TECHNICAL PROGRESS REPORT

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TECHNICAL PROGRESS REPORT FOR OFFICE OF ENVIRONMENTAL MANAGEMENT/SCIENCE PROGRAM

Project EMSP-1

Title:

Improved Risk Estimated from Carbon Tetrachloride

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Abstract

Carbon tetrachloride (CCl_4) has been used extensively within the Department of Energy (DOE) nuclear weapons facilities. Rocky Flats was formerly the largest volume user of CCl_4 in the United States, with 5000 gallons used there in 1977 alone. At the Hanford site, several hundred thousand gallons of CCl_4 were discharged between 1955 and 1973 into underground cribs for storage. Levels of CCl_4 in groundwater at highly contaminated sites at the Hanford. facility have exceeded the drinking water standard of 5 ppb by several orders of magnitude. High levels of CCl_4 at these facilities represent a potential health hazard for workers conducting cleanup operations and for surrounding communities. The level of CCl_4 cleanup required at these sites and associated costs are driven by current human health risk estimates which assume that CCl_4 is a genotoxic carcinogen. The overall purpose of these studies is to improve the scientific basis for assessing the health risk associated with human exposure to CCl_4. Specifically, we will determine the toxicokinetics of inhaled and ingested CCl_4 in F344/Crl rats, $B6C3F_1$ mice, and Syrian hamsters. We will also evaluate species differences in the metabolism of CCl_4 by rats, mice, hamsters, and man. Dose-response relationships will be determined in all these studies. This information will be used to improve the physiologically based pharmacokinetic (PBPK) model for CCl_4 originally developed by Paustenbach *et al.* (1988) and more recently revised by Thrall and Kenny (1996). We will also provide scientific evidence that CCl_4, like chloroform, is a hepatocarcinogen only when exposure results in cell damage, cell killing, and regenerative cell proliferation. In combination, the studies outlined in this proposal will provide the exact types of information needed to enable refined cancer risk estimates for CCl_4 under the new guidelines for risk assessment proposed by the EPA in April 1996 (U.S. EPA, 1996).

Recent Progress

Specific aims of this research are to: 1) evaluate species differences in the metabolism of CCl_4 by F344/Crl rats, $B6C3F_1$ mice, and Syrian hamsters ***in vivo.*** Extrapolations to man will be based on results of parallel ***in vitro*** studies using liver microsome preparations from these rodent

species and man; 2) determine the toxicokinetics of inhaled and ingested CCl_4 in rats, mice and hamsters; and 3) determine whether CCl_4 is a hepatocarcinogen only when exposure results in cell damage, cell killing, and regenerative cell proliferation.

For specific aims 1 and 3, rats, mice and hamsters were exposed to 0, 5, 20, or 100 ppm CCl_4 6 hours per day, 5 days per week for 1, 4, or 12 weeks. Weight gain was depressed in a dose-dependent manner in rats and hamsters, but not mice. Serum alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) were not elevated in any species exposed to 5 ppm CCl_4. After 1 week of exposure, ALT and SDH were elevated in mice > hamsters, but not in rats inhaling 20 ppm $CC1_4$. At 100 ppm, the ranking was hamsters >> mice > rats. Histopathologically, in animals exposed to 100 ppm CCl_4 for 1 week, hepatocellular necrosis and mitosis occurred in hamsters >> mice > rats. At 4 weeks, these responses were similar in hamsters and mice but less evident in rats inhaling 100 ppm. In animals exposed to 20 ppm for 1 or 4 weeks, these reactions occurred in mice > hamsters > rats. These preliminary data indicate that rats are more resistant to CCl_4 induced hepatocellular injury and cell proliferation than mice and hamsters. The effects of CCl_4 inhalation on hepatic microsomal metabolism of CCl_4 are currently being evaluated.

Future Direction and Expected Progress

- We will compare the rates of CCl_4 metabolism by rats, mice, and hamsters ***in vivo.*** Extrapolations to man will be based on parallel studies of the metabolism of CCl_4 by rat, mouse, hamster, and human liver slices ***in vitro.*** This work should be completed within FY1998.
- Using hepatic microsome preparations, we will determine the role of specific cytochrome P450 isoforms in CCl_4 mediation toxicity for laboratory animals and relate this to humans. The effects of repeated inhalation and ingestion exposure of CCl_4 on these isoforms will also be determined. This work is ongoing and should be completed within FY 1998.
- We will evaluate the toxicokinetics of acutely inhaled and ingested CCl_4 in rats, mice, and Syrian hamsters as a function of exposure concentration of ingested dose. The effects of previous inhalation exposure to or ingestion of CCl_4 on the toxicokinetics of subsequently inhaled or ingested $^{14}CCl_4$ will also be determined. This work will be initiated in early-to-mid FY1998. Tissue analyses will be completed within FY1998 so that information can be used in revising the PBPK model.
- We will revise the current PBPK model for CC4 based on information obtained from the above experiments. This work will be completed in FY1999.
- We will determine whether repeated ingestion of CCl_4 in drinking water at concentrations 100 and 1000 times the current drinking water standards results in hepatic cell injury, death, and regenerative proliferation. This work will be initiated in early FY1998 and completed before the end of the fiscal year.