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Carcinogenic and Toxicologic Effects of Inhaled Ethylene Oxide and Propylene Oxide in F344 Rats[1,2]

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*U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, 4676 Columbia Parkway, Cincinnati, Ohio 45226, and †Environmental Pathology Laboratories, Inc., P.O. Box 474, Herndon, Virginia 22070

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Carcinogenic and Toxicologic Effects of Inhaled Ethylene Oxide and Propylene Oxide in F344 Rats. LYNCH, D. W., LEWIS, T. R., MOORMAN, W. J., BURG, J. R., GROTH, D. H., KHAN, A., ACKERMAN, L. J., AND COCKRELL, B. Y. (1984). *Toxicol. Appl. Pharmacol.* **76**, 69–84. The chronic inhalation toxicity and carcinogenicity of ethylene oxide (EO) and propylene oxide (PO) were evaluated in a 2-year inhalation bioassay. Five groups of male weanling Fischer 344 rats, 80 per group, were exposed at 0 ppm (shared control; filtered air), 50 ppm EO, 100 ppm EO, 100 ppm PO, or 300 ppm PO (7 hr/day, 5 days/week) for 104 weeks. Body weights from rats exposed to EO and PO at all exposure concentrations were significantly reduced compared to controls. A statistically significant increase in mortality was observed in all groups of exposed rats compared to controls. Skeletal muscle atrophy in the absence of any sciatic nerve neuropathology was found in rats exposed at 100 ppm EO and 300 ppm PO. Statistically significant associations between EO exposure and an increased incidence of the following rat neoplasmas were observed: mononuclear cell leukemia, peritoneal mesothelioma, and mixed cell brain glioma. Among rats exposed to PO there was a dose-dependent increase in the incidence of complex epithelial hyperplasia in the nasal passages, and two adenomas were detected in the nasal passages of rats exposed at 300 ppm PO. The incidence of adrenal pheochromocytomas was elevated in both PO exposure groups, but not in a dose-related manner. All rat groups were affected by an outbreak of *Mycoplasma pulmonis* infection which occurred about 16 months into the study. This infection alone and in combination with the epoxide exposures affected the survival of rats in this study, and influenced the development of the proliferative lesions in the nasal mucosa of the PO-exposed rats. No treatment-related changes in any clinical chemistry or urinalysis indices were detected. PO exposure did not increase the incidence of the three neoplasmas associated with EO exposure; however, adrenal pheochromocytomas and proliferative lesions of the nasal cavity were increased in rats exposed to PO.

Ethylene oxide (EO, 1,2-epoxyethane, oxirane; CAS 75-21-8) and propylene oxide (PO, 1,2-epoxypropane, propane oxide; CAS 75-56-9) are high-volume reactive alkylating agents used primarily as intermediates in the chemical industry. The EO produced is converted primarily to ethylene glycol for use as

[1] A preliminary summary of these results was presented at the 21st Annual Meeting of the Society of Toxicology, Boston, MA, February 22–26, 1982 and at the AAMI Technology Assessment Conference on In-hospital Ethylene Oxide Sterilization, Arlington, VA, November 21–22, 1983. A portion of the final results were presented at the 23rd Annual Meeting of the Society of Toxicology, Atlanta, GA, March 12–16, 1984.

[2] Histopathologic tissue evaluations were conducted by Environmental Pathology Laboratories, Herndon, Va.

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antifreeze, with lesser amounts used to produce nonionic surfactants (Hine *et al.*, 1981). A small amount of the total EO production is used as a sterilant and fumigant in the health care field; however, this usage provides the greatest opportunity for occupational (inhalation) exposures (Glaser, 1979). PO is produced primarily for conversion into propylene glycol and other chemicals, with other usage as a fumigant, herbicide, preservative, and solvent (Hine *et al.*, 1981). The National Institute for Occupational Safety and Health (NIOSH, 1977) estimates that about 150,000 and 270,000 workers are occupationally exposed to EO and PO, respectively, in the United States. The current permissible exposure limits enforced by the Occupational Safety and Health Administration (OSHA) are 50 ppm (90 mg/m^3) for EO and 100 ppm (240 mg/m^3) for PO as an 8-hr time-weighted average. The standard for EO is currently being reevaluated by OSHA (1983) because of recent reports of adverse reproductive, mutagenic, and tumorigenic effects in both animals and humans.

The acute and subchronic toxicity of EO has been reported in a variety of animal species (Hollingsworth *et al.*, 1956; Jacobson *et al.*, 1956; Weil *et al.*, 1963). The acute and subchronic toxicity of PO has been reported by Rowe *et al.* (1956), Jacobson *et al.* (1956), and Weil *et al.* (1963). The primary acute effects reported following exposure to both epoxides were eye and respiratory irritation. The above studies served as the basis of the current OSHA standards, even though none of these studies evaluated the chronic toxicity or carcinogenicity of these epoxides.

More recent evidence from animal experiments has pointed out the mutagenic and carcinogenic potential of EO and PO (Wolman, 1979; Glaser, 1979; Yager and Benz, 1982; Dunkelberg, 1979, 1982; Bootman *et al.*, 1979; Hardin *et al.*, 1983). Recent human data have suggested the mutagenic (Garry *et al.*, 1979; Abrahams, 1980; Yager *et al.*, 1983), adverse reproductive (Hemminki *et al.*, 1982), and possibly carcinogenic potential (Hogstedt *et al.*, 1979*a,b*) of EO.

To evaluate the chronic toxicity and carcinogenic potential of EO and PO using an exposure route relevant to workers, a rodent bioassay was conducted in which male F344 rats were exposed intermittently to inhaled EO and PO for 2 years.

METHODS

Animals. Four hundred weanling male Fischer 344 rats (Fischer 344/HAPBR) were obtained from Harlan Industries (Indianapolis, Ind.)[4] and quarantined for approximately 2 weeks prior to the initiation of exposures. Rats were received in two shipments, 1 week apart. The initiation of EO and PO exposures was also staggered so that all rats were the same age at the start of the study. Rats were individually identified by ear and toe clipping, and randomly assigned to treatment groups. Only rats which were in good health and had gained weight during the quarantine period were utilized in the study. Animals were housed individually in stainless-steel cages which served as both housing and exposure cages. Feed (Ralston Purina 5001 pellet diet; Ralston Purina, Inc., St. Louis, Mo.) and water (automatic watering system) were available *ad libitum* except during the exposure period. Indented craft paper with no chemical pretreatment was used to collect excrement. Fluorescent lighting was on a 12-hr light/dark cycle (7 AM–7 PM).

Experimental design. Rats, 80 per group, were exposed at 0 (shared control), 50, or 100 ppm EO, and at 100 or 300 ppm PO for about 7 hr/day, 5 days/week for 2 years. Each chamber housed 80 rats plus 12 cynomolgus monkeys during the 7-hr exposure period. Rats and monkeys were housed in separate animal rooms during the periods of no oxide exposure, with control animals housed in separate rooms from the exposed animals to preclude any potential for oxide exposure due to off-gassing. Monkeys were included to evaluate the effects of oxide exposure on a variety of organ systems. Rats from the first shipment were exposed to EO, with the second-shipment rats exposed to PO. Forty rats from each shipment were assigned to a shared group of 80 control animals which were exposed in chambers to filtered air. The EO 50 ppm and PO 100 ppm concentrations were selected since they were the current federal workplace standards for EO and PO, respectively. EO 100 ppm and PO 300 ppm concentrations were chosen since they were approximate maximum tolerated airborne concentrations for both rats and monkeys.

Test materials. EO, 99.7% pure, was obtained from Union Carbide Corp., Chicago, Illinois, in cylinders under its own vapor pressure. PO, 98% pure, was obtained

[4] Mention of a product or company name does not constitute endorsement by NIOSH.

as a liquid from Matheson, Coleman and Bell, Norwood, Ohio. The purity of both epoxides was verified by gas chromatography at intervals throughout the exposure period. Purity was found to be greater than 99% for both epoxides at each analysis period.

Inhalation exposures. Animal exposures were conducted in 4.5-m^3 stainless-steel and glass inhalation chambers. Chambers were operated under dynamic flow conditions at a pressure of −0.25 cm water with tangential airfeed manifolds maintained at 40 liters/min. Chamber airflows provided 12 to 15 air changes/hr. Temperature and humidity were maintained at 23 ± 3°C and 50 ± 10%, respectively. PO exposure atmospheres were generated by metering liquid PO directly into the tangential filtered air intake. EO was vaporized using an Atinco EO vapor generator (Atlas Instrument and Manufacturing Co., Tulsa, Okla.), and the vapor was metered into the filtered air intake of the chamber. Chamber concentrations were monitored two to four times per hr using an infrared analyzer (Wilks Miran 1A or Miran 801; Foxboro Analytical, S. Norwalk, Conn.), with adjustments to the generation systems made during each exposure day to maintain the exposure concentrations at planned concentrations. Infrared analyzers were calibrated using certified gas standards obtained from Liquid Carbonic Corporation, Specialty Gas Division, Chicago, Illinois. Charcoal tube samples of the test atmospheres analyzed by gas chromatography verified that the chamber concentrations were within 10% of the target values, and that the control chamber atmosphere was free of EO and PO.

Clinical observations. Individual animal body weights for test and control groups were recorded weekly for the first 10 weeks, biweekly for the next 20 weeks (Weeks 11–30), monthly for the next 64 weeks (Weeks 31–95), and weekly for the last 10 weeks (Weeks 96–105). Rats were individually observed twice a day, once in the morning and once in the afternoon, for clinical signs, morbidity, and mortality.

Clinical studies. Hematology indices, including hematocrit, hemoglobin, RBC and WBC counts, clotting time, and differential counts, were evaluated at the 104 week termination. In addition, differential slides were prepared on all rats killed in a moribund condition during the last 6 months of the study as an aid in the detection of mononuclear cell leukemia. The following serum parameters were also evaluated at termination: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), blood urea nitrogen (BUN), creatinine (CRE), sorbitol dehydrogenase (SDH), and albumin/globulin ratio (AG). Urinalyses conducted at termination included measurements of acetone, albumin, glucose, blood, casts, crystals, WBC, RBC, bacteria, and epithelial cells.

Gross and histopathology. All rats which died or were killed (sodium pentobarbital, 35 mg/kg, ip) underwent a complete necropsy. The following tissues were weighed: lung, liver, kidneys, adrenals, spleen, testes, and brain. The ratios of organ to body weight and organ to brain weight were calculated for each animal. Complete gross examinations were performed on all animals, and a standard set of 34 tissues plus all gross lesions were fixed in 12% buffered formalin. Tissues from all animals in the study were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. A National Toxicology Program (NTP) quality assessment review of the histopathology and related technical procedures was performed by Clement Associates, Inc. (Washington, D.C.), and all tumor diagnoses were reviewed and confirmed by an NTP pathology working group.

Statistical evaluations. Group body weight comparisons were made using a multivariate analysis of covariance (MANCOVA). The body weights from each animal (until the death of the first animal of the groups being compared) served as multiple dependent variables, initial weight as the covariate, and exposure groups as the independent variable. If statistically significant results ($p < 0.05$) were obtained with the MANCOVA, comparisons of exposure groups at various time periods were assessed by the Duncan multiple range test.

Using the number of days survived on study, survival functions were computed and cumulative functions were plotted for each group. The actuarial method was used to compute the survival function (Berkson and Gage, 1950). The Lee–Desu statistic (Lee and Desu, 1972) was used for comparisons of survival functions.

For absolute organ weights, relative organ weights, hematology, and clinical chemistry parameters, a comparison of distributions of values was made for each variable using the Kruskal–Wallis test, followed by multiple comparisons if the initial test were significant at $p \leq 0.05$.

The incidences of mononuclear cell leukemia and peritoneal mesothelioma were analyzed for group differences by the Mantel–Haenszel Method. The method of Peto *et al.* (1980) was used to compare glioma incidences.

The incidences of other tumors or pathological lesions in the exposure groups were compared to the control groups by a χ^2 test. If a significant association was found between incidence and exposure, further analyses were completed to see if both exposure concentrations differed from the controls.

In all cases, except for RBC and WBC counts where differences in absolute numbers necessitated comparisons of the EO and PO groups to their individual uncombined control groups, each exposure group was compared to the combined rat control group.

RESULTS

Chamber Concentrations

The actual chamber concentrations were close to the targeted concentrations with little day-to-day variation. Chamber concentrations

for the EO exposure groups were 50.9 ± 2.2 ($\bar{X}$ ± SD) and 101.3 ± 3.5 ppm, respectively. Rats surviving the 2-year study were exposed to EO on 486 exposure days, with an average exposure duration of 6.9 hr/day. Chamber concentrations for the PO exposure groups were 101.4 ± 4.9 and 302.4 ± 7.8 ppm, respectively. PO rats were exposed an average of 6.9 hr/day for 488 days over the course of the 2-year study.

Animal Health Status

Approximately 8 months into the study some rats exhibited signs of murine pulmonary infections which did not appear to be related to EO or PO exposure. A decision was made to treat all rats in the study with oxytetracycline hydrochloride (Oxytet-10, Professional Veterinary Laboratories, Minneapolis, Minn.; Tetrachel-Vet, Rochelle Labs, Inc., Long Beach, Calif.). The tetracycline was added to the drinking water at a concentration of 500 mg/liter. Rats were treated for 3 weeks at the 8-month time period, for 2 weeks after 16 months of exposure when the infection appeared to become more severe, and for 3 weeks after 20 months of exposure. Inhalation exposures continued throughout the tetracycline treatment periods, except during the 16th month period when all rat exposures were suspended for 14 calendar days. *Mycoplasma pulmonis* was confirmed by serology during the 16th month disease outbreak.

Body Weights

Statistically significant differences in group mean body weights were seen in the EO-exposed rats (Fig. 1). By exposure Week 9 the EO 100 ppm group rats had significantly lower body weights than the controls, and these body weights remained lower for the remainder of the study. The EO 50 ppm group had significantly lower body weights than the control group by Week 14, and these body weights remained lower for the remainder of the study.

Group mean body weight differences were also seen in rats exposed to PO (Fig. 2). By Week 2 the PO 300 ppm group mean weight was significantly less than the control group, and this difference in body weight remained significant for the remainder of the study. After exposure Week 39, the group mean weight of the PO 100 ppm rats was significantly lower than the control group mean, and this difference continued for the remainder of the study.

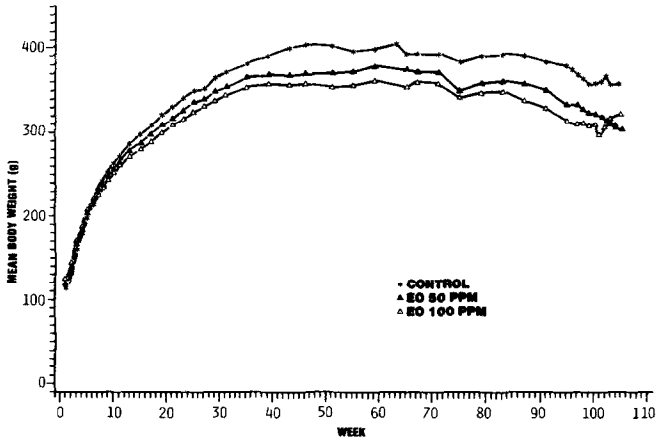

FIG. 1. Body weight curves of male F344 rats exposed to inhaled ethylene oxide (EO) for 2 years. Weight gain was statistically lower ($p < 0.05$) in the EO 100 ppm group (Week 9) and the EO 50 ppm group (Week 14) compared to the controls, and remained lower for the remainder of the study.

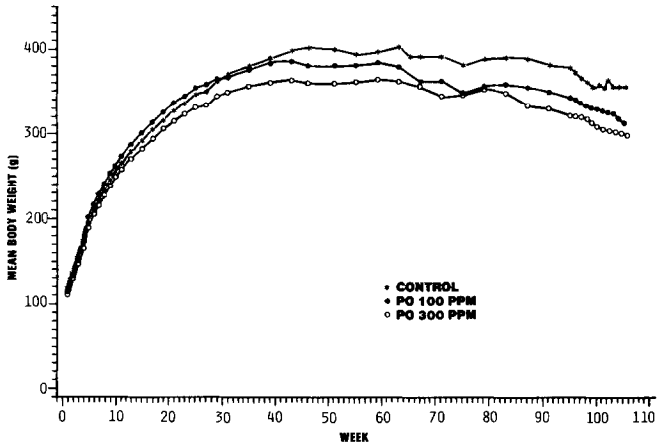

FIG. 2. Body weight curves of male F344 rats exposed to inhaled propylene oxide (PO) for 2 years. Weight gain was statistically lower ($p < 0.05$) in the PO 300 ppm group (Week 2) and the PO 100 ppm group (Week 30) compared to controls, and remained lower for the remainder of the study.

Survival Analysis

For the rats in the control group the first death occurred during Week 30; for the EO 50 ppm group Week 21; for the EO 100 ppm group Week 34; for the PO 100 ppm group Week 25; and for the PO 300 ppm group Week 67. Terminations of moribund rats were considered deaths, and the terminal kills as censored events. The median survival time was reduced for all exposed groups of rats compared to the control group (Figs. 3 and 4). The values for median survival time (days) on study were controls, 720; EO 50 ppm, 690; EO 100 ppm, 653; PO 100 ppm, 705; and PO 300 ppm, 675. The survival

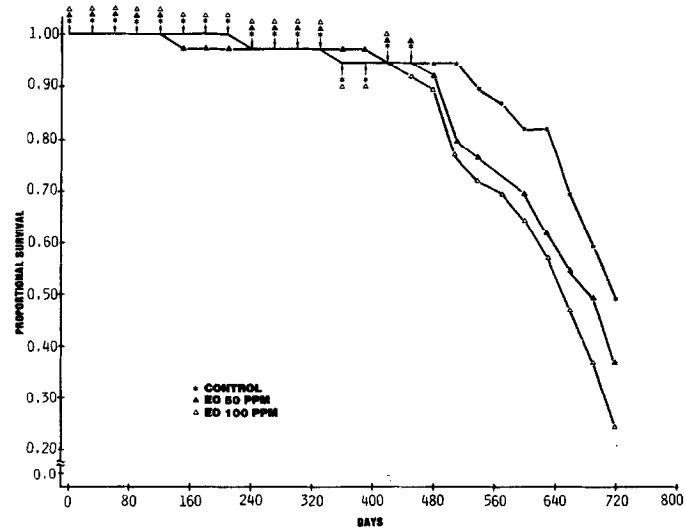

FIG. 3. Cumulative survival curves of male F344 rats exposed to inhaled ethylene oxide (EO) for 2 years. Survival of the EO 100 ppm group was significantly decreased compared to the control group ($p < 0.01$).

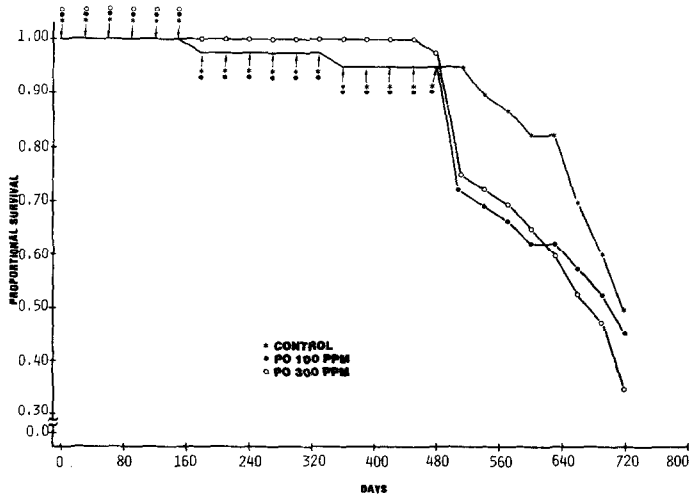

FIG. 4. Cumulative survival curves of male F344 rats exposed to inhaled propylene oxide (PO) for 2 years. Survival of the PO 300 ppm group was significantly decreased compared to the control group ($p < 0.01$).

curves for the EO 100 and PO 300 ppm rat groups (adjusted for multiple comparisons) were significantly different from the control group ($p \leq 0.01$). The results of these analyses must be interpreted with caution, as the survival rates reflect the toxicity of the epoxides as well as deaths due to the epizootic outbreak. Hence, the *M. pulmonis* infection alone and in combination with the epoxide exposures affected the survival of rats in this study.

Hematological Findings

No differences in the percentage of monocytes or eosinophils in differential counts were seen in any of the rat groups. Some significant differences in the percentage of neutrophils and lymphocytes were seen between control and exposed groups; however, these changes occurred in the absence of any statistically significant differences in total WBC counts, so these differences were not interpreted to be of toxicologic significance. For both groups of PO-exposed rats, hemoglobin concentrations were statistically elevated ($p < 0.025$) compared to the control group. In addition, for those animals for which the information was available, the WBC, RBC, hematocrit, and hemoglobin values for rats with and without mononuclear cell leukemia were compared within each exposure group. These results are discussed under Pathology.

Clinical Chemistry and Urinalysis Findings

These results reflect measurements taken on 15 rats/group at the termination of the study. A group difference was found for AST, with the values of the control group significantly lower ($p < 0.05$) than the EO 50 and EO 100 ppm groups. No other group differences were noted. Among the PO rats, the controls and the PO 300 ppm values for AST and SDH were significantly lower ($p < 0.05$) than the values of the PO 100 ppm rats. No other group differences were noted. No data analyses were performed on the rat urinalysis measurements; however, the frequency distributions appear uniform across groups.

Organ Weights

Organ weights and relative organ weights for each group of rats are presented in Tables

1 and 2. The results of analyses of the organ/brain weight ratios paralleled those of the organ/body weight ratios.

The increased lung and lung to body weight ratios probably reflect the *Mycoplasma* infection and the reduced body weights of the exposed animals. The kidney weight changes, seen only for the absolute weights, probably reflect smaller body weights of the exposed rats. The adrenal weight increases may be correlated with pathology of the adrenal gland (see next section). Testicular weight changes are not considered to be related to EO or PO treatment due to the extremely high incidence of interstitial cell tumors seen in aged F344 rats. The absolute brain weight reductions, although slight, were statistically significant, and may reflect the reduced body weights of the exposed rats. No consistent pattern in brain weight changes could be correlated with the observed pathology (see next section).

Gross and Histopathologic Findings

Rats exposed to EO. The incidence of pathological lesions and neoplasms in the various rat groups are presented in Tables 3 and 4, respectively. Rats exposed at 50 and 100 ppm EO had a higher incidence of inflammatory lesions of the lungs, nasal cavities, trachea, and internal ear. The lungs of these rats had an increased incidence of bronchiectasis, bronchial epithelial hyperplasia, and inflammatory changes consistent with the manifestations seen in chronic respiratory disease complex in rodents.

Rats exposed at 50 and 100 ppm EO had a high incidence of proliferative and degen-

TABLE 1

ABSOLUTE ORGAN WEIGHTS OF MALE RATS KILLED FOLLOWING 2-YEAR INHALATION EXPOSURES TO ETHYLENE OXIDE OR PROPYLENE OXIDE

Exposure group (ppm)	Organ					
	Lung	Liver	Kidneys	Adrenals	Testes	Brain
Control	2.97 ± 0.62[a] (2.20–5.42)[b] 2.85[c] (39)[d]	14.80 ± 4.22 (8.28–34.94) 14.85 (39)	3.59 ± 0.65 (2.25–5.26) 3.55 (39)	0.08 ± 0.02 (0.043–0.200) 0.08 (39)	5.03 ± 2.47 (1.39–12.54) 4.45 (39)	2.19 ± 0.12 (1.88–2.37) 2.20 (39)
EO50	4.23 ± 2.74 (1.91–14.48) 3.29 (27)	12.88 ± 2.98 (7.25–20.09) 12.64 (26)	3.14 ± 0.37* (2.15–3.87) 3.06 (27)	0.10 ± 0.02* (0.053–0.186) 0.10 (27)	5.51 ± 10.75 (0.68–58.65) 3.41 (27)	2.10 ± 0.11* (1.78–2.25) 2.12 (27)
EO100	3.18 ± 0.51 (1.91–4.19) 3.08 (15)	13.44 ± 2.26 (9.88–19.22) 13.28 (15)	3.14 ± 0.36* (2.57–3.84) 3.08 (15)	0.10 ± 0.02* (0.065–0.126) 0.09 (15)	3.89 ± 2.27 (0.97–9.56) 3.40 (15)	2.04 ± 0.13* (1.66–2.21) 2.03 (15)
PO100	4.48 ± 1.61* (2.39–8.61) 4.12 (33)	14.11 ± 3.08 (7.83–21.03) 14.38 (33)	3.20 ± 0.51* (2.08–4.18) 3.28 (33)	0.11 ± 0.11 (0.018–0.620) 0.08 (33)	3.17 ± 1.56* (0.88–6.37) 3.16 (33)	2.12 ± 0.11* (1.90–2.31) 2.10 (33)
PO300	3.41 ± 0.62* (2.54–5.33) 3.26 (27)	13.02 ± 2.63 (6.79–18.58) 12.88 (27)	3.03 ± 0.33* (2.15–3.53) 3.11 (27)	0.15 ± 0.37 (0.045–2.021) 0.08 (27)	3.27 ± 1.22* (1.27–5.75) 3.18 (27)	2.07 ± 0.11* (1.78–2.24) 2.08 (27)

[a] $\bar{x} \pm$ SD.
[b] Range.
[c] Median.
[d] Number of rats.
* Statistically significant difference versus controls, $p \leq 0.05$.

TABLE 2

ORGAN/BODY WEIGHT RATIOS OF MALE RATS KILLED FOLLOWING 2-YEAR INHALATION EXPOSURES TO ETHYLENE OXIDE OR PROPYLENE OXIDE[a]

Exposure group (ppm)	Organ					
	Lung	Liver	Kidneys	Adrenals	Testes	Brain
Control	8.96 ± 2.24[b] (6.35–17.89)[c] 8.44 (39)[d]	44.26 ± 11.92 (26.94–91.95) 43.24 (39)	10.79 ± 2.47 (7.73–20.96) 10.11 (39)	0.25 ± 0.08 (0.15–0.54) 0.23 (39)	15.17 ± 7.63 (4.67–36.14) 14.05 (39)	6.58 ± 0.78 (5.18–8.59) 6.34 (39)
EO50	15.31 ± 11.32* (5.52–57.69) 10.51 (27)	45.45 ± 13.12 (24.25–75.62) 43.70 (26)	10.98 ± 2.04* (7.19–15.05) 10.64 (27)	0.34 ± 0.12* (0.18–0.80) 0.31 (27)	17.82 ± 31.41 (2.43–173.0) 12.05 (27)	7.34 ± 0.95* (6.11–9.95) 6.94 (27)
EO100	10.50 ± 3.11 (6.10–18.53) 9.54 (15)	43.50 ± 8.41 (29.28–56.96) 43.27 (15)	10.15 ± 1.57 (6.78–12.90) 10.44 (15)	0.31 ± 0.06* (0.24–0.49) 0.30 (15)	12.40 ± 6.55 (2.97–24.77) 12.27 (15)	6.67 ± 1.30 (4.30–9.31) 7.12 (15)
PO100	15.59 ± 6.69* (7.31–33.63) 12.99 (33)	47.86 ± 10.94 (27.13–85.14) 46.84 (33)	10.82 ± 1.55 (6.93–13.17) 11.15 (33)	0.39 ± 0.43* (0.06–2.48) 0.31 (33)	10.54 ± 4.86* (3.56–21.59) 10.75 (33)	7.24 ± 1.01* (5.28–10.29) 6.69 (33)
PO300	11.99 ± 2.24* (8.51–18.83) 11.78 (27)	45.61 ± 8.98 (30.45–65.65) 41.57 (27)	10.64 ± 1.03 (8.92–13.42) 10.45 (27)	0.53 ± 1.25* (0.20–6.78) 0.27 (27)	11.42 ± 4.15* (5.48–20.18) 10.98 (27)	7.31 ± 0.67* (6.19–8.94) 7.22 (27)

[a] Organ weight/body weight × 1000.
[b] $\bar{x}$ ± SD.
[c] Range.
[d] Number of rats.
* Statistically significant difference versus controls, $p \leq 0.05$.

erative lesions of the adrenal cortex, consisting of vacuolation and hyperplasia or hypertrophy of the cells of the zona fascicularis. In some of these rats the areas of hyperplasia and hypertrophy formed distinct nodules which compressed the adjacent cortical tissue. The skeletal muscle myopathy observed in a higher incidence in the EO 100 ppm rats consisted of multifocal areas of atrophy and degeneration of skeletal muscle fibers. These changes were not accompanied by any change in the nerves which was detectable by light microscopy. The EO-exposed rats also had an increase in the number of rats with multifocal mineralization of the posterior layers of the choroid/sclera portion of the eye.

Other nonneoplastic alterations seen in exposed and control rats were typical of aged F344 rats. These changes included moderately severe kidney lesions and nonsupportive myocarditis and interstitial cardiac fibrosis.

When the data from all rats in the study were compared (interim deaths plus terminal kills), a statistically significant association of EO exposure with increased occurrence of mononuclear cell leukemia was found ($p = 0.03$) between the control and EO 50 ppm rats. The mononuclear cell leukemia was typical of the leukemia reported to occur in the F344 rat. The primary change was the accumulation of poorly differentiated mononuclear cells in the splenic red pulp sinuses, sinusoidal spaces of the liver, and the alveolar capillaries of the lungs. Hepatosplenomegaly was a common gross finding and focal to multifocal splenic fibrosis was usually observed. When comparing the control group with the EO 100 ppm group, a nonsignificant

TABLE 3

INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE F344 RATS EXPOSED TO INHALED ETHYLENE OXIDE OR PROPYLENE OXIDE FOR 2 YEARS

Organ	Exposure group (ppm)				
	Control	EO50	EO100	PO100	PO300
Adrenal glands					
Cortical nodular hyperplasia	0/78[a]	2/77	14/78*	0/78	0/80
Multifocal cortical vacuolation	0/78	25/77*	42/78*	0/78	0/80
Multifocal cortical hyperplasia	0/78	16/77*	36/78*	0/78	0/80
Brain					
Gliosis	0/76	2/77	4/79	0/78	0/78
Dilated ventricles	0/76	4/77	6/79	0/78	0/78
Focal hemorrhage	0/76	1/77	7/79	0/78	0/78
Eye					
Cataract, unilateral	2/77	3/79	9/78	1/79	1/80
Heart					
Myocarditis	42/78	23/78	29/80	34/80	37/80
Intestines					
Nematodiasis	12/78	17/79	17/78	8/79	13/80
Kidneys					
Chronic glomerulonephritis	71/77	63/78	56/77	76/79	77/79
Liver					
Bile duct hyperplasia	17/78	32/78	15/79	15/78	12/76
Pericholangitis	12/78	7/78	4/79	20/78	15/76
Lungs					
Acute bronchopneumonia	11/79	21/79*	30/80*	5/80	4/80
Chronic pneumonia, focal	6/79	28/79*	42/80*	66/80*	53/80*
Edema	0/79	14/79*	10/80*	6/80*	11/80*
Nasal Cavity					
Suppurative rhinitis	12/76	63/75*	62/75*	21/77*	44/78*
Complex epithelial hyperplasia	0/76	0/75	0/75	2/77	11/78*
Pancreas					
Atrophy	12/77	15/73	21/73	14/76	13/73
Prostate					
Prostatitis	33/78	9/79	4/78	35/79	43/79
Seminal vesicle					
Atrophy	14/78	16/74	16/76	11/77	9/80
Skeletal muscle					
Multifocal myopathy	7/77	10/78	43/70	1/78	25/78
Spleen					
Focal fibrosis	6/77	9/79	23/76*	11/78	12/78
Extramedullary hematopoiesis	34/77	53/79*	46/76*	3/76*	16/78*
Testes					
Atrophy	54/78	39/79	39/79	54/78	62/80
Trachea					
Tracheitis	2/79	16/79	13/78	63/80	62/80
Tracheobronchial lymph node					
Lymphoid hyperplasia	3/73	1/78	5/79	41/76	41/79

[a] All groups consisted of 80 male rats at the initiation of the study. Denominators less than 80 reflect tissues accidentally lost or tissues which could not be examined histologically due to autolysis.

* Statistically significant difference versus controls, $p < 0.05$.

TABLE 4

INCIDENCE OF NEOPLASTIC LESIONS IN MALE F344 RATS EXPOSED TO INHALED ETHYLENE OXIDE OR PROPYLENE OXIDE FOR 2 YEARS

Organ	Exposure group (ppm)				
	Control	EO50	EO100	PO100	PO300
Adrenal glands					
Cortical adenoma	3/78[a]	1/77	0/78	9/78	4/80
Pheochromocytoma	8/78	14/77	13/78	25/78*	22/80*
Brain					
Glioma (mixed cell)	0/76	2/77	5/79*	0/78	1/78
Astrocytoma	0/76	0/77	0/79	1/78	0/78
Body cavity					
Peritoneal mesotheliomas	3/78	9/79	21/79**	8/78	9/80
Bone (vertebral column)[b]					
Sarcoma	0/0	0/0	0/0	0/0	1/3
Osteosarcoma	0/0	0/0	0/0	0/0	1/3
Chondroma	0/0	0/0	0/0	0/0	1/3
Bone marrow					
Myeloid leukemia	0/79	0/77	1/76	0/78	0/79
Ear canal					
Osteosarcoma	1/76	0/56	0/56	0/57	0/55
Harderian gland					
Adenoma	1/77	0/76	0/70	0/74	0/78
Heart					
Neurimona	1/78	0/78	0/80	0/80	1/80
Intestines					
Sarcoma	0/78	0/79	1/78	0/79	0/80
Adenocarcinoma	0/78	0/79	0/78	0/79	2/80
Kidneys					
Hamartoma	0/77	1/78	0/77	0/79	0/79
Liver					
Hepatocellular carcinoma	0/78	0/78	0/79	0/78	1/76
Neoplastic nodule	2/78	3/78	2/79	1/78	1/76
Lungs					
Alveolar/bronchiolar adenoma	1/79	1/79	2/80	0/80	0/80
Alveolar/bronchiolar carcinoma	1/79	1/79	0/80	0/80	0/80
Adenocarcinoma-squamous metaplasia	1/79	1/79	0/80	0/80	0/80
Squamous cell carcinoma	0/79	1/79	0/80	0/80	0/80
Sarcoma, metastatic	2/79	0/79	1/80	1/80	1/80
Adenomatous polyps, bronchial	1/79	0/79	0/80	0/80	0/80
Nasal passages					
Osteosarcoma	0/76	0/75	0/75	0/77	1/78
Adenoma	0/76	0/75	0/75	0/77	2/78
Omentum[b]					
Lipoma	0/6	1/18	0/24	0/4	0/2
Fibrous histiocytoma	1/6	0/18	0/24	0/4	0/2
Palate[b]					
Squamous-cell papilloma	0/0	2/2	1/1	0/0	0/0

TABLE 4—*Continued*

Organ	Exposure group (ppm)				
	Control	EO50	EO100	PO100	PO300
Pancreas					
Islet cell adenoma	23/77	16/73	15/73	12/76	14/73
Islet cell carcinoma	2/77	1/73	0/73	0/76	0/73
Pituitary					
Carcinoma	4/73	0/66	0/67	3/70	5/72
Adenoma	44/73	20/66*	21/67*	29/70*	28/72*
Preputial gland					
Adenocarcinoma	1/69	0/74	0/73	0/72	0/73
Prostate					
Adenoma	5/78	0/79	0/78	1/79	1/79
Fibrosarcoma	1/78	0/79	0/78	0/79	0/79
Sarcoma	0/78	0/79	1/78	0/79	0/79
Skeletal muscle					
Reticulum cell sarcoma	0/77	0/78	1/70	0/78	0/75
Skin					
Squamous cell carcinoma	2/69	3/74	2/73	2/72	5/73
Keratoacanthoma	1/69	0/74	0/73	0/72	0/73
Basal cell carcinoma	0/69	1/74	0/73	0/72	0/73
Spleen					
Mononuclear cell leukemia	24/77	38/79*	30/76	18/78	19/78
Stomach					
Sarcoma	0/76	0/79	1/77	0/79	0/80
Subcutis					
Fibroma	1/69	3/74	6/73	1/72	1/73
Fibrous histiocytoma	1/69	1/74	0/73	0/72	0/73
Testes					
Interstitial cell tumor	58/78	50/79	42/79	50/78	54/80
Thorax[b]					
Carcinoma	0/0	1/2	0/0	0/0	0/0
Thymus					
Carcinoma	0/24	0/22	1/17	0/0	0/0
Thyroid glands					
C-Cell adenoma	5/77	7/79	3/77	0/79	0/80
C-Cell carcinoma	2/77	4/79	1/77	1/79	5/80
Follicular cell carcinoma	0/77	1/79	0/77	2/79	1/80
Parathyroid adenoma	0/77	1/79	1/77	1/79	1/80
Vertebra[b]					
Paravertebral carcinoma	0/0	1/1	0/0	0/0	0/3
Zymbal gland					
Squamous cell carcinoma	2/72	1/56	3/56	2/57	2/55
Sarcoma	1/72	0/56	0/56	0/57	0/55
Adenoma	1/72	0/56	1/56	0/57	0/55

[a] All groups consisted of 80 male rats at the initiation of the study. Denominators less than 80 reflect tissues accidentally lost or tissues which could not be examined histologically due to autolysis.

[b] Tissue not routinely examined in all rats.

* Statistically significant difference versus controls, $p < 0.05$.

** Statistically significant difference versus controls, $p < 0.01$.

association was found ($p = 0.15$). It should be noted that an excess mortality had occurred in the EO 100 ppm group (19% survival versus 49% of controls), and this mortality influenced the leukemia incidence of the high-dose rats. If the mononuclear cell leukemia incidence of only the terminal kill rats is compared for the EO 100 ppm group versus the controls, an exposure-related increased mononuclear cell leukemia association exists ($p < 0.01$). The WBC count was statistically increased in rats with leukemia in the EO 50 ppm group ($p = 0.02$), but not in the EO 100 ppm group ($p = 0.09$).

An association of EO exposure and increased incidence of peritoneal mesotheliomas was also found. Generally, the tumors were present on the tunica vaginalis surrounding the testes and epididymis and, on occasion, spread to the peritoneal surfaces of the organs within the peritoneal cavity. A significant association of exposure and increased occurrence was found for the EO 100 ppm group ($p = 0.002$) versus the controls, even in the presence of excess mortality. The EO 50 ppm group comparison did not indicate a significant association ($p = 0.13$). Using a test for trends (Armitage, 1971), there was not a departure from a linear trend, suggesting a proportional increase in mesotheliomas with increased exposures.

During histopathologic evaluation of brain tissue, several exposed rats were identified with mixed cell gliomas. None were found in the control group. The term glioma was used because the tumors contained mixed cell components with areas of both astrocyte and oligodendroglia cells within the same tumor. Two rats with gliomas were found in the 50 ppm EO group, one dying prior to termination. Five rats with gliomas were found in the 100 ppm EO group; four of these rats died prior to scheduled termination. Among the rats diagnosed with gliomas before Day 700 (one in the EO 50 and three in the EO 100 ppm group), neither mononuclear cell leukemia nor peritoneal mesotheliomas were found. Assuming that the gliomas occurred in a fatal context, trend analysis indicated a significant increase of gliomas with increased EO exposure. In addition to the gliomas observed, two additional rats exposed at 50 ppm EO and four additional rats exposed at 100 ppm EO had increased numbers of glial cells, termed gliosis. In these cases no evidence of inflammation nor neuronal degeneration or necrosis were found which would stimulate the glial response. Hence, these gliosis cases may represent incipient gliomas.

The incidences of other neoplasms, common in F344 rats, were generally comparable among the control and treated groups, and bore no apparent relationship to EO exposure. These neoplasms included pituitary adenomas, islet cell adenomas of the pancreas, pheochromocytomas of the adrenal gland, and interstitial cell tumors of the testes.

Rats exposed to PO. When compared to the control group of rats, rats exposed at 100 or 300 ppm PO had an increased incidence and greater degree of severity of inflammatory lesions of the lungs, nasal cavity, trachea, and middle ear—all characteristic of rodent chronic respiratory disease (See Table 3). In spite of the murine mycoplasmosis, a dose-dependent increase in the incidence of complex epithelial hyperplasia was detected in the nasal passages of the PO-exposed rats (2.6% in the PO 100 ppm group and 14.1% in the PO 300 ppm group). In addition, two rats exposed at 300 ppm PO had nasal cavity adenomas (Table 4). The proliferative lesions in the nasal mucosa appeared to be treatment related, but the degree to which their development was influenced by the intercurrent inflammatory disease cannot be ascertained. In addition, the incidence of adrenal pheochromocytomas was statistically elevated ($p < 0.05$) in the PO exposure groups, but not in a dose-related manner.

Skeletal myopathy was observed primarily in the PO 300 ppm rats, and consisted of multifocal areas of atrophy and degeneration. No lesions were noted in the sciatic nerves by light microscopy. Cardiomyopathy and

chronic glomerulonephritis, characteristic of aged F344 rats, were noted across all groups including the controls.

Mononuclear cell leukemia and peritoneal mesotheliomas similar to those described previously with EO were found in all treatment groups including the controls. The incidence of mesotheliomas was increased in the PO exposed rat groups, but not in a dose-related manner. Other neoplasms with comparable incidences in all rat groups included interstitial cell tumors of the testes, islet cell adenomas of the pancreas, and adenomas of the pituitary.

DISCUSSION

Exposures of F344 rats at 50 or 100 ppm EO for 2 years resulted in statistically significant declines in body weight gain and increased mortalities at both exposure concentrations. Statistically significant associations of tumor incidence and EO exposure were found for several rat neoplasms. The outbreak of the *M. pulmonis* clearly impacted upon the study. Rat survival was affected, especially among the exposed rats, and the incidences of typical murine pneumonia lesions in the lungs, middle ear, and nasal passages were increased. Nevertheless, we do not feel that the epizootic outbreak negates the major findings of the study as they have been verified by independent inhalation bioassays (Snellings *et al.*, 1981, for EO; NTP, 1983, for PO).

A statistically significant association was seen in the incidence of mononuclear cell leukemias, peritoneal mesotheliomas, and brain gliomas with increasing EO concentration. Mononuclear cell leukemia is common in aged F344 rats, with Goodman *et al.* (1979) reporting the background incidence from pooled male control F344 rats (100 weeks) to be 12.3%. The background incidence of peritoneal mesotheliomas is reported to be 2.3% by the same authors. The finding of increased mononuclear cell leukemia is in agreement with the findings of a statistically significant increase in mononuclear cell leukemia in female rats exposed at 100 ppm EO for 2 years in an industry-sponsored EO inhalation bioassay (Snellings *et al.*, 1981). A statistically significant increase in the incidence of peritoneal mesotheliomas in male rats exposed at 100 ppm EO was also reported by these investigators.

Brain gliomas, unlike the preceding neoplasms, are not common in aged F344 rats. Goodman *et al.* (1979) reported a glioma incidence of 0.22% in male F344 rats. This finding of a dose-dependent increase in glioma incidence has been verified following a reevaluation of all brain tissue slides in the industry-sponsored EO study (William Snellings, personal communication).

Mononuclear cell leukemia is rat related, and is believed to originate in the rat spleen (Moloney *et al.*, 1970, Moloney and King, 1973). It has also been reported in Wistar (Zawidska and Grice, 1971) and Sprague–Dawley rats (Abbott *et al.*, 1983). The disease is not identical to any human leukemia, although Ward and Reynolds (1983) have recently reported that the heterogeneity of mononuclear cell leukemia (or, as it is more recently termed, large granular lymphocyte leukemia) is similar to that seen in human non-Hodgkin's lymphomas (Borowitz *et al.*, 1981). An association between EO exposure and increased leukemia incidence in humans has been suggested (Ehrenberg and Hallstrom, 1967; Hogstedt *et al.*, 1979*a,b*). However, these studies were limited by small sample sizes, lack of complete exposure data, and some exposures to multiple chemicals. NIOSH (1981) concluded that these investigations suggest that an excess risk of cancer may exist for the EO workers studied. It should also be noted that Morgan *et al.* (1981) reported that 2 of 46 deceased workers who had formerly worked in an EO chemical plant developed brain/CNS cancer (not classified further by these authors).

Among rats exposed to PO at 100 or 300 ppm for 2 years, statistically significant declines in body weight gain and increased

mortality were observed at both concentrations. Increased mortality was also reported in rats exposed at 100 or 300 ppm PO in an industry-sponsored 2-year bioassay (DOW, 1982). Survival of F344 rats exposed at 200 or 400 ppm in the NTP bioassay (NTP, 1983) was not affected by PO exposure, although body weight and survival of B6C3F1 mice were affected by PO exposure in the same study. The finding of increased proliferative nasal lesions in rats exposed to PO at 100 or 300 ppm is in agreement with findings of the NTP bioassay. In the latter study, increases in the incidences of inflammation and squamous metaplasia were seen in the nasal passages of both species. Papillary adenomas (2 rats) and adenocarcinomas (2 mice), hemangiomas (8 mice), and hemangiosarcomas (7 mice) were found in the nasal turbinates of the animals exposed at 400 ppm PO.

In spite of body weight depressions and increased mortality in all four EO and PO rat exposure groups, a significant increased tumorigenic response (with the exception of the adrenal pheochromocytomas, which were not increased with increasing PO exposure) was seen only in the EO rats. This finding is in agreement with published mutagenicity data on EO and PO (Glaser, 1979; Wolman, 1979; Bootman *et al.*, 1979; Hardin *et al.*, 1983) which indicate that EO is a more potent mutagen than PO. The minimal tumorigenic response in rats exposed at 100 or 300 ppm PO by inhalation is in variance with published skin painting (Walpole, 1958), sc injection (Dunkelberg, 1979), and gavage studies (Dunkelberg, 1982). However, the finding of two adenomas in the nasal passages of rats exposed at 300 ppm is in agreement with the site of application increases in skin and forestomach tumors reported following PO administration by these authors. These results may be due to a local, repeated, irritant effect. The minimal tumorigenic response at 100 or 300 ppm PO by inhalation may be due to selection of an exposure concentration which was too low (300 versus 400 ppm in the NTP study) or to differences in the bioavailability of inhaled PO compared to direct application to skin or stomach.

In summary, a no-observed-effect level was not found for either EO or PO in this study. The finding of toxicity at both 50 ppm EO and 100 ppm PO, the current federal permissible exposure level (PEL) concentrations, indicate that both of these standards need to be reevaluated. OSHA (1983) is currently reevaluating the EO standard, and has recommended lowering the PEL for EO to 1 ppm. The ACGIH (1980) has recently lowered their recommended threshold limit values for EO to 1 ppm and for PO to 20 ppm. Data from the current study would support these actions, and point out the need for additional epidemiologic evaluations of workers occupationally exposed to EO and PO.

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