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Project Summary

Assessment of Mutagenic Potential of Mixtures of Organic Substances in Renovated Water

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This study was conducted to determine the presence of mutagenic activity in municipal wastewater and to evaluate the performance of available advanced wastewater treatment systems for removing such activity. Further, the distribution of the described activity among various classes of chemical compounds was studied in an attempt to identify the active fractions. The study was conducted utilizing rapid *in vitro* bioassays for detection of mutagenic activity and, therefore, was expected to provide a preliminary assessment regarding the mutagenic hazards linked to the potential potable use of reclaimed wastewater. The results of this investigation should be of value to the Environmental Protection Agency and others in identifying the problem areas and in setting priorities for in-depth and more specialized toxicological studies. Knowing that certain wastewaters have toxic properties, treatment systems may be designed to remove the toxic components or sources of such components, for example certain industries could provide pretreatment measures.

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

Introduction

A full toxicological investigation of the potential hazards from the consumption of renovated wastewater is necessary before considering it for potable reuse. Wastewaters may contain hazardous chemicals for which currently no criteria exist. Such pollutants may pass through wastewater treatment plants in trace amounts, so an evaluation of the toxicological properties of such effluents is warranted.

Methods

An investigation was undertaken to evaluate the performance of selected advanced wastewater treatment processes for removing (introducing) mutagenic substances and to determine the distribution of the detected activity among various classes of chemical compounds. The study utilized specially constructed strains of *Salmonella typhimurium* and *Saccharomyces cerevisiae* to assess mutagenic activity, and the mammalian cell — BHK21 Cl 13 (to a limited extent) to determine transforming activity. In an effort to recover the wide variety of organic contaminants present in wastewater and renovated wastewater, three independent concentration methods were utilized. These included sorption on polyurethane foam plugs, sorption on XAD resin, and solvent extraction. Conditions suitable

for recovery of organics of polyurethane foam were: wastewater temperature 62 C, flow rate 250 ml/min, with a sample volume of 10 l for secondary effluent and 20 l for intermediate or fully treated wastewater. The conditions used for XAD resin were: wastewater temperature 50 C, flow rate 100 ml/min, XAD-4 and XAD-8 columns (1 x 10 cm) connected in series, sample volume 5 l. For recovery of organics by liquid-liquid extraction, 15% methylene chloride in hexane (75 ml solvent/1 wastewater) was used. The crude organic mixtures recovered from wastewaters were separated into seven major chemical classes of organic compounds according to their solubility under acidic, basic, and neutral conditions, and each class was tested for mutagenic activity. The classes of compounds isolated were; ether insoluble, water soluble, basic, amphoteric, strong acid, weak acid, and neutral. Adsorption chromatography with silica gel columns was used to subfractionate the neutral fraction into aliphatic, aromatic, and oxygenated fractions.

A mutagenic response by the wastewater concentrates and separated fractions was observed with the Ames *Salmonella* liquid suspension assay which involved preincubation of the test mixture and *Salmonella* tester strain in liquid suspension prior to plating. Conventional plate incorporation and spot tests failed to detect mutagens in crude concentrates and separated fractions. For detection of low concentrations of active compounds in unconcentrated wastewater, the assay was performed by making filter sterilized test water into media (base agar layer). This modification of the assay permitted incorporation of up to 10 ml of test wastewater in the Ames assay (70% v/v). The amino acid histidine, which interferes with the Ames assay by artifactually increasing the number of revertants, was not detected in wastewaters. The yeast assay (forward and reverse mutagenesis, mitotic cross over, and gene conversion) lacked the sensitivity for detection of mutagens in wastewaters, wastewater concentrates, or separated fractions. Only a marginal response was obtained with strain S288C (forward mutagenesis assay) with some samples. The assay also lacked the capacity to detect mutagenic compounds which required mammalian metabolic activation. The BHK cell system also proved to be unsuitable for this study because of the excessive time requirement and its erratic behavior.

Physical-Chemical System

The influent of the physical-chemical AWT process at Piscataway, Maryland, in unconcentrated form, showed minimal mutagenesis in the base pair substitution mutants TA100 and TA1535 of *S. typhimurium.* The activity was lost by incorporation of mammalian liver enzymes in the assay. Wastewater samples collected after the liming/recarbonation steps showed increased activity over that of the influent suggesting that these treatment steps were capable of introducing mutagenic substances. Alternatively, the liming/recarbonation steps may have removed toxicants and/or masking agents, thereby permitting detection of mutagens already present in the influent. The increased activity observed with the partially treated sample was removed by breakpoint chlorination and/or carbon filtration. However, the overall response with the final effluent was no less than that shown by the influent wastewaters. Mutagens were not present as conjugates in wastewaters since the presence of the hydrolytic enzyme β-glucuronidase in the assay did not result in an increased mutagenicity. The organic mixtures recovered from wastewater by the concentration methods showed mutagenic activity, but not in the same tester strains as with unconcentrated wastewaters. Also, the activity could be detected only when the indicator organism was incubated with the concentrates in liquid suspension prior to the assay. These findings suggested that the concentration methods employed failed to recover all the active compounds from wastewaters. The recovered activity of the purified effluent was found to be distributed mainly in weak acid, basic, and aliphatic and aromatic classes of compounds. The combined activity of all the separated fractions was greater than that of the crude concentrate, suggesting masking of activity in this complex mixture, either because of toxicants or masking agents. The chemical separation scheme used in this investigation resulted in the uncovering of some of the activity but not all, since many of the separated fractions remained toxic to the test organisms. Only a small percent of the original organics, as measured by total organic carbon, was recovered by the three concentration methods and the results must be tempered by this fact.

Biological With Physical-Chemical System

The influent and effluent from Bay Park AWT Plant, at East Rockaway, New York, which utilized combined biological and physical-chemical treatment, showed mutagenic response in the base pair substitution mutants TA100 and TA1535. A comparison of the mutagenic response of AWT influent and effluent revealed that the AWT method employed at Bay Park, as was observed with the Piscataway treatment, not only failed to remove certain mutagenic substances but also added new mutagens to the final effluent. Unlike the results with unconcentrated wastewater, the organic mixtures recovered from Bay Park wastewater showed activity in the frameshift mutant TA1538. The mutagenic chemical classes derived from the influent were basic, water soluble, and amphoteric. Activity in these classes was partially reduced as a result of treatment but activity appeared in two new fractions: the weak acid and ether insoluble.

Industrial Waste Plant

Wastewaters from the physical-chemical Niagara Falls, New York AWT process, which consists of 60-70% industrial wastes, showed weak to moderate mutagenicity in base pair substitution and frameshift tester strains of *S. typhimurium.* Mammalian metabolic activation was required for mutagenesis in frameshift mutants. The mutagenic activity was higher in influent wastewater than in partially treated and final effluent suggesting removal of mutagens during treatment. There were, however, mutagens still present in the final effluent. The influent wastewaters at the point samples had undergone partial treatment including liming/pH adjustment, and the addition of mutagen during these treatment steps may have been responsible for increased mutagenic activity of the influent.

Domestic Wastes

Influent and AWT effluent sample from the rapid sand filtration process at Lake George failed to cause mutagenesis in the *Salmonella* strains tested, b

showed an inhibitory effect on the spontaneous reversion rate of strain TA98. Organic mixtures recovered from these wastewaters were also free of mutagens. The data revealed that microbial synthesis of mutagens did not occur following prolonged contact with the natural delta sand beds. Because of the absence of mutagens in the influent wastewater, efficiency of the process for removing mutagens could not be assessed. These wastewaters did not contain any industrial wastes and it is significant that wastewaters strictly of domestic origin do not have mutagenic properties.

Sand Filtration System

Information on removal of mutagens by sand filtration was obtained using simulated laboratory sand columns which were dosed with partially treated primary effluent from the heavily industrialized area of Niagara Falls. The primary effluent did not show mutagenic activity in any of the chemical fractions except for the strong acid fraction. This was surprising since the wastewaters from this facility, when tested in unconcentrated form, showed high mutagenic response. After filtration of the wastewaters, through one half of the sand column, the strong acid fraction became less mutagenic, but strong mutagenic activity appeared in the water soluble and ether insoluble fraction, and weak mutagenicity was noted in weak acids, basic, and amphoteric fractions. On continued contact with sand, mutagens of the amphoteric, weak acid, and basic classes were removed to a nondetectable level but mutagens of ether insoluble, water soluble, and strong acid classes were unaffected. The presence of several new classes of mutagens in the midpoint and final effluent, not detected in the influent, suggests synthesis of mutagens during the sand filtration process. Alternatively, the sand filtration process may have removed toxicants and/or masking agents thereby permitting expression of the mutagens originally present in the influent.

Conclusions

The study shows that the physical-chemical, biological, and sand filtration processes studied were unable to completely remove mutagenic activity from wastewater. In several instances, treatment steps themselves may contribute mutagens. Further toxicological and analytical studies are required to determine the nature of potentially hazardous compounds present in renovated wastewater.

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The complete report, entitled "Assessment of Mutagenic Potential of Mixtures of Organic Substances in Renovated Water," (Order No. PB 81-153 843; Cost: $11.00, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
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Telephone: 703-487-4650
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