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Project Summary

In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Fifteen Pesticides

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Fifteen pesticides being reviewed as part of the EPA Substitute Chemical Program were examined by SRI International by several *in vitro* test procedures, for the following:

- **Reverse mutation in *Salmonella typhimurium* strains TA1525, TA1537, TA98, and TA100 and in *Escherichia coli* WP2.**
- **Induction of mitotic recombination in the yeast *Saccharomyces cerevisiae* D3.**
- **Relative toxicity in DNA repair-proficient and repair-deficient strains of *E. coli* (strains W3110 and p3478, respectively) and of *Bacillus subtilis* (strains H17 and M45, respectively).**
- **Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).**

None of the fifteen pesticides demonstrated genetic activity in all six of the *in vitro* assays. Bioallethrin was the only pesticide that was mutagenic in the *S. typhimurium* reverse mutation assay. Manzate-D and manzate 200 increased both mitotic recombination in *S. cerevisiae* D3 and UDS in WI-38 cells. Dithane M-22, dithane M-45, ethylchrysanthemate, and zineb increased mitotic recombination in *S. cerevisiae* D3. DL-*cis/trans* chrysanthemic acid was genotoxic in the relative toxicity assay, being more toxic to the repair-deficient (*rec*$^-$) *B. subtilis* strain M45 than to the repair-proficient (*rec*$^+$) strain H17.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, 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

The Federal Insecticide, Fungicide, and Rodenticide Act designates the U.S. Environmental Protection Agency (EPA) as the governmental body responsible for the safety of all pesticides used in the United States. More recently, the Federal Environmental Pesticide Control Act (PL 92-516) strengthened EPA's regulatory responsibilities in the area of pesticides to include intra- as well as interstate commerce.

To be federally registered, a pesticide must have been determined not to be hazardous to health or to the environment when used according to its labeling restrictions. Thus, in accordance with new law as well as with specific directives included in Public Law 93-135, 1973, EPA is now conducting a thorough review of the implications of using alternative chemicals, including older registered pesticides, for pest control.

In the pesticide review process, EPA emphasizes the development of scientific criteria for evaluating the safety of compounds substituted for those pesticides found to be hazardous. In addition to review and evaluation of the literature on

pesticides and maintenance of liaison with industry and academia, the strategy program includes laboratory studies to obtain additional data. One of these laboratory programs is directed toward gathering mutagenesis data on a selected number of compounds.

EPA's program is responsive to one of the recommendations included in the President's Scientific Advisory Committee Report of September 1973, *Chemicals and Health*. In that document, the Committee recommended that "Regulatory agencies should take steps to insure that new scientific data raising the possibility of new or extended hazards from chemicals in use are subject to careful process of scientific review for merit interpretation."

Results and Discussion

The results of the *in vitro* microbiological and UDS assays of the fifteen compounds are summarized in Table 1. A positive response in these assays is defined as a reproducible dose-related increase in the effect being observed. A genotoxic or mutagenic effect was observed for eight of the fifteen pesticides tested. The eight pesticides that had a positive response in one or more of the assays were bioallethrin, DL-*cis/trans* chrysanthemic acid, dithane M22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200, and zineb. None of the pesticides tested were positive in all six of the *in vitro* assays. Bioallethrin was the only compound mutagenic in the *Salmonella*/microsome assay. Dithane M-22, dithane M-45, ethylchrysanthemate, manzate-D, manzate 200, and Zineb all increased mitotic recombination in *S. cerevisiae* D3, but only manzate-D and manzate 200 also induced unscheduled DNA synthesis in WI-38 cells. DL-*cis/ trans* chrysanthemic acid was genotoxic in the *B. subtilis* relative toxicity assay but was inactive in all other assays. Dose-response curves are presented for pesticides that give a positive response in the assays except for ther relative toxicity assays. Biphenyl, chlordimeform, NRDC-149, permethrin, polyram, resmethrin, and sumithrin were not genotoxic or mutagenic in any of the six assays we performed.

Microbiological Assays

Each pesticide was tested at least twice on separate days, using one plate per dose. The first experiment was a test over a wide range of doses to look for toxicity or mutagenicity. If no toxicity or mutagenicity was observed, the second experiment was conducted at higher concentrations. If mutagenicity was observed, a dose response was determined. An assay that gave a mutagenic response was always repeated to confirm that the results were reproducible.

Conclusions and Recommendations

Our results indicate that none of th fifteen pesticides tested in Phase III wer mutagenic or genotoxic in all six of the *i vitro* assays. Only two pesticides wer positive in more than one assay. The were manzate-D and manzate 200, whic increased mitotic recombination in *S cerevisiae* D3 and induced UDS in WI-3 cells. Dithane M-22, dithane M-45, ethyl chrysanthemate, and zineb increase mitotic recombination. The *Salmonella* microsome assay detected mutageni activity in only one pesticide, bioallethrir No pesticide was found to be mutageni with *E. coli* WP2. DL-*cis/trans* chrysan themic acid was genotoxic in the relativ toxicity assay.

It is recommended that the eight pesti cides that were positive in at least on assay be considered for further study t characterize more completely their poten tial hazards to human health. Although mutagenic response does not mean that chemical is harmful to humans, th combination of six separate assay sys tems greatly enhances the probability o detecting potentially hazardous chemi cals. It is apparent that no one assay i uniquely capable of detecting the spec trum of mutagenic events that differen chemical structures may cause.

Table 1. In Vitro *Mutagenesis: Summary Data for EPA Pesticides*

Pesticide	Salmonella typhimurium *(His⁺ Reversion)* -MA	+MA	Escherichia coli *WP2 (Try⁺ Reversion)* -MA	+MA	Saccharomyces cerevisiae *D3 (Mitotic Recombinants)* -MA	+MA	Escherichia coli *(Relative Toxicity)*	Bacillus subtilis *(Relative Toxicity)*	*UDS (DNA Repair)* -MA	+MA
Bioallethrin	-*	+	-	-	-	-	-	-	-	-
Biphenyl	-	-	-	-	-	-	-	-	-	-
Chlordimeform	-	-	-	-	-	-	-	-	-	-
DL-cis/trans *Chrysanthemic acid*	-	-	-	-	-	-	-	+	-	-
Dithane M-22	-	-	-	-	+	-	-	-	-	-
Dithane M-45	-	-	-	-	+	+	-	-	-	-
Ethylchrysanthemate	-	-	-	-	+	+	-	-	-	-
Manzate-D	-	-	-	-	+	+	-	-	+	-
Manzate 200	-	-	-	-	+	+	-	-	+	-
NRDC-149	-	-	-	-	-	-	-	-	-	-
Permethrin	-	-	-	-	-	-	-	-	-	-
Polyram	-	-	-	-	-	-	-	-	-	-
Resmethrin	-	-	-	-	-	-	-	-	-	-
Sumithrin	-	-	-	-	-	-	-	-	-	-
Zineb	-	-	-	-	+	+	-	-	-	-

**Negative response, -; positive response, +.*

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The complete report, entitled "In Vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Fifteen Pesticides," (Order No. PB 85-193 761/AS; Cost: $17.50, subject to change) will be available only from:
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