THE PERSISTENCE OF ETHION AND ZOLONE RESIDUES ON GRAPE FOLIAGE IN THE CENTRAL VALLEY OF CALIFORNIA

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The hazard of serious intoxication of agricultural field workers by organophosphate insecticide residues has led to the establishment of reentry intervals in California. In the attempt to extend this concept nationally, it has been found that there is a lack of relevant field data for setting of such standards. In an effort to expand knowledge of the relationship between foliar residue levels and occupational hazard, an examination of the persistence and transport of ethion, Zolone, and their oxygen analogs on grape foliage was conducted. Both dislodgeable and penetrated residues were followed for 28 days post-application for both insecticides while soil surface residues were followed for ethion only.

A marked difference is seen in the decay rates of the dislodgeable vs. the penetrated residues of ethion, the dislodgeable residues decaying more quickly. This difference is not apparent for Zolone. Of particular significance to worker hazard is the finding that the oxones of both ethion and Zolone in the dislodgeable residues reach a plateau after approximately seven days and do not degrade further by day 28.

In recent years it has become apparent that foliar residues of certain organophosphorus insecticides can present the hazard of intoxication to field workers engaged in activities involving substantial contact with the crop (Milby *et al.* 1964). In California, this problem was deemed sufficiently serious to cause the establishment of field re-entry intervals by the State Department of Agriculture in June 1971 (California State Department of Agriculture 1971). More recently the Occupational, Safety, and Health Administration (OSHA) has attempted to extend the re-entry concept to apply nationally (Federal Register 1973).

In OSHA hearings on their proposed standards, and similar hearings held by EPA, the lack of relevant field data has been repeatedly mentioned. Although the relationship

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between foliar residue levels and the extent of the occupational hazard is not currently known, it is clear that a knowledge of the persistence and availability of foliar residues presents an important first step in defining the potential hazard. With this need in mind a cooperative study, coordinated by Dr. J. Blair Bailey of the Division of Agricultural Sciences, University of California-statewide, was organized to investigate the persistence and transport of several organophosphate insecticides on grape foliage near Salida, California in July 1972. In this study both concentrate and dilute applications of the insecticides ethion (*0,0,0′,0′*-tetraethyl-*S,S′*-methylene-*bis*phosphorodithioate) and Zolone [*0,0*-diethyl-*S*-(6-chloro-2-oxobenzoxazolin-3-yl) methyl-phosphorodithioate] and Guthion [*0,0*-dimethyl-*S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl) phosphorodithioate were made to grapes. This paper reports the results of foliar residue persistence for ethion and Zolone and of surface soil residue persistence for a period of up to 28 days post-application.

Experimental

The experimental vineyard, comprised of 11.5 acres of Thompson seedless grapes, was divided into 24 experimental plots, each being 12 rows wide by approximately 1/16 mile long. Pesticides were applied only to the two center rows of each plot, thereby leaving approximately ten rows between adjacent treated portions of each plot. Wind speeds during application ranged from 11 to 15 km/hr. The spray history for the vineyard did not include any applications of the three insecticides used in this study during the 1972 growing season, a fact that was verified by the absence of ethion and Zolone in the pre-application samples.

Ethion, Zolone, and Guthion were applied, each as a concentrate and dilute application (Table I). Four replicates of each combination were randomly assigned to the 24 individual plots. One plot receiving each application was reserved for a study of the utility of washing for reducing foliar residues and neither these results nor Guthion results are included in the data presented here.

Sampling methods

Foliage. Leaf punches with dies 1.5 cm in diameter, and similar in design to those described for xeromorphic leaves (Gunther *et al.* 1973) were used to take leaf samples just prior to and directly following application of pesticide to the plots and at 1, 3, 5, 7, 14, 21, and 28 days post-application. On each of the sampling dates 275 leaf disks were taken from each experimental plot resulting in a sample of approximately ten g with a one-side surface area of 486 cm^2. Leaf punch samples were taken principally from the exterior of the vine canopy in the vicinity of berry clusters, since this area presented the principal area of picker contact during the harvesting of the crop. Also, most samples were taken between waist and eye level; the bulk of them being taken from about shoulder height. Every attempt was made to distribute the sampling locations evenly over the length of the plot row, and from both sides of the row. The last five vines at each end of the plot rows were excluded to eliminate effects that might arise from commencement and termination of spraying and by drift and overlap of the sprays from the adjacent plots.

Soil. Surface soil samples were taken with a soil scoop designed to take a horizontal slice of the soil surface about 6 mm thick and 77.5 cm^2 in area. By use of a sheetmetal perimeter barrier even the most flowable soils could be lifted out of place with minimal disturbance and emptied into a container. Four samples were taken for each replicate and all replicates for a given pesticide and application regimen were composited to produce one sample that consisted of sixteen scoopsful for each sampling date.

The surface soil sample was used because it was surmised that the potential for resuspension was greatest in this region of the soil and because run-off and overspray do not penetrate deeply into the soil. Downward percolation of the pesticide in the warm, dry growing season is apparently not rapid (Stewart *et al.* 1971). Mechanical disturbance by wind or movement of personnel and equipment through the field does not penetrate the soil surface to any great extent except when intentional soil disruption such as in plowing, discing, harrowing, or trenching is practiced. Since the scope of this study did not include the examination of the effect of such activities on the resuspension of pesticide residues deposited on orchard or vineyard floors, soil sampling was discontinued in replicates where major disturbances of soil surface, such as trenching for irrigation occurred.

Preservation of samples. All samples were labeled and placed on dry ice in the field as soon as they were collected. Thereafter they were kept at between – 11 and – 18°C until they could be extracted.

Materials and methods

Standards. Ethion and its monooxon [*S*-[[(diethoxyphosphinothioyl)thio] methyl] -*0*, *0*-diethylphosphorothioate] and dioxon (*0,0,0′,0′*-tetraethyl-*S,S′*-methylene-*bis*phosphorothioate) were supplied by the Niagara Chemicals Division, FMC Corporation, Middleport, New York. Zolone and Zoloxon [*0,0*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl-phosphorothioate] were obtained from Rhodia Inc., New York. Malathion [*S*-(1,2-dicarboethoxyethyl) *0,0*-dimethylphosphorodithioate], used as an internal standard for the gas chromatographic analysis of these pesticides (McNair and

Table I. *Pesticides application data*

Chemical	Formulation	Manufacturer	Amount used (concentrate)[a]	Amount used (dilute)[b]
Zolone	3 EC	Rhodia, Inc.	2-1/4 lb or 6 pt	3 lb or 1 gal
Ethion	8 EC	Niagara Division of FMC Corp.	1 lb or 1 pt	1-1/4 lb or 1-1/4 pt

[a] Actual ingredient per acre in 30 gal of water.
[b] Actual ingredient per acre in 200 gal of water.

Bonelli 1969) was provided by American Cyanamid Corporation, New York. All were ⩾ 98% pure and were used as anlytical standards without further purification.

Analytical reagents. Solvents used for extraction, transfer, and cleanup procedures were Mallinckrodt Nanograde. Column cleanup was performed with Florisil 60/100 mesh without special activation. Mallinckrodt granular anhydrous sodium sulfate was used for dehydration of extracts.

Equipment. Evaporations for reduction of solvent volumes or for solvent exchanges were carried out with a rotary vacuum evaporator. Glass columns for Florisil clean-up were 2.0 cm id by 15 cm length with a 200 ml solvent reservoir. Analysis was performed on a Varian Aerograph Model 1520 dual column gas chromatograph equipped with a rubidium sulfate alkali flame ionization detector (Varian Model 1351), operated according to the manufacturer's specifications. Carrier gas was dry nitrogen at a flow rate of 20 ml/min. Ethion and its oxons were chromatographed on 0.76 m X 2 mm column packed with 10% DC 200 on Chromosorb W, HP, 80/100 mesh. Injector temperature was 220°C, column oven 205°C, and detector bath 235°C. Zolone and its oxon were analyzed on a 0.41 m X 2 mm column packed with 10% DC 200 on Chromosorb W, HP, 80/100 mesh. Injector temperature was 230°C, column oven 225°C and detector bath at 245°C. Retention times for all of the compounds of interest under the above conditions are in Table II.

Residue extraction

Leaf punches. Prior to treatment each sample of leaf punches was allowed to warm to room temperature and soon thereafter was weighed and extracted. The leaf punches were washed using the procedure of Gunther *et al.* (1973) except that a one pint polyethylene bottle was used instead of a glass jar. These aqueous surface washes were back-extracted

Table II. *Gas chromatographic retention times*

Compound	Retention time on 0.76 m column at 205°C (min)	Retention time on 0.4 m column at 225°C (min)
Malathion	2.50	0.88
Ethion	7.25	–
Ethion monooxon	5.62	–
Ethion dioxon	4.62	–
Zolone	–	4.38
Zoloxon	–	3.25

with chloroform, dried over anhydrous sodium sulfate, reduced in volume and converted to benzene solution. This surface wash was designated as the dislodgeable residue.

Following surface washing for dislodgeable residue determination, the leaf punches were drained and homogenized twice with 25 g of anhydrous sodium sulfate in 100 ml of chloroform in a one-pint Waring blendor. The homogenates were filtered through Whatman No. 1 paper and the filtrates were evaporated almost to dryness and exchanged to benzene.

Soils. After thawing, a moisture determination was conducted on an aliquot of the soil sample by heating for 18 hr at 110°C. For residue extraction, an untreated aliquot of each soil sample was screened through a six-mesh sieve to remove sticks, stones, and plant materials and a 250 g portion of the sieved material extracted with 300 ml of chloroform in a one-quart Waring blendor.

Extract cleanup. Due to naturally occurring interferences, the surface washes of leaf punches containing ethion, all the leaf homogenate extracts, and all the soil samples required cleanup prior to chromatographic analysis. These samples were run through a column consisting of 10 g of Florisil sandwiched between two 10-g layers of anhydrous sodium sulfate. The column was prewashed with 50 ml of benzene and the sample applied to it in 10 to 15 ml of benzene. Ethion and its oxygen analogs were eluted with the following sequence of solvent mixtures: 220 ml of benzene; and 100 ml of 25% ethyl ether in benzene; 100 ml of 25% acetone in benzene; and 50 ml of acetone. All eluates were combined. For Zolone, the solvent sequence was 50 ml of benzene (discarded); followed by a further 150 ml of benzene; 100 ml of 8% ethyl ether in benzene; and 100 ml of 25% acetone in benzene. All eluates were combined.

The eluates were transferred into benzene solutions, made up to volume with benzene in volumetric flasks and fortified with a known quantity of malathion as an internal standard prior to gas chromatographic analyses.

Recoveries were determined for each pesticide and its oxon(s) separately by dosing untreated leaf disks with known amounts of each compound and immediately running them through the extraction procedure described above. Recoveries for surface wash procedure ranged from 97 to 102% for both ethion and Zolone and their respective oxygen analogs. Extraction of the homogenized leaf discs gave recoveries of 94 to 96% for the same compounds. Recoveries from soil were not determined.

Results

The decay curves for ethion residues in the dislodgeable form and in the leaf extracts for both concentrate and dilute applications are shown in Figures 1 to 4. Similar decay curves for the Zolone applications are presented in Figures 5 and 6. The ethion residues in surface soil samples are presented in Figures 7 and 8. In order to make a comparison between dislodgeable residue and leaf extract residue the concentration of residues has, in both instances, been based on the weight of the leaf punches (ppm).

The curves in all of these figures represent the means of data from three replicate plots for each particular pesticide treatment. An indication of the variation of each data point could be given by the coefficient of variation of each data point on each curve. However, rather than do this for all figures, which would introduce considerable difficulty in their interpretation, an average coefficient of variation has been calculated from the individual values of the coefficient of variation for each curve and this value has been indicated on the pertinent figure in parentheses following the compound name. For purposes of comparison, range bars are included on the ethion curve of Figure 3.

The initial residues, both dislodgeable and from leaf extracts, were essentially identical for the dilute and concentrate application for each insecticide in spite of the fact that the dilute applications used 25% more material (AIA) than was used in the concentrate applications (Figures 1 to 6). However, if one contrasts the levels of initial deposits of ethion with Zolone it is observed that the total initial deposits of Zolone exceed those of ethion by approximately threefold, while the corresponding application ratios averaged 2.33:1. An additional difference between the behavior of ethion and Zolone residues can be discerned at the time the first post-application samples were taken (one day post-

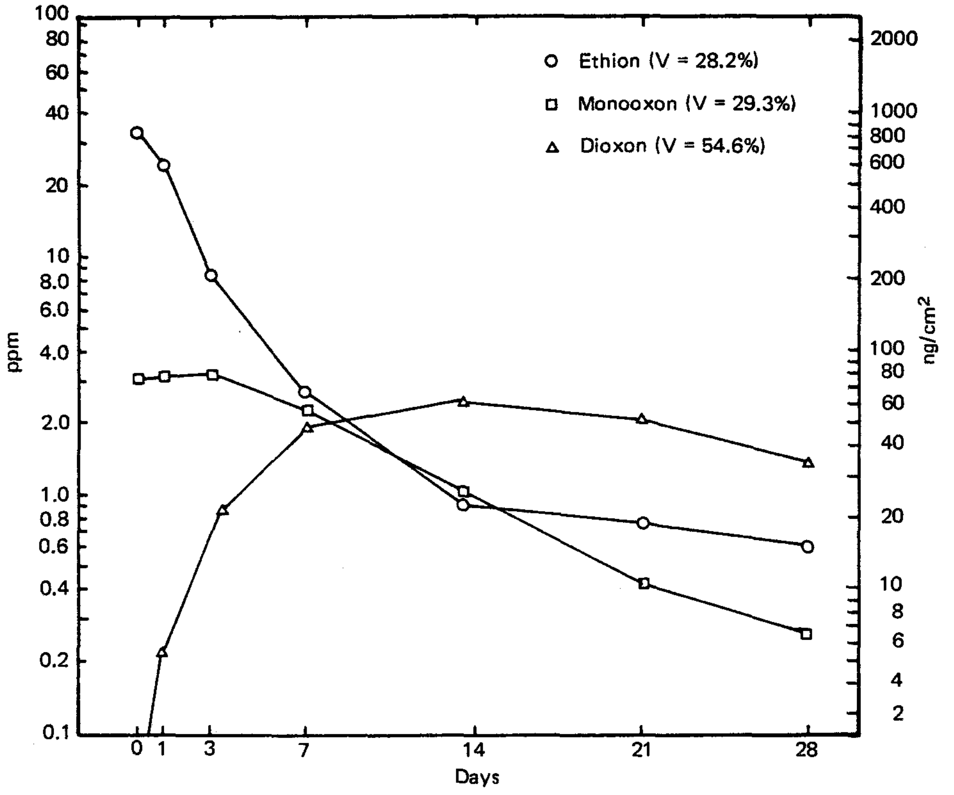

Fig. 1. Ethion concentrate dislodgeable residues.

application). The ethion residue levels on the leaf surface were markedly less than the corresponding leaf extract residues (Figures 1 to 4) while the opposite result was observed for Zolone (Figures 5 and 6).

The data presented in Figures 1 to 4 demonstrate that the decay of ethion both in the dislodgeable residue and the leaf extract is not first order but can in most instances be characterized by an initial rapid decay rate over a period of some three to seven days followed by a slower decay rate. The decay rate of Zolone, on the other hand (Figures 5 and 6), can be more convincingly characterized by a first order decay curve for both the dislodgeable and the leaf extract residues.

When one examines the fate of the dislodgeable residues in comparison with the fate of the leaf extract residues for ethion and Zolone some very significant differences in behavior can be discerned. For ethion, residue values for concentrate and dilute applications are consistent. However, the dislodgeable ethion residue decays far more rapidly than the

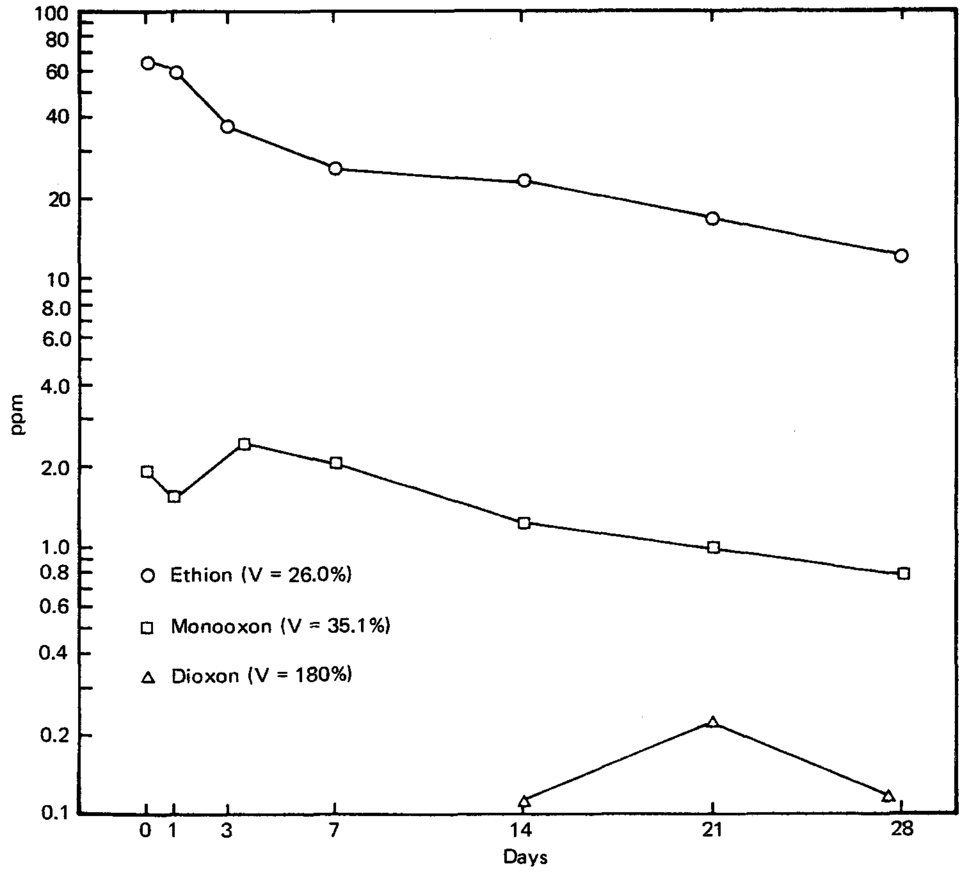

Fig. 2. Ethion concentrate – leaf extracts.

leaf extract residue. In dislodgeable residues the ethion monooxon appears at the time of pesticide application, possibly as a contaminant in the formulated material, and persists at that level for approximately three days, then begins to decay in an approximately first-order fashion. Almost immediately after pesticide application, ethion dioxon appears, increasing in concentration to between 7 and 14 days post-application and thereafter slowly decreasing in concentration.

In the leaf extracts, the ethion monooxon shows virtually the same decay behavior as ethion monooxon in the dislodgeable residue. Significantly, however, ethion dioxon was only detected in very small quantities between days 14 and 28 post-application for the ethion concentrate replicates.

The decay of the dislodgeable and leaf extract residues of Zolone are remarkably similar, especially after seven days post-application. It is also interesting to note that the decay rate of ethion in the leaf extract is almost identical with that of Zolone after the third day. Dislodgeable Zoloxon residues are consistently higher than Zoloxon residues from leaf extracts throughout the 28-day sampling period.

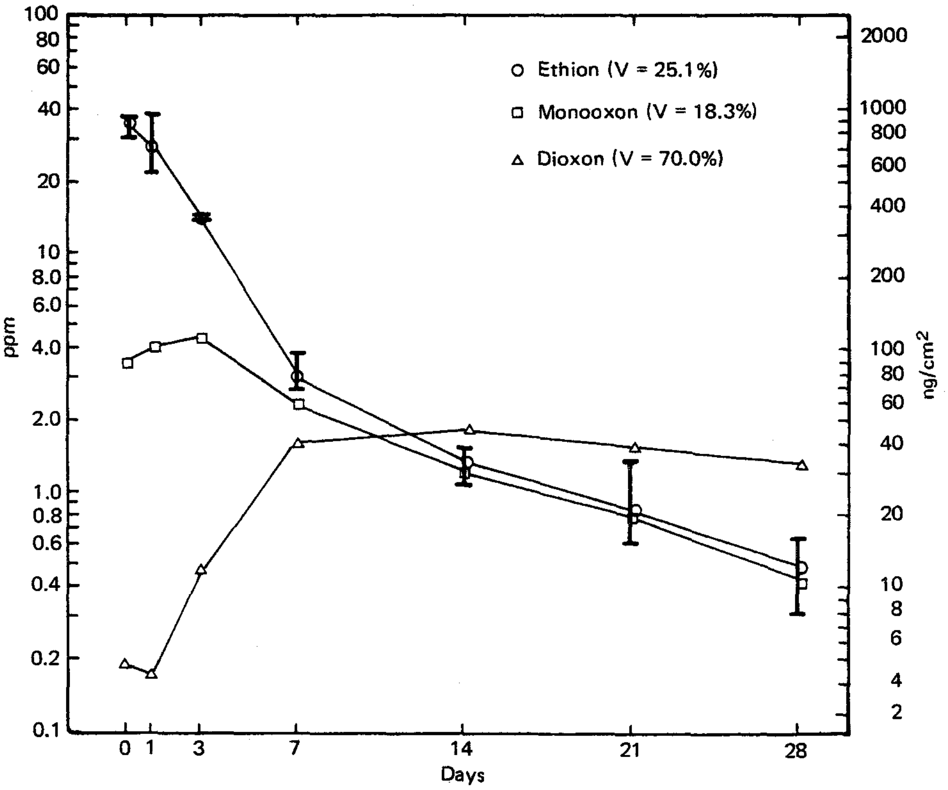

Fig. 3. Ethion dilute dislodgeable residues.

The ethion soil residues (Figures 7 and 8) show the same qualitative decay patterns as those for dislodgeable leaf residues (Figures 1 and 3).

Discussion

While it is extremely difficult to draw precise conclusions from field studies such as those reported in this paper, several pertinent observations can be made based on the results of this investigation.

There is distinct evidence that photodecomposition and/or oxidation are important factors in the degradation of the surface residues of ethion but are not as significant in the degradation of Zolone. This is evidenced by the higher decay rates of dislodgeable ethion residues compared with the rates of decay of leaf extract ethion residues. While incident light intensity measurements were not made during the study, climatic data

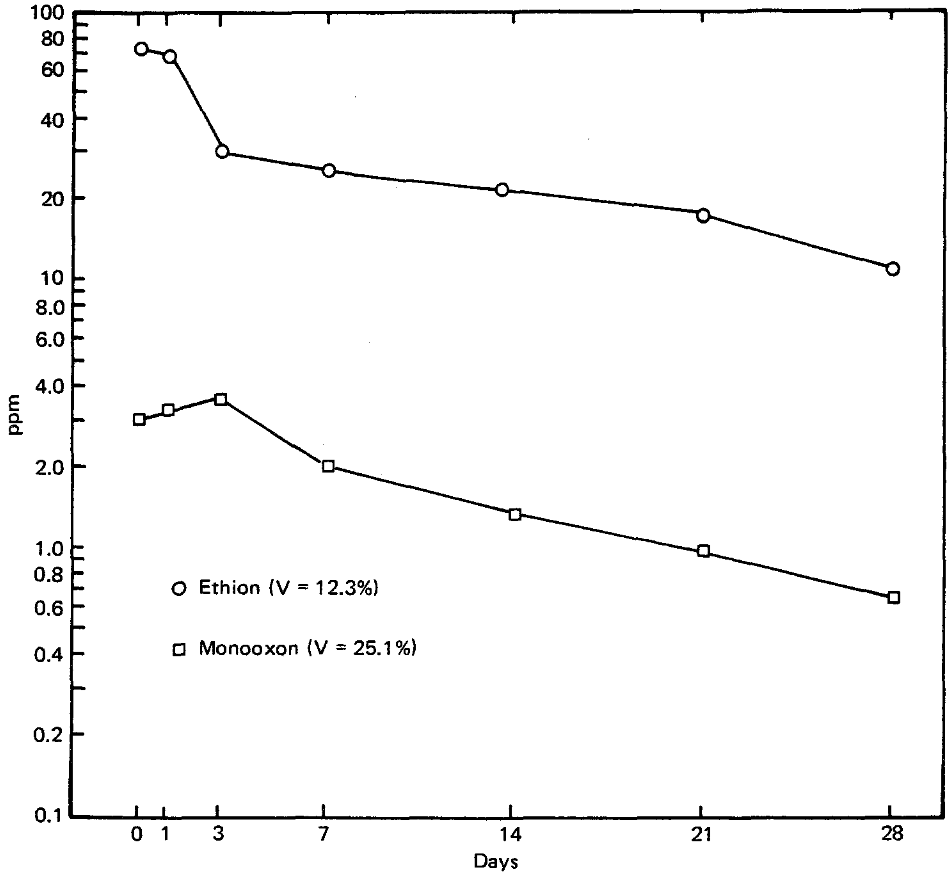

Fig. 4. Ethion dilute – leaf extracts.

indicate that cloud cover over the northern end of the San Joaquin Valley was minimal throughout the period of the experiment.

Observations of temperature (Figure 9) during the experimental period at the Stockton Airport, 18 miles northwest of the test site, provided data that tended to indicate the importance of temperature in the decay of ethion and Zolone residues. The most significant climatic change over the period was a hot period which peaked at three days post-application with a maximum of 114°F and an overnight minimum of 74°F. After a cooling trend that terminated 14 days post-application, temperatures again rose to the range of 95°F to 105°F for the remainder of the experiment. Daytime relative humidity ranged from 15 to 30% and overnight values were between 50 to 80%. The daily average relative humidity ranged from 30 to 50%.

The clearest evidence of temperature effects was the rapid decay of the ethion leaf and soil extract residues between the time of application to three days post-application. As shown in Figure 9, the temperature decreased at four days post-application and both the ethion leaf extract data of Figures 2 and 4 and the soil data of Figures 7 and 8 indicate a simultaneous lessening of ethion decay rates.

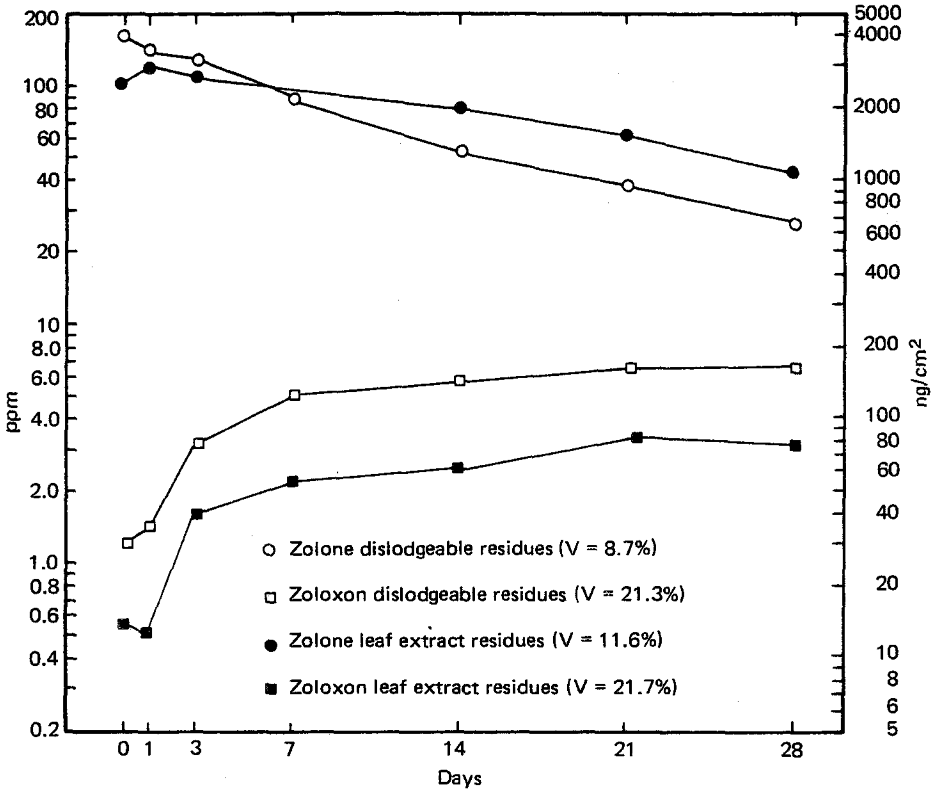

Fig. 5. Zolone concentrate residues.

Temperature effects on the levels of Zolone residues in leaf extracts do not appear to be as great as for ethion, however, such an effect may be masked by the apparent transport of Zolone between the surface and the interior of the leaf. The data suggest that over the week following application, Zolone was transported into the leaf and reached a surface/internal equilibrium between day 7 (Figure 6) and day 14 (Figure 5). In general, however, it appears that Zolone is less sensitive than ethion to environmental factors in the ranges experienced during the course of this study. These conclusions should, however, be regarded as tentative due to the statistical variability of the sampling and analytical techniques.

The behavior of the oxygen analogs of ethion and Zolone is as expected for products in a sequential reaction scheme. As the parent compound is oxidized the oxygen analogs are generated and if the generation rate of the oxon exceeds its decay rate the oxon will accumulate. This is well illustrated for ethion where the dioxon level reached a maximum at a time well after its precursor, the monooxon, (Figures 1, 3, 7, and 8). The accumulation of the oxygen analogs for both ethion and Zolone approached the levels of the parent compounds and in some instances surpassed them during the later stages of the experiment.

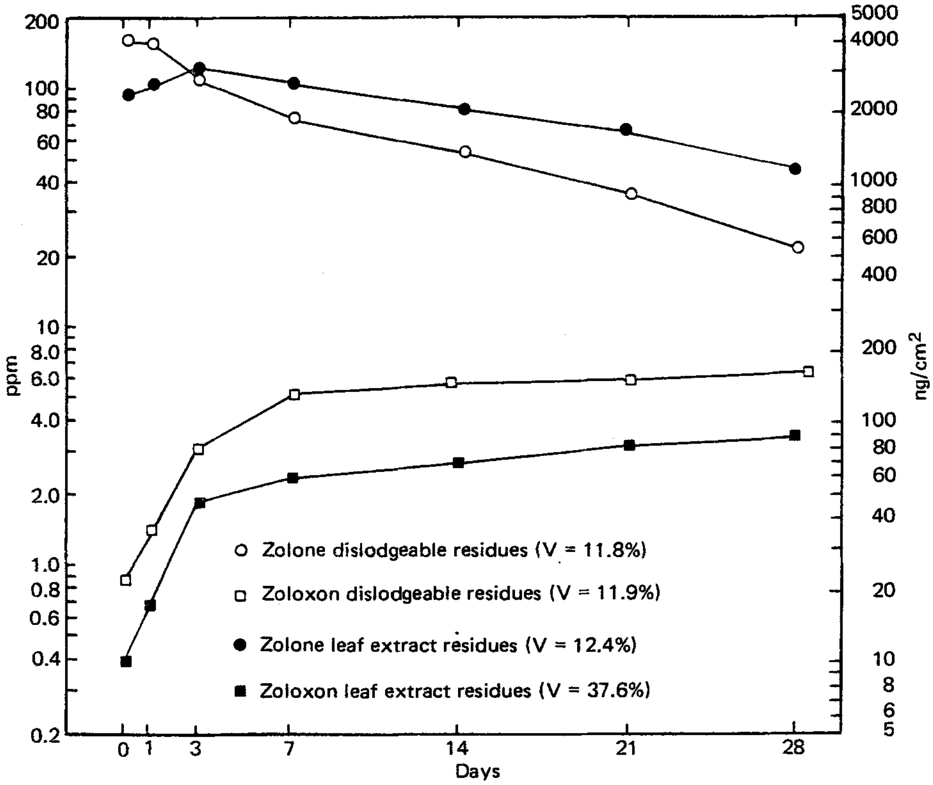

Fig. 6. Zolone dilute residues.

Conclusions

The implications of this study from the standpoint of worker safety are highly significant. There is little doubt that the dislodgeable residue is more closely correlated to worker exposure than the leaf extract residue and this study has shown the two residues to be quite different in quantity and character, particularly for ethion. Moreover, even when lesser differences are noted between the quantity and characteristics of dislodgeable and whole leaf extract residue (e.g., for Zolone) the fact that the dislodgeable Zoloxon residues are consistently higher than the Zoloxon residues from the leaf extracts are significant from the viewpoint of worker exposure.

The finding that the soil ethion residue level and character compares qualitatively to the level and character of dislodgeable residues on leaf surfaces suggests that soil dust on leaf surfaces may play a role in residue decay thereon and thus influence worker exposure. Finally this study points out the urgent need for the study of residue decay under conditions of controlled environmental variables – temperature, humidity, and

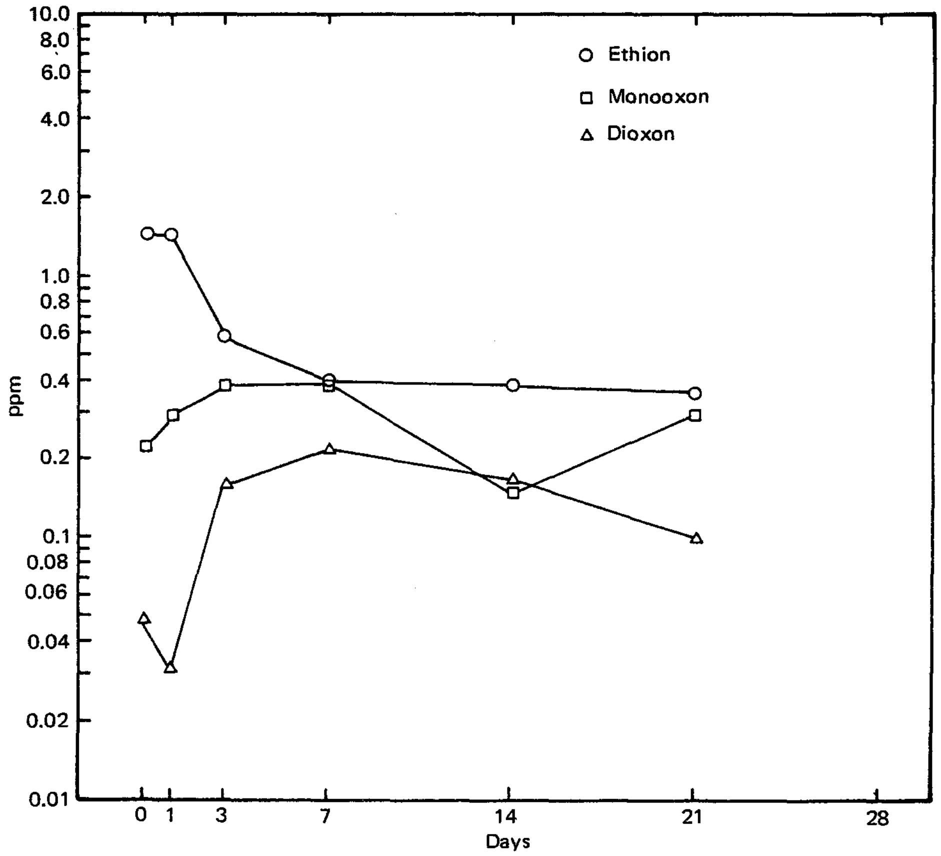

Fig. 7. Ethion concentrate soil residues.

light intensity – so that the role of these factors in the persistence of organophosphate pesticide residues on leaf surfaces in the presence and absence of soil dust may be elucidated.

Acknowledgments

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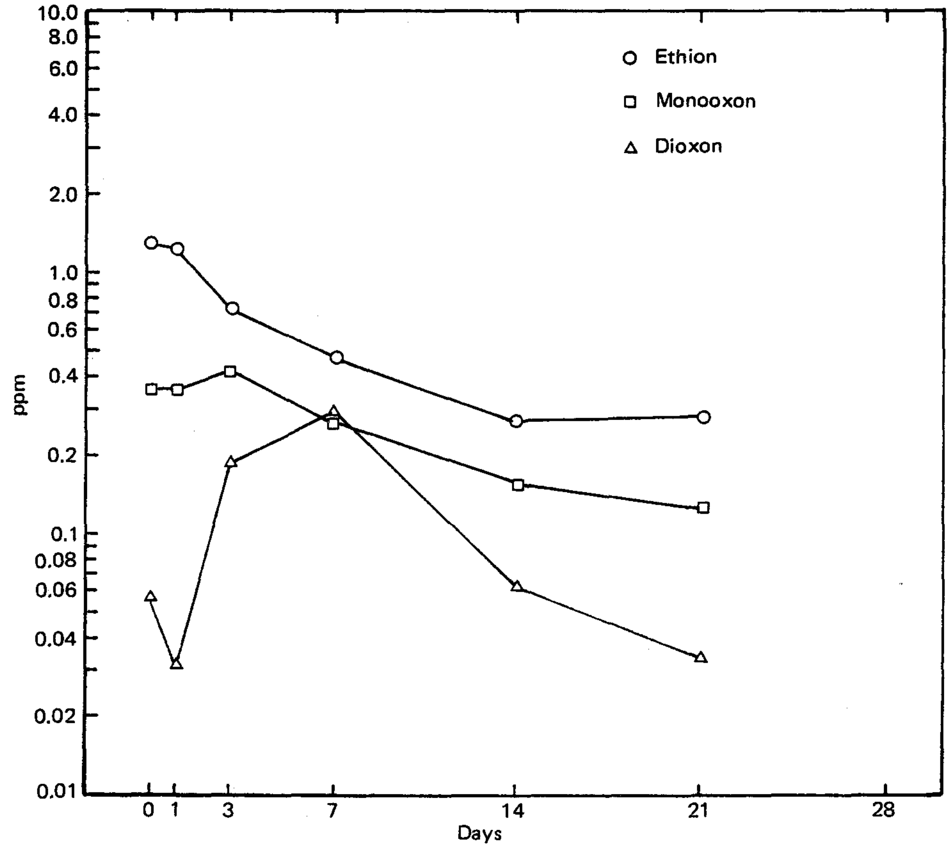

Fig. 8. Ethion dilute soil residues.

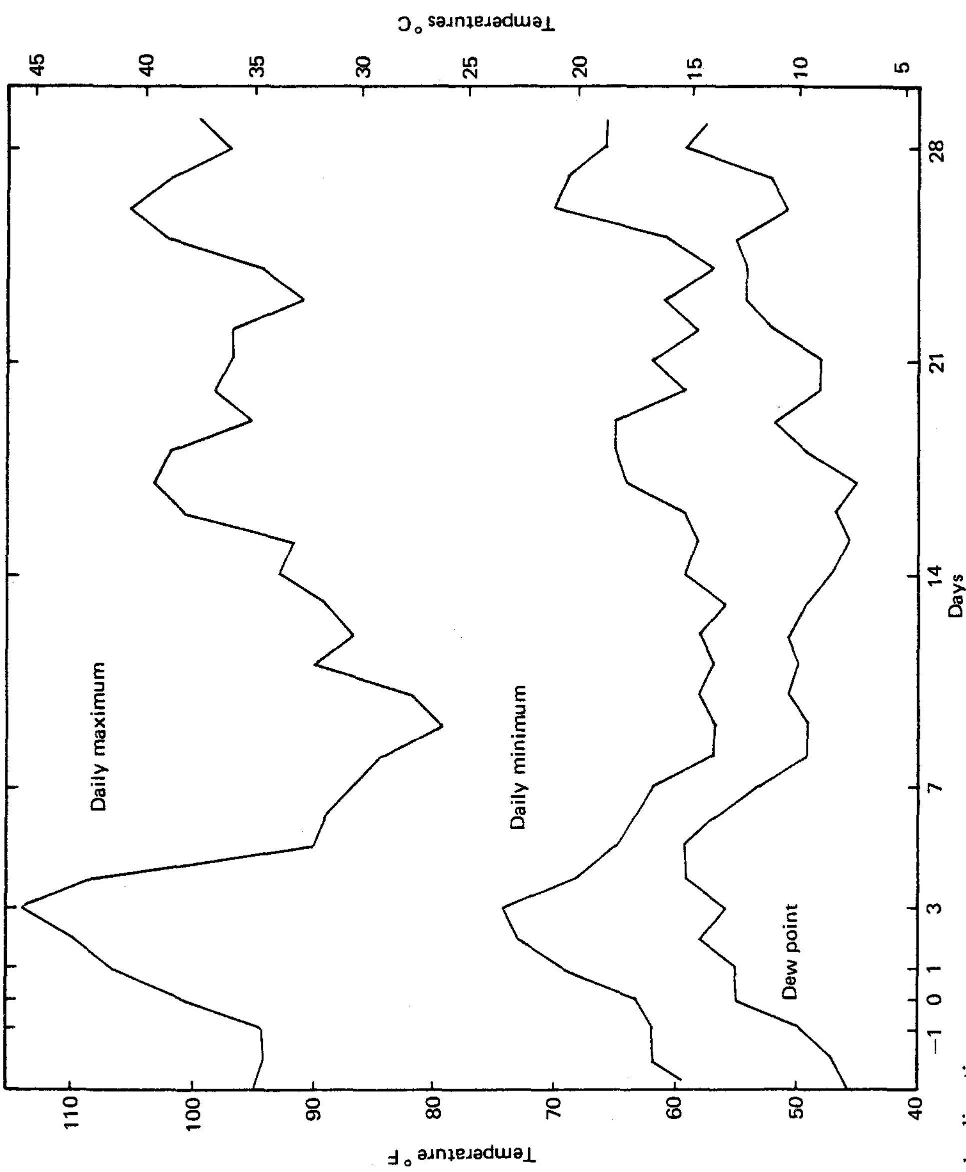

Fig. 9. Salida climatic survey.

References

California State Department of Agriculture: Order adopting and repealing regulations of the California State Department of Agriculture pertaining to economic poisons and injurious materials (Sacramento) (1971).

Federal Register: Occupational Safety and Health Standards (Chapter 17, part 1910) 38 (83) 10715 (1973).

Gunther, F. A., W. E. Westlake, J. H. Barkley, W. Winterlin, and L. Langbehn: Establishing dislodgeable pesticide residues on leaf surfaces. Bull. Environ. Contam. Toxicol. 9, 243 (1973).

McNair, H. M., and E. J. Bonelli: Basic Gas Chromatography, Varian Aerograph, Walnut Creek, California pp. 150, 151 (1969).

Milby, T. H., F. Ottoboni, and H. W. Mitchell: Parathion residue poisoning among orchard workers. J. Am. Med. Soc. 189, 351 (1964).

Steward, D. K. R., D. Chisolm, and M. T. H. Ragab: Long term persistence of parathion in soil. Nature 229, 47 (1971).

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