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

BD Thrall, LB Sasser, IR Schultz and RJ Bull

Molecular Bioscience Department
Pacific Northwest National Laboratory
Richland, WA
Problem

Regulatory levels for trichloroethylene (TCE) in the environment are based on previous findings of liver tumor induction in mice.

The metabolites proposed to contribute to the carcinogenicity of TCE are trichloroacetate (TCA) and dichloroacetate (DCA), both of which induce liver tumors in mice.

Whereas evidence for a direct mutagenic effect of TCA or DCA is weak, tumors induced by TCE have been reported to have a shift in the spectra of mutations in the H-ras oncogene as compared to spontaneously arising tumors. Shifts in mutation spectra have previously been associated with a genotoxic mode of action.

In assessing human risk from genotoxic carcinogens, a linear extrapolation to low dose exposures (assuming the irreversible accumulation of mutations) is the current default approach. For agents which do not act by a direct genotoxic mode of action, this approach can lead to regulatory levels and remediation costs which far exceed that necessary to protect human health.
The focus of this research is to evaluate whether mechanisms other than direct genotoxic activity can explain shifts in mutational spectra in tumors induced by TCE, TCA and DCA.

Understanding the mode of action can significantly impact the regulatory levels necessary to maintain human risk at acceptable levels.
Approach

**In vivo Toxicodynamics**

_Do the metabolites of TCE selectively stimulate the growth of tumors with a particular mutation?_

**Metabolism and Kinetics**

_Which carcinogenic metabolites are formed after exposure to different doses of TCE? Are effective carcinogenic levels of these metabolites formed?_

**Molecular/Cellular Biology**

_What concentrations of metabolites are effective at promoting the growth and survival of preneoplastic hepatocytes? What are the potential molecular targets that are affected?_
H-ras Mutation Frequency and Spectrum in Tumors Induced by TCA and DCA

*Data for historical controls and TCE are from Anna et al. (1994).*
Effect of Study Duration on the Frequency of H-ras Mutations in DCA-Induced Liver Tumors*

*Data shown are combined from this study, Anna et al. (1994) and Ferreira-Gonzalez et al. (1994).
Effect of DCA and TCA on Growth and Survival of VC- and AAF-Initiated Tumors

VC alone: 1.77 mm³ average tumor volume
AAF alone: 0.64 mm³ average tumor volume

VC or AAF (single dose) → Water, DCA (2 g/L) or TCA (2 g/L) → Sacrifice (18 wks)

VC+DCA
VC+TCA
AAF+DCA
AAF+TCA

Tumor Number
Tumor Volume

VC alone: 1.77 mm³ average tumor volume
AAF alone: 0.64 mm³ average tumor volume
What concentrations of DCA and TCA are effective at promoting the growth of initiated (preneoplastic) hepatocytes?

Soft Agar Culture for isolation of initiated (anchorage-Independent) hepatocytes

Score Colonies

Genotype/Phenotype

Exposure
DCA and TCA Promote the Growth of Initiated Hepatocytes In vitro

A single i.p. dose of genotoxic agent given at weaning promotes in vitro colony formation in hepatocytes isolated one month later, verifying that the colonies which form over agar are truly ‘initiated’ hepatocytes.

In vitro exposure of hepatocytes isolated from untreated mice to DCA or TCA is sufficient to promote colony formation, suggesting these chemicals promote the growth of spontaneously initiated cells.
The Effective Concentration of DCA for Promoting Clonal Expansion of Initiated Hepatocytes In vitro is Shifted by Prior Exposure to DCA In vivo

* Significant increase over untreated controls (p<0.05)
Metabolism and Kinetics

TCA, Chloral Hydrate and Trichloroethanol Formation After i.v. Dosing with TCE

DCA Blood Concentrations After i.v. or Gavage Dosing with TCE

- i.v. (100 mg/kg)  - Vehicle Control
- Gavage (1000 mg/kg)  - LOQ
Relationship Between DCA Blood Levels and Drinking Behavior

Diurnal Drinking Water Pattern
(Duffy et al. 1991)
Summary and Conclusions

The results indicate that neither an increased frequency nor a change in the spectra of H-ras mutations in mouse liver tumors are reliable indicator that the carcinogen acts by a genotoxic mode of action. The most apparent relationship observed was an increase in H-ras mutations as a function of study duration, suggesting that these mutations are a secondary process associated with development of the tumor, rather than a causal process.

Although levels of TCA which are effective in promoting clonal growth of initiated hepatocytes are formed after exposure to TCE, the levels of DCA were at or below the limit of quantitation (2 μM). However, peak DCA blood levels in mice treated with 0.5 g/L DCA in water (an effective carcinogenic dose) are also low (< 10 μM) due to rapid metabolic clearance. Therefore the extent to which DCA contributes to the carcinogenicity of TCE is unclear.

Initiation/promotion and cellular studies all support the hypothesis that the carcinogenic activity of both TCA and DCA is mediated by promoting the survival and growth of preexisting initiated cells in mouse liver.
References


Project Publications


