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Developmental Toxicology Evaluation of 1-Pentanol, 1-Hexanol, and 2-Ethyl-1-Hexanol Administered by Inhalation to Rats

B.K. NELSON,[1] W.S. BRIGHTWELL,[1] A. KHAN,[1] E.F. KRIEG, JR.,[1] and A.M. HOBERMAN[2]

ABSTRACT

As part of a large study evaluating the developmental toxicology of industrial alcohols, groups of approximately 15 Sprague-Dawley rats were exposed for 7 h/day on gestation days 1–19 to one of three alcohols at the highest concentrations we could generate as a vapor. Since these concentrations were not sufficiently high to be toxic to the maternal rats, lower levels were not included. These concentrations were 14,000, 3500, and 850 mg/m^3 1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol, respectively. Dams were weighed daily for the first week of exposure and weekly thereafter and were sacrificed on day 20. Fetuses were serially removed, blotted dry, examined for external malformations, sexed, weighed, fixed, and examined for visceral or skeletal defects. Pentanol reduced maternal feed intake and weight gain, but there were no significant decreases in fetal weights. Resorptions were slightly increased in the group exposed to 1-hexanol, and 2-ethyl-1-hexanol reduced maternal feed intake, but neither hexanol derivative produced malformations. Obviously, teratogenicity by other routes of exposure cannot be ruled out, particularly in view of the low volatility of these alcohols. However, the present studies indicate that inhalation of pentanol, hexanol, or 2-ethyl-1-hexanol at the stated concentrations can produce limited maternal toxicity, but none was teratogenic to rats.

INTRODUCTION

DUE TO THEIR EXTENSIVE USE as industrial solvents, a series of aliphatic alcohols are being investigated for developmental toxicity. The studies focus on structure-activity relationships among the primary alcohols with carbon chain lengths from 1 to 10, although some branched-chain derivatives are also included. The alcohols are administered by inhalation to reflect the route of exposure found in industry. The main concern is for selective developmental toxicity in which the conceptus is at greater risk than is the maternal animal. Consequently, the

[1]Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226.
[2]Argus Research Laboratories, Horsham, PA 19044.

initial concentrations were selected to demonstrate significant maternal toxicity, and subsequent exposure groups were administered lower concentrations. At high concentrations, methanol, the propanols, and 1-butanol are teratogenic to rats, whereas ethanol, 2-butanol, and tertiary butanol are not.[1-3] Malformations are typically seen only at concentrations that are also toxic to the maternal animals; this indicates a lack of selective toxicity to the developing conceptus.

As reported in the 1800s (Richardson, 1869), the overall toxicity of alcohols to adult animals increases as carbon chain length increases (up to around six carbons). Our studies have provided corroborative evidence for this finding, which is now seen as a principle or law.[4] For example, 20,000 ppm ethanol (two carbons) produced complete narcosis in the exposed animals, whereas 16,000 ppm had negligible effects. The reason 16,000 ppm had so little impact likely has to do with the ability of rats to metabolize ethanol very rapidly.[5] In contrast, 10,000 ppm 1-propanol or isopropanol (three carbons) was severely toxic to maternal animals, and this concentration of some butanol isomers (four carbons) was lethal to the exposed animals.[2,3]

At the outset of these studies of the alcohols, we recognized that our ability to generate alcohol vapors would be limited because the volatility of the alcohols decreases as carbon chain length increases. Additionally, there were few teratology studies of any of the alcohols except ethanol.[6] As the studies progressed, it became apparent that these alcohols were embryotoxic only at levels that were also maternally toxic.[1-3] Consequently, maternal toxicity became our most available indicator of the likelihood of developmental toxicity. However, exposures to alcohols with carbon chain lengths in excess of four, as reported in the present study, could not be generated at concentrations that produced obvious maternal toxicity, since volatility decreases substantially as chain length increases. Nonetheless, the studies were completed to provide data from comparable measurement systems on the entire series of straight-chain alcohols. These studies were thus intended to determine if the same relationship between maternal toxicity and embryotoxicity occurred in the longer chain alcohols, or if these alcohols produced selective developmental toxicity. Therefore, we exposed rats to the highest concentrations of longer chain alcohols that we could generate as a vapor. If teratogenicity had been observed, then lower concentrations would have been examined.

The present paper reports our results with 1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol. Pentanol and hexanol were selected because they have five and six carbons in the primary chain, respectively; 2-ethyl-1-hexanol was one of the branched-chain derivatives selected for evaluation because its primary metabolite, 2-ethylhexanoic acid, is an isomer of the human teratogen, valproic acid.[7] These alcohols are used as solvents and in inks, lubricating oils, and plasticizers.[8] An estimated 200,000–300,000 American workers are exposed to these alcohols.[9]

MATERIALS AND METHODS

The generation system for inhalation exposures has been illustrated and described in detail.[3] Exposures were conducted in 0.5 m^3 Hinners-type chambers having a dynamic airflow of approximately 0.5 m^3/minute, a temperature of 77 ± 2°F, and a relative humidity of 50 ± 15%. Reagent-grade 1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol were purchased from commercial sources. The purity of each was ≥99% as checked by gas chromatography using modifications of NIOSH Analytical Methods 1400, 1401, and 1402, respectively.[10] A constant flow of each alcohol was mixed with a known volume of heated compressed air, resulting in instantaneous vaporization of the alcohol. This vapor-air mixture was introduced into the mainstream of the chamber airflow upstream from an orifice, and the resulting turbulence produced uniform mixing of the test chemical throughout the exposure chamber.

The concentrations of each chemical were monitored continuously using a Miran 1A infrared analyzer, and were recorded every hour. Calibration checks of this analyzer were completed daily for the latter two chemicals by injecting samples representing 90% and 110% of the target concentration before and after the exposures were completed. Exposure concentrations were verified on a weekly basis using a secondary method of analysis. Charcoal tube samples were

drawn 2 days/week and analyzed by gas chromatography[10] with partial verification of these methods using spiked samples of known concentration.

The concentrations selected for study were the highest that we could generate as a vapor for each alcohol and maintain the chamber temperature below 80°F. These were 14,000 mg/m^3 for pentanol, 3500 mg/m^3 for hexanol, and 850 mg/m^3 for 2-ethyl-1-hexanol. Concentrations significantly higher than these resulted in aerosol production within the chamber. Approximately 15 rats were assigned without bias to each exposure group, with an equal number of sham-exposed controls for each two exposure groups. Rats were exposed for 7 h/day, with a ¼–½ h degassing period before the animals were removed from the chambers.

The experimental animals were VAF+ Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI; quarantined for 1–2 weeks and checked for common pathogens on receipt) weighing 200–300 g at the beginning of pregnancy. Environmental conditions included a 12:12 light-dark cycle, a temperature of 24 ± 2°C, and a relative humidity of 50 ± 10%. Breeder males from the same source were housed in standard metal cages equipped with automatic water dispensers. NIH-07 lab chow (Ziegler Bros., Garden, MA) and tap water (analyzed quarterly for impurities) were available ad libitum except when the pregnant females were in the exposure chambers.

Sperm-positive females (sperm presence = day 0) were placed in shoebox cages with cleaned, heat-treated sawdust bedding (Absorb-Dri; Tasty Foods, Cincinnati, OH). Total feed and water intake were measured once a week (namely, gestation days 7, 14, and 20). Females were weighed daily for the first week and weekly thereafter. From gestation days 1–19, females were placed in stainless steel wire mesh cages within the exposure chambers and exposed for 7 h/day to alcohol vapors.

On gestation day 20, pregnant females were individually weighed and euthanized by CO_2 asphyxiation. The uterus (with ovaries) was removed, and the number of corpora lutea of pregnancy, implantations, resorption sites, and live fetuses recorded. Fetuses were serially removed, examined for external malformations, blotted of excess fluids, weighed, and sexed. One-half of the fetuses were randomly selected, placed in 80% ethanol, and subsequently eviscerated, macerated in 1.5% KOH, stained with alizarin red S, and examined for skeletal malformations. The remaining fetuses were fixed in Bouin's solution and subsequently sectioned and examined for visceral abnormalities using the Wilson technique.[11]

Data were analyzed using multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA); $p \leq 0.05$ was accepted as statistically significant. Three separate analyses were performed using exposure group as the independent variable. For the litter data (numbers of corpora lutea, resorptions, females per litter and males per litter, and weight of females per litter and of males per litter), a one-way MANOVA/ANOVA design was used. If a significant MANOVA was observed, individual ANOVAs were performed, and if these were significant, Bonferroni corrections were made comparing the individual exposure group with controls. A second analysis, for the weight data, used a litter per exposure group × day ANOVA. A third analysis, for the feed and water consumption data, used a litter per exposure group × week MANOVA/ANOVA design. For the ANOVAs, the probabilities of all within-litter main effects and interactions were corrected with the Greenhouse-Geisser estimate of Box's epsilon.

RESULTS

As already stated, the highest concentrations that we could generate as a vapor (and maintain the chamber temperature below 80°F) were 14,000 mg/m^3 for pentanol (five carbons), 3500 mg/m^3 for hexanol (six carbons), and only 850 mg/m^3 for 2-ethyl-1-hexanol (six carbons in the primary chain). Means from the continuous monitoring were equal to these target values, with standard deviations not exceeding 5% of the means. Concentrations from the secondary monitoring method (gas chromatograph analysis of samples captured on charcoal tubes) were frequently 10–20% lower than the means from the primary method. However, results from

TABLE 1. SUMMARY OF DATA IN TERATOLOGY STUDY (LITTER MEAN ± SD)

Data	*1-Pentanol*	*1-Hexanol*	*2-Ethyl-1-hexanol*	*Control*
Mean maternal weight (g)				
Day 0	260 ± 22	253 ± 16	283 ± 18	243 ± 25
Day 7	267 ± 21	268 ± 12	286 ± 17	262 ± 24
Day 14	291 ± 23	296 ± 10	310 ± 17	291 ± 26
Day 20	348 ± 25	363 ± 13	371 ± 20	354 ± 32
Overall gain	88	110	88	111
Mean feed consumption (g)				
Week 1	92 ± 14	122 ± 11	97 ± 17	108 ± 14
Week 2	110 ± 10	136 ± 09	107 ± 12	124 ± 12
Week 3	99 ± 12	122 ± 10	113 ± 11	118 ± 10
Overall mean	100 ± 14[a]	127 ± 12[a]	106 ± 15[a]	117 ± 13
Mean water intake (g)				
Week 1	222 ± 33	201 ± 32	196 ± 32	204 ± 46
Week 2	223 ± 43	209 ± 24	203 ± 24	276 ± 93
Week 3	288 ± 45	242 ± 39	248 ± 30	265 ± 123
Overall mean	244 ± 51	217 ± 36	216 ± 36	248 ± 96
Mean corpora lutea per litter	13 ± 3	17 ± 1	15 ± 2	14 ± 4
Mean resorptions per litter	0.2	1.3[a]	0.3	0.4
Mean number females per litter	7 ± 1	7 ± 2	7 ± 2	8 ± 2
Mean number males per litter	5 ± 2	8 ± 3	7 ± 2	7 ± 2
Mean fetal weight (g)				
Female	3.11 ± 0.31	3.05 ± 0.20	3.02 ± 0.20	3.19 ± 0.20
Male	3.32 ± 0.26	3.19 ± 0.21	3.18 ± 0.30	3.28 ± 0.27

[a]Significantly different from control at $p < 0.05$.

spiked samples were often similarly below the concentrations supplied. Consequently, the figures from the primary monitoring method (infrared analyzer) are cited throughout this paper.

In contrast to the lower alcohols (four-carbon chain and below), sufficient concentrations of these alcohols could not be generated to produce obvious maternal toxicity. Further, subjective observations of the maternal animals did not provide evidence of toxicity. Overall feed consumption was lower than in controls (Table 1; approximately 10–15%) for both the pentanol- and 2-ethyl hexanol–exposed groups. Although the weight gain appeared to be lower in these same groups (Table 1), these differences did not reach statistical significance. There were no treatment effects on water intake, on the number of corpora lutea, or on the number or fetal weights of either sex. A slight but statistically significant increase in resorptions was seen in the hexanol-exposed group (1.3 versus 0.4 per litter for controls), although the resorption mean is still in the range seen in historical controls (up to 1.3 per litter).[12]

No malformations were observed in any group in the present study. Administration of each alcohol resulted in small (nonsignificant), reversible delays in ossification of the caudal vertebrae, sternum (first sternal center and xiphoid), metacarpals, and hindpaw phalanges. These variations are frequently seen with growth retardation, but significant effects on fetal weights were not observed in the present study.

DISCUSSION

The present study found no indication of malformations induced after exposure of pregnant rats to 14,000 mg/m^3 pentanol (five carbons), 3500 mg/m^3 hexanol, or 850 mg/m^3 2-ethyl-1-hexanol (both having six carbons in the primary chain). This latter alcohol was the only one previously examined for teratogenecity, and these results are of substantial interest because 2-ethylhexanoic acid (a metabolite of 2-ethyl-1-hexanol), is an isomer of the teratogen valproic acid. Ritter et al.[7] administered 6.25 or 12.5 mmol/kg (1 or 2 ml/kg; approximately 250 or 500 mg/kg) 2-ethyl-1-hexanol by oral gavage to groups of seven Wistar rats on gestation day 12. The percentage of fetuses dead or resorbed was not increased by either level, but the percentage of malformed survivors increased from 2 at the lower level to 22 at the higher level (compared with 0% in controls). Molar equivalent administration of 2-ethylhexanoic acid (6.25 and 12.5 mmol/kg) produced 1 and 68% malformations, respectively. These results were similar to the percentage of malformations produced by approximately one-half the dose of valproic acid. For comparison purposes, using assumptions detailed for ethanol,[5] we estimate that our daily dose of 2-ethyl-1-hexanol would have been approximately 24 mg/kg (0.6 mmol/kg). Thus, relatively high concentrations of 2-ethyl-1-hexanol (of the order of 10 times higher than those used in our study) could be teratogenic, but such concentrations cannot be generated as a vapor. Furthermore, since our exposures were designed to produce maximum concentrations under ideal experimental conditions, it is highly unlikely that concentrations as high as we achieved would be observed in occupational environments.

In summary, the volatility of the alcohols decreased rapidly with increasing carbon chain length and with substitutions on the primary chain. In the present study, we could not generate sufficient concentrations of either the five- or six-carbon alcohols to produce obvious maternal toxicity. This is at variance with that seen with carbon chains of one, two, three, and four, all of which were maternally toxic at concentrations that could be generated relatively easily. Based on our observation that teratogenicity occurs only in the presence of maternal toxicity following exposure to the short-chain alcohols, it is perhaps not surprising that no teratogenicity was observed with the longer chain alcohols, since significant maternal toxicity was not seen. Nonetheless, we conclude that high concentrations of 1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol were not teratogenic to rats when administered by inhalation at concentrations near the maxima that could be generated as a vapor.

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Address reprint requests to:
B. K. Nelson
Division of Biomedical and Behavioral Science
NIOSH C-24
4676 Columbia Parkway
Cincinnati, OH 45226-1998