

# Biodegradation of Mirex by Sewage Sludge Organisms

by

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Very few biodegradation studies on Mirex have been reported, and to the best of our knowledge no metabolites of Mirex have been detected. Using uniformly labelled  $^{14}\text{C}$ -Mirex, GIBSON et al. (1972) and MEHENDALE et al. (1972) found that rats do not degrade Mirex. The latter also found that Mirex was not metabolized by bean and pea root preparations or by rat, mouse and rabbit liver preparations. The apparent biodegradation of Mirex in sewage sludge is reported here.

## Materials and Methods

Uniformly labelled  $^{14}\text{C}$ -Mirex (0.1 mg, 1.2 uCi) (Mallinckrodt Nuclear) was incubated in the dark with 100 ml. of sewage sludge in 250 ml. Erlenmeyer flasks under aerobic and anaerobic conditions at 30°C. Controls composed of  $^{14}\text{C}$ -Mirex in sterilized sludge were maintained for both aerobic and anaerobic samples.

After 2.5 months of incubation all samples were extracted according to the method of YOSHIDA and CASTRO (1970). The hexane extract was transferred to a centrifuge tube, evaporated under nitrogen and purified by elution through a Florisil column using hexane. The eluate was concentrated to 0.2 ml., spotted on a silica gel thin layer plate, and developed to a height of 17 cm. with acetone:hexane (1:9, v/v). The plate was autoradiographed, and zones corresponding to Mirex and metabolite were scraped into scintillation vials for radioactivity determination.

## Results and Discussion

The autoradiograms of the thin-layer plates showed two distinct spots for the anaerobic samples but only one spot for the aerobic samples and the controls. The ratio of the counts of the Mirex zone ( $R_f$  of 0.74) to those of the metabolite zone ( $R_f$  of 0.65) was calculated for each sample as quantitative evidence of the presence of a small amount of a metabolic product.

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These data are presented in Table 1.

TABLE 1

Ratio of Radioactivity in Mirex and Metabolite Zones\*

Sample	Ratio
	Mirex/metabolite
Anaerobic Sludge	88
Anaerobic Control	761
Aerobic Sludge	636
Aerobic Control	470
Mirex Stock Solution	440

\* These experiments have been replicated 3 times using different samples of sludge. The data presented here are from one experiment and are representative of the 3 replications.

The data indicate that under the experimental conditions used sewage sludge microbes degrade Mirex under anaerobic conditions. The controls and the fact that the incubation was done in the dark eliminated the possibility that the non-Mirex spot could have been caused by chemical degradation or photolysis. The data for the Mirex stock solution and controls exclude the possibility that the metabolite resulted from autoradiolysis.

Structure determinations of the metabolite are in progress and efforts are also being made to isolate and identify Mirex-degrading microorganisms.

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