

PROTOCOL FOR A STUDY OF THE EFFECTS OF MATERNALLY INHALED
VINYL CHLORIDE ON RAT AND RABBIT EMBRYONAL AND FETAL
DEVELOPMENT

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INTRODUCTION

Vinyl chloride is widely used in the preparation of polyvinyl chloride resin, as a co-polymer in Saran and other plastics, as a solvent and as a chemical intermediate. A report of the effect of single exposures of mice, rats and guinea pigs to vinyl chloride by Mastromatteo et al, (1960) indicates that this compound has very low acute toxicity. Anesthesia is the primary significant effect of acute exposure to high concentrations (75,000-100,000 ppm). Use of vinyl chloride as a surgical anesthetic has been discouraged because of its undesirable effect on the circulatory system and its high flammability. The effect of repeated exposure of laboratory animals to vinyl chloride has been reported by Torkelson et al, (1961). Groups of animals were exposed 7 hours/day, 5 days/week for up to six months to either 500, 200, 100 or 50 ppm vinyl chloride in air. Detectable changes occurred at all but the lowest concentration. Repeated exposure for six months to 200 ppm resulted in histologic changes in the centrilobular area of the livers of rabbits but not in rats, guinea pigs or dogs. At 100 ppm, only slight

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2

liver enlargement was observed. In a study reported by Viola et al., (1971), rats were exposed to 30,000 ppm vinyl chloride vapor for 4 hours daily, 5 days/week for 12 months. Findings on these rats at or before the end of 12 months exposure included severe chronic hepatitis, interstitial pneumonia, as well as tumors of the skin, lungs and bones.

Reports of studies of the potential of vinyl chloride to have a deleterious effect on the developing embryo and fetus have not been found in the literature. The study described in this protocol has been designed to determine whether or not exposure of pregnant rats and rabbits has a deleterious effect on embryonal and fetal development.

EXPERIMENTAL PROCEDURES

A) Design

In an initial study, bred rats and rabbits will be exposed to twice the maximum excursion limit of vinyl chloride (TLV = 200 ppm). Twice the maximum excursion limit of vinyl chloride is 500 ppm ($200 \text{ ppm} \times 1.25 \times 2$). Rabbits will be exposed on days 6 through 18 and rats on days 6 through 15 of gestation for 7 hours on each day. Groups of 30 rats and 15-20 rabbits will be exposed. A group of control rats and rabbits will be exposed in a chamber to filtered room air. If exposure to 500 ppm causes no evidence of maternal toxicity, embryotoxicity or teratogenicity, additional groups of rats and rabbits will be

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exposed to 5,000 ppm on the same days of gestation. If maternal toxicity is observed at 500 ppm, additional higher dose levels will not be studied, regardless of the effect on the embryo and fetus. If embryotoxicity or teratogenicity is observed at 500 ppm, additional lower concentrations will be studied at half-fold decrements until no embryotoxicity or fetal toxicity is evident. Thus, concentrations of 250, 125 or 62.5 ppm, etc, will be studied if a significant embryotoxic effect is observed at 500 ppm in the initial experiment.

B) Exposure Procedure

Exposure of pregnant animals will be carried out in stainless steel dynamic chambers of 3.7 cubic meter volume. The chamber atmosphere will be generated by metering gaseous vinyl chloride at a known rate into a metered stream of air into the chamber. The concentration of vinyl chloride in the chamber atmosphere will be calculated from the ratio of material delivery rate and the total chamber air-flow rate. The analytical concentration will be determined by infrared spectrometry (Beckman IR10). The wave lengths for analysis will be 10.6 and 11.2 μ . The chamber concentration will be analyzed periodically during exposure. Combustion conductivity analysis will also be used to continuously monitor the exposure concentration.

C) Animals

Adult New Zealand white rabbits and Sprague-Dawley rats

will be used. The day of natural mating will be considered day zero of pregnancy. Animals will be housed individually in wire-bottom cages and maintained on commercially available laboratory animal chow ad libitum. Animals will not have access to water or food in the inhalation chamber during the exposure period. Food consumption will be measured at 3-day intervals during gestation.

D) Maternal Observations

Animals will be observed daily throughout the gestation period for indications of toxicity from the test material. The maternal body weight of rabbits will be recorded on days 6, 12 and 18 of gestation. The body weight of rats will be recorded on days 6, 10 and 16 of gestation. In addition, maternal body weights and the weight of the maternal liver will be recorded at the time of cesarean section, day 21 in rats and day 29 in rabbits.

E) Teratological Examination

On gestation days 21 and 29 in rats and rabbits, respectively, the pregnant females will be sacrificed by carbon dioxide inhalation and the fetuses will be removed by cesarean section. The following data will be recorded: 1) position and number of fetuses in utero; 2) number of live and dead fetuses; 3) number of resorptions; 4) number of corpora lutea; 5) individual pup weight and crown rump length and 6) gross external abnormalities.

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One-third of each litter will be examined immediately by dissection under a low-power microscope for evidence of soft-tissue abnormalities. Each pup in each litter will be eviscerated and sexed and placed in 95% ethanol, cleared and stained with Alizarin Red S for subsequent examination for skeletal anomalies.

F) Statistics

Statistical evaluation of the frequency of anomalies and resorptions among litters will be made by the Fisher Exact Probability test (Siegel, 1956). Analyses of maternal and fetal body weights and body measurements and liver weights will be made by an analysis of variance. Group means will be compared to controls using Dunnett's test (Steel and Torrie, 1960). The level of significance chosen for all cases is $P < 0.05$. The litter is considered the experimental unit of treatment and observation.

G) Estimated Cost

The estimated cost of this study for both species is \$8,000 + 10% for each concentration to be studied.

H) References

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