

# Some Effects of Sub-Acute Exposure to Toluene on Schedule-Controlled Behavior

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GLOWA, J. R. *Some effects of sub-acute exposure to toluene on schedule-controlled behavior.* NEUROBEHAV. TOXICOL. TERATOL. 3(4) 463-465, 1981.—Behavioral effects of repeated exposure to toluene have been assessed on mice responding under an FI 60-sec schedule of milk presentation in a hermetically sealed chamber. Sessions consisted of alternating 10 min periods where milk was available under the FI schedule and 25 min periods where responding had no scheduled consequences. Mice were exposed to 500, 1000 or 2000 ppm toluene, for the last 4 hrs of five consecutive daily sessions. At concentrations of 500 ppm toluene had little effect on responding, on any of the 5 days of repeated exposure. Concentrations of 1000 ppm consistently increased rates of responding during each session and each day of repeated exposure. Concentrations of 2000 ppm consistently decreased responding after the first exposure series during daily exposures; increases in responding were seen at the beginning of sessions during the initial days of exposure. Consistent concentration-dependent effects of toluene were obtained over 4-hr exposure periods with schedule-controlled responding in mice.

Toluene	Sub-acute exposure	Behavior	Schedule-controlled responding	Mice
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TOLUENE is a widely used industrial solvent that has also been recognized as having abuse potential [7]. For these reasons the biological effects of toluene have been intensively studied. Behavioral effects of toluene have also been investigated, some of these studies have monitored schedule-controlled responding immediately following exposure to that compound [9,10]. Studies of this type have shown that recovery from rate-decreasing effects of toluene is rapid and response rates often increase after exposure is terminated. Others have monitored behavior during acute exposure to toluene [3, 4, 5]. These studies have confirmed that increased response rates can result from exposure to toluene and have also shown a relationship between the behavioral effects of toluene and exposure concentrations. The present study assessed some behavioral effects of different concentrations and durations of exposure in mice repeatedly exposed to toluene.

## METHOD

### Subjects

Adult male white mice, descended from Charles River CD-1 strain, were maintained at 80% of their free-feeding weight (23.9-28.0 g) by controlled post-session feeding.

### Apparatus

The exposure chamber was a 25-quart pressure cooker (Wisconsin Aluminum Foundry, Manitowic, WI.) A complete description of the chamber and its operating characteristics has been published [5]. The chamber, which was

hermetically sealed when closed, had an injection port and a hot plate inside to vaporize the added solvent. A sampling port allowed the removal of air samples. Both ports were equipped with a teflon coated rubber septum. A magnetically coupled fan provided mixing.

An aluminum inner chamber (9×8×7 cm high) confined the mouse within the exposure chamber. A hole, 1 cm in dia. and 2 cm above the floor was drilled in the center of the front wall of the mouse chamber. Immediately behind this hole a beam of light traversed its horizontal diameter, falling on a photocell. The beam was broken when the mouse inserted the tip of its nose into the hole. A small stainless steel cup (0.025 ml) was located below the hole at floor level. A pump located outside the exposure chamber filled the cup with milk through a teflon tube. Above the hole were three small lights which could serve as visual stimuli; the chamber was also equipped with a speaker and white noise.

### Procedures

Each break of the light beam was counted as a response. Milk was delivered according to an FI 60-sec schedule; under that schedule, in the presence of flashing lights and no white noise the first response to occur after the elapse of a 60-sec interval produced milk. After each milk presentation there was a 5-sec timeout (TO). Eight consecutive FI 60-sec + TO 5-sec cycles constituted a series and series were separated by a 25 min inter-series TO. If 8 milk presentations did not occur within 10 min, the inter-series TO automatically occurred. During TO, as well as before and after sessions, the lights were off, white noise was on, and respond-

ing had no scheduled consequences. A daily session consisted of nine series. Sessions were conducted 6 days a week.

Total numbers of responses and time elapsed in each FI series were recorded and used to calculate rates of responding for each series of the day before exposure (day 0) as well as each of the 5 days of exposure. Rates of responding in the first series of each session were not included in tabulation of results. Rates in the next two (2nd and 3rd) series provided control values for daily sessions. Rates of responding during series 4 through 9 described the effects of toluene following 25 min through 4 hrs of exposure, respectively. Data are expressed as a ratio of individual rates of responding during a particular series to the rate of responding for that mouse during the control series of the day before exposure (day 0), averaged across mice. Following the non-exposure control day (day 0), mice were exposed to toluene on days 1 through 5. Toluene was introduced into the chamber at the end of the 3rd FI series (and hence 30 min before the 4th series), to obtain nominal concentrations of 500, 1000 or 2000 ppm. As the chamber was not opened during the remainder of the session chamber concentrations could be monitored. This was done by withdrawing 1 ml gas samples between each series and analyzing them by flame ionization in a Hewlett Packard 5830A Gas Chromatograph and 18850A terminal. Six mice were studied at 500 ppm and 1000 ppm, 5 mice were studied at 2000 ppm.

#### Agent

Toluol (Fluka AG, Switz.) of purity greater than 99.5% was used.

### RESULTS

#### Control Performance

During control experiments, the mean rate of responding over the 6 consecutive series following the control series averaged 0.93 of that control and showed no consistent trend throughout the session; the mean coefficient of variation in the individual series was 15.1% of control.

#### Effects of Toluene

Toluene was added to the chamber at the end of the 3rd FI series in amounts to give concentrations in the chamber of 500, 1000, or 2000 ppm. Figure 1 compares the effects of toluene on responding in the 4th series (thus after 25 min of exposure) through the 9th series (after approximately 4 hr of exposure) to control rates of responding on day 0 for each series during each day of exposure. Repeated daily exposure to 500 ppm toluene had little effect on rates of responding on any day. Initial exposure to 1000 ppm toluene (day 1) increased response rates to approximately twice those of initial control values throughout the session. Consistent increases in responding were observed during both control and exposure series on all subsequent days. With exposures to 2000 ppm, toluene increased response rates following the first 25 min of exposure (4th series) on the first two days, but decreased responding in subsequent series. By the 5th series responding was decreased more than 50% while in the 6th and subsequent series responding was typically decreased to less than 0.2. During the 3rd through 5th day of exposure no increases in responding were seen during early portions of the exposure sessions. Responding during daily control

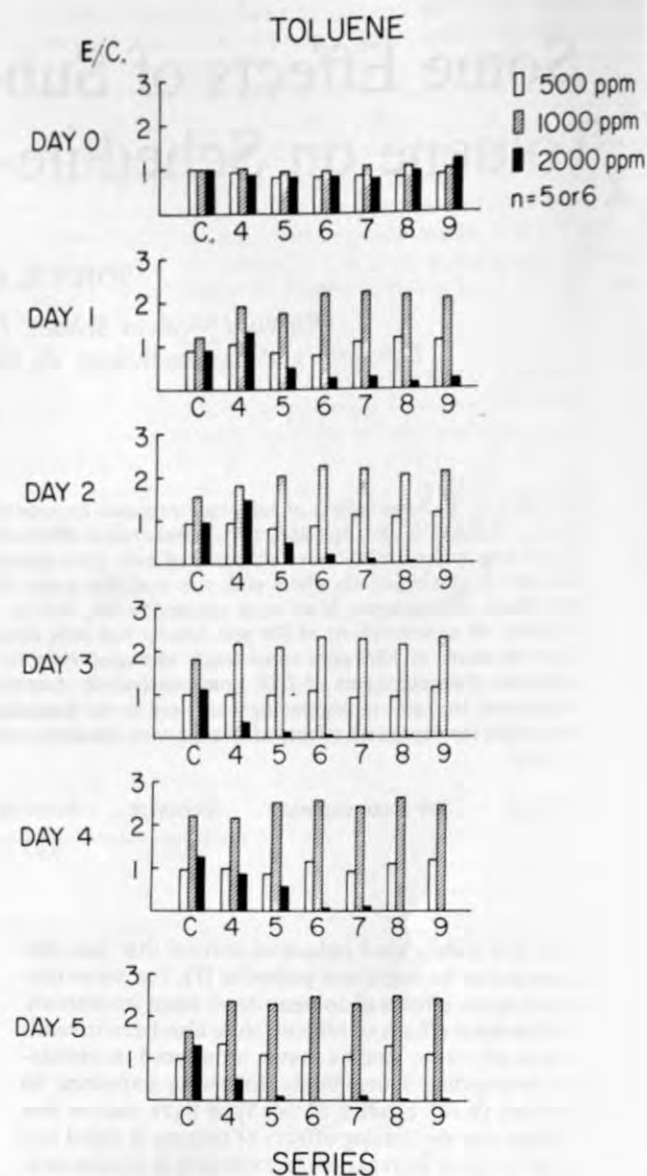


FIG. 1. Effects of repeated exposure to toluene on responding of mice maintained under an FI 60-sec schedule of milk presentation. Days are arranged vertically, on day 0 no exposure occurred, on days 1-5 mice were exposed to 500 ppm, 1000 ppm or 2000 ppm, for series 4 through 9 of each daily session. Series 2 and 3 constituted daily controls, the effects are expressed as a ratio of the rate of responding during a series to the rate of responding during the control series on day 0. Consecutive series are represented on the horizontal axis; the ratio of experimental to control (E/C) is represented on the vertical axis.

series was not greatly changed as a result of exposure to 2000 ppm toluene on the day before.

#### DISCUSSION

The present experiments describe some behavioral effects of repeatedly exposing mice to toluene when responding was maintained under an FI-60 sec schedule of milk presentation. Repeated exposure to 500 ppm toluene for 4 hr daily for 5 days had little behavioral effect, whereas repeated

exposure to twice that amount resulted in both a sustained increase in responding during exposures and also during control performances. The consistency of the behavioral effects obtained over 4 hr exposures to 500 and 1000 ppm was striking and confirms that stable behavioral effects can be obtained with toluene under the present conditions. However, with higher concentrations slightly longer exposure durations were required to produce a consistent behavioral effect. At these concentrations responding essentially ceased following approximately 2 hrs of exposure. These results demonstrate that reliable behavioral effects of organic solvents can be obtained with relatively simple and inexpensive means.

The present results are consistent with previous reports of the acute effects of exposure to toluene. Responding is often increased either during or following acute exposure to moderate concentrations [3, 4, 5, 9, 10]. In one previous report [5] responding of mice maintained under schedule conditions similar to those presently reported was maximally increased following acute exposure to 700–1000 ppm. Somewhat larger increases in responding were obtained during exposure to 1000 ppm in the present study. In that earlier study responding of mice was decreased approximately 50% by, depending upon the duration of the exposure, 1700–2400 ppm [5]. In the present study approximately 1 hr of exposure to 2000 ppm decreased responding 50%. The behavioral effects of many compounds change following repeated admin-

istration. Comparisons between acute and sub-acute exposures and the consistency of effect across sub-acute exposure days suggest that if the behavioral effects of toluene change with repeated exposure, exposure periods longer than those presently studied are required.

Near asymptotic blood levels are reached within 15–30 min of initial exposure to low (200 ppm or less) concentrations of toluene [1, 2, 8]. The behavioral effects presently obtained for exposure to 500 ppm and 1000 ppm are consistent with a fairly rapid initial rise to asymptotic blood levels of toluene within 25 min. When higher (2000 ppm) concentrations of toluene have been studied blood levels have continued to rise for as long as 3 hr [6]. The present behavioral effects of 2000 ppm exposures are consistent with an increase in blood levels of toluene over the first 2 hr of exposure. However, although consistent behavioral effects were obtained with 4-hr exposures to 500 ppm and 1000 ppm, the possibility that longer exposures might eventually result in a decrease in responding is not excluded. Studies of behavior during exposure to organic solvents are important not only in their own right, but also because they can effectively relate neurobehavioral toxicity to a variety of exposure parameters.

#### ACKNOWLEDGEMENTS

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