	38.0
		52.0	43.5	

* Sex ratio is expressed as mean of percent males per litter ± S.D.; percentage of all fetuses which were male is also shown.

litters produced was low in the groups exposed to styrene oxide during gestation (Table 17). A corresponding decrease in the mean number of implants per corpus luteum was not apparent (Table 22).

The percentages of litters of rats with resorptions were markedly lower (11% and 20%, respectively) in the low-air (significant) and low-low (not significant) groups than the 44% in the air-air group (Table 22). In the rabbits (Table 23), however, this measure, i.e., the percentage of litters with resorptions, was significantly increased in the group exposed to the low concentration; the fraction (3/4) was even larger in the high group. The incidence of resorptions was also increased from a value of 0.25 resorptions per litter in the control rabbits to 0.93 in low and 1.5 in the high groups, respectively, but the differences were not statistically significant.

Methyl Bromide

There were no marked differences in the values of any of these measures of fecundity or embryotoxicity attributable to exposures of rats (Table 24) or

TABLE 20. Fecundity, Embryotoxicity and Fetal Viability in Pregnant Rats Exposed Pregestationally and Gestationally to Butylene Oxide and/or Filtered Air. Results are expressed as mean ± S.D., except as noted.

Measure	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Number of Litters$_v$	35	32	28	33	36	31	30
Corpora Lutea/Dam^v	17.1 ± 3.9	15.8 ± 3.2	16.4 ± 4.8	15.9 ± 3.6	16.0 ± 4.2	15.3 ± 3.0	15.2 ± 2.4
Number Implants/Litter$_v$	13.5 ± 3.0	12.9 ± 3.6	12.0 ± 3.4	12.9 ± 3.9	12.8 ± 3.7	11.5 ± 4.0	13.7 ± 2.7
Implants/Corpus Luteum^v	0.81 ± 0.20	0.82 ± 0.21	0.77 ± 0.23	0.82 ± 0.23	0.80 ± 0.19	0.75 ± 0.26	0.90 ± 0.12
Number of Live Fetuses	459	385	322	405	434	346	388
Number of Dead Fetuses	1	0	0	0	0	0	0
Number of Resorptions	15	20	15	18	24	13	23
Live Fetuses/Litter	13.1 ± 3.2	12.3 ± 3.8	11.5 ± 3.4	12.3 ± 4.0	12.2 ± 3.8	11.2 ± 4.1	12.9 ± 2.9
Dead Fetuses/Litter$_v$	0.3 ± 0.17	0.0	0.0	0.03 ± 0.17	0.0	0.0	0.0
Resorptions/Litter^v	0.43 ± 0.78	0.59 ± 0.80	0.54 ± 0.84	0.58 ± 0.71	0.69 ± 1.3	0.42 ± 0.72	0.77 ± 1.0
% Early*	2.3	4.0	3.9	3.4	4.8	3.2	4.7
% Mid*	0.85	0.74	0.60	1.2	0.66	0.58	1.0
% Late*	0	0	0	0.24	0	0	0
Total %	3.15	4.74	4.5	4.84	5.46	3.78	5.7
Number of Litters with Resorptions	10	14	10	15	14	10	15
% Litters with Resorptions	28.6	43.8	35.7	42.4	36.1	29.0	50.0
Resorptions/Litters$_v$ with Resorptions	1.5 ± 0.71	1.4 ± 0.63	1.5 ± 0.71	1.3 ± 0.47	1.8 ± 1.6	1.4 ± 0.53	1.5 ± 0.83

* Calculated as percent of total implants.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 21. Fecundity, Embryotoxicity and Fetal Viability in Pregnant Rabbits Exposed to Butylene Oxide or Filtered Air From Day 1 Through Day 24 of Gestation. Results expressed as mean ± S.D., except as noted.

Measure	Gestational Exposure Level		
	Air*	Low*	High
Number of Litters	27	19	2
Corpora Lutea/Doe	9.5 ± 2.5	10.7 ± 2.2	10.0
Number of Implants/ Litter	7.6 ± 3.1	7.9 ± 3.0	6.5
Implants/Corpus Luteum	0.80 ± 0.26	0.74 ± 0.26	0.65
Number of Live Fetuses	182	138	8
Number of Dead Fetuses	1	3	0
Number of Resorptions	23	9	5
Live Fetuses/Litter	6.7 ± 3.1	7.0 ± 3.1	4.0
Dead Fetuses/Litter	0.07 ± 0.27	0.16 ± 0.38	0.0
Resorptions/Litter[v]	0.85 ± 1.3	0.47 ± 0.84	2.5
% Early†	4.4	2.7	23.1
% Mid†	1.5	2.0	15.4
% Late†	4.4	1.3	0.0
Total %	10.3	6.0	38.5
% Implants Resorbed[v]	9.9 ± 14.0	5.0 ± 8.3	38.1
Number of Litters with Resorptions	14	6	2
% Litters with Resorptions	51.9	31.6	100.0
Resorptions/Litters with Resorptions	1.64 ± 1.39	1.5 ± 0.84	2.5

* Data pooled for Replicates I and II.
† Calculated as percent of total implants.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 22. Fecundity, Embryotoxicity and Fetal Viability in Pregnant Rats Exposed Pregestationally and Gestationally to Styrene Oxide and/or Filtered Air. Results are expressed as mean ± S.D., except as noted.

Measure	Pregestational-Gestational Exposure Level			
	Air-Air	Air-Low	Low-Air	Low-Low
Number of Litters	34	12	28	5
Corpora Lutea/Dam[v]	14.0 ± 3.2	14.2 ± 3.0	12.4 ± 1.9	12.2 ± 0.84
Number of Implants/Litter	10.7 ± 3.2	8.8 ± 4.7	11.3 ± 2.3	9.2 ± 3.5
Implants/Corpus Luteum	0.79 ± 0.25	0.60 ± 0.30	0.89 ± 0.22	0.76 ± 0.29
Number of Live Fetuses	351	97	312	45
Number of Dead Fetuses	0	0	0	0
Number of Resorptions	24	9	4	1
Live Fetuses/Litter	10.3 ± 3.4	8.1 ± 4.3	11.2 ± 2.2	9.0 ± 3.7
Dead Fetuses/Litter	0.0	0.0	0.0	0.0
Resorptions/Litter[v]	0.71 ± 1.3	0.75 ± 0.87	0.14 ± 0.45	0.20 ± 0.45
% Early*	4.3	4.7	0.95	2.2
% Mid*	2.1	3.8	0.32	0.0
% Late*	0.0	0.0	0.0	0.0
Total %	6.4	8.5	1.27	2.2
Number of Litters with Resorptions	15	6	3	1
% Litters with Resorptions	44.1	41.7	10.7[s]	20.0
Resorptions/Litters with Resorptions	1.6 ± 1.6	1.5 ± 0.55	1.3 ± 0.58	1.0

* Calculated as percent of total implants.
s Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 23. Embryotoxicity and Fetal Viability in Pregnant Rabbits Exposed to Styrene Oxide or Filtered Air From Day 1 Through Day 28 of Gestation. Results are expressed as mean ± S.D., except as noted.

Measure	Gestational Exposure Level		
	Air	Low	High
Number of Litters	16	15	4
Corpora Lutea/Doe	11.9 ± 3.1	10.7 ± 2.0	10.8 ± 2.2
Number of Implants/Litter[v]	9.1 ± 3.6	9.3 ± 1.5	7.5 ± 2.7
Implants/Corpus Luteum	0.79 ± 0.29	0.89 ± 0.17	0.68 ± 0.14
Number of Live Fetuses	141	125	24
Number of Dead Fetuses	0	0	0
Number of Resorptions	4	14	6
Live Fetuses/Litter	8.8 ± 3.4	8.3 ± 1.5	6.0 ± 2.9
Dead Fetuses/Litter	0	0	0
Resorptions/Litter	0.25 ± 0.58	0.93 ± 0.88	1.5 ± 1.3
% Early	1.4	5.0	10.0
% Mid	0	0.72	6.7
% Late	1.4	4.3	3.3
Total %	2.8	10.0	20.0
Number of Litters with Resorptions	3	10	3
% Litters with Resorptions	18.8	66.7[s]	75.0[s]
Resorptions/Litters with Resorptions	1.3 ± 0.58	1.4 ± 0.70	2.0 ± 1.0

[s] Indicates statistically significant ($P \leq 0.05$) difference from air-air (control) value.

[v] Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

TABLE 24. Fecundity, Embryotoxicity and Fetal Viability in Pregnant Rats Exposed Pregestationally and Gestationally to Methyl Bromide and/or Filtered Air. Results are expressed as mean ± S.D., except as noted.

Measure	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Number of Litters	37	31	36	34	38	36	36
Corpora Lutea/Dam	14.9 ± 2.7	14.0 ± 2.3	15.0 ± 2.8	14.9 ± 2.6	16.4 ± 3.4	14.5 ± 2.6	14.8 ± 2.8
Number of Implants/Litter	12.2 ± 3.0	12.7 ± 2.1	12.9 ± 1.6	13.3 ± 2.3	13.4 ± 3.0	13.3 ± 2.1	13.2 ± 2.3
Implants/Corpus Luteum	0.83 ± 0.22	0.93 ± 0.18	0.88 ± 0.14	0.90 ± 0.14	0.83 ± 0.19	0.92 ± 0.11	0.90 ± 0.14
Number of Live Fetuses	427	379	456	430	484	466	466
Number of Dead Fetuses	1	0	0	1	0	0	0
Number of Resorptions	24	16	8	20	25	12	8
Live Fetuses/Litter v	11.5 ± 3.2	12.2 ± 2.4	12.8 ± 1.6	12.7 ± 2.2	12.7 ± 2.8	12.9 ± 2.1	12.9 ± 2.4
Dead Fetuses/Litter	0.03 ± 0.16	0.0	0.0	0.03 ± 0.17	0.0	0.0	0.0
Resorptions/Litter v	0.65 ± 1.4	0.52 ± 0.93	0.22 ± 0.42	0.56 ± 0.75	0.63 ± 1.2	0.33 ± 0.54	0.22 ± 0.54
% Early*	3.8	2.8	1.1	3.1	3.0	1.7	1.3
% Mid*	1.5	1.3	0.43	0.89	1.6	0.63	0.43
% Late*	0.0	0.0	0.22	0.22	0.20	0.0	0.0
Total %	5.3	4.1	1.8	4.2	4.8	2.3	1.7
Number of Litters with Resorptions	14	10	8	14	13	11	6
% Litters with Resorptions	37.8	32.3	22.2	41.2	34.2	30.6	16.7
Resorptions/Litters with Resorptions v	1.7 ± 1.9	1.6 ± 1.0	1.0 ± 0.0	1.4 ± 0.50	1.9 ± 1.4	1.1 ± 0.30	1.3 ± 0.52

* Calculated as percent of total implants.
v Denotes statistically significant ($P \leq 0.05$) differences in group variances for indicated measure.

rabbits (Table 25) to methyl bromide. There were several instances in altered variances in the rats (Table 24), but not in the rabbits.

A computer program was used to cross-tabulate the location of the resorbed implantation sites (classified as early, mid, and late) by position within the uterus. Live and recently dead fetuses were also included in these tabulations, in which uterine position was indicated numerically, as counted from the uterine to cervical end of each uterine horn. An example of these tabulations, which were prepared separately for each experimental group in the study, is shown in Figure 13. Copies of these records have been submitted to the Clearinghouse for Occupational Safety and Health Information, DTS, 4676 Columbia Parkway, Cincinnatti, Ohio, 45226. Inspection of these data disclosed no preferential site for resorption or death to occur.

FETAL MORPHOLOGY

Definition of Terms

An array of morphologic differences from "normal" were recorded in each experiment of this study (Tables 26-33). The usual minor differences in prevalent types between the rat and rabbit were noted, although few of the defects were severe in either species. In fact, most of the changes described are best considered morphologic variations which are often not considered noteworthy. The incidences of such variations may be consistently (although not necessarily significantly) elevated in the experimental groups, and may show a tendency toward increasing in a dose-dependent manner. When this occurs, it may provide subtle clues of altered embryogenesis (Palmer, 1977). As will be noted, such usually was not the case in this study.

The primary events tabulated include stunting, which was rarely overt. The cases indicated were "calculated stunted," which is operationally defined in our laboratory as fetuses with weights less than 67% of the litter mean, calculated after their exclusion (See McLaren and Michie, 1960). Several of the anomalies, transposition of the aorta or pulmonary vessels and frank situs inversus, are usually considered as true anomalies. These were seen only sporadically and showed no indication of being related to exposure. This was also true of most of the other external and visceral deviations, which are listed descriptively in the tables. A conscious effort was made to use descriptive terminology rather than more precise terminology with specific meanings. Thus, arthrogryposis was used to denote the observation of the external feature of persistent flexure of a joint (Table 30) and the absence of an associated skeletal change was indicated by a footnote. Likewise, dilated ureter was tabulated since it could not be determined if it was or was not hydroureter. The presence of changes such as enlargement of the cerebral ventricles were based on subjective judgements by the investigators. Such enlargements were not considered hydrocephalus unless accompanied by a thinning of the cerebrum.

The classification of skeletal changes, which were primarily variations, was based on the descriptions of Kimmel and Wilson (1973). An extra (14th) rib, at the Lumbar I position, was commonly noted as were rudimentary ribs at the same position. These were differentiated on the basis of whether they were greater or less than half the length of the 13th rib. The term incomplete

TABLE 25. Fecundity, Embryotoxicity and Fetal Viability in Pregnant Rabbits Exposed to Methyl Bromide or Filtered Air From Day 1 Through Day 24 of Gestation. Results are expressed as mean ± S.D., except as noted.

Measure	Gestational Exposure Level		
	Air	Low	High
Number of Litters	17	13	1
Corpora Lutea/Doe	9.3 ± 2.0	9.6 ± 3.0	11.0
Number of Implants/Litter	8.3 ± 2.5	8.0 ± 3.4	8.0
Implants/Corpus Luteum	0.89 ± 0.20	0.77 ± 0.26	0.73
Number Live Fetuses	128	85	8
Number Dead Fetuses	0	2+	0
Number of Resorptions	13	17+	0
Live Fetuses/Litter	7.5 ± 2.6	6.5 ± 3.8	8.0
Dead Fetuses/Litter	0.0 ± 0.0	0.15 ± 0.56	0.0
Resorptions/Litter	0.71 ± 1.5	1.3 ± 3.3	0.0
% Early*	3.6	13.5	0.0
% Mid*	1.4	1.0	0.0
% Late*	2.9	1.9	0.0
Total %	7.9	16.4	0.0
Number of Litters with Resorptions	7	6	0
% Litters with Resorptions	41.2	46.2	0.0
Resorptions/Litters with Resorptions	1.9 ± 1.9	2.8 ± 4.5	0.0

* Calculated as percent of total implants.

\+ Twelve of the 17 occurred in one litter which was completely resorbed; one litter consisted of a single resorption site.

NIOSH METHYL BROMIDE

FILE NONAME (CREATION DATE = 01/10/80)

* CROSSTABULATION OF
POSITION BY VIABLE
CONTROLLING FOR..
TRT VALUE.. 3
* *

	VIABLE					
COUNT / ROW PCT / TOT PCT	1 ALIVE	D-FULL	D-LATE	D-MID	D-EARLY	ROW TOTAL
	1	2	3	4	5	
POSITION 1	60	0	0	0	5	65
	92.3	0.0	0.0	0.0	7.7	14.4
	13.3	0.0	0.0	0.0	1.1	
2	65	0	0	0	1	66
	98.5	0.0	0.0	0.0	1.5	14.6
	14.4	0.0	0.0	0.0	0.2	
3	63	0	1	1	1	66
	95.5	0.0	1.5	1.5	1.5	14.6
	14.0	0.0	0.2	0.2	0.2	
4	59	0	0	2	1	62
	95.2	0.0	0.0	3.2	1.6	13.7
	13.1	0.0	0.0	0.4	0.2	
5	53	1	0	0	2	56
	94.6	1.8	0.0	0.0	3.6	12.4
	11.8	0.2	0.0	0.0	0.4	
6	49	0	0	1	1	51
	96.1	0.0	0.0	2.0	2.0	11.3
	10.9	0.0	0.0	0.2	0.2	
7	40	0	0	0	1	41
	97.6	0.0	0.0	0.0	2.4	9.1
	8.9	0.0	0.0	0.0	0.2	
8	26	0	0	0	1	27
	96.3	0.0	0.0	0.0	3.7	6.0
	5.8	0.0	0.0	0.0	0.2	
9	12	0	0	0	1	13
	92.3	0.0	0.0	0.0	7.7	2.9
	2.7	0.0	0.0	0.0	0.2	
POSITION 10	4	0	0	0	0	4
	100.0	0.0	0.0	0.0	0.0	0.9
	0.9	0.0	0.0	0.0	0.0	
COLUMN TOTAL	431	1	1	4	14	451
	95.6	0.2	0.2	0.9	3.1	100.0

Figure 13. Typical computer-generated cross-tabulation indicating the intrauterine location of living and dead fetuses and of resorption sites. This example is for the "low-air" exposure group of rats in the methyl bromide experiment. Fetal deaths are indicated by "D-Full" and resorptions by "D-LATE," "D-MID" and "D-EARLY."

TABLE 26. Incidence of External and Visceral Anomalies After Butylene Oxide Exposures of Rats. Results are expressed as number of fetuses affected/number litters affected; % litters affected are shown below.

Anomaly or Variation	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Number of Fetuses*/Litters	459/35	394/32	322/28	406/33	436/36	330/30	387/30
Number of Fetuses Examined for Visceral Anomalies	231	201	165	209	227	173	196
Number of Heads Examined for Internal/Skeletal Anomalies	225/234	196/198	159/163	203/203	216/220	167/163	195/192
Stunted			2/2 7.1			1/1 3.3	1/1 3.3
Kinked Tail				1/1 3.0		1/1 3.3	
Webbed Toes				1/1 3.0			
Transposed Aorta							1/1 3/3
Extra Hepatic Lobe (Rudimentary)	1/1 2.9						2/2 6.7
Absent Hepatic Lobe		1/1 3.1					
Hydronephrosis	1/1 2.9		1/1 3.6				1/1 3.3
Absent Renal Pelvis							1/1 3.3

* Skeletons of all fetuses were examined.

TABLE 27. Incidence of Skeletal Anomalies or Variations After Butylene Oxide Exposures of Rats. Results are expressed as number of fetuses affected/number litters affected; % litters affected are shown below. See Table 26 for numbers of fetuses examined.

Anomaly or Variation	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Extra Rib (Lumbar I)	31/12 34.3	10/8 25.0	11/7 25.0	10/7 21.2	25/14 38.9	13/6 20.0	19/10 33.3
Rudimentary Rib	108/25 71.4	100/25 78.1	88/21 75.0	82/21 63.7	96/26 72.2	75/23 76.7	113/26 86.7
"Twisted Rib"				1/1 3.0			
Unossified Sternebra (#6)	48/17 48.6	52/18 56.3	58/18 64.3	77/15 45.5	99/20 55.6	32/12 40.0	26/11 36.7
Unossified Sternebra*			2/2 7.1	1/1 3.0	10/6 16.7	1/1 3.3	2/2 6.7
Bipartite Sternebra*			1/1 3.6		3/2 5.6		
Scrambled Sternebra						1/1 3.3	
Doubled Centra*			1/1 3.6	1/1 3.0		1/1 3.3	
Extra Lumbar Vertebrae	8/3 8.6	1/1 3.1			1/1 2.8		
Ossification Defects:							
Interparietal	1/1 2.9	9/4 12.5	5/3 10.7	3/2 6.1	5/2 5.6	2/2 6.6	3/2 6.7
Supraoccipital	1/1 2.9	3/2 6.3	4/3 10.7	6/2 6.1	1/1 2.8	3/2 6.6	1/1 3.3
Metatarsal and Metacarpal							1/1 3.3

* Three or more sternebrae or centra involved.

TABLE 28. Incidence of External, Visceral, and Skeletal Anomalies After Butylene Oxide Exposures of Rabbits. Results are expressed as number of fetuses affected/number of litters affected; % litters affected are shown below.

Anomaly or Variation	Gestational Exposure Level		
	Air	Low	High
Number of Fetuses*/Litters	183/27	140/19	8/2
Number of Heads Examined for Internal/Skeletal Anomalies	91/92	73/67	4/4
Stunted	1/1 3.7	4/4 21.1	1/1† 50.0
Septal Defect	1/1 3.7		
Hypoplasia of Tail and Toe			1/1† 50.0
Absent Kidney (Unilateral)			1/1† 50.0
Bent Clavicle	1/1 3.7	1/1 5.3	
Fused Sternebrae		1/1 5.3	
Misaligned Sternebrae§	2/2 7.4		
Missing Lumbar Centra		1/1 5.3	
Missing Rib	1/1 3.7		
Extra Rib (Lumbar I)	31/16 59.3	40/12 63.2	6/2 100.0
Rudimentary Rib	17/10 37.0	26/12 63.2	
Incomplete Rudimentary Rib	5/4 14.8	2/2 10.5	
Incomplete Rib (Thoracic XII)	1/1 3.7		
Spade Rib	1/1 3.7		
Branched Ribs	2/2 7.4	1/1 5.3	
Ossification Defects:			
Bone Islands (Skull)	1/1 3.7		
Sternebra #6	2/1 3.7		

* All fetuses received both visceral and skeletal examination.
† Indicated defects occurred in one fetus.
§ Also were bipartite.

TABLE 29. Incidence of External, Visceral, and Skeletal Anomalies and Variations After Styrene Oxide Exposures of Rats. Results are expressed as number of fetuses affected/number of litters affected; % litters affected shown below.

Anomaly or Variation	Pregestational-Gestational Exposure Level			
	Air-Air	Air-Low	Low-Air	Low-Low
Number of Fetuses*/Litters	340/34	100/12	314/28	45/5
Number of Fetuses Examined for Visceral Anomalies	181	54	162	24
Number of Heads Examined for Internal/Skeletal Anomalies	175/165	48/52	158/156	21/24
Stunted	3/3 8.8	2/1 8.3	3/3 10.7	
Transposed Pulmonary Artery			1/1 3.6	
Hydrocephalus			3/2 7.1	
Extra Rib (Lumbar I)	21/12 35.3	14/5 41.7	24/11 39.3	6/2 40.0
Rudimentary Rib	104/23 67.7	25/5 41.7	60/23 82.1	12/3 60.0
Incomplete Rib (Thoracic XI)		1/1 8.3		
Extra Lumbar Vertebrae	14/9 26.5	8/5 41.7	4/4 14.3	9/1 20.0
Unossified Sternebrat	10/3 8.8	47/8s 66.7	4/3 10.7	16/2[s] 40.0
Misaligned Sternebrat	2/1 2.9		1/1 3.6	
Fused Sternebrat			1/1 3.6	
Dumbell Centrat		1/1 8.3		
Doubled Centrat		1/1 8.3		1/1 20.0
Ossification Defects:				
Metatarsal & Metacarpal		14/2§ 16.7		
Pubis		7/3§ 25.0		
Supraoccipital	2/2 5.9	6/5s 41.7	3/3 10.7	5/3s 60.0
Interparietal	7/5 14.7	3/3 25.0	13/9 32.1	3/2 40.0

* Skeletons of all fetuses were examined.
† Three or more sternebrae or centra affected.
§ General retardation was seen in two of the litters involved.
s Indicates statistically sifnificant ($P \leq 0.05$ difference from air-air (control) value.

TABLE 30. Incidence of External, Visceral, and Skeletal Anomalies After Styrene Oxide Exposures of Rabbits. Results are expressed as number of fetuses affected/number of litters affected; % litters affected shown below.

Anomaly or Variation	Gestational Exposure Level		
	Air	Low	High
Number of Fetuses*/Litters	141/16	125/15	24/4
Number of Heads Examined for Internal/Skeletal Anomalies	91/92	73/67	4/4
Arthrogryposis			1/1† 25.0
Hydronephrosis			1/1† 25.0
Blood Filled Bladder	1/1 6.3		
Enlarged Ventricle (Heart)	1/1 6.3		
Cyst Adjacent to Gallbladder	1/1§ 6.3		
Ovarian Cyst	1/1§ 6.3		
Extra Rib (Lumbar I)	44/8 50.0	32/11 73.3	11/4 100.0
Rudimentary Rib	14/9 56.3	15/9 60.0	4/2 50.0
Incomplete Rudimentary Rib	2/2 12.5	4/3 20.0	
Incomplete Rib (Thoracic XII)		2/2 13.3	
Spade Rib		3/2 13.3	
Fused Ribs		1/1# 6.7	
Ossification Defects:			
Interparietal		1/1 6.7	
Parietal	3/2 12.5		

* All fetuses received both visceral and skeletal examination.
† Both defects occurred in the same litter; no skeletal changes were associated with arthrogryposis.
§ Both defects occurred in one litter only.
# Incidence was 4/2, 13.3% if recently dead fetuses of one litter are included.

TABLE 31. Incidence of External and Visceral Anomalies After Methyl Bromide Exposures of Rats. Results are expressed as number of fetuses affected/number litters affected; % litters affected shown below.

Anomaly or Variation	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Number of Fetuses/Litters	426/37	378/31	461/36	432/34	483/38	464/36	464/36
Number of Fetuses Examined for Visceral Anomalies	223	192	230	221	241	237	236
Number of Heads Examined for Internal Anomalies	217	191	229	219	239	233	233
Stunted		1/1 3.2	1/1 2.8	2/2 5.9	1/1 2.6		1/1 2.8
Abnormal Foot Shape	1/1 2.7						
Kinked Tail	1/1 2.7					2/1 2.8	
Umbilical Hernia					1/1 2.6		
Mild Hydrocephalus	1/1 2.7		1/1 2.8		2/2 5.3		
Reduced Cerebral Size	1/1 2.7						
Cleft Palate	1/1 2.7				1/1 2.6		
Reversed Pulmonary Artery		1/1 3.2					
Situs Inversus		1/1 3.2					
Enlarged Ventricles (Cerebrum)			1/1 2.8		1/1 2.6	1/1 2.8	
Hemorrhagic Adrenal	2/1 2.7						
Hydronephrosis				2/1 2.9			
Dilated Ureter	1/1 2.7		1/1 2.8	2/1 2.9			

TABLE 32. Incidence of Skeletal Anomalies or Variations After Methyl Bromide Exposures of Rats. Results are expressed as number of fetuses affected/number litters affected; % litters affected shown below.

Anomaly or Variation	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Number of Fetuses/Litters Examined for Skeletal Anomalies	426/37	378/31	461/36	432/34	483/38	464/36	464/36
Number of Heads Examined for Skeletal Anomalies	209	187	232	213	244	241	231
Extra Rib, Lumbar I	8/5	11/7	3/3	21/12	9/5	14/10	8/5
	13.5	22.6	8.3	35.3	13.2	27.8	13.9
Rudimentary Rib	51/25	73/18	34/8	52/20	52/10	67/22	49/21
	67.6	58.1	22.2	58.8	50.0	61.1	58.3
Wavy Rib					1/1		3/2
					2.6		5.6
Bent Rib					2/1		6/1
					2.6		2.8
Extra Lumbar Vertebrae	3/1	2/1	1/1		1/1	3/2	
	2.7	3.2	2.8		2.6	5.6	
Unossified Sternebrae*	1/1	2/2	14/7	5/4		4/4	2/2
	2.7	6.5	19.4	11.8		11.1	5.6
Double Centra*		1/1		1/1			
		3.2		2.9			
Dumbell Centra*	2/1		3/2		3/3	1/1	
	2.7		5.6		7.9	2.8	
Misaligned Ilium					1/1		
					2.6		
Ilium Fused to Sacral Vertebral Arch						1/1	
						2.8	

*Three or more sternebrae or centra involved.

TABLE 32 (Continued)

Anomaly or Variation	Pregestational-Gestational Exposure Level						
	Air-Air	Air-Low	Air-High	Low-Air	Low-Low	High-Air	High-High
Ossification Defects:							
Metatarsal or Metacarpal				1/1	1/1	2/1	
				2.9	2.6	2.8	
Proximal Ends of Rib					2/2		1/1
					5.3		2.8
Vertebral Arch					1/1		
					2.6		
Lumbar Centra						1/1	
						2.8	
Atlas					1/1		
					2.6		
Pubis			4/1				
			2.8				
Ischium			1/1				
			2.8				
Supraoccipital	1/1	2/2	10/7	4/4	2/2		20/7
	2.7	6.5	19.4	11.8	5.3		19.4
Interparietal	6/5	13/9	9/7	5/5	9/8	5/5	20/14
	13.5	29.0	19.4	14.7	21.1	13.9	38.9
Parietal	4/3	8/4	6/5	3/2	20/11	10/4	11/7
	8.1	12.9	13.9	5.9	28.9	11.1	19.4
Frontal			1/1				

TABLE 33. Incidence of Visceral and Skeletal Anomalies and Variations After Methyl Bromide Exposures of Rabbits. Results are expressed as number of fetuses affected/number of litters affected; % litters affected shown below.

Anomaly or Variation	Gestational Exposure Level		
	Air	Low	High
Number of Fetuses*/ Litters	128/17	85/11†	8/1§
Number of Heads Examined for Internal/ Skeletal Anomalies	64/64	40/45	4/4
Small Lungs	1/1 5.8		
Extra Ribs	15/10 58.8	12/8 61.5	
Rudimentary Ribs	14/8 47.1	11/6 46.2	
Branched Ribs	2/2 11.8		
Missing Rib	1/1 5.8		
Spade Rib	1/1 5.8		
Misaligned Bipartite Sternebrae	2/2 11.8		
Double Centra		1/1 7.7	
Ossification Defect: Interparietal		1/1 7.7	

* All fetuses received both visceral and skeletal examination.
† All implantations of two litters were completely resorbed. The number of litters, therefore, is less than that indicated in Table 25.
§ No anomalies noted.

rudimentary rib was used when the proximal end was not visible. When this occurred at the Thoracic XII position, it was classified as incomplete (13th) rib. A number of less common rib defects were noted and are illustrated in Figure 14.

Sternebral and vertebral changes such as lack of ossification, misalignment, fusion, complete or incomplete doubling or dumbbell centra were tabulated only when the change was present in three or more adjacent sternebrae or vertebrae. The same criterion was used for sternebral fusion. An exception was made in the case of failure of ossification of Sternebra #6, which is routinely ossified. This failure is of interest since it was observed only in the butylene oxide experiments. The incidence was about 50% in all exposure groups in the rat but was present only in a single (control) rabbit litter. The other ossification defects tabulated were relatively minor--usually a reduced density or area of ossification--although bone islands were occasionally noted.

Butylene Oxide

Alterations of the nature or incidence of morphologic changes related to exposure were not noted in the rats (Tables 26 and 27) or in the rabbits (Table 28).

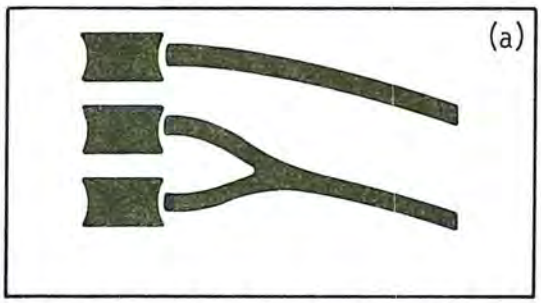
Styrene Oxide

Exposure of rats to styrene oxide resulted in a significantly increased fraction of litters with ossification defects of the sternebrae in the groups exposed during gestation (air-low and low-low) (Table 29). These exposures also resulted in a statistically significant increase in ossification defects of the supraoccipital bones. A comparable increase in these defects was not noted in the rabbits, which were exposed to lower concentrations (Table 30).

Methyl Bromide

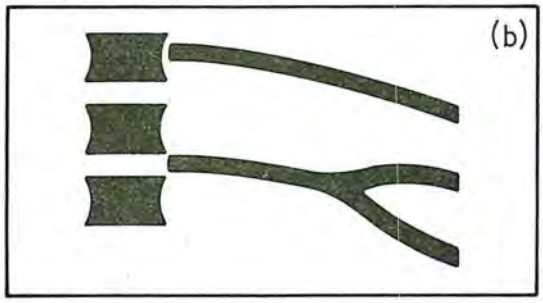
The variety of soft tissue changes noted in the methyl bromide experiments with rats was greater than with other materials (Table 31). These were seen in only one or two fetuses, including controls, and are not considered meaningful. Slight ossification defects (Table 32) also occurred in bones not affected in other experiments, but these also were without exposure-related pattern. No morphologic changes related to exposure of rabbits to methyl bromide were noted (Table 33).

FUSED - lateral-ventral joining of two adjacent ribs which

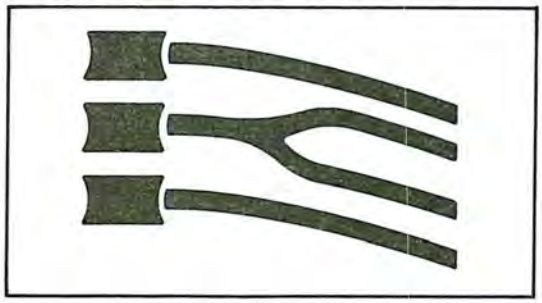


(a) clearly originate from two seperate vertebre or

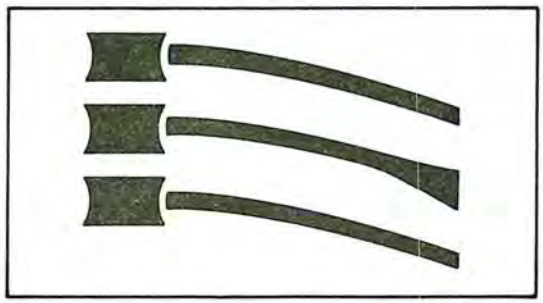
(b) appears to originate intervertebrally, with no ribs originating from the two adjacent vertebrae.



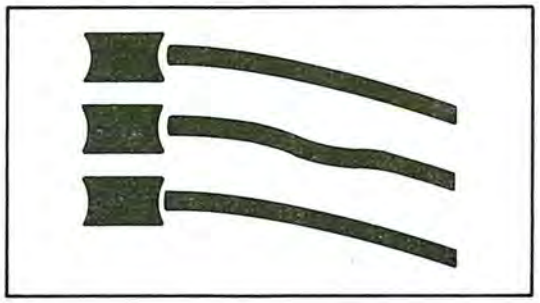
BRANCHED - lateral-ventral bifurcation of a rib which is single at its attachment to a single vertebra.



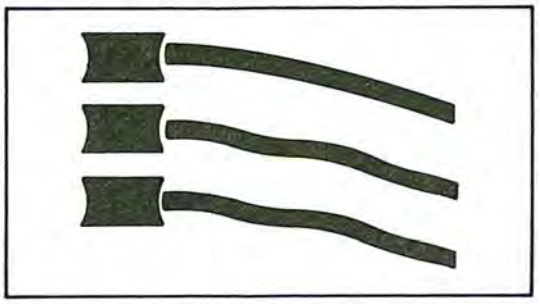
SPADE - a distinct "spade-shaped" enlargement toward the sternal end of the rib.



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.

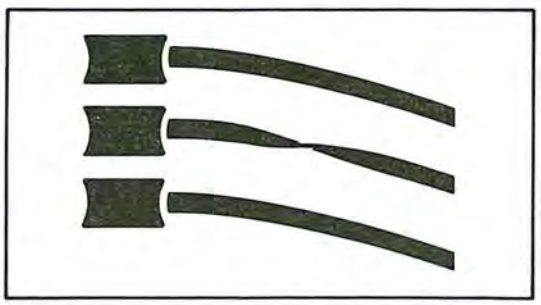


Figure 14. Diagrammatic representation of criteria used for classification of defects of the ribs. The vertebrae are indicated to the left and the defective ribs are shown as the central one or lower two of the ribs.

CONCLUSIONS

The most striking findings of this study were those associated with toxicity in the adult animals. Although these were primarily confirmatory of results previously reported by others, some aspects provide extensions of this prior knowledge. More important to the assessment of developmental toxicology--the primary objective of the present studies--they serve to place 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 more likely that all exposures 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 occupation environment which is safe for the pregnant worker will also be safe for her fetus.

There was little indication of maternal toxicity of butylene oxide or methyl bromide in the rat other than relatively trivial effects on weight gain and a few organ weights. Both of these agents were extremely toxic in the rabbit, killing 58 and 96% of those exposed to the high levels, respectively. The species differences with methyl bromide and the neurological effects on the rabbits are in accord with the limited information by Irish (1940). The few detectable embryotoxic effects with butylene oxide occurred in the rabbit at the concentration which was maternally lethal. Even at those toxic levels, methyl bromide was without remarkable embryotoxicity.

Styrene oxide was the only one of the three compounds which was overtly toxic in the rats, rapidly killing all animals at the 300 ppm level and producing substantial mortality at 100 ppm. Toxicity was also marked in the rabbit, producing mortality at 15 and 50 ppm. The histopathologic changes of the lung produced by styrene oxide tended to be more striking in the rat (metaplasia and hyperplasia) than in the rabbit, where these changes were not observed.

Although the differences were not statistically significant, pregestational exposure of the rat to styrene oxide appeared to reduce the number of corpora lutea. Gestational exposure of rats to 100 ppm of styrene oxide decreased fecundity by significantly increasing the loss of embryos prior to implantation. Preimplantation loss was not seen at the lower concentrations (15 and 50 ppm) used in the rabbit. These exposures tended to increase the frequency of resorptions in the rabbit but this was not observed in the rat. Fetal size (length and weight) tended to be decreased by exposure in both species, although the differences were usually not statistically significant. No clear

effect on morphologic integrity was seen although gestational exposure significantly increased the frequency of ossification defects of the sternebrae and occipital bones.

The developmental effects observed with styrene oxide are not surprising since they occurred in the presence of marked maternal toxicity. It may be considered that some manifestations of developmental toxicity, such as reduction of fetal size, are secondary to the effects on the maternal animals. Other effects, such as the reduction in corpora lutea, are clearly effects on the reproductive capacity of the exposed adult rat. Preimplantation loss and increased resorptions are usually regarded as indications of embryotoxicity but might be influenced by maternal factors. Thus, styrene oxide may be regarded as having reproductive and developmental toxicity at the levels studied, although the mechanisms involved have not been identified.

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