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NEUROPATHOLOGICAL EVALUATION OF MONKEYS EXPOSED TO ETHYLENE AND PROPYLENE OXIDE

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16. Abstract (Limit: 200 words)

The neuropathological effect of ethylene-oxide (75218) and propylene-oxide (75569) on monkeys was studied. Male monkeys were exposed to 0, 50, and 100 parts per million (ppm) ethylene-oxide and 0, 100, and 300ppm propylene-oxide at 6 hours per day, 5 days per week for 2 years. Brain, ulnar and sciatic nerves, and spinal cord tissue were examined histologically. No differences were found between controls and chemically treated animals, between sciatic and ulnar nerves, and between different levels of these nerves. The eight segments of spinal cord of control and experimental animals had no specific pathological differences. In the medulla oblongata of the brain, ethylene-oxide and propylene-oxide exposed monkeys had signs of axonal distrophy. Ethylene-oxide exposed monkeys also had signs of demyelination in this area of the brain. The author concludes that the concern expressed by OSHA about the allowable maximal amount of ethylene-oxide is strengthened by the result of the present study. Also, in view of the current great interest in the effect of chronic occupational exposure to the two compounds, additional studies would seem justified.

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri, under Contract No. 210-81-6004 with the National Institute for Occupational Safety and Health, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226. Dr. Kent Anger was the NIOSH Project Officer for this study.

The inhalation phase of the study was conducted at the NIOSH, Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226. The gross necropsies and tissue collection for the neuropathology study were performed by Dr. James Carter, Veterinary Pathologist, MRI, at the Robert A. Taft Laboratories from July 20 to July 23, 1981. The tissues were transported by Dr. Carter from NIOSH to Midwest Research Institute. Microscopic slides were prepared by Ms. Ellen Ellis, histology supervisor. Examination of the microscopic sections was performed by Dr. Helmuth Sprinz, Principal Investigator and Pathologist, MRI, and Dr. Howard Matzke, anatomist, Kansas University Medical Center.

The preparation of the slides were inspected on September 10, 1981 and the monthly reports were reviewed by the Quality Assurance Unit of MRI. The final report was reviewed and the data was audited on February 4 and 5, 1982. The work reported herein was found to meet the standards of the Good Laboratory Practice regulations (43 FR 59986, 1978). Specimens, raw data and reports are currently stored in the MRI archives.

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SUMMARY

A chronic inhalation toxicity study using the test compounds, ethylene oxide and propylene oxide, was performed in cynomolgus monkeys (Macaca fascicularis). Brain, spinal cord, sciatic nerve and ulnar nerve from ten monkeys were examined for neuropathology.

No treatment-related lesions were observed in the spinal cord, sciatic nerve or ulnar nerve. Axonal dystrophy was observed in the nucleus gracilis of all dose groups but was observed primarily in the treated animals. The second significant finding was the presence of demyelination in the very distal portion of the Fasciculus gracilis in one of two monkeys of both the low- and high-dose ethylene oxide groups.

I. INTRODUCTION

Ethylene oxide and propylene oxide are widely used industrial chemicals. They are utilized in the manufacture of glycols and polyester resins. Ethylene oxide is an important sterilizing agent for materials such as artificial joints and heart pacemakers which cannot be subjected to steam sterilization. Ethylene oxide has been reported to be a depressant of the central nervous system after even brief exposure to high concentrations. Ethylene oxide is stated to produce muscular atrophy, loss of smell and peripheral neuropathy after repeated exposures.[1] The Occupational Safety and Health Administration recently expressed concern that the present standard of 50 parts of ethylene oxide per 1 million parts of air may not adequately protect workers and currently is seeking scientific and economic data to determine if lower exposure limits are required (Federal Register, Vol 47, No. 17, January 26, 1982, pp. 3566 to 3571). According to reports received by NIOSH, propylene oxide has been reported to cause foot drop and leg weakness in humans exposed for several months. The Merck Index, 9th edition, lists Ethylene Oxide No. 3738 and Propylene Oxide No. 7645. They are listed in the NIOSH (1979) Registry of Toxic Effects of Chemical Substances, Vol I, under KX2450000 Ethylene Oxide and under TZ2975000, Propane 1,2-Epoxy (Propylene Oxide). Despite the great interest and importance of the subject, the possible neurotoxic effects of the two compounds have not been examined experimentally after chronic exposure to concentration which could exist in an occupational environment. Patty's Industrial Hygiene and Toxicology, 3rd revised edition, Vol IIA, 1981, lists only 25-year-old studies which no longer can be considered authoritative. To fill this void, the National Institute for Occupational Safety and Health initiated a chronic inhalation study in the Robert A. Taft Laboratories. The Midwest Research Institute provided a neuropathological evaluation of selected monkeys.

II. MATERIALS AND METHODS

A. Exposure

Male monkeys (Macaca fascicularis) (at the Robert A. Taft Laboratories, Cincinnati, Ohio) were exposed to 0-, 50-, and 100-ppm ethylene oxide, or to 0-, 100-, and 300-ppm propylene oxide at 6 hr/day, 5 days/week for a total of 24 months. Vapor concentrations were selected based on the relative toxicity of the test materials. The animals were tested bimonthly for alterations of nerve conduction velocities as well as changes in other organ systems.

B. Necropsy

At the end of the 24-month inhalation study, 10 monkeys were sacrificed, 2 each from the following groups: control, low and high dose of ethylene and propylene oxide, respectively. Gross necropsies and tissue collections were performed by Dr. James Carter of Midwest Research Institute with technical support by NIOSH personnel from July 20 to July 23, 1981, at the

Robert A. Taft Laboratories. There was no recovery period. The animals were subjected to gravity perfusion fixation in a deeply anesthetized state. Brains, spinal cords, ulnar and sciatic nerves were removed immediately and placed in fixative. The tissues were transported from Cincinnati to Kansas City by Dr. Carter. The fixing solution prepared at the Robert A. Taft Laboratories had the following composition: 160 g of sodium acetate dissolved in 1,760 ml of distilled water were mixed with 2,400 cc of 12% formalin. When tested in Kansas City, the fixing fluid contained 220 milliosmoles of sodium and had a pH of 6.9. As the total ionic strength of the fixing fluid is twice the sodium ion concentration, the solution was hypertonic. The specimens were processed as follows:

C. Peripheral Nerves

At the time of autopsy the proximal ends of both sciatic and ulnar nerves had been identified by a black suture. Four longitudinal blocks from four different sites were obtained from each nerve from each animal. They were labeled with the number of the animal, A for sciatic and B for ulnar nerve, and 1 for the most proximal portion of each nerve, 2 for a more peripheral portion of the proximal part of the nerve, 3 for the more proximal portion of the distal part of the nerve and 4 for the peripheral portion of the distal end of the nerve.

D. Spinal Cord

The spinal cord was divided into cervical, thoracic, lumbar and sacral portions; from each segment a more cranial and a more caudally situated block was taken and identified with the animal number and its position: Cervical A and B, Thoracic C and D, Lumbar E and F, and Sacral G and H.

E. Brain

A special brain macrotome was constructed at the Midwest Research Institute to assure reproducible serial sectioning. The brain stem 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 mid- and hindbrain. The platform of the macrotome was elevated to an angle of approximately 35 degrees so that the plane of section would correspond to the coordinates of the stereotaxic atlas of Shanta, Manocha and Bourne, Karger, Basel 1968.[2] The brains were cut serially at about 4-mm intervals in the coronal plane. The following blocks were obtained and so labeled for each animal identified by its number.

1. Motor and sensory cortex (frontal and caudal to the central sulcus)

2. Bulbus olfactorius

3. Olfactory tubercle, overlying basal ganglia, rostral to anterior commissure

4. Central operculum and insular cortex

5. Visual cortex to include area striata, Gennari's line and calcarine fissure

6. Basal ganglia at the level of the anterior commissure

7. Section caudal to the anterior commissure to include an optic tract and portions of the paraventricular and supraoptic nuclei.

8. Diencephalon at the level of the mammary bodies, including substantia nigra, red nucleus and third ventricle.

9. Nucleus of lateral and medial geniculate body and hippocampal formation.

10. Diencephalon and basal ganglia, to include dorsal, ventral and medial thalamus.

10a. Pretegmental region

11. Midbrain, level of superior colliculus

12. Midbrain, level of inferior colliculus

13. Upper pons, isthmus, with medial longitudinal fasciculus and reticular formation

14. Lower pons, level of cranial nerves VI and VII and inferior olivary nucleus

15. Lower pons, level as above, consisting of a cross section through the cerebellar hemisphere bordering the IV ventricle to include cortex and cerebellar nuclei (dentate)

16. Medulla oblongata, upper level

17. Medulla oblongata, at the level of the obex.

18. Medulla oblongata, lower level

19. Cerebellar cortex, vermis

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 in order to obtain the desired plane of section. A total of about 1,500 slides were prepared and examined both jointly using a

double-headed microscope and individually by the project director and his consultant. The slides initially were read as unknown, later the code was supplied by K. W. Anger, Ph.D.

III. RESULTS

A. Peripheral Nerve

The evaluation of the sections of peripheral nerves was seriously compromised by fixation artifacts which mimicked myelin degeneration. No differences could be discerned between controls and chemically treated animals, between sciatic and ulnar nerves and between different levels of these nerves. Despite the artifacts, evaluation of spinal cord and brain sections was possible.

B. Spinal Cord

The eight levels of spinal cord of control and experimental animals showed no specific pathologic findings.

C. Brain

The brain showed one incidental finding, the presence of melanin confirmed by the Fontana silver stain which was most marked in Control Monkey 77 where it also involved the operculum and insular cortex.

An abnormality heretofore not described in this species was present in the medulla oblongata, primarily in treated animals. It was restricted to the territory of the Nucleus gracilis and the most distal portion of the Fasciculus gracilis. It did not involve the cuneate tract, the cuneate nuclei, nor the sensory nerve and nucleus of the V nerve. The lesion, a classic example of axonal dystrophy, showed the following distribution in the group of ten monkeys.

Controls: A-77, A-82

A-77 - A single axonal body in the territory of N. gracilis, rated ± (trace). Single degenerating glial cells. No demyelination of Fasciculus gracilis.

A-82 - No axonal bodies. No demyelination. No abnormalities.

Low Propylene: A-37, A-66

A-37 - Multiple and varied axonal bodies strictly limited to area of N. gracilis, rated +++ (marked). No demyelination or other abnormalities.

A-66 - A few axonal bodies in the center of N. gracilis, rated + (slight).

High Propylene: A-62, A-239

A-62 - A few axonal bodies in area of N. gracilis, rated + (slight). No demyelination or other abnormalities.

A-239 - Rare, single axonal bodies on serial sectioning, rated ± (trace).

Low Ethylene: A-47, A-48

A-47 - A few axonal bodies in the center of N. gracilis diminishing caudally and rated + (slight). No demyelination.

A-48 - Multiple and varied axonal bodies in N. gracilis not quite as marked as in A-37, rated ++ to +++, and diminishing caudally. Demyelination of Fasc. gracilis in its terminal portion, rated ++ (moderate).

High Ethylene: A-76, A-89

A-76 - No axonal bodies on multiple sections. Severe demyelination of Fasc. gracilis, rated +++. The demyelination diminishing caudally extends into upper cervical cord.

A-89 - A few axonal bodies in the territory of N. gracilis, rated + (slight). No demyelination of Fasc. gracilis. No other abnormalities.

The axonal bodies in the monkeys were identical to those described by others.[3-9] In the monkeys the axonal dystrophy affected only the terminal axons of the fasciculus gracilis. Bodies were only found in the territory of the N. gracilis. Thus, the extent of the lesion was similar in affected animals and was not dose-related. Degeneration of neurons or atrophy of N. gracilis was not detected. No changes were observed in the spino-thalamic or spino-cerebellar tract. The cuneate nuclei and the mesencephalic nucleus of the trigeminal nerve were uninvolved.

The axonal bodies, when present, occurred symmetrically in the area of N. gracilis on either side in a similar degree of severity. They stained blue with the Gomori Trichrome, reddish with PAS and were argyrophilic with the Bodian silver stain. The bodies varied greatly in sizes from 2 to 60 μ in greatest diameter. They were, in general, round, or oval, or pyriform, but irregularly shaped bodies also were observed. The smaller ones were usually solid and stained uniformly. Some had a darker center and a lighter staining periphery. Intermediate- and large-sized bodies which had formed inside enlarged-up-to-giant-sized axons showed a variety of structures: (1) vacuolated bodies, some of which were filled with uniformly pale staining, finely reticulated material; (2) vacuolated bodies partially filled with broken-up or with solid spheroids; (3) bodies composed of numerous, fairly

tightly packed, deep-staining, individual, granular spheroids, measuring from 1 to 2 μ in diameter; (4) bodies composed of similarly packed, but more faintly staining, minute spheroids; (5) larger solid bodies of uniform staining intensity; (6) larger solid bodies showing varigated staining, such as a darker center and lighter periphery or the reverse, in some instances suggesting a laminated appearance; (7) bodies composed of a mixture of various sized individual spheroids, some of which were fused into a larger irregularly shaped structure. This great variety of size, shape and staining characteristics also has been noted previously. It has been studied in man where it was ascribed to different stages of formation, composition, reworking and fusion of axonal bodies.[10]

The second significant finding was the presence of demyelination in the very distal portion of the fasciculus gracilis in one of two monkeys of both the low- and high-dose ethylene oxide groups. No other instance of demyelination was seen. The combination of axonal dystrophy of N. gracilis with demyelination of the fasciculus gracilis has been described in humans.[9]

IV. DISCUSSION

For the first time, a unique type of axonal dystrophy has been observed in the cynomologus monkey. In two of the animals exposed to ethylene there was demyelination of the terminal fasciculus gracilis. Axonal dystrophy has been described in several animal species and, particularly, in the human. So far, it never has been associated with the chronic exposure to propylene or ethylene oxide vapors. As ethylene oxide is considered more toxic than propylene oxide, it is noteworthy that demyelination occurred only with exposure to the former. Individual differences in susceptibility seem to exist in that only one of the two animals of each group showed demyelination. If we can judge from a single animal, exposure to high-dose ethylene oxide was associated with the more severe degree of demyelination. In the same animal, demyelination was not associated with axonal bodies. Similar dissociations have been reported in humans.

The exact relationship between axonal dystrophy with the formation of axonal bodies and demyelination is still uncertain. In humans, the presence of axonal bodies alone has not been associated with clinical symptoms. Demyelination of the fasciculus gracilis should result in loss of proprioception of the hind limbs. Recovery should be possible if the cause of the demyelination is removed. In humans, this cannot be accomplished except in rare circumstances as axonal dystrophy and demyelination occurs in patients with longstanding illnesses and chronic malnutrion and malabsorption.

The largest body of literature on the subject concerns patients with cystic fibrosis or aged persons who sustained chronic weight loss. Recovery could be tested in the experimental animal. The irritating nature of the vapors for the exposed worker has been noted. It is possible, but hard to prove, that loss of smell, eye irritation, stress in general, caused malnutrition including vitamin E deficiency in the exposed monkeys. If this is so, then the vapors would be an indirect cause of the neurologic deficit.

Pentschew and Schwarz[3] experimentally produced axonal dystrophy and axonal bodies in adult rats through vitamin E dificiency. On the other hand, the epoxy compounds may have had a direct effect. Cowen and Olmstead[4] pointed out that similar lesions occurred spontaneously in conditions involving the metabolism of the axoplasm. One year later and 2 years after the publication of the paper by Pentschew and Schwarz, Sung[5] reported the association of axonal dystrophy and cystic fibrosis (mucoviscidosis). The relationship of these two conditions has been the subject of continuing interest.[8,9].

Yagashita[10] pointed out that axonal dystrophy may be seen in a variety of conditions, not all of which are primary brain diseases. In this respect, axonal dystrophy is nonspecific. This fact makes it also difficult to assign a direct cause-and-effect relationship between chronic vapor exposure and axonal dystrophy. We do not know the pathogenesis of axonal dystrophy in the exposed monkeys. Unfortunately, for technical reasons, we cannot entirely exclude peripheral nerve involvement. We cannot correlate the observed pathologic lesion to measurements of nerve conduction velocity. An examination of the spinal root ganglia would seem to be of importance as they are the source of the involved axons. Also, these ganglia lack the blood-brain barrier and could be a portal of entry of noxious substances.

In view of the current great interest in the effect of chronic occupational exposure to the two compounds, additional studies would seem justified. The concern expressed by OSHA about the allowable maximal level of ethylene oxide is strengthened by the result of the present study.

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