Parathion Residues on Apple and Peach Foliage as Affected by the Presence of the Fungicides, Maneb and Zineb

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The problem of hazard to crop workers who enter fields or orchards following application of toxic pesticides has been of considerable interest during recent years. Several outbreaks of illness resulting from crop reentry following pesticide application have occurred since QUINBY and LEMMON (1958) first reported pesticide residues as a cause of poisoning in crop workers; however, most of these "residue poisonings" have been relatively mild in nature.

The fact that many of the cases have occurred in workers whose field reentry was long enough after application that illness would not have been expected indicates the possibility of factors coming into play in certain situations that cause illness in workers in a particular crop at a particular time under specific conditions. Such conditions might produce a greater than normal amount of conversion products that are more toxic than the parent compound, or perhaps the life of the residue of the parent compound or conversion products is extended considerably, producing hazardous exposure conditions for a longer than normal period of time.

In past years parathion has, on rare occasions, caused illness in workers exposed to residues during hand fruit thinning operations in apple orchards in the State of Washington (QUINBY and LEMMON, 1958). Although such illnesses usually occurred in thinners who entered orchards within 48 hours after pesticide application, we have observed many thinning operations within that period without reported illness, indicating that time of entry following application may not be the only factor involved in crop reentry hazard. California investigators, on the other hand, have implicated parathion residues as the cause of illness in workers for periods up to 46 days after spraying of citrus crops (SPEAR, et al., 1975). In earlier research at this laboratory on attempts to determine the effect of multiple sprays as a factor, we were unable to detect any significant buildup of parathion or more toxic conversion products of that compound following repeated weekly applications to apple and peach trees (STAIFF, et al., 1975).

In a search for other factors that might produce more hazardous conditions for workers exposed to crop residues, the present study was carried out to determine if the presence of the fungicides, maneb (manganese ethylene-1,2-bisdithiocarbamate) or zineb

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(zinc ethylene-1,2-bisdithiocarbamate), on apple and peach foliage where parathion had been applied, affects the rate of decay of parathion residues and/or the formation of toxic conversion products such as paraoxon; thus producing more hazardous conditions for workers who entered treated areas. Maneb and zineb were chosen for this study since spray regimens in certain areas of the United States result in the possible combination of residues of these materials with parathion and the possibility that metals may affect the degradation of parathion or paraoxon.

MATERIALS AND METHODS

In one experiment three groups of four apple trees were sprayed weekly throughout an 8-week period during the summer of 1974. Group 1 was sprayed with a conventional 0.03% parathion solution, Group 2 was sprayed with the conventional 0.03% parathion solution followed by an application of 0.1% maneb solution, and Group 3 was sprayed with the conventional 0.03% parathion solution followed by an application of 0.1% zineb solution. Water-wettable powder formulations (25% parathion, 80% maneb, and 76% zineb) were used in making up the spray solutions. The trees were thoroughly covered to the point where the spray dripped from the foliage. The fungicide sprays were applied after the parathion solution had dried (approx. 1 hr). A similar study was also carried out on peach trees. The apple and peach trees were located in the same orchard and, at each parathion and fungicide application period, the appropriate trees were sprayed with the same tank of spray solution at approximately the same time of day in order to maintain similar application conditions. Although concentrations of the parathion, maneb and zineb sprays used were in the normal dosage range, the weekly spray schedule, as well as the application of the fungicides immediately following the parathion application, was not normal. The unusual spray schedule was intended to create extreme conditions in an effort to insure detectable residue changes should they occur as a result of the spray residue combinations. Leaf samples for residue analysis were taken at 1 day and 7 days after each application. One composite sample, representing 15 leaves taken at random from each of the 4 trees, was collected at each sampling period.

In another experiment, involving the same tree groups, parathion and paraoxon residues were determined at 1, 3, 7, 14, 21, 28, 38, and 45 days after the last application of pesticides. This was an extension of the above experiments beyond the 8-week multiple-spray period to determine degradation of residues over a prolonged period.

The average maximum daily temperature for the 8-week period during the repetitive apple and peach spraying was 31.9°C and the average minimum temperature was 13.2°C. The maximum and minimum temperatures during this period were 38.9°C and 2.2°C. Rainfall

during this period was minimal with measurable rainfall of 0.23 and 0.33 cm during two days of the second week and 0.03 and 0.10 cm on two days of the third week. During all of the experiments irrigation sprinklers were in operation for 24-hour periods at 10-day intervals. The sprinkling caused fairly heavy wetting of the lower branches of the trees but sampling from these lower branches was avoided as much as possible.

Foliage sampling was by use of a leaf punch with a diameter of 2.25 cm. Leaf disks were punched directly into glass bottles. The leaf disks (60 per composite sample) were then extracted with 50 ml of anhydrous benzene for 20 minutes on a wrist-action shaker. The extract was filtered, dried over anhydrous sodium sulfate, and stored at room temperature in amber bottles until analysis. A Varian Aerograph Model 1527C gas chromatograph equipped with a 250 mc tritium electron capture detector was used for analysis. Stainless steel columns, 1.83 m x 0.37 cm, packed with 4% SE-30/6% OV-210 on Varoport 80/100 mesh, were employed. Confirmation of representative samples for organophosphate response was accomplished on a Microtek MT-220 equipped with a Melpar flame photometric detector and a 1.83 m x 0.63 cm glass column packed with 4% SE-30/6% OV-210 on Chromosorb W HP, 80/100 mesh.

Additional analyses for detection of cholinesterase inhibiting activity that could not be attributed to paraoxon were carried out by an enzymatic assay based on the inhibition of bovine erythrocyte acetylcholinesterase. The pH Stat assay used was a modification of the method of NABB and WHITFIELD (1967). The original leaf extracts were evaporated to dryness under N_2 at 40°C to remove benzene, and reconstituted in ethanol. Ten μl of reconstituted extract was added to 0.9 ml of acetylcholinesterase solution (1 unit[a]/ml in water) and the mixture was incubated at 37°C for 1 hour. A portion of the incubation mixture (0.5 ml) was added to 4 ml of saline in a pH Stat and the mixture was brought to pH 8.0 by titration with 0.01 N Na OH. Acetylcholine perchlorate was added (0.5 ml of 0.001 M) and the rate of hydrolysis of the substrate was followed for 5 minutes. The activity of the incubation mixtures was compared to a control mixture that had been incubated with 10 μl of ethanol and the paraoxon equivalency of the concentrated extracts was determined from a standard inhibition curve. The standard inhibition curve was prepared using incubation mixtures containing from 0.25 to 10 ng of paraoxon per ml added in 10 μl of ethanol.

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[a] One unit of acetylcholinesterase will hydrolyze 1.0 μmole of acetylcholine per minute at pH 8.0 and 37°C.

RESULTS AND DISCUSSION

Residue levels of parathion on apple foliage at the end of 1- and 7-day periods following each weekly spray application are shown in Table 1. At 7 days after application the average parathion residues from conventional parathion spray were 22.1% of the levels found one day after application. At 7 days after application the average parathion residues from conventional parathion spray followed by maneb spray were 23.9% of the 1-day levels and the 7-day average parathion residues from conventional parathion spray followed by zineb spray were 20.9% of the 1-day levels. Considerable amounts of paraoxon were found in the foliage samples. The 7-day levels for paraoxon residues were found to be a significantly greater percent of the 1-day levels than was found for the corresponding parathion levels. This would seem to indicate either a greater persistence of paraoxon once it is formed or a continued buildup of paraoxon during the 7-day period. No measurable effect by the fungicides on the breakdown or alteration of paraoxon was observed.

Results of the similar study on peach trees are shown in Table 2. At 7 days after application the average parathion residue levels from conventional parathion spray were 12.5% of the levels found 1 day after application. At 7 days after application the average parathion residue levels from conventional parathion spray followed by maneb spray were 9.5% of the 1-day levels and the 7-day average parathion residues from conventional parathion spray followed by zineb spray were 9.9% of the 1-day levels. Although there was considerable reduction of parathion residues in all cases, there is an indication that both of the fungicides promoted a slightly greater percent reduction of parathion residues than if they had not been used. The production of paraoxon on peach was minimal, with levels below the limit of the sensitivity of the test in most instances. By comparing the data with that in Table 1 for apple, it can be seen that there was much less production of paraoxon on peach foliage than on apple foliage. This is a point of considerable interest in relation to crop reentry hazard. In all cases except one there was 100% breakdown of paraoxon levels on peach between days 1 and 7, and no measurable influence by the fungicides maneb and zineb on the breakdown of paraoxon was seen.

Results of a 45-day decay study of residues on apple and peach foliage following applications of parathion, and parathion plus maneb or zineb, are shown in Tables 3 and 4. As can be seen in the tables, there was essentially no difference in rate of decay of parathion or paraoxon on either type of foliage caused by the addition of the fungicides. While measurable amounts of parathion could be detected up to 38 days after application to apple trees, the amounts of paraoxon present beyond the 14-day period were below the level of detection. On peach foliage measurable amounts of parathion could be detected up to 28 days; however, paraoxon was not detected beyond the third day following application. Much less paraoxon was found on peach than on apple foliage.

TABLE 1

Gas Chromatographic Determination of Residues of Parathion and Paraoxon on Apple Foliage Following Spray Application of (1) Parathion, (2) Parathion Followed by Maneb, and (3) Parathion Followed by Zineb - During June 26 to August 14

Weekly periods	Days since application	Parathion recovered[a] (ng/cm^2)			Paraoxon recovered[a] (ng/cm^2)		
		0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb
0	prespray	el[b]	el	el	el	el	el
1	1	248	242	236	el	el	el
	7	81.2	69.0	80	13.4	19.6	5.8
2	1	638	522	404	35.2	13.4	40.8
	7	142.8	173.4	104.6	15.5	10.5	10.5
3	1	758	516	612	29	18.5	31.8
	7	90	96.2	81.2	17.6	14.3	8.8
4	1	542	596	552	19.3	11.4	12.6
	7	73.2	59	48.4	8.0	6.3	7.2
5	1	366	348	258	31.8	17.4	22.2
	7	82.8	86.6	81.2	7.9	7.9	13.4
6	1	314	322	538	14.7	15.5	17.2
	7	66	55.2	38.2	el	el	el
7	1	398	362	380	21.4	14.2	15
	7	84	114	78.6	8.8	el	13.4
8	1	524	534	442	21.4	13.4	24.2
	7	166	150	117.4	13.8	14.6	12.2

[a]Each value represents a composite sample of 60 leaves from 4 trees.

[b]Below lower limit of sensitivity of test.

TABLE 2

Gas Chromatographic Determination of Residues of Parathion and Paraoxon on Peach Foliage Following Spray Application of (1) Parathion, (2) Parathion Followed by Maneb, and (3) Parathion Followed by Zineb - During June 26 to August 14

Weekly periods	Days since application	Parathion recovered[a] (ng/cm^2)			Paraoxon recovered[a] (ng/cm^2)		
		0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb
0	prespray	el[b]	el	el	el	el	el
1	1	204	180.4	169.2	el	el	el
	7	23.6	15.7	27.8	el	el	el
2	1	183.2	155	176	el	el	el
	7	14.8	10.5	17.2	el	el	el
3	1	354	111	103	10.4	7.8	el
	7	14.2	11.3	7.1	el	el	el
4	1	352	128	268	7.8	6.3	6.3
	7	97.4	16.8	15.9	el	9.2	el
5	1	95.8	150	69.4	el	4.6	4.2
	7	9.85	5.8	7.35	el	el	el
6	1	226	109	93	6.8	4.2	4.2
	7	24.2	10.5	15.6	el	el	el
7	1	121	112	95.6	5	4.2	7.5
	7	14.7	14.7	9.6	el	el	el
8	1	418	326	189	10.5	8.8	13.4
	7	22.2	22.6	24.8	el	el	el

[a] Each value represents a composite sample of 60 leaves from 4 trees.

[b] Below lower limit of sensitivity of test.

TABLE 3

Gas Chromatographic Determination of Decay of Parathion and Paraoxon Residues on Apple Foliage Following Spray Application of (1) Parathion, (2) Parathion Followed by Maneb, and (3) Parathion Followed by Zineb - During August 14 to September 27

Days since spraying	Parathion recovered[a] (ng/cm^2)			Paraoxon recovered[a] (ng/cm^2)		
	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb
prespray[b]	84	114	78.6	8.8	el[c]	13.4
1	524	534	442	21.4	13.4	24.2
3	199	184	191	18.4	18.4	23.0
7	166	150	117	13.8	14.6	12.2
14	32.4	38.2	34.4	6.3	el	el
21	16.8	4.2	13.0	el	el	el
28	14.3	10.5	12.6	el	el	el
38	10.9	9.6	6.3	el	el	el
45	el	el	el	el	el	el

[a]Each value represents a composite sample of 60 leaves from 4 trees.

[b]Prespray sample obtained from foliage in morning before sprays applied; starting with 8th week of application as shown in Table 1.

[c]Below lower limit of sensitivity of test.

Several general observations were applicable to all the reported studies: On an average, half of the original parathion residue had disappeared before three days had elapsed. The pH Stat assay showed that no significant amounts of any cholinesterase inhibiting material other than paraoxon was present in any extract. No relationship was evident between the average weekly maximum or minimum temperature and the percent reduction in parathion residue or the percent conversion of parathion to paraoxon. No S-ethyl isomer of parathion could be detected on foliage, and no paraoxon

or S-ethyl isomer could be detected in either the freshly prepared spray solution or in the solution after it had been allowed to stand for a 24-hour period. These observations applied to both apple and peach foliage, not only where parathion alone was used, but also where maneb and zineb sprays had been added.

TABLE 4

Gas Chromatographic Determination of Decay of Parathion and Paraoxon Residues on Peach Foliage Following Spray Application of (1) Parathion, (2) Parathion Followed by Maneb, and (3) Parathion Followed by Zineb - During August 14 to September 27

Days since spraying	Parathion recovered[a] (ng/cm^2)			Paraoxon recovered[a] (ng/cm^2)		
	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb	0.03% Parathion	0.03% Parathion + 0.1% Maneb	0.03% Parathion + 0.1% Zineb
prespray[b]	14.7	14.7	9.6	el[c]	el	el
1	418	326	189	10.5	8.8	13.4
3	114	55.4	39.8	10.9	el	el
7	22.2	22.6	24.8	el	el	el
14	4.6	7.2	6.3	el	el	el
21	6.7	4.2	4.2	el	el	el
28	4.2	el	el	el	el	el
38	el	el	el	el	el	el
45	el	el	el	el	el	el

[a]Each value represents a composite sample of 60 leaves from 4 trees.

[b]Prespray sample obtained from foliage in morning before sprays applied; starting with 8th week of application as shown in Table 1.

[c]Below lower limit of sensitivity of test.

The data presented above indicates that under conditions of the experiment in this geographic area, which is somewhat typical of Pacific Northwest orchard conditions, there was no significant

effect by the fungicides maneb and zineb on the rate of parathion decay on apple foliage. However, with peach foliage there was a slightly greater reduction of parathion, on an average weekly basis, where the fungicides maneb and zineb were used than where parathion was applied alone. The data also indicates that there was no measurable difference in the decay rate of parathion, through the 45-day period of study, where these fungicides were used. Also, the fungicides did not seem to have an influence on the rate of degradation of paraoxon when it was formed on either apple or peach foliage. Thus, it appears that the presence of the fungicides maneb or zineb on apple or peach foliage with the pesticide parathion does not produce changes in residues sufficient to be considered a factor in producing more hazardous conditions for crop workers exposed to residues during thinning or picking activities in Pacific Northwest orchards.

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