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Determination of Chlorpyrifos and Its Major Breakdown Products in Technical Formulations

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Chlorpyrifos [0,0-diethyl-o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an insecticide which is formulated into various products such as Dursban©, Lorsban© and Ridlice©, used for the control of a number of ectoparasites including lice, on cattle and sheep. The mammalian oral toxicity LD50 ranges from 135-163 mg/kg for rats to 1000-2000 mg/kg for rabbits. The compound is rapidly detoxified in animals (Windholz *et al.* 1976; Worthing and Walker 1983) the metabolites being chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol and diethyl phosphorothioic acid and/or diethyl phosphoric acid (Sultatos and Murphy 1983; Sultatos *et al.* 1984). In cattle, metabolites are rapidly excreted into the urine and faeces (Everett 1982). Chlorpyrifos is therefore considered a comparatively safe insecticide for use on cattle. However, a number of instances have occurred where application of the formulated product has killed valuable stock (Everett 1982; Lein *et al.* 1982).

Technical formulations can contain two interesting impurities and/or breakdown products, sulfotep and 3,5,6-trichloro-pyridinol, the latter of which is significant from a toxicological viewpoint.

A procedure for the determination and confirmation of 3,5,6-trichloro-pyridinol along with chlorpyrifos and sulfotep, by gas chromatography - mass spectrometry is described. This procedure was used to analyse a number of chlorpyrifos formulations of varying ages, including one implicated in a number of cattle deaths. The latter formulation had been applied to 80 Holstein bulls of varying age, resulting in the death of 50 bulls. The remaining 30 required extensive veterinary treatment, which included the administration of atropine.

MATERIALS AND METHODS

All solvents and reagents used were analytical grade or equivalent. Chlorpyrifos (99.7%) and the major breakdown product: 3,5,6-trichloro-2-pyridinol (99.9%) were obtained from Dow Chemical Co., (Midland, Mich.).

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The chlorpyrifos formulation suspected of causing the death of some 50 head of breeding cattle was analysed as well as a number of other batches of the same formulation of varying age to determine whether any possible breakdown products had accumulated with storage under warehouse conditions. Samples were placed in screw-top glass jars and transported to the laboratory where they were examined by thin-layer chromatography, high-pressure liquid chromatography (HPLC), gas-liquid chromatography (GC) and GC-mass spectrometry.

Thin-layer chromatography (TLC) was used initially for separation and identification of chlorpyrifos and its breakdown products in the formulations. Glass plates coated with 0.25 mm of silica gel GF254 (Whatman) were used. Detection of chlorpyrifos and its potential breakdown products was achieved by preparing 2 mg per mL of each formulation in acetone and spotting 2 x 5 μL aliquots of each compound onto the TLC plate. After development of the chromatogram in a solvent mixture containing n-hexane: acetone, 4:1, the compounds were firstly observed under short wavelength ultraviolet light (254 nm), followed by spraying with a mixture consisting of the following: bromophenol blue (0.05 g in 10 mL acetone) diluted to 100 mL with a 1 w/v solution of silver nitrate in acetone: water (1:3). Blue spots on a white background were observed for the organophosphorus compounds after spraying with a second solution consisting of 1% v/v acetic acid in acetone. Rfs (x100) for chlorpyrifos and 3,5,6-trichloropyridinol were 95 and 36 respectively.

All formulation samples were subsequently analysed (in duplicate) by HPLC using the method of Skelly *et al.* (1981) (modified by substituting a Zorbax ODS column with a Spherisorb ODS column). In addition, each sample was examined by atomic absorption spectrometry for the presence of metallic ions such as copper, since these are very effective catalysts for the degradation of some organophosphorus esters (Cremlyn 1979).

Identification and/or confirmation of further breakdown products was carried out on a Hewlett-Packard 5970 MSD-5890 GC system. Each formulation sample was treated as follows prior to GC-MS: to 10 μL of each formulation sample 50 μL of bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added. The mixture was then allowed to stand for 10 minutes at room temperature. The reagents and solvents were then removed at room temperature using a gentle stream of dry nitrogen. The samples were then made up to 5 mL with dichloromethane. The mixture was analysed using a capillary GC-MS with the MS set up to selectively monitor the ions 253.9 and 322 for the presence of methylsilyl ether derivative of 3,5,6-trichloropyridinol and sulfotep respectively. Only those peaks which occurred at the correct retention times were accepted. Because the pesticide was present with the breakdown product during the derivatisation, it was necessary to determine if chlorpyrifos was affected by the silyl reagents. When the derivative was prepared at room temperature it was found that there was no apparent

reactions between the BSTFA and the chlorpyrifos. However prior to injection into the gas chromatograph is necessary to remove the reagents by gentle evaporation so that high temperature gas phase reaction are avoided. For later work the less reactive HMDS (hexamethyldesilazane) was substituted for the BSTFA. The concentrations of 3,5,6-trichloropyridinol in each sample was established by setting up a four point calibration graph at concentrations 1.88, 9.4, 47 and scan analyses were carried out to determine the presence of any other compounds for example, pyridyl phosphate.

RESULTS AND DISCUSSION

Analyses of the tissues of the dead cattle failed to reveal the presence of unchanged chlorpyrifos. This was probably due to rapid absorption and metabolism of the chlorpyrifos in the animals (Everett 1982). Metabolism of any remaining chlorpyrifos may have continued during the intervening time between autopsy and receival of samples at the laboratory, leading to further losses. Surviving animals had depressed blood cholinesterase levels and responded to treatment with atropine injections.

Table 1 lists the analyses of fifteen (15) chlorpyrifos formulations of varying ages (stored under warehouse conditions) including one formulation (sample #15), which had been stored in a farm shed and was implicated in the cattle deaths. It also contained a dark coloured residue composed of iron sulphides and elemental sulphur.

All formulation samples contained the major breakdown product: 3,5,6-trichloro-2-pyridinol in varying amounts (depending upon age and storage conditions) and small amounts (averaging 2 mg/mL) of sulfotep. The latter may represent an impurity produced during manufacture. Electron impact mass spectra of the 3,5,6-trichloropyridinol-TMS ether and sulfotep are shown in Figure 1.

However, only minute traces of the oxygen analogue chlorpyrifos oxon, was found in sample #15 and could not be detected in the other samples. These results are consistent with previous reports that the oxygen analogue is rarely found and if detected, constitutes only 1% of the chlorpyrifos level (Claborn *et al.* 1968; Ivey *et al.* 1978; Ivey and Palmer 1979; Ivey 1979). Accelerated degradation to trichloropyridinol and other breakdown products probably occurs through the effect of elevated temperatures (especially during the summer months), with the reactions being catalysed by iron in the presence of moisture.

A proposed scheme based on the work of Sultatos *et al.* (1984) and others, for the degradation of chlorpyrifos in formulations is shown in Figure 2. The order of toxicity in avian and mammalian systems of chlorpyrifos compared to its metabolite/breakdown products is pyridyl phosphate > 3,5,6-trichloro-

Table 1. Analyses of chlorpyrifos formulations.

Sample Number	Age of sample from time of manufacture (years)	Chlorpyrifos mg/g	3,5,6-trichloro[1]-pyridinol mg/g	Sulfotep[2] mg/ml	Iron content µg/g
(1)	5	449	5.7	1.7	73
(2)	5	455	4.0	2.3	79
(3)	4	440	3.8	2.2	85
(4)	4	457	3.0	2.3	83
(5)	4	458	1.9	2.0	88
(6)	3	445	2.0	2.0	87
(7)	3	458	1.0	2.0	86
(8)	2	464	1.4	1.7	81
(9)	1	443	1.2	1.9	82
(10)	1	447	1.3	1.6	87
(11)	1	442	<0.5	1.9	69
(12)	1	450	<0.5	1.5	79
(13)	1	451	<0.5	1.6	78
(14)	1	455	<0.5	1.6	63
(15)	2	298	138	6.5	115

[1] Average of three determination including quantitative mass spectrometic analysis (as the TMS - ether derivative).

[2] Estimated by mass spectrometry.

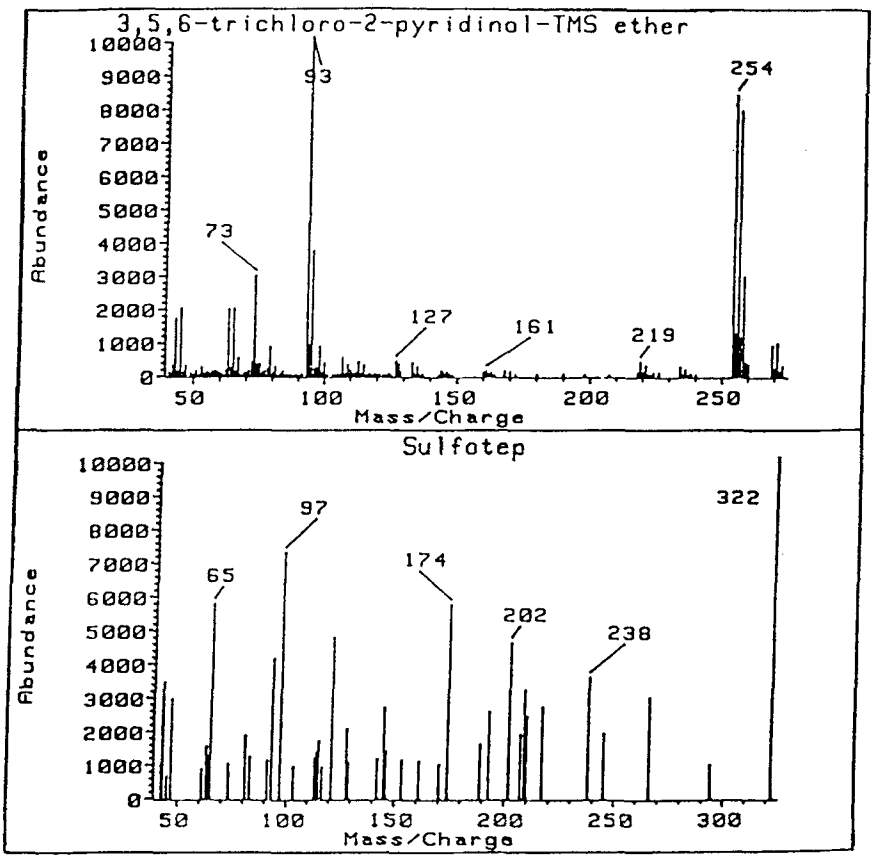

Figure 1. Electron impact mass spectra of 3,5-6-trichloro pyridinol-TMS ether and sulfotep.

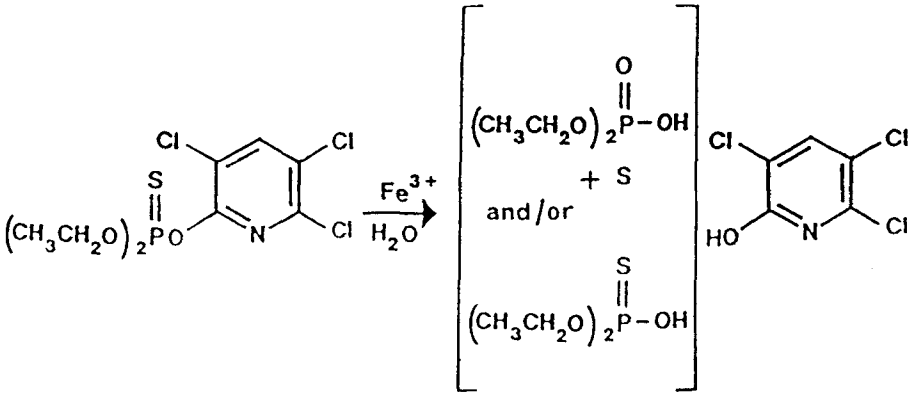

Figure 2. Degradation reaction of chlorpyrifos in formulations.

pyridinol > chlorpyrifos (Muscarella *et al.* 1984). It has also been shown that certain combinations of insecticides had a greater toxicity to insects than the more toxic component alone (Sumerford 1954; Street *et al.* 1966; Triolo and Coon 1966; Metcalf *et al.* 1966). Hence chlorpyrifos formulations can present a toxicological hazard to cattle, which is greatly increased with application of aged and/or poorly stored (eg. at elevated temperatures) formulations where appreciable amounts of the major breakdown product has accumulated.

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