Item ID Number	03287 ☐ **Not Scanned**
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Report/Article Title	Biodegradation of Chlorinated Dibenzodioxins and Dibenzofurans
Journal/Book Title	Research Review 1977
Year	1978
Month/Day	June
Color	☐
Number of Images	2
Description Notes	EPA-600/9-78-014.

SOURCE: RESEARCH REVIEW 1977. EPA-600/9-78-014. June 1978, pp 30-31.

(EPA Environmental Research Laboratory, Gulf Breeze Fl 32561)

rapidly reached equilibrium; however, the amount accumulated was directly proportional to the partitioning coefficient of the compound between seawater and the hexadecane film.

In the second phase of the research, tests using hexadecane as a basic surface film showed that the addition of alkyl benzene sulfonate, tannic acid, or Tween-80 all increased the concentration of DDT from the water column, i.e., 260 to 320 times that seen in the water column.

Effects of Pollutants on Microbial Activities in Estuarine Surface Films

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A group of compounds that earlier had reacted positively in mutagenicity tests (Ames systems) were used in studies of 9 surface slick isolates. In tests using the disc diffusion method, pentachlorophenol (PCP) and Captan inhibited the growth of 9 and 8 of the 9 isolates, respectively. Further tests with discs saturated with 0.1 ml of DMSO containing 100 µg of test substance showed that PCP inhibited only 3 of 9 isolates, whereas Captan and Captafol inhibited all 9 cultures. Future studies will establish the dose responses for selected inhibitory compounds on Ames strains and on surface slick isolates.

Previous studies suggest that heptachlor may alter the transport of hydrophobic materials into the cell. Therefore, studies undertaken in 1977 assessed the synergistic action of heptachlor and mutagenic compounds in the Ames system. Subinhibitory levels of chlordane (20 ppm) were found to be inhibitory in the presence of heptachlor (20 ppm). No enhanced mutagenicity was noted for combinations of heptachlor with BHC, Captafol, Captan, Carbaryl, chlordane, Kepone, mirex, and PCP. Preliminary results from surface film samples indicate the presence of toxic and possibly mutagenic substances.

Earlier, this investigation demonstrated preferential removal of naphthalene and biphenyl in a synthetic oil, using a heptachlor (1 mg/ml) system inoculated with either Candida lipolytica or C. maltosa. Work in 1977 examined the disappearance of naphthalene from a simplified system containing only hexadecane or hexadecane and biphenyl. Biphenyl appeared to stimulate utilization of hexadecane and naphthalene by C. lipolytica. Heptachlor reduced naphthalene utilization by both yeasts. Similar results were observed for a modified synthetic oil containing biphenyl, naphthalene, tetradecane, hexadecane, and pristane. Heptachlor (1000 ppm) or Kepone (10 ppm), when added into this system, caused a reduction in utilization of naphthalene and hexadecane.

Tests on effects of sublethal concentrations of pesticides on proteolytic activity showed that selected aromatic and chlorinated hydrocarbons caused no reduction in this enzyme activity.

Seventeen cultures were tested for their response to combinations of PCP, o-chlorophenol, naphthalene, 1-chloronaphthalene, heptachlor, and methoxychlor. Synergistic responses were often produced by combinations of 1-chloronaphthalene and heptachlor or methoxychlor (8 out of 10); napthalene and PCP and naphthalene and ortho-chlorophenol also inhibited these cultures.

Biodegradation of Chlorinated Dibenzodiozins and Dibenzofurans

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Chlorinated dibenzo-p-dioxins have long been recognized as possible by-products in the manufacture of certain chlorinated phenols (i.e. pentachlorophenol and 2,4,5-trichlorophenol). Interest in these compounds has increased since the discovery of highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in samples of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Further, chlorinated dibenzo-p-dioxins and dibenzofurans have been detected in commercial preparations of polychlorinated biphenyls. Many of these chlorinated compounds are used extensively in both industry and agriculture; therefore, the high level of toxicity of the chlorinated dibenzo-p-dioxins is an environmental concern.

This project seeks to determine reaction microorganisms that degrade dibenzofuran and dibenzodioxin, and to identify their chlorinated derivatives.

Microorganisms were collected from mud flats and algal mats at Port Aransas, Texas. Each sample was incubated in seawater containing either dibenzo-p-dioxin or dibenzofuran (0.5 g/ℓ). No growth was obtained after 10 weeks incubation at 25°C on a rotary shaker (250 rev/min). Six additional estuarine samples (collected later) also failed to yield any organisms that could grow by using either substrate as the sole source of carbon and energy. The same samples, however, yielded growing organisms that were able to use hexadecane as their sole source of carbon. Nine of these organisms were retained for co-oxidation studies.

Other laboratory cultures, however, were found to metabolize dibenzo-p-dioxin and dibenzofuran: a Beijerinckia species previously isolated through its ability to grow in biphenyl; a Pseudomonas species that degrades naphthalene; and Cunninghamella elegans, an organism isolated through ability to degrade crude oil from North Carolina estuary. These microorganisms would not grow in dibenzo-p-dioxin or dibenzofuran. However, when an alternative growth substrate was present, significant degradation of both aromatic compounds occurred.

Although no organism was found to use dibenzo-p-dioxin and dibenzofuran as a sole source of carbon and energy, these compounds were readily metabolized when an alternative carbon source was available.

Dibenzo-p-dioxin was oxidized to cis-1,2-dihydroxy-1,2-dihydrodibenzo-dioxin, which then forms a catechol. Studies have yet to determine whether the molecule can be degraded completely to naturally occurring products.

Dibenzofuran is attacked at two positions on the mol-

ecule, indicating that dihydrodiols are produced at the 1,2- and 3,4-positions. Identification of the degradation products posed significant analytical problems, although most have been solved. Preliminary evidence indicates that two catechols and two ring-fission products are produced by Beijerinckia. The broad specificity of this organism (in terms of its ability to metabolize the same molecule at different positions) heightens interest in forthcoming studies on the effects of chlorine substitution.

The fungus Cunninghamella elegans appears to metabolize aromatic compounds in a manner analogous to the mammalian liver. Mammals are known to produce arene oxides which, in some cases, can react with nucleic acids to initiate mutagenic changes. In the case of benzo[a]pyrene, a metabolite, trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, serves as a substrate for the formation of 9,10-epoxy-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (diol-epoxide), which is both mutagenic and carcinogenic. Although no studies have been reported on the mammalian metabolism of dibenzofuran, results obtained in this study indicate the formation of an arene oxide. These molecules, by rearrangement, are known to given phenols and undergo enzymatic hydration to form trans-dihydrodiols. Because C. elegans produces 2- and 3-hydroxydibenzofuran and 2,3-dihydroxy-2,3-dihydrodibenzofuran, the prior formation of 2,3-epoxydibenzofuran is indicated. Also, the stability of the dihydrodiol to acid treatment indicates that it is the trans-isomer.

Metabolites isolated in sufficient amounts will be tested for mutagenic activity in the Ames system by Dr. Eugene Goldschmidt at the University of Houston during the next reporting period.

Insecticide Persistence in Natural Seawater as Affected by Salinity, Temperature, and Sterility

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This investigation determined the effect of temperature, salinity, and sterility on the persistence and degradation of representative organophosphorus and chlorinated hydrocarbon insecticides (malathion, parathion, methyl parathion, diazinon, and methoxychlor).

Surface water samples of 0, 10, 20, and 28 parts per thousand (ppt) salinity were amended with the above insecticides and incubated in the dark at 30°, 20°, and 10°C under sterile and nonsterile conditions. Insecticide abatement was followed by electron-capture, gas-liquid chromatography.

No significant differences between sterile and nonsterile treatments were observed for any insecticide. The effect of increasing temperature was highly significant with regard to increased degradation of malathion, parathion, methyl parathion, and diazinon. Methoxychlor reflected the recalcitrance characteristic of the chlorinated hydrocarbon insecticides throughout 84 days of incubation and was not significantly affected by salinity, temperature, or sterility. Salinity effects varied among the four organophosphates: highly significant for malathion and diazinon, significant for methyl parathion, but not significant for parathion.

Malathion was the shortest-lived insecticide: half-lives at 30°C varied from approximately 11 days in fresh water to less than two days at 10, 20, or 28 ppt salinity. The disappearance rate of methyl parathion was second only to malathion and ranged from 27 days (half-life) in fresh water to 16 days at 28 ppt. In fresh water, a 45-day half-life for diazinon suggested a substantial resistance to degradation, especially at 30°C. In saline water, however, diazinon abatement was accelerated; 24 days half-life at 28 ppt salinity. Parathion, the most persistent organophosphate insecticide, reflected a half-life of at least 44 days regardless of salinity.

One bacterium, tentatively identified as Moraxella sp., was isolated from sediment by enrichment and proved capable of readily degrading malathion either as a primary carbon source or in the presence of peptone. Two bacteria were tested for the ability to degrade methyl parathion: one bacteria, possibly a Pseudomonas sp., proved capable of utilizing the insecticide with or without peptone; the other, a Moraxella, reflected no degradation of methyl parathion as the primary carbon source and only limited utilization of the insecticide in the presence of peptone. Neither bacteria screened for parathion metabolism was capable of insecticide degradation under conditions of this evaluation.

Work was completed September 30, 1977, on this two-year study. The final will be published early in 1978 in the EPA Ecological Research Series.

Feasibility of Using Bacterial Strains to Test for Environmental Carcinogens

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A rapidly growing amount of data is available concerning the mutagenic and carcinogenic properties of new chemicals and products manufactured for commerce in recent years. However, literature pertaining to mixtures, such as chemical wastes, is scarce and difficult to locate.

This grant produced a review of literature related to the feasibility of using bacteria as screening agents to detect cancer-causing agents in the environment. Mutagenicity data were also included in the literature search because of growing experimental evidence that most chemical carcinogens are mutagens and therefore many mutagens may be carcinogens.

Results of the investigation indicate that bacterial strains can be used to initiate a series of studies aimed at screening mixed wastes for potential mutagens and carcinogens. Findings will be published in the EPA Ecological Research Series in 1978.