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
(Extension to sheep)

SUMMARY REPORT (2)

1. Phoxim is an organophosphorous insecticide used in veterinary medicine for the control of mites, lice and other ectoparasites in pigs. Phoxim is also used in plant protection. 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 0.5 to 1 litre, to be repeated after 14 days.

   Phoxim has been evaluated by The Committee for Veterinary Medicinal Products (CVMP) previously for its use cattle, pigs, sheep and goats, but only for pigs provisional MRLs were established. 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 brain, observed in a 2-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.

   Currently phoxim is included in Annex III in according 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

2. An application has now been submitted for an extension of the MRLs for phoxim to sheep. For wash and spray treatment, a 500 to 1000 mg phoxim/l solution should be applied at approximate volumes of 2 to 3 litre for sheep, to be repeated after 14 days.

3. Metabolism and residues were studied in sheep. Six sheep (Clun x Suffolk, bodyweight 50±10 kg and 15 to 16 months of age) received a single topical application with ^{14}C-phoxim (uniformly phenyl ring-labelled, specific activity 1.56 MBq/mg) in N-butanol as vehicle at a dose of 25 mg/kg bw. Four additional sheep received the same test substance in N-methylpyrrolidone intravenously at a single dose of 1 mg/kg bw, in order to provide tissue and excreta samples for metabolite profiling in the event of (too) low levels of radioactivity being absorbed after topical application.

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The animals were slaughtered in groups of 1 male and 1 female at 4 and 8 hours after intravenous injection or 7, 21 and 28 days after topical dosing. At slaughter, samples of the application site (topically treated sheep), blood, liver, kidneys, muscle (fore and hind quarter), abdominal fat (omental and perirenal mixed) and subcutaneous fat (remote from the application site) were taken for analysis. Excreta were collected up to 7 days after application, and blood samples were taken before dosing and at 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours after dosing, where applicable, for all animals and then at 24 hourly intervals up to slaughter. All samples were analysed for total radioactivity, and if possible, further analysed for the parent compound and metabolites. The analytical techniques involved were LSC, TLC, HPLC (radiodetection and UV detection), LC-MS, and LC-MS/MS.

The following values for pharmacokinetic parameters for radioactivity concentrations in plasma following a single topical application to sheep were obtained: C_{max} (µg equivalents/g) 0.084 (n=3); T_{max} (hours) 192 (median, n=3); AUC_{0-168} (µg equivalents.h/g) 11.8 (n=3); AUC_{0-504} 31.4 (n=2); AUC_{0-672} 30.4 (n=1); k (hours) 0.0029 (n=2); t½ (hours) 243.2 (n=2).

At seven days the total recovery of radioactivity was in the range of 64 to 85%. The majority of the radioactivity was associated with the applications site (42 to 64% of the dose). Excretion via urine accounted for 7 to 11.5% of the dose and via faeces for 0.7 to 2.6% of the dose at 7 days. Phoxim was not detected in urine. The main metabolite in urine was cyanoxim and its glucuronide and sulphate conjugates, together accounting for approximately 68% of the radioactivity in urine. The metabolite profile in urine following intravenous injection was similar.

The highest concentrations of total radioactive residues were found in liver (ranging from 1603µg phoxim equivalents/kg at day 7 to 775 µg/kg at day 28) and fat (ranging from 2071 µg phoxim equivalents/kg at day 7 in abdominal fat to 542 µg/kg at day 28 in subcutaneous fat). Residues were less abundant in muscle and kidney, at day 28 the levels were down to 115 µg phoxim equivalents/kg in kidney and to 59 µg/kg in muscle. The average ratios of phoxim to total radioactive residues were approximately 0.75 for fat, 0.5 for muscle, and 0.1 for kidney. For liver, no ratio could be calculated, because the parent compound was not present in liver samples at any time point. The radioactivity in fat was well extractable, but in kidney and in muscle only approximately 30% was extractable, whereas the radioactivity in liver was hardly extractable (less than 5%, although at 4 hours after the intravenous dose 40% of the radioactivity in liver was extractable). The vast majority of the radioactivity in fat consisted of the parent compound. Other extractable metabolites in fat were not identified. Based on these findings, phoxim is considered the most appropriate marker residue for monitoring, which is in line with the MRLs already established for pigs.

4. Depletion of residues was studied in 10 male and 10 female Suffolk sheep (bodyweight 40.5 to 51 kg, approximately 15 months of age, sheared 4 weeks before treatment, mean wool length 15 mm at treatment) were dipped in an aqueous solution of phoxim that was prepared from the commercial formulation at a final nominal phoxim concentration of 500 mg per litre. Groups of 2 males and 2 females were slaughtered at 7, 21, 28, 35, and 49 days after dipping. Samples of liver, kidney, muscle (mixed sample of fore and hind quarter), and fat (subcutaneous fat, and a mixed sample of omental and perirenal fat) were taken. All samples were analysed for phoxim concentrations using an HPLC-UV technique which was the same as the proposed routine analytical method with validated limits of quantification of 200 µg/kg for fat and 25 µg/kg for all other tissues.

   In line with the results reported in studies, which were already assessed by the CVMP previous application, high levels (1532 µg/kg at 7 days) were found in fat, in particular in subcutaneous fat, in which phoxim was detectable up to the last time point of 49 days (66 µg/kg). Phoxim concentrations in muscle and kidneys were much lower (189 µg/kg, respectively 35.3 µg/kg at day 7), being only detectable up to 21 (kidney) and 28 (muscle) days. In liver samples, phoxim was not detectable at all.

5. A method for the determination of phoxim in ovine tissues is proposed. The reported limits of quantification are 200 µg/kg for fat and 25 µg/kg for other tissues. The method (HPLC with UV detection) is well described but not completely validated with respect to specificity (possible interference of other veterinary drugs: the method was tested with regard to possible interference of fenbendazole, enrofloxacin and diazinon, but not with regard to representatives of other classes of veterinary drugs commonly used in sheep) and linearity (only the linearity of the detector response is reported, data on the linearity of the method is lacking).

6. Phoxim was also evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1999 and, based on an ADI of 0-4 µg/kg bw, temporary MRLs were established for cattle, sheep, goats and pigs at values of 50 µg/kg for muscle, liver and kidney, 400 µg/kg for fat and 10 µg/kg for cows' milk.

Conclusions and recommendation

Having considered that:

- an ADI of 0.00375 mg/kg bw (0.225 mg/person) was established for phoxim,
- phoxim was considered the most suitable marker residue,
- after topical treatment of sheep, high concentrations of phoxim were found in fat, but much lower concentrations were found in kidney and muscle, and no phoxim was detected in liver,
- the ratio of marker to total residues was 0.75 for fat, 0.50 for muscle, and 0.1 for kidney,
- phoxim is extensively metabolised in liver and could not be detected in this tissue in the non-radiolabel residue depletion studies, therefore it was not considered necessary to establish MRLs for liver,
- an analytical method for sheep tissues was provided but not fully validated;

the Committee for Veterinary Medicinal Products recommends the inclusion of MRLs for phoxim in sheep tissues 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
Phoxim	Phoxim	Ovine	50 µg/kg 400 µg/kg 50 µg/kg	Muscle Fat Kidney	Not for use in animals from which milk is produced for human consumption. Provisional MRLs expire on 1.7.2001.

Based on these MRLs values, taking into account the estimated total residues in liver which are approximately 1.5 times higher than in fat, the daily intake will represent 72% of the ADI.

Before the Committee for Veterinary Medicinal Products can consider the inclusion of the MRLs for sheep tissues into Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant is requested to submit a fully validated method for the determination of phoxim in sheep tissues in accordance with the requirements of Volume VI, in particular with respect to specificity (possible interference with other commonly used veterinary drugs) and linearity of the whole method instead of the linearity of the detector response.