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Testing of selected workplace chemicals for teratogenic potential

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HARDIN BD, BOND GP, SIKOV MR, ANDREW FD, BELILES RP, NIEMEIER RW. Testing of selected workplace chemicals for teratogenic potential. *Scand j work environ health* 7 (1981): suppl 4, 66—75. The reproductive toxicity and teratogenic potential of 19 industrial chemicals have been investigated during the past 3 a. Preliminary studies utilizing intraperitoneal treatments of rats on days 1—15 of gestation have been conducted on the following ten chemicals: allyl chloride, bisphenol A, copper naphthenate, ethylene dibromide, hexachlorobutadiene, 2-mercaptobenzothiazole, methyl styrene, naphthalene, 2-nitropropane, and 1,2,3-trichloropropane. Studies utilizing inhalation exposure of rats and rabbits on days 1—19 and 1—24, respectively, of gestation have been conducted on the following nine chemicals: butylene oxide, carbon disulfide, 2-ethoxyethanol, ethyl benzene, methyl bromide, nitrous oxide, styrene oxide, tetrachloroethylene, and trichloroethylene. In the preliminary studies, evidence of teratogenic potential was seen with allyl chloride and bisphenol A, and fetal toxicity was found in the absence of maternal toxicity with methyl styrene and 2-nitropropane. In the inhalation studies, 2-ethoxyethanol was strongly embryotoxic at the higher exposure levels employed and was teratogenic at the lower concentration.

Key terms: allyl chloride, bisphenol A, butylene oxide, carbon disulfide, copper naphthenate, 2-ethoxyethanol, ethyl benzene, ethylene dibromide, hexachlorobutadiene, 2-mercaptobenzothiazole, methyl styrene, naphthalene, 2-nitropropane, nitrous oxide, reproductive toxicity, styrene oxide, teratogenesis, tetrachloroethylene, trichloroethylene, 1,2,3-trichloropropane.

Recently, it has become increasingly clear that occupational exposure to industrial chemicals may impair functional reproductive capacity or may affect the fetus. Consequently, teratogenesis testing has become an important part of the overall toxicology program of the National Institute for Occupational Safety and Health (NIOSH). Chemicals for teratogenesis testing were selected from lists of chemicals under consideration by NIOSH for future development of a criteria document. Current and projected usage or exposure and an evaluation of the existing data on each chemical were used as the selection criteria. During the past 3 a, 19 chemicals selected in this way were tested by either inhalation exposure of rats and rabbits or intraperitoneal injection of rats. The results of all 19 studies are summarized in this report, and detailed observations are presented for two of the chemicals.

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Methods and materials

Treatment by intraperitoneal injection

Probe studies were conducted with rats injected intraperitoneally with the test chemical dissolved or suspended in corn

oil. Initial dose-response studies with nonpregnant rats established, for each chemical, the maximum tolerated dose (MTD) defined as the dose at which there was no mortality, no marked signs of toxicity (such as unconsciousness), and less than a 10 % reduction (relative to controls) in body weight gain during or within two weeks following the course of 15 daily intraperitoneal injections. The chemicals tested and the doses used are shown in table 1.

After the MTD determinations, young adult female Sprague-Dawley rats (250—300 g) were caged with breeder males of the same strain. The females were examined daily for the presence of sperm in a vaginal lavage. The day sperm was detected was designated as day 1 of gestation. Inseminated females were randomly assigned to treatment or control groups. Treatment began on day 1 of gestation and continued through day 15. Controls were injected intraperitoneally with corn oil, while the test group received the test chemical in corn oil at the MTD. Each group was composed of 10—15 inseminated females individually housed in stainless steel wire mesh cages with free access to food and water.

On day 21 of gestation, the females were killed by decapitation and the uterine contents were examined. The individual fetuses were weighed, measured for crown-rump length, sexed, and examined for externally visible malformations. One-half to two-thirds of each litter was preserved in Bouin's fluid for internal examination by the Wilson method of free-hand razor-blade sectioning, and the balance of each litter was preserved in ethanol for clearing and skeletal staining with alizarin red. The internal organs of the maternal rats were examined grossly, and the brain, heart, lungs, liver, spleen, kidneys, adrenals, and ovaries were weighed and then preserved in 10 % formalin for histopathological examination.

Inhalation exposure

Inhalation studies were conducted with Wistar or Sprague-Dawley rats and New Zealand white rabbits, in both the NIOSH Cincinnati facilities and the contract research laboratories working under contract to NIOSH. Exposure concentrations were selected from published toxicity data. Recommended occupational exposure limits

Table 1. Intraperitoneal pilot teratology studies on rats.

Chemical	OSHA PEL (mg/m³) [a]	Dose [b]		Maternal toxicity [c]	Fetal toxicity [d]	Teratogenesis [e]
		mg/kg	mmol/kg			
Allyl chloride	3	80	1.05	+ [g]	+	+
Bisphenol A	None	125	0.55	+	+	? [i]
Copper naphthenate	None	10	[f]	— [h]	—	—
Ethylene dibromide	155	55	0.29	+	—	—
Hexachlorobutadiene	None	10	0.04	+	+	—
2-Mercaptobenzothiazole	None	200	1.20	—	—	—
Methyl styrene	480	250	2.12	—	+	—
Naphthalene	50	395	3.08	—	—	—
2-Nitropropane	90	170	1.91	—	+	—
1,2,3-Trichloropropane	300	37	0.25	+	—	—

[a] OSHA = Occupational Safety and Health Administration, PEL = permissible exposure limit.
[b] Administered to rats intraperitoneally in corn oil on days 1 through 15 of gestation.
[c] Reduced body weight gain or altered weights (absolute or relative) of two or more organs (statistically significant at $p < 0.05$).
[d] Reduced pre- or postimplantation survival, reduced fetal body weight or length (statistically significant at $p < 0.05$).
[e] Grossly visible external or internal (visceral or skeletal) malformations (statistically significant at $p < 0.05$).
[f] Equivalent dose in millimoles per kilogram not available because copper naphthenate is of variable composition and indefinite molecular weight.
[g] + Present.
[h] — Absent.
[i] ? Suggestive but not statistically significant results.

were used as the lower concentration when two exposure levels were employed. The chemicals tested and the concentrations used are shown in table 2. In most of the inhalation studies, two exposure levels were used, although the in-house nitrous oxide study and two contract studies (trichloro- and tetrachloroethylene) were restricted to a single concentration.

The experimental protocols varied somewhat between the in-house and contract studies and between studies conducted by different contractors. In most cases, the animals were exposed for 6 to 7 h/d on gestation days 1 to 19 (rats) or 1 to 24 (rabbits). The contract studies also included rat groups that were exposed to the test chemical for three weeks before the breeding to simulate an occupational exposure. The target number of litters per group was 30 for rats and 20 for rabbits; however, breeding difficulties in some studies reduced rabbit groups to approximately 15.

All the pregnant animals were sacrificed on the day before term (day 21 for rats, day 30 for rabbits), and the litters were collected as previously described. Maternal organs were also collected for histopathological examination. The fetal examinations were generally as described for the intraperitoneal studies, except that one contractor used the Staples method of dissection under magnification for the visceral examinations, with skeletal examination of all fetuses.

Results

Intraperitoneal injection

Summary results are shown in table 1. With eight of the chemicals tested (copper naphthenate, ethylene dibromide, hexachlorobutadiene, 2-mercaptobenzothiazole, methyl styrene, naphthalene, 2-nitrapropane, and 1,2,3-trichloropropane), treatment-related effects were limited to evidence of maternal or fetal toxicity. A statistically significant ($p < 0.05$) change in at least two maternal organ weights, either an increase or a decrease, was seen with three of these eight chemicals (ethylene dibromide, hexachlorobutadiene, and 1,2,3-trichloropropane). Fetal toxicity was reflected in a significant incidence ($p < 0.05$) of delayed fetal development in the 2-nitropropane and hexachlorobutadiene studies. The development of the heart was delayed by 1—2 d, and dilated renal pelvises and ureters were seen in the hexachlorobutadiene study. With methyl styrene, the incidence of resorptions was significantly increased ($p < 0.05$), and the fetal sex ratio was significantly altered ($p < 0.05$) with a deficit of female fetuses. No teratogenic effects were suggested for any of these eight chemicals, and no treatment-related histopathological changes were observed in maternal tissues.

With allyl chloride, maternal heart, liver, spleen, and kidney weights were significantly increased ($p < 0.05$), but there were no treatment-related histopathological changes. Fetal toxicity was reflected in a significant increase ($p < 0.05$) in resorptions in treated litters. No visceral or skeletal malformations were seen, but there was a significant incidence of fetuses from treated litters with edema ($p < 0.01$) and short snout with protruding tongue ($p < 0.05$). These defects were not seen in any of the control litters. John et al (5) recently reported an inhalation teratology study of allyl chloride using rats and rabbits that revealed no teratogenic activity. NIOSH is presently investigating possible explanations for the conflicting results of these studies, including identification of impurities in the allyl chloride used by NIOSH and the John group.

Results with bisphenol A are shown in tables 3 and 4. When it became apparent that very few of the females treated with 125 mg/kg (0.55 mmol/kg) were pregnant, four reserve females that had not been assigned to either control or experimental groups were bred and treated at 85 mg/kg (0.37 mmol/kg) (previously selected as the 5-d MTD for male rats in a dominant lethal study). As shown in table 3, the higher dosage significantly ($p = 0.0014$) impaired the establishment of pregnancy, and both doses caused a significant ($p < 0.02$) reduction in the number of live fetuses per litter. Fetal toxicity was evident as statistically significant ($p < 0.001$) dose-related reductions of fetal body weight and crown-rump length. An examination of maternal tissues revealed histocytosis and intraalveolar pigmented mac-

rophages in the lungs and peritonitis in the 125 mg/kg group.

Because of the small number of litters available in the treated groups, only tentative conclusions can be drawn regarding the possible teratogenic effects of bisphenol A. As shown in table 4, when data were analyzed with the litter as the experimental unit, the treated groups differed significantly from the controls in only two instances — in the 85 mg/kg group, litters containing fetuses with incomplete skeletal ossification ($p = 0.033$); in the 125 mg/kg group, litters containing fetuses with enlarged cerebral ventricles or hydrocephaly ($p = 0.011$). When the analyses were based on fetuses affected, rather than litters, there was a number of statistically significant differences (table 4). With two exceptions, all the abnormalities seen in the treated fetuses were relatively minor anomalies or variations of normal development and could be regarded as evidence of a toxicity-related delay in fetal development. However, imperforate anus occurred in three fetuses from a single litter treated with bisphenol A at 125 mg/kg. This and the other major malformation (hydro-

Table 2. Inhalation teratology studies on rats and rabbits.

Chemical	OSHA PEL (ppm) [a]	Exposure levels [b] (ppm)		
		Low		High
Butylene oxide	None	250		1,000
Carbon disulfide	20	20		40
2-Ethoxyethanol	200	160	(rabbits)	615
		200	(rats)	765
Ethyl benzene	100	100		1,000
Methyl bromide	C-20 [c]	20		70
Nitrous oxide	None		1,000	
Styrene oxide	None	15	(rabbits)	50
		100	(rats)	300
Tetrachloroethylene	100		500	
Trichloroethylene	100		500	

[a] OSHA = Occupational Safety and Health Administration, PEL = Permissible exposure limit.
[b] Rats and rabbits were exposed at the same concentrations except as noted for 2-ethoxyethanol and styrene oxide.
[c] Ceiling limit.

Table 3. Bisphenol A: Interaperitoneal injection observations from rats at sacrifice.

	Control	Treated	
		85 mg/kg (0.37 mmol/kg)	125 mg/kg (0.55 mmol/kg)
Number pregnant/number inseminated	11/12	4/4	3/12 [b]
Implants per female [a]	11 ± 3	8 ± 4	5 ± 3 [c]
Implants as percentage of corpora lutea	88.5	72.3	39.5
Living fetuses per female [a]	11 ± 2	7 ± 6 [c]	4 ± 5 [c]
Living fetuses as percentage of implants	96.7	85.3	73.3
Dead or resorbed implants per female	0.4	1.25	1.33
Dead or resorbed as percentage of implants	4.0	14.7	26.7
Fetal body weight (g) [a]	3.97 ± 0.23	2.58 ± 0.61 [d]	2.39 ± 0.25 [d]
Fetal crown-rump length (cm) [a]	3.9 ± 0.2	3.4 ± 0.3 [d]	3.2 ± 0.4 [d]
Sex ratio (M : F)	65 : 54	15 : 14	8 : 3

[a] Mean ± SD.
[b] Differs significantly from controls ($p = 0.0014$ by Fisher's Exact Test).
[c] Differs significantly from controls ($p < 0.02$ by Wilcoxon 2-Sample Test).
[d] Differs significantly from controls ($p < 0.001$ by the t-test).

cephaly) in the 125 mg/kg group may be indicative of a teratogenic effect of bisphenol A. A satisfactory evaluation of teratogenic potential will require a study with sufficient numbers of pregnant rats. NIOSH will be conducting such a study later this year.

Inhalation exposure

The results of the inhalation exposure are summarized in table 5. With six of the nine chemicals tested (butylene oxide, carbon disulfide, methyl bromide, nitrous oxide, styrene oxide, and tetrachloroethyl-

Table 4. Bisphenol A: Intraperitoneal injection, fetal observations from rats.

	Control		Treated			
			85 mg/kg (0.37 mmol/kg)		125 mg/kg (0.55 mmol/kg)	
External observations [a]						
Total examined	118	(11)	29	(3)	11	(3)
Total affected	3	(1)	0	(0)	3 [c]	(1)
Imperforate anus	0	(0)	0	(0)	3 [d]	(1)
Protruding tongue	3	(1)	0	(0)	0	(0)
Skeletal examination [a]						
Total examined	57	(11)	14	(3)	5	(1)
Total affected	11	(7)	10 [d]	(2)	5 [d]	(1)
Incomplete ossification	0	(0)	10 [d]	(2) [b]	5 [d]	(1)
Reduced sternebrae	3	(2)	10 [d]	(2)	5 [d]	(1)
Split centra	3	(2)	2	(1)	0	(0)
Dumbbell centra	4	(3)	2	(1)	1	(1)
Rudimentary 14th rib	1	(1)	0	(0)	0	(0)
Visceral examination [a]						
Total examined	61	(11)	15	(3)	6	(3)
Total affected	2	(1)	11 [d]	(2)	6 [d]	(3) [b]
Enlarged cerebral ventricles	2	(1)	11 [d]	(2)	5 [d]	(2)
Hydrocephaly	0	(0)	0	(0)	1	(1)

[a] Number of fetuses with number of litters in parentheses.
[b] Differs significantly from controls ($p \leq 0.05$ by Fisher's Exact Test).
[c] Differs significantly from controls ($p \leq 0.01$ by Fisher's Exact Test).
[d] Differs significantly from controls ($p \leq 0.001$ by Fisher's Exact Test).

Table 5. Inhalation teratology studies: Summarized results.

Chemical	Rats			Rabbits		
	Maternal toxicity [a]	Fetal toxicity [b]	Teratogenicity [c]	Maternal toxicity [a]	Fetal toxicity [b]	Teratogenicity [c]
Butylene oxide	— [d]	—	—	+ [e]	—	—
Carbon disulfide	—	—	—	—	—	—
2-Ethoxyethanol	+	+	+	+	+	+
Ethyl benzene	+	—	—	—	—	—
Methyl bromide	—	—	—	+	—	—
Nitrous oxide	—	—	—	—	—	—
Styrene oxide	+	+	—	+	—	—
Tetrachloroethylene	—	—	—	—	—	—
Trichloroethylene	—	—	—	—	—	? [f]

[a] Reduced body weight gain or altered weights (absolute or relative) of two or more organs (statistically significant at $p < 0.05$).
[b] Reduced pre- or postimplantation survival, reduced fetal body weight or length (statistically significant at $p < 0.05$).
[c] Grossly visible external or internal (visceral or skeletal) malformation (statistically significant at $p < 0.05$).
[d] — Absent.
[e] + Present.
[f] ? Suggestive but not statistically significant results.

ene), there was no evidence of a teratogenic effect. With trichloroethylene, there was no statistically significant change in the malformation rates in either species, but four cases (two fetuses in each of two litters) of external hydrocephaly were noted among the trichloroethylene-exposed rabbits (2). In the opinion of the contractor conducting this study, external hydrocephaly in rabbits is rare enough to raise some suspicion of a teratogenic response. Trichloroethylene had previously been tested through the inhalation exposure of rats and mice at a lower concentration (300 ppm) (8). The results of that study were negative for both species.

Two of the test chemicals (butylene oxide and methyl bromide) were highly toxic to maternal rabbits. Styrene oxide was highly toxic to both species, but rabbits were more sensitive than rats (9). Despite a significant increase in mortality among rabbits exposed to butylene oxide at 1,000 ppm, fetal toxicity was minimal in the litters of surviving females. With styrene oxide, it was necessary to abandon exposure at 300 ppm when 40 % of the rats died during or within 2 d following a single 7-h exposure at that concentration. Mortality was significantly ($p < 0.05$) increased at 100 ppm, but pregestational and gestational exposures were completed at this level. Fetal toxicity, but no teratogenic effects, were seen at 100 ppm. The styrene oxide levels for rabbits were reduced to 15 and 50 ppm, but maternal mortality was still increased in both exposure groups, significantly so ($p < 0.05$) in the 50-ppm group. There was a tendency towards increased prenatal mortality and reduced fetal size and body weight, but the differences were not statistically significant. In maternal tissues examined histopathologically, bronchiolar epithelial hypertrophy or hyperplasia with squamous metaplasia was seen in the lungs of rats exposed at 100 ppm. No other treatment-related pathological changes were noted.

The methyl bromide exposures had no apparent effect on rats. However, the rabbits exposed at 70 ppm began to lose weight and to show signs of distress after slightly over one week of exposure. Convulsive movements developed, followed by hind-limb paresis, and deaths began to occur on day 9 of gestation. Exposure was terminated after day 15 in an effort to prevent additional deaths in the 70-ppm group. The exposure of the control and 20-ppm groups was also terminated after day 15 to preserve comparability between groups of exposure duration. Nevertheless, deaths continued in the 70-ppm group through day 27 of gestation, reaching 96 % total mortality (24 of 25 rabbits). No maternally toxic, fetotoxic, or teratogenic effects were seen in the rabbits exposed at 20 ppm.

The rabbits exposed to ethyl benzene had a significantly ($p < 0.05$) reduced number of live kits per litter at both exposure levels, but the number of implantations per litter and the number dead or resorbed per litter did not differ from those of the controls (1). Thus the reduced number of live fetuses was not clear evidence of embryo- or fetotoxicity. Maternal toxicity in rats exposed at 1,000 ppm was reflected in increased liver, kidney, and spleen weights. A possible reduction in fertility as a result of exposure before breeding was observed in rats at both exposure levels. Eighty-nine percent of the sperm-positive females that had been exposed to filtered air pregestationally were subsequently found to be pregnant. In both of the groups exposed to ethyl benzene before breeding, only 77 % ($p < 0.05$) of the sperm-positive females were subsequently pregnant. The absence of a concentration-related difference in response at 100 and 1,000 ppm makes this observation less convincing than it would be had there been a differential response over this tenfold concentration range. There was a significant ($p < 0.05$) increase in the incidence of extra ribs in both of the rat groups exposed at 1,000 ppm during gestation and in the group exposed to filtered air pregestationally and at 100 ppm during gestation. While this is not itself regarded as a teratogenic response, Kimmel & Wilson (6) have suggested that an increased incidence of extra ribs may predict the potential for teratogenesis at higher levels of exposure.

Because of the unique properties of 2-ethoxyethanol (1), the exposure concentrations were lower than planned during the pregestational exposures of rats (150 and 650 ppm) and throughout the rabbit

exposures (160 and 615 ppm). Three groups of rabbits were exposed on days 1 through 18 of gestation. The exposures had originally been planned to continue until day 24 of gestation, but they were terminated early because the rabbits exposed at 615 ppm suffered severe anorexia and weight loss. Five of these rabbits died, but the survivors recovered quickly after the exposures were terminated. Rabbits ex-

Table 6. 2-Ethoxyethanol: Inhalation exposure, observations from rabbits at sacrifice.

	Exposure group [a]		
	Air	Low (160 ppm)	High (615 ppm)
Number pregnant/number inseminated	28/29	25/29	27/29
Implants per female [b]	9 ± 2	9 ± 2	8 ± 4
Implants as percentage of corpora lutea	82	86	77
Living fetuses per female [b]	9 ± 2	7 ± 2 [c]	0 [c]
Living fetuses as percentage of implants	97	78	0.0
Dead or resorbed implants per female [b]	0.3 ± 0.6	2 ± 1 [c]	8 ± 4 [c]
Dead or resorbed as percentage of implants	3	22	100.0
Fetal body weight (g) [b]	47 ± 7	44 ± 11	—
Fetal crown-rump length (cm) [b]	10.3 ± 0.5	10.1 ± 0.4	—
Sex ratio (M : F)	109 : 105	98 : 69	—

[a] Seven hours per day on days 1—18 of gestation.
[b] Mean ± SD.
[c] Groups differ significantly with an analysis of variance and Duncan's Multiple Range Test ($p < 0.001$).

Table 7. 2-Ethoxyethanol: Inhalation exposure, fetal observations from rabbit fetuses (number of litters in parentheses).

	Exposure group [a]					
	Air		Low (160 ppm)		High (615 ppm)	
Total examined	214	(24)	167	(23)	0	(0) [b]
Acrania	0	(0)	1	(1)	—	(—)
Spina bifida	0	(0)	1	(1)	—	(—)
Ventral wall defects [c]	0	(0)	4	(4) [i]	—	(—)
Fused aorta/pulmonary artery	0	(0)	5	(5) [i]	—	(—)
Cardiac dysmorphology	0	(0)	1	(1)	—	(—)
Renal changes [d]	0	(0)	5	(5) [i]	—	(—)
Forelimb flexure	0	(0)	3	(2)	—	(—)
Missing toenail	0	(0)	1	(1)	—	(—)
Scoliosis	0	(0)	1	(1)	—	(—)
Brachyury	0	(0)	1	(1)	—	(—)
Rib dysmorphology [e]	4	(4)	5	(5)	—	(—)
Supernumerary ribs	155	(23)	164	(23)	—	(—)
Vertebral variations [f]	76	(13)	160	(23) [i]	—	(—)
Sternebral variations [g]	2	(2)	16	(9) [i]	—	(—)
Altered fontanelle	0	(0)	2	(2)	—	(—)
Ossification defects [h]	4	(3)	3	(3)	—	(—)

[a] Seven hours per day on days 1—18 of gestation.
[b] All litters were totally resorbed.
[c] 2 (2) umbilical hernias; 1 (1) "split peritoneum"; 1 (1) omphalocoele and diaphragmatic hernia.
[d] 3 (3) cystic and ectopic; 1 (1) fused; 1 (1) unilateral agensis.
[e] Controls: 2 (2) "knobby", 2 (2) thickened distally; Exposed: 1 (1) fused, 1 (1) bent, 3 (3) thickened distally.
[f] Includes > 12 thoracolumbar, double centra, and extra lumbar arch.
[g] Includes accessory, bipartite, fused, misaligned, and scrambled.
[h] Includes rudimentary ribs without ossification of proximal end, missing interparietal, missing vertebrae.
[i] Differs significantly from controls ($p < 0.05$ by Fisher's Exact Test).

posed at 160 ppm also showed maternal toxicity in reduced food consumption and body weight gain and increased maternal liver weight. As shown in table 6, all litters in the 615-ppm group were totally resorbed, and in the 160-ppm group the number of live fetuses was significantly reduced, whereas resorptions were increased ($p < 0.001$). Fetal morphological examinations (table 7) revealed a significantly ($p < 0.05$) increased incidence of renal, cardiovascular, and ventral body wall defects in fetuses from the 160-ppm exposure group. Certain minor skeletal variations were also increased.

With rats, there were signs of maternal toxicity (reduced liver weight and increased lung and kidney weights) in those exposed at 765 ppm during gestation, but maternal toxicity was not seen at the 200-ppm level. As with rabbits, all the litters were totally resorbed in rats exposed during gestation at the higher 2-ethoxyethanol level (table 8), but the incidence of resorptions was not significantly increased at the lower exposure. Fetal toxicity at 200 ppm was evident in a significant reduction in fetal body weight and crown-rump length. Fetal morphological examinations (table 9) revealed a signifi-

Table 8. 2-Ethoxyethanol: Inhalation exposure, observations from rats at sacrifice.

	Pregestational-gestational exposure groups[a]						
	Air—air	Air—200 ppm	Air—765 ppm	150 ppm—air	650 ppm—air	150—200 ppm	650—765 ppm
Number pregnant/number inseminated	32/37	28/37	31/37	37/38	34/35	34/37	33/35
Implants per female[b]	13 ± 3	13 ± 3	12 ± 4	12 ± 3	13 ± 3	13 ± 3	13 ± 3
Implants as percentage of corpora lutea	80	70	80	80	80	80	80
Living fetuses per female[b]	12 ± 3	12 ± 3	0[c]	12 ± 3	12 ± 4	12 ± 3	0[c]
Living fetuses as percentage of implants	92	92	0.0	100	92	92	0.0
Dead or resorbed implants per female[b]	0.5 ± 0.7	1.0 ± 1.1	12.0 ± 2.9[c]	0.4 ±0.8	0.8 ± 1.4	0.9 ± 1.1	12.5 ± 3.0[c]
Dead or resorbed as percentage of implants	8	8	100	0	8	8	100
Fetal body weight (g)[b]	3.8 ± 0.3	3.0 ± 0.4[c]	—	3.9 ± 0.3	3.8 ± 0.3	3.2 ±0.3[c]	—
Fetal crown-rump length (cm)[b]	3.6 ± 0.2	3.3 ± 0.4[c]	—	3.7 ± 0.2	3.7 ± 0.2	3.4 ± 0.2[c]	—
Sex ratio (M : F)	183 : 205	143 : 181	—	240 : 204	201 : 216	189 : 213	—

[a] Seven hours per day for three weeks before breeding, and on days 1—19 of gestation.
[b] Mean ± SD.
[c] Groups indicated differ significantly from others with an analysis of variance and Duncans Multiple Range Test ($p < 0.05$).

Table 9. 2-Ethoxyethanol: Inhalation exposure, fetal observations from rat fetuses (number of litters in parentheses).

	Pregestational-gestational exposure groups[a]						
	Air—air	Air—200 ppm	Air—765 ppm	150 ppm—air	650 ppm—air	150—200 ppm	650—765 ppm
Total examined	386 (32)	324 (28)	0 (0)[b]	441 (37)	420 (33)	397 (34)	0 (0)[b]
General edema	1 (1)	1 (1)	— (—)	0 (0)	0 (0)	0 (0)	— (—)
Hydrocephaly	1 (1)	0 (0)	— (—)	0 (0)	0 (0)	0 (0)	— (—)
Anophthalmia	1 (1)	0 (0)	— (—)	0 (0)	0 (0)	0 (0)	— (—)
Cardiovascular defects[c]	0 (0)	6 (4)[e]	— (—)	0 (0)	0 (0)	2 (2)	— (—)
Polydactyly	0 (0)	0 (0)	— (—)	0 (0)	0 (0)	1 (1)	— (—)
Rib dysmorphology[d]	0 (0)	13 (6)[e]	— (—)	1 (1)	0 (0)	6 (6)[e]	— (—)
Supernumerary ribs	49 (15)	84 (21)[e]	— (—)	30 (16)	41 (15)	110 (24)[e]	— (—)
Reduced ossification	32 (17)	225 (26)	— (—)	17 (11)	22 (13)	275 (33)	— (—)
Sternebral variations	0 (0)	0 (0)	— (—)	1 (1)	0 (0)	1 (1)	— (—)

[a] Seven hours per day for three weeks before breeding, and on days 1—19 of gestation.
[b] All litters were totally resorbed.
[c] 1 (1) transposed pulmonary artery and 1 (1) retrotracheal pulmonary artery in 150—200 ppm; 6 (4) retrotracheal pulmonary artery in air—200 ppm.
[d] Includes bent, fused, knobby, and wavy ribs.
[e] Differs significantly from air—air controls ($p \leq 0.05$ by Fisher's Exact Test).

cantly ($p < 0.05$) increased incidence of cardiovascular and skeletal defects in fetuses from the 200-ppm exposure groups.

Discussion

The series of reported experiments yielded interesting and, in some cases, surprising results for several chemicals. The studies using intraperitoneal injection were regarded as preliminary since the group sizes were small and the treatment route was not comparable to occupational exposures. Despite the high doses administered and the nonphysiological treatment, there were no signs of adverse effects on fetal development with five of the ten chemicals, copper naphthenate, ethylene dibromide, 2-mercaptobenzothiazole, naphthalene, and 1,2,3-trichloropropane. No further teratogenesis studies are being planned for these chemicals. Fetal toxicity was seen with hexachlorobutadiene and 2-nitropropane, and embryo toxicity with methyl styrene, but no teratogenic effects were suggested. These three chemicals should be regarded as relatively low priority candidates for more extensive teratological screening by another route of administration, but NIOSH does not currently have any additional studies planned.

Results in the intraperitoneal series with allyl chloride and bisphenol A clearly suggested the need for further study. Bisphenol A significantly interfered with the establishment of pregnancy. Estrogenic activity had previously been reported (3, 4, 7) for bisphenol A, and this may be the mechanism by which implantation was blocked in rats treated with 125 mg/kg. There was a suggestion of a teratogenic effect, but the small number of treated litters available for examination precludes any firm conclusion. Currently, bisphenol A is the object of continuing NIOSH studies in which inhalation and dermal exposures are being used.

Allyl chloride administered intraperitoneally was toxic to both mother rats and their fetuses. In addition subcutaneous edema and a craniofacial defect (short snout with protruding tongue) occurred in the treated litters at a statistically significant rate. NIOSH had scheduled allyl chloride for inhalation teratology testing in rats and rabbits when John et al (5) reported negative results of a study in which rats and rabbits were exposed to allyl chloride at 30 or 300 ppm. The NIOSH inhalation study has now been suspended, at least temporarily, pending an evaluation of possible explanations for the conflicting results of the two studies. It appears that the allyl chloride tested by John et al was much purer than that used by NIOSH. Pilot tests with some of the impurities in the NIOSH sample will be considered.

The results with ethyl benzene were suggestive of a potential effect on fertility and possibly of a teratogenic potential above 1,000 ppm. Neither effect, however, was unequivocally demonstrated, and further studies are required for clarification. Currently NIOSH is not scheduling follow-up studies.

The inhalation studies of 2-ethoxyethanol provided clear evidence of severe embryotoxicity in both species at concentrations above 600 ppm. Maternal toxicity was also severe in rabbits at this higher (615 ppm) exposure level, and was present but less severe in rats exposed at 765 ppm. Fetal toxicity was significant in both species at concentrations near the federal occupational exposure limit (200 ppm). Maternal toxicity was slight in rabbits and absent in rats at the lower exposure levels. In addition the incidence of fetal malformations was significantly increased in both species at the lower exposures. These findings are disturbing in light of the number of persons NIOSH estimates are exposed to 2-ethoxyethanol in this country: 360,000. Furthermore, this is only one representative of a widely used class of solvents, the ethylene and diethylene glycol monoalkyl ethers and their acetates. Currently, NIOSH is attempting to identify a suitable human population for field studies, and laboratory screening studies of other members of this chemical class will be initiated in the coming year.

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