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# Project Summary

# The Evaluation of the Mutagenicity of Municipal Sewage Sludge

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**Samples of five municipal sewage sludges from Illinois cities have been subjected to a multiorganism testing program to determine the presence or absence of mutagenic activity. Chicago sludge has been the most extensively tested using the *Salmonella*/microsomal activation assay, the micronucleus test *Tradescantia*, the *wx* locus assay in *Z. mays*, and sister chromatid exchange induction in human lymphocytes. Mutagenic activity has been observed in the sludges from Chicago and Sauget, Illinois. Sludges from primarily domestic sewage do not apparently contain mutagens.**

***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

A major problem facing the United States is the disposal of the sludge resulting from the treatment of municipal sewage. There has been a substantial increase in the number and efficiency of sewage treatment plants in the past decade, particularly in suburban and rural areas, resulting in an increased amount of sludge requiring disposal. In addition, previously employed sludge disposal methods such as ocean dumping may be modified in the near future. The incineration of sludge requires expensive facilities and may result in increased air pollution An attractive alternative disposal method is the application of this organic matter to agricultural lands or its use in the reclamation of land stripped of its organic matter by surface mining.

There may, however, be problems associated with sludge disposal by land application. Analyses of sludge from the Calumet and Southwest treatment plants of the Metropolitan Sanitary District of Chicago showed that concentrations of several heavy metals were substantially enriched over typical soil compositions. Thus, in 1967, a large demonstration and research program was initiated to examine the feasibility of land application of digested sewage sludge from the Metropolitan Sanitary District of Chicago waste water treatment plants. A major objective was to examine compositional changes in soil, crops, and water arising from sludge-amended fields.

In the intervening period, there has been a great deal of study of the uptake and accumulation of heavy metals (most notably zinc and cadmium) in corn, the effects of these elements on soil bacteria, and the potential for transmission of heavy metals through a food chain that might result in impacts on the general public health if widespread use was made of sludge as a soil additive Zinc and cadmium have been found to accumulate in corn grain and leaf tissues. High levels of sludge treatment appear to inhibit the development of several inbred strains of corn but do not cause overt symptoms of metal toxicity. Long-term studies of food chain and field productivity effects of sludge are in progress.

Additional contaminants that may be present in municipal sewage sludge are organic compounds from industrial

processes or other anthropogenic activities. Of potential concern are compounds that are genetically active and may cause mutations, birth defects, or cancer. In a metropolitan area like Chicago there are major chemical, petrochemical, petroleum-refining, and other industries that utilize organic chemicals in large quantities. Other activities such as fossil fuel combustion produce genotoxic compounds that may find their way into the sanitary sewer system. Therefore, there exists the possibility that there are genotoxic compounds present in the sewage sludge that may be released into the environment by leaching into surface or groundwater or incorporated into the human food chain through the use of sewage sludge as a soil additive. In order to examine this question, samples of sewage sludge were obtained from several local sewage treatment plants that treat a variety of wastes ranging from only household wastes to combinations of industrial and domestic wastes. These sludge samples have been subjected to a multiorganism screening procedure to determine if mutagenic compounds are present in sludge and sludge-amended soil.

## Conclusions

This study has determined that mutagenic activity is present in some municipal sewage sludges. Sludges from Champaign, Chicago, Hinsdale, Kankakee, and Sauget, Illinois were studied. The Chicago municipal sewage sludge sample clearly has components that induce a variety of mutagenic responses. Although it is not possible to compare the quantitative responses in the various test organisms employed, the sludge or sludge extracts did induce a response in all of the species employed. The supernatant liquid after appropriate concentration was found to be mutagenic using the Ames *Salmonella* assay. Acetone, hexane, and chloroform:methanol extracts of the pellet contained promutagens that could be activated by mammalian microsomes (S-9). Acetone was a more effective solvent than hexane in the extraction of the promutagens from the sludge pellet. The results indicate that a moderately potent agent can be extracted from the sludge since, with microsomal activation, the material extracted from only 155 $\mu$l of whole Chicago sludge was able to induce twice the number of revertant colonies per plate of Ames strain TA98 compared to the background negative control plates.

The Champaign, Hinsdale, and Kankakee sludges showed a weak response from samples obtained by the further chemical fractionation of the chloroform:methanol extracts following activation Only the Sauget sample demonstrated direct acting mutagenic activity as well as very substantial mutagenicity after activation with mammalian hepatic microsomes.

In this project, the experiments using the *Tradescantia* micronucleus test encompassed the analysis of over 2.0 x $10^6$ meiotic cells that were individually scored for micronuclei. The project mean negative control frequency based on 2.0x $10^5$ tetrads was 3.53MCN/100 tetrads. The overall positive control (50 mm maleic hydrazide) frequency based on 4.7 x $10^4$ tetrads was 10.71 MCN/100 tetrads.

The *Tradescantia* data clearly indicate that whole or diluted Chicago sludge induces chromosome aberrations in meiotic cells. The aqueous phase is much less mutagenic than whole sludge and it appears that a major portion of the mutagenic agents remain in the sludge solids. The acetone fraction of the Chicago sludge gave a negative response in the micronucleus test. When whole sludge samples from each municipality except Sauget were evaluated an interesting pattern of response emerged. A ranking in order of decreasing mutagenic response is Chicago, Kankakee, Hinsdale and Champaign. When concentrated, whole sludge samples are evaluated, the same ranked order is maintained. However, the Chicago sludge is a much more potent mutagen than the Kankakee sludge samples. A one half dilution of whole Chicago sludge induced a mean value of 5.19 MCN/100 tetrads as compared to a value of 6.30 MCN/100 tetrads for the 13.3x concentration of Kankakee whole sludge. Neither the Hinsdale nor Champaign sludge samples induced a significant frequency of micronuclei in *Tradescantia* tetrads. The Sauget sample has not yet been tested with this assay. There is an apparent correlation of the cytogenetic potency of the sludge sample and the relative industrialization of the municipality.

In order to provide a mammalian endpoint, the ability of sludge extracts to induce sister chromatid exchanges (SCE's) in human peripheral lymphocytes has been examined. It is quite difficult to culture the cells in the presence of the extracts, and only partial results are currently available. We have discovered a direct-acting agent(s) in an acetone extract of Chicago sludge. A 100 $\mu$l sample of a 15-fold concentrated sample of this extract induced a statistically significant increase in the frequency of SCE s (p0.01) The control frequency was 0.21 $\pm$ 0.02 SCE/chromosome while the sample increased the frequency to 0.32 $\pm$ 0.02 SCE/chromosome. A positive control of 5 mM ethyl methane sulfonate (EMS) induced an SCE/chromosome frequency of 0.48 $\pm$ 0.02. This acetone extract is not directly mutagenic when tested with the *Salmonella* assay but also showed activation by mammalian S-9. The results also show that these acetone extracts are toxic to human lymphocytes at high concentrations. Further studies with this assay are in progress.

The *in situ* tests of sludge-amended soil using Chicago sludge and the maize *wx* locus assay demonstrate that mutagens are available to the plant when sludge is used as a soil amendment. Toxic as well as mutagenic responses were observed. The laboratory studies indicate that sludge can induce a substantial number of mutations at the *wx* locus in inbred Early-Early Synthetic as well as reverse mutation at the *wx-90* locus observed in the field studies. Champaign sludge did not demonstrate mutagenic activity in the forward mutation assay with Early-Early Synthetic. The other sludges were not tested in this assay because of time constraints. These studies indicate that mutagens present in sludge-amended soil are transported into a crop plant and can induce genetic damage in germ cells. However, these studies do not provide any indication of mutagenicity in the kernels grown on sludge-amended soil or transfer of the mutagens from the sludge to ground or surface waters. However, these results do suggest the need for further study of the possible adverse effects of land application of sludge from large industrialized cities.

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*The complete report, entitled "The Evaluation of the Mutagenicity of Municipal Sewage Sludge," (Order No. PB 83-264 077; Cost: $10.00, 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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