Mechanisms Involved in Trichloroethylene-Induced Liver Cancer: Importance to Environmental Cleanup

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Research Objective

Clean-up costs for chlorinated solvents found on DOE sites are most frequently driven by TCE because it is both the most wide spread contaminant and is generally present at the highest concentrations. Data that would permit increases in risk-based standards for TCE would reduce complex-wide clean up costs by hundreds of millions of dollars.

The objective of this project is to develop critical data for improving risk-based clean-up standards for trichloroethylene (TCE).

The project is organized around two interrelated tasks:

Task 1 addresses the tumorigenic and dosimetry issues for the metabolites of TCE that produce liver cancer in mice, dichloroacetate (DCA) and trichloroacetate (TCA). Early work had suggested that TCA was primarily responsible for TCE-induced liver tumors, but several, more mechanistic observations suggest that DCA may play a prominent role. This task is aimed at determining the basis for the selection hypothesis and seeks to prove that this mode of action is responsible for TCE-induced tumors. This project will supply the basic dose-response data from which low-dose extrapolations would be made.

Task 2 seeks specific evidence that TCA and DCA are capable of promoting the growth of spontaneously initiated cells from mouse liver, in vitro. The data provide the clearest evidence that both metabolites act by a mechanism of selection rather than mutation. These data are necessary to select between a linear (i.e. no threshold) and non-linear low-dose extrapolation models.

Research Progress

Task 1. Tumorigenesis studies: Originally, TCA was considered the active metabolite in inducing liver cancer with TCE. We have now sequenced codon 61 of the H-ras protooncogene in a total of 30 TCA-induced and 64 DCA-induced liver tumors. In the past year we sacrificed 47 mice that had been treated with 1 g/kg TCE in an aqueous emulsion (Alkamuls®) by gavage for 79 weeks. Forty-four tumors were recovered from these mice.

Sequencing of codon 61 of the H-ras gene has now been completed for all three chemicals. The lower mutation frequencies seen with DCA and TCE in aqueous vehicle are much more consistent with previous observations made with other tumor promoters. The mutation frequency observed with TCA is much higher than observed with other peroxisome proliferators. If the mutation spectra within tumors carrying a H-ras mutation are compared across compound, the results with DCA are more similar to that of TCE than that of TCA.

Since TCA has been shown to be a peroxisome proliferator and that mechanism has already been discounted as having relevance for humans, much of our work has focused on DCA. We have been

working on the use of magnetic resonance imaging of tumors as a means of demonstrating its mode of action. This work has shown that at high doses (2 g/L) the primary effect of DCA on tumorigenesis is to increase tumor growth rates. This was determined by examining the growth rates of tumors on mice maintained on treatment with the growth that occurred in animals whose treatment was suspended. Tumor growth rate essentially stopped when treatment stopped. Modeling of these data indicates that these data combined with previously reported effects of DCA on cell replication rates within tumors and measurements of suppressed apoptosis can completely account for the apparent "initiating" activity of DCA. These data indicate that DCA falls into a category of carcinogen that should be treated with non-linear methods to estimate safe doses.

Pharmacokinetic results: Issues related to the dosimetry of the DCA that is produced from the metabolism of TCE have become much clearer in the past year. There are two issues, 1) the amount of DCA that is generated from a given dose of TCE and 2) the amount of DCA that must be in the liver to induce a carcinogenic response. Data generated in the past year indicates that the area under the curve (AUC) projected for DCA from TCE based on its pharmacokinetic properties indicates a level that would be expected to give rise to a tumor response.

Task 2. Establishing selective advantage as a mode of action for DCA and TCA: The assay was used to measure the relative potency of DCA and TCA to promote the clonal growth of initiated hepatocytes. This was demonstrated early in this project by plating hepatocyte suspensions from untreated mice on soft agar and demonstrating that TCA or DCA caused a dose-dependent increase in anchorage-independent colonies. Growth of colonies could be demonstrated at concentrations of these metabolites that approximate those seen *in vivo*. The colonies produced with TCA and DCA reflected the different phenotypes of the tumors they induce *in vivo*.

In the past year, we have been able to simulate the effects of DCA *in vivo* on glycogen accumulation in isolated primary hepatocytes. We have yet to establish the mechanism for this accumulation and its relationship to the tumorigenic response. However, the demonstration of the growth response and glycogen accumulation suggests that mechanistic studies of DCA's effects can be conducted *in vitro* with some confidence that effects are reflecting *in vivo* effects of the chemical.

Implications

Most of the work performed since the start of FY00 has focused on the Symposium at 2000 Annual Society of Toxicology Meeting that will lay out EPA's findings and completing an interaction study between DCA and TCA. RJB has been asked to summarize the state of the mode of action information data that is available on TCE and its metabolites. These data form the basis for modification of the current MCL for TCE in drinking water. It is anticipated that this will require pressure on the OGWDW to put TCE on their regulatory calendar.

Information Access

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