The effect of inhaled benzene on embryonal and fetal development was assessed in
and rabbits CF-1 mice and New Zealand white rabbits were exposed to 0 or 500 p
benzene for 7 hr per day from days 6 through 15 (mice) and 6 through 18 (rabbit
gestation. Little evidence of maternal toxicity was seen in either species. Although some
signs of embryonal toxicity were observed in both mice and rabbits, a teratogenic effec
was not discerned in either species inhaling 500 ppm of benzene.

Embryotoxicity of inhaled benzene in mice and rabbits

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introduction

Benzene (benzol, C_6H_6) is used primarily as a starting material in the production of a wide variety of organic chemicals. Benzene is also inherently present in some industrial hydrocarbon solvents and in motor gasoline as a high octane component. Exposure to benzene vapors produces central nervous system depression."' Occupational exposure to high levels of benzene on a chronic basis has been associated with leukemia,[2-4] aplastic anemia,[5,6] and chromosomal aberrations.[7-9]

Several studies have been conducted in laboratory rats to evaluate the potential teratogenicity of inhaled benzene. In one study,"'' pregnant rats were exposed to 0, 10, 50, or 500 ppm of benzene for 7 hr per day from days 6 through 15 of gestation; a low incidence of fetal malformations (exencephaly, angulated ribs, and non-sequential ossification of forefeet) was observed among the litters of rats exposed to 500 ppm. suggesting a possible teratogenic effect at that concentration. No evidence of a teratogenic effect was found by other investigators who exposed rats to 300 to 2200 ppm of benzene for 6 hr per day from days 6 through 15 of gestation.[11] In a third study."') a teratogenic effect was not seen among rats inhaling 10 or 40 ppm of benzene for 6 hr per day from days 6 through 15 of gestation; however, increased embryolethality was noted at both levels of exposure.

The purpose of the study presented herein was to assess the effect of inhaled benzene on embryonal and fetal development in two additional species, the mouse and the rabbit.

materials and methods

Rcnzene (industrial grade), identified as ID95-01000-0070, was supplied for use in this study by Exxon Chemical Company U.S.A., Houston, Texas. However, on at least two days of exposure, but no more than 5 days. the test animals were exposed inadvertently to a sample of benzene obtained from Mallinckrodt Chemical Company; the Mallinckrodt benzene was identified as nanograde quality, Lot No. 1043B3.

Virgin CF-I mice (Charles River, Portage,, Michigan) and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) were housed in wire-bottomed cages in temperature- and humidity-controlled rooms with a 12 hr light-dark cycle. The animals were maintained on commercial laboratory chow (Ralston Purina Company, St. Louis. Missouri) and tap water *ad libitum* except while in the exposure chambers. Mice and rabbits were allowed at

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least two and three weeks, respectively. for acclimatization *to* the laboratory before use. The day on which a vaginal plug was observed in mice or the day on which rabbits were bred naturally **was** considered day zero of gestation.

Groups of 35 and 37 bred mice were exposed in chambers to filtered room air or 500 ppm of benzene, respectively, for 7 hr per day from days 6 through 15 of gestation. Concurrently, groups of 20 bred rabbits each were exposed in the same chambers to filtered room air or 500 ppm of benzene for 7 hr per day from days 6 through 18 of gestation.

Exposures were conducted under dynamic airflow conditions in stainless steel and glass Rochester-type chambers **of4.3**m^3 volume. The atmosphere was generated by metering liquid benzene at a controlled rate into a heated vaporization **flask.** The vapors were then blown into theairstream being drawn into thechamber. Concentrations in the chambers were analyzed daily throughout exposure by alternately sampling the experimental and control chambers at 60 and 30 minute intervals, respectively, using a Miran 1 infrared spectrophotometer at a wavelength of **3.25** microns. The mean (±S.D.) of the daily time-weighted average concentrations of benzene in the exposure chamber was 507±12 ppm.

Tlie animals were observed daily from day **6** of gestation. and were weighed at several intervals during *the* experimental period. Food and water consumptions were recorded during the experimental period at 3-day intervals for *the* mice and at daily intervals for the rabbits. On day 18 and 29 of gestation, the mice and rabbits, respectively, were sacrificed by carbon dioxide inhalation. The number and position of live, dead, and resorbed fetuses were noted. All fetuses were weighed, measured (crown-rump length), sexed, and examined for external alterations and cleft palate.

One-third of the fetuses of each litter, selected at random, was examined immediately for evidence *of* visceral alterations by dissection under a low power stereo microscope.[15] Heads of mouse fetuses which were examined for visceral alterations were placed in Bouin's solution and subsequently examined by the razor-section technique of Wilson.[13] All of the fetuses from each litter of mice and rabbits were cleared with KOH, stained with alizarin red-S,[14] and examined for skeletal alterations.

At the time of sacrifice, blood samples were obtained through an incision in the neck of individual rabbit fetuses from five different litters of the control and benzene-exposed groups. Hematologic determinations were not conducted on fetal mice since sufficient quantities of blood *to* perform the evaluations could not be obtained. Additional groups of 8 bred mice and 4 bred rabbits each were exposed concurrently with the teratology-study animals. Blood samples were obtained from mice by decapitation on day 16 of gestation and from rabbits by cardiac puncture on day 19 of gestation. The following parameters were analyzed in adult and fetal blood: packed cell volume, percent hemoglobin, red blood cell counts, and white blood cell counts.

The Wilcoxon test as modified by Haseman and Hoel[16] was used to evaluate the incidence of fetal alterations and resorptions. Maternal and fetal body weights, food and water consumption data.. and hematologic data were analyzed statistically by a one-way analysis of variance.[17] In all cases. the level of significance chosen was $p < 0.05$.

results

Exposure of bred miceand rabbits to 500 ppm of benzene for 7 hr per day had no significant effect on the dams' appearance, demeanor, body weight, or body weight gain. Food and water consumptions of pregnant mice were not altered by. inhalation of benzene. The amount of food and water consumed by pregnant rabbits exposed to benzene **was** significantly greater than that of the controls on a few days toward the end of the csposure period.

Observations made at the time of sacrifice of the dams are presented-in Table 1. Inhalation of 500 ppm of benzene did not significantly affect the incidence of pregnancy in mice or rabbits. No significant effect on the average number of live fetuses or resorptions per litter was discerned in either species. Mean fetal body weight but not crown-rump length was decreased significantly among litters of mice exposed to benzene. In rabbits, fetal body measurements were not altered significantly by exposure to benzene.

TABLE I
Observations at Sacrifice of Mice and Rabbits Exposed to 500 PPM of Benzene

	Mice		Rabbits	
	Control	Benzene	Control	Benzene
No of bred females	35	37	20	20
% Pregnant	74	81	90	95
No of lifters	26	30	18	19
Implantations/dam[A]	12±2	13±2	9±2	9±2
Fetuses/litter[A]	11±2	11±2	8±2	8±2
Resorptions/litter[A]	1.5±1.4	1.9L2.1	0.5±0.6	0.7±1.0
Fetal body weight g[A]	1.01±0.09	0.95±0.10[B]	394'57	385'50
Fetal crown rump length. mm[A]	24.1±1.3	23.8±1.1	97.2±7.5	95.1±6.0

[A]Mean of litter values ± standard deviation
[B]Significantly different from the control value, $p < 0.05$

The incidence of fetal malformations among litters of mice and rabbits is summarized in Table II. Among the offspring of mice exposed to benzene. no major malformation occurred at an incidence significantly different from that of the controls. Cleft palate was observed in single fetuses in both control and benzene-exposed groups of mice; in the latter case, the fetus with cleft palate also had asymmetric vertebrae **and** fused ribs. Significant increases in the occurrence of several minor skeletal variants (not considered to be malformations) were seen in litters of benzene-exposed mice, including delayed ossification of sternebrae, delayed ossification of **skull** bones, and unfused occipital bones of the skull.

In rabbits, exposure to benzene did not significantly alter the occurrence of fetal malformations, when considered individually or collectively. **A** single case of gastroschisis was noted in a litter exposed to benzene. Fused thoracic vertebrae accompanied by fused ribs were observed in another benzene-exposed litter. Another exposed fetus exhibited fused ribs only. No major malformations were observed among the control group. Two minor skeletal variants, i.e., lumbar spurs and the proportion of fetuses with 13 ribs (the normal number is 12 or 13), occurred significantly **less** often among the litters of rabbits exposed to benzene than among the control litters.

Table III shows the results of the hematologic evaluation following exposure to benzene. Hematologic values of adult bred mice and rabbits exposed to benzene were not significantly different from the control **values.** No significant hematologicchanges were evident among the fetal offspring of rabbits exposed to benzene. Hematologic evaluation of fetal mice

TABLE II
Fetal Malformations Among Litters of Mice and Rabbits Exposed to 500 PPM of Benzene

	Mice		Rabbits	
	Control	Benzene	Control	Benzene
	No Fetuses (Liners) Examined			
External Examination	279(26)	325130)	150(18)	152(191
Visceral Examination	97(26)	112(30)	56(18)	59(19)
Skeletal Examination[A]	253(24)	286(28)	142(17)	152(19)
	No Fetuses (Litera) Affected			
Total Malformations	1(1)[B]	1(1)[C]	0(0)	3(3)[D]

[A]Due to problems infixation several mouselitters and one rabbit litter were not examined for skeletal alterations
[B]Cleft palate
[C]Cleft palate fused ribs and asymmetric vertebrae in one fetus
[D]Gastroschisis fused ribs fused ribs and thoracic vertebrae

TABLE III
Hematologic Evaluation Following Exposure to Benzene

Species	Benzene (ppm)	PCV (%)	REC ×10⁶/mm³	Hgb g/100 mL	WBC ×10³/mm³
Mouse. adult[a]	0	50±2	8 6±0 4	16 3±0.6	7 2±1 1
	500	48±2	8 4±0.2	16.0±0 6	6 9±1 6
Mouse, fetal nor determined					
Rabbit, adult[a]	0	40±6	5911 1	13 3±2 4	6.9±2.9
	500	39±4	5 8±0.7	12.7±1 2	5.8±0 7
Rabbit, fetal[b]	0	44±6	3.7±0 4	12 2±1 4	0 65±0 13
	500	47±4	4 1±0.4	139'1 2	0 89±0 32

[a]Blood was collected on days 16 (mice) and 19 (rabbits) of geslation
[b]Blood was collected on day 29 of gestation
Values are expressed as the mean ± standard deviation

was not performed since a sufficient quantity of blood could not be obtained.

discussion

The results of the present study, as well as those of earlier teratology studies on inhaled benzene, are summarized in Table IV. In the present study, benzene was not teratogenic in either mice or rabbits inhaling500 ppm of the compound for 7 hr per day during the period of major organogenesis. The few malformations which were observed among the benzene-exposed litters were types that have been noted to occur spontaneously at a low incidence in previous control groups. In comparison, a possible teratogenic effect was reported in rats exposed to 500 ppm of benzene for 7 hr per day from days 6 through 15 of gestation.[11] However, in another study,[11] teratogenicity was not observed in rats inhaling up to 2200 ppm of benzene for 6 hr per day on the same days of gestation.

Although a teratogenic effect was not found, some evidence of embryotoxicity was noted in mice and rabbits exposed to benzene. In mice. increased incidences of delayed ossification of skull bone and sternebrae were observed among the offspring of dams exposed to benzene. This effect was associated with a decrease in the average fetal body weight. In rabbits. the only statistically significant evidence of an effect on embryonal or fctal development was a decrease in the occurrence of two minor skeletal variants: lumber spurs and the proportion of fetuses with 13 ribs.

TABLE IV
Summary of Results of Teratology Studies on Inhaled Benzene

Species	Benzene (ppm)	Maternal Weight Gain	Feral Body Weight	Resorptions	Skeletal Variants	Malformations
CF-1 Mouse	500		DEC		INC	
NZ Rabbit	500				INC	
S-D Rat[11]	10					
	50	DEC	DEC		INC	
	500	DEC	DEC		INC	INC[a]
S-D Rat[11]	100					
	300				INC	
	300[b]					
	2200	DEC	DEC		INC	
S-D Rat[11]	1o		-	INC		
	40		-	INC		

(-) = No significant difference compared to the control value. DEC = decreased compared to the control value.
INC = increased compared to the control value
[a]Possible reratogrnic effect (not statistically significant)
[b]The 300 ppm concentration was tested twice

In rats, increased frequencies of minor skeletal variants (delayed ossification of sternebrae and missing sternebrae) were reported in female but not male offspring of dams exposed to benzene.[11] The present study showed significant increases in the incidences of delayed ossification of sternebrae, delayed ossification of skull bones, and unfused occipital bones of the skull in litters of benzene-exposed mice. As in the above study,[11] these observations have been judged minor skeletal variations. not to be considered malformations.

Investigators have reported an increased incidence of resorptions among bred rats exposed for 6 hr per day to 10 or 40 ppm of benzene.[12] But, in two other teratology studies[10-11] on benzene in rats, no significant effect on the occurrence of resorptions was seen at levels of exposure as high as 500 and 2200 ppm of benzene. In the present study, inhalation of 500 ppm of benzene did not significantly affect the incidence of resorptions in either mice or rabbits.

The lack of teratogenicity in mice in the present study contrasts with an earlier study in which cleft palate was observed in three of fifteen liters of mice given a single subcutaneous injection of 3 mL benzene/kg body weight on day 13 of gestation.[11] However, it is possible that the cleft palates attributed to benzene were actually produced by the stress of such a massive subcutaneous injection; unfortunately, a vehicle control group was not included in that study. In the present study, one fetus with cleft palate was observed in each of the control and exposed groups.

The concentration of benzene tested in this study, 500 ppm, was markedly higher than the current OSHA standard of 10 ppm.[19] Moreover. the difference in dose between humans and animals may be even greater than that indicated by concentration alone. The amount of air inhaled per minute by mice and rabbits in proportion to their body weight is approximately 10 and 3-5 times, respectively, that of humans.[20] Thus, the present findings do not indicate a teratogenic hazard in humans exposcd to 10 ppm or less of benzene.

In summary, a teratogenic effect was not discerned in either mice or rabbits inhaling 500 ppm of benzene for 7 hr per day during the period of major organogenesis. The only evidence of embryotoxicity or fetotoxicity seen in this study was the altered occurrence of some minor skeletal variations, which in mice was associated with a decrease in fetal body weight.

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AN EVALUATION OF THE MANUSCRIPT, "CYTOGENETIC STUDY OF WORKERS EXPOSED TO BENZENE", BY DANTE PICCIANO, Ph.D.

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