# Influence of Herbicides on Insecticide Metabolism in Leaf Tissues

Fa-Yan Chang,* Gerald R. Stephenson, and Leon W. Smith

The influence of six herbicides on the metabolism of the insecticides 1-naphthyl methylcarbamate (carbaryl), *O*-ethyl *S*-phenyl ethylphosphonodithioate (Dyfonate), and diethyl mercaptosuccinate S ester with *O,O*-dimethyl phosphorodithioate (malathion), was studied in leaf discs of tomato or bean. The herbicides 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (linuron) and 3′,4′-dichloropropionanilide (propanil) inhibited the metabolism of Dyfonate and malathion in bean tissue, while carbaryl metabolism in tomato was inhibited by linuron but stimulated by isopropyl *m*-chlorocarbanilate (chlorpropham). Propanil did not inhibit the overall rate of disappearance of the carbaryl but did inhibit the conversion of one metabolite of carbaryl to another. 3,6-Dichloro-*o*-anisic acid (dicamba), 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (pyrazon) and *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (Kerb) did not affect the metabolism of the three insecticides.

There are numerous reports of herbicidal activity being influenced by insecticides (Chang *et al.*, 1971; Kaufman *et al.*, 1970). These interactions most commonly result in increases in phytotoxicity due to an inhibition of herbicide degradation by the insecticides (Kaufman *et al.*, 1970; Matsunaka, 1968; Swanson and Swanson, 1968; Yih *et al.*, 1968). Little is known about the influence of herbicides on insecticides when these chemicals are present together.

This study was initiated to determine the effects of various herbicides on the metabolism and persistence of several insecticides in plant tissues.

## MATERIALS AND METHODS

The insecticides studied were carbaryl, Dyfonate, and malathion. These chemicals were all labelled with carbon-14 and each had a radiochemical purity of at least 99%. Their chemical names, specific activities, and sources are listed in Table I, along with the names and sources of the nonlabeled herbicides. With the exception of pyrazon, the herbicides were all technical grade of high purity. Pyrazon was repurified by recrystallization before use.

Two plant species, bean (*Phaseolus vulgaris* L. cv. Red Kidney) and tomato (*Lycopersicon esculentum* Mill. cv. John Baer) were grown in soil in a growth chamber (16-hr day, 21° C and 8-hr night, 18° C). Leaf discs, 11 mm in diameter, cut from tomato leaves were used for the metabolism study with carbaryl, and leaf discs from the primary leaves of bean were used for the study with Dyfonate and malathion. Fifteen leaf discs constituted one sample and duplicate samples were used in each treatment.

Experimental procedures were similar to those reported previously (Chang *et al.*, 1971). Briefly, the radio-labelled insecticides were dissolved in 0.35 *M* mannitol with or without each nonlabelled herbicide and were fed to the freshly cut leaf discs by vacuum infiltration. The treated leaf tissues were removed from the treatment solution and incubated in a growth cabinet at 25° C in light (12,000 lux) for various lengths of time, depending on the experiment. The leaf tissues were then freeze-killed and extracted with ethanol. The insecticides and their metabolites were separated by thin-layer chromatography with 0.25 mm of silica gel G on glass plates. The radio-labelled compounds were located on the chromatogram plates by autoradiography and then quantified by liquid scintillation counting.

Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada.

## RESULTS AND DISCUSSION

In bean leaf discs which were not treated with the herbicide mixtures, only 31% of the malathion and 46% of the Dyfonate remained unchanged after 2 and 17 hr, respectively (Table II). The metabolism of carbaryl in tomato leaf discs was somewhat slower, with 76% of the parent compound remaining after 17 hr.

The radioactivity in these insecticide-treated leaf tissue extracts was resolved to at least two radioactive spots on the chromatogram which were distinguishable from the spots for the parent insecticides. The first radioactive spot on the chromatogram which had a lower $R_f$ value than the parent compound was referred to as Metabolite I; other radioactivity which remained at or near the origin and increased with time was referred to as Metabolite II.

Bourke *et al.* (1968) found that malathion was converted to five metabolites in red kidney bean seedlings. Thus, the two metabolite regions found on the chromatograms in our study could contain more than one metabolite, but chromatography using the solvent systems of Bourke *et al.* (1968) and those described by Kadoum (1970) failed to give any further separation of the radio-labelled metabolite products.

McBain *et al.* (1970) recently have reported the metabolic fate of Dyfonate in potato plants. After soil application, Dyfonate and several of its metabolic products were detected in the foliage as well as in the tubers. The major metabolites include *O*-ethylethanephosphonic acid and methyl phenyl sulfone or its conjugated products with sulfate and glycoside.

The metabolism of carbaryl has been studied extensively and has recently been reviewed by several authors (Casida and Lykken, 1969; Dorough, 1970; Kuhr, 1970). In plants as

**Table I. Insecticides and Herbicides Used and Their Source of Supply**

| Insecticides | Chemical name | Specific activity (mCi/mmol) | Source |
|---|---|---|---|
| Carbaryl carbonyl-$^{14}C$ | 1-Naphthyl methylcarbamate | 26.4 | Amersham Searle Corp. |
| Dyfonate[a] ring-$^{14}C$ | *O*-Ethyl *S*-phenyl ethylphosphonodithioate | 4.7 | Stauffer Chemical Co. |
| Malathion-1,2-succinyl-$^{14}C$ | Diethyl mercaptosuccinate, S ester with *O,O*-dimethyl phosphorodithioate | 4.6 | Amersham Searle Corp. |
| **Herbicides** | | | |
| Chlorpropham | Isopropyl *m*-chlorocarbanilate | | PPG Industries Inc. |
| Dicamba | 3,6-Dichloro-*o*-anisic acid | | Velsicol Chemical Corp. |
| Kerb[a] | *N*-(1,1-Dimethylpropynyl)-3,5-dichlorobenzamide | | Rohm & Haas Co. |
| Linuron | 3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea | | E. I. du Pont de Nemours & Co. |
| Propanil | 3′,4′-Dichloropropionanilide | | Rohm and Haas Co. |
| Pyrazon | 5-Amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone | | Badische Anilin- & Soda-Fabrik Corp. |

[a] Trade name.

well as insects and mammals, carbaryl is converted enzymatically into *N*-hydroxymethyl, 4-hydroxy, 5-hydroxy, and 5,6-dihydro-5,6-dihydroxy derivatives. These metabolites are largely conjugated with sugars and they are less toxic than carbaryl itself.

Kuhr and Casida (1967), studying the fate of carbaryl in bean, resolved six spots of carbaryl derivatives on thin-layer chromatograms; each of the metabolite spots contained several conjugated metabolites. In the present study only two radioactive regions were detected in addition to the region for the parent molecule carbaryl. In light of the work of Kuhr and Casida (1967), it is possible that each of the two metabolite regions may contain more than one metabolite.

**Influence of Herbicides on the Disappearance of the Parent Insecticide Compounds.** The rates of insecticide metabolism in leaf discs not treated with herbicides (Table II) were assumed to be the normal rates for the conditions reported and were arbitrarily considered to be 100%. The metabolism rates of the insecticides under the influence of the herbicides were reported as percentages of these controls (Figure 1).

Of the six herbicides examined, three (dicamba, pyrazon, and Kerb) showed no significant influence on the metabolism of any insecticide tested. However, linuron inhibited the metabolism of all the three insecticides tested. Propanil inhibited the metabolism of Dyfonate and malathion, but had no apparent effect on the overall disappearance of carbaryl. Carbaryl metabolism was stimulated by chlorpropham, but the metabolism of Dyfonate or malathion was not affected by this herbicide.

**Table II. Thin-Layer Separation of Radio-Labelled Compounds Present in Leaf Tissues after Treatment with $^{14}C$-Labelled Carbaryl (Tomato), Dyfonate (Bean), and Malathion (Bean)[a]**

| Insecticide | Incubation time, hr | Tlc solvent | % Radioactivity ($R_f$ values in parentheses) | | |
|---|---|---|---|---|---|
| | | | Parent compound | Metabolite I | Metabolite II[b] |
| Carbaryl | 17 | A | 76(0.62) | 5(0.33) | 19(0.00) |
| Dyfonate | 17 | B | 46(0.75) | 9(0.36) | 45(0.00) |
| Malathion | 2 | B | 31(0.50) | 56(0.29) | 13(0.00) |

[a] The ethanol extractable $^{14}C$-labelled compounds were separated on 0.25-mm silica gel G plates developed with solvent A, ether:hexane (4:1, v/v) or solvent B, hexane:acetic acid:ether (75:15:10, v/v/v). [b] Activity at origin which may represent more than one compound.

With the limited data available from this study, it is impossible to correlate inhibition of pesticide metabolism with the structure of the pesticides involved. However, it is interesting to note that two structurally similar herbicides, linuron and propanil, showed very similar inhibitory effects on the metabolism of Dyfonate and malathion. Conversely, in a previously reported study (Chang *et al.*, 1971) it was observed that both Dyfonate and malathion inhibited the metabolism of linuron and propanil.

The stimulation of carbaryl metabolism by chlorpropham

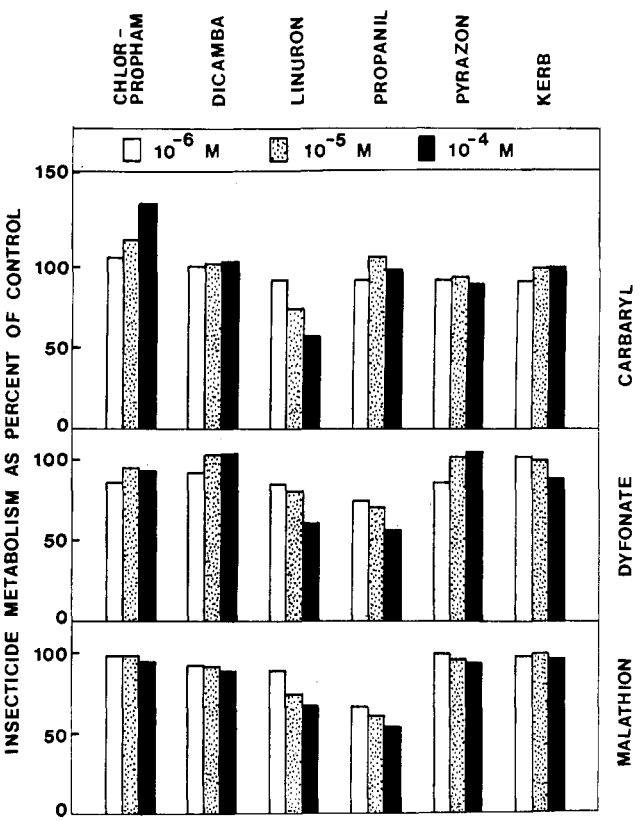


**Figure 1. The influence of herbicides on the disappearance of the insecticides, carbaryl (tomato), Dyfonate (bean), and malathion (bean). Each herbicide was examined at three concentrations ($10^{-6}$, $10^{-5}$, and $10^{-4}$ *M*). Coefficients of variability for these experiments ranged from 3.9 to 7.2%**

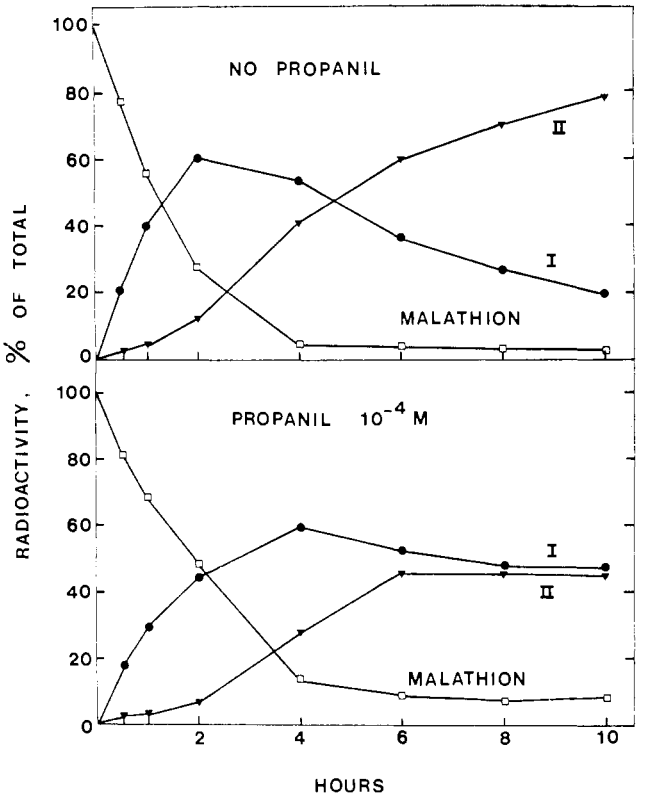


**Figure 2. Time course study of malathion metabolism in bean leaf tissues as influenced by propanil ($10^{-4}$ *M*). I represents metabolite of malathion at $R_f$ 0.29, while II represents metabolite(s) at origin**

(also a carbamate) is difficult to explain. It is possible that one compound (chlorpropham) may induce the formation of an enzyme in a plant tissue, which also metabolizes another similar compound (carbaryl). However, the validity of this explanation is weakened by the fact that the converse is not true, *i.e.*, in plants carbaryl had little effect on chlorpropham metabolism (Chang *et al.*, 1971) and in soil carbaryl is a competitive inhibitor of the chlorpropham hydrolyzing enzyme isolated from soil microorganisms (Kaufman *et al.*, 1970).

**Influence of Propanil on Malathion Metabolism—Time Course Study.** The influence of propanil on malathion metabolism in bean was studied in greater detail by examining the ratio of parent compound to metabolites in extracts after 0-, 0.5-, 1-, 2-, 4-, 6-, 8- and 10-hr treatments (Figure 2). The results clearly indicate that, in the control tissue without propanil, malathion disappeared very rapidly and was hardly detectable after 4 hr. Metabolite I ($R_f$ 0.29) increased in concentration from 0 to 2 hr and then decreased with a corresponding increase in the amount of Metabolite II at the origin. Although not indicated in the graph, with incubations longer than 17 hr, II was the only radioactive region detected. These results all strongly indicate a precursor–product relationship between malathion, metabolite I, and region II, respectively.

In the presence of propanil at $10^{-4}$ *M*, the disappearance of malathion in bean was inhibited (Figure 2). Nearly twice as much malathion remained unchanged after 2 and 4 hr in propanil-treated tissue compared to that remaining in tissue which was treated with only the insecticide. Furthermore, propanil strongly inhibited the subsequent conversion of metabolite I to those at region II.

**Influence of Propanil on Carbaryl Metabolism—Time Course Study.** The disappearance of parent carbaryl from tomato leaf tissue was not significantly affected by propanil (Figure 1). However, it was noticed that the comparative amounts of the metabolites at $R_f$ 0.33 (I) and the origin (II) did differ depending on the presence or absence of propanil. To investigate this further, tomato leaf discs treated with carbaryl and with or without propanil ($10^{-4}$ *M*) were sampled and analyzed at 4-hr intervals up to 24 hr. It was again apparent that propanil did not greatly affect the disappearance of parent carbaryl, but the ratio of metabolite I to region II was significantly affected at each incubation period (Figure 3). Here again, metabolite I appeared to be the precursor of those at region II, and the transformation from I to II was strongly inhibited by propanil. The significance of this influence is not clear, but it points out that herbicide insecticide interactions may occur at any step in the metabolic pathway and that some interactions may be overlooked, if only the disappearance of the parent compound is examined.

These results indicate that the herbicide linuron may increase the persistence of the insecticide carbaryl, when these two pesticides are simultaneously present in plant tissues. Similarly, linuron and propanil may increase the persistence of Dyfonate and malathion in plants. On the contrary, chlorpropham may decrease the persistence of carbaryl when these two chemicals are present in plants together. Since Dyfonate is quite persistent in the soil (Read, 1969) and is slowly metabolized by plants, further increasing the persistence in the plant by herbicides may not significantly influence insect control. On the other hand, malation is a very short-lived insecticide in plants and the presence of linuron or propanil in the plants may significantly increase its activity against insects.

Many noninsecticidal compounds have been found to be strong synergists of some insecticides (Casida, 1970). It is

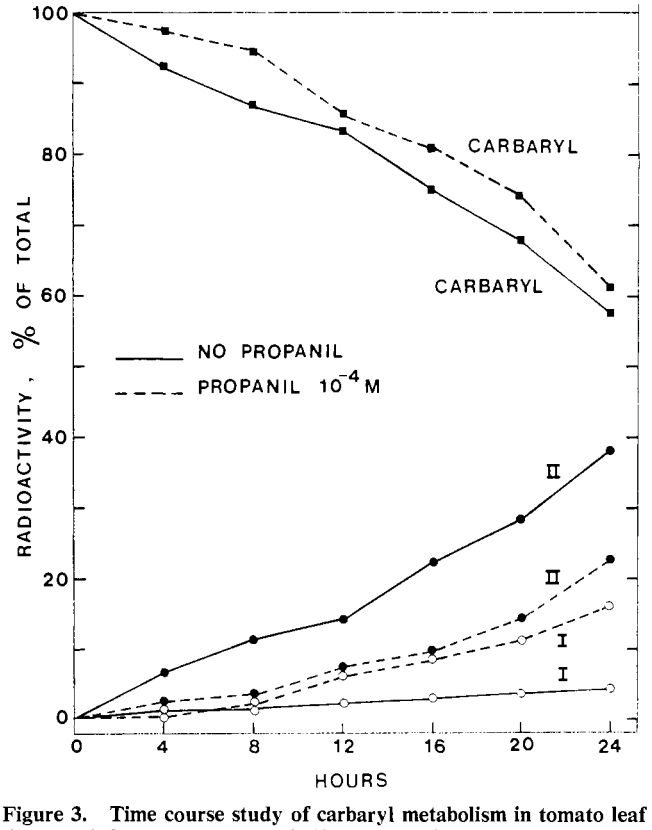


**Figure 3. Time course study of carbaryl metabolism in tomato leaf tissue as influenced by propanil ($10^{-4}$ *M*). I represents metabolite of carbaryl at $R_f$ 0.33, and II represents metabolite(s) at origin**

generally believed that the synergists act by inhibiting the detoxification of the insecticides (Barnes and Fellig, 1969; Bigley, 1966; Casida, 1970; Georghiou and Metcalf, 1961; Plapp and Tong, 1966). This study indicates that some herbicides such as linuron and propanil may also act as synergists with some insecticides by inhibiting the detoxification of the insecticides in the plants. On the other hand, the increased persistence of the insecticides by the simultaneous presence of herbicides in the plants may create greater residue problems when these pesticides are used together on crops for consumption by man and animal.

ACKNOWLEDGMENT

The technical assistance of Jane McLeod and Prasanta C. Bhowmik is greatly appreciated.

LITERATURE CITED

Barnes, J. R., Fellig, J., *J. Econ. Entomol.* **62,** 86 (1969).
Bigley, W. S., *J. Econ. Entomol.* **59,** 60 (1966).
Bourke, J. B., Broderick, E. J., Hackler, L. R., J. AGR. FOOD CHEM. **16,** 585 (1968).
Casida, J. E., J. AGR. FOOD CHEM. **18,** 753 (1970).
Casida, J. E., Lykken, L., *Ann. Rev. Plant Physiol.* **20,** 607 (1969).
Chang, F. Y., Smith, L. W., Stephenson, G. R., J. AGR. FOOD CHEM. **19,** 1183 (1971).
Dorough, H. W., J. AGR. FOOD CHEM. **18,** 1015 (1970).
Georghiou, G. P., Metcalf, R. L., *J. Econ. Entomol.* **54,** 231 (1961).
Kadoum, A. M., J. AGR. FOOD CHEM. **18,** 542 (1970).
Kaufman, D. D., Kearney, P. C., Von Endt, D. W., Miller, D. E., J. AGR. FOOD CHEM. **18,** 513 (1970).
Kuhr, R. J., J. AGR. FOOD CHEM. **18,** 1023 (1970).
Kuhr, R. J., Casida, J. E., J. AGR. FOOD CHEM. **15,** 814 (1967).
Matsunaka, S., *Science* **160,** 1360 (1968).
McBain, J. B., Hoffman, L. J., Menn, J. J., J. AGR. FOOD CHEM. **18,** 1139 (1970).
Plapp, F. W., Jr., Tong, H. H. C., *J. Econ. Entomol.* **59,** 11 (1966).
Read, D. C., *J. Econ. Entomol.* **62,** 1338 (1969).
Swanson, C. R., Swanson, H. R., *Weed Sci.* **16,** 481 (1968).
Yih, R. Y., McRae, D. H., Wilson, H. F., *Plant Physiol.* **43,** 1291 (1968).

*Received for review March 5, 1971. Accepted for publication May 6, 1971. This study was supported by grants from National Research Council of Canada.*