2,4-D (020)

EXPLANATION

2,4-D was evaluated for residues within the CCPR Periodic Review Programme by the JMPR in 1998. The Meeting recommended numerous MRLs including an MRL of 0.1 mg/kg for grapefruit and oranges based on four supervised trials of the minor pre-harvest use as a plant growth regulator, and recommended withdrawal of the existing CXL of 2 mg/kg for citrus fruits.

The 32nd Session of the CCPR in 2000 decided to retain the CXL for citrus fruits, as the governments of South Africa, Uruguay and the USA wished to accommodate post-harvest use, and Spain also preferred not to have MRLs for individual citrus fruits. The governments of the USA and Spain informed the CCPR that additional residue trials would be reported to the JMPR. The Netherlands and South Africa disagreed with the evaluation of the data on which the proposed separate MRLs for oranges and grapefruit was based (ALINORM 01/24, para 89).

The 2001 Meeting received information on GAP and supervised residue trials for the post-harvest use of 2,4-D on lemons and oranges.

USE PATTERN

The post-harvest use of 2,4-D isopropyl ester (common name 2,4-D-isopropyl) is registered on lemons in the USA and on citrus fruits in Uruguay. 2,4-D-isopropyl is applied in a water-wax emulsion or as a diluted flush or spray followed by waxing in the packing house to inhibit abscission of buttons on harvested fruits. The registered uses are shown in Table 1 where application rates are expressed as acid equivalents (kg ae/hl).

Table 1. Registered post-harvest uses of the 2,4-D isopropyl ester.

Crop	Country	Method, label instructions	Spray conc. (kg ae/hl)[1]	No.	WhP[2], days
Lemons	USA	Post-harvest packing house use to maintain healthy buttons on lemons. Added to wax emulsions on lemons waxed before storage. Otherwise as a special treatment (flush or spray) after the last fresh-water rinse.	0.042	1	-
Grapefruit Lemons Mandarins Oranges	Uruguay	Post-harvest, as wax or as a pre-waxing spray.	0.042	1	-

[1] Factor 0.84 (molecular weight 2,4-D acid 221, molecular weight 2,4-D-isopropyl 263.12)
[2] Withholding period

RESIDUES RESULTING FROM SUPERVISED TRIALS

Johnson and Strickland (1995). Two supervised trials on the post-harvest use of 2,4-D on lemons in California, USA, were reported to the 1998 JMPR. However there were too few trials for the estimation of a maximum residue level. The results are repeated in Table 2.

Table 2. Results of two US trials on the use of 2,4-D on lemons reported to the 1998 JMPR (Johnson and Strickland, 1995). Whole fruit analysed.

Site no./Location	Variety	Application rate		Date of treatment	Growth stage	Residue (mg/kg)	PHI or WHP (days)	Remarks
		g ae/ha (g ae/hl)	water (l/ha)					
Site 1 Tulare County, CA	Eureka	56 (1.3)	4456	12/6/94	Immature	0.06, 0.05 <0.05 (2)	0 7	pre-harvest
		(50)		12/15/94		0.42 0.29 0.61 0.41	0 28 56 112	post-harvest[1] in storage
Site 2 Ventura County, CA	Lisbon	56 (1.2)	4663	12/20/94	Immature	<0.05, 0.05 <0.05 (2)	0 7	pre-harvest
		(50)		12/28/94		0.54 0.4 0.52 0.5	0 28 56 112	post-harvest[1] in storage

[1] Fruits harvested 7 days after pre-harvest treatment were treated in storage.

Johnson and Strickland (2001). In 2001 six groups of Navel oranges and four of lemons were treated, and one group of each used as controls. Fifty individual Navel oranges and 50 lemons (1st 4 trials) and 50 Navel oranges (5th and 6th trials) were sprayed with a 0.05 kg ai/hl 2,4-D-isopropyl solution (0.042 kg ae/hl). Twenty oranges and 20 lemons were randomly collected from each trial. All trials complied with label directions and simulated standard industry practices. Duplicate solutions were prepared for duplicate treatments.

For post-harvest use 2,4-D-isopropyl is applied in a water-wax emulsion or as a diluted flush or spray followed by waxing in the packing-house. Trials conducted before preparation of the protocol indicated that 2,4-D residues were slightly higher after aqueous spray applications (mean 0.26 mg/kg) than after wax emulsions (mean 0.16 mg/kg). Post-harvest aqueous spray applications were therefore made to maximize potential residues.

Control samples were handled in the same way as treatment samples except that they were sprayed with a blank formulation. Each replicate consisted of 20 fruits weighing an average of 2.5 kg (Navel oranges) or 1.3 kg (lemons). All were frozen immediately after treatment and subdivided into two 10-fruit samples. The fruits were homogenized and two 10-g sub-samples from each batch were extracted and analysed for 2,4-D residues by gas chromatography (GLC).

The Navel oranges were harvested on 17 January 2001 and collected from the packing house on the next day. The Lisbon lemons were harvested on 16 January 2001 and collected the next day. The fruit were transported on the day of collection to the Sunkist Research Station and placed in a cold room (approximately 1.7°C). The fruit did not receive any pre-harvest 2,4-D treatments or post-harvest applications of any kind.

In the first four treatments on 23 January 2001 50 Navel oranges and 50 lemons were treated at the same time according to label directions and industrial practice. Two extra treatments were applied to the oranges. All the fruit were put through a 10% solution of a commercial foamer wash, rinsed with fresh water, and dried. Control fruit were then sprayed with a blank formulation, and the treated fruit at 0.05 kg ai/hl 2,4-D-isopropyl.

For each run the fruit were placed on a conveyor belt and rubber balls were used to push the sample through the line to ensure the samples maintained a constant flow rate. The treatment and control solutions were applied through four nozzles which coated the fruit as they passed underneath. The spray solution and wet fruit also coated several rows of plastic brushes that the fruit rolled through after being sprayed. The total contact time measured with a stopwatch was approximately 22

seconds. The fruit were next conveyed through foam brushes, where they were partially dried and then onto wax brushes to apply a commercial storage wax solution consisting of 12.5% storage wax and 87.5% soft water. Then the fruit were conveyed through a forced-air dryer (52 - 57°C) to dry the wax and samples were placed in plastic bags off the packing line, labelled, placed in a second plastic bag, and immediately placed in freezers. The next day samples were shipped on dry ice via overnight express for determination of the residues.

One approximately 50-ml sample of each control and treatment solution was collected immediately before each treatment and analysed to establish the actual concentration of active ingredient in the application solutions.

Analytical methods. 2,4-D in unpeeled raw citrus fruit was determined by a fully-validated method. All individual fruit included in each 10-fruit sample were homogenized. The ground samples were stored frozen at approximately -20°C. Two 10 g sub-samples of the homogenized sample were analysed. 2,4-D was isolated by extraction with 0.7 M NaOH for 1 hour at 100°C. An aliquot of the extract was acidified with sulfuric acid and extracted with ether. The 2,4-D in the extract was then converted to its methyl ester using a boron trifluoride/methanol solution. After water was added, the sample was taken up in hexane and analysed by gas chromatography with a mass selective detector (GLC-MSD). The limit of quantification (LOQ) for raw citrus fruit was 0.05 mg/kg. All samples were analysed within 20 days of treatment.

5-ml samples of application solution mixes were hydrolyzed in aqueous sodium hydroxide solution to convert any esters or salts of 2,4-D to the sodium salt. The sample was then acidified with sulfuric acid to convert all 2,4-D to the free acid, saturated with sodium chloride, and partitioned into ethyl ether. The ether was evaporated and the residue treated with boron trifluoride-methanol solution to convert 2,4-D acid to the methyl ester. Water was added to the reaction mixture and 2,4-D-methyl partitioned into hexane and determined by GLC-MSD. The concentration in the application solution was calculated as 2,4-D-isopropyl. The LOQ was 0.1 mg/kg for application solutions.

Results. Residues were calculated as acid equivalents in mg ae/kg. 2,4-D residues in all control orange and lemon samples were <0.05 mg/kg. Mean 2,4-D residues in orange samples treated with the 0.05 kg ai/hl 2,4-D-isopropyl spray solution were 0.2 mg/kg. The maximum residue found in any Navel orange sample was 0.27 mg/kg (Table 3). Mean residues in lemons treated with the 0.05 kg ai/hl 2,4-D-isopropyl spray solutions were 0.395 mg/kg, and the maximum residue found in any lemon sample was 0.65 mg/kg (Table 4).

Table 3. 2,4-D residues at day 0 in Navel oranges, whole fruit (Johnson and Strickland, 2001).

Sample No.	Treatment (kg ae/hl)	Treatment sample	Analytical sample	2,4-D residues (mg ae/kg)	
				measured	mean
101-014-05	Trial 1 0.042	1	1	0.154	0.16
			2	0.166	
101-014-06		2	1	0.270	0.24
			2	0.214	
101-014-07	Trial 2 0.042	1	1	0.235	0.22
			2	0.207	
101-014-08		2	1	0.252	0.22
			2	0.186	
101-014-09	Trial 3 0.042	1	1	0.144	0.16
			2	0.172	
101-014-10		2	1	0.179	0.2
			2	0.218	

Sample No.	Treatment (kg ae/hl)	Treatment sample	Analytical sample	2,4-D residues (mg ae/kg)	
				measured	mean
101-014-11	Trial 4 0.042	1	1	0.197	0.19
			2	0.189	
101-014-12		2	1	0.218	0.21
			2	0.204	
101-014-13	Trial 5 0.042	1	1	0.204	0.2
			2	0.194	
101-014-14		2	1	0.213	0.21
			2	0.210	
101-014-15	Trial 6 0.042	1	1	0.183	0.19
			2	0.203	
101-014-16		2	1	0.183	0.19
			2	0.192	

Table 4. 2,4-D residues at day 0 in whole lemons (Johnson and Strickland, 2001).

Sample No.	Treatment (kg ae/hl)	Treatment sample	Analytical sample	2,4-D residues (mg ae/kg)	
				measured	mean
101-014-17	Trial 1 0.042	1	1	0.337	0.33
			2	0.315	
101-014-18		2	1	0.654	0.6
			2	0.554	
101-014-19	Trial 2 0.042	1	1	0.297	0.33
			2	0.354	
101-014-20		2	1	0.382	0.41
			2	0.429	
101-014-21	Trial 3 0.042	1	1	0.338	0.36
			2	0.375	
101-014-22		2	1	0.352	0.37
			2	0.383	
101-014-23	Trial 4 0.042	1	1	0.404	0.44
			2	0.477	
101-014-24		2	1	0.310	0.33
			2	0.344	

The formulation blank solution used to spray control orange and lemon samples contained 0.145 mg/l 2,4-D-isopropyl. Mean 2,4-D-isopropyl concentration in the six treatment solutions ranged from 106% to 136% of the nominal concentration of 0.05 kg ai/hl (Table 5), and the mean for all six treatments was 121% of nominal.

Table 5. 2,4-D-isopropyl concentrations in applied solutions (Johnson and Strickland, 2001).

Sample No.	Solution	Analysis	2,4-D-isopropyl (kg ai/hl)	Treatment mean (kg ai/hl)	% of nominal
101-014-25	Control 1	1	0.000015	0.000014	
		2	0.000014		
101-014-26	Trial 1	1	0.075	0.068	136
		2	0.062		
101-014-27	Trial 2	1	0.062	0.061	122
		2	0.059		
101-014-28	Trial 3	1	0.058	0.062	124
		2	0.067		
101-014-29	Trial 4	1	0.054	0.054	108
		2	0.054		
		1	0.051		

Sample No.	Solution	Analysis	2,4-D-isopropyl (kg ai/hl)	Treatment mean (kg ai/hl)	% of nominal
101-014-30	Trial 5	2	0.056	0.053	106
101-014-31	Trial 6	1	0.060	0.064	128
		2	0.068		

APPRAISAL

2,4-D was evaluated for residues in a periodic review by the JMPR in 1998, and many MRLs were recommended. In the case of citrus fruits, the JMPR estimated a maximum residue level of 0.1 mg/kg for grapefruit and orange on the basis of four supervised trials conducted according to minor pre-harvest use as a plant growth regulator, and recommended withdrawal of the current CXL of 2 mg/kg for citrus fruit.

The CCPR at its thirty-second session in 2000 decided to retain the CXL for citrus fruits, as the Delegations of South Africa, Uruguay and the USA preferred to do so in order to accommodate post-harvest use. The Delegation of Spain also preferred the CXL to MRLs for individual commodities. Spain and the USA informed the CCPR that the results of additional trials would become available for the JMPR. The Netherlands and South Africa disagreed with evaluation of data for the proposed separate MRLs for orange and grapefruit (ALINORM 01/24).

The 2001 JMPR received information on trials conducted in Uruguay and the USA on citrus fruit by GAP and on supervised trials of post-harvest use of 2,4-D on lemons and oranges.

Residues of supervised trials

2,4-Dichlorophenoxyacetic acid isopropyl ester (2,4-D IPE) is currently registered and is applied after harvest to commercial citrus species in order to inhibit abscission of buttons on harvested fruit in Uruguay (grapefruit, orange, mandarin, lemon) and in the USA (lemon). The solutions of 2,4-D IPE can be applied as a treatment in a water–wax emulsion in packing houses or as a diluted flush or spray.

The 1998 JMPR evaluated two post-harvest trials on lemons in California conducted according to current GAP in Uruguay and the USA. The concentrations of residues in whole fruit were 0.54 and 0.61 mg/kg.

Post-harvest treatments were made to navel oranges (six trials) and lemons (four trials) with experimental packing-line equipment at a research centre in California in 2001. Applications were made in accordance with current label requirements in Uruguay and the USA at maximum rates. Commercial application and fruit handling practices were followed. The fruit were sprayed with 2,4-D IPE solution containing 0.05 kg ai/hl (2,4-D acid equivalent, 0.04 kg ai/hl). The concentrations of residues on whole orange fruit were, in ranked order (median underlined), 0.19, 0.2, <u>0.21</u> (2), 0.22 and 0.24 mg/kg. The concentrations on whole lemon fruit were, in ranked order, 0.37, 0.41, 0.44 and 0.6 mg/kg.

The Meeting acknowledged that the data for oranges (median, 0.21 mg/kg) and lemons (median, 0.49 mg/kg) were different. However, on the basis of Uruguayan use on oranges, grapefruit, mandarins and lemons, the Meeting decided to recommend an MRL for citrus fruits based on the whole data set. The concentrations on whole fruit after post-harvest treatment of oranges and lemons were, in ranked order, 0.19, 0.20, 0.21 (2), 0.22, <u>0.24</u>, <u>0.37</u>, 0.41, 0.44, 0.54, 0.60 and 0.61 mg/kg. The Meeting estimated a maximum residue level of 1 mg/kg for citrus fruit. As no data were submitted for the edible portion, the Meeting estimated an STMR value of 0.3 mg/kg, based on the residues in whole fruit.

Fate of residues during processing

The 1998 JMPR estimated processing factors of 0.1 for citrus juice and < 1 for citrus oil. The Meeting applied these factors to the STMR value of 0.3 mg/kg for citrus fruit and estimated STMR-P values of 0.03 mg/kg for citrus juice and 0.3 mg/kg for citrus oil.

Recommendations

On the basis of the data from supervised trials, the Meeting concluded that the residue concentrations listed below are suitable for establishing maximum residue limits and for assessing the IEDI.

Definition of the residue (for compliance with MRLs and for estimation of dietary intake): 2,4-D

Commodity		Recommendation, values in mg/kg			
CCN	Name	MRL		STMR, STMR-P[1]	HR
		New	Previous		
FC 0001	Citrus fruits	1 Po	–	0.3	
	Citrus juice			0.03	
	Citrus oil			0.3	
FC 0203	Grapefruit	W	0.1		
FC 0004	Oranges, Sweet, Sour	W	0.1		

W: The previous recommendation is withdrawn.
[1] As no data for edible portions were available, the STMR values are based on results for whole fruits.

Dietary risk assessment

Long-term intake

STMR or STMR-P values for 2,4-D were estimated by the current Meeting for citrus fruits and the processed commodities citrus juice and oil. Further STMR or STMR-P values were estimated by the 1998 JMPR for 22 commodities. When data on consumption were available, these values were used to estimate dietary intake. The results are shown in Annex 3.

The IEDIs for the five GEMS/Food regional diets, based on the estimated STMRs, were 3–20% of the ADI. The Meeting concluded that long-term intake of residues of 2,4-D from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The 2001 JMPR concluded that it was unnecessary to establish an acute RfD for 2,4-D. The Meeting therefore concluded that the short-term intake of 2,4-D residues is unlikely to present a risk to consumers.

REFERENCES

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