European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

PHOXIM
(Extension to laying hens)

SUMMARY REPORT (6)

1. Phoxim (CAS 14816-18-3) is an organophosphorous insecticide currently used in veterinary medicine for the control of mites, lice and other ectoparasites in pigs and sheep. 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. 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. In laying hens, phoxim is intended for treatment and control of the red mite. Cages are sprayed with 25 ml of a solution containing 2 g phoxim/l. The laying hens stay in the cages during treatment and will be exposed to phoxim.

   Phoxim has a very limited use in plant protection in the European Union.

   Phoxim was previously evaluated by the Committee for Veterinary Medicinal Products (CVMP) for its use pigs and sheep. 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 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	Porcine	20 µg/kg 700 µg/kg 20 µg/kg 20 µg/kg	Muscle Skin + fat Liver Kidney	
		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.

2. Following the evaluation of an application for the extension of phoxim to laying hens, phoxim was included in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

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Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Phoxim	Phoxim	Chicken	50 µg/kg 550 µg/kg 25 µg/kg 50 µg/kg 60 µg/kg	Muscle Skin + fat Liver Kidney Eggs	Provisional MRLs expire on 1.7.2005

Further to the establishment of provisional MRLs, additional data was submitted regarding the analytical method.

3. Metabolism and residues were studied in laying hens. Six female laying hens (Hisex, more than 26 weeks of age, bodyweight 1.4 to 1.9 kg) were dermally treated with 14C-phoxim (universally labelled in the phenyl ring, radiochemical purity greater than 97 %) diluted in butan-1-ol and in propan-2-ol to a final concentration of 200 mg active ingredient/ml, with a specific activity of 5.31 mCi/mmol (196.5 kBq/mg). The substance was applied at the base of the feathers on the back. Five of these animals were given a dose of 11.3 to 12.4 mg/kg bw, and one animal received a dose of 19.5 mg/kg bw. Another 12 hens were given the 14C-phoxim (in capsules) at doses in the range of 4.66 to 5.79 mg/kg bw to allow metabolic profiling. The birds given the dermal treatment were slaughtered 12 days after application. Of the orally treated birds, six were slaughtered at 6 hours after application and the remaining 6 at 7 days after application.

   Twelve days after dermal treatment, the highest mean concentrations of total radioactive residues were found in skin + fat from the application site (1460 µg phoxim equivalents/kg). Mean concentrations of total radioactive residues in other tissues (expressed as µg phoxim equivalents/kg) were: 270 µg/kg in kidney, 188 µg/kg in skin plus fat remote from the application site, 85 µg/kg in liver, and 2.9 to 16 µg/kg in muscle.

   The mean concentrations of the parent compound phoxim at 12 days after dermal treatment were: 99 µg/kg in skin + fat remote from the application site, 8.7 µg/kg in liver, and from non-detectable to 4.7 µg/kg in muscle. The concentration of phoxim was not determined in kidneys but in an additional cold residue study using the same design, the mean concentrations of phoxim in kidney was less than 2.4 µg/kg. Phoxim was proposed as the marker residue, in line with the evaluations of phoxim in pigs and sheep. The ratio of marker to total residues was 0.120 for liver, 0.01 for kidney, 0.178 for muscle, and 0.551 for skin plus fat. The composition of residues was investigated in excreta, in skin plus fat taken 12 days after dermal treatment, and in edible tissue (except kidney) taken 6 hours after oral treatment. All other edible tissues (including eggs) were stated to have residue concentrations that were too low for this purpose. From the data it can be concluded that the main route of metabolism is hydrolysis to z-cyanoxim and further conjugation to cyanoxim sulphate. This profile is similar to that proposed for rats, pigs, and sheep, although the glucuronide conjugate of z-cyanoxim and hippuric acid were also significant metabolites in the urine and tissues of these species.

   In eggs, the concentration of total radioactive residues reached a maximum of 69 µg phoxim equivalents/kg at 8 days after dermal treatment and gradually declined thereafter. The highest concentration of the parent compound, phoxim, (29 µg/kg) was also found at this time point. The average ratio of marker to total residues in eggs was 0.32.

4. Depletion of residues was studied in 28 female Hisex hens (bodyweight 1332 to 2028 g). The hens were dermally treated with phoxim, using a 2 g phoxim/l formulation. The formulation was sprayed within a distance of about 10 cm between the spray nozzle and the hen. Each hen received a spray volume of about 10 ml, equivalent to approximately 20 mg phoxim per hen. The treatment was repeated after 7 days. Groups of four birds were slaughtered at seven and 14 days after the second treatment. The remaining birds (n=20) were slaughtered at 21 days after the second treatment. The number of eggs examined at each time point ranged from 9 to 23.

The concentrations of phoxim was determined in liver, muscle, skin + fat, and in eggs, using the proposed routine analytical HPLC-UV method. Residues in kidneys were not investigated. Highest residues were found in skin plus fat ranging from 538 to 1496 µg/kg at 7 days after the second administration, declining to concentrations ranging from 398 to 579 µg/kg at 14 days and from 95.5 to 536 at 21 days. Lower levels were found in muscle, ranging from 7.77 to 23.5 µg/kg at 7 days, from 3.68 to 8.79 at 14 days, and from non-detectable to 9.73 at 21 days. Phoxim could not be detected in liver at any time point.

The concentration of phoxim in eggs reached a maximum of 12.3 µg/kg at 10 days after the second treatment, and declined gradually to 5.3 µg/kg at 20 days after second treatment. It was noted that although these were detectable concentrations, they were below the limit of quantification, which was set at 30 µg/kg, the lowest validation level used for eggs.

5. An HPLC method with UV detection is proposed for the monitoring of residues of phoxim in chicken tissues and eggs. The validated limits of quantification are 200 µg/kg for skin + fat, 25 µg/kg for liver, 10 µg/kg for muscle, and 30 µg/kg for eggs. The method was described in the ISO 78/2 format. For chicken kidney an LC-MS/MS method is proposed. This method is also well described and sufficiently validated. The limit of quantification for kidney is 25 µg/kg.

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. In 2002 the temporary MRLs for sheep, goats and pigs became final, and in 2004 the temporary MRLs for cattle and cows' milk were withdrawn. No MRLs were established for chickens and eggs.

Conclusions and recommendation

Having considered that:

- an ADI of 0.00375 mg/kg bw (0.225 mg/person) was previously established for phoxim,
- phoxim was considered the most suitable marker residue,
- after topical treatment of hens, high concentrations of phoxim were found in skin + fat, but much lower concentrations were found in muscle, and no phoxim was detected in liver or kidney,
- the ratio of marker to total residues at day 12 was 0.551 for skin + fat, 0.178 for muscle, 0.120 for liver, 0.01 for kidney, and 0.32 for eggs,
- phoxim has a very limited use in plant protection in the European Union,
- a validated analytical method for chicken tissues and eggs was provided;

the Committee for Veterinary Medicinal Products recommends the inclusion of phoxim 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	Chicken	25 µg/kg 550 µg/kg 50 µg/kg 30 µg/kg 60 µg/kg	Muscle Skin + fat Liver Kidney Eggs	

Based on these MRLs values, the maximum daily intake will represent 98 % of the ADI.