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Biphasic Dose-Response Relationship for Effects of Toluene Inhalation on Locomotor Activity[1]

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HINMAN, D. J. *Biphasic dose-response relationship for effects of toluene inhalation on locomotor activity.* PHARMACOL BIOCHEM BEHAV **26**(1) 65–69, 1987.—To investigate the effects of inhalation of toluene on spontaneous locomotor activity, rats were exposed to graded concentrations of toluene and locomotor activity was measured continuously before, during and after exposure. This study used a randomized, cross-over, graded-dose experimental design, with sham-exposure as the control. The locomotor activity pattern during toluene exposure depended upon the toluene concentration in the air. At the lowest effective concentration (5000 ppm) locomotor activity increased monophasically during exposure, and decreased monophasically during recovery. At higher concentrations (10,000–15,000 ppm) locomotor activity initially increased in a concentration-dependent manner. With continued exposure to the higher concentrations, locomotor activity decreased and eventually ceased at the highest concentration. Recovery from exposure to high concentrations of toluene was also biphasic. These results demonstrate that the behavioral responses to extremely high concentrations of toluene are characterized by biphasic actions as demonstrated both by analysis of concentration-response and time-action characteristics. Exposure to concentrations of toluene similar to those used in this study occurs during organic solvent abuse and glue sniffing in humans.

Toluene inhalation Glue sniffing Organic solvent abuse Locomotor activity

ORGANIC solvent abuse is a significant social problem, especially among adolescents [4, 15, 27, 30]. One frequently abused solvent is toluene, and toluene is preferred by solvent abusers due to its pleasant effects and lack of side effects [16]. Thus, investigation of the pharmacologic effects of toluene inhalation is important to understanding organic solvent abuse.

Abuse of organic solvents involves exposure to extremely high concentrations of solvents [4, 15, 19, 20]. However, the full dose-response curve should be investigated to understand the pharmacology of a drug. Some of the effects of toluene inhalation in experimental animals are: changes in locomotor activity [11, 14, 22], changes in open-field behavior [12], disruption of operant behaviors [8, 17, 31, 34], alteration of performance in the Sidman avoidance schedule [28], anticonvulsant activity [33], antipunishment activity [33], and self-administration of solvents [32]. Electrophysiologic studies demonstrated both excitatory and depressant effects of toluene exposure [1,29]. In addition, exposure to toluene produced hearing loss [22, 23, 25].

Varying effects of toluene on spontaneous locomotor activity in rats have been reported. In acute exposures, activity was increased in adult rats exposed briefly to high concentrations of toluene [11,12] or in mice exposed to lower concentrations of toluene for one hour [14]. Activity was decreased in weanling rats exposed to toluene subchronically [22]. Thus, it is possible that toluene inhalation produces a biphasic effect on spontaneous locomotor activity. To test this possibility, the dose-response curve for effects of toluene on locomotor activity was measured in this study. In previous studies [11, 12, 22], locomotor activity was measured during recovery from toluene inhalation. Analysis of behavior during recovery from solvent inhalation is complex due to the rapid drug elimination. The increase in locomotor activity could either be related to the rapid elimination of toluene [11], or to the actual level of toluene in the brain [14]. To test these two alternatives, in the present study locomotor activity was measured continously during exposure to toluene, as well as during recovery from exposure.

METHOD

Animals

Adult, male Long Evans hooded rats (300–400 g) were used. The rats had free access to food and water, and were housed in individual cages in a room with a 12 hour light/12 hour dark cycle (lights on from 7:00 a.m. to 7:00 p.m.).

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Inhalation Apparatus and Generation of Toluene Vapors

The rats were acutely exposed to toluene in a positive-pressure, whole-body inhalation chamber. This system was modified from inhalation systems previously described [2, 10, 13]. The chamber was constructed of Plexiglas and lined with Teflon to minimize adsorption of toluene to the surface of the chamber. The volume of the chamber was 19.25 liters, and the dimensions were 35×22×25 cm. The airflow rate was 15 liters/minute as measured by a Brooks model 1355 SHO-RATE 1500 flowmeter. The estimated time to reach 99% of equilibrium (t_{99}) for this system was 5.9 minutes

$$[t_{99}=(4.604 \times \text{chamber volume})/\text{airflow rate}].$$

Toluene vapors were generated using a universal vaporizer as previously described [12]. This custom-built vaporizer had a 2 liter-capacity stainless steel reservoir. Room air (at ambient room temperature) was filtered by a Balston DFU filter to remove dust and oil, and then bubbled through the toluene via a stainless steel mesh bubbler. The saturated toluene vapors were diluted with room air to produce the final concentration of toluene. Tubing and connectors were either stainless steel or Teflon-coated plastic to minimize adsorption of toluene. The toluene was reagent grade (purity >99%; J. T. Baker Co.).

The concentrations of toluene reported are nominal values (see [12]). The concentrations were calculated from the ratio of airflow through the vaporizer to the airflow of the room air diluent. Assuming that the toluene vapors were saturated, the concentration of toluene (in ppm) = (vapor pressure of toluene/atmospheric pressure) × 10,000. The actual concentrations of toluene in the air were not measured. However, the experimental design was a concentration-effect study with graded concentrations of toluene administered in a randomized order. Thus, day-to-day variation in the toluene concentration could not bias the results.

Procedures

This study used a randomized, cross-over, graded-concentration experimental design. Six rats were used. Each rat was exposed to sham-exposure and several concentrations of toluene (2 to 5 exposures per rat). None of the rats died during the course of the study. The order of presentation of toluene concentrations was randomized by Latin square.

Each exposure consisted of three phases: habituation, exposure and recovery. A single rat was placed in the inhalation chamber and the chamber was sealed with duct tape. During the habituation phase, room air was pumped through the inhalation chamber. During the exposure phase, toluene vapors were continuously pumped through the chamber. During the recovery phase, room air was again pumped through the chamber. The durations of the habituation and exposure phases were 30 and 60 minutes, respectively. The duration of the recovery phase was predetermined, and varied according to the concentration of toluene during the exposure. Preliminary studies showed that the recovery time following exposure to 2500 to 15,000 ppm toluene ranged from 30 to 90 minutes. During sham-exposure, room air was pumped through the inhalation chamber continuously for 2.5 hours.

Locomotor activity was monitored continuously throughout each exposure by placing the exposure chamber on a Model SE Animex activity monitor. The sensitivity of the activity monitor was calibrated each day prior to the exposures. The number of activity counts per 5 minutes was tabulated. In addition, the locomotor activity was qualitatively assessed by subjective observation of the behavior. All the studies were conducted between 12:00 noon and 6:00 p.m. (during the lights-on part of the light/dark cycle).

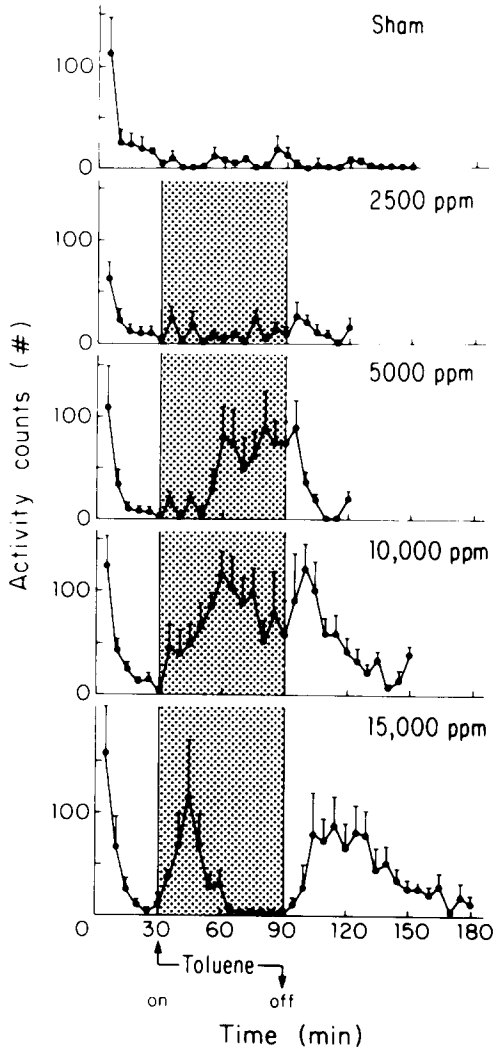

FIG. 1. Locomotor activity in rats before, during and after exposure to toluene. The time of exposure is indicated by the shaded area. Top panel: sham exposure. Bottom panels: increasing levels of toluene (levels indicated in the insets). Each point is the mean of 5–6 observations; the vertical bars are the s.e.m.

Data Analysis

The data were analyzed by analysis of variance (ANOVA, two-factor [treatments-by-subjects], mixed design with repeated measures [observation intervals]) and multiple *t*-tests [6].

RESULTS

Locomotor Activity During Habituation

During the 30-minute habituation period, locomotor ac-

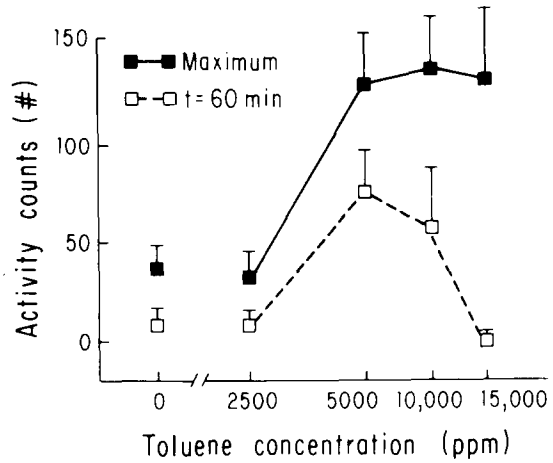

FIG. 2. Dose-response curves for effects of toluene on locomotor activity. Solid symbols are the maximum activity per 5 minutes during exposure (regardless of time). Open symbols are locomotor activity during the period t=55 to 60 minutes. Each point is the mean of 5–6 observations; the vertical bars are the s.e.m. The abscissa is toluene level (logarithmic scale).

tivity was initially high and decreased steadily with time (Fig. 1). Locomotor activity in the habituation period was not significantly different among the five exposure groups [ANOVA for treatment, $F(4,155)=0.76$, $p>0.05$]. After habituation, rats in the sham-exposure group consistently showed a low level of activity during the remainder of the observation period.

Locomotor Activity During Exposure to Toluene

Exposure to graded levels of toluene produced a dose-dependent increase in locomotor activity (Fig. 1) which was significant with respect to dose level, time and interaction factors [ANOVA, $F(4,311)=104.67$, $p<0.001$; $F(11,311)=4.19$, $p<0.001$; and $F(44,311)=6.05$, $p<0.001$, respectively]. Locomotor activity was not significantly different in the 2500 ppm toluene group than in the sham-exposure group ($p>0.05$ at each observation period, multiple t-test). At 5000 ppm toluene, locomotor activity initially was low, then increased monophasically and finally remained elevated during remainder of the exposure. At the two highest levels of toluene, a biphasic time-action curve was observed which showed a maximum increase in activity at approximately 30 and 15 minutes during exposure to 10,000 and 15,000 ppm toluene, respectively. At the highest level, all the rats eventually ceased spontaneous activity. At this level of exposure the rat was typically lying on its side, with vigorous myoclonic jerks (cf. [12] for description), but with no overt signs of cyanosis.

Subjective observation of the overt behavior of the rats during the exposure revealed that the increased locomotor activity was associated with extreme ataxia, headshakes and hindlimb myoclonus, with the rat continuously moving about the inhalation chamber in an apparently random manner (no stereotypic behaviors). No attempt was made to quantitate these behaviors either during the exposure or during the recovery. Quantitative assessment of these behaviors during recovery from toluene exposure was previously reported [12].

The biphasic effects of toluene are also demonstrated by examination of the dose-response curves (Fig. 2). The two dose-response curves in Fig. 2 were constructed based on different criteria. In one curve, the maximum locomotor activity observed during the exposure, regardless of the time of occurrence, was used as a measure of response. In the other curve, the locomotor activity at a fixed time (the end of the exposure) was used. In the curve showing the maximum locomotor activity the dose-response curve is a monophasic curve with a maximum effect at approximately 5000 ppm toluene. In the curve showing locomotor activity at a fixed time of observation the dose-response curve is biphasic with a maximum effect at 5000 ppm toluene.

Locomotor Activity During Recovery From Toluene

Activity patterns during recovery from toluene were dependent upon the level of toluene during exposure [Figs. 1 and 2; ANOVA for treatment, time and interaction factors, $F(4,155)=16.59$, $p<0.001$; $F(5,155)=0.84$, $p>0.05$; and $F(20,155)=1.71$, $0.05<p<0.1$, respectively]. During recovery from 5000 ppm toluene, locomotor activity decreased monophasically and recovery occurred in approximately 20 to 30 minutes. Recovery from the two highest levels of toluene was biphasic, with both the time to maximum activity and time to recovery dependent upon the level of toluene during exposure.

Subjective observation of the rats during the recovery revealed that the overt behavior was qualitatively similar to that occurring during the exposure: extreme ataxia, headshakes and hindlimb myoclonus, with continuous random movement about chamber. These behaviors gradually decreased with time until the rat typically went to a corner of the chamber and became quiescent (some rats appeared to go to sleep).

DISCUSSION

The results of this study clearly demonstrate that exposure to inhalation of toluene produces biphasic effects on spontaneous locomotor activity in rats. At the lowest effective level of toluene, locomotor activity increased monophasically with time and this increased activity was sustained through the rest of the exposure. At higher levels of toluene, the biphasic actions of toluene were observed. Initially, locomotor activity increased, with the time to peak dependent upon the level of toluene in the air. With continued exposure to a constant level of toluene, activity then decreased and eventually spontaneous activity ceased at the highest level. The biphasic actions of toluene inhalation were demonstrated both by analysis of dose-response and time-action characteristics.

Patterns of locomotor activity during recovery from acute exposure to toluene were related to the patterns observed during the exposure. Thus, at lower toluene levels, locomotor activity increased monophasically during exposure and decreased monophasically during recovery. At higher levels, biphasic responses occurred both during exposure and during recovery. During recovery from exposure to the higher levels, activity initially increased followed by a decrease to baseline levels of activity.

In the present study, only total locomotor activity during exposure to and recovery from toluene were measured. Locomotor activity is only one aspect of the behavioral syn-

drome associated with toluene exposure [11, 12, 14, 22]. It is possible that the biphasic dose-response curves result from a change in the relative contributions of various types of activity which constitute total motor activity. Further investigation will be required to examine more fully this issue.

These results are consistent with the hypothesis that locomotor activity during exposure is dependent on the level of toluene at the site of action, presumably the central nervous system (CNS). Thus, at low levels of toluene, locomotor activity is increased, whereas at higher levels this solvent-induced hyperactivity is attenuated. This hypothesis can account for the occurrence of a biphasic time-action curve both during exposure and during recovery, since the CNS levels of toluene would increase monophasically during exposure and decrease monophasically during recovery (cf. [3,5]).

The pattern of solvent abuse among humans is to inhale solvent vapors for only a few minutes to achieve the desired effects [4, 15, 20] which may last as long as 60 minutes following a single exposure [16]. During this period the individual could experience rapid changes in the levels of solvent in the CNS, since elimination of toluene from the brain following inhalation is very rapid [7, 24, 26]. Thus, it has been suggested that behavioral effects of toluene are related to the rapid elimination of toluene from the brain [11] rather than to the actual CNS level of toluene [5,14]. To address this issue, in the present study locomotor activity was measured both during exposure to toluene and during recovery. If the hyperactivity associated with toluene inhalation were a function of the rapid elimination of toluene, then the hyperactivity would be greater during the recovery than during the exposure.

The results showed that both the time-action curve during exposure and the dose-response curve were biphasic; biphasic time-action curves were also recorded during recovery from inhalation. Furthermore, the level of hyperactivity during the recovery was similar to the hyperactivity during the exposure. From these observations it can be concluded that the biphasic actions of toluene are not related to the rapid elimination of toluene, and are probably related to actual brain levels of toluene.

The results of the present study are consistent with previous studies demonstrating biphasic effects of toluene on other responses such as EEG and sleep-wake patterns [1,29], operant behaviors [17] and algesia [9]. Thus the biphasic actions of toluene may generalize to many pharmacodynamic effects. Accordingly, in comparative analysis of solvent abuse it is essential to define the level of exposure. This may explain why studies of solvent abuse in humans have not produced consistent results. For example, EEG patterns during solvent inhalation have been reported to be either increased slow wave activity [4,19], spikes and slow waves [4] or increased high frequency activity [21]. These apparently conflicting results may represent actions of solvent exposure measured at different parts of the dose-response curve.

In studies of inhalation exposure, both concentration and duration of exposure determine the dose-response characteristics [5,18]. These variables must be considered when comparing results from different studies. Thus, in the present study, biphasic responses to toluene were observed when rats were exposed to 2500–15,000 ppm toluene for one hour. In contrast, only increased locomotor activity occurred in mice exposed to 520–1400 ppm toluene for 1 hour [14] or in rats exposed to 2500–30,000 ppm toluene for 5–12 minutes [11,12]. Biphasic effects of toluene on rearing were recorded in rats exposed to 1000–4000 ppm toluene for 4 hours [29]. Decreased locomotor activity occurred in weanling rats exposed subchronically to 900–1400 ppm toluene for 14 hours/day [22]. These observations reinforce the conclusion that toluene inhalation produces biphasic effects on spontaneous behavior, and that the effects are dependent on both the concentration of toluene and the duration of exposure.

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REFERENCES

1. Arito, H., H. Tsuruta and K. Nakagaki. Acute effects of toluene on circadian rhythms of sleep-wakefulness and brain monoamine metabolism in rats. *Toxicology* **33:** 291–301, 1984.
2. Barrow, C. S. and D. E. Dodd. Ammonia production in inhalation chambers and its relevance to chlorine inhalation studies. *Toxicol Appl Pharmacol* **49:** 89–95, 1979.
3. Benignus, V. A., K. E. Muller, C. N. Barton and J. A. Bittikofer. Toluene levels in blood and brain of rats during and after respiratory exposure. *Toxicol Appl Pharmacol* **61:** 326–334, 1981.
4. Brozovsky, M. and E. G. Winkler. Glue sniffing in children and adolescents. *NY State J Med* **65:** 1984–1989, 1965.
5. Bruchner, J. V. and R. G. Peterson. Evaluation of toluene and acetone inhalant abuse. I. Pharmacology and pharmacodynamics. *Toxicol Appl Pharmacol* **61:** 27–38, 1981.
6. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics*. Glenview, IL: Scott, Foresman and Co., 1977.
7. Carlsson, T. and T. Lindquist. Exposure of animals and man to toluene. *Scand J Work Environ Health* **3:** 135–143, 1977.
8. Colotla, V. A., S. Bautista, M. Lorenzana-Jimenez and R. Rodriquez. Effects of solvents on schedule-controlled behavior. *Neurobehav Toxicol* **1:** Suppl 1, 113–118, 1979.
9. Contreras, C. M. and R. E. Bowman. Excitatory and hypoalgesic effects of toluene in the rat. *Bol Estud Med Biol* **32:** 31–38, 1982.
10. Drew, R. and S. Laskin. Environmental inhalation chambers. In: *Methods of Animal Experimentation*, edited by W. I. Gay. New York: Academic Press, 1973, pp. 1–41.
11. Gause, E. M., V. Mendez and I. Geller. Exploratory studies of a rodent model for inhalant abuse. *Neurobehav Toxicol Teratol* **7:** 143–148, 1985.
12. Hinman, D. J. Tolerance and reverse tolerance to toluene inhalation: Effects on open-field behavior. *Pharmacol Biochem Behav* **21:** 625–631, 1984.
13. Hinners, R. G., J. K. Burkart and C. L. Punte. Animal inhalation exposure chambers. *Arch Environ Health* **16:** 194–206, 1968.
14. Kjellstrand, P., B. Holmquist, I. Jonsson, S. Romare and L. Mansson. Effects of organic solvents on motor activity in mice. *Toxicology* **35:** 35–46, 1985.
15. Lewis, P. and D. Patterson. Acute and chronic effects of the voluntary inhalation of certain commercial volatile solvents by juveniles. *J Drug Issues* **4:** 162–175, 1974.

16. Massengale, O. N., H. H. Glaser, R. E. LeLieuve, J. B. Dodds and M. E. Klock. Physical and psychologic factors in glue sniffing. *N Engl J Med* **269:** 1340–1344, 1963.
17. Moser, V. C. and R. L. Balster. The effects of acute and repeated toluene exposure on operant behavior in mice. *Neurobehav Toxicol Teratol* **3:** 471–475, 1981.
18. Moser, V. C. and R. L. Balster. Acute motor and lethal effects of inhaled toluene, 1,1,1-trichloroethane, halothane, and ethanol in mice: Effects of exposure duration. *Toxicol Appl Pharmacol* **77:** 285–291, 1985.
19. Nylander, I. "Thinner" addiction in children and adolescents. *Acta Paedopsychiatr (Basel)* **29:** 273–284, 1962.
20. Press, E. and A. K. Done. Solvent sniffing. Physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. I. *Pediatrics* **39:** 451–461, 1967.
21. Press, E. and A. K. Done. Solvent sniffing. Physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. II. *Pediatrics* **39:** 611–622, 1967.
22. Pryor, G. T., J. Dickinson, R. A. Howd and C. S. Rebert. Neurobehavioral effects of subchronic exposure of weanling rats to toluene or hexane. *Neurobehav Toxicol Teratol* **5:** 47–52, 1983.
23. Pryor, G. T., J. Dickinson, E. Feeney and C. S. Rebert. Hearing loss in rats first exposed to toluene as weanlings or as young adults. *Neurobehav Toxicol Teratol* **6:** 111–119, 1984.
24. Pyykko, K., H. Tahti and H. Vapaatolo. Toluene concentrations in various tissues of rats after inhalation and oral administration. *Arch Toxicol* **38:** 169–176, 1977.
25. Rebert, C. S., S. S. Sorenson, R. A. Howd and G. T. Pryor. Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. *Neurobehav Toxicol Teratol* **5:** 59–62, 1983.
26. Sato, A., T. Nakajima, Y. Fujiwara and K. Hirosawa. Pharmacokinetics of benzene and toluene. *Int Arch Arbeitsmed* **33:** 169–184, 1974.
27. Sharp, C. W. and L. T. Carroll (Eds.). *Voluntary Inhalation of Industrial Solvents*. Rockville, MD: National Institute on Drug Abuse, 1978.
28. Shigeta, S., T. Misawa and H. Aikawa. Effects of concentration and duration of toluene exposure on Sidman avoidance in rats. *Neurobehav Toxicol* **2:** 85–88, 1980.
29. Takeuchi, Y. and N. Hisanaga. The neurotoxicology of toluene: EEG changes in rats exposed to various concentrations. *Br J Ind Med* **37:** 314–324, 1977.
30. Weisenberger, B. L. Toluene habituation. *J Occup Med* **19:** 569–570, 1977.
31. Weiss, B., R. W. Wood and D. A. Macys. Behavioral toxicity of carbon disulfide and toluene. *Environ Health Perspect* **30:** 39–45, 1979.
32. Wood, R. W. Reinforcing properties of inhaled substances. *Neurobehav Toxicol* **1:** Suppl 1, 67–72, 1979.
33. Wood, R. W., J. B. Coleman, R. Schuler and C. Cox. Anticonvulsant and antipunishment effects of toluene. *J Pharmacol Exp Ther* **230:** 407–412, 1984.
34. Wood, R. W., D. C. Rees and V. G. Laties. Behavioral effects of toluene are modulated by stimulus control. *Toxicol Appl Pharmacol* **68:** 462–472, 1983.