Neurotoxicology and Teratology, Vol. 13, pp. 83–90 © Pergamon Press plc, 1991 Printed in the U.S.A. 0892-0362/91 $3.00 + .00

Sensory-Evoked Potentials in Rats Chronically Exposed to Trichloroethylene: Predominant Auditory Dysfunction

CHARLES S. REBERT, VIRGINIA L. DAY, MICHAEL J. MATTEUCCI AND GORDON T. PRYOR

SRI International, LA117, 333 Ravenswood Ave., Menlo Park, CA 94025

Received 22 August 1989

REBERT, C. S., V. L. DAY, M. J. MATTEUCCI AND G. T. PRYOR. *Sensory-evoked potentials in rats chronically exposed to trichloroethylene: Predominant auditory dysfunction.* NEUROTOXICOL TERATOL 13(1) 83–90, 1991.—Sensory-evoked potentials (EPs) were studied in male Long-Evans and Fischer-344 rats in order to characterize the electrophysiological consequences of chronic inhalation exposure to trichloroethylene (TCE). Groups of ten Long-Evans rats were exposed to air or 1600 ppm or 3200 ppm TCE for twelve weeks and evaluated periodically with a multisensory test battery. Brainstem auditory-evoked response (BAER) amplitudes were depressed by TCE, whereas somatosensory and visual potentials remained normal. The effects on BAERs, which varied with tone intensity and frequency, suggested that TCE causes a predominantly high-frequency hearing loss. Comparable effects were obtained in both strains of rats and were like those previously observed following exposure to toluene.

Trichloroethylene Rats Sensory-evoked potentials Hearing loss Brainstem auditory-evoked response

THE chlorinated aliphatic hydrocarbon trichloroethylene (TCE) is primarily used for industrial degreasing operations, but has also been used in paint removers, as an anesthetic, a solvent for chemical extractions, a dry cleaning agent, and as a component of adhesives and lubricants (1, 43, 49). Worldwide annual production at one time approached a million tons (16). Industrial workers may be exposed to relatively low levels over extended periods of time (1, 16, 21) and there have been numerous instances of accidental exposures to levels [e.g., 800 to 1,000 ppm—e.g., Grandjean *et al.* (18)] much beyond the threshold limit value of 100 ppm per 8-hr day. In addition TCE may be self-administered at very high concentrations as a euphoriant (44,49). Its anesthetic properties insure that TCE produces a variety of acute effects on the nervous system (1,16), but its potency as a chronic neurotoxicant is still under debate. Clarification of this concern is important since TCE is one of the chemicals found abundantly at waste sites (R. Dyer, EPA, personal communication).

Given TCE's ubiquity as an industrial degreaser, the likelihood that workplace exposures to moderately high concentrations are not uncommon, and TCE's use for psychoactive effects at high concentrations, it would seem likely that, were it a potent neurotoxicant, more reports of neurotoxicity would have appeared in the medical literature and that graded manifestations would be evident. Thus, one must wonder at the causative factors underlying the several cases of severe neurotoxic reactions reported in humans (22,45). It is possible that, in some instances, the neurotoxicity is due to a degradation product such as dichloroacetylene formed from TCE in the presence of heat (1). Neuropathologic changes consequent to TCE exposures have been observed in dogs (2) and rabbits (4), but those studies must be considered with caution since at the time they were conducted it is possible that pure TCE was not used (49).

In gerbils chronically exposed to TCE by inhalation, no persistent postexposure neurobehavioral effects were observed except in the presence of trichloroethane which revealed a latent toxicity (20). Kulig (21), in a very systematic investigation, observed cumulative changes in several neurobehavioral parameters in rats, but the effects did not persist beyond the exposure period. Dorfmueller *et al.* (9) obtained no results indicative of maternal, embryonic, or developmental neural dysfunction associated with maternal exposures to 1800 ppm TCE for about 40 days. Thus, there remains considerable uncertainty concerning the neurotoxicity of pure TCE.

We, and others, have shown that electrophysiologic measurements such as the EEG and sensory-evoked potentials (EPs) provide reliable indications of persistent neurotoxicities when known neurotoxicants such as n-hexane, carbon disulfide, triethyltin, lead, mercury, etc., are administered to animals (6, 10, 11, 15, 23–25, 35, 36, 40). It seemed important, therefore, to determine if TCE neurotoxicity might be revealed by these techniques. Because TCE has been reported to be ototoxic to humans (47) and several solvents have been found to produce ototoxicity in rats (32, 34, 36) we examined tone intensity EP amplitude functions of the brainstem auditory-evoked response (BAER) to determine if TCE also produced hearing loss in rats. Long-Evans rats were used to examine the chronic effects of TCE and both Long-Evans and Fischer-344 rats were used to examine in more detail hearing loss suggested by results of the chronic experiment. The Fischer-344 rats were studied to confirm effects in the Long-Evans rats and to determine if effects in the two strains differed in any way.

TABLE 1

SUMMARY STATISTICS FOR PREP PIN2 AMPLITUDE IN RATS CHRONICALLY EXPOSED TO TRICHLOROETHYLENE

Condition n/Group	Week No.	Groups 1 = Control Mean (SE)	2 = 1600 Mean (SE)	3 = 3200 Mean (SE)	Significant ($p \leq 0.05$) t-Tests for Group Comparisons 1-2	1-3	2-3	df*	$F_{2,df}$
Baseline									
9,9,8	1	68.77 (8.91)	77.08 (8.12)	65.43 (8.97)					
9,10,10	2	64.19 (7.18)	71.63 (10.03)	67.60 (9.14)					
Exposure									
9,10,10	3	73.95 (6.91)	61.86 (6.46)	74.49 (6.74)					
9,9,9	5	70.16 (9.93)	64.37 (8.93)	58.49 (7.51)					
10,8,10	8	57.30 (7.93)	55.50 (6.76)	54.93 (7.37)					
9,8,9	11	54.62 (5.21)	59.87 (5.52)	46.99 (7.25)					
6,7,8	14	62.48 (4.76)	55.56 (9.53)	35.66 (3.94)		2.88	2.23	18	4.69
Recovery									
9,8,9	15	46.34 (7.01)	59.54 (8.08)	54.10 (7.61)					
8,8,9	17	56.59 (8.75)	61.66 (7.77)	47.62 (8.30)					

*df Based on pooled error term from ANOVA.

METHOD

Solvent

Mallinckrodt reagent grade trichloroethylene (labeled 99.8% pure) was used.

Subjects

Thirty male Long-Evans rats and fourteen male Fischer-344 rats (approximately 300 g, 100 days old at the time of exposure) were obtained from Simonsen Laboratories (Gilroy, CA). Before initiation of the exposures, the rats were housed three per cage in plastic cages (23 × 14 × 45 cm), with food and water available ad lib. The Fischer rats were studied after the chronic study of the Long-Evans rats was completed.

Surgical Procedures

The Long-Evans rats were implanted with three epidural electrodes consisting of 0–80 × 1/8-inch stainless steel screws that were placed over the olfactory bulb (reference electrode) and somatosensory (2 mm posterior and lateral to bregma) and visual (6 mm posterior and 3.5 mm right of bregma) cortices (35). The electrodes were attached by copper wire to a connector and the apparatus was cemented to the skull with dental acrylic. The surgery was carried out under sodium pentobarbital anesthesia (70 mg/kg IP); atropine sulfate (0.05 mg/kg IM) was used to inhibit mucous membrane secretions. Fischer-344 rats were not surgi-

TABLE 2

SUMMARY STATISTICS FOR PBAER RMS AMPLITUDE IN RATS EXPOSED TO TRICHLORETHYLENE

Condition n/Group	Week No.	Groups 1 = Control Mean (SE)	2 = 1600 Mean (SE)	3 = 3200 Mean (SE)	Significant ($p \leq 0.05$) t-Tests for Group Comparisons 1-2	1-3	2-3	df*	$F_{2,df}$
Baseline									
10,10,10	1	3.86 (0.37)	4.04 (0.27)	3.60 (0.25)					
10,10,10	2	3.06 (0.38)	3.79 (0.27)	3.16 (0.35)					
Exposure									
10,10,10	3	2.90 (0.35)	3.66 (0.33)	3.26 (0.40)					
10,9,10	5	2.71 (0.40)	3.19 (0.31)	2.00 (0.29)					
10,9,10	8	2.73 (0.31)	2.44 (0.30)	1.67 (0.29)		2.52	2.95	26	5.12
10,8,9	11	2.73 (0.33)	3.16 (0.38)	0.83 (0.20)		4.44	5.14	24	15.54
9,8,9	14	2.79 (0.40)	3.20 (0.39)	0.89 (0.21)		3.98	4.71	23	12.99
Recovery									
8,8,9	15	2.60 (0.49)	2.93 (0.37)	0.80 (0.17)		3.62	4.27	22	10.78
8,8,8	17	2.33 (0.55)	2.59 (0.40)	0.61 (0.14)		3.02	3.49	21	7.18

*df Based on pooled error term from ANOVA.

TABLE 3

SUMMARY STATISTICS FOR CBAER RMS AMPLITUDE IN RATS EXPOSED TO TRICHLOROETHYLENE

Condition n/Group	Week No.	Groups 1 = Control Mean (SE)	2 = 1600 Mean (SE)	3 = 3200 Mean (SE)	Significant ($p \leq 0.05$) t-Tests for Group Comparisons 1-2	1-3	2-3	df*	$F_{2/df}$
Baseline									
10,10,10	1	4.70 (0.40)	5.00 (0.31)	4.21 (0.38)					
10,10,10	2	4.63 (0.62)	4.86 (0.26)	4.73 (0.54)					
Exposure									
10,10,10	1	4.62 (0.60)	5.40 (0.39)	4.91 (0.61)					
10,9,10	2	4.70 (0.42)	4.72 (0.43)	3.25 (0.40)		2.52	2.49	26	4.214
10,9,10	3	4.42 (0.45)	3.88 (0.38)	2.42 (0.43)		3.37	2.41	26	6.082
10,8,9	4	4.45 (0.38)	4.51 (0.35)	1.71 (0.30)		5.76	5.56	24	21.405
9,8,9	5	4.37 (0.46)	4.13 (0.38)	2.00 (0.40)		4.11	3.59	23	10.113
Recovery									
8,8,9	1	4.10 (0.51)	3.65 (0.44)	1.86 (0.30)		3.84	3.06	22	8.39
8,8,8	2	3.58 (0.61)	3.61 (0.49)	1.47 (0.25)		3.14	3.18	21	6.66

*df Based on pooled error term from ANOVA.

cally implanted. Instead, they were anesthetized as above at the time of BAER recordings and 25-gauge needles were placed subcutaneously over the anterior portion of the nose and posterior skull along the midline.

Surgeries on the Long-Evans rats were carried out over the course of 11 days. Twenty-one days after the last surgery all the rats were examined electrophysiologically to verify that adequate EPs could be obtained. The first formal baseline tests were done thirteen days later, the day before the rats were placed in the inhalation chambers (this week was considered week 1 of the experiment). A second baseline test was conducted 7 days after the rats were placed in the chambers, the day before exposures began.

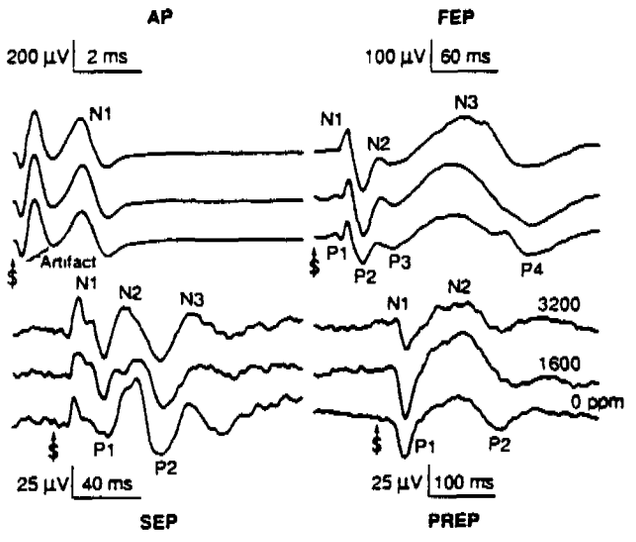

FIG. 1. Group-averaged EPs obtained from Long-Evans rats after the 14th week of the experiment (12th week of exposure) for control (bottom traces of each set of EPs) and the TCE-exposed rats. AP = compound action potential of the ventral caudal nerve. SEP = cortical shock (tail) EP. FEP = flash EP. PREP = pattern reversal EP. Only PREP amplitude differed among the groups (see Table 1).

Exposure Chambers and Schedule of Activities

The rats were housed and exposed in 62.5-liter, clear Plexiglas chambers (50 × 50 × 25 cm). One group of Long-Evans rats served as controls and was exposed to clean air at a continuous flow rate of 16–20 liters per min. A second group was exposed to 1600 ppm TCE. The remaining group was exposed to 3200 ppm TCE. A regulated volume of clean air was bubbled through the solvent and mixed with a larger volume of clean air to achieve the desired chamber concentrations. The chamber concentrations were verified daily by gas chromatography and generally remained within 10% of the intended concentrations.

The Long-Evans rats were exposed for 12 hours each day except on test days. The exposures lasted from 10:00 a.m. to 10:00 p.m. The lights were on from 8:00 a.m. to 8:00 p.m. Tests began 10 hr after the daily exposure ended. Exposures started at the beginning of the third week of the experiment. Electrophysiologic recordings were subsequently obtained at the end of Weeks 3, 5, 8, 11, and 14 of the experiment (after 1, 3, 6, 9, and 12 weeks of exposure). The exposures were terminated at the end of experimental Week 14, the day before the Week 14 test, and additional routine tests in the recovery period were obtained at the end of Weeks 15 and 17 of the experiment. Special auditory function tests were carried out during Week 16 of the experiment.

The Fischer-344 rats were exposed to air (n = 4), 2000 ppm (n = 5), or 3200 ppm (n = 5) TCE 12 hours a day for three weeks with the same light-dark and exposure schedule as the Long-Evans rats.

Electrophysiologic Measures

The latency and amplitude of the compound action potential (AP) of the ventral caudal nerve of the tail and various parameters (as described below) of brain-evoked potentials elicited by auditory, visual, and somatosensory stimulation were measured in Long-Evans rats. The rats were restrained in a specially de-

signed plastic holder that held the rat in correct orientation to the various sources of stimulation (37).

Tail Stimulation

To record the AP (1 Hz to 3 kHz recording bandpass) the tail was taped into a Plexiglas holder containing 25-ga needles that protruded about 1 mm into the ventral aspect of the tail. The recording electrode (referenced to another electrode 3 cm more proximal) was 6 cm from the stimulating cathode. The anode was 2 cm distal to the cathode, approximately 1 cm from the tip of the tail. Stimuli were 300 constant-current square waves of 3 mA and 50-μsec duration delivered at a rate of 19/sec. The cortical shock EP (SEP 1 Hz to 250 Hz recording bandpass) evoked from somatosensory cortex was elicited by 75 constant-current square waves of 3 mA and 50-μsec duration delivered to the tail at a rate of 1.3/sec.

Visual Stimulation

The flash EP (FEP, 1 Hz to 250 Hz recording bandpass) was elicited by 50 flashes of 10-μsec duration from a Grass PS-2 photostimulator (intensity setting of 8) presented at a rate of 1.3/sec. Pattern-reversal evoked potentials (PREPs, 1 Hz to 150 Hz recording bandpass) were elicited by 150 checkerboard reversals at a rate of 1.3/sec, using a Grass VPG 24 × 18 cm television monitor placed 8 cm in front of the rat. At that distance, checks in the center of the visual field subtended 14° at the 256-check setting. Contrast was set to maximum. Luminance from the screen, and of the light and dark checks separately, was determined with a Tektronix J6523 1° probe. When the probe covered four checks (two black, two white), luminance (average of 3 determinations) was 29.8 ft L. Dark checks were 2.9 ft L and light checks were 56.5 ft L (average of 5 determinations).

Auditory Stimulation

Two types of brainstem auditory-evoked responses were elicited by 1) 1000 clicks (square waves of 100-μsec duration applied to a high-frequency speaker, CBAER) or 2) 1000 tone pips (1-msec duration, 0.2 msec rise/fall times, PBAER) presented at a rate of 19/sec. Intensity of the click or pip was about 60 dB above the threshold for eliciting the BAER in control rats. The recording bandpass was 400 Hz to 6 kHz. The high highpass setting improved the signal-to-noise ratio and clarified early components. In separate tests, the intensity and frequency of the pips were varied to characterize an apparent hearing loss (vide infra).

Data Analysis

Due to time and technical constraints, acceptable data were acquired from 6 to 10 Long-Evans rats from each group during each test session. EPs were usually quantified by measuring latencies and peak-to-peak amplitudes of waveform components. Overall waveform amplitudes of BAERs were obtained by integrating the waveforms from the first through the fifth components, a procedure previously found useful in evaluating stimulus intensity-EP amplitude functions (36). Chronic effects were statistically evaluated by one-way analyses of variance (ANOVA) across groups at each measurement time. It was not possible to carry out a repeated-measures ANOVA across time because of unequal numbers of measurements at the different times. Significant F-ratios were followed by least-significant-difference *t*-test comparisons between each exposed group and the controls, using the residual degrees of freedom and error variance from the analyses of variance. Intensity-amplitude functions of PBAERs were evaluated by two-factor (Group × Intensity) repeated-measures ANOVAs for each frequency separately.

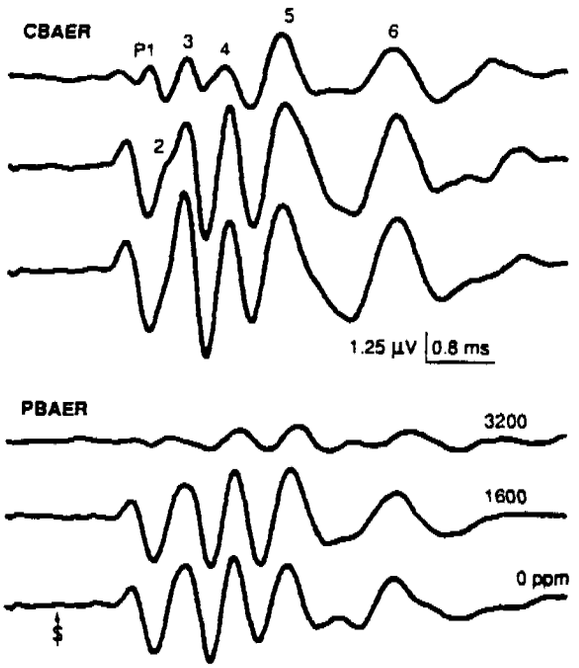

FIG. 2. Group-averaged CBAERs (upper) and PBAERs obtained from Long-Evans rats after the 14th week of the experiment (12th week of exposure) for control and TCE-exposed groups. The stimulus marker indicates the approximate time of arrival of sound to the ear.

RESULTS

Body Weight and Temperature

Mean body weights of the exposed Long-Evans groups remained comparable to control weights throughout the experiment. All rats gained weight; average weights increased from about 400 g to about 525 g over the course of the experiment. Colonic temperatures also remained normal in the exposed groups; temperatures were 37.8 (0.3 = SD), 37.5 (0.12), 37.7 (0.51)°C in control, 1600 ppm, and 3200 ppm groups, respectively, recorded after nine weeks of exposure.

Electrophysiologic Parameters

Group-averaged waveforms of Long-Evans rats obtained after 12 weeks of exposure for the AP, SEP, FEP, and PREP are shown in Fig. 1. Except for the PREP, the waveforms were comparable among the groups throughout the experiment and statistical analyses revealed no systematic differences across groups for any parameter of the AP, SEP, or FEP. There was a tendency for PREP amplitude to be smallest in the 3200 ppm group; for component P1N2 and the integrated amplitude score the F-ratios were significant, $F_{P1N2}(2,18) = 4.7$, $p = 2.3 \times 10^{-2}$; $F_{RMS}(2,18) = 3.7$, $p = 4.5 \times 10^{-2}$, at the last exposure test. However, there was a general trend toward reduced amplitude in all groups over the course of the experiment, and there were no significant differences in the recovery phase (Table 1).

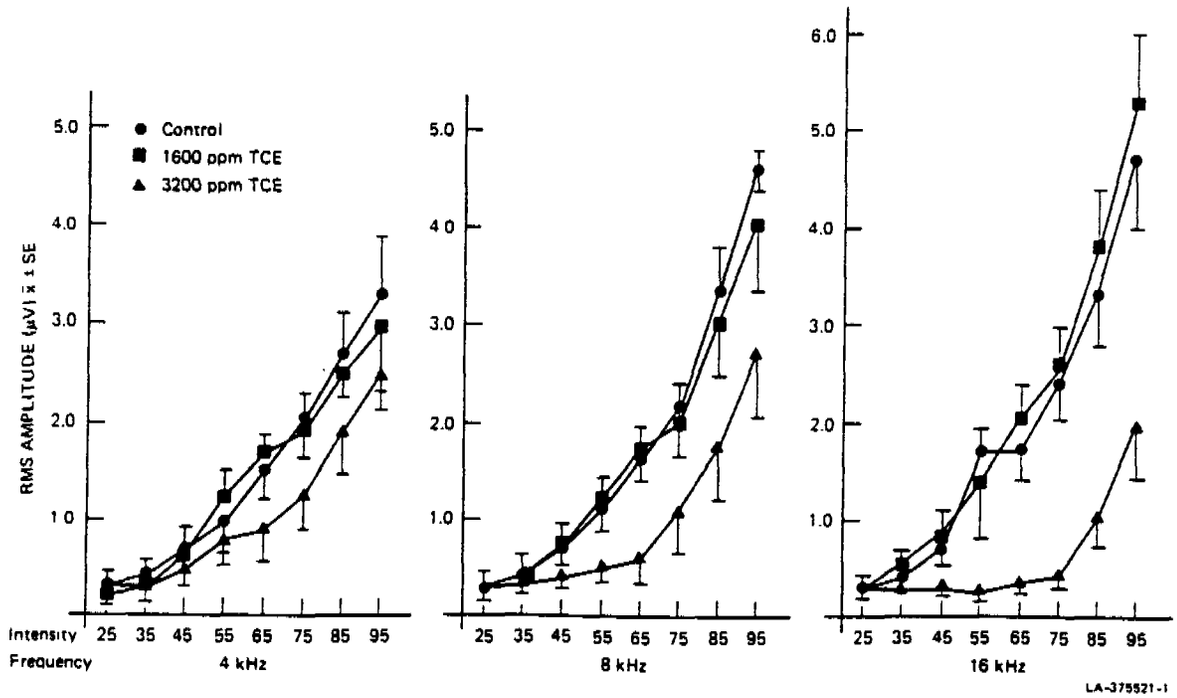

FIG. 3. Intensity-amplitude functions for pip-evoked BAERs at different frequencies in Long-Evans rats exposed to air or trichloroethylene (TCE) showing predominantly mid-to-high frequency hearing loss in rats exposed to 3,200 ppm TCE. These tests were conducted during the second week of the recovery period.

CBAER and PBAER group-averaged waveforms from Long-Evans rats are shown in Fig. 2. Amplitudes of all components were depressed in the 3200 ppm group, but the effect appeared to be more severe on the PBAER than the CBAER. This general effect was best reflected by a waveform integration score. Reductions of amplitudes were statistically significant for CBAERs by the second exposure test carried out after three weeks of exposure. At this time the overall F-ratio was significant for the CBAER, $F(2,26)=4.2$, $p=2.6\times10^{-2}$, but not for the PBAER. Follow-up tests indicated that only the 3200 ppm group differed from controls. However, the overall F-ratios for both responses were significant for all subsequent tests through the third week of the recovery phase [e.g., for the third week in recovery, $F_{PBAER}(2,21)=7.2$, $p=4.2\times10^{-3}$; $F_{CBAER}(2,21)=6.66$, $p=6.1\times10^{-3}$]. The effect was limited throughout to the 3200 ppm group (Table 2: PBAER; Table 3: CBAER).

Although CBAER amplitude decreased there was no effect on the latency of any component nor on the several interwave times (1–3, 3–5, 1–5). In contrast, increased latency of PBAER Component 5 and the 3–5 and 1–5 interwave times were statistically significant after nine weeks of exposure [e.g., $F_{p5}(2,24)=3.91$, $p=3.4\times10^{-2}$]. Again, the effect was limited to the 3200 ppm group.

To further examine the possible ototoxicity reflected by the decreased BAER amplitudes, tests were run using tone pips at frequencies of 4, 8, or 16 kHz and eight intensities ranging from 25 to 95 dB (machine settings), a range of intensities known to elicit a series of BAERs graded in amplitude (36). These results were analyzed by repeated-measures ANOVA (Intensity × Group) of RMS amplitudes for each frequency separately. In each case there was a highly significant effect of stimulus intensity ($Fs>55$, $ps<1.0\times10^{-5}$). At 4 kHz the Group × Intensity interaction was minimally significant, $F(14,126)=1.79$, $p=4.7\times10^{-2}$. Although only marginally significant, at almost all intensities PBAER RMS amplitude was smaller in the 3200 ppm group than in the others (Fig. 3). This effect was more pronounced in the 8-kHz condition and the interaction term was highly significant, $F(14,126)=2.89$, $p=8.2\times10^{-4}$. The largest effect was observed with the 16-kHz stimulus, $F(14,126)=6.08$, $p=4.5\times10^{-9}$. For all frequencies, the effect was confined to the high-exposure group.

The generality of TCE ototoxicity across rat strains was evaluated by testing its effect in Fischer-344 rats. The results (Fig. 4) were essentially the same as for the Long-Evans rats except that PBAER amplitudes at the 2000 ppm concentration were between the control values and those of the high-concentration group, whereas the Long-Evans rats exposed to 1600 ppm were unaffected. Interaction F-ratios (Intensity × Group) were as follows for 4-, 8-, and 16-kHz stimuli, respectively: $F_{4k}(14,63)=2.54$, $p=6.1\times10^{-3}$; $F_{8k}(14,70)=4.84$, $p=3.9\times10^{-6}$; $F_{16k}(14,70)=14.69$, $p=1.2\times10^{-15}$.

DISCUSSION

In general, the results of this experiment were similar to those of Kulig (21) in that there was no evidence to indicate that TCE is a potent neurotoxicant. As Kulig has pointed out with respect to the test of caudal nerve function—and, as we noted in the Introduction, the point is valid for the other tests as well—the EP measures are affected by a variety of known neurotoxicants. It has also been reported that the relative potencies of several anticancer drugs with respect to human neurotoxicity are similar in rats as evidenced by EPs (40). The lack of general neurotoxic effects in this experiment is not, therefore, due to an absence of

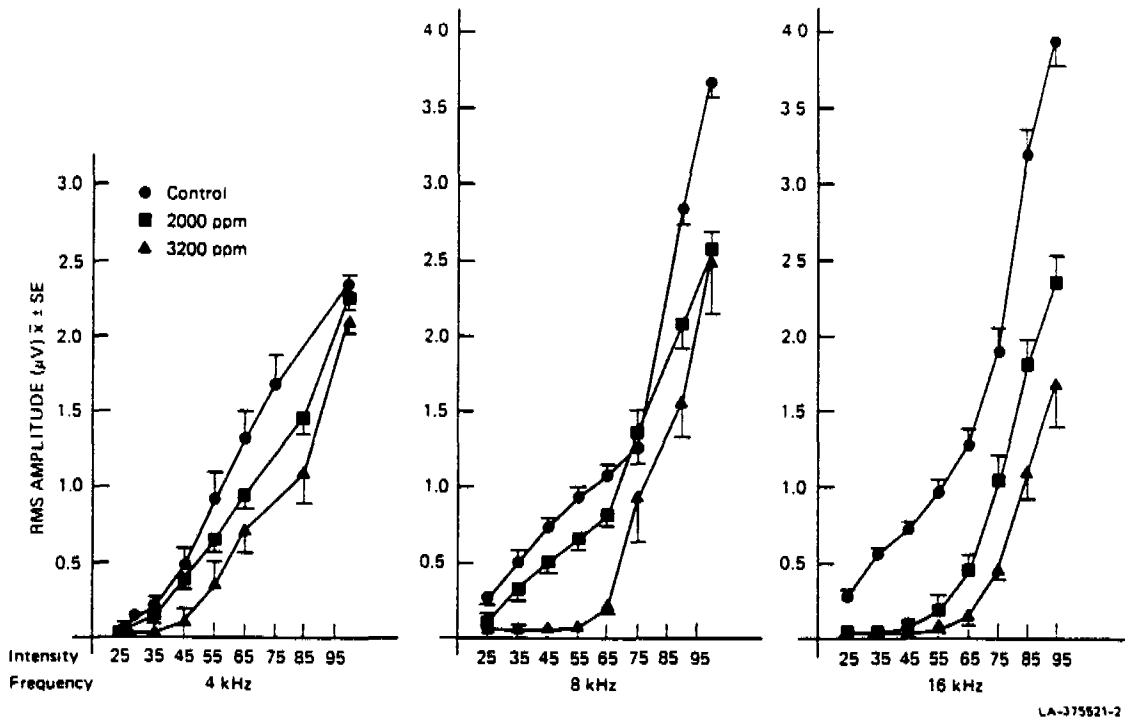

FIG. 4. Intensity-amplitude functions for pip-evoked BAERs of different frequencies in Fischer-344 rats exposed to trichloroethylene (TCE) showing predominantly mid-to-high frequency hearing loss in rats exposed to 2,000 and 3,200 ppm TCE. These tests were conducted one week after the end of the exposure.

sensitivity of the measures to neural dysfunction. However, this is not to say that with further procedural modifications or other tests, subtle neurotoxic effects of TCE might not be detected. For example, systematic manipulation of parameters of pattern reversal stimulation (7) might reveal persistent effects, as there was some indication that the PREP was affected during the exposures. Also, whereas routinely obtained EPs are not altered by toluene (12,31), the results of Dyer *et al.* (12) suggest that recovery cycle procedures might provide added sensitivity. Another factor of possible importance is the concentration of TCE used in this study. Typically, rats exposed to neurotoxicants gain weight more slowly than unexposed controls, or lose weight, but that was not the case in this investigation. Perhaps TCE levels sufficient to produce an effect on weight gain would also cause persistent neural dysfunction.

Although one would expect to reject the null hypothesis more than 5% of the time when a large number of variables are evaluated several times (i.e., inflated type 1 errors), in other experiments we have noted considerable consistency in EP parameters when several baseline tests were run on the same rats (37,39), with type 1 errors of about 7%, depending on the particular EP. In this study, for example, there were no indications of any systematic effects of treatments for the AP, SEP, or FEP. Of 63 comparisons for the AP none were significant at $p<0.05$; four of 99 comparisons were significant for the SEP but in no consistent pattern with respect to components or experimental variables; two of 108 comparisons were significant for the FEP. Overall, only 2.2% of comparisons for these three EPs were significant. We wonder, therefore, if statistical corrections for potentially inflated type 1 errors might not, in some cases, result in excessive type 2 errors.

Pryor (29) has shown that chronic inhalation exposure of rats to toluene at concentrations that produce no changes in EPs (except the BAER) induces abnormalities in gait and landing foot splay reminiscent of the cerebellar ataxia observed in heavy abusers of solvents (42). Because of certain similarities in the effects of TCE and toluene, e.g., they are both excitatory in the vestibular-oculomotor reflex test (48) and similarly ototoxic as shown here, we suspect that TCE might also induce gait abnormalities. Kulig (21) did not detect changes in a measurement of coordinated movements, but the method used did not allow evaluation of the relationships among the four limbs, an important dimension in Pryor's results.

As indicated by some reviewers (8,19) the American literature on solvent neurotoxicity, until recently, contained no systematic assessment of the effect of solvents on hearing, but reference to hearing loss associated with human exposures to solvents appeared in Europe as early as 1959 (28) and perhaps 1955 (5) and Gieldanowski (17) examined the effects of several alcohols on cochlear microphonics in cats in 1965. From most clinical/industrial reports of hearing loss associated with solvent exposures (3, 13, 14, 26, 27) it is not possible to definitively identify the responsible solvent(s) because the exposures generally involved mixtures. Thus, considerable interest in the experimental analysis of solvent ototoxicity was precipitated by the observations of Pryor *et al.* (32) and Rebert *et al.* (41) that toluene caused a mid-to-high frequency hearing loss in rats, measured behaviorally and electrophysiologically (BAER). The loss of hair cells in toluene ototoxicity has also been confirmed (30,46). Experimentally induced hearing loss in rats has now also been shown for xylene, styrene (34), carbon disulfide (36), and TCE. For TCE, our data suggests that the threshold concentration (12 hr per day) is around 2,000 ppm as the Long-Evans rats exposed to 1,600 ppm did not exhibit hearing loss, whereas the Fischer-344 rats exposed to 2,000 ppm

TCE did show a loss; both strains exposed to 3,200 ppm exhibited similar hearing loss. These results also indicate the ototoxicity is not peculiar to the albino rat. It is interesting to note that 11 weeks of exposure to the subthreshold TCE level (1,600 ppm) did not result in hearing loss, whereas only three weeks of exposure to 2,000 ppm TCE was ototoxic. Similar results have been obtained with toluene (33).

The mechanisms of solvent-induced ototoxicity are not known. A commonality of toluene and TCE is their unsaturated structure. Of a variety of aromatic and halogenated hydrocarbons tested for their effects on vestibulo-ocular postrotatory nystagmus (48), it was noted that, with very few exceptions, the major determinant of excitation or inhibition of the reflex was the presence or absence of chemical double bonds. Unsaturated structures produced excitation. We have similarly noted potent ototoxicity of double-bonded structures such as toluene, xylenes, styrenes, and trichloroethylene, but no, or minimal, ototoxicity of compounds such as dichloromethane, n-hexane, and methyl-ethyl-ketone. We recently observed that acute exposure to toluene enhances late components of the BAER (38) and we suggested that the effect could be mediated by differential hyperexcitability in various parts of the cochlea: such an effect might underlie chronic ototoxicity. That notion was supported by the observation that the amplitude enhancement was related to the frequency of the eliciting stimulus (click or tone pip). These conjectures suggest that many, but not all, solvents might produce hearing loss.

ACKNOWLEDGEMENTS

This research was supported by EPA Agreement CR-810275 and, in part, by NIDA Contract 271-87-3132. The authors express their appreciation to Dr. Robert S. Dyer, EPA, and Dr. Charles Sharp at NIDA, for their encouragement and support of this work, and to Rosie McCormick and Gloria Sonsona for typing the manuscript.

REFERENCES

1. Annau, Z. The neurobehavioral toxicity of trichloroethylene. Neurobehav. Toxicol. Teratol. 3:417–424; 1981.
2. Baker, A. B. The nervous system in tricholoroethylene (poisoning): an experimental study. J. Neuropath. Exp. Neurol. 17:649–655; 1958.
3. Barregard, L.; Axelsson, A. Is there an ototraumatic interaction between noise and solvents? Scand. Audiol. 13:151–155; 1984.
4. Bartonicek, V. J.; Brun, A. Subacute and chronic trichloroethylene poisoning: a neuropathological study in rabbits. Acta Pharmacol. Toxicol. 28:359–369; 1970.
5. Bock, E.; Sahanek, O.; Brockova, V.; Dostal, O.; Chytka, E. Prumyslove otrary trichlorethylenem. Prac. Lek. 7:12; 1955.
6. Boyes, W. K.; Dyer, R. S. Pattern reversal and flash evoked potentials following acute triethyltin exposure. Neurobehav. Toxicol. Teratol. 5:571–577; 1983.
7. Boyes, W. K.; Dyer, R. S. Pattern reversal visual evoked potentials in awake rats. Brain Res. Bull. 10:817–823; 1983.
8. Brown, R. D.; Feldman, A. M. Pharmacology of hearing and ototoxicity. Ann. Rev. Pharmacol. Toxicol. 18:233–252; 1978.
9. Dorfmueller, M. A.; Henne, S. P.; York, R. G.; Bornschein, R. L.; Manson, J. M. Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene. Toxicology 14:153–166; 1979.
10. Dyer, R. S. The use of sensory evoked potentials in toxicology. Fundam. Appl. Toxicol. 5:24–40; 1985.
11. Dyer, R. S.; Howell, W. E.; Reiter, L. W. Neonatal triethyltin exposure alters adult electrophysiology. Neurotoxicology 2:603–611, 1981.
12. Dyer, R. S.; Muller, K. E.; Janssen, R.; Barton, C. N.; Boyes, W. K.; Benignus, V. A. Neurophysiological effects of 30 day chronic exposure to toluene in rats. Neurobehav. Toxicol. Teratol. 6:363–368; 1984.
13. Ehyai, A.; Freemon, F. R. Progressive optic neuropathy and sensorineural hearing loss due to chronic glue sniffing. J. Neurol. Neurosurg. Psychiatry 46:349–351; 1983.
14. Fornazzari, L.; Carlen, P. L.; Wilkinson, D. A.; Kapur, B. Irreversible cortical and cerebellar impairment in toluene abusers. Neurology 32:A515; 1982.
15. Fox, D. A., Loundes, H. E.; Birkamper, G. G. Electrophysiological techniques in neurotoxicology. In: Mitchell, C. L., ed. Nervous system toxicology. New York: Raven Press; 1982:229–335.
16. Fuller, B. B. Air pollution assessment of trichloroethylene. Washington, DC: EPA-68-02-1495; 1976.
17. Gieldanowski, J. Badania nad toksycznym dzialaniem niektorych akoholi na narzad cortiego. Med. Pracy 16:448–451; 1965 (English summary).
18. Grandjean, E. The effects of short exposures to trichloroethylene in swimming performance and motor activities of rats. Am. Ind. Hyg. Assoc. 24.371–379. 1963.
19. Guth, P. S., Bobbin, R. P. The pharmacology of peripheral auditory processes: cochlear pharmacology. Adv. Pharmacol. Chemother. 9: 93–130; 1971.
20. Kjellstrand, P.; Lanke, J.; Bjerkemo, M.; Zetterquist, L.; Mansson, L. Irreversible effects of trichloroethylene exposure on the central nervous system. Scand. J. Work Environ. Health. 6:40–47; 1980.
21. Kulig, L. M. The effects of chronic trichloroethylene exposure on neurobehavioral functioning in the rat. Neurotoxicol. Teratol. 9:171–178; 1987.
22. Lawrence, W. H.; Partyka, E. K. Chronic dysphagia and trigeminal anaesthesia after trichloroethylene exposure. Ann. Intern. Med. 95: 710; 1981.
23. Mattsson, J. L.; Albee, R. R. Sensory evoked potentials in neurotoxicology. Neurotoxicol. Teratol. 10:435–443; 1988.
24. Mattsson, J. L.; Albee, R. R.; Eisenbrandt, D. L.; Chang, L. W. Subchronic neurotoxicity in rats of the structural fumigant, sulfuryl fluoride. Neurotoxicol. Teratol. 10:127–133; 1988.
25. Mattsson, J. L.; Alligood, J.; Koering, J.; Levin, S.; Miller, E. Early effects of methylmercury on the visual evoked response of the dog. Neurotoxicology 2:499–514; 1981.
26. Mettrick, S. A.; Brenner, R. P. Abnormal brainstem auditory evoked potentials in chronic paint sniffers. Ann. Neurol. 12:553–556; 1982.
27. Odkvist, L. M.; Bergholtz, L. M.; Ahlfeldt, H.; Andersson, B.; Edling, Ch., Strand, E. Otoneurological and audiological findings in workers exposed to industrial solvents. Acta Otolaryngol. Suppl. 386:249–251; 1982.
28. Pierangeli, C. E.; Carmi, G.; DiBrino, M. L'azione neurotossica del tetracloruro di carbonio sulle strutture centrali e periferiche della vie acustica e vestibolare. Clin. Otorinolarigoliat. 11:1–38; 1959 (English summary).
29. Pryor, G. T. Animal models for evaluating the neurobehavioral consequences of solvent exposure. Second meeting of the International Toxicology Association. Sitges, Spain: May, 1989.
30. Pryor, G. T., Dickinson, J.; Feeney, E.; Rebert, C. S. Hearing loss in rats first exposed to toluene as weanlings or as young adults. Neurobehav. Toxicol. Teratol. 6:111–119; 1984.
31. Pryor, G. T.; Dickinson, J.; Howd, R. A.; Rebert, C. S. Neurobehavioral effects of subchronic exposure of weanling rats to toluene or hexane. Neurobehav. Toxicol. Teratol. 5:47–52; 1983.
32. Pryor, G. T.; Dickinson, J.; Howd, R. A.; Rebert, C. S. Transient cognitive deficits and high-frequency hearing loss in rats exposed to toluene. Neurobehav. Toxicol. Teratol. 5:53–57; 1983.
33. Pryor, G. T., Rebert, C. S.; Dickinson, J.; Feeney, E. Factors affecting toluene-induced ototoxicity in rats. Neurobehav. Toxicol. Teratol. 6:223–238; 1984.
34. Pryor, G. T., Rebert, C. S.; Howd, R. A. Hearing loss in rats caused by inhalation of mixed xylenes and styrene. J. Appl. Toxicol. 7:55–61; 1987.
35. Rebert, C. S. Multisensory evoked potentials in experimental and applied neurotoxicology. Neurobehav. Toxicol. Teratol. 5:659–671; 1983.

36. Rebert, C. S.; Becker, E. Effects of inhaled carbon disulfide on sensory-evoked potentials of Long Evans rats. Neurobehav. Toxicol. Teratol. 8:533–541; 1986.
37. Rebert, C. S.; Davis, E. E.; Juhos, L. T.; Jensen, R. A.; Pryor, G. T.; Robin, D. E. Effect of acute respiratory acidosis on multimodality sensory evoked potentials of Long Evans rats. Int. J. Psychophysiol. 8:155–168; 1989.
38. Rebert, C. S., Matteucci, M. J.; Pryor, G. T. Multimodal effects of acute exposure to toluene evidenced by sensory-evoked potentials from Fischer-344 rats. Pharmacol. Biochem. Behav. 32:757–768; 1989.
39. Rebert, C. S.; Matteucci, M. J.; Pryor, G. T. Acute effects of inhaled dichloromethane on the EEG and sensory-evoked potentials of Fischer-344 rats. Pharmacol. Biochem. Behav. 34:619–629; 1989.
40. Rebert, C. S.; Pryor, G. T.; Frick, M. S. Effects of vincristine, maytansine, and *cis*-platinum on behavioral and electrophysiological indices of neurotoxicity in the rat. J. Appl. Toxicol. 4:330–338; 1984.
41. Rebert, C. S.; Sorenson, S. S.; Howd, R. A.; Pryor, G. T. Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. Neurobehav. Toxicol. Teratol. 5:59–62; 1983.
42. Rosenberg, N. L.; Spitz, M. C.; Filley, C. M.; Davis, K. A.; Schaumburg, H. H. Central nervous system effects of chronic toluene abuse—Clinical, brainstem evoked response and magnetic resonance imaging studies. Neurotoxicol. Teratol. 10:489–495; 1988.
43. Schwetz, B. A.; Leong, K. J.; Gehring, P. J. The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32:84–96; 1975.
44. Sharp, C. W.; Brehm, M. L., eds. Review of inhalants: Euphoria to dysfunction. NIDA Monograph 15. Washington, DC: U. S. Government Printing Office; 1977.
45. Steinberg, W. Residual neuropsychological effects following exposure to tricholorethylene (TCE): a case study. Clin. Neuropsychol. 3:1–4; 1981.
46. Sullivan, M. J.; Rarey, K. E.; Conolly, R. B. Ototoxicity of toluene in rats. Neurotoxicol. Teratol. 10:525–530; 1989.
47. Szulc-Kuberska, J. Zagadnienia xybrane z dziedziny przewleklych przemyslowych zatrue trojchloroetylenem Folia Med. Lodz. 16:67–90; 1972 (English summary).
48. Tham, R.; Bunnfors, I.; Eriksson, B.; Larsby, B.; Lindgrin, S.; Odkvist, L. M. Vestibulo-ocular disturbances in rats exposed to organic solvents. Acta Pharmacol. Toxicol. 54:58–63; 1984.
49. Waters, E. M.; Gerstner, H. B.; Huff, J. E. Trichloroethylene: I. an overview. J. Toxicol. Environ. Health 2:671–707; 1977.