TRICHLORFON
(METRIFONATE)

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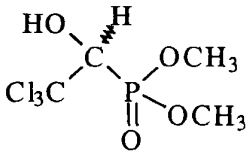
IDENTITY

Chemical Name: Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate (IUPAC & CAS name); CAS No. 52-68-6

Synonyms: Trichlorfon, Metrifonate

Chemical Structure:



Molecular formula: $C_4H_8Cl_3O_4P$
Molecular weight: 257.45

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: A racemic mixture of *cis*- and *trans*- Trichlorfon
Appearance: White crystals
Solubility: Water, 13.6; methanol, 107; ethanol, 87.6; acetone, 63.1; acetonitrile, 69.4; n-heptane, 0.15 (all in g/100ml at 25°C)
Melting point: 76-81°C
Stability: No information provided

RESIDUES IN FOOD AND THEIR EVALUATION

Introduction

The product is used for cattle and horses (as minor species). Trichlorfon is an old drug and a large part of the data was generated before the introduction of GLP. Nevertheless there is a substantial amount of new information provided that was mainly done in accord with GLP.

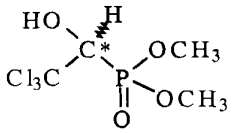
Conditions of use

Trichlorfon is an organophosphorus compound with insecticidal, acaricidal, and anthelmintic properties. It is used orally, topically or parentally for the control of parasites in various animal species. Humans may be treated orally with trichlorfon for infestations of *Schistosoma haematobium*, and it has some use in the treatment of Alzheimer disease. Trichlorfon is used as an insecticide on food crops and forests.

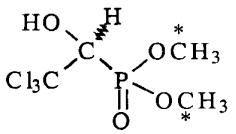
Cattle are treated orally or with aqueous pour-on, wash, or spray solutions at 50–75 mg/kg of body weight. Repeated dosing may be necessary. The preparations for use on horses are similar, but the oral dose is 35 mg/kg of body weight; one topical application for use on horses contains febantel.

PHARMACOKINETICS

Trichlorfon used in the pharmacokinetics studies was labelled in one of two carbons with ^{14}C as shown below.



^{14}C ethyl trichlorfon



^{14}C methyl trichlorfon

Laboratory Animals

Rat

[Ethyl-^{14}C]-trichlorfon was administered to groups of 5 male albino rats in a single dose of 10 mg/kg either orally, i.v., or intraduodenally. Additionally [methyl-^{14}C]-trichlorfon was administered orally at 10 mg/kg to 5 male rats. Pharmacokinetic measurements were made over a 48 hour period (Ahr and Siefert, 1992). In a second study rats weighing about 200 g were dosed orally either once or repeatedly for 21 consecutive days with 10 mg/kg BW [ethyl-^{14}C]-trichlorfon (Schwarz et al, 1994). Both studies were not compliant with GLP.

Absorption

The drug was rapidly absorbed following oral administration and reached peak concentrations in plasma usually in <1 hour. There were higher values for the AUC in the earlier 1992 study than in the 1994 study. Otherwise similar values for the parameters were obtained. After 21 repeat doses the t_{max} was the same (0.5 h) but other parameters were obviously increased; e.g. C_{max} = 10.4 mg/L; $t_{1/2}$ = 28.4 h and AUC = 494 mg.h /kg.

Elimination

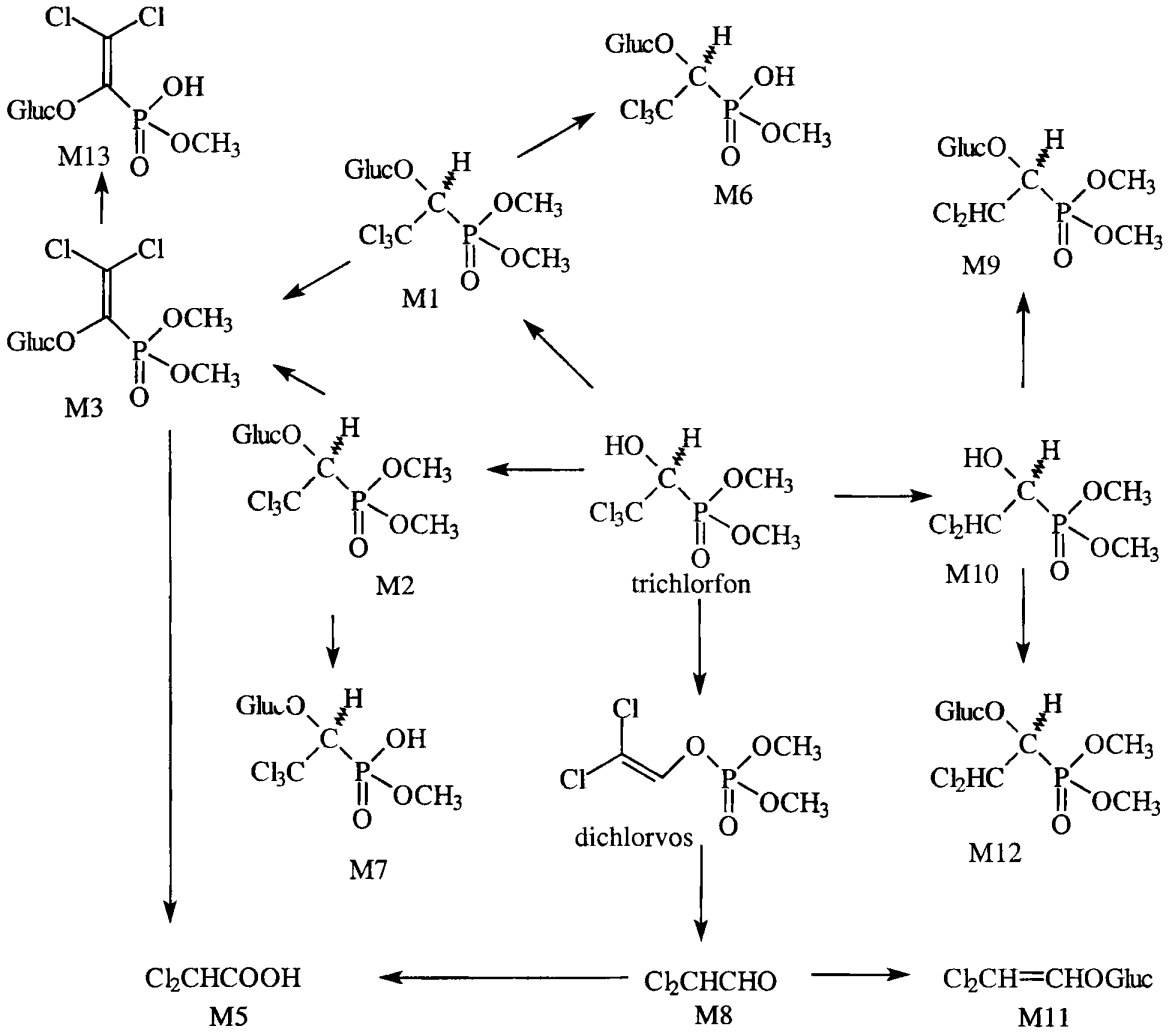
The radiolabelled trichlorfon was rapidly excreted into expired air, urine and faeces (Ahr and Siefert, 1992; Schwarz et al., 1994). The results are shown in table 1. The study also included a group of four rats with biliary cannulae and analysis of the data showed that there was clear evidence of enterohepatic recirculation following intraduodenal administration of 10 mg/kg BW [ethyl-^{14}C]-trichlorfon. Because of the extensive metabolism of the drug, the expired $^{14}CO_2$ was high (20-26% dose) and similar for both the different routes of administration or the different labelling position of the ^{14}C. Of the radioactivity not expired as $^{14}CO_2$, 43-54% of the dose was excreted in the urine and about 20% in the faeces. When repeated oral doses were administered, 39% and 38% of the total dose was excreted in the urine and faeces, respectively.

Metabolism

More than 20% of the dosed radioactivity is expired as $^{14}CO_2$ (Ahr and Siefert, 1992). This indicates extensive and rapid metabolism in rats. Metabolites were identified in the plasma, urine and bile by Boberg et al., (1993) using HPLC, MS, NMR, GC-MS and LC-MS.. They are: rearrangement to form dichlorvos, that is further metabolised to dichloroacetaldehyde and then dichloroacetic acid; replacement of a Cl by H to form the $CHCl_2$ group; formation of a glucuronide at the OH group; demethylation of the OCH_3 group to an OH group;.the two diastereoisomers, M1 and M2, are converted to M3 by removal of HCl; M3 is further metabolised to either M13 or dichloroacetic acid.

The metabolites except dichlorvos and parent drug, are assigned M numbers similar to those used by the sponsor. The diastereoisomers, M1 and M2 formed from trichlorfon and M9 and M12 formed from M10 have been identified. The biotransformation to M1 and M2 and further to M3 was a major pathway, however more M1 isomer than M2 isomer was found in plasma and urine indicating stereoselective glucuronidation. Metabolites M1, M2 and M3 were the most abundant metabolites. The main trichlorfon biotransformations are shown in Figure 1below.

Figure 1. Biotransformation of Trichlorfon in the rat.



Rabbits

Two complementary non-GLP compliant studies were carried out using rabbits. In both studies (Ahr et al., 1993, 1994), three NZ white rabbits were administered [ethyl-^{14}C]-trichlorfon as a single oral dose of 67 mg/kg BW. Three rabbits also received a single i.v. bolus of dichlorvos (Ahr and Zimmer, 1994). Pharmacokinetic parameters were determined for blood plasma and excreta using the total radioactivity or the concentrations of the (+) and (-) enantiomers of trichlorfon.

Absorption

The absorption of radiolabelled drug was rapid and complete (>99%). The peak concentrations of total radioactivity for the (-) trichlorfon isomer and the (+) trichlorfon isomer were 102, 16 and 1 mg/l plasma, respectively, and attained at 30, 15 and 25 min, respectively.

Elimination

[Ethyl-^{14}C]-trichlorfon was almost completely eliminated through the urine (99% of the dose) with only 1% of the dose in the faeces. This varies from the rat in that there is no evidence that the radioactivity would be expired as $^{14}CO_2$.

Metabolism

The metabolites found in both plasma and urine were broadly similar to those found in the rat (see table 2). The exceptions were the absence of parent drug in the rabbit plasma and urine and the presence of M13 (8.5% dose) in the urine. Ninety-three percent of the initial dose of radioactivity was accounted for in the metabolite profiles for rabbit urine, whereas only 53% was accounted for in rat urine.

Monkey

A GLP compliant study used three female Rhesus monkeys dosed orally with 5 mg/kg BW [ethyl-^{14}C]-trichlorfon. The same animals were administered the same i.v. dose in a cross-over design experiment (Cornelissen, 1993). The biotransformation of trichlorfon was carried out in a non-GLP compliant study by Boberg et al. (1994), using plasma and urine samples from the above study.

Absorption
The absorption of radiolabelled drug following oral dosing was rapid and almost complete (>83% of the dose). Peak plasma concentration was 6.8 mg.eq/l reached at 1 hour post dosing. In both treatments, radioactivity was initially rapidly eliminated, with an approximate.$t_{1/2}$ ~1.2 h., followed by a terminal elimination with a half-life of about 100 h.

Elimination
Radioactivity was excreted in the urine (> 75% of the dose) and was almost complete with 4 hours. 6.8% of the dose was in the expired air and only 2.3% in the faeces.

Metabolism
As found in the rat and rabbit, the major urinary metabolites were the enantiomers M1 and M2 and their degradation product, M3. Other metabolites were the same as for the rat with the exception of M14, a dichloroethanol glucuronide, a minor component. M14 is formed by the following route:

Trichlorfon → dichlorvos → dichloroacetaldhyde (M8) → dichloroethanol (M4) → dichloroethanol-glucuronide (M14).

Results of the pharmacokinetics and metabolites are shown in tables 1 and 2, respectively.

Table 1. Plasma pharmacokinetic parameters and excretion of ^{14}C measured as ^{14}C-trichlorfon equivalents in rats and in rabbits.

	i.v. ethyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral methyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral [ethyl-^{14}C]-trichlorfon	
						(-) isomer	(+) isomer
Pharmacokinetic Parameters							
Study	Rat (1992)	Rat (1992)	Rat (1994)	Rat (1992)	Rabbit (1993)	Rabbit (1994)	Rabbit (1994)
	105 ± 1.1	98 ± 1.2			350 ± 1.1[d]		
	158 ± 1.1	138 ± 1.3	69 ± 1.3		536 ± 1.1	14.5 ± 1.2	0.73 ± 1.5
	15.8	13.8	6.9		8.24	0.43	0.02
	1.21 ± 1.34				0.78 ± 1.2[c]		
	33.5 ± 1.1	27.7 ± 1.2	15.4 ± 1.1		64.4 ± 1.3	0.49 ± 1.53	0.85 ± 2.46
C_{max} (mg/L)	-	5.44 ± 1.1	4.6 ± 1.2	4.4 ± 1.1	102 ± 1.4	16.1 ± 1.5	0.93 ± 2.1
t_{max}(h)	-	0.51 ± 1.5	0.58 ± 1.37	0.33 – 0.67	0.5 ± 1.5	0.26 ± 2.22	0.42 ± 1.49
Excretion Characteristics							
Expired $^{14}CO_2$	20.3 ± 5.2[a]	20.6 ± 2.1[a]	not measured	25.9 ± 3.0[a]	not measured	not measured	Not measured
Urine (% dose)	47.4 ± 8.0[a]	46.1 ± 3.6[a]	42.9 ± 0.8[b]	54.4 ± 3.1[a]	99[d]	not measured	Not measured
Faeces (% dose)	21.9 ± 5.8[a]	21.2 ± 3.2[a]	19.6 ± 2.4[b]	9.1 ± 1.0[a]	1[d]	not measured	Not measured

Note. Pharmacokinetic parameters are geometric means ± SD; excretion characteristics are mean ± SD. The dose in rats is 10 mg/kg BW (n = 5); in rabbits, 67 mg/kg BW (n = 3). The (+) and (-) enantiomers were quantitated separately. The results for rats are taken from Ahr & Siefert (1992), Schwarz et al. (1994a); for rabbits, Ahr and Zimmer, (1994) and Ahr, et al., (1993)
Footnote. [a] measured over 48 hours, [b] measured over 24 hours, [c] measured over 1-3 h, [d] measured over 0-72 h.

Table 2. Metabolites of trichlorfon as percent radioactivity in plasma and as percent dose in the urine in test animals after an oral dose of [ethyl-^{14}C]-trichlorfon and in one cow after an oral dose of ^{32}P-trichlorfon.

Species	Rat		Rabbit		Monkey		Cattle	
Compound	Plasma 0.66h 5h	Urine 24h	Plasma 0.75h 1.5h	Urine 24h	Plasma 0.75h 1.5h	Urine 24h (n=2)	Blood 2 h 3h	Urine 12h
Trichlorfon	6.4 (-)	0.3			3.2 2.3	0.8	7.5 6.8	0.2
Dichlorvos						0.04		n.d.
M1	29.0 (-)	9.1	23.1 14.2	5.1		18.7		
M2	11.4 (-)	7.0	3.1 2.0	1.6		4.5		
M3	41.3 (-)	14.4	49.5 48.1	59.7	70.9 57.9	29.7		50
M4					2.1[t] 2.2[t]			
M5	1.3 5.0				8.4 10.7	0.4		
M6		0.8		0.2		1.0		
M7		1.0		0.6		4.6		
M8					2.2 3.4	2.8		
M9	3.1 (-)	0.4		0.4				
M10		1.0		see M9				
M11	see M9			see M9		3.4		
M12		0.3		0.2		0.3		
M13				8.5		2.4		
Others not identified	8.7 91.0	18.7	9.5 15.2	16.5	(-) (-)	3.9		15.8
% dose		52.9		92.8		73.3		66.0

Note. (-) is not found. See M9 means that compound was identified but co-eluted with M9. Footnote: [t] is tentative result

Food Producing Animals

Cattle: Topical Administration

The radiolabelled drug applied topically in the cattle studies was [ethyl-^{14}C]-trichlorfon. The purity was 98.6 - 98.7% and the specific activity was 2.11 GBq/mmol. The [ethyl-^{14}C]-trichlorfon was applied to the back of 8 calves at a target dose level of 40 mg /kg BW. The heads of the animals were restrained throughout the study period to prevent oral ingestion of the dose (Lynch and Speirs, 1998).

Absorption

14-49% of the dose was not available for absorption because of run off following application. Absorption of the test material through the skin was assessed by measuring total radioactivity in plasma at various times post dose and by measuring daily excretion of radioactivity in urine and faeces until 120 h post dose in two of the calves. Absorption into the systemic circulation was rapid with the maximum plasma concentration of total radioactivity observed at 4 h post dose in males and 6 h in females. Plasma concentrations of total radioactivity at this time (Cmax) represented 1.01 mg equiv./kg and 0.36 mg equiv./kg, in the two calves, respectively. Results indicate that plasma concentrations of total radioactivity declined in a biphasic manner, rapidly to 24 h and from 24 -120 h post dose thereafter more slowly. The plasma elimination half-life of total radioactivity over this period represented 124 h and 258 h in the two calves, respectively.

Elimination

Over 0-120 h post dose, excretion of total radioactivity was relatively low in the two calves, with 2.8% and 1.6% of the administered dose excreted in urine, and 3.3% and 0.3% of the administered dose in the faeces of the two calves, respectively. Taking into account that a significant portion of the administered dose was not available for absorption due to run-off, then the amount excreted as a percentage of the retained dose would be higher. Over 120 h post dose, 13.6% and 5.2% of the retained dose was excreted in urine and 16.2% and 1.0% of the retained dose in faeces of the two calves, respectively.

Metabolism

The tissues from the calves slaughtered on 1day post-dosing were investigated for metabolic profiles (Phillips and Johnson, 1998). Samples of muscle, liver and kidney were acidified with phosphoric acid and sequentially extracted with distilled water; acetonitrile; acetonitrile:water and 1% acetic acid in methanol. The non-extracted portion was extracted further following hydrolysis with a pepsin/HCl mixture. Fat was acidified with phosphoric acid and sequentially extracted with acetonitrile; acetonitrile:water and 1% acetic acid in methanol. The non-extracted portion was further extracted with acetone:acetonitrile and finally with acetonitrile:NaOH. The tissues collected for the calves killed on days 2, 3 and 5 were similarly evaluated (Phillips, 1998). The total residues (TR) and the percentage of TR that were extractable are shown in Table 3.

Table 3. The total residues and their extractability in tissues of calves treated with a pour-on preparation of [ethyl-^{14}C]-trichlorfon.

Tissue	Total Residues as µg eq./kg with percent extractable (in parentheses)			
	1 day [a]	2 day [c]	3 day [c]	5 day
Muscle distant	20-305 (90)			
close	488, 687 (88)			
Muscle Composite		218 (96)	185 (90)	92, 92 (94)
Liver	396, 779 (96)	1272 (83)	1947 (91)	824, 1056 (90)
Kidney	309, 622 (96)	732 (91)	1153 (92)	548, 579 (97)
Fat Renal	21, 23 (80)			
Omental	65, 18 (78)			
S.c. distant	84, 139 (86)			
S.c. close	1054, 882 (85)			
Fat Composite		807 (78)	950 (73)	119, 274 (78)

Note. Distant or close indicates samples were collected either distant from or close to the dose area. Composite muscle and fat included tissues distant and close to dosing area.

Footnote. [a] TR in individual animals with mean value for extraction; [c] composite tissue of two animals.

The extractable fractions were used for metabolite identification. This was difficult due to the complex nature of the residues, the large amount of polar material and several minor metabolites. In fat, trichlorfon and dichloroacetic acid were identified as major metabolites in all the day 1 samples. Dichlorvos was found in one of the day 1 fat samples. Liver and kidney contained mainly polar metabolites and other unknown metabolites. Muscle contained trichlorfon and a compound provisionally identified as desmethyl-dichlorvos. It was not possible to identify a ratio of a single compound, e.g. trichlorfon, to the total residues.

Cattle: Oral administration

A grub-infested, lactating dairy cow was administered ^{32}P labelled trichlorfon as an oral dose of 25 mg/kg BW (Robbins et al., 1956). Samples of blood, urine, faeces and milk were collected. The concentrations of total radioactivity, trichlorfon and dichlorvos were measured. Peak blood concentration (Cmax = 15.1 mg equiv./kg) was attained at 2 h post dosing of which 7.5% was trichlorfon. The trichlorfon was mostly eliminated (66% within 12 hours) in the urine. Only 0.26% of the radioactivity excreted was trichlorfon. A major metabolite accounted for 77% of the radioactivity excreted and was tentatively identified as M3. Less than 3% of the radioactivity was excreted in the faeces. Dichlorvos was not detected in any of the samples.

Goats

The metabolic fate of [ethyl-^{14}C]-trichlorfon following oral administration of a single dose of 8.56 mg/kg BW on three consecutive days was studied in two lactating goats (Chopade *et al.*, 1987). The goats were sacrificed 4 hours after the last dose and samples of tissues and milk were collected and subsequently analysed for their metabolite composition. Unmetabolised trichlorfon was detected in small amounts (6-7%) in muscle and kidney but was not present in liver, fat or milk. A large proportion of the radioactivity was incorporated into tissue proteins and sugars and accounted for 38%, 52% and 23% or the Total Residues in muscle, liver and kidney, respectively. The conjugates of M5 (see figure 1) accounted (as %TR) for 43% in muscle, 11% in liver, 44% in kidney and 70% in fat. Other metabolites, namely, desmethyltrichlorfon, desmethyldichlorvos, M1, M2 and M4, were identified at low levels. The metabolic pathways proposed for the goat have some similar pathways to those of the rat but other routes are different.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

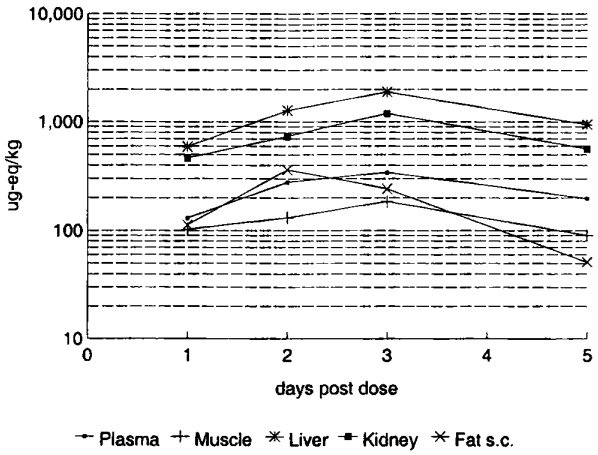
Cattle. Topical administration

[Ethyl-^{14}C]-trichlorfon was applied to the back of 8 calves at a target dose level of 40 mg /kg BW. The heads of the animals were restrained throughout the study period to prevent oral ingestion of the dose (Lynch and Speirs, 1998). One male and one female were sacrificed at each of 1, 2, 3 and 5 days post-dosing. Edible tissue samples and plasma samples were collected, stored at –20°C, and subsequently analysed for residues. Samples of fat, muscle and skin, close to and distant from the dose area were collected for analysis.

In the first few hours after dosing a large proportion (14-49%) of the dose ran off the animals and was not available for absorption. At slaughter, the animals were washed and the radioactivity in the washings was 4 -16% of the dose. The amount of dose still remaining on the skin was 8-28% and would continue to contribute to the tissue residues as the drug was absorbed. Only 2 and 6% of initial dose was estimated as absorbed in the two calves slaughtered at 5 days post-dosing, respectively.

The amount of radioactivity in plasma and tissues distant from the dose area reached a maximum 3 days and are depleting by five days post-dosing (see table 3 and figure 2). This may be due to the large part of the dose remaining on the skin - an essential requirement for prolonged ecto-parasiticidal activity.

Figure 2. Total residues (μg trichlorfon equiv./kg) in plasma and edible tissues of calves after dosing with a pour-on preparation of [ethyl-^{14}C]-trichlorfon at 40 mg/kg BW



Note. The values are the means for 2 calves. The values for muscle are the means for the combined values for both flank and round muscle.

Cattle. Oral administration

A grub-infested, lactating dairy cow was administered ^{32}P labelled trichlorfon as an oral dose of 25 mg/kg BW (see above) (Robbins et al., 1956). Samples of milk were collected. The concentrations of total radioactivity were measured. Peak milk concentration (Cmax = 2.3 mg equiv./kg) was attained at 18 hours post dosing of which only traces were identified as trichlorfon. Dichlorvos was not detected in any of the samples. The milk samples were subjected to bioassays to determine insecticidal activity to house flies. None of the samples had insecticidal activity, suggesting that the metabolic products were not toxic to insects.

Other residue depletion studies with unlabeled drug

Cattle

Numerous residue depletion studies using unlabelled drug administered either orally or topically were performed during 1959-69 (before GLP). The following cattle studies were submitted: Auer and Krebber (1998) and Aur et al., (1999) and 4 older non-GLP studies. The analytical methods used were not generally specific for trichlorfon or dichlorvos. Residue determination using the inhibition of cholinesterase enzyme activity would have been of particular interest. It may be possible to correlate the inhibition of cholinesterase activity with later results where it is certain that no residues of trichlorfon and dichlorvos and perhaps no biological activity are found.

Two new GLP compliant studies were submitted by the sponsors. The residues of trichlorfon and dichlorvos were measured in tissues and milk of cattle after topical application of unlabelled trichlorfon (Auer and Krebber, 1998). Sixteen German Pied adult cattle (8 male and 8 female) were treated with a single spray of 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. Groups of 2 males and 2 females were slaughtered at 12 hour, 1, 3 and 7 days after treatment. Liver and kidney samples were collected. Samples of muscle and fat were collected at sites distant and close to the area of application. The samples were analysed for residues of both trichlorfon and dichlorvos by a validated LC-MS/MS method (Krebber, 1998). No residues of trichlorfon were detected (LOD = 50 µg/kg) in muscle and fat distant from the dosing area nor in liver and kidney. No residues (LOD = 50 µg/kg) of dichlorvos were found in any tissue except in fat tissue close to the dose site taken from one animal at day 1. Samples of muscle and fat from this animal also contained residues of trichlorfon. The results for muscle and fat close to the dosing area are shown in Table 4. 12 hours after dosing residues were found in the subcutaneous fat close to the dose area in all four animals.

In the second study 8 dairy cows were treated with a single spray of a 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. (Auer et al., 1999). Four cows had high milk yields and four had low yields. Milk was collected and the concentration of trichlorfon measured by a validated LC-MS/MS method (Krebber, 1999). Milk samples were collected up to seven days post treatment. The results are shown in table 4. Trichlorfon, was excreted in the milk, primarily during the first 12 hours post-dosing. The highest level was 205 µg/kg in one cow sampled at 6 h post-dose. Residues were present just above the LOQ (25 µg/kg) in one cow at 24 hours and another cow at 36 hours but thereafter no residues were detected (LOD 2.5 µg/kg).

Table 4. Residues (µg/kg) of trichlorfon and dichlorvos in cattle tissues after a 40 mg/kg B.W. pour-on treatment of trichlorfon.

TISSUE and RESIDUE	6 hours	12 hour	1 day	1.5 days	2 days	3 days	7 days
Trichlorfon							
Muscle close to dosing site	-	<50	90 <50 (3)	-	-	<50	<50
Fat close to dosing site	-	173, 70, 51, 142	2350 <50 (3)	-	-	<50	<50
Milk	79±57	61±84	28 <25 (7)	29 <25 (7)	<25	<25	-
Dichlorvos							
Muscle close to dosing site	-	<50	<50	-	-	<50	<50
Fat close to dosing site	-	<50	142, <50 (3)	-	-	<50	<50
Milk	<25 (3) <2.5 (5)	<25 (1) <2.5 (7)	<2.5	<2.5	<2.5	<2.5	-

Horse

Three horses were administered a single oral dose of 35 mg trichlorfon /kg BW in a paste combination with 6 mg febental/kg BW. The horses were sacrificed 14 days later and samples of fat and muscle assayed for the sum of trichlorfon and dichlorvos measured as the common breakdown product, dimethyl phosphite, by gas chromatography. All residues were < LOD, 50 µg/kg (Dorn and Blass, 1982).

BOUND RESIDUES AND BIOAVAILABILITY

The majority of the tissue residues of [ethyl-^{14}C]-trichlorfon applied topically to cattle was extractable (see table 3). Following a more rigorous pepsin/HCl digest and extraction procedure for muscle, liver and kidney tissues (after the initial mild solvent extraction), the additional amount of total residues was 23 and 25% in muscle, 15 and 19% in liver and 5 and 17% in kidney (Phillips, 1998). The milder extraction procedure for fat resulted in 14-30% of the residues being non-extractable. The nature of the bound residues is unknown.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A validated GC-MS method for the identification and quantification of trichlorfon residues in the liver, kidney, muscle and fat of cattle, using d_6-trichlorfon as an internal standard, has been reported (Krebber, 1998). A modification of this method, using an external standard added to the sample matrix blank that has been taken through the isolation procedure has been validated for the analysis of trichlorfon residues in tissues and milk of cattle (Krebber, 1999a) and in the liver, kidney, muscle and fat of horse (Krebber, 1999b).

Trichlorfon residues are extracted from either tissues or milk with acetonitrile containing 0.1% formic acid. For liver, kidney and muscle, the combined extracts were diluted with water, extracted with dichloromethane, evaporated to dryness and reconstituted with n-heptane. For milk and fat, the acetonitrile extract was partitioned with n-heptane, the acetonitrile phase evaporated to dryness and reconstituted with n-heptane.

For sample clean-up, n-heptane extracts were added to a silica cartridge, washed with ethyl acetate-heptane (1:9) and trichlorfon eluted with ethyl acetate. For cattle tissue samples, the ethyl acetate elutate was evaporated, reconstituted with a small volume of ethyl acetate and the internal standard (d_6-Trichlorfon) added. For analyses where external the external standard was used, an extract derived by subjecting residue-free tissue or milk to the entire extraction procedure was fortified with trichlorfon immediately prior to GC-MS analysis. Trichlorfon was detected by selected ion monitoring at m/z 109 with selective ion monitoring at m/z 115 for d_6-trichlorfon.

Performance characteristics of the method

The method was validated for use over the linear range of detector response, 0.01-1 mg/kg for tissues and 0.05-2 mg/kg for milk. Detection in the selective ion monitoring mode, together with an established GC retention time ensure that the method has a high degree of specificity. Other performance characteristics of the method, using either internal or external standard are shown in Table 5.

Table 5. Recovery data for internal and external standard variations of the GC-MS trichlorfon residue method at different spiking concentrations.

Matrix	% Recovery (%SD)								
	Cattle-internal standard			Cattle-external standard			Horse-external standard		
	L*	M	H	L*	M	H	L*	M	H
Liver	76 (7.3)	83 (6.3)	84 (6.8)	81 (5.7)	81 (5.6)	85 (5.4)	81 (7)	70 (17)	73 (5)
Kidney	90 (2.4)	78 (8.4)	72 (8.8)	83 (7.6)	86 (7.8)	86 (10.4)	76 (15)	80 (11)	98 (6)
Muscle	88 (3.2)	86 (2.7)	83 (3.7)	96 (7.8)	88(14.9)	82 (9.9)	77 (14)	71 (10)	72 (19)
Fat	87 (13.0)	101 (4.2)	101 (4.0)	96(20.8)	112 (17.1)	97 (8.1)	78 (21)	101 (11)	87 (11)
Milk				75(13)	93 (12)	94 (18)			

Note. * = LOQ values are 0.05 mg/kg for tissue and 0.025 mg/kg for milk. L = 0.05 mg/kg for tissue (0.025 mg/kg for milk); M = 0.1 mg/kg (0.05 mg/L for milk); H = 0.2 mg/kg (0.1 mg/L for milk)

The limit of detection (LOD) of the method in cattle was 9 μg/kg for liver and fat, 6 μg/kg for kidney and fat and 2 μg/kg in milk. In the horse, using an external standard, the LOD was 20 μg/kg in liver, 16 μg/kg in kidney, 8 μg/kg in muscle and 9 μg/kg in fat. The limit of quantification (LOQ) was 50 μg/kg in all tissues and 25 μg/kg in milk. The recoveries obtained from each tissue and milk at the LOQ are shown in table 5.

This method proposed by the sponsor for routine surveillance purposes has no reported matrix interference. The non-commercial availability of the internal standard would require that the external standard method should be used or the introduction of a suitable surrogate standard investigated.

The possibility of determination of dichlorvos by this method has not been reported. It is possible that, give the chemical and physical properties of trichlorfon, that diclorvos residues could be detected and quantified as part of a multi-residue organophosphate (OP) screen used for OP residues by regulatory agencies.

APPRAISAL

Both oral and topical administration of trichlorfon is indicated for cattle, only an oral preparation is available for the horse. The new data for topical administration was developed in compliance with GLP. None of the older data for the oral administration is GLP compliant and is only of limited coverage.

The metabolic pathways for the rat and rabbit appear more substantiated than for ruminants. The biotransformations in the cow, goat or the horse have not been clearly established. It proved difficult to quantify the metabolites in the cattle administered [ethyl-14C]-trichlorfon (see table 2). Nevertheless, in all species there is evidence of rapid metabolism following absorption. Trichlorfon and dichlorvos account for very low percentages of the total residues in the major edible tissues and milk of cattle.

Both trichlorfon and dichlorvos have insecticidal activity and more information on the insecticidal properties of the other metabolites would have been valuable. It is not known whether the other metabolites are also toxic.

The persistence of the active compound at the site of pour-on application is necessary for long ecto-parasiticidal action. This persistence alters the pharmacokinetics of trichlorfon as a pour-on treatment compared with the oral administration. The levels of radioactivity in plasma and tissues distant from the pour-on dose area appear to peak at 3 days and are depleting by five days post-dosing (see table 3 and figure 2). The persistence of residues may be due to the continuing absorption of the large part of the dose remaining on the skin. Studies beyond a 5 day post-dosing period would have provided better information on the rate of depletion of the total residues. There was considerable variability between cattle in the amount of run-off dose, 14–49%. If similar large differences were observed under field conditions it could affect both efficacy and withdrawal times for individual cattle.

Numerous residue depletion studies using unlabelled drug administered either orally or topically were performed during 1959-69 (before GLP). The analytical methods were not generally specific for trichlorfon or dichlorvos. The sponsors have submitted chromatographic studies but not those using other end-point measurements such as the determination of residues using the inhibition of cholinesterase enzyme activity. It may possible to correlate the inhibition of cholinesterase activity with later results in which it is certain that no residues of trichlorfon and dichlorvos are found. Two new GLP compliant studies were submitted by the sponsors in which the residues of trichlorfon and dichlorvos were measured in tissues and milk of cattle after topical application of unlabelled trichlorfon. At intervals during a 7 day observation period, no residues of trichlorfon were detected (LOD = 50 μg/kg) in muscle and fat distant from the dosing area or in liver and kidney. No residues (LOD = 50 μg/kg) of dichlorvos were found in any tissue except in fat tissue close to the dose site taken from one animal at day 1. Samples taken from this animal of muscle and fat close to the site of application also contained residues of trichlorfon. The results for muscle and fat close to the dosing area are shown in table 4. Residues were found in the subcutaneous fat close to the dose area in all four animals. 12 hours after dosing. In the second study dairy cows were treated with a single spray of 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. Trichlorfon was excreted in the milk, mostly during the first 12 hours post-dosing. The highest level was 205 μg/kg in one cow sampled at 6 h post-dose. Residues were present just above the LOQ (25 μg/kg) in one cow at 24 hours and another cow at 36 hours but thereafter no residues were detected (LOD 2.5 μg/kg).

A validated GC-MS method for the identification and quantification of trichlorfon residues in the liver, kidney, muscle and fat of cattle, which uses d6-Trichlorfon as an internal standard, has been reported. A modification of this method using an external standard added to the sample matrix blank has been taken through the isolation procedure. It has been validated for the analysis of trichlorfon residues in tissues and milk of cattle (Krebber, 1999a) and in the liver, kidney, muscle and fat of horse (Krebber, 1999b). No interference from the matrix has been reported in this method, which is proposed by the sponsor for use in routine surveillance. Since the internal standard is not available commercially, either

the method involving external standard should be used or the introduction of a suitable surrogate standard should be investigated.

MAXIMUM RESIDUE LIMITS

The ADI is 0–0.02 mg/kg of body weight, equivalent to a maximum of 1200 μg per 60-kg person. In reaching its decision, the Committee considered the following:

1. Only trichlorfon and dichlorvos are of toxicological concern. Trichlorfon is a pro-drug, and dichlorvos is the only metabolite with effective insecticidal action.
2. Dichlorvos is very unstable and is not found in animal tissues or milk.
3. The metabolism of trichlorfon in target and laboratory animals is broadly similar.
4. Trichlorfon is metabolized so extensively and rapidly that the ratio of marker residue to total residues cannot be defined.
5. There is a suitable routine analytical method for determining trichlorfon, with an LOQ of 50 μg/kg for muscle, liver, kidney, and fat and 25 μg/kg for milk.
6. The concentrations of trichlorfon in tissues distant from the site of application were below the LOQ.
7. Within one day of administration of a pour-on preparation, the concentrations of residues in muscle and fat samples collected close to the site of application were above the LOQ in a few animals. By 3 days after administration, no residues were present in fat or muscle close to the site of administration.
8. Residues of trichlorfon were found at concentrations above the LOQ from the first three milkings after treatment, but thereafter the concentrations were below the LOQ. No residues of dichlorvos were found at concentrations greater than the LOQ (25 μg/kg) in any sample of milk.
9. The TMDI for total residues calculated for muscle, liver, kidney and fat in the pivotal radiodepletion study in calves was 150 μg, representing only 12.5% of the ADI.
10. Insufficient information was available to extend the MRLs to horses.

The MRLs for muscle, liver, kidney and fat should serve as guidelines only for the control of residues. Residues of trichlorfon were not detected in the residue depletion studies reviewed by the Committee. The Committee believes that residues in muscle, liver, kidney and fat would not be found at the limit of quantification of available analytical methods. MRLs were not allocated by the Committee for muscle, liver, kidney and fat considering that no detectable residues should be present in tissues from animals treated with trichlorfon when used in accordance with good practice in the use of veterinary drugs. Therefore, the LOQ may be used as guideline maximum residue concentrations in muscle, liver, kidney and fat by National governments. The guidance values are 50 μg/kg for muscle, liver, kidney and fat in cattle and an MRL of 50 μg/kg for milk measured as parent drug. The TMDI is 75 μg for milk.

The Committee requests the development of an analytical method for trichlorfon with LOQs at least one-half of the current values.

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