



## Project Summary

# Microbial Transformation Kinetics of Xenobiotics in Aquatic Environment

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Valid second-order rate equations will permit the wider use of mathematical models for predicting the persistence of organic chemicals in fresh and marine waters. In the laboratory study, the microbiological transformations of the butoxyethylester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), *p*-cresol,  $\alpha$ -naphthol and quinoline were examined to determine whether their degradation rates are reasonably described by second-order rate equations. Graphical analysis of the data with first-order log plots indicated that quinoline, *p*-cresol, and  $\alpha$ -naphthol were only transformed following a lag phase. The lag period was followed by a transformation phase where the detectable decrease in compound concentration could be described by a pseudo first-order rate equation and for which pseudo first-order rate constants could be determined. The transformation of 2,4-DBE, however, occurred immediately upon addition of the compound to sample waters.

Much of the variability in first-order constants for the different compounds could be accounted for in the range of average bacterial populations, measured during the transformation phase, that were used to calculate second-order rate constants. Second-order rate constants were clustered into groups that were statistically different. Within all but two groups the range in first-order rate constants was greater than the range in second-order constants.

Results of the study support the usefulness of the second-order transformation kinetics concept for describing the microbial transformation

of organic chemicals in the aquatic environment. The research points to a continuing need for efforts in the area of quantitative kinetic approaches to the predictive modeling of microbiological transformation in the environment.

*This Project Summary was developed by EPA's Environmental Research Laboratory, Athens, GA, 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

Estimates of the degradation rates and thus the persistence of organic compounds in a wide range of aquatic environments require an in-depth understanding of the microbiological transformation pathways (reaction sequences) of major compound classes and the effects of physical-chemical (environmental) parameters as well as microbiological population dynamics on the rates of these reactions. Although not complete, a wealth of information describing the metabolism of major compound classes, especially in the area of aerobic degradation, is available in the scientific literature. Anaerobic metabolism has not been characterized to the same degree, however.

The microbiological degradation and transformation rates of numerous organics have been determined in environmental samples. Unfortunately, much of this data base is sample specific and not transferable to other sites. Only in recent years have attempts been made to quantitate these rates so that they can be used to estimate the persistence of

specific organics in a range of environments. These recent studies have led to the development of mathematical models to estimate persistence of organics.

The continued development of viable predictive models of the degradation of organic compounds in the environment requires the interactions of the following two research areas:

- The development of environmentally applicable mathematical representations of the microbiological processes involved in the transformation and ultimate degradation of organic compounds
- The design and development of procedures to produce laboratory data compatible with the mathematical representations

Previously, these areas have developed independently in many respects. In the future, as microbiological degradation models become more sophisticated, it is essential that the two areas be investigated in an interactive manner, each process being directed, in turn, by the other as new information and methodologies are developed. This will ensure the most rapid and complete development of useful microbiological models.

This research approach does not preclude theoretical studies. Models, however, are generally of little practical value when no consideration is given to the availability of laboratory data to exercise the model, or worse, if no consideration is given to the development of laboratory techniques to provide the data. Also of little value are degradation studies in which the usefulness of the data as input for available or developing computer models has not been considered.

Continued efforts to integrate these two research areas will lead to better microbiological models and a better understanding of microbiological degradation in the environment. An important first step in this direction has been to use second-order rate equations (i.e., the product of a second-order rate constant, a biomass term and the compound concentration) to describe the microbiological transformation of organics in fresh and marine waters.

The potential utility of second-order equations arises from the aspect that, once reliable second-order rate constants are available, the transformation rates at specific study sites can be estimated with

reasonable accuracy from measurements of the biomass (bacteria). In most cases, these estimates are markedly less costly and labor intensive than the experimental determination of the transformation rate. Ultimately, the utility of second-order rate constants and equations in predicting the transformation rates of organics will depend on the reproducibility of second-order rate constants experimentally determined in different laboratories and on the range of compound types for which second-order rate equations will describe their transformation in aquatic environments.

In this study, the microbiological transformation of a series of organic substrates was examined to determine whether their rates of degradation are reasonably described by second-order equations. The compounds were  $\alpha$ -naphthol, quinoline, *p*-cresol, and the butoxyethylester of 2,4-dichlorophenoxyacetic acid.

## Discussion

The microbiological transformation rates of the four organic compounds added to natural water samples were examined in the laboratory. Graphical analysis of the data with first-order log plots indicated that transformation of these compounds occurred in two phases. The initial phase consisted of a lag period during which no decrease in compound concentration could be detected. Quinoline, *p*-cresol, and  $\alpha$ -naphthol, were only transformed following a lag phase. The transformation of 2,4-DBE occurred immediately upon addition of the compound to sample waters. The lag period was followed by a transformation phase where the detectable decrease in compound concentration could be described by a pseudo first-order rate equation and for which pseudo first-order constants could be determined.

The variability in first-order constants for the different compounds ranged from a low of 13.6-fold for 2,4-DBE to a high of 185-fold for quinoline. Much of the variability could be accounted for in the range in average bacterial populations, measured during the transformation phase, that were used to calculate second-order rate constants and from the observation that second-order rate constants could be clustered into groups that were statistically different.

The variability of second-order constants within these groups ranged from 1.18- to 36.14-fold, whereas the first-order constants ranged from 1.24- to

184.71-fold. Within all but two groups, the range in first-order rate constants was greater than the range in second-order constants.

Comparison of the second-order rate constants for 2,4-DBE and *p*-cresol and the second-order constants reported in an independent study indicated that, although the mean values were markedly different, the standard deviations were remarkably close in all cases. Either of two possible explanations can account for the difference in these values: there is a systematic difference between laboratory procedures or there were significant differences in the microbial populations in waters sampled by each of the laboratories and the differences are real.

Before a detectable decrease in compound concentration could be measured for three of the four compounds examined, a significant lag period was observed. This lag could have importance in the approach used in computer modeling of compounds that behave in this manner.

One of the primary interests in developing site independent second-order rate constants has been their use in exposure assessment modeling. The reproducibility of these constants for a number of compounds observed in our work and that of others emphasizes their utility in exposure assessment. The role of microbiological transformation or degradation of xenobiotics, however, cannot be limited only to second-order constants. The observation that second-order rate constants fall into groups suggests that the rate of transformation of xenobiotics in the environment may require a probability distribution function (i.e., transformation models may need to have a random variable format). The conservative alternative would be simply to use the smallest second-order rate constant determined for a given compound.

Although more data are needed with different sites and different compounds, these results do offer significant encouragement for prediction of microbial transformation rates in natural systems.

## Recommendations

A continued research effort in this area, which is a "kinetic approach" to the predictive modeling of microbiological transformations in the environment, should address the areas of adaption kinetics, reaction kinetics, and quantitation of biomass. Biomass is important in extrapolations from basic research

models to widely applicable predictive models that are normally constructed to use readily available or easily attainable data. For example, a compound-specific biomass measure can now be obtained by using a most-probable-number technique for enumerating microorganisms. The technique incorporates  $^{14}\text{C}$ -labeled substrates. In time, this procedure may displace the standard pour-plate methods currently being used.

Where second-order rate equations are applicable, the "kinetic approach" has rested on the ability of laboratory studies to determine an average second-order rate constant, using a number of different waters with different microbial populations. The average rate constants could then be used to predict degradation rates at other sites if the microbial population and organic substrate concentrations are known.

This approach also can be used when the degradation rate is described by alternative mathematical expressions. For example, if the Michaelis-Menten equation were used, one would simply determine the average values for  $K_m$ ,  $V_{\max}$ , and the bacterial population of a number of waters and then use these average values to estimate degradation rates in other waters where the substrate concentration and bacterial populations are known. The bacterial population in this case is used to set the value of  $V_{\max}$  from an average specific activity term ( $\text{sp. act.} = V_{\max}/\text{bacterial population}$ ) determined in the initial evaluation studies. Similar examples can be derived from other mathematical expressions of the degradation rate.

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*The complete report, entitled "Microbial Transformation Kinetics of Xenobiotics in Aquatic Environment," (Order No. PB 84-162 866; Cost: \$13.00, subject to change) will be available only from.*

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