Neurotoxicology and Teratology, Vol. 11, pp. 153–159. Pergamon Press plc, 1989. Printed in the U.S.A. 0892-0362/89 $3.00 + .00

Behavioral Teratology Investigation of l-Propanol Administered by Inhalation to Rats[1]

B. K. NELSON, W. S. BRIGHTWELL, B. J. TAYLOR, A. KHAN, J. R. BURG, E. F. KRIEG, JR. AND V. J. MASSARI

Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health 4676 Columbia Parkway, Cincinnati, OH 45226 and Department of Pharmacology, Howard University College of Medicine 520 W. Street, NW, Washington, DC 20059

Received 15 May 1988

NELSON, B. K., W. S. BRIGHTWELL, B. J. TAYLOR, A. KHAN, J. R. BURG, E. F. KRIEG, JR. AND V. J. MASSARI. *Behavioral teratology investigation of l-propanol administered by inhalation to rats.* NEUROTOXICOL TERATOL 11(2) 153–159, 1989.—Due to their structural similarity to ethanol, a human teratogen, and their widespread use in industry, a series of industrial alcohols are being investigated for developmental toxicity. This paper presents the results of exposures to 7000 ppm l-propanol, which is minimally toxic to maternal animals and produces a low incidence of teratogenicity, and to 3500 ppm l-propanol, which is not toxic to maternal rats and produces no teratogenicity. Propanol vapors or filtered air was administered for 7 hr/day to 15 pregnant Sprague-Dawley rats throughout gestation or to 18 male rats daily for 6 weeks. Tests of offspring were: a) ascent on a wire mesh screen, b) rotorod, c) open field and optically monitored activity, d) running wheel, e) avoidance conditioning, and f) progressive fixed ratio schedule of reinforcement. Brains from 10 rats per group were dissected into cerebrum, cerebellum, brainstem, and midbrain, and were assayed for protein, acetylcholine, dopamine, norepinephrine, serotonin, β-endorphin, Met-enkephalin, and substance P. Overall, the results indicate that exposure to high concentrations of l-propanol can affect fertility in exposed males (only 2 of 17 produced litters), but there were no consistent effects seen in the behavioral or neurochemical tests measured. This lack of effects is surprising based on predictions from the structural similarity of l-propanol to ethanol, and on long-standing observations that toxicity (to adult animals) increases with carbon chain length among the aliphatic alcohols.

Alcohols Propanol l-Propanol n-Propyl alcohol Teratology Reproductive toxicology Behavioral teratology Developmental toxicology Neurochemistry

ETHANOL is a teratogen in humans as well as animals (1–3). In animals, a route of exposure which produces high blood ethanol levels, such as gavage or liquid diet (34), also produces teratogenicity, although data are less compelling by routes which do not produce high blood alcohol levels [such as inhalation in our previous research (25,26) or when added to drinking water (34)]. Ethanol is one of a series of structurally-related alcohols which are widely used as industrial solvents, and to which workers could potentially be exposed, primarily by inhalation. Most alcohols have not been assessed for their ability to produce malformations or behavioral dysfunction following prenatal exposure. Since data from structurally-related compounds often serve to predict toxicity, the possibility that alcohols other than ethanol are also teratogenic raised concerns from both an applied, as well as a scientific, perspective. Furthermore, "Richardson's Law," based on toxicity data from several species of experimental animals, predicts that the toxicological properties of aliphatic alcohols increase with their molecular weight (32). By extension, this "Law" raises further concerns about the potential teratogenicity of heavier alcohols. Thus, due to their large potential exposure population and their structural similarity to a known teratogen, we began investigating the teratogenicity of a series of aliphatic alcohols.

Because of its close structural similarity to ethanol, l-propanol (n-propyl alcohol) was the first in the series (after ethanol) to be studied for behavioral teratogenicity. As an industrial solvent, l-propanol is used in polishing compounds, brake fluids, degreasing agents, antiseptics, and

[1]This research was conducted under the Good Research Practices program of the Division of Biomedical and Behavioral Science, which is based upon the 1978 guidelines of Good Laboratory Practices issued by the Food and Drug Administration. The animal facility is accredited by the American Association of Accreditation of Laboratory Animal Care. Mention of product or company names does not constitute endorsement by NIOSH.

food products. A 1983 review (35) concluded that l-propanol's narcotic effects are stronger than those of ethanol, but that its low volatility would not make it dangerous to humans following inhalation exposures. Occupational exposure standards of 200 ppm (time-weighted averages) have been established by the Occupational Safety and Health Administration (29 CFR 1910.1000) and recommended by the American Conference of Governmental Industrial Hygienists (30).

Developmental toxicity information on l-propanol is limited. Grant and Samson (12) compared the ability of ethanol and l-propanol to produce microcephaly in neonatal rats. Using an intragastric catheter, they administered 3–8 g l-propanol/kg body weight (mean blood levels of 212±120 mg%) to Long-Evans rats on postnatal days 5–8. On postnatal day 18, animals were sacrificed and the brains examined for microcephaly both by weight and biochemical measures. They found that microcephaly was produced by l-propanol, similar to that observed with ethanol (247±43 mg%) and teritiary-butanol (48±13 mg%). Their conclusion that l-propanol exposure during periods of brain growth retards brain development, as well as the close structural similarity of l-propanol and ethanol, suggested that behavioral dysfunction may occur in postnatal rats following prenatal exposures to l-propanol in this concentration range.

Only one study in the literature has investigated the teratogenicity of l-propanol. Nelson *et al.* (27) found that inhalation of 10000 ppm l-propanol for 7 hr/day on gestation days 1–19 [producing maternal blood levels of 6.6 mg/dl or mg%; interestingly, this was much lower than the blood level of 164 mg/dl seen in (nonpregnant) young rats weighing 110–120 g] was maternally toxic, increased resorptions, and was teratogenic. Externally, approximately one-third of the fetuses were malformed, having either a short or missing tail or ectrodactyly. Skeletal malformations (primarily rudimentary cervical ribs) and visceral malformations (primarily cardiovascular and urinary defects) were seen in each of the 12 surviving litters and in approximately one-fourth of the fetuses examined. At 7000 ppm l-propanol (blood levels of 4.2 mg/dl), maternal feed intake was slightly reduced from controls, but weight gain was not affected. Resorptions were not increased above control levels, but fetal weights were reduced by nearly 20% from controls, and the incidence of skeletal malformations was increased from that of controls (9 of 15 litters or 19 of 95 fetuses affected, compared with one fetus from a similar number of controls). At 3500 ppm l-propanol (blood levels of 2.5 mg/dl), no significant adverse effects were noted either in maternal animals or fetuses.

While these studies indicated that developmental toxicity can be seen at high levels of l-propanol, behavioral teratogenicity might be seen at much lower concentrations, as has been reported for other agents (33). 2-Ethoxyethanol, a glycol ether which is structurally related to propanol, was found to produce behavioral and neurochemical alterations in the offspring of rats. These alterations were seen at concentrations about half those which produced malformations (100 vs. 200 ppm administered for 7 hr/day during gestation) (22).

The Nelson *et al.* study (27) noted above served to identify doses for the present behavioral teratology study. This study followed a pattern in previous behavioral teratology studies (33), in which a high concentration was sought that would produce minimal maternal toxicity and no (or a low incidence of) malformations. For the low concentration, an absence of maternal and embryo/fetal toxicity was desired. Thus, concentrations of 7000 and 3500 ppm l-propanol were selected. There has been recent interest in the effects of paternal exposure on offspring development: ethanol (5) and a number of other drugs (4, 28, 36) have produced positive findings such as growth retardation, malformations, fetal death, and behavioral and neurochemical alterations. Therefore, the present study included not only females exposed to l-propanol throughout gestation, but also groups of males exposed to l-propanol and subsequently mated to nonexposed females.

METHOD

Inhalation Exposures

Exposures were conducted in 0.5 cubic meter Hinners exposure chambers (Charles Spengler and Associates, Cincinnati, OH) as previously described (27). Briefly, a micrometering pump controlled injection of a specified amount of reagent grade l-propanol (Matheson, Coleman, and Bell Manufacturing Chemists, Cincinnati, OH) into one inlet of a three-way valve. Through a second inlet, heated compressed air was introduced, and the liquid-air mixture was forced through the outlet into a Greensmith impinger for evaporation and mixing. The concentration was controlled by modifying the flow of the chemical and the temperature of the compressed air. This vapor mixture was introduced into the exposure chamber air flow upstream from an orifice plate, and the resulting turbulence provided uniform mixing of the vapor and air before it entered the chamber. Air flow was approximately 0.5 m^3 per minute. Chamber temperatures were maintained at 76±2° and humidity at 50±10%.

The concentration within the chamber was continuously monitored by a Miran 1A infrared analyzer (Wilkes/Foxboro Analytical, South Norwalk, CT) that had been calibrated within the concentration range being administered, and was connected to a stripchart recorder. Daily mean, range, and time-weighted average concentrations were calculated. These daily values were averaged for an overall mean for each concentration. In addition, the analyzer was interfaced with an Apple II+ computer which recorded five-minute l-propanol concentration means for each five-minute period.

For independent verification of exposure chamber concentrations, charcoal tube samples were collected from the chamber atmosphere. Sampling times varied from 10 to 30 min, and approximately 13 samples were collected over a two-day period each week. The samples were subsequently analyzed using standard methods (10). Bulk samples of propanol were analyzed by a gas chromatograph equipped with a flame ionization detector, and found to be of purity equivalent to that of the standard sample (>99%). Target exposure concentrations were 7000 and 3500 ppm. As exposures were conducted approximately three months apart, separate control groups were run for the two concentrations of propanol.

Experimental Animals

Virgin female Sprague-Dawley rats (VAF/plus; 175–200 g; Charles River Breeding Laboratories, Wilmington, MA) were acclimated to our animal quarter conditions during a one- to two-week quarantine. As maintained throughout the study, these conditions were: Lights on from 7:00 a.m. to 7:00 p.m., temperature of 24±2°C, humidity of 50±10%, NIH-07 rodent pellets (Zeigler Bros., Inc., Gardner, PA) and tap water available ad lib except when the animals were in the exposure chambers. Males from the same supplier

weighed over 300 g and were housed individually. Females were housed three per cage, in suspended wire mesh cages equipped with automatic water dispensers (Hoeltge Inc., Cincinnati, OH).

For breeding, females were placed individually with males. Each morning, the paper under each male's cage was examined for sperm plugs. If no plugs were detected, vaginal smears were taken. Females with sperm (gestation day 0) were assigned without bias to treatment groups and placed individually into polycarbonate shoe box cages with filter covers. Bedding was hardwood sawdust (Absorb-dri from Zeigler Bros., Gardner, PA). Maternal weights, feed intake, and water intake were measured over weekly intervals (viz., measurements on gestation days 0, 7, 14, and 21). On gestation days 1–19, exposed and sham-exposed females (target N=15/group) were placed in the exposure chambers for 7 hr/day, and were left in the chambers for an additional 15 min for degassing after vapor generation terminated.

Other males [N=18/group (consisting of three sets of six males with exposures beginning on succeeding weeks), initial weights approximately 450 g] were similarly exposed for 7 hr/day, 7 days/week, for 6 weeks, and weighed weekly. After two nonexposure days, they were mated (1:1) with unexposed virgin females for a maximum of 5 days. All except the last six males were discarded, and for comparison purposes, the females were handled in the same manner as sham-exposed females. Because of infertility observed in the males exposed to the high level of propanol, the final six exposed males were remated at biweekly intervals to see if the infertility was reversible. These animals were not included in the behavioral teratology phase.

On gestation day 21, females were placed in clean bedding, given a paper towel for nest construction, and left undistrubed until after parturition. Within 16 hr after parturition, the dam was weighed, as was her litter. From those that had nursed, four female and four male pups (±1) were arbitrarily selected and fostered to untreated dams which had delivered within the preceding 48 hr. Two pups from each of 5 foster litters were used as unhandled controls for neurochemistry (described later). All other exposed dams and extra pups, including those from the foster dams, were discarded. Although the occurrence was rare, litters not having at least three pups of each sex (i.e., an abnormal sex ratio) were discarded. Rats were weaned on day 25, and weighed individually on days 7, 14, 21, 28, and 35 (birth=day 0).

Behavioral Testing

Seven behavioral tests spanning postnatal days 10 through approximately 90 (Table 1) have been utilized in our laboratory to evaluate various central nervous system functions (neuromuscular ability, activity, and learning) at several stages of development (22, 23, 26). Female and male pups were selected randomly, ear-marked, and assigned to test groups on postpartum day 10. For each test, one female and one male were used from each litter. Investigators involved with the animal testing were not aware of the treatment groups to which individual subjects belonged. Details of the testing procedures have been published elsewhere (23,26). Briefly, the tests were as follows:

1. The Ascent test was conducted using a 6 mm-wire mesh screen inclined 70° from horizontal; it was administered for a maximum of 60 sec on days 10, 12, 14; time on the screen and distance climbed were measured.

2. The Rotorod test used a 9-cm (diameter) rod which was 10 cm long and positioned 48 cm above a sawdust-cushioned box; the rod surface was coated with sand permanently affixed to the rod's surface. The test was administered on days 21, 25, and 29 to the same rats used for the Ascent test. Testing began at 6 rpm, and this rate was increased at 3 rpm intervals until the animals had five unsuccessful trials. The maximum rpms attained for each day were used for data analysis.

3. The Open Field test consisted of a 1-m diameter floor marked into grids, housed in an enclosure which was ½ m high. One female and one male per litter were tested for 3 min/day in 3-day test blocks: 16, 17, 18; 30, 31, 32; 44, 45, 46; and 58, 59, and 60 days after birth. The number of grids entered during each three-day test block were summed for analysis.

4. An optical-digital animal activity monitor (Opto-Varimex©, Columbus Instruments International Corp., Columbus, OH), placed in an acoustically-shielded audiometric chamber, was used to assess activity in the same animals tested in the Open Field apparatus. This assessment occurred immediately following the Open Field test. The number of photobeam interruptions for 3 min/day for each of the 3-day test blocks was used as the measure of activity.

5. Running wheel activity was measured for approximately 24 hr on days 32–33. Two Wahman activity wheels were placed in a sound-attenuated chamber, and the data were summed and divided into day and night activity scores.

6. For avoidance conditioning, two BRS/LVE shuttle boxes were housed in sound-attenuated chambers, and each box had a 4-cm partition in the center. Scrambled electric shocks (0.7 mA) could be delivered to either side of the grid floor. The warning stimulus was a 5-sec tone (nondirectional cue). Times between trials varied between 15 and 45 sec, with a mean of 30 sec. Two sets of animals were used for this test—one set (those rats used for the Ascent and Rotorod tests) beginning on day 34, and the other set (the rats used in the Open Field and Opto-Varimex© tests) beginning on day 60. Animals were given 20 trials per day for a maximum of 14

TABLE 1

BEHAVIORAL TESTS AND DAYS OF TESTING IN BEHAVIORAL TERATOLOGY STUDY (SEE TEXT FOR EXPLANATION)

Behavioral Tests	Group*	Function Tested	Days of Age
1. Ascent on wire mesh	1	Neuromuscular	10, 12, 14
2. Rotorod	1	Neuromuscular	21, 25, 29
3. Open field and 4. Automated open field	2	Exploratory activity	16, 17, 18; 30, 31, 32; 44, 45, 46; 58, 59, 60
5. Activity wheel	2	Circadian activity	32–33
6. Avoidance conditioning	1,2	Aversive learning	Begun days 34, 60
7. Operant conditioning	3	Appetitive learning	Begun day 40

*Rats in the same group were administered the tests in the order shown.

TABLE 2

REGIONAL CONCENTRATIONS OF NEUROCHEMICALS IN BRAINS FROM 21-DAY-OLD PUPS FOLLOWING PRENATAL EXPOSURE TO 1-PROPANOL (MEAN ± SD)

Neuro-chemical	Maternal	Control	Unhandled Control	Maternal	Paternal	Control
		7000 ppm			3500 ppm	
Protein (mg/sample)						
CR*	10.74 ± 1.60	12.88 ± 1.10	10.64 ± 1.90	9.14 ± 1.11	8.76 ± 0.59	9.35 ± 1.80
CB†	5.79 ± 1.16	8.25 ± 2.98	5.19 ± 1.19	4.49 ± 0.90	4.57 ± 0.51	4.59 ± 0.53
BS‡	3.44 ± 1.09	4.53 ± 0.65	3.17 ± 0.87	3.05 ± 0.69	3.32 ± 0.29	3.08 ± 0.96
MB§	4.39 ± 1.35	5.63 ± 0.80	5.74 ± 1.23	3.54 ± 0.82	4.24 ± 0.39	3.99 ± 1.01
Acetylcholine (pmole/mg protein, × 10^{-2})						
CR	1.3 ± 1.2	0.6 ± 0.8	0.3 ± 0.5	1.0 ± 1.1	0.2 ± 0.4	0.2 ± 0.5
CB	0.2 ± 0.8	0.2 ± 0.4	1.6 ± 1.3	2.3 ± 2.7	1.2 ± 1.2	0.5 ± 0.8
BS	2.6 ± 3.0	0.6 ± 0.9	0.6 ± 0.7	1.2 ± 0.5	4.8 ± 4.8	1.1 ± 1.1
MB	0.6 ± 0.7	2.3 ± 2.4	1.4 ± 1.0	1.0 ± 1.1	5.0 ± 5.0	1.6 ± 1.5
Serotonin or 5-hydroxytryptamine (pg/μg protein)						
CR	14.55 ± 0.92	18.95 ± 2.84	15.67 ± 3.82	19.26 ± 8.55	14.93 ± 3.50	16.23 ± 4.81
CB	1.19 ± 0.98	3.02 ± 1.40	3.47 ± 1.84	3.50 ± 1.68	4.52 ± 1.56	3.58 ± 2.17
BS	9.27 ± 5.67	6.82 ± 2.82	8.41 ± 5.82	8.86 ± 3.82	10.30 ± 6.75	14.39 ± 9.56
MB	12.63 ± 3.65	19.87 ± 5.6	19.48 ± 6.41	19.57 ± 5.19	17.55 ± 4.30	21.22 ± 12.74
Dopamine (pg/μg protein)						
CR	1.55 ± 0.30	0.90 ± 0.28	1.97 ± 0.93	2.37 ± 0.68	2.33 ± 0.74	1.72 ± 0.83
CB	0¶	0	0	0	0	0
BS	0	0	0	0	0	0
MB	0.76 ± 0.36	0.44 ± 0.25	1.60 ± 1.06	0.99 ± 0.73	1.08 ± 0.55	1.73 ± 1.93
Norepinephrine (pg/μg protein)						
CR	0.28 ± 0.08	0.19 ± 0.04	0.36 ± 0.10	0.42 ± 0.09	0.46 ± 0.14	0.33 ± 0.11
CB	0.17 ± 0.13	0.09 ± 0.04	0.23 ± 0.11	0.29 ± 0.09	0.40 ± 0.14	0.33 ± 0.16
BS	0.87 ± 0.46	0.43 ± 0.11	0.67 ± 0.31	1.05 ± 0.36	1.25 ± 0.27	0.92 ± 0.24
MB	0.95 ± 0.16	0.64 ± 0.09	1.27 ± 0.35	1.61 ± 0.66	1.45 ± 0.28	1.32 ± 0.47
β-endorphin (pg/μg protein)						
CR	0.20 ± 0.11	0.24 ± 0.10	0.29 ± 0.14	0.18 ± 0.05	0.21 ± 0.05	0.18 ± 0.10
CB	0.079 ± 0.050	0.087 ± 0.035	0.117 ± 0.067	0.086 ± 0.055	0.130 ± 0.071	0.137 ± 0.033
BS	1.65 ± 1.43	0.75 ± 0.43	0.73 ± 0.75	0.63 ± 0.19	1.48 ± 1.19	0.73 ± 0.41
MB	0.52 ± 0.18	3.78 ± 2.41	3.12 ± 1.45	2.47 ± 2.24	2.80 ± 2.16	1.76 ± 1.48
Substance P (pg/μg protein)						
CR	0.10 ± 0.04	0.11 ± 0.03	0.13 ± 0.04	0.46 ± 0.24	0.52 ± 0.39	0.22 ± 0.19
CB	0.009 ± 0.009	0.007 ± 0.007	0.01 ± 0.01	0.048 ± 0.048	0.13 ± 0.09	0.057 ± 0.080
BS	0.23 ± 0.06	0.17 ± 0.05	0.23 ± 0.08	0.77 ± 0.47	0.79 ± 0.50	0.48 ± 0.31
MB	0.30 ± 0.12	0.31 ± 0.05	0.38 ± 0.11	0.98 ± 0.54	1.22 ± 0.70	0.31 ± 0.11
Met-enkephalin (pg/μg protein)						
CR	0.090 ± 0.014	0.093 ± 0.029	0.081 ± 0.009	0.117 ± 0.027	0.117 ± 0.025	0.080 ± 0.018
CB	0.002 ± 0.004	0.002 ± 0.004	0	0	0.002 ± 0.004	0.002 ± 0.004
BS	0.026 ± 0.028	0.016 ± 0.016	0	0.015 ± 0.014	0.022 ± 0.015	0.011 ± 0.014
MB	0.13 ± 0.09	0.13 ± 0.07	0.12 ± 0.03	0.08 ± 0.06	0.14 ± 0.04	0.08 ± 0.08

*Cerebrum, †cerebellum, ‡brainstem, §midbrain, ¶nondetectable.

days for learning (defined as reaching a criterion of four or fewer shocks/day for two consecutive days) and 10 days for extinction (two consecutive days of 16 or more nonresponse trials).

7. Operant conditioning was conducted in Coulbourn test cages which were housed in sound-attenuated cubicles. Each cage had response levers located on both sides of a centrally located water dipper and trough. The dipper was activated by a response which provided access to the water for 3 sec. A naive group of animals was magazine-trained for 30 min/day on days 40 and 41, and they were autoshaped overnight on day 41. The reinforced response lever was alternated on successive days, and the rats were placed on a progressive fixed-ratio schedule of reinforcement for 1.5 hr/day until they no longer responded sufficiently to receive reinforcement.

Neurochemistry

One female and one male which had received no prior testing were selected randomly from five litters and sacrificed by focussed microwave irradiation (6,18) on postnatal day 21 for analysis of protein and seven putative neurotransmitters: Acetylcholine (ACh), dopamine (DA), serotonin or 5-hydroxytryptamine (5-HT), norepinephrine (NE), substance P (sub P), β-endorphin (β-end), and Met-enkephalin (M-enk). Because of infertility in the paternal animals exposed to 7000 ppm l-propanol, no samples could be collected from this group. In place of this group, brains were taken from 10 offspring of foster dams which were not handled or weighed ("unhandled controls" in Table 2). These were compared with the brains from the regular sham-exposed controls to determine the effects of handling on brain neurochemistry. The brain was separated into four general brain regions: Cerebrum, cerebellum, brainstem and midbrain as previously described (23), and samples were frozen at −80°C until assayed.

Brain samples were homogenized by sonication in 8 ml of 0.1 N HCl. Aliquots were removed for determination of protein (2.0 μl) (16); ACh (25 μl) [(11) as modified (14,17)]; DA (50 μl) (8); NE (50 μl) (8); 5-HT (10 μl) (29); sub P (20 μl) (20); β-End (200 μl) (19); and M-enk (75 μl) (19). Samples were randomized for processing sequence, and the assays were performed using a single-blind procedure.

Statistical Analyses

Behavioral data were analyzed using multivariate analysis of variance (MANOVA) where the data fit normal distributional assumptions required for parametric analyses (7,15). However, nonparametric tests were thought to be more appropriate in the majority of cases, so an m-ranking procedure was used (7). Repeated measures analyses were conducted where appropriate. All groups at 7000 ppm l-propanol were included in one analysis, and those at 3500 ppm were included in a second analysis. Because of the time difference between exposures in the 7000 and 3500 ppm l-propanol groups, no direct comparisons between the groups were deemed justified. In all cases, $p \leq 0.05$ was required for significance. When the same group of animals was used for multiple comparisons, Bonferroni (7,21) corrections were made to adjust the probabilities required for significance.

The neurochemical data were also analyzed using MANOVA, followed by analysis of variance (ANOVA) if the MANOVA was significant. MANOVA is used to reduce the probability of making Type I errors when multiple dependent measures are analyzed. A Litter/Exposure Group × Region design was used for this analysis. Litter was nested within Exposure Group, and Region was treated as a within-litter variable. The Greenhouse-Geisser correction was used on all within-litter main effects and interactions. Because of the small number of degrees of freedom, probabilities of multivariate test statistics could not be calculated accurately for the main effect of Region and the Group × Region interaction. Consequently, the critical probability for each ANOVA (0.05) was divided by the number of dependent measures (8) to obtain a critical probability (0.0063).

RESULTS

Maternal/Paternal Observations

The exposures were maintained at the target concentrations throughout the study. For the 62 exposure days at 7000 ppm, the daily means (±SD) from hourly infrared analyzer readings averaged 7030(±80) ppm (daily means generated from the 5-min computer means were virtually identical), with charcoal tube samples averaging 7010(±60) ppm. At 3500 ppm, the exposure also continued for 62 days, with daily means averaging 3510(±20) ppm and charcoal tubes averaging 3510(±10) ppm.

The number pregnant/number bred were 17/17 for maternally-exposed rats, 2/16 for paternally-exposed rats, 18/18 for controls, and 38/40 for foster rats in the 7000 ppm l-propanol group. In spite of sperm plugs apparent for 16 paternally-exposed males (one male died as a result of fighting with a cage-mate, and another did not mate), only two litters resulted. One had a litter of 12, and the other had a litter of only two pups. Because of the infertility in the exposed male group, the last six exposed males were retained (the other males had been sacrificed before the infertility was noted) and mated at biweekly intervals. One of these was the male which produced the litter with 12 pups. The number of males which produced litters were as follows: In week 1, 1 of 6 produced litters (viz. the one just discussed); in week 3, 2 of 6; in week 5, 4 of 6; in week 7, 4 of 6; in week 9, 4 of 6; in week 11, 3 of 6; in week 13, 6 of 6; and week 15, 6 of 6. Thus, once a male sired a litter, he was generally fertile thereafter. At 3500 ppm l-propanol, the number pregnant/number bred was 17/18 for maternally-exposed rats, 17/18 for paternally-exposed rats, 18/18 for controls, and 52/56 for foster dams.

Although a group of sham-exposed males was not included, the lack of weight gain in the exposed males suggested that their weight gain was retarded during the first week of exposure, but likely normal thereafter. Weekly mean weights (±SD) for the six weeks the males were exposed to 7000 ppm propanol were 444(±40) g, 444(±38) g, 466(±36) g, 489(±38) g, 511(±38) g, and 528(±40) g. For those exposed to 3500 ppm propanol, the weights were 510(±24) g, 504(±26) g, 514(±29) g, 527(±30) g, 539(±32) g, and 554(±34) g. Weight gain of exposed females (compared using multivariate analysis) was not affected by exposure to l-propanol. Feed intake (compared using an m-ranking technique) was reduced ($p<0.05$) in the females exposed to 7000 ppm propanol (weekly means±SD for the exposed vs. control groups were 107(±13) vs. 134(±20) g, 122(±12) vs. 145(±18) g, and 137(±14) vs. 158(±18) g. At 3500 ppm l-propanol, there were no significant effects on maternal weight gain or feed intake. No significant differences were found among any of the groups for the number of live pups per litter, the length of gestation, the birth weights, or neonatal survival.

Examination of the offspring revealed that 2 of 15 litters from the 7000 ppm l-propanol maternally exposed group had several pups (2–3/litter) with crooked tails (noted soon after birth), and these defects persisted. There were no effects on offspring weight gain at either exposure concentration. Sex differences in weights became apparent on day 28.

Behavior

The behavioral data are not included because the control groups' data were published in a comparison of control groups from two studies (25) and because there were so few differences from controls in this study. The data can be obtained from the senior author.

At 7000 ppm l-propanol, exposed animals were not significantly different from controls on any of the tests. At 3500 ppm l-propanol, the only significant differences between ex-

posed animals and controls were in the activity measures. In the Open Field test, females from the paternally-exposed group were less active than the controls on days 44, 45, and 46. On the activity wheel, this same group was less active than the controls. In the optical activity monitor, males in the maternally-exposed group were less active than control males on days 44, 45, and 46. No other significant differences were observed.

Neurochemistry

The main effect of Exposure Group was not significant for either 7000, $F(4,16)=1.77$, $p=0.31$—including the unhandled control group, or 3500 ppm l-propanol, $F(6,16)=1.86$, $p=0.23$. As expected, there were significant differences between Regions for most neurochemicals (Table 2; statistical data not shown). None of the ANOVAs for the Group × Region interaction were significant.

DISCUSSION

The scarcity of effects seen in this behavioral teratology investigation of l-propanol is surprising. Based upon extensive work with ethanol and on limited research with other alcohols [specifically, 1-propanol administered to neonatal rats (12) and tertiary-butanol to pregnant mice (9)], 1-propanol seemed likely to produce behavioral teratogenic effects. We had previously reported that inhalation exposure to methanol was teratogenic in rats but that inhalation exposure to ethanol was not (24). Compared to controls, ethanol was found to produce quantitative changes in the steady state concentration of a small number of neurochemicals in offspring of rats exposed prenatally to high concentrations of ethanol (16,000 ppm for 7 hours/day). In spite of extensive literature reporting behavioral teratogenic effects of ethanol (1–3), however, no behavioral changes were seen in our study (26). The absence of behavioral effects was presumed to be due to the relatively low blood levels we achieved using inhalation exposures of ethanol (25,26).

In behavioral teratology research, behavioral effects have generally been seen at lower concentrations than those which produce structural malformations (33). As reported previously (27), 7000 ppm l-propanol, albeit producing low blood levels in mature rats, is a teratogenic concentration; this was confirmed by the litters in this study having crooked tails. Behavioral and neurochemical effects were, however, rare or nonexistent. The relatively high variability in the neurochemistry data may have precluded significance. It is unclear whether the lack of effects is a result of the tests, species selected, or the dosing regimen (including the low blood levels we obtained), or if l-propanol simply does not produce behavioral teratogenic effects. The behavioral tests used in the present study are typical of those used in the field (22), and have demonstrated sensitivity to the prenatal effects of the industrial solvent 2-ethoxyethanol via inhalation exposures in rats in this laboratory. The nonsignificance of l-propanol effects suggests that "Richardson's Law," which predicts that toxicity to adult animals increases with increasing molecular weight of the alcohols, does not apply to the developmental effects of this alcohol. Additional research will be needed to replicate and confirm this anomaly.

One finding from this study was unexpected: Exposure of male rats to 7000 ppm l-propanol for 6 weeks produced reversible infertility. This observation deserves replication using methods designed to detect direct effects on the sperm or ejaculate, and on the males themselves [e.g., (28, 31, 37, 38)]. The lack of such effects at 3500 ppm indicates that high concentrations are required to produce the observed infertility.

In summary, it seems unlikely that l-propanol will produce functional effects at levels lower than those required to produce malformations. As these levels generally are maternally toxic, the present results indicate that l-propanol is not a selective developmental toxicant, and exposure to concentrations of l-propanol currently permitted in the workplace will likely not result in behavioral teratogencity.

ACKNOWLEDGEMENTS

We thank Jeff McLaurin for his help during the exposures, and Debbie Taylor, Shirley Robertson, and Karl DeBord for their help on other technical aspects of the study.

REFERENCES

1. Abel, E. L. Fetal alcohol syndrome. vol. 1: An annotated and comprehensive bibliography. Boca Raton, FL: CRC Press; 1981.
2. Abel, E. L. Fetal alcohol syndrome. vol. 2: Human studies. Boca Raton, FL: CRC Press; 1982.
3. Abel, E. L. Fetal alcohol syndrome, vol. 3: Animal studies. Boca Raton, FL: CRC Press; 1982.
4. Adams, P. M.; Shabrawy, O.; Legator, M. S. Male-transmitted developmental and neurobehavioral deficits. Teratogenesis Carcinog. Mutagen. 4:149–169; 1984.
5. Anderson, R. A., Jr. The possible role of paternal alcohol consumption in the etiology of the Fetal Alcohol Syndrome. In: Fetal alcohol syndrome. vol. 3: Animal studies. Boca Raton, FL: CRC Press; 1982:83–112.
6. Butcher, S. H.; Butcher, L. L.; Harms, M. S.; Jenden, D. J. Fast fixation of brain *in situ* by high intensity microwave irradiation: application to neurochemical studies. J. Microwave Power 11(1):61–65; 1976.
7. Conover, W. J. Practical nonparametric statistics. 2nd ed. New York: Wiley and Company; 1981.
8. Coyle, J. T.; Henry, D. Catecholamines in fetal and newborn rat brain. J. Neurochem 21:61–67; 1973.
9. Daniel, M. A.; Evans, M. A. Quantitative comparison of maternal ethanol and maternal tertiary butanol diet on postnatal development. J. Pharmacol. Exp. Ther. 222:294–300; 1982.
10. Eller, P. M. NIOSH manual of analytical methods. 3rd ed. vol. 1, Method 1401. Washington, DC: DHHS (NIOSH) Publication No. 84–100; 1984.
11. Goldberg, A. M.; McCaman, R. E. The determination of picomole amounts of acetylcholine in mammalian brain. J. Neurochem. 20:1–8; 1973.
12. Grant, K. A.; Samson, H. H. n-Propanol induced microcephaly in the neonatal rat. Neurobehav. Toxicol. Teratol. 6(2):165–169; 1984.
13. Gros, C.; Pradelles, P.; Rouget, C.; Bepoldin, O.; Dray, F.; Fournie-Zaluski, M. C.; Roques, B. P.; Pollard, H.; Llorens-Cortes, C.; Schwartz, J. C. Radioimmunoassay of methionine- and leucine-enkephalins in regions of rat brain and comparison with endorphins estimated by radioreceptor assay. J. Neurochem. 31:29–39; 1978.
14. Hoover, D. B.; Muth, E. A.; Jacobowitz, D. M. A mapping of the distribution of acetylcholine, choline acetyltransferase, and acetylcholinesterase in discrete areas of rat brain. Brain Res. 153:295–306; 1978.

15. Hummel, T. J.; Sligo, J. R. Empirical comparison of univariate and multivariate analysis of variance procedures. Psychol. Bull. 76(1):49–57; 1971.
16. Lowry, O. H.; Rosebrough, J. J.; Farr, A. L.; Randall, J. L. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
17. McCaman, R. E.; Stetzler, J. Radiochemical assay for ACh: modifications for sub-picomole measurements. J. Neurochem. 28:669–671; 1977.
18. Merritt, J. H.; Frazer, J. W. Microwave fixation of brain tissue as a neurochemical technique: a review. J. Microwave Power 12(2):133–139; 1977.
19. Miller, R. J. The enkephalins. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Neuropeptides. New York: Plenum Press; 1983:107–207.
20. Mroz, E. A.; Leeman, S. E. Substance P. In: Jaffe, B. M.; Behrman, J. R., eds. Methods in hormone radioimmunoassay. 2nd ed. New York : Academic Press; 1979:121–138.
21. Muller, K. E. Design and analytical methods. In: Annau, Z., ed. Neurobehavioral toxicology. Baltimore: The Johns Hopkins University Press; 1986:404–423.
22. Nelson, B. K.; Brightwell, W. S.; Setzer, J. V.; Taylor, B. J.; Hornung, R. W.; O'Donohue, T. L. Ethoxyethanol behavioral teratology in rats. Neurotoxicology 2(2):231–249; 1981.
23. Nelson, B. K.; Brightwell, W. S.; Burg, J. R.; Massari, V. J. Behavioral and neurochemical alterations in the offspring of rats after maternal or paternal inhalation exposure to the industrial solvent 2-methoxyethanol. Pharmacol. Biochem. Behav. 20(2): 269–279; 1984.
24. Nelson, B. K.; Brightwell, W. S.; MacKenzie, D. R.; Khan, A.; Burg, J. R.; Weigel, W. W.; Goad, P. T. Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fundam. Appl. Toxicol. 5:727–736; 1985.
25. Nelson, B. K.; Brightwell, W. S.; Burg, J. R. Comparison of behavioral teratogenic effects of ethanol and n-propanol administered by inhalation to rats. Neurobehav. Toxicol. Teratol. 7(6):779–783; 1985.
26. Nelson, B. K.; Brightwell, W. S.; MacKenzie-Taylor, D. R.; Burg, J. R.; Massari, V. J. Neurochemical, but not behavioral deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol. Neurotoxicol. Teratol. 10(1): 15–22; 1988.
27. Nelson, B. K.; Brightwell, W. S.; MacKenzie-Taylor, D. R.; Khan, A.; Burg, J. R.; Weigel, W. W.; Goad, P. T. Teratogenicity of n-propanol and isopropanol administered at high inhalation concentrations to rats. Food Chem. Toxicol. 26(3): 247–254; 1988.
28. Robaire, B.; Trasler, J. M.; Hales, B. F. Consequences to the progeny of paternal drug exposure. In: Lobl, T. J.; Hafez, E. S. E., eds. Male fertility and its regulation. Boston: MTP Press Limited; 1985:225–243.
29. Saavedra, J. M.; Brownstein, M.; Axelrod, J. A specific and sensitive enzymatic-isotopic microassay for serotonin in tissues. J. Pharmacol. Exp. Ther. 186:508–515; 1973.
30. TLVs Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment with Intended Changes for 1986–1987. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 1986.
31. Trasler, J. M.; Hales, B. F.; Robaire, B. Chronic low dose cyclophosphamide treatment of adult male rats: effect on fertility, pregnancy outcome and progeny. Biol. Reprod. 34:275–283; 1986.
32. von Oettingen, W. F. The aliphatic alcohols: Their toxicity and potential dangers in relation to their chemical constitution and their fate in metabolism. Public Health Bulletin No. 281. Washington, DC: U.S. Govt. Printing Office; 1943:1–253.
33. Vorhees, C. V. Principles of behavioral teratology. In: Riley, E. P.; Vorhees, C. V., eds. Handbook of behavioral teratology. New York: Plenum Press; 1986:23–48.
34. Wiener, S. G. Nutritional considerations in the design of animal models of the Fetal Alcohol Syndrome. Neurobehav. Toxicol. 2:175–179; 1980.
35. Wimer, W. W.; Russell, J. A.; Kaplan, H. L. Alcohols toxicology. Park Ridge, NJ: Noyes Data Corp.; 1983:46–47.
36. Zenick, H. Mechanisms of environmental agents by class associated with adverse male reproductive outcomes. In: Lockey, J. E.; Lemasters, G. K.; Key, W. R., Jr., eds. Reproduction: The new frontier in occupational and environmental health research. New York: Alan R. Liss, Inc; 1984:335–361.
37. Zenick, H.; Blackburn, K.; Hope, E.; Baldwin, D. An evaluation of the copulatory, endocrinologic, and spermatotoxic effects of carbon disulfide in the rat. Toxicol. Appl. Pharmacol. 73:275–283; 1984.
38. Zenick, H.; Blackburn, K.; Hope, E.; Oudiz, D.; Goeden, H. Evaluating male reproductive toxicity in rodents: a new animal model. Teratogenesis Carcinog. Mutagen. 4:109–128; 1984.