Pharmacology Biochemistry & Behavior, Vol. 23, pp. 401–410, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Interactions Between Toluene and Alcohol[1]

GORDON T. PRYOR, ROBERT A. HOWD, EDWARD T. UYENO AND ANDREA B. THURBER

SRI International Menlo Park, CA 94025

Received 26 November 1984

PRYOR, G. T., R. A. HOWD, E. T. UYENO AND A. B. THURBER. *Interactions between toluene and alcohol.* PHARMACOL BIOCHEM BEHAV **23**(3) 401–410, 1985.—Weanling male Fischer-344 rats were exposed by inhalation to air or 2000 ppm toluene for 8 hours each day for 2 weeks. Subgroups had access to water or 6% alcohol as their only fluid sources, respectively. Rats exposed to both toluene and alcohol subsequently showed a marked preference for 6% alcohol in two-bottle choice tests that persisted for up to 20 days for some rats. Rats exposed to toluene without access to alcohol and control rats (exposed to air and water) showed a marked aversion to the alcohol solution, and only 2 of 12 rats forced to drink alcohol without exposure to toluene preferred alcohol in the preference tests. Exposure to both toluene and alcohol also caused greater inhibition of weight gain than exposure to either substance alone, accompanied by greater signs of organ toxicity as indicated by clinical blood chemistries. Exposure to toluene caused marked hearing loss as assessed by a behavioral technique (conditioned avoidance), and there was a trend toward enhancement of this ototoxic effect by forced consumption of alcohol.

Behavioral audiometry Clinical blood chemistry Enhanced alcohol preference Rats Solvent abuse Toluene/alcohol interactions Toluene-induced hearing loss

INHALATION of common industrial solvents for their euphoric effects is fairly widespread, especially among younger persons [2, 23, 31, 32]. Concurrent use of other drugs, especially alcohol and marihuana, is also common in this young population [4, 8, 16, 17, 36, 38]. Concern about possible unexpected toxic interactions among these substances is heightened by the possibility that early solvent use, alone or in conjunction with other substances, may promote subsequent abuse and consequent psychologic and/or physiologic dependence [13,16].

Geller *et al.* [11] recently reported that rats exposed for 10 minutes daily for 5 days to very high concentrations of toluene—a ubiquitous and highly preferred solvent of abuse [31,32]—increased their preference for alcohol. These provocative results appeared at a time when we were planning an experiment to examine the potential toxic interactions between toluene and alcohol and to see whether alcohol affected the hearing loss caused by exposure to toluene that we had recently discovered [24–27]. Therefore, although the exposure conditions were markedly different between the two experiments, we included tests of alcohol preference to examine the generality of the effect reported by Geller *et al.* [11]. The results of these tests were so striking in showing an increased preference for alcohol that they became the main focus of this report.

METHOD

Subjects

Male, weanling rats (23 days old) were obtained from Simonsen Laboratories Inc., Gilroy, CA. Upon arrival they were housed in the exposure chambers described below (12 rats per chamber). They were allowed to acclimate to the chambers for three days with food and water available ad lib. Temperature in the inhalation laboratory was controlled at 23±1°C, and the relative humidity was 40 to 60%. The lights were on from 0700 to 1900 hours.

Materials

Industrial grade toluene was purchased from Van Waters and Rogers. The material contained over 99% toluene, with less than 1.0% contamination by other hydrocarbons, including benzene (0.1%), octanes, and xylenes as measured by gas chromatography-mass spectroscopy. Absolute ethanol was diluted with distilled water to a 6% solution (v/v).

Exposures and Alcohol Access

After acclimation for 3 days, the rats were exposed to either filtered air or toluene in a 4-chamber exposure system. Each chamber, constructed of clear Plexiglas, measured 50×50×25 cm (62.5 liters). Pressurized, filtered air was metered through the chambers at a flow rate of 16 to 20 liters per minute. Toluene vapor was introduced into, and mixed with, the main air stream of two of the chambers by bubbling a diverted portion (controlled by flowmeters) of the air through the solvent. Solenoid valves and associated timers allowed precise and automatic control of the exposure duration. Chamber concentrations were monitored by gas chromatography of samples taken through septum ports located in the walls of the chambers. The exposures were from 0900 to 1700 hours daily for 14 or 15 days. Distilled water was the only source of fluid in one chamber of each condition (control or toluene exposure), and 6% alcohol was the only source of fluid in the other chamber of each condition. Access to the fluid was via two ballpoint drinking spouts at-

[1]This research was supported by NIDA Contract No. 271-80-3712. We thank Dr. C. W. Sharp of the NIDA for his continued support, advice and encouragement. We also thank Ms. J. Dickinson for technical assistance and Ms. R. McCormick for her excellent help in preparing the manuscript.

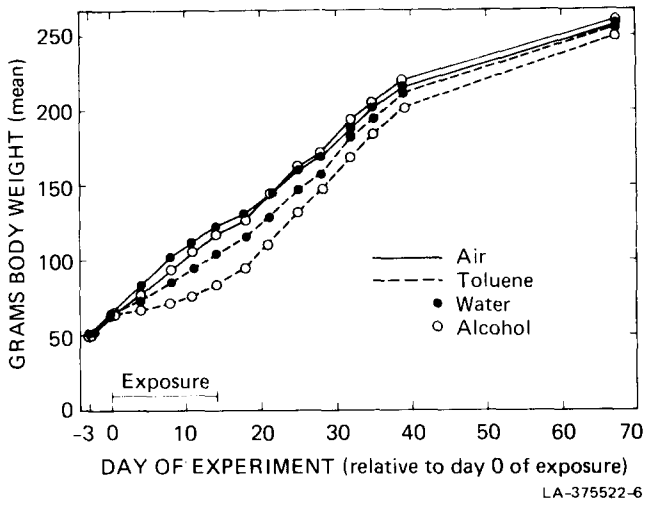

FIG. 1. Effect of exposure to toluene and alcohol, alone or together, on body weight. Standard errors are omitted to preserve clarity but were generally smaller than the symbols used to denote the means.

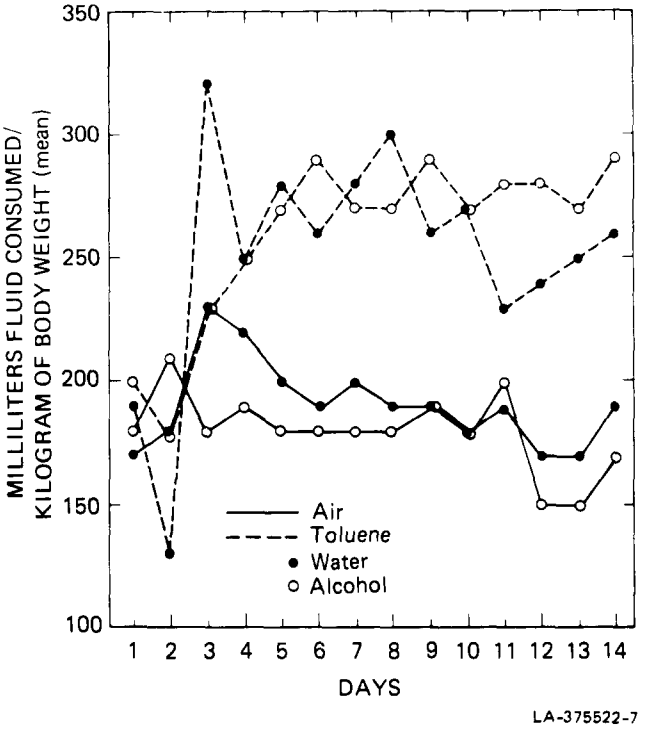

FIG. 2. Effect of exposure to toluene and alcohol, alone or together, on total fluid consumption per kilogram body weight.

tached by Tygon tubing to inverted 1-liter bottles suspended above the chambers. Fluid consumption per rat was measured by 24-hour weight differential of the bottles divided by the number of rats in the chamber. Food consumption was not measured. The rats were weighed upon arrival and at intervals throughout the experiment. One rat previously exposed to toluene and alcohol developed abnormal incisor growth that prevented it from eating normally. It was sacrificed after 10 days of alcohol preference testing.

Clinical Chemistry

After 14 days of exposure to filtered air or toluene, half of the rats in each chamber were removed, and a total of 300 μl of blood was sampled retro-orbitally from one eye using a 100-μl capillary pipette for puncture of the orbital sinus under light carbon dioxide anesthesia. The samples were centrifuged and the following constituents in plasma were measured using a Gemini™ Miniature Centrifugal Analyzer: glucose, blood urea nitrogen (BUN), creatinine, phosphorus, bilirubin, serum glutamate oxalacetate transaminase (SGOT), alkaline phosphatase, cholesterol, calcium, total protein, albumin, and Cl^-. Plasma Na^+ and K^+ were measured by flame photometry. The other half of the rats in each chamber were removed and processed as just described after 15 days of exposure (to allow the clinical chemistries to be done fresh).

Alcohol Preference

After blood was sampled, the rats were housed individually in standard plastic rat cages with wood shavings as bedding in the animal quarters. Two bottles, one containing distilled water and the other containing a 6% alcohol solution, were placed on each cage, with food available ad lib. Twenty-four-hour fluid consumption from each bottle was measured by weight differential. Spillage was usually less than 1 ml in 24 hours, including the losses that occurred when the bottles were removed for weighing and refilling. The positions of the bottles were alternated daily except on some weekends.

After 20 days the alcohol bottles were removed. Five days later they were returned, and preference was measured for 4 days. Then the rats were rehoused three per cage (no alcohol) and began training on a multisensory conditioned avoidance response (CAR) task. After training and testing for auditory response thresholds (see below) the rats were again rehoused individually and alcohol preference was measured for 4 days.

Multisensory CAR Task

The apparatus and procedures for training rats to perform a multisensory CAR task have been described in detail elsewhere [14]. Briefly, the rats learned to pull or climb a pole suspended from the ceiling of the test chamber to avoid or escape a 1-mA scrambled, constant current shock on the grill floor. The aversive current was preceded by one of three warning stimuli—an increase in the intensity of the test chamber light, a nonaversive current on the floor (100 μA), or a pure tone (4 kHz, 58.0 dB re 20 $\mu N/m^2$ SPL) from a loudspeaker located in the ceiling of the chamber. The stimuli were pulsed at the rate of 2.5 per second (200 msec on: 200 msec off). All rats were trained on all three stimuli in order to determine the extent to which any effects were sensory-specific. The three stimuli were presented randomly (one per trial) during each session. A response during the warning signal terminated the trial and was scored as a successful avoidance. A response during aversive shock also terminated the trial and was scored as an escape. Absence of any response was scored as an escape failure. A Digital

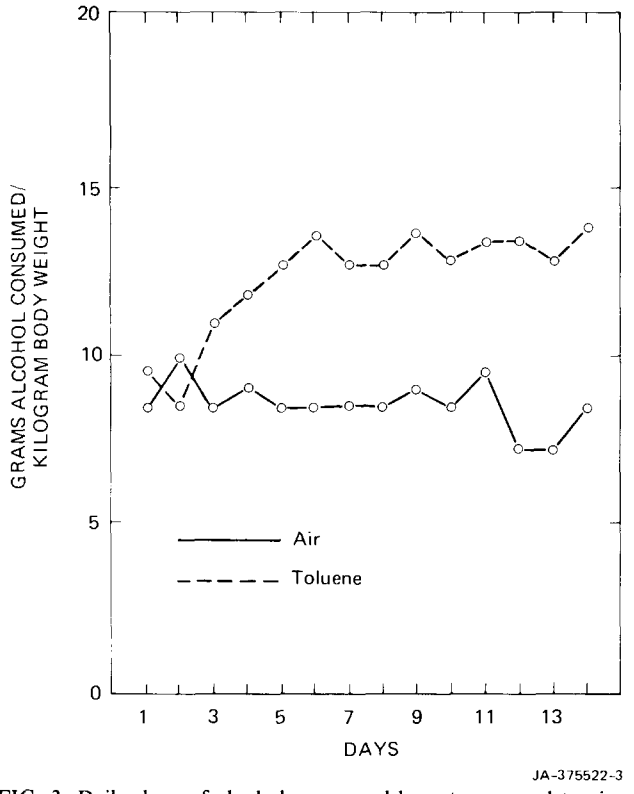

FIG. 3. Daily dose of alcohol consumed by rats exposed to air or toluene.

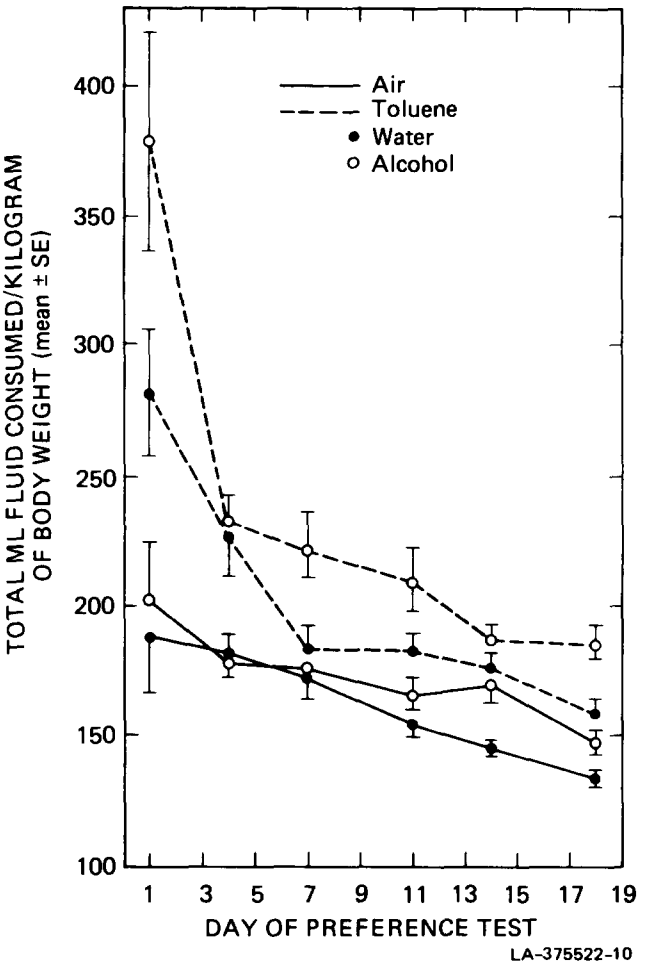

FIG. 4. Total fluid consumed per kilogram body weight during alcohol preference testing after exposure to toluene and alcohol, alone or together.

Equipment Corporation (Maynard, MA) PDP 8/F computer located in an adjoining room provided stimulus control and data collection for the 24 chambers that were operated concurrently.

Training proceeded in three phases. First, the rats were trained in a 30-trial session to escape aversive footshock. The shock was presented randomly in time and lasted 20 seconds in the absence of a response. Then the rats were given four daily 60-trial sessions to learn to avoid the footshock in the presence of the three warning stimuli. The duration of the warning stimulus was 10 seconds and the duration of the aversive shock was 20 seconds (in the absence of an avoidance response, the warning stimulus remained on with the aversive stimulus). The third phase consisted of two 60-trial sessions per day for three days. The warning stimulus interval was shortened to 5 seconds to reduce false positive responses. The frequency of the tone was increased during this phase to provide generalization training. The intensities were chosen so as to be above threshold even for the rats that were expected to have suffered toluene-induced hearing loss. The frequencies (kHz) and intensities (in parentheses, dB re 20 $\mu N/m^2$ SPL) were 4 (58.0), 8 (55.7), 12 (70.4), 16 (68.3), and 20 (66.4) for the first five sessions. On the sixth session the intensity of the 20-kHz tone was reduced to 37.6 dB as a preliminary test for hearing loss. Data from rats that failed to learn the escape response or who did not consistently perform the avoidance response to all three stimuli were not used in the analyses (two rats exposed to air and water, two exposed to air and alcohol, and one exposed to toluene and alcohol).

Behavioral Audiometry

Following training, generalization, and confirmation of the expected hearing loss caused by exposure to toluene in the preliminary test, this effect was characterized further by generating intensity/response functions to the tone at different frequencies. Auditory response thresholds were estimated by giving the rats a series of test sessions during which only the tone was used as the warning stimulus. Each session consisted of 60 trials, ten at each of five tone intensities and ten blank trials (a 5-second period preceding the aversive shock during which a response terminated the trial, but with no warning stimulus present). The different tone intensities and blank trials were presented randomly, and only one frequency was tested during a given 60-trial session. We defined the auditory response threshold as the estimated intensity at which 50% avoidance was expected for each rat at each frequency.

Tone intensities differed, depending on the frequency being tested. They were 40.4 to 63.8 dB at 4 kHz, 37.6 to 61.6 dB at 8 kHz, 24.0 to 46.9 dB at 12 kHz, and 20.5 to 43.5 dB at 20 kHz. The five intensities presented in a given session were approximately equally spaced on the dB scale and were

TABLE 1

FREQUENCY DISTRIBUTION OF ALCOHOL PREFERENCE SCORES OF RATS EXPOSED TO TOLUENE OR ALCOHOL, ALONE OR TOGETHER

Exposure	Fluid Available	Preference Test Days	Alcohol Preference Score 0–20	21–40	41–60	61–80	81–100
Air	Water	1–5	12	0	0	0	0
		6–10	12	0	0	0	0
		11–15	12	0	0	0	0
		16–20	12	0	0	0	0
	Alcohol	1–5	7	2	1	1	1
		6–10	9	1	0	0	2
		11–15	9	1	0	0	2
		16–20	10	0	0	0	2
Toluene	Water	1–5	12	0	0	0	0
		6–10	12	0	0	0	0
		11–15	12	0	0	0	0
		16–20	12	0	0	0	0
	Alcohol	1–5	1	0	0	4	7
		6–10	2	1	2	1	6
		11–15*	4	1	1	1	4
		16–20	6	1	0	0	4

Values are the numbers of rats in each group with average preference scores that were in the intervals indicated.

*One rat was sacrificed.

chosen to span the auditory response thresholds in control rats at each frequency. To obtain auditory response thresholds in many of the toluene-exposed rats at the higher frequencies, additional test sessions were conducted with higher intensities. The intensities for these test sessions were 53.1 to 76.3 dB at 12 kHz and 48.9 to 72.3 dB at 20 kHz.

Estimates of tone intensity were made using a General Radio Company Type 1511-C sound level meter set at A weighting. The microphone (General Radio Company Type 1560-PS) was positioned 1 cm from the loudspeaker. Because this microphone is not sensitive to frequencies above 10 kHz, the calibration curves obtained at 4 and 8 kHz, which were identical, were used to extrapolate the intensities at the higher frequencies based on the measured voltages delivered to the speaker (Realistic, 3.5-inch solid-state piezo super horn). There was no attempt to correct for attenuation in sound pressure level from the location of the microphone to the actual nonstationary position of a rat in the chamber. We consider these intensity estimates to be satisfactory for comparison purposes, but they do not represent true sound pressure levels at the rats' ears.

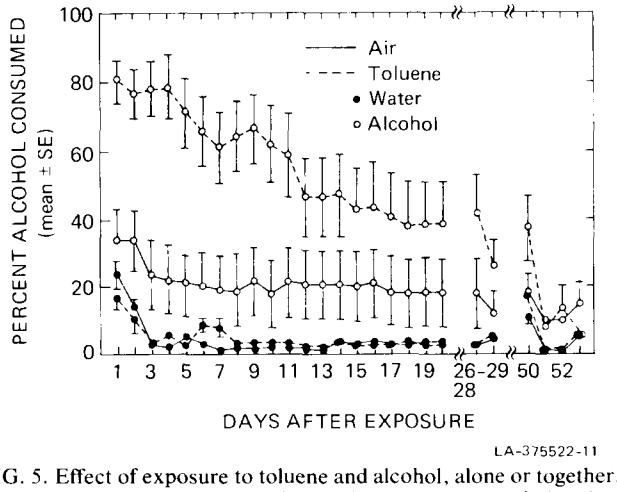

FIG. 5. Effect of exposure to toluene and alcohol, alone or together, on alcohol preference. Breaks in abscissa represent periods when only water was available.

Data Analysis

Alcohol preference was defined as the percentage of total 24-hour fluid consumption that was consumed as 6% alcohol solution. A 3-way, repeated measures analysis of variance (ANOVA) was used to analyze these data, although departures from the underlying assumptions were evident (lack of homogeneity of variance and non-normal distributions). Because of the magnitude of the effects noted, it is unlikely that this represents any serious error.

To estimate auditory response thresholds, the linear portion of the response curve around 50% avoidance was regressed on intensity, and the 50% point was interpolated. No correction for false positives (i.e., responses on blank trials) was made because there were no significant differences among groups in this regard. If an auditory response threshold could not be estimated, the highest intensity tested was arbitrarily assigned to that rat for that frequency.

Three-way, repeated measures ANOVAs were used to assess treatment effects (exposure to toluene and access to alcohol), changes over time (or over tone intensity and frequency), and their interactions [40]. Two-way ANOVAs were used for nonrepeated measures analyses and to assess

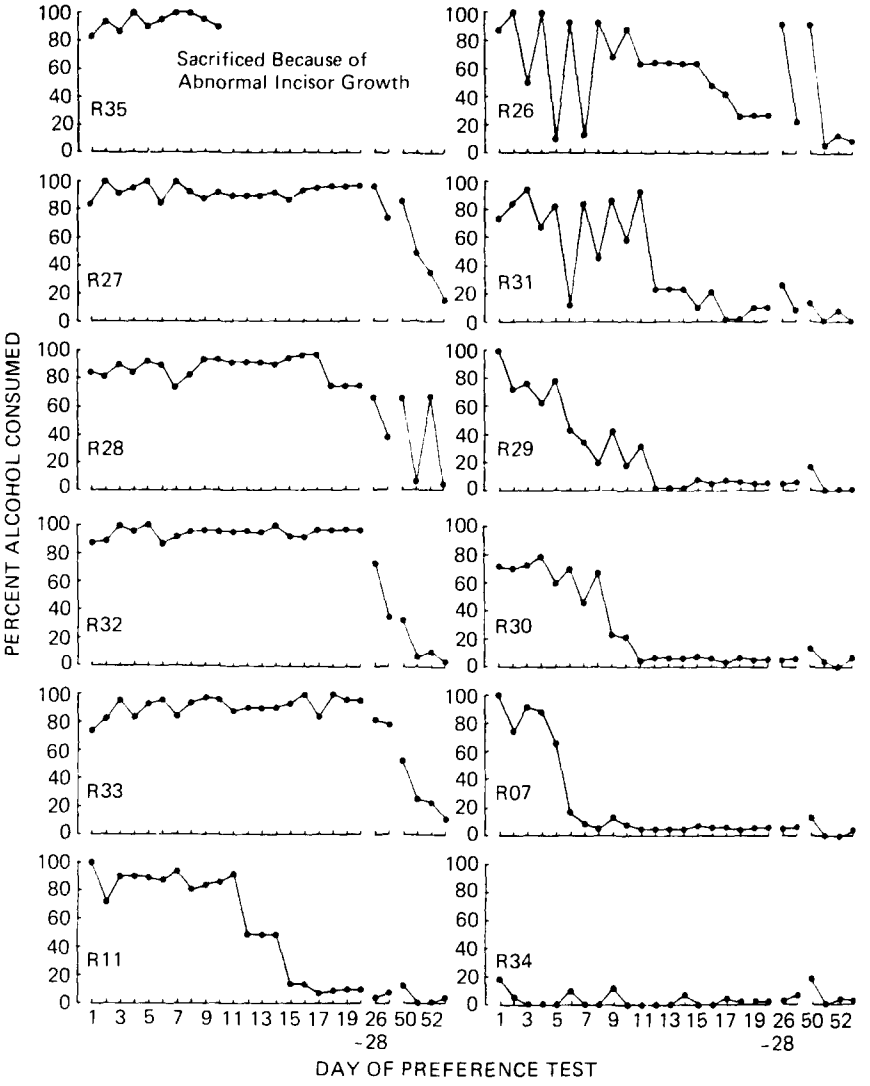

FIG. 6. Preference scores for individual rats exposed to toluene and forced to drink alcohol. Breaks in abscissa represent periods when only water was available.

each repeated measure separately when interactions between treatment and repeated measures resulted in F-ratios with $p<0.05$. Fisher's least-significant-difference (lsd) test was used in *a priori* comparisons between means of control and experimental groups subsequent to an F-ratio with $p<0.05$. Because a generally accepted method for controlling type I errors is not available for experimental designs of the type used, actual probability values are given for the ANOVA results where $p<0.05$. Results of the lsd tests are reported at $p<0.05$.

RESULTS

Body Weight

Figure 1 shows that weight gain was inhibited in all experimental groups compared with the control group exposed to filtered air with access to water (Toluene × Alcohol × Repeated Measures interaction, $F(13,559)=2.85$, $p=5.4\times10^{-4}$). The effect on body weight was least for the rats exposed to air and forced to drink alcohol and greatest for the rats exposed to toluene and forced to drink alcohol. For the group exposed to air and forced to drink alcohol, the differences from the control groups met the criterion of $p<0.05$ by lsd test only on Days 4 and 8. For the group exposed to toluene with access to water, the differences met this criterion on Days 4 through 21. For the group exposed to toluene and forced to drink alcohol, the differences had associated ps<0.05 on Days 4 through 35. This last group also differed from the group exposed to toluene without access to alcohol on Days 8 through 25. The more-than-additive interaction between exposure to both toluene and alcohol was reflected by interaction F-ratios (1,43) of 7.50, 8.34, 7.48, 6.77, and 4.40 (ps≤0.04 to 0.006) on Days 8, 11, 14, 18, and 21, respectively. The effects were reversible, and there were no differences in body weights among groups on Days 39 and 67.

Fluid Consumption During Exposure

Because the rats were group housed, individual fluid consumption could not be measured, thus prohibiting statistical

TABLE 2

EFFECT OF EXPOSURE TO TOLUENE, WITH OR WITHOUT ALCOHOL, ON PERFORMANCE OF A MULTISENSORY CAR TASK

Exposure	Fluid Available	n	Stimulus: Tone	Stimulus: Shock	Stimulus: Light
Air	Water	10	50.9 ± 5.5	86.4 ± 3.4	72.3 ± 2.6
	Alcohol	10	58.0 ± 8.0	87.5 ± 4.0	79.5 ± 5.4
Toluene	Water	12	17.9 ± 6.7*	84.6 ± 3.5	79.2 ± 2.9
	Alcohol	10	6.5 ± 1.5*	88.0 ± 2.4	73.5 ± 4.6

Values are the mean (±SE) percent avoidance. The frequency of the tone was 20 kHz (37.6 dB) for this test.

*$p<0.05$ compared with air/water control by lsd test following F-ratio associated with $p<0.05$.

analyses. However, group consumption was consistently less in the chambers with alcohol than in those with water and consistently greater in the chambers with toluene than in those with air. The effect was even more dramatic when expressed relative to body weight (Fig. 2). From these data it is also possible to estimate roughly the daily dose of alcohol consumed by the groups forced to drink alcohol (Fig. 3). Thus, the group exposed to air had a daily alcohol intake of about 8 or 9 g/kg throughout the exposure phase. The group exposed to toluene had a similar intake initially, but intake increased over about 6 days to stabilize at about 13 g/kg.

Alcohol Preference

Total fluid consumption per rat was very variable within all groups during the first few days of alcohol preference testing, presumably because the rats had to learn to deal with the two-bottle choice situation according to their particular preferences. Nevertheless, consumption was higher initially in the rats previously exposed to toluene, and a separate ANOVA for Day 1 revealed an $F(1,43)$ associated with previous exposure to toluene of 6.05 ($p=0.018$). This presumed carryover effect was gone by Day 2. When expressed relative to body weight (Fig. 4), total fluid consumption was markedly elevated in the groups exposed previously to toluene (toluene main effect, $F(1,43)=47.02$, $p=2.1\times10^{-8}$). This effect diminished over time as the rats gained weight to a greater extent than they increased their total fluid consumption (Toluene × Repeated Measures interaction, $F(5,215)=10.44$, $p=5.7\times10^{-9}$). Nevertheless, the differences from controls still had associated ps<0.05 by lsd test on Day 18 (ts$(43)=3.45$ and 7.22 for the groups previously exposed to toluene with or without alcohol, respectively).

Figure 5 shows that most of the rats previously exposed to toluene and forced to drink alcohol had a strong initial preference for alcohol that gradually diminished, on the average, over the 20-day test period. In contrast, the rats exposed to air and water or toluene and water rapidly developed a marked and persisting aversion to the alcohol solution. Some of the rats exposed to air and forced to drink alcohol strongly preferred the alcohol solution but most did not as reflected by the large standard errors. Although these data violated the underlying assumptions for use of ANOVA (as noted in the Method section), the Toluene × Alcohol × Repeated Measures interaction $F(19,817)$ was 3.11 ($p=9.2\times10^{-6}$).

The data are, perhaps, better represented by the frequency distributions shown in Table 1. During the first five days 11 of the 12 rats exposed to toluene and forced to drink alcohol had preference scores greater than 60%; the twelfth rat avoided the alcohol solution throughout. Preference eventually diminished to less than 40% for 6 of these rats but remained greater than 90% for the other 4 (one rat was sacrificed). Two rats forced to drink alcohol but not exposed to toluene had preference scores greater than 60% and this preference persisted throughout the 20 days of testing; the other ten rats rapidly developed a marked aversion to the alcohol solution. All rats avoided the alcohol solution after two periods of forced abstinence.

The individual drinking patterns of the rats exposed to toluene and forced to drink alcohol are shown in Fig. 6. The data for R26 and R31 are interesting, because their preference for alcohol was cyclic and may have reflected a position preference for some time before eventually rejecting the alcohol solution. Thus, these two rats apparently did not find the alcohol solution initially aversive, as did all of the controls. Also of note is the observation that of the 11 rats that initially preferred alcohol, this preference lasted a minimum of 5 days (RO7).

Multisensory CAR and Behavioral Audiometry

There were no differences among groups during acquisition of the multisensory CAR task or during generalization of the tone frequency from 4 to 20 kHz at high intensities. However, when the intensity of the 20-kHz tone was decreased from 66.4 to 37.6 dB as a preliminary test of the expected hearing loss associated with exposure to toluene, the rats exposed to toluene performed the tone CAR more poorly than either group exposed to air (Table 2; Toluene, $F(1,39)=47.37$, $p=3.1=10^{-8}$). Although performance by the group exposed to toluene and forced to drink alcohol was poorer than the corresponding group with access to water, the difference was not associated with a $p<0.05$. Nor did forced consumption of alcohol alone cause any changes in performance. There were no differences among groups in performance of the shock and light CARs or in escape failures.

To characterize the extent of hearing loss associated with exposure to toluene, intensity/response functions were generated using only the tone. There were no differences among

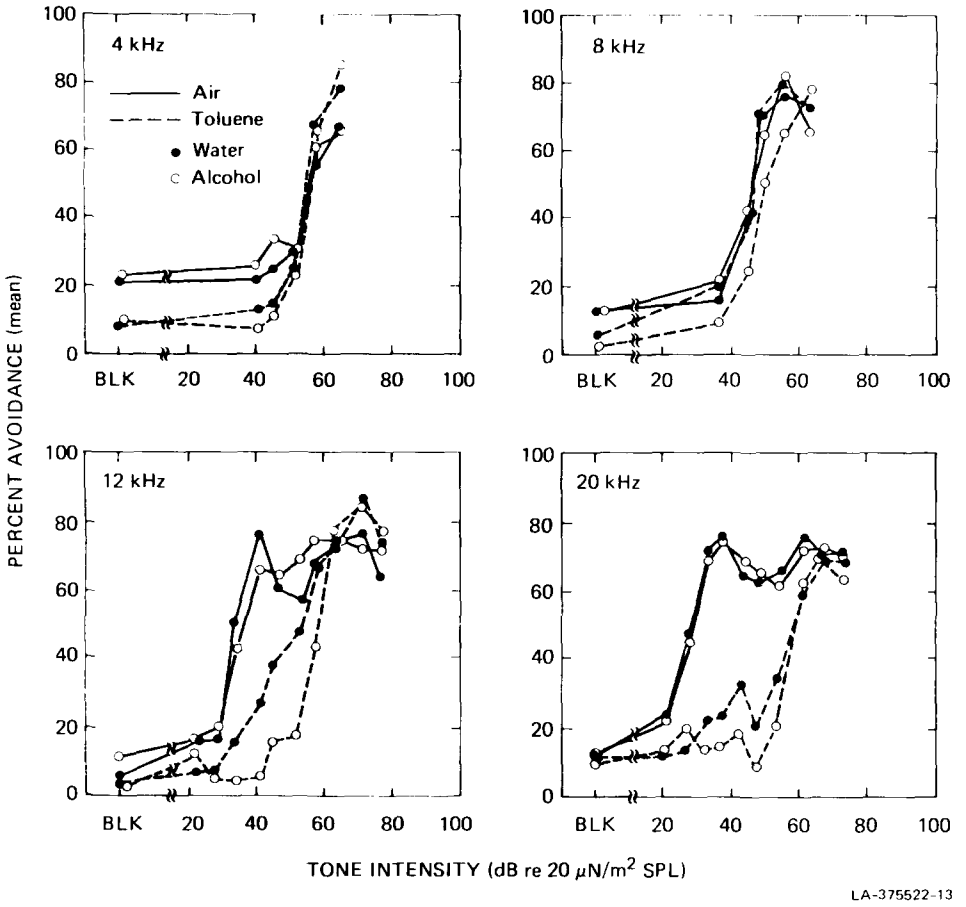

FIG. 7. Tone intensity/response functions of rats exposed to toluene and alcohol, alone or together. Standard errors are omitted to preserve clarity. Blk=blank trials.

groups in the intensity/response functions at 4 and 8 kHz (Fig. 7), although there was a trend for the rats exposed to toluene to make fewer false positives (responses on blank trials) than the rats exposed to air. Also the rats exposed to toluene and forced to drink alcohol performed the avoidance response at slightly lower levels than the other rats at all but the highest intensity of the 8-kHz tone. A clear indication of hearing loss caused by exposure to toluene was seen at 12 and 20 kHz (Toluene × Intensity, Fs(10,380)=4.15 and 18.70, respectively, $ps=1.8\times10^{-5}$ and 2.1×10^{-10}, respectively). The rats exposed to toluene and forced to drink alcohol were slightly more impaired than those exposed to toluene alone at 12 kHz ($ps\leq0.05$ at 41.0, 46.9, and 53.1 dB by lsd test). The two groups appeared equally impaired at 20 kHz.

Figure 8 shows the auditory response thresholds derived from the intensity/response functions. The Toluene × Frequency interaction, F(3,117), was 85.64 ($p<1.0\times10^{-11}$). Although the Toluene × Alcohol × Frequency interaction, F(3,117), was only 2.25 ($p=0.09$), a separate 2-way ANOVA at 12 kHz revealed a Toluene × Alcohol interaction, F(1,39), of 5.57 ($p=0.02$). A greater impairment at this frequency in the rats exposed to toluene and forced to drink alcohol than in those exposed to toluene alone was suggested by an associated $t(39)=3.46$, $p=0.0013$.

Blood Analysis

Nine of the 14 clinical blood chemistry measures were altered by exposure to toluene or alcohol, alone or in combination, as indicated by 2-way ANOVAs (Tables 3 and 4). Subsequent comparisons by lsd-test showed that exposure to alcohol alone did not affect any of the measures except for BUN, which was decreased compared with water controls. Similarly, exposure to toluene alone had only minor effects on cholesterol and albumin. However, the combination of exposure to both toluene and alcohol caused greater changes and on more of the measures than exposure to either alone.

DISCUSSION

The results of this experiment were striking in demonstrating a marked increase in the number of rats that strongly preferred a 6% alcohol solution over water after being exposed to toluene and forced to drink alcohol for 2 weeks. Thus, our results provide general support for those of Geller *et al.* [11], although markedly different exposure conditions were used. However, no such increased preference for alcohol was seen in the rats exposed to toluene and not forced to drink alcohol; indeed, they, like the controls, rapidly developed a marked aversion. Thus, our results suggest that exposure to toluene alone under the conditions tested is not sufficient for inducing enhanced alcohol preference.

TABLE 3

EFFECT OF EXPOSURE TO TOLUENE AND ALCOHOL, ALONE OR TOGETHER, ON CLINICAL BLOOD CHEMISTRIES

	Air		Toluene	
Measure	Water	Alcohol	Water	Alcohol
Glucose (mg %)	193.5 ± 5.9	199.6 ± 6.4	195.1 ± 4.6	182.0 ± 7.1
BUN (mg %)	20.1 ± 1.27	16.1 ± 0.71*	18.1 ± 0.64	9.0 ± 0.00*
Creatinine (mg %)	0.28 ± 0.03	0.28 ± 0.07	0.28 ± 0.04	0.28 ± 0.04
Phosphorus (mg %)	7.87 ± 0.17	7.76 ± 0.13	8.16 ± 0.21	7.43 ± 0.21*
Total bilirubin (mg %)	0.23 ± 0.02	0.26 ± 0.01	0.23 ± 0.02	0.33 ± 0.02*
SGOT (IU/L)	97.4 ± 6.45	103.6 ± 4.67	110.2 ± 6.30	116.7 ± 4.59*
Alkaline phosphatase (IU/L)	871.0 ± 25.8	792.5 ± 26.8	925.0 ± 34.7	614.5 ± 35.2*
Cholesterol (mg %)	50.5 ± 1.29	54.1 ± 1.07	55.7 ± 1.42*	68.1 ± 2.05*
Calcium (mg %)	10.4 ± 0.07	10.4 ± 0.09	10.5 ± 0.13	10.6 ± 0.12
Total protein (g %)	5.98 ± 0.11	5.86 ± 0.06	5.80 ± 0.06	5.90 ± 0.06
Albumin (g %)	4.13 ± 0.04	4.20 ± 0.08	4.34 ± 0.05*	4.51 ± 0.06*
Na^+ (meq/L)	145.4 ± 0.73	144.2 ± 0.55	146.0 ± 0.65	147.8 ± 0.81*
K^+ (meq/L)	6.77 ± 0.12	6.69 ± 0.14	6.80 ± 0.24	6.48 ± 0.19
Cl^- (meq/L)	99.9 ± 0.79	98.5 ± 0.87	98.7 ± 0.97	95.1 ± 0.72*

Values are the means (±SE) of 8 to 12 rats, except for BUN (n=2) and total protein (n=5) in the toluene/alcohol group, where insufficient sample was available for many of the rats.

*$p<0.05$ compared with air/water control by lsd-test following F-ratio associated with $p<0.05$.

Because of the adverse effect on weight gain, it could be argued that the caloric value of the alcohol was responsible for the marked increase in alcohol preference by the rats exposed to toluene and forced to drink alcohol [18]. We do not think that such an interpretation is correct for a number of reasons. First, food was freely available throughout the experiment, so that no externally imposed deprivation schedule was in effect. If the rats chose alcohol for its caloric value, they did so in preference to available food. This argument seems untenable, because 6% alcohol was aversive to the control rats. Second, no preference for alcohol was seen in the rats exposed to toluene but not forced to drink alcohol, although they too were inhibited in weight gain—albeit to a lesser extent than the rats exposed to both toluene and alcohol. Third, if the increase in alcohol was related to the decrease in body weight, then a negative correlation between these variables might be expected. However, the correlations between alcohol preference and body weight in the rats exposed to both toluene and alcohol on Days 1, 4, 7, 11, 14, and 18 of preference testing were 0.48, 0.03, 0.06, 0.07, −0.15, and −0.41, respectively (all $ps>0.05$). Moreover, the one rat in this group that consistently avoided alcohol had a body weight that was close to the average for this group, and loss of alcohol preference by other rats in this group was not related to the weight gain. Finally, Sampson and Falk [33] showed that food-restricted rats would select a noncaloric, 0.25%-saccharin solution over a previously preferred 5% alcohol solution or water, resulting in marked weight loss and eventual death.

Total fluid consumption during exposure was greater in the rats exposed to toluene than in those exposed to air, regardless of whether the fluid was water or alcohol. We have observed this effect in several previous experiments involving exposure to toluene (unpublished data), and a similar effect has been reported for xylene [30]. This effect diminished rapidly after the exposures to toluene ended in terms of total fluid consumed per rat. Expressed relative to body weight, the effect was greater and diminished more slowly as the rats gained weight without a proportionate increase in fluid consumption. The reason for this effect of toluene on fluid consumption is not known. Similarly treated rats excreted more urine than controls and the pH of the

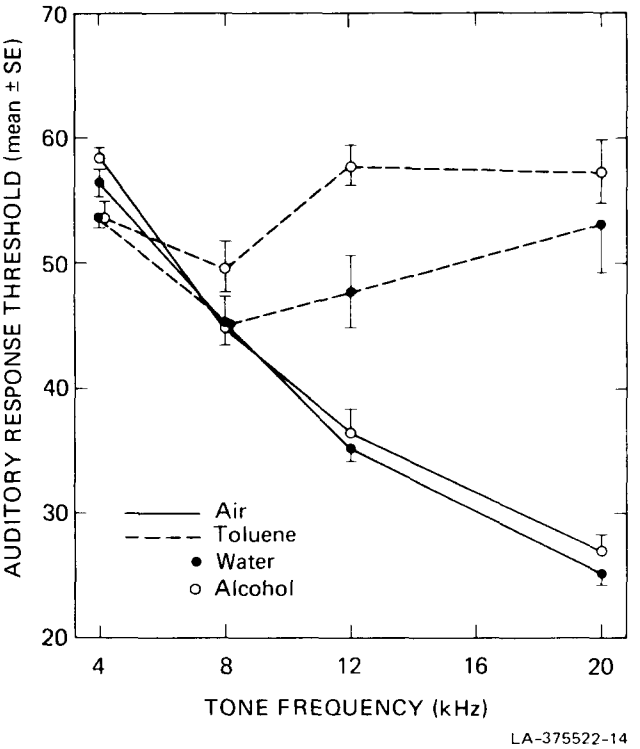

FIG. 8. Behavioral auditory response thresholds (dB re 20 $\mu N/m^2$ SPL) in rats exposed to toluene and alcohol, alone or together.

TABLE 4
RESULTS OF STATISTICAL ANALYSES OF BLOOD CHEMISTRY DATA

	Source of Variance					
	Toluene		Alcohol		Interaction	
Measure	F	p	F	p	F	p
Glucose (1,44)†	1.73	*	0.33	*	2.49	*
BUN (1,27)	2.01	*	17.10	3.1×10^{-4}	12.02	1.8×10^{-3}
Creatinine (1,44)	0.00	*	0.05	*	0.00	*
Phosphorus (1,44)	0.01	*	5.10	2.9×10^{-2}	2.79	*
Total bilirubin (1,44)	4.11	4.8×10^{-2}	11.41	1.5×10^{-3}	2.48	*
SGOT (1,43)	5.42	2.5×10^{-2}	1.38	*	0.00	*
Alkaline phosphatase (1,44)	4.02	*	39.56	1.3×10^{-7}	14.07	5.1×10^{-4}
Cholesterol (1,39)	35.01	6.8×10^{-7}	23.35	2.1×10^{-5}	14.34	5.1×10^{-4}
Calcium (1,43)	1.84	*	0.22	*	0.03	*
Total protein (1,32)	1.05	*	0.06	*	1.57	*
Albumin (1,40)	19.96	6.3×10^{-5}	3.36	*	1.71	*
Na^+ (1,43)	9.16	4.2×10^{-3}	0.10	*	5.02	3.0×10^{-2}
K^+ (1,43)	0.20	*	1.14	*	0.48	*
Cl^- (1,43)	7.39	9.4×10^{-3}	8.52	5.6×10^{-3}	2.00	*

*$p > 0.05$.
†Associated degrees of freedom in parentheses.

urine was more acidic because of the excess hippuric acid present (the major metabolite of toluene) (unpublished data). Thus, the increased fluid consumption may have been in response to decreased water retention. However, a more direct central effect on mechanisms regulating drinking cannot be ruled out and would also be consonant with the increased urination.

Regardless of why the rats exposed to toluene increased their fluid consumption, the result was to increase their daily dose of alcohol compared with that of the group exposed to air. Thus, the increased preference for alcohol by the rats exposed to toluene and forced to drink alcohol could, perhaps, be associated with their higher intake of alcohol during the exposure phase. Others have reported that alcohol preference is increased by pretreatment with alcohol at levels that exceed metabolic capacity and induce physical dependence [8, 19, 34, 37]. However, this result is not universal, and physical dependence per se does not appear to be a necessary or sufficient condition for voluntary intake of large amounts of alcohol by humans or animals [3, 6, 10, 12, 20, 21]. Another interpretation is that forced consumption of large amounts of alcohol allows the animal to experience the pharmacologic effects of alcohol, including its reinforcing properties [1, 5, 15, 35, 41]. Thus, any manipulation that increases the consumption of alcohol may also increase the probability of subsequent high voluntary intake. Such an interpretation is unlikely in the case of the results of Geller *et al.* [11]; although data on alcohol dosage were not presented, it is clear that some of their rats that subsequently increased their alcohol preference did not consume large, or even moderate, amounts of alcohol in conjunction with exposure to toluene. An intriguing possibility is that the pharmacologic effects of toluene per se, including its reinforcing properties [39], were experienced by their rats and that those effects were similar, if not identical, to those of alcohol. Indeed, solvent users have reported that the "high" obtained with solvents is similar to that caused by alcohol [7].

Another possible interpretation of the results of these experiments is that toluene altered gustatory and/or olfactory sensitivity in such a way as to lessen the aversive taste of the alcohol. Such an effect would promote alcohol consumption in a forced-drinking situation and, possibly, in a choice situation as well. Interestingly in this regard, a favorite route of inhalation by human solvent users is through the mouth rather than the nose [31,32], thus maximizing the potential for damage to gustatory receptors. Humans, like rats, generally have to overcome the taste of alcohol (by disguise, habituation, or sheer determination) before they can comfortably experience its pharmacologic effects.

The hearing loss caused by exposure to toluene was in complete agreement with our earlier findings [24–27]. Forced consumption of alcohol did not affect learning and/or performance of the multisensory CAR task, nor did it cause any hearing deficit. However, there was a tendency for forced consumption of alcohol to increase the severity of the hearing loss caused by toluene. Lack of clear evidence in this regard may have been due to the large magnitude of the hearing loss caused by toluene alone in this experiment, thus precluding a clear demonstration of potentiation because of the ceiling imposed by our test methods. Additional research with less intense schedules of exposure to toluene is needed to settle this issue.

Exposure to both toluene and alcohol caused greater organ toxicity, as evidenced by the clinical chemistry data, than exposure to either substance alone. The interpretation of this finding is unclear at this time. The enhanced effect could represent a real toxic interaction between toluene and alcohol. However, because the rats exposed to toluene consumed more alcohol than the rats exposed to clean air, the effect might have been caused by the increased dose of alcohol alone. Although there are reports of metabolic interactions between alcohol and solvents [9, 22, 28–30], further work is required before the potential toxic manifestations of such interactions are clarified.

REFERENCES

1. Amit, Z. and M. H. Stern. Alcohol ingestion without oropharyngeal sensations. *Psychon Sci* **15:** 162–163, 1969.
2. Barnes, G. E. Solvent abuse: A review. *Int J Addict* **14:** 1–26, 1979.
3. Begleiter, H. Ethanol consumption subsequent to physical dependence. In: *Alcohol Intoxication and Withdrawal*, vol 2, edited by M. M. Gross. New York: Plenum Press, 1975.
4. Berry, G. J., R. K. Heaton and M. W. Kirby. Neuropsychological assessment of chronic inhalant abusers: A preliminary report. In: *Voluntary Inhalation of Industrial Solvents*, edited by C. W. Sharp and L. T. Carroll. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
5. Carney, J. M., M. E. Llewellyn and J. H. Woods. Variable interval responding maintained by intravenous codeine and ethanol injections in the rhesus monkey. *Pharmacol Biochem Behav* **5:** 577–582, 1976.
6. Cicero, T. J. and B. R. Smithloff. Alcohol oral self-administration in rats: Attempts to elicit excessive intake and dependence. In: *Advances in Experimental Medicine and Biology*, vol 35, edited by M. M. Gross. New York: Plenum Press, 1973.
7. Cohen, S. Why solvents? In: *Voluntary Inhalation of Industrial Solvents*, edited by C. W. Sharp and L. T. Carroll. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
8. Deutsch, J. A. and H. S. Koopmans. Preference enhancement for alcohol by passive exposure. *Science* **179:** 1242–1243, 1973.
9. Elovaara, E., Y. Collan, P. Pfäffli and H. Vainio. The combined toxicity of technical grade xylene and ethanol in the rat. *Xenobiotica* **10:** 435–445, 1980.
10. Freund, G. Alcohol withdrawal syndrome in mice. *Arch Neurol* **21:** 315–320, 1969.
11. 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:** 933–937, 1983.
12. Goldstein, D. B. Rates of onset and decay of alcohol physical dependence in mice. *J Pharmacol Exp Ther* **140:** 377–383, 1974.
13. Gutiérrez, F., I. M. Hernández and S. Rábag. Psychological, familial, and social study of 32 patients using inhalants. In: *Voluntary Inhalation of Industrial Solvents*, edited by C. W. Sharp and L. T. Carroll. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
14. Howd, R. A. and G. T. Pryor. Effect of chronic morphine on the response to and disposition of other drugs. *Pharmacol Biochem Behav* **12:** 577–586, 1980.
15. Karoly, A. J., G. Winger, F. Ikomi and J. H. Woods. The reinforcing property of ethanol in the rhesus monkey. *Psychopharmacology (Berlin)* **58:** 19–25, 1978.
16. Korman, M. Clinical evaluation of psychological factors. In: *Review of Inhalants: Euphoria to Dysfunction*, edited by C. W. Sharp and M. L. Brehm. Rockville, MD: NIDA Research Monograph 15, DHEW Publication No. (ADM) 77-553, 1977.
17. Korman, M., F. Trimboli and I. Samler. A psychiatric emergency room study of inhalant abuse. In: *Voluntary Inhalation of Industrial Solvents*, edited by C. W. Sharp and L. T. Carroll. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
18. Lester, D. and E. X. Freed. Criteria for an animal model of alcoholism. *Pharmacol Biochem Behav* **1:** 103–107, 1973.
19. Maarfaing-Jallat, P. and J. Le Magnen. Induction of high voluntary ethanol intake in dependent rats. *Pharmacol Biochem Behav* **17:** 609–612, 1982.
20. Mello, N. K. and J. H. Mendelson. Experimentally induced intoxication in alcoholics: A comparison between programmed and spontaneous drinking. *J Pharmacol Exp Ther* **173:** 101–116, 1970.
21. Meyer, R. D., W. Staltman and G. E. Miller. Effect of ethanol dependence induced artificially in the rhesus monkey on the subsequent preference for ethyl alcohol. *Physiol Behav* **9:** 43–48, 1972.
22. Morvai, V. and G. Ungvary. Effects of simultaneous alcohol and toluene poisoning on the cardiovascular system of rats. *Toxicol Appl Pharmacol* **50:** 381–389, 1979.
23. Press, E. and A. K. Done. Physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. *Pediatrics* **39:** 611–622, 1967.
24. Pryor, G. T., J. Dickinson, E. Feeney and C. S. Rebert. Hearing loss in rats first exposed to toluene as weanlings or as young adults. *Neurobehav Toxicol Teratol* **6:** 111–119, 1984.
25. Pryor, G. T., J. Dickinson, R. A. Howd and C. S. Rebert. Transient cognitive deficits and high-frequency hearing loss in rats exposed to toluene. *Neurobehav Toxicol Teratol* **5:** 53–57, 1983.
26. Pryor, G. T., C. S. Rebert, J. Dickinson and E. M. Feeney. Factors affecting toluene-induced ototoxicity in rats. *Neurobehav Toxicol Teratol* **6:** 223–238, 1984.
27. Rebert, C. S., S. S. Sorenson, R. A. Howd and G. T. Pryor. Toluene-induced hearing loss in rats evidenced by the brainstem auditory-evoked response. *Neurobehav Toxicol Teratol* **5:** 59–62, 1983.
28. Rühimaki, V., K. Savolainen, P. Pfäffli, H. W. Sipple and A. Laine. Metabolic interactions between *m*-xylene and ethanol. *Arch Toxicol* **49:** 253–263, 1982.
29. Sato, A., T. Nakajima and Y. Koyama. Effects of chronic ethanol consumption on hepatic metabolism of aromatic and chlorinated hydrocarbons in rats. *Br J Ind Med* **37:** 382–386, 1980.
30. Savolainen, H., H. Vainio, M. Helojoki and E. Elovaara. Biochemical and toxicological effects of short-term, intermittent xylene inhalation exposure and combined ethanol intake. *Arch Toxicol* **41:** 195–205, 1978.
31. Sharp, C. W. and M. L. Brehm, editors. *Review of Inhalants: Euphoria to Dysfunction*. Rockville, MD: National Institute on Drug Abuse Research Monograph 15, DHEW Publication No. (ADM) 77-553, 1977.
32. Sharp, C. W. and L. T. Carroll, editors. *Voluntary Inhalation of Industrial Solvents*. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
33. Samson, H. H. and J. L. Falk. Alteration of fluid preference in ethanol-dependent animals. *J Pharmacol Exp Ther* **190:** 365–376, 1974.
34. Sinclair, J. D., S. Walker and W. Jordan. Alcohol intubation and its effects on voluntary consumption in rats. *Q J Stud Alcohol* **34:** 726–743, 1973.
35. Smith, S. G., T. E. Werner and W. M. Davis. Comparison between intravenous and intragastric alcohol self-administration. *Physiol Psychol* **4:** 91–93, 1976.
36. Stephens, R. C., S. C. Diamond, C. R. Spielman and D. S. Lipton. Sniffing from Suffolk to Syracuse: A report of youthful solvent use in New York State. In: *Voluntary Inhalation of Industrial Solvents*, edited by C. W. Sharp and L. T. Carroll. Rockville, MD: DHEW Publication No. (ADM) 79-779, 1978.
37. Tang, M. and J. L. Falk. Ethanol dependence as a determinant of fluid preference. *Pharmacol Biochem Behav* **7:** 471–474, 1977.
38. Wechsler, H. and D. Thurm. Teenage drinking, drug use, and social correlates. *Q J Stud Alcohol* **34:** 1220–1227, 1973.
39. Weiss, B., R. W. Wood and D. A. Macys. Behavioral toxicology of carbon disulfide and toluene. *Environ Health Perspect* **30:** 39–45, 1979.
40. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.
41. Yanagita, T. and S. Takahashi. Dependence liability of several sedative-hypnotic agents evaluated in monkeys. *J Pharmacol Exp Ther* **185:** 307–316, 1973.