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Toxicology of Maternally Ingested Carbon Tetrachloride (CCl_4) on Embryonal and Fetal Development and *in vitro* Fertilization in Mice

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ABSTRACT—Oral administration of carbon tetrachloride (CCl_4) for five consecutive days beginning on either day 1, 6 or 11 of pregnancy in mice had no effect on maternal body weight, liver and kidney weight or pregnancy. The various neonatal parameters e.g. pup weight and crown rump length were also not affected. No malformations were detected in any pup on day 1 post-partum. The development of pups (incisor eruption on day 11 and eye opening on day 14) was normal. CCl_4 at concentrations of 0.05 and 0.5 mM had no significant effect on *in vitro* fertilization while higher doses caused a significant decrease. A significant linear relationship between 1, 2, 5 and 10 mM CCl_4 and a decrease in the fertilization rate were found. Similarly all the doses of CCl_4 (1 to 10 mM) resulted in a significant increase in abnormal ovum forms. A significant linear relationship was found between the dose and percent of abnormal forms. Thus CCl_4 had no *in vivo* reproductive toxic effects following administration of 1/10 and 1/100 LD_{50} dose. However, addition of CCl_4 in culture medium in concentrations found in blood of lethally intoxicated rats, adversely affected the *in vitro* fertilization rate and caused an increased incidence in abnormal ova.

INTRODUCTION

Carbon tetrachloride (CCl_4) is a colorless volatile organic compound commonly used as an industrial solvent, a fumigant and in the manufacture of fluorocarbons. CCl_4 has been detected in ground and municipal water, air, aquatic organisms and food. Thus, it becomes a potential health hazard [7, 17, 21]. CCl_4 is readily absorbed and distributed to all organs following ingestion or inhalation. The liver is the main organ responsible for biotransformation and detoxification [13, 14]. Evidence for a genotoxic or mutagenic action of CCl_4 has largely been negative [9, 10].

Reproduction may be sensitive to a number of environmental factors, physical, chemical or emotional. Studies of reproductive effects of CCl_4 in the past have tended to use varying exposure routes and very high doses resulting in maternal toxicity and conflicting results [3, 4, 11, 15, 16].

The present study reports the effects of oral ingestion of CCl_4 on mice for 5 consecutive days beginning on either Day 1, 6 or 11 of pregnancy in B6D2F1 mice. In this strain the first 5 days of gestation are characterized by sequential cleavage of the fertilized oocytes that generates a hatched blastocyst. Implantation is completed on Day 5. Days 6 to 10 are characterized by organogenesis. Finally, Days 11 to 15 are characterized by growth of the fetus. These time periods were chosen in an attempt to isolate detrimental effects on various stages of development and to determine the most susceptible stage for CCl_4 insult. The effects of CCl_4 on *in vitro* fertilization was also evaluated.

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MATERIALS AND METHODS

Animals

Female B6D2F1 mice were obtained from one of two sources. Six week old females used for *in vivo* experiments were obtained from the Jackson Laboratories (Bar Harbor, ME). In some experiments, mice were bred on site from parental strains. All animals were housed in a 12 hr light: