



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PHOXIM (extension to sheep)

SUMMARY REPORT (4)

1. Phoxim is an organophosphorous insecticide used in veterinary medicine for the control of mites, lice and other ectoparasites. Phoxim is administered topically either as a wash, spray or pour-on. For wash and spray treatment of pigs, 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. 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.

This assessment relates to the extension of MRLs to sheep. For wash and spray treatment of sheep, a 500 to 1000 mg phoxim/l solution should be applied at appropriate volumes of 2 to 3 l/animal, to be repeated after 14 days. For dip treatment sheep are dipped for at least 30 seconds in a solution containing 500 mg phoxim/l.

Phoxim has also a very limited use as a pesticide in plant protection.

Phoxim has previously been evaluated by the Committee for Veterinary Medicinal Products (CVMP). 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 of Council Regulation (EEC) No. 2377/90 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

Recently the CVMP recommended the establishment of provisional MRLs for phoxim in ovine species 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

Further data were provided to support an entry of phoxim in Annex I of Council Regulation (EEC) No 2377/90 for sheep.

2. 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.

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 hour 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: $C_{\max}$ ($\mu\text{g equivalents/g}$) 0.084 (n=3); $T_{\max}$ (hours) 192 (median, n=3); $\text{AUC}_{0-168\text{h}}$ ($\mu\text{g equivalents.h/g}$) 11.8 (n=3); AUC_{0-504} 31.4 (n=2); AUC_{0-672} 30.4 (n=1); k (hours) 0.0029 (n=2); $t_{1/2}$ (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 1603 μg phoxim equivalents/kg at day 7 to 775 $\mu\text{g/kg}$ at day 28) and fat (ranging from 2071 μg phoxim equivalents/kg at day 7 in abdominal fat to 542 $\mu\text{g/kg}$ 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 $\mu\text{g/kg}$ 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.

3. 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 $\mu\text{g/kg}$ for fat and 25 $\mu\text{g/kg}$ for all other tissues.

In line with the results reported in studies, which were already assessed by the CVMP during the 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.

4. A method for the determination of phoxim in ovine tissues is proposed. The method (HPLC with UV detection) is well described in a format closely resembling ISO 78/2 and fully validated. The limits of quantification are 200 µg/kg for fat and 25 µg/kg for other tissues.
5. 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,
- a validated analytical method for sheep tissues was provided;

the Committee for Veterinary Medicinal Products recommends the inclusion of MRLs for phoxim in sheep tissues in Annex I 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.

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, which can be accepted as phoxim has a very limited use as pesticide in the European Union.