Vinyl Chloride: Inhalation Teratology Study in Mice, Rats and Rabbits

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These studies evaluated the effects of inhaled vinyl chloride monomer (VCM) on mouse, rat and rabbit embryonal and fetal development. Groups of pregnant CF-1 mice, Sprague-Dawley rats and New Zealand white rabbits were exposed to 500 ppm VCM for 7 hr daily during the period of major organogenesis. Subsequently, other groups of mice were similarly exposed to 50 ppm VCM, and rats and rabbits were exposed to 2500 ppm. While maternal toxicity was observed, exposure to VCM did not cause significant embryonal or fetal toxicity and was not teratogenic in any of the three species at the concentrations tested. Simultaneous exposure of some of the pregnant animals to VCM by inhalation plus 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to VCM alone in the three species. The fetal effects observed were similar to those reported for these three species following administration of ethanol without VCM exposure.

Introduction

Inhalation exposure to vinyl chloride monomer (VCM) has been shown to be carcinogenic in laboratory animals (*1*, *2*) and in humans (*3*). The carcinogenic potential of inhaled VCM following *in utero* exposure has been reported (*2*), but observations to determine the teratogenic potential in laboratory animals were not made. Epidemiologic studies (*4-6*) of incidence rates for malformations of the central nervous system among families of employees or residents in the vicinity of vinyl chloride polymerization facilities have not supported any evidence that VCM is teratogenic in humans. A series of studies were conducted in our laboratory to assess the hazard associated with exposure and to investigate the mechanism by which VCM might exert its toxic effects. The purpose of the studies described in this report was to assess the embryotoxic and teratogenic potential of inhaled VCM in mice, rats and rabbits.

The exposure levels tested in the teratology studies are presented in Table 1. In an initial experiment, groups of mice, rats and rabbits were exposed to 500 ppm of VCM, 7 hr daily on days 6-15 (mice and rats) or 6-18 (rabbits) of gestation. Subsequently, additional groups of rats and rabbits were exposed to 2500 ppm. For each concentration tested, concurrent control groups of mice, rats and rabbits were sham-exposed to filtered room air. Since previous studies in this laboratory indicated that the primary metabolic pathway for VCM is blocked by ethanol (*7*), it was considered possible that simultaneous administration of ethanol in the drinking water of animals exposed to VCM might alter its metabolism in a manner which would enhance its toxic or teratogenic potential. Thus, in this experiment some of the VCM-exposed animals were given 15% (v/v) ethanol in their drinking water during the same period of gestation.

Table 1. Teratology studies with vinyl chloride: levels of exposure.

Species	VCM concn, ppm	Ethanol concn, %
Mice	500	0
	500	15
	50	0
	50	15
Rats, rabbits	2,500	0
	2,500	15
	500	0
Mice, rats, rabbits	0	0

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The teratogenic potential of imbibed ethanol in mice, rats, and rabbits was previously studied in our laboratory and reported by Schwetz et al. (*8*). No teratogenic effects were observed when 15% ethanol was given in the drinking water to mice and rats on days 6-15 of gestation or to rabbits on days 6-18 of gestation, although retarded fetal growth and development were observed in mice and rats.

Methods

Female CF-1 mice (Carworth, Portage, Michigan) weighing 25 to 30 g, Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Michigan) weighing approximately 250 g and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) weighing 3.5-4.5 kg were used in this study. The day on which a vaginal plug was observed or the day on which sperm were seen in a vaginal smear was considered day zero of pregnancy for mice and rats, respectively. The day of natural mating was considered day zero for rabbits. Between daily exposures, animals were housed in wire-bottom cages in a room controlled for temperature, humidity and light cycle. Commercial laboratory animal food (Ralston Purina Co., St. Louis, Missouri) and tap water or tap water containing ethanol were available during the periods between exposure to VCM. Food consumption was measured at 3-day intervals for mice and rats and at 2-day intervals for rabbits. All animals were deprived of food and water during the 7-hr exposure period each day.

Exposure of bred animals was conducted in stainless steel chambers of 3.7 m^3 volume under dynamic conditions. The atmosphere of VCM was generated by diluting gaseous VCM with filtered room air at a rate calculated to give the desired concentration. Vinyl chloride monomer (chloroethylene) obtained from Matheson Gas Products, Joliet, Illinois was used for the exposures. The actual concentration was measured with an infrared spectrophotometer (Perkin Elmer 12A or Miran I) with a multipath gas cell.

All animals were observed daily throughout pregnancy and maternal body weights were recorded at several intervals during gestation. Pregnant mice and rats were sacrificed by carbon dioxide inhalation on day 18 and 21 of gestation, respectively. Pregnant rabbits were sacrificed on day 29 of gestation. The uterine horns were exteriorized through a midline incision in the abdominal wall and the number and position of live, dead and resorbed fetuses were noted. After being weighed, measured (crown-rump length) and sexed (mice and rats), the fetuses were examined for external anomalies. One-third of each litter was immediately examined for evidence of soft tissue anomalies by dissection under a low power microscope (*9*). The heads of those fetuses (mice and rats only) were preserved in Bouin's solution and examined for soft tissue but not skeletal anomalies (*10*). Rabbit fetuses were sexed on the basis of examination of internal genitalia. All fetuses were then eviscerated, preserved in alcohol and subsequently cleared and stained with Alizarin Red-S for examination for skeletal anomalies (*11*).

The Fisher exact probability test (*12*) was used to evaluate the incidence of resorptions among litters. Maternal and fetal body weights and body measurements and maternal liver weights were analyzed statistically, by an analysis of variance and Dunnett's test (*13*). The incidence of fetal anomalies was analyzed by the Wilcoxon test as modified by Haseman and Hoel (*14*). The group of animals which was exposed only to vinyl chloride served as the control for those animals which were exposed to vinyl chloride in combination with 15% ethanol in the drinking water. The controls for animals exposed to vinyl chloride and maintained on tap water without ethanol were exposed to filtered room air in exposure chambers which were similar to those used for exposure to vinyl chloride.

Results and Discussion

Maternal Toxicity

Exposure to 500 ppm VCM was maternally toxic to mice; deaths (5 of 29 bred females), decreases in the amount of weight gained during gestation, in food consumption and in absolute liver weight as compared to the air only controls were observed. Evidence of toxicity was not apparent among mice exposed to 50 ppm of VCM. The combination of VCM exposure with 15% ethanol in the drinking water significantly enhanced the toxicity as compared to VCM alone for both concentrations, though no deaths occurred in the 50 ppm VCM plus ethanol group of mice.

Rats exposed to 500 ppm VCM gained less weight than controls during gestation, but no other evidence of toxicity was observed at this level. One maternal death among 17 bred females, decreased food consumption and an increase in liver weight were observed at 2500 ppm VCM in rats. Ethanol given in combination with 2500 ppm VCM was more maternally toxic than exposure to VCM alone; the amount of body weight gained by pregnant rats during gestation and food consumption were significantly decreased in the group given the combi-

nation. The liver weight relative to body weight was increased in this group as compared to the rats exposed only to VCM, but no deaths were observed as a result of treatment with the combination. An ethanol group was not included among the rats exposed to 500 ppm of VCM.

Some deaths were observed among pregnant rabbits exposed to 2500 ppm VCM alone (one of seven bred females) or in combination with ethanol (3 of 19 bred females); however, other evidence of toxicity in rabbits consisted only of decreases in food consumption among those exposed to 500 ppm VCM alone and among rabbits given the combination of 2500 ppm plus 15% ethanol in the drinking water.

Among mice, rats and rabbits given 15% ethanol in the drinking water during gestation, Schwetz et al. (8) reported that maternal toxicity, as evidenced by decreased body weights, occurred in all three species.

Observations at the Time of Cesarean Section

Among litters of mice exposed to 500 ppm of VCM, the incidence of resorptions was increased; 13% of the implants were resorbed versus 7% in the concurrent air controls (Table 2). Historical control data from 801 litters of CF-1 mice in our laboratory show the mean percentage of implantations resorbed to be 11% with a range of 6-22%. Thus, both of these values are within the range observed for control groups of this strain. Litter size and fetal body weight were decreased at 500 ppm. These effects may have been secondary to the toxicity observed among pregnant dams at this exposure level. Toxic effects on the embryo or fetus were not observed among litters of mice exposed to 50 ppm VCM.

Ethanol in combination with VCM produced greater fetotoxicity than exposure to VCM alone at both levels. In the 500 ppm VCM plus ethanol group, a decrease in the percentage of pregnant mice was observed. This resulted in only seven litters, two of which contained only implantations which were resorbed. Fetal body measurements were decreased among the ethanol groups when compared to groups given either 50 or 500 ppm VCM alone.

No adverse effects on the percentage of pregnant dams or the incidence of implantations resorbed were observed among rats (Table 3). Exposure to

Table 2. Mice: observations at the time of cesarean section.

	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		no ethanol	15% ethanol		no ethanol	15% ethanol
Number of litters	21	20	16	26	19	7
Live fetuses/litter[a]	10 ± 4	11 ± 4	10 ± 4	12 ± 2	11 ± 2[b]	8 ± 6[c]
% Implantations resorbed	15(40/261)	8(18/238)	11(19/172)	7(26/351)	13(33/248)[b]	19(13/69)
Fetal body weight, g[a]	1.00 ± 0.11	1.02 ± 0.10	0.84 ± 0.14[c]	1.07 ± 0.06	0.99 ± 0.11[b]	0.78 ± 0.15[c]
Fetal crown-rump length, mm[a]	23.0 ± 1.9	24.2 ± 0.8[b]	22.4 ± 1.5[c]	23.7 ± 1.2	23.6 ± 1.0	21.2 ± 1.5[c]
% Pregnancy	57(21/37)	74(20/27)	57(16/28)	88(28/32)	72(21/29)	31(9/29)[c]

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

Table 3. Rats: observations at the time of cesarean section.

	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
Number of litters	28	31	19	16	16
Live fetuses/litter[a]	12 ± 2	12 ± 2	12 ± 2	13 ± 2	12 ± 2
% Implantations resorbed	1(4/342)	3(11/398)	4(9/238)	3(6/220)	4(7/195)
Fetal body weight, g[a]	5.67 ± 0.29	5.44 ± 0.38[b]	5.59 ± 0.27	5.62 ± 0.29	5.34 ± 0.32[c]
Fetal crown-rump length, mm[a]	42.6 ± 1.2	43.6 ± 0.8[b]	43.6 ± 1.5	43.3 ± 1.1	42.4 ± 0.9[c]
% Pregnancy	96(28/29)	94(31/33)	95(19/20)	100(17/17)	94(16/17)

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

2500 ppm VCM plus ethanol resulted in fetal body weights and crown-rump lengths which were lower than fetal body measurements among litters from rats exposed only to 2500 ppm VCM. In the group exposed to 500 ppm of VCM alone, fetal body weights were decreased as compared to concurrent air controls, though fetal crown-rump lengths were significantly increased.

In rabbits, the incidence of resorptions was significantly increased among litters given 2500 ppm VCM plus ethanol, where 53% of the implantations showed evidence of resorption versus 24% among

Table 4. Rabbits: observations at the time of cesarean section.

	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
Number of litters	18	19	11	5	16
Live fetuses/litter[a]	8 ± 1	7 ± 2[b]	6 ± 3	6 ± 4	4 ± 4
% Implantations resorbed	6(10/162)	9(14/150)	22(19/88)	24(10/42)	53(79/149)[c]
Fetal body weight, g[a]	35.23 ± 4.82	34.13 ± 4.17	36.46 ± 4.82	33.77 ± 4.48	32.48 ± 5.88
Fetal crown-rump length, mm[a]	91.0 ± 4.2	92.6 ± 5.0	92.6 ± 4.7	87.1 ± 5.2	87.7 ± 6.3
% Pregnancy	100(18/18)	95(19/20)	100(11/11)	86(6/7)	95(18/19)

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

Table 5A. Mice: incidence of fetal anomalies.

	No. fetuses (no. litters) examined					
	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		No ethanol	15% ethanol		No ethanol	15% ethanol
External examination	221(20)	220(20)	153(14)	325(26)	215(19)	56(5)
Soft tissue examination	74(20)	75(20)	50(14)	107(26)	73(19)	29(5)
Skeletal examination	221(20)	220(20)	153(14)	325(26)	215(19)	56(5)
Bones of the skull	147(20)	145(20)	103(14)	217(26)	142(19)	37(5)

Table 5B. Mice: incidence of fetal anomalies.

	% fetuses (% litters) affected					
	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		No ethanol	15% ethanol		No ethanol	15% ethanol
External examination						
Cleft palate	1(10)	1(10)	2(21)	0	1(5)	6(40)
Anopthalmia	0	0	0	0	0	2(20)
Exencephaly	0	0	0	1(8)	1(10)	2(20)
Soft tissue examination						
Thymus	0	0	4(7)	0	0	0
Skeletal examination						
Skull bones, unfused	0	0.7(5)	24(50)[a]	1(12)	5(21)	11(20)
Skull, delayed ossification	9(35)	8(37)	40(100)[a]	13(54)	30(58)[b]	70(100)[a]
Sternebrae, unfused	3(20)	3(25)	13(57)[a]	2(19)	9(42)[b]	34(80)[a]
Sternebrae, delayed ossification	7(50)	4(35)	44(100)[a]	1(12)	6(42)[b]	43(100)[a]
Vertebrae-lumbar spurs	4(35)	5(40)	2(21)	4(31)	3(21)	14(80)[a]
Vertebrae, forked atlas	0.4(5)	1(10)	4(36)[a]	0	0	4(20)
Vertebrae, delayed ossification	0	0	1(14)	0	0	5(40)[a]

[a]Significantly different from VCM-exposed group, $p < 0.05$.
[b]Significantly different from air-exposed control, $p < 0.05$.

those exposed only to VCM (Table 4). Only five litters were available for examination in the latter group. Litter size was decreased as compared to concurrent air controls among litters of rabbits exposed to the lower level of 500 ppm, but no effect on litter size resulted from exposure to 2500 ppm of VCM. The decreased mean litter size at 500 ppm most likely occurred because the rabbits in this group released fewer ova; the number of corpora lutea observed on the ovaries was lower among animals in this group.

In the studies by Schwetz et al. (*8*), a slight increase in the incidence of resorptions was observed among litters of rabbits, but not among litters of mice or rats given 15% ethanol in their drinking water during gestation. Decreases in fetal body measurements were observed by Schwetz et al. (*8*) among litters from both mice and rats maintained on drinking water containing ethanol. In the present study, decreases in fetal body measurements were more pronounced when ethanol was given in combination with VCM in these two species; however it is not clear if this apparent synergistic effect was mediated via metabolic interference in the maternal animal.

Incidence of Fetal Anomalies

Among litters of mice, no external or soft tissue anomalies were observed at a significantly higher incidence than the respective controls for any of the exposed groups (Table 5). Cleft palate was observed in 40%, or two of the five litters examined, at 500 ppm VCM plus 15% ethanol. This incidence is not statistically increased as compared to the group exposed to 500 ppm VCM alone. Due to the low percentage of pregnancy in the females, only five litters were available for examination in the 500 ppm VCM plus ethanol group. Among litters of mice exposed to 500 ppm VCM without ethanol in the drinking water, increased incidences of three skeletal variants indicative of delayed skeletal development were observed. Ethanol, when given in combination with VCM, caused a significant increase in the occurrence of a number of these skeletal variants at both levels of VCM exposure. Thus, the occurrence of delayed skeletal development coincided with those treatment regiments which were maternally toxic and resulted in decreased fetal body measurements.

Among rats exposed to 2500 ppm of VCM, the

Table 6A. Rats: incidence of fetal anomalies.

	No. fetuses (no. litters) examined				
				2500 ppm VCM	
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination	339(28)	387(31)	229(19)	214(16)	188(16)
Soft tissue examination	113(28)	129(31)	76(19)	73(16)	63(16)
Skeletal examination	337(28)	387(31)	229(19)	214(16)	188(16)
Bones of the skull	225(28)	259(31)	153(19)	141(16)	125(16)

Table 6B. Rats: incidence of fetal anomalies.

	% fetuses (% litters) affected				
				2500 ppm VCM	
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination					
Omphalocele	0	1(3)	0.4(5)	0	0.5(6)
Soft tissue examination					
Microphthalmia	0	0	0	0	2(6)
Dilated ureter	2(7)	2(6)	5(10)	27(50)[a]	5(19)[b]
Small kidney	0	0	0	0	2(6)
Skeletal examination					
Vertebrae, lumbar spurs	1(4)	9(52)[b]	14(68)	12(69)	35(69)[b]
Vertebrae, delayed ossification	0.3(4)	2(16)	7(53)	4(50)	21(81)[a]

[a]Significantly different from air-exposed control, $p < 0.05$.
[b]Significantly different from VCM-exposed group, $p < 0.05$.

incidence of a single anomaly, dilated ureter was significantly higher than controls (Table 6). Ethanol did not further increase the incidence of this anomaly. The incidence of dilated ureter was significantly lower in the ethanol group as compared to the VCM exposed group. Only minor skeletal variants were observed at an increased incidence among the exposed rats. Lumbar spurs occurred more often than controls among litters at 500 ppm, but not among those exposed to 2500 ppm VCM alone. This variant was again observed at an increased incidence among the litters of rats treated with the combination. The incidence of delayed ossification of vertebral centra was also increased in this group and is indicative of a slight delay in skeletal development. As in mice, these skeletal changes occurred in those treatment groups where the combination of high exposure levels of VCM and ethanol produced evidence of maternal toxicity and decreased fetal body measurements.

Among rabbits, external and soft tissue anomalies were observed at a low incidence in those groups exposed to 2500 ppm of VCM alone or in combination with ethanol (Table 7). These included a single fetus with a dilated cerebral ventricle at 2500 ppm and a single fetus with cleft palate from the group exposed to 2500 ppm plus 15% ethanol. A dilated renal pelvis was observed in two fetuses from a single litter in the latter group. Two additional fetuses from this group exhibited an enlarged atrium of the heart. Ossification of the fifth sternebra was delayed at the 500 ppm level, but not at the 2500 ppm level of exposure. Only four litters were available for examination at the 2500 ppm of VCM exposure level.

These data show that the combination of VCM exposure with ethanol in the drinking water was more toxic to the developing fetus, as it was in the maternal animal, than exposure to VCM alone. However, neither treatment regimen was teratogenic in the species tested. Fetal effects consisted of increased incidences of minor skeletal variants indicative of a delay in development in mice and rats. Similar skeletal changes (delayed ossification of sternebrae or vertebral centra and unfused sternebrae or bones of the skull) were observed by Schwetz et al. (*8*) in these two species given 15% ethanol in drinking water. Thus, the exposure to high concentrations of VCM in combination with ethanol in the drinking water produced toxic effects in the developing embryo or fetus which were similar to those produced by ethanol alone.

A similar lack of teratogenicity of VCM in mice and of VCM alone or in combination with ethanol in rats was reported recently by Ungvary et al. (*15*). Several experiments were conducted. Exposure to 1500 ppm for 24 hr/day during organogenesis was reported to have no teratogenic or fetal effects

Table 7A. Rabbits: incidence of fetal anomalies.

	No. fetuses (no. litters) examined				
				2500 ppm VCM	
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination	152(18)	136(18)	69(9)	32(4)	70(9)
Soft tissue examination	50(18)	47(18)	24(9)	10(4)	25(9)
Skeletal examination	152(28)	136(18)	69(9)	32(4)	70(9)

Table 7B. Rabbits: incidence of fetal anomalies.

	% fetuses (% litters) affected				
				2500 ppm VCM	
	No VCM no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination					
Cleft palate	0	0	0	0	1(11)
Soft tissue examination					
Dilated renal pelvis	0	0	0	0	8(11)
Dilated cerebral ventricle	0	0	0	10(25)	0
Enlarged atrium, heart	0	0	0	0	8(11)
Skeletal examination					
Sternebrae, delayed ossification	28(77)	38(94)[a]	20(44)	16(75)	24(67)

[a]Significantly different from air-exposed control, $p < 0.05$.

apart from an increase in fetal liver weight. Exposure during the third part of pregnancy produced no deleterious effects, whereas exposure during the early days of gestation increased fetal mortality and resulted in decreased fetal body weights. Exposure to vinyl chloride and simultaneous maintenance on a liquid alcoholic diet during the neurulation period produced evidence of skeletal retardation, but no malformations in rats. Though the exact days of gestation during which the different treatment regimens were employed were not stated by the author, the reported results are in apparent agreement with those from our laboratory.

A report by Mirkova et al. (*16*) summarized the fetal and postnatal effects following exposure of pregnant rats to 6.15 mg/m^3 of VCM by inhalation throughout the entire gestation period. An increase in early embryo deaths (immediately after blastocyst implantation) and a decrease in fetal body weight were reported by these authors. Observed anomalies in the offspring included generalized hematomas, (8-fold increase over controls) internal hydrocephalus (54.5% of the fetuses), encephalocele (2.53%), and variations of sternebral ossification (2.8% of the fetuses). Several postnatal effects indicative of hepatotoxicity and disturbances in the hepatobiliary system in the progeny following *in utero* exposure were also reported. The 6.15 mg/m^3 level of exposure is equal to only 2.5 ppm of VCM. The exact length of exposure periods and details of the testing methods employed, especially as pertains to vapor generation and analyses, were not reported by Mirkova et al.; thus no explanation for the differences in observed effects of VCM is apparent. The reported results are markedly inconsistent with those from our laboratory where a thousandfold increase in exposure level 2500 ppm was not embryolethal in rats, and similar fetal anomalies were not observed.

In summary, exposure of pregnant mice, rats or rabbits to VCM by inhalation at concentrations sufficiently high to cause maternal toxicity was not teratogenic in any of these species. Fetal effects consisted of delayed skeletal development in mice at 500 ppm, an exposure level which was maternally toxic, and an increase in the incidence of dilated ureter in rats following maternal exposure to 2500 ppm. In mice exposed to 500 ppm of VCM, the incidence of fetal resorptions was increased over concurrent air controls. The incidence of resorptions observed in this group was at the high end of the range for historical control groups in our laboratory.

Ingestion of 15% ethanol in the drinking water enhanced the toxicity of inhaled VCM. Fetal body measurements were decreased among mice and rats given the combination and increases in the occurrence of skeletal variants indicative of delayed development were observed in both species. The fetal effects observed were similar to those reported for these test species following administration of ethanol without VCM exposure. The incidence of resorptions was increased in rabbits given ethanol in combination with VCM, and maternal toxicity was enhanced by ingestion of ethanol in all three species.

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