INTRODUCTION
Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) at concentrations ranging from 10-50 mg/liter are commonly present in finished drinking water as by-products of chlorine disinfection. The widely used degreasers and solvents, trichloroethylene and tetrachloroethylene, are metabolized to DCA and TCA and to TCA respectively. DCA, TCA, trichloroethylene and tetrachloroethylene are carcinogenic in B6C3F1 mouse liver. The objective of this study was to determine the pathogenesis by which DCA and TCA induce liver tumors in B6C3F1 mice. An understanding of the mechanism could lead to a more accurate estimation of the carcinogenic hazard, if any, to humans exposed to these by-products of chlorine disinfection in finished drinking water. The proposed pathway examined was the ability to promote liver tumors in mice by enhancing cell proliferation. This pathway could result in a better understanding of the dose-response relationship for the induction of liver tumors by DCA and TCA in B6C3F1 mice and of the relevance of the carcinogenic activity in mice to human cancer risk assessment.

The pathways proposed for the carcinogenic activity of DCA and TCA include regenerative cell proliferation of preneoplastic hepatocytes in response to necrosis and peroxisome proliferation resulting in the formation of hydroxylated purine and pyrimidine bases in hepatic DNA. These two mechanisms could contain both a threshold and a possible greater sensitivity of mouse compared to human liver. Should the above pathogenic mechanisms for the chloroacetic acids be responsible for mouse liver carcinogenicity, a change in the current calculation of the maximum contaminant level guideline (MCLG) to accommodate these finding would be in order.

The specific objectives of this study were:
1. to demonstrate that DCA and TCA are tumor promoters and to determine the dose-response relationship for their promoting activity,
2. to determine in mouse liver the dose-response relationship of enhanced cell proliferation induced by DCA and TCA
3. to demonstrate that the liver becomes refractory to DCA and TCA enhancement of cell proliferation, and
4. to determine the enhancement by DCA and TCA of cell proliferation in preneoplastic/neoplastic lesions.

RESULTS AND DISCUSSION
Effect of DCA and TCA Upon Cell Proliferation in Murine Liver
DCA at 0.26, 0.86, and 2.6 gm/liter and TCA at 0.32, 1.06, and 3.2 gm/liter in the drinking water were evaluated for their ability to induce cell proliferation in the liver of female B6C3F1 mice. DCA exhibited a concentration-related increase in the BrDU-labeling index after 5 days of treatment. However, at 12 and 33 days, DCA failed to affect the BrDU-labeling index except for an apparent increase resulting from the exposure to the high dose of DCA
at 12 days. TCA increased the BrDU-labeling index only for the sacrifice on day 5 and with all three dose levels producing approximately the same increase. Hence, after 5 days of exposure to either DCA or TCA, the liver no longer responded with an increase in cell proliferation.

Tumor Promotion by DCA and TCA

The objective of this study was to demonstrate the tumor promoting activity of DCA and TCA. Methylnitrosourea (MNU) administered by intraperitoneal injection, and DCA or TCA administered in the drinking water, did not significantly induce foci of altered hepatocytes, hepatocellular adenomas or hepatocellular carcinomas. When animals were initiated with MNU, subsequent treatment with 2.6 gm/liter of DCA in their drinking water increased yield of both foci of altered hepatocytes and hepatocellular adenomas. By 221 days of treatment, all the MNU-initiated lesions susceptible to DCA appeared to have been promoted at least to the stage of foci of altered hepatocytes. Also, as the treatment of DCA continued from 221 days to 362 days, the yield of foci of altered hepatocytes decreased with an increase in the yield of hepatocellular adenomas, strongly suggesting the progression of foci stage to adenomas. When treatment of DCA was terminated at 221 days, there was a reduction in the yield of foci of altered hepatocytes and of hepatocellular adenomas.

Treatment of MNU-initiated mice with the high dose of TCA (3.2 gm/liter) significantly increased the yield of hepatocellular adenomas at 221 and 362 days and the yield of hepatocellular carcinomas at 362 days. The mid-dose of TCA (1.06 gm/liter) induced a significant number of hepatocellular adenomas and carcinomas at 362 days. Upon termination of TCA treatment at 221 days of exposure, the yield of tumors, i.e. hepatocellular adenomas + hepatocellular carcinomas, at 362 days (1.64 tumors/mouse) was the same as previously found at 221 days (1.30 tumors/mouse). However, the yield of hepatocellular carcinomas per mouse continued to increase from 0.2 (2/10) at 221 days to 0.73 at 362 days. Therefore, termination of TCA treatment did not result in regression of hepatocellular adenomas but rather their apparent progression to hepatocellular carcinomas.

SUMMARY AND CONCLUSIONS

This research project determined the ability of DCA and TCA to promote liver tumors in B6C3F1 mice and studied the relationship of tumor promoting activity to cell proliferation. The following results were obtained:

1. DCA and TCA promoted liver carcinogenesis in B6C3F1 mice. Promotion is defined as the enhancement of the progression of initiated cells to precancerous lesions and tumors.

2. Foci of altered hepatocytes and hepatocellular adenomas promoted by DCA regressed upon termination of treatment, while those of TCA do not regress but rather progressed to hepatocellular carcinoma.

3. DCA and TCA administration enhanced cell proliferation in the liver for only five days of treatment, after which no further enhancement was observed.

In conclusion, exposure to drinking water containing DCA at 0.86 gm/liter or TCA at 0.32 gm/liter appears insufficient to promote liver tumors in B6C3F1 mice and these concentrations could be considered thresholds for carcinogenic activity in B6C3F1 mice.