ETHOXYQUIN (35)

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EXPLANATION

Ethoxyquin was reviewed in 1999 under the Periodic Review Programme. That JMPR declined to recommend the use of the maximum residue level estimate for pears as an MRL because of uncertainty on the toxicity of the degradation products. The 2005 JMPR established an ARfD for ethoxyquin and noted that both the ARfD and the ADI were defined in terms of the parent and metabolites/degradates methylethoxyquin (MEQ), dihydroethoxyquin (DHEQ) and dehydromethylethoxyquin (DHMEQ). A study on the storage stability of ethoxyquin on pears and two new studies on the magnitude of the residue on pears (postharvest treatment) have been supplied (Northwest Horticultural Council, USA).

Stability of pesticide residues in stored analytical samples

A previously considered study (JMPR 1999) showed up to a 60% degradation of radiolabelled ethoxyquin on pears stored frozen (–2 °C) for 33 weeks.

A report on the stability of ethoxyquin on pears stored frozen for 42 days was provided to the Meeting (Schebler, 2007). An EC 480 g/kg formulation of ethoxyquin was used to prepare a dipping solution by dilution to a target concentration of 2700 mg/kg with 1% aqueous ascorbic acid/acetonitrile, 20:80 (v:v). The actual measured concentration was 3700 $\pm$ 1500 mg/kg based on three replicate aliquots from the treatment solution.

Pears (39) were fully immersed individually for 30 ± 5 seconds in the treatment solution. Each pear was allowed to air dry on a drying rack until visually dry. Treated pears were placed in sets of three into individual plastic bags, sealed, labelled as Treated Frozen (TF) and stored in the dark at -20 °C

Additional pears (39) were fully immersed individually for 30 ± 5 seconds in the treatment solution. Each pear was allowed to air dry on a drying rack until visually dry. Treated pears were individually wrapped in aluminium foil and placed in sets of three into storage bags and vacuum sealed. Each storage bag was placed into individual plastic bags, sealed, labelled as Treated Frozen Dark (TFD) and stored in the dark at –20 °C.

Control pear samples for both TF and TFD were also prepared.

At time zero and at intervals up to 42 days, bags of control, TF and TFD pears were removed from the frozen storage and prepared for analysis. The pears were chopped, frozen with liquid nitrogen and ground into powder. A subsample (10 g) was extracted with acetonitrile containing 0.5% (w/v) ascorbic acid.

Analysis was by HPLC with fluorescence detection (360 nm excitation, 432 nm emission) and external standard calibration (5). Response was linear over the range of 0.020 to 0.51 µg/mL, with a regression coefficient of 0.9998. The method was previously considered (JMPR 1999). No current recovery data (fortified pear controls) were provided in the present submission nor was a limit of quantitation demonstrated.

The results are summarized in Table 1. No values were provided for the untreated control samples.

Table 1 Stability of field-incurred ethoxyquin on pears stored frozen (–20 °C)

Storage Interval (days)	Type [a]	Concentration [b] (µg/g) (average)	Remaining [d] (%)
0	TF	48 [c], 2.7, 3.4 (3.0)	100
	TFD	0.85, 1.0, 0.79 (0.89)	100

Storage Interval (days)	Type [a]	Concentration [b] (µg/g) (average)	Remaining [d] (%)
1	TF	1.0, 1,3, 0.66 (1.0)	33
	TFD	2.3 [c], 0.83, 0.86 (0.84)	94
4	TF	1.0, 1.3, 0.66 (1.0)	33
	TFD	3.0, 3.6, 4.3 (3.6)	400
7	TF	3.7, 3.9, 3.5 (3.7)	120
	TFD	5.2, 5.9, 4.0 (5.1)	570
14	TF	5.9, 4.3, 4.8 (5.0)	170
	TFD	6.3, 4.7, 5.0 (5.4)	610
21	TF	3.2, 3.0, 2.6 (2.9)	97
	TFD	5.4, 4.8, 4.8 (5.0)	560
28	TF	2.8, 3.0, 1.3 (2.4)	80
	TFD	5.8, 5.7, 4.2 (5.2)	580
35	TF	4.3, 3.0, 3.5 (3.6)	120
	TFD	6.1, 5.2, 3.5 (4.9)	550
42	TF	6.5, 3.3, 4.3 (4.7)	160
	TFD	4.9, 4.1, 3.6 (4.2)	470

[a] TF = Treated pears stored frozen in plastic. TFD = Treated pears wrapped in foil and stored frozen in plastic (simulating storage in the absence of light).

[b] Analysis of 3 individually prepared pears.

[c] Discarded as outlier.

[d] Representative chromatograms were included, but no raw data.

USE PATTERN

Ethoxyquin is a fungicide applied as a postharvest treatment to fruit entering cold storage as a scald preventive agent. Uses are summarized below.

Commodity	Country	Formulation	Application Method	Application Rate
Pear	USA	EC 460 g/kg	Drench, Spray, brush bed, conveyor roll, impregnated paper wraps	2700 ppm drench, spray or brush OR (1) 1000 - 1500 ppm drench (2) 1200 - 1700 ppm line spray OR impregnated paper wraps. In all cases, total application must not exceed 2700 ppm.
Pear	USA	180 g/kg ready-to-use liquid	Electrofog	16.2 g ai/1000 kg fruit.

RESIDUES RESULTING FROM SUPERVISED TRIALS

A study was previously considered (JMPR 1999) and it was concluded that 14 trials were conducted at maximum GAP (2700 mg/kg aqueous or wax spray on a brush bed or conveyor rolls). The residues in ranked order were: 0.40, 0.66, 1.58, 1.72, 1.72, 1.79, 1.81, 1.90, 1.99, 2.03, 2.23, 2.26, 2.29 and 2.45 mg/kg.

The Meeting received a study on post-harvest treatment by spraying with ethoxyquin (Jackson and Strickland 1999). Twelve groups of pears (certified organic d'Anjou) were treated. An

additional two groups of pears from the same lot used for treatment were used as controls. During each treatment, 20 individual pears with a combined weight of approximately 3.6 kg were treated. Replication of treatments was achieved by replicating preparation of treatment solutions.

Pears were treated using a commercial EC formulation of ethoxyquin. The formulation contained 48.6% ethoxyquin as determined by analysis. Applications were made at the target rate of 2700 ppm active ingredient (ai) by performing a 1/200 (v/v) dilution of the EC with water. The actual spray concentrations for the 12 application mixtures ranged from 2425 to 2895 mg/L, average 2745 $\pm$ 120 mg/kg.

The 20 pears used for each spray application were placed in two plastic file boxes (about 1.8 kg per box) that had large openings between the plastic on the bottoms and sides. Pears were sprayed using a pressure of 1.4 kg/cm^2. To ensure complete coverage of the fruit, the pears were treated for 30 seconds, rotated 180° and treated for an additional 30 seconds (60 seconds total). An average of 2.6 L of the mix was applied to each group of 20 pears. Following treatment, pears were placed in plastic coated wire freezer baskets to drain and air dry.

To maintain stability of residues on treated pears, the stems were removed from the dry fruit and then individually wrapped in aluminium foil to minimize light degradation. The 10 pears comprising each sample were then placed in a plastic bag, and the bag was vacuum packed and sealed to remove as much air as possible. The samples were maintained frozen (–10 °C to -30 °C) until preparation for analysis. All pears were analysed within 20 days of treatment.

All 10 pears included in each sample were homogenized for analysis, and two 10 g subsamples from each were extracted and analysed for ethoxyquin residues using HPLC (Anon., 1997). The method was validated by the fortification, extraction and analysis of control homogenized pears with ethoxyquin (0.50, 1.00, 3.00 and 5.00 mg/kg). Recoveries ranged from 99% at 1 mg/kg to 108% at 0.5 mg/kg. The stated, but not demonstrated, LOQ was 0.1 mg/kg.

The results are summarized in Table 2.

Table 2 Residues on Pears from Post Harvest Spray Treatment with an Ethoxyquin EC Formulation at 2700 mg/L

Treatment Number	Ethoxyquin (mg/kg)	Average of Replicate Analyses (mg/kg)
1	1.40, 1.77	1.6
1	1.68, 1.39	1.5
2	1.96, 1.62	1.8
2	1.83, 1.52	1.7
3	2.23, 2.25	2.2
3	2.05, 2.41	2.2
4	1.82, 1.58	1.7
4	2.13, 1.87	2.0
5	2.38, 2.19	2.3
5	2.32, 1.89	2.1
6	1.73, 2.19	2.0
6	2.35, 2.54	2.4
7	1.94, 1.86	1.9
7	1.33, 1.78	1.6
8	1.74, 1.88	1.8
8	1.84, 1.76	1.8
9	2.19, 2.34	2.3
9	2.15, 2.15	2.2
10	1.55, 1.90	1.7

Treatment Number	Ethoxyquin (mg/kg)	Average of Replicate Analyses (mg/kg)
10	1.46, 1.40	1.4
11	1.49, 1.56	1.5
11	1.84, 1.61	1.7
12	2.01, 2.06	2.0
12	1.33, 1.40	1.4

A second study was conducted in which post harvest spray was combined with paper wrap treatment of pears (Jackson and Strickland 2001). Three replicate experiments were conducted, where replication included preparation of treatment solutions. Each treatment consisted of spraying 120 pears with a 1700 mg/L ethoxyquin aqueous mixture prepared from a 51% EC formulation. Actual mixture concentrations were 1575, 1710, and 1640 mg/L.

The pears (120) used for each spray application were placed in two plastic fruit picking lugs that had large openings between the plastic on the bottoms and sides. Pears were sprayed using a pressure of 1.4 kg/cm^2 for three minutes. Approximately 17 L of material were applied to each 120-pear sample. Following the spray treatment, pears were placed in plastic coated wire freezer baskets until they had drained and air dried.

Twenty treated pears (about 4 kg) were wrapped in untreated paper, and the remaining 100 pears were wrapped in paper impregnated with 1000 ppm ethoxyquin. Ten individual wraps from the same lot as used in the study were found to contain from 1000 to 1050 ppm ethoxyquin, and averaged 1020 ppm.

Twenty pears from each replicate were collected immediately following treatment (Day 0), 20 pears from each treatment were collected the day after treatment (Day 1 ambient conditions), and the remaining pears were placed in cardboard boxes in commercial controlled atmosphere storage. Actual storage temperatures varied from 33.4 °C to 35.0 °C during the storage interval. Twenty pears from each treatment were then removed following 7, 14 and 29 days of storage.

To obtain control samples, one set of 100 pears was treated with a spray application containing a formulation blank for the ethoxyquin product and wrapped using paper not impregnated with ethoxyquin. These pears were also stored for 0, 1, 7, 14 and 29 days.

Each 20-pear set was randomly divided into two 10-pear samples after collection. To maintain stability of residues on treated pears, upon sample collection each pear was individually wrapped in aluminium foil to minimize light degradation. The paper wrap was left on all pears during collection, freezing, and transport to the analytical laboratory for analysis. The 10 pears comprising each sample were then placed in a plastic bag, and the bag was vacuum packed and sealed to remove as much air as possible. Pears were maintained frozen (–10 ° to –30 °C) until preparation for analysis. Samples were analysed within 23 to 45 days of treatment.

All 10 pears comprising each sample subset were homogenized (frozen with peels), and two 10 g subsamples from each of the samples were extracted and analysed for ethoxyquin using HPLC (Anon 1997).

A total of 12 control pear samples fortified at various levels were assayed along with the treated samples to determine the percent recovery of ethoxyquin in pears. Concurrent recoveries ranged from 94% to 104% for fortifications of 0.14 to 1.8 mg/kg. All control pear samples contained < 0.1 mg/kg ethoxyquin.

Results are summarized in Table 3.

Table 3 Residues of ethoxyquin on Pears after post–harvest spray treatment (1700 mg/L) and enclosure in treated wrap paper (1000 ppm)

Days after Treatment	Treatment Number	Ethoxyquin (mg/kg)	Average of Replicate Analyses (mg/kg)
0	1 [a]	0.454, 0.380	0.42
		0.463, 0.350	0.41
	2 [a]	0.577, 0.570	0.57
		0.347, 0.439	0.39
	3 [a]	0.429, 0.495	0.46
		0.158, 0.126	0.14
0	1 [b]	0.898, 0.723	0.81
		0.759, 0.809	0.78
	2 [b]	0.621, 0.599	0.61
		0.990, 1.07	1.0
	3 [b]	1.12, 1.23	1.2
		0.865, 0.781	0.82
1 [c]	1	0.752, 0.729	0.74
		0.702, 0.738	0.72
	2	0.488, 0.434	0.46
		0.522, 0.645	0.58
	3	0.513, 0.543	0.53
		0.542, 0.541	0.54
7 [d]	1	0.618, 0.553	0.59
		0.769, 0.766	0.77
	2	0.598, 0.555	0.58
		0.368, 0.388	0.38
	3	0.612, 0.581	0.60
		0.560, 0.522	0.54
14	1	1.53, 1.19	1.4
		1.59, 1.40	1.5
	2	1.75, 1.46	1.6
		1.31, 1.21	1.3
	3	1.30, 1.03	1.2
		0.932, 0.936	0.93
29	1	0.258, 0.256	0.26
		0.238, 0.296	0.27
	2	0.274, 0.173	0.22
		0.154, 0.136	0.14
	3	0.370, 0.430	0.40
		0.501, 0.445	0.47

[a] Untreated wrapping paper.

[b] Treated wrapping paper.

[c] Ambient storage from treatment.

[d] Controlled atmosphere commercial storage.

A third study was reported for the post harvest thermofogging (aerosol fog) of pears with ethoxyquin (Jacobson 1997). A small-scale residue study was conducted at a simulated commercial pome fruit cold-storage facility (refrigerated container, 3.1 m^2, 5.6 m^3). Two bins of D' Anjou pears totalling approximately 600 kg were obtained from a commercial source. To make up a sample, six individual fruits were placed in a plastic mesh bag which was knotted with the excess mesh. These bags were interspersed among the loose pears in sixteen (16) of the 24 crates (30 × 41 × 61 cm) to be treated. The bags were nestled in fruit placed in plastic crates each containing nominally 25 kg of fruit. The crates were then stacked in the storage room simulating the placement of commercial-size bins in a controlled atmosphere warehouse. The 24 crates were arranged in two stacks of four high by three deep in an experimental room at the storage facility, with the crates containing sample bags distributed in the stacks as determined by a random number system.

The room was sealed and 17.8 % ethoxyquin (w/w) ready-to-use liquid formulation was applied using an electric thermal fogger (heater temperature 398 °C and the mixing chamber 168 °C) situated outside the room with its nozzle protruding into the room through an access hatch. The actual amount applied was 34.9 g of test substance or 6.21 g ethoxyquin ai This corresponds to a rate of 58.2 g formulation/metric ton. The test chamber temperature was 1.5 °C and relative humidity was 69%. On the day after the application, samples were taken from the treated crates.

Samples were frozen immediately after sampling and maintained frozen until analysis. All samples were extracted within 37 days of the test. Samples were analysed by HPLC with a fluorescence detector for residues of ethoxyquin (Anon 1997). Pears (control) were spiked in triplicate at 0.3, 0.5, 1.0, 2.0 and 3.0 mg/kg ethoxyquin. Individual spike recoveries ranged from 71% to 100%. For concurrent recoveries, 0.3 and 3 mg/kg ethoxyquin fortified control pears were prepared and analysed. The recoveries were 100% at both the 0.3 and 3.0 spike levels.

All samples (n = 4) were < 0.3 mg/kg. All control samples were also below the demonstrated LOQ (0.3 mg/kg).

APPRAISAL

The 1999 JMPR Meeting made no maximum residue level recommendation for pears due to uncertainty on the toxicity of the degradation products. An ARfD was established by the 2005 JMPR which and noted that both the ARfD and the ADI were defined in terms of the parent and metabolites/degradates methylethoxyquin (MEQ), dihydroethoxyquin (DHEQ) and dehydromethylethoxyquin (DHMEQ).

Methods of Analysis

Available analytical methods determine only parent ethoxyquin. There are no methods for the routine determination of MEQ, DHEQ and DHMEQ as needed for dietary risk assessment.

Previously reviewed studies (JMPR 1999) indicated there was up to a 60% conversion of radiolabelled ethoxyquin to the metabolites/degradates, including MEQ, DHEQ and DHMEQ. This occurred over a 33 week storage interval at –2 °C.

The Meeting concluded that total residues, for dietary intake assessment, may be estimated by multiplying the measured ethoxyquin residue by a factor of 2.5. This reflects the result of the radiolabelled degradation study and typical cold storage conditions for treated pears.

Stability of pesticide residues in stored analytical samples

Ethoxyquin on pears is unstable under conditions of frozen storage at –20 °C in plastic. The apparent concentration of ethoxyquin drops to 33% of the applied dose within one day, but returns to or exceeds 100% over the next 40 days. This may have been due to an interaction between the plastic container and ethoxyquin.

Ethoxyquin on pear is somewhat more stable when stored wrapped in foil in evacuated bags at –20 °C.

The Meeting concluded that pear samples being tested for ethoxyquin should be stored frozen and protected from oxygen to the extent possible. Pears should be prepared for analysis in as short a time as possible following collection.

Results of supervised residue trials on crops

Pear

The Meeting received studies of the post-harvest treatment of pears by spraying, a combination of spraying and wrapping in treated paper, and by thermofogging.

Twelve trials were conducted at the maximum USA GAP (Ethoxyquin EC, 2700 mg ai/L, brush or spray application). Residues in ranked order were: 1.6, 1.7 (2), 1.8 (2), 1.9, 2.0 (2), 2.2, 2.3 (2), 2.4 mg/kg.

Four trials were conducted at the maximum USA GAP (18% ethoxyquin, thermofog application, 16.2 g ai/1000 kg). Residues in ranked order were: < 0.3 (4) mg/kg.

Three trials were conducted at a rate slightly in excess of the maximum USA GAP (EC 460 g/kg, 1000–1500 ppm drench + impregnated paper wraps). The trials involved a spray at a concentration of 1700 ppm followed by wrapping with impregnated paper. The Meeting considered the trials to be within 120% of the maximum GAP and therefore acceptable. The residues in ranked order were: 1.2, 1.5, 1.6 mg/kg.

Based on the 12 post-harvest spray trials of pears, the Meeting estimated an STMR of 5 mg/kg (2.0× 2.5) and an HR of 6.0 (2.4× 2.5) and a maximum residue level of 3 mg/kg.

RECOMMENDATIONS

The Meeting estimated the maximum residue levels and STMR values and HR values shown below. The maximum residue levels are recommended for use as MRLs.

Definition of the residue (for compliance with MRL) for plant commodities: *ethoxyquin*

Definition of the residue (for estimation of dietary intake) for plant commodities: *ethoxyquin plus degradates methylethoxyquin (MEQ), dihydroethoxyquin (DHEQ) and dehydromethylethoxyquin (DHMEQ).*

Commodity		MRL, mg/kg		STMR	HR
CCN	Name	New	Previous	mg/kg	mg/kg
FP0230	Pear	3	W	5.0	6.0

DIETARY RISK ASSESSMENT

Long-term intake

The current maximum ADI for ethoxyquin is 0.005 mg/kg bw. The International Estimated Daily Intakes (IEDIs) of ethoxyquin based on the STMRs estimated for one commodity for the thirteen GEMS/Food cluster diets were in the range of 0% to 40% of the maximum ADI. The Meeting concluded that the long-term intake of residues of ethoxyquin resulting from its uses that have been considered by JMPR is unlikely to present a public health concern.

Short-term intake

An ARfD for ethoxyquin of 0.5 mg/kg bw was established by the 2005 JMPR. The IESTIs of ethoxyquin by the general population and by children were calculated for commodities for which HRs were estimated. The IESTI was 20% of the ARfD for the general population and 50% of the ARfD for children. The results are shown in Annex 4 of the 2008 Report of the JMPR.

The Meeting concluded that short-term intake of residues of ethoxyquin from its use on pears is unlikely to present a public health concern

REFERENCES

Code	Author	Year	Title, Institute, Report reference
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WP 00-01	Johnson, G.; Strickland, M.	2001	Magnitude of Residues in/on Fresh Pears After Post-Harvest Spray and Paper Wrap Treatments with Ethoxyquin: Lab Project Number: WP00-01. Unpublished study prepared by Western EcoSystems Technology.
101-11: 6608-102: 99-01	Johnson, G.; Strickland, M.	1999	Magnitude of Residues in/on Fresh Pears After Post Harvest Treatment with Ethoxyquin: Final Report: Lab Project Number: 101-11: 6608-102: 99-01. Unpublished study prepared by WEST, Inc., and Covance Labs., Inc.
06002.	Jacobson, S.	2007	Magnitude of Residue of Ethoxyquin on Pears Following Post-Harvest Fogging with Xedaquin A. Project Number: 06002. Unpublished study prepared by Compliance Services International. Determination of Ethoxyquin in Feeds by Liquid Chromatography: Collaborative Study," as described in the Journal of *AOAC* International Vol. 80, No.4, 1997, pp. 725-31.