TOXICOLOGY AND APPLIED PHARMACOLOGY 80, 487–495 (1985)

Effects of Toluene Inhalation on Behavior and Expired Carbon Dioxide in Macaque Monkeys[1]

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Received January 25, 1985; accepted May 14, 1985

Effects of Toluene Inhalation on Behavior and Expired Carbon Dioxide in Macaque Monkeys. TAYLOR, J. D., AND EVANS, H. L. (1985). *Toxicol. Appl. Pharmacol.* **80,** 487–495. Cynomolgus macaque monkeys received head-only exposure to 0, 100, 200, 500, 1000, 2000, 3000, and 4500 ppm toluene for 50 min while simultaneously tested for delayed matching-to-sample behavior, a test of cognitive functions. Response time increased and accuracy of matching decreased at 2000 ppm or more of toluene, indicating an attentional deficit but not specific memory effects. Behavioral indices exhibited monotonic concentration-related changes. Expired carbon dioxide (CO_2), the most sensitive index, displayed an inverted U-shaped concentration–effect curve, which increased at 100 ppm (the TLV) and decreased at 4500 ppm toluene. Changes in expired CO_2 provide new evidence of physiological changes at very low levels of toluene. These changes may indicate combined behavioral, respiratory, sensory, and metabolic effects. No behavioral measure exhibited either cumulative effects or tolerance to 4500 ppm during two 3-day exposures. However, both response time and expired CO_2 exhibited an acute, within-session tolerance. The results indicate that brief inhalation exposure to toluene impairs cognitive and motor abilities at concentrations below those causing overt signs, such as ataxia and intention tremoring.

Toluene (methyl benzene) is widely used in thinners and paints and as a substitute for the more toxic solvent benzene. Toluene lacks the carcinogenic potential of benzene and is not a significant respiratory irritant (Nielsen and Alarie, 1982). Occupational exposure to toluene has increased along with its commercial use (Cherry *et al.*, 1982). Toluene also has been voluntarily inhaled by humans (Iregren, 1982; Crites and Schuckit, 1979; Fornazzari *et al.*, 1983) and by experimental animals (Weiss *et al.*, 1979; Wood, 1978; Yanagita *et al.*, 1970). Toluene inhalation produces, in humans, symptoms of nervous system dysfunction (headache, dizziness, euphoria), and at higher concentrations, signs of neurological impairment (ataxia and intention tremoring) (Benignus, 1981; Von Oettinger *et al.*, 1942; Longley *et al.*, 1967). Because neurotoxic effects are the most prominent evidence of sublethal exposure to toluene, further study of the neurobehavioral effects of toluene inhalation is important.

Deficits in cognitive functions such as memory are frequently reported in humans exposed to solvents (Mutti *et al.*, 1984; Wilson, 1943; Knox and Nelson, 1966). However, most human studies consist of anecdotal case reports of poorly documented exposures (Tarsh, 1979; Weisenberger, 1977; Takeuchi *et al.*, 1981) and a few experiments limited to very low exposure levels (Astrand *et al.*, 1972; Veulemans and Masschelein, 1978; Dick *et al.*, 1984).

[1] A preliminary report of this work was presented at the 1985 Annual Meeting of the Society of Toxicology. (1985, *The Toxicologist* **5,** 23.)

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0041-008X/85 $3.00

Toluene inhalation has not been studied with animal models of memory. Most animal studies have employed schedule-controlled behavior in rodents to investigate tolerance, i.e., the diminution of toluene's effect with continued exposure (Moser and Balster, 1981; Yamawaki *et al.*, 1982), rate of responding (Glowa, 1981), and tranquilizer-like activity (Geller *et al.*, 1983; Wood *et al.*, 1984).

A disadvantage of investigating the behavioral effects of toluene in rodents is their limited capacity for complex cognitive behaviors which are important functions in both human and nonhuman primates. Wood (1978), using the squirrel monkey, found that these animals would press a lever for 15-sec deliveries of 1000 or 3000 ppm toluene. The nonhuman primate, therefore, is an important model, offering the opportunity to study complex cognitive functions at sublethal concentrations.

The purpose of this study was to examine the concentration–effect curve for acute toluene inhalation in the macaque monkey. Three types of effects were investigated. First, the effects of toluene on cognitive function were determined by the performance of monkeys on a delayed matching-to-sample test while being exposed to toluene. Few studies have evaluated behavior during, rather than after, inhalation exposure. Second, the effect of toluene on expired CO_2 was measured as a composite index of the monkey's behavioral, respiratory, cardiovascular, and metabolic activities. Toluene affected expired CO_2 of mice (Bushnell *et al.*, 1985). Third, we sought evidence of tolerance and sensitization following intermittent exposure as well as evidence of acute, within-session tolerance (Corfield-Sumner and Stolerman, 1978).

METHODS

Animals. Six adult feral female cynomolgus monkeys[3] (*Macaca fascicularis*) were housed individually in stainless-steel cages in a room automated for 12 hr of light and 12 hr darkness (lights on 6:00 AM–6:00 PM) and fed Purina Monkey Chow twice daily. The monkeys received free access to water during the last 3 hr of the light cycle and were deprived for at least 15 hr prior to each test session. Body weights ranged from 2.7 to 3.7 kg. All animal care conformed to NIH guidelines (National Institutes of Health, 1980). The monkeys were tested 50 min per day, Monday through Friday, throughout the experiment.

Two of the monkeys had previously received 100 ppm toluene and one monkey had received 1000 ppm toluene, each for 6 hr/day, 5 day/week, in a 90-day study 2 years previous to the start of this experiment. For economic reasons these previously exposed monkeys were selected for the present study. They did not differ in health or behavior from additional monkeys which had similar testing history but had never received toluene. No new differences between these two groups appeared over the course of the study reported below.

Apparatus. All toluene exposures and behavioral tests were conducted in a two-compartment, sound-attenuating, light-proof chamber, which was maintained under negative pressure (5 cm H_2O) and vented to a fume hood. The entire system is shown in Fig. 1. Filtered ambient air was passed over the toluene and was diluted with filtered ambient air to the desired concentration before passing into the lower, second compartment. Dilution air was controlled by a rotometer, which maintained the desired concentration to within 10% at all times. Ambient air entered the system under positive pressure via one half of a dual-head, noncontaminating diaphragm pump.[4] All parts of the exposure system were connected via flexible tubing,[5] either 1/4 or 3/16 in. i.d. The toluene concentration in the experimental air was measured and recorded continuously, 60 cm upstream of the head-only exposure helmet by an infrared gas analyzer.[6,7] A calibration curve for the vapor analyzer was generated by injecting known amounts of toluene into a closed-loop system, including the analyzer, of known volume. This procedure yielded meter readings for known concentrations of toluene. The linear equation which best fit these data was used to calculate the toluene vapor concentration for a given analyzer reading.

Each monkey was trained, with a leash and collar, to sit in a restraint chair of a type used previously for behavioral studies (Evans, 1975). The chair was made of clear acrylic to allow viewing the animal through a one-way peep hole in the test chamber. The chair was latched to the floor of the lower compartment facing a panel of opaque acrylic. Three stimulus-response keys (2.6 cm diameter) were centered horizontally (7.7 cm apart center to center) at eye level on the panel. The keys could be illuminated from the rear with red, yellow, or green light.

[3] Hazleton Research Laboratories, Reston, Va.

[4] Model 7530-60, Cole-Palmer, Chicago, Ill.

[5] No. 14-169-1K, Tygon, Fisher Scientific, Pittsburgh, Pa.

[6] MIRAN 1A, Foxboro Analytical, South Norwalk, Conn.

[7] Model 252A, Linear Instruments Corp., Reno, Nev.

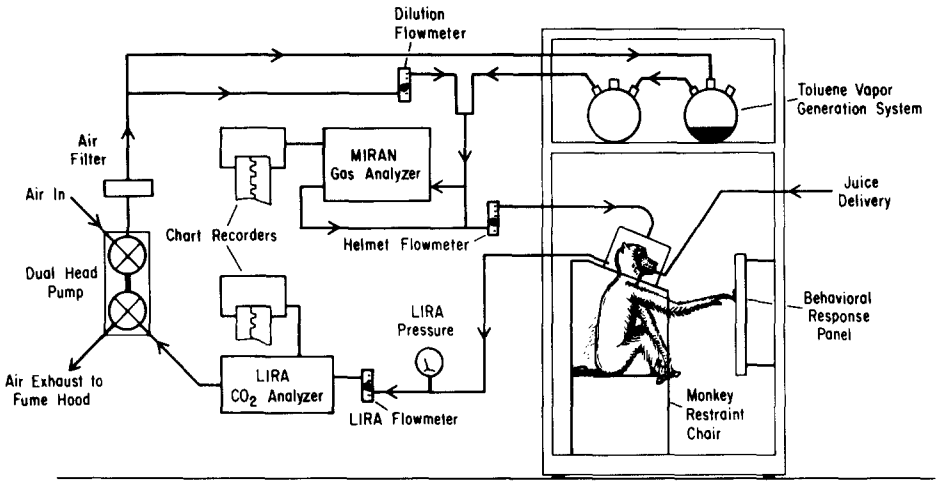

FIG. 1. Head-only exposure system. The top compartment (67 × 46 × 28 cm inside dimension) contained the vapor-generating apparatus, which consisted of a flask containing liquid toluene[12] and a second flask to ensure that the toluene remained in the vapor state. The lower compartment (67 × 46 × 85 cm inside dimension), in which the monkey was exposed, was equipped with continuous masking noise.[13] A houselight provided a low level of illumination sufficient to observe the monkey. Instruments were kept on a rack next to the chamber. Direction of air flow is indicated by the arrows.

Pressing each key with a force of approximately 0.5 N was required to register a response.

Inhalation exposures were performed by placing a cylindrical acrylic helmet (17.0 cm diameter, volume = 2.6 liter) over the monkey's head. The helmet rested flush on the neckplate of the chair and was held in place by screws. The helmet was not air tight and was therefore maintained under slight positive pressure so that the desired toluene concentration was ensured around the monkey's nose and mouth. The CO_2 measurement (see below) thus was not the animal's total output, but rather a representative sampling. The toluene atmosphere entered the helmet through the center of the top at 8.2 liter per min and was exhausted by five horizontal ports (1/8 in. diameter) at its base. The exhaust passed through a 3.5-liter mixing bottle and then through a CO_2 infrared analyzer[8] before being vented through the pump to the fume hood.

The CO_2 analyzer's manufacturer provided a calibration curve. Nitrogen and 0.2% CO_2 in nitrogen from tanks were passed through the analyzer before each use to ensure that the meter readings matched the manufacturer's calibration curve. The expired CO_2 of the monkey was measured continuously throughout each exposure session and recorded and integrated by a chart recorder.[7] Expired CO_2 was computed as milliliters per kilogram body weight$^{(0.75)}$ × minutes for the first and last 18 min intervals of the 50-min exposure session. These two 18-min intervals are referred to below as the first half and second half expired CO_2.

A stainless-steel spout, inside the helmet within reach of the monkey's mouth, delivered approximately 0.5 ml of grape juice[9] for each correct response made by the monkey. The apparatus was controlled and the data were recorded by a PDP8A computer[10] with SKED interface.[11]

Behavioral tests. A three-choice, variable-delay matching-to-sample task was used. Each trial began with presentation of a red flashing light on the center key (0.1 sec on, 0.1 sec off). Following the first response by the monkey on the center key, called an observing response, or the expiration of 10 sec without a response, the red flashing light was replaced on the center key by a randomly chosen colored light (red, yellow, or green), called the sample stimulus. The number of trials initiated by the monkey divided by the total number of trials presented defined the probability of responding. The sample stimulus remained on for 0.5 sec, after which, a randomly chosen variable retention interval began with no stimuli present. Following the retention interval of either 0 (actually 0.2), 4.0, or 16.0 sec, all three keys were illuminated, each with one of the three possible colors as comparison stimuli. The positions of the stimuli were randomized across trials. A response

[8] Model 303 LIRA analyzer, Mine Safety Appliances Co., Pittsburgh, Pa.

[9] Welchade, Welch Foods Inc., Concord, Mass.

[10] Digital Equipment Corp., Maynard, Mass.

[11] State Systems Inc., Kalamazoo, Mich.

[12] 99+% pure, Aldrich Chemical Co., Milwaukee, Wisc.

[13] Model 64651 noise generator, Ralph Gerbrands Co., Arlington, Mass.

on the key with the same color as the sample terminated the stimuli and produced 0.5 ml of grape juice. A response on either of the other two keys, or the expiration of 3.0 sec without a response, produced a 7.0 sec timeout during which there were no stimuli present. The time elapsed between the termination of the retention interval and a key press was called the response time. An incorrect choice, or a failure to respond, was followed by a repetition of the same sample-delay-comparison sequence. This correction trial occurred until the animal made a correct choice or until five consecutive errors occurred. The data from correction trials were not used in calculating the accuracy of matching, in order to eliminate possible bias due to the position of the stimuli. Data from all trials were used to calculate response time, probability of responding, and responses during the retention interval. The intertrial interval was 1.0 sec, and the session duration was 50 min.

Three-Day Tolerance. After stable baseline performance was established, the monkeys were divided randomly into two groups of three. One group was exposed to 4500 ppm toluene for 50 min on each of three consecutive days, while the second group received sham exposures to air on those days. Later, after having completed an acute concentration–effect protocol (see below), the 3-day tolerance experiment was repeated with the same groups. Neither tolerance nor cumulative effects were found, and on that basis the acute concentration–effect protocol was used.

Acute Concentration–Effect Study. Each monkey was exposed twice weekly, Tuesday and Friday, to one of six concentrations of toluene (0, 500, 1000, 2000, 3000, 4500 ppm). This procedure took 6 weeks. Further, each monkey received filtered ambient air during practice sessions on Monday and Thursday to ensure recovery of baseline. Each monkey received two exposures at each concentration, and exposures were randomized for all monkeys according to a repeated-treatments design. After the last exposure day of the acute concentration–effect determination, the monkeys were again subjected to the 3-day tolerance experiment described above.

Follow-up study of lower concentrations. Because expired CO_2 was significantly affected at the lowest concentration (500 ppm), additional acute exposures were conducted with air, 100, 200, and 500 ppm toluene. This follow-up study used the same protocol as in the acute-concentration–effect study. Exposures began 3 months after the last exposure in the 3-day tolerance study.

Data analysis. For each monkey, five dependent variables were measured: accuracy (percentage correct) of delayed matching, response time, responses during the retention interval, the probability of responding, and expired CO_2. Data were averaged across the exposures each monkey received at each toluene concentration and analyzed for each session half separately. The number of exposures ranged from one (100 and 200 ppm) to three (0 and 500 ppm). The means for accuracy were submitted to repeated-measures analysis of variance (Dixon, 1981) with dose, retention interval, and session half as factors. The means of the other four measures were submitted to repeated-measures analysis of variance with dose and session half as factors. A criterion of $p < 0.05$ was required for significance. Analyses that yielded significant interactions between factors were submitted to further analyses of the simple main effects of each factor (Kirk, 1968). A post hoc least significant difference (LSD) test compared the means of each concentration level to control, to determine the lowest concentration of toluene which significantly affected each measure or factor (Fisher, 1969). Baseline variability during sham exposures is shown as ±1 SE around the mean at 0 ppm in Figs. 2 and 4. The SEs were not shown elsewhere since the SEs, based on group means, invite inappropriate speculation as to the significance of the differences between points which are more properly tested with the within-subject repeated-measures design assessing the mean differences of each animal rather than group mean differences.

RESULTS

Three-day tolerance. There was no significant difference across days in accuracy of matching, response time, responding during delay, or probability of responding in either the first series of three daily exposures to 4500 ppm toluene or to the second 3-day series at the conclusion of the concentration–effect study. Table 1 shows the results for response time, the most sensitive behavioral index. Toluene-induced changes were still present on Day 3, but were not different from changes on Day 1. Recovery was complete in 24 hr (Table 1, Day 0 vs Day 4). Results were similar after the second 3-day series, indicating absence of tolerance or sensitization. All monkeys improved somewhat in accuracy of matching under air exposures between the first 3-day series and the second 3-day series, indicating that behavioral impairment produced by acute toluene exposure had neither long-lasting nor cumulative effects.

Accuracy of delayed matching. Under control conditions monkeys performed consistently throughout the 50-min session and accuracy of matching declined as a function of the retention interval. Under toluene exposure, accuracy was analyzed separately for the first 25 min and the second 25 min of the session. Toluene did not significantly affect accuracy in the first half of the session [$F(5, 25)$

TABLE 1

EFFECT OF TWO 3-DAY SERIES OF EXPOSURES TO TOLUENE ON RESPONSE TIME[a]

	Baseline[b] Day 0	Exposure[c] Day 1	Exposure[c] Day 2	Exposure[c] Day 3	Recovery[b] Day 4
First series[d]					
Toluene	1.37 ± 0.12	1.61 ± 0.15	1.67 ± 0.13	1.64 ± 0.07	1.39 ± 0.10
Air	1.39 ± 0.08	1.39 ± 0.07	1.45 ± 0.11	1.44 ± 0.07	1.38 ± 0.08
Second series					
Toluene	1.13 ± 0.08	1.55 ± 0.11	1.58 ± 0.08	1.63 ± 0.07	1.21 ± 0.03
Air	1.34 ± 0.10	1.28 ± 0.08	1.26 ± 0.07	1.34 ± 0.03	1.33 ± 0.08

[a] Data are mean response times in seconds (±SE, $N = 3$).
[b] All monkeys were exposed to air in baseline and recovery determinations.
[c] Exposure for 50 min daily to 4500 ppm toluene (3 monkeys) or air (3 monkeys) for 3 consecutive days.
[d] First series was determined after initial baseline training and before the acute concentration–effect study. Second series was determined after the concentration–effect study, approximately 7 weeks after the first series.

$= 1.83$, $p = 0.143$]. In the second half of the session, 4 of 6 monkeys failed to respond at 4500 ppm. Accuracy of matching data from the second half session was therefore analyzed only for concentrations of 0 to 3000 ppm toluene. Accuracy was impaired by toluene [$F(4, 20) = 6.81$, $p = 0.001$]. The impairment was significant at 2000 ppm and above at the 0 sec delay (Fig. 2). There was no overall significant interaction between retention interval and toluene concentration, nor was accuracy significantly affected at 4 and 16 sec delay due to the greater variability of those data.

Probability of responding. The probability that a monkey would make an observing response to initiate a trial was significantly reduced by toluene [$F(5, 25) = 28.71$, $p < 0.001$]. Figure 3A shows the within-session changes in this measure during each 50-min exposure to toluene. Post hoc LSD tests of the means for each concentration showed that a significant reduction occurred at toluene levels of 3000 ppm and higher for both halves of the session. Further, the response probability significantly declined within the exposure session [$F(1, 5) = 10.48$, $p = 0.023$]; and the interaction between session half and toluene level was significant [$F(5, 25) = 6.80$, $p < 0.001$], indicating that the within-session decrement became more severe with increasing concentrations of toluene. These latter two effects occurred mainly at 4500 ppm in the second half

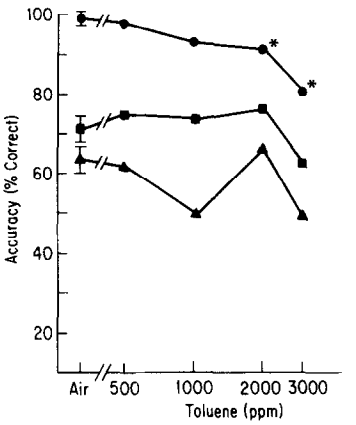

FIG. 2. Effects of toluene on accuracy of delayed matching during the second half of the exposure session. Accuracy was determined at each of three retention intervals (●, 0 sec; ■, 4 sec; ▲, 16 sec). Bars show ±1 SE around the mean accuracy during sham exposure to air, as an indication of baseline variability. Asterisks indicate points that are significantly different from air controls at the 0.05 level. Each monkey was exposed to every condition ($N = 6$). Data points from the follow up study (100 and 200 ppm) were not significantly different from air and were not plotted.

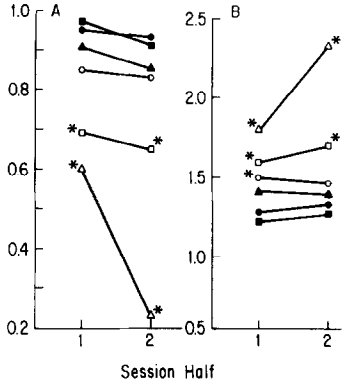

FIG. 3. Effects of toluene on two measures of delayed-matching performance with respect to session half: (A) probability of responding and (B) response time (sec). Asterisks indicate points which are significantly different from air controls in that half. ($N = 6$ for each point). (●, air; ■, 500 ppm toluene; ▲, 1000 ppm toluene; ○, 2000 ppm toluene; □, 3000 ppm toluene; △, 4500 ppm toluene.)

of the session, in which most monkeys remained awake, but were unresponsive.

Response time. The choice response time increased as a function of toluene concentration [$F(5, 25) = 19.58$, $p < 0.001$; Fig. 3B]. Response time was unique among the variables investigated for two reasons. First, of the behavioral measures tested, it was impaired by the least amount of toluene (2000 ppm) in the shortest amount of time, i.e., in the first half session. Second, it displayed differential effects across session halves which revealed a within-session tolerance to toluene. In the first half, response time was significantly increased by toluene concentrations at or above 2000 ppm, while in the second half, response time was increased by toluene levels at or above 3000 ppm. There was no significant effect across session halves but a significant interaction between session half and toluene concentration [$F(5, 25) = 4.41$, $p = 0.005$]. The source of the interaction is seen in Fig. 3B, which displays the changes in response time during the session with respect to toluene concentration.

Responding during retention interval. The number of responses during the retention interval was significantly decreased by toluene [$F(5, 20) = 3.42$, $p = 0.021$]. A post hoc LSD test showed that the effect occurred at 4500 ppm toluene, which, as stated previously, severely reduced the probability of all responding. There was no effect across session halves nor was there a significant interaction between dose and session half.

Expired CO_2. Toluene exposure resulted in (1) a significant main dose effect upon expired CO_2 [$F(7, 35) = 4.01$, $p = 0.003$], (2) a significant difference in expired CO_2 in the second half of the session relative to the first half [$F(1, 5) = 33.69$, $p = 0.002$], and (3) a significant interaction between session half and toluene concentration [$F(7, 35) = 6.87$, $p < 0.001$]. Analysis of the simple main effects revealed that each session half displayed an inverted U-shaped dose–response function (Fig. 4). Post hoc LSD tests showed that during the first 18 min of toluene exposure, expired CO_2 increased above air (0 ppm) values at 100 and 200 ppm and then decreased to below air controls at 4500 ppm toluene. During the second half of the session, expired CO_2 was significantly increased at 100 through 3000 ppm toluene and unchanged at 4500 ppm relative to 0 ppm. Post hoc LSD tests showed that expired CO_2 decreased in the second half relative to the first at 200 ppm toluene and increased in the second half relative to the first at all concentrations above 200 ppm (Fig. 4).

DISCUSSION

Four cognitive and motor skills in the monkey were affected in a concentration-dependent manner by acute toluene inhalation. Response time, accuracy of delayed matching, probability of responding, and responding during delay were first impaired at 2000, 2000, 3000, and 4500 ppm toluene, respectively. Expired CO_2, the most sensitive measure, increased at 100 ppm toluene, the lowest toluene concentration tested (Fig. 4).

This study investigated tolerance to toluene in the monkey for two reasons. First, tolerance characterizes the pharmacological effects of many substances which have abuse potential, and since toluene has been shown to have

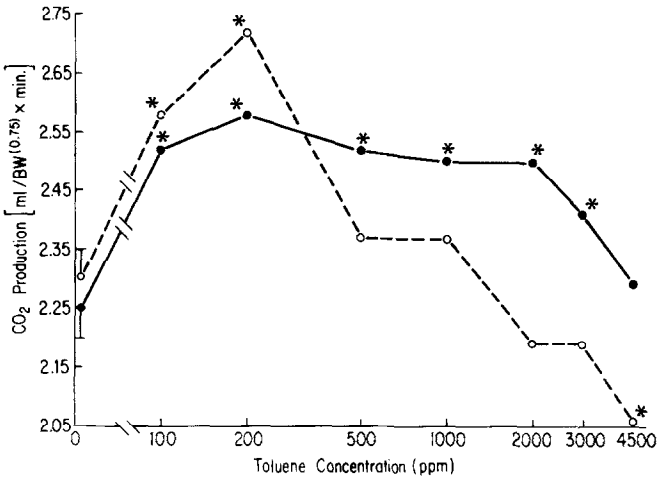

FIG. 4. Effects of toluene on expired CO_2 in the first 18 min (○, first half) and last 18 min (●, second half) of the 50-min session. Asterisks indicate points that are significantly different from 0 ppm for that session half. The y axis shows ml CO_2/kg body $wt^{0.75}$ × min.

abuse potential (Iregren, 1982; Crites and Schuckit, 1979; Fornazzari *et al.*, 1983), one might expect to see tolerance (Himnan, 1984). Second, the scheduling of exposure can influence the development of neurotoxicity (Pryor *et al.*, 1982).

The lack of either carry-over effects or tolerance permitted the use of a within-subject experimental design for the concentration–effect determination of twice-weekly acute toluene exposure. These results support evidence that workers' blood levels of toluene returned to baseline 2 days following occupational exposure (Konietzko *et al.*, 1980). The absence of tolerance in our two 3-day exposures also agrees with rodent studies with moderate toluene concentrations (Moser and Balster, 1981; Yamawaki *et al.*, 1982). A report of tolerance in the rat to repeated exposures used very high concentrations (unmeasured but in excess of 10,000 ppm; Himnan, 1984).

The chronic tolerance discussed thus far can be differentiated from an acute tolerance, which occurs rapidly, often during the course of a single exposure (Corfield-Sumner and Stolerman, 1978; Goldstein *et al.*, 1974). Response time exhibited acute tolerance to toluene (Fig. 3B). Additional evidence of acute tolerance is seen in the within-session decrement in toluene's effect on expired CO_2 at 4500 ppm (Fig. 4). Animal models of tolerance are important in understanding the mechanisms of chemicals which exhibit abuse potential.

Response time was increased by inhalation of 2000 ppm or more of toluene (Fig. 3B). These findings agree with Iregren (1982), who compared workers exposed to toluene with workers exposed to a mixture of solvents and with a control group. He found the toluene-exposed group performed less well in a test of simple reaction time than either of the other groups. Figure 3 also shows that the toluene-induced increase in response time and the decrease in probability of responding are nearly mirror images of each other.

Unrewarded responding during delay decreased at 4500 ppm toluene and was not affected by lower concentrations. This result is in agreement with Geller *et al.* (1979), who found responding during delay to increase in baboons during exposure to a mixture of industrial solvents, but not to low concentrations of these same solvents when administered alone.

This study confirms, in the monkey, what other studies have shown in man (Iregren, 1982; Fornazzari *et al.*, 1983): that toluene

leads to impairment of cognitive function. However, the decrement in accuracy of matching by monkeys exposed to toluene did not increase with increasing retention interval (Fig. 2). Therefore, the effect of toluene in monkeys was a deficit in attention rather than a specific effect upon short-term memory. This agrees with the deficit in visual vigilance in humans exposed to toluene (Dick *et al.*, 1984). Solvent-related memory deficits may be more readily revealed in workers who have had much less practice in the memory test than did our monkeys.

Expired CO_2 was a very sensitive indicator of toluene inhalation in the monkey. The inverted U-shaped concentration–effect curve shown by this measure is a result of behavioral, metabolic, and/or respiratory changes in the monkey. Inverted U-shaped concentration–effect curves also describe behavioral changes in the response rates of pigeons and rats during acute toluene exposure (Benignus, 1981; Miyagawa *et al.*, 1984). These curves suggest behavioral stimulation at lower doses and behavioral sedation at higher doses. Respiratory changes to toluene, in the form of a rapid and short-lasting irritant response, have been shown in mice (Nielsen and Alarie, 1982; Bushnell *et al.*, 1985) and might have further contributed to the decrease in expired CO_2 in the first half of exposure sessions to 4500 ppm toluene. Concentrations below 4500 ppm may have produced only changes in behavior and/or respiration which increased expired CO_2.

The present results show that toluene impairs cognitive function, particularly attention and visual-motor abilities. Most notably, response time was slowed after relatively brief exposures to concentrations as low as 2000 ppm. Considerably higher concentrations of toluene or benzene are required to produce respiratory irritation (Nielsen and Alarie, 1982) than are required to affect behavior (Figs. 2 and 3; Dempster *et al.*, 1984). These findings may provide insight and guidance concerning occupational exposures where concentrations of this magnitude are possible (Whitehead *et al.*, 1984; Riala *et al.*, 1984; Iregren, 1982) and concerning inhalant abuse, where higher concentrations are certain to occur.

ACKNOWLEDGMENTS

Submitted to the Program in Environmental Health Sciences, Graduate School of Arts and Sciences, in partial fulfillment of the requirements for the degree of Master of Science at New York University. Supported by Grant OH-00973 from NIOSH and by Research Grant ES-03461, Training Grant ES-07065, and Center Grant ES-00260, all three awarded by NIEHS. We thank Alan Monico for expert technical assistance, Dr. Philip Bushnell for advice and assistance throughout the experiment, Dr. Carroll Snyder and Dr. E. D. Palmes for their suggestions, and Eleanor Cordisco for secretarial assistance.

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