Studies on the Toxicity of Mixtures of Haloacetates and Ethanol in AML-12 Cells
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Abstract
The process of water chlorination results in production of different haloacetate by-products, such as dichloroacetate (DCA) and trichloroacetate (TCA). The compounds have been found to be hepatotoxic and hepatocarcinogenic in rodents through induction of oxidative stress (OS). Chronic ingestion of alcohol (ethanol) is also known to result in liver toxicity and cancer, with OS found to play an important role in that. This may suggest simultaneous exposure to mixtures of DCA, TCA and alcohol may result in interactive effects that will increase their hepatotoxicity, and to test this hypothesis, mixtures of DCA, TCA and alcohol were to be studied. However, we found in vivo tests in animals can be challenged by ethical issues, as for the large number of animals required to test various mixtures, and the length of time and high cost required to complete that. Alternatively, we used mouse liver cells (AML-12 cell line) as a screening model to test the effects of those compounds and their mixtures. Accordingly, various concentrations of each of the three compounds, as well as mixtures of the compounds were incubated with AML-12 cells for 48 h, and cells were then assayed for viability and various biomarkers of OS, including production of nitric oxide (NO), superoxide anion (O2-) and advanced oxidation protein products (AOPPs). DCA, TCA and ethanol were found to result in concentration-dependent decreases in cellular viability, with respective concentrations of 770 ppm, 930 ppm and 1.5% resulted in 20% decreases. Concentrations corresponding to 25% decreases in cellular viability by the compounds were used to design binary mixtures of the compounds, as well as a mixture of the three compounds to assess possible interactions between them. In general, binary mixtures containing DCA and TCA resulted in effects on cellular viability and induction of the tested biomarkers of OS that were additive, and binary mixtures of DCA (or TCA with ethanol), as well as a mixture containing the three compounds resulted in greater than additive effects on those biomarkers. These results indicate significant contribution of alcohol to the hepatotoxic effects of DCA and TCA and may suggest possible increase in the risk of hepatotoxicity from simultaneous consumption of chlorinated water and alcohol. The results may be used as basis for further investigation of that suggestion in vivo, in animals.