PRELIMINARY REPORT: VINYL CHLORIDE TERATOLOGY STUDY IN MICE, RATS AND RABBITS

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INTRODUCTION AND PURPOSE

Recent observations on the carcinogenic potential of vinyl chloride has stimulated the conduct of a series of studies in our laboratory to further assess the hazard associated with exposure to vinyl chloride as well as to investigate the mechanism by which vinyl chloride might exert untoward effects. The purpose of the studies described in this interim report was to assess the potential of vinyl chloride to have a deleterious effect on the developing embryo and fetus of laboratory animals.

The animals used in this study include CF-1 mice, Sprague-Dawley (Spartan substrain) rats and New Zealand white rabbits. The animals were exposed to 500 ppm vinyl chloride for seven hours each day. Mice and rats were exposed on days 6-15 of gestation while rabbits were exposed on days 6-18. Exposure of bred animals was conducted in stainless steel chambers of 3.7 cubic meter volume under dynamic conditions. Groups of control animals were exposed in a chamber to filtered room

air. The atmosphere of vinyl chloride was attained by metering gaseous vinyl chloride into a stream of air of known flow rate and subsequently into the chamber. The concentration of vinyl chloride in the chamber atmosphere was calculated from the ratio of material delivery rate and the total chamber airflow rate. The concentration of vinyl chloride in the chamber was determined and continuously monitored by infrared spectrometry.

In each species, the day of natural mating was considered day zero of pregnancy. Animals were housed in wire-bottom cages and were maintained on commercially available laboratory animal chow free choice. Animals did not have access to food or water while in the inhalation chambers.

The animals were observed daily throughout the gestation period for indications of toxicity from the test material. The fetuses of pregnant mice, rats and rabbits were delivered by cesarean section on gestation days 18, 21 and 29, respectively. Following examination of all pups for external abnormalities, one-third of each litter was examined immediately by dissection under a low power microscope for evidence of soft tissue abnormalities. Each pup in each litter was then eviscerated and sexed and placed in 95% ethanol, cleared and stained with alizarin red-S for subsequent examination for skeletal anomalies.

In addition to the above described groups of animals that were exposed to vinyl chloride alone, an additional group of bred mice was exposed to vinyl chloride and was given ethanol in addition to determine if the teratogenic potential of vinyl chloride was enhanced by simultaneous exposure to ethanol. The rationale for conducting this study was based on the results of previous studies in this laboratory of the metabolism of vinyl chloride. The primary and fastest pathway for vinyl chloride was blocked by ethanol. Therefore, blockade of this pathway may alter the metabolism in a manner which would produce different and/or more metabolites possessing greater toxicity. Also, Dr. Irving Seilikoff, Mount Sinai Hospital, has recently suggested an association between alcohol consumption and vinyl chloride exposure with the development of angiosarcoma in workers (personal communication). In the present study, mice were given ethanol in their drinking water at a concentration of 15%. In previous studies in our laboratory, such exposure to ethanol was not teratogenic in mice.

The results of this initial study will be used to determine the design of subsequent segments of this teratology study.

RESULTS AND DISCUSSION

The examination of the fetuses for skeletal malformations has not been completed at the present time. Therefore,

final conclusions regarding the effect of exposure of pregnant mice, rats and rabbits to 500 ppm vinyl chloride cannot be drawn at the present time. However, a summary of the preliminary results is given in the accompanying table.

Except for a slight but consistent decrease in food consumption among rabbits, no signs of toxicity were observed in the adult rats or rabbits either during the time of exposure or at the time of necropsy and cesarean section. No maternal deaths were observed and the pregnancy rate was comparable to that observed in the respective control groups. Exposure of rats to vinyl chloride had no effect on the number of implantation sites or on the incidence of fetal resorptions or litter size. The fetal body weight was slightly decreased and the fetal crown-rump length was slightly increased compared to these measurements in fetuses of control dams. No external or soft tissue malformations were observed among the rat fetuses. Among rabbits exposed to 500 ppm vinyl chloride, there was a slight decrease in the number of implantations with a resultant slight decrease in the average litter size compared to the control litters. There was no effect on the incidence of resorptions, although one litter was totally resorbed. There was no effect on fetal body measurements or the incidence of external or soft tissue malformations among the fetuses of rabbits.

Mice seemed to be more susceptible to vinyl chloride than either of the other 2 species in several respects. There was a slight decrease in the weight gain among mice exposed to vinyl chloride during gestation. In addition, maternal deaths were observed during the exposure period. Six deaths occurred among 30 exposed mice. Exposure of another group of 10 nonbred mice resulted in no deaths. Necropsy examination of the deaths among the bred mice did not reveal the cause of death. Tissues were saved for microscopic examination. The percent pregnancy among mice exposed to vinyl chloride was slightly lower than among control mice. The normal range of pregnancy rate among previous teratology studies with mice is from 85 to 100%. The 72% pregnancy rate among the vinyl chloride-exposed mice is just slightly lower than the lower limits of the expected pregnancy range. Exposure to vinyl chloride resulted in a slight increase in the incidence of resorptions among mice. There was a very slight decrease in the average litter size compared to the control values; fetal body weights were also slightly lower than controls. The incidence of external and soft tissue malformations was not different than among litters of control mice.

Concomitant treatment of mice with ethanol in the drinking water during the days of exposure to vinyl chloride appeared

to accentuate the effect of exposure to vinyl chloride alone. The maternal weight gain during pregnancy was significantly lower than among mice exposed to vinyl chloride alone. The pregnancy rate was decreased in comparison to the vinyl chloride-exposed mice, the air control mice and mice which received ethanol alone in an earlier study. In contrast to these enhanced toxic manifestations, the incidence of maternal deaths was not increased by concomitant ethanol treatment. There was a significant decrease in the number of implantation sites among pregnant mice exposed to vinyl chloride and ethanol and 2 of the litters were completely resorbed. There was a significant decrease in the litter size as well as fetal body measurements compared to mice exposed to vinyl chloride alone. The incidence of external malformations among mice exposed to vinyl chloride and ethanol was not different from that among mice exposed to vinyl chloride. However, the incidence of cleft palate was significantly increased among mice receiving ethanol plus vinyl chloride.

Based on the results accumulated up to the present time, it appears that exposure of bred mice, rats and rabbits to 500 ppm vinyl chloride during the period of organogenesis did not result in a teratogenic response. Mice appeared to be slightly more susceptible to the toxic effect of vinyl chloride than either rats or rabbits. Simultaneous exposure of mice to vinyl chloride by inhalation and ethanol in the

drinking water resulted in toxic effects greater than that observed among mice exposed to vinyl chloride alone. It must be kept in mind, however, that these are preliminary conclusions and final conclusions will be drawn only upon completion of the examination of the fetuses for skeletal malformations. The effect to exposure to 500 ppm will be used to determine the experimental design of the remaining portions of this teratology study.

SUMMARY - VINYL CHLORIDE TERATOLOGY STUDIES

	Rats	Rabbits	Mice	
Vinyl chloride, 500 ppm, 7 hr/day	yes	yes	yes	yes
Ethanol - 15% in drinking water	no	no	no	yes
Gestation days of treatment	6-15	6-18	6-15	6-15
No. maternal deaths/No. treated	0/33	0/19	6/30 (20%)	4/30 (13%)
% Pregnancy	94 (control = 96)	95 (control = 100%)	72 (control = 88)	31 (control = 88)
No. litters examined	31	18	19	5
Maternal weight gain	--[a]	--	sl. dec.	dec.
Maternal food consumption	--	dec.	--	dec.
Maternal liver weight - absolute	--	--	sl. dec.	dec.
- relative	--	--	--	dec.
Implantation sites/dam	--	sl. dec.	--	dec.
% Resorptions	--	--	sl. inc.	--
Litters totally resorbed	0/31	1/19	0/19	2/7
Litter size	--	sl. dec.	sl. dec.	dec.
Fetal body weight	dec.	--	sl. dec.	dec.
Fetal crown-rump length	inc.	--	--	dec.
External malformations	--	--	--	--
Visceral malformations	--	--	--	inc.[b]
Skeletal malformations	NC	NC	NC	NC

[a] -- = no change, dec. = decrease, inc. = increase, sl. = slight, NC = exam not completed

[b] cleft palate