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Effects of Acetone and Toluene Vapors on Multiple Schedule Performance of Rats[1,2,3]

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GELLER, I., R. J. HARTMANN, S. R. RANDLE AND E. M. GAUSE. *Effects of acetone and toluene vapors on multiple schedule performance of rats.* PHARMAC. BIOCHEM. BEHAV. 11(4) 395–399, 1979.—Six rats were trained to press a lever for a liquid food reward on a multiple fixed ratio–fixed interval (FR–FI) schedule of reinforcement. When lever-pressing rates became relatively stable, the animals were exposed to 150 ppm of either acetone or toluene for duration times of ½, 1, 2 and 4 hr. Exposures were conducted at least three weeks apart. Acetone produced minimal changes on the FR–FI responding during the ½ hr exposure. During the 1 hr exposure period, both FR and FI rates increased while during the 2 hr exposure, both FR and FI responses decreased below control levels. During the 4 hr exposure FI responses approximated control levels for 2 rats and were above the control level for the third animal while FR rates were below controls for 2 of the 3 subjects. Rate changes under toluene were generally qualitatively similar to those produced by acetone. An initial enhancement of FR and FI rates occurred during the shorter exposure periods followed by a decrease in rates during the longer exposure periods.

Acetone Toluene Multiple fixed ratio–fixed interval schedule

NON-MEDICAL use of volatile hydrocarbons has been known for many years. Substances which have been abused via the inhalation route include plastic cement, airplane glue, cleaning fluid, paint thinner, anti-freeze, gasoline, nail polish remover and lighter fluid [25]. Chemical constituents of these substances which probably are responsible for their abuse potential are toluene, acetone, benzene, xylene, ethyl ether, alcohol and amyl nitrite. The chemicals are central nervous system (CSN) depressants that produce an initial stimulatory effect sometimes followed by euphoria and hallucinations [10].

The occurrence of glue or solvent sniffing-induced cardiac arrest or peripheral polyneuropathies [1, 11, 12, 19, 20, 26] has generated a number of animal studies [4,18]. These investigations have been concerned with extremes in toxicity rather than subtle alterations of behavior not evident through gross observation of animals during or following exposures to solvent vapors.

The application of operant technology for the study of substances abused through inhalation, allows for detection of CNS effects at minimal concentrations of a chemical prior to reaching a level which could result in irreversible pathological damage. Studies such as these are exemplified by the work of Beard and Wertheim [5] and Armstrong *et al.* [2] and more recently by the work of many noted investigators in the field of operant conditioning [24].

In the present investigation acetone and toluene were evaluated for effects on a multiple fixed ratio–fixed interval schedule of reinforcement. This allowed for comparison of different exposure durations in the same animals under two conditions of food reinforcement.

METHOD

The subjects were six male Holtzman, Sprague-Dawley rats, housed individually and maintained at 80% of their original starting weights. They were trained in Skinner boxes obtained commercially and manufactured according to our specifications. The boxes were designed so that they could be placed in an exposure chamber which permitted the gases within the box to be maintained at the desired concentration ± 10%.

Rats were trained to press a lever for a liquid food reward on a multiple fixed ratio (FR), fixed interval (FI) schedule of reinforcement. During the fixed interval component, a clicker was activated and lever responding was reinforced once every 2 min. A tone stimulus signaled the FR component which required 60 responses to produce one reward. The components of the FR–FI schedule, of 10 min duration, alternated throughout a 1-hr session. Total FR responses were recorded for each of three, 10-min periods during an experimental session. Responses during each 2-min FI segment were broken down into eight 15 sec bins. These were totalled for the three 10-min FI segments of the session.

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Duration of exposures to the gases were 1/2, 1, 2 and 4 hr. Exposures were separated by a minimum of 3-week intervals. Behavioral sessions were of 1 hr duration. For the 1/2 hr exposure, animals were subjected to the acetone or toluene atmosphere during the first 30 min of the test session. For the 2- and 4-hr gas exposures, the behavioral session was conducted during the final exposure hour.

In an attempt to correlate behavioral effects with cumulative dose of vapor inhaled by the animal, twelve rats were exposed to acetone vapor at a concentration of 150 ppm in a large Plexiglas chamber. Blood was taken at 30, 60, 120 and 240 min, and concentrations of acetone in blood were determined by gas chromatography using a modification of the Wallace and Dahl headspace technique for blood alcohol [22].

Atmospheres of acetone or toluene were generated according to the vapor saturation technique [7], a method used for substances that are liquid at room temperature. The method involves bubbling air through a gas washing bottle containing the liquid to be vaporized. The gas washing bottle is kept in a constant temperature bath. In passing through the liquid, the air becomes saturated with vapor which is then directed to the air intake ducts of the exposure chamber. Changing the flowrate by means of a fine metering valve or changing the temperature of the constant temperature bath allows a large range of pollutant concentrations to be produced in the exposure chamber. The technique is simple and works well for liquids with relatively low boiling points.

Flame ionization gas chromatography was used to monitor the concentration of the organic volatile atmospheres. Figure 1 shows the concentrations of gases for the 4 hr exposure of acetone and toluene. The charts indicate that once the desired exposure levels were reached they remained relatively stable.

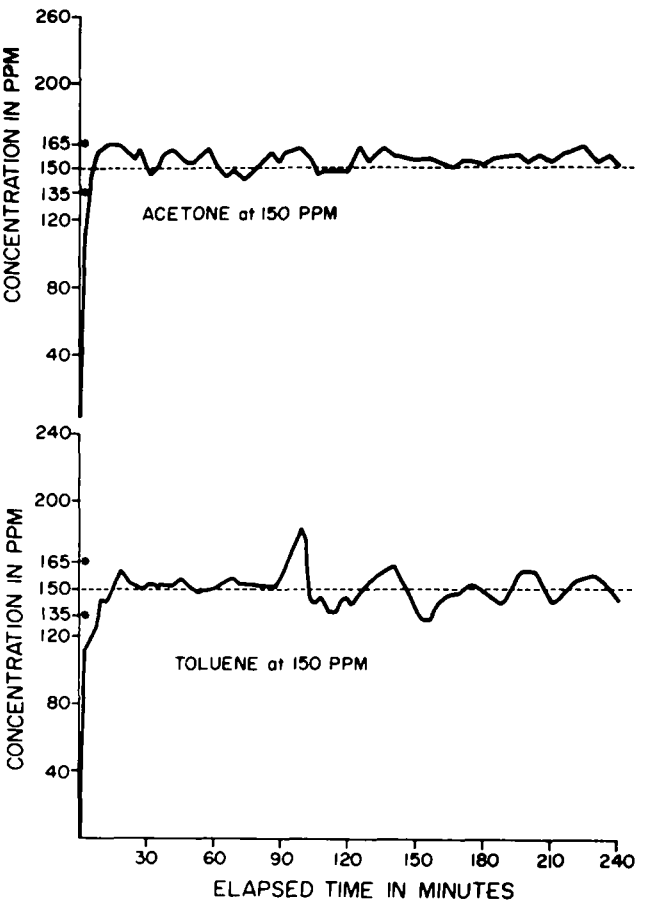

FIG. 1. Exposure chamber concentrations of acetone and toluene during 4-hr exposures.

RESULTS

The effects of exposure of rats to 150 ppm acetone are shown in Fig. 2. The data are expressed as percent of control. The control levels represent an average of 4 pre-exposure control sessions for each rat. The brackets represent standard errors. During the 1/2 hr exposure the effects on FR and FI rates were minimal. During the 1 hr exposure both FI and FR rates increased, while during the 2-hr exposure they were below control values. During the 4-hr exposure, FR rates were below controls for two rats while FI responses approximated control values for two rats and were above control for the third rat.

Results of the acetone blood level studies are shown in Fig. 3. It is apparent that under these exposure conditions, circulating levels of acetone increase gradually over a two-hour period to plateau at approximately 1.2 mg%. This steady-state level is maintained for the remainder of the exposure although the range of variability appears to increase, probably reflecting differences in rates of elimination of acetone through urination or exhalation by individual animals.

The rate changes which occurred under toluene exposure were generally similar to those observed for acetone (Fig. 4).

Both FR and FI rates increased during the shorter exposure durations and decreased during the longer durations. During the 1/2-hr exposure the FR rate was reduced significantly below the control range for one rat. During the 1 hr exposure FR rate increased for all animals and further increased for a single rat during the 2-hr exposure. FR rate was reduced below the control level for 2 rats during the 2-hr exposure and for all subjects during the 4-hr exposure. FI rate was also reduced for one rat during the 1/2-hr exposure while during the 1-hr exposure FI rates increased above control for all three rats. FI responding was unchanged for two rats and below baseline for one rat during the 2-hr exposure. During the 4-hr exposure FI rates were below control for two rats and above control for one.

Cumulative response curves for the acetone and toluene exposures revealed the following: the curves for toluene were shifted to the left of control during the 1/2, 1 and 2-hr exposure thereby indicating a shortening of IRT's and a loss of FI scallops. The curve for the 4-hr exposure was shifted to the right of the control curve.

For the acetone exposures, the curves for 1/2, 1 and 4 hr were shifted to the left while the 2-hr curve was shifted to the right of control.

DISCUSSION

For the acetone vapor exposure, the time course of behavioral effects appears to reflect, to some degree, the time course observed for the blood levels of acetone (Figs. 2 and 3). At the 30 min time point, there are minimal, if any, effects upon either FR or FI rates for any of the 3 animals; at this time, blood levels of acetone are also only about one-third of

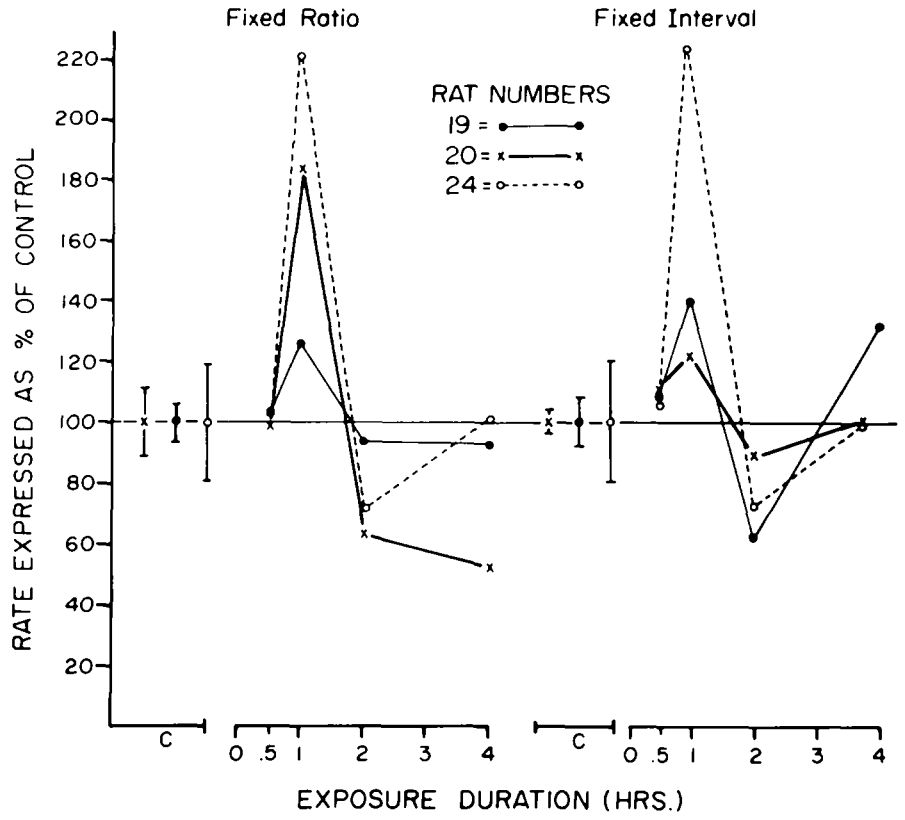

FIG. 2. Effects of inhalation of 150 ppm acetone by rats on fixed ratio–fixed interval response rates as a function of exposure duration. (Ordinate: rates of responding expressed as percent of average control rate. Abscissa: duration of exposures in hours. The points and brackets above C represent the mean ± SE for control rates of responding. Controls represent 4 determinations for each of 3 rats.)

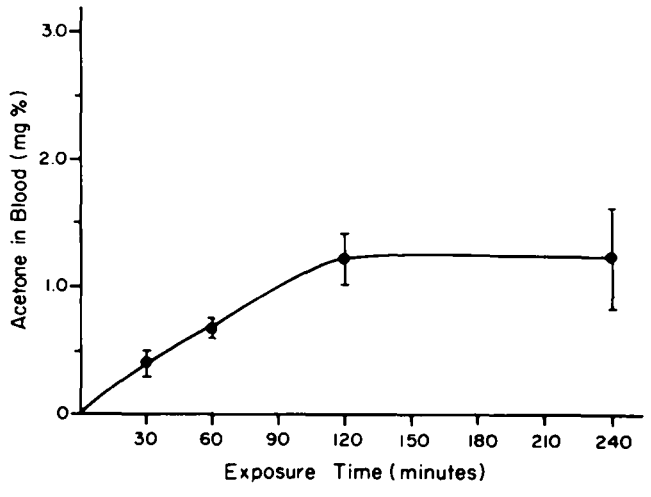

FIG. 3. Concentrations of acetone in blood of rats inhaling 150 ppm acetone as a function of exposure duration. (Brackets show ± SE.)

the maximum, apparent steady-state value. After 1 hr of exposure, both FR and FI rates have peaked, while blood levels have reached half-maximal value. At 2 hr into the 4 hr exposure, both FR and FI rates have decreased below control baseline; blood levels appear to have reached maximum levels at 2 hr, but the range of variability between individual animals has increased, probably due to differences in rates of acetone elimination. The trend observed for the 2 hr point appears to persist through the remaining 2 hr of exposure; at 4 hr, 1 of 3 animals exhibits an FR rate below its baseline range, while another of the 3 animals exhibits an FI rate above its control range. Again, at 4 hr, the level of acetone in blood appears to remain at the steady-state level, although the variability range between animals has increased further. The observed effects are suggestive of an initial response to inhaled acetone characterized by excitability or hyperirritability which correlates with increasing, but sub-maximal blood levels of acetone; this initial phase is succeeded by an adaptation or correction phase which may be related to lengthening of interresponse times and a shifting of the cumulative response curves to the right of control when the steady-state blood levels of acetone are attained. This biphasic response to inhaled acetone is suggestive of that produced by administration of a general depressant such as phenobarbital which at low doses enhances variable-interval response rates of rats and at higher doses decreases the response rates [9].

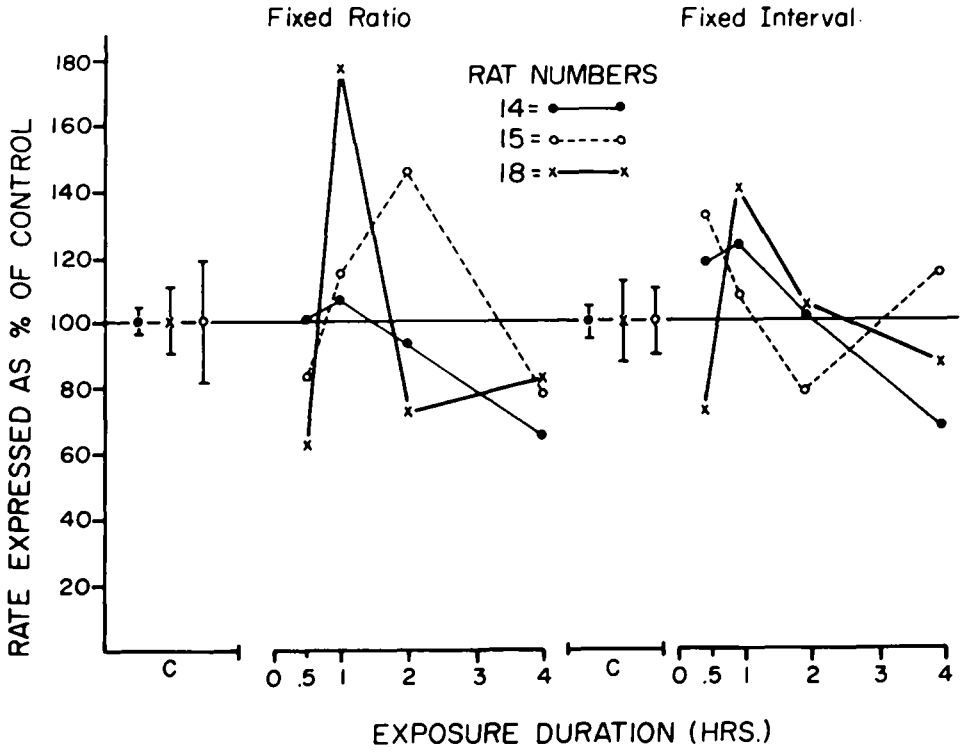

FIG. 4. Effects of inhalation of 150 ppm toluene by rats on fixed ratio–fixed interval response rates as a function of exposure duration. (Ordinate: rates of responding expressed as percent of average control rate. Abscissa: duration of exposures in hours. The points and brackets above C represent the mean ± SE for control rates of responding. Controls represent 4 determinations for each of 3 rats.)

Effects of toluene vapor inhalation upon the human central nervous system have been recognized for many years [21]. The reported effects of toluene inhalation for 4 hr, include a progression from drowsiness, headache, and dilation of pupils (50 ppm); through fatigue, incoordination, muscular weakness, and nervousness (100 ppm); confusion, paresthesias of skin, nausea, insomnia, and restless sleep (200 ppm); all symptoms appeared to worsen progressively through 800 ppm, with exhilaration and dizziness being experienced at the 600 ppm level. These effects were apparently not due to a non-specific irritant action or odor since no irritation of mucous membranes was experienced, and the olfactory nerve was rapidly paralyzed [21]. Intoxication and unconsciousness have been reported for high levels of exposure [14,15].

Toluene, along with other hydrocarbon solvents, is considered to act as a narcotic in acute exposure situations, with rapid onset of narcosis upon inhalation of high vapor concentrations [6]. Lewis and Patterson [13] have reported that toluene first stimulates and later depresses the nervous system. Because of the neuroactivity of toluene, the threshold limit value (TLV) for toluene exposure is 100 ppm, with a short term exposure limit concentration of 150 ppm, and a maximum allowable concentration (MAC) of 200 ppm [8].

A review of the toluene literature reveals that relative to the reported TLV, high exposure concentrations of toluene have been employed in animal studies. Doses for acute toxicity studies have ranged from 5300 pm to highs of 20,000 ppm and above [6].

Our results reported herein indicate that atmospheric concentrations of 150 ppm toluene produce the stimulatory phase of CNS depression within the initial 30 min of exposure, and that the depressive phase then sets in with performance being reduced below normal levels by 2 hr and remaining subnormal for the remaining 2 hr of a 4 hr exposure. There appear to be individual differences between animals which could either condense or expand the time frame of the initial stimulatory response somewhat.

Weiss *et al.* [23] have evaluated toluene for effects on a counting like behavior in the pigeon. Their reported effects are qualitatively similar to ours in that they found both enhancement and depression of response rates; response rates were increased at 400, 800 and 1600 ppm toluene, while 3200 ppm first produced depression and then enhancement of rate. The extreme differences in the exposure concentrations required to produce similar effects in the two studies, could be due to differences in toluene sensitivities between pigeon and rat; differences in sensitivities between the tests employed; or differences in the methods of exposure. Weiss *et al.* [23] first exposed their animals and then conducted the behavioral tests after removing the animals from the test atmosphere, while in our experiments behavioral testing was carried out during the exposure periods. Several studies have indicated that blood and tissue concentrations of toluene drop rapidly within 30 min after cessation of exposure to levels between 10 and 50% of exposure levels, depending upon the exposure concentration employed [16,17]. The drop in tissue concentrations is exponential, and the animals

in the Weiss *et al.* experiment worked up to 100 reinforcements which required approximately 30 min during control; therefore it would appear likely that the actual dose responsible for the observed behavioral effects is from 10 to 50% of their nominal exposure dose. If this, in fact, should turn out to be the case, the results of these investigators may agree quite well with those we are reporting here.

The time course of blood concentrations was not determined for toluene in these experiments as it was for acetone. The pharmacokinetic relationships for inhaled toluene are obviously quite complex. Toluene is known to be rapidly absorbed upon inhalation and partitioned into lipid depots throughout the body [3, 16, 17]. The high fat/blood partition coefficient of 113 [3] insures that the blood concentration of toluene remains low compared to a substance such as acetone, and the steady-state level in blood is more rapidly established than for acetone, while tissue concentrations may continue to increase in proportion to lipid content throughout exposure to toluene vapor. These relationships have been established for normal humans and animals; the behavioral studies reported here employed food-deprived animals which had been brought to 80% of normal body weight. These lean (male) animals possess virtually no fatty deposits anywhere in their bodies; normal tissue lipids (of much lower partition coefficient for toluene) therefore represent the only storage reservoir for inhaled toluene. Under these conditions, the uptake rates and equilibrium concentrations of toluene may be markedly altered. It is planned to assess these factors in future experiments.

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