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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

AZAMETHIPHOS

SUMMARY REPORT (2)

1. Azamethiphos is an organophosphorus insecticide which acts by inhibition of cholinesterase activity. In veterinary medicine, it is used in fish farming to control external parasites of the Atlantic Salmon. The application rate for this use is 0.1 to 0.2 mg/litre as a bath treatment.

   Azamethiphos is currently included in Annex III of Council Regulation (EEC) No 2377/90, in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Azamethiphos	Azamethiphos	Salmonidae	100 µg/kg	Muscle and skin in natural proportions	Provisional MRLs expire on 1.6.1999

   Azamethiphos is also used as a pesticidal spray for control of flies and cockroaches in warehouses and other buildings.

2. In rats and lactating goats, azamethiphos was very well absorbed after oral administration and was rapidly metabolised and excreted. The oral bioavailability was close to 100% in both species. The pattern of elimination of the administered radioactivity depended on the position of the radiolabel in the molecule. When rats were given azamethiphos ^{14}C-labelled in the methylene group at an oral dose of 5 mg/kg bw, 41%, 4% and 35% of the administered radioactivity was recovered from the urine, the faeces and in expired air, respectively, within 24 hours of dosing. However when the substance was ^{14}C-labelled in the pyrimidine moiety 85 to 98% of the orally administered radioactivity was recovered from the urine, 1 to 6% from the faeces and 0.01 to 0.05% from the expired air, respectively, within 24 hours of dosing. In goats given an oral dose of [3a-^{14}C]-pyridyl azamethiphos, equivalent to 9 mg/kg feed, approximately 80% of the administered radioactivity was recovered from the urine, almost all of this within 12 hours of dosing. Less than 0.5% of the administered dose was recovered in the milk. The substance was widely distributed to the tissues but residues in all tissues of both rats and goats were rapidly depleted.

   Azamethiphos was poorly absorbed after topical administration. In rats treated topically with 10, 100 or 1000 µg of [3a-^{14}C]-pyridyl azamethiphos/cm^2 of skin (equivalent to 0.1, 1.0 or 10 mg/rat), absorption over a 10-hour exposure period corresponded to 19%, 4% and 1% of the administered dose, respectively. Goats were killed 29 hours or 120 hours after topical treatment with [3a-^{14}C]-pyridyl azamethiphos; the total recovery of radioactivity from blood, tissues, excreta and milk accounted for only 2.3% and 2.9% of the administered dose, respectively.

   Unmetabolised azamethiphos in rat excreta accounted for less than 0.5% of the administered radioactivity. No residues of azamethiphos were detected in goat urine. In both species, the major urinary metabolites were identified as 3-amino-3-hydroxy-5-chloropyridine and its glucuronide and sulphate conjugates. The residues in tissues could not be charcterised due to the low concentrations of radioactivity present.

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3. Azamethiphos was of moderate acute oral toxicity to mammals but of high acute oral toxicity to birds. Acute oral LD_{50} values of 1040 to 1180 mg/kg bw and 1452 mg/kg bw were obtained in rats and mice, respectively but the acute oral LD_{50} in quail was 91 mg/kg bw. The substance 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 the 50% wettable powder used in fish farming; the acute oral and dermal LD_{50} values in rats were 2413 mg/kg bw and greater than 3100 mg/kg bw, respectively. In the acute studies, the signs of toxicity were consistent with cholinesterase inhibition. Azamethiphos was an eye irritant in the rabbit but 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 a 13-week repeated-dose study in dogs, groups of 4 and 5 male and female Beagles were fed diets containing 0, 30, 300 or 3000 mg azamethiphos/kg feed. The concentrations of 30 and 300 mg/kg feed corresponded to 1.1 and 11.0 mg/kg bw/day in males and to 1.2 and 12.8 mg/kg bw/day in females, respectively. Administration of the top dose level of 3000 mg/kg feed was interrupted on days 5 to 14 and then reduced to 1000 mg/kg feed on day 36 due to severe loss of bodyweight. Emesis and reduced plasma and erythrocyte (but not brain) cholinesterase activities were observed in all azamethiphos treated groups and so a NOEL was not established. A second 13-week study was carried out in which groups of 4 male and 4 female Beagles were fed diets containing 0 or 10 mg azamethiphos/kg feed, corresponding to 0, 0.26 and 0.33 mg/kg bw/day in males and females respectively; no substance-related effects were observed. In a 52-week study in dogs, groups of 4 male and 4 female Beagles were fed diets containing 0, 10, 100 or 1000 mg azamethiphos/kg feed. These doses corresponded to 0, 0.26, 2.72 and 31.5 mg/kg bw/day in males respectively and to 0.24, 2.86, 28.43 mg/kg bw/day in females, respectively. At 1000 mg/kg feed, there were clear reductions in brain, plasma and erythrocyte cholinesterase activities. Brain cholinesterase activity was not depressed at lower doses. At 100 mg/kg feed, plasma and erythrocyte cholinesterase activities were depressed but the reductions were not considered to be biologically significant. Therefore, 100 mg/kg feed, equivalent to 2.72 and 2.86 mg/kg bw/day in males and females, respectively was the NOAEL.

   Significant reductions in plasma and erythrocyte cholinesterase activities were also observed in a 13-week repeat-dose study in which groups of 25 male and 25 female Sprague-Dawley rats were fed diets containing 0, 30, 300 or 3000 mg/kg feed, equivalent to 0, 2, 20 and 241 mg/kg bw/day. At the top dose level erythrocyte cholinesterase activity remained significantly reduced even after the rats were maintained on untreated diet for a 28-day recovery period. Brain cholinesterase activity was not monitored. Consequently no conclusions could be drawn from this study regarding a NOEL. It was not considered necessary for the study to be repeated because a satisfactory NOAEL had been established in the dog, the species normally the most sensitive to the anticholinesterase activity of organophosphorus compounds, and because brain cholinesterase activity was monitored in a 2-year study in rats.

5. 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.

6. 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 rats were subsequently carried out in accordance with the principles of GLP. In one study, azamethiphos was administered in the diet at concentrations of 0, 40, 200 or 1000 mg/kg feed, equivalent to 0, 2.7, 13.3 and 65.2 mg/kg bw/day in males and to 0, 3.0, 14.8, 71.3 mg/kg bw/day in females respectively. In the other study, azamethiphos was administered in the diet at concentrations of 0, 2, 40, 200 or 1000 mg/kg feed, equivalent to approximately 0, 0.1, 2 to 3, 10 and 50 mg/kg bw/day. In these studies, there were no significant effects on mating or fertility.

   However, pup weights were reduced at dose levels which also caused reductions in parental bodyweight gain. The dose level of 40 mg/kg feed (equivalent to approximately 2 to 3 mg/kg bw/day) was considered to be the NOEL based on reduced bodyweight. No effect on brain cholinesterase activity was observed at any dose level. The NOAEL for plasma and erythrocyte cholinesterase inhibition was considered to be 40 mg/kg feed (equivalent to approximately 2 to 3 mg/kg bw/day).

7. Two teratology studies were carried out in rats. In the first study, groups of 22 to 29 female Sprague-Dawley rats were given daily oral gavage doses of 0, 25, 75 or 150 mg/kg bw/day from days 6 to 15 of gestation. Signs of maternal toxicity, including reduced food consumption, were evident at 150 mg/kg bw/day. There was no evidence of teratogenicity but an increase in the incidence of delayed ossification was observed at 150 mg/kg bw/day; the NOEL for foetotoxicity was 75 mg/kg bw/day. In the second study, groups of 26 to 29 female Sprague-Dawley rats were given daily oral gavage doses of 0, 1, 75 or 200 mg/kg bw/day from days 6 to 15 of gestation. Maternal toxicity (including salivation, lethargy, diarrhoea and reduced bodyweight gain) was observed at 200 mg/kg bw/day. There was no evidence of teratogenicity or foetotoxicity at any dose level.

   Teratology studies were also carried out in the chinchilla (daily oral gavage doses of 0, 2.5, 7.5 or 15 mg/kg bw/day were administered from days 6 to 18 of gestation) and the New Zealand White rabbit (daily oral gavage doses of 0, 2, 12 or 36 mg/kg bw/day were administered from days 7 to 19 of gestation). Due to the deaths of 4 dams, the top dose level of 36 mg/kg bw/day was reduced to18 mg/kg bw/day). 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 also caused maternal toxicity. The NOEL for foetotoxicity in the chinchilla was 7.5 mg/kg bw/day. In the study in New Zealand White rabbits, there was no evidence of foetotoxicity at any dose level. However maternal toxicity (reduced bodyweight gain) was observed at 12 mg/kg bw/day; the NOEL for maternal toxicity was 2 mg/kg bw/day.

8. Azamethiphos was mutagenic in several *in vitro* assays. The substance was an indirectly acting mutagen in *Salmonella typhimurium* TA 100 and induced DNA damage in mammalian cells (human fibroblasts and rat hepatocytes) *in vitro*. Azamethiphos induced an increase in convertants in *Saccharomyces cerevisiae* D7 and demonstrated transformative properties in BALB/3T3 mouse embryo fibroblasts. An equivocal result was obtained in the *in vitro* mouse lymphoma assay for gene mutation.

   Four *in vivo* mutagenicity assays were carried out and all gave negative results. However, the protocols for three of these studies were not completely satisfactory and the fourth study used an assay which had not been well validated. The mating schedule used in the dominant lethal assay in male mice (administered oral doses of 0, 120 or 360 mg/kg bw) did not cover the full period of germ cell maturation. Too small group sizes were used in the nucleus anomaly test in which Chinese hamsters were administered two doses in the range 33.5 to 135 mg/kg bw, 24 hours apart, and bone marrow samples taken 24 hours after the second dose. Too small group sizes were also used in the sister chromatid exchange assay in which Chinese hamsters were given single oral doses of 0, 33.5, 67 or 134 mg/kg bw and bone marrow samples taken 24 hours after dosing. The unscheduled DNA synthesis assay in rabbit testes is an assay which has not been well validated; in this study rabbits were given single intraperitoneal injections of 2.5, 5 or 10 mg azamethiphos/kg bw. It was concluded from these data that azamethiphos was probably not genotoxic *in vivo*.

9. In a study carried out in accordance with the principles of GLP, groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing 0, 20, 200 or 1500 mg/kg feed, equivalent to approximately 0, 0.8, 8.0 and 63 mg/kg bw/day in males and to 0, 1.1, 11.2 and 89 mg/kg bw/day in females, respectively. Satellite groups of 20 animals per sex and dose and 10 animals per sex and dose were maintained on the same diets and used for blood monitoring and interim kill. There was no evidence of carcinogenicity or specific target organ toxicity. Brain cholinesterase activities were reduced at the top dose. Although some depression of plasma and erythrocyte cholinesterase activities was apparent at 200 mg/kg feed, the reductions were of no toxicological significance. Therefore, 200 mg/kg feed (equivalent to 8.0 and 11.2 mg/kg bw/day in males and females, respectively) was a NOAEL.

A poorly conducted (pre-GLP) carcinogenicity study was carried out in which groups of 60 male and 60 female CD-1 mice were fed diets containing target concentrations of 0, 20, 100 or 500 mg/kg feed. There was no increase in tumour incidence in this study and no evidence of any target organ toxicity. However, survival was poor and the achieved dietary concentrations were significantly below the target concentrations. In a more recent study carried out in accordance with GLP, groups of 51 male and 51 female CD-1 mice were fed diets containing 0, 50, 500, 1500 or 4000 mg/kg feed, corresponding to 0, 6.2, 60.2, 183.4 and 491.4 mg/kg bw/day in males and to 0, 7.7, 76.2, 219.7 and 582.9 mg/kg bw/day in females, respectively for 102 and 104 weeks. In this study, erosive or ulcerative lesions of the gastrointestinal tract (frequently associated with anaemia) were common causes of death at the top dose level of 4000 mg/kg feed. There was no compound-related increase in tumour incidence. Overall, it was concluded that azamethiphos was not carcinogenic.

10. In a study carried out in 1978, groups of 30 hens were given either a single dose of 66 mg azamethiphos/kg bw or two doses at the LD_{50} value, 21 days apart. The birds treated at twice the LD_{50} value were also given intramuscular injections of atropine. There were no clinical signs or histopathological changes indicative of delayed neurotoxicity. However a substantial number of tissues were lost to autolysis. Another study was carried out in 1991 to a more modern protocol. Some aspects of the study were not completely satisfactory. However there was no evidence of delayed neurotoxicity. No biochemical assays were conducted for neuropathy target esterase (NTE) in either of these studies. It was noted that at the time of the first assessment of azamethiphos there were no validated assays for neuropathy target esterase and the OECD guidelines did not include such tests. The absence of such information was considered acceptable in this case in the light of the absence of detectable residues in salmon muscle and skin.

11. Azamethiphos was a skin sensitiser in two separate studies in guinea pigs. Cases of skin sensitisation were also reported in a small number of pesticide spray operators.

12. An ADI of 0.025 mg/kg bw, by applying a safety factor of 100 to the rounded NOAEL of 2.5 mg/kg bw/day in the dog was established. 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. A similar NOEL (2 to 3 mg/kg bw/day) was established in a 2-generation study in rats, based on reduced bodyweight; in this study, the same dose level was considered to be a NOAEL for cholinesterase inhibition.

13. 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 of salmon with ^{14}C-azamethiphos, formulated as the 50% wettable powder, at a nominal concentration of 0.2 mg/litre in the water, for one hour, mean total residues in muscle were 20 µg equivalents/kg and depleted to 4 µg equivalents/kg, 12 hours after the end of treatment. Over the same time period, mean total residues in salmon skin depleted from 117 µg equivalents/kg to 16 µg equivalents/kg.

    Because of the low residue concentrations, the nature of the residues in salmon muscle was not investigated. Dissected portions of tissues such as skin and liver were homogenised and extracted but the large amounts of co-extracted fish materials and the low amount of radioactivity present precluded characterisation of metabolites. Because significant concentrations of radioactivity were present in bile, the characterisation of metabolites in bile was attempted. The major metabolite, accounting for more than 50% of the radioactivity in bile fluid, was the glucuronic acid conjugate of 2-amino-3-hydroxy-5-chloropyridine. This metabolite had been identified as one of the major metabolites of azamethiphos in rat and goat urine.

14. Atlantic salmon were treated with a commercial 50% wettable powder formulation of azamethiphos at a nominal concentration of 0.2 mg/litre in the water for one hour. The water temperature during the experiment was in the range 11.1 to 13.2°C. Fish were caught at intervals of 1 and 12 hours, and 1, 2, 3 and 7 days after the end of treatment. Although it was intended to sample 10 fish per time point, only 8 fish and 9 fish were taken on days 2 and 3 respectively because some of the fish escaped. Residues in muscle and skin were analysed for azamethiphos using the proposed routine analytical method based on HPLC with UV detection. Residues of azamethiphos in all samples were below the limit of detection (20 µg/kg), at all time points.

15. The routine analytical method for the determination of residues of azamethiphos in salmon tissues was based on HPLC with UV detection and had been described in the ISO 78/2 format. The method was of appropriate specificity and it had been shown that residues of dichlorvos, trichlofon, and the azamethiphos metabolite 2-amino-3-hydroxy-5-chloropyridine did not interfere in the assay. The limit of quantification was 42 µg/kg for salmon muscle and for salmon skin. Validation data for muscle with skin in natural proportions were not provided and data for accuracy and precision were provided at only 2 concentrations.

Conclusions and recommendation

Having considered that:

- an ADI of 0.025 mg/kg bw (i.e. 1.5 mg/person) was established for azamethiphos,
- immediately after the end of treatment, the amount of total residues likely to be ingested by consumers represent less than 20% of the ADI,
- residues of azamethiphos in fish muscle and skin were always below the limit of detection, even in fish caught within one and 12 hours of treatment;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for azamethiphos and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Azamethiphos	Salmonidae	