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Project Summary

Reduction of Toxicity to Aquatic Organisms by Industrial Wastewater Treatment

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The specific goal of this research was to conduct 24-hour static acute bioassays with "untreated" influent and "treated" effluent using fathead minnows *(Pimephales promelas)* and water flea *(Daphnia magna)* to biologically evaluate the effectiveness of industrial wastewater facilities in reducing effluent toxicity to aquatic organisms. Of primary interest to the EPA was an evaluation of the capacity of the wastewater treatment facilities of the pesticide industry for reducing toxicity. To accomplish the stated goal, on-site 24-hour static acute toxicity tests were performed during ten consecutive workdays at seven industrial sites. Five of the test sites are defined as pesticide manufacturers, while the remaining sites consisted of an organo-chemical manufacturer and a bleached-kraft paper mill. The effectiveness of the treatment plants was determined by performing static acute toxicity tests with the fathead minnow *(Pimephales promelas)* and the water flea *(Daphnia magna)* on samples of the wastewater collected before and after treatment. Results of the studies are expressed in terms of both median lethal concentrations (LC50's) as percent effluent and lethal units.

The data from these studies indicate that the wastewater treatment plants provided an average efficiency of 98% in reducing the toxicity of "untreated" wastewaters. Neither species tested proved to be a more sensitive indicator of toxicity, though a larger data base is required to make a valid appraisal.

Of interest was the observation that while some wastewater treatment facilities provide good efficiency (98+%) in reducing toxicity, the resulting effluent still represented a relatively high number of lethal units. This was a result of the fact that the "untreated" influent entering the waste treatment system contained a very high level of lethal units and a subsequent 98% reduction of that level still resulted in a toxic wastewater.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, 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

In 1977, the United States Environmental Protection Agency was charged by Congress under the Federal Water Pollution Control Act Amendments (PL-95-217) to develop a program which would protect aquatic life by eliminating the discharge into the nation's waterways of toxic pollutants in toxic amounts. EG&G, Bionomics' personnel performed on-site toxicity testing at seven industrial wastewater treatment facilities throughout the United States. The data generated from these studies will aid EPA in developing effluent discharge guidelines related to the toxicity to aquatic organisms of industrial wastewater streams.

The specific goal of this research was to conduct 24-hour static acute bioassays with untreated influent and treated effluent using fathead minnows *(Pimephales promelas)* and water flea *(Daphnia magna)* to biologically evaluate the effectiveness of industrial wastewater facilities in reducing effluent toxicity to aquatic organisms.

To accomplish this task, it was necessary to evaluate the toxicity of the wastewaters before and after biological and/or chemical treatment procedures. Results of the acute toxicity studies are expressed in both the conventional format as median lethal concentrations (LC50's) and as lethal units, a concept which is similar to that of toxic units as described by Esvelt, Kaufman, and Selleck (1971). The lethal unit concept, as it applies to effluents, is the concentration of the effluent divided by the 24-hour LC50. This unitless expression of toxicity may be ideally suited for use with wastewater discharges of diversified effluents and can be incorporated into the regulatory process of controlling and limiting toxic discharges.

Of primary interest to EPA was an evaluation of the capacity of the wastewater treatment facilities of the pesticide industry for reducing toxicity. The Agency's rationale in selecting this industry was based on the premise that discharges from pesticide manufacturers might be expected to be relatively toxic, and perhaps not readily degraded even during biological treatment. The industries which participated in this study were Monsanto Company, Muscatine, Iowa; Mobay Chemical Company, Kansas City, Missouri; Monsanto Company, Luling, Louisiana; and Diamond Shamrock Corporation, Green Bayou, Texas. Most chemical manufacturing complexes are not limited to the production of pesticides alone. These sites were selected because they had segregated pesticide wastewater treatment facilities which do not (for the most part) receive wastewater from other manufacturing processes, and because the majority of the treatment systems were candidates for best available technology economically achievable (BAT) systems or portions thereof. In addition to the above four pesticide manufacturers, studies were conducted with a complex organochemical effluent at Union Carbide Corporation, South Charleston, West Virginia; a complex organo-pesticide effluent at E.I. duPont Nemours & Company, Inc., LaPorte, Texas; and a bleached-kraft paper mill effluent from International Paper Company, Georgetown, South Carolina.

Technical Approach

On-site testing was carried out at each site in one of EG&G, Bionomics' Mobile Aquatic Toxicology Laboratories. Unless otherwise stated, procedures used in conducting the on-site tests followed "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (EPA, 1975). All raw data generated from these studies are permanently stored in the archives at EG&G, Bionomics, Wareham, Massachusetts.

The majority of the wastewater treatment systems evaluated consisted of the following (generalized) treatment steps: coarse screening of particulates, pH neutralization, activated sludge digestion, and clarification. The specific design of the wastewater treatment plants varied from one site to the next depending on the nature and composition of the wastewater being treated. Wastewater samples, which were generally representative of the major contributory pesticide wastewater stream entering the treatment plant (untreated influent) and the final discharge stream from the wastewater treatment plant (treated effluent), were collected at each site.

Depending on the site, one or two untreated influent wastewater streams were tested. In certain instances, direct access to an incoming wastewater stream was not possible until after it had already entered some portion of the treatment system (e.g., grit basin or pH neutralization basin). At these sites the influent samples were obtained immediately prior to the next step in the treatment process. The complexity of some wastewater treatment plants made it impossible to test influent samples which were wholly representative of the total pesticide wastewater load entering the treatment system. This was a result of multiple wastewater streams entering the treatment plant at different stages in the treatment process.

Treated effluent samples were obtained at sampling points located immediately after the last step in the treatment process. Since many of the sites hav multiple wastewater treatment systerr which are utilized for treating othe manufacturing processes, the treate effluent tested in these studies repre sented only the wastewater discharge directly from the pesticide wastewate treatment system. These samples wer collected prior to dilution with othe process wastewater streams or non contact cooling waters and, therefore are not representative of the company' total discharge entering the receivin system.

Twenty-four hour static acute toxicit tests were performed daily on sample of untreated influent and treated effluer at each of the study sites (Mobay Chemi cal, only treated effluent) over te consecutive test days. Fathead minnov tests were conducted on wastewate samples collected at two samplin periods each day while daphnid test were performed on alternating day beginning with test day 1. At four of th sites, *Salmonella* Bacterial Mutagenesi Assays (Ames Tests) were performed o samples of the treated effluent.

Grab samples of each wastewate (influent and effluent) were obtaine twice a day (9:00 a.m./3:00 p.m.) for ter consecutive days to coincide with th test regimentation. A sample of eacl wastewater was taken prior to the firs day of testing in order that preliminar range-finding tests could be conducte with each test species. At certain sites personnel from the host company collected duplicate or split samples of the wastewater with the laboratory personnel from EG&G, Bionomics.

A characterization of the chemica and physical parameters of the dilutior water and treated and untreated effluen wastewaters was performed daily a each site. On each test day, samples o reconstituted dilution water were analyzed for total hardness, total alkalinity, specific conductance, pH, temperature, and total residual chlorine. Similarly, aliquots of each wastewater sample collected during each ten-day study were analyzed for dissolved oxygen concentration (DO), pH, specific conductance, temperature, and total residual chlorine. These measurements were made on samples of the wastewaters brought back to the mobile laboratory, but prior to temperature adjustment.

During each test, the pH, dissolved oxygen concentration, and temperature were measured in the control, high middle, and low test concentrations prior to the addition of the test organisms and at the termination of the test. Specific conductance was monitored similarly, but only at the initiation of testing.

Mortality data derived from each definitive test were used to calculate a median lethal concentration (LC50) and its 95% confidence limit utilizing the moving average angle method (Harris, 1959). The data are presented as both LC50 values (percent wastewater) and lethal units (100/LC50). The LC50 is the calculated nominal concentration of the wastewater in diluent water which produces mortality of 50% of the test animal population at the stated time of exposure; i.e., 24-hour LC50. In those instances where LC50 values could not be calculated due to <50% mortality in 100% wastewater, the lethal units have been derived through graphical interpolation of the data on log x probit paper and provide the most probable toxicity concentration (Esvelt, et al., 1971).

Results and Discussion

In the present study, the results of toxicity studies indicate a higher level of lethal units present in the wastewaters of the pesticide formulation, both before and after treatment as compared to the wastewaters of pulp and paper mill and organo-chemical plants (Table 1). A sufficient data base is not available to determine whether these observations are valid overall for the industries of concern. The average efficiency of the wastewater treatment plants in reducing the toxicity of the "untreated" wastewater was 98% (range, 92-100%). Excluded in the average were Mobay Chemical Company and E.I. duPont. Mobay was excluded since influent tests were not performed, and consequently the percent efficiency of the treatment system could not be determined. E.I. duPont was excluded from the calculation since a true representation of the toxicity of the entire influent wastewater load could not be determined.

The results of ten consecutive days of biological testing at each site indicate that the toxicity of the treated effluent samples remained relatively constant from day to day. The average number of lethal units present in the treated effluents, as determined from tests with fathead minnows, was 2.1 with a range of 0-6 lethal units. Similarly, *Daphnia magna* tests provide a mean of 6.5 lethal units with a range of 0-34. As would be expected, the toxicity of the untreated influent samples was extremely variable on a day-to-day basis. From the fathead minnow studies, the untreated influent contained a mean of 93 lethal units (range, 4.6-373), while *Daphnia magna* acute tests produced a mean of 261 lethal units (range, 1.7-782).

The results of the Ames Test performed on the treated effluent water samples, collected on test days 1, 3, 6, 7, and 9 at Monsanto (Luling, La.), indicate a weak, but consistent, increase in the reversion index of tester strain TA1535. The dose-related increase was present both with and without metabolic activation, but was most pronounced in the presence of rat liver microsomes.

A confirmation study of the His^+ phenotype of revertant colonies of strain TA1535 at the highest dose level indicated that 91% of the colonies were true revertants. This result supports the conclusion that the observed increase in reversion index of TA1535 is real and not an artifact of the experiment. The Ames Test showed no significant increase in the reversion index of tester strains, with or without metabolic activation, in treated effluent samples collected at Monsanto, Muscatine, Iowa; Diamond Shamrock, Green Bayou, Texas; or Mobay Chemical, Kansas City, Missouri.

Toxicity of the treated effluent is relative to the toxicity of the untreated influent. For example, if an influent contained 300 lethal units and underwent biological and/or chemical treatment which has an efficiency of 99%,

Table 1. **Mean Lethal Units for Fathead Minnows and **Daphnia magna** Exposed to Untreated Influent and Treated Effluent from Industrial Wastewater Treatment System.*

	Fathead Minnow			***Daphnia magna***		
Industry	*Influent*	*Effluent*	*% Toxicity Reduced*	*Influent*	*Effluent*	*% Toxicity Reduced*
Union Carbide (South Charleston)	10.0	0	100.0	12.0	0	100.0
Monsanto (Muscatine)	47.0	1.5	97.0	41.0	1.3	97.0
Mobay Chemical (Kansas City)	—	—	—	3.8	4.4	—
Monsanto (Luling)	74.0	6.0	92.0	88.0	3.1	96.0
Diamond Shamrock (Green Bayou)	360.0	1.2	99.7	130.0	2.6	98.0
E.I. du Pont (La Porte) #1	4.5	2.4	-**	4.8	3.5	-**
#2	140.0			770.0		
International Paper (Georgetown)	2.8	0	100.0	1.7	0	100.0

$$\text{*Lethal units} = \frac{100}{\text{24-hr LC50/EC50 (percent waste)}}$$

***Unable to make valid comparison*

then the treated effluent would still contain 3 lethal units. This would be equal to an LC50 value of 33% effluent. Conversely, if an influent having moderate toxicity is subjected to the same treatment efficiency, the resulting effluent will probably be non-toxic or of a very low toxicity. The significance of this observation must be viewed in terms of what additional engineering steps have been applied to the effluent between the time the wastewater leaves the treatment system and enters the receiving water. Dilution with other treated process streams and non-contact cooling water must be taken into consideration in both designing wastewater treatment systems and regulating their discharge.

The Mass Emission Rate, as proposed by Esvelt, et al. (1971), provides a useful method of incorporating lethal units and flow rates in assessing the impact of a wastewater stream on the total ecology of the receiving water. To determine the mass emission rate for a stream, one must first determine the relative toxicity (RT) of the wastestream which is defined as the product of the toxicity concentration (expressed as lethal units) and the flow rate.

$$RT = Tc \times \text{Flow Rate}$$

The mass emission rate then for a receiving water (Tc_2) is defined as the relative toxicity divided by the combined waste and dilution water flow.

$$Tc_2 = \frac{RT}{\text{wastewater flow rate} + \text{dilution water flow rate}}$$

At present, the mass emission rate for a receiving water is a somewhat nebulous value requiring further regulatory definition. If a program for water quality management is to be developed, which considers the toxic properties of wastes, then additional information must be obtained on the efficiency of the wastewater treatment systems. The utilization of a study program similar to that carried out under the present contract is one method by which the minimum efficiency of a wastewater treatment plant, under normal operating conditions, could be determined. If the toxicity of an effluent could be correlated with one or more chemical parameters which can be monitored continuously, then a mechanism would evolve by which regulators and plant operators could monitor the discharge of toxic wastes on a real time basis before they had a chance to cause damage to the environment.

In this study, neither the fathead minnow nor the water flea proved to be routinely more sensitive than the other in tests with the "untreated" or "treated" wastewaters. Since an adequate data base has not been developed for these species to the vast majority of industrial effluents, it is suggested that both the fathead minnow and daphnids be tested. On the other hand, should an on-going biomonitoring program be instituted for an industrial discharge in which both species provide similar results, then future testing might be limited to only one species. *Daphnia magna* are attractive for this purpose from the standpoint that their use can greatly reduce the economic burden to an industry for such a program. Daphnids are relatively easy to culture, require no capital investment, and can be used by plant personnel to generate required data. It may also be assumed that with such a biomonitoring tool available to them, industry will take it upon themselves to incorporate its use on a regular basis in monitoring the efficiency of their wastewater treatment systems.

Finally, the use of the lethal unit concept should be applied to all effluent toxicity studies. LC50 values as percent effluent do not provide an accurate assessment of the impact of the wastewater. This is especially true for highly toxic wastes. The mean of a number of LC50 tests may not equal the mean from the same tests expressed as lethal units. A highly toxic effluent when converted to toxic units will skew the mean upwards and as a result will more accurately represent the toxic load entering the environment.

For those effluents resulting in less than 50% mortality in 100% effluent, the most probable toxicity concentration (expressed as lethal units) is equally important in determining mass emission rates.

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The complete report, entitled "Reduction of Toxicity to Aquatic Organisms by Industrial Wastewater Treatment," (Order No. PB 81-222 366; Cost: $8 00, subject to change) will be available only from:
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