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Effect of Exposure to High Concentrations of Toluene on Ethanol Preference of Laboratory Rats[1]

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GELLER, I., R. J. HARTMANN AND E. M. GAUSE. *Effect of exposure to high concentrations of toluene on ethanol preference of laboratory rats.* PHARMACOL BIOCHEM BEHAV **19**(6)933–937, 1983.—Male and female Holtzman Sprague Dawley rats were given 10-minute exposures to high concentrations of toluene twice a week at 10–30 days of age. The rate of acquisition of ethanol preference for these rats did not differ significantly from litter-mate sham exposed controls. Once ethanol preference curves were established, the rats were exposed daily over a 5-day period to high concentrations of toluene. An increase in ethanol intake occurred in most of the rats irrespective of early toluene exposures at 10–30 days of age.

Rats Toluene Ethanol preference

ABUSERS of volatile solvents are known to co-abuse a number of other drugs. These include sedatives, hypnotics, stimulants, narcotics, hallucinogens, marijuana and alcohol [4]. Of these drugs, abuse of alcohol has been reported to occur simultaneously with solvent abuse [1]. It has also been reported that in a junior high school population heavy alcohol drinkers were more likely to be "glue-sniffers" than were light alcohol drinkers [11]. Toluene is the principal intoxicant in many of the products that are involved in solvent sniffing [6].

It may also be relevant that alcohol consumption is extensive in populations that are exposed to solvents in the workplace [8]. It has been suggested that ingestion of ethanol in combination with inhalants may significantly alter the toxicity of the inhalant [8,10]. Inhalant exposure may also modify either the intake or the effects of ingested ethanol.

The present study was designed to investigate ethanol preference of laboratory rats as a function of toluene abuse. Exposures to high concentrations of toluene for short periods of time were used in a protocol modelled upon the human solvent-alcohol abuse situation.

METHOD

The animals were 15 male and 15 female Holtzman Sprague Dawley rats. Half of the animals in this study were from a group previously exposed to high concentrations of toluene twice a week during the period of 10–30 days of age; the other half were sham exposed litter-mate controls. At 33 days of age the rats were placed in individual 9×15×18 inch cages and were kept in a laboratory with ambient temperatures of 21–24 degrees C on an unrestricted diet of lab chow. Light-dark cycles of 12 hr dark and 12 hr light were maintained during each 24-hr period. Water and an ethanol solution were available at all times in 100 ml calibrated drinking tubes mounted on the back or on either side of the cages, with their spouts protruding into the cages approximately 1½ inches above the floor level. The 3 bottle-2 fluid choice method [5] was used to prevent the animals from selecting a fluid based on a position preference. Amounts of fluid consumed per 24-hr period were recorded. Drinking tubes were washed, refilled and rotated on a daily basis. The starting ethanol concentration for all rats was a 5% v/v solution. After 6 weeks, the concentration was increased to 8% v/v and after 2 more weeks it was increased to 10% v/v. In the event a particular concentration was rejected by any rat, the animal was maintained on that concentration. The rejection criterion was arbitrarily set as ethanol intake equal to 25% or less of total fluid intake.

In order to evaluate the effects of toluene exposure on ethanol preference, only those rats that drank less than 50% of total fluid intake as ethanol solution were used. These animals were exposed to extremely high concentrations of toluene once each afternoon during a 5-day period. In most cases exposures were at 36,000 ppm. The use of such high concentrations was based on the review of Press and Done [6] who reported that "glue-sniffers" are generally exposed to as much as 10,000 ppm before becoming unconscious. Comparable exposure conditions for rats were determined experimentally [2].

A large 20.662 liter desiccator fitted with a gas sampling port on the lid was used as a static exposure chamber. Toluene was introduced from a syringe onto filter paper lining the

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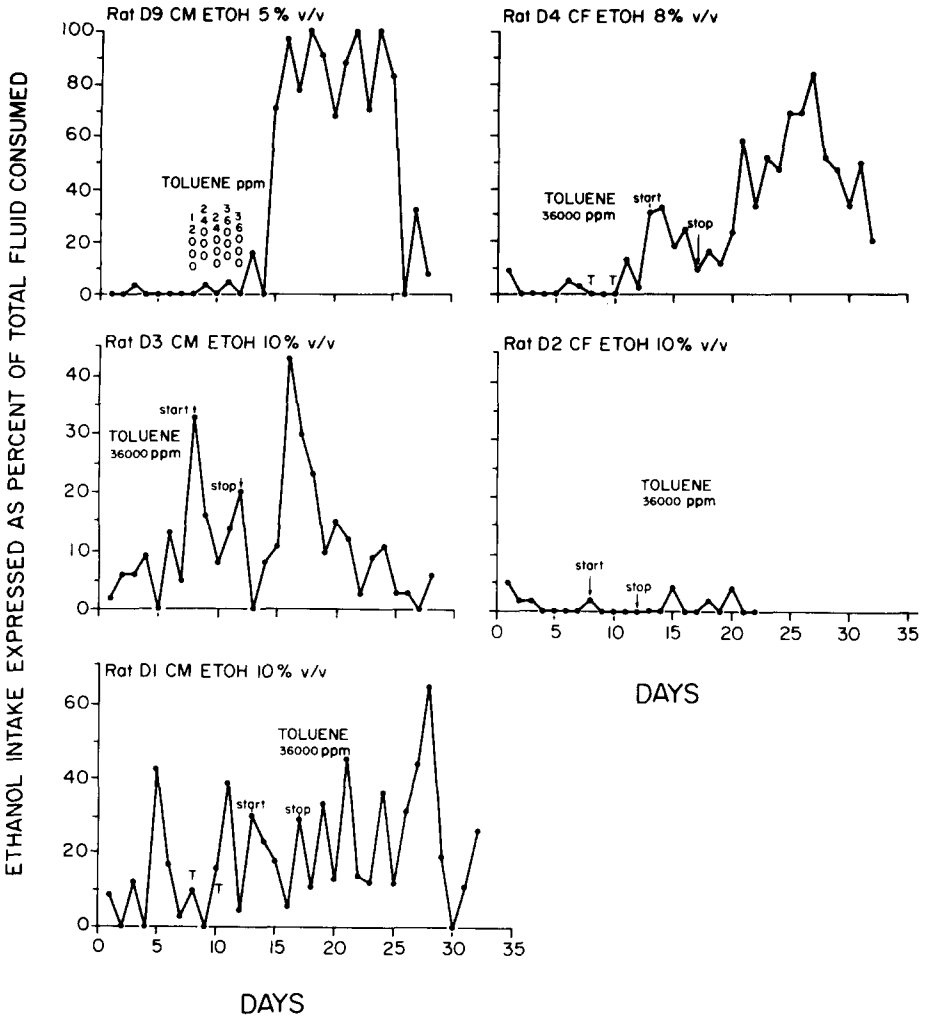

FIG. 1. Effects of five daily 10-minute exposures to toluene on ethanol intake of laboratory rats. Ethanol intake is expressed as a percentage of total fluid consumed. CM=males with no previous toluene exposures. CF=females with no previous toluene exposures.

walls of the desiccator. Simultaneously with the introduction of the toluene a rat was placed in the chamber for 10 minutes. The atmosphere was sampled (0.15 ml) at 1.3-min intervals and analyzed for toluene concentration by gas chromatography. After completion of the toluene exposures, control data were obtained by placing rats into the exposure chamber for 10 minutes per day over a 5-day period and following ethanol intake over a period of at least 12 days.

RESULTS

The rates of acquisition of ethanol preference were examined statistically for evidence of an effect of the early (10–30 days of age) exposure to toluene. The data were analyzed by the repeated measures model, using sex and dose as between factors and weekly means of the percentages of ethanol as the within factor. The BMDP4V program was used for the calculations. No effects of dose, sex or dose-sex interactions were found, nor were there any dose-trial, sex-trial or dose-sex-trial interaction effects. Although not statistically significant, there did appear to be a trend toward higher ethanol consumption by males that received the early toluene exposures compared to the sham exposed controls. However, this trend was not seen for the females.

Toluene exposure data obtained after ethanol preference had been established are shown in Fig. 1. The three control males (cm) and two control females (cf) were exposed at the doses indicated. Ethanol intake is expressed as a percentage of total fluid consumed during a 24-hr period. Ethanol intake increased in 4 of the 5 rats as a function of exposure to toluene. Prior to exposure, rats D4 and D9 drank minimal amounts of an ethanol solution. During the exposure period, ethanol intake did not increase for rat D9 but on the third post-exposure day a precipitous increase of ethanol intake occurred, an effect which persisted over a 10-day period. Two acute 10-minute exposures (T) to 36,000 ppm toluene had no effect on the ethanol preference of rats D1 or D4. During the 5 day exposure period for rat D4, ethanol intake increased slightly on the first 2 exposure days and decreased during the following 3 exposure days. Intake again increased on the fourth day post exposure, reached a maximum on the tenth post-exposure day and then decreased gradually during

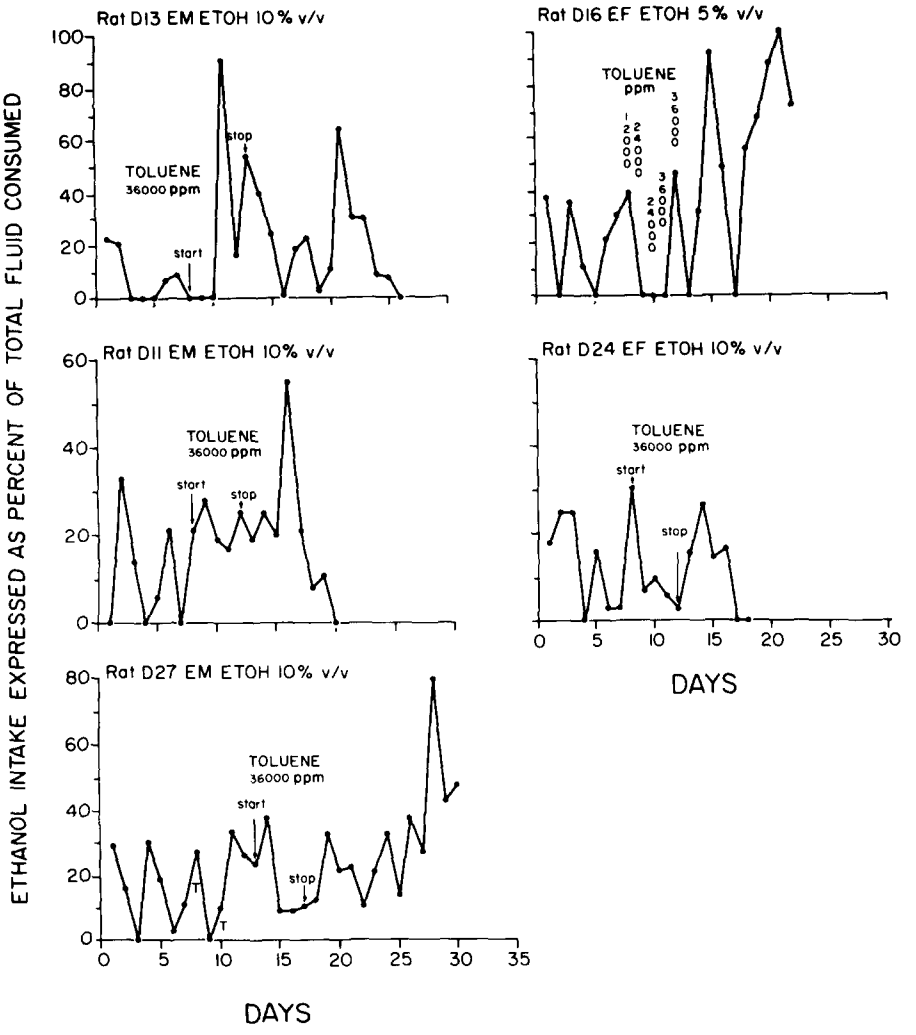

FIG. 2. Effects of five daily 10-minute exposures to toluene on ethanol intake of laboratory rats. Ethanol intake is expressed as a percentage of total fluid consumed. EM=males previously exposed to toluene at 10–30 days of age. EF=females previously exposed to toluene at 10–30 days of age.

the following 5-day period. During the 7 days pre-toluene exposure, a maximum amount of ethanol solution consumed. by rat D3 was no more than 13% of the total fluid consumed. The value on the first exposure day reached 33% and on the fourth post-exposure day a maximum of 43%. Ethanol intake returned to baseline values during the following 5-day period. During and after 5 days of exposure to toluene, ethanol preference for rat D1 increased above the values obtained during 6 of the pre-exposure days. Although the value for one of the control days reached 42%, the general trend during and after toluene exposure was an increase in ethanol preference which reached a maximum of 64%, on the eleventh post-exposure day. Exposure to 36,000 ppm of toluene over a 5-day period had virtually no effect on ethanol preference of rat D2.

Figure 2 contains data for rats that had been exposed to 36,000 ppm toluene twice a week during the period of 10–30 days of age. Although pre-toluene preference baselines are generally higher than those of the rats represented in Fig. 1, the toluene-induced increase of ethanol preference also occurred in these animals. The maximum preference value for rat D13 was 23% during the control period and reached a value of 91% on the fourth exposure day. Ethanol preference remained above control values on the first 3 post-exposure days, then decreased to control levels and again increased to a high of 64% on the ninth post-exposure day. Pre-exposure control values for rat D16 ranged from 0 to 38%. Exposure of the rat to toluene, at the doses indicated, resulted in a gradual increase to 92% on the third post-exposure day and 100% on the ninth post-exposure day. Ethanol preference for this animal did not return to the pre-exposure preference baseline. The maximum pre-toluene control baselines for rats D11 and D27 approximated 30%. Following exposures to 36,000 ppm of toluene over a 5-day period, ethanol preference reached a value of 55% for rat D11 on the fourth post-exposure day and then gradually returned to controls levels. Rat D27 received two acute exposures of 36,000 ppm toluene prior to the 5-day exposure treatment. The second acute exposure for rat D27 was followed by an increased ethanol intake on the first post-exposure day. The maximum increase in ethanol intake for rat D27 occurred on the eleventh post-exposure day. Ethanol preference for this rat did not

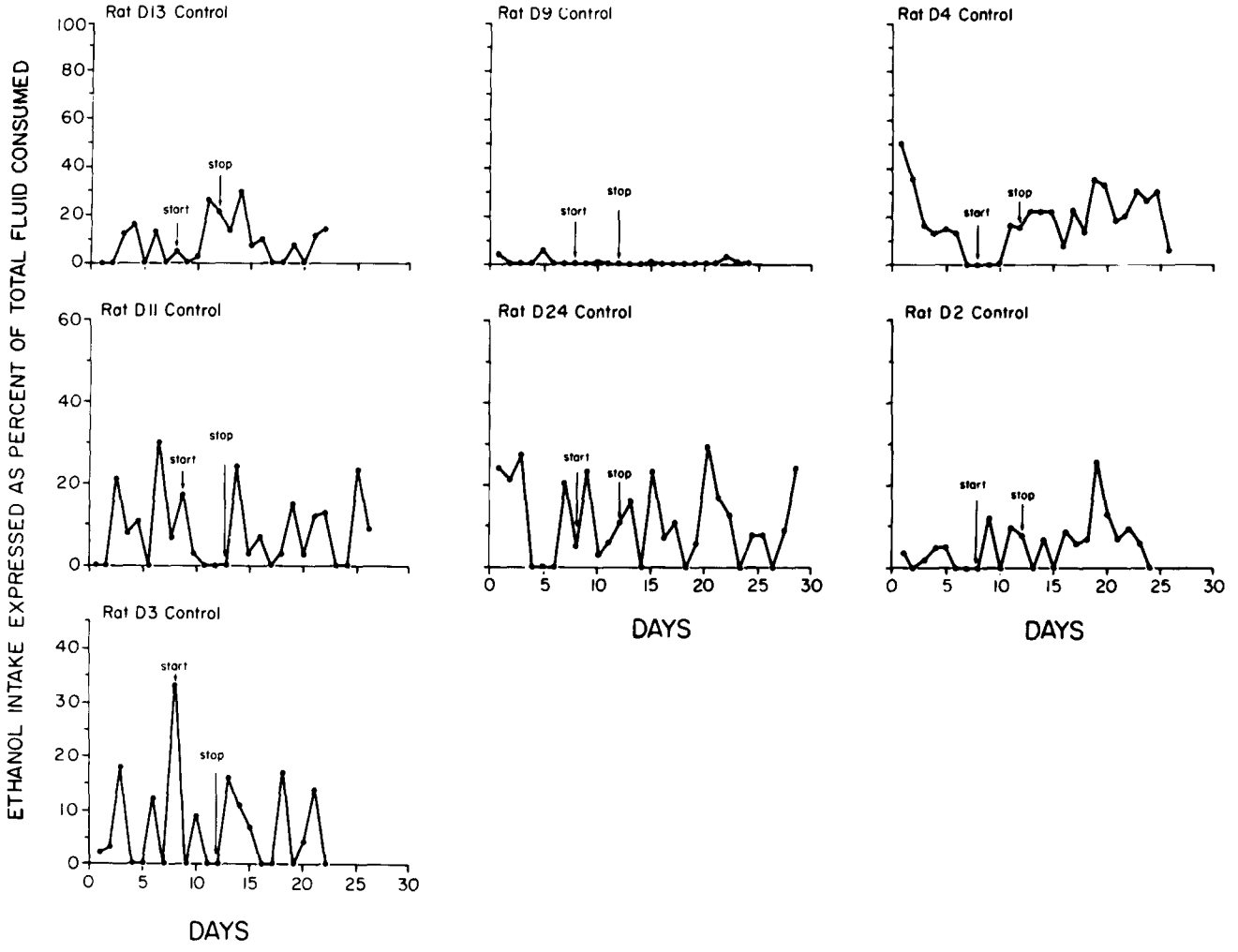

FIG. 3. Effects of five daily 10-minute sham exposures on ethanol intake of laboratory rats. Ethanol intake is expressed as a percentage of total fluid consumed. Ethanol concentrations for these animals are as indicated in Figs. 1 and 2.

return to the pre-exposure control baseline. Although the data for Rat D24 are not as striking as for the other rats, ethanol preference values obtained on the first exposure day and on the second post-exposure day were higher than any of the pre-exposure control period.

Control data were not obtained for all of the rats since some of the animals did not recover their pre-toluene control baselines. Figure 3 contains control data for 7 of the rats. The maximum ethanol preference for D13 under sham exposure conditions was 29% as compared to a maximum of 91% during toluene exposure (Fig. 2). Sham exposure control values for D9 remained below 5%, while under toluene the levels reached 100%. Data for rat D4 indicate a peak value for ethanol preference of 35% seven days after the sham exposure as compared with a toluene-induced increase of ethanol preference to a maximum value of 85%. The fresh air control values for rat D11 remained below the 30% value which the animal reached 2 days prior. The 5-day fresh air control for this rat was conducted soon after the toluene exposure and the pre-control value of 30% might be due to residual effects of toluene exposure. In any case the control peak values of 24% and 32% are far below the peak value of 60% reached by this animal 4 days after exposure to toluene. Control data for D24 show a peak value of 29% on day 20 which was slightly below the 33% reached on the second day post-toluene. Ethanol preference of D2 reached a maximum of 13% during the sham exposure and peaked at 24% five days later. Although toluene exposures appeared to be ineffective for this rat, perhaps the control data might reflect the presence of residual toluene in the CNS since the sham exposures were conducted 9 days after the last toluene exposure. As was shown in Fig. 2, the peak effect for rat D3 after toluene exposure was 45% while the control data indicate a peak value of 33% on the first day of the sham exposure. It is difficult to account for the high control value in this rat since the sham exposures occured over 1 month post toluene when all toluene should have been out of the CNS.

DISCUSSION

The findings of this investigation clearly demonstrate that daily exposures of rats to extremely high doses of toluene for 10-minute intervals over a 5-day period resulted in increased ethanol preference. Control exposures to fresh air were ineffective in this regard. The effect occurred in both male and female rats irrespective of whether they had experienced

toluene exposures during the period from 10–30 days of age. The increase in ethanol preference occurred for some animals during the exposure period and for others anywhere from 2 to as many as 10 or 11 days post exposure. These observations with rats might provide an insight into the excessive use of alcoholic beverages by individuals that are exposed to a variety of solvents on a daily basis in the abuse situation or in an industrial setting.

A number of other investigators have conducted studies of ethanol and solvent interactions. Savolainen *et al.* [10] exposed rats to 300 ppm xylene for 6 hr per day, 5 days per week over a 2-week period. The animals had access to an ethanol solution (15% v/v) during this time. During the second week of exposure the xylene-exposed rats drank more ethanol solution than did the controls. Behavioral evaluation indicated a potentiation of xylene effects by ethanol. The investigators also reported that the accumulation of xylene in perirenal fat which they observed in the xylene-exposed animals was lowered considerably in the xylene animals that had access to ethanol. This observation might suggest that the increase in ethanol intake may have resulted in a more rapid excretion of xylene.

In another study [8], it was shown that ingestion of a moderate dose of ethanol prior to exposure to approximately 147 or 274 ppm xylene caused a marked alteration in the kinetics of xylene. The blood xylene level increased 1.5- to 2-fold and urinary methylhippuric acid, the xylene metabolite, decreased by 50%, thereby suggesting that ethanol decreased the metabolic clearance of xylene. The authors speculated that the disturbance in xylene kinetics was probably due to an inhibition of microsomal metabolism by ethanol.

Sato *et al.* [9] reported that toluene disappeared more rapidly from the blood of rats treated with ethanol than from the blood of control animals. They interpreted their findings to mean that chronic ethanol consumption accelerates the in vivo metabolism of toluene in the rat. However, they also found that ethanol inhibited the in vitro metabolism of toluene. The amount of ethanol in the body with other chemicals may be a critical determinant of whether it has a stimulating or inhibiting effect on drug-metabolizing enzymes. [2].

Pyykko *et al.* [7] studied the uptake, distribution and elimination of labelled toluene in various tissues of rats following inhalation. Evaluation of brain/blood ratios 12 hr post exposure revealed a value of 1.72, indicating a greater amount of toluene in brain than in blood. We are not aware of any data on the possible presence of toluene, even at low levels, in the brains of animals exposed to the high concentrations used in the present studies. If one assumes that there are low levels of toluene in brain or other tissues of rats as long as 2 weeks following exposure to high concentrations of toluene, this would provide a basis for considerable speculation concerning the observed toluene-induction of higher ethanol intake. Just as in the xylene studies cited above, rats might be increasing ethanol intake in order to potentiate a possible "high" when the low levels of toluene are still present in the brain. Perhaps the rats have increased ethanol intake in response to an effect on the clearance of toluene as previously reported [9]. Although the present research doesn't provide any direct evidence to support these speculations, it does offer a number of approaches to studies on the mechanism of these effects.

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