KRESOXIM-METHYL

[FIGURES 1-13 are reproduced at the end of this monograph]

IDENTITY

ISO common name: kresoxim-methyl (ISO-proposed).

Chemical name

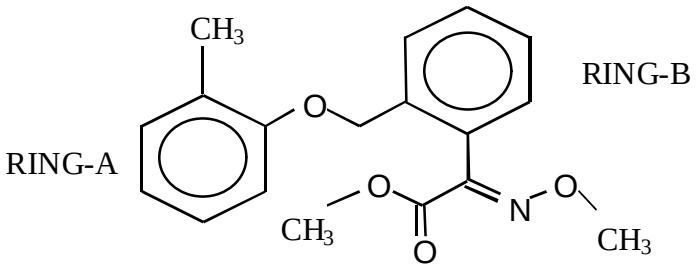
IUPAC: methyl (*E*)-methoxyimino-[α-(*o*-tolyloxy)-*o*-tolyl]acetate
methyl (*E*)-2-methoxyimino-[2-(*o*-tolyloxymethyl)phenyl]acetate

CA: (*E*)-α-(methoxyimino)-2-[(2-ethylphenoxy)methyl]benzeneacetate

CAS registry no: 143390-89-0

Synonyms: LAB 242 009
BAS 490 F

Structural formula:



Molecular formula: $C_{18}H_{19}NO_4$

Molecular weight: 313.36

PHYSICAL AND CHEMICAL PROPERTIES

Pure active ingredient

Physical State: white crystalline solid

Odour: mildly aromatic

Melting point: 101.6-102.5 (99.7% purity).

Boiling point: No boiling or sublimation of kresoxim-methyl up to 310 °C decomposition temperature using OECD method 113.

Relative Density: 1.258 at 20 °C using EEC method A 3.1.4.3

Solubility in water:	kresoxim-methyl (99.4%) 2.00 ± 0.08 mg/l at 20 °C (using EEC method A6) free acid 91 mg/l at 20 °C
Solubility in organic solvents (99.7%, 20°C)	1.7 g/kg in n-heptane 111 g/kg in toluene 939 g/kg in dichloromethane 14.9 g/kg in methanol 217 g/kg in acetone 123 g/kg in ethyl acetate
Vapour pressure:	kresoxim-methyl: 2.3 x 10^{-6} Pa at 20 °C (extrapolated). (*z*)-isomer (490M1): <1 x 10^{-5} Pa
Octanol/water partition coefficient:	kresoxim-methyl (99.4%): log K_{ow} = 3.40 at 25°C free acid: log K_{OW} = 0.15 (pH 7; 20 °C).
Dissociation constant:	kresoxim-methyl: pKa >2 and <12; titration for acidic range by OECD method 112. Dissociation in alkali claimed not to be possible because of structure. free acid: pKa = 4.2 (20 °C).
Surface tension (99.7%)	72.3 mN/m at 20°C. 0.5% (saturated) solution (EEC method A5)

Satisfactory IR, NMR and Mass Spectra of kresoxim-methyl (99.7%) were submitted.

(Binninger, 1995; BASF, 1994; Moult, 1996; Redecker, 1990, 1991; Gückel, 1992a,b; Türk 1994a,b,c; Kröhl, 1994 and Loeffler, 1992).

Technical material

Purity:	91% w/w minimum
Appearance:	light-brown powder
Physical state:	solid

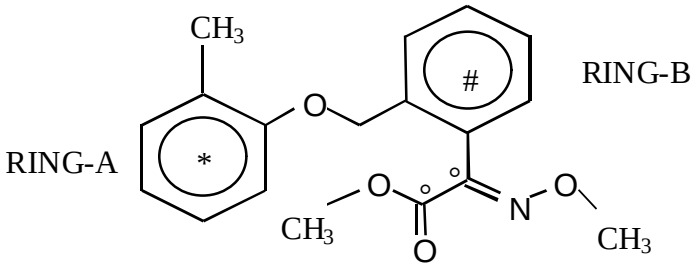
Formulations

Kresoxim-methyl is a broad spectrum fungicide structurally related to Strobilurin A, a natural product of the wood-decaying fungus *Strobilurus tenacellus*. It is a member of a new class of biologically active compounds - the strobilurins. It is formulated as an SC, WG or SE.

METABOLISM AND ENVIRONMENTAL FATE

Radiolabelled test materials

Kresoxim-methyl was labelled in one of three positions shown below.



Key to radiolabel positions:

* [*phenoxy*-U-^{14}C]kresoxim-methyl
\# [*phenyl*-^{14}C]kresoxim-methyl
° [^{13}C]kresoxim-methyl

A single page summary of the metabolism of kresoxim-methyl in animals and plants was submitted (Ammermann *et al.,* 1992) but was not used in the evaluation.

Animal metabolism

Rats. In a series of experiments on male and female Wistar rats (Kohl, 1994) the test substance was administered either orally or intravenously according to the dosing regime detailed in Table 1. Rats from groups A to D were used to determine metabolite patterns in faeces and urine, and those from dosing groups DY (phenyl-labelled) and DX (phenoxy-labelled) were used for the isolation and identification of urinary and faecal metabolites. HPLC was used to resolve the metabolites which were then identified by comparison with HPLC standards, mass spectrometry and NMR.

Table 1. Dose groups used in the metabolism studies on rats each containing 3-10 animals of each sex.

Group	Dose regime
A	Single i.v. dose 5 mg/kg bw (phenyl label). Urine and faeces analysed.
B	Single oral dose 50 mg/kg bw (phenyl label). Urine and faeces analysed.
C	Oral doses 50 mg/kg bw/day (14 daily doses no label, one dose phenyl label). Urine and faeces analysed.
D	Single oral dose 500 mg/kg bw (phenyl label). Urine and faeces analysed.
DX	Single oral dose 500 mg/kg bw (phenyl label, with 30% ^{13}C label on side chain). Urine and faeces analysed.
DY	Single oral dose 500 mg/kg bw (phenoxy label). Urine and faeces analysed.

All major and most minor metabolites were isolated, with a total of 33 metabolites (including conjugates) identified. Code numbers and chemical structures are given in Figure 13, following the section on plant metabolism.

Up to 75% of the oral dose in groups B-D was excreted unchanged in the faeces. No unchanged parent compound was detected 3.5-4 hours after oral dosing in plasma, liver or kidneys, and none was identified in the bile or urine, indicating that kresoxim-methyl is rapidly and completely metabolized after absorption from the G.I. tract.

The metabolite composition in plasma indicates that the ester cleavage, which leads to the free acid 490M1, is the fastest and most important biotransformation step which is assumed to occur

as a first-pass reaction. Accordingly, after intravenous dosing a considerable quantity of unchanged parent compound (16% of the dose) was found in the urine of female rats.

Most of the initially formed 490M1 is further metabolized, mainly by hydroxylation of the aromatic phenoxy ring and the aryl-CH_3 group. Thus, after oral administration, the metabolites 490M2 "alcohol acid", 490M9 "phenol-acid" and their glucuronides were generally the predominant final products in the excreta and in the plasma, liver and kidneys. In female rats these subsequent reactions seem to be less pronounced, since females generally excreted higher proportions of 490M1.

Mainly in males, the cleavage of the benzyl ether bridge contributes to a certain extent to the biotransformation. Fragments containing the phenyl ring were mainly recovered in the form of metabolites 490M6, 490M20, and 490M16. At the highest dose level and in the 14-day study 490M16 became relatively more important in the urine of males than females.

The phenoxy ring is further oxidized to monohydroxybenzyl alcohol, dihydroxybenzyl alcohol and salicylic acid, but these metabolites were found only as the sulfate conjugates (490M41, 490M42, 490M43 and 490M22) in the urine, together with the glucuronide of *o*-cresol, 490M37.

Glucuronated conjugates were detected in notable quantities in the bile, 490M26, 490M33, 490M25, 490M39, 490M29 being found in the largest quantity in the bile of both sexes.

The metabolite patterns in the excreta of groups B and C were qualitatively and quantitatively similar, so it would appear there was no induction of metabolic pathways by pre-dosing with kresoxim-methyl.

On the basis of these observations it is concluded that the phase I biotransformation of kresoxim-methyl is characterized by six metabolic reactions as shown in Figure 1: Cleavage of (1) the ester, (2) the oxime ether and (3) the benzyl ether bonds; (4) hydroxylation of the phenoxy ring *para* to the existing oxygen substituent, (5) oxidation of the aryl-methyl group to the benzyl alcohol and (6) its subsequent oxidation to the corresponding carboxylic acid. The (*E*)-(*Z*)-isomerization of the oxime ether group of the parent molecule is assumed to be a non-enzymatic reaction catalysed by light and/or acids. The combination of these reactions and the conjugation of the hydroxy compounds to form glucuronides or sulfates leads to the observed large number of metabolites.

Goats. In a study in 1991, either [^{13}C]- or [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity >98% and 94.6-97.6% respectively) was given orally by catheter to two goats (species not reported). Goat A was dosed with ^{14}C-labelled material at 7.1 ppm in the feed for 5 consecutive days. Goat B was dosed with a mixture of ^{13}C and ^{14}C-labelled material corresponding to an average of 450 ppm in the feed. During the dosing period blood, milk, urine and faeces were collected. Goats A and B were slaughtered 23 and 4 hours after the last dose respectively and liver, kidney, muscle, fat, bile and gastrointestinal tract were removed. Milk production appeared not to change significantly throughout the study. The total radioactivity was determined by LSC after combustion. The urine and milk samples were stored for 2 months and tissue samples for 1 month before analysis.

The distribution of radioactivity is shown in Table 2. Milk contained in total 0.031% (goat A) and 0.027% (goat B) of the total radioactivity administered. Goat A excreted 69.5% of the dose in the urine and 18.1% in the faeces, and goat B excreted 59.3% in the urine and 24.5% in the faeces. After slaughter the highest residue (mg/kg parent equivalent) was found in the kidneys and bile. In the low-dose goat A the residues in the milk, muscle and fat were below 0.01 mg/kg. The liver contained 0.038 mg/kg and kidneys 0.142 mg/kg.

Table 2. The distribution of radioactivity in goats dosed with [*phenyl*-^{14}C]kresoxim-methyl.

Sample	Goat A, 7.1 ppm feed		Goat B, 454 ppm feed	
	mg/kg as parent	% of TRR	mg/kg as parent	% of TRR
Milk, individual samples	0.001-0.003	0.001-0.005	0.068-0.316	0-0.004
Milk, total	0.025	0.031	2.74	0.027
Liver	0.04	0.06	6.81	0.07
Kidneys	0.15	0.03	14.04	0.02
Fat	0.001	0.01	0.33	<0.005
Muscle	0.001	0.01	0.22	0.01
Bile	0.49	0.03	146	0.03
Stomach lining	0.06	0.17	11.98	0.25
Stomach contents	0.18	1.09	95	8.90
Intestine lining	0.11	0.23	4.64	0.08
Intestine contents	1.13	3.10	48	1.56
Stomach/intestine cleaning water	0.03	0.09	6.84	0.44
Urine in bladder	2.15	0.91	482	0.26
Urine excreted Total (% of dose)	2.5-5.5	10.3-16.9 69.51	222-713	2.1-11.4 59.31
Faeces excreted Total (% of dose)	0.9-4.9	0.7-5.3 18.08	108-312	0.7-5.7 24.52
Blood	0.01	0.03	1.89	0.05
'Vomited' material (cud)[1]	0.03	<0.005	1.0	0.10
Tray water	0.0	<0.005	0.02	<0.005
Cage cleaning	0.11	1.08	17.63	1.47
TOTAL		94.48		97.10

[1]The report states that this material was collected from the mouth and oesophagus and is considered to be from regurgitation during normal digestion.

The identification of the radioactive compounds in various extracts from the high-dose goat is shown in Table 3. The radioactivity was measured by combustion and LSC. Urine samples were analysed directly by CI-MS, with preparative HPLC in some cases. Faeces samples were extracted with methanol followed by water. Pooled daily milk samples were extracted with methanol, centrifuged and filtered. Methanol was evaporated from the supernatant to leave the aqueous phase. Water and acetone were added and any precipitate was filtered and washed with acetone. The residues were redissolved in acetonitrile /water /formic acid and analysed by HPLC. All other samples were extracted with methanol followed by water, centrifuged and subjected to solvent partition (dichloromethane, hexane/acetonitrile and ethyl acetate separately or in combination). The extracts were further purified by silica and/or preparative HPLC where necessary. The residual precipitates were either incubated with pronase and extracted with HCl (liver) or ammonia (muscle and liver) and further purified by ion exchange or size exclusion chromatography, or were solubilized with Biolute S or toluene. Extracts were analysed by HPLC with a scintillator detector. Identification of compounds in extracts of milk and edible tissues was achieved by chromatographic comparison with extracts of urine.

The extraction of radioactivity by methanol and water ranged from 63% of the TRR in liver to 98% in kidney. The main metabolites were 490M1 (14% of the TRR in liver, 26% in muscle, present in milk), 490M2 (11% in liver, 34% in kidney), and 490M9 (10% in muscle, 63% in milk, present in fat). Minor metabolites identified were 490M6 in fat, liver and kidney, 490M18 in all tissues and 490M19 in muscle and liver. Other radioactive peaks characterized by HPLC accounted for 18% of the TRR in liver, 7% in kidney, 3% in fat, 16% in milk and 3% in muscle.

The three major metabolites were formed by cleavage of the methyl ester bond to yield the free acid 490M1, followed by side-chain hydroxylation to 490M2 and aromatic ring hydroxylation to 490M9. Minor metabolites were generated by cleavage of the oxime ether bond to 490M18, conjugation with glycine to 490M19, a combination of ring hydroxylation and oxime ether cleavage to 490M56, and by cleavage of the phenyl ether bond to 490M6. Ring hydroxylation without ester cleavage resulted in 490M15 which was found in the faeces only (Giese, 1992; Mayer, 1994).

Table 3. Compounds identified in goat tissues, milk and excreta after dosing with [*phenyl*-^{14}C]kresoxim-methyl at a level of 450 ppm in the feed. Levels expressed as mg/kg parent compound.

Sample	Extract	490-M6 mg/kg	490-M2 mg/kg	490-M9 mg/kg	490-M18 mg/kg	490-M19 mg/kg	490-M1 mg/kg	Polar mg/kg	Other extracted mg/kg	Total residual mg/kg
Urine[1]		2.1	31.7	27.7	2.1	0.3	31.5			
Faeces	methanol[2]	3.4	10.8	27.8	1.8	0.8	10.2	4.2	5.4	6.2
Milk	acetone		0.04	0.12			0.003		0.03	0.007
Muscle	EtAc[3]								0.02	
	DCM[4]								0.14	
	Hexane[5]							0.01	0.009	0.06
	ACN[3]		0.03	0.017	0.017	0.015	0.05			
	Residual			0.006			0.005		0.048	
Fat	ACN	0.03	0.08				0.08			0.06
	Hexane		0.006	0.001	0.002		0.004		0.012	
	Residual							0.033	0.04	
Liver	DCM		0.51	1.86			0.84	0.12		2.82
	EtAc		0.03	0.05	0.12					
	water	0.03	0.02	0.03	0.005	0.003	0.01	0.23		
	Residual	0.22	0.13	0.007	0.094	0.02	0.09	1.51		0.75
Kidney	methanol	0.44	4.56	4.04			2.92		0.59	0.28
	DCM								10.05	
	EtAc								1.27	
	water	0.04	0.05				0.03	0.20	0.22	
	Residual		0.007		0.03		0.02	0.19		0.034

[1]Also contained 0.2 mg/kg 490M56
[2]Also contained 12.1 mg/kg 490M15 and 1.3 mg/kg parent
[3]ethyl acetate
[4]dichloromethane
[5]acetonitrile
[6]Also contained 0.024 mg/kg parent

The metabolic pathways of kresoxim-methyl in goats are shown at the end of this monograph in Figure 2.

Hens. In a study carried out in 1992, [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity >99%) was fed to 5 hens (ISA strain) in six daily doses equivalent to 10 ppm in the feed and to 10 hens at 180 ppm in the feed. The hens were dosed in the morning before feeding but after collecting eggs and excreta. About 3 and 23 h after the final dose for the high- and low-dose groups respectively, samples were taken and stored up to 1 month before analysis. Sub-samples were combusted and analysed by LSC.

From the low dose 88.2% of the total administered radioactivity was recovered, 82.6% of which was excreted, and from the high dose 87% was recovered with 71.55% excreted. About 23 h after the final dose of 10 ppm total radioactive residues of 0.009, 0.065 and 0.082 mg/kg kresoxim-methyl equivalents were found in the skin, kidneys and liver respectively. The radioactivity in the eggs was 0.012 and 0.215 mg/kg and did not appear to reach a plateau during the study. The distribution of radioactivity is shown in Table 4.

Table 4. The distribution of radioactivity in hens dosed with [*phenyl*-^{14}C]kresoxim-methyl.

Sample	Time, h	^{14}C from 10 ppm dose		^{14}C from 180 ppm dose	
		mg/kg as kresoxim-methyl	%dose	mg/kg as kresoxim-methyl	% dose
Eggs	0-24	Nd	nd	0.100	0.02
	24-48	0.009	0.03	0.086	<0.01
	48-72	0.007	0.02	0.131	0.02
	72-96	0.009	0.02	0.164	0.02
	96-120	0.011	0.03	0.215	0.04
	120-123	-	-	-	-
	120-143	0.012	0.04	-	-
Eggs pooled[1]		0.009		0.155	
Skin	At slaughter	0.01	<0.01	0.68	<0.01
Kidney		0.06	0.01	6.36	0.04
Liver		0.08	0.02	6.98	0.14
Fat		<0.005	-	0.76	<0.01
Muscle		0.002	-	0.20	<0.01
GI tract			0.59		10.77
Balance					
Tissues			0.6		10.9
Excreta			82.6		71.5
Excreta pooled[1]		5.48		115.16	
Cage washings			4.9		5.2

[1] Used for characterization

Samples were extracted with methanol and water. Bound radioactivity was released by pronase treatment. The radioactivity was characterized by liquid/liquid partition and analysed by HPLC. The compounds in the excreta, eggs and edible tissues were isolated and identified as far as possible.

Methanol extracted 57-59% of the TRR in the skin and liver derived from the low dose, and a further 6%-9% was extracted by water. After 180 mg/kg dose, methanol extracted >75% of the TRR in the tissues and eggs from the high dose and a further 2-6% was extracted from the tissues by water. Pronase liberated 60-90% of the unextracted radioactivity from the eggs of the high-dose group and 21% from the tissues. Samples from the high-dose group were used for characterization and identification.

The main metabolites identified in the liver were the isomeric glucuronides 490M63 and 490M67 (9.2% of the TRR), 490M28 (13.7%) and 490M9 (20.1%). The main metabolites in muscle were the sulfates 490M51 and 490M66 (20.0% of the TRR) and 490M60 plus 490M20 (8.6%), in skin the parent (10.7% of the TRR) and 490M58 (10.4%), in fat the parent (41.2% of the TRR) and the non-polar 490M15 (16.6%) and 490M48 (7.7%), and in eggs the sulfates 490M51 and 490M66 (15.7% of the TRR), the glucuronide 490M31 (9.7%), 490M48 (11.4%) and the unchanged parent (8.3%). The results are shown in Table 5, and the metabolic pathways in poultry proposed by the manufacturer in Figure 3 (Burke, 1994; Grosshans, 1994c).

Table 5. Metabolites detected in hens dosed with [*phenyl*-^{14}C]kresoxim-methyl at a level equivalent to 180 ppm in the feed in six daily doses.

Sample	490-M1	490-M5	490-M6	490-M9	490-M8/11	490-M14	490-M15	490-M16	490-M20/60	490-M24	490-M25/26	490-M28	490-M31	490-M33	490-M46	490-M47	490-M48	490-M50/52
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Liver		0.30		1.35		0.18	0.18	0.16				0.92		0.20		0.21		
Muscle		0.006			0.002		0.004		0.015		0.004	0.003			0.007	0.003	0.003	0.008
Skin		0.02	0.015	0.03			0.03			0.01					0.04	0.01	0.04	
Fat							0.12			0.01					0.03		0.06	0.02
Eggs	0.005										0.004		0.012	0.005 (+M39)		0.003	0.014	0.006

Sample	490-M51/66	490-M55/65	490-M56	490-M57	490-M58	490-M59	490-M63/67	490-M64	Parent	Characterized (pronase incubated)	Characterized (polar)	Characterized (solvent)	Total Residual
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Liver					0.08	0.05	0.62			1.08	0.23	1.09	0.23
Muscle	0.03			0.003	0.01	0.004			0.005	0.03	0.01	0.019	0.007
Skin	0.03	0.0017		0.01	0.08	0.06			0.08	0.03	0.06	0.12	0.04
Fat	0.01		0.02		0.01	0.006			0.31	0.03	0.02	0.04	0.01
Eggs	0.02						0.003	0.004	0.01	0.03			0.008

Plant metabolism

Apples. In German studies carried out in 1991-93, [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity 96-98%), formulated as BAS 490 00F, was applied to 5 year old apple trees (variety Mutsu) in three different treatment patterns as follows (Hofmann, 1992a-c; Grosshans, 1994a).

(1) "Leaf application": 6 spray applications of 400 g ai/ha, PHI 14 days. The manufacturer stated that apples were present at the later leaf applications and were not protected from the spray.

(2) "Early application": 2 applications of 400 g ai/ha at the beginning of the vegetation period, PHI 149 days.

(3) "Fruit treatment": 2 applications of 800 g ai/ha to apples only, PHI 14 days.

Samples were stored frozen at -18°C for 1 month before analysis. Peel and pulp were extracted separately with methanol. The extractable radioactivity was characterized by solvent partition, TLC and HPLC. The compounds were isolated by HPLC and their structures elucidated by MS and/or chromatographic comparison with reference compounds. Acceptable chromatograms and spectra were submitted. To characterize the unextracted radioactivity, the unextractable radioactive residue was treated with dilute aqueous ammonia and lignin and cellulose fractions were separated. Individual residues in the peel and pulp were recalculated on a whole-apple basis. Extraction and characterization schemes are shown in Figures 4 and 5.

The calculated total radioactive residues in the whole apples (Table 6) were 0.36 mg/kg as kresoxim-methyl from the leaf application, 0.04 mg/kg from the early application and 0.84 mg/kg from the fruit treatment. The radioactivity remained mainly on the peel (89% to 98% of the TRR) with very low translocation to the pulp. Of the calculated TRR in the whole fruits, 94% to 98% could be extracted with methanol (Table 7). After liquid/liquid partition, the extractable radioactivity was predominantly found in the ethyl acetate phase (80% to 94% of the TRR). The unextractable ^{14}C in the peel after leaf application amounted to 4.5% of the TRR and an additional 2.2% could be released with aqueous ammonia. Part of the unextractable radioactivity was associated with lignin (3.1% of the TRR).

Table 6. The distribution of radioactivity in apples treated with phenyl-labelled [^{14}C]kresoxim-methyl. Measured and calculated TRR, parent equivalents.

Application	Application rate, kg ai/ha	PHI, days	Sample	Measured TRR[1], mg/kg	Calculated TRR[2], mg/kg
Leaf	6 x 0.4	14	fruit pulp	0.06	
			core	0.05	
			peel	1.39	2.16
			leaves	18.52	
			branches	1.73	
			total pulp (flesh + core)		0.03
			whole apple		0.36
Early	2 x 0.4	149	fruit pulp	0.01	
			core	0.04	
			peel	0.05	0.29
			leaves	1.03	
			branches	0.41	
			total pulp (flesh + core)		0.005
			whole apple		0.04
Fruit treatment	2 x 0.8	14	fruit pulp	0.02	
			core	0.02	
			peel	5.68	6.30
			leaves	0.23	
			total pulp (flesh + core)		0.02
			whole apple		0.84

[1]Total measured by combustion [2]Sum of extractable and unextractable radioactivity

Table 7. Fractionation of ^{14}C in whole apples after treatment with phenyl-labelled [^{14}C]kresoxim-methyl.

Fraction	Leaf application		Early application		Fruit treatment	
	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
TRR	0.359	100.0	0.041	100.0	0.837	100.0
MeOH extract	0.341	94.9	0.038	94.3	0.822	98.2
Unextractable residue	0.018	5.1	0.002	5.7	0.015	1.8
MeOH extract						
Ethyl acetate phase	0.317	88.1	0.033	80.0	0.784	93.6
Aqueous phase	0.025	6.9	0.005	12.8	0.027	3.2

The extracts from all three trials showed similar patterns of compounds (Table 8) and contained predominantly unchanged kresoxim-methyl (74-93% of the TRR). In extracts of the leaf application apples kresoxim-methyl (78.3% of the TRR), its isomer 490M0 (3.3%), the acid 490M1 (3.0%) and conjugates of 490M2 (1.8%) and 490M9 (2.1%) were all identified by MS.

Overall about 94% of the extractable residues from the leaf application (88.4% of the TRR) could be identified. The remaining extractable radioactivity, which was spread over several peaks, was too little for identification (0.0015 to 0.0045 mg/kg) and was characterized by chromatographic properties.

Table 8. Characterization of extractable residues in apples, calculated on a whole-apple basis.

Fraction	Metabolite identity	Leaf application		Early application		Fruit treatment	
		mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
Al	{	0.0045	1.2	0.0011	2.7		
A2	{only	0.0015	0.4	0.0002	0.5	0.0002	<0.1
A3	{character-	-	-	-	-	0.0001	<0.1
A4	{ized as	-	-	-	-	0.0003	<0.1
A5	{polar	0.0044	1.2	0.0016	4.0	0.0008	0.1
A6	{	0.0026	0.7	0.0011	2.6	-	-
A7	conjugates	0.0188	5.2	0.0025	6.1	0.0088	1.1
A7 after enzyme treatment:							
Uncleaved A7	conjugates	0.0014	0.4				
Aglycone A7/1	490-M2	0.0065	1.8				
Aglycone A7/2	490-M9	0.0076	2.1				
Aglycone A7/3	isomer of 490-M9	0.0033	0.9				
A8	490-Ml	0.0106	3:0	0.0008	1.9	0.0118	1.4
A9*	mw 313	0.0057	1.6	0.0006	1.4	0.0032	0.4
A10	Parent	0.2810	78.3	0.0301	74.0	0.7788	93.0
All	(*Z*)-isomer	0.0117	3.3	0.0005	1.2	0.0182	2.2

* the manufacturer stated that A9 was also found in the wheat metabolism study "but the position of the OH-group group is unclear."

Wheat. In German studies carried out in 1990-91, [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity 97-99.7%, formulated as BAS 490 00F), was applied twice to wheat (variety Star) at growth stages 29 and 52, at either 0.25 or 1.25 kg ai/ha. Samples were stored at -20°C for 1-4 months before analysis. Milled samples were combusted and analysed by LSC to determine the total radioactive residue (TRR).

Extraction and characterization schemes are shown in Figures 7-9. The samples were first extracted with methanol and subsequently with dilute aqueous ammonia. The extractable radioactivity was characterized by solvent partition, solid-phase extraction, TLC and HPLC. The compounds were isolated by HPLC and their structures elucidated by MS and/or chromatographic comparison with reference compounds. The unextractable radioactive residue was treated with enzymes, acids and bases, and fractionations of lignins, cellulose, protein, and starch was followed by further subdivision of the starch fraction.

The distribution of the total radioactive residues is shown in Table 9. After treatment at 0.25 kg/ha 30-99% of the TRR in the separate parts of the plants could be extracted with methanol, with the lowest proportion extracted from mature grain (Table 10). The subsequent extraction with dilute aqueous ammonia released an additional 31% of the TRR from the grain and 16% from the straw. Most of radioactivity in the ammonia extracts was recovered by chromatography on phenyl SPE cartridges. After partition between ethyl acetate and water most of the radioactivity extracted with methanol was found in the ethyl acetate phase (85-99% of the TRR). The unextractable activity was equivalent to 0.27 mg/kg in the straw (2.9% of the TRR) and 0.025 mg/kg in the grain (38.8%).

Table 9. The distribution of radioactivity in wheat treated with phenyl-labelled [^{14}C]kresoxim-methyl. Measured and calculated TRR, parent equivalents.

Application rate, kg ai/ha	PHI, days after 2nd treatment (days after 1st treatment)	Sample	Measured TRR[1], mg/kg	Calculated TRR[2], mg/kg
0.25	-56 (0)	plant	8.06	12.01
	-1 (55)	plant	2.10	1.31
0.25	0 (56)	plant	7.72	5.26
	64 (120)	grain	0.06	0.06
		glumes	1.87	2.67
		straw	12.92	9.21
		roots	1.14	
1.25	-56 (0)	plant	53.00	75.48
	-1 (55)	plant	6.07	11.84
1.25	0 (56)	plant	53.78	29.69
	63 (119)	grain	0.28	0.26
		glumes	10.82	18.43
		straw	44.8	61.39
		roots	3.17	

[1]Total measured by combustion
[2]Sum of extractable and unextractable radioactivity (MeOH)

Table 10. Extraction of radioactivity from wheat with methanol and ammonia (cf. Figure 6).

Sample	PHI days after 2nd treatment	Primary extraction		Partition of MeOH extract				Secondary extraction				SPE separation of ammonia extract			
		Methanol extract (E1)		Ethyl acetate extract (EE)		Aqueous phase (EW)		Ammonia extract (E2)		Unextractable residue (R2)		Application eluate (EA)		Methanol eluate (EM)	
		mg/kg	% of TRR	mg/kg	% of E1	mg/kg	% of E1	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
0.25 kg/ha															
Forage	-56	11.96	99.6	9.64	98.8	0.11	1.2	0.02	0.2	0.03	0.3	0.001	<0.1	0.02	0.2
Forage	-1	1.22	93.2	0.81	88.7	0.10	11.3	0.06	4.8	0.05	3.7	0.002	0.1	0.05	4.2
Forage	0	5.13	97.5	3.70	85.9	0.61	14.1	0.09	1.7	0.06	1.2	0.001	<0.1	0.08	1.5
Glumes	64	1.44	54.1	1.09	86.6	0.17	13.4	0.95	35.6	0.27	10.1	0.075	2.8	0.88	33.1
Straw	64	7.55	82.0	6.73	92.3	0.56	7.7	1.49	16.1	0.27	2.9	0.049	0.5	1.43	15.6
Grain	64	0.02	30.4	0.02	85.1	0.003	14.9	0.02	31.1	0.02	38.8	0.005	7.8	0.014	21.7
1.25 kg/ha															
Forage	-56	75.26	99.7	66.83	99.8	0.14	0.2	0.10	0.1	0.10	0.1	0.002	<0.1	0.09	0.1
Forage	-1	11.40	96.3	9.86	96.4	0.37	3.5	0.30	2.5	0.19	1.6	0.007	0.1	0.29	2.4
Forage	0	29.24	98.5	24.09	98.2	0.43	1.8	0.32	1.1	0.17	0.6	0.007	<0.1	0.30	1.0
Glumes	63	13.77	74.7	10.74	92.6	0.85	7.4	3.36	18.3	1.03	5.6	0.170	0.9	3.11	16.9
Straw	63	53.70	87.5	57.75	96.3	2.23	3.7	6.23	10.1	1.44	2.3	0.203	0.3	6.00	9.7
Grain	63	0.13	48.1	0.12	91.6	0.011	8.4	0.07	26.7	0.07	28.3	0.020	7.6	0.04	16.4

Table 11. Characterization of extractable residues in wheat treated with 0.25 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl.

Frac-tion	Identity	Forage, day of 1st treatment		Forage, 55 days after 1st treatment		Forage, day of 2nd treatment		Straw, 64 days after 2nd treatment		Grain, 64 days after 2nd treatment	
		mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
1		-		-		-		0.017	0.2	-	
2		-		-				0.011	0.1	0.001	1.5
3		-		-				0.027	0.3	0.0036	5.6
4		-		-				0.101	1.1	0.0025	3.9
5		-		0.015	1.1			0.026	0.3	0.0009	1.4
6		-		-		0.007	0.1	0.134	1.5	0.0027	4.2
7	conj[1]	-		-		0.007	0.1	0.148	1.6	-	
8	conj[1]	-		0.107	8.2	0.072	1.4	0.451	4.9	0.0020	3.1
9	conj[1]	-		0.029	2.2	0.019	0.4	0.875	9.5	0.0008	1.2
10	conj[1]	-		0.033	2.5	-		0.174	1.9	0.0002	0.3
11		-		-		-		-		0.0007	1.1
12		-		0.016	1.2	-		0.096	1.0	0.0015	2.3
13		-		-				-			
14		-		-				0.052	0.6	-	
15		-		-				0.045	0.5	-	
16		-		-				0.014	0.1	0.0021	3.3
17		-		-				0.011	0.1	-	
18		-		-				0.022	0.2	-	
19		-		-				0.033	0.4	-	
20	490M1	0.247	2.1	0.039	3.0	0.063	1.2	0.126	1.4	-	
21	490M17	-		0.037	2.8	0.031	0.6	0.329	3.6	0.0005	0.8
22		-		-				0.011	0.1	-	
23	Parent	11.506	95.8	0.980	74.8	4.889	92.8	5.923	64.3	0.0111	17.2
24		0.120	1.0	0.005	0.4	0.031	0.6	-		-	
25	490M0	0.108	0.9	0.016	1.2	0.087	1.7	0.359	3.9	0.0002	0.3
26		-		-		-		-		0.0010	1.5
27								-		0.0006	1.0
Total			99.8		97.4		98.9		97.6		48.7

[1]Conjugates include 490M2 and 490M9 (see text for levels)

The extracts of corresponding samples from the two treatment groups showed qualitatively similar patterns of compounds (see also Table 12). After treatment at 0.25 kg/ha the main radioactive residue was the unchanged parent at levels of 5.9 mg/kg in mature straw and 0.98 mg/kg in forage 1 day before the second treatment. The following compounds were also identified in the straw and -1 day forage: the (*Z*)-isomer 490M0, the acid 490M1, and conjugates of 490M2 and 490M9. The mature grain contained 0.011 mg/kg (17.2% of the TRR) of the parent and a variety of metabolites constituting 0.3-5.6% of the TRR. Enzymatic cleavage of the conjugate fractions 7-10 yielded the following respective levels of the acids 490M2 and 490M9 from the lower rate: 0.03 and 0.103 mg/kg in 55-day forage, 0.39 and 1.04 mg/kg in mature straw from the 0.25 kg/ha treatment; 0.019 and 0.003 mg/kg in mature grain from the 1.25 kg/ha treatment. Samples from the higher rate treatment contained higher relative and absolute amounts of the parent compound (Table 12).

Table 12. Characterization of extractable residues in wheat treated with 1.25 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl.

Fraction	Identity	Forage, day of 1st treatment		Forage, 55 days after 1st treatment		Forage, day of 2nd treatment		Straw, 64 days after 2nd treatment		Grain, 64 days after 2nd treatment	
		mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
1		-		-		-		-		-	
2		-		-		-		-		-	
3		-		-		-		-		0.0115	4.3
4		-		-		-		0.107	0.2	0.0076	2.9
5		-		0.011	0.1	-		0.186	0.3	-	
6		-		0.025	0.2	-		0.210	0.3	0.0023	0.9
7	conj	-		0.033	0.3	0.033	0.1	0.360	0.6	0.0010	0.4
8	conj	-		0.419	3.5	0.232	0.8	2.083	3.4	-	
9	conj	-		0.081	0.7	0.072	0.2	2.053	3.3	0.0218	8.3
10	conj	-		-		-		0.215	0.3	-	
11		-		-		-		-		-	
12		-		-		-		-		0.0032	1.2
13		-		-		-		-		0.0009	0.4
14		-		-		-		-		-	
15		-		-		-		-		-	
16		0.009	<0.1	-		-		-		-	
17		-		-		-		-		-	
18		0.005	<0.1	-		-		-		-	
19		-		-		-		-		-	
20	490M1	0.528	0.7	0.389	3.3	0.369	1.3	0.743	1.2	0.0009	0.4
21	490M17	-		0.308	2.6	0.263	0.9	2.202	3.6	0.0031	1.2
22		-		-		-		-		-	
23	Parent	73.30	97.1	10.22	86.4	27.92	94.0	50.73	82.6	0.1034	39.5
24		0.828	1.1	-		0.292	1.0	-		-	
25	490M0	0.677	0.9	0.194	1.6	0.351	1.2	0.805	1.3	-	
26		-		-		-		-		-	
W27		-		-		-		-		-	
Total			99.8		98.7		99.5		97.1		59.5

Fractionation of the unextractable radioactivity from the 0.25 kg/ha treatment (R2 residue, Table 13) in straw indicated that 2.1% of the TRR was associated with lignin and only 0.2% was incorporated into cellulose. In the grain 31.7% of the TRR was associated with or incorporated into starch, of which 9.4% of the TRR could be converted into $^{14}CO_2$ by fermentation of the glucose with yeast. The metabolic incorporation of ^{14}C in the glucose was thought to be caused by mineralization of the parent or its metabolites in soil and assimilation of the $^{14}CO_2$ by the plants at late growth stages. Lignin contained 7.9% of the TRR, protein 6.3% and cellulose 1.6%.

Table 13. Fractionation of unextractable ^{14}C in wheat treated with 0.25 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl. See Figures 6-8 for details of fractionation schemes.

Fraction		Straw		Grain	
		mg/kg	% of TRR	mg/kg	% of TRR
Total radioactive residue El + Rl		9.52	100.0	0.07	100.0
MeOH extract	El	7.69	80.8	0.02	26.8
Residue	R1	1.83	19.2	0.05	73.2
Ammonia extract	E2	1.62	17.0	0.02	33.8
Residue	R2	0.32	3.3		
HCl extract of R2	E3	0.02	0.2		
EDTA extract of residue R3	E4	0.01	0.1		

Fraction		Straw		Grain	
		mg/kg	% of TRR	mg/kg	% of TRR
Enzyme extract of R4	E5	0.07	0.8	0.004	5.6
1M HC1-hydrolysis of R5	E6	0.03	0.3		
6M HCl hydrolysis of R5	E7	0.01	0.1		
NaOH hydrolysis of R7	E8	0.09	1.0		
Total dietary fibre extract of R5	E9	0.03	0.3	0.02	25.4
Lignin, cellulose, protein and starch fractionation					
TRR		9.213.	100.0	0.06	100.0
MeOH extract	El	7.551	82.0	0.02	31.7
Extraction residue	R1	1.662	18.0	0.04	68.3
Ammonia extract	E2	1.486	16.1	0.02	31.7
Extraction residue	R2	0.269	2.9	0.02	39.7
Lignin	R11/15	0.198	2.1	0.005	7.9
Cellulose	R12/16	0.020	0.2	0.001	1.6
Protein	E2B			0.004	6.3
Starch	E13B			0.02	31.7
Fermentation of glucose from starch					
Starch fraction				0.02	32.2
Ethylene glycol				0.0003	0.5
14[$C0_2$] traps				0.006	9.4
Yeast supernatant				0.003	4.6
Yeast				0.003	5.2

Table 14. Characterization of unextractable residues in wheat treated with 1.25 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl.

Fraction		Straw		Grain	
		mg/kg	% of TRR	mg/kg	% of TRR
Lignin, cellulose, protein and starch fractionation					
TRR		61.39	100.0	0.30	100.0
MeOH extract	El	53.70	87.5	0.15	50.0
Residue	R1	7.69	12.5	0.15	50.0
Ammonia extract of R1	E2	6.23	10.1	0.07	23.5
Residue	R2	1.44	2.3	0.07	24.8
Lignin	R11/15	0.95	1.6	0.01	4.4
Cellulose	R12/16	0.07	0.1	0.05	18.5
Protein	E2B			0.008	2.7
Starch	E13B			0.002	0.7

Overall, >95% of the extractable residue could be identified or characterized in the forage and straw, and about 50% in the grain from the 0.25 kg/ha treatment. An additional 47.5% of TRR in grains was incorporated into or associated with natural products. The remaining extractable radioactivity, which was split into many peaks was too low for identification and was characterized only by chromatographic properties (Hofmann, 1991a,b; Grosshans, 1994b).

Grapes. In US studies carried out in 1991 (Nelsen *et al.*, 1995) phenoxy- and phenyl-labelled kresoxim-methyl (radiochemical purities 98.8 and 99.2%), formulated as BAS 490 F, were applied five times to mature Muscatine grape vines (variety Carlos) at rates of 0.5 kg/ha per application. Grapes were harvested 14 days after the final treatment. Samples were stored at <0°C for 1-7 months before analysis. Whole grapes were rinsed with methanol and then homogenized. The total radioactive residue was calculated at the sum of the ^{14}C in the methanol rinse and in the homogenized washed grapes, determined by combustion.

The extraction scheme is given in Figure 9. Homogenized grapes were extracted with either methanol or acetone/water. Residues in homogenates, grape rinses and unwashed samples were separated and identified by SPE, TLC, HPLC with UV and radiometric detection, GC-MS, and enzyme hydrolysis (β-glucosidase and hesperidinase). Confirmation of identifications by HPLC and TLC was by co-chromatography.

No significant differences were observed between the results for phenoxy- and phenyl-labelled kresoxim-methyl. About 40% of the TRR (1.6-1.91 mg/kg) was removed as surface residue by the methanol rinse (Table 15). There were no significant differences between the proportions extracted by methanol and acetone/water, indicating that cleavage of the molecule was unlikely. Less than 10% of the TRR remained unextracted. The total recovery of residues in the extracts used for characterization was about 90% of the TRR and the unextractable residue contained about 5%.

Table 15. Radioactive residues in grapes treated with phenoxy- and phenyl-labelled [^{14}C]kresoxim-methyl (mg/kg as parent equivalents based on weight of unwashed grapes).

Sample	Phenoxy label		Phenyl label	
	% of TRR	mg/kg	% of TRR	mg/kg
Methanol rinse	34.7, 40	1.32, 1.60	32.1, 40.5	1.13, 1.91
Homogenized rinsed grapes	65.3, 60	2.51, 2.40	67.9, 59.5	2.49, 2.81
TRR	100	3.83, 4.00	100	3.62, 4.72

Table 16. Fractionation of radioactive residues in grapes treated with phenoxy- and phenyl-labelled [^{14}C]kresoxim-methyl.

Sample	Phenoxy label		Phenyl label	
	Acetone/water	Methanol, mean %	Acetone/water	Methanol, mean %
TRR, mg/kg	3.83	3.94	3.62	4.35
% in rinse	34.7	38.2	32.1	37.7
% in homogenized grapes	52.4	50.7	56.7	52.0
% of TRR extracted	87.1	88.9	88.8	89.7
% unextractable	4.3	6.3	4.5	5.8
% of TRR recovered	91.4	95.1	93.3	95.5

Parent kresoxim-methyl was the main residue, 55.38% of the phenoxy TRR and 57.35% of the phenyl (Table 17). The alcohol-acid 490M2 was also prominent at 13.76% and 8.86% of the TRR (sum of free and conjugated). After β-glucosidation, levels of the phenolic acid 490M9 were 5.75% and 4.38% of the TRR. Small amounts of free 490M9 were originally present, at 1.25% and 1.4% of the TRR. The enzymatic conversion of conjugates to aglycones by ß-glucosidase was 85-88%. Approximately 3.5% and 5% of the TRR remained unidentified as polar or conjugated metabolites. A mixture of over 20 unhydrolysed conjugates and other polar materials were individually below 0.05 mg/kg. Those from the phenyl label were unaffected by the additional hesperidinase hydrolysis.

There were no major qualitative differences between the two labels.

Table 17. Characterization of radioactive residues in grapes treated with phenoxy- and phenyl-labelled [^{14}C]kresoxim-methyl.

Identity	phenoxy label		phenyl label	
	%TRR	mg/kg	%TRR	mg/kg
Unknown polar			0	0
Unknown polar			0.59	0.027
Unknown polar			1.40	0.066
Unknown polar	0.56	0.023	0.28	0.013

Identity	phenoxy label		phenyl label	
	%TRR	mg/kg	%TRR	mg/kg
Conjugate	3.18	0.127	2.78	0.130
490m2[1]	13.76	0.550	8.86	0.418
490m9[1]	5.75	0.230	4.38	0.206
490m54[1]	2.07	0.083	1.37	0.064
490M1[1] (acid)	0.48	0.019	0.13	0.006
Unknown	0.72	0.029	0.83	0.039
Unknown	1.03	0.041	1.11	0.052
Parent	55.38	2.215	57.35	2.707
Z-isomer	3.49	0.139	3.75	0.177
Unknown	0.58	0.023	0.42	0.019
(Background	3.70	0.148	2.98	0.140)
Total	90.70	3.63	86.23	4.07

[1]Sum of free and conjugated

Rotational crops. In trials in Germany during 1992/93 (Hoffmann, 1993a,b; Grosshans, 1994d) [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity 97-99%), was applied to bare soils at 0.3 kg/ha. After ageing for 30 days, the soils were diluted with untreated soil in the ratio of 1:9 in order to simulate ploughing. 30 days later spring wheat (Star, Ralle varieties), green beans (Maxi, Marona), carrots (Nantaise Frühbund) and lettuce (Ultra, Ovation) were sown or planted. Crops were harvested at interim stages to identify metabolites (as the final harvest samples would contain low levels of residues) and at maturity. Samples were stored at -18°C before analysis by combustion and LSC. The samples were apparently not washed before analysis.

The samples were extracted with methanol and then with dilute aqueous ammonia. The extractable radioactivity was separated by liquid/liquid partition (cyclohexane/ethyl acetate/water) and HPLC. Conjugates were cleaved by enzymes (hesperidinase and β-glucoside) and the structures of isolated metabolites and aglycones elucidated by chromatographic comparison with reference compounds.

The highest total radioactive residues found in the crops at harvest in mg/kg were 0.15 in wheat straw, 0.007 in wheat grain, 0.21 in bean forage, 0.009 in green beans, 0.006 in carrot roots, 0.047 in carrot foliage and 0.01 in lettuce heads (Table18). Methanol extracted 57 to 77% of the TRR and the subsequent dilute aqueous ammonia extraction released additional 20% to 34%. The total extraction was >85% of the TRR.

Table 18. The distribution of radioactivity in rotational crops after treatment of soil with 0.3 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl. (Measured and calculated TRR, parent equivalents).

Sample	Type	Days after sowing or planting	TRR[1], in crops, mg/kg equiv	TRR[2], in crops, mg/kg equiv	TRR (column 4) as % of initial radioactivity in soil (mean 0.93 mg/kg)
Soil	initial		0.918, 0.83, 1.05		
Soil	after 30 days ageing		0.05, 0.066, 0.06		6.3
Wheat forage	interim	42, 61	0.067, 0.232	0.05	
Wheat straw	final harvest	124, 125	0.119, 0.068	0.077-0.146	10.0
Wheat grain	final harvest	124, 125	0.006, 0.007		0.7
Soil	final harvest	124, 125	0.050, 0.029		4.2
Green beans (pods)	interim	26, 56, 75	0.36, 0.004, 0.004	0.057-0.195	
Green beans (beans plus pods)	final harvest	77	0.009		1.0

Sample	Type	Days after sowing or planting	TRR[1], in crops, mg/kg equiv	TRR[2], in crops, mg/kg equiv	TRR (column 4) as % of initial radioactivity in soil (mean 0.93 mg/kg)
Bean forage	final harvest	77	0.21		22.6
Soil	final harvest	77	0.042		4.5
Carrots (plant)	interim	50	0.025	0.026	
Carrots (foliage)	interim	52, 90	0.061, 0.010		
Carrots (roots)	interim	52, 89, 90	0.052, 0.005, 0.003		
Carrots (foliage)	final harvest	111	0.047	0.035	5.0
Carrots (roots)	final harvest	111	0.006		0.6
Soil	final harvest	111	0.036		3.9
Lettuce (leaves/head)	interim	26, 30, 49, 56	0.053, 0.051, 0.02, 0.028	0.03-0.032	
Lettuce (head)	final harvest	66	0.010		1.0
Lettuce (roots)	final harvest	66	0.231		24.8
Soil	final harvest	66	0.032		3.4

[1]Measured by combustion
[2]Sum of extractable and unextractable radioactivity

In extracts of bean forage, carrot forage and lettuce conjugates were the main radioactive residues, accounting for about 30-40% of the TRR. Enzymatic treatments of the extracts and/or isolated conjugate fractions produced mainly the aglycones 490M2 and 490M9. The rest of the extractable radioactivity in these samples gave rise to several radioactive peaks representing up to 0.043 mg/kg (22% of the TRR). Mature lettuce also contained 9.4% of the TRR (0.003 mg/kg) as the unchanged parent compound and carrot forage 12.7% (0.005 mg/kg) as 490M1.

Wheat straw contained small amounts of the free hydroxy metabolites 490M2 and 490M9 (together about 10% of the TRR). Enzyme treatment produced 4.8% of the TRR as 490M2 and 10.4% as 490M9. The parent compound was detected in straw at 1.5% of the TRR (0.001 mg/kg). The results are shown in Table 19.

The results shown in Table 18 suggest that there is a slight uptake from soil into the crops, particularly wheat straw, bean forage and lettuce roots. Wheat straw and lettuce heads contained the parent compound at 0.15% and 0.09% of the initial radioactivity in the soil (Hofmann, 1993a,b; Grosshans, 1994d).

Table 19. Characterization of radioactivity in rotational crops after treatment of soil with 0.3 kg/ha phenyl-labelled [^{14}C]kresoxim-methyl. Where two figures are shown they are the results of separate uptake studies.

HPLC Peak	Identity	Wheat straw		Wheat forage		Bean forage		Carrot forage		Lettuce	
		mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
1				0.003	6.0	0.002, 0.001	0.9, 1.4			0.003	8.9
2		0.01	7.6					0.0015	5.7		
3		0.001	0.9							0.0005	1.8
4		0.001	1.2			0.001	1.3	0.003	8.6		
5				0.009	17.1	0.043, 0.008	21.9, 13.4	0.002	6.9	0.001, 0.0006	4.2, 1.9
6		0.006	7.8								
7		0.001	1.1	0.003	6.3						

HPLC Peak	Identity	Wheat straw		Wheat forage		Bean forage		Carrot forage		Lettuce	
		mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR
8		0.021	16.0			0.017, 0.001	8.5, 2.4				
9		0.017, 0.004	13.2, 4.8	0.005	10.9						
10	unknown	0.034, 0.01	26.5, 12.5	0.017	33.5	0.008, 0.004	4.2, 6.8	0.0007, 0.001	2.0, 3.2		
11		0.003	3.8								
12	conjugates	0.015, 0.004	11.6, 5.2								
13	conjugates	0.003	3.7	0.002	4.5	0.071, 0.018	36.7, 30.9	0.015, 0.004	43.6, 13.9	0.012, 0.015	41.5, 50.8
14		0.001	1.8			0.007, 0.008	3.4, 14.3	0.002, 0.004	5.7, 14.5	0.004	12.7
15						0.015, 0.002	7.7, 3.6				
16											
17								0.0003	1.1		
18	490M2	0.011, 0.001	8.5, 1.6								
19	490M9	0.0014	1.8							0.0006	2.1
20											
21		0.0008	1.1					0.0002	0.8		
22	409M1			0.001	1.5			0.004	12.7		
23		0.001	1.4								
24	Parent	0.001	1.5							0.003	9.4
25	(*Z*)-isomer										

Metabolic pathways in plants

The pathways proposed by the manufacturer for the metabolism of kresoxim-methyl in apples, wheat and following crops and in grapes are shown in Figures 10 and 11 respectively.

The manufacturer concludes that conjugates of 490M-2 and 490M-9 are incorporated into the starch and protein of wheat grain and the lignin of straw, and are further incorporated as bound residues in following crops.

Summary of metabolism

A total of 53 metabolites or degradation products of kresoxim-methyl have been identified in studies with rats, hens, goats, apples, wheat and grapes. A number of these have been identified in domestic animals and crop plants but not in rats: 16 in hens, 3 in goats and 1 in grapes.

Of these, only two are likely to occur in food commodities at significant levels (>0.05 mg/kg). These are 490M18, which was found at significant levels in several tissues in goats, and 490M54 which was found at significant levels in grapes. 490M18 is a postulated intermediate in rats.

The code names and chemical structures of the metabolites and degradation products are given in Figure 12 (at end of evaluation).

Environmental fate in soil and water/sediment systems

All the studies were carried out according to national or international guidelines and GLP.

Laboratory soil degradation studies

(a) In a study according to BBA guidelines Limbergerhof sandy loam soil was treated with [*phenoxy*-^{14}C]kresoxim-methyl (radiochemical purity (*Z*)- + (*E*)-isomers 100%, 0.5 mg/kg) and incubated at 40% maximum water holding capacity in the dark at 20°C. Volatile compounds were trapped in ethylene glycol, 0.5M H_2SO_4 and 0.5M NaOH. Soil samples were taken at nine intervals during 183 days and extracted with methanol and methanol/water. After clean-up the extracts were analysed by LSC and TLC and the identities of the compounds confirmed by HPLC and MS.

Recoveries were >92% of the applied radioactivity (AR) for the first 63 days but subsequently dropped to about 80% of the AR, which was attributed to loss of CO_2 and sorption of radioactivity to the filter paper during extraction. The degradation of kresoxim-methyl was very rapid (DT90 <3 days) and the free acid 490M1 reached 84% of the AR. This was subsequently degraded with a first-order half-life of 38 days. At the end of the experiment cumulative CO_2 production reached 27% of the AR whilst bound residues had reached a plateau of 47%. The bound residues were further examined and most of the radioactivity was found in the humin fraction. The only other compound identified during the experiment was 490M0 (the (*Z*)-isomer of kresoxim-methyl) and levels of this were very low in comparison with 490M1. No other compound exceeded 2.5% of the AR (Kellner, 1994a).

(b) In a study according to EPA guidelines Holly Springs sandy loam soil was treated with [*phenoxy*-^{14}C]kresoxim-methyl (radiochemical purity 95% (*E*)-isomer, >99% (*E*)- + (*Z*)- isomers, 0.5 mg/kg) and incubated at 75% water content in the dark at 20°C. Volatile compounds were trapped in ethylene glycol and 0.5M NaOH. Soil samples taken at nine intervals during 363 days were extracted and analysed as above.

Recoveries of radioactivity were 73-100% of the AR. Further work showed that the missing radioactivity was recoverable from the filter paper and was from the same compounds as in the main extract. The degradation of kresoxim-methyl was very rapid (DT90 about 2 days). The free acid 490M1 reached 66% of the AR and was subsequently degraded with a first-order half-life of 131 days. At the end of the experiment the cumulative CO_2 represented 2% of the AR. Bound residues reached a peak of 41% of the AR at day 272 and then decreased. Small amounts of the diacid 490M4 (max 3.3% of the AR) and 490M0 (4.4% of the AR) were also detected (Kellner, 1994b).

(c) This study was similar to (a), but the Limbergerhof sandy loam soil was treated with phenyl-labelled kresoxim-methyl (radiochemical purity >99%, 0.5 mg/kg). Volatile compounds were trapped in ethylene glycol and 0.5M NaOH. Soil samples were taken at ten intervals during 181 days and extracted and analysed as before.

Recoveries of radioactivity were >91% of the AR. The degradation of kresoxim-methyl was again very rapid (DT90 about 2 days). The free acid reached 81% of the AR before degradation with a first-order half-life of 57 days. The cumulative production of CO_2 was 43% of the AR. Bound residues reached a plateau of 36% of the AR with most of the radioactivity in the humin fraction. Other unidentified compounds reached a maximum of 1.6% of the AR (Kellner, 1994c).

(d) In a study according to BBA and Danish guidelines Speyer 2.2 sandy loam, Limbergerhof sandy loam, Limbergerhof clay loam and Versuchsstation sandy loam were treated with kresoxim-methyl (0.5 mg/kg) and incubated at 40% maximum water holding capacity in the dark at 20°C. Samples of the Limbergerhof sandy loam were also incubated under the same conditions at 0.05 mg/kg, and at 0.5 mg/kg with soil at 20 and 60% maximum water holding capacity and at 10 and 30°C. Soil samples taken at six intervals during 100 days were extracted by refluxing with buffered aqueous alkaline solution to hydrolyse the ester. After clean-up the acid was quantified by GLC. The mean

recovery was 83% and the limit of determination 0.005 mg/kg. First-order half-lives for the combined parent ester and acid are shown in Table 20.

Table 20. First-order half-lives of the combined kresoxim-methyl and its free acid metabolite (Keller 1993a).

Soil	Application rate, mg/kg	Temp., °C	% of max. water holding capacity	Half-life, days
Speyer 2.2 (sandy loam)	0.5	20	40	59 (r^2=0.95)
Limbergerhof (clay loam)	0.5	20	40	425 (r^2=0.25)
Versuchsstation (sandy loam)	0.5	20	40	22 (r^2=0.98)
Limbergerhof (sandy loam)	0.5	20	40	25 (r^2=0.99)
Limbergerhof (sandy loam)	0.5	20	20	192 (r^2=0.87)
	0.5	20	60	24 (r^2=0.99)
	0.5	10	40	122 (r^2=0.98)
	0.5	30	40	22 (r^2=0.94)
	0.05	20	40	20 (r^2=0.98)

(e) This study complied with Danish criteria. Limbergerhof sandy loam soil treated with 0.5 mg/kg [*phenoxy*-^{14}C]kresoxim-methyl (radiochemical purity (*E*)- + (*Z*)- isomers >99%, (*Z*)-isomer about 5%) was incubated at 40% maximum water holding capacity in the dark at 20°C under a stream of nitrogen. Anaerobic conditions were regularly checked. Volatile compounds were trapped in ethylene glycol, 0.5M H_2SO_4 and 0.5M NaOH. Soil samples taken at seven intervals during 100 days were extracted and analysed as before.

Recoveries of radioactivity were 85-98% of the AR; the losses could be accounted for by sorption to filter paper during the extraction procedure. The degradation of kresoxim-methyl was very rapid (DT90 <3 days). The free acid 490M1 reached a maximum of 84% of the AR, but its subsequent degradation was too slow to calculate a half-life. At the end of the experiment the cumulative CO_2 accounted for 2.6% of the AR and bound residues for 20%. No other volatile compounds were detected (Kellner, 1993a).

(f) In a companion study Limbergerhof sandy loam soil was sterilized, treated with [*phenyl*-^{14}C]kresoxim-methyl (radiochemical purity >98%, (*Z*)-isomer about 2%) at 0.5 mg/kg and incubated at 40% maximum water holding capacity in the dark at 20°C. Soil samples taken at five intervals during 181 days were analysed.

Recoveries of radioactivity were 87-120% of the AR. Kresoxim-methyl was so slowly degraded that after 181 days 68% was still present (degradation was too variable to calculate a half-life). The free acid 490M1 and bound residues (up to 4.8% of the AR) accounted for the remaining radioactivity. CO_2 was not measured (Kellner, 1992).

Soil dissipation under field conditions

(a) In a study according to BBA guidelines kresoxim-methyl (300 g ai/ha) was applied to bare soil at four sites in Germany (Niederhofen clay loam, Birkenheide sandy loam, Oberding clay loam and Brockhausen clay loam) in May 1992. Twenty soil cores were taken from each site at 0, 14, 28, 60, 100 and 182 days. These were sectioned into 0-10 and 10-25 cm layers and corresponding layers combined. Samples were extracted with iso-octane/phosphate buffer (1:1) and analysed by GLC for kresoxim-methyl only or combined kresoxim-methyl and 490M1, with procedural recoveries of 110% and 79% respectively and a limit of determination of 0.01 mg/kg in both cases. The samples were stored up to 18 months before analysis and there was some degradation of kresoxim-methyl during this period. CLICK HERE to continue