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Developmental Toxicology Assessment of 1-Octanol, 1-Nonanol, and 1-Decanol Administered by Inhalation to Rats

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ABSTRACT

As part of a large study evaluating the developmental toxicology of industrial alcohols administered by inhalation, groups of approximately 15 pregnant female Sprague-Dawley rats were exposed for 7 h/day on gestation days 1-19 (sperm = 0) to one of three long-chain alcohols at the maximum concentrations that could be generated as a vapor. These concentrations were 400 mg/m^3 1-octanol, 150 mg/m^3 1-nonanol, and 100 mg/m^3 1-decanol. Dams were weighed daily for the first week of exposure, and weekly thereafter. On gestation day 20, rats were sacrificed. Fetuses were serially removed, blotted dry, examined for external malformations, sexed, weighed, placed in appropriate fixatives, and subsequently examined for visceral or skeletal abnormalities. No treatment-related effects were observed in pregnant females, frequency of resorptions, fetal weights, or skeletal/visceral malformations. Thus, long-chain alcohols at these vapor concentrations appear not to be toxic as evidenced by these fetal and maternal parameters.

INTRODUCTION

ALCOHOLS HAVE A WIDE VARIETY OF USES depending on chemical and physical properties, including use as solvents, cosolvents, and chemical intermediates. Industrial exposure to the alcohols is widespread because of these diverse uses.[1] The teratogenicity of ingested ethanol is well established, both in humans and in experimental animals.[2] The paucity of data concerning the developmental toxicity of alcohols other than ethanol led us to focus on structure–activity relationships in a series of studies to investigate the developmental toxicology of a homologous series of alcohols with carbon chain lengths ranging from 1 (methanol) through 10 (1-decanol). We selected inhalation as the route of exposure to investigate the potential adverse effects following a typical route of occupational exposure. Previous reports on the developmental toxicity of various alcohols administered to rats for 7 h/day by inhalation include those for methanol (which was found to produce malformations at 10,000 and 20,000 ppm) and ethanol (which was found not to produce malformations at 20,000 ppm[3]), *n*- and isopropanol (both of which produced malformations at both 7000 and 10,000 ppm[4]), 1-, 2-, and *t*-butanol (of which only the highest concentration of 1-butanol [8000 ppm] produced defects[5]), and 1-pentanol, 1-hexanol, and 2-ethyl-1-hexanol (none of which produced developmental toxicity at the concentrations administered[6]). The present report addresses a study evaluating the developmental toxicology of the long-chain alcohols, including 1-octanol, 1-nonanol, and 1-decanol administered by inhalation to rats.

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These three alcohols are used in a variety of applications, but the common use of all three alcohols is in the manufacture of plasticizers. Because of low volatility, significant exposure to these particular alcohols via inhalation is probably infrequent. Since our study was addressing structure–activity relationships among the alcohols, the majority of which are found as vapors inhaled in the workplace, these three alcohols were presented via inhalation. Because of their low volatility, the maximum concentrations that could be generated as a vapor, while maintaining the chamber temperature below 80°F, were quite low.

METHODS

The generation system used for inhalation exposures in this study was the same as that described previously,[3–6] and discussed in detail.[7] Briefly, exposures were conducted in 0.5 m^3 Hinners-type chambers, having a dynamic air flow of approximately 0.5 m^3/min. The vapor generation equipment was housed above the exposure chambers in sealed glove boxes, which were maintained under negative pressure to prevent any vapor leakage into room air. A constant flow of alcohol was mixed with a known volume of heated compressed air, resulting in vaporization of the alcohol. This vapor/air mixture was introduced into the chamber airflow system upstream from the orifice plate. The resulting turbulence downstream from the orifice plate produced a uniform mixing of the test chemical throughout the exposure chamber.

Reagent-grade 1-octanol, 1-nonanol, and 1-decanol were purchased from commercial sources (1-nonanol [lot No. 7328] from EM Science, Cherry Hill, NJ; 1-octanol and 1-decanol [both lot No. A15B] from Eastman Kodak Co., Rochester, NY). Samples of each chemical were analyzed for purity by gas chromatography using modifications of NIOSH analytical methods 1401 and 1402.[8]

Exposure concentrations of each alcohol near the animal breathing zone within the chamber were recorded continuously by a Miran 1A general purpose infrared analyzer which was calibrated within ±50% of the target concentrations [i.e., two data points above the target concentration (125% and 150% of the target concentration) and two data points below the target concentration (50% and 75% of the target concentration), with a minimum of three samples per data point]. Calibration checks of this analyzer were conducted daily before the exposures began by injecting samples representing 90% and 110% of the target chemical concentrations. The results indicated that all of the calibration checks for 1-octanol and 1-nonanol, and 87% of those for 1-decanol, were within 5% of the target concentrations; all of the checks for 1-decanol were within 10% of the target concentration. Charcoal tube samples were collected two days per week and analyzed using gas chromatography to provide a confirmatory analysis by a second method.[8] Spiked samples were also submitted to evaluate the accuracy of the gas chromatographic analytical results. Periodically, air samples were collected from the control chamber, which received only filtered room air, to determine if any of the alcohols could be detected in the control chamber. The exposure chamber concentration, temperature, and relative humidity were recorded each hour during an exposure day. From these hourly readings, the daily mean, range, and time-weighted average concentrations were calculated.

The concentration selected as a target for each alcohol was the highest concentration which could be generated as a vapor at an average daily chamber temperature 70–80°F. These concentrations were 400 mg/m^3 for 1-octanol, 150 mg/m^3 for 1-nonanol, and 100 mg/m^3 for 1-decanol. Generation of exposure concentrations higher than the target levels resulted in aerosol production within the chamber.

Approximately 15 rats were assigned to each exposure group and to a sham-exposed control group which served as a comparison group for both 1-octanol and 1-nonanol. At the time the control group was sham-exposed, preliminary work had been accomplished with 1-decanol, and we expected that the 1-decanol exposure would be completed about the same time as the sham exposures to the control group. Equipment failure and unanticipated problems in achieving stable exposure concentrations, however, delayed the decanol exposures for almost one year. Data from the 1-decanol group, therefore, were compared with a composite control group consisting of the 11 control groups employed in previous alcohol studies by this author.[9]

To make efficient use of the inhalation chambers, females were assigned without bias to groups as they became pregnant, but controls were run simultaneously with exposed animals in all cases (except for decanol, as discussed previously). Animal breeding and housing conditions were the same as those described previously (e.g., Refs. 5

and 6). Briefly, sperm-positive females (sperm presence = day 0) were placed in shoebox cages with clean, heat-treated sawdust bedding. Total feed and water intake were measured at weekly intervals on gestation days 7, 14, and 20. Females were weighed daily during the first week of exposure, and weekly thereafter. On gestation days 1-19, the pregnant females were placed in stainless steel wire mesh cages within the exposure chambers and exposed to alcohol vapors generated for 7 hr/day (1-octanol and 1-nonanol) or 6 h/day (1-decanol; the shorter exposure duration was due to the extended time required to generate the target concentrations for 1-decanol). Buildup and decay curves of the target concentrations within the chambers were steep and similar. Total exposure time, therefore, was within a few minutes of the time periods indicated above, allowing these exposure periods to be treated as 7- (or 6-) h blocks.

On gestation day 20, females were individually weighed and euthanized by CO_2 asphyxiation. The uterus and ovaries were removed, and the numbers of corpora lutea of pregnancy, implantation sites, resorption sites, and live fetuses were recorded. Fetuses were serially removed, examined for external malformations, blotted of excess fluids, weighed, and sexed. One-half of the fetuses were selected randomly, placed in 80% ethanol, and subsequently eviscerated, macerated in 1.5% KOH, stained with alizarin red-S, and examined for skeletal malformations and variations. The remaining fetuses were fixed in Bouin's solution and subsequently sectioned and examined for visceral abnormalities using the technique of Wilson.[10]

Statistical analyses varied by the type of observations. A one-way multivariate analysis of variance (MANOVA)/analysis of variance (ANOVA) design was used for the number of corpora lutea, number of resorptions, number of female pups, number of male pups, mean weight of female pups, and mean weight of male pups. If the MANOVA was significant, then individual ANOVAs were completed. Maternal weights (with each dam = litter), collected on several days of gestation as described above, were analyzed as Litter/Group × Day ANOVA. Litter was nested within Group, and Day was a within-litter variable. A Litter/Group × Week ANOVA also was used for the feed and water intake data, based on group means of weekly consumption. For both of the latter analyses, the Greenhouse–Geisser estimate of epsilon was used to adjust the degrees of freedom of the within-litter main effects and interaction. Fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution,[11] or an ANOVA; the Kruskal–Wallis test was used if a nonparametric analysis was more appropriate.

RESULTS

The maximum mean concentrations that we could generate as a vapor under our experimental protocol were relatively low. Means ±SD from the infrared analyzer (charcoal tubes) were: 402 ±8 (373 ± 18) mg/m^3 for 1-octanol; 145 ± 5 (135 ± 6) mg/m^3 for 1-nonanol; and, 100 ± 3 (57 ± 7) mg/m^3 for 1-decanol. (The discrepancy between the two methods of analysis for 1-decanol likely are associated with decanol's extremely high boiling point and low vapor pressure, which make vapor generation and monitoring difficult.) Concentrations obtained with the infrared analyzer (viz., 100 mg/m^3) are assumed to be correct for two primary reasons: calibration of the analyzer was verified daily, and research conducted in an attempt to determine the reasons for the discrepancy between the analyzer and charcoal tube results suggested that approximately 40% of the decanol was absorbed by the glass wool in front of the charcoal in the sampling tubes, and the glass wool was not included in the analyses of the charcoal tubes. Consequently, it is our judgement that the infrared analyzer concentrations are valid, and these concentrations are cited throughout this report. Temperature and relative humidity remained quite consistent across experiments, with means ±SD of 79.5 ± 1.2°F and 42 ± 6% for 1-octanol, 81.1 ± 1.1°F and 48 ±3% for 1-nonanol, and 78.0 ± 1.0°F and 37 ± 4% for 1-decanol.

Inhalation exposure of pregnant rats to 400 mg/m^3 1-octanol or to 150 mg/m^3 1-nonanol did not produce detectable maternal or fetal toxicity compared with controls (Table 1). As noted above, the lack of a concurrent control group for comparison with the 1-decanol treatment group (due to problems encountered in generating decanol vapors) necessitated a metaanalysis of data from 11 control groups used previously in our five-year investigation of 13 alcohols.[9] No significant effects were found for the 1-decanol exposures compared with the overall mean (of means) of these 11 control groups (using the variance of these means as the error term for this analysis).

TABLE 1. SUMMARY OF DATA IN TERATOLOGY STUDY (LITTER MEANS ± SD)[a]

	1-octanol	*1-nonanol*	*1-decanol*	*control*[b]
Mean maternal weight (g)				
Day 0	257 ± 24	275 ± 19	281 ± 34	273 ± 17
Day 7	276 ± 26	293 ± 17	297 ± 26	286 ± 17
Day 14	302 ± 24	318 ± 20	329 ± 28	315 ± 17
Day 20	368 ± 30	381 ± 27	392 ± 42	378 ± 24
Overall gain	111	106	111	105
Mean feed consumption (g)				
Week 1	150 ± 16	117 ± 09	129 ± 23	126 ± 39
Week 2	131 ± 15	131 ± 18	143 ± 16	142 ± 23
Week 3	119 ± 10	129 ± 13	130 ± 14	129 ± 17
Overall mean	133 ± 19	126 ± 15	134 ± 17	132 ± 28
Mean water intake (g)				
Week 1	250 ± 34	218 ± 25	287 ± 54	234 ± 85
Week 2	246 ± 31	240 ± 30	313 ± 97	261 ± 61
Week 3	271 ± 30	271 ± 36	320 ± 78	287 ± 60
Overall mean	256 ± 33	243 ± 37	307 ± 78	261 ± 72
Mean corpora lutea/litter	14.8 ± 3.6	13.5 ± 2.7	13.8 ± 3.0	14.9 ± 2.1
Mean resorptions/litter	0.4 ± 0.6	0.7 ± 0.9	0.5 ± 0.8	0.5 ± 0.8
Mean number females/litter	7.9 ± 2.6	6.0 ± 2.3	6.3 ± 3.0	6.9 ± 1.7
Mean number males/litter	5.9 ± 2.5	6.7 ± 2.5	6.8 ± 2.4	6.6 ± 1.7
Mean fetal weights (g)				
Female	3.44 ± 0.64	3.43 ± 0.37	3.30 ± 0.61	3.12 ± 0.31
Male	3.56 ± 0.80	3.48 ± 0.55	3.46 ± 0.65	3.22 ± 0.36

[a]No significant differences were detected.

[b]Data from concurrent controls only; data from the combined controls over the five years of study (used for the decanol comparisons) are available from the authors (and the majority are published in Ref. 9).

DISCUSSION

Concentrations of octanol, nonanol, and decanol which could be generated as a vapor produced little effect on maternal rats or their fetuses. Since dermal exposure was not assessed directly in this study, it is unclear how large a quantity of these alcohols would be absorbed through the skin. As noted in a recent review,[12] unless the skin is washed, less volatile solvents (such as the alcohols used in the present study) may remain for a longer duration on the skin and consequently may undergo greater absorption than more volatile solvents.

The present studies represent the first developmental toxicology evaluations of these three alcohols. Because of the low volatility of these three alcohols, a developmental toxicology evaluation of these alcohols at higher inhalation concentrations was not possible. The structural similarities among the alcohols (the structure–activity relationships observed among these alcohols is discussed in a separate publication[9]), and the developmental toxicity found for methanol, ethanol, 1-propanol, isopropanol, and 1-butanol,[3–5] suggest that the long-chain alcohols may be developmentally toxic at higher biological concentrations (i.e., delivered doses) than could be generated in this study. Inhalation, however, would not likely be a route of administration by which developmental toxicity would be observed because of the low volatility of these alcohols. Theoretical data on higher concentrations, therefore, would have only limited application. Based on the results of the present studies, we conclude that inhalation exposures of rats to 1-octanol, 1-nonanol, or 1-decanol at the concentrations used in this study do not produce developmental toxicity.

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