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NEUROBEHAVIORAL ASSESSMENT OF CHRONIC LOW-LEVEL METHYL BROMIDE EXPOSURE IN THE RABBIT

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The research reported here was intended to identify the concentration at which methyl bromide begins to produce neurotoxic effects in the rabbit, a species known to be sensitive to this compound. Rabbits were exposed via inhalation to 27 ppm methyl bromide over a period of 8 mo for a total exposure duration of 900 h. Biweekly neurobehavioral tests, consisting of the latency rates of the ulnar and sciatic nerves and the amplitude of the eyeblink reflex of the orbicularis oculi muscle, failed to uncover any untoward consequences of the exposures. The rabbits gained weight and otherwise appeared to be healthy. In contrast to reports available in the literature, these findings suggest that long-term exposures to methyl bromide, in the present concentration range, are tolerated by this species. Also detailed in this report is the course of recovery of a separate group of rabbits previously given subchronic exposures to 65 ppm methyl bromide. These animals developed severe neuromuscular losses and had impaired blink reflexes and body weights. The symptoms partially subsided within 6–8 wk after removal from the exposures, suggesting that recovery from a nonfatal but seriously debilitating exposure is possible.

INTRODUCTION

Neurobehavioral impairment is a prominent feature of toxic exposures to methyl bromide. Even brief inhalation exposure to high concentrations of this fumigant can produce diverse neurological symptoms characterized by abnormal reflexes, incoordination, ataxic gait, auditory and visual disturbances, and muscular weakness (Shield et al., 1977). Memory loss, seizures, mental confusion, and depression have also been reported, but sensorimotor impairment, especially in the lower limbs, appears to dominate the clinical picture (von Oettingen, 1944). Exposures of longer duration to

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low levels of methyl bromide can produce tremor and paresthesias, in addition to the neurological responses typical of acute exposures (Rathus and Landy, 1961). Many of the symptoms subside spontaneously (Greenberg, 1971; Kantarjian and Shaheen, 1963), but the improvement may require up to several months to stabilize, and residual motor impairment may persist if the initial disturbance was profound (Collins, 1966; Hine, 1969).

Clinical reports of accidental exposures in humans provide the bulk of the evidence on the neurotoxicity of methyl bromide. Only one laboratory study, conducted more than 40 yr ago, has examined animals systematically in order to obtain comparative dose-effect information. In this study, Irish et al. (1940) examined a wide range of inhalation exposure conditions in rats, rabbits, guinea pigs, and monkeys, using visual inspection to assess behavioral deficits. They observed that nearly 60% (34 of 58) of the rabbits surviving exposures to 33 ppm developed hindlimb paralysis during the 6-mo exposure period. This was the lowest concentration at which definite neurobehavioral effects could be detected in the rabbit, and the remaining species were unaffected at concentrations of 33 ppm and below. The impact of this finding is apparent in the fact that the American Conference of Governmental Industrial Hygienists (ACGIH) cites it (1980) in support of its recommended threshold limit value (TLV) of 5 ppm for methyl bromide. Because both the behavioral and chemical analytical methods available to Irish and his associates in 1940 have since been improved, a closer examination of the neurobehavioral effects of exposures to methyl bromide in rats and rabbits was undertaken by Anger et al. (1981). They used conduction velocity and electromyography to assess neurological impairment, and infrared analyses to quantify exposure concentrations. Their results confirmed some of the differences in species sensitivity reported by Irish et al. (1940), and described for the first time the rapid development of neurobehavioral effects seen in rabbits within the first few weeks of exposure to 65 ppm methyl bromide. More specifically, they found that the rabbit's eyeblink reflex declined in amplitude after 1 wk (30 h) of exposure to 65 ppm of methyl bromide. By wk 3 (90 h) of exposure, hindlimb paralysis was minimally apparent in one exposed animal, and by wk 4, each exposed animal exhibited severe locomotor losses accompanied by clear myoneural decrements in the fore and hind limbs.

The present study was undertaken to extend these findings to longer-term exposures in rabbits, and to probe within the reported minimally effective exposure range between 17 and 33 ppm (Irish et al., 1940). We include data, not previously presented by Anger et al. (1981), that describes the course of neurobehavioral recovery observed after cessation of the 65-ppm exposures.

MATERIALS AND METHODS

Eight adult male New Zealand White rabbits, weighing 2.3–2.7 kg at the start of the experiment, were obtained from King's Wheel Rabbitry (Mt. Vernon, Ohio). After a 2-wk quarantine period, 6 animals were randomly assigned to the exposure group, and 2 animals to the control condition. During the study the animals were housed in the chambers used for exposures, and given *ad libitum* access to food (Purina Laboratory Rabbit Chow, Ralston Purina Co., St. Louis, Mo.) and water when exposures were not in progress. Due to a lack of exposure chamber space, only 2 "control" animals were included in the experiment; they served as a point of reference rather than for purposes of statistical comparisons.

Exposures

Inhalation exposures to methyl bromide vapor were administered in 3 standard 0.5-m^3 stainless-steel chambers (Charles Spengler and Associates, Cincinnati, Ohio). The control rabbits breathed filtered room air delivered to an identical chamber. The rabbits were exposed in pairs, the individual animals being separated by stainless-steel partitions within each chamber. Details of the procedures used to generate the test atmospheres were reported previously (Anger et al., 1981). Briefly, a 10%/90% mixture of 99% pure methyl bromide gas in air (Wright Bros., Cincinnati, Ohio) was directed from a storage cylinder via Teflon tubing through flow meters for calibration, and then to the intake plenum of each chamber, where it was diluted with room air to produce a target concentration of 25 ppm methyl bromide. Air samples were obtained automatically from each chamber every 12 min and analyzed by a Miran II infrared (IR) analyzer (Foxboro-Wilkes, Norwalk, Conn.) using an automatic zero circuit that reset the baseline for changing humidity levels, since water vapor and methyl bromide absorb at similar wavelengths. The average concentration throughout the course of exposures was 26.6 ppm (SD 4.4), and the mean of confirmatory charcoal tube samples, processed independently by the National Institute for Occupational Safety and Health (NIOSH) (1977) PCA & M method S-372 at the Utah Biomedical Test Laboratory (Salt Lake City, Utah), was within 1 ppm of the mean of the IR readings for the periods sampled. Conditioned room air was maintained at approximately 22°C. The exposures were administered for 7.5 h/d, 4 d/wk, during the normal daylight working schedule of the laboratory until an accumulated exposure duration of 900 h had been completed.

Testing

The neurobehavioral tests examined (1) the latency rates of the sciatic and ulnar nerves, and (2) the amplitude of the eyeblink reflex of the orbicularis oculi muscle, which traverses a facial trigeminal reflex arc. The tests

were administered once before the start of the exposure regime and biweekly thereafter. Thus, 16 test blocks were generated, one for the preexposure condition and one after each of 15 60-h exposure periods. Each test block began approximately 36 h after termination of the preceding exposure period and consisted of 2 test days. Reflex tests were administered on the first day to unanesthetized rabbits, and latency rates were assessed on the second day in anesthetized rabbits.

Stimulation of the sciatic and ulnar nerves was achieved by applying a 0.2-msec, positive-going square wave of supramaximal intensity (Grass Model S88 biomedical stimulator, Grass Instruments, Quincy, Mass.) to the proximal nerve segment, and the resulting muscle response was displayed on a Tektronix 5441 storage oscilloscope (Tektronix, Dayton, Ohio) from which response latency was recorded. The latency was then expressed as an inverse proportion of the distance between the stimulating and recording electrodes, and corrected for variation in skin temperature (DeJesus et al., 1973). The derived test score, referred to by us as "latency rate" (Cohen and Brumlik, 1968), thus represents the compound influence of conduction velocity in the stimualted nerve segment and transmission time across the myoneural junction. The test scores obtained in this manner were then entered into separate analyses of variance for the sciatic and ulnar nerves. This technique duplicates the method actually used by Anger et al. (1981), who reported their procedure as a traditional measure of motor-nerve conduction velocity.

Eyeblink reflex responses were obtained from rabbits tested individually in a Plexiglas restraining cage located in a brightly illuminated audiometric test booth. Monopolar needle electrodes were inserted under the skin adjacent to the right lateral canthus and superior palpebral portion of the orbicularis oculi muscle of the eye. A ground electrode was placed at the base of the right pinna. After a brief warm-up and calibration period, eyeblink reflexes were elicited at regular 7-s intervals by an air puff directed to the right cornea, until a total of 50 stimuli were delivered. The unfiltered electromyograph (EMG) response was amplified by a Disa type 14A-11 electromyograph (Disa Electronics, Franklin Lakes, N.J.) and fed through a sample-and-hold circuit (Coulbourn Instruments, Lehigh, Pa.), which detected the positive-going peak in the signal over a 100-ms poststimulus period. The resulting voltage was recorded by an IBM Series/I computer (IBM Corp., Atlanta, Ga.), which averaged the 50 response amplitudes to provide a single measure of reflex strength for each rabbit in each test session. The mean difference in reflex amplitudes between the preexposure test and the last test in wk 30 was then evaluated using a *t*-test.

RESULTS AND DISCUSSION

Mean latency rates of the sciatic and ulnar nerves of the exposed animals failed to exhibit the anticipated decline characteristic of peripheral

nervous system impairment produced by methyl bromide (Fig. 1). In fact, a slight but anomalous increase in the scores was obtained. Five of the 6 exposed animals ($t = 4.24$, $df = 5$, $p < 0.01$) and both control animals showed this change in the sciatic nerve response. Similar changes were observed for the ulnar nerve in 4 of the 6 exposed animals ($t = 1.86$, $df = 5$, $p < 0.06$) and 1 of the controls. However, these increases should not properly be attributed to methyl bromide, because the literature [including Anger et al. (1981), who used the same rabbit strain and laboratory equipment as in the present study] suggests an outcome of impaired conduction rather than its improvement over time.

Results of the serial reflex testing also failed to reveal reliable differences that could be attributed to the exposures (Fig. 2). The exposed and control animals exhibited consistent blink amplitudes through the first 90 h of the exposure, which was followed thereafter by a consistent decline in responding of approximately 35% in the exposed animals and 46% in the controls. A t-test conducted on the mean scores of the 6 exposed animals indicated that there was a significant decline in responding between the preexposure test and the last exposure test ($t = 5.39$, $df = 5$, $p < 0.01$). However, the 2 control animals exhibited a similar decline, and examination of the individual records revealed that blink amplitudes decreased in each of the 8 animals over this period. This inherent decline in reflex amplitudes with repeated testing, known as behavioral habituation (Thompson

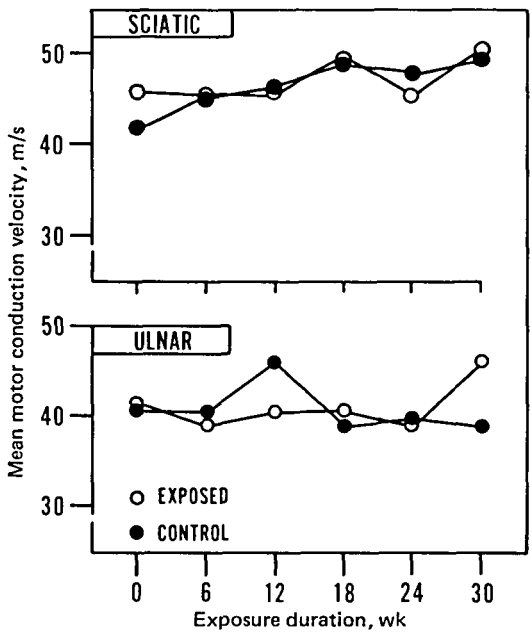

FIGURE 1. Effects of methyl bromide exposure on mean sciatic and ulnar nerve latency rates, expressed in m/s. The abscissa depicts test points between 0 and 900 exposure hours.

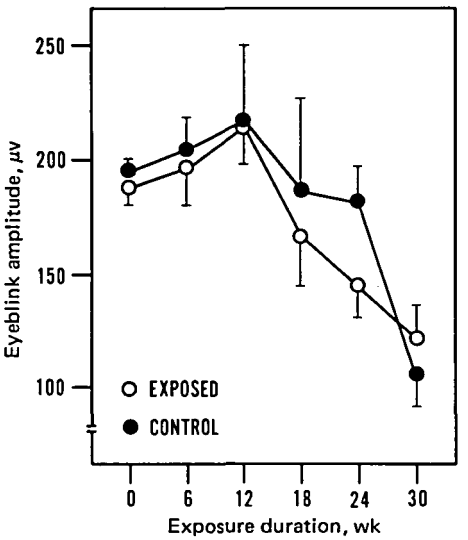

FIGURE 2. Effects of methyl bromide exposure on eyeblink amplitudes, expressed in μv. Each point is the mean ± SE.

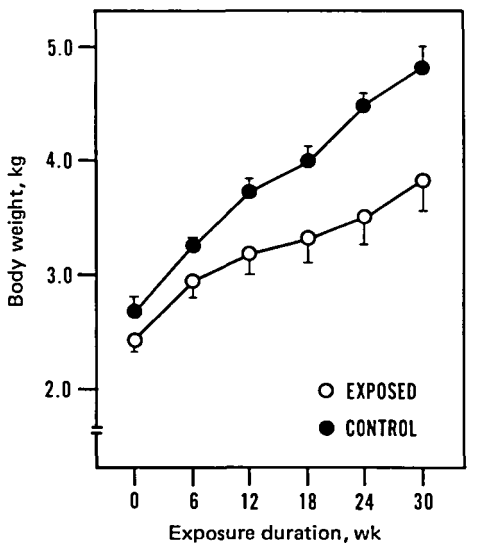

FIGURE 3. Effects of methyl bromide exposure on body weights, expressed in kilograms. Each point is the mean ± SE.

and Spencer, 1966), would be expected after 16 test sessions or more than 800 stimulus repetitions. Such interfering effects of habituation have been noted before for other chemicals (D'Arcy and Harpur, 1977), and they may have masked any subtle late impact of the present exposures. However, the roughly equivalent habituatory decline seen in the 2 control animals is reason to be cautious in accepting this conclusion. The more straightforward conclusion is that the methyl bromide exposures failed to influence this response measure.

Rabbits in the exposed group gained steadily in weight from a mean of 2.4 kg (SD 0.1) in the preexposure period to a mean of 3.8 kg (SD 0.7) in the final exposure week (Fig. 3). The control animals also gained weight over this period, from a mean of 2.7 kg (SD 0.1) to a final mean weight of 4.8 kg (SD 0.3). The observed difference in mean weight gain between exposed and control animals approached statistical significance ($t = 1.84$, $df = 6$, $p < 0.06$), but this is difficult to interpret because of the small number of control animals. Our failure to observe any systematic changes in feeding or grooming behaviors of the sort observed by Anger et al. (1981) suggests that the present weight differences should not be attributed to the exposures.

Our failure to find reliable neurobehavioral impairments at an exposure concentration of 27 ppm is problematic for our understanding of the much earlier finding of Irish et al. (1940), in which obvious motor losses were reported in rabbits exposed to 33 ppm methyl bromide. We must register concern that, if 33 ppm produced paralysis in nearly two-thirds of the rabbits so exposed by Irish, then 27 ppm would be expected to have some effect on the electrophysiological measures used in the present experiment. We suggest three independent, but not mutually exclusive, sources for this discrepancy. First, it may be that differences in the strain of the rabbits used led to variation in the induction of impairment by the chemical treatment. This is a somewhat attractive explanation because it permits the question to be forthrightly addressed by additional research, but it is not entirely satisfactory because previous results (Anger et al., 1981) would suggest that the response of the New Zealand White rabbit to exposures of 65 ppm is substantially the same as that of the (unspecified) strain used by Irish and his associates. Second, it is possible that concentrations of 27 and 33 ppm span the threshold level for the occurrence of neurobehavioral impairment by methyl bromide. This explanation would provide an unusually precise identification of the threshold level producing neurotoxicity, but it assumes, incorrectly, that the studies shared common procedures. Since this is not the case, it would be premature to conclude that the defining exposure characteristics of methyl bromide neurotoxicity had been uncovered. A more likely cause of the discrepant results obtained for the exposure range between 27-ppm and 33-ppm exposures is that the methods used to confirm the exposure concentraitons produced covert variation in the levels used by Irish and his co-workers, which in turn

influenced the neurobehavioral outcome. In the present study the exposures were precisely monitored to within a mean of less than 3 ppm of the target. On the other hand, Irish et al. do not discuss the details of their analytic procedures (see, for example, Irish and Adams, 1940). This simpler explanation of the discrepancy is the most acceptable because it does not confound the sensitivity of the behavioral methods used with the magnitude of the exposures, but it has implications for our understanding of methyl bromide that are different from those of the previous explanations. The work of Irish et al. would suggest that exposure concentrations between 17 and 33 ppm are potentially neurotoxic. Our interpretation of the present results is that concentrations near the upper bound of this range can be experienced in chronic exposures without obvious impairment, at least in the rabbit.

In marked contrast to the animals exposed to 27 ppm methyl bromide, the rabbits previously exposed by Anger et al. to 65 ppm showed severe impairments in neuromotor performance, body weight, and general appearance after 100 h of exposure. Subsequent to the report by Anger et al. (1981), 3 of these animals were examined 6–8 wk after termination of the exposures in order to assess the persistence of the observed neurobehavioral losses. Latency rates for the sciatic nerve, which had declined during the exposures by 18% to a mean of 34.9 m/s (SD 2.4), recovered to 42.2 m/s by wk 6 postexposure. The mean rates for the ulnar nerve declined by 26% to 38.3 m/s (SD 3.4) over the exposure period, but improved only slightly thereafter to 40.8 m/s (SD 7.0). The mean blink reflex amplitudes in these 3 animals showed the greatest proportionate decrement during the exposures, declining by 69% to a mean of 94.3 μv (SD 24.9). Eight weeks after termination of the exposures, this measure had improved to a mean of 133.9 μv (SD 0.2). The rabbits showed almost no increase in body weight during the exposures, changing from a mean of 2.3 kg (SD 0.02) to 2.4 kg (SD 0.02), a gain that is smaller than is characteristic of young adult rabbits. However, by wk 6 of recovery their mean weight had improved to 3.4 kg (SD 0.03). Thus it appears that exposures to 65 ppm methyl bromide, producing obvious paralysis and electrophysiological signs of motor impairment, require an extended period for recovery, which, in the present instance, was not complete after 6–8 wk. This aspect of our results, obtained after a controlled exposure to methyl bromide, substantially agrees with the clinical observations of others (e.g., Collins, 1966), made after accidental exposures.

In summary, our results suggest first, that neurological impairment is not a prominent fetaure of chronic exposure to low levels (27 ppm) of methyl bromide, and second, that higher levels (65 ppm) produce severe neuromotor anomalies, which, however, admit to at least partial recovery within a somewhat protracted period after removal from the toxic environment.

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