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Ethion Distribution in Florida Valencia Oranges

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When pesticide is applied to a citrus grove, part of the spray deposits on the fruit surface. Over time, some of this surface deposit penetrates into the pulp (Gunther 1969).

The Codex Alimentarius Commission (established by FAO and WHO in 1963) and the U. S. Environmental Protection Agency set pesticide tolerances for citrus fruit based on whole fruit (pulp plus peel) residues. Their present citrus tolerance for ethion is 2.0 ppm in the whole fruit (Carman and Lichty 1986). In 1969, the U.S. and Canada had a 2.0 ppm tolerance for ethion in whole fruit; the Netherlands had a 0.5 ppm tolerance (Gunther 1969). These tolerances are ultimately based on the acceptable daily intake (ADI), the amount considered safe from the total daily consumption of a particular pesticide from all dietary sources. The ADI set by the Codex Alimentarius Commission is 0.001 mg/kg bodyweight for ethion plus its oxygen analogues. Gunther (1969) studied the distribution of many pesticides in California citrus. With the exception of systemic pesticides, residues were low in pulp and resided primarily in peel. This raises the question whether tolerances should be based on whole fruit residues, a method which may seriously overestimate consumed (pulp) residues.

The purposes of this experiment were to determine the distribution of ethion, ethion monoxone, and ethion dioxone between peel and pulp of Florida Valencia oranges and to assess whether the ethion concentration for the entire fruit was representative of ethion concentration in the consumed portion.

MATERIALS AND METHODS

An 18-year-old, 20 acre Valencia on rough lemon rootstock grove was selected for this experiment. Ethion 4EC (4 lb AI/gal) had been applied at a rate of 120 oz ethion 4EC, combined with 1% FC 435-66 spray oil, in 1000 gal of water per acre in July 1982 or 1.7 kg AI/acre (3.75 lb AI/A). On April 14, 1983 (day -4), ethion was applied to four random 48-tree plots of mature Florida Valencia oranges at a rate of 12 oz ethion 4EC and 0.5% FC 435-66

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spray oil/800 gal water/A or 0.17 kg AI/A (0.375 lb AI/acre). The same plots were resprayed 4 days later (day 0) with 96 oz ethion 4EC/800 gal water/acre (1.36 kg AI/A; 3 lb AI/A) because the first treatment had been mistakenly applied at 0.125 the prescribed rate. Treatments were applied with airblast equipment. This grove had mature fruit on the trees, had bloomed 2 weeks prior to the application and also had grape-sized immature fruit on the same trees.

Ten mature fruit were clipped at random from each of the four replicated plots at days -4 (1 hr prespray), -3, 1, 3, 7, 9, 14, 21, 28, and 35. Fruit samples were peeled by hand with a fruit knife to remove the peel from the pulp. Five longitudinal cuts were made and the six peel sections were removed using the hand and the sharp edge of the knife. Although a 'cleaner' method has been recommended by Gunther (1969), the knife procedure used here more closely resembles consumer practices. Pulp and peel sample weights were taken, beginning with day 9, replicate D. Peel was sectioned into small pieces and the seeds were removed from pulp to aid in blending. Blending of pulp or peel was done with a commercial Waring blender at low rpm for 60 sec. Peel samples required periodic manual stirring to reintroduce peel to the blade area. A hundred gram aliquot of each plot sample was stored in Ziploc™ bags at -20°C until extraction.

On August 3, 1983 (day 107 after the final application), 10 immature Valencia fruit were clipped at random from each of the four replicated plots along with an unreplicated sample of 10 unsprayed immature fruit from an unsprayed area of the experimental grove. Previous to this date, the peel could not have been separated from the pulp in these immature fruit. Extraction of the peel and the pulp of immature fruit was by the same method as for mature fruit.

Fifty grams of the sample (pulp or peel) were extracted with 100 ml 80:20 methylene chloride:acetone by blending in a 250 ml Sorvall omni-mixer blending cup at high rpm for 1 min. Extracts were decanted through silanized glass wool into a 500 ml separatory funnel. The glass wool and powder funnel were rinsed with 50 ml of extraction solvent and the extraction procedure was repeated on the debris in the blender cup. Fifty ml of water was added to the blender for each peel extraction to aid in solvent partitioning. No water was added to pulp extractions. The second extraction was filtered into the separatory funnel, the solvent and water layers were allowed to separate, and the solvent layer was carefully drawn off into a 500 ml boiling flask. The extract was reduced almost to dryness on a rotary evaporator at 40°C and transferred in three rinses into 10 ml of isooctane for gas-liquid chromatography. Ethion, ethion monoxone, and ethion dioxone were quantified on a Tracor 550 dual column gas chromatograph equipped with a Melpar flame photometric detector (phosphorous mode). The silanized glass column used for detection of ethion and ethion monoxone was 1.8 m x 2 mm ID packed with 5% OV-210 on 80/100 mesh Gas-Chrom Q™ (Applied

Science Laboratories, Inc., State College, PA). In order to detect ethion dioxone at high sensitivity, all samples were reanalyzed with a column length of 46 cm. Operating conditions were: N_2 carrier gas 40 ml/min; air 100 ml/min; H_2 60 ml/min. Oven, detector, inlet, and outlet temperatures were 190°, 285°, 205°, and 200°C, respectively. All injections were 5 ul. Retention times were 6.8 and 9.2 min for ethion and ethion monoxone, respectively, with the 1.8 m column, and 3.6 min for ethion dioxone with the 46 cm column. Recoveries (four replications, mean ± S.E.M.) of standards from fortified blended peel at 1 ppm ethion and ethion monoxone and 1.5 ppm ethion dioxone were: 96 ± 4% for ethion, 72 ± 4% for ethion monoxone, and 73 ± 10% for ethion dioxone. Recoveries of standards from fortified blended pulp were: 87 ± 2% for ethion, 76 ± 3% for ethion monoxone, and 85 ± 2% for ethion dioxone. Based on a GLC sensitivity limit of 0.5 ng for ethion, ethion monoxone, and ethion dioxone, with a 5 ul injection, the quantitative detection limit of this method for all three compounds was 0.02 ppm. Observed quantities between 0.02 ppm and 0.01 ppm are reported as a trace; those below 0.01 ppm are reported as non-detectable. Standard ethion (95.1%), ethion monoxone (90.4%), and ethion dioxone (55%) were supplied by FMC Corporation, Middleport, NY 14105.

RESULTS AND DISCUSSION

Total ethion levels in whole mature fruit declined from 1.8 ppm 3 days after all spraying had ceased to 0.6 ppm 35 days thereafter (Table 1). The mature fruit sample collected on day -4, one hour before the 1/8X application, contained ethion in the peel only at 1.6 ppm. This mature, harvestable crop, treated 9 months prior to sampling, contained ethion pulp residues below 0.02 ppm (unquantifiable trace level) and no detectable ethion monoxone or ethion dioxone. The sample collected on day -3, contained 0.7 ppm ethion in the peel. The ethion peel residues of the pre- and postapplication samples were not statistically different ($p < 0.05$), owing to the large sampling variance in the 1.6 ppm value.

The second application of ethion on day 0, plus the residual ethion from the July 1982 application and the 1/8X application, resulted in ethion levels in the peel on day +1 of 6.1 ppm. Gunther (1969) found an ethion residue in Valencia peel of 6.4 ppm 30-40 days after an application of 1.5 lb AI/acre, about 1/4 the rate applied here. The day +1 pulp residue of 0.16 ppm ethion probably resulted from the application on day 0. Ethion declined by day +7 to levels of 3.3 ppm in peel and 0.02 ppm in pulp. These levels persisted over the next 2 weeks. Ethion monoxone remained below detectable limits (0.02 ppm) in the pulp, but appeared on day +3 in the peel, reaching a maximum of 0.23 ppm on day +21, and showed little attenuation even by day +35. Ethion dioxone was undetectable in both peel and pulp throughout the experiment.

Previous studies of the distribution and persistence of ethion in citrus peel and pulp have not been undertaken in Florida. A com-

Table 1. Ethion concentration in peel and pulp of mature Valencia oranges (ppm, ug/g)

Day	Peel (ppm)		Pulp (ppm, ug/g)		Whole fruit
	Ethion (E)	Monoxone (M)	Ethion	Monoxone	E+M(ppm)
- 4[a]	1.6 ± 0.7[b]	ND[c]	Tr[d]	ND	0.4
- 3	0.7 ± 0.1	ND	Tr	ND	0.2
+ 1	6.1 ± 0.9	ND	0.16 ± 0.01	ND	1.6
+ 3	7.0 ± 0.5	0.15 ± 0.00(4)	0.09 ± 0.01	ND	1.8
+ 7	3.3 ± 1.0	0.10 ± 0.03	0.02 ± 0.01	ND	0.9
+ 9	4.5 ± 0.3	0.16 ± 0.01	0.02 ± 0.01	ND	1.2
+14	3.9 ± 0.2	0.19 ± 0.02	0.03 ± 0.00(3)	ND	1.0
+21	3.5 ± 0.2	0.23 ± 0.04	0.02 ± 0.00(1)	ND	0.9
+28	2.0 ± 0.2	0.16 ± 0.01	Tr	ND	0.5
+35	2.3 ± 0.2	0.21 ± 0.02	Tr	ND	0.6

[a]Prespray, [b]Mean ± standard error, four replications of 10 fruit each, [c]None detected (< 0.01 ppm), [d]Trace (0.01 - 0.02 ppm). Ethion dioxone was not detected in peel or pulp.

parison of our results can be made to the persistence of ethion on citrus leaf surfaces from a study conducted by Nigg et al. (1977) in Florida. By day +7 postspray, the surface ethion concentrations (ng/cm^2) were near 50% of day +1 levels for a Nov.-Dec. experiment but only about 10% of day +1 levels for a May-June experiment. This compares with a corresponding figure of 54% ethion reduction in peel and 13% in pulp by day 7 post-application (Table 1). By day +21, ethion leaf surface concentrations had declined to about 10% of day +1 levels for the Nov.-Dec. experiment and to non-detectable levels by day +21 in the May-June experiment. The corresponding +21 day percentages from Table 1 are 57 and 13%. Persistence of ethion within the peel of an orange clearly exceeds ethion persistence on the leaf surface.

Ethion monoxone in the Nigg et al. (1977) leaf study reached a maximum on day +1 postspray or sooner in both experiments, declining to about 40% of maximum by day +14 in the Nov.-Dec. experiment and 20% in the May-June experiment. Maximum levels of monoxone in peel (Table 1) had not been reached at day +14. Ethion monoxone appears to persist longer in orange peel than leaf surfaces. Very little ethion dioxone was detected in the Nigg et al. (1977) leaf study and none was detected in this study. Another investigation by Nigg et al. (1979) showed very little penetration from peel into pulp in five different mature malathion-treated Florida citrus fruit varieties. Malathion levels in fruit peel were a few ppm while only traces were detected in fruit pulp, consistent with the results obtained here for ethion. No malaoxon was found in peel or pulp. Carmargo et al. (1982) also found no insecticide residues penetrating into the pulp of carbophenothion and phenthoate-treated Hamlin oranges in Brazil.

Recording total (wet) weights of peel and pulp for each 10-fruit sample of mature fruit allowed a more direct determination of the

proportion of ethion residing in the peel and in the pulp. These weights were taken from day +9, replicate D, onward. Table 2 presents these data. Table 3 gives the same data for samples collected prior to sample 9D, but with the assumption that the peel weights and pulp weights were 55 and 168 g, respectively, the mean values from Table 2. The data from these tables suggest that the pulp contains a very small percentage of the whole fruit ethion, ranging from 7.8 $\pm$ 1.8% one day postspray to 2.4 $\pm$ 0.2% one week postspray and thereafter. With a 2.4% partitioning of ethion into the pulp, the CODEX whole fruit ethion tolerance of 2 ppm (~ 446 ug ethion) would imply a consumed level of only 0.06 ppm (0.024 x 446 ug ÷ 168 g). Alternatively, a 2 ppm tolerance level for the consumed portion (~ 336 ug ethion) would translate into a 63 ppm ([336 ug ÷ 0.024] ÷ 223 g) tolerance for the whole fruit. This analysis is based, of course, on the assumption that none of the peel is consumed, whereas small amounts of peel may be consumed in marmalade, pastry, etc., and further that customary application and harvesting procedures are followed. Generalization of these results to other varieties of citrus is unwarranted since peel characteristics vary with variety.

At day +107, immature fruit contained ethion at 0.045 $\pm$ 0.006 ppm (mean $\pm$ SEM, four replications) within the peel, but ethion was undetectable (<) in the pulp. No ethion monoxone or ethion dioxone was detected in either the peel of pulp of the immature fruit. These immature fruit would have been due for harvest in April or about 1 year after application. Samples of immature fruit from unsprayed portions of the experimental grove were uniformly negative for all three compounds.

Treated immature fruit and treated mature fruit samples were collected from the same trees. The much lower residue concentration in the immature peel vs. the mature peel samples resulted in part from the fact that immature fruit samples were collected on day 107 postspray, allowing more time for ethion dissipation than was the case with the mature peel ($\leq$ 35 days) and in part from three applications of ethion to the mature fruit versus one application to the immature fruit. But another factor is the greater opportunity for residue dilution in the immature fruit resulting from normal fruit growth. Allen (1976) has modeled the growth of Valencia oranges in this region of Florida. While the increase in peel surface area of the mature Valencias over 35 days is minimal, immature Valencias typically have 80 times the surface area on August 3 as on April 14, according to Allen's study. This factor alone is enough to account for the ethion concentration difference between mature and immature fruit peel observed here.

The application date in this study was a typical treatment period, but ethion is not a popular pesticide for use in April. Ethion is normally applied once to Florida Valencia oranges in mid-June to late July when immature fruit are one-third to one-half their mature surface area, with harvest occurring the following April to mid-June. This allows considerably more time

Table 2. Ethion in peel and pulp of whole mature Valencia oranges based on peel and pulp weight

Day	Replication[a]	Weight[b] Peel (g)	Weight[b] Pulp (g)	Total Ethion Peel (ug)	Distr. (%)	Pulp (ug)	Distr. (%)
9	D	45.68	152.55	188.6	97.5	4.88	2.5
14	A	61.80	192.90	240.1	97.7	5.59	2.3
	B	62.43	200.41	274.9	97.7	6.47	2.3
	C	49.32	144.38	159.4	97.2	4.66	2.8
	D	45.09	147.99	175.2	98.4	2.87	1.6
21	A	48.81	153.30	178.9	98.1	3.51	1.9
	B	52.87	167.00	199.4	98.2	3.52	1.7
	C	60.27	167.95	220.6	98.7	2.96	1.3
	D	58.74	186.35	159.1	98.0	3.28	2.0
28	A	53.94	168.70	102.2	> 96.7	Tr[c]	< 3.3
	B	56.91	167.68	136.6	> 97.5	Tr	< 2.5
	C	51.73	162.99	103.5	> 96.9	Tr	< 3.1
	D	57.71	168.28	97.2	> 96.5	Tr	< 3.5
35	A	53.81	164.26	121.2	> 97.3	Tr	< 2.7
	B	55.13	168.44	103.4	> 96.7	Tr	< 3.3
	C	57.51	174.12	170.1	> 98.0	Tr	< 2.0
	D	56.78	174.61	118.3	> 98.5	ND[d]	< 1.5
		55+5[e]	168+15[e]				

[a]Ten fruit per replication, [b]Mean weight per fruit, [c]Trace (< 0.02 ppm), [d]None detected (< 0.01 ppm), [e]Mean ± standard deviation

for residue dissipation and/or dilution through growth than for the mature fruit of Tables 1-3. Most mature Valencia oranges have been harvested by July, but can still be on the same tree with immature fruit during the mid-June to late July treatment period. The mature fruit ethion peel and pulp concentrations reported in this study reflect a "worst-case" situation. The day -4 data better reflect residues reaching the consumer, consistent with normal grower practices in Florida.

Pesticide residues determined in citrus pulp may theoretically be a result of the peeling process. Contamination of fruit pulp during the peeling process is difficult to avoid. However, from the data in Table 1, the loss in peel residue from day +1 to day +21 was less than 50%. For the same period, the pulp residue decreased almost 90%. For the period +3 to +21 days, the peel residue loss was about 50%; the pulp residue loss was about 75% for this same period. If the pulp residues were due strictly to contamination by the peel, the peel and pulp losses should be approximately equal. We consequently assume that the pulp residues reported here were penetrated residues.

Citrus fruits are not impermeable to pesticides and some penetration into the edible portion is to be expected. Gunther (1969) presents a long list of pesticides which penetrated into the pulp of oranges and lemon, but at very low levels. Brown and Albrigo

Table 3. Calculated ethion in peel and pulp of whole mature Valencia oranges

Day	Replication[a]	Total Ethion[b] Peel (ug)	Distr. (%)	Pulp (ug)	Distr. (%)
-4	A	158.4	> 99.3	Tr[c]	< 0.7
	B	140.3	> 99.2	Tr	< 0.8
	C	30.0	> 96.3	Tr	< 3.7
	D	18.4	> 94.0	Tr	< 6.0
-3	A	28.1	> 96.1	Tr	< 3.9
	B	46.9	> 97.7	Tr	< 2.3
	C	34.6	> 96.8	Tr	< 3.2
	D	33.4	> 96.7	Tr	< 3.3
1	A	368.0	93.4	26.04	6.6
	B	394.4	94.4	23.52	5.6
	C	237.6	88.7	30.24	11.3
	D	Sample lost	--	24.36	--
3	A	408.1	96.3	15.78	3.7
	B	451.0	95.7	20.51	4.3
	C	341.0	96.4	12.62	3.6
	D	342.7	95.9	14.83	4.1
7	A	24.3	> 97.7	ND[d]	< 2.3
	B	265.4	97.8	5.88	2.2
	C	207.3	> 99.5	Tr	< 0.5
	D	220.8	97.8	4.87	2.2
9	A	269.7	> 99.8	ND	< 0.2
	B	283.9	> 99.8	ND	< 0.2
	C	220.0	96.3	8.37	3.7

[a]Ten fruit per replication, [b]Assuming peel weight = 55 g, pulp weight = 168 g; see Table 2, [c]Trace (< 0.02 ppm), [d]None detected (< 0.01 ppm)

(1972) found 0.005 ppm of benomyl in the pulp of sprayed fruit after 1 day and 0.109 ppm after 14 days. Fruit treated post-harvest by dipping contained 0.14 ppm benomyl in the pulp after 14 days (Brown 1974). Residues of imazalil were 0.2 ppm in the pulp of Valencia oranges after 3 days and 0.6 ppm after 42 days (Brown et al. 1983).

In this experiment, however, ethion penetrated into the pulp of Valencia oranges at very low levels, even in a "worst case" situation. These residues were well below the 'whole fruit' tolerances adopted by most countries and were below all 'whole fruit' tolerances by day 7. These pulp residues disappeared rapidly. Since the mature fruit in this experiment were physiologically inactive, similar disappearance of ethion pulp residues may also occur after harvest.

Under normal usage in Florida (see day -4, Table 1), only unquantifiable residues of ethion would be found in the pulp of fruit eaten fresh by the consumer. This level may be reached well ahead of harvest, probably within 90 days after treating

immature fruit.

Whole citrus fruit regulations for the CODEX countries (1978) allow a maximum limit of 2 ppm of ethion plus ethion monoxone in the whole fruit. The Dutch Ministry of Health has exercised its option to reduce this allowable level to 0.1 ppm. But according to the data presented here, a whole fruit level of 2 ppm translates into a 0.02 ppm level (or lower) in the consumable pulp 1 week postspray and thereafter.

For regulatory purposes, fruit penetration data should be produced with the same amount of formulation and varying amounts of water to match dilute to ultralow volume applications. If these data so indicate, then pesticide residue tolerances should be based on consumed pulp only, rather than on whole fruit. Otherwise, they will have no basis in fact for consumer safety.

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