ETHOXYQUIN (035)

EXPLANATION

Ethoxyquin, an antioxidant preservative, was first evaluated in 1969. It was originally identified by the 1990 CCPR as a candidate for re-evaluation (ALINORM 91/24, Appendix V). It was scheduled by the 1994 CCPR for re-evaluation by the 1994 JMPR (ALINORM 91/24A, Appendix VI, Annex 1), but removed from the 1994 JMPR schedule at the 1994 CCPR because the Committee was informed that the manufacturer was not supporting the existing CXLs for apples and pears. The US and UK delegations indicated that data might be supplied. The CCPR recommended deletion of the existing CXLs if no information became available by the 1995 Session (ALINORM 95/24, para 104).

At the 1995 CCPR, the delegation of the USA opposed deletion of the CXLs and informed the Committee that residue data on pears and a full toxicological data package would be available for the JMPR in 1996. Ethoxyquin was, therefore, scheduled for toxicological and residue reviews by the JMPR in 1998 and 1999 respectively. It was decided to postpone the withdrawal of the CXL for pear until the 28th session of the CCPR (ALINORM 95/24A, paragraph 89). The CCPR in 1996 and 1997 postponed discussion on deletion of the CXL for pear (ALINORM 97/24, para 41; ALINORM 97/24A, para 49).

The 1998 JMPR conducted a review of the toxicology of ethoxyquin. The present evaluation is within the CCPR Periodic Review Programme.

Information on residue chemistry and environmental fate was provided by the Northwest Horticultural Council (USA). The governments of Germany and The Netherlands submitted additional information.

IDENTITY

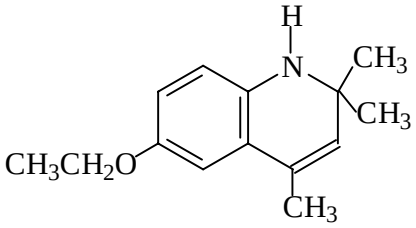
ISO Common name: ethoxyquin

Chemical name
- IUPAC: 1,2-dihydro-2,2,4-trimethylquinolin-6-yl ethyl ether
- CA: 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

CAS Registry No.: 91-53-2

Synonyms: ethoxyquine
ethoxychin (Czech)

Structural formula:



Molecular formula: $C_{14}H_{19}NO$

Molecular weight: 217.31

Physical and chemical properties

Pure active ingredient:

Appearance: brown slightly viscous liquid (Wojcieck, 1996)

Vapour pressure: $3.46x10^{-2}$ Pa ($2.60x10^{-4}$ mm Hg) at 25 C (Schetter and Kogovsek, 1996)
$1.18x10^{-1}$ Pa ($8.83x10^{-4}$ mm Hg) at 35 C
$3.32x10^{-1}$ Pa ($2.49x10^{-3}$ mm Hg) at 45 C

Boiling point: 150 C at 747 mm Hg (Wojcieck, 1996)

Octanol/water partition coefficient: 283 (log P_{ow} = 2.45) at 25 C (Lorence, 1996b)

Solubility at 25 C:

water	170 mg/l (Lorence, 1996c)
acetonitrile	462 g/l
n-octanol	452 g/l

Hydrolysis at 25 C:
pH 5 half-life = 3.7 days (Reynolds and Campbell, 1995)
pH 7 half-life = 6.7 days
pH 9 half-life = 9.3 days
Major degradation products (identified by LC-MS result from demethylation, desethylation at the ether linkage, and dimerization.

Technical material

Minimum purity: 97% (Schetter, 1996)

Stability: stable for 14 days at 55°C (Wojcieck, 1996) 15% loss under exposure to light for 14 days (Wojcieck, 1996)

Impurities: 6 impurities, each <0.5% w/w

Formulations

The commercially available formulation is an EC (emusifiable concentrate).

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

No studies were reported. None are required, as the only use of ethoxyquin is on pears post-harvest and neither pears nor pear by-products are significant animal feed items.

The 1998 JMPR reviewed the metabolism of ethoxyquin in rats. Parent ethoxyquin was not found in the urine, and only traces were found in faeces, liver, kidneys and adipose tissue, from rats given an intravenous dose. Some metabolites were identified in the urine and bile. The major metabolic pathway was O-de-ethylation at C-6 (yielding a phenol) and conjugation. The metabolic products in rats are different from those in plants (see below).

Plant metabolism

Anjou pears were washed with water and air-dried. A total of 144 pears, each 200 $\pm$ 30 g, were dipped for about 30 seconds in a solution prepared from an aqueous mixture of ring-labelled [^{14}C]ethoxyquin (unspecified amount, $\geq$93% purity by HPLC, radiochemical purity $\geq$99%, specific activity 98.6 mCi/mmol), Deccoquin 305 Concentrate (Elf Atochem, 52.3% ethoxyquin, 3,828 mg [^{12}C]ethoxyquin), and [2,4-^{13}C]ethoxyquin (1,277 mg, purity $\geq$98.5% by HPLC). The mixture was diluted to 257 ml with distilled water. The specific activity was 3659 dpm/μg or 1.65 μCi/mg. The [^{13}C]ethoxyquin served as a marker (+ 2 mass units) for GC-MS analyses. The pears were air-dried for 2 hours, and stored in an incubator at -2 $\pm$ 2.0°C and relative humidity >95% with constant air circulation. Incoming air was passed through a saturated sodium chloride solution and outgoing air was passed through a Tenax-TA trap.

The maximum label dosing concentration is 2.7 mg/ml. The test solution contained about 20 mg/ml, so its concentration was approximately 7.5 times the maximum label rate.

Eight pears were removed from storage at intervals of 0, 2, 7 and 14 days and 6, 8, 10, 12, 16, 20, 24, 28, and 33 weeks. Each of the pears was washed with methanol (70-100 ml) and the rinses analysed by LSC. Two whole pears were sliced, frozen with liquid nitrogen, and ground. The remaining six pears were peeled, and their combined peels and pulps were separately frozen with liquid nitrogen and ground. Triplicate aliquots of peel, whole pear, and pulp were combusted and the total radioactivity in each sample was measured.

Peel, pulp, and whole pear samples were extracted with methanol/water/chloroform (2.2:1:1) and the post-extraction solids (PES) were extracted with chloroform. All the extracts from each sample were combined, methanol/water and chloroform layers were separated and the ^{14}C in each fraction and the PES were determined. The nominal limit of detection was 0.01 mg/kg as ethoxyquin. The organic and aqueous extracts were then concentrated and analysed by HPLC and TLC.

The post-extraction solids from 33-week pulp, peel, and whole pear were each incubated in acetate buffer at 37°C with cellulase for a total of 26 hours. The hydrolysate was filtered, the aqueous fraction (Aq-1) was extracted with ethyl acetate to give EtOAc-1 and Aq-2, and the remaining solids (PES-2) were refluxed with 0.1 N NaOH for one hour to yield PES-3 and Aq-3. Aq-3 was extracted with ethyl acetate to yield Aq-4 and EtOAc-2.

Portions of the [^{14}C]ethoxyquin dosing solution were subjected to oxidation in air and by horseradish peroxidase. The products were extracted and analysed by TLC or HPLC.

HPLC was used both to analyse extracts and to isolate unknown degradation products. The instrumentation consisted of Nucleosil 5 C-18 100A, 4.6 x 250 mm column with a UV detector (254 nm) and a flow-through radioactivity detector in series and. Two mobile phase systems were used: (1) an isocratic mixture of acetonitrile and water (65:35) and (2) programmed elution from acidic water (0.25 ml H_3PO_4/l; A) to acetonitrile (B). The gradient was programmed from 100% A at 3 minutes to 100% B at 21 minutes. The flow rate for both systems was 1 ml/min. In some cases, the eluates were collected and analysed by LSC.

One-dimensional TLC was used to profile, quantify and purify extracts, with both normal-phase Si-60 F_{254} silica gel and reversed-phase Si-C-18F silica gel plates. Ethoxyquin and other reference standards were co-chromatographed as appropriate. The seven solvent systems used were detailed. The developed plates were examined with a radio-imaging system and non-radioactive spots were visualized with UV light.

Gas chromatography with a radioactivity monitor (RAM) and a flame ionization detector (FID) in parallel was used for the qualitative analysis of extracts and isolated unknowns. The column was a Restek Rt_x-1, 15 m x 0.25 mm i.d. Isolated unknowns were subsequently analysed by GC-MS on a similar column. Both quadrupole and magnetic sector instruments (EI mode) were used.

The total radioactive residues in the methanol rinses and extracts of peel, whole pear, and pulp at each sampling are shown in Table 1. The registrant provided adequate raw data to validate the findings.

The radioactivity in the rinses ranged from about 85% of the TRR in the whole pear on day 0 to about 10-20% after 12 weeks. The radioactivity in the rinsed peel reached about 35-50% of the TRR during weeks 10-33, and that in the pulp ranged from <2% on day 0 to 40-50% at weeks 12-24. The TRR in rinsed whole pears increased from 15% of that in unrinsed pears on day 0 to about 85-90% at weeks 12-33. This indicates translocation of the radioactive residue from the surface to the peel and pulp over a 12-week period.

The peel, pulp, and whole fruit extracts and post-extraction solids from each sampling were analysed for total radioactive residues. In the peel, 55-60% of the TRR was in the chloroform extract, about 20% in the methanolic aqueous extract, and about 25% in the PES. There was no pattern of changing distribution with time. Similar distributions with no consistent pattern of change with post-treatment interval were found in the pulp and whole pears.

The distribution of radioactive residues in the 33-week post-treatment samples of whole pear, pulp, and peel is shown in Table 2.

The methanol rinses at various intervals after treatment analysed by TLC and HPLC showed extensive degradation of ethoxyquin. Normal-phase TLC of 0-day, 2-day, and 6-week rinses revealed four distinct radioactive regions, including one attributable to the parent compound and one at the origin. Similar results were obtained when the TLC was conducted in an inert argon atmosphere. The HPLC radiochromatogram of the 0-day rinse showed ethoxyquin and an unknown eluting about 3.5 minutes earlier at a ratio of 1:4.2, unknown:ethoxyquin. HPLC radiochromatograms of the 28-day methanol rinse showed ethoxyquin, the early-eluting unknown, and at least 3 late eluters, including a major peak at approximately 34.5 minutes (1.4:1, unknown:parent). The 8-week radiochromatogram showed the 34.5 minute peak as the major component (2.2:1.0, unknown:parent). In a total of 6 discrete peaks two components were eluted before the parent and three components after it. The 33-week rinse showed only the 34.5 minute peak (62%) and a small amount of ethoxyquin (6%).

Table 1. Distribution of radioactivity in rinses and fractions of pears at intervals after treatment.

Post-treatment interval	Whole pear			Pulp			Peel (rinsed)	
	Rinse , % of TRR	Rinsed fruit, % of TRR	Total conc., mg/kg[1]	Rinse, % of TRR[2]	Rinsed pulp, % of TRR	Pulp, mg/kg	Peel , % of TRR	Peel, mg/kg
0 days	85.6	14.4	21.3	84.2	1.53	0.370	14.3	19.7
2 days	61.2	38.8	19.5	65.4	7.25	2.33	27.3	45.3

Post-treatment interval	Whole pear			Pulp			Peel (rinsed)	
	Rinse , % of TRR	Rinsed fruit, % of TRR	Total conc., mg/kg[1]	Rinse, % of TRR[2]	Rinsed pulp, % of TRR	Pulp, mg/kg	Peel , % of TRR	Peel, mg/kg
7 days	50.5	49.5	24.0	52.1	15.7	4.67	32.2	51.1
14 days	50.0	50.0	24.4	54.1	19.4	4.52	26.6	34.7
28 days	40.7	59.3	26.4	37.3	30.1	8.92	32.6	51.8
6 weeks	26.4	73.6	26.7	35.7	33.8	8.45	30.5	41.0
8 weeks	19.4	80.6	19.9	26.9	36.7	9.01	36.4	46.7
10 weeks	15.8	84.2	23.2	16.7	37.3	11.4	46.0	73.3
12 weeks	14.9	85.1	20.9	20.8	45.5	11.0	33.8	44.1
16 weeks	11.1	88.9	18.8	15.8	40.6	9.72	43.6	55.4
20 weeks	20.7	79.3	16.1	18.1	45.2	12.6	36.7	54.9
24 weeks	12.5	87.5	25.9	10.5	49.0	14.3	40.5	60.4
28 weeks	14.5	85.5	21.4	16.8	44.1	13.7	39.1	71.3
33 weeks	8.22	91.8	17.2	12.6	37.2	9.42	50.2	75.5

[1] As ethoxyquin. Average 21.8 $\pm$ 3.4 mg/kg, n = 14.
[2] Rinse of whole fruit, before peeling

The 32-minute unknown HPLC peak from the 8- and 33-week post-treatment rinses (the retention time had decreased with use of the column) was isolated by HPLC. Normal-phase TLC showed it to be the spot of highest R_f. The RAM gas chromatogram revealed a retention time of 22.7 minutes, compared to a 13.2-minute retention time for ethoxyquin. The GC-MS spectra of the isolated unknown in the 8- and 33-week rinses were identical. There was a molecular ion at m/z 432 and the ^{13}C compound at m/z 434. As the molecular weight of ethoxyquin is 217, the unknown mass spectrum is consistent with an ethoxyquin dimer (2 x 217-2). The relative abundances of ^{12}C and ^{13}C make it likely that the ^{13}C dimer would contain only one ^{13}C monomer. The fragment of greatest abundance was m/z 417, formed by loss of a CH_3 group. Other significant fragments were at m/z 173 and 201. No further isolations or identifications of the minor degradation products were attempted.

Table 2. Distribution of the radiolabel in the methanol rinse, extracts, and hydrolysates of pears 33 weeks after treatment.[1]

Fraction	Peel (TRR = 94.4 mg/kg as ethoxyquin)		Whole fruit (TRR = 17.2 mg/kg)		Pulp[3] (TRR = 9.42 mg/kg)	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Methanol rinse	20.0	18.9	8.22	1.41	-	-
Chloroform extract	50.7	47.8	45.8	7.86	38.3	3.61
Water/methanol extract	11.8	11.1	27.0	4.63	23.0	2.17
PES-1[2]	22.6	21.3	35.7	6.12	34.2	3.22
Organic extract of cellulase hydrolysate	1.09	1.03	0.402	0.069	0.544	0.0513

Fraction	Peel (TRR = 94.4 mg/kg as ethoxyquin)		Whole fruit (TRR = 17.2 mg/kg)		Pulp[3] (TRR = 9.42 mg/kg)	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Aqueous extract of cellulase hydrolysate	1.60	1.51	1.59	0.273	2.03	0.192
PES-2	22.3	21.0	35.89	6.16	31.0	2.92
Aqueous extract of base hydrolysate	13.3	12.5	22.2	3.81	23.8	2.24
PES-3	8.49	8.01	9.01	1.55	4.12	0.388
TOTAL (%)	107.8		115.4		93.4	

[1] Recalculated without normalization; registrant normalized each processing step to 100%
[2] PES-1 was sequentially hydrolysed with cellulase and 0.1 N NaOH (reflux, 1 h)
[3] Pulp constituted 80.7% (w/w) of the pear, peel 13.6%, 5.7% not identified

The methanol rinse of the 33-week post-treatment fruit (containing 1.4 mg/kg ethoxyquin equivalents) was reacted with methyl iodide (CH_3I) to methylate any amine groups. The product mixture was analysed by HPLC. The ethoxyquin dimer peak remained unchanged, but a new peak appeared at about 19 minutes. Both compounds were isolated and analysed by GC-MS. The 32-minute peak had the spectrum identified as ethoxyquin dimer. The 19-minute compound showed a base peak at m/z 446, 14 mass units higher than the base peak of the dimer. The registrant attributed the 19-minute peak to a C-N dimer and the 32-minute peak to an N-N dimer. A C-N dimer would have one secondary amino group available for methylation (+14); an N-N dimer could not be methylated; a C-C dimer would have two secondary amino groups available for methylation (+28, m/z 460).

The methanol rinses showed decreasing amounts of ethoxyquin with increasing storage periods as follows: day 0 58% of the TRR or 12.4 mg/kg, day 28 15% of the TRR or 3.87 mg/kg, week 8 3.9% or 0.77 mg/kg, and week 33 0.49% or 0.085 mg/kg.

The chloroform extracts of peel, pulp, and whole pear from various samples from day 0 to 6 weeks were analysed by normal-phase TLC. No ethoxyquin was detected at any post-treatment interval. The radiochromatograms showed polar material at the origin and an unknown with a lower R_f value than ethoxyquin. The pulp extracts also contained an unknown with a higher R_f than ethoxyquin. Extracts of a control pear fortified with the dosing solution and of a fortified solvent blank both showed substantial decomposition of ethoxyquin. The HPLC radiochromatograms of the chloroform extracts showed no parent compound and a complex product mixture, even from the 0-day sample. The 33-week peel extract showed 3 major late-eluting peaks (21% at 27.3 min, 28% at 30.6 min, and 11% at 31.8 min). The 33-week whole pear extract showed similar peaks representing 18%, 16%, and 11% of the TRR. The 32-min peak from the 33-week chloroform extracts of whole fruit, peel, and pulp was isolated by HPLC and analysed by GC-MS. The three spectra corresponded to that of the dimer isolated from the methanol rinse (molecular ion m/z 432 and base peak m/z 417). The GC-MS spectrum of the 30.6 min peak had a molecular ion m/z 416 and base peak m/z 401, suggesting a dimeric structure less one CH_4 unit. Attempts to purify the extracts by TLC resulted in decomposition.

The methanol/water extracts of 12-week pear and 33-week whole fruit, peel, and pulp samples were purified by SPE and analysed by TLC. Reverse-phase TLC showed only material at the origin. Normal-phase TLC revealed one smeared zone. No parent compound was evident. Extracts were also analysed by HPLC. The radiochromatograms showed a complex pattern of dispersed radioactivity, with no parent or other distinct major peaks. An extract of 10-week post-treatment pulp contained three late-eluting peaks that corresponded approximately in retention time to the 3 peaks from the chloroform

extracts of 33-week peel and whole pears. Attempts to isolate the components by TLC caused decomposition.

Cellulase digestion of the PES from the 33-week whole fruit, peel, and pulp released 2.6%, 2.7%, and 2.0% of the TRR respectively. An appropriate control (digestion of the sample without cellulase) was not run, and the activity of the cellulase was not demonstrated. Ethyl acetate extraction of the hydrolysis mixture recovered $\leq$1% of the TRR in all cases.

Subsequent base hydrolysis of the post-extraction solids released 14%, 25%, and 23% of the TRR from the peel, pulp, and whole fruit respectively. Very little of this radioactivity (<2%) was extracted from the aqueous phase by ethyl acetate. The aqueous extracts (Aq-4) were analysed by HPLC. All showed one major peak (>90% of the injected ^{14}C) in the 18-20-minute region. The registrant attributed this to a C-N or C-C dimer on the basis only of retention time. In the whole pear 100% of the radioactivity in the water fraction (22% of the TRR, or 3.8 mg/kg) was attributable to the dimer. The compound was not isolated.

About 40% of the TRR in whole pears after 33 weeks was identified as a mixture of C-N and N-N ethoxyquin dimers (62% x 8.22% in the methanol rinse + 28% x 45.8% in the chloroform extract + 100% x 22.2% in the base hydrolysate). About 0.5% of the TRR was identified as ethoxyquin, and an additional 2% was released by cellulase. About 27% of the TRR was characterized as a water-soluble complex mixture of polar compounds in the methanol/water extract, and about 7% of the TRR (from the chloroform extract) might be attributed to monomers such as dehydrodemethyl-ethoxyquin, methyl-ethoxyquin, and dihydro-ethoxyquin, but this identification was tentative and based solely on HPLC retention time patterns.

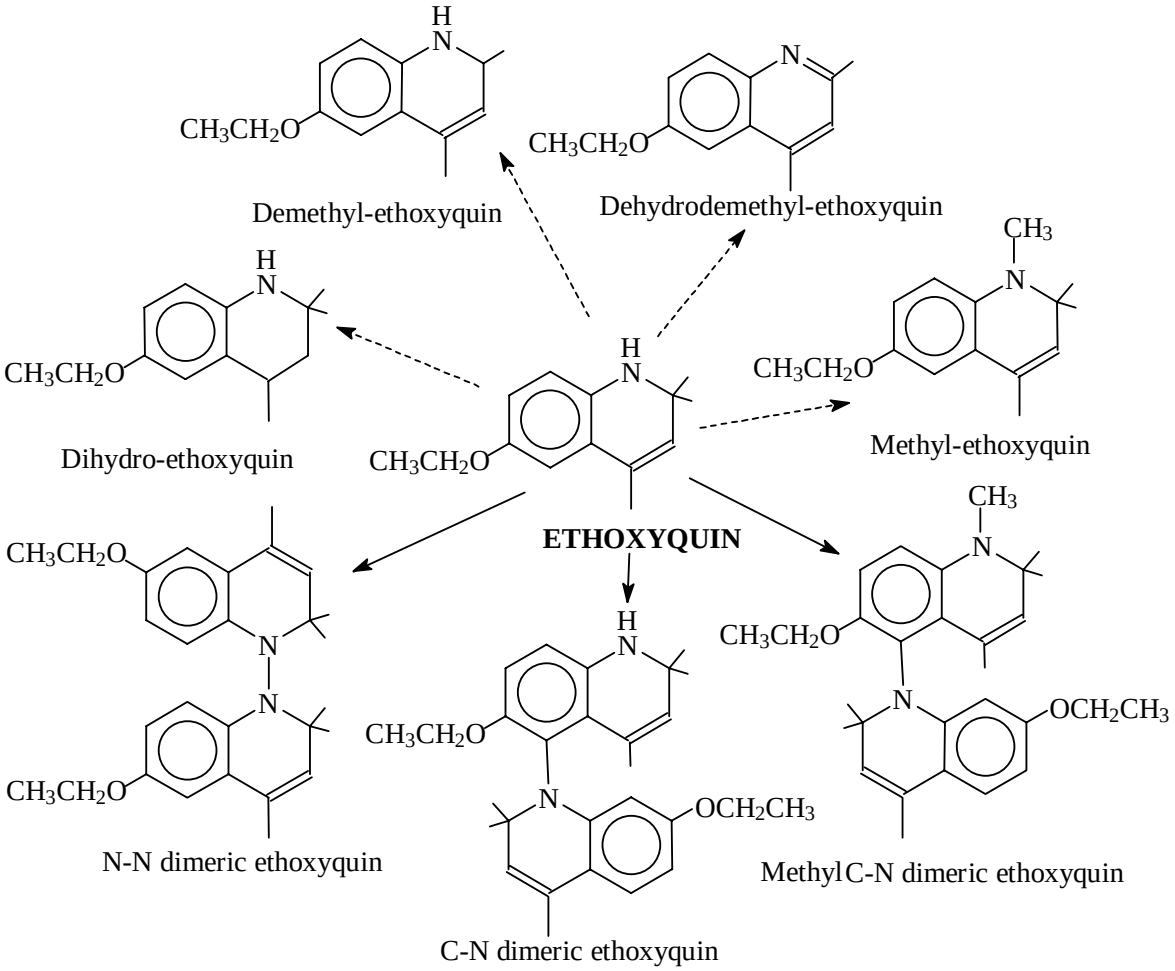
Various experiments were conducted on the dosing solution to identify possible degradation products. A methanol solution of [^{14}C]ethoxyquin was stirred in the air at room temperature for 2.5 days. The product mixture was analysed by TLC and HPLC. The latter showed peaks at 16.4, 17.8 and 35 min in addition to ethoxyquin at 20 min. The two early-eluting compounds were isolated and subjected to GC-MS. The 16 min compound showed four GC-MS peaks on the total ion chromatogram (spectrum nos. 665, 685, 699, and 723) at 11-12.5 min. Spectrum 665 showed a parent ion at m/z 219 and a base peak at m/z 204, consistent with dihydro-ethoxyquin. Spectrum 685, a major peak, had the same spectrum as ethoxyquin and may be from an ethoxyquin isomer. Spectrum 699 had a parent ion at m/z 201 and a base peak at m/z 173. This is consistent with 1-dehydro-2-demethylethoxyquin (Figure 1). Spectrum 723 had a parent ion at m/z 231 (ethoxyquin + 14), suggesting a methylated ethoxyquin (Figure 1).

The 17.8-minute HPLC peak from the oxidation of ethoxyquin was shown to be due to two compounds by GC-MS. Spectrum 686 was identical to that of ethoxyquin. Spectrum 699 had a parent ion at m/z 201, and was presumed to be the dehydrodemethyl-ethoxyquin found in the 16-minute HPLC peak. The compounds identified in the 16-18-minute retention time window may be among those found at similar retention times in the methanol rinses and chloroform extracts from the treated pears at the shorter storage periods, but this is speculative.

Treatment of [^{14}C]ethoxyquin with horseradish peroxidase (5 h, ambient temperature) transformed it to a more polar product whose HPLC retention time did not correspond to any of those in the pear extracts. No further work was conducted.

Figure 1. Proposed metabolic pathways of ethoxyquin in pears.

Dashed arrows indicate metabolism to products that have not been isolated or definitely identified.



Environmental fate

No studies were reported. As the only use of ethoxyquin is as a post-harvest dip treatment for pears exposure of the soil or ground water to ethoxyquin is unlikely. Rotational crops studies also have no practical benefit.

Analytical methods

In the US FDA Pesticide Analytical Method I (PAM Vol. II, Pesticide Reg. Sec. 180.178, 1975) peel or pulp (12 g) is extracted with iso-octane (200 ml) and 10% sodium carbonate (5 ml). The filtered iso-octane extract (50 ml) is partitioned with 0.5 N HCl (3 x 25 ml), the pH of the HCl extract is adjusted with 50% KOH, and the alkaline aqueous solution is extracted with iso-octane (3 x 25 ml). The combined iso-octane extracts are washed with water (25 ml), diluted to 100 ml and analysed in a photofluorimeter. The analysed solution (25 ml) is shaken with 0.04% potassium permanganate (15 ml) and the optical

density of the quenched iso-octane is measured. The second photofluorimeter reading is subtracted from the first. Calibration is with external standards containing known concentrations of ethoxyquin in iso-octane. The method was validated for pears at the limit of determination of 0.25 mg/kg.

The method was validated by dipping a Bosc pear in a solution of radiolabelled and natural-abundance ethoxyquin. The total ethoxyquin concentration was about 0.25%. The pear was dipped for 30 seconds, air-dried, ground with liquid nitrogen into a powder and stored on dry ice. Aliquots were radioassayed.

Three aliquots of the powder were analysed according to the PAM method. Aliquots of the extracts and washes were radioassayed and the final iso-octane extract was analysed with a luminescence/fluorescence detector. Excitation and emission maxima were 382 and 413 nm respectively.

The recovery of the radiolabel at each step of the procedure and a comparison of the results based on radioactivity with those based on fluorescence are shown in Table 3.

Table 3. Recovery of radiolabelled ethoxyquin by PAM Method 1 and comparison of results by measurement of radioactivity and by measurement of fluorescence.

Sample	% of total radiolabel				µg ethoxyquin in sample	
	Original iso-octane extract	Iso-octane after partition with HCl	Alkaline water after extraction with iso-octane	Final iso-octane extract	By radioactivity	By fluorescence
#1	72.4	1.1	2.0	69.8	565	976
#2	68.3	0.8	1.6	66.0	518	875
#3	85.0	1.1	1.4	82.2	677	1177
Mean	75.2	1.0	1.7	72.7	587	1009

The government of The Netherlands submitted two official methods for the determination of ethoxyquin in food commodities (Ministry of Health, Welfare and Sport, 1996). The first is a multi-residue GLC method. The sample is extracted with either ethyl acetate or acetone. Acetone extracts are partitioned with a second solvent, such as methylene chloride. There is no clean-up or derivatization. The gas chromatograph is equipped with a DB-1, DB-5, or DB-1701 capillary column.

The second method is specialized for ethoxyquin, and has been applied to apples and pears. A homogenized sample (25 g) is extracted with 100 ml hexane, 10 ml 10% sodium carbonate, and 10 ml 10% sodium ascorbate solution. The mixture is centrifuged and the hexane layer is retained. The hexane extract is analysed by HPLC on a 250 x 4.6 mm i.d. column packed with Polygosil 60-5, with a fluorescence detector (excitation 355 nm, emission 440 nm). The mobile phase is 1% 1-propanol in hexane at 1.0 ml/min. The recovery is reported to be 104 $\pm$ 3% for apples and 100 $\pm$ 0.8% for pears, at a fortification concentration of 2.1 mg/kg, n = 10. The limit of determination is stated to be 0.01 mg/kg.

A modified AOAC method (AOAC, 1987) has been used in residue trials by the Northwest Horticultural Council. Ten g of homogenized pear is mixed with 0.5 g ascorbic acid in a centrifuge tube and blended with acetonitrile (100 ml, 30 seconds). An aliquot of the extract is analysed by the standard AOAC HPLC procedure. The method was validated at 0.5 mg/kg.

Stability of pesticide residues in stored analytical samples

No data were presented. The pear results of the metabolism study indicate that ethoxyquin is not stable on frozen pears. The proportion of the total radioactive residue attributable to ethoxyquin decreased rapidly at 0°C, being 58% on the day of treatment, 15% after 28 days, 4% after 56 days, and 0.5% after 231 days.

Definition of the residue

The current definition is ethoxyquin. The pear metabolism study has shown that ethoxyquin is readily converted to dimers, polar water-soluble compounds, and the degradation products dehydrodemethyl-ethoxyquin, methyl-ethoxyquin and dihydro-ethoxquin. Even on the day of application, 42% of the ethoxyquin had been degraded.

The Meeting concluded that the residue for compliance with MRLs should be defined as ethoxyquin. The residue for the estimation of dietary exposure cannot be defined until the toxicities of the plant degradation products are known.

USE PATTERN

In the USA, a solution containing 2700 mg/kg ethoxyquin can be applied as a spray on a brush bed or conveyor rolls, or combined in a pack-out wax treatment. It is used post-harvest on pears to prevent scald.

Ethoxyquin has no uses on agricultural crops in Germany or The Netherlands.

RESIDUES RESULTING FROM SUPERVISED TRIALS

Two post-harvest trials were conducted with Anjou pears in the state of Washington, USA, in 1996. The pears were in cold storage before the study. The pears were first soaked in a sodium phenyl orthophosphate bath and dried. The pears were passed through a commercial packing-line on a brush bed conveyor. In each trial ethoxyquin was applied as a wax spray at a concentration of 2700 mg/kg. An SC containing 52.2% ethoxyquin was diluted at 5 ml to 1000 ml water, to give about 0.26 kg ai/hl. The solution was discharged at a rate of 580 ml/min, which provided thorough coverage of the pears. A separate solution was prepared for each trial. The pears were passed through a drier, and duplicate samples of 16 treated and untreated pears were collected in each trial. The pears were halved, immediately frozen, and analysed within 18 days of collection.

The pears were analysed by the US FDA Pesticide Analytical Manual Vol. II method. A P&K Model 650-10S fluorimeter was used, with excitation and emission maxima of 359 nm and 430 nm respectively. Calibration was with external standards, 0.0075–0.18 µg/ml. Linear regression analysis with a forced zero gave typical r^2 values of 0.999. Two sets of validations were conducted before analysing the test samples, with control samples fortified with ethoxyquin at 0.25–5 mg/kg, and recoveries were also determined concurrently with the analysis of treated samples. The recoveries are shown in Table 4. The limit of determination is assumed to be 0.25 mg/kg.

Twelve additional trials in Washington State were conducted in 1999 by the Northwest Horticultural Council. A 51.3% SC was diluted with water to give a nominal 2700 mg ai/l. Organically grown pears stored at 0°C were warmed to room temperature and divided into groups of 20. The 20 pears used in each trial were placed in two plastic file boxes with large openings in the sides and bottom. The pears were sprayed with the 2700 mg/kg solution through four disc cone nozzles located 7.7 cm above the

fruits and spaced 2 cm apart. The spray was released at 1.3 bar. The pears were sprayed for 30 seconds, rotated 180°, and treated for a further 30 seconds. About 2.6 l was applied to each box of twenty.

Two groups of twenty pears were treated with a formulation blank as controls before running the ethoxyquin trials. Each trial was run with an independently prepared treatment solution. The application equipment was cleaned between treatments by rinsing the pressurized tank and nozzles with clean water.

Each group of twenty treated pears was allowed to drain dry and then divided into two sub-groups of 10 pears each. The stems were removed and the pears were individually wrapped in aluminum foil. Each group of ten was placed in a plastic bag, and the bags were vacuum packed and stored frozen pending analysis.

The pears were analysed by the AOAC method. Each group of ten was homogenized after freezing in liquid nitrogen and two 25 g sub-samples were taken from each trial for analysis. All samples were analysed within 14 days of collection. Both control samples contained <0.01 mg/kg. Validation and concurrent recoveries are shown in Table 4. The validated limit of determination was 0.5 mg/kg.

Table 4. Recovery of ethoxyquin from fortified pears.

Fortification, mg/kg	Recovery, %
Validation by PAM Method II	
0.25	107
0.5	104
1	81
3	90
5	98
0.25	107
0.5	86
1	97
3	75
5	94
Concurrent recoveries by PAM Method II	
0.5	69
3	76
5	86
0.25	99
0.5	92
3	89
0.25	129
0.5	91
3	98
0.25	99
0.5	92
3	89
Mean	92.4 $\pm$ 14.6%

Fortification, mg/kg	Recovery, %
Validation by AOAC method	
0.50	99
0.50	108
1.0	99
1.0	101
3.0	104
3.0	101
5.0	103
5.0	103
Concurrent recoveries by AOAC method	
0.566	104
5.44	95.8
1.07	95.3
3.11	84.2
1.03	106
2.96	97.6
0.949	92.3
1.00	92.5
Mean	96.0 $\pm$ 6.9 %

The results of the analyses of the treated pears are shown in Tables 5 and 6 .

Table 5. Residues of ethoxyquin on pears on the day of treatment with a wax containing 2700 mg/kg ethoxyquin applied with a brush bed conveyor.

Trial no.	Sample no.	Ethoxyquin, mg/kg
WA-48	1	0.37, 0.44 (0.40)
	2	0.25, 0.38 (0.32)
Average WA-48		0.36
WA-49	1	0.72, 0.61 (0.66)
	2	0.67, 0.66 (0.66)
Average WA-49		0.66

Table 6. Residues of ethoxyquin on pears on the day of treatment with an aqueous spray at a nominal concentration of 2700 mg/l.

Trial no.	Solution concentration, mg/l	Sample no.	Ethoxyquin, mg/kg
1	2400	1	1.39, 1.76 (1.58)
		2	1.69, 1.38 (1.54)
Average #1			1.56
2	2800	1	1.96, 1.61 (1.79)
		2	1.82, 1.51 (1.67)
Average #2			1.73
3	2800	1	2.22, 2.24 (2.23)

Trial no.	Solution concentration, mg/l	Sample no.	Ethoxyquin, mg/kg
		2	2.04, 2.40 (2.22)
Average #3			2.22
4	2900	1	1.81, 1.55 (1.68)
		2	2.12, 1.86 (1.99)
Average #4			1.84
5	2700	1	2.38, 2.19 (2.29)
		2	2.32, 1.89 (2.11)
Average #5			2.20
6	2900	1	1.73, 2.19 (1.96)
		2	2.35, 2.54 (2.45)
Average #6			2.20
7	2800	1	1.94, 1.86 (1.90)
		2	1.33, 1.78 (1.56)
Average #7			1.73
8	2900	1	1.74, 1.88 (1.81)
		2	1.84, 1.76 (1.80)
Average #8			1.80
9	2800	1	2.18, 2.33 (2.26)
		2	2.14, 2.14 (2.14)
Average #9			2.20
10	2800	1	1.54, 1.90 (1.72)
		2	1.45, 1.39 (1.42)
Average #10			1.57
11	2700	1	1.48, 1.55 (1.52)
		2	1.84, 1.60 (1.72)
Average #11			1.62
12	2800	1	2.00, 2.05 (2.03)
		2	1.32, 1.39 (1.36)
Average #12			1.70

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

No information.

In processing

There are no major processed commodities of pears in international trade.

Residues in the edible portion of food commodities

No information.

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

The government of The Netherlands reported that 500 apples in commerce were analysed in the period 1994–1996. None contained residues at or above the MRL, 3 mg/kg. Five had residues <0.05 mg/kg, the limit of determination.

NATIONAL MAXIMUM RESIDUE LIMITS

The Meeting was informed of the following national MRLs.

The Netherlands	
Apples	3 mg/kg
Pears	3 mg/kg
Other food Commodities	0.05 mg/kg (limit of determination)
USA	
Apples	3 mg/kg (pre-harvest and post-harvest use)
Pears	3 mg/kg (pre-harvest and post-harvest use)

APPRAISAL

Ethoxyquin, an antioxidant preservative, was first evaluated in 1969 and re-evaluated for toxicology in the CCPR Periodic Review Programme in 1998. It was scheduled by the 1995 CCPR for a periodic review of residue chemistry in 1999. The deletion of the CXL for pears treated post-harvest, the only use with a Codex MRL, was postponed pending the toxicological and residue chemistry reviews.

Data on residues and environmental fate were provided by the Northwest Horticultural Council (USA) and additional information was reported by the governments of Germany and The Netherlands.

Animal metabolism

No studies were reported. Since the only use is for the post-harvest treatment of pears and pears are not a ruminant or poultry feed item, studies of metabolism in ruminants and poultry are not needed.

At the 1998 Meeting, the WHO Core Assessment Group reviewed the metabolism of ethoxyquin in rats. Parent ethoxyquin was not found in the urine and only traces were found in the faeces, liver, kidneys and adipose tissue of rats given an intravenous administration. Some metabolites were identified in the urine and bile. The main metabolic pathway was *O*-de-ethylation, yielding a phenol, and conjugation .

The metabolic products in rats are different from those in plants.

Plant metabolism

Anjou pears were dipped in a solution containing unlabelled and [^{14}C]ethoxyquin (ring-labelled) at a concentration of 20 mg/ml, 7.5 times the maximum label rate. The pears were dipped in the solution for 30 seconds, air-dried for 2 hours and stored in an incubator at –2°C and relative humidity >95% with

constant air circulation. Eight pears were removed from storage at intervals of 0, 2, 7 and 14 days, and 6, 8, 10, 12, 16, 20, 24, 28 and 33 weeks.

The eight pears at each sampling were washed with methanol, two were sliced and ground at liquid nitrogen temperature, and the remaining six were peeled and the combined peels and pulps separately frozen and ground.

The whole pears, peel and pulp were each extracted with methanol/water/ chloroform (2.2:1:1). The post-extraction solids from the three 33-week samples were sequentially treated with cellulase and refluxing 0.1 N NaOH.

The samples were analysed by LSC, TLC, HPLC, GLC and GC-MS. The total radioactive residue (TRR) remained constant at 22 $\pm$ 3 mg/kg over the 14 sampling intervals, but the distribution among rinse, pulp and peel changed dramatically. The rinse contained 86% of the TRR on day 0 and this decreased to 50% on day 7 and 8.2% in week 33. The composition of the residue in the rinse changed from 58% ethoxyquin on day 0 to 0.49% in week 33. The residue in the peel increased from 14% on day 0 to 46% in week 10 and then fluctuated between 34 and 50% of the TRR. The residue in the pulp increased from 1.5% on day 0 to 16% on day 7 and to 49% in week 24. Thus, the radiolabelled residue was substantially translocated into both the peel and pulp.

The radiolabelled residue was readily isolated from the pear samples by a combination of solvent rinse, organic and aqueous solvent extractions, cellulase hydrolysis and base hydrolysis. In the pears stored for 33 weeks after treatment, 8% of the TRR was removed by a methanol rinse, 46% was extracted by chloroform, 27% was extracted by methanol/water,
2% was released by cellulase and 23% was released by mild base hydrolysis. The final post-extraction solid contained 9% of the TRR. Similar results were obtained at other storage intervals.

A significant proportion of the radiolabelled residue was identified by a combination of TLC, HPLC and GC-MS. In the pears stored for 33 weeks about 40% of the TRR (6.8 mg/kg as ethoxyquin) was identified as a mixture of C-N and N-N dimers. Only 0.5% (0.09 mg/kg) was identified as parent ethoxyquin. An additional 2% was characterized as released by cellulase and 27% of the TRR (4.6 mg/kg) was characterized by HPLC and TLC as a complex mixture of water-soluble polar compounds. Air oxidation of [^{14}C]ethoxyquin produced a residue that yielded TLC and HPLC chromatograms similar to those of rinses and extracts of [^{14}C]ethoxy- quin-treated pears. The residue is composed of degradation products (dehydrodemethyl-ethoxy- quin, *N*-methyl-ethoxyquin, dihydro-ethoxyquin) and this residue may constitute 7% of the TRR (1.2 mg/kg) in whole pears stored for 33 weeks.

The Meeting concluded that the metabolism and degradation of ethoxyquin on pears is adequately understood. Ethoxyquin is rapidly degraded or metabolized and the residue, but not ethoxyquin itself, is translocated into the pulp. Less than 0.5% of the total radioactive residue was ethoxyquin (in the methanol rinse) in treated pears stored frozen for 33 weeks.

No information was reported to the Core Assessment Group on the toxicology of the plant degradation products. They formed rapidly and were not observed in the rat metabolism study. The Meeting agreed not to recommend any MRLs, and recommended the withdrawal of the single existing CXL, until the toxicology of the degradation products in plants is known.

Environmental fate

No studies on environmental fate were reported, but none are required because ethoxyquin is used only in controlled indoor situations where entry into soil or water is very unlikely.

Analytical methods

The official US enforcement method consists in extraction of the whole fruit, peel, or pulp with iso-octane, clean-up by partition, and analysis with a photofluorimeter. The method was validated with labelled and unlabelled ethoxyquin, and used in pear trials at a limit of determination of 0.25 mg/kg.

The two official enforcement methods in The Netherlands are extraction and analysis by capillary column GLC (multi-residue method) and extraction with n-hexane and analysis by HPLC with a fluorescence detector. Acceptable recoveries were reported.

An AOAC HPLC method was validated at 0.5 mg/kg and used for data collection.

The Meeting concluded that adequate analytical methods exist for the determination of ethoxyquin in fruits for both enforcement and data collection.

Stability of residues in stored analytical samples

No studies were conducted, but the results of the metabolism study clearly indicate that ethoxyquin is unstable on pears stored frozen. Almost 50% of the ethoxyquin is lost on the day of application and 85% is lost by day 28 of frozen storage.

Definition of the residue

The current definition is ethoxyquin. The metabolism study has shown that ethoxyquin is readily degraded to dimers and probably to demethyl-ethoxyquin, methyl-ethoxyquin, dehydrodemethyl-ethoxyquin and dihydro-ethoxyquin.

The Meeting concluded that the residue for compliance with MRLs should be defined as ethoxyquin. The residue for the estimation of dietary exposure cannot be defined until the toxicities of the plant degradation products are known.

Residues resulting from supervised trials.

Fourteen trials on pears were conducted in the USA. GAP specifies post-harvest treatment with a 2700 mg/kg aqueous or wax spray on a brush bed or conveyor rolls. The trials were conducted at this rate, two with brush conveyor application of wax and twelve with an aqueous spray. Samples were frozen immediately and analysed within 14-18 days of treatment. The metabolism study indicates that a substantial loss of ethoxyquin (perhaps about 60%) may have occurred during storage.

The residues in rank 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 estimated an STMR of 1.86 mg/kg and a maximum residue level of 3 mg/kg, but could not recommend the maximum residue level for use as an MRL.

Fate of residues in storage and processing

No storage or processing studies were reported, but ethoxyquin has been shown to be unstable on frozen pears and would be even more unstable on pears stored at temperatures above 0°C.

RECOMMENDATIONS

The Meeting concluded that an MRL could not be recommended. An STMR cannot be recommended in the absence of an MRL.

Definition of the residue for compliance with MRLs: ethoxyquin. The residue for the estimation of dietary intake cannot be defined until the toxicities of the degradation products in plants are known.

Commodity		MRL, mg/kg		STMR, mg/kg
CCN	Name	New	Previous	
FP 0230	Pear	W	3 Po	-

FURTHER WORK OR INFORMATION

Desirable

1. Studies of ruminant or poultry metabolism.

2. A study of the stability of residues in stored analytical samples, with samples taken at intervals of hours up to 24 hours and then on alternate days.

DIETARY RISK ASSESSMENT

Chronic intake

No intake could be estimated because the Meeting recommended withdrawal of the single existing CXL.

Acute intake

The 1998 JMPR concluded that an acute RfD for ethoxyquin is unnecessary. This conclusion was based on a determination that the pesticide is unlikely to present an acute toxicological hazard, and residues are therefore unlikely to present an acute risk to consumers.

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