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# Dislodgable Residues of Dialifor and Phosalone and Their Oxygen Analogs Following a Reported Worker-injury Incident in the San Joaquin Valley, California

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Dialifor [0,0-diethy S-(2 chloro-1-phthalimidoethyl)-phosphorodithioate] a product of Hercules, Inc. and phosalone [0,0-diethyl S-(6-chlorobenzoxazolon-3-yl) methyl phosphorodithioate] a product of Rhodia Chem., Chipman Division, were found on grape foliage following a reported worker-injury episode in Madera County, California. Shortly after the time of the reported incident, random sampling of grape foliage was done in numerous fields throughout the county by inspectors of the California State Department of Food and Agriculture (DFA). Residue analysis showed that three fields in which the workers had been harvesting grapes had high levels of dialifor and two of the three fields also had phosalone residues. The leaf samples initially analyzed by the DFA laboratory were extracted with hexane for rapid analysis so the residues found were not considered to be only dislodgeable residues according to the method of Gunther et al. 1973. These results were used primarily for field identification in relation to possible exposure. Knaak et al. 1978a, reported 118 workers had significant depressed blood cholinesterases, but only three of the workers required hospitilization.

Wide variation of residues among samples was noted by DFA. This fact along with the large numbers of individually treated plots with various possible combinations of pesticides present resulted in the DFA requesting services of the university. Some three weeks after the incident, foliage samples were taken by the University. This report describes the conditions of the sampling activities and the analyses.

## Sample Collection and Analysis

A light rain had begun to fall at the time of the sampling and although the foliage was wet, the sampling was continued. A total of three fields were sampled, one was 80 acres (field I), a second was 30 acres (field II) and the third was less than 10 acres (field III). 100 leaf punches were taken from each row sampled for the full length of the row with a leaf punch having a surface area of 2.5 $cm^2$ (Gunther et al. 1973). Fields I and II were sampled every 10th row starting with row 5. In field III (consisting of 17 rows), three rows were skipped two rows sampled,

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three skipped, two sampled, etc. until all of the field was sampled. The jars containing the samples were immediately chilled in ice until extracted a few hours later using a slightly modified procedure of Gunther _et. al_. (1973).

Gas chromatography using standard silicone columns did not separate the oxygen analogs from dialifor and phosalone, however, a Florisil column was successfully employed for this separation prior to glc analysis. A 1.5 cm o.d. x 10 cm chromatographic column was prepared from bottom to top with a small plug of glass wool, 1.0 cm of anhydrous sodium sulfate, 5g of Florisil and 1.5 cm of anhydrous sodium sulfate. The packed column was prewashed with 15 ml of benzene and the washing discarded. The concentrated extract, ca. 2 ml, was quantitatively transferred to the column using 5 ml of benzene followed by 70 ml of 6% ethyl ether in benzene. This fraction contained dialifor and phosalone. An additional 30 ml of 12.5% acetone in benzene was used to elute off the two oxygen analogs. Figure 1 shows chromatograms of the two fractions using gas chromatography under the conditions described below.

Analyses were conducted on a Tracor Model 550 gas chromatograph equipped with a flame photometric detector, and a Hewlett Packard Model 3380 integrator. A 1.8 m x 2 mm id. glass column was packed with a 10% SE-30 on 60/80 mesh Gas Chrom. Q. Column temperature was maintained at 215° with the injection and detection temperature at 205° and 190° respectively. The nitrogen carrier gas flow rate was 40 ml/min and air and hydrogen flow rates as required for optimum performance. The retention time of phosalone and dialifor under these conditions was ca. 4.0 and 5.2 min respectively and for the oxygen analogs, 3.0 and 4.2 min.

The recoveries of dialifor, phosalone and their respective oxygen analogs added to grape leaf tissues void of any organophosphate containing pesticides ranged between 83 to 100 percent when fortified at the 0.1 ppm level.

## Results and Discussion

The insecticides and their time of application for each of the three fields sampled as reported to the agricultural commissioners office are shown in Table 1.

Both dislodgeable and penetrated residues were determined on all samples, however, primary attention was centered on the dislodgeable residues since it is these residues that are commonly associated with worker-exposure. Considerable effort was devoted to the analysis of the oxygen analogs of dialifor and phosalone. Table 2 shows the mean and standard deviation for all four compounds analyzed. Residues were expressed in ppm and ng/cm$^2$ and even though the leaves were wet at the time of sampling due to the rain, the variability between these two expressions in the three fields was negligible. This would indicate that the residues were either not influenced by the rain or any differences caused were common throughout the three fields. The standard deviation between samples was large indicating strip treating of the respective chemicals on a row by row basis. It was also noted that the

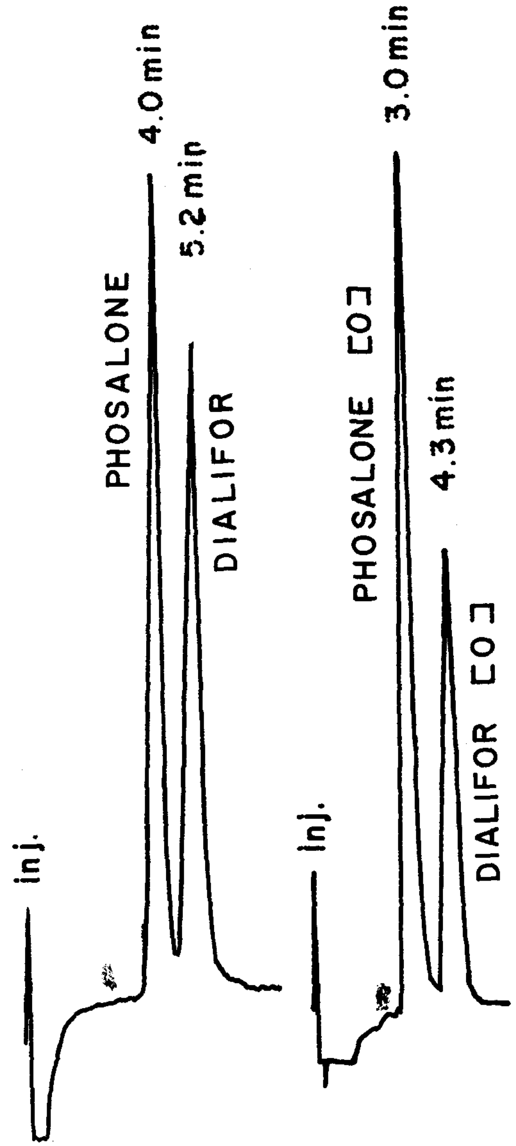

Figure 1. Gas chromatographic response for 4 ng each of: A, phosalone and dialiflor;B, phosolone oxygen analog and dialiflor oxygen analog, following separation on a Florisil column.

phosalone residues on a row by row basis did not in any way correlate with the dialifor residues. Therefore, if the variability was due only to sampling and the washing of the leaves by the rain, one would expect a very similar pattern in relative amounts of residue of the two compounds. However, such was not the case. This suggests that spot treatment of the insecticides within the field and even repeated application where insect infestation was the greatest may have occurred.

The most interesting finding was the relative amounts of the oxygen analog of dialifor found on the leaf surface. Since this oxygen analog was not expected in any appreciable amounts, due in part to a previous study on citrus (Westlake et. al. 1971) such levels were of considerable interest and may have contributed substantially to the illness of the farm workers. It would be difficult to explain the cause of worker injury on the basis of the residues. However, these figures may mean little due to the weather and time elapsed between the poisoning episode and our sample collection. Comparisons of this data with DFA laboratory analyses collected earlier (Knaak et. al. 1978a) would assume that the residue levels were much higher at the time the workers were exposed. Levels of dialifor reported by DFA from those same fields sampled shortly after the incident and extracted with hexane were often several times greater than what is reported here. Little is known about the toxicity of the oxygen analog, but one would expect these analogs to be several times more toxic than the parent, as is true with most oxygen analogs of organophosphate insecticides. For example, if the residue of the oxygen analog is ten times more toxic than dialifor, an exposure of 20 ng/cm$^2$ could be equivalent to 200 ng/cm$^2$ of dialifor. We don't know this to be true but it is known that the oxygen analog is approximately 20 times more efficient than dialifor in inhibiting red cell cholinesterase. This study indicates that the oxygen analog of dialifor should be measured on foliage and dermal toxicity studies should be conducted. This would seem particularly important because this chemical is known to be quite persistent as compared to most organophosphate insecticides. The dislodgeable or surface bound residues of phosalone were approximately the same as dialifor although the oxygen analogs were barely detectable. Since phosalone has been used for several years in California on grapes without any reported incidents of injury and its acute toxicity is less than dialifor ($LD_{50}$ for male rats 120 mg/kg, female rats 135-170 mg/kg) it would not in itself be a strong suspect as a primary cause of the worker poisoning unless it should potentiate the toxic effect of dialifor. Recent studies with workers exposed to phosalone and its oxygen analog (Knaak et. al. 1978b, Spear and Popendorf, 1977) in excess of the levels to which workers were exposed in this incident had shown no adverse effects including depressed cholinesterase levels.

More research needs to be done with dialifor and its oxygen analog but we feel that the oxygen analog should be given special attention as a possible major contributor to worker injury in fields containing dialifor residues.

TABLE 1
Insecticides applied to fields sampled and reported to the county Agricultural Commissioners office prior to 9/28/76

| Field | Approx. Acres | No. of Rows Sampled | Reported Spray History | | | |
|---|---|---|---|---|---|---|
| | | | 6/2-5/76 | 6/13/76 | 7/28/76 | 7/31/76 |
| I | 80 | 24 | Phosalone | Kryocide | Omite 25 rows only | Dialafor |
| II | 30 | 6 | 6/5-10/76<br>Phosalone<br>Kryocide | 7/29-31/76<br>Dialifor<br>Omite | | |
| III | <10 | 6 | 6/20/76<br>Dialifor<br>Kryocide | 7/31/76<br>Dialifor<br>Omite | | |

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## TABLE 2

### Amount of Dislodgeable Residues
### Taken from Grape Fields (Madera California) on September 28, 1976

| Field | Chemical | ppm | | $ng/cm^2$ | |
|---|---|---|---|---|---|
| | | Mean | Standard Deviation | Mean | Standard Deviation |
| I | Phosalone | -- | -- | -- | -- |
| | Phosalone[O]* | -- | -- | -- | -- |
| | Dialifor | 8.62 | 4.98 | 107.0 | 62.3 |
| | Dialifor[O]* | 1.74 | 1.24 | 21.6 | 15.2 |
| II | Phosalone | 4.78 | 3.53 | 51.6 | 38.8 |
| | Phosalone[O]* | 0.83 | 0.35 | 8.98 | 3.74 |
| | Dialifor | 5.60 | 2.90 | 60.3 | 30.4 |
| | Dialifor[O]* | 2.20 | 0.97 | 22.7 | 10.6 |
| III | Phosalone | 10.87 | 7.07 | 111.8 | 74.2 |
| | Phosalone | 1.39 | 0.82 | 14.0 | 7.66 |
| | Dialifor | 9.87 | 7.84 | 99.6 | 75.6 |
| | Dialifor[O]* | 2.59 | 2.13 | 26.2 | 20.4 |

*[O] refers to the oxygen analogs of the respective compounds.