PHOXIM

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INTRODUCTION

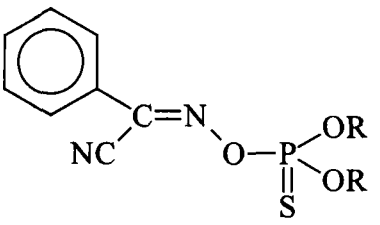
Phoxim has not previously been reviewed by the Committee, but was previously reviewed by the Joint Meeting on Pesticide Residues (JMPR), which established an ADI and recommended MRLs based on horticultural use. As the sponsor has indicated that it does not intend to continue to support horticultural use of phoxim, the JMPR has recommended that the previously established MRLs for such use should no longer be supported. The use of phoxim as a veterinary product has therefore been referred to the Committee, which received data for such use for number of food animal species, including cattle, pigs, sheep, goats and rabbits. Most of the studies provided were not conducted according to current GLP standards.

IDENTITY

Chemical name: Diethyl O-(α-cyanobenzylideneamino)thiophosphate (IUPAC)
4-Ethoxy-7-phenyl-3, 5-dioxa-6-aza-4-phosphaoct-6-ene-8-nitrile 4-sulfide;
phenylglyoxylonitrile oxime O,O-diethyl phosphorothioate (CAS)

Synonyms: PHOXIM Baythion, Bay 5621, Bay 77488, Sebacil, Volaton, SRA 7502

Structural formula:



$R = CH_2CH_3$ phoxim
$R = CH_3$ phoxim methyl

CAS number: 14816-18-3

Molecular formula: $C_{12}H_{15}N_2O_3PS$

Molecular weight: 298.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Phoxim

Appearance: Pale yellow oil.

Boiling point: 102°C ($bp_{0.01}$).

Refractive Index: 1.5405 (n^{20}_{D}).

Solubility: Freely soluble in alcohols, ketones, aromatic hydrocarbons and chlorinated aliphatic hydrocarbons; slightly soluble aliphatic hydrocarbons, vegetable oils and mineral oils; nearly insoluble in water.

Optical rotation: Optically inactive

Ultraviolet maxima: Not reported.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of Use

General

Phoxim is an ectoparasiticide of the organophosphate group used for the control of Psoroptes-, Sarcoptes- and Chorioptes mites, biting and sucking lice, sheep keds, flies, ticks and fly maggots in wounds. Species for which registrations have been granted include cattle, pigs, sheep, goats and horses, but some countries have excluded the use of phoxim in lactating animals. Phoxim is sold as a 50% (w/v) formulation for dilutions as a wash, spray or dip and as a 7.5% (w/v) pour-on formulation.

Dosage

As a wash or spray treatment, phoxim is typically applied either as a single treatment or in two treatments at a 7-day interval. The formulated product (50 g phoxim/100 mL) is diluted, using 10 mL of per 10 L of water, and sufficient liquid is utilised to ensure that the total body of the animal has been moistened. The concentration of phoxim may be doubled for treatment of severe mange infestation. When used as a plunge dip, typically 1 L of the formulated product is diluted in 1000 L of water. A pour-on formulation (7.5 g phoxim/100 mL) is also available for treatment of pigs for mange or lice infestation and is applied at a recommended dose of 0.4 mL of formulated product per kg body weight.

PHARMACOKINETICS AND METABOLISM

Metabolism in Laboratory Animals

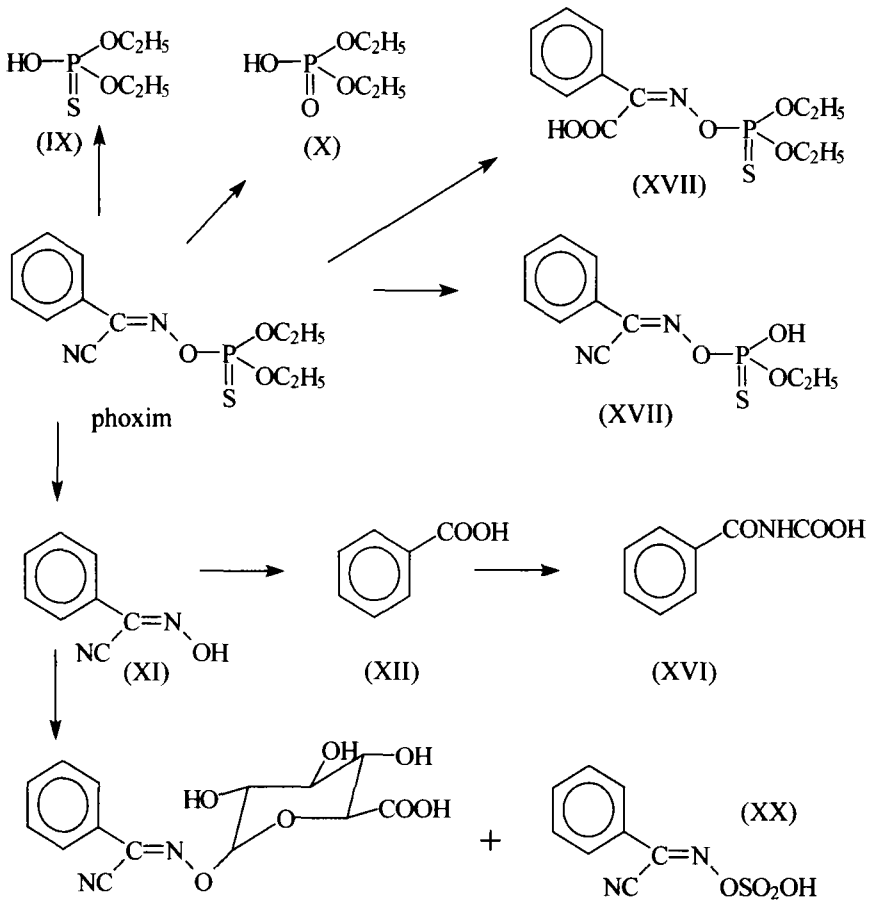
General

The proposed phoxim metabolic pathways are presented in Figure 1. Phoxim metabolism has been summarised in mice and rats for studies in which the compound was administered with two different radiolabels in its structure (Ecker, 1982). The two labels used were ^{14}C, incorporated into the benzene ring, and side chain ^{32}P-labelled phoxim. In a study with rats, 3 individual administration groups were used consisting of 5 rats each. A total of 93.6 ±5.1% of the ^{14}C labelled phoxim was excreted during the first 24 hours after oral administration of a single dose at 10 mg/kg body weight to male rats. The amount excreted in female rats after equal dose was slightly lower (85.6 ±7.5%). At 10 days post administration, the excretion was practically complete when over 90% of the excreted ^{14}C label was found in the urine, with the remainder in faeces. The total amount of ^{14}C-labelled phoxim excreted (89.9 ±3.9%) during 10 days after a dose of 1 mg/kg BW was slightly lower compared to the higher dose. No data were available on the metabolite

profile in rat urine. The urinary metabolite profile in white mice was investigated 24 hours after administration of ^{32}P-phoxim at 114 mg/kg BW and 30 hours after administration 955 mg/kg BW (Vinopal and Fukota, 1971). Practically all of the radioactivity excreted could be related to metabolites X, XVII, IX and XVIII (Figure 1) of which 85% was diethyl phosphoric acid (X) and O,O-diethyl thiophosphoric acid (IX), after the lower dose and 87% was diethyl phosphoric acid (X), "phoxim carbonic acid" and O,O-diethyl thiophosphoric acid (IX) after the higher dose.

In another study, containing both long-term and short-term exposures, [^{14}C, ^{3}H]- doubly labelled phoxim methyl was given orally to rats at 1 mg/kg body weight (Puhl, 1976). The compound was labelled with ^{14}C in the benzene ring and with ^{3}H in the O-methyl group in the long-term study. Only ^{14}C-labelled phoxim was used for the short-term (tissue) study. In the long-term study, 2 male and 2 female Sprague-Dawley albino rats were used, with an average weight of 245g. The animals were kept in metabolic cages, allowing collection of urine, faeces and expired air. Urine and faeces samples were collected at 4, 8, 12, and 24 hours and at subsequent 24 hourly intervals until the sacrifice of the rats. The air traps were sampled and changed at 24-hour intervals. In the short-term study 12 male and 12 female rats were used. Two rats of each sex were sacrificed at 1, 2, 4, 8, 12, and 24 h after administration of the compound. Urine, faeces, tissue, blood, and plasma samples were obtained at the time of sacrifice. Quantitative radiocarbon analysis was performed by use of liquid scintillation counting. Thin layer chromatography was used for separations of the metabolites and the bands identified by autoradiography. The bands were then subjected to liquid scintillation counting. The results showed that the majority of urinary metabolites were polar compounds based on the TLC characteristics and poor extractability into chloroform. Dimethylphosphate, dimethylphosphorothioate and an unknown third metabolite accounted for practically all excreted ^{3}H radioactivity. Over 90% of the ^{14}C-labelled metabolites were recovered in the urine. Metabolites designated as "B" and "E" appeared to be the major metabolites but these metabolites could not be identified. It was suggested that the metabolite "E" is not a pure compound but a mixture of which one component being a conjugate of phenylglyoxylnitrile oxime, one of the major metabolites found in liver.

Figure 1. Metabolic pathways of phoxim



Metabolism in Food Animals

Cattle

In a non-GLP study, a single lactating dairy cow was treated with ring-labelled ^{14}C-phoxim methyl by oral administration of a capsule containing 0.2 mg/kg BW of phoxim (Puhl, 1976). The metabolism and excretion were studied after a single and after five consecutive daily oral administrations of the compound to the cow. Urine, milk, faeces and blood samples were collected after the single and multiple dose administrations. Phoxim methyl was rapidly absorbed and the radioactivity was excreted mainly in urine. The phoxim methyl was mainly converted to hippuric acid, indicating a complex metabolic pathway. The other minor metabolites were not identified. Amounts of radioactivity recovered in milk indicated rapid depletion of residues over several milkings, following the single treatment. The cow was sacrificed 5 hrs after the last administration and tissue samples were obtained. Combustion analysis of tissue samples indicated residues of phoxim methyl as follows: kidney, 0.62 mg/kg; liver, 0.38 mg/kg; muscle, 0.03 mg/kg; fat, 0.02 mg/kg.

Pigs

In the report by Klein and Weber (1988), biotransformation of ^{14}C-phenyl-labelled phoxim was described in pigs in a GLP-study. Two animals were administered a single oral dose at 5 mg/kg. The animals were sacrificed at 24 and 72 h after administration. An additional untreated animal was sacrificed before the study in order to determine the background radioactivity of the tissues and to study the extraction procedures. The ^{14}C-phenyl-labelled phoxim was administered to the 2 animals together with unlabelled drug in a ratio of 1.5:1. The animals received approximately 2 mCi in a dose of 5 mg ^{14}C-labelled phoxim/kg BW.

A urine sample was collected at 8 h and urine and faecal samples were collected at 24, 48, and 72 h following administration of ^{14}C-phenyl-phoxim. Venous blood samples were collected from one pig at 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 56, and 72 h and the other at 0.5, 1, 2, 3, 8, and 24 h after administration of the compound. Liver, kidney, muscle, loin, subcutaneous fat and blood were removed at sacrifice and stored frozen pending analysis.

As in laboratory animals and in the cow, phoxim methyl was rapidly absorbed in the pig and the majority of the radioactivity was excreted in urine. The faecal excretion appeared slightly higher at later time points but the minimal number of test animals do not allow any further conclusions concerning routes of excretion. The major metabolite in urine (almost 90%) was identified as cyanobenzaldoxime, which was excreted as its glucuronic acid conjugate. The fraction of total administered radioactivity found in the tissue was much below 1%. At 24 h, total phoxim residues in edible tissues were: liver, 0.60 mg/kg; kidney, 0.35 mg/kg; muscle, 0.05 mg/kg; fat, 1.32 mg/kg. At 72 hrs, total phoxim residues were: liver, 0.37 mg/kg; kidney, 0.12 mg/kg; muscle, 0.02 mg/kg; and fat, 0.68 mg/kg.

The pharmacokinetic analysis showed rapid absorption of the radioactivity. This is apparently consistent with rapid absorption of phoxim. However, the other pharmacokinetic parameters, such as total clearance, renal clearance and mean residence time (MRT) were obviously determined for the total radioactivity and not specifically for the parent compound and do not provide any information on the pharmacokinetics of phoxim methyl. The pharmacokinetic analysis presented may be representative for any combination of metabolites. Therefore, it is very difficult to conclude that the pharmacokinetic model of best fit would be a two-compartment model because the two phases observed may relate to two different compounds. Furthermore, determination of a parameter such as clearance requires knowledge of bioavailability, which was not determined in the present report.

TISSUE RESIDUE DEPLETION STUDIES

Radio-labelled Residue Depletion Studies

No studies were reported in which target animals were treated using a radio-labelled phoxim formulation typical of those used in current veterinary applications.

Other Residue Depletion Studies (with Unlabelled Drug)

General Comment

The results reported in the studies discussed in this section were not corrected for analytical recovery.

Cattle

Four yearling cattle (250 to 370 kg BW) were sprayed with a formulation containing 1000 mg/L phoxim, with the treatment repeated at 8 days (Terblanche, 1978a). Insufficient data were provided in the report to calculate an approximate dose rate in mg/kg. The animals were slaughtered in pairs at 14 and 28 days following the second treatment and samples of liver, kidney, muscle and fat were collected for analysis. Samples were also collected from an untreated animal which served as a control. Analysis was by gas chromatography with a nitrogen-phosphorus detector, following extraction and clean-up of homogenised tissue. Analytical recoveries were approximately 92 - 104% and a limit of detection of 0.01 mg/kg. No details on the extraction, clean-up or conditions of chromatographic analysis were provided. Fat samples from the animals slaughtered at 14 days contained residues of 0.32 and 0.37 mg/kg, respectively, while residues in fat from both animals slaughtered at 28 days were 0.02 mg/kg. Residues in all other tissues were below the limit of detection. The study was not conducted to GLP.

Lactating Cattle

Three dairy cows each received two treatments 8 days apart with a spray formulation containing 1000 mg/L phoxim (Terblanche, 1978b). Milk samples were collected at 12 h and 1, 3, 7 and 14 days following the second treatment and were analysed by gas chromatography with a nitrogen-specific detector. Analytical recoveries from fortified controls were 90 - 95% at 200 µg/kg and the limit of detection was 2 µg/kg. Residues found were as follows: 12 h, 220-420 µg/kg; day 1, 120-200 µg/kg; day 3, 8-15 µg/kg; day 7, <2 µg/kg; day 14, <2 µg/kg.

In a subsequent study, three lactating cows were each sprayed twice at an 8 day interval with a dipwash formulation containing 500 mg/L phoxim (Terblanche, 1979a). Milk samples collected at 12 h, 1, 2 and 3 days following the second treatment were analysed as in the previous study. Residues found were as follows: 12 h, 80-210 µg/kg; day 1, 22-55 µg/kg; day 2, 6-20 µg/kg; day 3, 4-10 µg/kg.

A third study was reported in which 5 cows each received a single treatment with a spray containing 500 mg/L phoxim (FAO, 1985). Milk samples were collected at morning and evening milkings for 13 days following treatment. Subsequently, the same cows received two treatments with the same formulation at an interval of 6 days, after which milk samples were collected for 5 days following the second treatment. Following the initial treatment, residues reported in milk samples were as follows: 0.5 day, 40-80 µg/L; day 1, 10-20 µg/L; day 1.5, 8-10 µg/L; day 2, not detected to 2 µg/L; subsequent milkings, not detected. Residues in samples collected following the two treatments separated by 6 days were as follows: 0.5 day, 2-4 µg/L; day 1, 2-16 µg/L; day 1.5, 2-5 µg/L; day 2, 1-2 µg/L; day 2.5, 1-5 µg/L; day 3, not detected at 1 µg/L; subsequent samples, not detected. No explanation was provided for the discrepancy in residue levels observed between the single treatment and two treatment experiments.

None of the above studies were conducted to current GLP standards.

Pig

Twenty-two pigs (average weight 40 kg) were treated in a GLP study with phoxim as a pour-on formulation at a dose corresponding to 30 mg/kg BW, with a second treatment 14 days following the initial application (Greife *et al*, 1991). Groups of 4 animals were slaughtered at 7, 14, 21, 28 and 35 days following the second treatment. Samples of muscle (semimembranosus), belly fat, liver and kidney were collected, as well as muscle, skin and fat from the application area (M. longissimus dorsi). After extraction and partitioning using acetonitrile and hexane, extracts were cleaned up on silica gel and analysed by liquid chromatography with UV detection. Additional details on the methodology are provided in the discussion in the Methods Section (see Heukamp, 1991). No residues of phoxim were detected in muscle from the application area (from days 7-35) or from other portions of the carcass (from days 7-21), nor were residues found in liver or kidney samples at day 7. Samples of these tissues for the remaining slaughter dates were not tested. Residues were found in skin and fat samples and these are summarised in Table 1.

Table 1. Phoxim residues found in skin and fat from pigs administered two treatments of phoxim at a 14-day interval at an estimated dose rate of 30 mg/kg BW.

Withdrawal time (days after 2nd application of phoxim)	Phoxim Residues (mg/kg)		
7	0.492 ± 0.083	0.511 ± 0.136	0.346 ± 0.030
14	0.218 ± 0.072	0.222 ± 0.095	0.107 ± 0.028
21	0.135 ± 0.095	0.108 ± 0.093	0.049 ± 0.034
28	0.046 ± 0.023*	0.034 ± 0.008	0.023 ± 0.011
35	0.023 ± 0.008	n.d. - 0.033	n.d. - 0.012

* Mean calculated for only 3 animals; a result of 0.441 mg/kg in fat from one animal was rejected as an outlier. Other samples from this animal were consistent with results for other animals and were not excluded. n.d. = not detected

Nine pigs received a pour-on application of phoxim applied along the dorsal midline at a dose of 30 mg/kg BW, repeated after 14 days. The pigs were slaughtered in groups of 3 animals at 7, 14 and 28 days following the second application (Gyr, 1985). An untreated pig was used as a control. No detectable residues were present in any liver, kidney or muscle samples at the detection limit of 0.01 mg/kg. In fat samples, residues were as follows: day 7, 0.3-1.1 mg/kg; day 14, 0.05-0.10 mg/kg; day 28, 0.06-0.09 mg/kg. The study was not conducted to current GLP standards.

In an earlier study, 4 pigs were sprayed with a solution containing 1000 mg/L of phoxim, with a repeat application after 8 days (Terblanche, 1978c). The pigs were slaughtered in pairs at 14 and 28 days after the second application. Liver, kidney, muscle and fat were collected for analysis from the 4 treated pigs and from an untreated control. Following extraction and clean-up, samples extracts were analysed by gas chromatography using a nitrogen-phosphorus detector. Recoveries were 78-82% for phoxim from fortified liver, kidney and muscle and 100% for fat, with a limit of detection of 0.01 mg/kg for phoxim in all tissues. No residues were detectable in any liver, kidney or muscle samples. In pigs slaughtered 14 days following the second treatment, phoxim residues in fat were 0.04 and 0.05 mg/kg, respectively, while residues in fat from the two pigs slaughtered 28 days after the second treatment were <0.01 mg/kg and 0.13 mg/kg, respectively.

Subsequently, an experiment was conducted in which 6 pigs received a spray application of 500 mg/L phoxim, repeated after 1 week (SABS, 1980). Pigs were slaughtered in groups of 3 animals at 7 and 14 days following the second application, with fat collected for analysis. Following Soxhlet extraction and clean-up, samples were analysed by gas chromatography with a nitrogen-phosphorus detector. No phoxim residues were found in excess of the limit of detection of 0.05 mg/kg. Neither of these two studies was conducted according to current GLP standards.

Sheep

In a non-GLP study, nine sheep were sprayed twice at an interval of 8 days with a solution containing 1000 mg/kg phoxim, with groups of 3 sheep each slaughtered at 7, 14 and 21 days following the second treatment (Terblanche, 1978d). Liver, kidney, muscle and fat samples from these sheep, plus from an untreated control sheep, were analyzed following extraction and clean-up by gas chromatography using a nitrogen-phosphorus detector. The method had a claimed limit of detection of 0.01 mg/kg and recoveries using fortified tissues were 92% for 1 mg/kg phoxim in fat and, at a fortification level of 0.50 mg/kg, were 88%, 100% and 88% in muscle, liver and kidney, respectively. Residues found in tissues from the treated animals are summarised in Table 2.

Table 2. Residues in edible tissues of sheep following two spray treatments 8 days apart with a solution containing 1000 mg/L phoxim (3 animals per treatment group).

Withdrawal period (days)	Phoxim residues (mg/kg)			
	fat	muscle	Kidney	liver
7	2.40 - 2.75	0.03 - 0.07	<0.01 - 0.07	<0.01
14	0.74 - 1.45	<0.01 - 0.03	<0.01 - 0.03	<0.01
21	0.11 - 0.62	0.02 - 0.04	<0.01	<0.01

Nine sheep were divided into groups of 3 each, with two groups (groups 1 and 2) receiving two spray treatments at an interval of 8 days with 500 mg/L phoxim , while group 3 received phoxim at 1000 mg/L in two spray treatments also 8 days apart (Terblanche, 1979b). Groups 1 and 2 were slaughtered at 14 and 21 days, respectively, after the second treatment, while group 3 was also slaughtered at 21 days after the second treatment. Samples of liver, kidney, muscle and fat were analyzed for phoxim residues, following extraction and clean-up, by gas chromatography using a nitrogen-phosphorus detector (SABS, 1979). The method had a claimed limit of detection of 0.01 mg/kg, with analytical recoveries from fortified tissues reported as follows: fat, 105%; muscle, 88%; liver, 65%; kidney, 75%. No residues were detected in tissues from a sheep used as a control, or in muscle, kidney or liver from the treated sheep. Residues in fat samples from sheep in the three treatment groups were as follows: Group 1, 0.17-0.66 mg/kg; Group 2, 0.03-0.17 mg/kg; Group 3, 0.20-0.52 mg/kg. The study was not conducted to current GLP standards.

In another non-GLP study, 18 sheep were divided into groups of 6 animals which received a plunge-dip treatment with phoxim for 1 minute in a tank containing, respectively, 1000, 2000 or 3000 mg/L phoxim solution (Hopkins *et al.*, 1980). Within each group, 3 sheep were treated 21 days prior to slaughter and 3 sheep were treated 45 days prior to slaughter. One untreated sheep was slaughtered as a source of control tissue. No residues of phoxim were found in liver, kidney or muscle samples from any of the treated animals using a gas chromatographic method of analysis with flame photometric detection in the phosphorus mode (limit of detection, 0.05 mg/kg). In fat samples, residues ranged from 1.6 - 2.8 mg/kg in sheep slaughtered 30 days after treatment at 3000 mg/L and from <0.05-1.0 mg/kg at 45 days post-treatment. With dip treatment at 2000 mg/L, residues in fat at 30 days were 1.2-1.8 mg/kg and at 45 days were <0.05 - 0.6 mg/kg. In the 1000 mg/kg treatment group, no residues were detectable in fat at 45 days, while at 30 days residues were <0.05 - 0.7 mg/kg.

Twelve sheep were divided into two equal groups which received, respectively, 500 mg/L phoxim as a plunge dip (single treatment) or 1000 mg/L phoxim as a spray solution, repeated after 7 days (Schröder, 1988). The animals were slaughtered in groups of 3, at 28 and 35 days after the plunge dip or the second spray treatment. Only 2 sheep were included in the day 28 group for the spray treatment due to the death of one animal from causes not related to the treatment during the study. Analysis of fat samples for phoxim and its oxygen analogue by a gas chromatographic method with a limit of detection of 0.05 mg/kg for both phoxim and the oxygen analogue revealed no traces of the analogue in any samples. For the plunge dip treatment samples, residues declined from <0.05 - 0.18 mg/kg at day 28 to <0.05 mg/kg at day 35. For samples from animals that received the spray treatment, residues were <0.05 and 0.05 mg/kg at day 28 and <0.05 to 0.10 mg/kg at day 35. This study also did not meet current GLP standards.

One study was also conducted to assess the effect of phoxim treatment of lactating sheep (Hapke, 1980). Following treatment of 6 lactating sheep in a plunge dip containing 500 mg/L phoxim, milk was collected for the next 3 days and tested for residues using a liquid chromatographic (HPLC) method with a claimed limit of detection of 0.03 mg/L. No phoxim residues were detected in any of the samples.

Goats

Nine goats were sprayed twice, at an interval of 8 days, with a solution containing 1000 mg/L phoxim (Terblanche, 1978e). The goats were divided into groups of 3 animals each, which were slaughtered at 7, 14 and 21 days, respectively, following the second treatment. Liver, kidney, muscle and fat samples from the nine goats and an untreated control animal were tested for phoxim residues by a gas chromatographic method using a nitrogen-phosphorus detector (limit of detection, 0.01 mg/kg). Analytical recoveries from fat at 1 mg/kg were 79%, while recoveries from liver, kidney and muscle at 0.5 mg/kg were 102, 90 and 98%, respectively. In the day 7 samples, liver and kidney from one goat and kidney from a second goat contained 0.01 mg/kg phoxim. All other liver and kidney samples for this and the other sampling dates contained <0.01 mg/kg phoxim. In muscle, phoxim residues were: day 7, 0.01 - 0.05 mg/kg; day 14, 0.01 - 0. 2 mg/kg; day 21, <0.01 - 0.03 mg/kg. In fat, phoxim residues were: day 7, 0.53 - 0.85 mg/kg; day 14, 0.16 - 0.46 mg/kg; day 21, 0.08 - 0.10 mg/kg. This study was not conducted to current GLP standards.

Rabbits

Sixteen rabbits were treated on 7 consecutive days with a lotion containing 0.1% phoxim using a topical application in the ears, corresponding to 1 mg phoxim per day (Marcotrigiano, 1991). Animals were sacrificed, in pairs at 1, 3, 5, 7, 10, 15, 21 and 28 days following the final treatment, with samples of liver, kidney, muscle and fat collected for analysis. Samples were also collected from two other rabbits, which did not receive the phoxim treatment. Analysis was conducted by liquid chromatography (HPLC) with UV detection, with a claimed limit of detection of 0.005 mg/kg for phoxim and mean recoveries of 76%. Maximum residues were found in kidney at day 1 (0.13 mg/kg), while residues peaked in other tissues at day 3 (fat, 0.13 mg/kg; liver, 0.08 mg/kg; muscle, 0.01 mg/kg). By day 7, residues were at or approaching the detection limit in all tissues. The experiment was repeated using a spray formulation, at an estimated

dosage of 1 mg/rabbit/day. Highest residues were found in day 1 samples (fat, 0.14 mg/kg; liver, 0.11 mg/kg; kidney, 0.10 mg/kg; muscle, not quantifiable). Residues depleted rapidly and were not detectable in any tissues at day 7. The study did not meet GLP requirements.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Two distinct analytical approaches have been developed for the determination of phoxim residues, with earlier studies using gas chromatography and more recent studies using liquid chromatography as the preferred analytical technique. In the earliest method reported by the sponsor (Blass and Reigner, 1978), homogenised liver, kidney, muscle or fat tissue was extracted with hexane, partitioned with acetonitrile to remove lipids, then cleaned up on a column packed with basic alumina. Phoxim residues were eluted with benzene and analysed by gas chromatography using a nitrogen-phosphorus (alkali flame) detector. It was noted that the gas chromatography column packed with 2% SE-30 on Chromosorb GAW, DMCS, required conditioning by heating and by frequent injection of the test substance. A limit of detection of 0.04 mg/kg was claimed for the method, with analytical recoveries in the range 75-86%. The report does not specify the species from which tissues were tested.

A subsequent report described a method used in analytical work conducted by the South African Bureau of Standards (SABS, 1979). This method was applicable to muscle, liver, kidney and fat from sheep and goat. The procedure was essentially the same as that described above, with hexane extraction, partitioning with acetonitrile and clean-up on alumina. Analysis was by gas chromatography with nitrogen-phosphorus detector, using an equivalent column packing material to that described above. The claimed limit of detection was 0.05 mg/kg for all tissues. This method was apparently also applied in studies involving cattle, milk from cattle and pigs (Terblanche, 1978a-c, 1979a).

A variation on the previous methodology was used in a depletion study of phoxim in sheep. The use of a more selective flame photometric detector enabled the analysis of samples with less clean-up than required when an alkali flame detector was selected, as in the above reports (Hopkins *et al*, 1980). In this study, tissue samples were extracted with hexane, after the addition of anhydrous sodium sulphate to absorb water. The hexane extract was concentrated, partitioned with acetonitrile, with the final extract injected into the GC without further clean-up. The limit of detection achieved was 0.05 mg/kg, with recoveries ranging from 73 - 99% at 1 mg/kg.

The first reported use of liquid chromatography (HPLC) was in a study on phoxim residues in pig tissues (Gyr, 1985). Samples of liver, kidney, muscle and fat were extracted and partitioned between acetonitrile and hexane, following which extracts were analysed by liquid chromatography (HPLC) with UV detection at 285 nm. Unlike HPLC methods described subsequently, no additional clean-up using solid phase extraction was reported. Recoveries were 82-90% for all tissues at 0.1 mg/kg and from 85-90% at 1 mg/kg fortification levels, with a claimed limit of detection of 0.01 mg/kg.

A method using liquid chromatography (HPLC) with UV detection for the analysis of phoxim in swine tissues is similar to the methods reported using gas chromatography, in that partitioning between hexane and acetonitrile is followed by clean-up on alumina (Maasfeld, 1990). Liver, kidney and muscle were initially extracted with acetonitrile, then partitioned with hexane, while fat samples were homogenized with hexane and partitioned with acetonitrile. In either case, the acetonitrile phase was concentrated and cleaned up on an alumina column, after which phoxim was eluted with dichloromethane, taken to dryness, then taken up in acetonitrile and water for HPLC analysis. The limit of determination of the method was 0.01 mg/kg, with detection limits of 0.002-0.003 mg/kg for tissues. Analytical recoveries determined at 0.01 and 0.05 mg/kg fortification levels were 67-68% for fat, 72% for kidney, 52-55% for liver and 71-75% for muscle. For 5 replicate determinations at each fortification level in each tissue, relative standard deviation was <10%.

A similar approach for the analysis of swine tissues has also been reported in which, following extraction and partitioning as described above, the extract was cleaned up on a silica gel packing instead of alumina (Heukamp, 1991). In this procedure, elution of phoxim residues from the silica gel was performed with hexane/dichloromethane (50/50, v/v), after which the extract was dried and reconstituted in acetonitrile/water for analysis. the limit of determination was again 0.01 mg/kg for all tissues, with limits of detection reported from 0.002-0.003 mg/kg for the various tissues. Analytical recoveries were improved for kidney (82-108%) and fat (77-86%), but less so for muscle (52-89%) and liver (56-69%). A revised edition of the method is also available in a more recent report (Krebber and Heukamp, 1995).

APPRAISAL

A number of factors require consideration by the Committee in recommending the MRLs for veterinary use of phoxim. The Committee established an ADI of 0 - 4 µg/kg BW per day, equivalent to 240 µg per day for a 60 kg person and, because phoxim is not currently planned to be used for crop protection; the whole ADI is available for veterinary usage.

The total residue represented by the marker residue can not be determined from available studies, which also did not permit a complete assessment of the toxicity of the metabolites. As no other residues were detected or identified in the depletion studies, the marker residue is parent phoxim. Depletion studies demonstrate that residues in treated animals, other than rabbits, are at very low concentrations immediately following treatment and subsequently in all tissues except fat. MRLs recommended for muscle, liver and kidney are based on 2x the LOQ of available analytical methods (0.01 mg/kg). Based on depletion studies with unlabelled compound, the target tissue is fat in all species.

MRLs for fat are based on the GLP study in pigs, using an estimate based on the mean plus 3 standard deviations, and are extended to other species based on the similar results obtained in available depletion studies. MRLs for sheep could be extended to goats, based on the similar results observed in the available studies in the two species. The Joint Meeting on Pesticide Residues (JMPR) had previously recommended an MRL of 0.01 mg/kg for phoxim in milk from cattle.

Suitable analytical methodology is available for determining phoxim at the recommended MRLs for edible tissues.

On the basis of the maximum observed residues in cattle, pigs, sheep and goats, and in milk from cattle treated with phoxim by the recommended topical routes of administration, the Committee recommended temporary MRLs for edible tissues of cattle, pigs, sheep and goats of 20 µg/kg in muscle, 20 µg/kg in liver, 20 µg/kg in kidney, 400 µg/kg in fat and 10 µg/kg in cattle whole milk, expressed as parent compound. The MRLs as recommended would result in a daily maximum intake of 44µg of phoxim (Table 3).

Table 3. Theoretical Maximum Daily Intake (TMDI) of Phoxim Residues

Food Item	MRL (µg/kg)	Food Basket (g)	TMDI (µg)
Muscle	20	300	6
Liver	20	100	2
Kidney	20	50	1
Fat	400	50	20
Milk	10	1500	15
Total:			**44**

The Committee required the following data for evaluation in 2002:

1. Radiolabel studies to relate the marker residue to the total residue in ruminants and pigs following topical application of the formulated product.

2. GLP residue studies using the current recommended treatments in sheep and cattle.

3. A study to confirm distribution of the marker residue in rabbits.

4. Validation of available methodology for phoxim residues in tissues of sheep, cattle, goats, rabbits and in milk from cattle.

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