The European Agency for the Evaluation of Medicinal Products

EMEA/MRL/001/95-FINAL

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AZAMETHIPHOS

SUMMARY REPORT (1)

1. Azamethiphos is an organophosphorus insecticide which acts by inhibition of cholinesterase activity. It is used as a pesticidal spray for control of flies and cockroaches in warehouses and other buildings. In veterinary medicine, it has been proposed for use in fish farming to control external parasites of the Atlantic Salmon. The proposed application rate for this use 0.1 to 0.2 ppm as a "bath" treatment.

2. In rats, azamethiphos was very well absorbed after oral administration and was rapidly metabolised and excreted. The pattern of elimination of the administered radioactivity depended on the position of the radio-label in the molecule. Elimination was recovered mostly from the expired air when the radio-label was in the methylene group and mostly from the urine when the radiolabel was in the pyridine moiety. There was evidence that the major metabolic pathway was similar in rats and salmon and involved degradation to 2-amino-3-hydroxy-5-chloropyridine followed by glucuronic and sulphuric acid conjugation. Azamethiphos was poorly absorbed after topical administration in all the species studied.

3. Azamethiphos was of moderate acute oral toxicity to mammals but of high acute oral toxicity to birds. It was of lower mammalian toxicity when applied topically, reflecting the low percutaneous absorption of the substance. The substance was of low acute oral and dermal mammalian toxicity when formulated as a 50% wettable powder. The signs of acute toxicity were consistent with cholinesterase inhibition. Azamethiphos was an eye irritant in the rabbit. The 50% wettable powder formulation was only a mild eye irritant.

4. Azamethiphos is prone to degradation in animal feeds. In the early repeat-dose studies, achieved test substance intakes were on the low side until allowances were made for this instability. In 13-week repeat-dose studies in dogs, a no-effect level of 0.26/0.33 mg/kg bw per day was established in males/females respectively, based on reductions in plasma and erythrocyte cholinesterase activities. In a 52-week study in dogs, no reduction in brain cholinesterase activity was observed at a dose level of 2.72/2.86 mg/kg bw in males/females respectively. There was some reduction in erythrocyte cholinesterase activity at this dose level, but not at the lower dose level of 0.26/0.24 mg/kg bw per day, for males/females respectively.

5. Significant reductions in plasma and erythrocyte cholinesterase activities were also observed in a 13-week repeat-dose study in rats. At the top dose level (3000 ppm) cholinesterase activity was reduced even after the rats were maintained on untreated diet for a 28-day "recovery" period. No no-effect level was established for the inhibition of blood cholinesterase activity. Brain cholinesterase activity was not monitored.

6. Azamethiphos had a very low therapeutic margin of safety in the target species, salmon. Consequently the drug must be used with great care, paying attention to the correct dose level, since over-dosing could result in the death of the target species.

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7. In a 3-generation reproduction study in rats, there were no significant effects on reproductive parameters but the study was carried out under unsatisfactory conditions and so it was not possible to place much reliance on the results. Two 2-generation studies in the rats were subsequently carried out in accordance with the principles of GLP. There were no significant effects on reproductive parameters though pup weights were reduced at dose levels that also caused reductions in parental body weight gain. 40 ppm (2 mg/kg bw per day) was a no-effect level based on reduced body weight and 2 ppm (0.1 mg/kg bw per day) was a NOEL based on erythrocyte cholinesterase inhibition. No effect on brain cholinesterase activity was observed at dose levels of up to 1000 ppm (approximately 50 mg/kg bw).

8. Two teratology studies were carried out in rats. There was no evidence of teratogenicity in either study. The no-effect level for foetotoxicity was 75 mg/kg bw in one study but there was no evidence of foetotoxicity at doses up to 200 mg/kg bw in the second study which used a different strain of rat. Teratology studies were also carried out in the chinchilla and the NZW rabbit; there was no evidence of teratogenicity in either study. Reduced foetal weights and an increased incidence of delayed ossification in the chinchilla study indicated foetotoxicity at dose levels that were maternally-toxic. The no-effect level for foetotoxicity was 7.5 mg/kg bw per day In the study in NZW rabbits, there was no evidence of foetotoxicity at the top dose level of 18 mg/kg bw per day a dose level that was maternally-toxic; the no-effect level for maternal toxicity was 2 mg/kg bw.

9. Azamethiphos was mutagenic in several in vitro assays. The substance was an indirectly acting mutagen in *S. typhimurium* TA 100 and induced DNA damage in mammalian cells *in vitro*. Azamethiphos induced an increase in revertants in *S. cerevisiae* D7 and demonstrated transformative properties in BALB/3T3 mouse embryo fibroblasts. An equivocal result was obtained in the mouse lymphoma assay.

10. Four in vivo mutagenicity assays were carried out and all gave negative results. However the protocols for three of these studies could be criticised. The mating schedule used in the dominant lethal assay did not cover the full period of germ cell maturation, and small group sizes were used in the nucleus anomaly test and the sister chromatid exchange assay. The UDS assay in rabbit testes is an assay which has not been well validated.

11. Several organophosphorus compounds have been reported to give positive results in mutagenicity assays in vitro but to give no significant positive results in vivo. (For example, see IARC Monograph Volume 53 page 288 on Dichlorvos: Genetic and related effects.) The rapid metabolism may be one reason why the positive in vitro activity is not expressed *in vivo*.

12. Two carcinogenicity studies were carried out in the rat. In the first (pre-GLP) study, survival at termination was below 50% in all the male groups. Cholinesterase activity was reduced at all dose levels. There was no evidence of carcinogencity. Survival at termination was below 50% in all groups except the top dose females in the second (GLP) study in rats in which doses of up to 1500 ppm were administered in the feed. There was no compound-related increase in tumour incidence. A no-effect level of 0.8 and 1.1 mg/kg bw per day was established for males and females respectively, based on reduced plasma and erythrocyte cholinesterase activity. Brain cholinesterase activity was not reduced at this dose level, nor at the higher dose level of 8.0/11.2 mg/kg bw for males/females respectively.

13. A non-GLP carcinogenicity study in the mouse was flawed by poor survival at termination though survival at week 94 was at least 50% in all groups. There was no increase in tumour incidence in this study. Poor survival also marred a GLP study in which erosive or ulcerative lesions of the gastrointestinal tract (frequently associated with anaemia) were common causes of death at the top dose level of 4000 ppm. These lesions were not observed in the earlier study which used lower dose levels There was no compound-related increase in tumour incidence in the GLP study; on the contrary, tumour incidence was reduced at the top dose level probably reflecting the reduced survival in that group. Overall, it was concluded that azamethiphos was not carcinogenic.

14. Special studies were carried out to investigate the potential of azamethiphos for delayed neurotoxicity and to investigate the effectiveness of various antidotes. Azamethiphos did not induce delayed neurotoxicity and atropine was shown to be an effective antidote in countering the anticholinesterase effects.

15. Azamethiphos was a sensitiser in the standard guinea pig test recommended by the OECD. Cases of skin sensitisation were also reported in a small number of pesticide spray operators.

16. The proposed use of azamethiphos in fish farming means that deliberate contamination of the aquatic environment will occur. Azamethiphos is fairly toxic to several aquatic species including lobster larvae. However it degrades fairly rapidly in seawater. Nevertheless, great care should be observed and precautions taken to minimise environmental contamination.

17. An ADI of 0-0.025 mg/kg bw, by applying a safety factor of 100 to the NOAEL of 2.5 mg/kg bw in the dog. This was considered to be the most appropriate end-point on which to base the ADI because brain cholinesterase activity was not reduced. Although there was some depression in erythrocyte cholinesterase activity at this dose level, the magnitude of the depression was not indicative of an adverse toxicological effect.

18. The absorption of azamethiphos following the topical treatment of salmon was low and there was no bioaccumulation. Depletion of total azamethiphos-related residues in salmon was rapid. Residue depletion was faster in muscle than in other tissues such as liver and skin. Immediately after treatment, "total" residues in muscle were in the region of 0.02 mg/kg. Residues of unmetabolised azamethiphos in muscle and skin were below 0.02 mg/kg within 1 hour of treatment.

19. Because of the low residue concentrations, the nature of the residues in salmon tissues was not investigated. An investigation into the nature of the residues in salmon bile indicated that the major metabolic pathway was similar in rats and salmon and involved degradation to 2-amino-3-hydroxy-5-chloropyridine and subsequent conjugation.

20. The Committee agreed to elaborate the following provisional MRL:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Azamethiphos	Azamethiphos	Salmonidae	100 µg/kg	Muscle and skin in natural proportions	Provisional MRL expire on l.6.1997

Based on this MRL, it was calculated that consumer intake of azamethiphos from fish would account for less than 5% of the ADI calculated above.

21. The routine analytical method for the determination of residues of azamethiphos in salmon tissues was based on HPLC with UV detection. The method was of appropriate specificity and some data on precision had been obtained at a concentration of 40 µg/kg indicating that this may be the Limit of Quantitation.

However the limits of detection (LOD) and quantitation (LOQ) had not been correctly determined in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community. It was agreed that the MRLs should remain provisional pending satisfactory determination of the LOD and LOQ.

The information on the determination of the LOD and LOQ should be provided by 1 June 1996.