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.

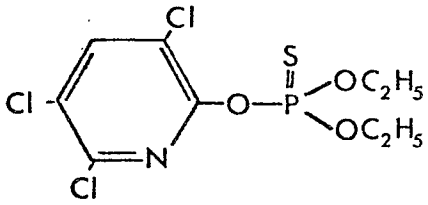
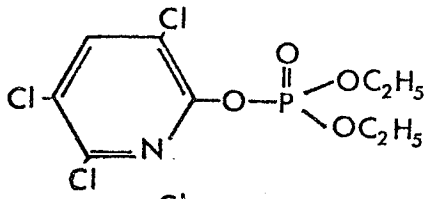
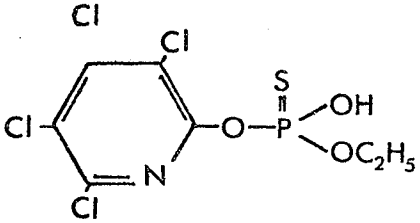
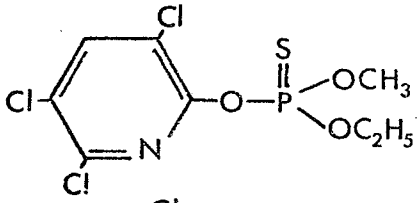
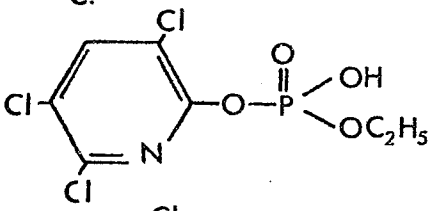
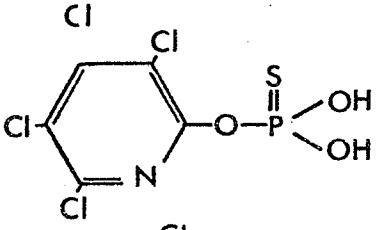
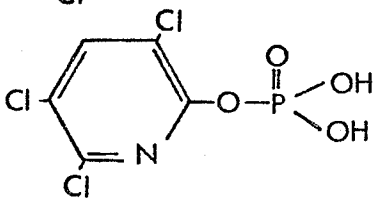
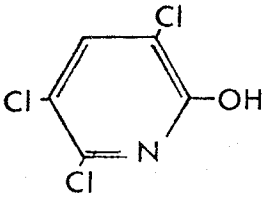
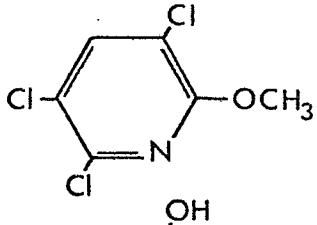
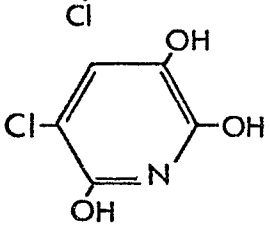
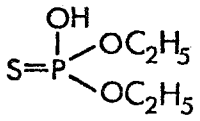
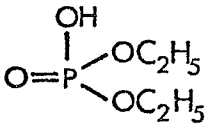
Structure	Code	Chemical Name
	I	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos)
	II	diethyl 3,5,6-trichloro-2-pyridyl phosphate (O-analog)
	III	O-ethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
	IV	O-ethyl O-methyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
	V	ethyl 3,5,6-trichloro-2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

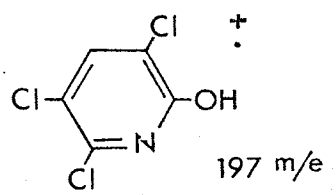
and 15 hr in sewage water. It was observed that chlorpyrifos in sewage water did not degrade completely, but equilibrated at a residual level near 0.003 mg/l. An adsorption-desorption equilibrium between water and organic matter was proposed to account for this residual level, and for the extended six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

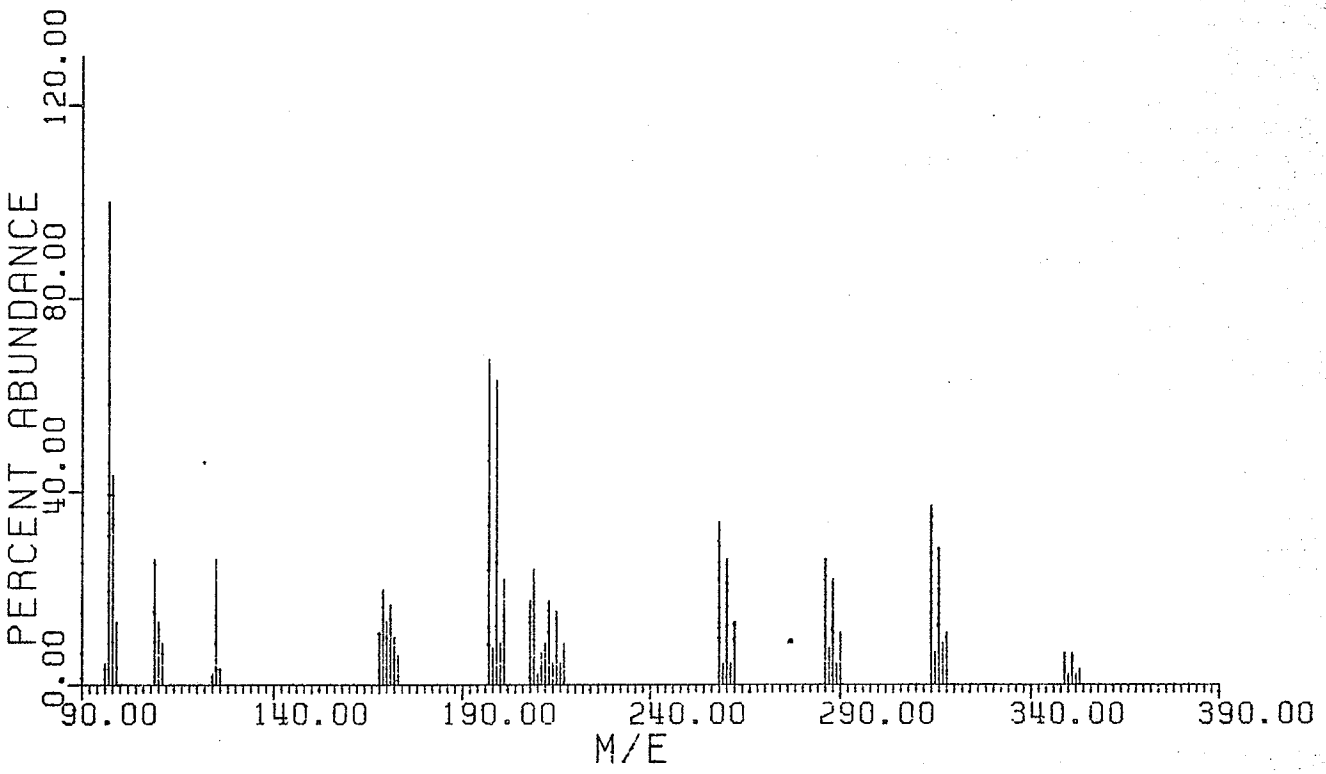


FIGURE 1. CHLORPYRIFOS MASS SPECTRUM (LORES, 1977).

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.

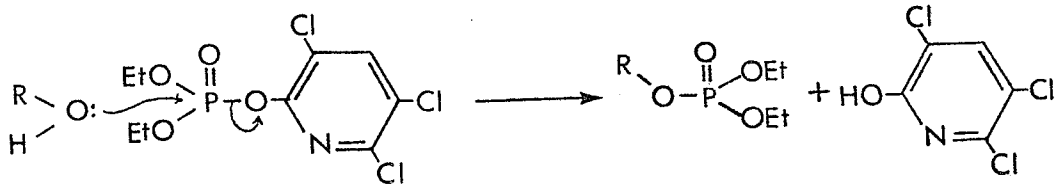
Eto (1974) has pointed out that two prerequisites are necessary for phosphorylating activity: 1) decreased p pi - d pi contribution to the P-OAr bond (i.e., decreased double bond character), and 2) increased positive charge on the phosphorus atom.

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,



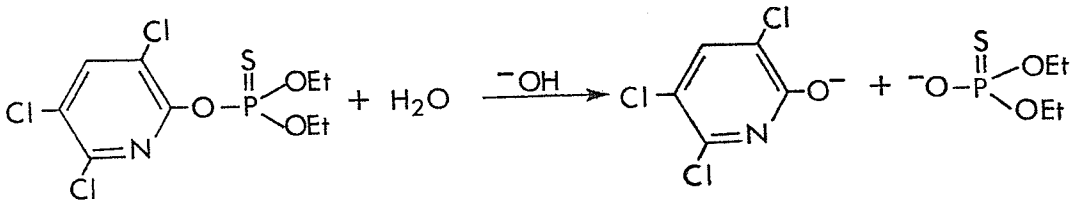
where ROH is the acetylcholinesterase molecule, the OH being that of serine (Eto, 1974).

(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.

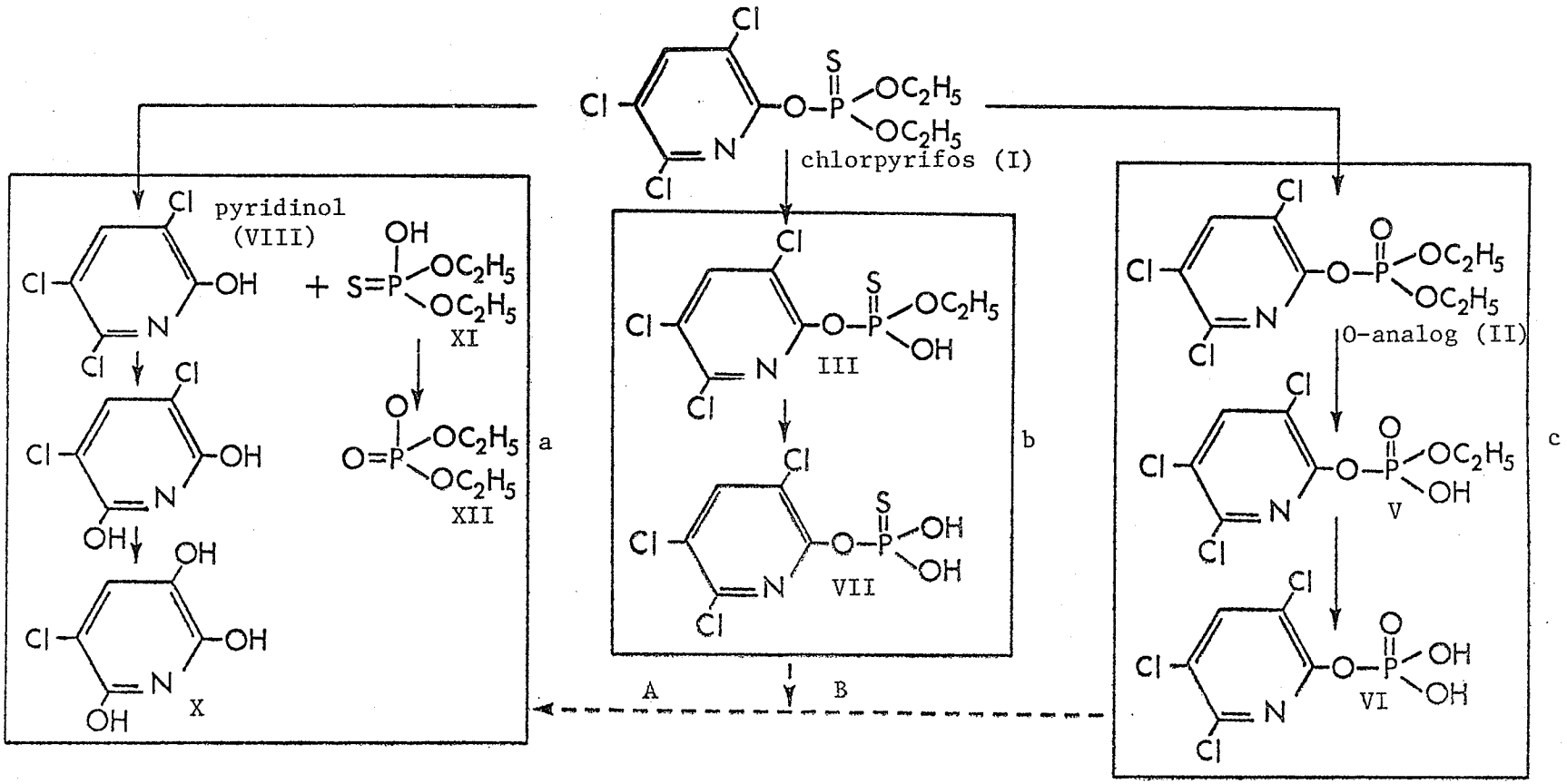
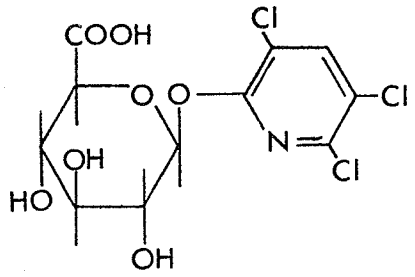


Figure 2. Diagramatic representation of reported chlorpyrifos metabolites (from Table 3). See Table 1 for names corresponding to Roman numerals.

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,