FUNDAMENTAL AND APPLIED TOXICOLOGY 12, 469–479 (1989)

Lack of Selective Developmental Toxicity of Three Butanol Isomers Administered by Inhalation to Rats

B. K. NELSON,[1] W. S. BRIGHTWELL, A. KHAN, J. R. BURG, AND P. T. GOAD[2]

Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, Ohio 45226, and Intox Laboratories Inc., 1 Intox Drive, Redfield, Arkansas

Received March 31, 1988; accepted September 22, 1988

Lack of Selective Developmental Toxicity of Three Butanol Isomers Administered by Inhalation to Rats. NELSON, B. K., BRIGHTWELL, W. S., KHAN, A., BURG, J. R., AND GOAD, P. T. (1989). *Fundam. Appl. Toxicol.* **12,** 469–479. As part of an ongoing study of the developmental toxicology of industrial alcohols, this report presents the results of the teratology assessments of 1-butanol, 2-butanol, and t-butanol administered by inhalation to rats. Groups of approximately 15 Sprague–Dawley rats were exposed at 8000, 6000, 3500, or 0 ppm 1-butanol, 7000, 5000, 3500, or 0 ppm 2-butanol, or 5000, 3500, 2000, or 0 ppm t-butanol for 7 hr/day on Gestation Days 1–19 (sperm = 0). In each case, the highest concentration was selected to produce maternal toxicity. Dams were sacrificed on Gestation Day 20, and fetuses were individually weighed, tagged, and examined for external malformations. One-half of the fetuses were stained and examined for skeletal abnormalities, and the other half were examined for visceral defects using the Wilson technique. For each butanol isomer examined, the highest concentration (and the intermediate in some cases) was maternally toxic, as manifest by reduced weight gain and feed intake. Even at a maternally toxic dose, and in spite of a dose-dependent reduction in fetal weights for each isomer, the only teratogenicity observed was a slight increase in skeletal malformations (primarily rudimentary cervical ribs), seen with the highest concentration of 1-butanol. Thus, although teratogenicity was observed at 8000 ppm 1-butanol, and developmental toxicity was observed with each of the butyl alcohol isomers studied, concentrations 50 times the current permissible exposure limits for these three butanol isomers do not produce teratogenicity in rats.

Alcohols are widely used as industrial solvents. Because of the well-recognized teratogenicity of ethanol via the oral route in animals and humans, and the dearth of reproductive toxicity information on most other alcohols, we have undertaken a large study on the teratogenicity of this class of industrial solvents. As most occupational/environmental exposures involve inhalation, our study utilized that route of exposure. It is directed toward the identification of structure–activity relationships, primarily the normal alcohols having carbon chain lengths up to 10. We have previously reported that inhalation exposure to methanol is teratogenic in rats at relatively high concentrations, but ethanol exposure is not (Nelson *et al.*, 1985), and that both *n*-propanol and isopropanol are teratogenic at high concentrations (Nelson *et al.*, 1988). The present paper reports results with *n*-butyl alcohol (1-butanol), *sec.*-butyl alcohol (s-butanol; 2-butanol; 1-methyl-1-propanol), and *tert.*-butyl alcohol (t-butanol; 2-methyl-2-propanol).

Butanol and its isomers are used extensively as solvents for paints, lacquers, coat-

[1] To whom correspondence and reprint requests should be addressed.

[2] Present address: Ayerst Research Laboratories, Chazy, NY 12921.

0272-0590/89 $3.00

ings, and resins, as extractants in the manufacture of antibiotics, hormones, and vitamins, and as intermediates in the manufacture and synthesis of a variety of compounds (Rowe and McCollister, 1982). Approximately 1.8 million (1-butanol) and 50,000 (2-butanol and t-butanol) people are potentially exposed to these alcohols occupationally (NIOSH, 1980). The compounds are not particularly toxic, having LD50 values from 3 to 6 g/kg in experimental animals (Rowe and McCollister, 1982). Legal occupational exposure limits as 8-hr time-weighted averages (TWA) are 100 ppm for *n*- and t-butyl alcohol and 150 ppm for 2-butanol (29 CFR 1910.1000). The 8-hr TWA TLVs are 50 ppm for 1-butanol and 100 ppm for s- and t-butyl alcohol (ACGIH, 1986).

Reproductive toxicology information is lacking for these solvents. Only two studies are reported in the literature, and both are on t-butanol. Since t-butanol does not undergo biotransformation by alcohol and aldehyde dehydrogenases, it has been used in attempts to determine the mechanism of action of ethanol. Daniel and Evans (1982) compared the effects of prenatal ethanol and t-butanol on postnatal development. Swiss–Webster (Cox) mice were given 1.0, 0.75, or 0.5% t-butanol-derived calories, 3.6% ethanol-derived calories, or 0% (controls) in a liquid diet (Bioserv) on Gestation Days 5–19, with all groups pair-fed to the 1.0% t-butanol group. Pups from four fostered and four nonfostered litters were tested on a variety of preweanling tests. Statistical analyses used the individual, rather than the litter, as the unit of comparison. Maternal intake of 1.0% t-butanol reduced food consumption and maternal weight gain, and the number of stillborn pups was increased in a dose-related manner. The authors concluded that t-butanol was approximately five times more potent than ethanol in producing a developmental delay in postnatal physiological and psychomotor performance.

Grant and Samson (1982) compared ethanol and t-butanol for their ability to induce microcephaly in the neonatal rat. Using an artificial rearing technique (the so-called "pup-in-a-cup" technique) from Postnatal Days 4 to 18, they administered selected doses of ethanol or t-butanol on Days 4–7. Offspring from six litters were cannulated on Day 4, and one-half of the pups from each litter received alcohol in their milk formula or milk only. The alcohol dosages were equated by membrane-to-buffer partition coefficients to produce blood levels of approximately 250 mg ethanol or t-butanol per 100 ml blood. Microcephaly was apparent in both alcohol groups.

Thus, the developmental toxicity of t-butanol has been investigated to a small extent, but the other isomers have not been studied to date. The present paper reports our investigation of the developmental toxicity of three butanol isomers administered by inhalation to rats.

METHODS

Experimental Animals: Housing Conditions and Procedures

Virgin female Sprague–Dawley rats (176–200 g) specified to be free of *Mycoplasma*, Sendai virus, and internal and external parasites (Charles River Breeding Laboratories, Wilmington, MA)[3] were acclimated to a 12-hr light/dark cycle, a temperature of 24 ± 2°C, and humidity of 50 ± 10% after quarantine for 1–2 weeks. Breeder males weighing over 300 g from the same source were housed individually under similar conditions in 32 × 41 × 18-cm stainless-steel wire-mesh cages equipped with automatic water dispensers (Hoeltge Inc., Cincinnati, OH). Purina or NIH-07 lab chow (Ziegler Bros., Garden, MA) and tap water were available *ad libitum*, except when pregnant animals were in the exposure chambers.

For mating, virgin 200- to 300-g females were placed individually with breeder males. Each morning, the litter paper under each male's cage was examined for sperm plugs; if no plugs were detected, vaginal smears were taken. Females with sperm (Day 0 of gestation) were placed individually into 30 × 34 × 17-cm polycarbonate cages having autoclavable polyester filter covers. Bedding consisted of cleaned, heat-treated sawdust from a local supplier (Absorb-Dri, from Tasty Foods, Cincinnati,

[3] Mention of trade names does not imply endorsement by NIOSH.

OH). Weekly food and water intake, along with maternal weights, were measured on Gestation Days 0, 7, 14, and 20. Females were also weighed each morning for the first week of exposure. From Gestation Days 1 to 19, females were transported from the animal quarters to the exposure chambers in their homecage shoe boxes with filter tops in place. Females were placed into 13 × 25 × 18-cm compartments in stainless-steel wire-mesh caging within the exposure chambers. Controls were placed in similar caging within an adjacent exposure chamber for the same hours as the exposed animals. Exposures were conducted 7 hr/day, and the animals were left in the chambers for degassing for approximately ½ hr after vapor generation was terminated. They were then removed and returned in their homecages to the animal quarters where the water bottles were replaced.

On Gestation Day 20, pregnant females were individually weighed and euthanized by CO_2 asphyxiation. The entire uterus (with ovaries attached) was removed, and corpora lutea, resorptions (classified as early, middle, or late), and live fetuses were counted. Fetuses were serially removed, examined for external malformations, blotted of excess fluids, and weighed, and external sex was determined.

One-half of the fetuses were randomly selected, placed into 80% ethanol, and subsequently eviscerated, macerated in 1.5% KOH, stained in alizarin red S, and examined for skeletal malformations and variations. The other half were placed in Bouin's solution and subsequently examined for visceral malformations and variations using a razor blade cross-sectioning technique (Wilson, 1965).

Inhalation Facility and Procedures

The inhalation exposures were conducted in 0.5-m^3 Hinners-type exposure chambers (Charles Spengler and Associates, Cincinnati, OH). The vapor generation equipment was housed above the exposure chambers in glove boxes which were maintained under negative pressure to prevent any leakage of contaminants into the room. Reagent-grade 1-butanol, 2-butanol, or t-butanol (Curtin Matheson Scientific, Cincinnati, OH) was placed into a flask. A low-flow pump (RP Model lab pump; Fluid Metering Inc., Oyster Bay, NY) circulated liquid from the reservoir flask into a 10-ml syringe contained within the flask such that the syringe was constantly overflowing. Thus the syringe provided a constant head of chemical for a second pump (controlled by a micrometer adjustment) which injected the specified amount of liquid into a three-way valve which was attached to a Greensmith impinger. Heated compressed air was introduced through the second inlet of the three-way valve. Alcohol evaporation was controlled by regulating the preheating of compressed air. The impinger provided increased contact time between the air and the liquid to ensure total evaporation. In generation of high concentrations, glass beads were also placed at the bottom of the impinger to further increase the heat transfer area between the alcohol and the compressed air. This vapor and air mixture was introduced into the chamber airflow upstream of the orifice plate. The turbulence and pressure drop created by the orifice plate provided uniform mixing downstream of the vapor and air before the mixture entered the chamber. Airflow through the chambers provided approximately one air change per minute.

The concentration within the chamber was monitored continuously with a Miran 1A general-purpose infrared analyzer (Wilks/Foxboro Analytical, South Norwalk, CT) which was calibrated within the range to be tested. The Miran 1A was connected to a strip chart recorder for continuous recording of the concentration throughout the day. On an hourly basis, the chamber concentration, temperature, and humidity were recorded on a daily observation sheet. At the end of each day, the strip chart was attached to the data sheet, and the mean, range, and time-weighted average concentrations were calculated. At the conclusion of the study, these daily values were averaged for an overall study mean for each concentration. In addition, the infrared analyzer used to monitor chamber concentrations was interfaced with an Apple II+ computer which displayed and recorded 5-min means of chemical exposure concentrations. These means were averaged each hour to give hourly and subsequent daily means, which were also used to calculate a study mean for each concentration.

Samples of the bulk chemical were analyzed by gas chromatography for purity. In addition, charcoal tube samples were collected from the chamber atmosphere for independent verification of chamber concentrations. Sampling times varied from 10 to 30 min in duration, and samples were collected at the rate of 5–10 per week. The samples were independently analyzed by NIOSH analytical methods (NIOSH, 1977—No. S62 for 1-butanol and t-butanol; NIOSH, 1977—No. S53 for 2-butanol). The NIOSH Division of Physical Sciences and Engineering, Arthur D. Little, Inc. (Cambridge, MA), and Southern Research Institute (Birmingham, AL) provided purity analyses of the bulk chemicals and analyzed the charcoal tube samples.

Selection of concentrations for initial pilot exposures to evaluate toxicity of the butanol isomers was based on our results with the propanols (Nelson *et al.*, 1988). Five to six nonpregnant females were exposed for 7 hr to 9000 or 10,000 ppm of each butanol isomer. Depending on the subjective toxicity observed in these animals, a similar number of other animals were exposed to lower concentrations in the pilot phase. For the teratology phase, the high concentration was selected to be maternally toxic, but not lethal, and two lower concentrations were included. Based on the pilot exposures, 15–20 sperm-positive females were assigned without bias to groups and exposed to 0, 3500, 6000, or 8000 ppm 1-butanol; 0, 3500,

5000, or 7000 ppm 2-butanol; and 0, 2000, 3500, or 5000 ppm t-butanol.

Statistical Analyses

For the maternal data, multivariate analysis (with baseline as covariate) was used for weight comparisons across groups. The group differences in food and water intake were analyzed by multivariate analysis of variance. A Kruskal–Wallis test was used for group comparisons of corpora lutea per animal. For the fetal data, analysis of variance was used to compare fetal weights across groups and sex. Group comparisons of the variables including litter size, percentage alive/litter, percentage normal/litter, and percentage females/litter were made using the Kruskal–Wallis test. For the variables including skeletal malformations, skeletal variations, visceral malformations, visceral variations, external malformations, and nonnormal fetuses, the number of litters with one or more of the variables of interest was compared between groups using Fisher's exact test. The results of the tests were adjusted for multiple comparisons, when appropriate, using the Bonferroni technique. A probability of $p \leq 0.05$ was required for significance.

RESULTS

The measured purity of each of the butanol isomers was ⩾ 99%. Throughout the exposures, concentrations of each isomer were easily generated and were uniform. The mean concentrations ± SD (number of exposure days) from the daily infrared readings of 1-butanol were 8000 ± 80 (25 days), 6000 ± 80 (21 days), and 3510 ± 20 ppm (40 days); charcoal tube results were typically 10–15% lower (means were 7700, 5960, and 3000 ppm). For 2-butanol, the infrared means were 6990 ± 50 (22 days), 4990 ± 100 (24 days), and 3540 ± 110 ppm (23 days); means from charcoal tubes were 6270, 3900, and 3180 ppm. Although these charcoal tube results at the higher and lower levels were about 10% lower than the infrared means, the one at the midlevel was approximately 20% lower than the infrared mean. For t-butanol, the infrared means were 5030 ± 100 (23 days), 3510 ± 30 (22 days), and 2200 ± 20 ppm (42 days); charcoal tube results were typically only slightly lower than the infrared means (means were respectively 4500, 3500, and 2010 ppm).

No contaminants were detected in the charcoal tubes collected from the control chambers. When rounded to the appropriate number of significant digits, the means from the infrared analyzer (including those recorded by computer) were equivalent to the target concentrations. As these means represent the results from continuous monitoring (as opposed to the periodic charcoal tube samples), these are the concentrations cited throughout this paper.

Maternal and General Toxicity Observations

Very high concentrations of the butanol isomers in the pilot study produced different effects, depending on the isomer. For 1-butanol, 9000 ppm produced death in two of six nonpregnant rats (one after 1 day and another after 2 days of exposure). Exposure to 10,000 ppm 2-butanol produced death in all five nonpregnant rats exposed for only 1 day. Exposure of six nonpregnant rats to 10,000 ppm t-butanol for one day produced severe narcosis in all animals, and death in five of the six.

At 8000 ppm, 1-butanol produced narcosis in approximately one-half of the maternal animals (observed subjectively), and this was selected as the high concentration for the teratology study. With 2-butanol, 7000 ppm produced narcosis in all animals, and they had not recovered completely the following day; this was selected as the high concentration for the teratology study of 2-butanol. No effects were detected in rats exposed to 6000 ppm 1-butanol. Rats exposed to 5000 ppm 2-butanol were partially narcotized, with locomotor activity impaired. This same level of t-butanol produced narcosis in all exposed animals, and was selected as the high concentration for the teratology study. At 3500 ppm 1-butanol or 2-butanol, animals were not visibly affected. However, both 5000 and 3500 ppm t-butanol produced an unsteady gait at

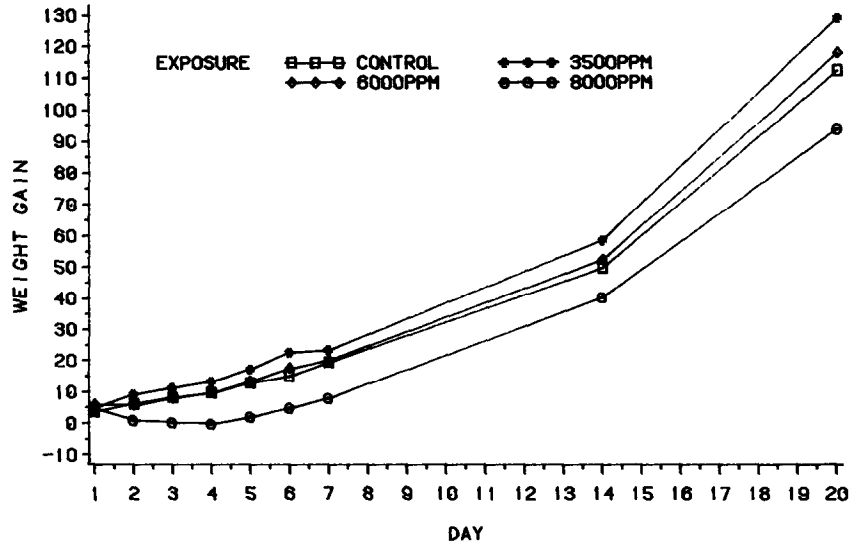

FIG. 1. Weight gain in dams exposed to 8000, 6000, 3500, or 0 ppm 1-butanol throughout gestation.

the end of the 7-hr exposure; all animals responded to a tap on the cage, but locomotor activity was impaired. In fact, after exposure to 2000 ppm t-butanol, the animals were also unsteady. Thus, 1-butanol was (subjectively) the least toxic to maternal animals, 2-butanol was of intermediate toxicity, and t-butanol was toxic at lower concentrations than were the others.

In general, objective measures of maternal toxicity increased in a dose-related manner for all three butanol isomers. Figures 1–3 depict weight gain, using the mean of daily weights for the first week and weekly weights thereafter. Statistical comparisons utilized analysis of variance with initial weight as a covariate (ANCOVA) for comparing treatment groups with their respective controls for weekly weights. These were followed by multivariate analyses (MANCOVA) to compare the entire gestational weight gain.

The highest concentration of 1-butanol (8000 ppm) produced mortality in 2 of 18 rats and reduced weight gain, but the statistical significance disappeared when adjusted for multiple comparisons. For 2-butanol, there were no mortalities in pregnant rats, but weight gain was significantly reduced by all three concentrations. The significant reduction appeared to occur at the highest concentration for all three weeks, but only for Weeks 1 and 3 at the two lower concentrations. Only the 5000 ppm t-butanol group was significantly lighter than controls.

Somewhat parallel with weight gain, food consumption was significantly reduced by at least the highest concentration of each isomer (Table 1). For 1-butanol, consumption was reduced both at 6000 and 8000 ppm (overall means were 382 g for controls versus 320 and 332 g, respectively). With 2-butanol, food consumption was reduced in all treatment groups (overall means were 408 g for controls and 364, 352, and 288 g for treatment groups in order of increasing concentration). Regarding food consumption, only the 5000 ppm t-butanol group consumed significantly less than their respective controls, and the significance was only in the first 2 weeks (overall, the means were 361 g for controls versus 278 g at 5000 ppm t-butanol). Water intake (data not shown) increased as pregnancy progressed and was generally higher, though not significantly, in treatment groups than in controls.

Fetotoxicity also generally increased with concentrations of each of the butanol isomers.

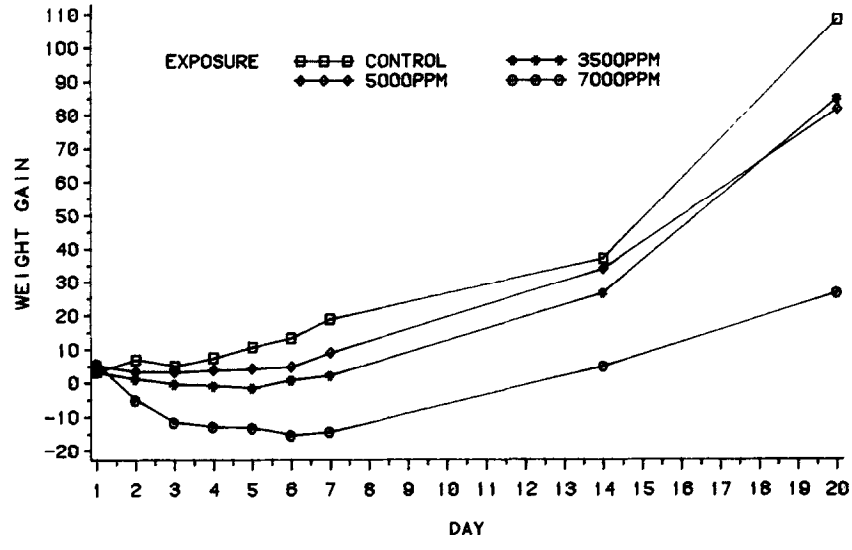

FIG. 2. Weight gain in dams exposed to 7000, 5000, 3500, or 0 ppm 2-butanol throughout gestation.

Tables 2–4 present the mean numbers of corpora lutea, resorptions, and live fetuses, the mean weights of female and male fetuses, the numbers of litters examined, malformations, and variations, and the percentages of litters normal for both skeletal and visceral examinations. The number of corpora lutea was not affected. The number of live fetuses was significantly reduced and resorptions were increased only at the highest concentration of 2-butanol. However, fetal weights were slightly depressed at the high and intermediate concentrations of all three isomers, as well as at the lowest concentration of t-butanol.

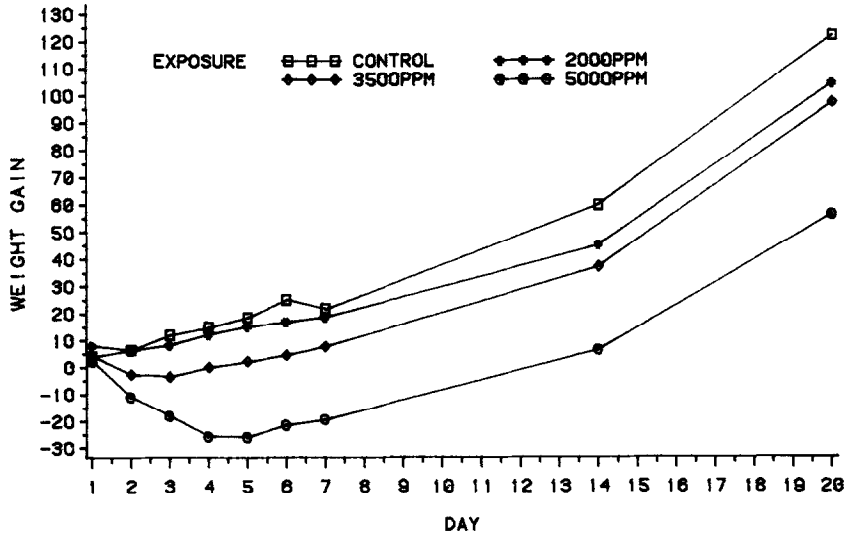

FIG. 3. Weight gain in dams exposed to 5000, 3500, 2000, or 0 ppm t-butanol throughout gestation.

TABLE 1

MEAN MATERNAL FEED INTAKE (g)

	Week 1	Week 2	Week 3
1-Butanol			
Control	124 ± 15	134 ± 21	124 ± 16
3500 ppm	142 ± 17	142 ± 16	133 ± 16
6000 ppm	102 ± 24*	108 ± 12*	110 ± 09*
8000 ppm	103 ± 24*	118 ± 17	111 ± 17
2-Butanol			
Control	150 ± 12	132 ± 14	126 ± 15
3500 ppm	132 ± 10*	119 ± 11*	113 ± 13
5000 ppm	118 ± 25*	122 ± 22	112 ± 17*
7000 ppm	88 ± 47*	101 ± 16*	99 ± 11*
t-Butanol			
Control	103 ± 24	135 ± 11	123 ± 14
2000 ppm	117 ± 17	121 ± 16*	116 ± 12
3500 ppm	93 ± 18	131 ± 12	126 ± 13
5000 ppm	66 ± 27*	97 ± 14*	115 ± 11

* Significantly different from respective control ($p < 0.05$).

No external malformations were observed. The majority of skeletal malformations were rudimentary cervical ribs. Skeletal variants generally increased with increasing concentrations of all three butanol isomers. The lowest concentration of each isomer was not associated with any defects in the exposed animals. Variations seen were typical of fetotoxicity, particularly reduced ossification. The only case in which the percentage of normal fetuses was reduced from control levels was at 8000 ppm 1-butanol, where rudimentary cervical ribs were observed. Occasional visceral malformations (e.g., ventricular septal defect, hydronephrosis) were seen, as were variations (e.g., enlarged brain ventricles, dilated renal pelvis), but the incidences were not significantly affected by treatment with any of the butanol isomers.

DISCUSSION

In this inhalation teratology study of three butanol isomers in rats, only slight teratogenicity was observed. In spite of evidence of both maternal toxicity (reduced weight gain and food intake) with at least the highest level of each isomer and fetotoxicity (reduced weights and increased variations), the only statistically significant increase was in skeletal malformations at the highest concentration of 1-butanol. This increase in malformations at a concentration not associated with a significant reduction in maternal weights (although two animals died and feed intake was reduced from control levels) suggests a possible selective developmental toxicity for this alcohol. The increases in variations seen at high concentrations of the other alcohols, associated with maternal toxicity, are indicative of developmental toxicity. As the concentrations were at least 50 to 100 times higher than permissible occupational exposure concentrations, and the lowest concentrations generally produced no effects at all, it is highly unlikely that any teratogenic effects would be detectable at the permissible occupational exposure concentrations in the parameters we examined in rats.

The presence of teratogenicity at the highest concentration of 1-butanol (8000 ppm) suggests selective developmental toxicity for this alcohol. Although feed intake was reduced from control levels at this concentration, maternal weight gain was not significantly compromised. Further, since the other butanol isomers produced maternal toxicity but not teratogenicity, maternal toxicity per se was probably not responsible for the teratogenicity observed. The dose-related decrease in fetal weights and apparent increase in skeletal variants at 6000 ppm 1-butanol suggest developmental toxicity at this concentration. However, no significant deviations from control were noted at 3500 ppm 1-butanol. For 2-butanol, the increase in resorptions, along with dose-related effects on fetal weights and variations, is indicative of developmental toxicity at the higher concentrations, although there were no significant effects at 3500 ppm. Tertiary butanol also evidenced developmental toxicity, with effects seen at

TABLE 2

FETAL OBSERVATIONS AFTER MATERNAL EXPOSURE TO 1-BUTANOL

	1-Butanol (ppm)			
	0	3500	6000	8000
Number pregnant/number bred	15/17	13/15	18/18	15/16
$\bar{x}$ Corpora lutea/litter	16 ± 2[a]	16 ± 2	17 ± 2	17 ± 2
$\bar{x}$ Resorptions/litter	1.1 ± 1.4	0.8 ± 1.0	0.9 ± 0.8	1.7 ± 1.7
$\bar{x}$ Live fetuses/litter	14 ± 3	15 ± 2	15 ± 1	13 ± 3
$\bar{x}$ Fetal weight (g)				
Female	3.3 ± 0.27	3.2 ± 0.18	2.9 ± 0.30*	2.4 ± 0.23*
Male	3.4 ± 0.31	3.4 ± 0.18	3.0 ± 0.31*	2.6 ± 0.25*
% Female/litter	49 ± 9	56 ± 11	48 ± 10	47 ± 12
Skeletal observations, litters (fetuses)				
No. examined	15 (102)	12 (85)	18 (129)	15 (98)
No. malformations	0	4 (5)	5 (8)	9 (16)
No. variations	14 (43)	11 (24)	17 (52)	25 (75)
% Fetuses normal	100 ± 0	94 ± 3	94 ± 3	85 ± 4*
Visceral observations, litters (fetuses)				
No. examined	15 (106)	13 (97)	18 (134)	15 (96)
No. malformations	0	0	2 (2)	4 (8)
No. variations	4 (7)	6 (8)	4 (6)	8 (19)
% Fetuses normal	100 ± 0	100 ± 0	99 ± 1	92 ± 4

[a] ± SD.

* $p < 0.05$ when compared with appropriate control.

all concentrations, although these were also associated with maternal toxicity.

The low teratogenic potential of the butanols in this study contrasts with the results seen with methanol (Nelson *et al.*, 1985) and the propanols (Nelson *et al.*, 1988). From a structural standpoint, two of the butanol isomers included in the present study could also be considered as derivatives of propanol, viz., 2-butanol (1-methyl-1-propanol) and t-butanol (2-methyl-2-propanol). Whereas maternally toxic concentrations of both *n*-propyl alcohol and isopropyl alcohol produced teratogenicity in rats (Nelson *et al.*, 1988), neither of the derivatives investigated in the present study produced such effects. This may be due to differences in such properties as the physiochemical nature of the alcohols (including their ability to cross biological membranes) or metabolic considerations. It appears, therefore, that the teratogenic potential of these propanol derivatives is more similar to that found after butanol exposure than after propanol exposure, at least in their effects on the parameters studied here.

For comparison with other studies and with occupational exposures, it is useful to estimate the absorbed daily dose in mg/kg/day. Using a minute volume of 45 ml for rats during the 420 min of daily exposure, it can be estimated that the rats inhaled approximately 18.9 liters of vapor during an exposure day.

TABLE 3

FETAL OBSERVATIONS AFTER MATERNAL EXPOSURE TO 2-BUTANOL

	2-butanol (ppm)			
	0	3500	5000	7000
Number pregnant/number bred	15/16	16/16	14/15	11/15
$\bar{x}$ Corpora lutea/litter	16 ± 2[a]	17 ± 1	16 ± 2	16 ± 2
$\bar{x}$ Resorptions/litter	1.5 ± 1.3	1.6 ± 1.4	1.5 ± 0.9	3.8 ± 2.2*
$\bar{x}$ Live fetuses/litter	14 ± 2	15 ± 2	14 ± 3	10 ± 3*
$\bar{x}$ Fetal weight (g)				
Female	3.1 ± 0.22	2.9 ± 0.20	2.6 ± 0.23*	1.4 ± 0.18*
Male	3.3 ± 0.23	3.1 ± 0.22	2.7 ± 0.25*	1.5 ± 0.12*
% Female/litter	50 ± 12	60 ± 13	52 ± 12	56 ± 13
Skeletal observations, litters (fetuses)				
No. examined	15 (102)	14 (104)	14 (93)	11 (53)
No. malformations	1 (1)	1 (1)	4 (4)	2 (2)
No. variations	13 (33)	13 (40)	12 (32)	11 (53)*
% Fetuses normal	99 ± 1	99 ± 1	95 ± 2	97 ± 2
Visceral observations, litters (fetuses)				
No. examined	15 (106)	14 (105)	14 (98)	11 (57)
No. malformations	1 (2)	1 (1)	2 (4)	1 (1)
No. variations	3 (3)	7 (14)	6 (14)	11 (52)
% Fetuses normal	98 ± 2	99 ± 1	96 ± 3	98 ± 2

[a] ±SD.

* $p < 0.05$ when compared with appropriate control.

It can also be assumed that no more than one-half of the butanols in the inspired air would be absorbed (Astrand *et al.*, 1976). Thus, at 10,000 ppm (30 mg/liter) the rats would inhale about 567 mg (18.9 liters × 30 mg/liter) of butanol, and absorb approximately 283 mg/day. Using an approximate body weight of 280 g (Day 15), this is an approximate daily absorbed dose on the order of 1000 mg/kg (283 mg/280 g × 1000). A series of such calculations leads to estimated daily absorbed doses of 800, 600, and 350 mg/kg at 8000, 6000, and 3500 ppm 1-butanol; 700, 500, and 350 mg/kg at 7000, 5000, and 3500 ppm 2-butanol; and 500, 350, and 200 mg/kg at 5000, 3500, and 2000 ppm t-butanol. Experimental studies with human subjects (Astrand *et al.*, 1976) exposed at 100 and 200 ppm 1-butanol suggest that daily doses absorbed by workers exposed at the permissible exposure levels (100 ppm) would not exceed 280 mg/kg. This is approximately the amount absorbed at the lowest concentrations included in the present study.

The results of the present research can be compared with those of Daniel and Evans (1982) for t-butanol, although the route and duration of exposure were different, as was the species, and their study included only postnatal observations. Daniel and Evans (1982) administered three to nearly 7 g/kg/day, so their doses were 10 to 30 times higher than those in the present study. Further, the small number of litters, the limited testing of

TABLE 4

FETAL OBSERVATIONS AFTER MATERNAL EXPOSURE TO t-BUTANOL

	t-Butanol (ppm)			
	0	2000	3500	5000
Number pregnant/number bred	15/16	18/20	15/15	13/15
$\bar{x}$ Corpora lutea/litter	16 ± 2[a]	16 ± 2	16 ± 2	16 ± 2
$\bar{x}$ Resorptions/litter	1.1 ± 1.2	1.2 ± 1.1	0.9 ± 1.0	1.1 ± 0.9
$\bar{x}$ Live fetuses/litter	13 ± 2	13 ± 4	15 ± 2	14 ± 2
$\bar{x}$ Fetal weight (g)				
Female	3.2 ± 0.23	2.9 ± 0.20*	2.8 ± 0.20*	2.2 ± 0.34*
Male	3.4 ± 0.21	3.1 ± 0.19*	3.0 ± 0.24*	2.3 ± 0.34*
% Females/litter	56 ± 16	53 ± 13	50 ± 12	46 ± 16
Skeletal observations, litters (fetuses)				
No. examined	15 (96)	17 (104)	14 (103)	12 (83)
No. malformations	0	0	2 (2)	2 (4)
No. variations	10 (18)	14 (35)	14 (53*)	12 (76*)
% Fetuses normal	100 ± 0	100 ± 0	98 ± 1	95 ± 4
Visceral observations, litters (fetuses)				
No. examined	15 (100)	17 (116)	14 (102)	12 (83)
No. malformations	1 (1)	1 (1)	2 (4)	1 (1)
No. variations	6 (6)	4 (4)	6 (6)	12 (27)
% Fetuses normal	99 ± 1	99 ± 1	96 ± 3	99 ± 1

[a] ±SD.

* $p < 0.05$ when compared with appropriate control.

animals (in terms of both the number of tests and the ages of testing), and use of the number of pups rather than the number of litters as the basis for statistical analyses all make interpretation of the Daniel and Evans results difficult. Nonetheless, our observations of dose-related decreased fetal weights and increased skeletal variations, in the absence of apparent maternal toxicity, are indicative of developmental toxicity. Although we did not allow any litters to survive postnatally, it may be that this developmental toxicity would have been reflected in decreased survivability of these offspring, consistent with the observations of Daniel and Evans.

These studies indicate that if sufficiently high daily doses (3200 mg/kg) of t-butanol are administered to experimental animals, adverse effects on developing tissues would be expected. However, such high doses would likely not be achievable by inhalation, since an exposure concentration of 30,000 ppm would be required to produce such effects. Since the highest concentration of 1-butanol used in the current study approached that in saturated air, it is unlikely that adverse effects from 1-butanol would occur after occupational inhalation. Absorption of liquid 1-butanol through exposed skin does not appear to occur at a sufficiently rapid rate to be potentially teratogenic (DiVincenzo and Hamilton, 1979).

Investigators have also proposed pharmacokinetic models to describe the distribution,

biotransformation, and elimination of 2-butanol (Dietz *et al.*, 1981) and structure–activity relationships among the alcohols (Kier and Hall, 1982). These suggest a similar lack of adverse effects following human exposure to s- and t-butanol.

In summary, inhalation of a high concentration of 1-butanol (8000 ppm) was teratogenic to rats, and was associated with maternal toxicity. High concentrations of each of the isomers examined produced developmental toxicity. However, it is apparent that 1-butanol, 2-butanol, and t-butanol are not strongly selective developmental toxicants in rats, at least in the parameters examined here. That is, if teratogenic effects were to be observed, they would likely occur only in the presence of maternal toxicity. Our results suggest that concentrations of these butanol isomers that are currently permitted in the workplace would likely not produce developmental toxicity in rats.

ACKNOWLEDGMENTS

We thank S. K. Robertson, D. R. MacKenzie-Taylor, B. J. Taylor, and Jeff McLaurin for their excellent technical help in the conduct of this research, Ms. Pam Schumacher for preparation of the manuscript, Dr. Robert Mason for his thoughtful review of the manuscript and his additions to the Discussion, and Dr. W. Kent Anger for his continuing support and reviews of the manuscript.

REFERENCES

ACGIH (1986). *TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment with Intended Changes for 1985–1986.* ACGIH, Cincinnati, OH.

ASTRAND, I., OVRUM, P., LINDQVIST, T., AND HULTENGREN, M. (1976). Exposure to butyl alcohol: Uptake and distribution in man. *Scand. J. Work Environ. Health* **2,** 165–175.

DANIEL, M. A., AND EVANS, M. A. (1982). Quantitative comparison of maternal ethanol and maternal t-butanol diet on postnatal development. *J. Pharmacol. Exp. Ther.* **222**(2), 294–300.

DIETZ, F. K., RODRIQUEZ-GIAXOLA, M., TRAIGER, G. J., STELLA, V. S., AND HIMMELSTEIN, K. J. (1981). Pharmacokinetics of 2-butanol and its metabolites in the rat. *J. Pharmacokin. Biopharmacol.* **9**(5), 553–576.

DIVINCENZO, G. D., AND HAMILTON, M. L. (1979). Fate of *n*-butanol in rats after oral administration and its uptake by dogs after inhalation or skin application. *Toxicol. Appl. Pharmacol.* **48,** 317–325.

GRANT, K. A., AND SAMSON, H. H. (1982). Ethanol and tertiary butanol induced microcephaly in the neonatal rat: Comparison of brain growth parameters. *Neurobehav. Toxicol. Teratol.* **4,** 315–321.

KIER, L. B., AND HALL, L. H. (1982). Structural influences and mechanisms of toxic effects of alcohols and their derivatives. *Bull. Environ. Contam. Toxicol.* **29,** 121–126.

NELSON, B. K., BRIGHTWELL, W. S., MACKENZIE, D. R., KHAN, A., BURG, J. R., WEIGEL, W. W., AND GOAD, P. T. (1985). Teratological assessment of methanol and ethanol at high inhalation levels in rats. *Fundam. Appl. Toxicol.* **5,** 727–736.

NELSON, B. K., BRIGHTWELL, W. S., MACKENZIE, D. R., KHAN, A., BURG, J. R., WEIGEL, W. W., AND GOAD, P. T. (1988). Teratogenicity of *n*-propanol and isopropanol administered at high inhalation concentrations to rats. *Food Chem. Toxicol.* **26,** 247–254.

NIOSH (1977). *NIOSH Manual of Analytical Methods, Part II, Standards Completion Program Validated Methods,* 2nd ed., Vol. 2. Chaps. S53 and S62. U.S. Dept. of Health, Education, and Welfare (NIOSH) Publication No. 77-157A, Cincinnati, OH.

NIOSH (1980). *Quarterly Hazard Summary Report by Estimated Number of Population, 08/06/80.* NIOSH, Cincinnati, OH.

ROWE, V. K., AND MCCOLLISTER, S. B. (1982). Alcohols. In *Patty's Industrial Hygiene and Toxicology* (G. D. Clayton and F. E. Clayton, Eds), 3rd ed., Vol. 2C, pp. 4527–4708. Wiley, New York.

WILSON, J. G. (1965). Methods for administering agents and detecting malformations in experimental animals. In *Teratology: Principles and Techniques* (J. G. Wilson and J. Warkany, Eds.), pp. 262–277. Univ. of Chicago Press, Chicago.