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Effects of Toluene Inhalation on Carbon Dioxide Production and Locomotor Activity in Mice[1]

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Effects of Toluene Inhalation on Carbon Dioxide Production and Locomotor Activity in Mice. BUSHNELL, P. J., EVANS, H. L., AND PALMES, E. D. (1985). *Fundam. Appl. Toxicol.* **5**, 971–977. Rapid and noninvasive tests of locomotor activity (LA) and carbon dioxide production (minute volume expired CO_2, or $\dot{V}_ECO_2$) in mice were sensitive to the effects of inhaled toluene. Compared to sham exposures, toluene at 100 ppm had no effect on LA or $\dot{V}_ECO_2$; at 1000 and 3000 ppm, LA increased during exposure, while $\dot{V}_ECO_2$ was suppressed for 6 to 24 min at the beginning of exposure. In a nominal 10,000-ppm exposure, toluene levels were increased from 1000 to 10,500 ppm in 60 min. At these levels, toluene abolished LA at concentrations above 8000 ppm, and suppressed $\dot{V}_ECO_2$ throughout exposure. During recovery from toluene-induced narcosis, both LA and $\dot{V}_ECO_2$ were elevated above control. In other studies, groups of mice inhaled toluene daily at 0, 100, 1000, or 3000 ppm, 5 hr/day for 8 or 90 days, and were tested individually 30 to 90 min after termination of exposure. Under these conditions, toluene decreased postexposure $\dot{V}_ECO_2$ for 1–2 weeks, altered the weekly pattern of change in $\dot{V}_ECO_2$, and did not affect LA. No effects of repeated, daily exposure to toluene were observed on body weight. These results demonstrate the utility of the present method to detect changes in LA and metabolic rate resulting from toluene inhalation, and suggest that different mechanisms are involved in the behavioral and metabolic responses to toluene inhalation.

Toluene has narcotic properties and is prevalent in gasoline, inks, paints, and adhesives. In humans, it produces euphoria at low doses and stupor, unconsciousness, and coma at high doses (Benignus, 1981). Human exposure to toluene may be occupational or recreational, as this solvent is reported to produce a pleasant euphoria with few side effects (Massengale *et al.*,1963). Its abuse potential may be enhanced by its low apparent irritancy to humans (von Oettingen *et al.*, 1942; Carpenter *et al.*, 1944) and mice (Nielsen and Alarie, 1982). However, few animal models of solvent inhalation have been developed, and the prevalence of toluene exposure indicates the need for careful evaluation of its health effects to the population at risk.

The systemic toxicity of inhaled toluene is reportedly low, with CNS effects predominating (NAS, 1981; Benignus, 1981). Acute exposure to toluene vapor produces a biphasic response in a variety of behavioral measures, depending upon dose and length of exposure. At low vapor concentrations (≤2000 ppm) or short exposures (less than 30 min) toluene increased locomotor activity (LA) (Yamawaki and Sarai, 1982), operant response rates (Weiss *et al.*, 1979; Glowa, 1981; Moser and Balster, 1981; Wood *et al.*, 1983), and sensitivity to shock and heat (Contreras and Bowman, 1982). Higher concentrations typically suppress behavior: thus, rats' operant response rates declined after inhalation of toluene at concentrations exceeding 6000 ppm for 30 min (Moser and Balster, 1981) or 3,000 ppm for 4 hours (Wood *et al.*, 1983). Operant responding for milk also decreased in mice inhaling 2000 ppm toluene (Glowa, 1981).

Chronic exposure to toluene vapor can induce visual and auditory dysfunction in human glue sniffers (Ehyai and Freemon, 1983)

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and high-frequency hearing loss in rats (Pryor *et al.*, 1983) associated with changes in brainstem auditory evoked responses (Rebert *et al.*, 1983) and cochlear hair cell loss (Sullivan *et al.*, 1984). Electroencephalographic and sleep disturbances have also been reported following chronic toluene inhalation (Hisanaga and Takeuchi, 1983).

Locomotor activity and minute volume expired CO_2 ($\dot{V}_ECO_2$) in mice provide indices of behavioral and metabolic activity in response to pharmacologic and environmental challenges (Bushnell *et al.*, 1983, 1984, 1985). These indices appear to be well suited to the study of toluene inhalation due to its narcotic and pharmacokinetic properties. Thus, changes in arousal and respiratory rate, mediated by parts of the brainstem normally sensitive to anesthetic and narcotic agents, may affect both LA and $\dot{V}_ECO_2$ as the solvent is inhaled. In addition, the rapid uptake and elimination of toluene means that its CNS effects should appear and fade rapidly with changes in toluene vapor concentration. Repetitive, on-line determinations of response concurrent with exposure are advantageous in the quantification of effect of this compound. Finally, the low irritant potency of toluene (Nielsen and Alarie, 1982) indicates that minimal ventilatory responses to toluene vapor will occur, and thus any locomotor and metabolic responses to toluene will be minimally influenced by such defensive reactions.

In addition to acute effects, it is possible that longer lasting effects of repeated toluene inhalation may be apparent in these measures. Either cumulative effects, akin the hearing loss resulting from chronic toluene inhalation (Pryor *et al.*, 1983), or tolerance to repeated exposure (Himnan, 1984) may be detectable in changes in LA and $\dot{V}_ECO_2$ to repeated toluene exposure.

We report here experiments in mice to determine (1) the effects of toluene inhalation at concentrations ranging from the 8-hr time-weighted average threshold limit value (TLV) of 100 ppm (ACGIH, 1984) to a clearly narcotizing level of 10,000 ppm on concurrently determined LA and $\dot{V}_ECO_2$ and (2) the effects of repeated, 5-hr daily exposures to atmospheres of toluene at concentrations from 100 to 3000 ppm on LA and $\dot{V}_ECO_2$ determined 30 to 90 min after termination of exposure.

MATERIALS AND METHODS

Subjects

Adult male C57BL/6J mice,[2] weighing 20–30 g, were housed in groups of four in acrylic cages (13 × 28 × 17 cm) on pine chip bedding.[3] Except when removed for testing, all animals lived in a colony room maintained on a 12:12 light:dark cycle with light onset at 6 AM. The room was ventilated with a one-pass air supply with 12–15 air changes per hour, which maintained the temperature at 27 ± 1°C and the relative humidity between 45 and 65%. Rodent lab chow[4] and water were available *ad libitum*. Animal care practices conformed to standards promulgated by NIH (1980).

Apparatus

The apparatus has been described in detail elsewhere (Bushnell *et al.*, 1983, 1985). Briefly, it consisted of eight mouse chambers in an isolation unit, each with an ir photobeam to detect LA. CO_2 concentrations were measured by two ir CO_2 analyzers,[5] assorted plumbing, and two integrating chart recorders[6] interfaced to a PDP/8a computer[7] with a SKED system.[8] Gas flow and pressure were maintained by vacuum pumps and critical orifices at 1.3 liters/min and 4–6 cm H_2O vacuum, respectively. Solvent vapor concentrations were determined with an ir spectrophotometer.[9]

Procedures

Generation of toluene vapor. For concurrent toluene exposure and testing (Experiment 1), saturated toluene vapor was generated by passing room air over the surface of warmed, high-purity toluene.[10] This vapor was then diluted with room air and passed through four of the eight indi-

[2] Jackson Labs, Bar Harbor, Maine.
[3] Beta Chip, Northwestern Products, Warrensburg, N.Y.
[4] Ralston Purina, St. Louis, Mo.
[5] LIRA, Mine Safety Appliances, Pittsburgh, Pa.
[6] Linear Instruments, Reno, Nev.
[7] Digital Equipment, Maynard, Mass.
[8] State Systems, Kalamazoo, Mich.
[9] MIRAN 1A, Foxboro Analytical, North Haven, Conn.
[10] Spectrophotometric grade (99+%), Aldrich, Milwaukee, Wisc.

vidual mouse test chambers; concentrations were assayed on-line every 6 min from one of the four chambers. For all other experiments, toluene vapor was generated by nebulizing the liquid at room temperature followed by dilution with air. Vapor of appropriate concentration was then passed through 1-m^3 Laskin inhalation chambers (Drew and Laskin, 1973), in which concentrations were monitored at 30 min intervals.

Locomotor activity measurement. Interruptions of the ir photobeam by the mouse were counted by the computer and normalized by square root transformation prior to analysis.

$\dot{V}_ECO_2$ measurement. CO_2 concentrations in the outflowing air of each mouse chamber were integrated over periods of 1.5 min in each 6-min sampling cycle. Airstream CO_2 concentrations were converted to minute volume expired CO_2 ($\dot{V}_ECO_2$) by multiplying the airstream CO_2 concentration (ml/liter) by total flow (liters/min). These volumes were then normalized to the metabolic mass (Kleiber, 1947) of each animal (body weight in kg raised to the 0.75 power) (see Bushnell *et al.*, 1985, for further details).

Exposure Protocols

In Experiment 1, LA and $\dot{V}_ECO_2$ of individual mice were measured concurrently with toluene inhalation in the individual test chambers. Sixteen mice were divided randomly into two groups of eight. One group inhaled toluene vapor at 10,000, 100, 1000, and 3000 ppm for 72 min on successive days; the other served as an air control (sham exposure) group. After appropriate toluene concentrations (up to 3000 ppm) were obtained in the test chambers, the mice were introduced into the vapor and data collection began 1 to 2 min thereafter. In the 10,000-ppm condition, the mice were placed in the chambers prior to generation of vapor for safety reasons; thus the concentration climbed exponentially from 0 to 10,500 ppm over the course of the exposure period ($t_{1/2}$ = 15 min).

In Experiments 2 and 3, separate groups of four or six mice were placed, without food or water, in 20 × 20 × 28-cm stainless-steel mesh exposure cages, which were put into the Laskin inhalation chambers prior to vapor generation. Vapor was maintained at the appropriate concentrations (±10%) for 5 hr, after which generation was stopped and the chambers were cleared of vapor for 30 min. Mice were then removed and tested individually for 24 min, beginning 0, 30, or 60 min thereafter (30, 60, or 90 min after termination of vapor generation). In Experiment 2, 14 mice were exposed to air for 4 days (sham exposure baseline), to 3000 ppm toluene for 5 days, to air for 2 days, and to 3000 ppm toluene for 3 final days. A control group (n = 14) was exposed simultaneously to air each day. In Experiment 3, mice (n = 8/group) were exposed to air, 100, 1000, or 3000 ppm toluene 5 days/week for 12 weeks. Each animal was weighed weekly including two pretreatment baseline weeks. Food and water consumption were not monitored. Equipment failure precluded collection of $\dot{V}_ECO_2$ data during Weeks 3–5.

Statistical Analysis

The statistical analysis utilized the analysis of variance and covariance program BMDP2V (Dixon, 1981), followed by simple main effects tests within significant interactions (Kirk, 1968) and Dunnett's tests (Myers, 1966) to compare experimental means to control. In the figures, points marked with asterisks differ from control at $p < 0.05$. In the postexposure tests (Experiments 2 and 3), no effect of the delay after exposure was observed; thus the data were pooled across delay times for subsequent analysis.

RESULTS

Experiment 1: Toluene Inhalation Concurrent with Determination of LA and $\dot{V}_ECO_2$

LA in control mice declined during air exposures (Fig. 1) and similarly in mice inhaling 100 ppm toluene. At 1000 ppm toluene, LA began to increase above control after 60 min of toluene inhalation; at 3000 ppm, the increase began after 12 min of toluene inhalation and continued throughout exposure. At 10,000 ppm, LA declined in parallel with controls for 24 min, then fell precipitously, reaching zero for all mice after 48 min of exposure to toluene. Analysis of covariance (using preexposure baseline scores as covariates) on these data yielded significant effects of toluene concentration [$F(4,42) = 17.05$, $p < 0.001$],

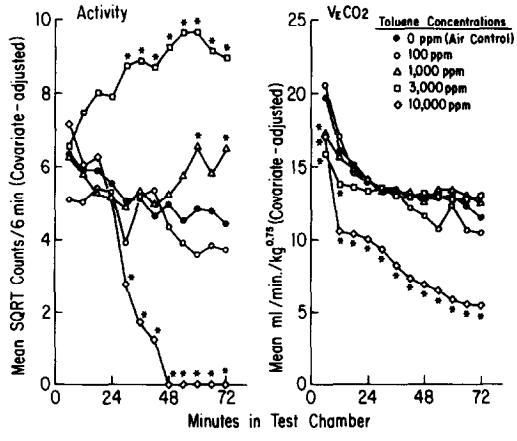

FIG. 1. Locomotor (left) and metabolic (right) responses of mice (n = 8 each) to graded doses of inhaled toluene. Asterisks indicate values differing significantly from control at $p < 0.05$.

intervals [$F(11,473) = 5.67$, $p < 0.001$], and the toluene concentration × intervals interaction [$F(44,473) = 5.44$, $p < 0.001$].

$\dot{V}_E CO_2$ (Fig. 1) changes paralleled those of LA only at 10,000 ppm. At 1000 and 3000 ppm, an initial drop in $\dot{V}_E CO_2$ was observed, despite normal or increasing LA. No effect on $\dot{V}_E CO_2$ was observed at 100 ppm toluene. An analysis similar to that for LA showed significant effects of toluene [$F(4,42) = 24.89$, $p < 0.001$], intervals [$F(11,473) = 64.04$, $p < 0.001$], and the toluene × intervals interaction [$F(44,473) = 3.38$, $p < 0.001$].

Recovery from 10,000 ppm toluene is detailed in Fig. 2. Toluene concentrations (top panel) fell to 2000 ppm by 54 min after vapor generation was stopped. Recovery of $\dot{V}_E CO_2$ (Fig. 2, lower panel) began as soon as the toluene concentration dropped below 10,000 ppm [toluene × intervals interaction, $F(11,66) = 19.48$, $p < 0.001$], while LA remained uniformly zero (Fig. 2, middle panel) for 24 min, or until the toluene concentration fell below 6000 ppm [toluene × intervals interaction, $F(11,66) = 15.23$, $p < 0.001$]. Continued recovery was characterized by increases above control in both LA and $\dot{V}_E CO_2$.

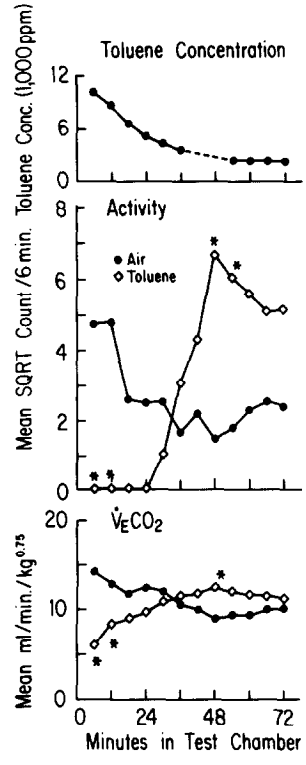

FIG. 2. Recovery from exposure to 10,000 ppm toluene. Toluene concentrations (top) fell from 10,500 to 2000 ppm 54 min after vapor generation was stopped. LA (middle) remained uniformly zero for 24 min, or until the vapor concentration fell below 6000 ppm, after which all animals became hyperactive as they recovered from narcosis. $\dot{V}_E CO_2$ levels (bottom) began to recover as soon as the vapor concentration fell below 10,000 ppm, and continued to increase for 48 min. The dashed line shows toluene concentration values estimated by interpolation between 36 and 54 min after the end of solvent generation.

Experiment 2: Daily Toluene

Exposure to 3000 ppm toluene for 5 hr/day for 8 days had no effect on LA measured 30–90 min after exposure to toluene vapor, but significantly [$F(2,38) = 16.23$, $p < 0.001$] reduced $\dot{V}_E CO_2$ by about 30% (Fig. 3). Postexposure $\dot{V}_E CO_2$ returned to normal on days when the test animals were exposed to air (Days 6 and 7 in Fig. 3), and was again suppressed upon reinstatement of toluene exposure on Days 8–10.

Experiment 3: Subchronic Toluene

Toluene inhalation exerted no overall effect on body weight. Mean ±SE weights (g) at the end of the 12-week exposure period follow: control, 26.2 ± 1.02; 100 ppm, 26.9 ± 1.02; 1000 ppm, 27.2 ± 1.1; 3000 ppm, 25.6 ± 0.6.

$\dot{V}_E CO_2$ levels tended to fall below control following exposure to all toluene concentrations (Fig. 4). However, the reduction in $\dot{V}_E CO_2$ was significant only at 3000 ppm, and only during Week 1 [main effect of toluene, $F(3,28) = 0.44$; toluene × weeks interaction, $F(24,224) = 1.62$, $p < 0.04$]. LA was not affected.

In addition, the suppression of $\dot{V}_E CO_2$ by toluene was more pronounced early in each week of exposure than late in the week [toluene × day-of-week interaction, $F(3,28) = 7.58$, $p < 0.001$]. This effect became apparent when the data were averaged across all weeks of the experiment (Fig. 5), and showed that, while

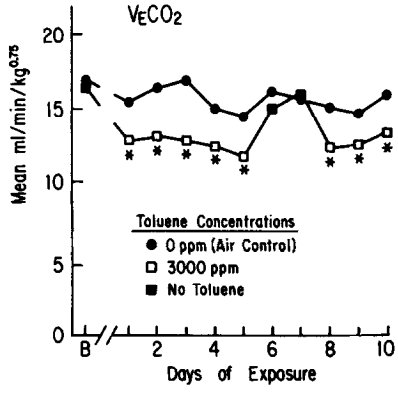

FIG. 3. Effects of repeated exposure to toluene on postexposure $\dot{V}_ECO_2$. Ten daily exposures (5 hr/day) to 3000 ppm toluene reduced $\dot{V}_ECO_2$, as measured 30–90 min after the end of toluene inhalation, by about 30% each day. $\dot{V}_ECO_2$ was normal on days when the mice were exposed to air (Days 6 and 7).

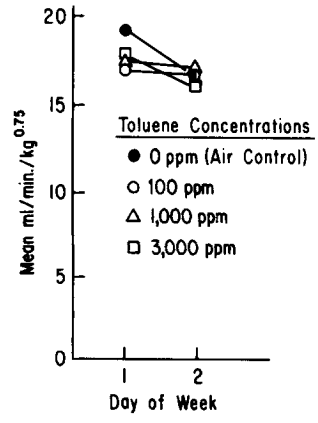

FIG. 5. Effect of daily toluene inhalation on $\dot{V}_ECO_2$ as a function of the day of the week. Values represent means averaged across the 12 weeks of exposure shown in Fig. 4.

the postexposure $\dot{V}_ECO_2$ of control mice typically declined during the week, that of the mice exposed to toluene at 100 and 1000 ppm was relatively low throughout the week. The 3000 ppm group showed a reduction in $\dot{V}_ECO_2$ from Monday to Thursday that was smaller than control, but still significant.

DISCUSSION

A prominent feature of the mouse's response to toluene inhalation was the divergent effects on LA and $\dot{V}_ECO_2$. This divergence indicates that these endpoints are not simply redundant measures of an animal's activity. Thus, in Experiment 1, inhalation of toluene at concentrations below 3000 ppm increased LA in a cumulative dose-dependent fashion (Fig. 1), yet decreased initial $\dot{V}_ECO_2$ levels in a strictly concentration-dependent manner (Fig. 1). Similarly in Experiments 2 and 3, postexposure $\dot{V}_ECO_2$ was suppressed in the absence of change in LA (Figs. 3 and 4).

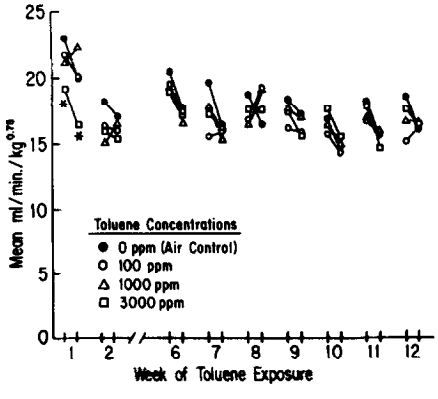

FIG. 4. Effect of 90 daily toluene exposures (5 hr/day, 5 days/week) on postexposure $\dot{V}_ECO_2$ as a function of weeks, showing two values per week.

$\dot{V}_ECO_2$ covaried with LA only when LA was suppressed by acute toluene inhalation at narcotizing concentrations above 3000 ppm. This pattern of response parallels similar effects of pentobarbital (Bushnell *et al.*, 1983, 1985). In both cases, narcosis was accompanied by ataxia and a fall in $\dot{V}_ECO_2$ (Fig. 1), and recovery by increasing LA and $\dot{V}_ECO_2$ to supranormal levels as in Fig. 2. Such a multiphasic behavioral response to barbiturates has been described previously (Bushnell *et al.*, 1975). This response pattern probably reflects differential sensitivity of inhibitory and excitatory brainstem centers, the former becoming depressed at lower narcotic concentrations than the latter (Brazier, 1961). This mechanism explains the hyperactivity and hyperexcitability associated with subnarcotic intoxication and possibly the attraction of solvent abuse to some people.

The biphasic toluene concentration–effect function for LA is also consistent with previous reports showing (1) behavioral activation resulting from pretest inhalation of toluene up to 3000 ppm (Weiss *et al.*, 1979; Moser and Balster, 1981; Wood *et al.*, 1983), (2) increased operant responding during inhalation of 1000 ppm toluene (Glowa, 1981), and (3) decreased responding during inhalation of 2000 ppm toluene (Glowa, 1981). The increased LA observed here at 1000 to 3000 ppm toluene probably represents activation of a variety of behaviors which would likely interfere with the performance of goal-directed operant behavior, as found by Glowa.

In contrast to the cumulative and biphasic effects of toluene on LA, the effect of toluene on $\dot{V}_ECO_2$ was immediate and monophasic; that is, $\dot{V}_ECO_2$ was immediately suppressed by toluene inhalation in a dose-related manner (Fig. 1). With continued exposure to 1000 and 3000 ppm, $\dot{V}_ECO_2$ returned to control levels after 6 and 24 min of exposure, respectively, despite increasing cumulative toluene dose.

The reasons for the suppression of metabolic rate in response to inhalation of toluene vapor are not known, but may involve any of a number of physiological adjustments in response to this vapor. Since toluene vapor is relatively nonirritating and does not inhibit ventilation in mice (Nielsen and Alarie, 1982), it is unlikely that the suppression of $\dot{V}_ECO_2$ was due simply to reduced ventilation rate. Nevertheless, the metabolic response to toluene resembles that induced by Formalin vapor, a powerful irritant (Jaeger and Gearhart, 1982). This similarity suggests that suppression of metabolic rate may represent part of a generalized defensive response to intoxication or physical trauma, and may be useful as an index of such responses.

In studies of repeated daily exposures to toluene (Experiments 2 and 3) LA and $\dot{V}_ECO_2$ were evaluated 30 to 90 min after toluene exposure. This exposure protocol did not change LA but did decrease $\dot{V}_ECO_2$ for 1–2 weeks after each exposure to 3000 ppm toluene (Figs. 3 and 4).

The suppression in $\dot{V}_ECO_2$ after inhalation of 3000 ppm toluene showed no sign of attenuation across eight exposures in Experiment 2 (Fig. 3). However, the fact that $\dot{V}_ECO_2$ suppression occurred for only the first week in Experiment 3 suggests the development of tolerance to repeated inhalation of 3000 ppm toluene (Fig. 4). Attenuation of the normal within-week decline in $\dot{V}_ECO_2$ by toluene at concentrations as low as 100 ppm (Fig. 5) may also be interpreted in terms of tolerance. Thus, toluene at 100 and 1000 ppm consistently suppressed $\dot{V}_ECO_2$ early in the week, following a weekend without exposure, but was ineffective late in the week, after 3–4 daily exposures. This within-week pattern suggests that tolerance to the solvent could develop each week and fade each weekend. It is likely that the regularity of the weekly exposure regimen in the 90-day study facilitated the development of this within-week effect, while the shorter exposure protocol in Experiment 2 was too brief to induce this form of tolerance.

In any case, it may be noted that $\dot{V}_ECO_2$ was suppressed in mice exposed to 100 ppm toluene (Fig. 5), which is the current 8-hr time-weighted average TLV for occupational exposure (ACGIH, 1984). Further work is needed to determine the importance of this effect, and to clarify its implications for human health. It is also apparent that a change in metabolic rate can be a sensitive index of intoxication in test animals, and should be examined in more detail to determine its role in the physiology of chemical intoxication.

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