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Tolerance and Reverse Tolerance to Toluene Inhalation: Effects on Open-Field Behavior[1]

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HINMAN, D. J. *Tolerance and reverse tolerance to toluene inhalation: Effects on open-field behavior.* PHARMACOL BIOCHEM BEHAV 21(4) 625–631, 1984.—Rats were exposed by inhalation to extremely high concentrations of toluene vapors twice daily for six weeks, as an animal model of organic solvent abuse. At preset intervals during repeated exposure, the rats were exposed to test concentrations of toluene and effects on behavior in an open field were measured. Concentration-effect curves were determined during Weeks 4 to 6 of repeated exposure. Tolerance to toluene was measured as a decreased response to the test exposure and a shift of the concentration-effect curve to the right. Reverse tolerance was measured as an increased response to the test exposure and a shift of the concentration-effect curve to the left. Results demonstrated that the effects of repeated exposure to toluene showed behavioral selectivity: tolerance developed to ataxia, hindlimb myoclonus, and inhibition of rearing, whereas reverse tolerance developed to headshakes and increased locomotor activity.

Toluene inhalation Glue sniffing Organic solvent abuse Tolerance Reverse tolerance Open-field behaviors

ORGANIC solvent abuse is a significant problem, especially among adolescents [1, 4, 15, 27, 32]. Solvent abuse often involves inhalation of a mixture of organic solvent vapors, and the individual components of these mixtures may have differing pharmacologic and toxicologic properties. Thus, in experimental studies it is important to evaluate each component separately. Toluene is a component of many commonly abused inhalant substances and is the substance of choice among solvent abusers because of its pleasant high and relative lack of side effects [17]. Furthermore, toluene does not cause peripheral neurotoxicity, whereas such toxicity is a significant effect of other organic solvents [5, 14, 28]. Thus inhalation of toluene is an appropriate model for investigating pharmacologic aspects of solvent abuse.

Abuse of organic solvents often involves repeated exposure to very high concentrations of solvent vapors [1, 15, 19, 20]. Some solvent abusers inhale the solvents on a daily basis for long periods of time [13,29]. However, little is known about quantitative changes in pharmacologic responses to inhalant drugs during chronic exposure to high concentrations of solvent vapors. Tolerance to glue sniffing in humans has been reported [32]. Chronic abusers reported that with repeated abuse they required more tubes of glue to experience the desired psychotropic effects. Alternatively, accumulation of solvents in the body may occur during chronic exposure. Accumulation in adipose tissue could occur, because elimination of solvents from adipose tissue is very slow [23,26]. Such accumulation could quantiatively alter the response to subsequent exposures.

Drug tolerance is measured experimentally in two ways [12]. Tolerance is indicated if the pharmacologic response to a standard dose is decreased following chronic administration of that drug. A more complete analysis of drug tolerance can be accomplished by evaluating the dose-response curve for the drug. Tolerance is demonstrated if the dose-response curve for the drug in chronically dosed animals is shifted to the right compared to drug-naive animals, indicating that more drug is required to produce a given pharmacologic response. Conversely, reverse tolerance is measured by an increase in the response to the standard dose and a shift in the dose-response curve to the left.

Recently, several animal models of organic solvent abuse have been reported, using behavioral or electrophysiologic measures of drug response. Behavioral effects of toluene inhalation include disruption of operant behaviors [6, 18, 33, 35], decreased locomotor activity [21], and self-administration of solvents [34]. Electrophysiologic studies indicated that toluene inhalation produced a mixture of excitatory and depressant effects on the central nervous system [8,30] and impairment of hearing as evidenced by analysis of auditory-evoked potentials [25]. Hearing loss was also be demonstrated by behavoral audiometric procedures [22].

In the present study, the effect of inhalation of toluene on

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open-field behaviors in rats was used as an animal model for solvent abuse. The exposure paradigm was designed to mimic human solvent abuse. The typical pattern of solvent abuse among humans is to inhale high concentrations of solvent vapors for only a few minutes to achieve the desired psychotropic effects [1, 15, 20]. The psychotropic effects may last for 30 to 60 minutes following a single episode [1,20]. Thus, in the present study, rats were exposed briefly to high concentration toluene vapors in a head-only exposure chamber. Effects of a test exposure to toluene on open-field behaviors were measured at intervals during repeated exposures, and concentration-effect curves for toluene were measured after three weeks of repeated exposures, in order to evaluate the development of tolerance and reverse tolerance.

METHOD

Adult, male Long Evans hooded rats (250–400 g) were used. The rats had free access to food and water and were housed in individual Plexiglas cages in a room with a 12 hour light/12 hour dark cycle in effect. The lights were on from 7:00 a.m. to 7:00 p.m. Three groups of rats were used. One group (n=10) was exposed to toluene only by the acute exposure paradigm. Two other groups were used in the repeated exposure study: a toluene-exposure group (n=10) and a sham-exposure group (n=6). Each of these groups was exposed to two exposure paradigms: the daily exposure paradigm and the test exposure paradigm.

Acute Exposure Paradigm

Each rat was restrained in a specially constructed, tubular restrainer and exposed to toluene or room air for 5 minutes in an individual head-only exposure chamber (modified from systems previously described [9, 11, 24]). The chamber was constructed of Plexiglas and lined with teflon to minimize adsorption of toluene to the surface of the chamber. The chamber volume was 2 liters, and the dimensions were 10×10×20 cm. The air-flow rate was 2.5 liters/minute as measured by a model 1355 Brooks SHO-RATE 150 flowmeter. Open-field behaviors were recorded for 5 minutes beginning immediately after exposure. Each rat was exposed to four concentrations of toluene or room air (sham exposure), with at least three days between consecutive exposures. The order of presentation of the toluene concentrations and sham exposure was randomized by Latin square.

Daily Exposure Paradigm

Groups of four or five rats were individually restrained and exposed to toluene vapors (10,000 ppm) or room air in a multiple, head-only inhalation chamber. The chamber volume was 6 liters (30×10×20 cm), and the air flow rate was 6 liters/minute. Each exposure lasted 15 minutes, and exposures were repeated twice daily (at approximately 10:00 a.m. and 6:00 p.m.), for five days per week.

After the morning exposure, the rats were returned to their individual home cages, which were placed on Animex model SE activity meters. Activity counts were recorded for 5 minutes beginning immediately after the exposure.

Test Exposure Paradigm

Each rat was restrained and exposed to toluene or room air in the individual head-only exposure chamber as described above. On Days 1, 6, 13, and 20 (Weeks 0, 1, 2, and 3) all rats were exposed for 5 minutes to the test exposure paradigm, and open field behaviors were recorded immediately following exposure. Rats in the sham-exposure group were exposed to room air, and rats in the toluene-exposure group were exposed to 10,000 ppm toluene.

Concentration-Effect Study

During the concentration-effect study (Weeks 4 to 6 of repeated exposure) the rats were exposed to the daily exposure paradigm (sham- or toluene-exposure) twice daily for 20 consecutive days, except that on alternate mornings the rats were exposed to a test concentration of toluene. All rats were exposed individually to each of four concentrations of toluene or room air (sham exposure) for 5 minutes, and open-field behaviors were recorded immediately after the exposure. In these studies, the observations were made in a blind fashion with regard to whether the rat was from the sham-exposure or toluene-exposure group. The order of presentation of the toluene concentrations and room air was randomized by Latin square.

Normal Open-Field Behaviors After Repeated Exposures

Sixteen to 20 hours after the final daily exposure to toluene or sham exposure, each rat was placed in the open field. The standard open-field behaviors plus the number of fecal pellets dropped were recorded for 5 minutes.

Open-Field Test

Open-field behaviors were observed in a Plexiglas box (40×50×25 cm) and were recorded for 5 minutes immediately following the exposure. Behaviors recorded were: righting reflex (+,0), ataxia (+,0), headshakes (#), hindlimb myoclonus (+0), rearing (#), and grooming (min). The righting reflex was measured by placing the rat in the supine position and noting whether it assumed the prone position with all four paws touching the floor. Loss of the righting reflex was recorded when the rat failed to regain the prone position within 30 seconds. Ataxia was recorded as a quantal response (all-or-nothing) and was scored when the rat staggered while walking or fell when attempting to rear or groom. Locomotor activity was measured simultaneously by placing the observation box on an Animex model SE activity meter.

Generation of Toluene Vapors

Toluene vapors were generated using a universal vaporizer. This custom-built vaporizer was a 2-liter-capacity reservoir made of stainless steel. Room air, prefiltered by a Balston DFU filter to remove oil and dust, was bubbled through the toluene (J. T. Baker Co.; reagent grade, purity > 99%) via a stainless steel mesh bubbler. The saturated toluene vapors were diluted with room air to produce the final concentration in the exposure chambers. All tubing and connectors were either teflon-coated plastic or stainless steel to minimize adsorption of toluene.

All concentrations reported are nominal values. These values were calculated based on the ratio of air flow through the vaporizer to the air flow of the room air diluent. It was assumed that the toluene vapors generated by the vaporizer were saturated [saturation (in ppm) = (vapor pressure of toluene ÷ atmospheric pressure) × 10,000]. Actual concentrations of toluene in the air in the exposure chambers were not measured. However, during repeated exposures, the

TABLE 1

ACUTE CONCENTRATION-EFFECT STUDIES

	Concentration of toluene ($\times 10^3$ ppm)					EC50* ($\times 10^3$ ppm)
	0	2.5	5	10	15	
Loss of righting (%)	0	0	0	0	70‡	13.5
Ataxia (%)	0	0	30	90‡	70‡	6.4
Hindlimb myoclonus (%)	0	30	40	70‡	80‡	6.3
Rearing (#)	17.0 ± 2.6	12.4 ± 1.6	9.0 ± 2.3	5.9 ± 0.8‡	9.0 ± 1.7†	5.2
Grooming (%)	100	80	20‡	30‡	10‡	3.6
Headshakes (#)	0.7 ± 0.3	0.2 ± 0.1	2.1 ± 0.6	4.1 ± 1.1‡	3.0 ± 0.7†	6.2
Activity (#)	91.4 ± 20.6	116.6 ± 16.4	135.0 ± 16.7	147.1 ± 21.2	186.6 ± 24.2‡	6.3

*EC50—effective concentration in 50% of animals (quantal responses) or concentration that produced a 50% change in response (graded responses).

†$p<0.05$ (multiple t-test for graded responses, z-test for quantal responses) compared to sham exposure.

‡$p<0.01$ (multiple t-test for graded responses, z-test for quantal responses) compared to sham exposure.

n=10 rats per group.

technical aspects of the exposure were the same on each day. Moreover, during the concentration-effect study, the order of presentation of the graded concentrations of toluene was randomized by Latin square, and the order of testing the sham-exposed or toluene-exposed rats was also randomized. Thus, any daily variation in the actual concentration of toluene could not bias the concentration-effect curves for either exposure group.

Statistical Analysis

Graded data were analyzed by analysis of variance (two-factor, mixed design with repeated measures) and multiple Student's t-tests. Quantal data were analyzed by log-probit analysis [16], and by the nonparametric z-test for significant differences between proportions [3].

RESULTS

Acute Effects of Toluene Inhalation

Exposure to toluene in the concentration range 2,500 to 15,000 ppm for 5 minutes caused concentration-dependent changes in open-field behaviors in the hooded rat (Table 1). Toluene inhalation caused severe ataxia and decreased grooming and rearing. After exposure to the highest concentration of toluene, there was a brief period (<2 min) during which the righting reflex was absent.

Two abnormal motor patterns were observed during recovery from exposure to toluene that were not observed following sham exposures: hindlimb myoclonus and severe headshakes. Hindlimb myoclonus was a particularly striking overt effect of toluene inhalation. The myoclonus consisted of rhythmic movement of one hind limb, that resembled kicking or scratching, but that appeared to be involuntary and purposeless. Usually, the myoclonus involved only one hind limb, although in some rats it alternated from side to side. An episode of myoclonus typically lasted for 5 to 10 seconds. In severe cases, myoclonus lasting 30 seconds or more occurred and continued while the rat walked around the field on three legs.

The second abnormal motor pattern was severe headshakes involving only the head and neck but not the trunk.

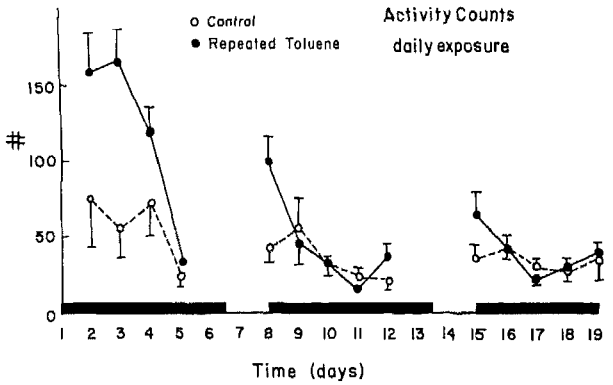

FIG. 1. Activity counts in the home cage following daily exposure paradigm. Number of rats (n)=6 in sham-exposure group, 10 in toluene-exposure group. Solid horizontal bar represents repeated daily exposure. Data are mean±s.e.m.

Mild headshakes occurred only rarely in the control observations. Headshakes following toluene exposure were sometimes so severe that they caused the rat to lose its balance.

A concentration-related increase in spontaneous locomotor activity occurred in the toluene-exposed rats. This locomotor activity was qualitatively different from normal locomotor or exploratory activity. In toluene-exposed rats the locomotor activity was characterized by exaggerated running and stumbling, with no sniffing, rearing, or grooming.

Observations During Daily Exposures

Rats exposed to toluene initially showed more activity than sham-exposed rats when they were returned to their home cages (Fig. 1; ANOVA, for treatment, $F(1,14)=5.60$, $p<0.05$; for treatment by trials interaction, $F(3,42)=5.12$, $p<0.005$). However, tolerance developed rapidly to this increase in activity, and after five days of exposure there were no significant differences between toluene- and sham-exposed rats (multiple t-test, $p>0.05$). In Weeks 2 and 3,

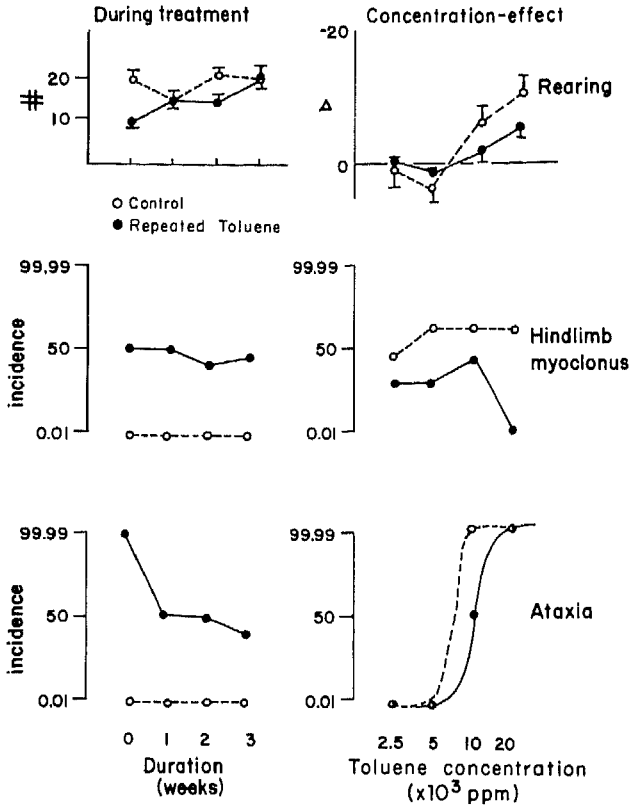

FIG. 2. Development of tolerance to toluene. Effects of test exposures during repeated exposure are shown in the left panels and the concentration-effect curves are shown in the right panels. The top panels show the effects of toluene on rearing as total number of rears (left) and difference from sham-exposure (right); the data are mean±s.e.m. The middle and bottom panels show the effects of toluene in hindlimb myoclonus and ataxia, respectively; the data are % incidence on a probability scale. The abscissas for the concentration-effect curves are on a logarithmic scale; n=6 in sham-exposure group, 10 in toluene-exposure group.

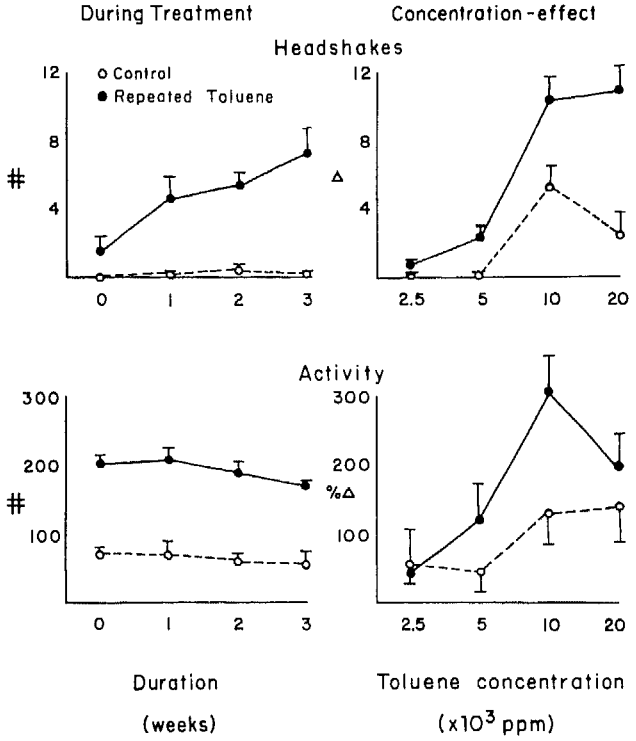

FIG. 3. Development of reverse tolerance to toluene. The effects of test exposures during repeated exposure are shown in the left panels and the concentration-effect curves are shown in the right panels. The top panels show the effects of toluene on headshakes as total number (left) and as difference from sham exposure (right). The bottom panels show the effects of toluene on locomotor activity as total number of activity counts (left) and as % change from sham exposure (right). The data are mean±s.e.m. The abscissas for the concentration-effect curves are on a logarithmic scale; n=6 in sham-exposure group, 10 in toluene-exposure group.

activity in the home cage following the daily exposure was not significantly different in sham- and toluene-exposed rats ($F(1,14)=1.36$, $p>0.05$ and $F(1,14)=0.46$, $p>0.05$ for Weeks 2 and 3 respectively). The treatment by trials interaction was significant for Week 2, $F(4,56)=3.99$, $p<0.005$, but not for Week 3, $F(4,56)=0.08$, $p>0.05$.

Effects of Test Exposure: Development of Tolerance and Reverse Tolerance

Open-field behaviors following the test exposures in the sham-exposed rats were very consistent between Weeks 0, 1, 2, and 3 (Figs. 2 and 3, Table 2). No significant changes in rearing, headshakes, or locomotor activity were recorded between the four tests ($F(3,15)=2.03$, 1.65, and 0.69 for rearing, headshakes, and activity, respectively, $p>0.05$ in each case; ANOVA calculated for sham-exposed rats only).

In contrast, the responses to toluene changed progressively during repeated exposures. Inhibition of rearing and ataxia decreased after the first week of exposure, and the responses remained constant thereafter (Fig. 2; Table 2). On the other hand, the number of headshakes following toluene exposure increased progressively with repeated exposure (Fig. 3; Table 2). The incidence of hindlimb myoclonus and effects on grooming and locomotor activity did not change during repeated exposure (Figs. 2 and 3; Table 2).

Concentration-Effect Studies Following Repeated Exposures

Concentration-effect curves for inhibition of rearing and the incidence of ataxia were shifted to the right in the toluene-exposed rats compared to the sham-exposed rats, whereas the curve for hindlimb myoclonus was shifted to the right and downward (Fig. 2; Table 2). In contrast, the concentration-effect curves for headshakes and locomotor activity were shifted to the left and upward in the toluene-exposed compared to the sham-exposed rats (Fig. 3; Table 2). The concentration-effect curve for inhibition of grooming did not change after repeated daily exposure to toluene (Table 2). Loss of the righting reflex occurred only in response to the highest concentration of toluene tested (20,000 ppm). The incidences of loss of the righting reflex were 5/6 and 7/10 for the sham-exposed and toluene-exposed groups, respectively. This difference was not significant ($p>0.05$, z-test).

Normal open-field behaviors measured 16 to 20 hours

TABLE 2

TOLERANCE AND REVERSE TOLERANCE TO TOLUENE

	Analysis of variance*					
	Observations during repeated exposure†			Concentration-effect study‡		
Behavior	Source	F	p	Source	F	p
Rearing	Treatment	1.47	n.s.	Treatment	0.38	n.s.
	Duration	8.00	<0.001	Concentration	21.44	<0.001
	Interaction	5.76	<0.005	Interaction	3.41	<0.025
Headshakes	Treatment	12.42	<0.005	Treatment	10.92	<0.005
	Duration	6.18	<0.005	Concentration	7.75	<0.025
	Interaction	3.23	<0.001	Interaction	1.28	n.s.
Activity	Treatment	166.96	<0.001	Treatment	1.80	n.s.
	Duration	3.33	<0.05	Concentration	15.15	<0.001
	Interaction	0.61	n.s.	Interaction	2.88	n.s.
Grooming	Treatment	111.62	<0.001	Treatment	1.68	n.s.
	Duration	0.03	n.s.	Concentration	43.28	<0.001
	Interaction	0.27	n.s.	Interaction	1.28	n.s.

*Two factor mixed design with repeated measures; degrees of freedom=(1,14) for treatment, and (3,42) for duration, concentration and interaction.

†Responses to test exposure on Days 1, 6, 13, and 20 of repeated exposure.

‡Responses to various concentrations of toluene during Weeks 4–6 of repeated exposure.

TABLE 3

NORMAL OPEN-FIELD BEHAVIORS AFTER REPEATED EXPOSURES

Behavior*	Sham-exposure group (n=6)	Toluene-exposure group (n=10)	p
Rearing (#)	18.7 ± 3.0	15.2 ± 2.1	n.s.
Grooming (min)	1.2 ± 0.4	1.0 ± 0.1	n.s.
Activity counts (#)	87.6 ± 15.3	54.4 ± 8.0	<0.05
Headshakes (#)	0.3 ± 0.3	0.2 ± 0.1	n.s.
Defecation (#)	0.2 ± 0.2	1.1 ± 0.5	n.s.

*Open-field behaviors were measured 16–20 hours after the final daily exposure to toluene or sham exposure. Data are mean ± s.e.m.

after the final exposure (sham or toluene) were not significantly different between the two groups except that the toluene-exposed rats showed significantly less locomotor activity than the sham-exposed rats (Table 3). The toluene-exposed rats gained significantly less weight during repeated exposure than the sham-exposed rats (51±6 g and 76±10 g, respectively, $p<0.05$, Student's t-test).

DISCUSSION

Acute inhalation of toluene vapors in a head-only inhalation chamber caused marked, concentration-related alterations in spontaneous behaviors of rats as measured in the open-field test. Toluene decreased rearing and grooming, and increased locomotor activity, associated with concentration-related ataxia. Exposure to toluene also induced two abnormal motor patterns not observed following sham exposures: hindlimb myoclonus and severe headshakes. Within the concentration range investigated, there were clear concentration-related increases in both of these behaviors. The highest concentrations of toluene caused a transient loss of the righting reflex, lasting less than 2 minutes after cessation of exposure to toluene. This loss of the righting reflex was particularly notable for two reasons. During the loss of righting, there frequently were isolated myoclonic jerks involving both hind limbs and fore limbs in vigorous whole body kicking movements. Also, during the period of loss of the righting reflex, the auditory startle response remained intact or was exaggerated (unpublished observations).

These acute effects of toluene inhalation on spontaneous behaviors were observed during recovery from brief exposures to very high concentrations of toluene in a head-only exposure chamber. This type of exposure paradigm was

specifically designed as an animal model to mimic patterns of solvent abuse in humans. During glue sniffing or solvent abuse, humans inhale toluene vapors estimated to be as high as 10,000 ppm for a few minutes in order to "self-titrate" to a desired level of intoxication [20]. The subjective effects of euphoria and hallucinations may last for 30 to 60 minutes following a single inhalational episode [1,17].

The duration of the behavioral effects of acute exposure to toluene was not determined in the present study. However, the results of preliminary studies indicated that the duration of the overt behavioral effects of toluene inhalation was approximately 15 to 30 minutes following the test exposure paradigm (5 minutes exposure), and 30 to 60 minutes following the daily exposure paradigm (15 minutes exposure). These estimates are consistent with reports of the duration of action of toluene following brief exposures [2,18]. Also, the results of pharmacokinetic studies demonstrate that toluene is rapidly eliminated following inhalational exposure [23,26].

The observed effects of toluene on open-field behaviors are consistent with previous reports of acute effects of toluene on behavior and neurophysiologic responses. Available evidence indicates that toluene and other organic solvents exert a mixture of excitatory and depressant effects on functions of the central nervous system depending on the concentration. Exposure to low levels of toluene produce primarily excitatory effects on the central nervous system such as hyperalgesia [7], increased fast activity in the electroencephalogram (EEG) [30], and increased wakefulness or arousal [30]. Higher levels of exposure to toluene produce predominantly central nervous system depressant effects such as disruption of operant behaviors [6, 18, 33, 35], hypoalgesia [7], slowing of the EEG pattern [30], and increased time spent in sleep [30].

The studies cited above reveal the effects of acute exposure to toluene on behavioral and neurophysiologic responses. In contrast, effects of repeated or chronic exposure to toluene have been reported. The primary purpose of the present study was to investigate the development of tolerance to toluene during repeated inhalational exposure. The results demonstrate that both tolerance and reverse tolerance develop, and that responses to repeated inhalation of toluene show marked behavioral selectivity. Thus tolerance to ataxia and inhibition of rearing was demonstrated by a decrease in response to the test exposure during repeated exposure and by a shift in the concentration-effect curve. Tolerance to hindlimb myoclonus was demonstrated only in the concentration-effect study.

On the other hand, reverse tolerance to headshakes was demonstrated by an increased response to the test exposure and a shift of the concentration-effect curve to the left with a higher ceiling. Reverse tolerance to increased activity was demonstrated in the concentration-effect study.

Tolerance to inhalation of toluene has not been previously demonstrated quantitatively. In one study, mice exposed repeatedly to toluene were not tolerant to its effect on operant behavior when measured following termination of repeated exposure [18]. However, it is possible that any tolerance had dissipated at the time of testing. In fact, the mice were slightly more sensitive to toluene [18], which may suggest reverse tolerance.

Both quantitative and qualitative changes in responses to repeated inhalation of toluene have been reported, although none of the studies systematically examined the development of tolerance. Rats repeatedly exposed to toluene showed less motor activity than control rats but with continued exposure this effect abated [21], which could be interpreted as development of tolerance. Similarly, repeated exposure to toluene decreased the threshold for chemically-induced seizures, and this effect also abated with continued exposure [31]. Repeated exposure to extremely high concentrations of toluene produced complex quantitative and qualitative changes in EEG responses to subsequent exposure to toluene [8]. However, it is not possible to determine whether these changes were related to development of tolerance since this possibility was not specifically investigated.

Two general mechanisms of tolerance have been identified: dispositional and functional tolerance [12]. In dispositional tolerance, the rate of elimination of a drug increases during chronic administration. Consequently, when a standard dose of drug is given to a tolerant animal a lower peak drug level is achieved at the site of action, and the duration of drug action is shortened. In functional or cellular tolerance, the drug produces less pharmacologic response in the tolerant animals than in drug-naive animals even though the drug levels at the site of action are equivalent in both groups.

The present results do not indicate whether the tolerance that develops during repeated inhalation of toluene is functional or dispositional. In order to distinguish between the two types of tolerance it is necessary to measure the brain levels of toluene and its elimination in toluene- and sham-exposed rats. In addition, measurement of behavioral responses at equivalent brain levels of toluene is required to fully analyze the development of tolerance to toluene. Toluene has been shown to induce drug metabolizing enzymes in the liver of chronically exposed rats, and the levels of toluene in adipose tissue decreased during chronic exposure [10]. However, brain levels of toluene were not changed during chronic exposure [10].

Tolerance to toluene developed rapidly during the first week of repeated exposure, then remained constant for the remainder of the study. On the other hand, reverse tolerance developed slowly and progressively throughout the duration of the study. This difference in the time courses of development of tolerance and reverse tolerance suggests that the mechanisms of tolerance and reverse tolerance are different.

Tolerance developed to increased locomotor activity during repeated exposure (Fig. 1), but reverse tolerance to increased locomotor activity was demonstrated during the concentration-effect study (Fig. 3). These are not contradictory results, since the experimental conditions in the two studies were different. Locomotor activity during repeated exposures was measured in the rats' home cages, a familiar environment. During the concentration-effect study, locomotor activity was measured in the open field, a novel environment. Thus, these results indicate that the effects of repeated exposure to toluene show behavioral selectivity depending on the particular behavior measured.

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