

R & D REPORT

DOW CHEMICAL U.S.A.

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CRI NUMBER

LABORATORY REPORT CODE

HET K-002661-(11)

DATE ISSUED

August 3, 1978

REV
8(E)

DEPARTMENT

Toxicology Research Laboratory

LAB. NO.

PROBLEM NO.

91 91 81 01 11 21 11 11 01 9

TITLE

Embryotoxicity of Inhaled Benzene in Mice and Rabbits

47

PAGES
IN FULL
REPORT

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report
is:☐ INTERIM☒ FINAL

and mainly:

☒ NEW☐ REVIEW

DATA REFERENCES (book and page):

(Refer also to earlier related reports and publications.)

PATENT STATUS: ☐ disclosure submitted ☐ case filed ☒ no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

The effect of inhaled benzene on embryonal and fetal development was assessed in mice and rabbits. CF-1 mice and New Zealand white rabbits were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Little evidence of maternal toxicity was discerned in either species. Exposure to benzene altered the incidence of some minor skeletal variants among the offspring of both species. In mice, a significant decrease in fetal body weight was noted. Although some evidence of embryotoxicity was seen in both species, a teratogenic effect was not observed in either mice or rabbits inhaling 500 ppm of benzene.

DO 136413
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EMBRYOTOXICITY OF INHALED BENZENE IN MICE AND RABBITS

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This work was sponsored jointly by Exxon Corporation and
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DO 136414
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EMBRYOTOXICITY OF INHALED BENZENE IN MICE AND RABBITS

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Summary

The effect of inhaled benzene on embryonal and fetal development was assessed in mice and rabbits. CF-1 mice and New Zealand white rabbits were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Little evidence of maternal toxicity was discerned in either species. Exposure to benzene altered the incidence of some minor skeletal variants among the offspring of both species. In mice, a significant decrease in fetal body weight was noted. Although some evidence of embryotoxicity was seen in both species, a teratogenic effect was not observed in either mice or rabbits inhaling 500 ppm of benzene.

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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 used as an industrial solvent and as an anti-knock agent in gasoline. Acute exposure to benzene vapors produces central nervous system depression (Browning, 1965). Occupational exposure to high levels of benzene on a chronic basis has been associated with leukemia (Aksoy et al., 1972a; 1974; Berlin, 1974), aplastic anemia (Saita, 1973; Aksoy, 1972b), and chromosomal aberrations (Pollini and Colombi, 1964; Vigliani and Forni, 1969; Forni et al., 1971). The OSHA-proposed standard for an occupational exposure to benzene for 8 hours per day, 5 days per week is 1 ppm (OSHA, 1977).

Several studies have been conducted in laboratory animals to evaluate the potential teratogenicity of benzene. Watanabe and Yoshida (1970) reported cleft palate, agnathia, and micrognathia among the offspring of mice given a massive subcutaneous injection of 3 ml benzene/kg body weight on day 13 of gestation. In another study, pregnant rats were exposed to 0, 10, 50, or 500 ppm of benzene for 7 hours per day from days 6 through 15 of gestation (unpublished data,

Hazleton Laboratories Corp., Vienna, Va.). A low incidence of 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. In contrast, Green et al., (1978) found no evidence of a teratogenic effect in Sprague-Dawley rats inhaling 300 or 2200 ppm of benzene for 6 hours per day from days 6 through 15 of gestation.

The purpose of the study presented herein was to assess the effect of inhaled benzene on embryonal and fetal development in two additional species. For these studies, pregnant CF-1 mice and New Zealand white rabbits were exposed to 500 ppm of benzene by inhalation for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Since the development of leucopenia has been demonstrated in rats, guinea pigs, and rabbits following repeated exposure to benzene vapors (Wolf et al., 1956), blood samples from the female mice and rabbits and from rabbit fetuses were collected for hematologic evaluation. This work was sponsored jointly by Exxon Corporation and The Dow Chemical Company.

MATERIALS AND METHODS

Test Material. Benzene, identified as ID95-01000-0070, was supplied by Exxon Chemical Company U.S.A., Houston, Texas. The test sample was analyzed at the Analytical and Information Division, Exxon Research and Engineering Company, Linden, New Jersey (Table 1). On at least two days of exposure, however, 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. Samples of both the Exxon and Mallinckrodt benzenes have been retained for possible comparative analysis.

Test Animals. Virgin CF-1 mice (Charles River, Portage, Michigan) and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) were housed in wire mesh cages in a room controlled for temperature ($70 \pm 3^{\circ}\text{F}$), humidity ($45 \pm 5\%$), and light cycle (12 hrs light and dark). The animals were maintained on commercial laboratory chow (Ralston Purina Company, St. Louis, Missouri) and tap water free choice except while in the exposure chambers. Mice and rabbits were allowed at 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 was considered day zero of gestation. The animals were uniquely identified by means of an ear punch (mice) or a metal ear tag (rabbits).

Experimental Design. Groups of 35 and 37 bred mice were exposed in chambers to filtered room air or 500 ppm of benzene, respectively, for 7 hours 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 hours per day from days 6 through 18 of gestation.

Inhalation studies were conducted under dynamic airflow conditions in stainless steel and glass Rochester-type chambers of 4.3 cubic meter volume. The atmosphere was generated by metering liquid benzene at a known rate into a heated vaporization flask and then into the airstream being drawn into the chamber. Concentrations achieved 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 I infrared spectrophotometer at a wavelength of 3.25μ .

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Standard samples were prepared daily by injecting known quantities of benzene into 100 liter plastic bags. The time weighted analytical concentration of benzene in the exposure chamber was 507 ± 12 ppm (mean $\pm$ standard deviation).

Maternal Observations. The animals were observed daily from days 6 of gestation until sacrifice for indications of toxicity from the test material. The mice were weighed on days 6, 8, 10, and 16 of gestation; the rabbits were weighed on days 6, 8, 10, 12, and 19 of gestation. In addition, maternal body and liver weights were recorded at the time of cesarean section, day 18 and 29 of gestation for mice and rabbits, respectively. Food and water consumptions were recorded during the experimental period at 3-day intervals for the mice and daily intervals for the rabbits.

Fetal Examination. On day 18 and 29 of gestation, the mice and rabbits, respectively, were sacrificed by carbon dioxide inhalation. The uterine horns were exteriorized through a mid-line incision in the abdominal wall, and the number and position of live, dead, and resorbed fetuses were noted. The uterus of each apparently non-pregnant female was stained with a 10% solution of sodium sulfide and examined for evidence of early resorption sites (Kopf et al., 1964); this procedure

was conducted solely for the purpose of determining the incidence of pregnancy, not the incidence of resorptions.

After being weighed, measured (crown-rump length), and sexed, all fetuses were examined for external alterations and cleft palate. One-third of the fetuses of each litter, selected at random, was examined immediately for evidence of soft-tissue alterations by dissection under a low power stereo microscope (Staples, 1974). The head of each mouse fetus which was examined for soft-tissue alterations was placed in Bouin's solution and subsequently examined by the razor section technique of Wilson (1965). All of the fetuses in each litter were cleared and stained with alizarin red-S (Dawson, 1926) to permit examination for skeletal alterations.

Hematologic Evaluation. At the time of cesarean section 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. The following parameters were analyzed: packed cell volume, hemoglobin content, red blood cell counts, and white blood cell counts. Hematologic determinations were not conducted on fetal mice since sufficient quantities of blood to perform the evaluations could not be obtained.

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Additional groups of eight bred mice and four bred rabbits each were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation. Blood samples were obtained from maternal mice by decapitation on day 16 of gestation and from maternal rabbits by cardiac puncture on day 19 of gestation. The following parameters were analyzed: packed cell volume, hemoglobin content, red blood cell counts, white blood cell counts, and differential white blood cell counts.

Statistical Evaluation. The Wilcoxon test as modified by Haseman and Hoel (1974) was used to evaluate the incidence of fetal alterations and resorptions. Maternal and fetal body weights, maternal liver weights, food and water consumption data, and hematologic data were analyzed statistically by a one-way analysis of variance (Steel and Torrie, 1960). In all cases, the level of significance chosen was $p < 0.05$.

RESULTS

Maternal Toxicity. Exposure of bred mice and rabbits to 500 ppm of benzene for 7 hours per day had no significant effect on the dams' appearance, demeanor, body weight, body weight gain, or liver weight (Tables 2 and 3). Food and water consumptions of pregnant mice were not altered by inhalation of benzene (Table 4). In comparison, the amount of food and water consumed by the pregnant rabbits exposed to benzene was significantly greater than that of the controls on a few days toward the end of the exposure (Tables 5 and 6).

Embryo- or Fetotoxicity. Inhalation of 500 ppm of benzene did not significantly affect the incidence of pregnancy in either mice or rabbits (Tables 7 and 8). No significant effect on the average number of live fetuses per litter or on the incidence of resorptions was discerned in either species.

Mean fetal body weight but not crown-rump length was decreased significantly among litters of mice exposed to benzene (Table 7). In rabbits, neither fetal body measurement was altered significantly by exposure to benzene (Table 8).

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Fetal Alterations. Among the offspring of mice exposed to benzene, no major malformation occurred at an incidence significantly different from that of the controls (Table 9). 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 exhibited asymmetric vertebrae as well as forked and fused ribs. Significant increases in the occurrence of several minor skeletal variants 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 (Table 10). A single case of gastroschisis was noted in a litter exposed to benzene. Fused thoracic vertebrae accompanied by fused and forked 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., 13 rib(s) and lumbar spurs occurred significantly less often among the litters of rabbits exposed to benzene.

Hematologic Evaluation. Exposure to 500 ppm-of benzene from days 6 through 18 of gestation did not produce any significant hematologic changes among the fetal rabbits (Table 11). Hematologic evaluation of fetal mice was not performed since a sufficient quantity of blood could not be obtained. Hematologic values of adult bred mice and rabbits exposed to benzene were not significantly different from the control values.

DISCUSSION

Benzene was not teratogenic in either mice or rabbits inhaling 500 ppm of the compound for 7 hours per day. 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 hours per day from days 6 through 15 of gestation (unpublished data, Hazleton Laboratories Corp. Vienna, Va.); however, Green et al. (1978) did not observe teratogenicity in rats inhaling up to 2200 ppm of benzene for 6 hours per day on the same days of gestation.

The lack of teratogenicity observed in the present study contrasts with the findings of Watanabe and Yoshida (1970); in that study, cleft palate was observed in three of fifteen litters of mice given a single subcutaneous injection of 3 ml benzene per kg body weight on day 13 of gestation. However, it is possible that the low incidence of cleft palate which was attributed to benzene by Watanabe and Yoshida was actually produced by the stress of such a massive subcutaneous injection; unfortunately, a negative 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.

Although a teratogenic effect was not discerned, some evidence of fetotoxicity was noted in mice inhaling benzene. Increased incidences of delayed ossification of skull bones and sternebrae were observed among the offspring of mice exposed to benzene. This effect was associated with a decrease in the average fetal body weight. In rabbits, the only evidence of an effect on embryonal or fetal development was a decrease in the occurrence of two minor skeletal variants: 13 ribs (the normal number is 12 or 13) and lumbar spurs.

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Green et al. (1978) reported increased frequencies of delayed ossification of sternebrae and missing sternebrae in female but not male offspring of rats exposed to benzene. A similar correlation between the occurrence of minor skeletal variants and the sex of the fetus was not observed in the present study.

The concentration of benzene tested in this study, 500 ppm, was markedly higher than the OSHA-recommended standard of 1 ppm (OSHA, 1977). However, the margin of safety may be even greater than indicated by exposure level alone since the amount of air inhaled by mice and rabbits in proportion to their body weight is approximately 5 and 10 times, respectively, that of humans (Handbook of Respiration, 1971). Thus, the findings of the present study, along with those of Green et al. (1978) do not indicate that benzene is uniquely hazardous to the unborn at levels of exposure which might be encountered in the workplace.

In summary, a teratogenic effect was not discerned in either mice or rabbits inhaling 500 ppm of benzene for 7 hours per day during the period of major organogenesis. Exposure to

500 ppm of benzene resulted in some minor variations in the development of the fetal skeleton in both species, but serious embryotoxicity or fetotoxicity was not observed.

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TABLE 1

RESULTS OF ANALYTICAL TESTING
BENZENE - ID 95-01000-0070^a

<u>TEST</u>	<u>RESULTS</u>	
<u>Color, Acid Washed</u> (ASTM D848)	Sample Layer No. 0	
	Acid Layer No. 1	
<u>Color, Platinum-Cobalt</u> (ASTM D 1209)	Pt-Co 5	
<u>Distillation, ASTM D350</u>		
760 mm Hg @ 26°C	5%	80.0°C
	10%	80.1
	20%	80.1
	30%	80.3
	40%	80.3
	50%	80.3
	60%	80.3
	70%	80.3
	80%	80.3
	90%	80.3
	95%	80.3
	IBP	79.4
	FBP	80.6
	Recovery	97.5%
	Loss	2.4%
	Bottoms	0.1%
<u>Sulfur Content, Total</u> (AM-S 60.23)	<1 ppm	
<u>H₂S Rating</u>	>0.0, <0.5	
<u>Specific gravity @ 60/60°F</u>	0.88437	
<u>G.C. For Trace Impurities</u>		
Total Non-Aromatics	68 ppm	
Toluene	199 ppm	
<u>Bromine Index, ASTM D2710</u>	4.15	
<u>Corrosion, Cu Strip, ASTM D 130</u>	1A - 3 hrs. @ 212°F	
<u>Acidity, ASTM D1613</u>	Basic Sample	

^aAs reported by J. M. Mulhall, Analytical and Information Division, Exxon Research and Engineering Company, Linden, New Jersey, 07036.

TABLE 2

BODY AND LIVER WEIGHTS OF PREGNANT MICE EXPOSED TO BENZENE BY INHALATION

	Control	Benzene ^a
Number of dams ^b	26	30
Maternal body weight (g) on gestation		
day 6	34+2 ^c	34+3
8	34+2	34+2
10	36+3	36+2
16	45+4	46+3
18	50+4	50+4
Maternal weight gain (g) on gestation		
days 6-7	0.4+2	-0.3+2
8-9	2+2	2+2
10-15	9+4	9+2
16-17	6+2	5+1
Total	17+4	16+3
Liver weight on day 18 of gestation		
absolute ^d	3.08+0.38	3.16+0.38
relative ^e	61.1+5.4	62.5+5.9

^aMice were exposed to 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bData from non-pregnant mice were not included in these analyses.

^cMean $\pm$ S.D.

^dg, mean $\pm$ S.D.

^emg liver/g body weight, mean $\pm$ S.D.

No values were significantly different from control values by a one-way analysis of variance, $p < 0.05$.

See Table A-1 for individual animal data.

TABLE 3

BODY AND LIVER WEIGHTS OF PREGNANT RABBITS EXPOSED TO
BENZENE BY INHALATION

	Control	Benzene ^a
Number of does ^b	18	19
Maternal body weight (kg) on gestation		
day 6	4.06±0.31 ^c	3.93±0.29
8	3.96±0.20	3.88±0.35
10	4.02±0.28	3.89±0.29
12	4.06±0.32	3.90±0.33
19	4.10±0.34	4.00±0.28
29	4.17±0.35	4.12±0.27
Maternal weight gain (kg) on gestation		
days 6-7	-0.05±0.06	-0.07±0.15
8-9	0.02±0.06	0.02±0.12
10-11	0.20±0.03	0.02±0.10
12-18	0.08±0.11	0.09±0.08
19-28	0.07±0.16	0.11±0.16
Total	0.11±0.25	0.19±0.17
Liver weight on day 29 of gestation		
absolute ^d	98±16	105±10
relative ^e	23.7±4.0	25.5±2.4

^aRabbits were exposed to 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bData from non-pregnant rabbits were not included in these analyses.

^cMean ± S.D.

^dg, mean ± S.D.

^eg liver/kg body weight, mean ± S.D.

No values were significantly different from control values by a one-way analysis of variance, $p < 0.05$.

See Table A-2 for individual animal data.

TABLE 4

FOOD AND WATER CONSUMPTION OF PREGNANT MICE EXPOSED TO BENZENE BY INHALATION

	<u>Control</u>	<u>Benzene^a</u>
Number of dams ^b	26	30
Amount of food consumed ^c daily during gestation		
days 6-8	6+1 ^d	6+1
9-11	6+2	6+2
12-14	7+2	7+2
15-17	8+2	8+1
Amount of water consumed ^c daily during gestation		
days 6-8	10+2	9+2
9-11	12+3	11+2
12-14	13+2	12+2
15-17	14+2	14+2

^aMice were exposed to 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bData from non-pregnant mice were not included in these analyses.

^cExpressed as grams/mouse/day.

^dMean $\pm$ S.D.

No values were significantly different from control values by a one-way analysis of variance, $p < 0.05$.

TABLE 5

FOOD CONSUMPTION OF PREGNANT RABBITS EXPOSED TO BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
Number of does ^b		18	19
Amount of food consumed ^c on gestation			
day	6	121+36 ^d	142+53
	7	144+34	143+46
	8	146+35	161+36
	9	149+42	160+46
	10	148+60	160+24
	11	132+29	156+43
	12	140+35	139+27
	13	134+29	143+23
	14	142+33	146+23
	15	139+50	159+41
	16	134+31	162+37 ^e
	17	124+43	166+36 ^e
	18	136+44	164+39
	19	140+52	188+41 ^e
	20	159+40	167+46
	21	143+42	141+53
	22	125+42	129+60
	23	118+48	118+63
	24	111+60	108+48
	25	98+64	98+44
	26	86+66	105+49
	27	85+52	111+42
	28	100+53	123+45

^aRabbits were exposed to 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bData from non-pregnant rabbits were not included in these analyses.

^cExpressed as grams/rabbit/day.

^dMean $\pm$ S.D.

^eSignificantly different from control value by a one-way analysis of variance, $p < 0.05$.

DO 136436
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TABLE 6

WATER CONSUMPTION OF PREGNANT RABBITS EXPOSED TO BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
Number of does ^b		18	19
Amount of water consumed ^c on gestation			
day	6	187+110 ^d	142+128
	7	268+64	246+100
	8	298+79	300+72
	9	278+63	295+76
	10	281+75	295+65
	11	258+46	269+76
	12	271+80	264+50
	13	267+78	298+97
	14	278+65	297+52
	15	242+60	292+62 ^e
	16	270+85	290+38
	17	237+84	287+84
	18	233+88	327+64 ^e
	19	252+75	338+69 ^e
	20	270+78	311+93
	21	256+88	274+101
	22	256+84	265+98
	23	295+126	249+112
	24	272+109	239+98
	25	234+129	256+86
	26	237+103	245+73
	27	237+136	231+73
	28	201+100	277+77 ^e

^aRabbits were exposed to 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bData from non-pregnant rabbits were not included in these analyses.

^cExpressed as grams/rabbit/day.

^dMean $\pm$ S.D.

^eSignificantly different from control value by a one-way analysis of variance, $p < 0.05$.

DO 136437
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TABLE 7

OBSERVATIONS AT CESAREAN SECTION AMONG MICE
EXPOSED TO BENZENE BY INHALATION

	<u>Control</u>	<u>Benzene^a</u>
No. of bred females	35	37
No. maternal deaths	(0/35)	(0/37)
Percent pregnant ^b	74%(26/35)	81%(30/37)
Percent pregnant, total ^c	86%(30/35)	89%(33/37)
Proportion of pregnant animals detected by sulfide stain ^d	13%(4/30)	9%(3/33)
No. litters examined	26	30
Implantation sites/dam ^e	12 _± 2	13 _± 2
No. fetuses/litter ^e	11 _± 2	11 _± 2
Resorptions/litter ^{e,f}	2 _± 1	2 _± 2
Reporptions/implantations ^f	12%(39/318)	15%(56/381)
Litters with resorptions ^f	69%(18/26)	73%(22/30)
No. litters totally resorbed ^f	0	0
Resorptions/litters with resorptions ^f	2.2(39/18)	2.6(56/22)
Dead fetuses/total fetuses	(0/279)	0.3(1/325)
Sex ratio, M:F	50:50	52:48
Fetal body weight, g ^g	1.01 _± 0.09	0.95 _± 0.10 ^h
Fetal crown-rump length, mm ^g	24.1 _± 1.3	23.8 _± 1.1

^aMice were exposed to 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bNumber of females with visible implantation sites at the time of cesarean section/total number of bred females.

^cNumber of females with implantation sites as observed either visually at the time of cesarean section or after staining the uterus with sodium sulfide stain/total number of bred females.

^dNumber of females with implantation sites detected only after staining the uterus with sodium sulfide/total number of females with implantation sites.

^eMean _± S.D.

^fResorptions detected by sodium sulfide staining were not included in these calculations.

^gMean of litter means _± S.D.

^hSignificantly different from control value by a one-way analysis of variance, p<0.05.

See Table A-3 for individual animal data.

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TABLE 8

OBSERVATIONS AT CESAREAN SECTION AMONG
RABBITS EXPOSED TO BENZENE BY INHALATION

	Control	Benzene ^a
No. of bred females	20	20
No. maternal deaths	(0/20)	(0/20)
Apparent pregnancy rate ^b	90%(18/20)	95%(19/20)
Total pregnancy rate ^c	90%(18/20)	95%(19/20)
Proportion of pregnant animals detected by sulfide stain ^d	(0/18)	(0/19)
No. litters examined	18	19
Corpora lutea/dam ^e	9+1	10+2
Implantation sites/dam ^e	9+2	9+2
Pregnancy wastage ^{e,f}	1+1	1+1
No. fetuses/litter ^e	8+2	8+2
Resorptions/litter ^{e,g}	1+1	1+1
Resorptions/implantations ^g	5%(9/168)	7%(13/182)
Litters with resorptions ^g	44%(8/18)	47%(9/19)
No. litters totally resorbed ^g	0	0
Resorptions/litters with resorptions ^f	1.1(9/8)	1.4(13/9)
Dead fetuses/total fetuses	(0/150)	(0/152)
Sex ratio, M:F	59:41	45:55
Fetal body weight, g ^h	39.43+5.67	38.46+5.05
Fetal crown-rump length, mm ^h	97.32+7.54	95.12+6.02

^aRabbits were exposed to 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bNumber of females with visible implantation sites at the time of cesarean section/total number of bred females.

^cNumber of females with implantation sites as observed either visually at the time of cesarean section or after staining the uterus with sodium sulfide stain/total number of bred females.

^dNumber of females with implantation sites detected only after staining the uterus with sodium sulfide/total number of females with implantation sites.

^eMean $\pm$ S.D.

^fPregnancy wastage = number of corpora lutea minus number of implantations.

^gResorptions detected by sodium sulfide staining were not included in these calculations.

^hMean of litter means $\pm$ S.D.

No values were significantly different from control values by a one-way analysis of variance or by Fisher's exact probability test (incidence data), $p < 0.05$. See Table A-4 for individual animal data.

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TABLE 9

INCIDENCE OF ALTERATIONS AMONG LITTERS OF MICE EXPOSED TO
BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
		No. Examined Fetuses (Litters)	
External Examination		279(26)	325(30)
Soft Tissue Examination		97(26)	112(30)
Skeletal Examination		253(24) ^b	286(28) ^b
Bones of the Skull		165(24)	189(28)
		% Affected (No. Affected)	
<u>External Alterations</u>			
Cleft palate*	F ^C	0.4(1)	0.3(1)
	L	4(1)	3(1)
Subcutaneous hemorrhage, head region	F	0	0.3(1)
	L	0	3(1)
<u>Soft Tissue Alterations</u>			
Slightly dilated 3rd ventricle of brain	F	0	1(1)
	L	0	3(1)
Subcutaneous hemorrhage, head region	F	1(1)	0
	L	4(1)	0
Subcutaneous hemorrhage, around brain	F	0	1(1)
	L	0	3(1)
<u>Skeletal Alterations</u>			
Sternebrae -			
delayed ossification	F	10(24)	18(51) ^d
	L	42(10)	64(18)
fused	F	0	1(3)
	L	0	11(3)
unfused	F	6(15)	10(29)
	L	38(9)	43(12)
extra site of ossification	F	4(9)	1(2)
	L	12(3)	7(2)

TABLE 9 (Continued)

INCIDENCE OF ALTERATIONS AMONG LITTERS OF MICE EXPOSED TO
BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
misshapen (#6)	F	0	0.4(1)
	L	0	4(1)
extra cartilage	F	1(2)	0
	L	4(1)	0
misplaced cartilage	F	1(2)	0
	L	8(2)	0
Ribs -			
extra	F	12(30)	12(35)
	L	58(14)	46(13)
forked or fused*	F	0	0.3(1) ^e
	L	0	4(1)
irregular ossification	F	0	0.3(1)
	L	0	4(1)
Vertebrae -			
asymmetric*	F	0	0.3(1) ^e
	L	0	4(1)
lumbar spurs	F	6(14)	5(15)
	L	42(10)	43(12)
delayed ossification of centra	F	0	0.3(1) ^e
	L	0	4(1)
delayed ossification of cervical arches	F	0	0.3(1) ^e
	L	0	4(1)
forked atlas	F	1(2)	0.5(1)
	L	8(2)	4(1)
misshapen atlas	F	0	0.5(1)
	L	0	4(1)
delayed ossification of atlas	F	0	0.5(1) ^e
	L	0	4(1)

TABLE 9 (Continued)

INCIDENCE OF ALTERATIONS AMONG LITTERS OF MICE EXPOSED TO
BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
Skull -			
delayed ossification	F	17(28)	34(64) ^d
	L	58(14)	68(19)
foramen, occipital or parietal	F	0	1(2)
	L	0	7(2)
unfused occipital	F	1(1)	5(10) ^d
	L	4(1)	21(6)
unossified, occipital	F	0	1(2)
	L	0	7(2)
misshapen, interparietal	F	0	0.5(1)
	L	0	4(1)
TOTAL MALFORMATIONS	F	0.4(1)	0.5(1)
	L	4(1)	4(1)

^aMice were exposed to 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bTwo litters from the control group and two litters from the benzene group were not examined for skeletal alterations. The fetuses from these litters were inadvertently mixed together during the staining procedure.

^cF = fetuses; L = litters.

^dSignificantly different from control value by a modified Wilcoxon test, $p < 0.05$.

^eThis alteration occurred in the single fetus with cleft palate.

*Considered to be a major malformation.

See Table A-5 for individual litter data.

TABLE 10

INCIDENCE OF ALTERATIONS AMONG LITTERS OF RABBITS EXPOSED TO
BENZENE BY INHALATION

		<u>Control</u>	<u>Benzene^a</u>
		No. Examined Fetuses (Litters)	
External Examination		150(18)	152(19)
Soft Tissue Examination		56(18) _b	59(19)
Skeletal Examination		142(17) _b	152(19)
		% Affected (No. Affected)	
<u>External Alterations</u>			
Gastroschisis	F ^c	0	0.6(1)
	L	0	5(1)
<u>Soft Tissue Alterations</u>			
Cystic calculus attached to gall bladder	F	0	2(1)
	L	0	5(1)
Fused innominate and left carotid arteries	F	88(49)	81(48)
	L	100(18)	100(19)
Satellite vessels off aorta, innominate or carotid arteries	F	18(10)	32(19)
	L	33(6)	47(9)
<u>Skeletal Alterations</u>			
Sternebrae -			
delayed ossification	F	89(126)	83(126)
	L	100(17)	100(19)
extra site of ossification	F	1(1)	0
	L	6(1)	0
fused	F	0	0.6(1)
	L	0	5(1)
unfused	F	0	1(2)
	L	0	11(2)
Ribs -			
13th rib or ribs	F	63(89)	43(65) ^d
	L	94(16)	89(17)
lumbar spur or spurs	F	5(7)	2(3) ^d
	L	41(7)	11(2)

TABLE 10 (Continued)

INCIDENCE OF ALTERATIONS AMONG LITTERS OF RABBITS EXPOSED TO
BENZENE BY INHALATION

		Control	Benzene ^a
fused or forked	F	0	1(2)
	L	0	11(2)
Vertebrae -			
fused vertebrae and hemi-centra, thoracic region	F	0	1(1)
	L	0	5(1)
misshapen or dumbbell-shaped centra	F	0	3(4)
	L	0	21(4)
unfused centra	F	0	1(2)
	L	0	11(2)
extra site of ossification between vertebrae	F	1(1)	0
	L	6(1)	0
Skull -			
extra suture line within parietal bone	F	0.7(1)	1(2)
	L	6(1)	11(2)
foramen - parietal or interparietal	F	2(3)	4(6)
	L	18(3)	32(6)
TOTAL MALFORMATIONS	F	0	2(3)
	L	0	16(3)

^a Rabbits were exposed to 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^b Due to problems in fixation, one litter of 8 fetuses was not examined for skeletal alterations.

^c F = fetuses; L = litters.

^d Significantly different from control value by a modified Wilcoxon test, $p < 0.05$.

*Considered to be a major malformation.

See Table A-6 for individual litter data.

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TABLE 11

HEMATOLOGIC EVALUATION OF FETUSES AND DAMS EXPOSED TO BENZENE

	N	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)				
						Neut	Lymph	Mono	Eosin	Baso
Fetuses ^a										
- Rabbit										
Control	5	43.6 $\pm$ 5.5	3.7 $\pm$ 0.4	12.2 $\pm$ 1.4	0.65 $\pm$ 0.13	Not Determined				
Benzene-exposed	5	46.7 $\pm$ 3.5	4.1 $\pm$ 0.4	13.9 $\pm$ 1.2	0.89 $\pm$ 0.32					
- Mouse										
Not determined										
Bred females ^b										
- Rabbit										
Control	4	39.9 $\pm$ 6.4	5.9 $\pm$ 1.1	13.3 $\pm$ 2.4	6.9 $\pm$ 2.9	46	46	4	1	3
Benzene-exposed	4	38.9 $\pm$ 3.5	5.8 $\pm$ 0.7	12.7 $\pm$ 1.2	5.8 $\pm$ 0.7	44	52	2	1	1
- Mouse										
Control	8	49.7 $\pm$ 1.5	8.6 $\pm$ 0.4	16.3 $\pm$ 0.6	7.2 $\pm$ 1.1	22	74	2	2	0
Benzene-exposed	8	48.1 $\pm$ 1.6	8.4 $\pm$ 0.2	16.0 $\pm$ 0.6	6.9 $\pm$ 1.6	28	69	2	2	0

^aBlood was collected on day 29 of gestation from the offspring of rabbits exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 18 of gestation.

^bBred female mice and rabbits were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 (mice) and 6 through 18 (rabbits) of gestation; blood was collected on days 16 (mice) and 19 (rabbits) of gestation.

No value differed significantly from the control value by a one-way analysis of variance, $p < 0.05$.

TABLE A-1
INDIVIDUAL BODY AND LIVER WEIGHTS OF PREGNANT MICE EXPOSED TO BENZENE BY INHALATION*

Animal Number	Maternal Body Weight (g)					Maternal Body Weight Gain (g)				Maternal Liver Weight (Day 18)	
	Day(s) of Gestation									Absolute g	Relative (g/kg Body Weight)
	6	8	10	16	18	6-8	8-10	10-16	16-18		
46-8000	36	33	33	39	42	3	0	6	3	2.67	63.6
46-8001	31	31	32	46	50	0	1	14	4	3.00	60.0
46-8013	34	36	37	47	51	2	1	10	4	3.10	60.8
46-8014	37	37	39	47	51	0	2	8	4	3.34	65.5
46-8015	35	37	40	49	51	2	3	9	2	2.91	57.1
46-8016	35	37	40	49	54	2	3	9	5	3.77	69.8
46-8018	36	35	37	46	51	-1	2	9	5	2.57	50.4
46-8021	34	35	37	49	54	1	2	12	5	3.37	62.4
46-8062	35	35	35	42	50	0	0	7	8	2.83	56.6
46-8063	35	36	38	46	56	1	2	8	10	3.60	64.3
46-8064	34	30	34	39	47	-4	4	5	8	3.23	68.7
46-8065	33	33	35	46	55	0	2	11	9	3.27	59.4
46-8066	33	33	35	41	47	0	2	6	6	2.86	60.8
46-8067	35	34	35	43	51	-1	1	8	8	3.10	60.8
46-8074	35	36	35	47	54	1	-1	12	7	3.73	69.1
46-8075	33	35	37	47	55	2	2	10	8	3.32	60.4
46-8077	33	34	40	48	52	1	6	8	4	2.54	48.8
46-8082	35	37	36	42	48	2	-1	6	6	3.00	62.5
46-8083	30	31	29	52	57	1	-2	23	5	3.62	63.5
46-8089	27	30	29	34	37	3	-1	5	3	2.33	63.0
46-8090	30	32	33	38	42	2	1	5	4	2.88	68.6
46-8091	31	36	36	46	51	5	0	10	5	2.83	55.5
46-8097	33	32	35	44	50	-1	3	9	6	NDB ^b	NDB ^b
46-8102	35	33	36	46	51	-2	3	10	5	2.71	53.1
46-8103	36	36	38	46	51	0	2	8	5	3.19	62.6
46-8104	34	32	35	46	52	-2	3	11	6	3.12	60.0
Mean ± S.D.	34 ± 2	34 ± 2	36 ± 3	45 ± 4	50 ± 4	0.4 ± 2	2 ± 2	9 ± 4	6 ± 2	3.08 ± 0.38	61.1 ± 5.4

*Mice were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bThe liver weight was inadvertently not determined for this animal.

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TABLE A-1 (Continued)

Exposure Level: 500 ppm

Animal Number	Maternal Body Weight (g)					Maternal Body Weight Gain (g)				Maternal Liver Weight (Day 18)	
	Day(s) of Gestation									Absolute	Relative
	6	8	10	16	18	6-8	8-10	10-16	16-18	g	(g/kg Body Weight)
46-8003	40	39	39	50	56	-1	0	11	6	3.76	67.1
46-8004	36	35	34	47	52	-1	-1	13	5	2.91	56.0
46-8022	35	35	37	46	49	0	2	9	3	3.02	61.6
46-8023	36	36	39	44	50	0	3	5	6	3.13	62.6
46-8024	31	32	34	41	45	1	2	7	4	3.01	66.9
46-8025	32	29	34	41	46	-3	5	7	5	2.76	60.0
46-8026	31	30	31	39	43	-1	1	8	4	2.48	57.7
46-8027	34	31	36	45	51	-3	5	9	6	3.15	61.8
46-8028	34	35	37	50	55	1	2	13	5	3.00	54.6
46-8029	36	35	39	50	56	-1	4	11	6	3.16	56.4
46-8068	32	33	37	46	51	1	4	9	5	2.81	55.1
46-8069	37	34	35	41	45	-3	1	6	4	2.51	55.8
46-8071	36	36	38	45	51	0	2	7	6	3.23	63.3
46-8072	39	37	41	52	55	-2	4	11	3	3.09	56.2
46-8078	36	33	39	50	57	-3	6	11	7	3.62	63.5
46-8080	34	31	34	44	50	-3	3	10	6	2.62	52.4
46-8081	36	37	39	45	51	1	2	6	6	3.52	69.0
46-8084	30	31	33	46	50	1	2	13	4	3.11	62.2
46-8086	31	33	33	43	48	2	0	10	5	2.82	58.8
46-8087	35	34	35	45	52	-1	1	10	7	3.03	58.3
46-8088	34	34	36	45	51	0	2	9	6	3.30	64.7
46-8092	31	30	36	44	47	-1	6	8	3	3.28	69.8
46-8093	29	32	34	49	54	3	2	15	5	3.82	70.7
46-8094	34	37	38	45	47	3	1	7	2	3.29	70.0
46-8095	33	35	38	48	52	2	3	10	4	3.83	73.6
46-8106	36	35	36	45	50	-1	1	9	5	3.08	61.6
46-8107	37	38	40	47	50	1	2	7	3	3.67	73.4
46-8108	37	37	39	51	56	0	2	12	5	3.82	68.2
46-8110	35	33	35	42	47	-2	2	7	5	2.80	59.6
46-8112	35	35	36	45	49	0	1	9	4	3.15	64.3
Mean ± S.D.	34 ± 3	34 ± 2	36 ± 2	46 ± 3	50 ± 4	0.3 ± 2	2 ± 2	9 ± 2	5 ± 1	3.16 ± 0.38	62.5 ± 5.9

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TABLE A-2

INDIVIDUAL BODY AND LIVER WEIGHTS OF PREGNANT RABBITS EXPOSED TO BENZENE BY INHALATION^a

Exposure Level: 0 ppm

Animal Number	Maternal Body Weight (g)					Day(s) of Gestation	Maternal Body Weight Gain (g)					Maternal Liver Weight (Day 29)		
	6	8	10	12	19		29						Absolute (g)	Relative (g/kg Body Weight)
								6-8	8-10	10-12	12-19	19-29		
47-8005	5.03	ND ^b	4.93	5.02	5.22	5.04	--	--	.09	.20	-.18	99	19.64	
47-8006	3.91	ND	3.89	3.92	3.95	4.08	--	--	.03	.03	.13	74	18.14	
47-8007	4.13	ND	4.08	4.09	3.87	3.94	--	--	.01	-.22	.07	101	25.63	
47-8008	3.83	ND	3.82	3.86	3.98	4.14	--	--	.04	.12	.16	97	23.43	
47-8031	3.90	3.86	3.85	ND	3.86	3.98	-.04	-.01	--	--	.12	102	25.63	
47-8032	4.12	4.04	4.03	ND	3.89	3.44	-.08	-.01	--	--	-.45	97	28.20	
47-8033	3.99	3.77	3.94	ND	3.99	4.13	-.22	.17	--	--	.14	113	27.36	
47-8038	3.82	3.82	3.90	3.91	4.00	4.26	0	.08	.01	.09	.26	85	19.95	
47-8039	4.00	3.99	3.93	3.96	3.97	4.06	-.01	-.06	.03	.01	.09	98	24.14	
47-8041	4.13	4.14	4.15	4.21	4.33	4.43	+.01	.01	.06	.12	.10	123	27.77	
47-8046	4.43	4.31	4.32	4.35	4.33	4.40	-.12	.01	.03	.02	.07	68	15.45	
47-8047	4.03	4.00	4.04	4.01	4.11	4.17	-.03	.04	-.03	.10	.06	82	19.66	
47-8048	3.82	3.80	3.81	3.83	3.97	4.15	-.02	.01	.02	.14	.18	115	27.71	
47-8049	3.97	3.91	3.96	4.00	4.09	4.10	-.06	.05	.04	.09	.01	96	23.41	
47-8054	4.01	3.99	3.99	3.98	4.14	4.25	-.02	0	-.01	.16	.11	84	19.76	
47-8055	4.38	4.36	4.30	4.28	4.51	4.76	-.02	-.06	-.02	.23	.25	125	26.26	
47-8056	3.81	3.81	3.83	3.81	3.90	3.84	0	.02	-.02	.09	-.06	111	28.91	
47-8057	3.76	3.65	3.65	3.67	3.72	3.89	-.11	0	.02	.05	.17	101	25.96	
Mean ± S.D.	4.06 ±0.31	3.96 ±0.20	4.02 ±0.28	4.06 ±0.32	4.10 ±0.34	4.17 ±	-0.05 ±0.06	0.02 ±0.06	0.02 ±0.03	0.08 ±0.11	0.07 ±0.16	98.39 ±15.67	23.72 ±4.02	

^aRabbits were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bNot determined for this animal

TABLE A-2 (Continued)

Exposure Level: 500 ppm

Animal Number	Maternal Body Weight (g)					Day(s) of Gestation	Maternal Body Weight Gain (g)					Maternal Liver Weight (Day 29)	
	6	8	10	12	19		29	6-8	8-10	10-12	12-19	19-29	Absolute (g)
47-8009	3.73	ND*	3.71	3.72	3.83	4.08	—	—	.01	.11	.25	119	29.17
47-8010	3.84	ND	4.01	4.01	4.04	4.16	—	—	0	.03	.12	118	28.37
47-8011	3.91	ND	3.83	3.87	4.10	4.36	—	—	.50	.23	.26	90	20.64
47-8012	3.79	ND	3.82	3.60	3.78	3.81	—	—	-.22	.18	.03	95	24.93
47-8034	3.99	4.01	3.97	ND	3.94	4.06	.02	-.04	—	—	.12	100	24.63
47-8035	4.03	4.01	4.00	ND	4.12	4.17	-.02	-.01	—	—	.05	101	24.22
47-8036	3.79	3.94	3.74	ND	4.03	3.99	.15	-.20	—	—	.04	94	23.56
47-8037	4.05	4.05	4.03	ND	4.12	4.12	0	-.02	—	—	0	105	25.49
47-8042	3.68	3.62	3.65	3.63	3.76	4.06	-.06	.03	-.02	.13	.30	104	25.62
47-8043	3.90	3.83	3.87	3.90	3.92	4.39	-.05	.02	.03	.02	.47	122	27.79
47-8044	3.89	3.96	3.80	3.96	3.89	4.15	.07	-.16	+.16	-.07	.26	106	25.54
47-8045	3.72	3.46	3.71	3.70	3.77	3.93	-.26	.25	-.01	.07	.16	104	26.46
47-8050	4.82	4.74	4.80	4.67	4.75	4.86	-.08	.06	-.13	.08	.11	101	20.78
47-8051	4.17	4.08	4.15	4.16	4.29	4.13	-.09	.07	.01	.13	-.16	98	23.73
47-8052	3.48	3.41	3.45	3.45	3.60	3.58	-.07	.04	0	.15	-.02	99	27.65
47-8053	4.12	3.62	3.91	4.10	4.23	4.03	-.50	.29	.19	.13	-.20	97	24.07
47-8059	3.92	3.92	3.91	3.92	4.09	4.31	0	-.01	+.01	.17	.22	126	29.23
47-8060	4.29	4.19	4.17	4.30	4.29	4.32	-.10	-.02	.13	-.01	.03	111	25.69
47-8061	3.55	3.45	3.47	3.49	3.53	3.75	-.10	.02	.02	.04	.22	101	26.93
Mean ± S.D.	3.93 ±0.29	3.88 ±0.35	3.89 ±0.29	3.90 ±0.33	4.00 ±0.28	4.12 ±0.27	-0.07 ±0.15	0.02 ±0.12	0.02 ±0.10	0.09 ±0.08	0.11 ±0.16	104.79 ±10.00	25.50 ±2.42

*Not determined for this animal

TABLE A-3

INDIVIDUAL LITTER SUMMARY OF OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION
OF MICE EXPOSED TO BENZENE BY INHALATION^a

Exposure Level: 0 ppm

ANIMAL NUMBER	IMPLANTATIONS	RESORPTIONS	LITTER SIZE	SEX RATIO M:F	BODY WEIGHT (g)	CROWN-RUMP LENGTH (mm)
46-8000	11	2	9	6:3	0.84	23.4
46-8001	13	1	12	6:6	1.02	25.1
46-8013	13	2	11	8:3	1.00	24.3
46-8014	10	0	10	3:7	1.05	24.4
46-8015	13	0	13	5:8	0.97	23.9
46-8016	13	5	8	1:7	1.14	25.2
46-8017	Non-Pregnant					
46-8018	13	1	12	4:8	1.01	22.3
46-8019	Non-Pregnant					
46-8020	Non-Pregnant					
46-8021	13	1	12	7:5	1.11	24.8
46-8062	12	0	12	7:5	1.03	25.1
46-8063	13	0	13	7:6	1.11	25.8
46-8064	9	2	7	3:4	0.90	24.4
46-8065	14	1	13	7:6	1.13	26.1
46-8066	9	0	9	5:4	1.16	26.1
46-8067	12	1	11	6:5	1.07	25.3
46-8074	14	3	11	4:7	0.97	24.3
46-8075	12	0	12	7:5	1.09	25.0
46-8076	Implantation sites detected only after sodium sulfide stain					
46-8082	13	2	11	6:5	0.96	23.9
46-8083	13	2	11	4:7	1.07	24.9
46-8089	6	2	4	3:1	1.00	24.2
46-8090	8	2	6	4:2	0.82	21.4
46-8091	14	4	10	6:4	0.93	22.9
46-8096	Implantation sites detected only after sodium sulfide stain					
46-8097	15	4	11	5:6	1.03	23.1
46-8098	Non-Pregnant					
46-8099	Implantation sites detected only after sodium sulfide stain					
46-8100	Non-Pregnant					
46-8101	Implantation sites detected only after sodium sulfide stain					
46-8102	14	1	13	6:7	0.83	21.6
46-8103	12	3	9	3:6	0.96	21.8
46-8104	13	0	13	6:7	1.07	24.2
46-8077	16	0	16	10:6	0.98	23.6
MEAN±SD	12±2	2±1	11±2	50:50	1.01±0.09	24.1±1.3

^aMice were exposed to 0 or 500 ppm benzene for 7 hrs/day on days
6-15 of a gestation.

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TABLE A-3 (continued)

Exposure Level: 500 ppm

ANIMAL NUMBER	IMPLANTATIONS	RESORPTIONS	LITTER SIZE	SEX RATIO M:F	BODY WEIGHT (g)	CROWN-RUMP LENGTH (mm)
46-8002	Implantation sites detected only after sodium sulfide stain					
46-8003	13	1	12	5:7	1.03	24.5
46-8004	16	0	16	10:6	0.82	22.8
46-8022	12	1	11	5:6	1.02	24.8
46-8023	13	2	11	6:5	0.92	23.0
46-8024	8	1	7	6:1	1.14	25.0
46-8025	11	0	11	7:4	0.96	24.3
46-8026	13	4	9	5:4	0.93	24.3
46-8027	13	0	13	5:8	0.93	24.3
46-8028	14	2	12	5:7	1.07	24.7
46-8029	14	0	14	9:5	1.04	24.9
46-8068	14	1	13	8:5	0.96	24.4
46-8069	10	3	7	3:4	0.80	22.2
46-8070	Implantation sites detected only after sodium sulfide stain					
46-8071	11	1	10	4:6	0.98	24.7
46-8072	15	2	13	7:6	0.96	24.0
46-8073	Non-Pregnant					
46-8078	14	1	13	8:5	0.90	23.2
46-8079	Removed from study due to leakage of water bottle					
46-8080	14	0	14	5:9	0.76	22.0
46-8081	7	1	6	3:3	0.96	24.7
46-8084	16	3 ^a	12	6:6	1.04	24.9
46-8085	Non-Pregnant					
46-8086	11	0	11	6:5	1.02	24.5
46-8087	13	2	11	4:7	0.90	22.9
46-8088	12	2	10	8:2	0.83	22.0
46-8092	10	3	7	2:5	0.85	22.8
46-8093	14	0	14	7:7	0.92	23.3
46-8094	14	4	10	7:3	0.80	22.0
46-8095	16	6	10	4:6	0.89	23.2
46-8105	Implantation sites detected only after sodium sulfide stain					
46-8106	14	3	11	7:4	0.88	22.4
46-8107	15	10	5	4:1	1.16	25.6
46-8108	12	2	10	5:1	1.12	24.9
46-8109	Non-Pregnant					
46-8110	9	0	9	6:3	0.86	22.6
46-8111	Non-Pregnant					
46-8112	13	1	12	2:10	0.97	24.0
MEAN±SD	13±2	2±2	11±2	52:48	0.95±0.10 ^b	23.8±1.1

^aOne fetus was dead at the time of cesarean section

^bStatistically significant by Dunnetts' Test p<0.05

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TABLE A-4

INDIVIDUAL LITTER SUMMARY OF OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION
OF RABBITS EXPOSED TO BENZENE BY INHALATION^a

Exposure Level: 0 ppm

Animal Number	Corpora Lutea	Implantations	Pregnancy Wastage	Resorptions	Litter Size	Sex Ratio M:F	Fetal Body Weight (g)	Crown-Rump Length (mm)
47-8005	10	12	0	1	11	8:3	39.9	100.3
47-8006	10	9	1	1	8	3:5	42.8	101.1
47-8007	9	8	1	1	7	4:3	36.4	95.8
47-8008	9	9	0	0	9	6:3	44.0	103.7
47-8031	12	9	3	1	8	6:2	36.3	90.0
47-8032	11	11	0	1	10	5:5	24.9	75.2
47-8033	10	9	1	2	7	1:6	39.3	90.3
47-8038	11	11	0	0	11	7:4	38.5	97.2
47-8039	10	10	0	0	10	5:5	34.2	93.9
47-8041	8	7	1	0	7	5:2	46.4	106.2
47-8046	10	9	1	0	9	4:5	41.6	100.2
47-8047	8	8	0	0	8	5:3	38.7	95.6
47-8048	8	8	0	0	8	6:2	39.1	99.1
47-8049	8	8	0	0	8	6:2	36.8	96.2
47-8054	8	5	3	1	4	0:4	51.2	108.4
47-8055	9	9	0	0	9	6:3	45.2	105.7
47-8056	8	8	0	0	8	6:2	39.7	98.2
47-8057	9	9	0	1	8	6:2	34.7	94.7
MEAN±SD	9±1	9±2	1±1	0.5±0.6	8±2	59:41	39.4±5.7	97.3±7.5

^aRabbits were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-18 of gestation

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TABLE A-4 (Continued)

Exposure Level: 500 ppm

<u>Animal Number</u>	<u>Corpora Lutea</u>	<u>Implantations</u>	<u>Pregnancy Wastage</u>	<u>Resorptions</u>	<u>Litter Size</u>	<u>Sex Ratio M:F</u>	<u>Fetal Body Weight (g)</u>	<u>Crown-Rump Length (mm)</u>
47-8009	9	6	3	1	5	0:5	50.3	107.4
47-8010	7	8	0	1	7	1:6	36.5	96.9
47-8011	15	14	1	0	14	7:7	37.3	96.9
47-8012	9	9	0	0	9	7:2	38.7	99.0
47-8034	10	6	4	1	5	2:3	33.0	84.4
47-8035	8	8	0	0	8	2:6	40.5	91.0
47-8036	7	7	0	0	7	2:5	40.6	94.6
47-8037	9	5	4	0	5	3:2	46.2	99.3
47-8042	10	10	0	0	10	7:3	40.0	98.4
47-8043	9	8	1	0	8	2:6	43.8	103.0
47-8044	11	11	0	2	9	3:6	39.9	98.6
47-8045	11	11	0	1	10	5:5	29.4	87.8
47-8050	15	12	3	4	8	6:2	40.4	97.6
47-8051	10	10	0	1	9	7:2	36.0	89.7
47-8052	7	8	0	0	8	5:3	36.3	91.2
47-8053	10	9	1	0	9	3:6	33.4	85.2
47-8059	8	7	1	0	7	2:5	31.0	90.2
47-8060	10	10	0	1	9	4:5	37.1	97.2
47-8061	7	7	0	2	5	1:4	40.3	98.9
MEAN±SD	10±2	9±2	1±1	0.7±1.0	8±2	45:55	38.5±5.0	95.1±6.0

TABLE A-7

HEMATOLOGIC EVALUATION OF PREGNANT MICE EXPOSED TO BENZENE BY INHALATION^a

Animal Number	PCV Percent	RBC $\times 10^6/\text{mm}^3$	Hgb g/100 ml	WBC $\times 10^3/\text{mm}^3$	WBC Differential Count (%)				
					Neut	Lymph	Mono	Eosin	Baso
<u>Control:</u>									
24	47.0	8.0	15.4	7.6	39	56	3	2	0
25	50.0	9.0	16.9	8.9	22	76	2	0	0
26	49.0	8.1	16.3	8.5	20	75	4	1	0
27	51.5	8.9	17.3	7.2	7	89	2	2	0
40	50.0	9.0	15.9	5.9	15	82	1	2	0
41	48.5	8.1	16.1	6.9	20	79	1	0	0
42	50.0	9.0	16.2	6.6	24	72	4	0	0
43	49.0	8.7	15.9	7.6	30	66	0	4	0
44	52.0	8.7	16.7	5.7	26	68	3	3	0
Mean $\pm$ S.D.	49.7 $\pm$ 1.5	8.6 $\pm$ 0.4	16.3 $\pm$ 0.6	7.2 $\pm$ 1.1	22	74	2	2	0
<u>Benzene-exposed:</u>									
77	49.5	8.3	16.8	9.6	18	81	1	0	0
78	47.0	8.4	15.8	7.0	31	66	2	1	0
79	47.0	8.2	14.9	6.9	26	74	0	0	0
80	48.0	8.4	16.1	4.5	20	77	2	1	0
93	49.0	8.5	15.9	7.3	36	57	1	5	0
94	50.0	8.7	16.4	5.2	23	74	2	1	0
95	45.0	8.0	15.4	6.9	29	69	1	1	0
96	49.0	8.7	16.3	7.9	37	56	3	3	0
Mean $\pm$ S.D.	48.1 $\pm$ 1.6	8.4 $\pm$ 0.2	16.0 $\pm$ 0.6	6.9 $\pm$ 1.6	28	69	2	2	0

^aBred female mice were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 15 of gestation; blood was collected on day 16 of gestation.

No value differed significantly from the control value by a one-way analysis of variance, $p < 0.05$.

TABLE A-8

HEMATOLOGIC EVALUATION OF RABBIT FETUSES AND DAMS EXPOSED TO BENZENE BY INHALATION

Animal Number	PCV	RBC	Hgb	WBC	WBC Differential Count (%)				
	Percent	$\times 10^6/\text{mm}^3$	g/100 ml	$\times 10^3/\text{mm}^3$	Neut	Lymph	Mono	Eosin	Baso
Fetuses^a									
Control:									
47-8031	43.0	3.9	12.7	0.52	Not Determined				
47-8032	35.0	3.1	9.9	0.53					
47-8033	48.0	4.0	13.6	0.75					
47-8046	43.0	3.6	12.1	0.66					
47-8047	49.0	3.8	12.6	0.81					
Mean $\pm$ S.D.	43.6 $\pm$ 5.5	3.7 $\pm$ 0.4	12.2 $\pm$ 1.4	0.65 $\pm$ 0.13					
Benzene-exposed:									
47-8034	49.0	3.7	14.3	1.14	Not Determined				
47-8035	44.5	4.2	13.3	0.67					
47-8036	45.5	3.9	13.3	0.48					
47-8037	43.0	3.9	12.7	0.88					
47-8050	51.5	4.7	15.7	1.26					
Mean $\pm$ S.D.	46.7 $\pm$ 3.5	4.1 $\pm$ 0.4	13.9 $\pm$ 1.2	0.89 $\pm$ 0.32					
Bred Females^b									
Control:									
602	34.0	5.0	11.0	6.7	66	27	4	1	2
80	37.5	5.3	12.3	5.2	18	78	2	0	2
810	49.0	7.4	16.7	11.0	68	21	8	2	1
122	39.0	5.9	13.2	4.6	32	59	1	0	8
Mean $\pm$ S.D.	39.9 $\pm$ 6.4	5.9 $\pm$ 1.1	13.3 $\pm$ 2.4	6.9 $\pm$ 2.9	46	46	4	1	3
Benzene-exposed:									
133	35.0	5.0	11.4	4.9	54	42	2	2	0
4006	43.5	6.7	14.2	6.0	46	51	2	1	0
187	39.0	6.2	12.9	5.6	30	64	2	1	3
698	38.0	5.5	12.3	6.6	47	49	3	0	1
Mean $\pm$ S.D.	38.9 $\pm$ 3.5	5.8 $\pm$ 0.7	12.7 $\pm$ 1.2	5.8 $\pm$ 0.7	44	52	2	1	1

^aBlood was collected on day 29 of gestation from the offspring of rabbits exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 18 of gestation.

^bBred female rabbits were exposed to 0 or 500 ppm of benzene for 7 hours per day from days 6 through 18 of gestation; blood was collected on day 19 of gestation.

No value differed significantly from the control value by a one-way analysis of variance, $p < 0.05$.

TABLE A-5

INDIVIDUAL LITTER SUMMARY OF FETAL ALTERATIONS AMONG LITTERS OF MICE EXPOSED TO BENZENE BY INHALATION^a

Exposure Level = 0 ppm

ANIMAL NUMBERS	NUMBER OF FETUSES EXAMINED																												TOTAL FETUSES	TOTAL LITTERS
	46-8000, 01, 13, 14, 15, 16, 18, 21, 62, 63, 64, 65, 66, 67, 74, 75, 77, 82, 83, 89, 90, 91, 97, 102, 103, 104,																													
EXTERNAL EXAMINATION	9	12	11	10	13	8	12	12	12	13	7	13	9	11	11	12	16	11	11	4	6	10	11	13	9	13	279	26		
SOFT-TISSUE EXAMINATION	3	4	4	3	4	3	4	4	4	3	4	3	4	4	4	5	4	4	3	3	3	4	4	4	4	97	26			
SKELETAL EXAMINATION	0 ^b	12	11	10	13	8	0 ^b	10 ^c	12	13	7	13	9	8 ^c	11	12	16	11	11	4	6	10	11	13	9	13	253	24		
BONES OF THE SKULL	0 ^b	8	7	7	9	5	0 ^b	8	8	9	4	9	6	4	7	8	11	7	7	1	3	7	7	9	5	9	165	24		
NUMBER OF FETUSES AFFECTED																														
Total Major Malformations	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1		
EXTERNAL ALTERATIONS																														
Cleft Palate*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1		
Subcutaneous hemorrhage, head region	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
SOFT TISSUE ALTERATIONS																														
Slightly dilated 3rd ventricle of brain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Subcutaneous hemorrhage, head region	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1		
Subcutaneous hemorrhage, around brain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
SKELETAL ALTERATIONS																														
Sternebrae -																														
delayed ossification	0	0	1	0	0		0	0	0	3	0	0	0	2	0	1	2	1	0	5	0	0	6	2	1	24	10			
fused	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
unfused	0	1	0	3	0		0	0	0	2	0	1	0	1	0	0	1	0	0	0	0	1	4	1	0	15	9			
extra site of ossification	0	0	0	0	0		0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	7	9	3			
misshapen (#6)	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
extra cartilage	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1			
misplaced cartilage	0	0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2			
Ribs -																														
extra	1	1	0	0	0		2	2	3	1	3	0	2	0	1	2	0	0	0	0	0	1	0	2	4	5	30	14		
forked or fused*	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
irregular ossification	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Vertebrae -																														
asymmetric*	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
lumber spurs	1	1	0	0	0		1	1	3	0	0	0	0	0	1	0	0	0	0	0	0	2	0	2	1	1	14	10		
delayed ossification of centra	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
delayed ossification of cervical arches	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
forked atlas	0	1	0	0	1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2		
misshapen atlas	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
delayed ossification of atlas	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Skull -																														
delayed ossification	2	1	0	2	1		2	0	0	1	0	0	0	2	0	0	1	1	1	3	0	1	9	1	0	28	14			
foramen, occipital or parietal	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
unfused occipital	0	0	0	1	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1			
unossified, occipital	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
misshapen, interparietal	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

^aMice were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bTwo litters from the control group and two litters from the benzene group were not examined for skeletal alterations. The fetuses from these litters were inadvertently mixed together during the staining procedure.

^cSome skeletons from litters 8021 and 8067 are missing from the skeletal examination because they were inadvertently damaged during the staining procedure.

^dConsidered to be a major malformation.

TABLE A-3 (Continued)

INDIVIDUAL LITTER SUMMARY OF FETAL ALTERATIONS AMONG LITTERS OF MICE EXPOSED TO BENZENE BY INHALATION^a

Exposure Level = 500 ppm

NUMBER OF FETUSES EXAMINED																																					TOTAL FETUSES	TOTAL LITTERS
ANIMAL NUMBER:	46-8003	04	22	23	24	25	26	27	28	29	68	69	71	72	78	80	81	84	86	87	88	92	93	94	95	106	107	108	110	112								
EXTERNAL EXAMINATION	12	16	11	11	7	11	9	13	12	14	13	7	10	13	13	14	6	13	11	11	10	7	14	10	10	11	5	10	9	12			325	30				
SOFT-TISSUE EXAMINATION	4	5	4	4	3	4	3	4	4	3	4	3	3	4	4	5	3	4	4	4	3	3	5	3	3	4	3	3	3	4			112	30				
SKELETAL EXAMINATION	12	0 ^b	11	11	7	11	0 ^b	12 ^c	12	3 ^c	13	9 ^c	10	13	13	14	6	13	11	11	10	7	14	10	10	11	5	10	9	12			286	28 ^b				
BONES OF THE SKULL	8	0	7	7	4	7	0	8	8	3	9	4	7	9	9	9	3	9	7	7	7	4	9	7	7	7	2	7	6	8			189	28 ^b				
NUMBER OF FETUSES AFFECTED																																						
Total Major Malformations	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
EXTERNAL ALTERATIONS																																						
Cleft Palate ^a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
Subcutaneous hemorrhage, head region	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
SOFT TISSUE ALTERATIONS																																						
Slightly dilated 3rd ventricle of brain	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
Subcutaneous hemorrhage, head region	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
Subcutaneous hemorrhage, around brain	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
SKELETAL ALTERATIONS																																						
Sternebrae																																						
delayed ossification	2		1	4	0	1		0	2	0	2	3	2	0	0	10	1	0	0	1	3	2	1	8	2	3	0	0	3	0			51 ^d	18				
fused	0		0	0	0	1		0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3				
unfused	2		1	1	0	0		0	0	0	0	0	0	1	0	6	0	1	0	6	5	2	0	0	2	1	0	0	1	0			29	12				
extra site of ossification	0		1	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		2	2				
misshapen (#6)	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1				
extra cartilage	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
misplaced cartilage	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
Ribs -																																						
extra	1		0	0	0	0		7	0	0	3	1	1	0	1	0	0	1	4	0	0	1	2	3	0	0	1	0	0	9			35	13				
forked or fused ^a	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ^e	1				
irregular ossification	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
Vertebrae -																																						
asymmetric ^a	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ^e	1				
lumbar apure	0		0	0	1	0		0	0	0	2	0	1	0	1	0	1	0	2	0	1	1	0	2	0	1	1	0	0	1			15	12				
delayed ossification of centra	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ^e	1				
delayed ossification of cervical arches	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ^e	1				
forked atlas	0		0	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
misshapen atlas	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1				
delayed ossification of atlas	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 ^e	1				
Skull -																																						
delayed ossification foramen, occipital or parietal	0		0	2	0	2		1	3	0	0	2	4	4	3	9	1	4	1	3	4	3	8	3	0	3	0	0	4	0			64 ^d	19 ^d				
unfused occipital	0		0	0	0	0		0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	2	1	3	0	0	1	0		10	6				
unossified, occipital	0		0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2				
misshapen, interparietal	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1				

^aMice were exposed to 500 ppm benzene for 7 hrs/day on days 6-15 of gestation.

^bTwo litters from the control group and two litters from the benzene group were not examined for skeletal alterations. The fetuses from these litters were inadvertently mixed together during the staining procedure.

^cSome skeletons from litters 8027 and 8069 are missing from the skeletal examination because they were inadvertently damaged during the staining procedure.

^dSignificantly different from control value by a modified Wilcoxon test, $p < 0.05$.

^eThis alteration occurred in the single fetus with cleft palate.

^fConsidered to be a major malformation.

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TABLE A-6

INDIVIDUAL LITTER SUMMARY OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS INHALING BENZENE BY INHALATION^a

Exposure: 0 ppm

ANIMAL NUMBER	NUMBER OF FETUSES EXAMINED																	TOTAL	TOTAL		
	47-8005, 06, 07, 08, 31, 32, 33, 38, 39, 41, 46, 47, 48, 49, 54, 55, 56, 57,																	FETUSES	LITTERS		
External Examination	11	8	7	9	8	10	7	11	10	7	9	8	8	8	4	9	8	8	150	18	
Soft Tissue Examination	4	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	56	18	
Skeletal Examination	11	8	7	9	0 ^b	10	7	11	10	7	9	8	8	8	4	9	8	8	142	17 ^b	
NUMBER OF FETUSES AFFECTED																					
TOTAL MAJOR MALFORMATIONS:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
External Alterations																					
Gastroschisis ^a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Soft Tissue Alterations																					
Cystic calculus attached to gall bladder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Fused innominate and left carotid arteries	4	3	2	3	3	3	3	4	3	1	2	3	2	3	2	3	3	2	49	18	
Satellite vessels off aorta, innominate or carotid arteries	0	2	0	0	0	0	1	1	3	0	0	0	0	1	0	2	0	0	10	6	
Skeletal Alterations																					
Sternbrae -																					
delayed ossification	11	6	7	9		10	6	10	10	6	6	5	8	8	4	4	8	8	126	17	
extra site of ossification	0	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	
fused	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
unfused	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ribs -																					
13th rib or ribs	9	4	4	5		1	5	10	9	0	9	7	2	6	3	7	4	4	89	16	
lumbar spur or spurs	0	0	1	0		1	0	1	0	1	1	0	0	0	0	0	1	1	7	7	
fused or forked ^c	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Vertebrae -																					
fused vertebrae and hemi-centra, thoracic region ^a	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
misshapen or dumbbell-shaped centra	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
unfused centra	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
extra site of ossification between vertebrae	0	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	
Skull -																					
extra suture line within parietal bone	0	0	0	0		1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	
foramen - parietal or interparietal	0	0	1	0		1	0	0	0	0	0	0	0	1	0	0	0	0	3	3	

^aRabbits were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bDue to problems in fixation, one litter of 8 fetuses was not examined for skeletal alterations.

^cConsidered to be a major malformation.

TABLE A-6 (Continued)

INDIVIDUAL LITTER SUMMARY OF FETAL ALTERATIONS AMONG LITTERS OF RABBITS EXPOSED TO BENZENE BY INHALATION^a

Exposure: 500 ppm

ANIMAL NUMBER	NUMBER OF FETUSES EXAMINED																				TOTAL FETUSES	TOTAL LITTERS
	47-8009	10	11	12	34	35	36	37	42	43	44	45	50	51	52	53	59	60	61			
External Examination	5	7	14	9	5	8	7	5	10	8	9	10	8	9	8	9	7	9	5	152	19	
Soft Tissue Examination	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	59	19	
Skeletal Examination	5	7	14	9	5	8	7	5	10	8	9	10	8	9	8	9	7	9	5	152	19	
<u>NUMBER OF FETUSES AFFECTED</u>																						
<u>TOTAL MAJOR MALFORMATIONS:</u>	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	3	3	
<u>External Alterations</u>																						
Gastrochisis ^a	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	
<u>Soft Tissue Alterations</u>																						
Cystic calculus attached to gall bladder	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	
Fused innominate and left carotid arteries	2	2	4	3	3	3	1	2	2	3	2	2	2	3	3	2	3	3	3	48 ^c	19	
Satellite vessels off aorta, innominate or carotid arteries	0	2	2	0	2	3	0	0	2	2	0	0	0	2	2	0	2	0	0	19	9	
<u>Skeletal Alterations</u>																						
<u>Sternumbrae -</u>																						
delayed ossification	4	5	14	8	5	5	5	3	10	8	8	8	7	3	5	8	7	9	4	126	19	
extra site of ossification	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
fused	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	
unfused	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	
<u>Ribs -</u>																						
13th rib or ribs	5	1	5	3	1	7	5	0	5	0	3	4	4	7	3	5	1	1	5	65 ^c	17	
lumber spur or spurs	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	3 ^c	2	
fused or forked	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	2	2	
<u>Vertebrae -</u>																						
fused vertebrae and hemi- centra, thoracic region ^a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	
misshapen or dumbbell- shaped centra	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	4	4	
unfused centra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	2	
extra site of ossification between vertebrae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<u>Skull -</u>																						
extra suture line within parietal bone	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	2	
foramen - parietal or interparietal	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	1	0	1	6	6	

^aRabbits were exposed to 0 or 500 ppm benzene for 7 hrs/day on days 6-18 of gestation.

^bDue to problems in fixation, one litter of 8 fetuses was not examined for skeletal alterations.

^cSignificantly different from control value by a modified Wilcoxon test, $p < 0.05$.

^dConsidered to be a major malformation.

DO 136459
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