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ARTICLES

Fruit Residue Data and Worker Reentry Research for Chlorthiophos Applied to California Citrus Trees

Yutaka Iwata,* James B. Knaak, Glenn E. Carman, Margarete E. Düsch, and Francis A. Gunther

Chlorthiophos [*O*-[2,5-dichloro-4-(methylthio)phenyl] *O,O*-diethyl phosphorothioate] was applied to California citrus trees. Residue methodology for the analysis of chlorthiophos, its sulfoxide, its sulfone, its oxon, its oxon sulfoxide, and its oxon sulfone on and in citrus fruit, on foliage, and in soil dust is presented. Residue dissipation curves obtained for these three substrates are given. Dermal dose–cholinesterase response curves for the six compounds are given for rats. These data are used to calculate safe residue levels on foliage. On the basis of the dislodgeable foliar residue data, a 70-day reentry interval is proposed.

Chlorthiophos [Celathion; *O*-[2,5-dichloro-4-(methylthio)phenyl] *O,O*-diethyl phosphorothioate], on the basis of preliminary results, appeared to be a potentially useful insecticide for the control of the California red scale, *Aonidiella aurantii* (Mask.), which is one of the major citrus pests in California. Consequently, a study was initiated to obtain residue data for chlorthiophos and any of its five cholinesterase (ChE)-inhibiting oxidation products which may be present after application of chlorthiophos to citrus trees. Figure 1 shows the chemical structures of these compounds. Fruit, dislodgeable foliar, and soil dust residues were obtained to assist in the setting of a fruit tolerance for consumer protection and of a safe reentry interval for protection of agricultural workers who may engage in prolonged and extensive contact with the treated foliage. the oral LD_{50} values for mouse, rat, and rabbit are 140, 13, and 20 mg/kg, respectively, and the dermal LD_{50} values for rat and rabbit are 58 and 48 mg/kg, respectively (Muacevič, 1976). Due to the high acute toxicity of chlorthiophos, dermal dose–ChE response data were generated for chlorthiophos and each of its five oxidation products. These data were used to calculate safe residue levels on foliage and, in conjunction with the dislodgeable residue data generated in this study, to calculate safe reentry intervals.

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EXPERIMENTAL SECTION

Treatment and Sampling. Mature orange trees were located on the University of California Citrus Research Center, Riverside, CA. Celathion 40WP formulation was supplied by EM Industries, Inc., Elmsford, NY, which represents Celamerk GMBH and Co. KG, Ingelheim, Germany.

Each plot consisted of four rows of six trees each. Three replicate plots were treated for each of two treatment rates. Applications were made by using an oscillating boom spray rig on Aug 8, 1980, at rates of 4.8 and 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1} [5.3 and 10.6 kg of AI (178 hL)$^{-1}$ ha^{-1}]. Samples were collected from the eight trees in the center of each plot. At each sampling, 1 fruit was removed from each quadrant from each of 8 trees to yield a 32-fruit sample. At each sampling, 5 leaf disks were collected from each of 8 trees as described by Gunther et al. (1973) to give a 40 leaf disk sample representing 5 disks per octant. At

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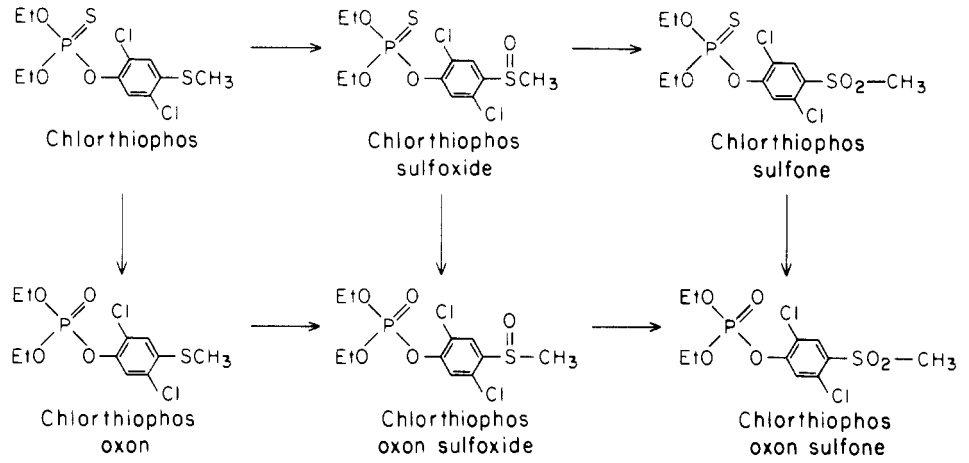

Figure 1. Chemical structure of chlorthiophos and five of its ChE-inhibiting oxidation products.

each sampling a soil dust sample was collected by vacuuming through a 100-mesh screen as described by Spencer et al. (1977) from 1 octant from each of 8 trees such that all 8 octant positions were represented by the composite sample.

Processing. *Fruit.* The procedure of Iwata et al. (1981) was used. Briefly, a 100-g sample of chopped rind or pulp was macerated with 300 mL of acetone, and the macerate was vacuum-filtered through a Büchner funnel. A 50-mL aliquot of the extract was shaken with 50 mL of benzene. The benzene phase was separated and removed by using a rotary evaporator, and the residue was redissolved in 5 mL of benzene. Final calculations assumed that the total extract was 380 mL based on 300 mL of acetone plus 80 mL of water from 100 g of substrate.

The following column chromatographic procedure was adapted from that of Bowman and Beroza (1968) which was used for fenthion. A 22-mm i.d. Kontes Chromaflex column was successively packed with a 1-cm layer of Na_2SO_4, 4 g of 60–200-mesh silica gel, and another 1-cm layer of Na_2SO_4. After the column was prewetted with 20 mL of 50% hexane in benzene, the 5-mL benzene extract was added. The column was successively eluted with 50 mL of 50% hexane in benzene (chlorthiophos), 50 mL of benzene (sulfone), 75 mL of 1% acetone in benzene (sulfoxide and oxon), 50 mL of 5% acetone in benzene (sulfone oxon), and 75 mL of 15% acetone in benzene (sulfoxide oxon); the compound eluted by each fraction is denoted in parentheses. The solvent from each of the five eluates was removed, and each resultant residue was dissolved in acetone for gas chromatographic (GC) analysis.

Dislodgeable Residues. The procedure of Iwata et al. (1977) was used. Briefly, the procedure entailed shaking the sample 3 times, each time with 100 mL of water containing a surfactant, and then extracting the combined aqueous wash twice, each time with 50 mL of CH_2Cl_2 to recover residues for analysis. The CH_2Cl_2 was removed, and the residue was dissolved in 5 mL of benzene and column chromatographed as described above for fruit samples.

Soil Dust. The procedure of Iwata et al. (1979) was used. Briefly, the procedure entailed shaking a 10-g dust sample with 10 mL of 10% aqueous acetone and 10 mL of hexane for 20 min. The extract was filtered through Na_2SO_4 and analyzed after column chromatographic fractionation as described above for fruit samples.

Method Validation. *Fruit.* Chopped orange rind and pulp samples were each fortified in the blender jar prior to the addition of the acetone to obtain procedural recoveries. All six compounds sought were added to the substrate at the same ppm level of fortification. Percent recoveries and standard deviations based on three replicate samples after fortification at 5, 1, and 0.1 ppm (0.2 ppm for oxon sulfoxide and oxon sulfone) for rind and 0.1 ppm for pulp were respectively 113 ± 10, 104 ± 8, 109 ± 4, and 100 ± 5 for chlorthiophos, 111 ± 4, 108 ± 2, 103 ± 3, and 115 ± 6 for the sulfoxide, 97 ± 13, 109 ± 4, 103 ± 4, and 103 ± 7 for the sulfone, 106 ± 5, 105 ± 3, 117 ± 1, and 121 ± 3 for the oxon, 113 ± 9, 111 ± 2, 113 ± 10, and 102 ± 14 for the oxon sulfoxide, and 101 ± 10, 95 ± 6, 92 ± 16, and 87 ± 9 for the oxon sulfone. No corrections were applied to the field sample data based on these results as all recoveries averaged over 85%.

A number of tests were conducted by using laboratory-treated oranges prior to the field application tests. Fruits were individually immersed for about 10 s in a mixture containing 1.0 g of Celathion 40WP and 100 mg each of the sulfoxide and the oxon sulfoxide in 800 mL of water and were air-dried for 24 h before processing. The Celathion mixture was equivalent to a field spray mix of 1.0 lb of formulation/100 gal of water. All fruits were washed to remove surface residues prior to use in subsequent tests.

Since field samples often cannot be processed immediately due to the number of samples involved, subsamples of laboratory-treated whole fruits were stored in a cold room at 8 °C for 2 and 7 days to check residue stability. Residue levels and standard deviations for three replicate samples of unstored and 2- and 7-day-stored fruits were respectively 3.7 ± 0.2, 3.8 ± 0.3, and 3.5 ± 0.1 ppm for chlorthiophos, 0.77 ± 0.05, 0.50 ± 0.02, and 0.68 ± 0.02 ppm for the sulfoxide, and 0.46 ± 0.10, 0.19 ± 0.03, and 0.22 ± 0.04 ppm for the oxon sulfoxide. Although no significant change in residue levels occurred for chlorthiophos and its sulfoxide, the oxon sulfoxide levels were significantly affected. Samples in this study were processed within 1.5 days of collection. These tests indicate that collection of a large number of samples to satisfy statistical requirements is self-defeating unless the samples are processed quickly.

Since immediate sample extraction with acetone would increase processing time, field samples were peeled, chopped in a Hobart food chopper, and frozen for later extraction. Aliquots of 100 g each of chopped rind prepared from laboratory-treated fruits were kept under frozen storage for 2, 4, and 6 weeks to check residue stability. Residue levels and standard deviations for three replicate samples of unstored and 2-, 4-, and 6-week-stored samples were respectively 3.7 ± 0.2, 3.7 ± 0.2, 3.9 ± 0.5, and 3.8 ± 0.2 ppm for chlorthiophos, 0.77 ± 0.05, 0.62 ± 0.06, 0.68 ± 0.02, and 0.72 ± 0.04 ppm for the sulfoxide, and 0.46 ± 0.10, 0.35 ± 0.02, 0.34 ± 0.02, and 0.40 ± 0.07 ppm for the oxon sulfoxide. The data show that the three

compounds were stable over the 6-week period tested.

Frozen, stored samples are extracted with acetone and again stored to await final processing by the analyst. Thus, stability of residues in acetone extracts needed to be confirmed. Three replicate rind samples were each fortified at 1.0 ppm with each of the six compounds. Samples were analyzed immediately, and aliquots were removed and analyzed after 2, 4, and 6 weeks of storage at 8 °C. Percent recoveries after no storage and 2, 4, and 6 weeks of storage, respectively, were 103 ± 2, 104 ± 4, 103 ± 7, and 103 ± 6 for chlorthiophos, 106 ± 4, 110 ± 2, 115 ± 4, and 118 ± 4 for the sulfoxide, 101 ± 1, 106 ± 9, 105 ± 3, and 117 ± 3 for the sulfone, 105 ± 4, 105 ± 3, 104 ± 7 and 115 ± 3 for the oxon, 106 ± 2, 106 ± 6, 110 ± 5, and 102 ± 13 for the oxon sulfoxide, and 115 ± 6, 110 ± 9, 114 ± 10, and 103 ± 9 for the oxon sulfone. Thus, short-term storage of acetone extracts was not detrimental.

For estimation of the extraction efficiency of the method, rind from the laboratory-treated fruits was used. The filter cake remaining after acetone blending was Soxhlet-extracted by using methanol–chloroform (13:87 azeotropic mixture). Soxhlet extraction (no thimble, only glass wool) was conducted for 2 h and again for another 2 h with fresh solvent. Three samples were used. The blending procedure yielded 3.7 ± 0.2 ppm, and the first 2-h Soxhlet extraction gave an additional 0.52 ± 0.04 ppm for chlorthiophos. The blending procedure yielded 0.77 ± 0.05 ppm, and the first 2-h Soxhlet extraction gave an additional 0.12 ± 0.01 ppm for the sulfoxide. Levels in the second 2-h extracts were <0.01 ppm. Too much interference was present to allow quantification of the oxon sulfoxide in the Soxhlet extracts. Thus, the blending procedure used was 87% efficient for both chlorthiophos and its sulfoxide. No corrections were made to the field sample data based on these results. So that the thermostability of the two compounds under the 2-h Soxhlet heating conditions could be checked, 25 μg of each compound was added to the boiling flask and a control rind sample was extracted. Recoveries were 103 ± 4% for chlorthiophos and 102 ± 11% for its sulfoxide, showing that neither compound was degraded under the extraction conditions.

Dislodgeable Residues. Since no meaningful fortifications of leaves were possible, the only tests conducted were for recovery of material added to aqueous leaf washes. Additions of 400, 40, and 4 μg were made and were equivalent to 1.0, 0.10, and 0.010 μg of residues/cm^2. Percent recoveries for three samples fortified at these three levels were respectively 96 ± 4, 83 ± 1, and 96 ± 5 for chlorthiophos, 105 ± 5, 107 ± 7, and 102 ± 3 for the sulfoxide, 100 ± 3, 107 ± 4, and 95 ± 4 for the sulfone, 108 ± 9, 96 ± 4, and 105 ± 6 for the oxon, 110 ± 3, 107 ± 2, and 119 ± 7 for the oxon sulfoxide, and 93 ± 11, 98 ± 12, and 97 ± 3 for the oxon sulfone. No corrections were made to the data based upon these results. All field samples were processed on the day of sampling.

Soil Dust. So that the adequacy of the extraction procedure described above could be checked, 10-g soil dust samples were mixed with cleaned sand and Soxhlet-extracted for 4 h by using an azeotropic acetone–hexane (59:41) mixture (Spencer et al., 1977). Table I shows the results for 3-, 10-, 17-, 31-, and 42-day samples extracted by the above-described shaking method and the Soxhlet procedure. Results were essentially identical for the two procedures. The shaking procedure was considered more practical for processing the collected samples.

Analysis. Chlorthiophos and its oxidation products were analyzed by gas chromatography with a Hewlett-Packard N/P ionization detector. A 60 cm by 4 mm i.d. glass column packed with 10% OV-1 coated on 60–80-mesh Gas-Chrom Q was used with a flow rate of 30 mL/min and a 230 °C column temperature.

Table I. Recovery of Chlorthiophos and the Oxidation Products (ppm) from Soil Dust by Two Extraction Methods

compound	day[a]	extraction method: Soxhlet	extraction method: shaking
chlorthiophos	pretreat	<1	<1
	3	150	150
	10	42	51
	17	49	49
	31	15	22
	42	19	22
chlorthiophos sulfoxide	pretreat	<1	<1
	3	96	104
	10	74	62
	17	95	100
	31	68	70
	42	74	92
chlorthiophos sulfone	pretreat	<1	<1
	3	4	5
	10	6	6
	17	8	9
	31	11	16
	42	15	25
chlorthiophos oxon	pretreat	<1	<1
	3	13	9
	10	1	<1
	17	1	2
	31	<1	<1
	42	<1	<1
chlorthiophos oxon sulfoxide[b]	3	23	23
	10	15	13
	17	27	25
	31	24	
	42	33	29
chlorthiophos oxon sulfone[b]	3	6	7
	10	3	4
	17	6	5
	31	7	7
	42	8	7

[a] Orange trees located on the University of California Citrus Research Center, Riverside, CA, were treated on Aug 8, 1980, by using Celathion 40WP. [b] Sample extracts were subjected to alkaline hydrolysis and the diethyl phosphate formed was analyzed as its pentyl ester. The extracts from pretreatment samples were fortified at 0.1 ppm each of the oxon sulfoxide and oxon sulfone prior to column cleanup and fractionation of eluates. Sample results have been corrected for recovery as determined from fortified extracts. Recoveries were 120% for the oxon sulfoxide and 106% for the oxon sulfone.

The fourth and fifth eluates obtained by column chromatography on silica gel, as described earlier, contained the sulfone and sulfoxide of chlorthiophos oxon, respectively. Due to interfering background peaks, it was impossible to analyze the extracts for the two compounds directly when field-weathered samples were analyzed. For the dislodgeable foliar residue samples, these compounds were analyzed by the 4-(*p*-nitrobenzyl)pyridine colorimetric procedure of Gunther et al. (1980). For the fruit and soil samples, the two compounds were each separately hydrolyzed to diethylphosphoric acid (DEP) and then analyzed as the pentyl ester after treatment with ethereal diazopentane. This procedure, which is an adaptation of an approach by Shafik et al. (1971), is given below.

After the solvent was removed from the column eluate, the residue was transferred to a 15-mL graduated tube. After removal of the solvent used to transfer the residue, 3 drops of 10% (w/v) aqueous NaOH solution and 1 mL

of acetone were added. The mixture was vortex-mixed and heated at 80 °C for 30 min; two additional vortex mixings were done during the heating period. After removal of any remaining acetone with a gentle air stream, 0.5 mL of a saturated NaCl solution, 0.5 mL of 5 N HCl, and 2 mL of 1:1 (v/v) acetonitrile–ether solution were added, and the total contents were vortex-mixed for 1 min. A 1-mL aliquot of the organic extract was transferred to a second 15-mL graduated tube, and ethereal diazopentane reagent (Shafik et al., 1973) was added until there remained a persistent yellow color. After the mixture was allowed to stand for 15 min, acetone was added to adjust the volume as needed, and the sample was analyzed by GC. With eluates obtained from 0.05-ppm-fortified orange rind, recoveries for three samples were 47 ± 2% for the oxon sulfone and 69 ± 4% for the oxon sulfoxide. All sample values were corrected for recovery based upon parallel fortified samples.

DEP pentyl ester was analyzed by gas chromatography with a 1.2 m by 4 mm i.d. glass column packed with 10% OV-1 coated on 60–80-mesh Gas-Chrom Q and operated at 170 °C and an 80 mL/min flow rate. A flame photometric detector was used.

For further work in dislodgeable residues, the pentyl ester method is recommended. Recoveries from aqueous leaf washes fortified with 400, 40, and 4 μg and representing residues of 1.0, 0.1, and 0.01 μg/cm², respectively, were for three samples 86 ± 5, 93 ± 9, and 55 ± 1%, respectively, for the oxon sulfoxide and 77 ± 8, 68 ± 4, and 49 ± 3%, respectively, for the oxon sulfone. Fortified samples should be run to correct for low recoveries.

Analytical Standards. Standards were provided by EM Industries, Inc. The chlorthiophos standard was a mixture of three isomers: 71.1% 2,5-dichloro-4-methylthio, 14.9% 4,5-dichloro-2-methylthio, and 12.3% 2,4-dichloro-5-methylthio. Standards of all the oxidation products were of the 2,5-dichloro-4-methylthio isomer only. Purity was 96.5% for the sulfoxide, 99% for the sulfone, 99% for the oxon, 91.2% for the oxon sulfoxide, and 96.7% for the oxon sulfone. All GC quantification used the peak height method. Standard curves were linear and passed through the origin.

Dermal Toxicity. Studies were conducted on the analytical standards described above by using the procedure for Knaak et al. (1980). Briefly, the hair was clipped from the backs of male albino rats. The test compound was applied to a 25-cm² area of the exposed skin by using 1 mL of acetone as a carrier. Three animals were treated for each dosage tested. Animals were sacrified after 72 h, and the ChE activity in the red blood cells was determined.

RESULTS AND DISCUSSION

Chlorthiophos is an organophosphorus (OP) insecticide that produces five additional ChE-inhibiting compounds under field conditions (see Figure 1). No published residue studies involving this insecticide were found. With the available phosphorus-selective detectors, the residue analysis for chlorthiophos and its oxidation products is, in principle, straightforward. Actual sample analyses, however, are very time consuming since the six compounds have to be separated into five eluate fractions by using column chromatography and then six analyses have to be made. Furthermore, due to interfering peaks obtained on the gas chromatograms of extracts of field samples, both the sulfoxide and sulfone of chlorthiophos oxon needed to be separately hydrolyzed to diethyl phosphate and then further converted to the pentyl ester to allow satisfactory quantification. Use of the pentyl ester allowed for quantification of lower residue levels due to the sharper GC

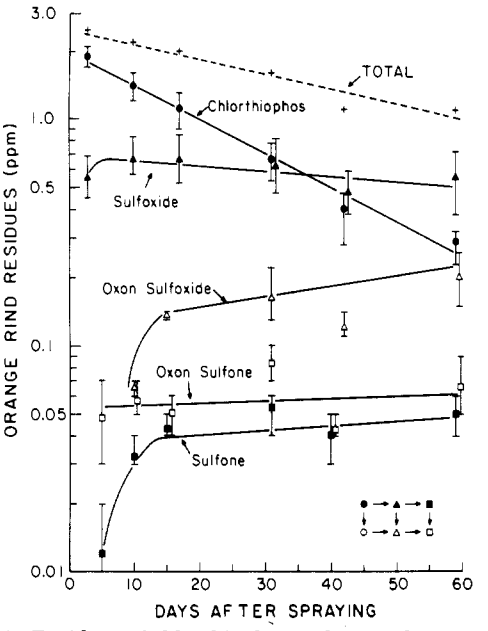

Figure 2. Residues of chlorthiophos and its oxidation products on and in orange rind after application of Celathion 40WP at a rate of 4.8 lb of AI (1900 gal)$^{-1}$ acre^{-1}. Vertical lines indicate the range of values found for six replicate field samples. Treatment was made on Aug 8, 1980, to trees located on the Citrus Research Center, Riverside, CA.

peaks obtained for this compound. No effort was made to conduct a residue study on the three geometrical chlorthiophos isomers in the formulation used since they are not readily resolved. Quantification was based on a single unresolved peak resulting from all three isomers. Hild et al. (1978) reported on the glass-capillary gas chromatographic properties of the three geometric chlorthiophos isomers (2,5-dichloro-4-methylthio, 4,5-dichloro-2-methylthio, and 2,4-dichloro-5-methylthio) and their corresponding sulfones.

Residue Data. *Fruit.* Rind was removed from the edible portion (pulp) of the oranges, and the rind and pulp were analyzed separately. Figures 2 and 3 show the rind residue levels after application of chlorthiophos to orange trees at 4.8 and 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1}, respectively. Residue values are for unwashed fruits as might be found at a roadside stand and thus represent a worst-case situation as some of the residues are likely to be surface residues adhering to the dust on the fruit. Chlorthiophos and its sulfoxide constituted the principal residues. Chlorthiophos residues slowly declined while its sulfoxide residues remained relatively unchanged over the 60-day experimental period. No chlorthiophos oxon was found above the 0.01-ppm level sought. It is speculated that either the most facile oxidation is the formation of the sulfoxide or the oxon, when formed, is rapidly further oxidized to its sulfoxide.

Residues of chlorthiophos, its sulfoxide, its sulfone, and its oxon were below the 0.01-ppm level in the edible portion of the fruit for all sampling dates. Residues of the oxon sulfoxide and the oxon sulfone ranged between 0.01 and 0.06 ppm. However, since these latter values were found for all sampling dates from 3 to 59 days postapplication, it was concluded that these values were background values and that actual residue levels for the two compounds were also below 0.01 ppm. Since Valencia oranges are 18.7 ± 6.3% rind by weight (Gunther, 1969) and since all residues are in the rind, whole-fruit residue levels are one-fifth the rind values.

Dislodgeable Foliar Residues. Dislodgeable residues on

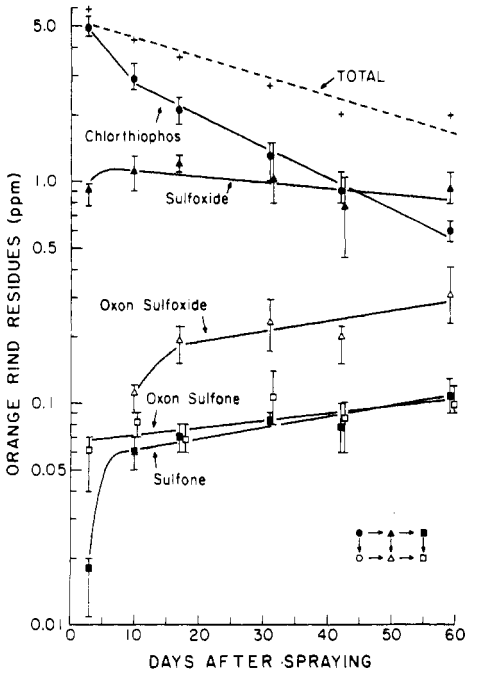

Figure 3. Residues of chlorthiophos and its oxidation products on and in orange rind after application of Celathion 40WP at a rate of 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1}. Vertical lines indicate the range of values found for six replicate field samples. Treatment was made on Aug 8, 1980, to trees located on the Citrus Research Center, Riverside, CA.

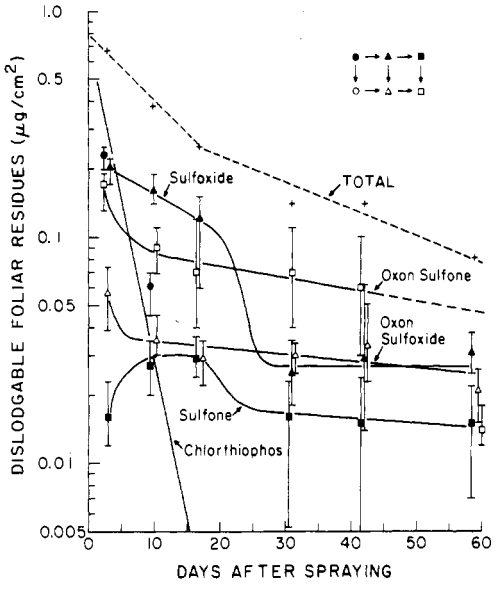

Figure 4. Dislodgeable residues of chlorthiophos and its oxidation products an orange foliage after application of Celathion 40WP at a rate of 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1}. Vertical lines indicate the range of values found for six replicate field samples. See the explanation given in the text regarding the anomalously high oxon sulfone residue values. Treatment was made on Aug 8, 1980, to trees located on the Citrus Research Center, Riverside, CA.

fruit and foliage are the residues available for dermal absorption by workers who come into extensive and prolonged contact with treated trees.

Figure 4 shows the dislodgeable residues resulting from an application of chlorthiophos at a rate of 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1}. Chlorthiophos disappeared rapidly from the treated foliage. The 3-, 10-, and 17-day samples showed 0.23 ± 0.02, 0.061 ± 0.008 and <0.003 μg/cm^2, respectively. No oxon was found above the 0.01 μg/cm^2 level sought.

The oxon sulfoxide and oxon sulfone were determined

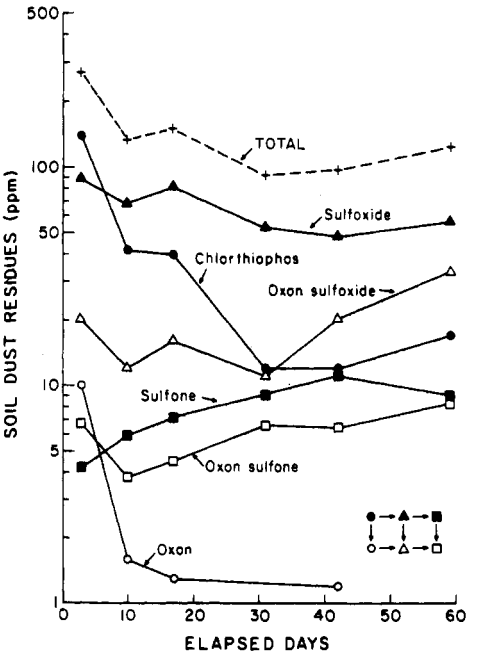

Figure 5. Residues of chlorthiophos and its oxidation products in the mobile soil dust collected from an orange grove. Trees had been treated at 9.5 lb of AI (1900 gal)$^{-1}$ acre^{-1}. Each datum point is the mean value obtained for three replicate field plot samples. Treatment was made on Aug 8, 1980, to trees located on the Citrus Research Center, Riverside, CA.

by using the colorimetric procedure of Gunther et al. (1980). The values for the oxon sulfone are believed to be too high since the residue levels found were greater than that of the oxon sulfoxide. Oxidation of the sulfoxide moiety to the sulfone would not be expected to be facile. The data are also inconsistent with the results obtained for rind residues (Figures 2 and 3) and soil dust residues (Figure 4) which were obtained by using a GC procedure. The very rapid drop of sulfoxide residues between 17 and 31 days was also unexpected due to the somewhat discontinuous nature of the residue level change. The only rainfall was an immeasurable trace recorded 10 days postapplication. For additional data, quantification of the oxon sulfoxide and the oxon sulfone should be done by using the pentyl ester GC method for best results.

Soil Dust. Residues resulting from spray drift and runoff from plant surfaces onto the mobile soil dust have been proposed as a source of toxicants to workers that move through the treated citrus grove. Toxicant-bearing dust could potentially become airborne through the action of wind or mechanical agitation and deposit on tree foliage or on the workers. Collection of soil dust residue data has become an integral part of recommendations for data acquisition in reentry studies. However, to date no use has been made of the data collected for a number of insecticides. Furthermore, unlike tree foliage, the soil surface characteristics of a citrus orchard are highly variable, depending on cultural practices and local soil characteristics. This makes the collected data even more intractable to meaningful interpretation. The actual importance of soil dust residues has not been completely verified. From data generated in parathion-treated orange groves, Spear et al. (1977) concluded that the dermal dose data obtained indicated that soil dust played a relatively unimportant role in the immediate exposure process; since ~80% of the dermal paraoxon dose obtained by the volunteers appeared to be deposited above the waist, the foliar residue was considered the more appropriate indicators of worker hazard.

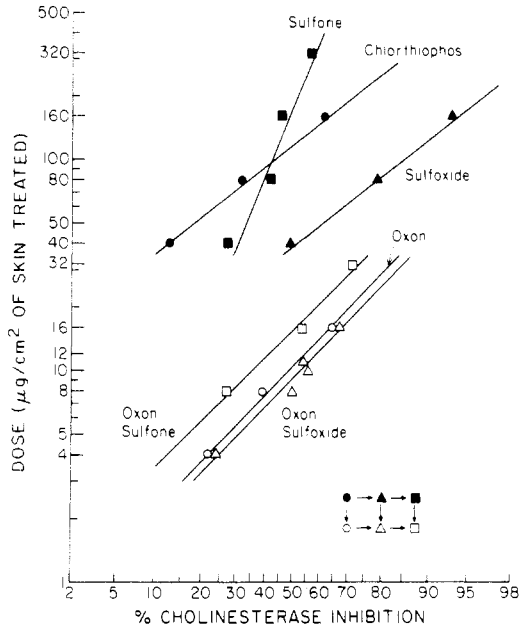

Figure 6. Percent red blood cell ChE inhibition in rats sacrificed 72 h after treatment of 25 cm^2 of skin surface with chlorthiophos or one of its five oxidation products.

Figure 5 shows the residue data for the fine (<100 mesh), dry soil dust collected from beneath the skirts of treated orange trees. Data are quite good considering the very difficult task of obtaining a representative sample at each sampling date when constrained to the use of a limited number of trees. Data points in Figure 5 are connected simply to show the qualitative trend in the data; smooth curves were too difficult to draw without indicating trends which did not necessarily exist due to the use of an insufficient number of data points. The residue levels found are not unusual and compare favorably with soil residues found for phenthoate (Iwata et al., 1981) and methidathion (Iwata et al., 1979). The results with respect to the various relative proportions of oxidation products parallel the data found for rind residues (Figures 2 and 3) obtained for unwashed fruits. Figure 5 shows the rapid disappearance of chlorthiophos oxon and confirms that it is formed and that it rapidly disappears by conversion into another product. Spencer et al. (1980a,b) have reported on the role of ozone and ultraviolet light in the formation of paraoxon from parathion.

Dermal Toxicity. Dermal absorption of toxic materials is considered to be the principal cause of ChE depression among workers who engage in extensive and prolonged contact with insecticide-treated foliage. Thus, dermal toxicity data are needed for the parent insecticide and all its ChE-inhibiting alteration products which may be found as postapplication residues on foliage. Figure 6 shows the dermal dose–ChE response curves for rats sacrificed 72 h after treatment of 25 cm^2 of skin surface with chlorthiophos or one of its five oxidation products. The data are for red blood cell (RBC) ChE because it is a better index of exposure than plasma ChE. Small quantities of OP compounds give greater inhibitory response of RBC ChE, and greater slopes for dose–response curves are obtained. Values for the slopes of the dose–response curves in Figure 6 are given in Table II.

Calculation of Allowable Residue Levels on Foliage. Safe reentry levels ideally should be based on actual field experiments using human subjects. Kahn (1979) has outlined a guide for the performance of field studies to establish safe reentry intervals for OP insecticides. However, the logistics of the desired experiments are formidable, and the experiments are further burdened with a high cost and ethical considerations. Thus, it is desirable to obtain as much use of the available field experiment data as possible.

Table II. Establishment of Safe Residue Levels ($\mu g/cm^2$) on Citrus Tree Foliage Using the Results of Dermal Dose–ChE Response Curves and Field Reentry Studies according to Knaak et al. (1980)

insecticide or alteration product[a]	slope of dose–response curve[b]	ED_{50}, $\mu g/cm$, of total body surface[c]	relative toxicity[d]	safe level on foliage, $\mu g/cm$[e]
chlorthiophos sulfoxide	2.5	3.1	9.4	0.19
chlorthiophos	2.4	8.8	27	0.54
paraoxon	2.3	0.33	1	0.02[f]
chlorthiophos oxon sulfone	2.0	1.2	3.6	0.07
chlorthiophos oxon sulfoxide	1.9	0.69	0.29	0.03
parathion	1.3	2.4	1	0.09[f]
azinphosmethyl	0.9	25	1	3.0[g]
chlorthiophos sulfone	0.8	13	0.53	1.6

[a] Reference compound is underlined; this compound is one for which actual field safety information is available. [b] Slopes derived from Figure 6 for chlorthiophos and its alteration products. Data used to construct the figure were statistically analyzed according to the Log-Probit Analysis procedure of Finney (1972). [c] ED_{50} in $\mu g\ cm^{-1}$ multiplied by 25 cm^2 (treated area) and divided by 325 cm^2 (total surface area of the rat). [d] ED_{50} of the compound under investigation divided by the ED_{50} of the underlined reference compound. [e] Relative toxicity multiplied by the established safe level of the reference as determined by actual reentry studies. [f] Spear et al. (1977). [g] Richards et al. (1978).

Knaak et al. (1980) proposed a method for setting reentry levels for new compounds based upon available field study data. Calculations of safe levels for chlorthiophos and its oxidation products on foliage calculated according to Knaak et al. (1980) are given in Table II. On the basis of the slope of the dose–response curves generated for ChE inhibition resulting from dermal absorption (Figure 6), compounds are grouped with other compounds that have similar slope values. One compound in the group must have established safety information, and its serves as a reference standard. The relative toxicity of the new compound relative to the reference compound is obtained by dividing the ED_{50} of the new compound by that of the reference compound. This factor multiplied by the empirically derived safe level of residues on foliage of the reference compound gives the calculated safe level for the new compound.

Thus, the safe levels for chlorthiophos, its sulfoxide, its sulfone, its oxon sulfoxide, and its oxon sulfone are 0.54, 0.19, 1.6, 0.03, and 0.07 $\mu g/cm^2$ on foliage, respectively. The oxon was disregarded as it was not found on foliage above the 0.01 $\mu g/cm^2$ level (Figure 4). These calculated levels can be used to set reentry intervals by reference to a dislodgeable residue curves such as Figure 4. The dislodgeable residue curve should be representative of actual field levels.

A difficulty in the above approach is that only one toxic compound is assumed to be present rather than a mixture of several toxic compounds. One is forced to make a reasoned evaluation of the total data. Since chlorthiophos

Table III. Procedure for Establishing Safe Levels ($\mu g/cm^2$) of Total Thions Plus Oxons of Chlorthiophos on Citrus Tree Foliage according to Knaak and Iwata (1981)

days after spraying	residues, $\mu g/cm^2$ thion[a]	oxon[b]	thion + oxon	thion plus oxon × RT[c]	(thion + oxon)/(thion + oxon × RT) × SL for thion,[d] $\mu g/cm^2$
20	0.12	0.11	0.23	0.60	0.07
40	0.04	0.09	0.13	0.43	0.06
60	0.04	0.07	0.11	0.37	0.06

[a] Since no chlorthiophos is present at or after 20 days, thion residues are the sum of chlorthiophos sulfoxide and sulfone. Values were obtained from Figure 4. [b] Since no chlorthiophos oxon is present at or after 20 days, oxon residues are the sum of chlorthiophos oxon sulfoxide and sulfone. Values were obtained from Figure 4. [c] RT (relative toxicity) is the ED_{50} of the chlorthiophos sulfoxide divided by the ED_{50} of the oxon sulfoxide. This RT differs in definition from that in Table II. [d] SL (safe level) for the thion is 0.19 $\mu g/cm^2$ as given in Table II for the most toxic thion, chlorthiophos sulfoxide.

reaches its calculated safe level about 1 day postapplication, its sulfoxide reaches its safe level about 5 days postapplication, and its sulfone is always far below its safe level, only the oxon sulfoxide and oxon sulfone needed to be considered. The safe level for each of these two compounds is reached about 30 days postapplication. As a first approximation, a 60-day reentry interval could be set since both compounds have dropped to about 0.02 $\mu g/cm^2$ by this time.

Knaak and Iwata (1981) proposed a method for dealing with total dislodgeable residues. Calculations based on this method are given in Table III for actual residues found on foliage after 20, 40, and 60 days postapplication as shown in Figure 4. Since no chlorthiophos was present at or after 20 days, the sulfoxide and the sulfone residues were added together and designated "thion". Since no oxon was found, the oxon sulfoxide and the oxon sulfone residues were added together and designated "oxon". Total thion and total oxon residues are assumed to be sulfoxide and oxon sulfoxide, respectively, since these are the most toxic compounds of each pair present. The oxon is converted to thion equivalents by using the ratio of the relative toxicity (ED_{50}) of one to the other. The data are used to arrive at a safe level of 0.06 $\mu g/cm^2$ total residues which translates to 70 days based on the *total* residue curve given in Figure 4.

The U.S. Environmental Protection Agency dealt with the reentry question in its May 5, 1981, draft of "Guidelines for Registering Pesticides in the United States; Subpart K; Exposure Data Requirements: Reentry Protection" (U.S. Environmental Protection Agency, 1981; Adams, 1981). A no effect level (NOEL) derived from data required under the guideline's Subpart F is required. A correlation between the foliar dislodgeable residues and the whole body dermal dose and a dislodgeable residue dissipation curve, both obtained under field conditions which approximate the intended use of the insecticide, is required.

The EPA document contains a model for the calculation of a reentry interval. According to the model, an allowable exposure level (AEL) is calculated from the lowest of the NOELs for the pesticide. The NOEL values would include the results, for example, of cholinesterase inhibition, teratogenicity, and reproductive-effect tests. A safety factor (SF) is used (10 is frequently used for cholinesterase inhibitors but other factors have been used), and a dermal penetration (DP) of the pesticide residues is assumed to

Table IV. Calculation of Reentry Intervals according to EPA Guidelines (U.S. Environmental Protection Agency, 1981) with Slight Modifications[a]

day	compound ratio[b]	NOEL,[c] $\mu g\ kg^{-1}\ day^{-1}$	AEL,[d] $\mu g\ kg^{-1}\ day^{-1}$	total dose,[e] $\mu g/h$	reentry level,[f] $\mu g/cm^2$
20	4:1:1:3	391	39.1	342	0.08
40	2:1:2:4	304	30.4	266	0.06
60	2:1:2:3	309	30.9	270	0.06

[a] The modification involves taking into account all toxic residues present on the foliage and using a total toxic residue level curve. [b] This is the ratio of sulfoxide:sulfone:oxon sulfoxide:oxon sulfone present on foliage as shown in Figure 4. [c] No effect level (NOEL) calculated from data from dermal dose–ChE response curve. NOEL = $ED_{10}(25\ cm^2)/0.23$ kg/day. Predicted $ED_{10} = P_1/ED_{10,1} + P_2/ED_{10,2} + \ldots + P_N/ED_{10,N}$, where P = proportion of component in mixture (Finney, 1972). ED_{10} values were extrapolated from Figure 6. ED_{10} for chlorthiophos, its sulfoxide, its sulfone, its oxon, its oxon sulfoxide, and its oxon sulfone was 35, 12, 4, 2, 2, and 3.5 $\mu g/cm^2$, respectively. [d] Allowable exposure level (AEL) = NOEL/SF. Safety factor (SF) = 10. [e] Total dose = (AEL)(body weight, 70 kg)/(duration, 8 h/day). [f] From total dose determine reentry level from graph of whole-body dermal dose ($\mu g/h$) vs. dislodgeable foliar residues (ng/cm^2) from data of Popendorf (1980) as abbreviated by U.S. Environmental Protection Agency (1981).

be 100% (i.e., 1.00) unles shown otherwise. Further assumptions are an 8-h working day and a 70-kg individual. The AEL in micrograms per hour is then (NOEL)(70)/(SF)(DP)(8).

For citrus, EPA utilizes an abbreviated form of the dermal dose–field residue correlation of Popendorf (1980). This correlation gives the allowable foliar residue level in $\mu g/cm^2$. This level is then used to obtain a reentry interval based on field dissipation curves.

Calculations based on EPA guidelines are given in Table IV. A number of modifications were made, however. Whereas EPA utilizes a NOEL, calculations herein were made by using an extrapolared ED_{10} value obtained from a dermal dose–ChE-inhibition graph for rats (Figure 6). Calculations also took into account all the toxic residues known to be present on foliage. The calculations indicated a safe level of 0.06 $\mu g/cm^2$ for total residues on foliage, and this value translates based on Figure 4 to a 70-day reentry interval. A value calculated based solely on the toxicity of chlorthiophos was 1.3 $\mu g/cm^2$; this value is higher than any value obtained in Figure 4.

It is obvious that the assumptions upon which the final values are based are numerous. The validity of some of the assumptions are tenuous. However, one must choose between inaction and attempting a first approximation to the sought answer. Three methods for calculating reentry intervals have been proposed, and calculations based on these proposals have been presented. All three methods support an ~70-day reentry time for chlorthiophos total residues. The agreement is not altogether surprising since the same basic principles, assumptions, and background data have been infused in varying degrees into all three approaches. The 70-day time is lengthy but not extraordinary; in California, parathion under certain usage conditions has a 60-day reentry interval. Regional variations in the reentry interval can be accommodated in that the whole-body dermal dose vs. dislodgeable foliar residue correlation and/or the dislodgeable residue dissipation curves can be generated for use on a regional basis. The data presented are valid for the experimental area in California, and it is recognized that the reentry period will

vary in other citrus growing areas.

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Potential Benlate Fungicide Exposure during Mixer/Loader Operations, Crop Harvest, and Home Use

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Potential exposure to Benlate fungicide was determined for three different agricultural use situations involving different types of exposure. Total exposure to benomyl was minimal during the mixing of Benlate for aerial application or during reentry into a treated field for crop harvest or during home use. Average potential dermal exposures for these three situations were 26, 12, and <1 mg of benomyl, respectively, with the major portion of the exposure on the hand and forearm areas. The average potential respiratory exposures for the three use situations were 0.08, 0.003, and 0.003 mg of benomyl, respectively. On the basis of the low dermal and respiratory toxicity of Benlate, these values do not contribute to a significant body dose.

Benlate fungicide, which contains benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolylcarbamate] as the active ingredient, is widely used to control a range of fungus diseases affecting over 30 different fruits, vegetables, field crops, and ornamentals. This paper addresses the question of potential human exposure to Benlate under a variety of use situations representing the extremes of potential exposure. These situations encompass mixing procedures for aerial application, reentry into treated fields, and home use (garden, ornamental, and greenhouse). They were selected in order to evaluate the total use pattern safety of Benlate.

Determination of potential exposure to pesticides has previously been studied in detail by Durham and Wolfe (1962). The procedures established in their studies have been successfully applied and reported by other investigators in the area of pesticide exposure (Staiff et al., 1975; Popendorf et al., 1979; Spear et al., 1977; Durham et al., 1972; Wolfe et al., 1959, 1961, 1975). By use of these established techniques in this study, Benlate potential dermal exposure was assessed by attaching absorbent pads to various parts of the body or clothing. Cotton gloves were worn to assess exposure to the hands. Respiratory exposure was monitored by the use of filter pads in specially modified respirators.

The results from this research indicate only minimal benomyl exposure in each of the three use situations studied. Maximum values, as expected, were noted in the mixing of Benlate prior to aerial application. In this use situation, the average dermal exposure was 26 mg of benomyl and the average total respiratory exposure was 0.08 mg of benomyl per mixing cycle.

EXPERIMENTAL SECTION

The materials and methods used in all three test situations have been described in detail by Durham and Wolfe (1962). All samplings for the measurement of potential exposure were collected under actual use conditions.

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