The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AMITRAZ (pigs)

SUMMARY REPORT (3)

1. Amitraz is a formamidine acaricide and insecticide used on top fruit, cotton and hops, and as a veterinary medicine for the treatment of ectoparasites in pigs, cattle, sheep, goats and dogs. It may be applied topically, as a spray, dip or pour-on.

2. For the target animal pig amitraz has been previously assessed according to the requirements of Council Regulation (EEC) No 2377/90. The measures taken according to this assessment have been published in Commission Regulations (EEC) No 3093/92 and No 1430/94. A provisional ADI of 0-0.003 mg/kg bw and provisional MRLs for porcine muscle of 0.05 mg/kg and for liver and kidney of 0.2 mg/kg based on the sum of amitraz and metabolites which are measured as 2,4-dimethylaniline, were established (expiry date 1 July 1996). Information to complete the toxicological evaluation and residue depletion data, including a validated analytical method, was required. This information has now been provided.

3. Amitraz interacts with octopamine receptors in the central nervous system of ectoparasites, inducing increased neuronal activity, abnormal behaviour, detachment and death. Clinical effects in mammals are due to its α-2-adrenoceptor agonist activity.

4. Amitraz is rapidly and well absorbed after oral administration and eliminated from most tissues within a few days. Amitraz is rapidly metabolized and excreted, mainly in the urine. The metabolism of amitraz proceeds via hydrolysis to N-(2,4-dimethylphenyl)-N'-methyl formamidine and 2,4-dimethyl formanilide. These metabolites still contain the 2,4-dimethylaniline (2,4-DMA) moiety. The end product is 4-amino-3-methylbenzoic acid which is rapidly conjugated and excreted. This metabolism pattern is qualitatively similar in the rat, mouse, cat, dog, baboon, cow and human. In urine of these species 4-amino-3-methylbenzoic acid (free and conjugated form) is most predominant (>70%). Other metabolites like N-(2,4-dimethylphenyl)-N'-methyl formamidine and 2,4-dimethyl formanilide constitute no more than 10% each.

   Amitraz is poorly absorbed by the dermal route.

5. Administered orally, amitraz is of moderately to low toxicity in acute toxicity tests with rats and mice. The LD_{50}-values in rats and mice are respectively 400-938 and >1600 mg/kg bw. Amitraz is of low toxicity after inhalation in rats (LC_{50} is 65 mg/l). Acute toxicity effects from amitraz treatment include lethargy, hyperexcitability, CNS depression, and hypothermia.

6. In a number of subchronic oral toxicity studies with amitraz in mice, rats and dogs, dogs appeared to be the most sensitive species with CNS depression as the most predominant effect (NOEL 0.25 mg/kg bw/day). Subchronic toxicity studies with metabolites of amitraz showed that 4-amino-3-methylbenzoic acid is far less toxic than amitraz, while the toxicity of N-(2,4-dimethylphenyl)-N'-methyl formamidine on a molar base is comparable to that of the parent compound.

7. From reproduction toxicity studies with rats (1- and 3-generation) and teratogenicity studies with rats and rabbits it was shown that amitraz influences mean litter size and viability of the young in doses that were toxic to the dams. Amitraz showed no teratogenic potential. The overall NOEL for developmental toxicity was 1.5 mg/kg bw/day.

8. In an adequate set of *in vitro* and *in vivo* mutagenicity tests, covering different genotoxic endpoints, amitraz and metabolites were regarded as non-genotoxic. In carcinogenicity studies with mice and

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rats amitraz was regarded as non-carcinogenic (the increased incidence of hepatocellular tumours in female mice was not considered to be significant to human health).

9. From a double blind randomized cross-over study with male volunteers receiving single doses of 0.0625 or 0.125 mg amitraz/kg bw, a NOEL of 0.125 mg/kg bw could be established.

10. Based on the NOEL of 0.25 mg/kg bw/day from the 2-year dog study, and using a safety factor of 100, an ADI of 0.003 mg/kg bw can be established. This figure has been rounded in accordance with the ADI established by the JMPR (1984, 1990) based on the same toxicity data.

11. Part of the ADI for amitraz is already occupied by consumption of food commodities of plant origin since amitraz is also used for plant protection purposes. Based on the recently established EU MRLs for the consumption of products from plant origin, the theoretical estimate of the total maximum daily intake (TMDI) is about 70% of the ADI. This leaves only 30% of the ADI (54 µg/day for a 60 kg person) for products of animal origin.

12. In pigs, residues can be found in all tissues after topical administration (spray or pour-on) of amitraz. After repeated spraying over the entire body surface, the highest residue concentrations were found in skin + subcutaneous fat (declining from 1.6 mg/kg amitraz equivalents after a withdrawal time of 1 day, to 0.013 mg/kg at day 21). Lower levels were found in liver, kidney, muscle and renal fat (0.012-0.03 mg/kg at day 1). Also repeated pour-on administration on the dorsal mid-line of the animals revealed the highest residue concentrations in processed skin (declining from 1 mg/kg amitraz equivalents after a withdrawal time of 1 day, to 0.08 mg/kg at day 14 and 21). Lower levels were found in liver (0.3 mg/kg at day 1, 0.22 mg/kg at day 3 and ≤ 0.1 mg/kg thereafter), kidney (0.24 mg/kg at day 1 and ≤ 0.08 mg/kg thereafter), muscle and fat (≤ 0.1 mg/kg at all time points).

13. In line with the recently established EU MRLs for amitraz for food products of plant origin, the marker residue for amitraz for food commodities of animal origin is now defined as "sum of amitraz and all metabolites containing the 2,4-DMA moiety, expressed as amitraz".

14. The proposed routine analytical GC method (with nitrogen selective detection) for the determination of amitraz in pig tissues is in accordance with the requirements laid down in Volume VI. It is based on hydrolysis of amitraz and all its derivatives containing the 2,4-DMA moiety to 2,4-DMA, and the results can be expressed as amitraz. The limit of quantification is 50 µg/kg (as amitraz) for all matrices.

Conclusions and recommendation

- having set an ADI of 0.003 mg/kg (180 µg/person) for amitraz;
- having considered the depletion profiles of residues of amitraz in pigs;
- having checked the availability of a validated analytical method for residues monitoring purposes;
- as the concentration of residues in muscle was extremely low, and below the LOQ of the analytical method, no muscle MRL was considered necessary.

The Committee recommends the inclusion of amitraz into Annex I of Council Regulation (EEC) No 2377/90 for porcine 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-DMA moiety, expressed as amitraz	Porcine	400 µg/kg	Fat and Skin	
			200 µg/kg	Liver, Kidney	

Based on these MRLs, consumer intake of residues from meat would represent approximately 28% of the ADI calculated in paragraph 10 and, taking into account pesticide use, this ADI would not be exceeded.