DRIED CHILLI PEPPERS

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EXPLANATION

The question of pesticide residues in dried chilli peppers was on the agenda of CCPR on several occasions.

The 2004 JMPR evaluated the information available on the water content of dried chilli peppers, and taking into account the CCPR decision on applying a generic factor for conversion of residues in fresh peppers to dried chilli peppers, a generic drying factor of 10 was used for making recommendations for maximum residue levels in dried chilli peppers.

The delegation of the Republic of Korea opposed the proposed Codex MRL(step 8) for dried chilli peppers at the 38th Session of the CCPR and offered to submit compound specific processing factors which were much lower than the default factor (10) used by JMPR.

The effect of drying of chilli peppers on the residues of azinphos-methyl, chlorfenapyr, clothianidin, diazinon, diethofencarb, EPN, folpet, imidacloprid, indoxacarb, metalaxyl, methomyl, methoxyfenozide, tetraconazole, and vinclozolin were reported by the Republic of Korea (Kee-Sung Yung, 2007). The results were evaluated by the present Meeting.

Analytical methods

Methods for the determination of pesticide residues

Sampling and sample preparation

About 15 kg of fresh red peppers were harvested one day after the last application and transported to the analytical laboratory on the day of sampling. About 4 kg of fresh red peppers were homogenised in a blender and kept in frozen at -20 °C until further processing.

Extraction

Procedure 1: azinphos-methyl, chlorfenapyr, clothianidin, diazinon, EPN, folpet, imidacloprid, indoxacarb.

A ten-gram portion of the homogenised fresh red peppers was homogenized with 100 ml acetone for 2 min at 10,000 rpm with a high-speed homogenizer, and then the extract was filtered through Büchner funnel. The baker and the filter cake were rinsed with 50 mL acetone.

Five grams of the dried and ground red peppers was mixed with 10 mL distilled water and kept for 1 h. After that, the extraction was carried out as in the case of fresh peppers.

The combined filtrate was quantitatively transferred to a 1 L separatory funnel, and after adding 400 mL of distilled water and 100 mL of saturated sodium chloride solution, it was partitioned with 2 × 50 mL of dichloromethane. The combined dichloromethane extract was dried over 20 g of anhydrous sodium sulphate layer, collected in a 250 mL distilling flask and evaporated just to dryness with a rotary vacuum evaporator at 40 °C. The residue was dissolved with 5 mL n-hexane:dichloromethane (8:2, v/v) mixture and was subjected to Florisil column chromatography.

Procedure 2: Diethofencarb

Same as procedure 1, except 2 × 50 mL n-hexane was used in the partitioning step instead of dichloromethane.

Florisil column chromatography

A chromatographic column (1 cm × 20 cm) was packed with 5 g 60 – 100 mesh Florisil, topped with about 2 cm layer of anhydrous sodium sulphate (granular, 0.63 – 2.0 mm), pre-washed with 50 mL of *n*-hexane. When the hexane layer reached the top of the packing, 5 mL of the concentrated extract was transferred onto the column. The sample extract tube and the column were rinsed with 2 × 3 mL hexane – dichloromethane/8:2, v/v mixture. The rinsate was also transferred onto the column. The column was eluted first with solvent mixture, which was discarded. The pesticides were eluted with a 2nd solvent mixture. The eluate was evaporated just to dryness at 40 °C. The residue was taken up in a solvent suitable for chromatographic determination. The variations applied for the various compounds are shown in Table 1.

Table 1. Florisil column cleanup of peppers extracts

	Rinsing	1st elution	2nd elution	Conc. extract	Chromatography
Azinphos methyl	2×3 mL M1	50 mL M1	60 mL M2	2 mL M3	GLC
Chlorfenapyr	2×3 mL M1	40 mL M1	60 mL M4	2 mL M3	GLC
Clothianidin	2×3 mL M1	50 mL M5	35 mL M6	2 mL M7	HPLC
Diazinon	2×3 mL M1	50 mL M1	60 mL M2	2 mL M3	GLC
Diethofencarb	2×3 mL M1	50 mL M2	50 mL M8	2 mL M7	HPLC
EPN	2×3 mL M1	50 mL M1	50 mL M4	2 mL M3	GLC
Folpet	2×3 mL M1	40 mL M1	60 mL M4	2 mL M3	GLC
Imidacloprid	2×3 mL M1	50 mL M8	50 mL M9	2 mL M7	HPLC
Indoxacarb		10 mL M10	35 mL M10	2 mL M7	HPLC
Metalaxyl		50 mL M2	50 mL M8	2 mL M3	GLC
Methoxyfenozide		30 mL M11	30 mL M11	2 mL M7	HPLC
Tetraconazole		50 mL M2	50 mL M9	2 mL M3	GLC
Vinclozolin		50 mL M1	50 mL M4	2 mL M3	GLC

M 1: mixture *of n*-hexane:dichloromethane (8:2, v/v)

M 2: mixture of *n*-hexane:dichloromethane:acetonitrile (48.5:50:1.5, v/v/v)

M 3: mixture of *n*-hexane:acetone (8:2, v/v)

M 4: mixture *of n*-hexane:dichloromethane:acetonitrile (49.65:50:0.35, v/v/v).

M 5: mixture of acetonitrile:acetone (96:4, v/v)

M 6: mixture of acetonitrile:acetone (55:45, v/v)

M 7: acetonitrile

M 8: mixture of *n*-hexane:dichloromethane:acetonitrile (45:50:5, v/v/v)

M 9: mixture of dichloromethane:acetonitrile (50:50, v/v)

M 10: mixture of *n*-hexane:ethyl acetate (7:3, v/v

M 11: mixture of *n*-hexane:ethyl acetate (75:25, v/v)

Based on the data from the preliminary analysis, the final solutions were appropriately diluted with the same solvent which was used to take up the materials after cleanup.

Chromatographic analysis

The compounds amenable to GLC analysis were eluted on a DB-5 fused-silica capillary column (30 m × 0.25 mm, 0.25 µm) and detected with ECD or NPD (metalaxyl).

The HPLC analysis was performed on Supelcosil™ LC 18 column (4.6 mm × 250 mm, 5 µm spherical).

Methomyl was analysed with a fluorescent detector after separation on a carbamate column and post column derivatisation.

The mobile phase was acetonitrile:water in all cases. Their compositions and the UV detection wavelengths are given in Table 2.

Table 2. Conditions for HPLC elution and UV detection of compounds

	Acetonitrile:water (v/v)	UV detection (nm)
Clothianidin	20:80	265
Diethofencarb	70:30	210
Imidacloprid	30:70	270
Indoxacarb	70:30	310
Methoxyfenozide	60:40	220
Methomyl	70:30	FLD

Analysis of methomyl

A ten-gram portion of the homogenised fresh red peppers or a five-gram portion of powdered red peppers (with 10 mL water) was homogenized with 100 ml acetonitrile. The concentrated extract was partitioned with hexane in the presence of saturated sodium chloride solution, then the methomyl residue was extracted with dichloromethane. After drying with anhydrous sodium sulphate, the solvent was evaporated and the residual material was taken up in 5 mL mixture of methanol:dichloromethane (4:96, v/v) and cleaned-up with solid-phase extraction (SPE) cartridge (Strata™ NH2, 1,000 mg/6 ml, 55 μm). The cartridge was conditioned with 5 ml dichloromethane, and the concentrated sample extract was transferred into the cartridge. The methomyl was eluted with 5 mL Mixture of methanol:dichloromethane (4:96, v/v) and the eluate was evaporated with a weak stream of nitrogen. The residue was redissolved in 2 ml of acetonitrile and analysed with HPLC-fluorescence detector (HPLC-FLD).

Recovery and LOQ values

Recovery tests were performed at 0.2 and 1 mg/kg levels in three replicates. The LOQs claimed were 0.02 mg/kg for all pesticides in fresh red peppers and 0.04 mg/kg in dried red peppers. The report on the validation of the method was not submitted. The recoveries of the pesticides in fresh red peppers and dried red peppers ranged from 81.9 to 114% and from 82.6 to 108%, respectively. The results are summarised in Table 3.

Stability of residues in stored samples

The stability of residues during storage was tested by spiking 3 × 10 g of fresh red peppers and 3 × 5 g of powdered red peppers with individual pesticide standard solutions equivalent to 0.5 mg/kg. The samples were stored at -20 °C until analyzed. The period of storage stability test was not reported, and concurrent recovery studies were not carried out. The percentages of survived residues are presented in Table 3, together with the analytical recoveries obtained during the prior validation of the method.

Table 3. Analytical recoveries of the test pesticides in fresh red chilli peppers, and percentage of residues survived after storage

Pesticide	Spike level mg/kg	Analytical recovery, % [a]		Storage, days [b]	Survived residue, %	
		Fresh	Dried		Fresh [c]	Dried [d]
Azinphos-methyl	0.2	98.11±1.39	98.28±1.98	11	88.85±1.16	85.48±1.53
	1.0	89.94±1.14	84.81±0.65			
Chlorfenapyr	0.2	103.09±3.54	105.99±1.55	13	90.99±3.94	101.03±0.64
	1.0	95.88±1.70	95.45±2.68			
Clothianidin	0.2	98.81±1.80	92.34±0.68	23	92.19±0.84	97.56±0.56
	1.0	96.98±0.76	98.38±0.90			
Diazinon	0.2	104.51±3.38	96.90±7.08	18	85.70±1.28	100.43±1.96
	1.0	81.89±4.88	97.93±0.56			
Diethofencarb	0.2	100.01±1.28	96.30±1.05	16	86.54±2.11	90.64±2.70
	1.0	91.96±0.19	90.12±1.89			
EPN	0.2	108.91±3.50	108.59±4.33	23	100.96±0.97	100.23±5.04
	1.0	102.77±1.88	108.36±7.97			
Folpet	0.2	112.39±0.92	111.33±1.07	12	90.73±7.35	114.58±8.92
	1.0	114.89±1.29	109.18±7.23			

Pesticide	Spike level mg/kg	Analytical recovery, % [a]		Storage, days [b]	Survived residue, %	
		Fresh	Dried		Fresh [c]	Dried [d]
Imidacloprid	0.2	100.62±4.60	101.51±3.39	14	101.16±0.16	109.60±0.51
	1.0	97.39±2.76	101.73±2.31			
Indoxacarb	0.2	101.96±3.61	101.43±6.92	21	84.20±1.86	92.60±4.16
	1.0	103.78±2.00	84.83±3.81			
Metalaxyl	0.2	102.32±0.63	102.32±0.63	25	103.86±1.57	102.86±1.89
	1.0	104.09±3.83	104.09±3.83			
Methomyl	0.2	85.45±0.94	82.62±1.90	30	97.88±1.50	97.46±1.56
	1.0	95.45±3.44	96.96±1.44			
Methoxyfenozide	0.2	93.50±0.13	92.39±0.48	19	97.97±0.19	94.86±1.85
	1.0	96.98±0.13	99.62±0.40			
Tetraconazole	0.2	85.20±1.39	107.40±3.03	15	102.29±6.07	91.41±1.74
	1.0	95.85±3.39	90.83±4.27			
Vinclozolin	0.2	103.08±3.62	100.72±2.01	20	93.78±1.51	87.54±1.16
	1.0	96.32±0.24	90.53±2.00			

a - Average of three replicate measurements with standard deviation

b - Storage period of fresh chilli paprika samples in deep-freezer

c - Residues measured after the storage

d - Storage periods were not reported

Determination of water content of peppers

The procedure specified by the Korean Food Code for dried chilli peppers was used: about 100 g portion of fresh red chilli peppers, after removal of its peduncle part, was pre-dried for 35 h at 60 °C. The pre-dried red peppers were then subjected to drying for 5 h at 105 °C. After cooling in a desiccator for 30 min, weight loss was measured to calculate the water content (Kee-Sung Kyung, 2007).

The water content of powdered red peppers was determined by drying 5 g at 105 °C for 5 h. The water content of fresh and dried peppers was determined in triplicate.

The water content (W%) was calculated from the weight before (m_b) and after drying (m_a) as follows:

$$W\% = 100 \times \frac{m_b - m_a}{m_b}$$

The water content of fresh red chilli peppers and powdered red chilli peppers was 84.0 ± 0.6 % and 31.2 ± 0.5%, respectively. Details of the individual studies were not reported.

Field trials

Field trials were performed in 2006 at two sites located in Chungbuk Province, a major cultivation area of red peppers in the Republic of Korea. Each experimental field consisted of 3 replicate plots (15× 50 m) separated by a buffer zone of 2.5 m. Within one plot the treated rows were separated by a 1 m buffer zone. The pepper seedlings of daeheung and daetong varieties were transplanted 30 cm apart in Field 1 and 2, respectively. Peppers were cultivated according to the conventional methods widely used in Korea.

The last two pesticide applications were performed with a backpack-type sprayer with standard nozzle 10 days apart at site 1, and 4 days apart at site 2 with higher rates than authorised to obtain sufficient residue levels for processing studies. The pesticide formulations and spray concentrations are given in Table 4.

Table 4. Pesticide products and their spray concentrations applied in the field trials

Pesticide	Form. type	ai content (%)	PHI days (No. of applications) max [a]	Spray solution (g, mL/10L water)	
				Registered	Applied
Azinphos-methyl	WP	25	15 (4) [b]	10	40
Chlorfenapyr	SC	10	3 (3)	5	10
Clothianidin	SC	8	3 (3)	5	10
Diazinon	EC	34	14 (1)	12.5	25
Diethofencarb	WP	25	7 (4)	10	20
EPN	EC	45	60 (2) [b]	10	20
Folpet	WP	50	2 (3)	20	40
Imidacloprid	WP	10	3 (4)	5	10
Indoxacarb	WP	10	5 (5)	5	10
Metalaxyl	WP	25	21 (2) [b]	20	40
Methomyl	WP	45	7 (2)	6.5	13
Methoxyfenozide	SC	21	7 (3)	5	10
Tetraconazole	EW	12.5	3 (3)	5	10
Vinclozolin	WG	47	14 (4) [b]	10	20

a - Number of maximum applications are given in brackets.

b - Not registered in Korea

Residues in fresh and dried red peppers

The residues measured in fresh and dried peppers in the Korean trials are summarised in Table 5.

Table 5. Residues measured in fresh and dried red chilli peppers

Pesticide	Field	Residues in red peppers [mg/kg] [a]		Processing Factor[b], pf	Conc. Factor[c], cf	pf/cf
		Fresh	Dried powder			
Azinphos-methyl	1	1.27±0.02	2.46±0.04	1.9	4.3	0.45
	2	0.93±0.04	2.71±0.03	2.9	4.3	0.68
Chlorfenapyr	1	3.04±0.35	15.37±0.52	5.1	4.3	1.2
	2	3.66±0.52	15.24±2.05	4.2	4.3	0.97
Clothianidin	1	2.95±0.04	7.63±0.29	2.6	4.3	0.60
	2	2.99±0.04	9.20±0.04	3.1	4.3	0.72
Diazinon	1	1.49±0.21	6.80±1.01	4.6	4.3	1.1
	2	2.01±0.24	6.02±1.37	3.0	4.3	0.70
Diethofencarb	1	4.35±0.02	10.68±0.09	2.5	4.3	0.57
	2	4.56±0.02	11.38±0.05	2.5	4.3	0.58
EPN	1	0.92±0.06	1.70±0.26	1.8	4.3	0.43
	2	0.81±0.15	2.82±0.61	3.5	4.3	0.81
Folpet	1	6.51±0.42	16.47±0.80	2.5	4.3	0.59
	2	4.52±0.22	20.59±1.48	4.6	4.3	1.1
Imidacloprid	1	1.75±0.02	4.33±0.10	2.5	4.3	0.57
	2	3.09±0.02	5.28±0.05	1.7	4.3	0.40
Indoxacarb	1	2.36±0.05	5.76±0.22	2.4	4.3	0.57
	2	3.01±0.08	9.69±0.52	3.2	4.3	0.75
Metalaxyl	1	2.16±0.01	6.65±0.11	3.1	4.3	0.72
	2	2.31±0.08	7.56±0.05	3.3	4.3	0.76
Methomyl	1	1.80±0.10	6.05±0.25	3.4	4.3	0.78
	2	1.78±0.05	5.21±0.11	2.9	4.3	0.68
Methoxyfenozide	1	0.98±0.04	3.01±0.27	3.1	4.3	0.71
	2	1.10±0.01	3.55±0.03	3.2	4.3	0.75

Pesticide	Field	Residues in red peppers [mg/kg] [a]		Processing Factor[b], pf	Conc. Factor[c], cf	pf/cf
		Fresh	Dried powder			
Tetraconazole	1	2.41±0.22	6.69±0.82	2.8	4.3	0.65
	2	7.19±0.77	16.39±1.12	2.3	4.3	0.53
Vinclozolin	1	4.75±0.94	10.93±2.47	2.3	4.3	0.53
	2	6.48±1.12	10.76±2.73	1.7	4.3	0.39

a - Mean values of triplicate measurements with standard deviations

b - Processing factor = (residue in dried peppers, mg/kg)/(residue in fresh peppers, mg/kg)

c - Concentration factor calculated from the average water content of fresh (84.04%) and dried (31.25%) peppers. Dry matter content of 100 g fresh peppers: 100-84.04=15.96 g. Total weight of dried peppers containing 31.25% water: 15.96/(1-0.3125)=23.21 g; cf=100/23.21=4.31

Water content of fresh and dry peppers

The water content of various varieties of fresh sweet and chilli peppers and in ground dried peppers varies substantially depending on the varieties and the conditions of drying and processing. In order to obtain information on the expected range of water content of various species of peppers, and the dried products obtained from them, the available information was collected from Australia/New Zealand, Germany, Japan and USA. The EPA/OPP database specifies that chilli peppers are non-bell peppers (peppers, sweet). The water content of non-bell peppers varies from 86 – 88 %.

The official water content of dried peppers shows much larger variation from 1.7% in Japan to 12% for spices allowed by the German standard. Examples, given in Table 6, illustrate how the concentration factor is affected by the water content of fresh and dried products.

Table 6. The affect of the water content of fresh and dried peppers on the concentration factor (cf)

	Water content,%		Dry matter in 100g fresh peppers	Weight [a] of dried peppers	Cf.	Ref.
	Fresh	Dried				
Chilli peppers						
Red chilli peppers	84.04	31.25	15.96	23.21	4.3	Kyung
Peppers, hot chili, red, raw	88	7.79	12	13.01	7.7	USDA
Spices, paprika	88	9.54	12	13.27	7.5	USDA
EPA/OPP chilli peppers	86	9.54	14	15.48	6.5	EPA
Chilli peppers	88	7.8	12	13.02	7.7	AUS/NZ
Chilli peppers fruits (raw)	75	1.7	25	25.43	3.9	Japan
Chilli peppers (raw),	93.1	3.8	6.9	7.17	14	Japan
Chilli peppers	86	12	14	15.91	6.3	Germany
Peppers, sweet						
Peppers, sweet green, raw	93.89	10	6.11	6.79	15	USDA
Sweet red paprika	91.1	10	8.9	9.89	10	Japan
Peppers, green, shape of chili peppers	91.4	3.8	8.6	8.94	11	Japan
Bell peppers	93	12	7	7.95	13	Germany
Peppers, sweet green, raw	93.89	12	6.11	6.94	14	USDA/G
Peppers, bell	92.4	12	7.6	8.64	12	AUS/NZ/G

a - Weight of dried material obtained from 100 g fresh material taking into account the water content reported in dried peppers.

References USDA/G and AUS/NZ/G indicates water content in fresh peppers and dried peppers taken from two data bases for illustration of possible scenarios.

REFERENCES

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