

TEBUCONAZOLE (189)

First draft prepared by Dr C Anagnostopolous, Benaki Phytopathological Institute, Athens, Greece

EXPLANATION

Tebuconazole is a triazole fungicide in the DMI (demethylation inhibitor) class. Tebuconazole was first evaluated by JMPR in 1994 (T, R). The latest residue evaluation was conducted in 2017 (R).

The 2010 JMPR review of tebuconazole reaffirmed an ADI of 0–0.03 mg/kg bw and established an ARfD of 0.3 mg/kg bw. The residue definition for compliance with the MRL and for estimation of dietary exposure for plant and animal commodities is parent tebuconazole. The residue is not fat soluble.

Tebuconazole was scheduled at the Fiftieth Session of the CCPR for the evaluation of additional uses by the 2019 Extra JMPR. The meeting received residue studies to support the uses in citrus fruits.

RESIDUE ANALYSIS

Analytical methods

The analytical method HW-002-P09-01 (HW-002-P09-01) was used to measure residues of tebuconazole in orange fruit, pulp, oil and juice. Tebuconazole residues are extracted by adding 3:1 v/v acetone:water to an aliquot of plant matrix followed by blending. An isotopic internal standard (IS) is added to the extract and the sample is capped and mixed. The sample was then filtered and vialled. An aliquot of the sample was analysed by LC-MS/MS using a C18 column and the determination was performed in ESI positive MRM mode, however only one MRM transition was monitored instead of two. Since the method was used for data collection in the residue trials, the confirmation of the target analyte which is tebuconazole is not questioned due to the absence of a 2nd transition. Validation was evaluated by recovery from spiked samples. The limit of quantitation was 0.01 mg/kg. Mean recovery values for the individual sample materials and spiking levels (spiking level 0.01–10 mg/kg) were in the range of 80–118% (relative standard deviations 0.8–13.6%). The recoveries obtained during the validation of the method for all 4 matrices are summarized in Table 1 below.

Table 1 Recoveries for tebuconazole in/on various orange commodities (method HW-002-P09-01)

Sample /method	Spike level [mg/kg]	No of tests	Mean recovery [%]	Recovery range [%]	RSD [%]
Orange, fruit	0.01	3	109	107-111	2.2
	0.10	3	104	99-106	3.9
	10.0	3	106	103-110	3.5
Orange, dried pulp	0.01	3	106	106-108	0.8
	0.10	3	105	105-107	1.0
	50.0	3	114	112-118	2.9
Orange, oil	0.01	3	111	98-119	10.7
	0.10	3	85	80-94	8.5
	150	3	103	100-105	2.6
Orange, juice	0.01	3	114	113-116	1.1
	0.1	3	109	106-113	3.4

USE PATTERN

The use pattern relevant for the residue data submitted for evaluation by the present JMPR meeting are summarized in Table 2. Tebuconazole EW 25 is an oil-in-water emulsion (EW) formulation containing 250 g ai/L of the active substance.

Table 2 Registered use of tebuconazole on citrus

Crop	Country	Formulation Type	Application method	No.	Interval (min)	Growth stage	Kg ai/hL	PHI days
Citrus	ES	EW	Post-harvest (drench spray)	1	-	Fruits	0.1	0

RESULTS OF SUPERVISED RESIDUE TRIALS ON CROPS

Citrus fruits

Eight residue trials were conducted in Germany in 1996 (RA-2076/96), with Tebuconazole 250 EW to determine the residues of tebuconazole on mandarin (4) and oranges (4) following one post-harvest spray application. The harvested fruit were at growth stage BBCH 79–89 at the time of application.

Fruits were placed in eight crates in single layers. Each crate contained between 233 to 280 mandarins or 135 to 207 oranges. Tebuconazole 250 EW, an oil-in-water emulsion, was applied at a concentration of 0.4% product (0.1% active ingredient; 0.1 kg ai/hL). The homogeneity of the application solution in the whole area of the fruits was verified with filter paper evenly spaced between the crates. Fruits were stored in dark at 4 °C.

Samples were collected at 0 day and 3, 7 and 13 (14) days after the treatment. The maximum storage period of deep-frozen samples before analysis was up to 273 days.

Residues of tebuconazole were analysed using Methods 00462 and 00462/E001. The LOQ was 0.05 mg/kg for tebuconazole in fruit, peel and pulp. Procedural recoveries for tebuconazole in fruit, peel and pulp were performed at 3 spiking levels (0.05, 0.5 and 5 mg/kg). The average recoveries were 77–89%. The RSD was ≤13%. The full dataset on oranges and mandarins is presented in Table 3 for mandarins and in Table 4 for oranges.

Table 3 Results of residue trials conducted in 1996 in Germany with Tebuconazole 250 EW on mandarins after indoor post-harvest treatment

Crop Variety	Application			Residues			Reference
	No	kg/hL (ai)	GS	Portion analysed	DALT (days)	tebuconazole (mg/kg)	
Satsumas	1	0.1	85	fruit	0	0.48	RA-2076/96 60272/8 0272-96
					3	0.43	
					7	0.33	
					13	0.35	
				pulp	3	<0.05	
					13	<0.05	
peel	3	1.4					
	13	1.3					
Mandarin (Clemons)	1	0.1	83	fruit	0	0.38	RA-2076/96 60578/6 0578-96
					3	0.36	
					7	0.35	
					13	0.32	
				pulp	3	<0.05	
					13	<0.05	
peel	3	1.1					
	13	1.5					

Crop Variety	Application			Residues			Reference
	No	kg/hL (ai)	GS	Portion analysed	DALT (days)	tebuconazole (mg/kg)	
Mandarin (<i>Clause-llina</i>)	1	0.1	89	fruit	0	<u>0.46</u>	RA-2076/96 60804/1 0804-96
					3	0.35	
					7	0.38	
					13	0.32	
				pulp	3	<0.05	
					13	<0.05	
peel	3	1.3					
	13	1.4					
Mandarin (<i>Nova</i>)	1	0.1	79	fruit	0	<u>0.40</u>	RA-2076/96 60807/6 0807-96
					3	0.35	
					7	0.33	
					13	0.30	
				pulp	3	<0.05	
					13	<0.05	
peel	3	1.1					
	13	1.2					

Table 4 Results of residue trials conducted in 1996 in Germany with Tebuconazole 250 EW on oranges after indoor post-harvest treatment

Crop Variety	Application			Residues			Reference
	No	kg/hL (ai.)	GS	Portion analysed	DALT (days)	tebuconazole (mg/kg)	
Orange (<i>Navel</i>)	1	0.1	85	fruit	0	0.22	RA-2076/96 60273/6 0273-96
					3	0.23	
					7	0.25	
					14	<u>0.27</u>	
				pulp	3	<0.05	
					14	<0.05	
peel	3	0.82					
	14	0.83					
Orange (<i>Lanetate</i>)	1	0.1	82	fruit	0	<u>0.28</u>	RA-2076/96 60577/8 0577-96
					3	0.25	
					7	0.28	
					14	0.20	
				pulp	3	<0.05	
					14	<0.05	
peel	3	1.2					
	14	1.2					
Orange (<i>Navelina</i>)	1	0.1	87	fruit	0	0.20	RA-2076/96 60806/8 0806-96
					3	0.23	
					7	<u>0.25</u>	
					14	0.20	
				pulp	3	<0.05	
					14	<0.05	
peel	3	0.92					
	14	0.84					

Crop Variety	Application			Residues			Reference
	No	kg/hL (ai.)	GS	Portion analysed	DALT (days)	tebuconazole (mg/kg)	
Orange (<i>New Hall</i>)	1	0.1	89	fruit	0	0.23	RA-2076/96 60808/4 0808-96
					3	0.27	
					7	0.25	
					14	0.20	
				pulp	3	<0.05	
					14	<0.05	
				peel	3	0.91	
					14	0.90	

Fate of residues in processing

Oranges (marmalade, juice)

Two processing studies were carried out in Germany (RA-3076/96) in order to determine the residues of tebuconazole in the processed commodities juice and marmalade. Tebuconazole EW 250 (an oil-in-water emulsion) was applied at a spray concentration of 0.4% product (0.1 kg ai/hL) in 240 L/ha wax, i.e. 0.024 g tebuconazole/m²) on harvested fruits in two different residue trials. Oranges for processing were collected 3 days after treatment. Fruit samples were processed into marmalade and juice, simulating both household and commercial practice. Processed samples were stored for up to 223–250 days before analysis. Residues of tebuconazole were determined according to Method Nos. 00462 and 00462/E001. The LOQ was 0.05 mg/kg. The processing procedures for peeled fruits, marmalade and juice are described below.

Preparation of peeled oranges: Orange samples were peeled with a knife. Peel and pulp were separated and homogenized in the presence of dry ice.

Preparation of Orange Marmalade: Orange fruits were washed and peeled with a knife. Subsequently the peel was cut into small strips and the fruit pulp was minced with a mixer and subsequently passed through a strainer to separate pulp waste and fruit puree. Sugar, gelling agent and the peel strips were added to the fruit puree. The orange marmalade was heated to 98–100 °C for about 3 minutes. After cooking, the marmalade samples were taken and stored at -18 °C.

Preparation of orange juice (pasteurized): Orange fruits were washed and peeled with a knife. The peeled oranges were pressed into pulp waste and raw juice. After pressing the raw orange juice was pasteurized at temperatures up to 85 °C. After pasteurization, juice samples were taken and were stored at -18 °C.

The results of the trials are summarized in Table 5. No residues of tebuconazole above the LOQ were found in the control sample.

Table 5 Results of marmalade and juice (pasteurized) processing trials conducted in 1996 in Germany with Tebuconazole 250 EW on oranges

Crop Variety	Residues		Processing factor	References
	Portion analysed	Tebuconazole (mg/kg)		
Orange (<i>Navelina</i>)	Fruit (RAC)	0.23	<i>not calculated*</i> <0.22	RA-2076/96 60806/8 0806-96
	juice	<0.05		
	marmalade	<0.05		
Orange (<i>New Hall</i>)	Fruit (RAC)	0.27	<i>not calculated*</i> 0.63	RA-2076/96 60808/4 0808-96
	juice	<0.05		
	marmalade	0.17		

*PF was not calculated since fruits were peeled before processing.

Citrus (pomace, oil, juice)

One processing study was conducted in Southern California (RAHWN001) in order to determine the residues of tebuconazole in the processed commodities dried pulp (pomace), pasteurized juice and oil. Tebuconazole 250 g/L EW was applied at a spray concentration of 0.4% product (0.1 kg ai/hL) on harvested fruits. Following treatment, the treated fruit was placed into cold storage for two to three days to simulate commercial post-harvest storage practices. Following cold storage, the fruit (RAC) samples were removed and triplicate subsamples were taken. The remaining oranges were processed to generate the processed commodities of dried pulp (pomace), oil, and juice (pasteurized). Samples were stored up to 103 days before analysis. Residues of tebuconazole were determined according to method No. HW-002-P09-01. The LOQ was 0.01 mg/kg. Mean procedural recoveries were 88–114% with RSD below 14% for all citrus matrices. The processing procedures for dried pulp (pomace), oil, and juice (pasteurized) are described below:

Preparation of oil: Before processing fruits were cleaned with a rotating brush and washed with warm water. From oil extraction, fruits were passed through a scarifier to scarify the flavedo (epicarp), and the collected flavedo and oil-water emulsion were passed through a mesh to separate the water emulsion from the flavedo fragments. The water emulsion was passed through a separator to separate the oil.

Preparation of juice (pasteurized): An aliquot of the scarified oranges was transferred to a juicer to separate the juice from the peel. The collected juice was screened and pasteurized (89 °C, 15sec.)

Preparation of dried pulp (pomace): The collected peel from the juicing process along with the scarified flavedo was combined to generate the wet peel/pulp. Calcium oxide was added and the limed peel was pressed and then dried to below 10% moisture.

The results of the trial are summarized in Table 6. In orange fruit, average residues of tebuconazole were at 5.6 mg/kg (3 days after treatment). After processing, residues were 40.2 mg/kg in dried pulp (pomace), 137 mg/kg in oil and 0.036 mg/kg in juice. No residues of tebuconazole above the LOQ were found in the control samples, except for oil (0.067 mg/kg).

Table 6 Results of dried pulp (pomace), oil, and juice (pasteurized) processing trials conducted in USA in 2012 with tebuconazole 250 EW in/on oranges

Crop Variety	Residues			References
	Portion analysed	Tebuconazole (mg/kg)	Processing factor	
Orange (Valencia)	Fruit (RAC)	5.6		RAHWN001 HWN001-12PA
	juice	0.036	<i>not calculated*</i>	
	oil	137/0.067**	24.5	
	pomace, dried	40.2	7.2	

* PF was not calculated since fruits were peeled before processing

** residues in control

The Meeting received two processing studies for oranges. An overview of the available processing factors derived in the current evaluation is presented in Table 7 below.

Table 7 Overall summaries of the available Processing Factors in oranges

RAC	Processed Commodity	Processing Factors [best estimate]
oranges	marmalade	<0.22, 0.63 [0.63]
	oil	24.5
	pomace, dried	7.2

APPRAISAL

Tebuconazole is a triazole fungicide in the DMI (demethylation inhibitor) class. Tebuconazole was first evaluated by JMPR in 1994 (T, R). The latest residue evaluation was conducted in 2017 (R).

The 2010 JMPR review of tebuconazole reaffirmed an ADI of 0–0.03 mg/kg bw and established an ARfD of 0.3 mg/kg bw. The residue definition for compliance with the MRL and for estimation of dietary exposure for plant and animal commodities is parent tebuconazole. The residue is not fat soluble.

It was scheduled at the Fiftieth Session of the CCPR for the evaluation of additional uses by the 2019 Extra JMPR. The Meeting received additional residue studies to support the additional uses in citrus fruits.

Methods of analysis

One new analytical method (HW-002-P09-01) was submitted that was used in the processing studies and is considered suitable for the determination of tebuconazole residues in orange fruits, dried pulp, oil and juice. The method is based on a simple extraction with 3:1 v/v acetone:water followed by determination with LC-MS/MS. The LOQ of the method is set at 0.01 mg/kg.

Stability of residues in stored analytical samples

Storage stability studies were not provided to the current Meeting. The 2011 Meeting concluded that residues of tebuconazole are stable in high-acid commodities for at least 30 months in frozen storage. Samples considered by the current meeting were stored for up to 273 days (ca. 9 months).

Results of supervised residue trials on crops

In Spain, tebuconazole is registered for post-harvest use on citrus fruits as a drench spray with a concentration of 0.1 kg ai/hL; no withholding period is specified. Four trials each for mandarins and oranges were conducted approximating the Spanish GAP. For post-harvest treatment the variability is expected to be significantly less than that of field trials thus four trials can be considered sufficient.

Mandarins (Subgroup of)

In mandarins (whole fruit), residues of tebuconazole were (n=4): 0.38, 0.40, 0.46 and 0.48 mg/kg. Residues in pulp were < 0.05 (4) mg/kg.

The Meeting estimated a maximum residue level of 0.7 mg/kg (mean + 4SD) in mandarin subgroup. Based on residues in pulp, the Meeting estimated a STMR of 0.05 mg/kg and HR of 0.05 mg/kg in mandarin subgroup.

Oranges, Sweet, Sour (subgroup of)

In oranges (whole fruit), residues of tebuconazole were (n=4): 0.25, 0.27 (2), and 0.28 mg/kg. Residues in pulp were < 0.05 (4) mg/kg, and residues in peel were (n=4): 0.83, 0.91, 0.92, and 1.2 mg/kg.

The Meeting estimated a maximum residue level of 0.4 mg/kg (mean + 4SD) in orange, sweet, sour (subgroup). Based on residues in pulp and peel, the Meeting estimated a STMR of 0.05 mg/kg and HR of 0.05 mg/kg in orange, sweet, sour (subgroup) pulp and a STMR of 0.915 mg/kg and HR of 1.2 mg/kg in orange peel.

Fate of residues during processing

The Meeting received processing studies for oranges. In one study (RA-3076/96), fruits were peeled prior to processing, which is not reflective of commercial processing, where whole fruits are pressed to obtain juice. Since residues of tebuconazole are on the surface of the fruits, peeling removed a significant amount of the residue that otherwise could have been transferred to the juice. In a second study (RAHWN001), oranges were scarified prior to juicing. This also removed a significant amount of the surface residue that could otherwise be transferred to juice. Therefore, the Meeting decided not to use either study to estimate a processing factor for citrus juice. The processing factors derived from

the processing studies and the resulting recommendations for STMR-Ps, HR-Ps, and/or maximum residue levels are summarized in the table below.

Processing (Transfer) Factors from the Processing of Raw Agricultural Commodities (RACs) with Field-Incurred Residues from Foliar Treatment with tebuconazole

RAC	Processed Commodity	Processing Factors [best estimate]	RAC MRL	Processed Commodity MRL	RAC STMR	Processed Commodity STMR-P
oranges	marmalade	<0.22, 0.63 [0.63]	0.4	--	0.27	0.17
	oil	24.5	0.4	10	0.27	6.6
	pomace, dried	7.2	0.4	3	0.27	1.9

Estimated maximum and mean dietary burdens of farm animals

The Meeting estimated the contribution from citrus pulp (dry) to the livestock dietary burden and based on the small increase by 0.8 ppm of dry matter diet, in relation to the maximum dietary burden estimate from the 2011 JMPR (54 ppm of dry matter diet), no change to the residue situation in animal commodities is expected. The Meeting confirms its previous recommendations for animal commodities.

RECOMMENDATIONS

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

The residue definition for compliance with the MRL and for dietary risk assessment for plant and animal commodities is parent tebuconazole.

The residue is not fat soluble.

CCN	Commodity, subgroups	Recommended MRL (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
FC 0003	Mandarins (subgroup of)	0.7 (Po)	-	0.05	0.05
FC 0004	Oranges, Sweet, Sour (subgroup of)	0.4 (Po)	-	0.05	0.05
AB 0001	Citrus pulp, Dry	3 (dw)	-	1.9 (median residue)	-
OR 0001	Orange oil, edible	10	-	6.6	-
	Orange peel	-	-	0.915	1.2
	Orange marmalade			0.17	

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for tebuconazole is 0–0.03 mg/kg bw. The International Estimated Daily Intakes (IEDI) for tebuconazole was estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2019 Extra JMPR Report.

The IEDIs ranged from 1–5% of the maximum ADI. The Meeting concluded that long-term dietary exposure to residues of tebuconazole from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The ARfD for tebuconazole is 0.3 mg/kg bw. The International Estimate of Short Term Intakes (IESTIs) for tebuconazole were calculated for the food commodities and their processed commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2019 Extra JMPR Report.

The IESTIs were 0–1% (children) and 0% (general population) of the ARfD. The Meeting concluded that acute dietary exposure to residues of tebuconazole from uses considered by the present Meeting is unlikely to present a public health concern.

REFERENCES

Report number	Author(s)	Year	Title, Source, Company name, Report No., Date, GLP status published or not
HW-002-P09-01	Stoughton, S. M.	2009	Analytical method for the determination of residues of tebuconazole in plant matrices using HPLC/MS/MS. Bayer CropScience LP, Stilwell, KS, USA. Bayer AG, Crop Science Division, Method No.: HW-002-P09-01, Edition Number: M-367751-01-1. No GLP, Unpublished
RA-2076/96	Allmendinger, H.	1998	Quantitation of residues of tebuconazole on mandarin and orange after post-harvest treatment of Folicur (250 EW). Bayer AG, Leverkusen, Germany. Bayer AG, Crop Science Division, Edition Number: M-023612-01-2. Unpublished.
RA-3076/96	Allmendinger, H. and Walz-Tylla, B.	1998	Quantitation of residues of tebuconazole in processed commodities of orange after post-harvest treatment of Folicur (250 EW). Bayer AG, Leverkusen, Germany. Bayer AG, Division Crop Science, Edition Number: M-008702-01-1. Unpublished
RAHWN001	Lenz, C. and Freeseaman, P.	2012	Tebuconazole 250 EW - Magnitude of the residue in/on orange processed commodities following post-harvest treatment. Bayer CropScience LP, RTP, NC, USA. Bayer AG, Crop Science Division, Edition Number: M-440417-01-1. Unpublished.