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Project Summary

The Isolation and Identification of Electrophilic Mutagens Produced During Chlorine Disinfection

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The chlorination of organic materials present in natural waters and in wastewaters generates many direct acting mutagenic and potentially carcinogenic products. The reaction of nucleophilic reagents with the unidentified mutagenic electrophiles present in disinfected water and with a number of known electrophilic compounds as a means of generating stable addition products that would aid in the isolation, chromatographic separation, and in the identification of the mutagens via mass spectrometry was investigated. Of the nucleophiles investigated for this purpose diethyldithiocarbamate proved to be the most suitable. It was found to react with most of the electrophiles, but the mass spectra of the resulting products while providing unique ions suitable for selective ion monitoring, were not suitable for structure determination. Nucleophilic functionalities bonded to solid supports were also investigated for the purpose of selectively isolating electrophilic mutagens from aqueous samples and while several were found that would remove the electrophiles, no suitable means of removing them from the supports was found.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Chlorination of the naturally occurring organics in water generates a variety of new products. Based upon results obtained from biological assays, it has been determined that some of these products are mutagenic. Many of the compounds arising from the chlorination process require no enzymatic activation to exhibit their mutagenic activity. These direct acting mutagens are relatively unstable compounds that will attack a nucleophilic reactant that possesses a readily available source of electron density (e.g., non-bonded electrons, pi systems, anions). Such electrophiles as alkylhalides, epoxides, low molecular weight aldehydes, and halogenated carbonyl systems are representative of this electrophilic reactant type that form during chlorination and that may interact with a nucleophile to undergo such reactions as substitution. In the situation where the nucleophile is a molecule of significant biological importance, the properties of the newly formed product are likely to be adverse.

It was known from earlier investigations that an external reactive nucleophilic reagent such as thiosulfate eliminated the mutagenic activity detectable in chlorinated wastewater with the Ames *Salmonella* assay. The presumed mode of reaction was through reduction and nucleophilic addition. The results of preliminary studies indicated that it may be possible to develop an

analytical methodology in which nucleophilic reagents would be reacted with the electrophiles in the sample to form products that would exhibit distinctive properties that would aid in their isolation and identification. Moreover, such an analytical scheme using nucleophilic tags would render the electrophilic mutagens harmless to laboratory personnel and also make them more amenable to manipulation.

The primary mode of compound identification currently used in these types of studies is gas chromatography/mass spectrometry (GC/MS). Therefore the labeled compounds must be sufficiently volatile to allow a GC separation without a significant probability of decomposition. It was also anticipated that physical or chemical properties of the covalently bound label (e.g., acidic or basic functionality, lipophilicity, fluorescence, radioactivity or electrochemical properties) would incorporate distinctive detection characteristics that would aid in the isolation of enriched fractions from the remainder of the complex mixture. The nucleophilic substances evaluated are listed in Table 1.

Another method for the selective isolation of electrophilic substances from water that was investigated was an insoluble polymeric matrix into which had been incorporated a removable nucleophilic label. The change in physical and chemical state (e.g., liquid to solid, reactive to non-reactive) would allow a ready separation from the complex matrix remaining dissolved in the water or merely adsorbed to the polymer surface. Moreover, it was hoped that this procedure would trap the more reactive mutagenic materials that might otherwise elude discovery. Cleavage of the modified electrophiles from the polymer will be accomplished by mild chemical procedures to give a cohort of organic materials that could be identified by conventional mass spectral techniques. The polymeric reagents evaluated are listed in Table 2.

Results

The underlying concept that reactive mutagenic electrophiles present in mixtures would react with nucleophilic probes resulting in decreased biological activity is strongly supported by this work. Of the nucleophiles tested, diethyldithiocarbamate (DEDTC) affected the most rapid reduction of mutagenicity. Cyanide and nitrogen bases including N-methyl-2-mercaptoimidazole, 2-mercaptopyridine, 2-mercaptopyrimidine and thiazole caused a much less dramatic decrease.

DEDTC emerged as the prime candidate for labeling of aqueous electrophilic mutagens. Positive features of DEDTC included: 1) the label high nucleophilic reactivity, 2) mass spectral patterns evolved (viz. 116/148/149) that were very characteristic of labeled dithiocarbamate, 3) the derivatives had a relatively high (15,000) UV extinction coefficient useful for HPLC analysis, 4) DEDTC is readily available and is inexpensive, 5) the increased hydrophobic nature of the derivatized DEDTC improved extraction efficiency, 6) a wide variety of DEDTC derivatives are suitably volatile for GC separation, and 7) no interference was observed with the Ames *Salmonella* assay for mutagenicity.

The examination of chlorinated waste water samples, concentrates of a chlorinated drinking water and samples of chlorinated humic acids demonstrated the presence of a host of labeled compounds. The dissimilarity of ion chromatograms for m/z = 116 (base fragment for DEDTC) from these different samples suggests that the number and proportion of reactive electrophiles can vary greatly with sample type. Labeling with DEDTC was shown by this work to be valuable for qualitative screening of mixtures and for trace analysis of known electrophiles by single-ion monitoring. However, the complete analysis of the unknown electrophile content of samples is not possible. While the number and relative concentration of electrophiles, even at trace concentrations in a complex matrix is readily available from the single-ion chromatographic data, the absence of molecular ion and high-mass fragments for many compounds often prevents their positive identification.

The difficulty encountered in achieving sufficient separation of the trace amounts of the these labeled electrophiles from the complex baseline to obtain unambiguous spectra by GC/MS prompted the development of a nucleophile with basic functionality so that the matrix could be simplified by pH controlled extraction or ion-exchange chromatography. N-methyl-2-mercaptoimidazole was chosen as the derivatizing agent because model adducts from standard small organic halides possessed adequate volatility and simple mass spectral characteristics. Isolation and analysis of the basic fraction from the mixture of the imidazole and a chlorinated waste water sample by

Table 1. *Monomeric Nucleophiles Examined as Tagging Reagents*

Nucleophile	Reason for Selection
Cyanide	Relatively good nucleophile, radio-label is available, only a minimum increase in molecular weight so products should have a high probability of being volatile.
Thiocyanate	Relatively good nucleophile, radio-label is available.
Acetate	Modest nucleophile, represents potential component of buffers.
Mercaptoacetic acid	Sulfhydral is a good nucleophile.
p-N,N-Dimethylaminoaniline	Products should be electrochemically active for easy detection, tertiary amine should allow selective acid extraction or isolation ion-exchange column.
Diethyldithiocarbamate	Excellent nucleophile, products should be volatile and provide characteristic MS fragments.
Sodium Benzene Sulfinate	Good nucleophile, recognized reagent for quinone analysis.
Benzoylhydrazide	Recognized reagent for carbonyl analysis.
Phenylhydrazine	Recognized reagent for carbonyl analysis.
Phenylsulfonylhydrazide	Recognized reagent for carbonyl analysis.

Table 2. Polymeric Nucleophilic Reagents Tested

Polvmer-Bound Nucleophile	Reason for Selection
Controlled Pore Glass/Thiol (CPG/Thiol) Pierce Chemical Co.	Commercially available, good nucleophile, structural analogy to glutathione, cleavage from the polymer is possible by acidic, or perhaps, enzymatic hydrolysis.
Polystyrene-bound sulfinate	Good nucleophile, known reagent type for quinones, reductive methods are known for cleavage of the sulfone products, easy to prepare.
Polystyrene-bound dithiocarboxylate	Excellent nucleophile, known facile hydrolysis of the bound thioester addition products, easy to prepare.
Polystyrene-bound dithiocarbamate	Excellent nucleophile, enhanced cleavage properties, analogy possible to monomeric carbamates used in other studies.

GC/MS provided mass spectra which were not easily interpreted. High resolution mass spectral analyses were not attempted, but could perhaps prove useful in identifying these types of compounds.

Dimethylaminobenzenethiosulfonate was synthesized and found to react with known halides to yield substances that possessed stable molecular ions. However, even when the label was extensively purified, products representing both the expected thiosulfonates and the corresponding sulfones were observed, thereby complicating the analysis. Despite this, the thiosulfonate was applied to a concentrate of a chlorinated drinking water known to contain Ames positive mutagens and was shown to reduce the mutagenic response. Analysis of the products of this reaction resulted in only a few spectra which were clearly label-related.

Three modified polymers were successfully synthesized and characterized. These solids were shown to remove electrophiles from aqueous samples efficiently. Moreover, one of these polymers (the dithiocarboxylate) was shown to remove the majority of mutagenic activity from a chlorinated drinking water sample. The electrophiles could not be extracted from the polymers to any degree by organic solvents or water. Attempts to cleave the label-electrophile bonds for analysis proved inefficient; although the percent recovery of electrophiles from the polymer was as high as 20 per cent in isolated cases, lower values were much more typical.

Conclusions

Diethyldithiocarbamate labeling has been shown to be a valuable method for qualitative screening of mixtures and for trace analysis of known electrophiles by single-ion monitoring GC/MS. However, the complete analysis of unknown electrophile content of samples is not possible using this method. While the number and relative concentrations of electrophiles, even at trace concentrations in a complex matrix is readily determined from the single-ion GC/MS data, the absence of molecular ions and high-mass fragments for many of the DEDTC-labeled electrophiles prevents their positive identification.

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The complete report, entitled "The Isolation and Identification of Electrophilic Mutagens Produced During Chlorine Disinfection," (Order No. PB 89-214 118/AS; Cost: $15.95, subject to change) will be available only from:
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