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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PHOXIM

SUMMARY REPORT (1)

1. Phoxim is an organophosphorous insecticide used in veterinary medicine for the control of mites, lice and other ectoparasites in cattle, pigs, sheep and goats. Phoxim is also used for plant protection, in tea production. As a veterinary drug, phoxim is administered topically either as a wash, spray, pour-on or dip. For wash and spray treatment, a 500 to 1000 mg phoxim/l solution should be applied at approximate volumes of 3 to 4 litres for cattle, 0.5 to 1 litre for pigs and 2 to 3 litres for sheep, to be repeated after 14 days. For dip treatment of sheep, animals are dipped for at least 30 seconds in a solution containing 500 mg phoxim/l. For pour-on treatment of pigs, a solution of 75 mg phoxim/ml is applied topically at a dose of 30 mg phoxim/kg bw, to be repeated after 14 days.

2. The insecticidal activity of phoxim is mediated through inhibition of cholinesterase. In *in vitro* experiments it was shown that mammals are less sensitive to phoxim compared to insects, which is probably due to differences in metabolism. The oxo-analogue of phoxim (PO-phoxim) is 100 to 1000 times more insecticidally potent than phoxim itself. This metabolite was found in fly extracts, but was not identified in mice, rats, rabbits, pigs or calves.

3. Pharmacokinetic studies were performed in mice, rats and pigs following oral administration and in pigs following dermal administration. After oral administration to rats at a dose of 10 mg/kg bw and to pigs at a dose of 5 mg/kg bw, phoxim was rapidly and completely absorbed from the gastrointestinal tract, with peak plasma levels within 30 minutes in the rat and 2 hours in the pig. Phoxim was also rapidly distributed to the tissues and organs of rats and pigs, with highest concentrations found in fat (pigs), kidney and liver. Phoxim was quickly eliminated in mice, rats and pigs, mainly via the urine (more than 80%) and to a lesser extent in faeces (less than 12.5%). Following a single pour-on treatment at a dose of 100 mg/kg bw, the dermal bioavailability of phoxim was low (1.2 to 2.9%).

4. The metabolism of phoxim was studied in flies, mice, rats, rabbits, pigs and calves. Apart from some qualitative and quantitative differences in metabolism between different animal species, it can be concluded that the main degradation steps in rats, pigs and rabbits involved hydrolysis of the phosphor ester bond and de-alkylation, rendering (conjugates of) cyanobenzaldoxime (further detoxified to hippuric acid in rats and pigs and to benzonitrile in rabbits) and desethyl (PO-)phoxim, respectively. In the mouse and calve (and to a lesser extent also in the rabbit), oxidation of the nitrile group was an important route of degradation, rendering phoxim carboxylic acid. The oxo-analogue of phoxim, which is responsible for the high insecticidal activity, was found in fly extracts but not in mammals, although this compound may be a short-lived intermediate as desethyl PO-phoxim was tentatively identified in rat plasma.

5. A large number of acute oral toxicity studies with phoxim revealed LD_{50} values ranging from 19.6 to 40 mg/kg bw in chickens, from 250 to greater than 1126 mg/kg bw in guinea pigs, rabbits, cats and dogs, and from 1248 to 10349 mg/kg bw in mice and rats.

6. In a 3-week dermal toxicity study in rabbits with doses of 0, 0.5 or 15 mg/kg bw/day (intact and abraded skin), the cholinesterase activity was inhibited in both erythrocytes and in the brain at the low dose (abraded skin) and the high dose (intact and abraded skin). The dermal NOAEL (intact skin) and LOAEL (abraded skin) for rabbits was 0.5 mg/kg bw/day.

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7. Oral repeated dose toxicity in mice was studied after oral administration for 42 days at dietary doses of 0, 5, 30, 150 or 750 mg/kg feed, equivalent to 0, 3, 18, 85 and 443 mg/kg bw/day for males and 0, 3, 20, 100 and 509 mg/kg bw/day for females. The cholinesterase activity in brain was depressed at all doses (without a dose-response relationship and may be due to fortuitous higher results of the determinations in controls compared to historical controls). Alkaline phosphatase activity and total protein content were increased in high dose males.

   Another feeding study in mice was performed using doses of 0, 0.5, 1.0, 5 or 30 mg/kg feed for 56 days, equivalent to 0, 0.28, 0.55, 2.8 and 17.8 mg/kg bw/day for males and 0, 0.35, 0.66, 3.1 and 23 mg/kg bw/day for females. In this study no effects on brain cholinesterase were observed up to the highest doses tested. The absolute and relative liver weights were increased in females at 23 mg/kg bw/day. For mice, an oral NOAEL of 5 mg/kg feed (equivalent to 3.1 mg/kg bw/day) could be established, based on increased liver weights in females.

8. Repeated dose oral toxicity in rats was studied following gavage administration during 21 days at dose levels of 0, 5 or 50 mg/kg bw/day. Erythrocyte cholinesterase activity was inhibited from 5 mg/kg bw/day onwards. Cholinesterase activity in brain was lowered in females at the highest dose of 50 mg/kg bw/day.

   In another study in rats following gavage administration during 30 days at dose levels of 0, 2, 5 or 15 mg/kg bw/day, erythrocyte cholinesterase activity was also inhibited in both males and females from 5 mg/kg bw/day onwards. Determinations on cholinesterase activity in brains were not performed.

   Gavage administration of phoxim to rats during 10 weeks at doses of 0, 100, 250, 500, 750 or 1000 mg/kg bw/day resulted in relatively high mortality rates without a clear dose-response relationship. Increased liver and kidney weights, and decreased cholinesterase activity in (whole) blood were observed at all dose levels.

9. The oral repeated dose toxicity of phoxim was also studied in two 3-months feeding studies in young rats.

   Phoxim was administered to rats at dietary concentrations of 0, 4, 12, 40 or 120 mg/kg feed for 3 months, equivalent to 0, 0.4, 1.2, 4 or 12 mg/kg bw/day. The cholinesterase activity in erythrocytes was decreased from 4 mg/kg bw/day onwards. The cholinesterase activity in brain was not determined and histopathology was not performed. The weights of the thyroid glands in high dose males and the kidney weights in high dose females were increased.

   Another 3-month feeding study was performed in rats at doses of 0, 5, 15, 50, 150 or 500 mg/kg feed, equivalent to 0, 0.5, 1.5, 5, 15 and 50 mg/kg bw/day. The cholinesterase activity in erythrocytes was inhibited from 5 mg/kg bw/day onwards (activity in brain was not determined). Liver weights were increased in both sexes at the two highest doses. In both studies, some organ weights were increased (thyroid, adrenals, kidney, liver) at higher doses, thyroid weights were increased in high dose females and kidney weights were increased at 15 mg/kg bw/day (females) and 50 mg/kg bw/day (males and females).

   For rats, an oral NOAEL of 15 mg/kg feed (equivalent to 1.5 mg/kg bw/day) could be established, based on the inhibition cholinesterase activity in the erythrocytes (in absence of determinations in the brain).

10. Oral repeated dose toxicity of phoxim in dogs was investigated in three 3-month dietary studies.

    In a limited study, phoxim was administered to dogs for 3 months at dietary doses of 0, 2, 5 or 10 mg/kg feed, equivalent to 0, 0.05, 0.125 and 0.25 mg/kg bw/day. Bodyweight gain appeared to be slightly reduced in males of the highest dose group. However, as this effect was not observed at a 3-month interim measurement during a two year oral toxicity study in dogs, using higher dosages and larger group sizes, this effect was discounted. Inhibition of cholinesterase activity (more than 20%) was observed in plasma of all treated animals, but not in erythrocytes. The activity in brain was not determined and histopathology was not performed.

The second 3-month feeding study used dietary doses of 0, 50, 200 or 1000 mg/kg feed, equivalent to 0, 1.25, 5 and 25 mg/kg bw/day. Increased relative liver weights were observed in males at the highest dose and in females from 1.25 mg/kg bw/day onwards. In high dose females reduced food intake resulted in weight loss over the whole study period. Cholinesterase activity in erythrocytes was inhibited at doses of 5 mg/kg bw/day and higher (activity in brain was not determined).

In another 3-month feeding study in rats using doses of 0, 0.3, 1 or 2 mg/kg feed, equivalent to 0, 0.0075, 0.025 and 0.05 mg/kg bw/day, plasma cholinesterase activity was reduced at the two highest doses. The cholinesterase activity in erythrocytes was not changed and that in brain was not determined.

The oral LOEL for dogs was 1.25 mg/kg bw/day, based on increased relative liver weights.

11. In a limited 6-month oral toxicity study in monkeys, in which phoxim was administered by gavage at doses of 0, 0.2, 0.65 or 2 mg/kg bw/day, a reduction of the cholinesterase activity in erythrocytes was the only effect observed at the highest dose. Autopsy and histopathology were not performed.

12. Long-term toxicity was studied in rats following oral administration of phoxim at dietary doses of 0, 15, 75 or 375 mg/kg feed for 24 months, equivalent to 0, 0.78, 3.96 and 17.69 mg/kg bw/day for males and 0, 1.08, 5.38 and 27.19 mg/kg bw/day for females. The cholinesterase activity in erythrocytes was decreased at all dose levels, but the activity in brain was lowered at the highest dose only. The absolute adrenal weights were decreased at the two highest doses in both sexes.

    After long term administration to rats, an oral NOAEL of 15 mg/kg feed (equivalent to 0.78 mg/kg bw/day) could be established, based on decreased adrenal weights.

13. Long-term oral toxicity was also studied in dogs treated with phoxim at dietary concentrations of 0, 0.1/0.3, 15 or 75 mg/kg feed for 24 months, equivalent to 0, 0.0025/0.0075, 0.375 and 18.75 mg/kg bw/day. Cholinesterase activity in erythrocytes was inhibited at 0.375 and 18.75 mg/kg bw/day, but the activity in brain was decreased at the highest dose only. Liver damage was evident at 18.75 mg/kg bw/day, as the liver weights and alanino-amino transferase and alkaline phosphatase were increased, cholesterol was decreased, livers were darker, and hepatocyte alterations were observed at this dose level.

    After long term administration to dogs, an oral NOEL of 15 mg/kg feed (equivalent to 0.375 mg/kg bw/day) could be established, based on effects on the liver and on inhibition of cholinesterase activity in the brain.

14. In a three generation reproduction study in rats at oral doses of 0, 15, 75 or 375 mg/kg feed, equivalent to 0, 0.75, 3.75 and 18.75 mg/kg bw/day, no parental toxicity or embryo-/foetotoxicity was observed at any dose level. The lactation index (ability of dams to nourish their offspring) was reduced at the highest dose in the F_{3b} generation only. The NOEL was therefore 75 mg/kg feed, equivalent to 3.75 mg/kg bw/day.

15. Teratogenicity studies by oral gavage were performed in rats (0, 30, 100 or 300 mg/kg bw on gestation days 6 to 15) and rabbits (0, 12, 36 or 72 mg/kg bw on gestation days 6 to 18). In the rat study, the only effect observed was a decreased body weight gain in high dose animals, revealing a NOEL for maternal toxicity of 100 mg/kg bw/day. No effects were seen on embryos or foetuses and no malformations were found. In the rabbit study, effects were seen on the embryos (increased resorption rate) and foetuses (reduced mean foetal weight) at the highest dose, which was also toxic to the dams (clinical signs, reduced food consumption and bodyweight gain). No malformations were observed. The NOEL for both maternal toxicity and embryo-foetotoxicity was 36 mg/kg bw/day in rabbits. The studies in rats and rabbits showed that phoxim is not teratogenic.

16. The mutagenic properties of phoxim were studied in a number of *in vitro* (*Salmonella*-microsomal assay in bacteria, gene mutation test in yeast, and a chromosomal aberration test in human lymphocytes) and *in vivo* (micronucleus test and dominant lethal test in mice) tests. The bacterial tests and the *in vitro* tests for gene mutations in yeast were all negative. A positive result was obtained in the *in vitro* cytogenetic assay in human lymphocytes, at one cytotoxic dose level and in absence of metabolic activation system, but not in its presence. Phoxim tested negative in the two *in vivo* tests. The balance of evidence suggests that phoxim is not genotoxic *in vivo*.

17. Phoxim was tested in an oral carcinogenicity study in B6C3F1 mice (this strain is sensitive for liver tumours), receiving dietary doses of 0, 1, 5, 150 or 450 mg/kg feed for 2 years, equivalent to 0, 0.47, 2.38, 66.88 and 198.16 mg/kg bw/day. Bodyweights were increased in females at the two highest doses. Cholinesterase activity in brain was decreased in females at the two highest doses and in males only at the highest dose. Liver toxicity was apparent at the highest dose (higher cholesterol levels, lower bilirubin levels in males, higher alkaline phosphatase and alanino-amino transferase levels in females, some hepatic eosinic and basophilic foci in males and a slight increase in adenomas in females). At 66.88 mg/kg bw/day, no effects on the livers were seen apart from increased blood cholesterol levels in both sexes. A NOAEL of 5 mg/kg feed, equivalent to 2.38 mg/kg bw/day was established, based on increased body weights, increased blood cholesterol and inhibition of brain cholinesterase activity. Phoxim is considered not carcinogenic in the mouse.

18. In a study for delayed neurotoxicity, hens were orally (gavage) treated with phoxim at a dose of 50 mg/kg bw, twice at an interval of 21 days. The hens were observed for up to 42 days after the last administration, and were killed one week thereafter. Most signs of toxicity disappeared within 5 days following treatment, and the hens were normal from the 26th observation day onwards. At termination, no paralysis or delayed effects were observed. Histopathological examination of the brain, spinal chord and the distal and proximal part of the *nervi ischiadici* showed no indications for neurotoxic effects. Biochemical assays for neuropathy target esterase were not provided, however taking into account the overall conclusions on the toxicological data, such information was not considered necessary.

19. In the maximisation test in guinea pigs, phoxim was shown to be a moderate sensitiser. A number of limited studies on irritation showed that phoxim was only slightly irritating to the skin and eyes.

20. The toxicology of phoxim was previously evaluated by the Joint (FAO/WHO) Meeting on Pesticide Residues in 1982 and 1984. An ADI of 0.001 mg/kg bw was established, based on the lowest oral NOEL of 0.05 mg/kg bw/day as found in dogs and applying a safety factor of 50.

    Phoxim was also recently discussed by the Joint (FAO/WHO) Expert Committee on Food Additives, however the final results of the assessment were not available to the Committee for Veterinary Medicinal Products (CVMP).

21. The CVMP considered 0.375 mg/kg bw/day as the overall oral NOEL. This NOEL was based on effects on the liver and a reduction of the acetylcholinesterase activity in the brains, observed in the two year feeding study in dogs. Based on this NOEL and applying a safety factor of 100 an ADI of 0.00375 mg/kg bw (i.e. 0.225 mg/person) was established.

22. Radiolabelled phoxim was administered orally in gelatin capsules to two pigs at a single dose of 5 mg/kg bw. The animals were slaughtered 24 and 72 hours after administration (only one animal per time point). Highest radioactive residues were found at 24 hours after administration: 1320 µg equivalents/kg in fat, 600 µg equivalents/kg in liver, 350 µg equivalents/kg in kidney and 50 µg equivalents/kg in muscle. At 72 hours, tissue concentrations were approximately half these values. In the tissues only phoxim and cyanobenzaldoxime could be identified. Quantification was only possible in fat (limit of quantification not given). Phoxim was found in fat (90% of radioactivity in fat), loin and muscle. Cyanobenzaldoxime was found in muscle, loin and liver.

    In a radiolabel study in seven pigs, phoxim was dermally administered as a pour-on at a dose of 100 mg/kg bw. The dermal bioavailability was 1.2 to 2.9%.

23. In a GLP residue study in pigs, the commercial pour-on product was applied at the recommended dose (30 mg/kg bw) along the dorsal backline of twenty animals. Treatment was repeated after 14 days. The animals were slaughtered (4 per group) at 7, 14, 21, 28 and 35 days following the second application. Phoxim concentrations were determined by high pressure liquid chromatography (HPLC) in samples of liver, kidney, muscle and abdominal fat, as well as in edible tissues at the site of application (skin, muscle and back fat). The limit of quantification was 10 µg/kg. Residues were only detectable in samples of fat and skin, with no differences between concentrations in abdominal fat and back fat. In fat the mean residue concentrations declined from 502 µg/kg at 7 days, via 220 µg/kg at 14 days, 121 µg/kg at 21 days, 86 µg/kg at 28 days, to 23 µg/kg at 35 days. The mean phoxim concentrations in skin were somewhat lower, declining from 346 µg/kg at 7 days, via 107 µg/kg at 14 days, 49 µg/kg at 21 days, 23 µg/kg at 28 days, to 11 µg/kg at 35 days.

24. A total of two limited residue studies in pigs following two spray treatments (interval 7 to 8 days) with a 50% emulsifying concentrate formulation with a concentration of 500 or 1000 mg phoxim/l were provided. Animals were slaughtered (2 per group) at 14 and 28 days following spraying with 1000 mg/l, and at (3 per group) 7 and 14 days following spraying with 500 mg/l. After the 1000 mg/l application, residues were found in fat (without skin) only (40 and 50 µg/kg at 14 days and 0.13 and lower than 10 µg/kg at 28 days). After the 500 mg/l application only residues in fat (without skin) were studied, revealing no detectable levels of phoxim in any of the samples.

25. A number of limited tissue residue studies were provided following pour-on (one study in pigs), spray (one in cattle, two in sheep, one in goat) and dip treatment (two in sheep), all at the recommended doses. In addition, limited data were provided on residues in milk after spray (two studies in cattle) and dip (one study in sheep) treatment at the recommended dose. In the tissue residue studies in cattle, pigs and sheep (dip treatment), phoxim was detectable in fat tissue only (highest values: 130 µg/kg (pigs 14 days), 37 µg/kg (cattle 14 days) and 700 µg/kg (sheep 30 days). Spray treatment of sheep revealed the highest residues in fat (2750 µg/kg at 7 days) and much lower levels in muscle and kidney (70 µg/kg at 7 days). After spraying of goats, the highest residues were found in fat (850 µg/kg at 7 days), and much lower levels were found in muscle (50 µg/kg at 7 days). Milk residue studies in cattle showed that highest residues (220 to 420 µg/l) were found at the first milking after treatment, declining to undetectable levels (lower than 2 µg/l) at 7 days after treatment. Phoxim was not detectable (lower than 30 µg/l) in sheep milk after dip treatment at the recommended dose.

    All these studies used only 2 or 3 animals per time point. The study reports were very limited, as details on the animal phase (e.g. product specification, application volume, method of sampling) and the analytical method were lacking. Therefore, these studies are inadequate for the establishment of maximum residue limits.

26. Based on the data provided in the oral administration study in pigs (5 mg/kg bw) phoxim can be retained as the marker residue. It can be estimated that, on the basis of total radioactivity, after 72 hours the total intake of residues by the consumer will be 34% of the ADI. In comparison to the recommended treatment (topical, 30 mg/kg bw, 1.2 to 2.9% dermal bioavailability) this is considered to be a worst case estimation. Therefore, no additional studies to substantiate the choice of phoxim as maker residue and no additional data to establish the ratio of marker residue to total residues are requested in this particular case.

27. For the routine determination of phoxim residues in edible tissues of pigs, an HPLC-UV method was provided. This method was well described, in a format closely resembling ISO 78/2. However, the method was not validated completely, in particular with respect to specificity, accuracy (liver), limits of quantification (liver) and detection, applicability and susceptibility. The limits of quantification were 10 µg/kg for all edible tissues. The same method was proposed for the cattle, sheep and goat, but for these target animals no validation data were provided.

28. For the determination of phoxim residues in cattle milk and in edible tissues of sheep and goats, gas chromatography (GC) methods were proposed, which were not validated.

Conclusions and recommendation

Having considered that:

- an ADI of 0.00375 mg/kg bw (i.e. 0.225 mg/person) was established,
- after dermal treatment of pigs, phoxim was only detected in skin+fat
- in pigs fat phoxim represents 90% of the total residues,
- due to the low concentrations in the other edible tissues (liver, muscle and kidney) the ratio of marker residue to total residues could not be established,
- based on the results from oral administration phoxim was considered to be the most suitable marker residue,
- no adequate residue depletion data were provided in target species other than pigs,
- an analytical method for pig tissues is available but not fully validated,
- no suitable analytical method was provided for tissues and milk of other target animal;

the Committee for Veterinary Medicinal Products recommends the inclusion of phoxim in Annex III to 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
Phoxim	Phoxim	Porcine	20 µg/kg 700 µg/kg 20 µg/kg 20 µg/kg	Muscle Skin+fat Liver Kidney	Provisional MRLs expire on 1.1.2001

Based on these MRLs values, the daily intake will represent about 22% of the ADI.

Before the Committee can consider the inclusion of phoxim for pigs in Annex I to Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The analytical method proposed for monitoring purposes for pigs should be fully validated according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community; in particular data must be provided on the limit of detection (at least 20 samples for each relevant matrix), the stability of samples during storage and the accuracy of the method for liver samples. The limit of quantification for liver samples should be confirmed by additional supporting data regarding the accuracy and the repeatability of the method at the proposed limit of quantification and possible interference with other veterinary medicinal products should be investigated. The method should be described in an internationally recognised format (e.g. ISO 78/2).