PHOXIM

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ADDENDUM

To the phoxim monograph prepared by the 52nd meeting of the JECFA and published in FAO Food and Nutrition Paper 41/12

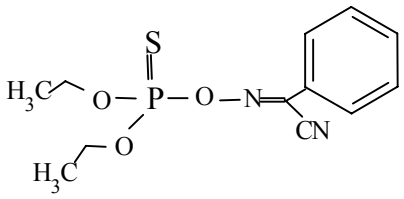
IDENTITY

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:



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_D^{20}

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.

INTRODUCTION

At the 52nd meeting of the Committee, an ADI of 0 - 4 μg per kg body weight was established. Temporary MRLs of 20 μg/kg in muscle, liver and kidney tissues and 400 μg/kg in fat for cattle, pigs, sheep and goats were recommended. The sponsor indicated that no horticultural use of phoxim was intended. The Committee requested further information concerning:

1. The results of studies using radiolabelled phoxim to determine the proportion of the total residues accounted for by the marker residue in ruminants and pigs following topical application of the formulated product.
2. The result of residue-depletion studies conducted in accordance with Good Laboratory Practice in cattle and sheep given the current recommended treatments.

3. Validation of available analytical methods for the detection of phoxim residues in tissues of cattle, sheep, goats and in cow's milk.

The Committee received at the present meeting data concerning metabolism and residue depletion of ^{14}C-Phoxim in sheep tissues, validation of the method for determination of phoxim concentrations in sheep tissues, sheep tissue residues of phoxim following dipping, investigation of the specificity of the routine analytical method and validation of the method of analysis for determination of phoxim concentrations in swine tissues.

RESIDUES IN FOOD AND THEIR EVALUATION

General

Phoxim is an ectoparasiticide of the organophosphate group used for the control of mites (Psoroptes, Sarcoptes, Chorioptes), 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 to wash, spray or dip, and as a 7.5% (w/v) pour-on formulation.

Dosage

As a wash or spray treatment, phoxim would typically be applied as a single treatment, or in two treatments at a 7-day interval, using 10 mL of the formulated product (50 g phoxim/100 mL) per 10 L of water, using sufficient liquid 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 above 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.

METABOLISM

Sheep

The proposed phoxim metabolic pathways were presented in the previous evaluation (JECFA, 1999). The study submitted to the present evaluation on metabolic fate of phoxim in sheep using ^{14}C-Phoxim by Winwick (1999) was performed in compliance to present GLP standards. The study used ^{14}C-Phoxim carrying the radiolabel universally in the phenyl ring of the molecule. The specific activity was 39.68 μCi/mg. The 10 sheep, Clun × Suffolk, consisted of 5 males and 5 females. Their age range was 15-16 months and weight 50 ±10 kg. The animals were kept in individual housing 14 days prior to drug administration and moved to metabolic cages 3 days prior to administration. The cages allowed collection of urine and faeces separately. The sheep were fed twice daily with concentrate ration prepared in advance for the entire study. Water was available *ad libitum*.

The animals were divided into two groups receiving the drug via dermal or intravenous administration. Group 1 (dermal application) consisted of 6 sheep and Group 2 (intravenous administration) of 4 sheep. Because these animals were used also for the residue depletion study, they were killed in groups of 2 (one male and one female) after 4 and 8 hours following intravenous administration and after 7, 21 and 28 days following cutaneous application.

Individual doses of ^{14}C-Phoxim were prepared by mixing the solution with non-labelled phoxim, Volaton (Sebacil) VL80, solution. The cutaneous administration of the compound was performed on a shaved dorsal area sized about 10% of body surface area. The area was covered with a bandage following administration.

Urine and faeces were collected after intravenous administration until slaughter and after cutaneous application from 0-8 hours, 8-24 hours and thereafter at 24 hour interval up to 7 days. Serial plasma samples were taken during the first day and thereafter once daily until slaughter. Tissue samples were collected at slaughter.

Radioactivity was measured by use of liquid scintillation counting. The samples were also analyzed using a high performance liquid chromatography (HPLC) method as well as a thin layer chromatography (TLC) method. A liquid chromatography/mass spectrometry method was described for the identification of the metabolites.

Table 1 demonstrates the recovery of radioactivity after topical administration (using bandages). The radioactivity was measured at the application area and in the excreta as a function of time.

Table 1. Recovery of radioactivity (% of dose) from sheep following a single topical application of ^{14}C-phoxim (25 mg/kg)

Sample	Sheep No. (sacrifice time in days)					
	1M (7)	2F (7)	3M (21)	4F (21)	5M (28)	6F (28)
Bandage extracts (24h)	7.1	7.9	13.7	14.7	8.8	12.8
Application site swabs	9.5	17.9	8.5	9.9	8.8	6.0
Application site extracts	45.0	42.1	37.7	37.1	43.2	32.1
Application site digest	2.6	4.0	4.1	6.0	4.7	3.5
Total application site	57.1	64.0	53.0	56.7	56.7	41.6
Urine:						
Day 1 (0-8 h)	0.4	0.6	0.3	0.2	0.2	0.2
Day 1 (8-24 h)	1.0	0.7	1.4	1.3	0.8	1.1
Day 2	2.0	1.5	1.6	1.3	1.1	1.2
Day 3	1.9	2.2	1.5	1.3	1.2	1.4
Day 4	1.7	1.3	1.6	1.3	1.1	1.4
Day 5	1.8	1.2	1.2	1.2	1.0	1.2
Day 6	1.4	1.5	1.2	1.1	0.9	1.1
Day 7	1.3	1.2	1.2	1.0	0.9	1.1
Total urine	**11.5**	**10.2**	**10.0**	**8.7**	**7.2**	**8.7**
Faeces:						
Day 1 (0-4 h)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Day 1 (4-24 h)	0.1	0.2	0.1	<0.1	<0.1	0.1
Day 2	0.3	0.4	0.3	0.1	0.1	0.1
Day 3	0.5	0.4	0.2	0.1	0.1	0.1
Day 4	0.4	0.4	0.2	0.2	0.2	0.2
Day 5	0.4	0.5	0.2	0.1	0.1	0.1
Day 6	0.4	0.3	0.2	0.1	0.1	0.1
Day 7	0.4	0.4	0.1	0.1	0.1	0.1
Total faeces	**2.5**	**2.6**	**1.3**	**0.7**	**0.7**	**0.8**
Cage wash	**0.1**	**0.1**	**0.2**	**0.1**	**0.1**	**0.1**
Total recovery	**78.3**	**84.8**	**75.5**	**77.2**	**73.5**	**64.0**

The data in Table 1 indicate that a significant portion of the radioactivity could be recovered at the application site (65-77%) during the first 7 days after application. The portion excreted in the urine appears to decrease slowly, as a function of time but excretion in faeces remains stable during the whole period. However, the major route of excretion of radioactivity is via urine while excretion via faeces appears to be an insignificant route.

The data indicated that nine metabolites were detected by use of HPLC in sheep urine collected during 7 days following topical administration by use of HPLC. Out of these, five were unknown. The amount of the metabolites was apparently given as relative detector response (Table 2a and 2b, p 72f). According to the data, reference material existed only for phoxim, z-cyanoxim, benzoic acid and hippuric acid. The table text does not indicate which detector system was used for these data i.e. if the relative amount of metabolites is a function of the radioactivity measurement, mass selective detection or UV absorption. However, based on Table 1 it appears that detection of radioactivity was used. The cyanoxim-glucuronide, z-cyanoxim and cyanoxim-sulphate appeared to be the major metabolites. No parent phoxim could be recovered in urine.

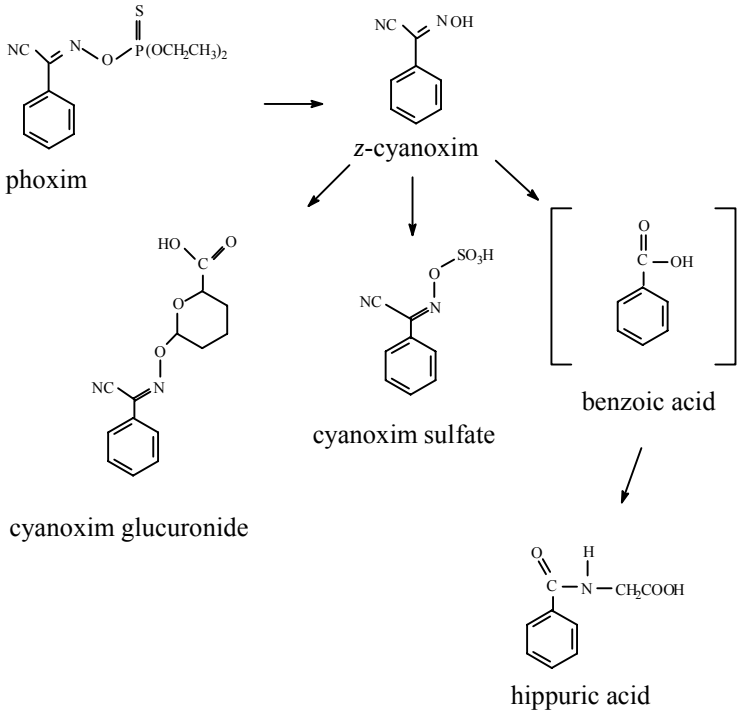


Figure 1. Proposed metabolic pathway for phoxim in sheep.

As can be observed, the total amount of metabolites excreted in the urine at this time consisted of only approximately 10% of the total administered dose. It can be seen that the two animals (2F and 4F) with the highest amount of z-cyanoxim, had also the lowest relative amounts of its cyanoxim-glucuronide and cyanoxim-sulfate indicating a slower metabolism in these animals.

Table 2a Proportion of radioactive components (by HPLC) excreted in the urine of sheep during 7 days after topical application of ^{14}C-phoxim (25 mg/kg)

Metabolite (identity)	**% chromatogram: Sheep No**					
	1M	2F	3M	4F	5M	6F
Met 1 (unknown, polar)	8.2	6.9	6.4	9.0	5.9	9.6
Met 2 (unknown)	2.5	3.0	3.4	9.1	3.3	5.5
Met 3 (hippuric acid)	2.6	2.5	3.6	4.8	2.0	4.6
Met 4 (cyanoxim-glucuronide)22.6	8.0	19.5	ND	14.2	17.8	
Met 5 (unknown)	ND	ND	ND	ND	ND	ND
Met 6 (z-cyanoxim)	11.5	40.3	15.1	41.3	25.6	16.0
Met 7 (unknown)	ND	ND	ND	ND	ND	ND
Met 8 (cyanoxim-sulphate)	36.3	23.6	32.2	17.3	34.2	33.2
Phoxim	ND	ND	ND	ND	ND	ND
Met 9 (unknown, polar)	6.6	5.3	7.0	4.9	4.6	5.9
Others	9.7	10.4	12.8	18.6	10.2	7.4
Total chromatogram (%)	**100**	**100**	**100**	**100**	**100**	**100**

Table 2b. Proportion of radioactive components (by HPLC) excreted in the urine of sheep during 7 days after topical application of ^{14}C-phoxim (25 mg/kg)

Metabolite (identity)	% dose: Sheep No.					
	1M	2F	3M	4F	5M	6F
Met 1 (unknown, polar)	0.9	0.7	0.6	0.8	0.4	0.8
Met 2 (unknown)	0.3	0.3	0.3	0.4	0.2	0.5
Met 3 (hippuric acid)	0.3	0.3	0.4	0.4	0.1	0.4
Met 4 (cyanoxim-glucuronide)2.6	0.8	2.0	ND	1.0	1.5	
Met 5 (unknown)	ND	ND	ND	ND	ND	ND
Met 6 (z-cyanoxim)	1.3	4.1	1.5	3.6	1.8	1.4
Met 7 (unknown)	ND	ND	ND	ND	ND	ND
Met 8 (cyanoxim-sulphate)	4.2	2.4	3.2	1.5	2.5	2.9
Phoxim	ND	ND	ND	ND	ND	ND
Met 9 (unknown, polar)	0.8	0.5	0.7	0.4	0.3	0.5
Others	1.1	1.1	1.3	1.6	0.7	0.6
Total chromatogram (%)	**11.5**	**10.2**	**10.0**	**8.7**	**7.0**	**8.6**

Based on the data obtained after intravenous administration, the metabolic pattern was similar to that observed following topical administration. However, proportionally the portion of z-cyanoxim in urine was much smaller that than after topical application, indicating that the metabolic pathway from z-cyanoxim to cyanoxim-glucuronide and cyanoxim-sulfate was faster after this route of administration.

Pharmacokinetics of ^{14}C-phoxim could be only partially characterized from the present data set.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

These data were obtained using the same animals that were used to study phoxim metabolism in sheep and, consequently, were used for the radiolabelled residue depletion studies as well. The animal housing, treatment protocols and slaughter times are described above. The sheep were killed by use of sodium pentobarbitone for anaesthesia followed by exsanguination. The following tissue samples were taken: application site, liver, kidneys, muscle, abdominal fat (omental and perirenal) and subcutaneous fat. The subcutaneous fat was taken from site far from the application site.

The following tables describe the total radioactivity and phoxim concentrations in the appropriate tissues. The table text does not really indicate what is the unit of these measurements.

Table 3. Concentrations of radioactivity in sheep tissues following a single topical application of ^{14}C-phoxim (25 mg/kg)

Tissue	Sheep No. (sacrifice time-days)					
	1M (7)	2F (7)	3M (21)	4F (21)	5M (28)	6F (28)
Liver	1.603	1.561	1.038	0.449	0.374	0.775
Kidney	0.401	0.345	0.113	0.096	<0.059	0.115
Muscle	0.137	0.116	0.074	0.047	<0.060	0.059
Fat-subcutaneous	1.828	1.853	0.679	0.414	0.147	0.542
Fat-omental/parietal	2.071	1.566	0.538	0.321	0.099	0.350
Whole-blood	0.145	0.153	0.146	0.094	0.069	0.142
Plasma	0.105	0.098	0.043	0.057	0.030	0.076

Results are expressed as µg equivalent phoxim/g tissue

Table 4. Concentrations of radioactivity in sheep tissues following a single intravenous administration of ^{14}C-phoxim (1 mg/kg)

Tissue	Sheep No. (sacrifice time-hours)					
	7M (4)	8F (4)	Mean	9M (8)	10F (8)	Mean
Liver	0.545	0.637	0.591	0.337	0.208	0.273
Kidney	0.406	0.604	0.505	0.282	0.265	0.274
Muscle	0.452	0.450	0.451	0.202	0.119	0.161
Fat-subcutaneous	0.420	0.418	0.419	0.277	0.371	0.324
Fat-omental/parietal	0.473	0.571	0.522	0.521	0.495	0.508
Whole-blood	0.147	0.144	0.146	0.062	0.060	0.061
Plasma	0.117	0.110	0.114	0.058	0.064	0.061

Results are expressed as μg equivalent phoxim/g tissue

Table 5. Concentrations of phoxim in sheep tissues following a single topical application of ^{14}C-phoxim (25 mg/kg)

Tissue	Sheep No. (sacrifice time-days)					
	1M (7)	2F (7)	3M (21)	4F (21)	5M (28)	6F (28)
Liver	ND	ND	ND	ND	ND	ND
Kidney	0.007	0.006	ND	0.014	ND	0.014
Muscle	0.068	0.038	0.027	0.034	ND	0.022
Fat-subcutaneous	1.431	1.481	0.543	0.328	0.118	0.618
Fat-omental/parietal	1.507	1.180	0.401	0.223	0.059	0.204

Results are expressed as μg phoxim/g tissue
ND: below the limit of detection; liver 0.002 μg/g, kidney 0.003 μg/g, muscle 0.006 μg/g, fat 0.003 μg/g

Table 6. Concentrations of phoxim in sheep tissues following a single intravenous administration of ^{14}C-phoxim (1 mg/kg)

Tissue	Sheep No. (sacrifice time-hours)					
	7M (4)	8F (4)	Mean	9M (8)	10F (8)	Mean
Liver	ND	ND	ND	ND	ND	ND
Kidney	0.007	0.007	0.006	0.005	ND	-
Muscle	0.266	0.221	0.244	0.127	0.073	0.100
Fat-subcutaneous	0.242	0.254	0.248	0.226	0.279	0.253
Fat-omental/parietal	0.278	0.376	0.327	0.412	0.400	0.406

Results are expressed as μg phoxim/g tissue
ND: below the limit of detection; liver 0.002 μg/g, kidney 0.003 μg/g, muscle 0.006 μg/g, fat 0.003 μg/g

Table 7. Total residues and residues of parent compound in tissues following topical application of ^{14}C-phoxim to sheep (25 mg/kg bw)

Tissue	Withdrawal time from treatment to slaughter (days)	Sheep identification number (sex)	Total residues ^{14}C-phoxim (mg equiv./kg)	Residues phoxim parent compound (mg/kg)	Ratio (total residue/parent compound)
Fat (subcutaneous)	7	1 (M)	1.828	1.431	1.28
		2(F)	1.853	1.481	1.25
	21	3(M)	0.679	0.543	1.25
		4(F)	0.414	0.328	1.26
	28	5(M)	0.147	0.118	1.25
		6(F)	0.542	0.618	1.14
Fat (omental/ perirenal)	7	1 (M)	2.071	1.507	1.37
		2(F)	1.566	1.180	1.33
	21	3(M)	0.538	0.401	1.34
		4(F)	0.321	0.223	1.44
	28	5(M)	0.099	0.059	1.68
		6(F)	0.350	0.204	1.72
Muscle	7	1 (M)	0.137	0.068	2.01
		2(F)	0.116	0.038	3.05
	21	3(M)	0.074	0.027	2.74
		4(F)	0.047	0.034	1.38
	28	5(M)	<0.060	ND	---
		6(F)	0.059	0.022	2.68
Kidney	7	1 (M)	0. 401	0.007	57.3
		2(F)	0.345	0.006	57.5
	21	3(M)	0.113	ND	---
		4(F)	0.096	0.014	6.86
	28	5(M)	<0.059	ND	---
		6(F)	0.115	0.014	8.21
Liver	7	1 (M)	1.603	ND	---
		2(F)	1.561	ND	---
	21	3(M)	1.038	ND	---
		4(F)	0.449	ND	---
	28	5(M)	0.374	ND	---
		6(F)	0.775	ND	---

ND = not detected --- = not calculated

The ratio of total radioactive residue to phoxim (TRR/ phoxim) was 1.14 - 1.72 in fat tissue, 1.38 -3.05 in muscle tissue and 6.86 - 57.5 in kidney tissue after topical application. The respective values (mean) after intravenous administration were 1.25 - 1.69, 1.61 - 1.85, and 84.2. The TRR/phoxim ratio did not seem to be time dependent.

The relative concentration of total radioactive residue appeared to be highest in the kidney and lowest in the subcutaneous fat tissue.

The phoxim concentration in fat tissue in this study was in good correlation with the earlier non-GLP studies reported in the previous evaluation (JECFA, 1999).

Residue depletion studies with non-radioactive phoxim

A tissue residue depletion study was conducted using 11 male and 11 female Suffolk cross sheep (Robinson, 1999). The study was conducted in compliance with current GLP standards. The treatment with phoxim was performed by immersion of the animals in an aqueous phoxim solution in a commercial dipping tank. The tank contained 1247 liter of phoxim solution at 500 mg active ingredient/liter. The animals were dipped in this solution for at least one minute.

No clinical signs of illness were observed during the study period. The animals weighed 40.5-51.0 kg at the beginning of the study. The sheep were shorn 4 weeks before the dipping treatment. Complete information concerning the housing

and feeding of the animals was provided. The animals were killed at 7, 21, 28, 35, and 49 days after the treatment. Two of the animals served as untreated controls. Macroscopic *post mortem* examination was performed at slaughter. Phoxim tissue concentrations were monitored by use of a validated HPLC method described below.

Phoxim residues were not found in any of the liver sample. The phoxim concentration in kidney obtained from 4 sheep killed 7 days post administration were 35.3, 30.8 and 31.0 μg/kg while one of the samples was below the validated LOQ. One kidney tissue sample obtained 21 days post-administration had trace phoxim concentration below the validated LOQ and no phoxim was detected in all the other kidney tissue samples. The respective values in muscle were 189.0, 75.1, 84.6 and 88.9 μg/kg at 7 days after administration. Traces of phoxim were found in 3 samples at 21 days and 2 samples at 28 days after administration but all these values were below the validated LOQ.

Phoxim was found in sheep omental/parietal fat tissue at 7 days after phoxim administration. the concentrations in 4 animals were 1136, 1066, 1635, and 1823 μg/kg. Traces of phoxim were found in all fat samples collected at all slaughter times after 7 days, but all of the detected concentrations were below the validated LOQ.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Three studies were provided for evaluation of the analytical methods concerning phoxim residues in tissues. One study concerned analysis of residues in sheep tissue, one in swine tissues and one the potential interference caused by other pharmaceutical compounds in the sample. First analytical method for phoxim was based on gas chromatography (Blass and Riegner, 1978). The present methods are very similar to the HPLC method by Krebber and Heukamp (1995) and discussed in the 52nd meeting (JECFA, 1999).

Sheep

The report of Gutteridge (1999a) describes validation of the method for analysis of sheep tissue. The study was performed in compliance to present GLP standards. The method is based on HPLC separation and UV light detection. Initially the analyte was extracted from the tissue by use of acetonitrile or hexane followed by a clean up phase using silica solid phase cartridges.

The study was performed using fortified sheep tissue. The claimed limits of detection (LOD) were 2.0 μg/kg in liver, 3.0 μg/kg in kidney, 5.5 μg/kg in muscle and 3.0 μg/kg. The limit of quantification (LOQ) was validated at 25 μg/kg in liver, kidney and muscle tissue and at 200 μg/kg in fat tissue. These values represent half the concentration of the present temporary MRLs. The method was validated for linearity, precision, specificity, limit of detection, limit of quantitation, accuracy and day-to-day precision.

The liver, kidney or muscle samples (10 g) were homogenized and extracted with acetonitrile. The extract was then centrifuged and the supernatant collected. The acetonitrile extraction was repeated and the supernatants combined. After filtration hexane was added and after shaking the phases were allowed to separate. The acetonitrile phase was collected and evaporated to dryness.

The fat sample (10 g) was homogenized with hexane. The sample was then centrifuged and the supernatant collected and filtered. Acetonitrile was then added, mixed thoroughly. After separation, the acetonitrile phase was then collected. The acetonitrile treatment was repeated twice. The acetonitrile fractions were combined and evaporated.

The dry residue is dissolved in hexane and used for solid phase extraction. The cartridge is conditioned with hexane and the sample is loaded. The sample container is rinsed with dichloromethane/hexane (10/90) and charged on the cartridge. The sample is then eluted with dichloromethane/hexane (50/50) and used for HPLC.

The chromatographic system contains a gradient pump and an UV detector set at 281 nm. The separation was performed using reversed phase C_{18} column and the solvent system was based on acetonitrile and a water gradient program.

Detector response linearity was determined at concentrations from 50 ng/mL to 900 ng/mL (n=10 for highest and lowest concentration) and the coefficient of determination was 0.997098 and the correlation coefficient was 0.99855. The relative standard deviation was 4.89% and 1.07% for the lower and higher concentration. The extracts from phoxim free tissue did not indicate any interfering peaks nor did peaks of enrofloxacin, fenbendazole or diazinon interfere. Diazinon standard solution caused a peak at the same retention time as phoxim but could not be detected after the present extraction procedure. The limits of detection were determined by extraction (n=20) from various tissues. These values were given above. The LOQ in this study was set at 25 μg/kg for liver, kidney and muscle and 200 μg/mL for fat. The accuracy (% recovery) determined in three occasions was 75.5-99.2% and assay precision (%) 1.36-16.2%. The assay accuracy and precision were very similar also among different analysts. The stability of the final extract was also examined. The results showed that phoxim was stable at room temperature for 48 hours and at -20 °C for 2 months except for liver, where the concentration was reduced by 60% during that time. The method uses standard laboratory chemicals. The method appears sensitive, specific and rugged enough to qualify as a regulatory method for residue control programs. The changes made to the original method were irrelevant.

Swine

A study concerning validation of the analytical method for swine tissue was performed (Gutteridge, 1999b). Also this study was in compliance with present GLP standards. The sample pre-treatment and the chromatographic procedures were identical to those described above for sheep tissue analysis. Therefore, only the relevant method performance data are evaluated here.

The LOD in muscle, liver, kidney, and skin/subcutaneous fat was 1.6, 2.3, 1.3, and 3.3 µg/kg, respectively. Detector response linearity was determined at concentrations from 50 ng/mL to 900 ng/mL (n=9 for lowest and n=10 for the highest concentration) and the coefficient of determination was 0.998503 and the correlation coefficient was 0.99925. The relative standard deviation was 5.27% and 1.34% for the lower and higher concentration. The original requirement for system precision was set at 5.0%. The obtained values were, however, considered to be acceptable. The extracts from phoxim free tissue did not indicate any interfering peaks nor did peaks of enrofloxacin, oxytetracycline or diazinon. As indicated earlier for the method for sheep tissue residues, diazinon standard solution caused a peak at the same retention time as phoxim but did not extract under these conditions. The limits of detection were determined by extraction (n=20) from various tissues. These values were given above. The LOQ in this study was set at 10.0 µg/kg for liver, kidney and muscle and 350 µg/mL for skin/subcutaneous fat. It is not clear why the validation was performed at an MRL of 700 µg/kg for fat, considering that the temporary MRL for pig fat set by the 52nd JECFA was 400 µg/kg. The accuracy (% recovery) determined in three occasions was 67.1-88.8% and assay precision (%) 2.65-18.9%. The assay accuracy between two different analysts was similar for all tissues and different occasions (74.2-91.5%). The precision was very similar also among different analysts. However, there was a considerable difference between the precision in muscle, liver and kidney tissue (7.17-12.2%) and fat tissue (2.46-5.87%). The stability of the final extract was also examined in a similar manner as was done in the method validation for sheep tissues. The results showed that phoxim was stable at room temperature for 48 hours and at -20 °C for 2 months except for liver, where the concentration was reduced to less than 70% during in 4 weeks and to 41% after 8 weeks. The method uses standard laboratory chemicals. The method appears sensitive, specific and rugged enough to qualify as a regulatory method for residue control programs. The changes made to the original method were irrelevant.

Specificity of the analytical method

An additional study concerning the specificity of the routine analytical method was performed (Robinson, 2000). This study, which was also in compliance with present GLP standards, determined the specificity of the method in both sheep and pig tissues. Altogether 14 different pharmaceutical compounds were investigated for their chromatographic response in the used routine HPLC method. The compounds used were trimethoprim, oxfendazole, spectinomycin, lincomycin, ceftiofur, tylosin tartrate, ampicillin, sulfadiazine, levamizole, oxytetracycline, flumethrin, doramectin, amitraz, and valnemulin. These compounds were injected to the HPLC at 1000 ng/mL while the phoxim concentrations varied from 20 to 1000 ng/ml. None of the compounds included in this study interfered with the phoxim analysis.

APPRAISAL

Phoxim was previously reviewed by the 52nd JECFA. Data were provided on the phoxim tissue residue depletion in two GLP studies. One of the studies used ^{14}C-phoxim while the other used non-labelled phoxim. Analytical methods for detection of phoxim concentrations in tissues for sheep and swine were provided. A study of the specificity of the analytical method was also performed. All these studies were conducted according to current GLP standards. The committee had requested radiolabel studies relating the marker residue to total residue in ruminants and swine. Only one study in one ruminant species, sheep, was submitted. A GLP residue depletion study in cattle, as requested by the 52nd JECFA, was not submitted. No studies concerning rabbits were obtained. Validation of the current methodology concerning cattle, goat and rabbit tissues as well as milk was not performed.

The cyanoxim-glucuronide, z-cyanoxim and cyanoxim-sulphate appeared to be the major metabolites in sheep. No parent phoxim could be recovered in the liver. However, phoxim related radioactivity (phoxim equivalent) was highest in the liver at 21 and 28 days post-administration. The studies (Table 7) indicated that total residues in kidney and muscle tissues were 8.2 and 2.7 times higher than the marker residue (phoxim) at 21 and 28 days after phoxim administration. This comparison could not be performed for liver, because parent phoxim could not be detected. The total residues in fat tissues were not more than 1.72 times higher than the phoxim concentration. However, the metabolites observed in these studies were considered to have no toxicological significance.

The analytical method for sheep was validated at a LOQ of 25 µg/kg in liver, kidney and muscle tissue and 200 µg/kg in fat tissue. These values are in accordance with the temporary MRLs. However, the LOQ for swine fat tissue was set at 350µg/kg, although the temporary MRL for this tissue is 400 µg/kg. Generally, it is preferred to validate residue control methods at a LOQ of half of the MRL (as it was done in the method for sheep tissues).

MAXIMUM RESIDUE LIMITS

Based on the new data submitted, the Committee recommended MRLs based on the following considerations:

- The ADI of 0-4 μg/kg bw corresponds to a maximum daily intake of 0.24 mg for a 60-kg person.
- The marker residue is parent phoxim.
- The metabolites were considered to be of no toxicological significance and were therefore not taken into account in the determining the MRL.
- The target tissue in all species is fat.
- A suitable analytical method is available for monitoring compliance with the proposed MRLs.
- The additional data received by the Committee allowed it to establish permanent MRLs only for sheep, swine and goat.
- As no new data were provided on cattle, temporary MRLs were recommended for this species.

The Committee recommended MRLs for edible tissues of sheep, swine and goat of 50 μg/kg in muscle, 50 μg/kg in liver, 50 μg/kg in kidney, and 400 μg/kg in fat, expressed as phoxim. Temporary MRLs of 50 μg/kg in muscle, 50 μg/kg in liver, 50 μg/kg in kidney, 400 μg/kg in fat and 10 μg/kg in milk were recommended for cattle and in cow's milk.

Recommended MRLs for phoxim.

Tissue	MRL (μg/kg)	Food Basket (g)	TMDI (μg)
Muscle	50	300	15
Liver	50	100	5
Kidney	50	50	2.5
Fat	400	50	20
Milk	10	1500	15
Total			57.5

The Committee requested a residue-depletion study in cattle, conducted in compliance with GLP, to be made available by 2004.

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