TOXICOLOGY AND APPLIED PHARMACOLOGY 68, 260–267 (1983)

Triethyltin Decreases Maximal Electroshock Seizure Severity in Adult Rats[1]

D. A. FOX[2] AND S. V. DOCTOR

Division of Toxicology, Department of Pharmacology, The University of Texas Medical School, Houston, Texas 77025

Received July 31, 1982; accepted January 11, 1983

Triethyltin Decreases Maximal Electroshock Seizure Severity in Adult Rats. FOX, D. A., AND DOCTOR, S. V. (1983). *Toxicol. Appl. Pharmacol.* **68**, 260–267. Acute and subacute treatment of adult rats with triethyltin bromide (TET) caused dose-dependent and time-dependent decreases in maximal electroshock seizure (MES) severity. This decrease in excitability was characterized by both a decrease in the percentage of animals exhibiting a maximal seizure and a corresponding decrease in the extension durations and an increase in the flexion durations. Acutely treated rats received (ip) 0, 1, or 5 mg/kg TET while subacutely exposed (po) received 0, 1, 5, or 10 ppm TET in the drinking water for 10 days. Experiments were designed so that the total consumed dose of TET, on a milligram per kilogram basis, equaled that in the acute experiment. No alterations in body weight were observed in either experiment. Acutely, the onset of action of TET was detectable within 0.5 hr. For the 1 mg/kg group, the effect peaked between 4 and 24 hr and completely recovered by 72 to 96 hr. For the 5 mg/kg group, the marked effect peaked at 4 hr, however, no recovery was observed. Subacute exposure for 1 to 2 days produced marked decreases in MES severity which were still present in the 5- and 10-ppm groups 14 days after cessation of exposure. Comparison of the onset and recovery data in the acute and subacute experiments revealed a close correspondence in similarly dosed rats. Comparison with other MES data from our laboratory revealed that adult rats were more sensitive to TET than adult mice or developing rats. Additionally, the MES test was able to detect subtle functional alterations in the central nervous system at lower doses of TET than previously reported neurobehavioral evaluation procedures.

Organotins are a group of biologically active organometallics that are being increasingly used as biocides, preservatives, catalysts, and polymer stabilizers (van der Kerk, 1976). In adult animals, acute exposure to sublethal doses of triethyltin (TET) produces reversible, dose-related neurophysiological, behavioral, and neurochemical alterations. Gerren *et al.* (1976) noted that mice exposed to 5 to 8 mg/kg TET displayed a decrease in the frequency of the electroencephalogram (EEG), an increase in the latency of the primary component of the visual evoked potential, and a decrease in spontaneous locomotor activity which were observed within 15 min followed by a complete recovery within 24 hr. In rats, small changes (Gerren *et al.*, 1976; Reiter *et al.*, 1981) in spontaneous locomotor activity were found following 1 to 3 mg/kg TET while slight, if any, changes in the EEG were found following an acute dose of 1 mg/kg TET. In contrast, a single dose of either 1 or 5 mg/kg

[1] This work was supported in part by NIEHS Toxicology Training Grant ES07090, NIOSH Grant OH07085, and a University of Texas Biomedical Research Grant. It was presented at the 20th annual meeting of the Society of Toxicology, San Diego, Calif., March 1–5, 1981.

[2] Send correspondence to: Dr. Donald A. Fox, Neurotoxicology Laboratory, College of Optometry, University of Houston, Houston, Tex. 77004.

0041-008X/83 $3.00

TET in mice produced a marked anticonvulsant effect, which was detectable within 30 min, peaked at 4 hr and completely recovered by 96 hr (Doctor and Fox, 1982a). Similar doses of TET have caused *in vivo* neurochemical alterations, such as decreased brain glucose metabolism (Cremer, 1970), decreased levels of whole brain norepinephrine and serotonin (Moore and Brody, 1961), and inhibition of oxidative phosphorylation in rat brain (Brody and Moore, 1962).

The maximal electroshock seizures (MES) test is an experimental tool which has been utilized by the pharmaceutical industry for over 35 years to screen potential grand mal-type anticonvulsant drugs. Its usefulness is based on the fact that the technique assesses the overall excitatory–inhibitory (i.e., convulsant–anticonvulsant) balance in the cerebrospinal axis (Purpura *et al.*, 1972). In addition, the MES test has been demonstrated to be a sensitive and quantifiable indicator of toxicant-induced CNS alterations (Woolley, 1970; Fox *et al.*, 1979; Fox, 1982; Doctor and Fox, 1982a,b, 1983) and to provide insight into the mechanisms by which neurotoxicants may act (Woolley, 1970; Fox *et al.*, 1979; Fox, 1982).

The present series of experiments was designed to examine the acute and subacute effects of TET in adult rats by employing the MES test. The major goals of this project were to compare the sensitivity of: (1) adult rats to equivalent doses of TET administered acutely and subacutely, (2) the MES test, relative to other neurobehavioral tests, in detecting alterations following TET exposure, (3) adult rats and adult mice dosed with the same doses of TET (Fox, 1982; Doctor and Fox, 1982a), and finally (4) adult and developing rats administered similar doses of TET (Doctor and Fox, 1983).

METHODS

General Experimental Design

Female Long–Evans hooded rats (240 to 260 g) obtained from Charles River Breeding Company (Wilmington, Mass.) were housed singly in hanging stainless-steel bottom cages in a room maintained on a 12:12 hr light:dark cycle commencing at 0700 hr and having a constant temperature (21 ± 1°C). The rats were provided Purina rat chow (Code No. 5001) *ad libitum* and depending upon the particular experiment or phase of experiment (detailed below), the rats were either provided water, 2% ethanol, a NaBr solution, or TET solution *ad libitum*. Groups of 8 to 10 animals were utilized for each dose level of TET administered. Two replications were performed.

Since 20 to 35% of all rats MES tested do not exhibit maximal (grade 5) seizures following electrical stimulation (Woodbury and Davenport, 1952; Fox *et al.*, 1979), it was necessary to exclude all rats exhibiting less than a grade 5 seizure prior to the initiation of the actual experiment. This step is especially important when the tested compound possesses anticonvulsant properties (e.g., TET). Two weeks prior to the start of TET exposure, all rats were MES tested (i.e., screened), and only those exhibiting a maximal seizure were used for these experiments.

In the acute exposure experiment, TET-Br in a 2% ethanol vehicle was injected (ip). Doses were 0, 1, or 5 mg/kg with the control group receiving either tap water or ethanol. Injections were given at the same time each day, between 0800 and 0900 hr, to avoid circadian rhythm effects. All of the animals were seizure evaluated 0.5, 4, 22, and 72 to 96 hr following injection.

In the subacute exposure experiment, TET-Br (Alpha Products, Danvers, Mass.) in a 2% ethanol vehicle was added to the drinking water to yield solutions of 1, 5, and 10 ppm TET. Controls received either tap water, 2% ethanol, or a 10-ppm NaBr drinking solution. The exposure period lasted for 10 days followed by a 14-day recovery period in which all rats received tap water. All rats were MES tested daily during the exposure period and during the first week of recovery, and also on Days 11 and 14 of the recovery period. Fluid consumption and body weight were monitored daily throughout the entire experiment so that the mean daily dose and total dose of TET could be computed.

MES Testing

A Model G electroshock stimulator with three timers was used to elicit and time the MES patterns (Wahlquist Instrument Co., Salt Lake City, Utah). The methods used for eliciting and scoring the MES patterns were modified from techniques described by Woodbury and Davenport (1952) and Fox *et al.* (1979). Briefly, disk electrodes were lightly coated with electrode cream (Hewlett–Packard Redox-Cream) and placed on the animals scalp approximately over the medial portion of each temporal cortex between the ears and the eyes. A sine wave (60 Hz) stimulus of 1 mA/g body wt was administered with a 0.2-sec duration. The timers, attached to the electroshock stimulator, were activated with the delivery of the shock and

stopped manually at the termination of each phase of the seizure. The indexes measured in this study were forelimb flexion (FF), forelimb extension (FE), hindlimb flexion (HF), and hindlimb extension (HE).

The MES pattern in rats is characterized by the sequential appearance of graded clonic–tonic seizures of increasing severity: grade 1, clonus; grade 2, tonic FF; grade 3, tonic FF followed by FE; grade 4, tonic FF and HF followed by FE; and grade 5, tonic FF and HF followed by FE and HE.

Statistical Analyses

Statistical analysis of the fluid consumption data and the duration data was performed by an overall unweighted mean's analysis of variance (ANOVA). Statistical significance levels reported in the tables are based on Duncan's test as described by Winer (1971). The χ^2 tests, used to evaluate seizure grade distributions, were performed according to methods described by Seigel (1956).

RESULTS

Adult rats exposed to TET, acutely and subacutely, displayed a dose-dependent decrease in MES severity as assessed by seizure grade distributions and duration of seizure phases. No alterations in body weight were observed in either experiment.

Acute Experiment

In the control group of rats, 100% had grade 5 (maximal) seizures at 0.5, 22, and 72 to 96 hr following administration of the 2% ethanol vehicle while 75% had grade 5 seizures at 4-hr postvehicle administration (Table 1). At no time were the forelimb or hindlimb seizure phase durations different within the control group (Table 2). The mean (±SE) control MES seizure phase durations (sec) were as follows: FF (1.3 ± 0.1), FE (12.2 ± 0.3), HF (5.2 ± 0.6), and HE (8.1 ± 0.9).

Compared to controls, an acute injection of TET (1 or 5 mg/kg) produced a dose-dependent and time-dependent decrease in MES severity. This effect was characterized by both a decrease in the percentage of animals exhibiting a maximal seizure (shift to the left in the seizure grade distribution) as seen in Table 1 and a corresponding decrease in the extension durations and an increase in the flexion durations (Table 2). The low dose of TET produced a mild, but significant, seizure grade shift that peaked between 4 and 24 hr. During this time interval, approximately 20% of the rats still exhibited HE while approximately 60% exhibited HF (Table 1). In contrast, the 5 mg/kg dose of TET caused a marked seizure grade shift such that HE was eliminated during the entire testing period (0.5 to 96 hr), and HF was abolished at 4 hr, the peak time of effect (Table 1). As seen in Table 2, the low dose of TET produced (i) at 0.5 hr, a 33% increase and a 15% decrease in FF and FE durations, respectively, and (ii) at 4 hr, a 64% increase and a 25% decrease in FF and FE durations, respectively. The high dose of TET produced (i) at 0.5 hr, and 83% increase and

TABLE 1

DISTRIBUTION OF ADULT RATS EXHIBITING VARIOUS MAXIMAL ELECTROSHOCK SEIZURE GRADES FOLLOWING AN ACUTE EXPOSURE TO TRIETHYLTIN (TET)

Time after exposure (hr)	Treatment (mg/kg)	Grades (%)			
		2	3	4	5
0.5	0 (EtOH)	0	0	0	$100^{a,b}$
	1	0	8	50	$42^{a,j}$
	5	14	36	50	$0^{b,j}$
4	0 (EtOH)	0^{j}	0	25^{n}	$75^{c,k}$
	1	0^{m}	15	69^{n}	15^{k}
	5	$50^{l,m}$	50	0	0^{c}
22	0 (EtOH)	0	0^{f}	0	$100^{d,e}$
	1	0	23^{o}	54	23^{d}
	5	0	$64^{f,o}$	36	0^{e}
72 to 96	0 (EtOH)	0	0	0^{h}	100^{g}
	1	0	8	17^{i}	75
	5	0	8	$92^{h,i}$	0^{g}

Note. Values presented are the percentages of animals exhibiting each seizure grade with 12 to 15 subjects per TET treatment group. The effects of TET treatment were evaluated statistically by χ^2 analysis of values for rats within the same seizure grade. Values sharing the same superscript differed at the following levels of significance.

$^{a-j}$ $P < 0.001$.

$^{j-m}$ $P < 0.01$.

^{n,o} $P < 0.05$.

TABLE 2

DURATIONS (sec) OF FLEXION AND EXTENSION FOLLOWING ACUTE TRIETHYLTIN (TET) EXPOSURE IN ADULT RATS

Time after exposure (hr)	Treatment (mg/kg)	Forelimb flexion	Forelimb extension	Hindlimb flexion 4	Hindlimb flexion 5	Hindlimb extension
0.5	0 (EtOH)	1.2 ± 0.1[a]	12.5 ± 0.4[b]	—	4.6 ± 0.4	9.1 ± 0.4
	1	1.6 ± 0.1[a]	10.6 ± 0.5[b]	11.3 ± 0.6	4.5 ± 0.3	8.8 ± 0.6
	5	2.2 ± 0.9[a]	8.7 ± 0.7[b]	10.3 ± 0.5	—	—
4	0 (EtOH)	1.4 ± 0.1[c]	11.9 ± 0.4[d]	12.4 ± 0.3	5.6 ± 1.0	7.4 ± 0.9
	1	2.3 ± 0.2[c]	8.9 ± 0.4[d]	10.6 ± 0.4	—	—
	5	3.7 ± 0.5[c]	7.2 ± 0.9[d]	—	—	—
22	0 (EtOH)	1.2 ± 0.1[e]	12.1 ± 0.3	—	5.2 ± 0.4	8.0 ± 0.6
	1	1.5 ± 0.1[f]	10.8 ± 0.4	11.6 ± 0.5	5.5 ± 0.5	7.7 ± 1.1
	5	2.4 ± 0.2[e,f]	9.9 ± 0.8[d]	12.6 ± 1.2	—	—
72 to 96	0 (EtOH)	1.2 ± 0.1[g]	12.1 ± 0.3[i]	—	5.2 ± 0.4	8.0 ± 0.6
	1	1.5 ± 0.1[h]	12.0 ± 0.4[j]	—	6.1 ± 1.1	7.6 ± 1.2
	5	2.5 ± 0.2[g,h]	9.8 ± 0.5[i,j]	12.3 ± 0.5	—	—

Note. Values presented are $\bar{x}$ ± SE of all seizures with 7 to 15 subjects per TET treatment group. Duration data were analyzed with an overall unweighted ANOVA. Statistical significance levels reported are based on posthoc multiple comparisons by Duncan's test. Groups sharing the same superscript differed at the 0.05 level of significance. Hindlimb flexion 4 data reflect the data from animals exhibiting only a grade 4 seizure following the administration of TET. A dash indicates that this seizure pattern did not occur at this time after TET exposure.

a 30% decrease in FF and FE durations, respectively, (ii) at 4 hr, a 164% increase and a 39% decrease in FF and FE durations, respectively, and (iii) at 72 to 96 hr, a 108% increase and a 19% decrease in FF and FE durations, respectively.

Subacute Experiment

The subacute experiment was designed so that the total dose of TET, on a milligram per kilogram basis, consumed during the 10-day exposure period equaled those administered during the acute experiment (i.e., 0, 1, and 5 mg/kg). By exposing adult rats to 0-, 1-, and 5-ppm TET drinking solutions, the 10-day total exposure doses were 0, 1.24, and 5.37 mg/kg (Table 3). A 10-ppm exposure dose, which caused a decrease in fluid consumption, gave a total dose of 7.02 mg/kg. No changes in body weight were observed between control and treatment groups during the experiment.

In the combined control groups (i.e., EtOH and NaBr), 100% of the rats exhibited grade 5 seizures throughout the experiment (Table

TABLE 3

FLUID CONSUMPTION, MEAN DAILY DOSE, AND TOTAL DOSE OF TRIETHYLTIN (TET) BROMIDE FOLLOWING 10 DAYS OF EXPOSURE IN DRINKING WATER

Exposure level (ppm)	Fluid consumption (ml/rat/day)	Mean daily dose of TET (mg/kg/day)	Total dose of TET (mg/kg)
0 (EtOH)	29.2 ± 1.0[a]	0	0
0 (NaBr)	29.4 ± 2.3[b]	0	0
1	30.3 ± 0.7	0.12	1.24
5	26.2 ± 1.3	0.54	5.37
10	17.3 ± 2.1[a,b]	0.70	7.02

Note. Values presented are $\bar{x}$ ± SE with 10 to 16 subjects per TET treatment group. Fluid consumption data were analyzed by a one-way ANOVA. Statistical significance levels reported here are between TET treatments and are based on posthoc multiple comparisons by Duncan's test. Groups sharing the same superscript differed at the 0.01 level of significance.

4). Although the flexion durations were slightly shorter and the extension durations were slightly longer in the NaBr group, compared to the EtOH group, no between group differences were generally observed (Tables 5 and 6). The mean (±SE) control MES seizure phase durations (sec)—FF (1.0 ± 0.2), FE (12.7 ± 0.5), HF (4.1 ± 0.6), and HE (9.7 ± 0.7)—were not different from the controls in the acute experiment.

Subacute exposure to 1, 5, or 10 ppm TET produced a dose-dependent and time-dependent decrease in MES severity. As noted previously, this anticonvulsant effect was characterized by both a shift to the left in the seizure grade distribution (Table 4A) and a corresponding decrease in the extension durations and an increase in the flexion durations (Tables 5A and B). One day of exposure to 5 or 10 ppm TET produced a large seizure grade shift such that 50% of the rats no longer exhibited HE. A similar effect was seen after only 2 days of exposure to 1 ppm TET (not shown). By the 10th day of exposure to TET, HE was almost totally abolished in the 1, 5, and 10 ppm group (Table 4A). Examination of Tables 5A and B reveals correlative increases in FF and HF and decreases in FE and HE following exposure to 5 or 10 ppm TET.

Partial recovery following cessation of exposure to TET was immediate (e.g., 1 day); however, slight decreases in MES severity were still observable 14 days postexposure in the 5- and 10-ppm groups. Approximately 40% of the rats in these two groups exhibited submaximal seizures (Tables 4B and 6). These two points, the immediacy of recovery and the long-term effects, are most apparent by examination of the seizure grade distribution data in Table 4B. In fact, examination of the seizure phase duration data in Table 6 on Day 14 without observation of the distribution data in Table 4B would lead to the erroneous conclusion that recovery was complete.

Comparison of the onset and recovery data in the acute and subacute experiments reveals that there is a close correspondence in simi-

TABLE 4

DISTRIBUTION OF ADULT RATS EXHIBITING VARIOUS MAXIMAL ELECTROSHOCK SEIZURE GRADES DURING AND FOLLOWING SUBACUTE EXPOSURE TO TRIETHYLTIN (TET)

A. Day of exposure	Exposure level (ppm)	Grades (%) 2	3	4	5	B. Day of recovery	Exposure level (ppm)	Grades (%) 2	3	4	5
1	0	0	0	0	100	1	0	0	0	0	100
	1	0	0	21	79		1	11	22	11	56*
	5	0	0	45*	55*		5	22	0	66***	11***
	10	0	0	50**	50**		10	20	0	50**	30***
2	0	0	0	0	100	6	0	0	0	0	100
	1	0	7	50**	43**		1	17	0	0	83
	5	0	10	70**	20**		5	11	0	45*	44**
	10	0	20	50**	30**		10	0	11	33*	56*
10	0	0	0	0	100	14	0	0	0	0	100
	1	9	9	82***	0***		1	17	0	0	83
	5	20	0	80***	0***		5	0	0	44*	56*
	10	20	0	70***	10***		10	0	0	33*	67*

Note. Values presented are the percentages of animals exhibiting each seizure phase with 7 to 16 subjects per TET treatment group. The effects of TET treatment were evaluated statistically by χ^2 analysis of values for rats within the same seizure grade. Values with an asterisk(s) differed from the paired control at the following level of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

TABLE 5

DURATION (sec) OF FLEXION AND EXTENSION DURING TRIETHYLTIN (TET) BROMIDE EXPOSURE

A. Day of exposure	Exposure level (ppm)	Forelimb flexion	Forelimb extension	B. Day of exposure	Exposure level (ppm)	Hindlimb flexion	Hindlimb extension
1	0 (EtOH)	1.1 ± 0.1	12.8 ± 0.4[a,b]	1	0 (EtOH)	4.2 ± 0.5	9.8 ± 0.7
	0 (NaBr)	1.2 ± 0.1	13.4 ± 0.5[c,d,e]		0 (NaBr)	3.9 ± 0.2	10.6 ± 0.5
	1	1.2 ± 0.1	12.4 ± 0.3[c]		1	4.9 ± 0.6	8.8 ± 0.6
	5	1.0 ± 0.1	11.6 ± 0.3[a,d]		5	4.1 ± 0.4	9.0 ± 0.7
	10	1.2 ± 0.2	11.3 ± 0.5[b,e]		10	2.8 ± 0.2	10.6 ± 0.3
6	0 (EtOH)	1.3 ± 0.2[p]	12.0 ± 0.2[f]	6	0 (EtOH)	4.2 ± 0.4	9.1 ± 0.5
	0 (NaBr)	0.9 ± 0.1[q,r]	12.8 ± 0.3[g,h]		0 (NaBr)	3.6 ± 0.3[a]	10.1 ± 0.4[b]
	1	1.3 ± 0.1[s]	12.0 ± 0.5[i]		1	4.8 ± 0.4	9.9 ± 0.9
	5	1.8 ± 0.2[p,q,s]	10.4 ± 0.3[f,g,i]		5	—	—
	10	1.5 ± 0.2[r]	11.0 ± 0.4[h]		10	5.3 ± 0.4[a]	8.0 ± 0.5[b]
10	0 (EtOH)	1.0 ± 0.1[t,u]	12.2 ± 0.3[j,k]	10	0 (EtOH)	4.9 ± 0.5[c]	8.4 ± 0.6
	0 (NaBr)	1.0 ± 0.1[v,w]	12.1 ± 0.3[l,m]		0 (NaBr)	3.4 ± 0.2[c]	9.6 ± 0.2
	1	1.2 ± 0.1[x]	12.4 ± 0.4[n,o]		1	—	—
	5	1.7 ± 0.1[t,v,w]	9.8 ± 0.6[j,l,n]		5	—	—
	10	2.3 ± 0.2[u,w,x]	9.2 ± 0.5[k,m,o]		10	—	—

Note. Values presented are $\bar{x} \pm$ SE with 7 to 16 subjects per TET treatment group. Duration data were analyzed by an overall unweighted ANOVA. Statistical significance levels reported are based on posthoc multiple comparisons by Duncan's test. Groups sharing the same superscript differed at the 0.05 level of significance. A dash indicates that this seizure pattern did not occur at this time after TET exposure.

TABLE 6

DURATION (sec) OF FLEXION AND EXTENSION DURING RECOVERY FROM TRIETHYLTIN (TET) BROMIDE

A. Day of recovery	Exposure level (ppm)	Forelimb flexion	Forelimb extension	B. Day of recovery	Exposure level (ppm)	Hindlimb flexion	Hindlimb extension
1	0 (EtOH)	1.2 ± 0.1[a,b]	12.4 ± 0.4[g]	1	0 (EtOH)	4.6 ± 0.6	9.2 ± 0.9
	0 (NaBr)	0.9 ± 0.2[c,d]	13.2 ± 0.3[h,i]		0 (NaBr)	3.5 ± 0.4[a]	10.4 ± 0.5[b]
	1	1.1 ± 0.1[e,f]	12.0 ± 0.4[j]		1	5.3 ± 0.9	7.8 ± 0.6
	5	1.9 ± 0.1[a,c,e]	10.3 ± 0.9[g,h,j]		5	—	—
	10	2.2 ± 0.5[b,d,f]	11.3 ± 0.4[i]		10	6.6 ± 0.7[a]	6.9 ± 0.9[b]
6	0 (EtOH)	1.1 ± 0.1	12.8 ± 0.4[j,k]	6	0 (EtOH)	4.2 ± 0.5	9.8 ± 0.7
	0 (NaBr)	1.1 ± 0.1	12.7 ± 0.3[l,m]		0 (NaBr)	4.3 ± 0.4	9.5 ± 0.5
	1	1.1 ± 0.1	12.3 ± 0.5[n]		1	4.5 ± 0.7	9.0 ± 1.0
	5	1.4 ± 0.2	10.4 ± 0.9[j,l]		5	4.4 ± 0.8	8.7 ± 1.4
	10	1.7 ± 0.5	10.0 ± 0.8[k,m,n]		10	3.9 ± 0.4	8.7 ± 0.6
14	0 (EtOH)	1.0 ± 0.1	12.7 ± 0.4	14	0 (EtOH)	4.2 ± 0.4	9.8 ± 0.5
	0 (NaBr)	1.1 ± 0.1	12.7 ± 0.4		0 (NaBr)	4.3 ± 0.4	9.5 ± 0.4
	1	1.1 ± 0.1	12.3 ± 0.4		1	4.5 ± 0.5	9.0 ± 1.2
	5	1.3 ± 0.2	11.2 ± 0.7		5	3.8 ± 0.5	9.2 ± 1.5
	10	1.4 ± 0.2	11.9 ± 0.7		10	3.7 ± 0.6	10.2 ± 0.9

Note. Values presented are $\bar{x} \pm$ SE with 3 to 16 subjects per TET treatment group. Duration data were analyzed by an overall unweighted ANOVA. Statistical significance levels reported here are based on posthoc multiple comparisons by Duncan's test. Groups sharing the same superscript differed at the 0.05 level of significance.

larly TET dosed (exposed) rats. For example, exposure to 5 or 10 ppm TET for 1 day (i.e., 0.54 or 0.70 mg/kg) results in an immediate anticonvulsant action similar to that caused by a 1 mg/kg dose of TET. Additionally, the overall pattern of recovery following 10 days of exposure to 1 or 5 ppm TET (i.e., 1.24 or 5.37 mg/kg total dose) is similar to that following an acute dose of 1 or 5 mg/kg TET (see Tables 1 and 4).

DISCUSSION

The results of this investigation demonstrated that exposure of adult rats to TET, either acutely or subacutely, produces a dose-dependent anticonvulsant effect as assessed by the MES test. This effect is characterized by both a decrease in the percentage of animals exhibiting a maximal seizure and a corresponding decrease in the extension durations and an increase in flexion durations. Additionally, there is a close correspondence between the onset and recovery values observed following acute and subacute exposure to TET.

Previous neurobehavioral testing procedures have demonstrated that TET is a potent neurotoxicant (Gerren *et al.*, 1976; Amochaev *et al.*, 1979; Squibb *et al.*, 1980; Reiter *et al.*, 1981; Tilson and Burne, 1981). However, the results from the present investigation demonstrate that the MES test is able to detect subtle functional alterations in the CNS at lower doses of TET and at earlier time points than previously reported. This finding probably reflects the the fact that the MES test is sensitive to perturbations in water, electrolyte, and acid–base balance as well as disturbances in energy metabolism and neurotransmitter functioning (Purpura *et al.*, 1972; Siesjo, 1978). Alterations in all of these parameters have been reported following TET exposure (Moore and Brody, 1961; Cremer, 1970; Selwyn *et al.*, 1970; Torack *et al.*, 1970; Fox, 1982). Thus, any or all of these neurotoxic effects of TET may be responsible for the observed decreases in MES severity.

Comparison of the anticonvulsant effects of an acute dose of TET (i.e., 1 or 5 mg/kg) in adult rats and mice reveals that the rat is a more sensitive species, especially with regard to the duration of action of TET (Fox, 1982; Doctor and Fox, 1982a). Rats dosed with 1 mg/kg TET exhibit an immediate anticonvulsant effect which peaks at 4 hr and is not fully recovered by 96 hr. In contrast, this dose has a relatively small effect on adult mice. Rats and mice dosed with 5 mg/kg TET, on the other hand, exhibit a similar time course of onset and peak action. However, the recovery following this acute injection is slower and incomplete in rats compared to mice (Tables 1 and 2; Doctor and Fox, 1982a).

A comparison of the sensitivity of developing rats dosed with TET to adult rats dosed with similar doses of TET reveals that the neonates are less sensitive then the adults. However, it should be noted that an absolute comparison of the acute effects following a single dose of TET is difficult because the MES response in the neonates at 10 days of age is not as well developed (or as characterized) as in the adults. Thus, a comparison of the shifts in seizure grade distribution or alterations in the durations of individual seizure phases is not possible until approximately 21 days of age, the age at which the MES response is almost fully mature (Fox *et al.*, 1979). After a subacute exposure of approximately 1 mg/kg TET, adult rats exhibit no HE whereas 67% of the 21-day-old neonates still exhibit HE. Similarly, a subacute dose of approximately 4 to 5 mg/kg TET to adult rats completely eliminates HE whereas 30% of the neonates still exhibit this response. A lower sensitivity of the neonates to TET, compared to the adult, is also observed if the flexion and extension durations are compared. Thus, in contrast to the conclusion reached by Watanabe (1977), neonatal rodents are less sensitive to the neurotoxic effects of TET than are adult rodents.

The biochemical mechanisms of action accounting for the present findings are unknown. Several previous *in vivo* neurochemical studies have demonstrated alterations following TET administration (Moore and

Brody, 1961; Brody and Moore, 1962; Cremer, 1970). However, these neurochemical effects do not correlate temporally with the results from the present study. Recent experiments in our laboratory suggest that TET preferentially interacts with the α-adrenergic and gabaergic transmitter systems to produce its anticonvulsant effect (Fox, 1982). Other studies which focused on the inhibition of carbonic anhydrase by TET as a possible mechanism proved negative (Doctor and Fox, 1982b).

In conclusion, results from these studies show that acute and subacute doses of TET produce marked anticonvulsant effects. The MES test appears to be more sensitive in detecting neurobehavioral changes following TET exposure than previously published methodologies. In comparison with our previous studies in mice (Doctor and Fox, 1982a), results from this study reveal that rats are more sensitive to TET than mice. And finally, data from this experiment and from other work (Doctor and Fox, 1983) demonstrate that adult rats are more sensitive to TET than neonatal rats.

ACKNOWLEDGMENTS

The authors thank M. Brower and R. Crenshaw for their technical assistance. Ms. Tommie Jackson and Ms. Linda Haygood are thanked for their expert secreterial assistance.

REFERENCES

AMOCHAEV, A., JOHNSON, R. C., SALAMY, A., AND SHAH, S. N. (1979). Brain stem auditory evoked potentials and myelin changes in triethyltin-induced edema in young rats. *Exp. Neurol.* **66,** 629–635.

BRODY, T. M., AND MOORE, K. E. (1962). Biochemical aspects of triethyltin toxicity. *Fed. Proc.* **21,** 1103–1106.

CREMER, J. E. (1970). Selective inhibition of glucose oxidation by triethyltin in rat brain *in vivo*. *Biochem. J.* **119,** 95–102.

DOCTOR, S. V., AND FOX, D. A. (1982a). Effects of organotins on maximal electroshock seizures (MES) responsiveness in mice. I. Tri(n-alkyl)tins. *J. Toxicol. Environ. Health* **10,** 43–52.

DOCTOR S. V., AND FOX, D. A. (1982b). On the role of carbonic anhydrase in the anticonvulsant effects of triethyltin. *Experientia* **38,** 824–825.

DOCTOR, S. V., AND FOX, D. A. (1983). Immediate and long-term alterations in maximal electroshock seizure responsiveness in rats neonatally exposed to triethyltin bromide. *Toxicol. Appl. Pharmacol.* **68,** 268–281.

FOX, D. A. (1982). Pharmacological and biochemical evaluation of triethyltin's anticonvulsant effects. *Neurobehav. Toxicol. Teratol.* **4,** 273–278.

FOX, D. A., OVERMANN, S. R., AND WOOLLEY, D. E. (1979). Neurobehavioral ontogeny of neonatally lead-exposed rats. II. Maximal electroshock seizures in developing and adult rats. *Neurotoxicology* **1,** 149–170.

GERREN, R. A., GROSWALD, D. E., AND LUTTGES, M. W. (1976). Triethyltin toxicity as a model for degenerative disorders. *Pharmacol. Biochem. Behav.* **5,** 299–307.

MOORE, K. E., AND BRODY, T. M. (1961). The effect of triethyltin on tissue amines. *J. Pharmacol. Exp. Ther.* **132,** 6–12.

PURPURA, D. P., PENRY, J. K., TOWER, D., WOODBURY, D. M., AND WALTER, R. (1972). *Experimental Models of Epilepsy—A Manual for the Laboratory Worker.* Raven Press, New York.

REITER, L. W., KIDD, K., HEAVER, G., AND RUPERT, P. (1981). Behavioral toxicity of acute and subacute exposure to triethyltin in the rat. *Neurotoxicology* **2,** 97–112.

SEIGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill, New York.

SELWYN, M. J., DAWSON, A. P., STOCKDALE, M., AND GAINS, N. (1970). Chloride-hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes mediated by trialkyl- and triphenyltin compounds. *Eur. J. Biochem.* **14,** 120–126.

SIESJO, B. K. (1978). *Brain Energy Metabolism.* Wiley, New York.

SQUIBB, R. E., CARMICHAEL, N. G., AND TILSON, H. A. (1980). Behavioral and neuromorphological effect of triethyl tin bromide in adult rats. *Toxicol. Appl. Pharmacol.* **55,** 188–197.

TILSON, H. A., AND BURNE, T. A. (1981). Effects of triethyl tin on pain reactivity and neuromotor functions of rats. *J. Toxicol. Environ. Health* **8,** 317–324.

TORACK, R., GORDON, J., AND PROKOP, J. (1970). Pathobiology of acute triethyltin intoxication. *Int. Rev. Neurobiol.* **12,** 45–86.

VAN DER KERK, G. J. M. (1976). Organotin chemistry: past, present and future. In *Organotin Compounds: New Chemistry and Applications* (J. J. Zuckerman, ed.), pp. 1–25. American Chemical Society, Washington, D.C.

WATANABE, I. (1977). Effect of triethyltin on the developing brain of the mouse. In *Neurotoxicology* (L. Roizin, H. Shiraki, and N. Grcevic, eds.), pp. 317–325. Raven Press, New York.

WINER, B. J. (1971). *Statistical Principles in Experimental Design.* McGraw-Hill, New York.

WOODBURY, L. A., AND DAVENPORT, V. D. (1952). Design and use of a new electroshock seizure apparatus, and analysis of factor altering seizure threshold and patterna. *Arch. Intern. Pharmacodyn. Ther.* **92,** 97–107.

WOOLLEY, D. E. (1970). Effects of DDT and of drug-DDT interactions on electroshock seizures in the rat. *Toxicol. Appl. Pharmacol.* **16,** 521–532.