

## 5.10 DIFENOCONAZOLE (224)

### RESIDUE AND ANALYTICAL ASPECTS

Difenoconazole was evaluated by the JMPR for the first time in 2007 when an ADI of 0–0.01 mg/kg bw and an ARfD of 0.3 mg/kg bw were established, and maximum, supervised trial median and high residue levels were recommended for a range of commodities. Additional studies on residues in banana, passion fruits, beans with pods, papaya, ginseng and almonds were evaluated by the present Meeting.

#### *Methods of analysis*

The analytical methods used for the determination of difenoconazole residues in samples derived from supervised trials, submitted for evaluation to the present Meeting, had already been considered by the 2007 JMPR. These methods are based on GC separation and pulse flame photometric detection (PFPD), ECD or MS detection. The validity of the results was supported by validation data on representative crops and results of concurrent recovery studies.

Fresh ginseng root and ginseng processed products were extracted with acetonitrile, partitioned either with dichloromethane or n-hexane, cleaned up on Florisil or silica gel column, and determined applying capillary column GC and ECD. The methods were validated before the analysis of the samples. Additional recovery studies were performed at the same time when the treated or processed samples were analysed. The average recoveries based on minimum 5 replicates ranged between 89 and 105% with repeatability of  $\leq 7.4\%$ . The limits of quantification were between 0.002–0.007 mg/kg for fresh ginseng root, and 0.007–0.04 for various ginseng products.

The freezer storage stability studies carried out with fresh ginseng and ginseng processed products showed that the residue was stable for the longest period (135 days) for which the samples were stored at or below -20 °C. The studies reported by the 2007 JMPR cover the other sample materials evaluated by the present Meeting.

#### *Results of supervised trials on crops*

The original labels with translation were provided only for the countries where the trials had been carried out.

The reports on supervised trials were made available for the Meeting. Some of the trials had not been conducted according to GLP. However, the documentation of the trials was sufficient for evaluation of the results.

#### *Banana*

A national use pattern in China permits a foliar application of difenoconazole EC 25 (250 g/L) on bananas at a dilution rate of 2000 to 3000 which equates to spray solution concentrations of 8.33 to 12.5 g ai/hL with a PHI of 42 days. Multiple applications are permitted with a maximum of three treatments at 10-day intervals.

Difenoconazole formulated as a 250 g/L EC was applied to banana plants (four trials) as a foliar application 3 or 4 times at the maximum GAP rate of 12.5 g ai/hL (2000 $\times$  dilution) and 3 or 4 times at a spray rate of 25 g ai/hL (double rate) in China. Samples were taken at 35 and 42 days. According to the typical practice in China, the applications were performed by spraying, from the ground using manual knapsack or mechanised equipment, directly upwards to the banana plants.

The difenoconazole residues in whole fruit samples treated according to the maximum GAP rate, in ranked order, were: 0.12, 0.18, 0.33 and 0.41 mg/kg.

Residues from 4 treatments at 12.5 g ai/hL rate were: 0.25, 0.29, 0.3, and 0.47 mg/kg. The residues from 3 or 4 treatments were not significantly different indicating that the first treatment made at least 82 days before sampling did not influence the final residue level. The trials performed with 3 and 4 applications were conducted side-by-side, therefore they are not independent and the results cannot be combined.

The residues in banana pulp derived from the same trials were substantially lower. For the 3 application trials the residues were: 0.045, 0.051, 0.052, and 0.11 mg/kg.

No correlation between the residue in pulp and whole banana could be established based on the results.

The 2007 JMPM evaluated residue data derived from ground and aerial treatments carried out with much lower dosage rate (GAP 8 × 0.1 kg/ha, PHI 0 day) than the Chinese trials, and resulted in lower residue values: < 0.02 (6), 0.02, 0.03, 0.04, 0.06, 0.07 and 0.13 mg/kg.

The two residue populations are significantly different and could not be combined.

The Meeting concluded that four residue trials, reflecting the Chinese GAP and growing conditions, was not sufficient for the estimation of residue levels.

#### *Papaya*

To protect fruits from pests and diseases a 3-day PHI is required during harvesting period for continuously fruiting crops like papaya in the Equatorial countries of Africa.

As part of the field trials conducted within the Pesticide Initiative Programme, aiming to provide data for the establishment of import tolerance in the European Union, difenoconazole was applied 6 times during the growing season at 60 g ai/ha at about 14-day intervals at two sites in Côte d'Ivoire. The application conditions (dosage, interval between applications and PHI) were based on the requirement to achieve adequate control of papaya diseases, but were not supported by a label or official declaration of approved use. Samples were collected at 3 and 7 days after the last three applications. The residues measured in samples taken at day 3 were: < 0.05, 0.05, 0.06, 0.07, 0.12 and 0.13 mg/kg.

Residues in samples taken 7 days after the last treatment were: < 0.05, < 0.05, < 0.05; 0.06, < 0.05 and 0.10 mg/kg.

Taking into account the rapid decrease of residues it is most likely that only the last application affects the residue levels in fruits. The residues taken from the same site after repeated treatments can be considered independent.

Based on the 3-day PHI and 0.06 kg ai/ha application rate which provided efficient control of diseases to protect the crop, the Meeting estimated a maximum residue level, STMR and HR values of 0.3, 0.065 and 0.13 mg/kg, respectively.

#### *Passion fruit*

A national use pattern in Brazil permits up to four foliar applications of difenoconazole EC 25 (250 g/L) on passion fruit at a rate of 5 g ai/hL or between 0.01 and 0.04 kg ai/ha with a PHI of 14 days.

In four Brazilian trials the applications were performed within GAP (1 treatment with -25% dosage rate) and the samples taken at 7 days after the treatment contained residues below the LOQ of 0.01 mg/kg with one exception (0.04 mg/kg).

Where the trials were conducted at 2.5–5 times maximum GAP rate the residues in all samples taken at 7 or 14 days were below the limit of quantification (0.01–0.05 mg/kg).

The difenoconazole residues in whole fruit collected at 7 or 14 days PHI were, in ranked order: < 0.01 (6), < 0.02 (2), 0.04, < 0.05 (2) mg/kg.

Taking into account that up to 5 times GAP dose rate did not lead to residues at or above 0.05 mg/kg at shorter intervals than the recommended PHI, the Meeting estimated a maximum residue level, an STMR value and HR value of 0.05, 0.01 and 0.04 mg/kg, respectively.

#### *Legume vegetables*

National use pattern in Portugal permits 2 foliar applications of difenoconazole EC 25 (250 g/L) at 12 to 14 days intervals on beans at a rate of 0.125 kg ai/ha with a PHI of 7 days. Eight trials in beans and one in peas were conducted in Italy and South France.

The results were evaluated against the Portugal GAP. The difenoconazole residues in beans or peas with pods were, in ranked order: 0.01, 0.03, 0.04, 0.05, 0.07, 0.09, 0.17, 0.31 and 0.50 mg/kg.

The Meeting estimated a maximum residue level, STMR value and an HR value for difenoconazole in beans and peas of 0.7, 0.07 and 0.5 mg/kg, respectively.

#### *Ginseng*

The GAP in Korea permits 4 or 5 foliar applications at 10 day intervals with 0.027 kg ai/hL or 0.053 kg ai/hL spray concentration and 7 or 14 days PHI, respectively.

Ready to harvest ginseng plantations of 4–6 years old were treated with difenoconazole EC formulation (0.053 kg ai/hL) 4 times at 10-day intervals in three typical ginseng growing areas in the Republic of Korea.

Three root samples were collected 14 days after the last application from each field. The residues in fresh ginseng root were: 0.0063, 0.011, < 0.02 (3), 0.038, 0.04, 0.10, and 0.36 mg/kg.

The Meeting estimated a maximum residue level, HR and STMR of 0.5 mg/kg, 0.36 mg/kg and 0.02 mg/kg, respectively.

#### *Tree nuts*

The US GAP permits up to two foliar applications of difenoconazole EC 250 (232 g/L) on almonds at a rate of 91 to 128 g ai/ha with a PHI of 14 days.

Six trials were conducted with four foliar applications at a rate of 127 g ai/ha. Samples of mature almond were collected at 14 days after the last application. Residues in all almond nutmeat samples were below the limit of quantification (0.01 mg/kg).

The US GAP permits up to two foliar applications of difenoconazole EC 250 (232 g/L) on pecans at a rate of 91 to 128 g ai/ha with a PHI of 14 days.

Trials were conducted with four applications at a rate of 0.129 kg ai/ha. Residues in 6 pecan nutmeat samples were < 0.01 (5) and 0.02 mg/kg.

Based on the mutually supporting residue data, the Meeting estimated a maximum residue level, STMR value and an HR value for difenoconazole in tree nuts of 0.03, 0.01 and 0.02 mg/kg, respectively.

#### *Animal feed*

##### *Beans and peas forage*

Residues in bean forage following the treatments according to GAP (2 × 0.125 kg/ha, PHI 7 days) were: 0.28, 0.31, 0.75, 0.76, and 0.85 mg/kg.

The Meeting estimated an STMR value and high residue for difenoconazole in bean forage 0.75 and 0.85 mg/kg, respectively.

#### *Almond hulls*

The residues in almond hulls derived from trials complying with the total seasonal rate as specified by US GAP (0.51 kg/ha) in ranked order, were: 0.53, 0.83, 1.04, 1.44, 1.93 and 3.22 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and high residue for difenoconazole in almond hulls of 6, 1.24 and 3.22 mg/kg, respectively. Estimated derived from use of the NAFTA and OECD calculators was 6 mg/kg.

#### *Fate of residues during processing*

Fresh ginseng roots were dried or extracted with ethanol to produce powdery material. The processing was carried out independently from samples obtained from three plots treated at each of the three different sites. The average processing factors were calculated from the results obtained from the three replicate plots. The best estimate for the processing factor for dried ginseng is 3.28. The ethanol extract of dried ginseng resulted in a wide range of processing factors (2.7–18.44) which made the obtaining of a single best estimate impossible. The apparent numerical processing factor for the water extract is 2. However, this estimate is very uncertain as it is based on the LOQ values of processed and fresh ginseng. In one study where the LOQ value was sufficiently low the results indicated a processing factor of 1.1

Consequently the Meeting could only estimate a processing factor of 3.3 for dried ginseng.

#### *Residues in animal commodities*

The 2007 JMPR evaluated two animal transfer studies carried out with Holstein dairy cows administering difenoconazole at 1 ppm (1×), 3 ppm (3×), 5 ppm (5×), 10 ppm (10×) and 15 ppm (15×) in the dry-weight diet for 29–30 consecutive days. The Meeting concluded that the two feeding studies were generally in good agreement of transfer factors, and decided to use the study with the 1 and 3 ppm feeding levels as most closely bracketing the dietary burdens.

#### *Livestock dietary burden*

The residues in almond hull and bean forage evaluated by the present Meeting contributed substantially to the beef and dairy cattle dietary burden calculation based on the maximum portion of agricultural commodities in animal feed (FAO, 2009). Dietary burden calculations for beef cattle, and dairy cattle are provided in Annex 6. The Japanese animal diet contained only soya bean seed of those commodities for which the JMPR estimated highest and median residues. The residues in soya bean seed resulted in an animal dietary burden of 0.00 ppm on dry matter bases, therefore those values are not included in the summary below.

	Livestock dietary burden, difenoconazole, ppm of dry matter diet					
	US/CAN		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.62	0.48	1.85	0.81	2.0	1.77 <sup>b</sup>
Dairy cattle	0.80	0.31	2.42 <sup>a</sup>	1.15	2.14	1.82 <sup>c</sup>

<sup>a</sup> Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat and milk.

<sup>b</sup> Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

<sup>c</sup> Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

### ***Animal commodities, MRL estimation***

For MRL estimation, the residues in the animal commodities are the sum of difenoconazole and CGA 205375 (1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol)) expressed as difenoconazole.

#### *Cattle*

For maximum residue level estimation, the high residues in the tissues were calculated by interpolating the maximum dietary burden of 2.42 ppm (in 2007 it was 2.10 ppm) between the relevant feeding levels (1 and 3 ppm) from the dairy cow feeding study and using the highest tissue concentrations from individual animals within those feeding groups.

The STMR values for the tissues were calculated by taking the STMR dietary burden (1.82 ppm) between the relevant feeding levels (1 and 3 ppm) from the dairy cow feeding study and using mean residue of the 3 animals.

In the following table, dietary burdens are shown in round brackets (), feeding levels and residue concentrations from the feeding study are shown in square brackets [] and estimated concentrations related to the dietary burdens are shown without brackets.

Dietary burden (ppm)	Milk	Muscle	Liver	Kidney	Fat
Feeding level [ppm]	Milk	Muscle	Liver	Kidney	Fat
<b>MRL</b>					
MRL dairy cattle (2.42) [1, 3]	Mean < 0.005 [<> 0.005, < 0.005]	highest 0.021 [<> 0.01, 0.026]	highest 0.121 [0.051, 0.15]	highest 0.0178 [<> 0.01, 0.021]	highest 0.031 [0.015, 0.038]
<b>STMR</b>					
STMR dairy cattle (1.82) [0, 1, 3]	Mean < 0.005 [0, < 0.005, < 0.005]	mean < 0.01 [0, < 0.01]	mean 0.041 [0, 0.045]	mean < 0.01 [0, < 0.01]	mean 0.012 [0, 0.013]

The data from the cattle feeding studies were used to support mammalian meat and milk maximum residue levels.

Residues in the milk were below LOQ (0.005 mg/kg) for all samples from the 1 ppm and 3 ppm feeding groups, so the dietary burdens (2.42 and 1.82 ppm) were taken as a proportion of the 3 ppm to calculate the residues resulting from the dietary burdens.

For muscle, the residue arising from a dietary burden of 2.42 ppm was 0.021 mg/kg, while the residue resulting from a dietary burden of 1.82 ppm was < 0.01 mg/kg. For fat, the residue arising from a dietary burden of 2.42 ppm was 0.031 mg/kg, while the residue resulting from a dietary burden of 1.82 ppm was 0.012 mg/kg.

The Meeting confirmed its previous recommendation for a maximum residue level for difenoconazole in mammalian meat (fat) of 0.05 mg/kg. The Meeting estimated STMR and HR values for meat (fat) of 0.012 and 0.031 mg/kg respectively. The Meeting estimated STMR and HR values for meat (muscle) of 0.01 and 0.021 mg/kg respectively.

The residues in milk were below the limit of quantification at 1 and 3 ppm feeding level. The Meeting estimated a maximum residue level of 0.005\* mg/kg and STMR value of 0.005 mg/kg for milk.

For liver, the residue arising from a dietary burden of 2.42 ppm was 0.121 mg/kg, while the residue resulting from a dietary burden of 1.82 ppm was 0.041 mg/kg. The Meeting confirmed its

recommendation for a maximum residue level of 0.2 mg/kg, and estimated an STMR value and an HR value for difenoconazole in liver of 0.041 and 0.12 mg/kg, respectively.

For kidney, the residue arising from a dietary burden of 2.42 ppm was 0.018 mg/kg, while the residue resulting from a dietary burden of 1.82 ppm was < 0.01 mg/kg. Although the residue levels in kidney were somewhat below those in liver, the Meeting decided that it was preferable to have an animal offal MRL which would be supported by the liver data.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in mammalian edible offal of 0.2, 0.041 and 0.12 mg/kg, respectively.

The Meeting withdrew its previous recommendations for STMR values of 0.043 mg/kg and HR values of 0.11 mg/kg for edible offal (mammalian), and HR values of 0.019 for meat (muscle) and 0.028 mg/kg meat (fat).

## **DIETARY RISK ASSESSMENT**

### ***Long-term intake***

The evaluation of difenoconazole resulted in recommendations for MRLs and STMR values for raw and processed commodities. Where data on consumption were available for the listed food commodities, dietary intakes were calculated for the 13 GEMS/Food Consumption Cluster Diets. The results are shown in Annex 3.

The IEDIs in the thirteen Cluster Diets, based on estimated STMRs were 1–10% of the maximum ADI (0.01 mg/kg bw). The Meeting concluded that the long-term intake of residues of difenoconazole from uses that have been considered by the JMPR is unlikely to present a public health concern.

### ***Short-term intake***

The IESTI of difenoconazole calculated on the basis of the recommendations made by the JMPR represented 0–3 % of the maximum ARfD (0.3 mg/kg bw) for children and 0–2 % for the general population.

The Meeting concluded that the short-term intake of residues of difenoconazole resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.