Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 665–671, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00046-4

Behavioral Effects of Trichloroethylene and Tetrachloroethylene in Mice

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Received 15 January 1996; Revised 26 December 1996; Accepted 24 January 1997

UMEZU, T., YONEMOTO, Y. SOMA AND T. MIURA. *Behavioral effects of trichloroethylene and tetrachloroethylene in mice.* PHARMACOL BIOCHEM BEHAV **58**(3) 665–671, 1997.—This study was performed to clarify the toxicological profiles of trichloroethylene (TRCE) and tetrachloroethylene (TECE) when they are administered intraperitoneally in mice. The ED_{50} for loss of righting reflex were 2596 mg/kg in TRCE and 4209 mg/kg in TECE. TRCE and TECE impaired bridge test performance at 500 and 2000 mg/kg, respectively. An operant behavior performance was also inhibited by TRCE at 1000 mg/kg and by TECE at 2000 mg/kg. Both TRCE and TECE exhibited anticonflict effects in a Vogel-type task at 500 mg/kg. This effect was confirmed by the finding that TRCE exhibited anticonflict action in a Geller-type paradigm at 250 mg/kg and more, as did TRCE did at 1000 mg/kg. These results show that TRCE and TECE affect various behaviors in mice and suggest that conflict behaviors are one of the most sensitive behavioral indicators of the effects of these substances. The toxicological profiles of TRCE and TECE with respect to behavioral effects were very similar, and they can be classified in a single category. © 1997 Elsevier Science Inc.

Trichloroethylene Tetrachloroethylene Righting reflex Bridge test Operant behavior Conflict behaviors Mice

TRICHLOROETHYLENE(TRCE) and tetrachloroethylene (TECE), chlorinated aliphatic hydrocarbons, are widely used in industry as degreasers, dry cleaning agents, paint removers, solvents for chemical extraction and components of adhesives and lubricants (16,39,45). TRCE is also used as an anesthetic and analgesic in medicine (32). Industrial workers are often exposed to relatively low levels of TRCE and TECE vapors and sometimes accidentally exposed to high levels (5,8,13,24,25,37). In addition, pollution of ground water by these compounds has occurred and they are now recognized as contaminants of drinking water (7,31,42). Therefore, there are many opportunities for accidental and even unknown exposure to these substances, sometimes by inhalation of vapors and sometimes by consuming polluted drinking water, and their health effects have been of increasing concern.

One of the characteristics of chlorinated aliphatic hydrocarbons is high lipophilicity. Thus, these compounds are readily transported to the central nervous system after exposure. TRCE causes loss of equilibrium, coordination and consciousness. Humans who were chronically exposed to low levels of TRCE complained of headache, dizziness, drowsiness, fatigue and deficits in psychomotor performance (30,33,40,43). Acute exposure to high levels of TECE results in depression of the central nervous system (CNS), hypotension and anesthetic death. Several investigators have shown that exposure to lower levels caused by abnormality in the modified Rhomberg test, light-headedness, headache, speech difficulty and sleepiness (38,39).

Therefore, at least some of the symptoms caused by TECE appear to be the same as those caused by TRCE. To assess the health effects of chemicals, it is necessary to clarify the dose–response relationship for each symptom. Although there are many reports on health effects of these substances, dose–response relationships between TRCE or TECE and their CNS-related symptoms are not well established.

In the field of pharmacology and toxicology, chemicals are characterized by profiles of their actions. Although there is a great deal in the literature on the toxicological effects of TRCE on CNS functions (1–4,6,11,12,14,15,17,18,26,35,36), its dose–response profile with respect to animal behavior has not

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been well characterized. Furthermore, the effects of TECE on animal behavior are even less well known (27). Therefore, to our knowledge, a comparison of the behavioral effect of TRCE and TECE has not been made, and thus it is unclear as to whether or not the toxicological profiles of these two chemicals are similar.

Information about interspecies variations are important for the evaluation of chemical effects on health. However, only limited information about the species differences on the behavioral effects of TRCE and TECE are available. The LD_{50} of TECE was 3000 mg/kg in mice and 1900 mg/kg in dogs when administered by intraperitonial (IP) injection (29). The LD_{50} of TECE were 4700 mg/kg in mice and 2100 mg/kg in dogs when given IP. Most previous studies on the behavioral effects of TRCE have been made only in rats. To understand more fully the interspecies differences in the effects of these chemicals, behavioral tests of the effects should be performed on other animals. We chose to investigate the behavioral effects of TRCE and TECE in mice because many behavioral methods used in rats are applicable to mice and because the many studies of mice will make it possible to examine the genetic factors that may effect sensitivity to these substances.

In the present study, we examined effects of TRCE and TECE on CNS functions by using various behavioral tests in mice.

MATERIALS AND METHODS

Animals

Animals used in this study were ICR strain male mice (Clea Japan, Tokyo), 8 weeks old and 31–40 g in body weight at the start of each experiment. Each of 10 animals was housed in a Plexiglas cage that had a stainless steel wire mesh top with wooden-flake bedding. Commercial solid food (Clea Japan) and tap water were available ad libitum in experiments 1 and 2. Animals in experiments 3–5 were subjected to restricted feeding or water deprivation. The room for breeding animals was artificially illuminated by fluorescent lamps on a 12-h light 12-h dark schedule (light period: 7 AM–7 PM), and the room temperature was regulated to 25 ± 1.0°C.

All experiments in this study were performed with the approval of the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies, Japan.

Chemicals

The chemicals used were TRCE and TECE (Wakeo Pure Chemical Ind., Osaka). Chemicals were dissolved in olive oil (Wako Pure Chemical Ind.) and administered IP in a fixed volume of 1 ml/100 g body weight, regardless of dose.

Experimental Procedures

The present study consists of 5 experiments for evaluation of neurobehavioral effects of intraperitoneal administration of TRCE and TECE.

Experiment 1: Righting Reflex Test. Seventy animals were used in this test and were divided into 7 groups. Ability of animals to right after being inverted was used as a measure of anesthetic effect. One hour after injections of TRCE and TECE, the number of mice that could not get up was counted.

Experiment 2: Bridge Test. Bridge test was performed to examine effects on equilibrium and coordination in animals. One hundred sixteen animals were used in this test and were divided into 8 groups. Each animal was used only once. A wooden rod, 15 mm in diameter, was fixed horizontally 30 cm above the floor. Animals were put on the rod, and the staying time, 300 s maximum, on the rod was measured. After 10 trials for acquisition of ability to stay on the rod, challenge tests were made 30 min after administration of TRCE or TECE.

Experiment 3: Effects of TRCE and TECE on operant behavior. This experiment was made to clarify effects of TRCE and TECE on learned behavior. Fourteen animals were used repeatedly in this experiment. They were divided into two groups (n = 6 for TRCE and n = 8 for TECE). Their food was restricted to induce hunger. Magazine training was then started, in which lever pressing in a skinner box allowed access to food pellets of 20 mg each in a continuous schedule of reinforcement, that is a fixed ratio of 1 (FR 1). When they could recognize the relationship between lever pressing and food gain, the reinforcement schedule was changed to discontinuous schedules, that is, at first to FR 5, then to FR 10, and finally to FR 20. After achievement of the final schedule, the reinforcement schedule was fixed at FR 20, and animals were trained at this ratio every day. Each session lasted for 1 h, and the sessions were done from Monday through Friday every week. After establishment of a stable baseline response (after about 3 weeks of training), the challenge test sessions were inserted into the schedule. TRCE, TECE or their vehicle olive oil were administered on Tuesday and Friday every week. First, vehicle administration was performed. Then, the effects of TRCE or TECE were examined in the order from low dose to high dose. Thirty minutes after administration, the number of lever presses during a 1-h session was recorded. On nonexperimental days, animals were trained without any treatment, and the stability of behavioral baseline was checked.

The apparatus used in this experiment consisted of an operant chamber, a schedule controller and a data recorder (GT-8510, GT-8005. GT-7715, respectively; O'hara & Co., Tokyo) (19). The chamber was made of acrylu fiber and aluminum boards with a dimension of 180 (W) × 90 (D) × 100 (H) mm. A stainless steel lever was vertically set in the side wall of the chamber. A saucer for food pellets was set in the same wall. The floor was made up of stainless steel grid, and it was wired to pass an electric current in a conflict schedule (see description for experiment 5). A speaker for presenting a warning stimulus was set in the center ceiling of the chamber. This speaker also was used for a conflict experiment.

Experiment 4: Conflict test (I). Conflict tests were devised originally to evaluate anticonflict effects of anxiolytics and have been used as a screening method for anxiolytic agents. Chemicals such as general depressants also show anticonflict effects. Thus, conflict tests were used to evaluate psychoactive effects of TRCE and TECE.

Thirty four animals divided into 2 groups (n = 15 for TRCE and n = 19 for TECE) were used repeatedly in an experiment employing a modified method of Vogel et al. (44). The apparatus was 5 sets of Plexiglas chambers [180 (W) × 100 (D) × 120 (H)] and a recorder (VC-3002-L and VC-2050-L; O'hara & Co.) A water bottle was set in each chamber and animals could drink water from a spout. The numbers of licks of a spout by animals were recorded simultaneously in each chamber for 40 min. Every 20th lick was punished by an electric shock (50 V, ca. 0.2 mA, 50 Hz AC, duration = 0.3 s) through the grid, which constituted the floor of the chamber.

Each mouse was subjected to the test weekly on the same day. For 2 days before the test, animals were deprived of water to induce thirst. On the test day, animals were put individually into chambers 20 min after administration of TRCE,

TECE, or olive oil, and the number of punishments during 40 min was recorded.

Experiment 5: Conflict test (II). In this experiment, 20 animals were used and were divided into 2 groups ($n = 10$ each for TRCE, $n = 10$ for TECE) and used repeatedly in each experiment.

The apparatus used in this experiment was the same as that used in experiment 3. Animals were trained under a MULT FR 20/FR 20-punishment schedule of food reinforcement, which was a modified method (19) established by Geller and Seifter (9) after magazine training. The schedule consisted of 4 pairs of a safe period of 5 min and an alarm period of 5 min, each period was alternated. Thus, each session lasted 40 min. During the safe period, the mouse's lever pressing was reinforced by food pellets at FR 20 without electric shock. During the alarm period, every 20th lever pressing was coupled with an electric shock (50–90 V, ca. 0.3 mA, 50 Hz AC, duration = 0.3 s) preceded by a warning stimulus (tone signal; 800 Hz, 90 dB) as the punishment for taking food. After establishment of stable baseline responses during the safe and alarm periods, animals showed high response rates during the safe period (about 30 counts/min) and low response rates during the alarm period (about 3 counts/min). Subsequently, challenge testing sessions were performed in which TRCE or TECE was administered to animals at intervals of 3–4 days 20 min before the start of the test session. During the safe and alarm periods, response rates were examined. Tests were performed following vehicle administration alone, and then the effects of TRCE or TECE were examined in order from low dose to high dose. On nonexperiment days, animals were trained without any treatment, and the stability of behavioral baseline was checked.

Statistical Analyses

The ED_{50} was calculated by a profit method (46).

Overall differences of medians of all treatments in experiment 2 were examined by the Kraskal-Wallis test followed by comparisons between the control and each treatment by the Steel multiple comparison procedure (two-tailed). Because the same animals were used repeatedly in experiments 3–5, overall differences of median in each experiment were examined by the Friedman test, followed by comparison between the control and each treatment by the Steel multiple comparison test (two-tailed), except for the data from experiment 5. From the results of experiment 4, anticonflict effects of TRCE and TECE were expected in experiment 5. Thus, a one-tailed Steel comparison was made to test for significance in the data of experiment 5. Five percent was used as the significant level (46).

TABLE 1

INCIDENCE OF LOSS OF RIGHTING REFLEX IN MICE AFTER ADMINISTRATION OF TRICHLOROETHYLENE (TRCE) AND TETRACHLOROETHYLENE (TECE)

TRCE				
Dose (mg/kg, IP)	2000	4000	5000	
Loss of righting reflex	2/10	9/10	10/10	
TECE				
Dose (mg/kg, IP)	2000	4000	6000	8000
Loss of righting reflex	0/10	5/10	8/10	10/10

Each group consisted of 10 animals. Loss of righting reflex at each dose is shown by the number of incidents per 10 animals.

RESULTS

Experiment 1

Effects of TRCE and TECE on righting reflex in mice were determined by counting the number of mice that could not get up 1 h after administration (Table 1). Based on these data, ED_{50} of TRCE and TECE were calculated to be 2596 mg/kg (95% confidence interval: 1987.0–3164.5 mg/kg) and 4209 mg/kg (95% confidence interval: 3196.1–5026.6 mg/kg), respectively.

Experiment 2

To examine the effects of TRCE and TECE on equilibrium and coordination, animals were placed on a rod, and the staying time was measured. As shown in Fig. 1a, the staying

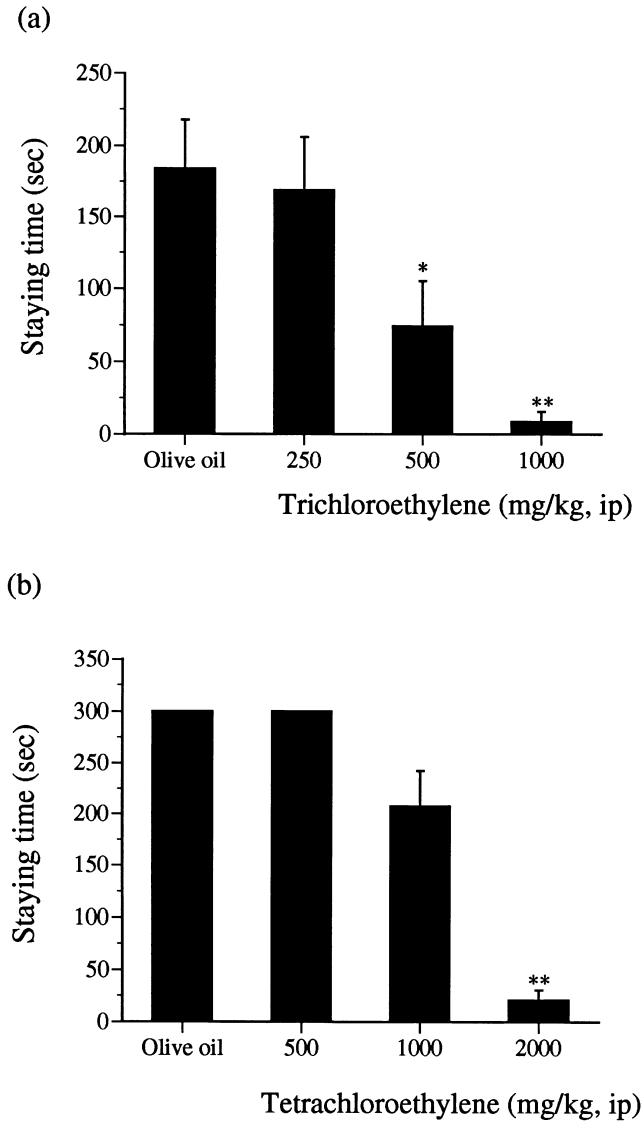

FIG. 1. Effects of TRCE (a) and TECE (b) on staying time in the bridge test 30 min after administration. Columns indicate mean values, and vertical lines indicate standard errors ($N = 13$–15 for each column). Significant differences as compared with olive-oil-treated control value (*$p < 0.05$, **$p < 0.01$, respectively; Steel multiple comparison test).

time of TRCE-treated groups decreased in a dose-dependent manner. Overall differences among the medians of all treatments in the TRCE groups was significant [Kraskal-Wallis test $\chi_0^2 = 26.54 > \chi^2(3, 0.05) = 7.81$]. Steel multiple comparison revealed that TRCE of 500 mg/kg or more significantly reduced staying time of mice on the rod (Fig. 1a). Overall comparison of TECE treated groups also showed significant differences ($\chi_0^2 = 29.57 > \chi^2(3, 0.05) = 7.81$), and multiple comparison revealed TECE at 2000 mg/kg significantly reduced the staying time (Fig. 1b).

Experiment 3

An operant behavior was examined to determine the effects of TRCE and TECE on learned behavior. The overall response rate of lever pressing to gain food access decreased significantly ($Q_0 = 19.60 > \chi^2(4, 0.05) = 9.49$) following TRCE administration. Multiple comparison showed that TRCE at 1000 mg/kg suppresses response rate significantly (Fig. 2a).

The overall response rate also decreased significantly after TECE administration ($Q_0 = 25.36 > \chi^2(5, 0.05) = 11.07$). TECE at 2000 mg/kg significantly suppressed response rate (Fig. 2b).

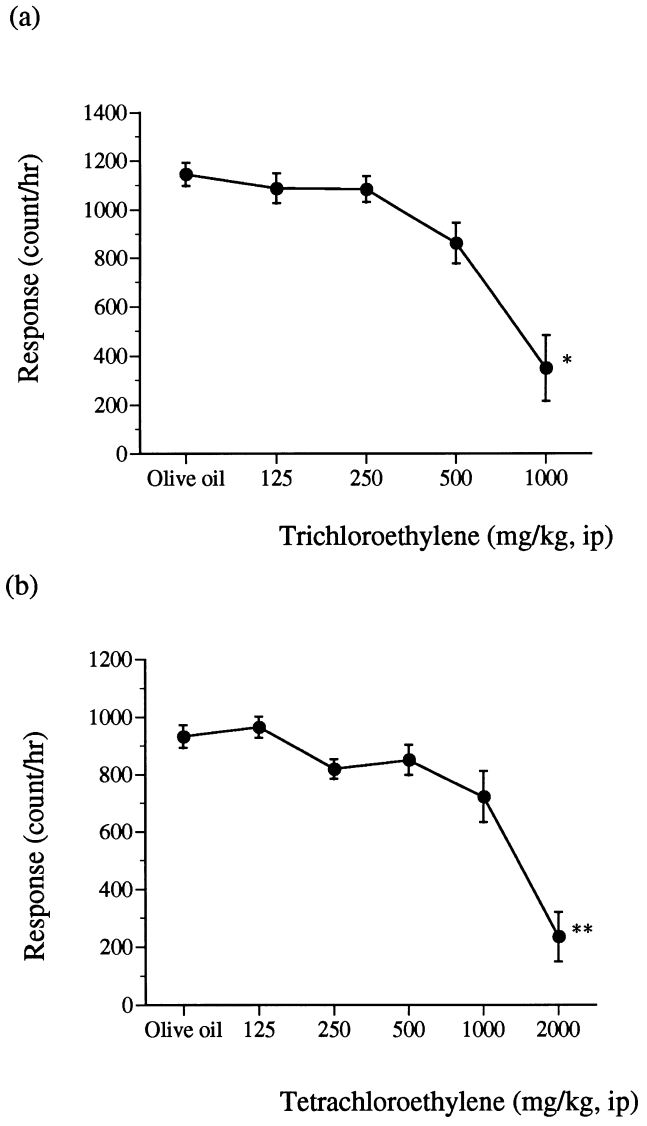

FIG. 2. Effects of TRCE (a) and TECE (b) on response rates of operant behavior (lever pressing) under FR 20 30 min after administration. Closed circles indicate mean values, and vertical lines indicate standard errors (a: $N = 6$, b: $N = 8$, for each point). Significant differences as compared with olive-oil-treated control value (*$p < 0.05$, **$p < 0.01$, respectively; Steel multiple comparison test).

Experiment 4

In conflict tests, the dose–response curves of TRCE and TECE were bell-shaped.

The number of punishments animals received after TRCE administration increased apparently at 500 mg/kg or greater; that is, anticonflict effects occurred at these doses. The difference was statistically significant ($Q_0 = 11.12 > \chi^2(3, 0.05) = 7.81$)(Fig. 3a).

TECE had no effect at 250 mg/kg but showed anticonflict effects at 500 mg/kg. This change was significant ($Q_0 = 24.71 > \chi^2(4, 0.05) = 9.49$) (Fig. 3b). At higher doses, however, the effect was not significant.

Experiment 5

Figure 4, the overall response rates during the safe period (unpunished response; closed circles) and during the alarm period (punished response; closed triangles) after administration of TRCE (Fig. 4a) and TECE (Fig. 4b).

TRCE had no significant effect on the response rates during the safe period at all doses examined ($Q_0 = 5.44 < \chi^2(4, 0.05) = 9.49$). However, TRCE at 62.5 mg/kg and more produced increased response rates during the alarm period. The differences among all treatments were significant ($Q_0 = 26.74 > \chi^2(4, 0.05) = 9.49$). Multiple comparison revealed that the response rates at 250 mg/kg and greater were significantly different from that of the control value (olive oil administration).

The Friedman test showed a significant difference in the overall response rates during the safe period for TECE ($Q_0 = 13.26 > \chi^2(5, 0.05) = 11.07$). However, the steel comparison showed no difference between the vehicle-treated control and each dose of TECE. However, TECE had a significant effect ($Q_0 = 18.57 > \chi^2(5, 0.05) = 11.07$) on the response rates during the alarm period. The change at 1000 mg/kg was statistically significant (Fig. 4b) when compared with the vehicle-treated control.

DISCUSSION

To our knowledge, only lethal effects of TRCE and TECE have been described in mice [LD_{50} of TRCE and TECE were 3000 mg/kg and 4700 mg/kg, respectively, when administered IP (29)]. Therefore, the present study was performed to examine the behavioral effects elicited by these compounds in mice.

TRCE and TECE have an anesthetic action in humans. In animals, a similar effect is indicated by loss of righting reflex. The present study confirms that loss of righting reflex occurs in mice with administration of TRCE and TECE, and ED_{50} were calculated to be 2596 and 4209 mg/kg, respectively. An anesthetic action of TRCE was reported in rats when they were exposed to its vapor at 4000 ppm (17). The effect of TRCE gas exposure at this concentration in rats may be comparable to that of intraperitoneal administration of 2000–3000 mg/kg in mice, although there is a species difference between mice and rats (23,28,41).

In the present study, a bridge test was applied to mice to examine effects on equilibrium and coordination. The staying

time of mice on the rod was reduced by administration of TRCE at 500 mg/kg and more and TECE at 2000 mg/kg. Thus, TRCE and TECE at those doses affect equilibrium and coordination.

There are several reports on the effects of TRCE on operant behaviors in rats. Grandjean (11) reported that rope climbing motivated by food was not affected by exposure to 200 and 800 ppm TRCE. Kishi et al. (17) showed that the conditioned avoidance response, which was reinforced by electric shock, was reduced by exposure to 250 ppm TRCE. However, to our knowledge, effects of TECE on operant behaviors have not been examined. The present study revealed that both TRCE and TECE reduced response rates of a food-motivated operant behavior, lever pressing, in mice at 1000 and 2000 mg/kg, respectively. Therefore, it is likely that TECE and TRCE depresses a learned behavior, although anesthetic effects of these two chemicals were not observed at these doses.

The Vogel acute conflict task (44) has been used to evaluate anticonfict effect of drugs in animals, mainly rats. In the present study, this method was applied to mice and demonstrated that both TRCE and TECE exhibit anticonflict action. These two chemicals probably have psychoactive actions against anxiety.

This finding was confirmed by the results of another experiment using the Geller-Seifter paradigm (9). This method has been applied to mice, and effects of various psychoactive drugs have been examined (19–22). The present study showed that both TRCE and TECE increased response rates during the alarm period in which lever pressing to gain food pellets

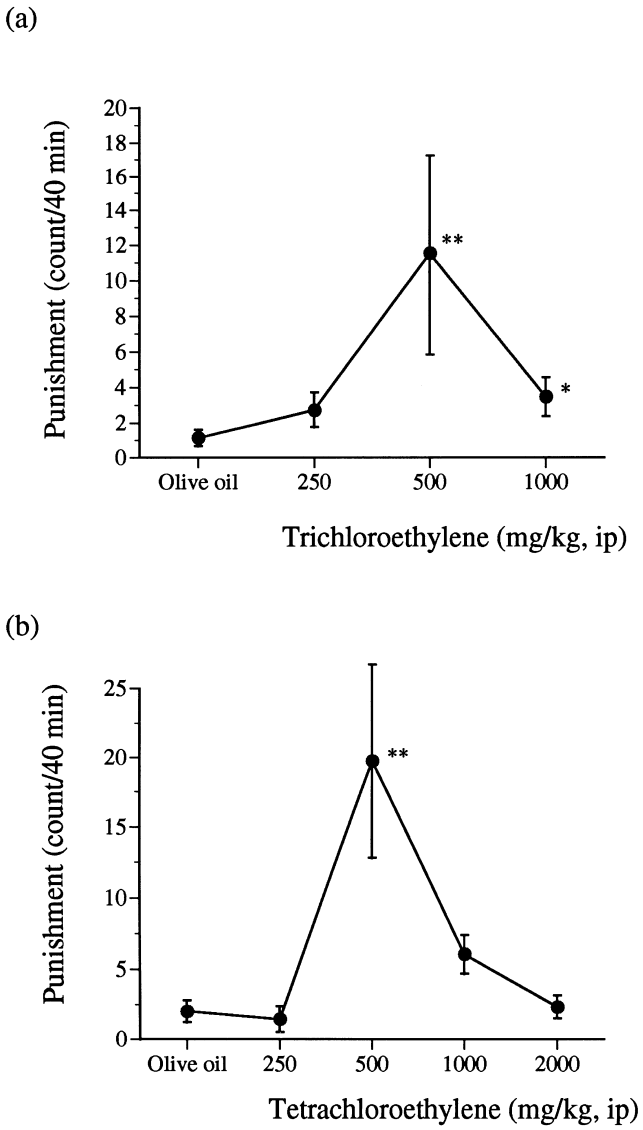

FIG. 3. Effects of TRCE (a) and TECE (b) on the Vogel-type conflict behavior. Closed circles denote means of number of punishments (electric shock) that animals received during the test (40 min) 20 min after administration, and vertical lines indicate standard errors (a: $N = 15$, b: $N = 19$, for each point). Significant differences as compared with olive-oil-treated control value ($*p < 0.05$, $**p < 0.01$, respectively; Steel multiple comparison test).

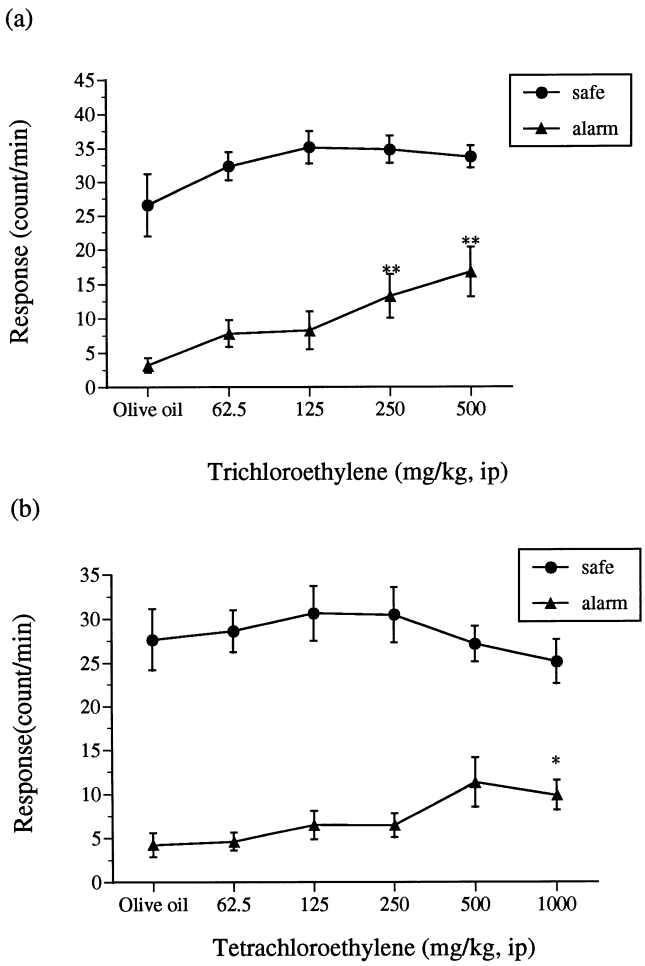

FIG. 4. Effect of TRCE (a) and TECE (b) on Geller-type conflict behavior. Closed circles indicate mean response (lever pressing) rates during the safe (nonpunishment) period and closed triangles denote mean response rates during the alarm (punishment) period under MULT FR 20/FR 20-punishment schedule 20 min after administration. Vertical lines indicate standard errors (a: $N = 10$, b: $N = 10$, for each point). Significant differences as compared with olive-oil-treated control value ($*p < 0.05$, and $**p < 0.01$, respectively; Steel multiple comparison test).

was punished by electric shock. One of the advantages of the Geller-type conflict paradigm is that it is possible to examine the effect of drugs on both an unpunished response (lever pressing) and a punished response (lever pressing) at the same time in the same animals. The present study demonstrated that 250–500 mg/kg of TRCE and 1000 mg/kg of TECE increase the punished lever-pressing rate without affecting the unpunished lever-pressing rate. These results indicate that punished lever pressing is more sensitive than unpunished lever pressing to TRCE and TECE. The results of TRCE and TECE are similar to those of benzodiazepine anxiolytics and barbiturates (9,19,22). This method is probably one of the most sensitive tests for evaluating effects of these substances at lower doses because these substances exhibited the anticonflict action without nonspecific actions on the unpunished lever pressing. This possibility is supported further by the present results in which the operant behavior (lever pressing) was suppressed and bridge test performance was inhibited at higher doses of TRCE and TECE.

Based on the results of the present study, the toxicological profile of TECE is similar to that of TRCE. Thus, they may be classified in the same category, although TRCE is at least 2 times more potent than TECE. These behavioral profiles are similar to those of well-studied chemicals such as gaseous anesthetics and aliphatic alcohols. There are many hypotheses on the mechanisms of gaseous anesthetics and aliphatic alcohols on the central nervous system (10). However, most of these hypotheses come from the general nature of these substances, lipophilicity. That is, it has been believed that the first step in the actions of these substances is to be dissolved in the cell membrane of neurons and thereafter to depress neural activities. Because TRCE and TECE also have high lipophilicity, their actions on behaviors might involve the same mechanism. This speculation is supported by the report (34) in which TRCE decreased action potentials of squid axons in proportion to its concentration.

ACKNOWLEDGEMENT

We thank Dr. M. S. Grace of the University of Virginia for his helpful suggestions on this paper.

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