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# Project Summary

# Mutagenistic Testing of Industrial Wastes From Representative Organic Chemical Industries

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**The general applicability of the Ames test for screening wastewater samples was investigated. Application of the Ames test to raw and treated wastewaters from representative organic chemical industries involved the investigation of several problems: (1) the feasibility of using the Ames test to detect mutagens in wastewater samples, (2) the relative effectiveness of various waste treatment processes, (3) the mechanics of establishing an Ames testing program, and (4) the economics of using the test in routine environmental screening.**

**Samples collected from 14 industrial sites were analyzed using the Ames procedure. Results were interpreted on the basis of relative increases in revertant colonies on test plates as compared to control spontaneous reversion plates. A "positive," sample consisted of six replicate test plates with an average count of at least twice the control value. Of 28 samples tested, 6 were interpreted as positive and 22 were interpreted as negative.**

***This Project Summary was developed by Robert S. Kerr Environmental Research Laboratory, Ada, OK, 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

An increasing concern about the possible introduction of carcinogens into the environment has resulted in a search for a simple, sensitive, and reliable method for the detection of these chemicals. The Ames test, developed by Bruce Ames, of the University of California, Berkeley, has achieved considerable attention among industries and governmental agencies interested in routine screening for potential carcinogens in water supplies.

The Ames test was originally designed to determine the ability of a specific compound to cause mutations. Since most carcinogens are also mutagens, a correlation has been made between positive results in the test and potential carcinogenicity. Test strains of bacteria, supplemented with extracts of rat liver to simulate mammalian metabolism, respond readily to the presence of most mutagens in minute quantities. The test is currently being investigated as a possible method for detecting mutagens in industrial effluents.

In conducting this study, the general applicability of the Ames test to screening wastewater samples was investigated. The problems involved in preparing samples for testing and in interpreting the results are markedly different for environmental samples as

opposed to pure compounds or extracts. The test was applied to raw and treated industrial wastewaters, in line with several objectives: (1) the determination of the feasibility of using the Ames test to detect carcinogens in the environment, (2) the relative effectiveness of various waste treatment procedures in removing carcinogenic substances, (3) the mechanics of establishing an Ames testing program, and (4) the economics of using the test for screening.

## Methods

Raw and treated wastewater samples collected from 14 industrial sites by the Environmental Protection Agency's Robert S. Kerr Environmental Research Laboratory were analyzed using the Ames procedure. The industrial sites represented plants involved in petroleum refining and production of organic chemicals, pesticides, wood preservatives, rubber, and pharmaceutical products.

The Ames test was designed to determine whether a pure compound can cause bacteria to mutate. The significance of this test system lies in the correlation between the ability to cause mutations in bacteria and the ability to cause mutations in mammals, including humans. Testing chemicals on animals is an expensive and time consuming process. Therefore, it is hoped that the much simpler Ames test can give valid preliminary information about mutagens.

The test works by inducing mutations in the areas of the bacterial chromosome that have already been mutated. To appear on test plates, histidine-biotin deficient mutants must regain the capacity to synthesize these compounds. This is accomplished when the mutagenic compound reacts with the genetic material, reversing the original mutations. Since this phenomenon occurs with some regularity in the absence of mutagens, the background "spontaneous reversion" rate must be determined before and during Ames testing. It is generally accepted that a given amount of material is considered to have mutagenic activity if test plates incorporating that dose show at least twice as many colonies as the average number of spontaneous revertants for the bacteria used.

## Results and Discussion

Results were interpreted on the basis of relative increases in revertant colonies on test plates as compared to control spontaneous reversion plates. For a sample to be scored positive, six replicate test plates had to give an average count of at least twice the control value. Of 28 wastewater samples tested, 6 were interpreted as positive (4 raw and 2 treated) and 22 were interpreted as negative.

Positive results were anticipated because some of the molecules generated by organic chemical industries have shown positive results in pure compound testing. In industrial wastewaters, however, the exact chemicals present and their individual concentrations are unknown. It is probable that some mutagens may be present in a sample, but in concentrations below the threshold of detection using these methods.

Some workers attempting to use the Ames test to screen wastewaters have approached the concentration problem by extracting the samples with methylene chloride. This was not done in this study because methylene chloride is a known carcinogen, and handling it increases the hazard and the time needed in sample preparation; because it is a mutagen, *any* residue of methylene chloride remaining in the extracts could alter the results of the testing. By not using the extraction procedure, these objections were eliminated while others were raised. The lack of concentration in test samples may lead to negative scores that might be positive if extracts were tested. Also, it is obvious that only the soluble phases of the samples are tested; any mutagens in or on particulate matter have been removed prior to testing.

In reviewing the validity of using the Ames test as a screening procedure, it is important to recognize that a potential exists for obtaining false results. The Ames test is a much more complex bioassay than it appears; with such a large number of controls necessary, even the rigid maintenance of quality control standards allows a margin of error to remain.

The potentials for false results (positive or negative) are always present in this type of bioassay; they cannot be totally eliminated. Any attempt at minimizing these potentials will detract from the value of the test as a rapid screening tool, but will certainly increase the confidence in the data. It is apparent that the experimental design used in this project was suitable only for preliminary screening of samples. It is not reasonable to classify a sample as mutagenic or not on the basis of this test alone.

Alternative bioassays, similar to the Ames test, have been developed for detecting mutagenic activity of pure compounds. These assays use yeasts, other bacteria, or tissue culture cells as the test organisms. If the Ames testing procedure outlined in this project is coupled with one or more of these assays, it may prove to be very useful as a screening technique. Ames testing should not be used as the only screening test for determining mutagenic activity of wastewaters.

## Conclusions

It is possible to detect positive mutagenesis in at least some wastewater samples with the procedure used in this project. Because the mutagenic agents are so dilute in wastewater, negative results obtained from these tests do not necessarily indicate the absence of mutagens. There is also a potential for obtaining false positives using this procedure. Therefore, it is not reasonable to classify a sample as positive or negative on the basis of this test alone.

While the test does not appear to require much sophistication, it requires a large laboratory used exclusively for Ames testing. The initial expense of establishing a laboratory for screening samples by Ames testing will be high. Once the laboratory is equipped and staffed with people who have had time to perfect the necessary technique, routine testing will be inexpensive when compared to animal studies.

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***John E. Matthews** is the EPA Project Officer (see below).*
*The complete report, entitled "Mutagenistic Testing of Industrial Wastes from Representative Organic Chemical Industries," (Order No. PB 81-155 574; Cost: $6.50, subject to change) will be available only from:*
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*5285 Port Royal Road*
*Springfield, VA 22161*
*Telephone: 703-487-4650*
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