The European Agency for the Evaluation of Medicinal Products

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DIAZINON (DIAMPYLATE)

SUMMARY REPORT

1. Diazinon (Diampylate) is an organophosphate insecticide used both as a veterinary medicinal product and for plant protection purposes. It acts as a cholinesterase inhibitor. For the treatment of animals the formulations are diluted in water and applied either as a dip, spray or wash.

2. Diazinon has moderate acute oral toxicity to mice and rats. The clinical signs observed were consistent with cholinesterase inhibition and included sedation, tremors, convulsions and ataxia. It is classified by the World Health Organisation as moderately hazardous.

3. Subchronic and chronic oral toxicity studies in mice, rats, dogs and monkeys were conducted. A NOEL of 0.015 mg/kg bw/day was established based on inhibition of brain cholinesterase activity in a one year study in dogs.

   In an oral 90-day study in rats the NOEL was 0.3 mg/kg bw (5 mg/kg dietary concentration), based on inhibition of brain cholinesterase activity at 250 mg/kg and above.

4. In a carcinogenicity study in mice, diazinon was administered at dietary concentrations of 0, 100 or 200 mg/kg over 103 weeks. There was no evidence of carcinogenicity.

5. In a carcinogenicity study in rats, diazinon was administered at dietary concentrations of 0, 400 or 800 mg/kg for 103 weeks. There was no evidence of carcinogenicity.

6. In a long term toxicity/carcinogenicity study, rats were maintained on a diet containing diazinon at concentrations of 0, 0.1, 1.5, 125 or 250 mg/kg for up to 99 weeks. The NOEL was 1.5 mg/kg (equal to 0.07 mg/kg bw/day), based on inhibition of erythrocyte and brain cholinesterase at 125 mg/kg and above. There was no evidence of carcinogenicity.

7. Twelve mutagenicity studies with different end points were conducted in prokaryotes and eukaryotes in vivo and in vitro. It was concluded that Diazinon is not mutagenic.

8. A multigeneration reproduction study was conducted in rats using dietary concentrations of 0, 10, 100 or 500 mg/kg. The NOEL was 10 mg/kg (equivalent to 0.5 mg/kg bw/day), based on a reduction in parental bodyweight gain in the Fl generation and a reduced survival rate and reduced bodyweight of Fl pups at 100 mg/kg.

9. Two teratogenicity studies were conducted in rats, in which Diazinon was orally administered at dose levels of 0, 10, 15, 20, 50 and 100 mg/kg bw/day. Maternal toxicity, indicated by weight loss and decreased food consumption was evident at 100 mg/kg bw/day. Effects on fetuses at this dose level consisted of retarded ossification and increased incidence of rudimentary ribs. The NOEL was 20 mg/kg bw/day, based on maternal toxicity and fetotoxicity. There was no evidence of teratogenicity.

10. A teratogenicity study in rabbits conducted with oral doses of 0, 7, 25 or 100 mg/kg bw/day revealed clinical signs of maternal toxicity, increased mortality and reduced bodyweight gain at 100 mg/kg bw/day. The NOEL was 25 mg/kg bw/day. There was no evidence of teratogenicity.

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11. Experimental evidence of a teratogenic potential of diazinon was found in studies on chicken embryos. However, as chicken embryo affords no parallel with the anatomical and physiological relationship existing between the pregnant mammal and her conceptus, diazinon is not considered a teratogen of relevance to mammals.

12. A neurotoxicity study performed with hens treated firstly at dose levels of 28 mg/kg bw/day (protected by atropine pre-treatment) and observed for three weeks and again with a single dose of 13 mg/kg bw diazinon and observed for a further three week period. This study did not reveal evidence of delayed neurotoxicity.

13. Diazinon was evaluated in four human male volunteers who received 0,025 mg/kg bw/day of diazinon in capsules for 34 - 36 days. There were no consistent treatment-related effects on plasma or erythrocyte cholinesterase activity, blood chemistry or urinalysis. No clinical effects were reported. The NOEL was 0.025 mg/kg bw/day. The NOEL determined in the study with four human male volunteers is supported by a second oral toxicity study with a 50 W formulation in 6 human test subjects conducted in 1966. A dose level of 0.025 mg active ingredient/kg bw/day was administered to 3 test persons for 43 days. Erythrocyte cholinesterase was not affected. At a dose level of 0.02 mg/kg bw/day, administered to 3 persons on 37 consecutive days, erythrocyte cholinesterase was not affected either.

14. Based on NOEL of 0.02 mg/kg bw/day from the human volunteers study, and a safety factor of 10, the Acceptable Daily Intake is 0 - 0.002 mg/kg bw. This ADI is the same as that set by the Joint Meeting on Pesticides Residues, (JMPR) in 1993.

15. In mammals, diazinon is rapidly and almost completely absorbed from the intestinal tract. Dermal absorption is also high in rats, Metabolism studies using radiolabelled compound have been conducted in rats and dogs as well as in the sheep and goat in compliance with GLP. Diazinon is readily metabolised, principally by cleavage of the pyrimidinyl phosphorous ester band and eliminated predominantly via the urine. In rats administered dermally with doses of 1 and 10 mg/kg bw, 73-81 % of the dose was recovered in the urine within 72 hours.

16. In sheep topically treated with an exaggerated high dermal dose (2270 mg of radiolabelled diazinon in an acetone solution) for 3 consecutive days and sacrified six hours after the final application, parent compound was the only significant residue found in fat (approx. 85% of total residues), a major residues in muscle (approx. 60%) and a minor residue in kidney (6%) and liver (4%). Two pyrimidinyl metabolites were the major metabolites in kidney and liver at this time. However these compounds show no acetylcholinesterase inhibition power and more then 10 times lower acute LD50 toxicity values in rats compared to parent compound. Moreover, these compounds were also found in rats and dogs, where inhibition of cholinesterase was identified as the principal effect of diazinon administration. Parent compound was therefore the marker residue.

17. In goats treated orally for 4 days with 4-5 mg/kg bw and slaughtered 24 hours after the final dose, parent compound was the major residue in fat (approx. 68%) while the pyrimidinyl metabolites accounted for 40% of total residues in liver, 60% in kidney and 75% of muscle radioactivity.

18. Milk residues have been studied in cows, goats and sheep, using untabelled drug. Animals were subjected to a spray formulation of diazinon. In both cow and goat milk diazinon levels were similar during the first four milkings after treatment; thereafter the decrease a residues in goat milk was slower than in cows. In both species, concentration of diazinon was less than 0.02 mg/kg after 3 days. In sheep, lower levels of diazinon were found in milk. Again concentrations declined to 0.02 mg/kg or below 3 days after treatment.

19. Studies using unlabelled drug have been performed in cattle, sheep, goats and pigs.

    In sheep dipped at a concentration of 250 mg/kg diazinon, residues in liver were below 0.02 mg/kg from the third day post treatment; below 0.02 mg/kg in kidney from the 7th day, below 0.02 mg/kg on day 14 in muscle and below 0.7 mg/kg in fat on day 14.

In goats sprayed with 600 mg/kg diazinon, residues were below 0.02 mg/kg in liver, kidney and muscle at 1, 3 and 7 days post treatment respectively. On day 7 all fat levels were below 0.7 mg/kg.

In pigs sprayed once or twice with an interval of 10 days with an emulsion containing 250 mg/kg diazinon residues were below 0.01 mg/kg in liver and kidney, 1 day after treatment. Muscle was below 0.02 mg/kg from day 3 while fat levels were below 0.02 mg/kg at this time also.

In cattle sprayed with an emulsion containing 600 mg/kg diazinon, liver residues were below 0.02 mg/kg in liver at day 1 post treatment. Residues in kidney and muscle were below 0.02 mg/kg at day 7 post treatment while residues in fat were below 0.7 mg/kg at this time also.

20. A validated Gas Chromatography (G.C.) method for the determination of Diazinon in animal tissues is available. This method has a limit of quantitation of 0.01 mg/kg. An alternative Gas Liquid Chromatography (G.L.C.) is also available for the determination of Diazinon in milk. This method has been fully validated with a limit of quantitation of 0.008 mg/kg.

Conclusion and recommendation:

Bearing in mind that Diazinon is used in vegetable crop protection as well as being a veterinary medicinal product in food producing animals, the maximum residue limits (MRLs) set for tissues and products of animal origin must also allow for a contribution from vegetable origin within the overall ADI;

The Committee for Veterinary Medicinal Products recommends that Diazinon be entered into Annex I of Council Regulation (EEC) No 2377/90 and that MRLs for Diazinon be set as indicated in the following table:

Pharmacologically active substance	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Diazinon	Diazinon	Bovine	700 µg/kg	Fat	
		Ovine Caprine	20 µg/kg	Muscle Liver Kidney Milk	
		Porcine	700 µg/kg	Fat	
			20 µg/kg	Muscle Liver Kidney	

The MRLs will account for approximately 62% of the ADI; the remaining 38% being compatible with the Estimated Maximum Daily Intake (EMDI) of residues of Diazinon from vegetable sources.