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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AMITRAZ (Bees)

SUMMARY REPORT (2)

1. Amitraz is a formamide insecticide and acaricide. It is used as a pesticide mainly on top fruit, cotton and hops. In veterinary medicine it is applied topically as a spray, dip, or pour-on to pigs, cattle and sheep for the control of ectoparasites. In apiculture, amitraz is used as a sustained-release strip containing 500 mg amitraz which is suspended in the hive for the treatment of *Varroa* disease. The recommended dose is 2 strips per hive for a period of 6 weeks. Other formulations (sprays, aerosols) are also used in some Member States.

   Currently amitraz is included in Annexes I and III of Council Regulation (EEC) No. 2377/90 as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Amitraz	Sum of amitraz and all metabolites containing the 2,4-DMA moiety, expressed as amitraz	Porcine	400 µg/kg 200 µg/kg 200 µg/kg	Skin + Fat Liver Kidney	
		Bovine	200 µg/kg 200 µg/kg 200 µg/kg 10 µg/kg	Fat Liver Kidney Milk	
		Ovine	400 µg/kg 100 µg/kg 200 µg/kg 10 µg/kg	Fat Liver Kidney Milk	

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Amitraz	Sum of amitraz and all metabolites containing the 2,4-dimethylaniline moiety, expressed as amitraz	Bees	200 µg/kg	Honey	Provisional MRL expires on 1.7.1999

The information requested for the establishment of final MRLs for amitraz in honey has now been provided.

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2. The product had no effect on bee mortality, size of the brood-comb, hive activity, hive weight or the size of the honey harvest when used at twice the indicated dose. At 5 times the indicated dose, hive activity was slightly reduced on very hot days.

3. In mammals, amitraz is rapidly and well absorbed after oral administration and eliminated from most tissues within a few days. Amitraz is rapidly metabolised and excreted, mainly in the urine. The metabolism of amitraz is qualitatively similar in the rat, mouse, cat, dog, baboon, cow and human, proceeding via hydrolysis to N-(2,4-dimethylphenyl)-N'-methyl formamide and 2,4-dimethyl formanilide. These metabolites still contain the 2,4-dimethylaniline moiety. The end product is 4-amino-3-methylbenzoic acid which is rapidly conjugated and excreted. Amitraz is poorly absorbed by the dermal route.

4. No pharmacokinetic or metabolism data were provided for the target species, bees. According to the FAO/WHO Joint Meeting on Pesticide Residues, amitraz was rapidly metabolised in plants to the same metabolites which were found in animals: N-(2,4-dimethylphenyl)-N'-methyl formamide and 2,4-dimethyl formanilide. Because the same metabolic pathways have been shown to occur in a wide range of animal and plant species, it would be logical to assume that the same metabolic pathways occur in bees. Therefore, the marker residue for honey should be the same as that already agreed for other species, i.e. the sum of amitraz and all metabolites containing the 2,4-dimethylaniline moiety, expressed as amitraz.

5. The Committee calculated an ADI for amitraz of 0.003 mg/kg bw per day, based on a NOEL of 0.25 mg/kg bw from a 2-year study in dogs, using a safety factor of 100. This figure was rounded in accordance with the ADI established by the FAO/WHO Joint Meeting on Pesticide Residues (1984, 1990), based on the same toxicity data. The Committee noted that the theoretical maximum daily intake (TMDI) arising from the pesticide use of amitraz was 126 µg/day leaving only 54 µg/day to cover all of the veterinary uses.

6. Following treatment of hives with 500 mg amitraz strips according to the recommended rate and dosing period, samples of honey, wax, pollen and propolis were taken from 4 hives for analysis of residues of amitraz. The samples were taken during mid-treatment, on the last day of treatment, and 3, 7, 14, 21 and 30 days after treatment was withdrawn. The analytical method used HPLC and measured only residues of unmetabolised amitraz. Residues of amitraz in all honey and wax samples taken up to 7 days after the strips were withdrawn were below the limits of detection (5 µg/kg for honey and 2500 µg/kg for wax). Consequently the later samples were not analysed. Residues of amitraz were found in only one sample of propolis taken mid-treatment (750 µg/kg); the limit of detection for propolis samples was 600 µg/kg. Residues of amitraz were also found in 2 samples of pollen which were taken mid-treatment (321 and 1050 µg/kg); the limit of detection for pollen was 200 µg/kg.

7. A second study was carried out in which 4 hives were treated with 500 mg amitraz strips for the recommended rate and dosing period. Samples of honey were taken at mid-treatment and on the day the strips were removed from the hives. Residues of 2,4-dimethylaniline in the samples were determined using a semi-quantitative assay based on thin layer chromatography. There were no detectable residues in any of the samples. The limit of detection was 200 µg/kg. Only a brief summary of this non-GLP study was provided.

8. According to a published report, hives were treated with a 1.6% amitraz formulation applied as an aerosol. The hives were treated either at the recommended rate of 40 mg amitraz or at double the recommended rate (80 mg/hive). Treatment was made during November and samples of bees, honey and wax were taken for analysis during the active season (i.e. the following March, May, July and August). The samples were analysed by a method which converted residues of amitraz and its metabolites to 2,4-dimethylaniline which was derivatised with heptafluoro butyric anhydride prior to determination by gas chromatography. The limit of quantification of the assay was 1 µg/kg. No

residues were detected in the bees. No residues were detected in wax samples from the treatment made at the recommended rate but small amounts of residues were found in wax samples taken during March from the double treatment (up to 61 µg/kg). Residues were also found in honey samples taken during March from the double treatment (up to 12 µg/kg).

9. The previously submitted residue depletion studies carried out using the strips containing 500 mg amitraz probably underestimated the magnitude of the residues present because adequate account of metabolites was not taken. The Committee noted that a number of other formulations based on amitraz had been used in beehives in the EU. Depending on the dosing regime, these could result in residues in honey which were higher that those indicated in the current application. A published paper reported residues of up to 500 µg/kg in samples of mixed honey from Eastern European sources, though it was not stated whether the residues were of amitraz or amitraz plus metabolites.

10. A recently submitted residue depletion study was carried out during 1998 in accordance with the principles of GLP. Two strips containg 500 mg amitraz were suspended in each of 6 hives for a period of 6 weeks. The hives had been treated with the product previously, twice a year, for 3 successive years. Samples of honey and wax were removed at intervals and analysed using the proposed routine analytical method based on gas chromatography. The highest mean residues in honey, 225 µg/kg, expressed as amitraz, were found 2 days after the end of treatment. The mean residues in honey declined to 103.5 µg/kg at 4 days after the end of treatment and 75 µg/kg at 15 days after the end of treatment. Residues in wax were much higher and did not correlate with the residues in honey. Mean residues in wax were 44 700 µg/kg, expressed as amitraz, 2 days after the end of treatment. Although the mean residues in wax depleted to 4 700 µg/kg at 10 days after the end of treatment, there was an apparent resurgence in mean residues in wax to 72 900 µg/kg at 15 days after the end of treatment.

11. The proposed routine analytical method was based on gas chromatography with electron capture detection. Residues of amitraz and its metabolites containing the 2,4-dimethylaniline moiety were converted to to 2,4-dimethylaniline prior to derivatisation and quantification. The method was described in the ISO 78/2 format and was satisfactorily validated. The limit of quantification was 50 µg/kg, expressed as amitraz, for both honey and wax. It was shown that residues in honey were stable during storage for up to 4 months at -20°C but were not stable when stored at +25°C.

Conclusions and recommendation

Having considered that:

- a toxicological ADI has been set at 0.003 mg/kg bw (i.e. 180 µg/person) for amitraz,
- the analytical method for the determination of residues of amitraz and its metabolites containing the 2,4-dimethylaniline moiety in honey was validated in accordance with VolumeVI of the Rules Governing Medicinal Products in the European Community;

the Committee for Veterinary Medicinal Products recommends the inclusion of amitraz in Annex I of Council Regulation (EEC) No. 2377/90 for honey in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Amitraz	Sum of amitraz and all metabolites containing the 2,4-dimethylaniline moiety, expressed as amitraz	Honey bees	200 µg/kg	Honey	

Based on these MRL values, the daily intake of amitraz from honey will represent approximately 2% of the ADI.