THE UNIVERSITY OF MANITOBA

CHLORPYRIFOS: Degradation in Pond Water, and the Extraction of the O-analog and Pyridinol Metabolites from Water.

by

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CHLORPYRIFOS: Degradation in Pond Water, and the Extraction of the O-analog and Pyridinol Metabolites from Water

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ABSTRACT

Part 1 contains a comprehensive literature survey dealing with the behavior and analysis of chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) and metabolites in four environmental systems: soil, plant, animal, and water.

A study of the degradation of chlorpyrifos in outdoor artificial pools was conducted jointly with G. Rawn, M.Sc., 1977 (Rawn, 1977). Two formulations of chlorpyrifos were examined; the 2.5% slow-release formulation, and the 48% emulsifiable concentrate formulation. The 'half-lives' observed for these formulations were 14±4 and 5±3 hours respectively. The concentration-time data was analyzed by three mathematical models, including a power rate law, a hyperbolic rate model, and a power function. The data was best described by the power function, followed by the hyperbolic and power rate models.

In Part 3, a method was developed for the tap water extraction of chlorpyrifos and two important metabolites; the O-analog (diethyl 3,5,6-trichloro-2-pyridyl phosphate), and the pyridinol (3,5,6-trichloro-2-pyridinol). The latter compound was determined as its methylation product, employing diazomethane as the derivatizing agent. Average extraction efficiencies for chlorpyrifos, the O-analog, and the pyridinol were 84±7, 82±3, and 77±10% respectively.

ACKNOWLEDGEMENTS

Helpful advice was received from many people during the course of this work. A special thanks goes to my advisor, Dr. G. R. Barrie Webster who always had time for my problems.

Financial support from the National Research Council is gratefully acknowledged.

Standards of chlorpyrifos and metabolites were kindly supplied by Dow Chemical Company.

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PREFACE

Chlorpyrifos, the active ingredient in Dursban insecticide (Dow Chemical Co.), is effective in the control of mosquito and blackfly larva. With respect to this application, two important problems were examined relating to the environmental impact of this compound.

1) Experiments were conducted to examine the persistence of chlorpyrifos in natural pond water (Part 2). This study was conducted throughout the summer of 1975, using outdoor pools located at Glenlea, Manitoba.

2) Effort was directed towards the development of a method for the simultaneous determination of chlorpyrifos and two important metabolites in water (Part 3).

All abbreviated chemical names are identified with a bracketted Roman numeral when encountered in the text for the first time. The reader is referred to Table 1 which contains the IUPAC name and molecular structure of these compounds.

Table 1. Chemical Names and Structures of CHLORPYRIFOS and Metabolites.

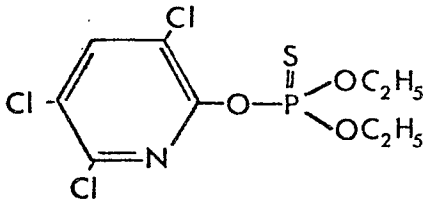
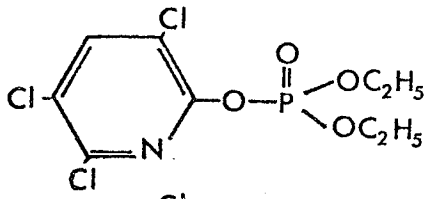
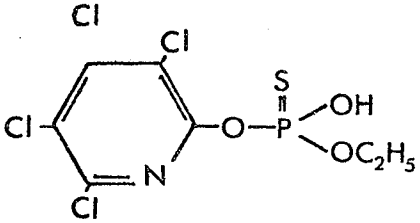
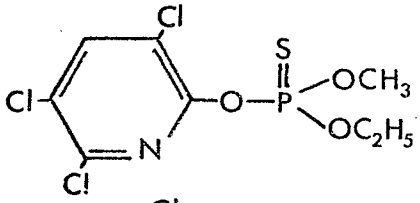
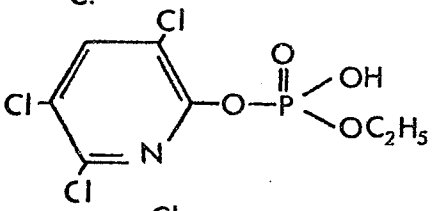
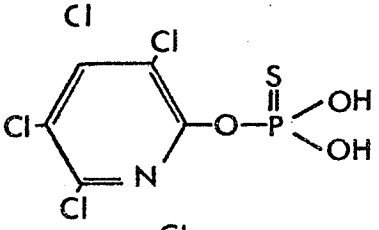
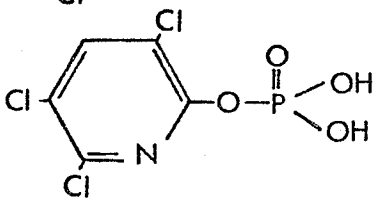
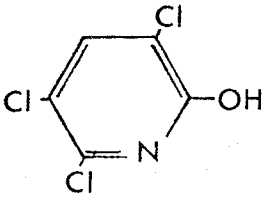
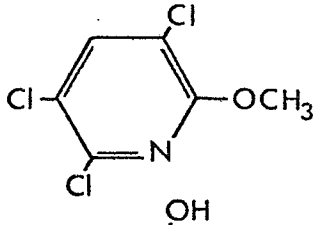
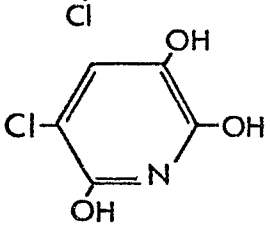
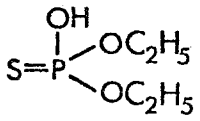
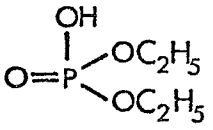
Structure	Code	Chemical Name
	I	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos)
	II	diethyl 3,5,6-trichloro-2-pyridyl phosphate (O-analog)
	III	O-ethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
	IV	O-ethyl O-methyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
	V	ethyl 3,5,6-trichloro-2 pyridyl phosphate
	VI	O-3,5,6-trichloro-2-pyridyl phosphorothioate
	VII	3,5,6-trichloro-2-pyridyl phosphate
	VIII	3,5,6-trichloro-2-pyridinol (pyridinol)

Table 1 (continued)

Structure	Code	Chemical Name
	IX	3,5,6-trichloro-2-methoxy-pyridine (Me-pyridinol)
	X	5-chloro-2,3,6-trihydroxy pyridine
	XI	O,O-diethyl thiophosphate
	XII	O,O-diethyl phosphate

PART 1. Literature Review.

(1.1) INTRODUCTION

Chlorpyrifos (I) is a broad-spectrum insecticide. At present it is used for the control of mosquito and blackfly larva (Eto, 1974), cattle ticks, stored product insects (Claborn et al., 1968b; and Gutenman et al., 1968), and pest insects of wheat and other cereal crops (McDonald, 1972; and McDonald and Swailes, 1971). This diversity of applications covers four basic environmental systems: soil, plant, animal, and water. These four systems will be reviewed with two themes in mind: 1) the behavior of chlorpyrifos in the environment, and 2) extraction and analysis of chlorpyrifos and metabolites.

(1.2) CHLORPYRIFOS IN THE ENVIRONMENT

(1.21) SOIL

The soil ecosystem provides a complex medium for pesticide activity. Two of the important types of reactions typical of soil are: adsorption reactions, e.g., pesticide adsorption onto the clay lattice surface, and biological reactions, such as pesticide degradation by soil bacteria.

It has been shown that pesticide degradation takes place largely via bacterial metabolism or related enzymatic reactions, although Getzin and Rosenfield (1968) have estab-

lished evidence that nonbacterial, chemical degradation may be significant in certain cases. The rate of pesticide degradation will control the persistence of biological activity in the soil (duration of effectiveness against soil insects). The desired length of this period is largely crop-dependent (Harris and Hutch, 1970). For example, cabbage maggot (Hylemya brassicae) is a serious pest of cruciferous crops. In radishes, a highly effective, short-residual material is all that is required. In contrast, the longer growing season of cabbage requires a moderately residual pesticide.

Chlorpyrifos has been found to be "slightly residual" in the soil by a comparative study of sixteen insecticides (Harris and Hitchon, 1970). In their experiment, zero percent mortality was recorded four weeks after application for the test insect (first-instar nymphs of a common field cricket, Acheta pennsylvanicus) on sandy soil at room temperature. In a second comparative study of seven insecticides, chlorpyrifos was again found to be "slightly residual", with zero percent mortality observed eight weeks after treatment in sandy soil (24°), employing mature full grown individuals of F. candida as the test insect.

Soil temperature has been shown by Thompson (1970) to be a significant factor in the persistence of chlorpyrifos. For the test insect F. candida, zero percent mortality was observed after eight weeks in sandy soil at 24°, and increased to 100% after the same time when the soil

temperature was lowered to 13°. Indeed, 20 weeks after treatment, 50% mortality of the test insect was still observed at this lower temperature.

Soil moisture content has been shown by Harris and Hitchon (1970) to affect pesticide activity in the soil. These workers compared sixteen insecticides applied to dry sandy loam, moist sandy loam, and muck soils. The pesticide activity ratios for the test insect Acheta pennsylvanicus ranged from 1 to 100 (Table 2) with chlorpyrifos having an intermediate value of 10, i.e., 10 times more active in moist soil than dry soil. These ratios may also be used as a comparative index of soil adsorption; higher activity ratios indicating stronger adsorption to soil particles. Thus, chlorpyrifos would be a moderately adsorbed pesticide with respect to the sixteen compounds tested.

In an overall soil activity comparison by Harris and Hitchon (1970), using as criteria; contact toxicity to flies and crickets, toxicities in dry and moist soils, and toxicity in muck soil, chlorpyrifos appeared to be 'most promising as a soil insecticide.' Of the insecticides tested, the bioactivity of chlorpyrifos in soil was exceeded

Table 2. Influence of soil moisture on the bioactivity of candidate insecticides studied by Harris and Hitchon (1970).

Insecticide	ppm in soil[2] showing activity[1]		
	Moist	Dry	Ratio (dry/moist)
methomyl	50	50	1
C-10015	50	100	2
AC 47470	10	50	5
AC 47031	10	50	5
Mobam	10	50	5
domethoate	10	50	5
aldrin	0.1	1	10
Dursban	0.5	5	10
bromophos	5	50	10
AC 43064	5	50	10
mercarbam	50	500	10
Di-syston sulfoxide	50	500	10
C-8874	10	500	50
diazinon	0.5	10	100
Mocap	1	100	100
disulfoton	5	500	100

[1] An insecticide was classed as 'active' when the percent mortality exceeded 10% for the test insect Acheta pennsylvanicus.

[2] Sandy loam soil was used, moist = 12% water, dry = 0% water.

only by that of aldrin (Table 2).

(1.22) PLANT

In plant metabolism studies with corn and beans using [^{36}Cl] chlorpyrifos, it was found that only a small amount of applied radioactivity (1-2%) was translocated into the plant (Smith, Watson, and Fischer, 1967). Labeled chlorpyrifos was applied directly to the leaf, and after three days about 80% of the applied chlorpyrifos was presumably lost due to volatilization, while about 18% remained at the site of application.

Chemical breakdown of the transported chlorpyrifos (1-2%) appeared to be quite extensive. Analysis of the treated leaves showed the presence of traces of [^{36}Cl] chlorpyrifos with significant quantities of [^{36}Cl] pyridinol (VIII) and [^{36}Cl] chloride. This would suggest that hydrolysis and dehalogenation were occuring at the site of application, and the decomposition products were being transported into the plant (Smith et al., 1967a). The importance of UV light in these reactions is discussed in section 1.321.

(1.23) ANIMAL

In two independent studies utilizing rats (Smith et al., 1967) and a lactating cow (Gutenman et al., 1968) it was found that chlorpyrifos was hydrolyzed and eliminated in waste excretion to the extent of 100% and 62% respectively. Chlorpyrifos was found to be degraded during elimination to

diethylthiophosphate (VI) and diethylphosphate (XII) in the lactating cow, and to [^{36}Cl] trichloropyridyl phosphate (VII) and [^{36}Cl] pyridinol (VIII) in rats.

The parent compound (chlorpyrifos) was not detected in milk or waste excretion of the cow, and only trace amounts of chlorpyrifos were found in the feces and urine of the rats. Furthermore, autopsies of the rats revealed that chlorpyrifos accumulated in fat (in which it is more soluble) where it persisted with a half-life of 62 hours.

(1.24) WATER

The hydrolytic stability of chlorpyrifos in water is an important factor due to the wide-spread use of chlorpyrifos as a mosquito larvicide. Schaefer and Dupras (1970) have shown that chlorpyritos exhibits significant residual activity in polluted waters while possessing limited stability in water in the absence of organic matter.

From their concentration-time data, a half-time (section 2.3321) of 6-7 hours (38°) was calculated for chlorpyrifos in pure water,

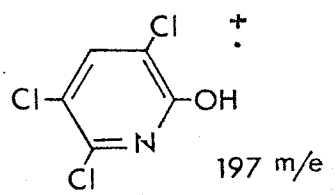
and 15 hr in sewage water. It was observed that chlorpyrifos in sewage water did not degrade completely, but equilibrated at a residual level near 0.003 mg/l. An adsorption-desorption equilibrium between water and organic matter was proposed to account for this residual level, and for the extended six week control period of chlorpyrifos in sewage water.

(1.3) CHEMISTRY OF CHLORPYRIFOS

Knowledge of the chemistry of chlorpyrifos is fundamental in understanding its mechanism of action and metabolism.

Chlorpyrifos was discovered by Dow Chemical Company in 1965 (Kenaga, 1965). It is a white crystaline solid; mp 42.5-43°; and highly soluble in most organic solvents, but almost insoluble in water (0.4 mg/l; Brust, 1966). It is stable except under strong alkaline or acidic conditions. The hydrolysis rate is generally enhanced by the catalytic action of cupric ion (Eto, 1974).

The mass spectrum of chlorpyrifos using electron impact ionization has been reported by Lores (1977), and Luke (1976). As shown in Figure 1, the base peak was the pyridinol moiety as shown below,



197 m/e

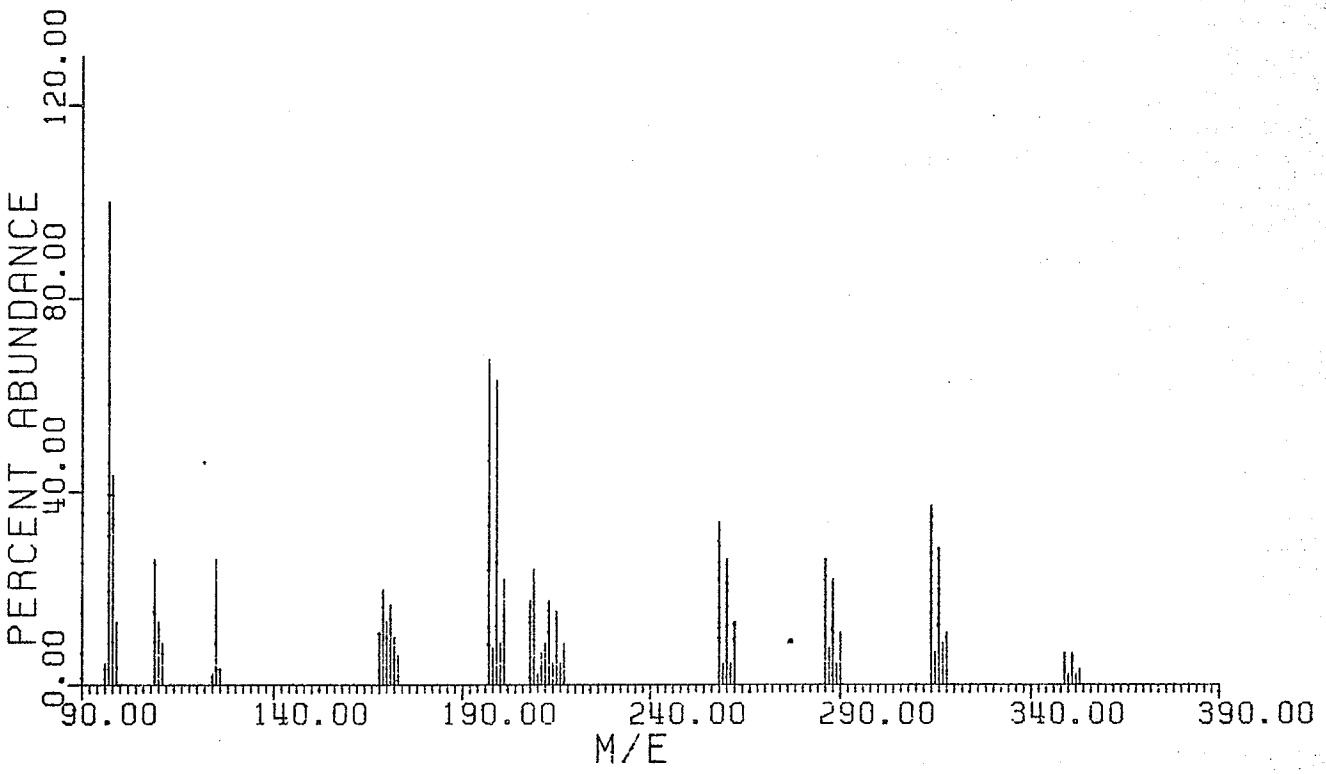


FIGURE 1. CHLORPYRIFOS MASS SPECTRUM (LORES, 1977).

while only a small parent ion was observed.

(1.31) MODE OF ACTION

Pentavalent phosphorus esters have phosphorylating and alkylating properties. Insecticidal and mammalian toxicity is generally accepted as due to the phosphorylation of acetylcholinesterase (Eto, 1974). Inactivation of this enzyme prevents the breakdown of acetylcholine, a neurohormone, causing impairment of the central nervous system.

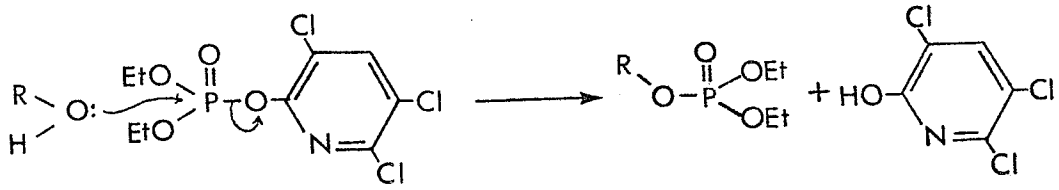
Eto (1974) has pointed out that two prerequisites are necessary for phosphorylating activity: 1) decreased p pi - d pi contribution to the P-OAr bond (i.e., decreased double bond character), and 2) increased positive charge on the phosphorus atom.

P pi - d pi bonding is a result of p electrons from oxygen overlaping with empty d orbitals of phosphorus. This is the configuration of P=O, P=S, P=C, P=N, and P=Se bonds, but a small component is also observed with P-OR bonds (Eto, 1974). For chlorpyrifos, however, this would be reduced by the electron withdrawing effect of the chlorinated pyridine ring.

Smith et al., (1967) have demonstrated that the O-analog of chlorpyrifos is the actual acetylcholinesterase inhibitor. This metabolite satisfies the second prerequisite for phosphorylating activity more so than chlorpyrifos. The greater electronegativity of oxygen compared to sulfur serves to decrease electron density about phosphorus, fa ci-

litating nucleophilic attack at this centre.

The phosphorylation reaction might be drawn as follows,



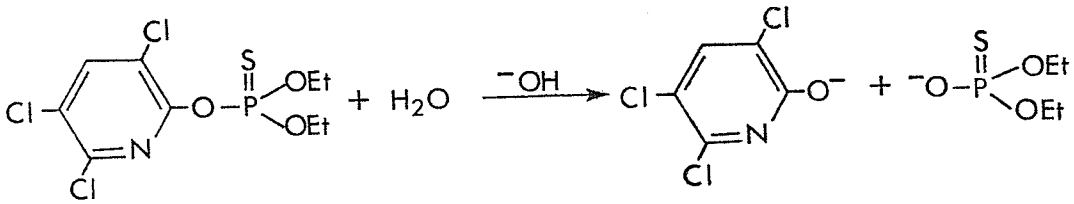
where ROH is the acetylcholinesterase molecule, the OH being that of serine (Eto, 1974).

(1.32) DEGRADATION

Reported metabolic degradation products of chlorpyrifos found in a wide diversity of biological systems are listed in Table 3. The data from this table is presented in flow diagram form in Figure 2.

(1.321) The Pyridinol

Chlorpyrifos hydrolyses in acid and basic media. In the latter media, the pyridinol and O,O-diethyl phosphorothoic acid (XI) are formed as a result of ArO-P bond cleavage (Brust, 1966).



Chlorpyrifos is more resistant to acid hydrolyzes. In this case, the reaction products are the same as those obtained from base hydrolysis, but are present in the protonated

Table 3. Reported degradation products of chlorpyrifos.

Reference	Substrate	Degradation Products[1]
Hutacharern '75	termite	O-analog(II); pyridinol(VIII)
Mann '71	bird	pyridinol
Dishburger '72	bird	pyridinol
Smith '67b	rat(urine)	3,5,6-trichloro-2-pyridyl phosphate(VII); pyridinol
Smith '67a	plant	pyridinol; ethyl-3,5,6-trichloro-2-pyridyl phosphate(V); 3,5,6-trichloro-2-pyridyl phosphate; dechlorinated pyridinol
	soil	pyridinol
Struble '73b	wheat	O-analog
Lores '77	liver (human)	O,O-diethyl O-4-thiomethyl-3,6-dichloro-2-pyridyl phosphorothioate
McKellar '76	milk	pyridinol
Gutenman '68	cow(milk,	diethyl thiophosphate(XI);
Bakke '76	rat (urine)	glucuronide of pyridinol; glycoside of pyridinol; pyridinol
Smith '66	fish	O-ethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate(III); ethyl 3,5,6-trichloro-2-pyridyl phosphate; pyridinol
Metcalf '73	water,alga, snail,larvae, fish	pyridinol
Smith '67b	rat(urine, feces)	3,5,6-trichloro-2-pyridyl phosphate; pyridinol

[1] See Table 1 for structures corresponding to Roman numerals.

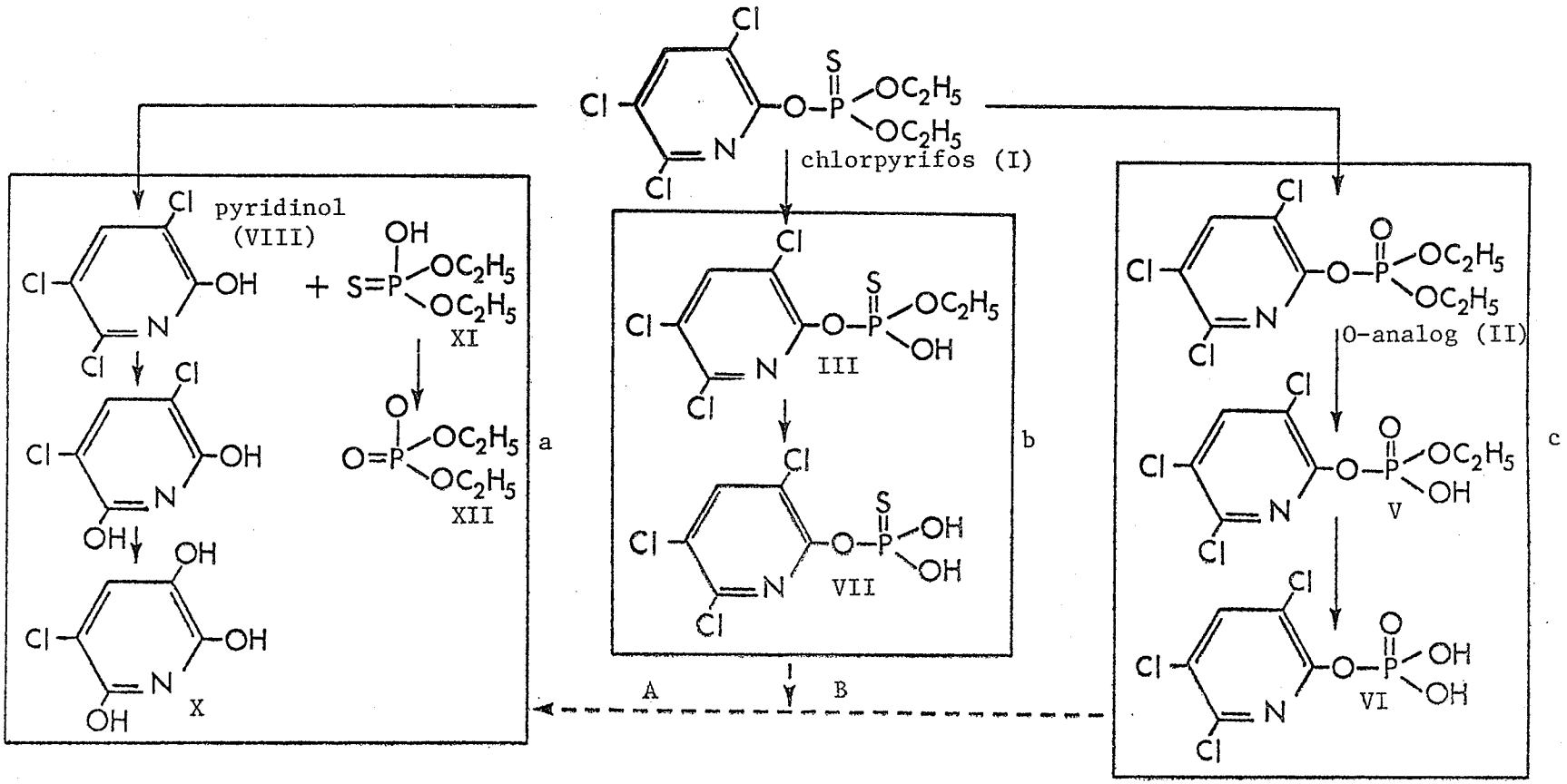
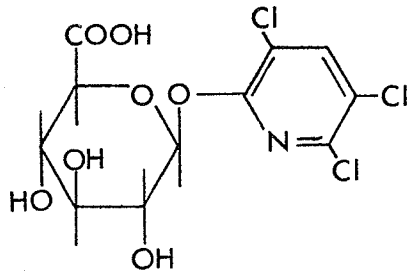


Figure 2. Diagramatic representation of reported chlorpyrifos metabolites (from Table 3). See Table 1 for names corresponding to Roman numerals.

form.

As mentioned in section 1.23, Gutenman et al., (1968) observed that 68% of chlorpyrifos fed to a Holstein cow was accounted for in the urine as the two metabolites: diethylthiophosphate(XI), and diethylphosphate(XII). Although this work did not include analysis for the pyridinol, it is implied that 68% of chlorpyrifos was metabolized to the pyridinol and the other two compounds.

Elimination of ingested chlorpyrifos as the pyridinol (18%) in rat urine was also reported by Smith et al., (1967b). In this study the majority of chlorpyrifos (70%) was eliminated as trichloropyridyl phosphate (VII). In a recent paper, Bakke et al., (1976) showed that this metabolite was incorrectly identified, and was actually the glucuronide of the pyridinol as follows:



In studies of chlorpyrifos metabolism in the rat and the cow, the mechanism of degradation was not discussed. Two possibilities are evident: acid hydrolysis in the digestive tract, and enzymatic breakdown in the liver and kidney. Two factors substantiate the latter process: 1) chlorpyrifos is not degraded by cow rumen fluid (Gutenman et al., 1968), and 2) in rats, chlorpyrifos is rapidly absorbed from the digestive system and circulated thoughout the body (Smith et al., 1967b).

When ring-labeled chlorpyrifos was applied to the leaves of corn and beans, only 1-2% of the radioactivity was translocated into the plant (section 1.22). Smith (1968) found that only 1-2% of solid chlorpyrifos was decomposed when exposed to artificial sunlight. Assuming the decomposition product was the pyridinol, the above translocation of radioactivity may be explained, due to the increased water solubility of the pyridinol compared to chlorpyrifos. In support of this assumption, Smith, in the same paper, found that alcoholic solutions of chlorpyrifos were decomposed to the pyridinol when exposed to UV light. Dechlorination was also observed, with the formation of diols and triols as shown in block 'a' of Figure 2.

In general, the pyridinol was found to be the major metabolite in the animal systems reviewed. In a study of chlorpyrifos metabolism in fish, several P-containing metabolites were observed as well as the pyridinol. The latter metabolite was mostly present at the end of the experiment,

and apparently was slowly decomposed by dehalogenation and by cleavage of the ring (Smith et al., 1966). This process is indicated by arrows A and B in Figure 2.

(1.322) P-containing Metabolites

The O-analog (II) is the most important toxic metabolite of chlorpyrifos. As mentioned earlier it is the active anticholinesterase agent.

The O-analog was not detected in plant, rat, or cow metabolism studies. In termites, however, it was found to be the major metabolite. When these insects were topically treated, the O-analog level reached 18% of all residues after 16 hr (Hutacharern and Knowles, 1975). This difference in metabolism by insects accounts for the high toxicity of chlorpyrifos to insects compared to animals (Kaemmerer and Buntenkotter, 1973; and Kenaga, 1965).

Conversion of chlorpyrifos to the O-analog in insects is probably similar to the general reaction of phosphorothioates cited by Fukuto (1957), i.e., an enzymatic process in the presence of oxygen.

$$(RO)_2-\overset{S}{\overset{\|}{P}}-OR' \xrightarrow[\text{enzyme}]{(O)} (RO)_2-\overset{O}{\overset{\|}{P}}-OR'$$

Other P-containing metabolites as shown in blocks 'b' and 'c' of Figure 2 have been reported, but are of considerably lesser importance. Smith et al., (1967b) described trich-

loropyridyl phosphate (VII) as a major metabolite found in rat urine (section 1.23). However, as discussed in the previous section, this metabolite was identified by mass spectrometry as the glucuronide of the pyridinol by Bakke et al., (1976).

When fish were placed in water containing 300 mg/l chlorpyrifos, two des-ethyl metabolites were detected in fish tissue after 48 hr: ethyl (trichloropyridyl) phosphate (V) and ethyl (trichloropyridyl) phosphorothioate (III) (Smith et al., 1967b). Similarly, where an excessive amount of chlorpyrifos was ingested, Lores (1977) reported an unusual thiomethyl metabolite in a human liver sample (Table 3). The presence of these metabolites in fish and human tissues do not necessarily indicate major metabolic pathways since unusual pathways may have been induced by high concentrations of chlorpyrifos (Lores, 1977).

(1.4) ANALYTICAL METHODOLOGY

Pesticide residue analysis involves three basic processes: extraction, cleanup, and quantitative analysis (usually by gas-liquid chromatography).

Extraction is perhaps the most complex of these three steps. It is very much dependent on the type of substrate, and, as seen from Tables 4, 5, and 6, chlorpyrifos and metabolites have been extracted from a wide diversity of substrates. To simplify the discussion of extraction, these substrates have been assigned to four general groups: soil,

plant, animal, and water.

Reported sample cleanup methods were generally restricted to two types: liquid-liquid partitioning, and column chromatography. Sample cleanup will be discussed with respect to these two topics.

The section on quantitative analysis is concerned with gas-liquid chromatography (GLC). Special attention is paid to the pyridinol which requires derivatization to convert it into a more volitile form for GLC.

(1.41) EXTRACTION

Published extraction results for chlorpyrifos, the O-analog, and the pyridinol are listed in Tables 4, 5, and 6.

Extraction of pesticide residues in general, requires a high degree of association between the substrate and extracting solvent to mobilize the pesticide material. This degree of association is largely a function of substrate-solvent polarity. Thus non-polar substrates such as fat were extracted with non-polar solvents such as hexane, whereas plant materials were extracted with more polar solvents such as methylene chloride. This trend is displayed in the following section dealing with extraction

Table 4. Reported extraction results- Chlorpyrifos.

Reference	Substrate	Method	Solvent[1]	% Recovery
Bowman '68	grass	blend	B	49
	grass	soxhlet	10%C/M	100
Bowman '67	corn,grass	blend	B	97
Braun '74	veg. tissue	blend	Al/B	97
Claborn'68b	fat	blend	H/Al	87
Claborn '68a	cow muscle,liver kidney,heart, brain,spleen	blend	A/celite	87
Dusch '70	water	acidify,H_2SO_4	H	92
	mud	tumble	A	110
	vegetation	blend	A	80
	fish	maceration	Al	75
	duck	maceration	Al	93
	insects	maceration	Al	100
	crustacea	maceration	Al	85
Faust '75	water	--[2]	MC	92
Getzin '68	soil	shake	A/H	--
Gutenman '68	milk	blend	A/B	95
	feces	blend	A/B	82
	urine	blend	A/B	120
Marganian '72	biota,sand, mud,water	blend	Al	--
McKellar'76	milk,cream	SiG[3]	MC	88
Rice '68	water	shake	MC	92
	silt	blend	MC	82
Schaefer '70	water	shake	MC	94
Schaefer '69	water	partition(pH1)	C	--
Struble '73b	wheat plants	soxhlet	B	94
	wht. kernels	grind & shake	B	86
Uk '72	flies	grind	A	100
	flies	grind	H	96
Wetters '71	soil	shake	A	94
	water	shake	MC	92
Winterlin '68	rice & pasture	blend	C	104
	water	--	B	92
	mud,inverts, fish	--	C	93

[1] A=acetone, B=benzene, C=chloroform, H=hexane, Al=acetonitrile, MC=methylene chloride.
[2] not given.
[3] sample absorbed onto an excess of silica gel.

Table 5. Reported extraction results- the O-analog.

Reference	Substrate	Method	Solvent[1]	% Recovery
Bowman	grass	blend	B	47
'68	grass	soxhlet	10%C/M	100
Bowman '67	corn,grass	blend	B	88
Braun '74	veg. tissue	blend	A1/B	83
Claborn'68b	fat	blend	A1/B	92
Claborn	cow muscle,	blend	A/celite	70
'68a	liver,	blend	A/celite	0
	heart,	blend	A/celite	30
	kidney,	blend	A/celite	18
	brain,	blend	A/celite	62
	spleen,	blend	A/celite	58
	milk	blend	A/celite	88

[1] see Table 4 for solvent key.

Table 6. Reported extraction results- the pyridinol.

Reference	Substrate	Method	Solvent[1]	% Recovery
Braun '74	veg. tissue	blend	A1/B	84
McKellar	milk,	acidify,		
'76	cream	shake	B	83

[1] see Table 4 for solvent key.

methodology.

(1.411) Soil

Table 4 contains several methods for extracting chlorpyrifos from soil. Perhaps the simplest and most rapid method was that reported by Wetters (1971). A 20 g soil sample was vigorously shaken with acetone (20 ml) for twenty minutes. The extraction mixture was centrifuged and an aliquot of acetone was analyzed directly by GLC.

Getzin and Rosenfield (1968) modified this method by adding hexane to the acetone/soil system and performing a second extraction. This step would help to remove chlorpyrifos bound to non-polar organic matter components such as fats and waxes (Taylor and Ashcroft, 1972).

Dusch (1970) extracted chlorpyrifos from mud (a soil suspension) with acetone. The aqueous acetone extract was filtered and partially evaporated to remove acetone. The remaining aqueous solution was then partitioned with hexane followed by direct GLC analysis.

Other solvents were also used to extract chlorpyrifos from soil including acetonitrile, chloroform, and methylene chloride.

Marganian (1972) extracted mud samples by blending with acetonitrile followed by a petroleum ether partition and GLC analysis.

Winterlin (1968) extracted chlorpyrifos from mud by shaking with chloroform. An interfering peak was removed by silica gel column cleanup.

Rice and Dishburger (1968) extracted air dried silt with methylene chloride. This extract was evaporated to dryness and redissolved in hexane, followed by multiple acetonitrile/hexane partitions in which the hexane was extracted three times. The collective acetonitrile extract was then evaporated and the residue dissolved in hexane for silica gel column cleanup.

Several of the above methods have incorporated a second solvent partition after the initial extraction. This is a form of sample cleanup as discussed in section 1.42.

(1.412) Plant

Bowman and Beroza (1967) extracted chlorpyrifos and the O-analog from corn and grass by blending with benzene. Struble (1973) used the same extraction technique for the analysis of these compounds in wheat. Both authors employed silica gel chromatography to remove undesired coextractives.

Winterlin (1968) used chlorcform to extract chlorpyrifos from rice and pasture grass samples. Sweep co-distillation was used for sample cleanup.

The above three methods have two things in common, 1) coextractive compounds were present after cleanup (Bowman and Beroza (1967) reported this problem only for low level analyses), and 2) analyses were performed with a flame photometric detector (FPD) in the phosphorus (P) mode.

Phosphorus is incorporated by the plant into many types of compounds, e.g., nucleic acids, phospholipids, coenzymes NAD and NADP, ATP, etc., (Devlin, 1969). The occurence of

P-containing coextractives is, then, not unlikely. The electron capture detector (ECD) responds poorly or not at all to some P compounds (Bowman et al., 1968), while offering excellent sensitivity to chlorpyrifos, and the O-analog and pyridinol metabolites. Use of this detector as opposed to the FPD might have helped to alleviate the problem of plant coextractives.

Extraction of P-containing insecticides from field treated crops was reviewed by Bowman et al., (1968). These authors found that with six P insecticides and their metabolites, best recoveries were obtained with an exhaustive soxhlet extraction using 10% methanol/chloroform.

In a comparison between blending with benzene (5 min.) and 10% methanol/chloroform soxhlet extraction (2 hr) for corn, chlorpyrifos recoveries were found to be approximately 16% higher for the latter method. In a similar comparison with Coastal Bermuda grass, a 4 hr soxhlet extraction with 10% methanol/chloroform extracted 51% more chlorpyrifos and 53% more O-analog than the benzene blend extraction. As shown in Tables 4 and 5, these results contradict an earlier paper by Bowman and Beroza (1967) in which recoveries of 97 and 88% were reported for chlorpyrifos and its O-analog respectively using the benzene blend technique to extract Coastal Bermuda grass.

Braun (1974) reported a method for determining chlorpyrifos, the O-analog , and the pyridinol in vegetable tissue. As seen in Figure 3, the vegetable sample was initially

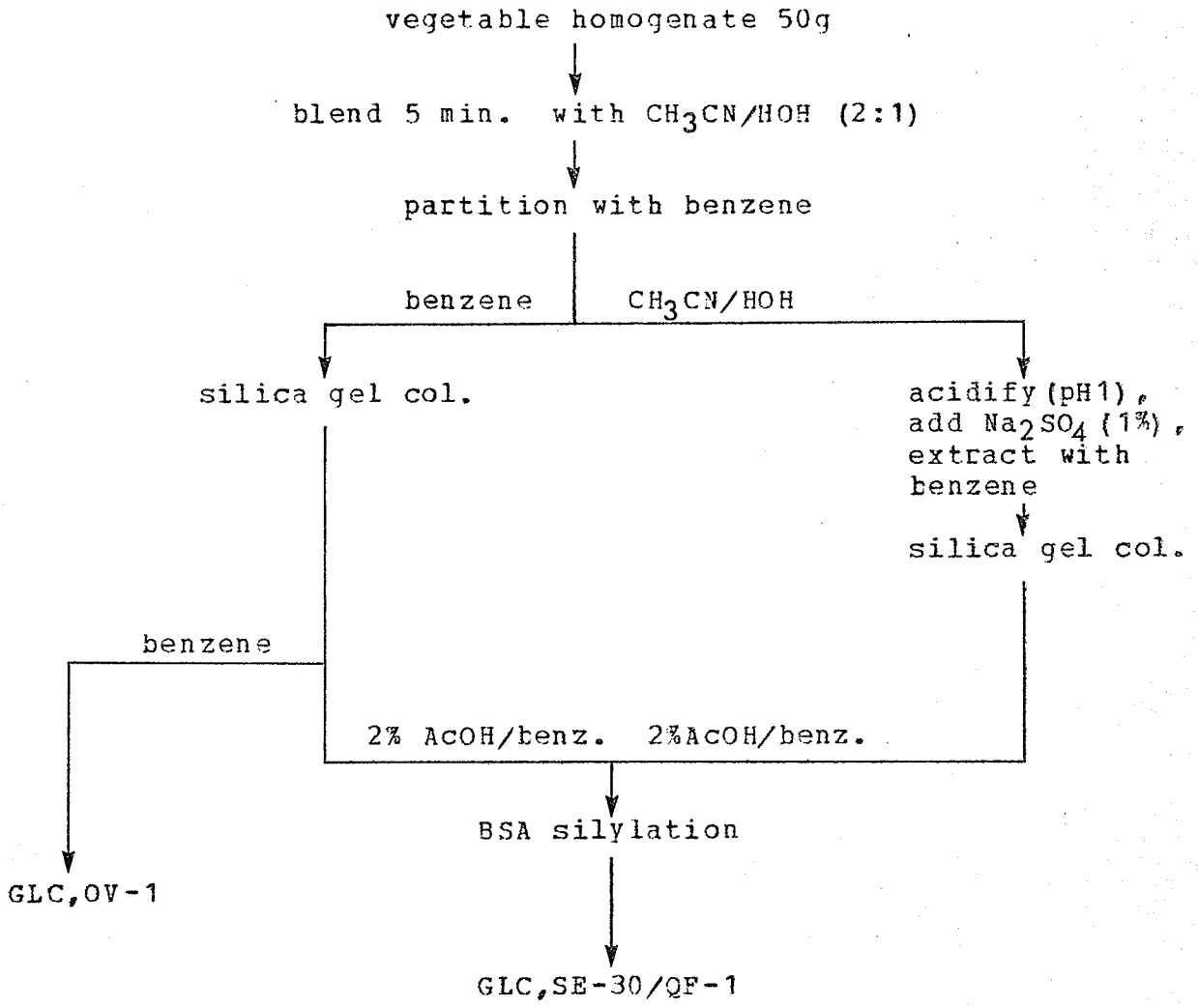


Figure 3. Extraction of chlorpyrifos, the O-analog, and the pyridincl from vegetable tissue (Braun, 1974). See text for description.

blended with acetonitrile/water (2:1, v:v) followed by a benzene partition to extract chlorpyrifos and the O-analog. The aqueous acetonitrile phase was then acidified to pH 1 to protonate the pyridinol, which was extracted by a second benzene partition. Silica gel column cleanup was employed, as discussed in section 1.422.

(1.413) Animal

Claborn et al., (1968b; 1968a) and Ivey and Claborn (1968) extracted chlorpyrifos and its O-analog from milk and several tissues of the Holstein cow including fat, muscle, liver, heart, kidney, and brain. In each case both compounds were extracted by the same method.

Fat samples were extracted with hexane, while all other tissues were initially extracted with acetone. The latter tissues were re-extracted with hexane to mobilize chlorpyrifos and the O-analog from the small amount of fat that was present in these 'non-fatty' tissues (Figure 4). The second important step in the extraction of fatty and non-fatty tissue was the hexane-acetonitrile partition. Chlorpyrifos and its O-analog partition into the more polar acetonitrile, leaving non-polar coextractives in the hexane layer. The acetonitrile fractions were evaporated and made up in 1 ml hexane for column cleanup.

Extraction results of the O-analog (Table 5) were low for liver, heart, and kidney tissue samples. It was postulated that an enzymatic reaction occured when the O-analog was blended with these tissues, resulting in either decomposi-

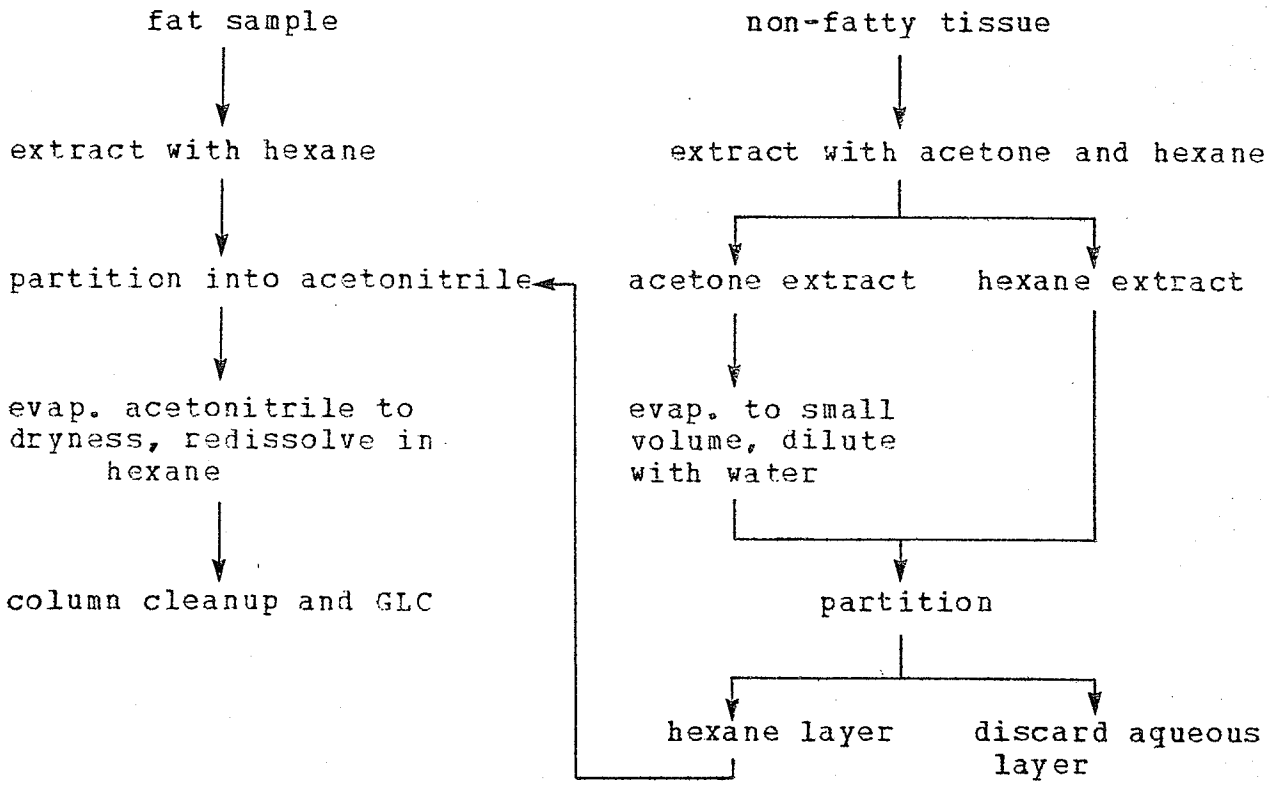


Figure 4. Extraction of chlorpyrifos and the O-analog from fat and non-fatty tissue (Claborn et al., 1968b).

tion, or the formation of a conjugate (Ivey and Claborn, 1969).

Dusch et al., (1970) extracted chlorpyrifos from fish samples using acetonitrile. Extraction of fish and fat samples is similar in that both methods must accomodate excessive amounts of lipid material. To remove these undesirable coextractives, Dusch employed a coagulation solution (1.25 g ammonium chloride plus 2.5 ml of phosphoric acid per litre of water). After cooling of the treated acetonitrile extract solution, the lipid material precipitated and was removed by filtration.

Claborn (1968a) and Ivey and Claborn (1968) determined chlorpyrifos and its O-analog in milk separately, by absorbing the sample into an excess of silica gel. The free-flowing powder was packed into two separate columns. Chlorpyrifos was eluted with 10% methylene chloride/hexane, which was evaporated and the residue dissolved in hexane for GLC analysis. The O-analog was eluted with hexane. Column cleanup was necessary for this extract before analysis.

McKellar (1976) analyzed milk and cream samples for chlorpyrifos and its O-analog by Claborn's method, but also reported a method (Table 6) for extracting the pyridinol metabolite. As shown in Figure 5, the milk or cream sample was acidified to protonate the weekly acidic pyridinol hydroxy group. Salt was also added to further decrease its solubility in milk. The protonated pyridinol was then extracted by benzene partition.

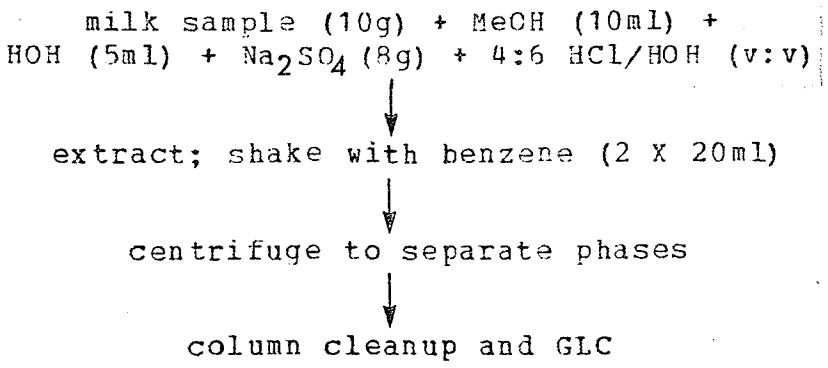


Figure 5. Extraction of the pyridinol from milk (McKellar, 1976).

(1.414) Water

In general, water is the least offensive substrate of those mentioned in this section from the point of view of coextractives. This is reflected in the methods discussed below for extracting chlorpyrifos from water, all of which did not employ column cleanup.

Methylene chloride was used by several workers to extract chlorpyrifos from pond water (Wetters, 1971; Rice and Dishburger 1968; Schaefer, 1970). The methylene chloride extract was evaporated and the residue dissolved in hexane for GLC analysis. In each case, recoveries of over 90% were reported. Schaefer (1969) extracted chlorpyrifos with chloroform, but did not report the percent recovery.

Dusch (1970) used hexane as the extracting solvent, i.e., eliminating the evaporation step mentioned above. This simplification was also accomplished by Winterlin (1968) who used benzene to extract chlorpyrifos from pond water.

Recoveries greater than 90% were reported for both hexane and benzene extractions.

Extraction of chlorpyrifos metabolites from water has not been reported using GLC analysis. Metcalf et al., (1973) extracted the O-analog and the pyridinol from water but employed thin-layer chromatography (TLC) and scintillation counting for quantitative analysis.

(1.42) SAMPLE CLEANUP

Generally, two types of cleanup procedures were employed for removing undesirable coextractives: liquid-liquid partitioning, and column chromatography.

(1.421) Liquid-Liquid Partition

Most of the methods for determining chlorpyrifos and its metabolites incorporated a liquid-liquid partition using solvents of different polarities such as hexane and acetonitrile. The principle involved is the difference in solubility of pesticide and coextractives in the polar or non-polar phase, the degree of separation of pesticide from coextractives being proportional to this difference. Complete separation, however, is seldom observed, since ccextractive materials are rarely of homogeneous nature, tending to distribute between polar and non-polar phases. Conversely, the pesticide may also be soluble in both immiscible phases (Bowman and Beroza, 1965).

This behavior was excellently displayed by Beroza et al., (1969) in their countercurrent distribution studies with a

variety of pesticides, sample extracts, and solvent systems. Of the pesticides and sample extracts examined, most were found to be soluble or slightly soluble in both phases of the solvent systems used, indicating that a complete separation of pesticide from substrate coextractives in one or two partitions would not be possible in most cases.

Liquid-liquid partitioning is useful for 'coarse' sample cleanup before column chromatography. An example of this is the extraction of animal tissue for chlorpyrifos shown in Figure 4. The hexane extract was partitioned into acetonitrile, leaving lipophilic coextractives in the hexane layer. In the method of Braun (1974) for vegetables (Figure 3), the extracting solvent, acetonitrile/water, was partitioned with benzene. In this case most of the plant coextractives remained in the more polar phase (aqueous acetonitrile).

(1.422) Column Chromatography

Florisil (mainly magnesium silicate), silica gel, and alumina are polar adsorbents and are used most frequently for cleanup of pesticide residues.

Table 7 is a list of cleanup columns reported for chlorpyrifos and metabolites. Silica gel was the most widely used adsorbent, the deactivated and 'as received' forms being used most frequently. Florisil and acidic alumina were reported only once for the analysis of chlorpyrifos and the pyridinol respectively.

Activation of polar adsorbents is accomplished by heating to remove adsorbed water (100-200° for several hours),

Table 7. Reported cleanup methods.

Reference	Column[2]	Solvent[1]	Substrate	Compound
Bowman '67	SiG 10g	B	corn,	chlor[4]
	+Na_2SO_4	A	grass	O-analog
Braun '74	SiG 10g	B	veg. tissue	chlor
	A	2%AcOH/B		O-analog[3], pyridinol
Claborn '68	SiG 9g 12%DA	8%MC/H	animal tis.	chlor
	SiG 12g			
	12%DA	HOH sat. MC	animal tis.	O-analog
Dusch '70	SiG	20%MC/H	veg. tissue	chlor
	Flor 8cm A	A	duck, insects	chlor
McKellar'76	SiG 8g	MC/H	milk,	chlor
	+Na_2SO_4	MC	cream	O-analog
	AcAl 4g A	40%HCl/HOH		pyridinol
Rice '68	SiG 9g		water,	
	+Na_2SO_4	8%MC/H	silt	chlor
Struble	SiG 16g	40%MC/H	wheat	chlor
'73b	12%DA	HOH sat.MC		O-analog
Uk '72	SiG 1g	B/H 1:1	insects	chlor
Winterlin	SiG	20%C/H	mud,fish,	
'68			inverts	chlor
Lores '77	SiG	B/H	liver (human)	see Table 3

[1] Solvent key: A=acetone, B=benzene, C=chloroform, Al=acetonitrile, MC=methylene chloride, AcOH=acetic acid.

[2] Column codes: SiG=silica gel, Flor=florisil, AcAl=acidic alumina, A=activated, DA=deactivated.

[3] eluted as the pyridinol, an hydrolysis product.

[4] chlor=chlorpyrifos.

whereas deactivation is accomplished by the accurate addition of water. Partially deactivated materials are often preferable to very active ones since even the transference of highly active adsorbents is liable to result in alteration of activity of the transferred material. In addition, covering of the most active sites in an adsorbent results in more homogeneous adsorptive power and consequently less tailing on elution (Morley, 1966).

Versino *et al.*, (1971) compared florisil and silica gel columns using malathion, parathion, and their *O*-analogs. For the solvent systems used, no significant difference was observed for the parent compounds, although the *O*-analogs were not eluted from the silica gel column.

In the method of Braun (1974) for vegetable tissue, the chlorpyrifos *O*-analog was eluted from silica gel as its hydrolysis product, the pyridinol, with 2% AcOH/benzene. This *O*-analog-derived pyridinol was derivatized and analyzed by GLC as shown in Figure 3. Metabolically-derived pyridinol was also analyzed in this method; column cleanup was performed on a separate silica gel column and the pyridinol

was eluted with the same solvent (2% AcOH/benzene).

(1.43) GLC ANALYSIS

Listed in Table 8 are GLC columns reported for the analysis of chlorpyrifos and metabolites. Of the liquid phases used, the order of popularity is DC-200 (4), SE-30 (3), OV-17 (2), SF-96 (2), and Carbowax-20M (2), where the bracketted numbers indicate the frequency of use (not including mixed columns).

Two problems have generally been associated with the cochromatography of chlorpyrifos and its O-analog: 1) resolution of the two compounds, and 2) obtaining optimum sensitivity for the O-analog. Characteristic of many P=O compounds, the O-analog binds more strongly to the column, reducing sensitivity, and often eluting as a tailing peak. This is largely due to an increase in polarity when sulfur is replaced by the more electronegative oxygen.

Several workers have compared different columns for their ability to resolve chlorpyrifos and its O-analog. Struble (1973a) examined several Carbowax and Ucon liquid phases. Ucon 75-H-90000 gave the best resolution of chlorpyrifos and its O-analog as well as providing the highest sensitivity for the O-analog.

Watts (1968) compared 10% DC-200, 10% DC-200/15% QF-1, and 2% diethylene glycol succinate (DEGS). The 10% DC-200 column gave poor resolution, while offering the best sensitivity for the O-analog. The mixed-phase column, on the

Table 8. Reported GLC columns used for analysis of chlorpyrifos and metabolites.

Reference	Column	Compound(s) Analyzed[2]
Bowman '67	5% DC-200	chlor, O-analog
Bowman '70	5% OV-101	chlor, O-analog
	5% OV-17	chlor, O-analog
	5% OV-210	chlor, O-analog
	5% OV-225	chlor, O-analog
Braun '74	4% SE-30/6% QF-1	pyridinol(TMS[1])
	5% OV-1	chlor
Claborn '68	5% SF-96	chlor
	8% DC-200	O-analog
Cochrane '76	3% Silar-5CP	chlor, O-analog
	3% OV-17	chlor, O-analog
Dusch '70	3% Carb[3] 20M	chlor
Gutenman '68	10% DC-200	chlor
Lores '77	2% OV-17/2% OV-210	chlor
Marganian '72	5% UCON-W98	chlor
McKellar '76	5% DC-200	chlor, O-analog, pyridinol(TMS[1])
Rice '68	5% SE-30	chlor
	5% SF-96	chlor
Schaefer '69	1% SE-30	chlor
Struble '73	5 % UCON 75-H-90000	chlor, O-analog
Struble '73a	5% UCON 50-H-5100	chlor, O-analog
	5% Carb[3] 20M	chlor, O-analog
	5% Carb[3] 20M TPA	chlor, O-analog
Uk '72	3% SE-30	chlor
	3% QF-1	chlor
Watts '69	10% DC-200	chlor, O-analog
	2% DEGS	chlor
	10% DC-200/15%QF-1	chlor, O-analog
Wetters '71	11% OV-17/11% QF-1	chlor

[1] TMS=trimethylsilyl derivative.
[2] chlor=chlorpyrifos.
[3] Carb=Carbowax.

other hand, provided better separation at the expense of loss of sensitivity for the O-analog (about 6 fold).

Of the OV phases examined by Bowman et al., (1970), OV-210 provided the best resolution of chlorpyrifos and the O-analog (O-analog response data were not given).

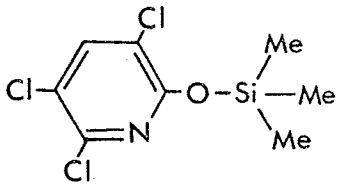
McKellar et al., (1976) separated chlorpyrifos, the O-analog, as well as the pyridinol (as the BSA derivative) on a 5% DC-200 column. Braun (1974) determined all three compounds in vegetable tissue, but, as previously discussed, analyzed the O-analog as the pyridinol (BSA derivative). Braun chromatographed chlorpyrifos on a 4% SE-30/6% QF-1 mixed-phase column, whereas the derivatized pyridinol was analyzed separately on a 5% OV-1 column.

(1.431) Derivatization of the Pyridinol with Diazomethane

The pyridinol is not chromatographable under conditions used for chlorpyrifos due to its low volatility. This may be increased by attaching an electron-donating group to the 2-hydroxy oxygen, such as a methyl or trimethylsilyl moiety.

As seen in Table 6, GLC analysis of the pyridinol has been reported by two authors. Both McKellar et al., (1976) and Braun (1974) reacted the pyridinol with N,O-bis(trimethylsilyl) acetamide (BSA) to synthesize the trimethyl-

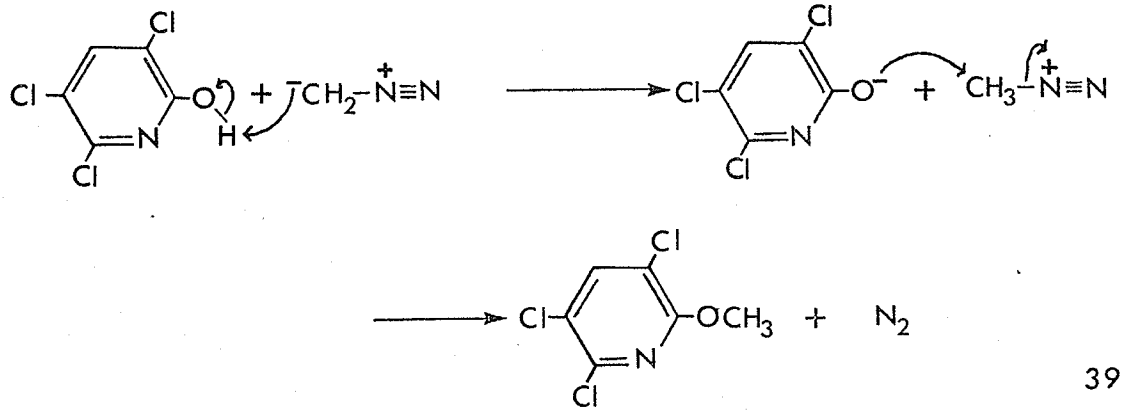
silyl derivative.



Methylation is another important derivatization reaction. It is commonly employed to esterify carboxylic acids, using diazomethane or methanolic boron trifluoride (Drozd, 1975).

Methylation of phenols has been reported with diazomethane (Smith, 1937; Khan, 1975; Hilgetag and Martini, 1972; Fieser and Fieser, 1967). The reaction is usually quantitative, and takes place under mild conditions in an inert medium such as ether, alcohcl, or chloroform (Hilgetag and Martini, 1972).

Reaction velocity increases with the acidity of the OH group, indicating that the reaction mechanism may involve protonation of the diazomethane carbon (Smith, 1937): the methylation reaction might be written as follows.



Diazomethane was successfully employed in this study for methylating the pyridinol metabolite of chlorpyrifos.

PART 2.

Chlorpyrifos Degradation in Pond Water.

(2.1) INTRODUCTION

Chlorpyrifos has been shown to be effective in mosquito control as a larvicide. This section contains a study of the chemical stability of chlorpyrifos in pond water, an important factor in effective larval control.

In the summer of 1975, a cooperative study was conducted at Glenlea Manitoba in which man made pools were fortified with chlorpyrifos. Water samples were collected throughout the summer to monitor the change in chlorpyrifos concentration. Field work and bioassay analysis of water samples was conducted by Gary Rawn (Entomology Department, University of Manitoba). GLC analysis of water samples was conducted by the author.

(2.2) MATERIALS and METHODS

Pesticide grade organic solvents were obtained from Caledon Laboratories Inc. Anhydrous sodium sulfate was obtained from Fisher Scientific Co., and was washed with hexane before use. An analytical standard of chlorpyrifos for GLC analysis, as well as the 2.5% slow release and 48% emulsifiable concentrate formulations of chlorpyrifos were provided by Dow Chemical Company of Canada Ltd.

(2.21) ARTIFICIAL POOLS

Each pool at Glenlea was formed from a wooden frame with dimensions of 1 m square by 30 cm deep and sunk into the ground to a depth of approximately 15 cm.

Inside the wooden frame was placed a lining of 4 mil polyethylene, which was covered with a 5 cm layer of sod (sod was held on the sides by wooden wedges between sod pieces).

A rectangular plot of twelve such pools was fabricated with a minimum distance of 1.8 m separating each pool.

During the degradation experiment, pool volumes were maintained at 150 l. The water source was a large polyethylene-lined dugout containing spring melt water.

(2.211) Pool Fortification

Listed in Table 9 are the fortification rates for each pool as well as the type of formulation used. At the bottom of this table, dates from spiking to last sampling are also included.

Table 9. Chlorpyrifos fortification levels of individual pools at Glenlea.

Pool	Fortification Rate (lb/acre)	(mg/l)	Formulation[1]
1	0.050	0.0383	2.5% SR
2	0.0	0.0	control
3	0.050	0.0383	48% EC
4	0.025	0.01916	2.5% SR
5	0.050	0.0383	48% EC
6	0.250	0.1916	2.5% SR
7	0.050	0.0383	2.5% SR

Date of fortification to date of last sampling; August 18 to September 11 1975.

[1] 2.5% SR= 2.5% Active ingredient granular slow release. 48% EC= 48% emulsifiable concentrate.

From the percent active ingredient as stated by the manufacturer, weights of 48% emulsifiable concentrate and 2.5% slow release granular formulations were calculated to fortify each pool to the desired level. Fortification was achieved by applying the correct weight of formulation evenly into each pool without stirring.

(2.212) Sampling

Sampling was accomplished by combining five 100 ml samples taken at the corners and centre of each pool. Each sample was collected with a 100 ml beaker attached to a long-handled clamp.

Three 100 ml aliquots of this 500 ml mixture were pipetted into 170 ml silanized medicine bottles, and stoppered with teflon-lined caps. Two of these samples were

submitted for GLC anaysis, while the remaining 100 ml water sample was bioassayed using Culex tarsalis.

(2.22) CHLORPYRIFOS ANALYSIS

(2.221) Bioassay

Twenty-four 4th instar larvae of Culex tarsalis were added to the 100 ml water sample immediately upon arrival in the laboratory. The sample bottle containing the larvae was then stored in the dark for 24 hours following which, the percent mortality was recorded.

The C. tarsalis colony originated from the Glenlea Research Station and was reared in the laboratory. Female adults were blood fed with Japanese Quail (Coturnix Coturnix Japanica) in plexiglass cages (30 cm^3) maintained at 25° and 73% relative humidity. The eggs deposited were allowed to pupate in photographic trays. Pupae were transferred to the plexiglass cages where they developed to the larval stage which was fed sixty mesh liver powder (Rawn, 1977).

(2.222) Gas-Liquid Chromatographic Analysis

Pond water samples were extracted immediately upon arrival in the laboratory such that the time between sampling and extraction was approximately one hour.

(2.2221) Extraction

The method of Wetters (1973) was employed to extract chlorpyritos from water, with minor modifications (Figure 6). Water samples (100 ml) were extracted with methylene chloride by gentle rocking rather than shaking. The latter procedure produced an emulsion that in some cases would not settle.

Methylene chloride extracts were drained into silanized medicine bottles (170 ml) fitted with teflon-lined caps. Pond water extracts were stored at -40° awaiting GLC analysis.

Removal of water from the methylene chloride extract was accomplished by shaking with a small amount of sodium sulfate. This was done until no visible water remained.

The dried extract was poured into a 100 ml round-bottom-flask (RBF), being careful not to transfer any sodium sulfate. The contents of this flask were evaporated to ca. 20 ml to make room for the medicine bottle rinses (2 X 15 ml hexane). The hexane/methylene chloride extract was then evaporated just to dryness. Both evaporations were done on a rotary evaporator with the flask immersed in a 35-40° water bath. The pressure of the system was reduced with a tap aspirator.

The residue was dissolved in 5.0 ml hexane and analyzed

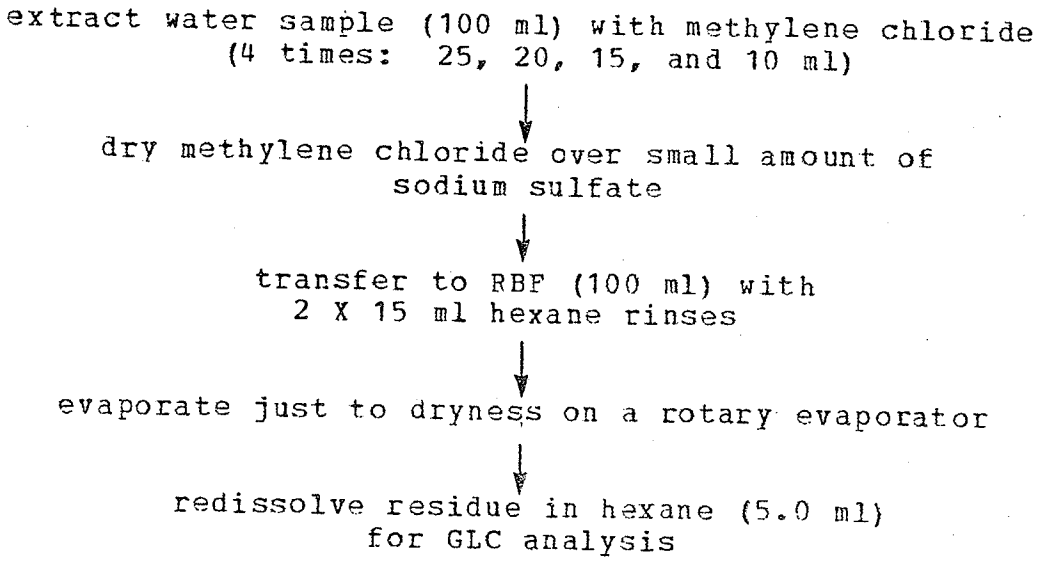


Figure 6. Extraction of chlorpyrifos from water.

directly by GLC (Figure 6).

(2.2222) GLC

Listed below are the GLC parameters used for sample analysis.

Instrument: Varian 2400.

Column: 5% DC-200/15% QF-1 on Gas Chrom Q, 80-100 mesh, 122 cm X 4 mm ID glass, nitrogen carrier, 50 ml/min.

Detector: Electron capture, ^{3}H.

Temperatures: oven 200°, injection port 223°, detector 220°.

Standard curves were made weekly to establish the linear range of the electron capture detector (ECD). All samples were analyzed qualitatively to determine the dilution required, if any, for the chlorpyrifos peak to remain in the linear range with a 2 µl injection.

The external standard method was employed, with all runs being made at an attenuation of $32X10^{-10}$ amps and with a constant injection volume of 2 µl. The injection sequence was as follows: standard, 4-5 samples, standard, 4-5 samples...

Each injection was made in duplicate, although under unstable conditions more injections per sample were required. With each injection the sample identification, attenuation, injection volume (µl), and dilution data were recorded. Chlorpyrifos concentration was calculated from a standard curve using peak height. External standard injec-

tions were used to correct for day-to-day changes in detector response.

(2.23) EXTRACTION EFFICIENCY DETERMINATION

Standards of chlorpyrifos were made from technical grade chlorpyrifos and the emulsifiable concentrate, the same formulation used in the field experiments;

Technical standard- 0.0500 g AI/100 ml acetone.

Emulsifiable concentrate standard- 0.0497 g AI/100 ml acetone, where AI= active ingredient.

Distilled water and pond water were fortified with these standards by adding 42 to 360 µl directly to 1 litre of water to obtain application rates of 0.02, 0.04, and 0.2 mg/l. After a two hour equilibration period at room temperature, 100 ml samples were extracted (Figure 6).

(2.3) RESULTS and DISCUSSION

(2.31) EXTRACTION EFFICIENCY

Chlorpyrifos extraction efficiency results from pond water and distilled water are presented in Table 10.

Recovery of chlorpyrifos from distilled water appeared to decrease as the fortification rate increased. At the lowest rate of 0.02 mg/l, essentially all of the chlorpyrifos was recovered, whereas at the highest rate (0.2 mg/l) 67±4% was extracted. This phenomenon was observed in other tap water

extraction experiments as discussed in section 3.37.

Such a trend was not observed in pond water results (section 3.37). The average recovery of all rates was 57±3% (99% confidence limits). Although this value was low, its reproducibility justified the use of this extraction method in the pond water persistence study of chlorpyrifos.

Table 10. Chlorpyrifos extraction efficiency results.

Fortification Rate (mg/l)		Percent Recovery			
		Distilled Water	n	Pond Water	n[1]
0.02	technical[3]	98±8	4	60±4	4
0.04	"	82±23	6	49±3	4
0.20	"	67±3	6	60±2	4
0.02	EC[4]	102±6	6	52±5	20
0.04	"	72±4	8	57±8	17
0.20	"	67±4	10	51±4	11
Average[2]				57±3	

[1] n = number of results averaged.
[2] 99% confidence limits.
[3] technical grade standard of chlorpyrifos.
[4] 48% emulsifiable concentrate formulation of chlorpyrifos.

(2.32) STORAGE DEGRADATION

As mentioned earlier, Glenlea water samples were extracted immediately upon arrival in the laboratory, and the methylene chloride extracts were stored at -40°. The duration of this storage period was of the order of 10 months.

An experiment was conducted to determine the stability of chlorpyrifos in methylene chloride extracts stored at this temperature. Several water samples were taken from pool 6 (fortified to 0.2 mg/l), 72 hours after chlorpyrifos application. The extracts from these samples were stored at -40°, and retrieved after various storage periods for analysis.

As shown in Table 11, no significant storage degradation of methylene chloride extracts occured.

Table 11. Storage degradation of chlorpyrifos extracts.

Storage Time (days)	Concentration (ng/ml)	n[1]
0	2.6±.9	5
28	2.5±.5	2
49	2.9±.4	2
61	2.1±.7	5
81	1.7±.5	3
220	2.5±.5	5

[1] n = number of results averaged.

(2.33) POND WATER ANALYSES

The degradation of chlorpyrifos in pond water was monitored by bioassay and GLC analyses.

(2.331) Bioassay Results

Bioassay results for pools 1, and 3-7 are shown in Figures 7-12. Zero percent mortality was observed throughout the study for the control pool (2).

Considerable data scatter was observed in pool 1 and 7 results (Figures 7 and 8). Regressional analysis was employed in these two cases to fit a curve through the data points. A third order polynomial was chosen due to the 'S' shaped degradation curve generally observed for the majority of pools.

Bioassay results of pools 3-5 (Figures 9-11) showed a distinct pattern, with less data scatter. This allowed a line to be drawn by hand through the data points. The validity of these curves, however, in areas of rapid percent mortality change with time may be questionable due to a lack of data in these regions.

Pools 1, 3, 5, and 7, (Figures 7-10) were fortified to the level recommended for larval control (0.04 mg/l); pools 1 and 7 being treated with the slow release (SR) formulation, and pools 3 and 5 with the emulsifiable concentrate (EC) of chlorpyrifos. The LD^{50} ($LD^{50} = LD_{50}$) concentration was reached at 125±4 hr (n=2) for pools 3 and 5 (EC), and at

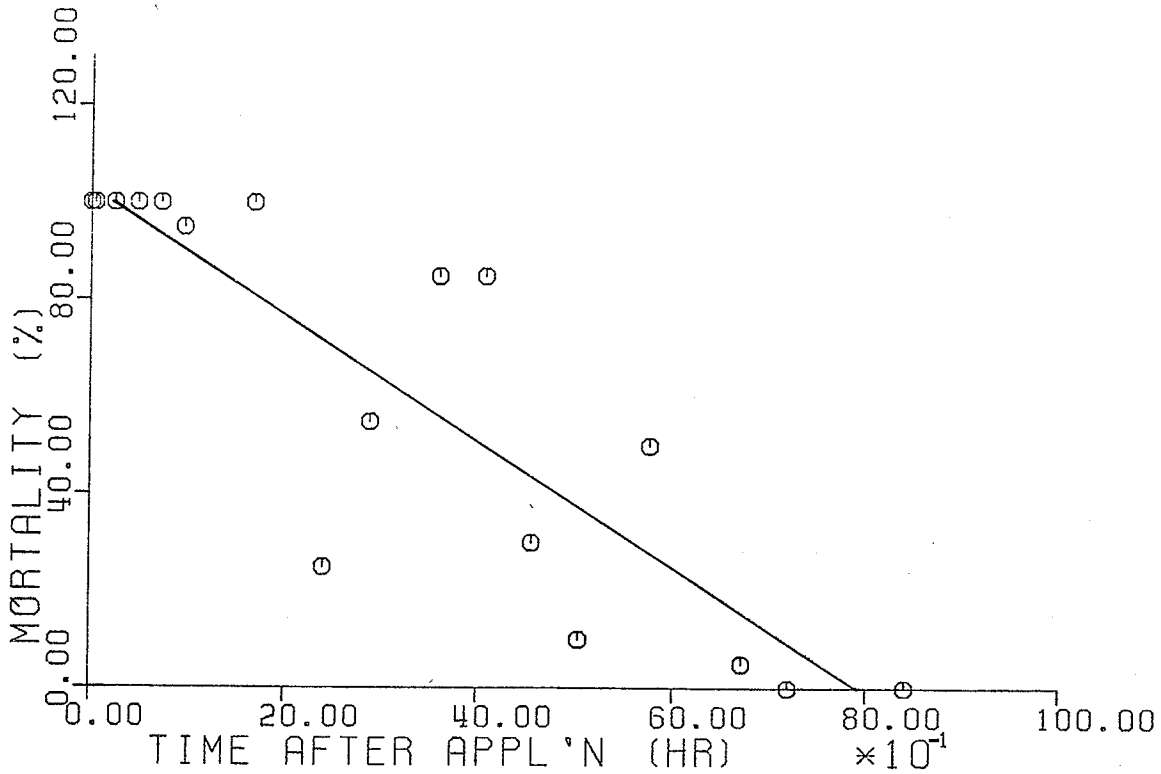


FIGURE 7. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 1, 0.04 MG/L, SR.

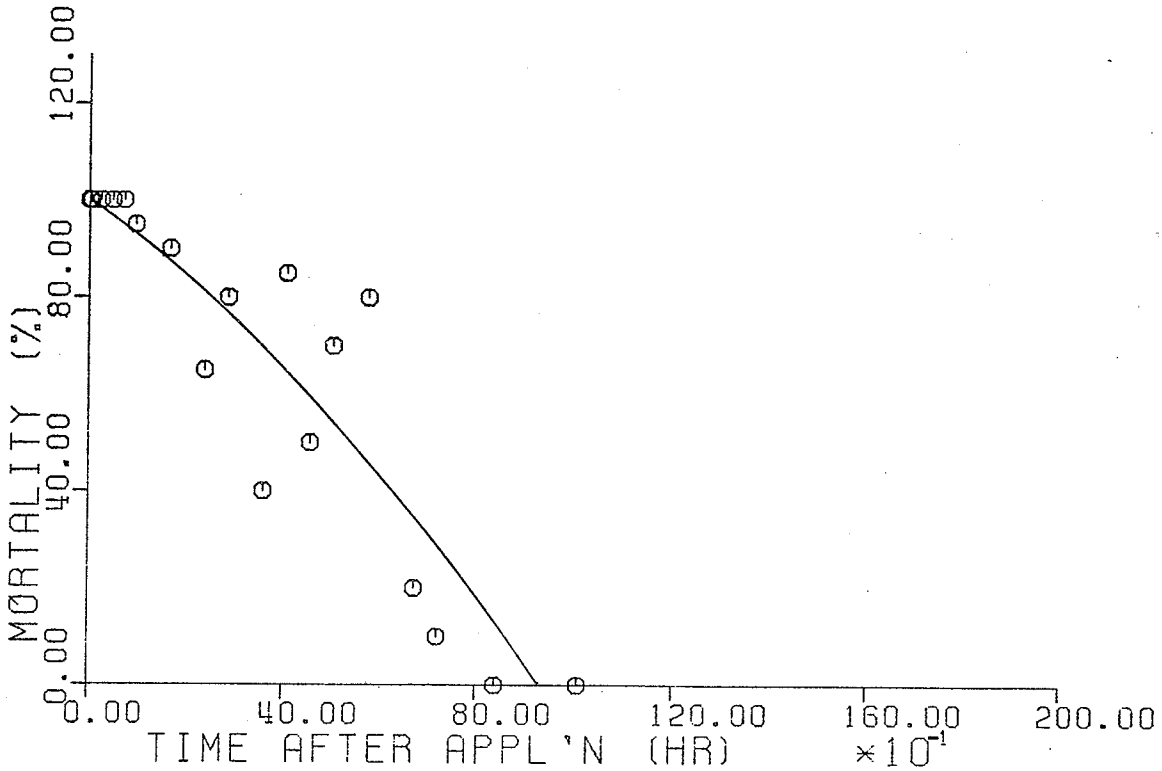


FIGURE 8. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 7, 0.04 MG/L, SR.

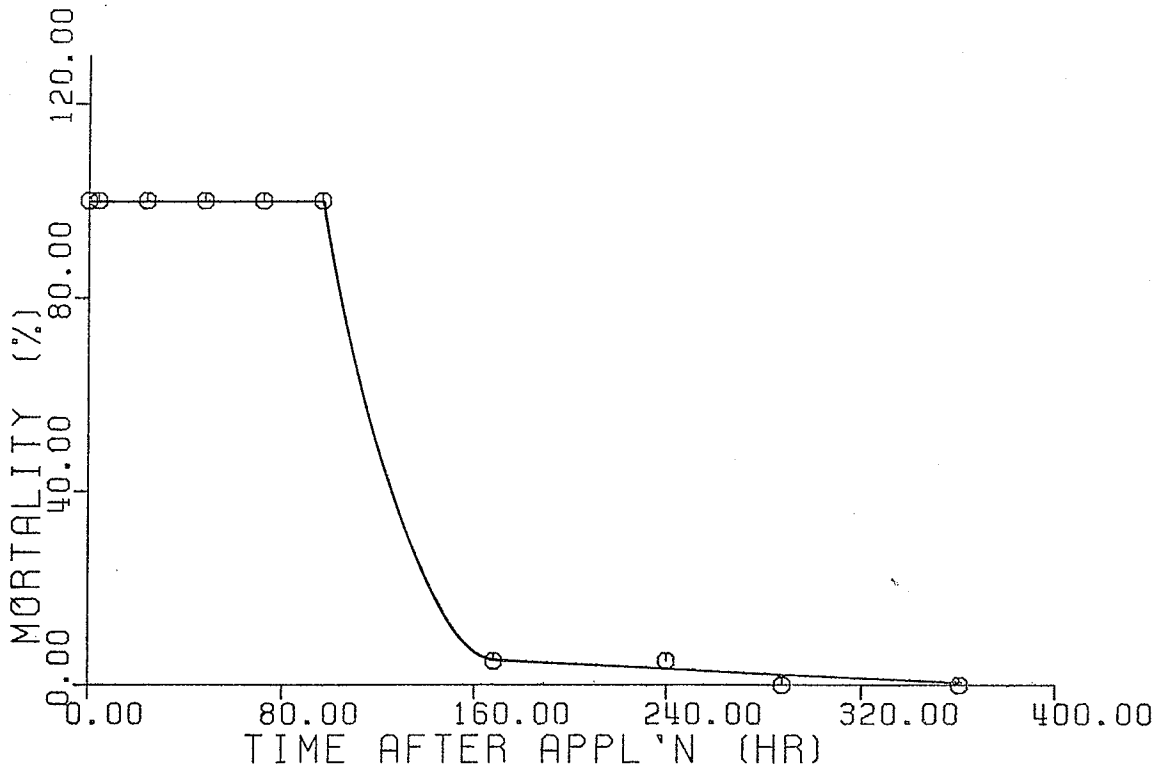


FIGURE 9. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 5, 0.04 MG/L, EC.

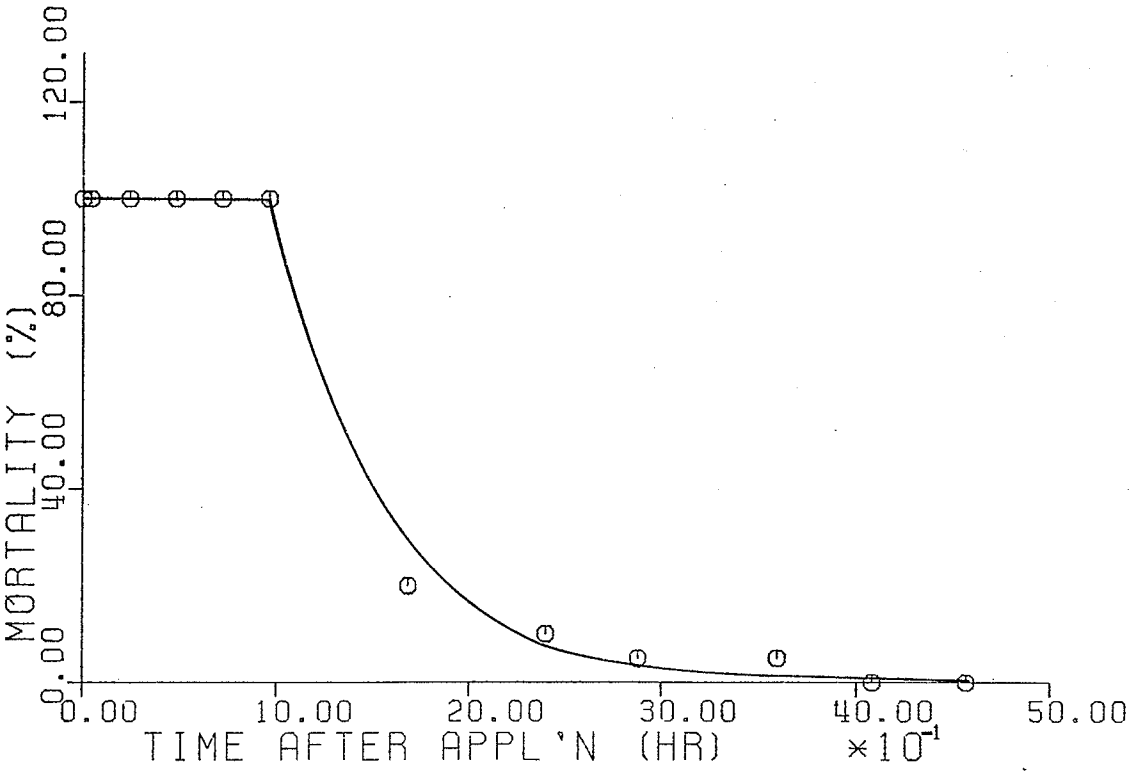


FIGURE 10. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 3, 0.04 MG/L, EC.

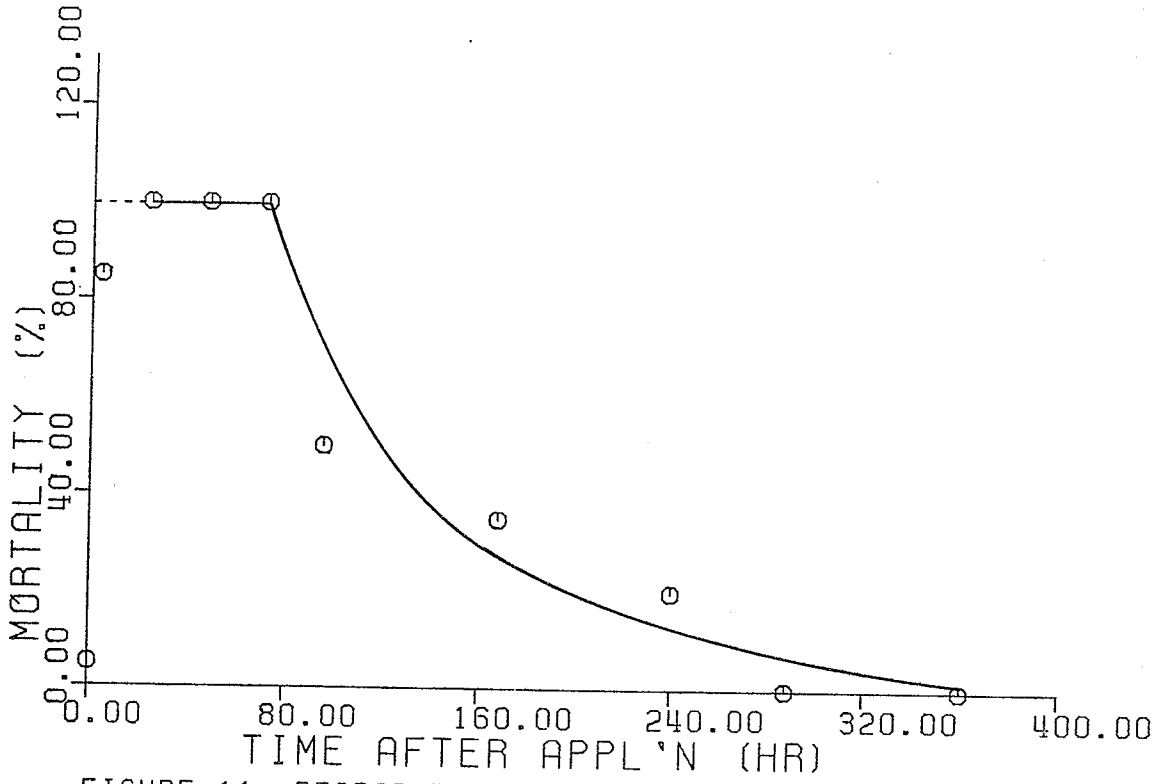


FIGURE 11. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 4, 0.02 MG/L, SR.

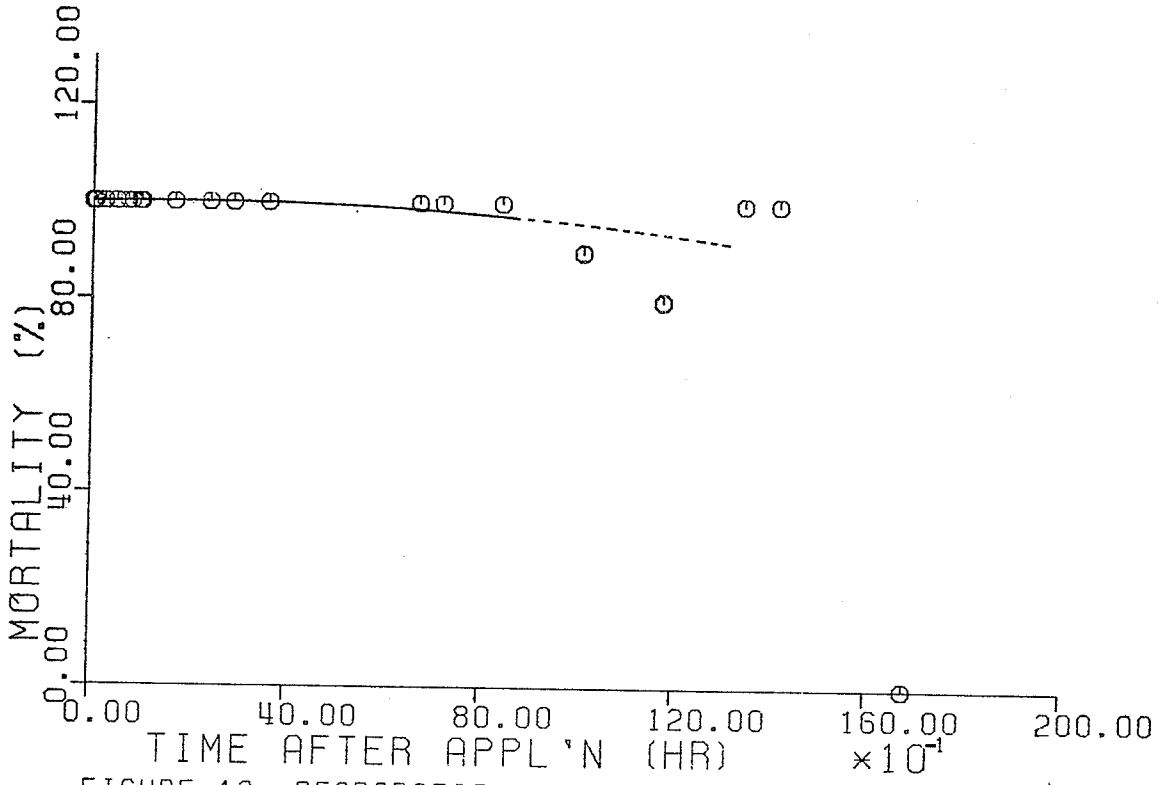


FIGURE 12. DEGRADATION OF CHLORPYRIFOS IN POND WATER; BIOASSAY RESULTS. POOL 6, 0.20 MG/L, SR.

478±98 hr (n=2) for pools 1 and 7 (SR). Zero percent mortality was not observed until 860±100 hr (n=2) for the latter pools, and occured much sooner for pools 3 and 5; approximately 300 hours (n=2, n is the number of results averaged, i.e., one from each pool). These data adequately displayed the effectiveness of the 2.5% slow release formulation over the 48% emulsifiable concentrate formulation of chlorpyrifos.

Pools 6 and 4 were fortified with 5 and 0.5 times the recommended rate respectively (0.2 and 0.02 mg/l, 2.5% slow release formulation). The higher dosage of pool 6 resulted in 100% mortality for nearly the entire sampling period (58 days), whereas pool 4, fortified to half the recommended level, attained the LD^{50} concentration at approximately 100 hours after chlorpyrifos application (Figures 11 and 12). It is interesting to note that half the recommended dosage of the 2.5% slow release formulation degraded to the LD^{50} concentration at about the same time as the full dosage of the 48% emulsifiable concentrate formulation.

(2.332) Gas-Liquid Chromatography Results

GLC results of chlorpyrifos degradation in pond water are shown for each pool in Figures 13-19. Each data point in these figures represents an average of four results. The control pool (Figure 19) showed an erroneous chlorpyrifos concentration at t=240 hours. This, however, was not reflected in the fortified pools and was attributed to sample contamination during analysis.

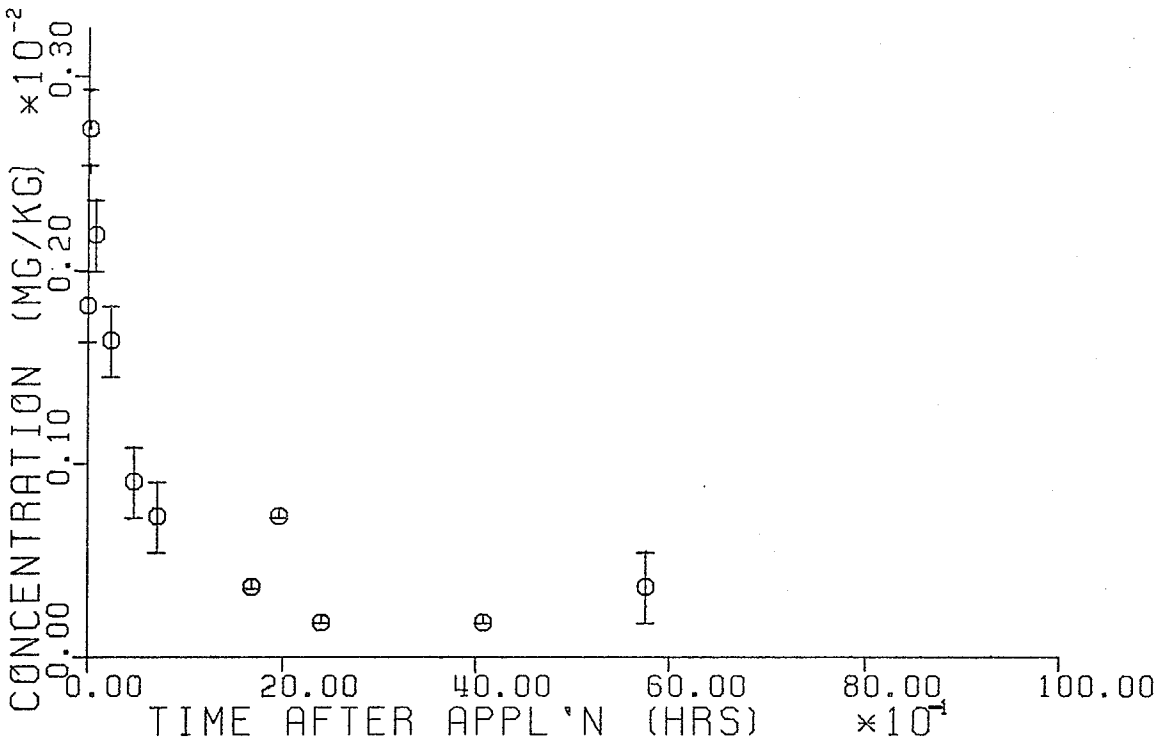


FIGURE 13. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 1, 0.04 MG/L, SR.

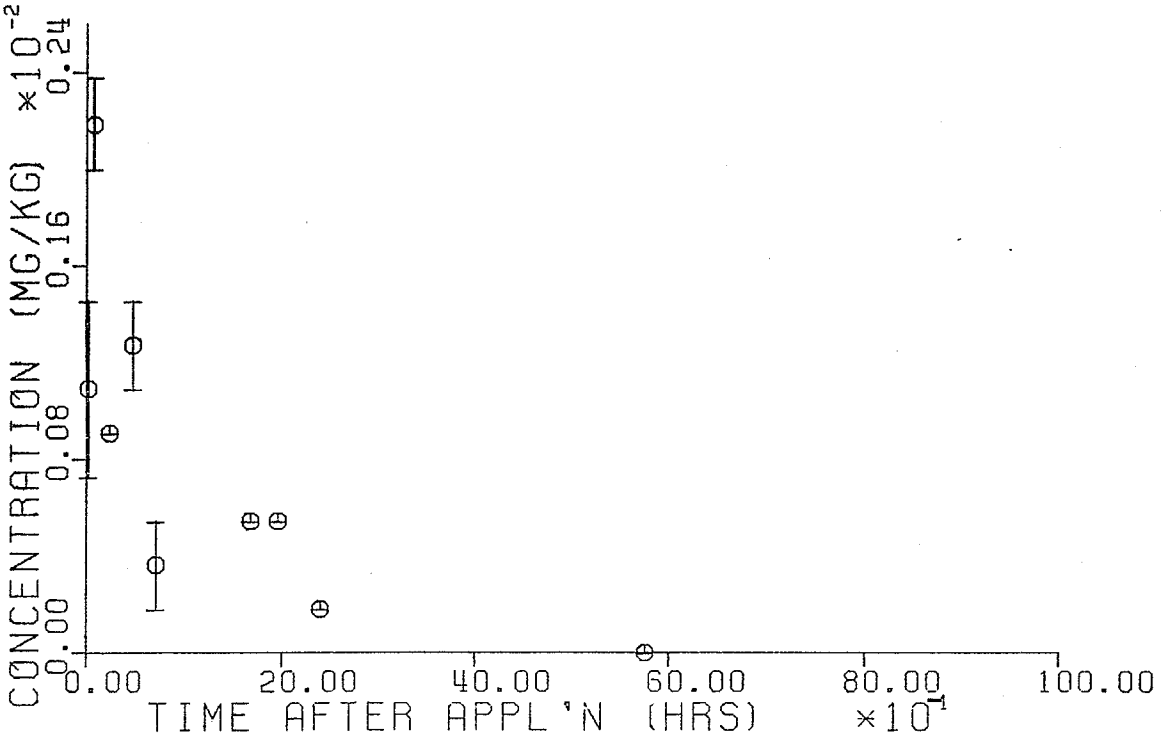


FIGURE 14. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 7, 0.04 MG/L, SR.

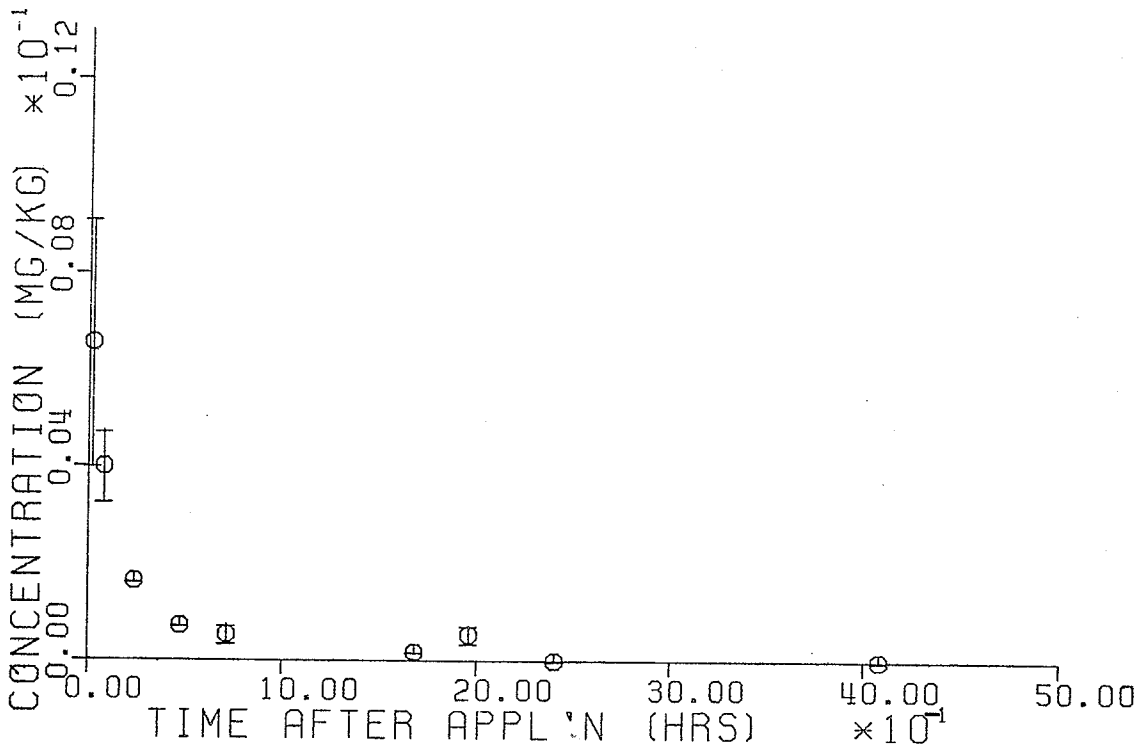


FIGURE 15. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 5, 0.04 MG/L, EC.

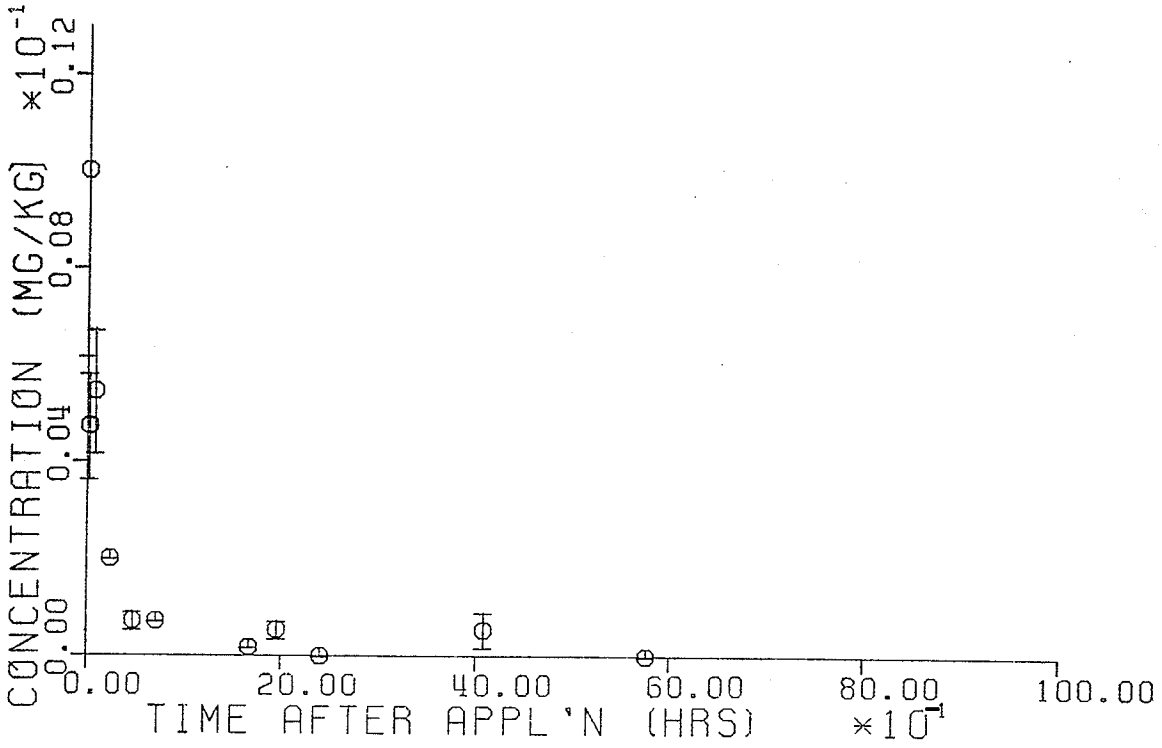


FIGURE 16. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 3, 0.04 MG/L, EC.

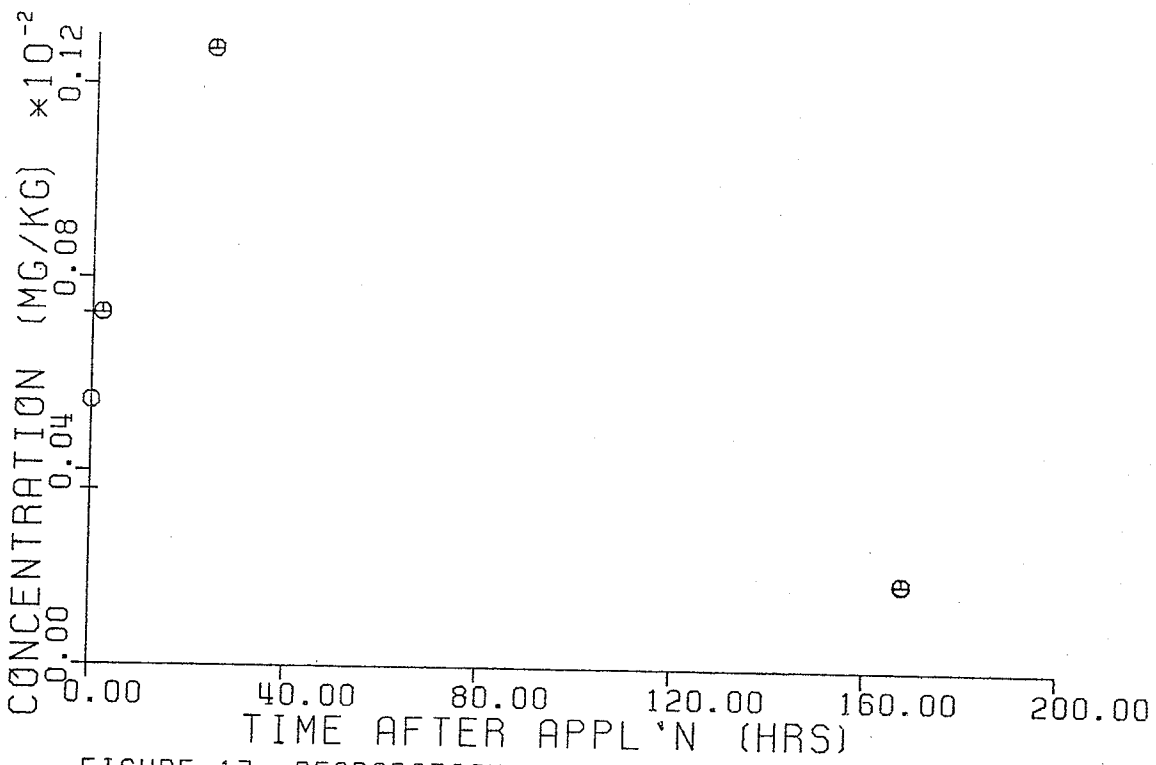


FIGURE 17. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 4, 0.02 MG/L, SR.

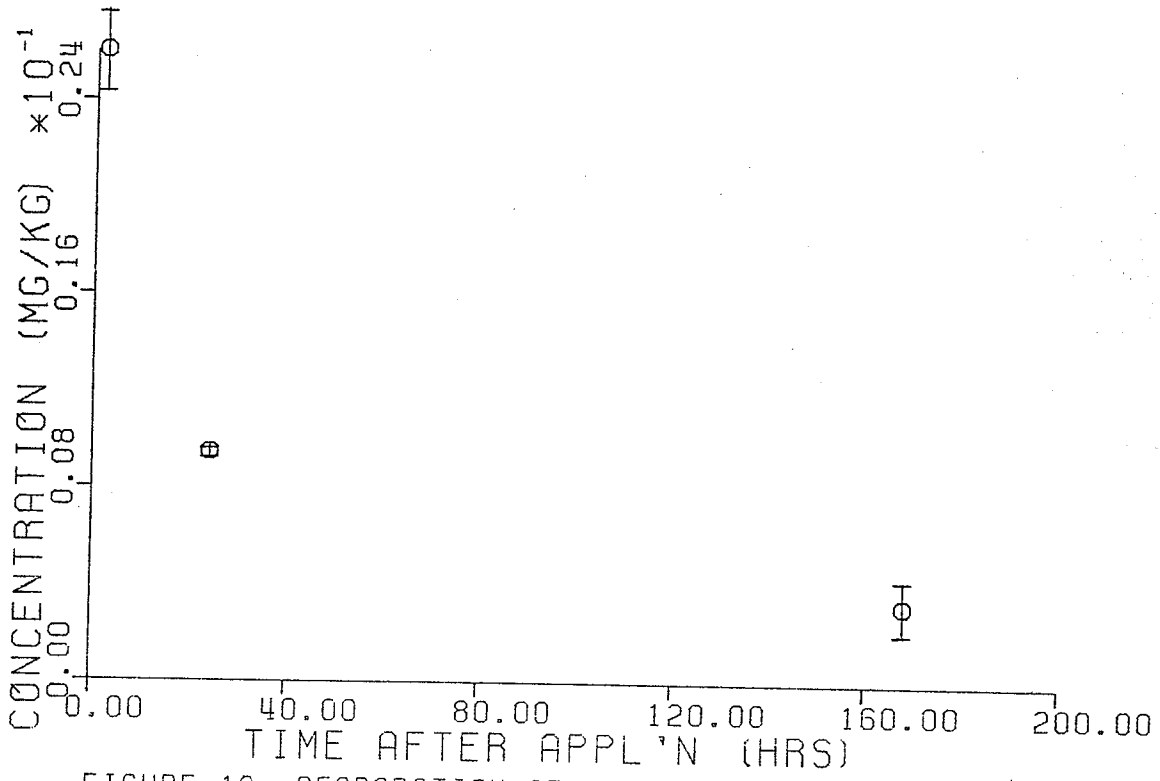


FIGURE 18. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 6, 0.20 MG/L, SR.

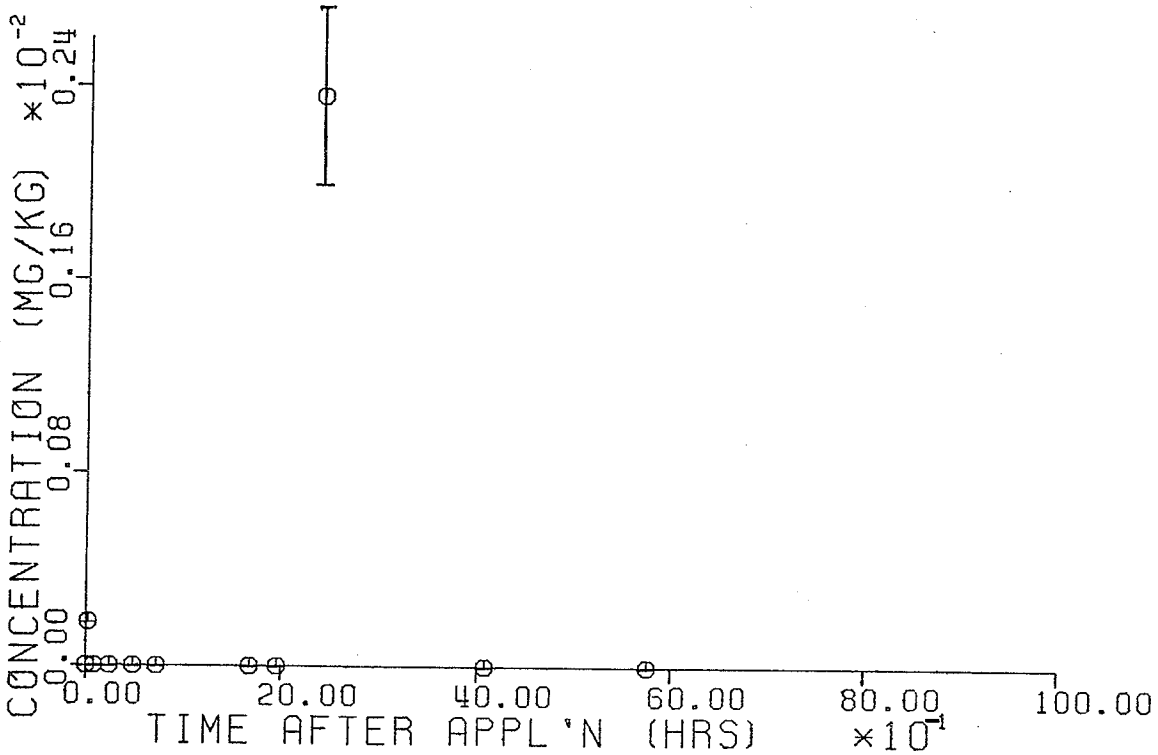


FIGURE 19. DEGRADATION OF CHLORPYRIFOS IN POND WATER; GLC RESULTS. POOL 2, CONTROL.

Results of chlorpyrifos degradation, in general, showed a non-linear concentration-time relationship which is best illustrated in Figure 13. Mathematical characterization of this relationship was somewhat hampered by the variation observed in the results. This was present to a moderate extent in pools fortified to 0.04 mg/l (pools 1,3,5, and 7; Figures 13-16), while more seriously affecting pool 4 results (0.02 mg/l, Figure 17).

(2.3321) Half-Time

The term half-time is used in the following text to indicate the time required for the chlorpyrifos concentration to reach half the initial value, i.e., half of the concentration at time = 0. This should not be confused with half-life, which is calculated from a kinetic equation. The general form of this equation may be written as

$$c = F(t)$$

where c is concentration, and t is time. The function F(t) may be a function derived from one of several kinetic relationships, such as first order or enzyme kinetics. Such a function for the degradation of chlorpyrifos in outdoor pools would be difficult to formulate due to the many factors influencing this process (section 2.3322).

The half-time of chlorpyrifos in each fortified pond was calculated from a power function, fit to the data by a least-squares program as discussed in the next section. As shown in Table 12, the half-time of pools fortified with the slow release formulation (14±4 hr, n=2) was approximately

twice that of pools fortified with the emulsifiable concentrate formulation (5±3 hr, n=2), at the 0.04 mg/l rate. This difference was also reflected in the bioassay results (section 2.331); i.e., the LD^{50} concentration was maintained in pools fortified with the slow release formulation for more than twice as long as those fortified with the emulsifiable concentrate formulation (at the 0.04 mg/l rate).

A half-time of 15 hours was estimated for chlorpyrifos in sewage water (approximately 20-30°) from the data of Schaefer and Dupras (1970, section 1.24). This is considerably longer than that reported in this study for the degradation of chlorpyrifos (emulsifiable concentrate) in pond water (5±3 hr, average water temperature = 14°). It is probable that higher levels of organic matter present in sewage water significantly elevated the concentration of reversibly bound chlorpyrifos, creating the same effect as the slow-release formulation. This might be represented by the following model

$$\begin{array}{ccc} C & \longrightarrow & M \\ \Uparrow\Downarrow & & \\ AC & & \end{array}$$

where C is dissolved chlorpyrifos, AC is adsorbed chlorpyrifos, and M represents metabolites.

(2.3322) Degradation Models

Disappearance of chlorpyrifos in pond water is a complex process, influenced by several factors such as photodegradation, temperature, adsorption, microbial population, and pH. The complexity of a mathematical model incorporating these variables precluded its application to the observed degradation patterns. Three simpler models, however, were tested, including two kinetic (mechanistic) rate models, and one empirical (regressional) equation.

Each mechanistic rate model represents a specific type of reaction mechanism as discussed below. By comparing the 'fit' of each model to the concentration-time data, it was possible to comment qualitatively on the importance of these two reaction mechanisms in the overall degradation of chlorpyrifos in pond water.

It is also important to note that, unlike a regressional equation, each constant employed in a mechanistic model has a specific physical meaning, such as a rate constant or reaction order. Literal interpretation of these constants cannot be made in the case of chlorpyrifos degradation in outdoor pools due to the complexity of the degradation process. To indicate this, constants such as 'reaction order' and 'rate constant' will be used with quotation marks in the following text.

The power rate model is shown below,

$$-\frac{dc}{dt} = kc^d = kc \text{ if } d=1 \qquad (1)$$

where c is concentration, k is the rate constant, and d is the reaction order (Model 1). In the case where d = 1, the equation takes the form of the familiar first order expression.

The power rate law arises from the situation where reaction follows activation of molecules, singularly or in groups, by collision. It usually applies to reactions in homogeneous solution at constant temperature (Hamaker, 1972).

Equation 1 may be manipulated to obtain the following linear form,

$$\frac{c^{1-d}}{1-d} = -kt + Ki \qquad (2)$$

where Ki is the constant of integration. The 'y=ax+b' form of this equation permitted the correlation coefficient (r^2) to be calculated for each pool, which is an indication of the goodness of fit.

First, however, the 'reaction order' (d) was determined numerically. This involved itterating equation 2 between d = 0.1 and 4.1 by steps of 0.1, calculating a value of r^2 for each step. Correlation coefficient maxima were observed for each pool (except pool 4), at which point optimum values of d were chosen (Table 12).

The second mechanistic relationship (Model 2) was the

hyperbolic rate model,

$$\frac{-dc}{dt} = \frac{k_1 c}{k_2 + c} = \frac{k_1 c}{k_2} \quad \text{if } k_2 >> c \qquad (3)$$

where c is concentration, t is time, k_1 is a maximum rate approached with increasing concentration, and k_2 is a combination of several rate constants, similar to K_m of the Michaelis-Menten kinetic equation.

Indeed, equation 3 closely resembles Michaelis-Menten kinetics, and, unlike the power rate model, reflects an equilibrium between a reactant and a catalyst (Hamaker, 1972).

Transformation into a finite difference form gives

$$\frac{2}{c_i + c_{i+1}} = \frac{k_1}{k_2} \frac{t_{i+1} - t_i}{c_i - c_{i+1}} - (1/k_2) \qquad (4)$$

or

$$\frac{1}{\bar{c}} = \frac{k_1}{k_2} \frac{\Delta t}{\Delta c} - (1/k_2) \qquad (5)$$

where i is itterated from 1 to the number of data points (by 1), $\bar{c}$ is the average concentration between two consecutive data points, and Δt and Δc are the change in time and concentration respectively between the same two data points. The above equation was derived assuming $dc/dt = \Delta c/\Delta t$ at $\bar{c}$. Delta values were calculated from data points in chronolog-

ical order, starting at t = 0 hours. This created problems due to the data variability; i.e., concentration did not decrease with time in all cases. Values of $-\Delta c/\Delta t$ which were negative or zero were eliminated from regressional analysis, causing a noteable reduction in the number of useable data points compared to the other models tested (Table 12, 'n' columns).

A power function (Model 3) was chosen as the empirical or regressional model,

$$c = mt^b \qquad (6)$$

where b and m are constants, c is concentration, and t is time. The linearized form is

$$\ln(c) = b[\ln(t)] + \ln(m) \qquad (7)$$

Concentration-time data for each pool (Appendix 3) was analyzed by a linear regression program to yield correlation coefficients (r^2) for each of the three linearized models as shown in Table 12.

Excessive variability in the correlation results (stemming from that found in the concentration-time data) allowed only a qualitative evaluation of the three models tested. Average correlation coefficients for pools fortified to 0.04 mg/l (pools 1,3,5, and 7; Figures 13-16) were 0.6±0.1, 0.7±0.3, and 0.8±0.2 for Models 1, 2, and 3 respectively. No significant difference was observed between the emulsifi-

Table 12. GLC results of chlorpyrifos degradation in pond water. Half times for each pool as well as correlation coefficients for Models 1, 2, and 3.

Pool	Portification (mg/l)		Correlation Coefficients (r^2)[6] M1	n[2]	M2	n	M3	n	'Order' (d)[3]	Half Time (hr)
1	0.04	SR[4]	0.60	11	0.44	6	0.82	10	0.9	16.2
7	0.04	SR	0.62	8	0.98	4	0.53	8	1.7	11.1
3	0.04	EC[5]	0.38	9	0.97	6	0.78	8	1.4	3.1
5	0.04	EC	0.65	7	0.42	6	0.90	7	1.6	7.0
4	0.02	SR	0.97	4	--	1	0.41	3	4.1	12.2
6	0.20	SR	1.00	3	--	2	0.99	3	2.8	9.8
AVERAGES										
	0.04	SR+EC	0.6±.1	4	0.7±.3	4	0.8±.2	4		
	0.04	EC	0.5±.2	2	0.7±.4	2	0.8±.1	2		
	0.04	SR	0.61±.01	2	0.7±.4	2	0.7±.2	2		

[1] calculated from Model 3. Average temperature = 14°.
[2] n at the top of the table is the number of data points used to calculate r^2. n for the averages is the number of results averaged.
[3] d is the 'reaction order' in Model 1.
[4] SR = 2.5% slow release formulation of chlorpyrifos.
[5] EC = 48% emulsifiable concentrate formulation of chlorpyrifos.
[6] M1, M2, and M3 = Models 1, 2, and 3.

able concentrate and slow release formulations, allowing both to be included in these averages; i.e., n=4. It was not possible to assign a preference to any one of the degradation models due to the high standard deviations observed. The correlation coefficients of all models, however, were fairly high, which was especially significant in the case of the mechanistic models (1 and 2). This provided evidence that enzymatic or surface reactions (Model 2), as well as molecular collision and activation reactions (hydrolysis and photolysis: Model 1) were both important in the overall disappearance of chlorpyrifos in pond water.

Pools 4 and 6 (Figures 17 and 18) were fortified to 0.02 and 0.2 mg/l respectively with the 2.5% slow release formulation (0.5 and 5 times the recommended rate). Results from these pools were not considered due to lack of data (pool 6), and excessive data variability (pool 4).

As mentioned earlier, the 'reaction order' (d) of Model 1 was estimated numerically for each pool (Table 12). The average 'order' of pools fortified at 0.04 mg/l (pools 1,3,5, and 7) was 1.4±0.4. As found for the correlation coefficients, no relationship was observed between formulation and 'reaction order', permitting the above average to include results of both formulations.

PART 3.

Extraction of chlorpyrifos Metabolites from Water.

(3.1) INTRODUCTION

The object of this study was to establish a method for the simultaneous determination of chlorpyrifos and metabolites in water. This was accomplished to the extent that 1) chlorpyrifos, the O-analog (II), and the pyridinol (VIII) were extracted separately by the same method, and, 2) chlorpyritos and the pyridinol were simultaneously determined in tap water. Development of this method was concerned with three major areas, extraction, cleanup, and analysis (GLC), each one capable of accomodating all three compounds. Only one GLC method was reported which satisfied this condition (McKellar, 1976), while reported extraction (Tables 4, 5, and 6) and cleanup (Table 7) methods were developed to accomodate, at most, chlorpyrifos and the O-analog simultaneously.

The method of Wetters (1971) for extracting chlorpyrifos from water provided the foundation for developing a method to extract chlorpyrifos and metabolites from water. The only major modification necessary was acidification of the water sample to make the pyridinol soluble in the extracting solvent, methylene chloride.

Most reported cleanup techniques used 8-16 g of adsorbent and up to several hundred milliliters of eluting solvent. Because of the relatively small volumes of water extracted in the present study (50-100 ml), and the homogeneous nature of

water compared to other substrates, it was feasible to 'streamline' the reported cleanup procedures considerably. Florisil was chosen as the adsorbent mainly for its high porosity, unlike silica gel, allowing rapid unaided column flow. Special considerations were required to elute the more highly adsorbed O-analog and methylated pyridinol (this derivative is referred to as the Me-pyridinol (IX), in the following text).

The O-des-ethyl (III) metabolite of chlorpyrifos was also investigated, but could not be extracted from water by the methods employed (Appendix 1).

(3.2) MATERIALS AND METHODS

(3.21) SOLVENTS AND REAGENTS

Pesticide grade methylene chloride, benzene, and cyclohexane were obtained from Caledon Laboratories Inc. An ether solution of diazomethane was prepared from diazald (Aldrich Chem., Co., Ltd.). Florisil (Floridin Co.) was activated (120°, 6 hr) and deactivated by adding 10% water (w:w) followed by thorough mixing by rotation. Glacial acetic acid (BDH Chemicals) was used to prepare 2% AcOH/benzene (v:v). Sulfuric acid obtained from Allied Chemical Co., was used for an aqueous 10% solution (v:v).

(3.22) FORTIFICATION OF TAP WATER

Standards of chlorpyrifos, the O-analog, and the pyri-

dinol (100 mg/l) were used to fortify tap water for extraction experiments. Volumes of 100 ml to 500 ml were fortified with 50 to 500 µl of standard depending on the concentration desired.

Fortification was accomplished by adding the required amount of standard, by syringe, directly to a volumetric flask. The standard solvent was evaporated with a gentle stream of nitrogen, and tap water was then added to the mark. After end-over-end mixing for several minutes the fortified water was extracted immediately.

(3.23) EXTRACTION METHOD

Water samples (50 ml) were extracted with methylene chloride (Figure 20). Each of the four extractions was performed by shaking vigorously for 1 minute. Emulsions were usually a problem with the first extraction, decreasing in intensity with successive extractions. After allowing a settling time of about 15 minutes methylene chloride extracts were carefully drained into a 125 ml round-bottom flask (RBF).

The total methylene chloride extract was evaporated to about 1 ml (rotary evaporator, 40° water bath), and transferred to a 5 ml centrifuge tube with 2 x 1 ml and 2 x 0.5 ml hexane rinsings. The hexane/methylene chloride extract was then placed in a 40° water bath and evaporated with a gentle stream of nitrogen to about 0.5 ml, followed by derivatization.

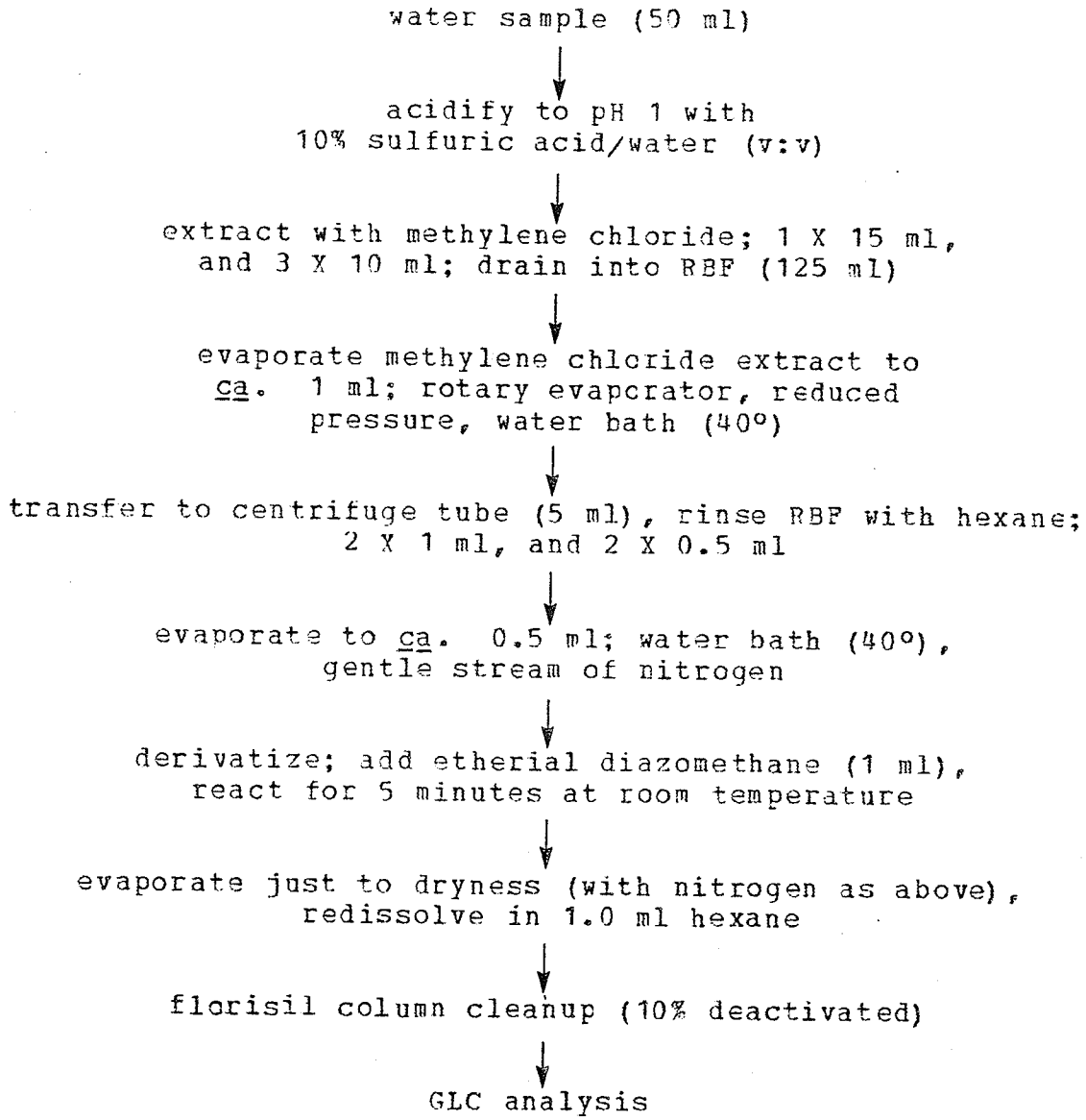


Figure 20. Extraction and analysis of chlorpyrifos and metabolites in water.

(3.231) Derivatization

All methylene chloride extracts were reacted with diazomethane regardless of whether they contained the pyridinol. As discussed in section 1.431, derivatization with diazomethane serves to methylate the pyridinol for GLC analysis. Chlorpyrifos and the O-analog were also subjected to this step to ensure the applicability of the method to the simultaneous determination of chlorpyrifos and metabolites.

Derivatization was performed by adding 1 ml etherial diazomethane (or sufficient quantity to maintain a yellow colour in the reaction mixture) to the water extract contained in ca. 0.5 ml hexane/methylene chloride.

After 5 minutes at room temperature the reaction mixture was evaporated just to dryness (40° water bath, gentle nitrogen flow) and the residue dissolved in 1 ml hexane for column cleanup or direct GLC analysis.

(3.232) Micro-Column Cleanup

A silanized glass wool plug was inserted into a disposable Pasteur pipet which was then packed to 5 cm with 10% deactivated florisil (0.5-0.6 g). The column was washed with 2 ml of 1:1 hexane/benzene (v:v) and loaded with the derivatized water extract or standard dissolved in 1 ml hexane.

When applied singly to the florisil column, chlorpyrifos was eluted with hexane/benzene 1:1 (v:v, 6 ml), and the O-analog and Me-pyridinol with 2% AcOH/benzene (v:v, 5 ml).

Cleanup of extracts containing chlorpyrifos and the Me-pyridinol were conducted by first eluting chlorpyrifos in 6 ml of hexane/benzene 1:1, followed by the Me-pyridinol in 5 ml of 2% AcOH/benzene, without changing the receiver.

After loading the column, the meniscus was allowed to reach the florisil before adding hexane/benzene. This solution was similarly allowed to drain before eluting with 2% AcOH/benzene as in the case of extracts containing chlorpyrifos and the Me-pyridinol.

All collected eluates were evaporated just to dryness in a water bath (35-40°) under a gentle stream of nitrogen. The residue was dissolved in hexane for GLC analysis.

(3.233) Gas-Liquid Chromatography

Listed below are the GLC conditions used throughout this study.

Instrument: Varian model 2400

Column: 5% OV-101/5% OV-210 on 80-100 mesh chromosorb W HP, 102 cm x 2 mm ID; oven, 180° (200° for the O-analog); nitrogen carrier, 86 ml/min.

Injection port: Teflon-lined septum, 220°

Detector: ^{3}H electron capture (EC), 220°.

GLC methodology was the same as that described in section 2.2222, the only difference being that in addition to chlorpyrifos, the Me-pyridinol and O-analog were also analyzed.

Adequate column conditioning was required to obtain consistent results for the O-analog, as found for many P=O compounds (Bowman and Beroza, 1967). Conditioning consisted of several injections of the O-analog (4 ng) until constant peak heights were obtained (usually after 4-6 injections). To maintain this conditioning, the O-analog was injected at least every 10 to 15 minutes.

GLC standards of the Me-pyridinol were prepared by the method used for derivatizing sample extracts (section 3.231). The desired amount of the pyridinol, contained in 1 ml of methylene chloride, was derivatized in a 5 ml volumetric flask by adding diazomethane (1 ml) and reacting for 5 minutes. The contents of the flask were then evaporated under a gentle stream of nitrogen and hexane was added to the mark.

(3.24) CONFIRMATION

GLC peaks of chlorpyrifos and metabolites were confirmed by 1) comparison of retention time to a standard, and 2) gas chromatography/mass spectroscopy. A standard of the Me-pyridinol was not available, and was synthesized on a milligram scale with diazomethane. In this synthesis, 40 mg of the pyridinol were dissolved in 1 ml acetone. Enough etherial diazomethane solution was added to maintain a yellow colour. After reacting for 5 minutes the solution was slowly evaporated to dryness under nitrogen leaving small crystals of reaction product.

A small crystal of this product was dissolved in 1 ml hexane for qualitative GLC analysis. Two peaks were observed with retention times of 0.4 (compound 4), and 1.8 minutes (compound 5). These components were separated on a florisil column as discussed in Appendix 2.

(3.241) NMR and Mass Spectra

Mass spectra of chlorpyrifos, the O-analog, and Me-pyridincl standards were obtained on a Finnigan model 1015 mass spectrometer using an ionization energy of 70-80 eV. Samples were introduced via direct inlet probe heated to 100°.

The NMR spectrum of the Me-pyridinol standard was obtained on a Varian A-50/60A instrument. Tetramethylsilane was added as an internal reference.

A Dupont Dimaspec GC/MS was employed for confirmation of GLC peaks obtained from extracts of fortified water.

Conditions:

Cclumn: 5% OV-101/5% OV-210 on Chromosorb W HP, 86 cm x 4 mm ID; oven, 200°; helium carrier, 40 ml/min.

Injector temp: 220°

Separator: glass jet, 220°

Ionization: electron impact (70 eV); source temp., 220°

(3.3) RESULTS AND DISCUSSION

(3.31) GAS-LIQUID CHROMOTOGRAPHY

Before each extraction experiment, a standard curve was made which was used to calculate percent recovery and to determine the linear range of the electron capture detector. Linear ECD response was typically observed over a range of 0.1-1 ng for chlorpyrifos and the Me-pyridinol, and 1-8 ng for the O-analog. Peaks obtained from the extraction of fortified water samples were maintained within this range by diluting or concentrating the extract as necessary. The minimum detectable limits (2 X noise level) were approximately 0.01 ng for chlorpyrifos and the Me-pyridinol, and 0.3 ng for the O-analog.

Retention times for the Me-pyridinol, chlorpyrifos, and the O-analog were 1.8, 5.2, and 3.6 minutes respectively (chlorpyrifos and the Me-pyridinol were chromatographed at 180°, and the O-analog at 200°). Separation of all three compounds injected simultaneously is shown in Figure 21. Temperature programming (180-195° at 2°/min) was employed to increase the resolution of the O-analog: retention times were 1.7, 4.4, and 6.2 minutes for the Me-pyridinol, chlorpyrifos, and the O-analog respectively.

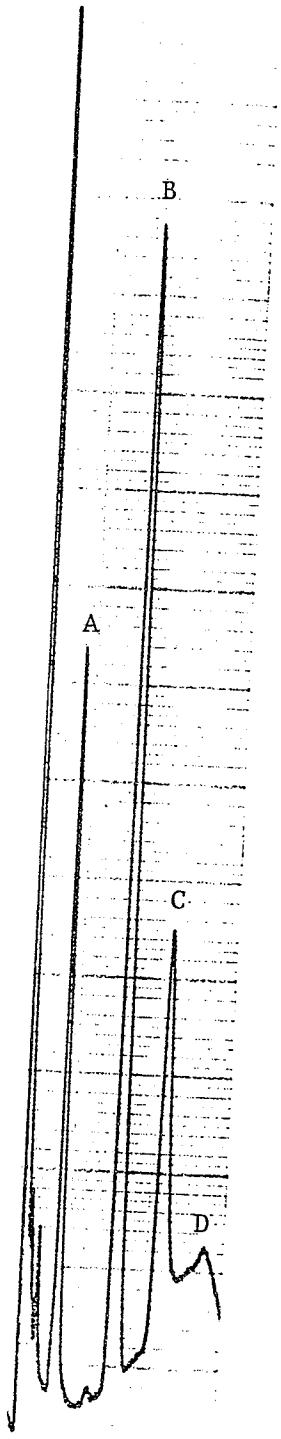


Figure 21. GLC separation of chlorpyrifos and metabolites using temperature programming; 180°-195° A 2°/min. A = the Me-pyridinol (0.5 ng, IX), B = chlorpyrifos (0.5 ng), C = the O-analog (2 ng, II), D = end of temperature programmed run.

(3.32) CONFIRMATION

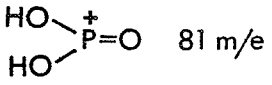
Discussed below are the mass spectra of standards of chlorpyrifos, the O-analog, and the Me-pyridinol. Mass spectra of GLC peaks (obtained on a GC/MS instrument) were compared to these spectra for confirmation.

(3.321) Chlorpyrifos

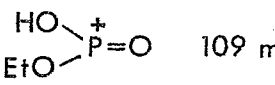
Figure 23 shows the mass spectrum of chlorpyrifos. Fragmentation agrees with that reported by Luke (1976) and Lores (1977) as shown in Figure 1.

(3.322) The O-Analog

Fragmentation of the O-analog, shown in Figure 22, was similar to that of chlorpyrifos, with base peaks at 81 and 109 m/e,



81 m/e



109 m/e

and a weak parent ion at 333 m/e.

(3.323) The Pyridinol and Me-pyridinol

The mass spectrum of the major product of Me-pyridinol synthesis (Appendix 2) revealed a large parent ion at 211 m/e corresponding to Me-pyridinol (Figure 24). Similarly, a large parent ion at 197 m/e was also observed for the pyridinol (Figure 25). Subsequent fragmentation of these compounds followed the proposed patterns shown in Tables 13 (Me-pyridinol), and 14 (pyridinol). In these schemes, a

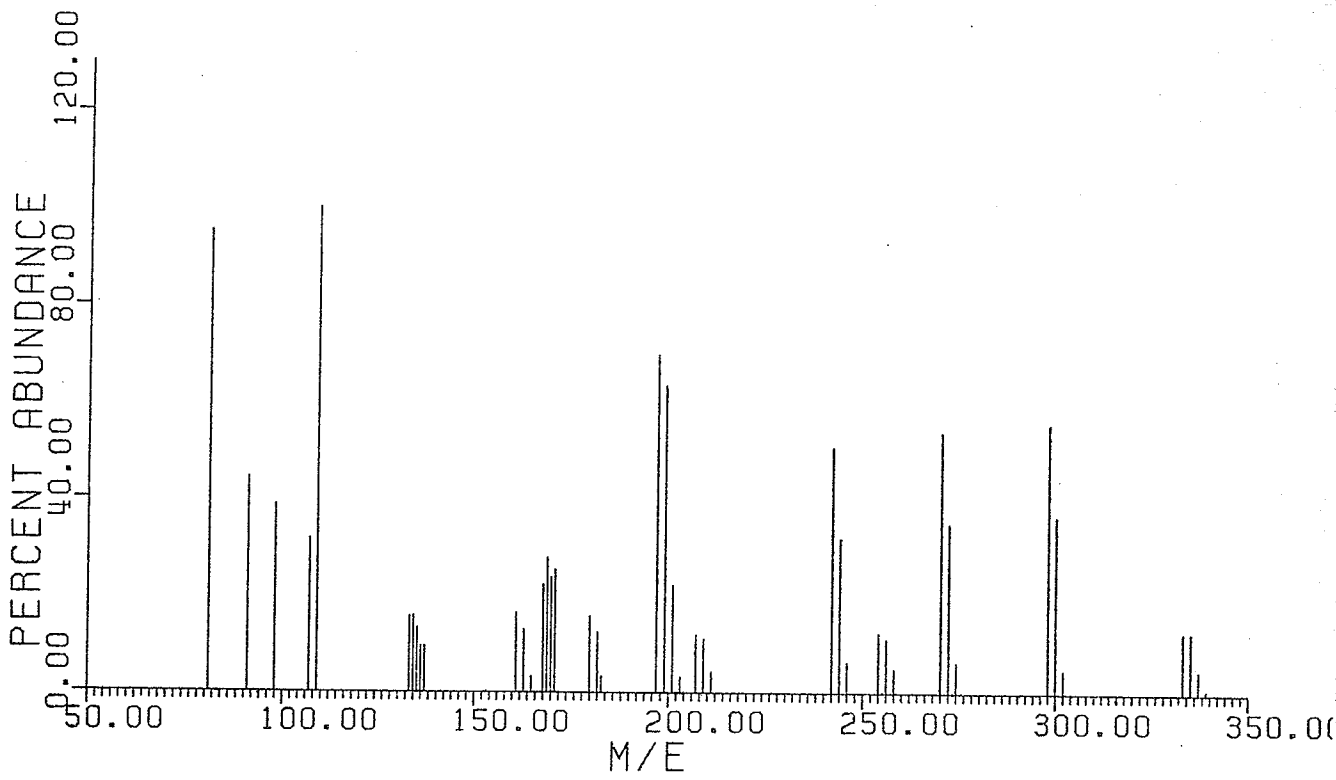


FIGURE 22. Ø-ANALØG (II) MASS SPECTRUM.

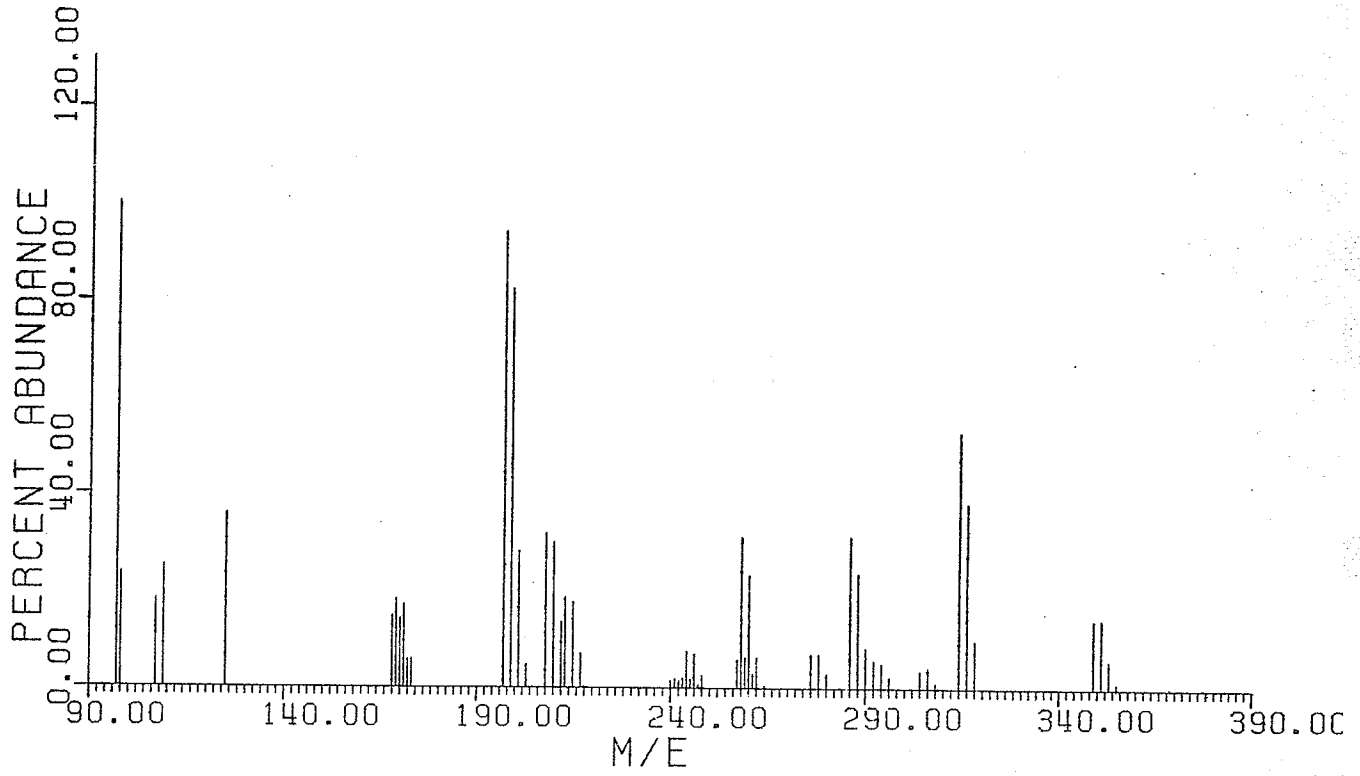


FIGURE 23. CHLØRPYRIFØS MASS SPECTRUM.

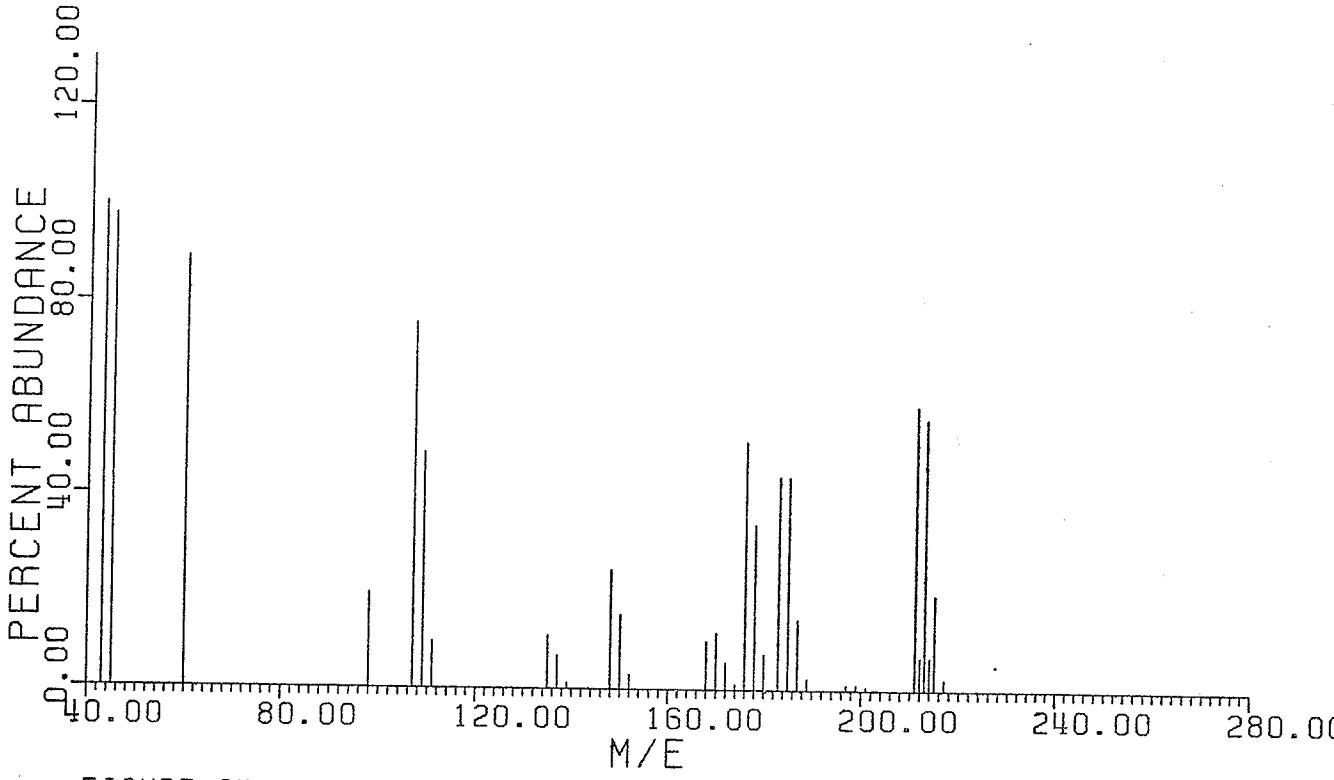


FIGURE 24. ME-PYRIDINOL (IX) MASS SPECTRUM.

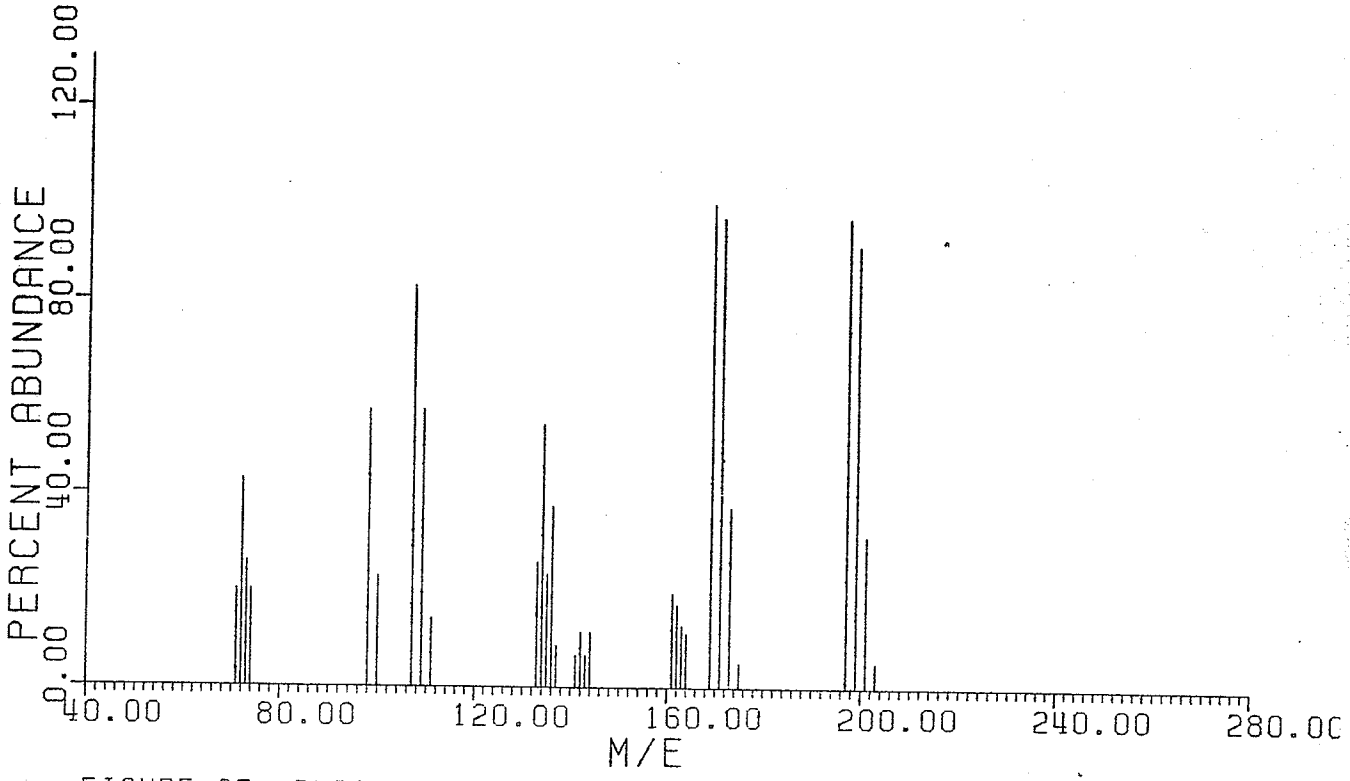


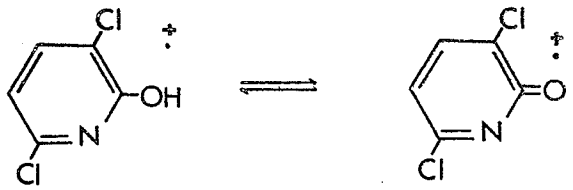
FIGURE 25. PYRIDINOL (VIII) MASS SPECTRUM.

methyl or hydride shift was proposed, followed by the loss of CO and the formation of a five-membered pyrrole structure.

The mass spectrum of the pyridinol has not been reported in the literature surveyed. However, studies have been published on related pyridine compounds which support the proposed fragmentation pattern.

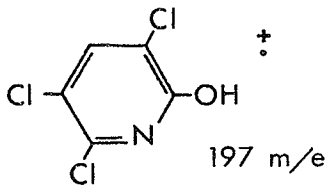
Considering the pyridinol fragmentation pattern in Table 14, several facts substantiate the possible existence of the pyridone structure (a); 1) pyridone-hydroxypyridine tautomerism (below) has been observed in aqueous media for 6-chloro-2-pyridone (Katritzky et al., 1967).

2) a similar rearrangement was reported by Tou (1971) to occur in the mass spectrometer for 3,6-dichloro-2-pyridinol:



3) because of resonance stabilization, it would be predicted

that the pyridinol fragment



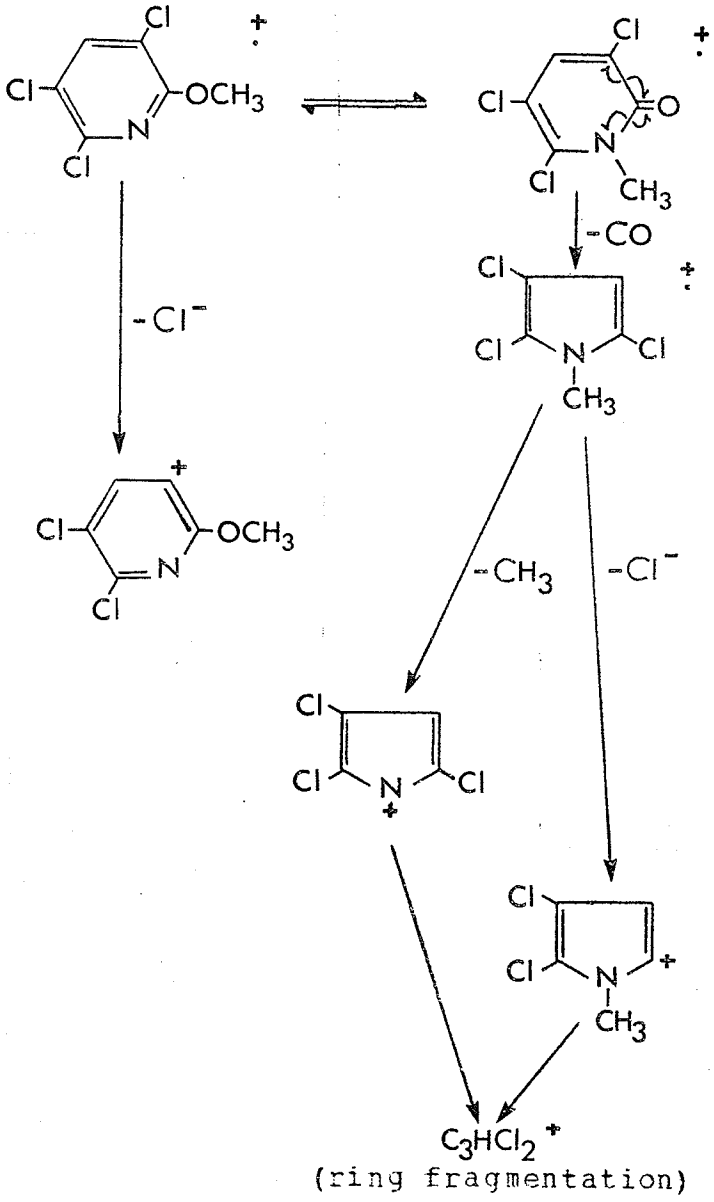
would appear as an intense peak in the O-analog and chlorpyrifos mass spectra. This was, in fact, observed. The next step proposed was the loss of CO from the parent ion. This has been reported for 2-pyridinol (Magnestian et al., 1975), although the products formed were not stated. The proposed mechanism of CO loss is shown to occur via homolytic cleavage of the pyridone structure (Table 14, structure 'a'), although this has not been reported.

The only difference between the pyridinol and Me-pyridinol fragmentations is that a methyl rather than a hydride shift was proposed (Table 13). This has not previously been reported for the Me-pyridinol. Fragmentation via loss of OCH_2, as found for 2-methoxypyridine (Stenhagen and Abrahamsson, 1974), was not observed; i.e., no peak was observed at 181 m/e.

Strong supporting evidence for the proposed Me-pyridinol fragmentation pattern (as for the pyridinol) was provided by the peak isotopic abundance patterns for each fragment containing chlorine (natural abundance for ^{35}Cl and ^{37}Cl is 100:32.5). The ratios of these peaks (Tables 13 and 14) are in agreement with those reported by McLafferty (1973).

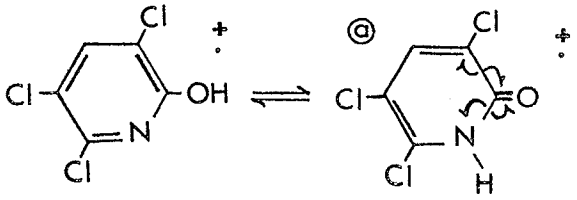
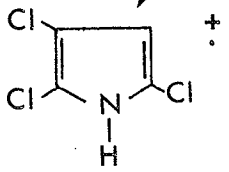
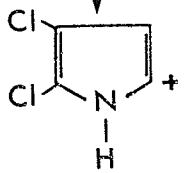
The NMR spectrum of the Me-pyridinol contained two peaks

Table 13. Proposed Me-pyridinol fragmentation.

Fragments	Peaks m/e	Peaks ht. (cm)[1]	Ratios of Peak Hts.
	211	2.4	1
	213	2.3	0.96
	215	0.8	0.33
	217	0.1	0.04
	183	1.8	1
	185	1.8	1
	187	0.6	0.33
	189	0.1	0.06
	176	2.1	1
	178	1.4	0.67
	180	0.3	0.14
	168	4.1	0.85
	170	4.8	1
	172	2.3	0.48
	174	0.5	0.10
	148	10.1	1
	150	6.3	0.62
	152	1.2	0.12
$C_3HCl_2^+$ (ring fragmentation)	107	3.1	1
	109	2.0	0.65
	111	0.4	0.13

[1] Scale (attenuation) is not constant.

Table 14. Proposed pyridinol fragmentation.

Fragments	Peaks m/e	Peaks ht. (cm)[1]	Ratios of Peak Hts.
	197	3.5	1
	199	3.3	0.94
	201	1.2	0.34
	203	0.2	0.06
	169	3.6	1
	171	3.5	0.97
	173	1.3	0.36
	175	0.2	0.06
	134	2.0	1
	136	1.4	0.70
	138	0.2	0.10
$C_3HCl_2^+$ (ring fragmentation)	107	2.9	1
	109	2.0	0.69
	111	0.4	0.14

[1] Scale (attenuation) is not constant.

(Figure 26). Resonance at 4.01 δ corresponded to methoxy protons, whereas the resonance of the single aromatic hydrogen was observed at 7.74 δ. Although the NMR spectrum of the Me-pyridinol was not reported in the literature surveyed, the above assignments were made by comparison with spectra of similar compounds as shown in Table 15.

(3.33) FLORISIL MICRO-COLUMN CLEANUP

Column cleanup recovery was evaluated by loading florisil micro-columns with standards of chlorpyrifos, the O-analog, and the Me-pyridinol (in hexane). Elution results are shown in Table 16.

Recovery of chlorpyrifos was not found to depend on column loading within the range investigated. The average amount eluted over three application rates (0.4, 1.0, and 10 µg) was 98±5%, in 5 ml of hexane/benzene 1:1 (v:v).

The elution technique for the O-analog and Me-pyridinol was developed from reported silica gel cleanup methods. Braun (1974) eluted the pyridinol and O-analog from silica gel with 2% AcOH/benzene (v:v). The latter compound, however, was hydrolyzed 'on column' to the pyridinol, requiring a separate column for each compound (Figure 3). With florisil, the O-analog was found to hydrolyze only partially; 81±2% of 5 µg was recovered with 2% AcOH/benzene (6 ml). The pyridinol which is created does not interfere with the simultaneous analysis of the pyridinol and the

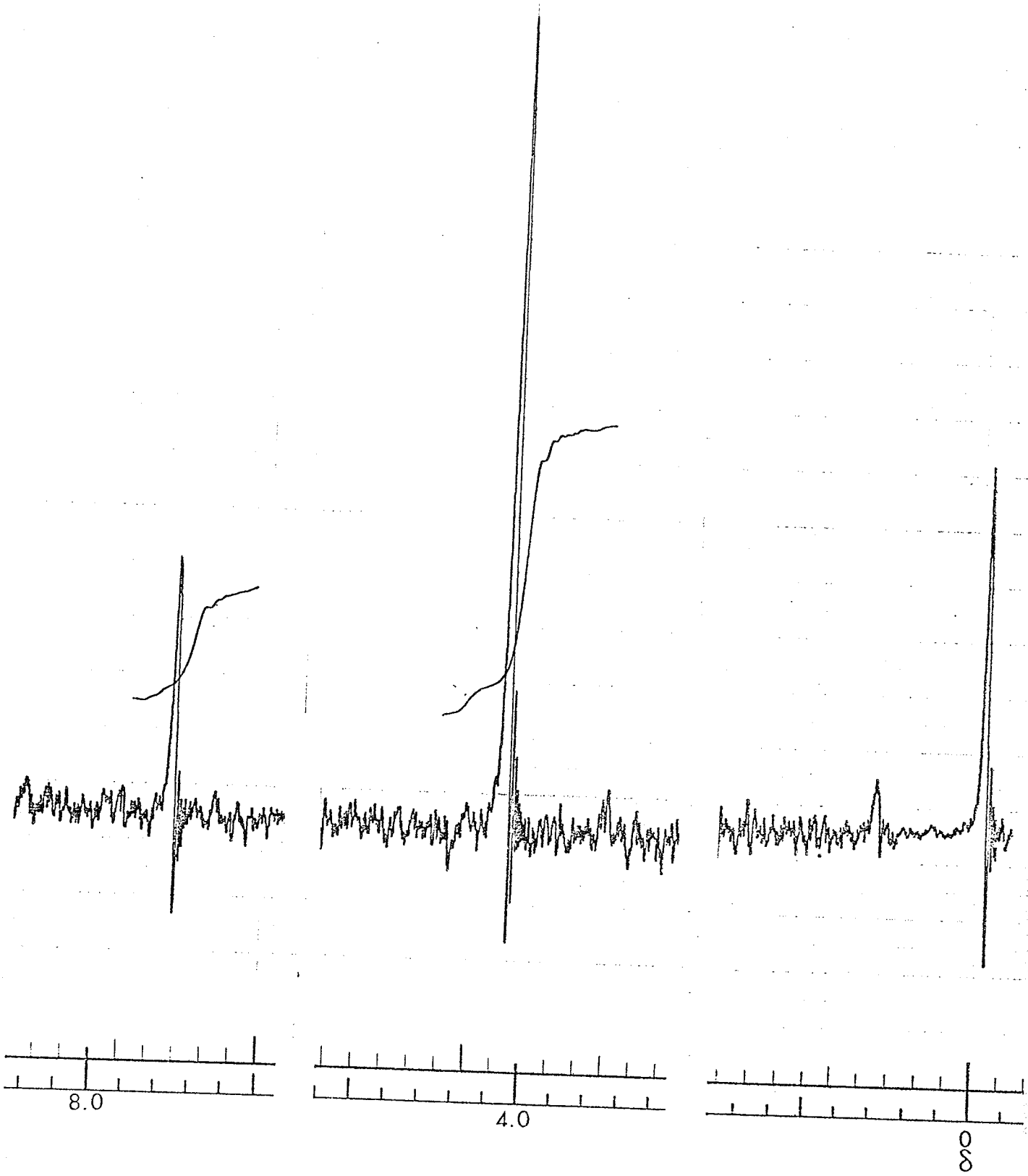


Figure 26. NMR spectrum of synthesized Me-pyridinol (IX).

Table 15. Reported NMR Spectra of Compounds Related to the Me-pyridinol (IX).

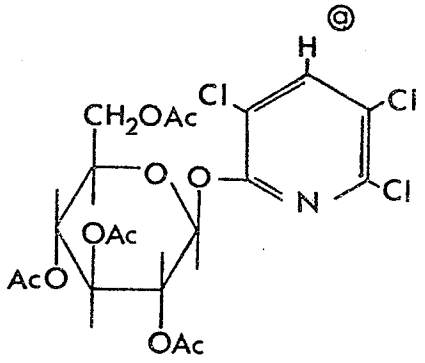
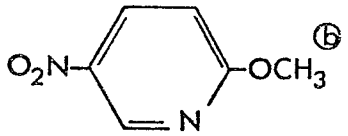
Compound	Resonance (δ)	Reference
 3,5,6-trichloro-2-pyridyl-tetra-o-acetyl-β-D-glycopyranoside	a = 7.72	Bakke, 1976
 2-methoxy-5-nitro pyridine	b = 4.03	Stadtler, 1967

Table 16. Elution of chlorpyrifos, the O-analog, and the Me-pyridinol from florisil.

Compd.[4]	Loading (ug)	Solvent[3]	% Recovery per Fraction[1] 1	2	3	4	5	6	Total
chlor	0.4	B/H	0	38±2	52±3	8±2	0	0	98±2
	1	B/H	0	40±4	59±10	0	0	0	99±7
	10	B/H	0	32±5	60±7	6±3	0	0	98±6
Me-pyr	1	MC	1±1	39±2	34±1	11±1	4±1	0	89±1
	5	MC	0	53±10	36±6	11±3	4±2	0	104±5
	10	MC	0	58±22	44±8	10±3	4±1	0	116±9
	47	2%AcOH/B	0	2±1	69±7	24±4	1±1	0	96±3
	21	2%AcOH/B	--[2]	--	--	--	--	--	99±3
	9	2%AcOH/B	--	--	--	--	--	--	94±5
	0.2	2%AcOH/B	--	--	--	--	--	--	80±9
O-an	1	MC	0	0	17±3	6±1	0	0	23±2
	5	MC	0	8±7	18±7	7±5	3±4	0	36±6
	11	MC	--	11±8	28±15	6±1	2±0	0	47±6
	5	2%AcOH/B	0	0	30±3	42±4	8±1	1±0	71±2
	10	2%AcOH/B	--[2]	--	--	--	--	--	76±8

[1] fraction volume = 1 ml. Standard deviations are for averages of two values (two columns).

[2] individual fractions were not collected. Elution volume = 6 ml.

[3] Solvent key: B/H=benzene/ hexane, 1:1 (v:v); MC=methylene chloride; 2%AcOH/b=2% acetic acid/benzene (v:v).

[4] Compound key: chlor = chlorpyrifos, O-an = O-analog, Me-pyr = Me-pyridinol (IX, Table 1).

O-analog in water because no derivatization takes place after column cleanup (Figure 20).

Acetone, methanol, and 2% AcOH/methanol were also investigated for their ability to elute the O-analog from florisil. At a column loading of 1 µg, the O-analog was not eluted in 10 ml of acetone or methanol. A small amount (36%) was recovered in 10 ml of 2%AcOH/methanol.

The Me-pyridinol was eluted from florisil with 2% AcOH/benzene over a loading range of 0.2-47 µg. An average of 92±8% was recovered over this range in an elution volume of 5 ml (Table 16).

Methylene chloride was reported for the elution of the O-analog from silica gel by McKellar et al., (1976), and Struble and McDonald (1973b). Recoveries from florisil, however, were incomplete; at a column loading of 11 µg, 47±6% was eluted, decreasing to 23±2% at the lowest loading of 1 µg. The Me-pyridinol, however, was totally recovered from florisil in 4 ml of methylene chloride (Table 16).

Results of column cleanup evaluation led to the choice of hexane/benzene 1:1 and 2% AcOH/benzene as elution solvents. Effort was then directed to extracting chlorpyrifos and metabolites from water.

(3.34) INDIVIDUAL RECOVERIES FROM TAP WATER

Chlorpyrifos, the O-analog, and the pyridinol were extracted from tap water individually, by the same method as outlined in Figure 20. Recoveries were conducted at three

rates, each rate being duplicated. Each extract was analyzed twice by GLC so that there were a total of four results per fortification rate.

Extraction results of chlorpyrifos and metabolites are presented in Tables 17-19. No significant relationship was observed between fortification rate and recovery, although, considering other data, this may not be the case for chlorpyrifos, as discused in section 3.37.

Average recoveries over all fortification rates were 84±7, 82±3, and 77±10% for chlorpyrifos, the O-analog, and the pyridinol respectively (99% confidence limits).

(3.341) Methylene Chloride/Water Emulsion

As mentioned in section 3.23, methylene chloride/water emulsions posed a problem in the extraction method. This was most serious in the first 15 ml methylene chloride extraction where only 8-10 ml of this volume could be recovered without visible water contamination.

Experiment 3 of the chlorpyrifos extractions employed a centrifugation step to clear the emulsion, allowing recovery of the total amount of methylene chloride in each extraction. This was accomplished by draining the emulsion into a clean glass test tube which was centrifuged for 5 minutes at full speed (International Clinical centrifuge). The separated phases were gently poured back into the separatory funnel and the methylene chloride drained. The centrifuge tube was rinsed with the next methylene chloride extraction volume.

Table 17. Recovery of chlorpyrifos from tap water.

Experiment	Fortification Rate (mg/l)	Recovery (%)	n[1]
1	0	0	2
	0.001	72±4	4
	0.041	87±10	4
	0.206	64±15	4
average		74±12	
2	0	0	2
	0.001	105±7	4
	0.041	112±4	4
	0.210	46±7	4
average		88±36	
3	0	0	2
	0.010	84±11	4
	0.040	88±2	4
	0.200	88±5	2
average		87±6	

[1] n is the number of results averaged; n=4, duplicate extraction and analysis; n=2, only 1 extraction was performed.

Table 18. Recovery of the pyridinol (VIII) from tap water.

Experiment	Fortification Rate (mg/l)	Recovery (%)	n[1]
1	0	0	2
	0.005	67±6	4
	0.254	108±11	4
	0.495	95±14	4
2	0	0	2
	0.005	82±3	4
	0.254	85±14	4
	0.495	109±8	4
3	0	0	2
	0.005	98±6	4
	0.130	83±4	4
	0.260	65±8	2

[1] see Table 17.

Table 19. Recovery of the O-analog (II) from tap water.

Experiment	Fortification Rate (mg/l)	Recovery (%)	n[1]
1	0	0	2
	0.01	64±18	4
	0.10	83±7	4
	1.00	85±2	4
2	0	0	2
	0.005	80±5	4
	0.100	83±6	2
	1.00	85±12	4
3	0	0	2
	0.01	77±6	4
	0.10	80±3	4
	0.40	80±4	2

[1] see Table 17.

Average chlorpyrifos recoveries for experiments 1, 2, and 3 were 74±12, 88±36, and 87±6% respectively. The reduced standard deviation of the latter result indicates the importance of eliminating the extraction emulsion. With pond water, on the other hand, it was found that anti-emulsion measures were not only desireable, but necessary. This was due to the formation of severe and sometimes irreversible emulsions, probably caused by the presence of suspended organic matter (section 2.2221).

(3.35) SIMULTANEOUS ANALYSIS

Chlorpyrifos and metabolites have been extracted individually by the same method with acceptable results, and it is logical that these compounds could be determined in the presence of each other. In partial verification of this statement, chlorpyrifos and the pyridinol were successfully coextracted from tap water. Recoveries (Table 20) were comparable to those found for these compounds extracted individually; average recoveries of all three fortification rates were 78±15, and 68±12% for chlorpyrifos and the pyridinol respectively. No relationship was observed between extraction efficiency and fortification rate for the pyridinol. Chlorpyrifos, on the other hand, showed a reduced extraction efficiency of 61±9% at the highest fortification level (0.2 mg/l), as discussed in section 3.37.

Table 20. Simultaneous extraction of chlorpyrifos and the pyridinol from tap water.

Compound	Fortification Rate (mg/l)	Recovery (%) [1]
Experiment 1		
chlorpyrifos	0.001	73±5
	0.041	86±11
	0.206	67±10
pyridinol	0.005	63±3
	0.254	65±14
	0.495	84±6
Experiment 2		
chlorpyrifos	0.001	99±4
	0.041	90±7
	0.210	55±8
pyridinol	0.005	79±6
	0.130	67±4
	0.260	52±8

[1] no chlorpyrifos or pyridincl (VIII) was detected in the controls. Each recovery is an average of 4 results (duplicate extraction and GLC analysis).

(3.36) APPLICATIONS

The major application of this method was for the determination of chlorpyrifos and metabolites in pond water, where the former is used for mosquito larva control. Unlike tap water, pond water contains suspended organic matter, which is a source of compounds that may interfere with GLC analysis. To ensure that such interferences would be removed by florisil chromatography, a pond water blank was extracted (Figure 20) and the extract analyzed before and after cleanup. The pond water extract before cleanup contained several interfering peaks as shown in Figure 27 (chromatogram 'a'). This extract was loaded onto a florisil micro-column and eluted with hexane/benzene 1:1 (6 ml) followed by 5 ml of 2% AcOH/benzene. Eluates were collected in 1 ml fractions and analyzed by GLC (2% AcOH/benzene fractions were evaporated under nitrogen and the residue dissolved in 1 ml of hexane). Pond water coextractives were eluted in fraction 5 of 2% AcOH/benzene (5th ml) as shown in chromatogram 'b' of Figure 27. All other fractions of both solvents were essentially free of coextractives as shown in the chromatogram of fraction 4 of 2% AcOH/benzene (Figure 27, 'c').

Referring to the florisil elution patterns of the O-analog and Me-pyridinol (Table 16), only small amounts of these compounds were eluted in the 5th 1 ml fraction of 2% AcOH/benzene (ca. 4%). Thus, with careful monitoring of the 2% AcOH/benzene elution volume, the florisil column cleanup method could be adapted to pond water analysis, with

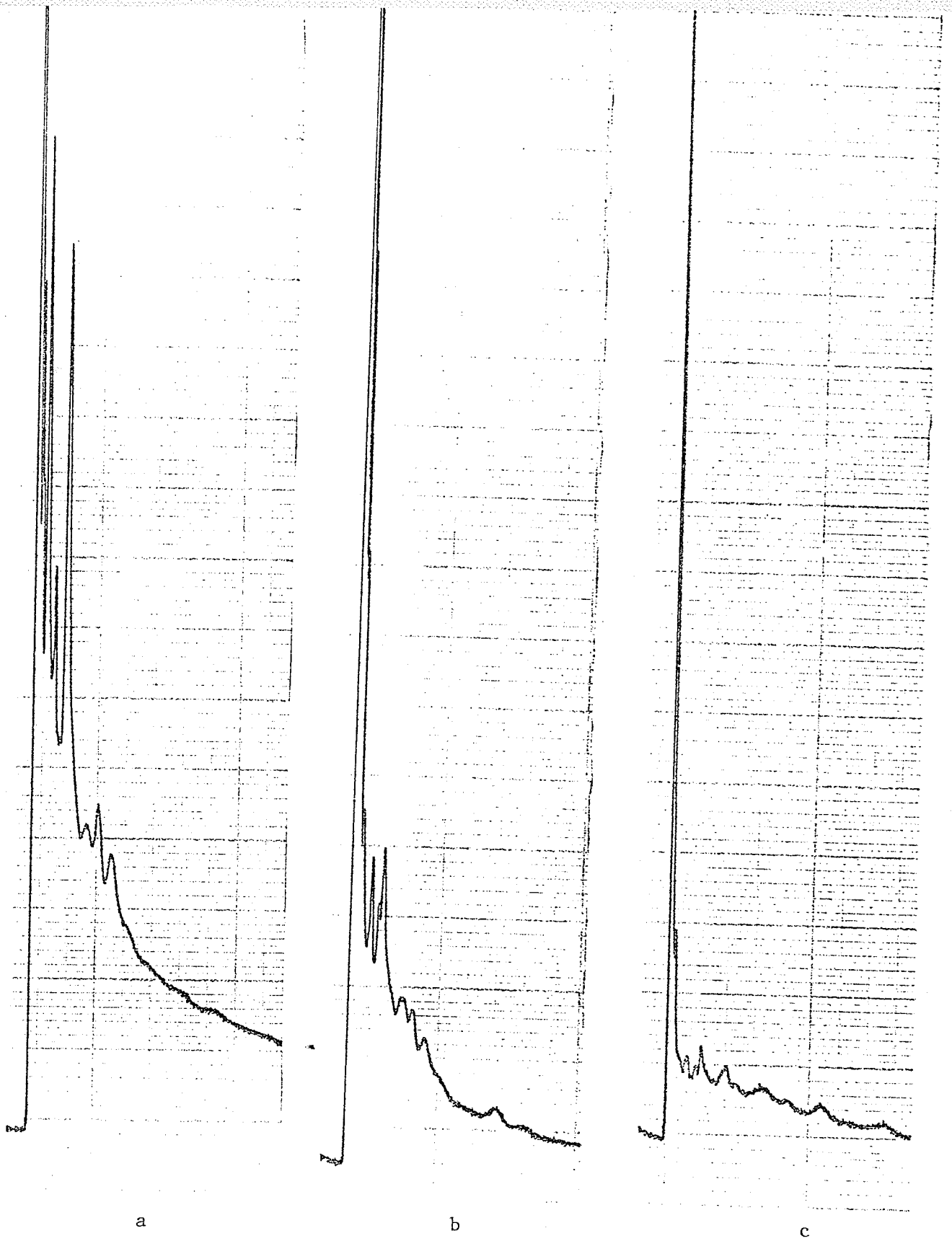


Figure 27. Florisil cleanup of pond water extract;
a) before cleanup, b) 5th 1 ml fraction of 2:1 ACOH/benzene,
c) 4th 1 ml fraction of 2:1 ACOH/benzene. Discussed in text.

only small losses of the O-analog and pyridinol metabolites.

Application of this method to other substrates such as vegetation and soil may be feasible with several modifications. This possibility is more easily examined if the method is broken down into its basic steps; 1) extraction, 2) derivatization, 3) column cleanup, and 4) GLC analysis.

Step 1: Simultaneous extraction of chlorpyrifos, the O-analog, and the pyridinol from vegetable tissue has been reported by Braun (1974). McKellar et al., (1976) extracted the pyridinol from milk, also using the same basic method to coextract chlorpyrifos and the O-analog (Tables 4, 5, and 6). In both methods, the sample was acidified to facilitate partitioning of the pyridinol into the extraction solvent. In general, it may be possible to adapt many of the reported chlorpyrifos and O-analog extraction methods (Tables 4 and 5) to include the pyridinol by lowering the pH of the extraction system.

Step 2: Derivatization of the pyridinol in tap water extracts (before column cleanup) was successfully accomplished using diazomethane. Application of this method to other substrates would almost certainly require that derivatization be performed in the presence of considerably more coextracted materials, depending on the substrate. Derivatization under these circumstances allows any reactive coextractives to be methylated along with the pyridinol. This may create several new interferences. Some may be lost during evaporation, but many may require removal by column

cleanup. The type and amount of coextractives present may also hinder the derivatization reaction. This problem may be eliminated by performing column cleanup before derivatization as discussed below.

Step 3: The florisil cleanup method was shown to remove undesirable coextractives from derivatized pond water extracts. However, a minor amount of carry-over of coextractives was observed in the 2% AcOH/benzene fractions containing the O-analog and Me-pyridinol. Thus, for substrates containing more interfering compounds, such as soil and vegetation, this cleanup procedure may be unsatisfactory. Several modifications might be proposed, including; 1) an increase in the amount of florisil, 2) the use of silica gel instead of florisil, and 3) performance of the derivatization step after column cleanup.

The latter two modifications would require changes in the eluting solvents. Several solvents have been reported for the elution of chlorpyrifos and the O-analog from silica gel, while elution of the Me-pyridinol has not been reported from silica gel (Table 7).

By performing derivatization after column cleanup, the problem of new interfering compounds created by the methylation reaction is eliminated. However, this modification requires that the pyridinol rather than the Me-pyridinol be eluted from the florisil column. In this case, 2% AcOH/benzene would not be suitable due to the partial hydrolysis of the O-analog to the pyridinol.

Step 4: GLC analysis would require no modifications assuming an efficient column cleanup had been developed.

(3.37) CHLORPYRIFOS RECOVERIES

In general, low chlorpyrifos recoveries (Table 21) from tap water were observed at the highest fortification rate of 0.2 mg/l. Chlorpyrifos recoveries were collected from three fortification experiments (Table 21):

Experiment 1) extraction of chlorpyrifos from tap water in conjunction with the chlorpyrifos degradation study (Part 2).

Experiment 2) extraction of chlorpyrifos in conjunction with the simultaneous determination of chlorpyrifos and metabolites in water (Part 3).

Experiment 3) coextraction of chlorpyrifos and the pyridinol by the method of Experiment 2.

Chlorpyrifos recoveries at the 0.20 mg/l concentration were approximately 25% lower than those at the lower fortification rates. It is possible that loss of chlorpyrifos occurred during rotary evaporation of methylene chloride extracts under reduced pressure.

Adsorption of chlorpyrifos on the glassware used for rotary evaporation may have caused a reduction in the vapour pressure of chlorpyrifos at the lower fortification levels. At the highest level (0.20 mg/l), more than enough chlorpyrifos may have been present to satisfy the surface adsorption requirement, causing an increase in the vapour pressure of chlorpyrifos. This is substantiated by the fact

that the round-bottom-flasks used in this step were not silanized. (The vapour pressure of chlorpyrifos at 35° is 8.9×10^{-5} mm Hg, Brust 1966.)

The 0.20 mg/l fortification level is relatively close to the solubility of chlorpyrifos in water (0.4 mg/l). Reactions favouring the removal of chlorpyrifos from solution, i.e., adsorption onto container walls, may also have been significant at this concentration during the fortification process.

Table 21. Results of chlorpyrifos extraction from tap water: collected from Parts 2 and 3.

Experiment[1]	Fortification Rate (mg/l)	% Recovery	n[2]
1	0.02	98±8	4
	0.04	82±23	6
	0.20	67±3	6
2	0.001	87±17	12
	0.044	96±14	12
	0.20	66±21	12
3	0.001	86±18	12
	0.040	88±9	12
	0.20	61±9	12

[1] see text for description of experiments.
[2] n is the number of results averaged.

(4) SUMMARY

In Part 2 a study of the degradation of chlorpyrifos in outdoor artificial pools was conducted jointly with G. Rawn, M.Sc., 1977 (Rawn, 1977). Two formulations of chlorpyrifos were examined; the 2.5% slow-release formulation, and the 48% emulsifiable concentrate formulation. The 'half-lives' observed for these formulations were 14±4 and 5±3 hours respectively. The concentration-time data was analyzed by three mathematical models, including a power rate law, a hyperbolic rate model, and a power function. The data was best described by the power function, followed by the hyperbolic and power rate models. Correlation coefficients for the latter two models, however, were fairly high (0.6±0.1, and 0.7±0.3 respectively) indicating that hydrolytic as well as enzymatic or catalytic reactions were important in the degradation of chlorpyrifos in pond water. A correlation coefficient of 0.8±.2 was calculated for the power function.

In Part 3, a method was developed for the tap water extraction of chlorpyrifos and two important metabolites; the O-analog (diethyl 3,5,6-trichloro-2-pyridyl phosphate), and the pyridinol (3,5,6-trichloro-2-pyridinol). The latter compound was determined as its methylation product, employing diazomethane as the derivatizing agent. Average extraction efficiencies for chlorpyrifos, the O-analog, and the pyridinol were 84±7, 82±3, and 77±10% respectively. That the method could be applied to the simultaneous extraction

of chlorpyrifos and metabolites was partially verified by the coextraction of chlorpyrifos and the pyridinol metabolite. Results of these experiments were similar to those reported for the individual extraction of chlorpyrifos and the pyridinol from tap water.

APPENDIX 1: The O-des-ethyl Metabolite of Chlorpyrifos.

As discussed in section 1.322, the O-des-ethyl metabolite of chlorpyrifos (structure III, Table 1) was reported in fish subjected to a high concentration of chlorpyrifos. It was also stated that the des-ethylation pathway of metabolism is of minor importance in mammals. The object of this work was to determine the stability of the O-des-ethyl in water.

This compound was analyzed by GLC as its methylation product (Me-O-des-ethyl, structure IV, Table 1). A stock solution (122 mg/l) was prepared by dissolving the potassium salt of the O-des-ethyl in acetone (25 ml) plus a drop of concentrated HCl. A dilution of this stock solution (1 mg/l in acetone) was derivatized with diazomethane by the method discussed in section 3.231 for the pyridinol. GLC analysis of the methylation product revealed three peaks eluting at 1.8 (compound 1), 4.4 (compound 2), and 6.6 minutes (compound 3). (See section 3.233 for GLC conditions; oven at 180°.) Compound 1 was identified by GLC retention time and mass spectroscopy as the Me-pyridinol (IX). Compound 2 was identified from its mass spectrum (Figure 28) as the Me-O-des-ethyl. The identity of compound 3 was unknown. The mass spectrum of this compound (Figure 29) did not show the pyridinol fragment at 197 m/e (section 3.323), found in

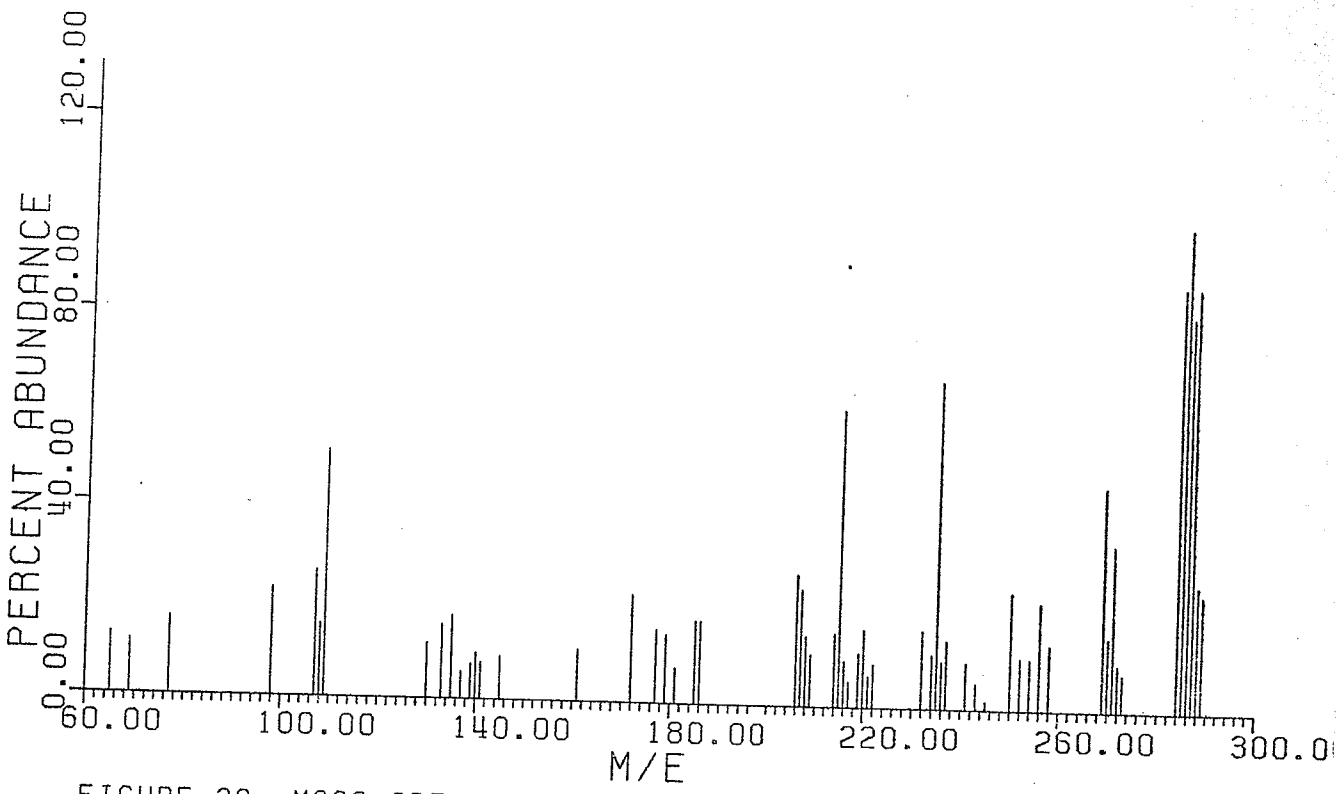


FIGURE 29. MASS SPECTRUM OF COMPOUND 3 (UNKNOWN).

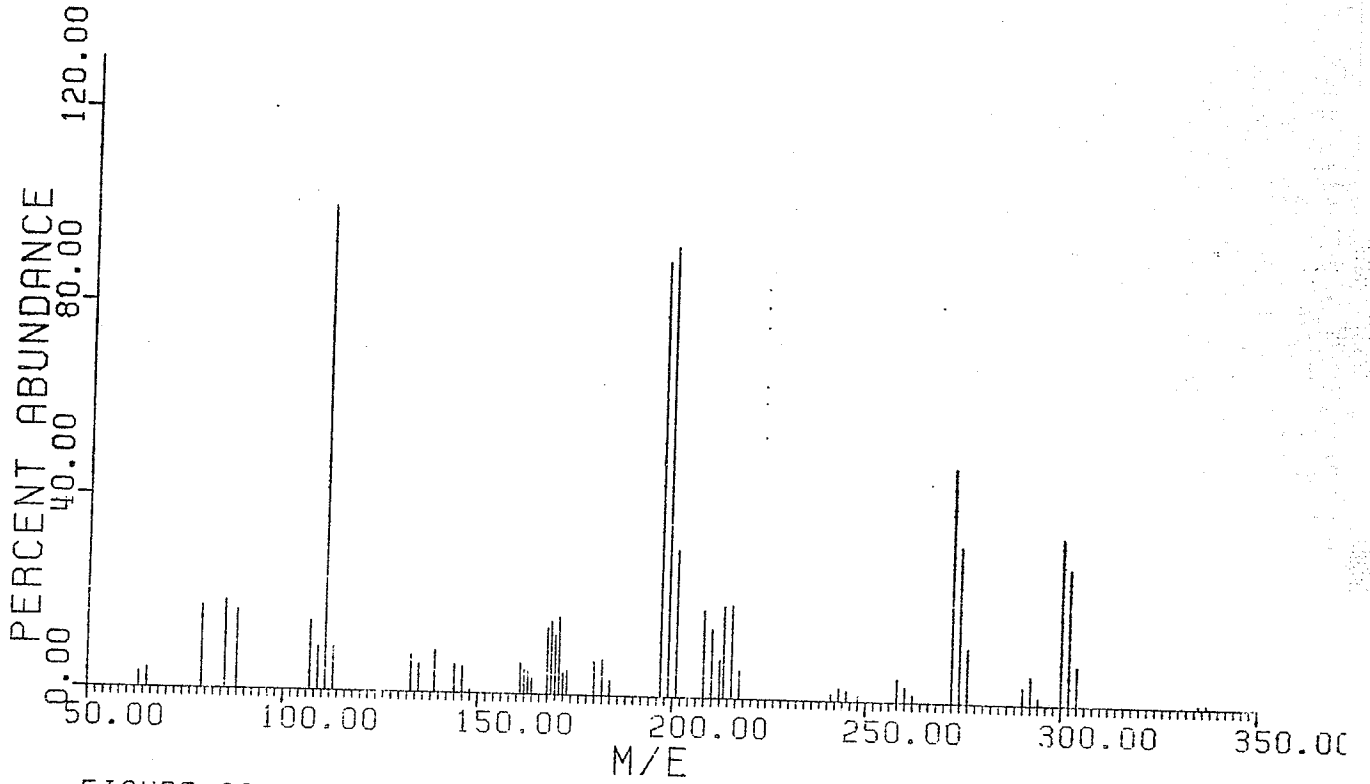


FIGURE 28. MASS SPECTRUM OF THE O-DES ETHYL (III) METHYLATION PRODUCT (ME-O-DES-ETHYL, IV).

the mass spectra of chlorpyrifos, the O-analog, the Me-pyridinol, and Me-O-des-ethyl chlorpyrifos. The isotopic abundance pattern typical of compounds containing three chlorine atoms was also not observed. The possibility that fragments at 285 and 269 m/e contained two chlorine atoms was investigated; the average peak height ratios (1 : 0.82 : 0.51) were in poor agreement with predicted values (1 : 0.65 : 0.11).

Compounds 1 and 3 may have been present as impurities in the O-des-ethyl standard, the purity of which was not stated by Dow Chemical Co., or they may have been produced during dissolution and derivatization.

An experiment was then conducted to determine if O-des-ethyl chlorpyrifos could be extracted from water. An aqueous solution was made by dissolving 0.002 g of the O-des-ethyl potassium salt in tap water (25 ml). This solution was diluted to 1 mg/l with tap water and extracted with methylene chloride according to the method described in Part 3 (Figure 20), with the following exceptions: 1) column cleanup was not performed, and 2) extracts were analyzed before and after derivatization with diazomethane.

No compounds were detected by GLC analysis of extracts before derivatization. After derivatization, however, two peaks were observed, corresponding to the Me-pyridinol (compound 1), and the unknown (compound 3). No Me-O-des-ethyl was detected.

To check the possibility that acidification to pH 1 may

have caused acid hydrolysis of the O-des-ethyl, several extractions were performed where fortified water samples were acidified over a pH range of 4-7 with 1% aqueous acetic acid. Analysis of these derivatized extracts did not show the presence of the Me-O-des-ethyl compound; only the Me-pyridinol, and compound 3 were present, as found for the pH 1 extraction. Similar results were obtained when other extracting solvents were tried, including hexane, benzene, and chloroform.

The ratio of

$$\frac{\text{peak height}}{\text{equivalent nanograms of O-des-ethyl injected}}$$

was used as an indication of whether compounds 1 and 3 were produced in water fortified with the O-des-ethyl compound. For the O-des-ethyl chlorpyrifos GLC standard these ratios were about 1.3 and 1.2 for compounds 1 and 3 respectively. For the water samples fortified with 1 mg/l of the O-des-ethyl compound, they were 1.1 and 0.7 for compounds 1 and 3 respectively.

As mentioned earlier, compound 1 was confirmed as the Me-pyridinol. Its extraction efficiency was established as 77±10% by the method employed. Using this value, the corrected ratio for compound 1 in water was calculated as 1.1/0.77 = 1.4±.2. Therefore, within experimental error, the ratios for the GLC standard (1.3) and water sample were

equal, indicating that compound 1 (the Me-pyridinol) was not produced when water was fortified with the O-des-ethyl compound.

For compound 3, the above defined ratio was significantly lower for the water sample (0.7) than for the GLC standard (1.2). Interpretation of these results was hampered by the fact that the extraction efficiency of compound 3 was unknown. Depending on this value, compound 3 may or may not have been produced when water was fortified with the O-des-ethyl compound.

It was concluded that O-des-ethyl chlorpyrifos was not extracted from tap water with the methods employed. Evidence to support the chemical breakdown of O-des-ethyl chlorpyrifos in water was inconclusive; it was possible that compound 3 may have been produced in water fortified with the O-des-ethyl compound. It was also feasible that O-des-ethyl chlorpyrifos degraded into compounds which were not detected.

APPENDIX 2: Purification of the Me-pyridinol.

Separation of the pyridinol methylation reaction products (compounds 4 and 5) was performed on a florisil column. Activated florisil (12 g) was packed into a glass tube (2 cm X 30 cm) fitted with a stopcock to control solvent flow. (Column packing was retained with a glass-wool plug.) The column was rinsed with 40 ml of hexane/benzene 1:1 (v:v) and loaded with the methylation product contained in hexane (2 ml). Compound 4 was eluted with 30 ml of this solution. The cclumn was then eluted with 175 ml of 2% AcOH/benzene (v:v). Compound 5 was contained in the last 75 ml of this volume.

APPENDIX 3: Chlorpyrifos concentration-time data for outdoor pools; GLC results.

Pool	Conc. (ng/ml)	Time (hr)	Pool	Conc. (ng/ml)	Time (hr)	Pool	Conc. (ng/ml)	Time (hr)
1	1.8	0	2	0.0	0	3	10.0	0
	2.7	2		0.2	2		4.7	2
	2.2	8		0.0	8		5.5	8
	1.6	24		0.0	24		2.0	24
	0.9	48		0.0	48		0.7	48
	0.7	72		0.0	72		0.7	72
	0.4	168		0.0	168		0.2	168
	0.8	196		0.0	196		0.5	196
	0.2	240		2.3	240		0.0	240
	0.2	408		0.0	408		0.5	408
	0.4	576		0.0	576		0.0	576
4	0.6	0	5	6.6	2	6	26.0	2
	0.7	2		4.0	8		9.5	24
	1.3	24		1.6	24		3.3	168
	1.8	168		0.7	48	7	1.1	2
				0.5	72		2.2	8
				0.2	168		0.9	24
				0.5	196		1.3	48
				0.0	240		0.4	72
				0.0	408		0.5	168
							0.5	196
							0.2	240
							0.0	576

APPENDIX 4: Chlorpyrifos percent mortality data for outdoor pools.

Time (hr)	Percent Mortality in Pools 1-7						
	1	2	3	4	5	6	7
0	100	0	100	5	100	100	85
4	100	5	100	85	100	100	100
24	100	0	100	100	100	100	100
48	100	7	100	100	100	100	100
72	100	0	100	100	100	100	100
96	95	0	100	50	100	100	100
168	100	0	20	35	5	100	95
240	25	0	10	20	5	100	90
288	55	0	5	0	0	100	65
360	85	0	5	0	0	100	80
408	85	0	0			100	40
456	30	0	0			100	85
524	10	0				100	50
576	50	0				100	70
672	5	0				100	80
720	0	0				100	20
840	0	0				100	10
1008		0				90	0
1176		0				80	0
1344		0				100	
1416		0				100	

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