Environmental Fate of Dialifor and Formation of Its Oxygen Analogue following Application on Grape Vines in the San Joaquin Valley, California

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Dialifor (Torak) insecticide formulation was applied at the highest permissible dosage to California grapes in the San Joaquin Valley. Dislodgeable foliar residues are reported for determining potential exposures of agricultural workers who may come into contact with the treated foliage under California conditions using normal recommended practices. Experimental plots which had been dusted just before and after application and nutrient additives to the formulation were evaluated for their effect on the pesticide persistance and oxygen analogue formation. Although the dissipation of dialifor was slower than that of many organophosphorus compounds, the dust and nutrient treatments had little if any influence on dissipation or oxygen analogue formation. The results from this study also question the cause of a reported California worker injury episode and lends credence to the possibility of a misapplication as the primary cause of injury. Fruit sampled 120 days postapplication contained residue under the established tolerance of 1.0 ppm.

Studies associated with fieldworker exposure to foliar insecticide residues have been reported by many investigators, particularly when cholinesterase-inhibiting compounds have been used to protect crops from insect damage. Many of these studies were designed to develop proper methodology for sampling, handling, and extracting dislodgeable residues. Many environmental factors which can give cause, either directly or indirectly, to worker injury along with the physiological effects have also been studied and reviewed by Gunther et al. (1977) and Kilgore et al. (1977).

The study reported in this communcation resulted from an investigation involving a reported illness episode among 118 fieldworkers harvesting grapes in the San Joaquin Valley of California (Knaak et al., 1978). Dialifor (Torak) and phosalone (Zolone) residues were found on the foliage where the workers had been working. The report asserts that dialifor had been applied at the rate of 1.0 lb/acre in 30 gal of water between 15 and 40 days earlier by using a Kinkelder air-blast sprayer. It also states that 0.06 $\mu g/cm^2$ dislodgeable residue would be considered a safe level and a period of at least 65 days is required before this safe level would be reached. Phosalone residues were supposedly not responsible for the illness in the fieldworkers. Dialifor residues taken from different fields at time of harvest varied considerably but one field had residues of dialifor on leaves amounting to 0.54 $\mu g/cm^2$. Other fields reported to have been harvested 7–9 days later contained dislodgeable residues of 0.14–0.16 $\mu g/cm^2$, and it was these latter fields where the workers reported being ill. Winterlin et al. (1978) also found a large variation in the dislodgeable residues. Their sampling of the foliage did not occur until several weeks later, and the sampling was carried out during a light rain. Average dislodgeable residues found from each of three fields ranged between 0.06 and 0.11 $\mu g/cm^2$ for dialifor and 0.05 and 0.11 $\mu g/cm^2$ for phosalone. The oxygen analogue residues were also analyzed, and residues averaged 0.02 $\mu g/cm^2$ for the dialifor oxygen analogue and phosalone oxygen analogue residues averaged 0.01 $\mu g/cm^2$. Little is known about the toxicity of the oxygen analogue of dialifor, but if one could assume the analogue to be several times more toxic, as is true with many other organophosphate insecticides, the levels found, though much lower, may have contributed to the poisoning episode. Knaak et al. (1978) further reported a dissipation study of dialifor on grapes conducted in the Coastal Range of California. Two separate applications of dialifor were applied to the vines with one set of plots sprayed with a 2-pt solution containing 1.0 lb of active ingredient (AI) of dialifor in 25 gal of water/acre. A second set of plots was sprayed with a dilute solution of dialifor containing 1 lb of AI in 100 gal of water/acre. The concentrated spray was applied with a Windmill 350 sprayer while the more dilute application was applied with an over-the-vine AMC double inverted U-boom sprayer. The degradation of the dislodgeable residue from the foliage 28 days postapplication, though measurably different with the two types of application, did result in residues of 0.37 and 0.2 $\mu g/cm^2$ for 25 and 100 gal/acre, respectively. The initial deposit, however, was approximately the same with 1.9 and 2.1 $\mu g/cm^2$, respectively. These results were considerably different from a dissipation study conducted by Maddy (1977 in the San Joaquin Valley. He reported that a 1.0 lb 100 gal^{-1} $acre^{-1}$ application applied with a modified speed sprayer gave an initial dislodgeable residue of 0.41 $\mu g/cm^2$ which is ~20% the amount found in the Coastal Range study. Knaak et al. (1978) attributed this difference in initial deposits and dissipation with different kinds of spray equipment and dust to the foliage or "inert" material in formulations. Reported studies with parathion by Gunther et al. (1977) and others would support the latter concept.

The study reported in this communication was designed to determine the possible influence dust particulate might have on residue degradation and oxon formation. The dust factor was subdivided into dust on the foliage just prior to the time of application (pre) and dust deposited on the foliage following an application (post). The influence of nutrients incorporated into the tank mixture at the time of application was also determined. This is a common practice for some growers. Dislodgeable foliar and penetrated residue data are reported for four foliar conditions to provide a basis for determining potential exposures of workers during the postapplication period. All applications were made at approximately the same time with the same spray rig and application rate. Residues of the oxygen analogues were also measured following postapplication.

EXPERIMENTAL SECTION

Application. Treatments were made on mature Thompson seedless grapevines located near Ripon, CA, on June 7, 1977, using a Windmill spray rig (Gervan Distributing Co., Modesto, CA). Torak G formulation (dialifor) was applied to give 1 lb of AI 100 gal^{-1} $acre^{-1}$ [1.12

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kg (9.35 hL^{-1}) ha^{-1}]. A sample of the Torak G formulation was taken from the spray rig tank at the time of application for analysis of purity and concentration. Dust used in this study was prepared from sieving Visalia silt loam through a 100-mesh screen, the same soil type as that reported in a 1974 worker-poisoning episode (Adams et al., 1976). The dust was applied to the foliage by using a hand-operated Hudson duster. The preapplication vines were sprayed with a fine water mist by using a hand sprayer just prior to dusting in order for the dust to adhere to the foliage and to prevent unwanted drift of the dust particles to other plots.

Nutrients added to the spray tank consisted of Ortho Nutrient Spray D, 4.0% P_2O_3, 3.25% Mg, 0.82% Cu, 1% Fe, 1.79% Mn, and 12.79% Zn as zinc sulfate monohydrate. The application rate was 4 lb of AI (100 $gal)^{-1}$ $acre^{-1}$ (4.5 kg ha^{-1}).

Phosalone was applied to the control plot at the time of the dialifor application and to all vines on Aug 6, 1977, 60 days following application of dialifor. The formulation was a 25% wettable powder (WP) and was applied at the same rate and by using the same equipment as described for dialifor.

Cultivation of the plots by disking was withheld until just prior to the phosalone treatment. No further cultivation or treatments were carried out until harvest.

Plots. The vineyard selected for this study consisted of 78 vines for each of 36 rows. Starting with row 3, only each fifth row was treated. Each row was divided into three equal parts and each part was randomly selected for an individual treatment. The control plot consisted of two rows located just south and downwind four rows from the treated plots. Each individual treated plot consisted of 26 vines, but only the center 12 vines were sampled, leaving 7 vines on each side which were not sampled. For each experimental treatment there were three replicate plots which were randomly selected throughout the field. The total number of individually treated plots amounted to 18 plus controls.

treatment no.	dust treatment	nutrient treatment
A	none	none
B	none	nutrient
C	preapplication	none
D	preapplication	nutrient
E	postapplication	none
F	postapplication	nutrient

Sampling. Twelve leaf disks (six from each side of the row) were taken from each of twelve vines about 1.0–1.5 m above ground by using a 2.5-cm^2 punch. Punched samples were taken from the leaves nearest the fruit and evenly divided between inside and outside the vines. Samples were collected on −1, 0, 1, 3, 7, 10, 14, 21, 29, 42, 63, 84, and 120 days postapplication. Each sample consisted of a total area of 720 cm^2, and calculations were based on both sides of the leaf. All samples were immediately placed on ice following collection until extracted for dislodgeable (surface) and total residues.

Fruit. At the time of the 120-day foliage sampling, fruit samples were collected from each of the six rows and analyzed for total residues. Approximately 1 kg of grapes was taken from in each row and composted in each of the three treated blocks. Samples in transit were stored on ice until transferred into a freezer for storage at −26 ± 3 °C until processing.

Environmental Monitoring. A recording hydrothermograph (Model 594, Friez Instrument Division, Bendix Aviation Corp.) was used for measuring temperature and humidity, and a radiometer connected to a recorder (Model 7155A, Hewlett-Packard, Inc.) was employed for measuring light intensity, during daylight hours.

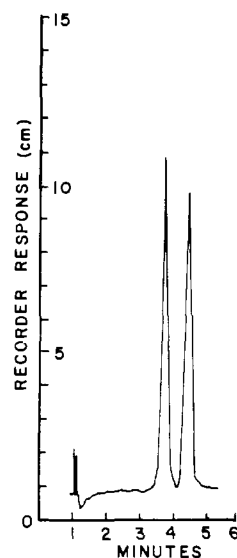

Figure 1. Response of 5 ng each of dialifor and dialifor oxygen analogue by the flame photometric detector. Retention times were 5.2 min for the oxygen analogue and 6.8 min for dialifor.

Extraction and Analysis. The samples were extracted by using a modified method of Gunther et al. (1973). Briefly, the leaf punches were weighed by using pretarred 8-oz jars and shaken 3 times with 75, 50, and 25 mL of water, respectively, containing a small quantity of wetting agent. The combined washes were extracted 3 times with 75, 50, and 50 mL of chloroform, respectively.

Penetrated residues (total extraction subsequent to the dislodgeable extraction) were determined for the 14-, 21-, 29-, and 42-day samples. The extraction procedure involved a Polytron extraction with 75 mL of chloroform and ~30 g of anhydrous sodium sulfate added to the leaf disk, homogenized for 2 min, decanted, and homogenized again with 75 mL of chloroform. Sample cleanup and separation of the oxygen analogue from dialifor involved a Florisil PR column. The eluting solvents were 6% (v/v) ethyl ether in benzene for the parent compound, followed by 12.5% (v/v) acetone in benzene for the oxygen analogue.

Frozen fruit samples were chopped and mixed in the presence of dry ice by using a Hobart food chopper. The homogeneous mixture (100 g) was transferred to a blender and blended with 400 mL of chloroform, followed by filtration through anhydrous sodium sulfate. The collected extract was then cleaned up by using a Florisil column as described previously for "penetrated" residue.

All sample extracts were analyzed by a gas chromatograph equipped with a flame photometric detector (526 nm). In the analysis of dialifor and its oxygen analogue a 2 mm i.d. × 90 cm, silanized, borosilicate column was packed with 10% SE-30 on Gas-Chrom Q, 60–80 mesh. The injector, column, and detector temperatures were 220, 220, and 190 °C, respectively. Nitrogen carrier gas flow was 50 mL/min. Minimum detectable levels of dialifor and its oxygen analogue were <1 ng.

Figure 1 shows the resolution obtained for dialifor and its oxygen analogue.

Preparation of Dialifor Oxygen Analogue. Dialifor oxygen analogue was not available at the time of this study. Therefore, preparation was accomplished by reacting dialifor with *m*-chloroperoxybenzoic acid to form the oxygen analogue (Blinn, 1964). Purification was accomplished by using high-performance liquid chromatography (Waters Associates) equipped with a 30-cm preparative column

packed with 10 μBondapak C_{18} (Waters Associates). The solvent mixture was 53% methanol in water. The oxygen analogue fraction was collected in a 250-mL separatory funnel. The solvent was then extracted 5 times with 50 mL of chloroform, and the extracts were combined into another 250-mL separatory funnel. The chloroform was washed 2 times with 25 mL of water to remove any extracted methanol. The chloroform fraction was filtered through anhydrous sodium sulfate into a 250-mL round-bottom flask, and the solvent was then evaporated to dryness. The sample was dissolved in 53% methanol-water and reinjected into the liquid chromatograph to determine purity by using recycled chromatography. Recycling the sample through the liquid chromatograph revealed a contamination product. Therefore, it was necessary to collect the sample from the liquid chromatograph and extract it a second time with chloroform according to the procedure as described above. The final collection from the liquid chromatograph revealed no other peaks, and the final purity (>98%) was determined by using gas chromatography and a Finnegan 3200E gas chromatograph-mass spectrometer.

RESULTS AND DISCUSSION

Formulation Analysis. For determination of the actual amount of dialifor applied to the vines, each newly prepared spray tank solution of the Torak G formulation was sampled and stored on ice. The formulation contained no detectable oxygen analogue (<4 mg/L). The actual calculated amount of active dialifor applied to the vines was 1.08 ± 0.09 lb/acre.

The control plot was five rows downwind from the dialifor-treated plots, and samples collected with 30 min following application showed less than 2% drift. Since there were four rows of untreated vines between the control and the dialifor-treated rows, the maximum amount of contamination between treatments that would be expected in this study would therefore be 2%.

Dislodgeable Residues. One of the primary causes for fieldworker injury in California from fields sprayed with organophosphate insecticides has been the formation of toxic products and particularly the oxygen analogue(s). The factors primarily responsible for oxygen analogue formation are sunlight, dust, and possibly ozone from polluted air (Spencer et al., 1975; Spear et al., 1977a,b, 1978) under dry California summers.

The maximum and minimum air temperature were taken at the research plot along with humidty and ultraviolet radiation. The year was typically hot and dry during the daytime and through the growing season with no measurable precipitation through the duration of the study. Warmest temperatures were recorded in late June and remained relatively constant through August. Figure 5 shows the maximum and minimum temperatures and relative humidity for the first 63-day period of this study.

Spear et al. (1978) were able to show from chamber studies that the paraoxon production index is a function of dust level and ozone concentration. Obviously, during a field study, the investigator has little influence over ozone concentration except that our study was conducted very close to two large metropolitan areas and a heavily trafficked freeway in the central valley of California. Although Spear et al. (1977b) have suggested that ozone from air pollution may be responsible for toxic oxon formation, the role of ozone is still not clear as Iwata et al. (1979) found no correlation between ozone concentration and oxon formation of methidathion field studies conducted in three different geographical areas in California. However, it has been generally agreed that the longer the insecticide level

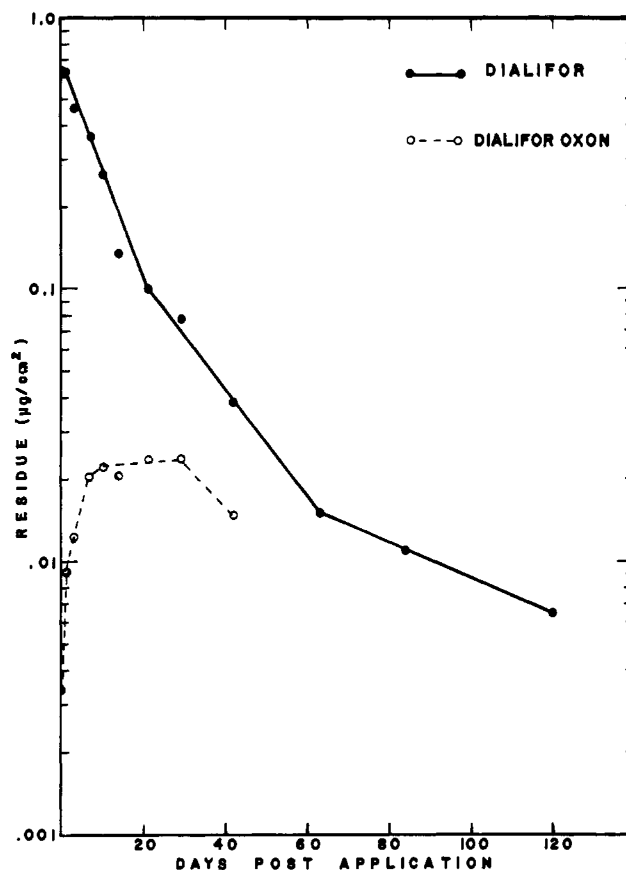

Figure 2. Dissipation curves for dislodgeable foliar residues of dialifor (closed symbols) and dialifor oxon (open symbols) following application to grape vines void of dust and nutrient additives at time of application.

stayed high, the greater amount of oxon formed. Since oxon levels relative to the applied chemicals is usually greater for soil that for foliar surface, leaf dust should be studied as an environmental factor influencing oxon formation.

During the winter and spring months of the year this study was conducted, California had experienced heavy rains and above-average rainfall; therefore, the foliage from the vines were very clean prior to application. Dusting of the leaves with a hand duster resulted in dust on the foliage in excess of 150 μg/cm^2.

Figures 1, 2, 3, and 4 are semilogarithimic plots of foliar dislodgeable residues of dialifor and its oxygen analogue for each treatment. All data points and the lines connecting the points represent the trend in dialifor dissipation and oxon formation. Samples were periodically analyzed throughout the growing season including the 120-day harvest samples. The plot was not cultivated until day 62 to avoid any possible ground disturbance which in turn could have resulted in a heavy deposit of dust on the foliage. However, a heavy growth of weeds and insect infestation made cultivation and spraying of phosalone necessary on day 63. By this time, dislodgeable residues of dialifor had declined to ~0.01 μg/cm^2. The phosalone application did prevent us from analyzing the oxygen analogue of dialifor following this period in time due to the relative large amounts of phosalone which had the same retention time and masked the very low levels of dialifor oxygen analogue on the gas chromatograph. Figure 1 shows the dislodgeable residues of dialifor and its oxygen analogue void of any dust or nutrient treatment. Each data point represents the mean of three samples. Dialifor is more persistent than many organophosphorus com-

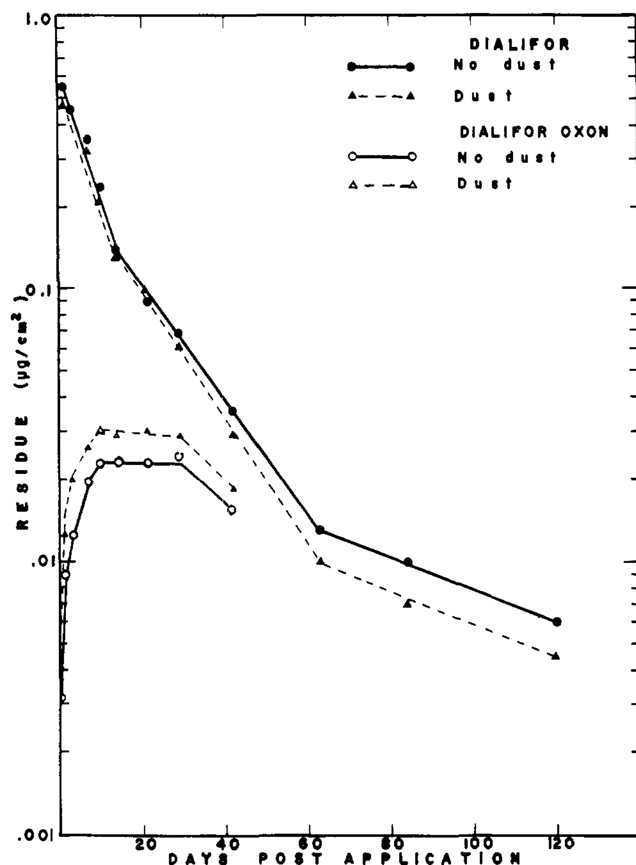

Figure 3. Dissipation curve for dislodgeable foliar residues of dialifor (closed symbols) and dialifor oxon (open symbols) before and after dust application to grape foliage.

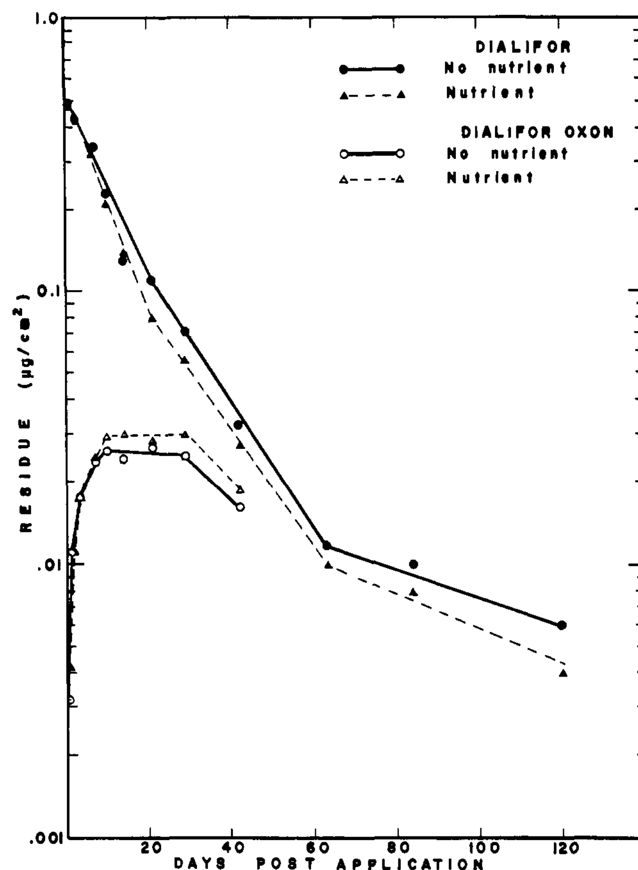

Figure 4. Dissipation curve for dislodgeable foliar residues of dialifor (closed symbols) and dialifor oxon (open symbols) with and without nutrient additives to the formulation.

pounds with a half-life of ~10 days. Fifteen days was the original California worker safety time interval, and the data from this study would indicate that this time period would result in dislodgeable residues of $\sim ^1/_3$ the original deposit on the foliage of 0.15 $\mu g/cm^2$. The oxygen analogue formation was low regardless of the treatment. Like most organophosphorus compounds, the oxon of dialifor started to form shortly after application. During the first 10 days, while the dialifor residue was relatively high, the oxon levels built up, but after this time the levels remained constant for several days as the oxon formation was matched by oxon dissipation. Following day 29 the oxon levels declined at approximately the same rate as dialifor. Mean values of the oxon never exceeded 0.03 $\mu g/cm^2$ in any of the treatments, and of all the samples analyzed, none were as high as 0.05 $\mu g/cm^2$. These data are similar to that reported by Winterlin et al. (1978), who reported means of 0.022–0.026 $\mu g/cm^2$ for dialifor oxon and 0.06–0.11 $\mu g/cm^2$ for dialifor from samples taken many days after a reported worker injury incident. The greatest difference between these two studies was the postapplication period. The 1978 study was reported to have been 60 days from time of application to harvest according to the records by Agriculture Commissioner Office while in our recent study only about 20–30 days would have elapsed since the last application to obtain a dialifor residue of 0.06–0.11 $\mu g/cm^2$. Samples collected by the California Department of Food and Agriculture at the time of the incident contained residues of 0.11–0.45 $\mu g/cm^2$ (Knaak et al., 1978). According to data taken from the current study, this would mean less than 5 days had elapsed since the dialifor application, assuming only one application had been made according to the label. Knaak et al. (1978) state that 0.06 $\mu g/cm^2$ is a possible safe reentry level. Data shown in Figure 1 would assume this level could be reached sometime between 30 and 34 days postapplication.

The reports by Adams et al. (1976), Gunther et al. (1977), Spencer et al. (1977), and Iwata et al. (1975) would clearly indicate worker safety studies should include foliar dust as an important environmental factor when studying dissipation of organophosphorus compounds and oxon formation. Nutrient additives such as zinc sulfate have also been reported to influence oxon formation (Popendorf and Leffingwell, 1978). All these factors were included in this study and are graphically shown in Figures 3 and 4. Each data point on the dust plot in Figure 3 represents 12 samples and is the mean of all the dusted foliage samples regardless of the pre- or postdusting application. The plot void of dust represents the mean of six samples. On the basis of foliar dust alone, there was little if any effect on degradation or oxon formation. Figure 5 illustrates the difference between the pre- and postdusting application. Of the two treatments, the predusting did appear to have a slightly greater influence on the formation of the oxygen analogue as compared to the postdust treatment. However, due to field variables and other factors, the difference between the dust treatments can be considered quite minimal. Oxon formation is a result of a complex process and generally results in a wide variations within a set of field replicates. Perhaps the most significant discovery from this set of data was that the rate of dialifor dissipation and oxygen analogue formation/dissipation between the dust and no dust treatments was in all practicality the same. This was particularly interesting in light of previous reported studies with organophosphate insecticides (Adams et al., 1977; Popendorf and Leffingwell, 1978).

Figure 4 shows the dialifor and the oxon data as influenced by nutrients. Each data point represents the mean

Table I. Penetrated Residues in Grape Leaves

days postapplication	penetrated residues, μg/cm²: no treatment, dialifor	no treatment, oxon	dust treatment, dialifor	dust treatment, oxon	nutrient treatment, dialifor	nutrient treatment, oxon	$\bar{x}$
14	0.1801		0.1428		0.1166		0.1401
		0.0065		0.0072		0.0070	0.0071
21	0.0990		0.0670		0.0606		0.0715
		0.0043		0.0038		0.0039	0.0039
29	0.0830		0.0448		0.0399		0.0521
		0.0043		0.0039		0.0038	0.0039
42	0.0521		0.0319		0.0275		0.0351
		0.0027		0.0033		0.0030	0.0031

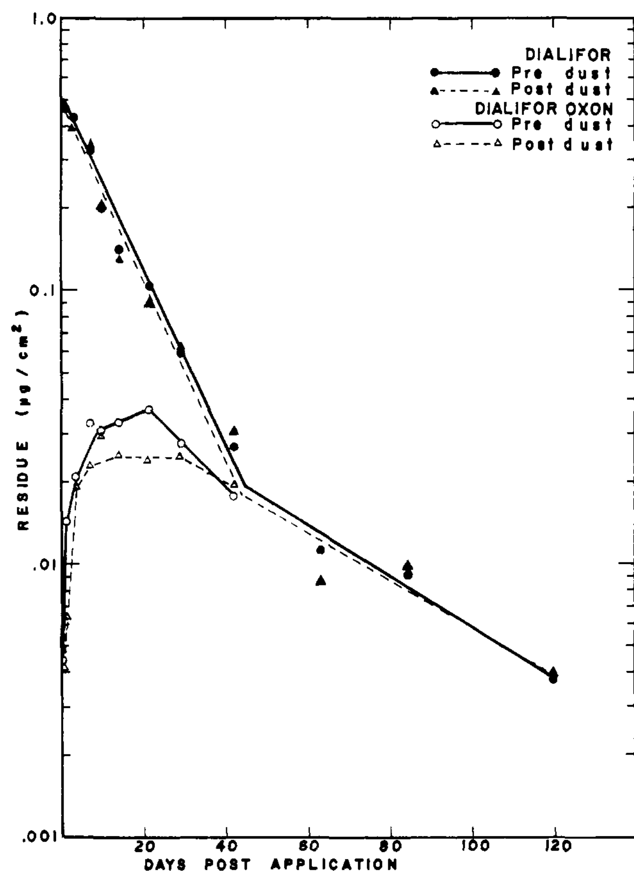

Figure 5. Dissipation curve for dislodgeable foliar residues of dialifor (closed symbols) and dialifor oxon (open symbols) following preapplication and postapplication of dust to the grape foliage.

of nine samples; half of the samples were sprayed with nutrients and the other half were not. The dissipation of dialifor for both treatments was well within experimental error and no major differences could be detected. Only a slight increase in the oxon formation could be attributed to the nutrient application which paralleled the dust factor. Again the variation between samples could tend to nullify the importance of this factor in an overall evaluation of worker exposure.

Penetrated Foliar Residues. Penetrated residues for dialifor and the oxon were determined on days 14, 21, 29, and 42 postapplication. The residues in the leaves were determined on the punched leaf disks after the dislodgeable residues were removed. Data from the penetrated portion of the leaf disks are given in Table I. There was considerably more variability between samples compared to the dislodgeable residues as is typical of other reported studies (Iwata et al., 1979). In spite of the irregularity, the penetrated residues of dialifor did compare very closely in magnitude with the dislodgeable residues. Dialifor oxon, on the other hand, was present at much lower levels.

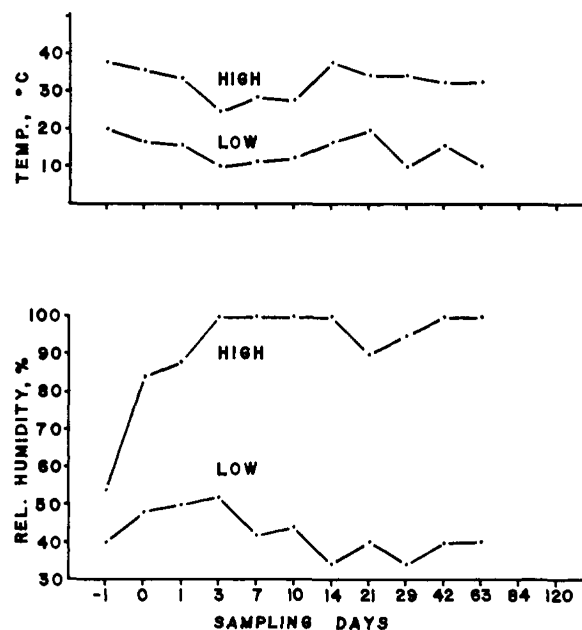

Figure 6. Periodic maximum and minimum air temperatures and relative humidity recorded at the time of foliage sampling.

Residues within the leaf tissue are not considered a potential threat to workers, and it is generally believed this aspect of a study has little relevance to worker safety except where it may be a factor in oxon formation.

Fruit Residues. Mature fruit was analyzed 120 days postapplication for total residue, and of the six samples analyzed the mean value was 0.018 ppm for dialifor with the largest level found at 0.024 ppm. This was considerably less than the 1.0-ppm tolerance established for grapes in 1976. No detectable dialifor oxon (<0.01 ppm) was found in any of the samples.

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Metabolism of Deethylatrazine, Deisopropylatrazine, and Hydroxyatrazine by the Soluble Fraction (105000*g*) from Goose Liver Homogenates

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Incubation of deethylatrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine] and deisopropylatrazine [2-chloro-4-(ethylamino)-6-amino-*s*-triazine] with the soluble fraction (105000*g*) from goose liver homogenates resulted in the formation of the corresponding hydroxy analogues. No dealkylation of hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] occurred when it was incubated with the enzyme preparation. The data suggest that, in the metabolism of atrazine by the soluble fraction from liver homogenates, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues rather than by partial N-dealkylation of hydroxyatrazine.

The metabolism of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] has been studied extensively in these laboratories. Residues of unchanged atrazine and some hydroxy and dealkylated metabolites were found to be present in excreta and various body tissues after chickens had been fed an atrazine-fortified ration (Foster and Khan, 1976; Khan and Foster, 1976). Partial N-dealkylation and hydrolysis were proposed as major pathways for atrazine metabolism in the chicken (Foster and Khan, 1976; Khan and Foster, 1976). Further investigations showed that the soluble fraction (105000*g*) from chicken liver homogenates contains a heat-labile, glutathione-dependent enzyme(s), which metabolizes atrazine in in vitro incubations (Foster et al., 1979). This was accomplished by conjugation with glutathione and subsequent hydrolysis with partial N-dealkylation to the hydroxy and dealkylated analogues. The major metabolic pathway was shown to proceed via enzymatic hydrolysis. Similar studies with enzyme preparations from goose, pig, and sheep liver homogenates indicated that in vitro metabolism of a mixture of atrazine and simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] proceeded via partial N-dealkylation accompanied by hydrolysis (Khan et al., 1979). However, hydrolysis to the corresponding hydroxy analogues appeared to be slower than partial N-dealkylation.

The present study is a continuation of these investigations concerning metabolism of *s*-triazines by an enzyme preparation from liver homogenates. It was of special interest to ascertain whether, in the in vitro metabolism of atrazine by the enzyme preparation, the formation of 2-hydroxy partially N-dealkylated metabolites occurs by the hydrolysis of the respective 2-chloro analogues or by the partial N-dealkylation of hydroxyatrazine.

Animal Research Institute (T.S.F. and M.H.A.) and Chemistry and Biology Research Institute (S.U.K.), Research Branch, Agriculture Canada, Ottawa, Ontario K1A 0C6.

Three atrazine metabolites, namely, deethylatrazine [2-chloro-4-amino-6-(isopropylamino)-*s*-triazine], deisopropylatrazine [2-chloro-4-(ethylamino)-6-amino-*s*-triazine], and hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], were incubated with the soluble fraction (105000*g*) from goose liver homogenates. Reaction products were extracted and identified by gas chromatography (GC).

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada) and used as received. Reference standards of deethylatrazine, deisopropylatrazine, and hydroxyatrazine were gifts from Ciba Geigy Ltd., Switzerland.

Stock solutions of deethylatrazine (195.9 μg/mL) and deisopropylatrazine (202.0 μg/mL) in acetone and hydroxyatrazine (41.2 μg/mL) in methanol were stored in the dark at room temperature. Gas chromatographic analyses of these solutions under the conditions described below indicated a single peak in each case. All other chemicals were analytical grade or the purest grade available.

Enzyme Preparation. Livers were obtained from male Chinese geese after they had been killed by cervical dislocation. The soluble fraction (105000*g*) was prepared as described previously (Khan et al., 1979) and used as the enzyme preparation. It was prepared fresh on the day each series of incubations was carried out.

In Vitro Incubations. Incubations were carried out in 50-mL glass-stoppered Erlenmeyer flasks. A typical incubation mixture contained enzyme preparation (4.5

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