NEUROPHYSIOLOGICAL AND NEUROPATHOLOGICAL EVALUATION OF PRIMATES EXPOSED TO ETHYLENE OXIDE AND PROPYLENE OXIDE

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Over 500 000 workers in the United States are exposed to ethylene oxide and propylene oxide. These two solvents are used as chemical intermediates, as well as components in the manufacture of fumigants and food preparation. The neurophysiologic and neuropathologic effects of these two organic oxides were investigated in five groups of 12 primates after exposure to 50 or 100 ppm ethylene oxide, 100 or 300 ppm propylene oxide, or no chemical (sham-exposed). Animals were exposed for 7 h/day, 5 days/wk for 24 months. Body weights, electroencephalograms, and motor nerve conduction velocities of the sciatic and ulnar nerves were assessed six times throughout the exposure period. Although the monkeys exposed to 100 ppm ethylene oxide had significantly lower mean weights, nerve conduction velocities did not differ significantly among the groups. Following termination of exposures, ten animals (two from each exposure group) were

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2. Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; ANOVA, analysis of Variance; EEG, electroencephalogram; EtO, ethylene oxide; IARC, International Agency for Research on Cancer; MANCOVA, multivariate analysis of covariance; MAP, muscle action potential; MCV, maximum motor nerve conduction velocity; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; PO, propylene oxide; RMS, root mean square power; TWA, time-weighted average.

3. Key words: axonal dystrophy, demyelination, EtO, neurotoxicity, nonhuman primates, PO.

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sacrificed for neuropathological examinations. Multiple axonal bodies were found in the nucleus gracilis in seven of eight oxide-exposed animals, and demyelination was found in two monkeys exposed to ethylene oxide. In contrast, a single axonal body was found in one of the two sham-control monkeys. However, the lack of a dose-response relationship suggests that this effect may not be related to oxide exposure. In a follow-up study, nerve conduction velocity and neuropathology were assessed in the remaining monkeys seven years after exposure terminated, but again, treatment-related effects could not be detected.

INTRODUCTION

Ethylene oxide (EtO) and propylene oxide (PO) are two widely used industrial chemicals. It is estimated that the worker populations exposed to EtO and PO number about 270 000 and 421 000, respectively, in assorted chemical, manufacturing, food processing, and hospital occupations (National Institute for Occupational safety and Health [NIOSH], 1983). EtO is used primarily as a chemical intermediate in the manufacture of ethylene glycol, glycol ethers, ethanolamine, and polyethylene glycol (Hine et al., 1981). EtO also is used in fumigation and in the sterilization of food products and medical equipment. PO is used principally as an intermediate in the manufacture of propyle and polypropylene glycol, which subsequently are used to produce polyester resin. PO also is used as a solvent in pharmaceutical and food formulation and as a humectant. EtO and PO are relatively stable in liquid form, but the vapors are highly flammable; EtO is subject to explosive decomposition at 3 to 100% concentration.

The U.S. Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for EtO was 50 ppm at the start of this study in 1979 (OSHA, 1971). However, in 1984 OSHA lowered the PEL, an 8-hour time-weighted average (TWA), to 1 ppm, with a short-term limit (15 minutes) TWA of 5 ppm (29 CFR 1910.1047) (OSHA, 1984). In 1983, NIOSH recommended an exposure limit of 0.1 ppm for EtO (cited in NIOSH, 1989). The reduction in the TWA was based on reports that EtO was mutagenic and carcinogenic in animals and potentially a human carcinogen. Based on repeated references in the literature to the neurotoxicity of EtO across species, Beliles and Parker (1987) used brain tumor incidence in two separate rodent inhalation studies to estimate the risk based on this end point. PO has an OSHA PEL of 20 ppm (29 CFR 1910.1000), which was lowered from 100 ppm in 1989 (OSHA, 1989). The American Conference of Governmental Industrial Hygienists (ACGIH) 8-hour Threshold Limit Values are 1 ppm and 20 ppm for EtO and PO, respectively (ACGIH, 1996); this group has designated EtO as an A2 substance (suspected human carcinogen).

Both oxides are considered irritants and depressants of the central nervous system (Hine et al., 1981), and both have been reported to have carcinogenic potential, including leukemia (Hogstedt et al., 1979; NIOSH, 1981; Theiss et al., 1981). Also, the International Agency for Research on Cancer (IARC) has concluded that EtO is a human carcinogen (IARC, 1994).

Gross et al. (1979) published the first report linking peripheral neuropathy in humans to high EtO exposure. Four sterilizer operators who were exposed to EtO concentrations >700 ppm for periods ranging from two weeks to two months developed neurologic disorders, including sensory-

motor peripheral neuropathy. Slowed EEGs, convulsions, clumsiness, unstable gait, ataxia, reduced nerve conduction velocity, weakness, reduced sensitivity in fingers and toes, and reduced reflexes were among the neurological signs found in these workers. Improvement was marked after two weeks with no EtO exposure, but two workers continued to show decreased conduction velocity. Problems with EtO neurotoxicity persist. Brashear et al. (1996) recently reported evidence of peripheral and central nervous system toxicity in operating room nurses and technicians who wore surgical gowns sterilized with EtO. Airborne EtO concentrations were not reported, but the EtO level in the gowns 18 days poststerilization was 298 ppm.

Neurotoxicity has not been detected at low exposure concentrations. In a study by Joyner (1964), the medical records of 37 chemical operators, reportedly exposed to 5 to 10 ppm of EtO for an average of 10.7 years, were compared to unexposed plant workers. This comparison produced no evidence of neurotoxic effects. In fact, the exposed group had fewer health problems than the comparison group.

Few experimental studies have examined the neurotoxic effects of these organic oxides. Hollingsworth et al. (1956) exposed rats, guinea pigs, and rabbits of each sex, as well as female mice and one male and one female monkey to various concentrations of EtO for a period of from ten days to about seven months in a range-finding study. At about 200 ppm and higher, peripheral sensory and motor neuropathy was noted in rats, rabbits, and monkeys, but at about 100 ppm and below, signs of neurotoxicity were not seen; no specific neurobehavioral tests were conducted. Jacobson et al. (1956) found similar effects of EtO in rats, mice, and dogs. At 100 ppm or below, no signs of neurotoxicity were evident, but EtO at 292 ppm and above was associated with hindlimb weakness, muscle atrophy, tremors, and mortality. Rowe et al. (1956) exposed two groups of rats, guinea pigs, rabbits, and two female monkeys to 102 or 195 ppm PO for approximately six months, but observed no paralysis or outward signs of peripheral neuropathy. These early animal and human studies have been reviewed and summarized by O'Donoghue (1985).

More recent studies have shown that higher exposure concentrations of both EtO and PO can produce central and peripheral sensory axonopathy. Chronic exposure (5–20 years) to EtO was correlated with impairment on selected neurobehavioral tasks and with slowed sural maximum motor nerve conduction velocity (MCV) in eight hospital workers (Estrin et al., 1987). Although the average exposure concentration was not calculated, peak breathing-zone exposures of about 200 ppm were recorded over a 6-year period. A series of studies by Ohnishi et al. (1985, 1986, 1988) found that rats exposed to EtO at 250 or 500 ppm for 13 to 36 weeks, or to PO at 1500 ppm for seven weeks developed central-peripheral distal axonal degeneration of the primary sensory neurons. These sensory changes occurred in the absence of significant muscle atrophy.

The present study of oxide neurotoxicity was one component of a more comprehensive evaluation of the chronic inhalation toxicity of EtO and PO (Lynch et al., 1984a, 1984b). In contrast to previous studies, concentrations and exposure conditions more likely to be found in the workplace and a larger group of nonhuman primates were employed.

METHODS

Sixty adult male *Macaca fascicularis*, obtained from the wild and therefore of unknown age, were assigned randomly to five treatment groups consisting of 12 cynomolgus monkeys each. At the onset of the study, the mean weights for the various exposure groups were: 5.26 kg (SD = 0.57) for the control group; 5.39 kg (SD = 0.45) for 50 ppm EtO; 5.21 kg (SD = 0.74) for 100 ppm EtO; 5.47 kg (SD = 1.04) for 100 ppm PO; and 5.39 kg (SD = 1.19) for 300 ppm PO. The mean weight differences were not statistically significant. During the 24-month exposure period, body weights were recorded prior to each testing session. When not being exposed or tested, the animals were housed in stainless steel primate cages (77.5 cm x 60 cm x 60 cm) and provided with Purina monkey chow (Ralston Purina Company, St. Louis, Missouri) once a day and water *ad libitum*.

EtO, 99.7% pure was obtained from Union Carbide Corporation, Danbury, Connecticut in cylinders under its own vapor pressure. PO, 98% pure, was obtained 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 >99% for both epoxides at each analysis period.

Study Phases

The study was conducted in two phases. Phase 1 of the project examined the neurotoxic effects of EtO and PO at two exposure levels: the pre-1989 OSHA PEL and a concentration that approximated the maximum tolerated dose in monkeys. Specifically, the monkeys were exposed 7 h/day, 5 days/wk for 24 months to 50 and 100 ppm EtO, and 100 and 300 ppm PO; sham-exposed monkeys served as the control group. Neuropathological examinations were performed on a subset of ten of these animals at the end of the exposure period.

Because of published reports suggesting that EtO exposures in the concentrations used in this study may lead to the development of leukemia (NIOSH, 1981), a second phase was added to the study. The remaining animals from phase 1 that had not been sacrificed for neuropathological examination were retained an additional seven years for observation, with no further exposure, to explore possible leukemic effects. Body weight, MCV, and neuropathology were evaluated in ten of these animals at the end of the seven-year retention period.

Procedures

In phase 1, body weight, electroencephalographic (EEG), and MCV measurements were made prior to initiation of EtO, PO, or sham exposures and served as a baseline against which to evaluate oxide-induced changes. During the 24-month-exposure period, these functions were tested five times at variable intervals, the first occurring three months after exposures began. A sixth test occurred within two days after the termination of exposure. Fifteen minutes prior to each test session, primates were administered a 10 mg/kg mixture (Banknieder et al., 1978) of ketamine (Ketalar, Parke Davis Company Incorporated, Morris Plains, New Jersey) and xylazine (Rompun, Haver-Lockhart Laboratories, Denver, Colorado) intramuscularly. Sedated animals

were then transferred from the animal quarters to the neurobehavioral testing laboratory in a portable carrying cage (Harford Systems, Incorporated, Aberdeen, Maryland), where they were weighed and MCV testing was conducted.

Neuropathological examinations were conducted in ten animals, two from each exposure group, at the end of the 24-month-exposure period (phase 1) and at the termination of the study (phase 2).

Inhalation Exposures

Monkeys were exposed in groups of 12, using five 3.5 m^3 stainless steel and glass chambers (Charles Spangler, Incorporated, Cincinnati, Ohio). The chamber temperature and relative humidity were maintained at 75°F ± 3° and 50% ± 10%, respectively. The oxide concentration of each chamber was monitored on-line every 30 minutes with a Miran 1A Infrared gas analyzer (Foxboro Analytical, S. Norwalk, Connecticut). The infrared analyzer was calibrated with certified gas standards obtained from Liquid Carbonic Corporation, Chicago, Illinois. The target exposure concentrations were 50 and 100 ppm for EtO, and 100 and 300 ppm for PO. A group of 12 monkeys, exposed only to room air in the chambers, served as sham-exposed controls for the oxide groups. Charcoal tube samples of the test atmospheres analyzed by gas chromatography verified that the chamber concentrations were within 10% of target values and that the control chamber atmosphere was free of EtO and PO.

Body Weight Measurement

Body weight measurements were made weekly for the first two months and monthly thereafter for the remainder of the study. Analyses of variance (ANOVA) were used to compare baseline weights across groups. For evaluating weight changes during the exposure period, initial weight was treated as a covariate and exposure groups as the independent variable in multivariate analyses of covariance (MANCOVA). Significant group differences were further analyzed at specific time periods using Duncan's multiple range test.

MCV Testing

MCV testing was conducted using a procedure similar to that described by Johnson (1980) and Johnson et al. (1977). The right sciatic nerve was stimulated from two locations, S-1 and S-2. The cathode electrode (stainless steel needle) was inserted through the skin near the sciatic nerve and popliteus muscle for S-1 stimulation and near the extensor digitorum longus for S-2 stimulation. For ulnar stimulation, the cathode electrode was placed near the right extensor carpi radialis longus muscles proximal to the elbow and the ulnar notch. The anode in each case was a stainless steel needle electrode inserted subdermally into the tail (within 2 to 3 cm of the base) for sciatic stimulation and subdermally in the right shoulder area for ulnar stimulation.

The muscle action potential (MAP) from nerve stimulation was obtained by inserting the active cathode needle electrode into the extensor digitorum brevis muscle; the indifferent electrode was placed approximately 1 cm proximal to the active electrode. The ground electrode was inserted subdermally between the stimulating and recording electrodes to minimize the stimulus artifact for MCV measurements.

Ulnar MCV was measured by inserting the recording electrode into the flexor digitorum brevis muscle; the indifferent electrode was placed 1 cm proximal to this muscle, with the ground electrode inserted between the stimulating and recording electrodes. The MAP displayed from supramaximal stimulation of the sciatic or ulnar nerves was captured from the oscilloscope by a single sweep of the time-base trigger.

Since MCV decreases at lower body temperatures, nerve conduction data were adjusted using a temperature correction equation (deJesus, 1973). Using a telethermometer (model 43TA ,Yellow Springs Instrument Company, Incorporated, Yellow Springs, Ohio), surface limb temperatures were recorded prior to measurement of the MCV by placing a surface disc probe on the skin at a point between the point of stimulation and the recording electrode. Temperatures were required to be within 1°C of normal (35.0°C for the sciatic nerve, and 36.0°C for the ulnar nerve) for MCV to be recorded. Animals possessing skin temperatures below the described range were wrapped in a blanket until skin temperatures of 35°C were regained before MCV testing was started.

The same procedure was used to evaluate MCV in ten monkeys at the end of phase 2 of the study, seven years after termination of exposure to the oxides.

MANCOVA was used in analyzing the MCV data. For each variable, measurements taken at each test session were used as the dependent variable. The session 1 measurement (baseline) was used as a covariate. The EtO treatment groups and PO treatment groups were then compared with the control group. The independent variable was the exposure group: the EtO 50 and 100 ppm groups, the PO 100 and 300 ppm groups, and the control group. If an overall group difference was found, univariate ANOVA was conducted to ascertain group and session differences. These statistical procedures were used for data from both phases 1 and 2.

Electroencephalograms

EEG recordings were obtained using a polygraph with notch filters (model R-611, Beckman Instruments, Incorporated, Fullerton, California). Subdermal needle electrodes (Grass type E2B, Grass Medical Instruments, Quincy, Massachusetts) were positioned about 1 cm from the midline suture at four locations: (1) over the left cranial hemisphere; (2) over the central and occipital sutures; (3) at the vertex; and (4) 1 cm in front of the central suture (with each position referenced to the left earlobe). The ground needle electrode was inserted at the bridge of the nose. Seven minutes of EEG activity from each animal were recorded on magnetic tape at 1 7/8 in/s using a recorder (model 5600C, Honeywell, Incorporated, Minneapolis, Minnesota). EEG activity was evaluated by spectral density analysis. A modified PDP-12 (Digital Equipment Corporation, Maynard, Massachusetts) computer program was used to sample three 32-second epochs of EEG activity and to compute spectral density (power spectra) for each epoch. The three epochs of EEG activity were recorded at the beginning, middle, and end of each seven minute EEG sampling period. The mean (N=3) spectral estimate was evaluated in terms of the percentage of electrical energy in the four common EEG bands, (i.e., delta [0.25–4Hz], theta [4–8Hz], alpha [8–14Hz], and beta [14–32Hz]). The total root mean square (RMS) power, calculated from the mean spectrogram for each EEG lead, was used to characterize overall EEG amplitude.

Neuropathology (Phase 1)

In the week following the termination of the exposures, two animals from each of the five groups were sacrificed; the remaining animals were maintained for phase 2. The animals were selected for sacrifice on the basis of MCV results, one animal with a slower velocity (e.g., 31.9 m/s) and one animal with a moderate velocity (e.g., 81.5 m/s). The animals were first deeply tranquilized with the ketamine/xylazine mixture and then anesthetized with sodium pentobarbital (Nembutal Sodium, Abbott Laboratories, North Chicago, Illinois) about 20 minutes before necropsy. Animals were perfused with normal saline (gravity feed) and then with a fixing solution consisting of 160 g of sodium acetate dissolved in 1.76 l of distilled water, to which 2.4 mL of 12% formalin had been added. This perfusate inadvertently contained 220 mmol of sodium per kilogram water (by flame photometry) at a pH of 6.9. The brain, spinal cord, and sciatic and ulnar nerves then were removed and placed in the fixative.

A special brain macrotome was constructed according to the recommendations of Shaltenbrand and Bailey (1969) to assure reproducible serial sectioning. The brainstem was divided by a single transverse section just anterior to the oculomotor roots and the anterior margins of the anterior colliculi, thus separating the cerebrum from the mid- and hindbrain. The platform of the macrotome was elevated to an angle of approximately 35° so that the plane of the section would correspond to the coordinates of the stereotaxic atlas of Shanta et al. (1968). The brains were cut serially at intervals of about 4 mm in the coronal plane.

Tissues were embedded in paraffin, cut, and stained routinely with Gomori Trichrome and Weil-Weigert. Sections of brain frequently had to be reoriented and recut to obtain the desired section plane. Bielschowsky's Silver and PAS stains were used to confirm the presence of axonal bodies in selected sections.

The following blocks were obtained for each animals (1) motor and sensory cortex; (2) bulbus olfactories (frontal and caudal to the central sulcus); (3) olfactory tubercle, overlying basal ganglia, rostral to anterior commissure; (4) central operculum and insular cortex; (5) visual cortex, to include the area striata, Gennari's line, and the calcarine fissure; (6) basal ganglia at the level of the anterior commissure; (7) section caudal to the anterior commissure, to include the optic tract and portions of the paraventricular and supraoptic nuclei; (8) diencephalon at the level of the mammillary bodies, including substantia nigra, red nucleus, and third ventricle; (9) the nucleus of the lateral and medial geniculate bodies and the hippocampal formation; (10) diencephalon and basal ganglia, to include the dorsal, ventral, and medial thalamus; (11) pretegmental region; (12) midbrain, at the level of the superior colliculus; (13) midbrain, at the level of the inferior colliculus; (14) upper pons, isthmus, with the medial longitudinal fasciculus and reticular formation; (15) lower pons, cranial nerves VI and VII, and inferior olivary nucleus; (16) lower pons, at the same level as (15), consisting of a cross section through the cerebellar hemisphere bordering the fourth ventricle, to include the cortex and cerebellar nuclei (dentate); (17) medulla oblongata, upper level; (18) medulla oblongata, at the obex; (19) medulla oblongata, lower level; and (20) cerebellum at the vermis.

Sections also were taken from the cranial and caudal aspects of the spinal cord and divided into cervical, thoracic, lumbar, and sacral portions. The ulnar and sciatic nerves were each divided into four segments representing: (1) the most proximal part of each nerve; (2) a more peripheral portion of the proximal part of the nerve; (3) a more proximal portion of the distal part of the nerve; and (4) the peripheral portion of the distal end of the nerve.

Neuropathology (Phase 2)

In phase 2, ten monkeys, two from each exposure group and the control group, were randomly selected, sacrificed, and sections of brain, spinal cord, ganglia, muscle, and peripheral nerves were embedded in resin (glycol methacrylate). One micrometer sections were stained with hematoxylin and Lee stain as a general tissue stain, modified Luxol Fast Blue for myelin, and Bielschowsky's stain for axons and neurofilaments. Gomori's trichrome stain was used on the skeletal muscle and peripheral nerve sections. Larger portions of brain and medulla oblongata were embedded in paraffin and stained with hematoxylin and eosin stain or LFB stain. The remaining procedures were the same as those outlined above for phase 1.

RESULTS

Body Weight

Following the initiation of exposures, the mean weights of all five groups declined below baseline. With the exception of the EtO 100 group, mean weights recovered to baseline levels within 5–13 weeks. Results in the 50 ppm EtO group and both PO groups did not differ statistically from those found in the control group, with each group showing a 12.0% increase in mean body weight over the two years of exposure. The 100 ppm EtO group, however, did not recover but remained at about 95% of baseline weight throughout the exposure period. By week 25, the EtO group weight was significantly lower than controls (MANCOVA, $p < 0.05$) and remained lower throughout the phase 1 (Lynch et al., 1984c). This decreased mean weight relative to controls and PO-exposed animals continued in the animals that were retained during the subsequent 7-year-observation period (phase 2).

MCV

Figure 1 presents MCV data for sciatic-tibial nerves in the EtO, PO, and control groups over the 24-month-exposure period. There was no significant difference between oxide exposure groups and controls. For two animals in the 100 ppm EtO group, however, a large reduction in sciatic MCV was found after 12 months of exposure. These two animals had normal MCVs of 59.2 and 73.7 m/s at the onset of the study, but these MCVs declined below 40 m/s by the end of 12 months. These MCVs remained stable at this level for the remaining 12 months of exposure. The multivariate analyses of MCVs in EtO–exposed animals were: sciatic-tibial nerve, $F(12, 48) = 1.18$, $p = 0.32$; and ulnar nerve, $F(12, 48) = 0.47$, $p = 0.92$. For the PO–exposed animals, the multivariate analyses were: sciatic-tibial nerve, $F(12, 46) = 1.07$, $p = 0.40$; and ulnar nerve, $F(12, 46) = 1.56$, $p = 0.14$. MCV data obtained from ten monkeys in phase 2 (at the end of study year #7), were not statistically significant for either the sciatic nerve or the ulnar nerve ($p = 0.19$).

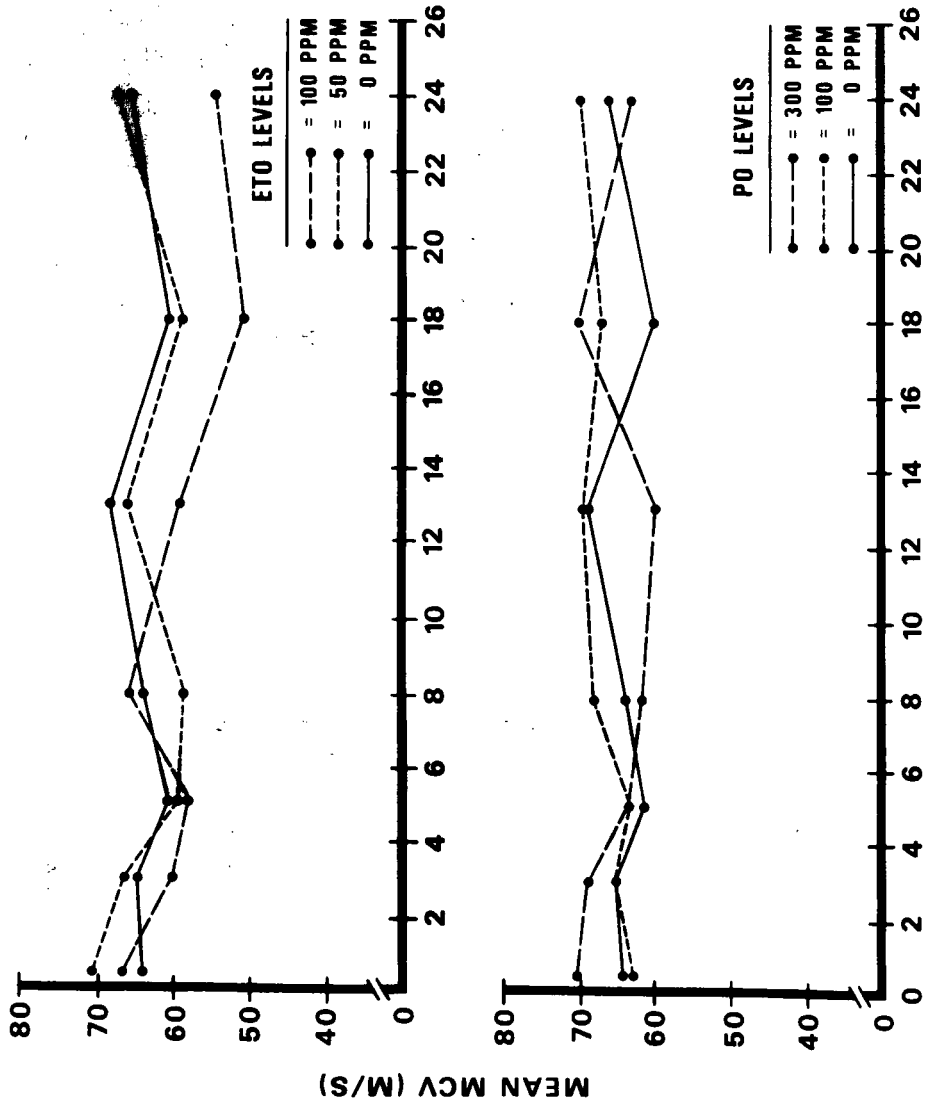

FIGURE 1. Effects of EtO (upper panel) and PO (lower panel) on the MCV of the sciatic-tibial nerve of monkeys during phase 1.

Peak-to-peak amplitude in response to nerve stimulation was also unaffected by the exposures and did not differ among exposure conditions in phases 1 and 2 of the study. The multivariate analysis for EtO vs control comparisons were $F(12, 48) = 1.41$, $p = 0.19$ for the sciatic nerve, and $F(12, 48) = 1.59$, $p = 0.13$ for the ulnar nerve. Multivariate PO vs control comparisons were $F(12, 44) = 0.40$, $p = 0.96$ for the sciatic nerve and $F(12, 44) = 0.50$, $p = 0.90$ for the ulnar nerve. Multivariate analyses for the animals retained in phase 2 also were not significant, with EtO vs control comparisons failing to reach significance for the sciatic nerve ($p = 0.23$) and the ulnar nerve ($p = 0.27$), and PO vs control comparisons also being nonsignificant for the sciatic nerve ($p = 0.84$) and the ulnar nerve ($p = 0.45$). No univariate analyses were performed for individual sessions because the multivariate analyses were not significant.

Electroencephalograms

Figure 2 presents lead 2–vertex EEG results from phase 1 of the study (EEGs were not measured in phase 2). EtO and PO exposure resulted in no detectable between-group effects on EEG; however, RMS power declined in all groups over the first 12 months of exposure. The percentages of delta, theta, alpha and beta EEG activities did not vary significantly from session to session or between groups as assessed by a multivariate analysis. A similar lack of effect was found for RMS power. An analysis of occipital lead EEGs also found no group differences.

Neuropathology (Phase 1)

As the total ionic strength of the fixative was twice that of the intracellular sodium ion concentration, the perfusate was hypertonic, resulting in perfusion fixation artifacts. The artifacts mimicked demyelination in peripheral nerve sections and therefore precluded interpretation of that tissue. However, spinal cord and brain tissues were not compromised. Evaluations of spinal cord, cerebral white matter, cortex, nuclei, and tracts were conducted using a single-blind procedure by neuropathologists at the Midwest Research Institute, Kansas City, Missouri. Lesions of indicating axonal dystrophy, as defined by Pentschew and Schwartz (1962) and Cowen and Olmstead (1963), were present in the area of the medulla oblongata, restricted to the nucleus gracilis of the oxide-exposed animals, but not the controls. Figure 3 depicts representative axonal swellings found in these evaluations. Axonal bodies in the nucleus gracilis were counted on each side. Severity of the lesion was graded according to Sung (1964), based on the work of Fujisawa (1967) as follows: (1) negative/trace (0–1 body in one nuclear area only); (2) slight (1–5 bodies in each nuclear area); (3) moderate (6–10 bodies), or (4) severe (≥11 bodies in each nuclear area). Using this grading scheme, results for each of the two animals for the various exposure and control conditions were: 50 ppm EtO, slight, severe; 100 ppm EtO, negative, slight; 100 ppm PO, severe, slight; 300 ppm PO, slight, slight. One control animal was rated negative/trace, and the other control animal had no axonal bodies. In addition, demyelination in the extreme distal portion of the fasciculus gracilis was found in two EtO monkeys, one from each exposure group. Demyelination was not seen in animals from the PO groups or in the control group. Statistical analyses of these data were not conducted because of the small sample sizes.

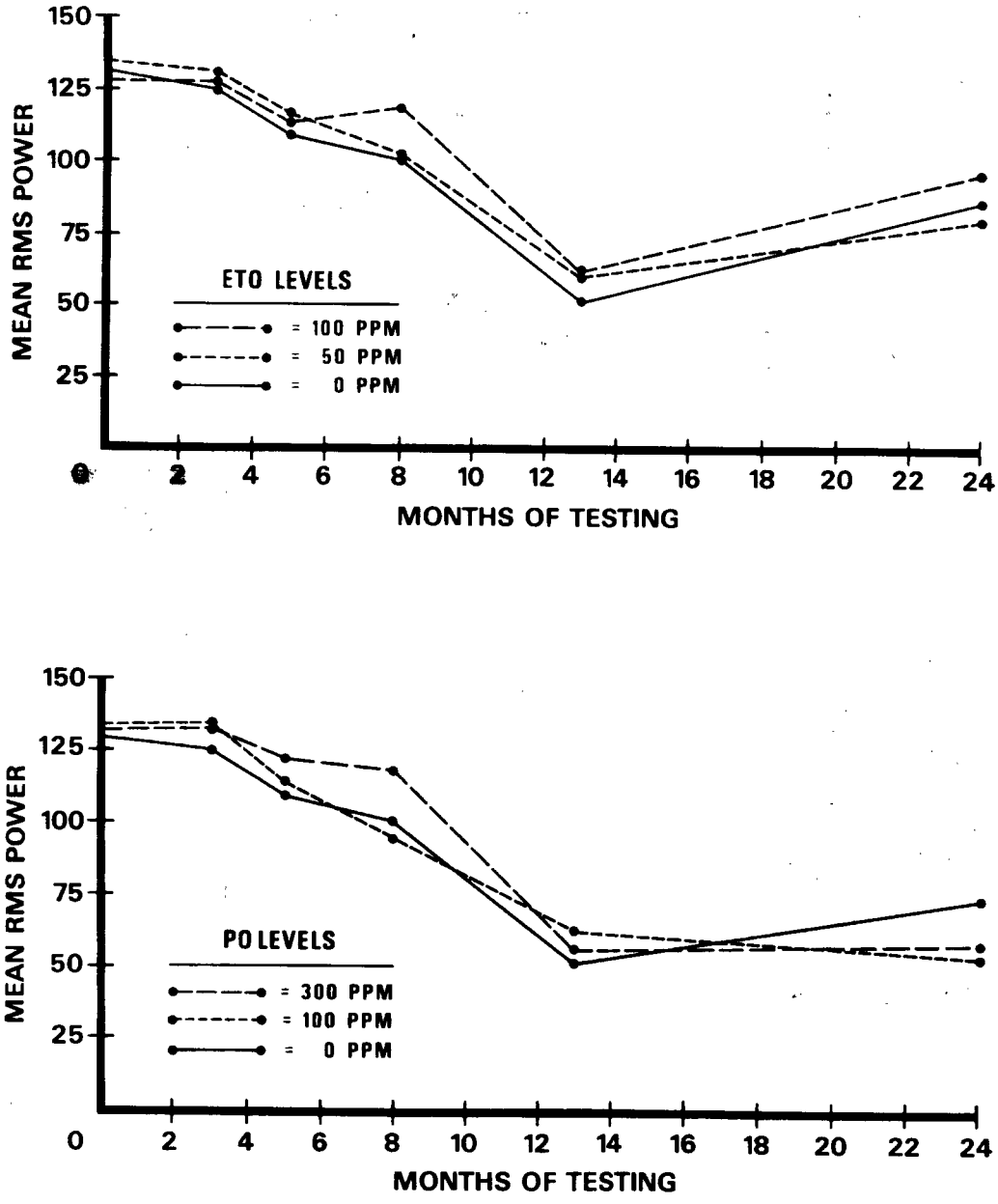

FIGURE 2. Effects of EtO (upper panel) and PO (lower panel) on the RMS power of EEG lead 2 (i.e., vertex) during phase 1.

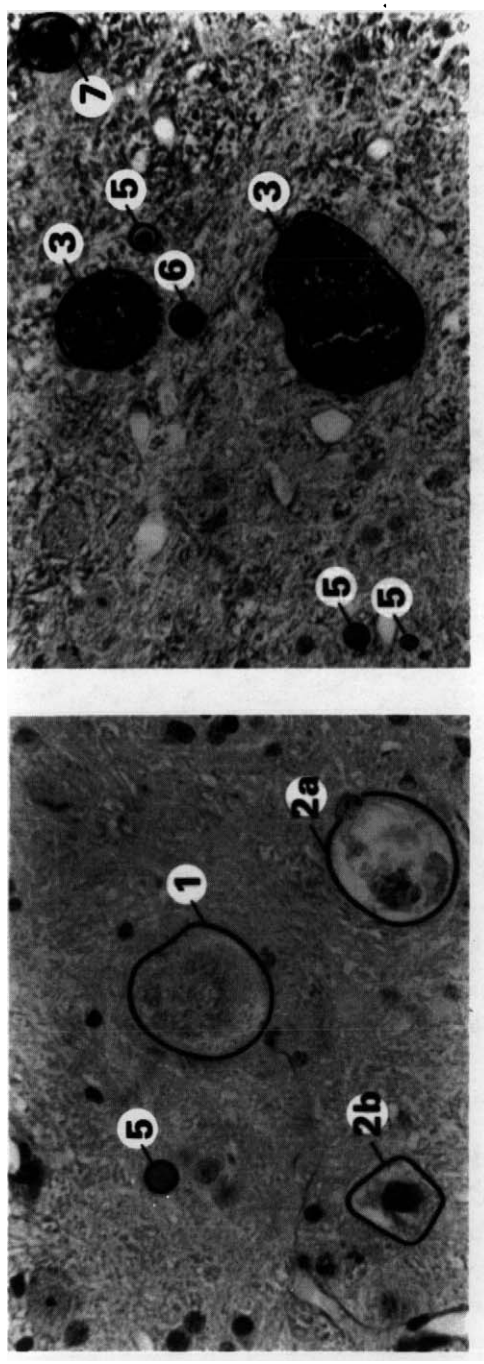

FIGURE 3. Typical histopathological findings (phase 1). The intermediate and giant axonal swellings included: (1) vacuolated bodies (some of which were filled with uniformly pale-staining, finely reticulated material); (2) vacuolated bodies partially filled with broken [a] or solid [b] spheroids; (3) bodies composed of numerous deep-staining, individual, granular spheroids, measuring from 1 to 2 μm in diameter; (4) bodies composed of similarly packed, but more faintly staining, and smaller spheroids; (5) solid bodies; (6) solid bodies showing variegated staining (such as a dark center and light periphery, or the reverse condition, suggesting a laminated appearance); (7) bodies composed of a mixture of various sized individual spheroids, some of which were focused into a larger, irregularly shaped structure.

Neuropathology (Phase 2)

The follow-up neuropathology assessments were conducted by Experimental Pathology Laboratories, Incorporated, Herndon, Virginia, seven years after the exposure terminated. Microscopic grading of axonal bodies in the nucleus gracilis were the same as those employed by the Midwest Research Institute. The results were: 50 ppm EtO, slight, moderate; 100 ppm EtO, slight, moderate; 100 ppm PO, moderate, moderate; 300 ppm PO, moderate, trace; controls, slight, moderate.

DISCUSSION

Under the conditions of this study, no significant neurophysiological effects were found in nonhuman primates following chronic exposures to 50 or 100 ppm EtO or 100 or 300 ppm PO using measures of MCV and EEG. These results are consistent with the research of Hollingsworth et al. (1956) and Rowe et al. (1956) showing that similar exposures to concentrations of PO (102 and 195 ppm) and EtO (49 and 113 ppm) in the range used in this study did not result in peripheral neuropathy in monkeys.

Relative to controls, no effects of EtO or PO on EEG activity were detected. The decline in RMS power in all control and exposed monkeys is likely an age-related effect. However, the possibility exists that the anesthetic, a mixture of ketamine and xylazine, which is known to be an effective muscle relaxant, could have masked an effect of PO and EtO on EEGs. Celesia and Chen (1974), for example, reported that ketamine induces a shift toward higher EEG frequencies in monkeys.

Phase 1 neuropathology examinations found axonal dystrophy in the nucleus gracilis in three of four EtO, four of four PO, and one of two control monkeys evaluated. Cavalier and Gambetti (1981) described similar findings in humans after exposure to EtO. However, the small number of monkeys examined, the lack of a dose-response relationship, the finding of no differences between PO and EtO groups, as well as the finding of a trace of dystrophy in one of two control animals, make it difficult to attribute these effects to oxide exposure.

In phase 2 neuropathology, excessive deposits of lipofuscin and a high frequency of axonal spheroids (eosinophilic axonal swelling) were seen in most of the monkeys evaluated. Lipofuscin was found at one or more brain, brainstem, or spinal cord sites in all ten monkeys. Spheroids were found, primarily in the gracile and cuneate nuclei, in eight of the ten animals. There were no group differences, however, and these findings could be related to aging.

Despite the lack of statistical differences between groups, two monkeys in the 100 ppm EtO group showed substantial decreases in MCV over the first 12 months of exposure. One of these monkeys was selected for neuropathological evaluation, and severe demyelination of the fasciculus gracilis was noted. The demyelination, diminishing caudally, extended into the upper cervical cord. The MCVs of these two animals remained relatively stable at a reduced velocity over the second 12 months of EtO exposure. The relationship between these findings to the observations of nerve conduction velocity declines in sterilizer operators (Gross et al., 1979; Zampollo et al., 1984; Schroder et al., 1985) cannot be assessed. Zampollo et al., (1984) however, reported

neurotoxicity in only 2 out of 12 sterilizer operators, suggesting that some individuals in an exposed population may be more susceptible to the neurotoxic effects of EtO. A number of authors have reported improvement in some indices of neurotoxicity following cessation of exposure to EtO which could explain the absence of neurological and neuropathological effects in the phase 2 assessments (Gross et al., 1979; Kuzuhara et al., 1983; Schroder et al., 1985; Crystal et al., 1988.)

The fact that neurotoxic effects of EtO and PO exposure have been reported in rats (Ohnishi et al., 1985, 1986, 1988) but not seen in monkeys in the present study probably reflects the higher exposure concentrations (250–500 ppm EtO; 1500 ppm PO) used by these authors. Rats were exposed concurrently with the monkeys in the present study for 24 months and no signs of neurotoxicity were observed in these animals exposed to either oxide at any of the exposure concentrations. In summary, no statistically significant group differences in neurophysiological or neuropathological end points were found in monkeys exposed for 24 months at 50 or 100 ppm EtO or 100 or 300 ppm PO and evaluated immediately following cessation of exposures and again seven years later.

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