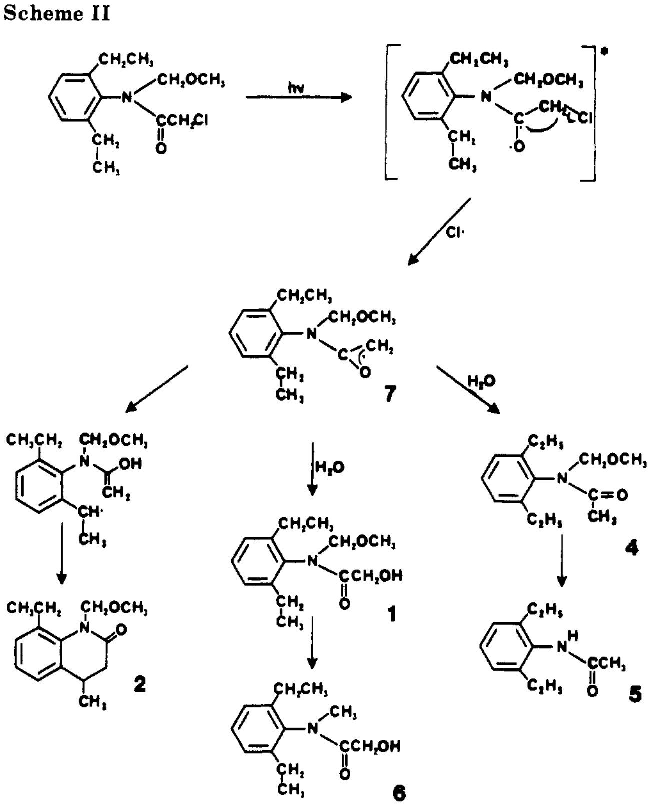
Scheme II



other minor products were observed but not isolated or identified; however, from the autoradiogram, it is clear that neither the carbonyl nor the ring carbons were removed during in the first few steps. Further photodegradation does occur, and after 1 h of irradiation only four very polar compounds were observed. Isolation and identification of these final products have proven to be complicated and are the subject of another study.

Ozonation, although somewhat slower than UV irradiation, appears to add another facet to decomposition. Ring cleavage, which probably enhances the degradation of alachlor, has been observed in ozonolysis. A good indication that ring cleavage does occur is that, after 1 h of treatment with ozone, none of the carbonyl label was depleted but nearly 30% of the ring label was lost. Additional evidence for ring cleavage was found in the NMR spectrum of one of the compounds where no aromatic protons were detected.

The addition of oxygen, the removal of chlorine, and the opening of the aromatic ring in these processes gave rise to compounds that were more biolabile than the parent material, as was demonstrated by results from soil experiments. The degradation of unaltered alachlor was very slow under these conditions, but the products of ozonolysis or photolysis were metabolized very rapidly. Although complete conversion of the products to CO_2 was not observed, it can be argued that much of the remaining label was incorporated into the biomass of the soil microorganisms.

In this study, we have examined separately the effects of aqueous photolysis and ozonation on alachlor and have found that each has an important role in the overall degradation scheme. Our work is continuing in an effort to optimize the combined use of photolytic ozonation with microbial metabolism which, we believe, will provide an efficient and economical pesticide waste treatment process to the agricultural community.

Registry No. Alachlor, 15972-60-8; **1**, 56681-55-1; **2**, 116149-24-7; **4**, 74886-79-6; **5**, 16665-89-7; **6**, 116149-23-6.

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Metal Complexes of Glyphosate

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Insoluble complexes of glyphosate with iron(III), copper(II), calcium, and magnesium ions are formed at near-neutral pH. This suggests a mechanism for the inactivation of glyphosate in contaminated groundwater.

Glyphosate (*N*-(Phosphonomethyl)glycine, trade name (Monsanto) Roundup) is a nonselective, broad-spectrum, postemergent herbicide. Part of its appeal is due to its relatively rapid degradation by microorganisms in soils to nonphytotoxic products (Hoagland and Duke, 1982). These products can even include beneficial phosphorus- and nitrogen-containing species (Rueppel et al., 1977). Its

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mammalian toxicity is relatively low (Franz, 1979), but nevertheless a concern in areas where significant amounts can reach the groundwater or other sources of drinking water, particularly in light of the high aqueous solubility of the isopropylamine salt (Hoagland and Duke, 1982), in which form it is usually applied.

Even though bacterial decomposition processes take place within a time frame of weeks or months (Rueppel et al., 1977), the herbicidal activity of glyphosate appears to be degraded more rapidly in soils (Hensley et al., 1978). Sprankle et al. (1975) postulated that this inactivation may be caused by binding to soil through the phosphonate group. In fact, glyphosate has three groups (amine, carboxylate, phosphonate) that should coordinate strongly to metal ions, particularly to hard transition-metal ions (Pearson, 1963), at near-neutral pH, where both the carboxylate and phosphonate will be deprotonated. The ability to coordinate strongly as a tridentate ligand (or even tetradentate if the phosphonate coordinates through two oxygens) places glyphosate in an almost unique position among herbicides. In addition, almost all of its principal metabolites should form strong complexes with transition-metal ions, and probably with other metal ions such as Ca, Mg, and Zn.

We are particularly concerned with the potential environmental impact on groundwater. Since complexation with the metal ions available in significant concentrations in groundwater could be expected to occur with near certainty, this study was initiated to study the properties of these complexes in order to assess the implications for control of glyphosate contamination. No such specific studies on glyphosate complexes have appeared in the literature, although metal ion influences on herbicidal activity, and the influence of glyphosate on physiological processes involving metal ions, have been studied (Duke et al., 1983; Hoagland and Duke, 1982; Hensley et al., 1978). Complexation by molecules similar to glyphosate, including (aminomethyl)phosphonic acid (Carter et al., 1967; Glowiak et al., 1980) and glycinebis(methylphosphonic acid) (Karadakov and Ivanova, 1979), has been investigated. These studies confirm the ability of phosphonates to coordinate strongly to metal ions and lead us to suspect even stronger complexation by a tridentate glyphosate dianion.

EXPERIMENTAL SECTION

Glyphosate (free acid = H_3glyp) was provided by the Monsanto Chemical Co. It was recrystallized from water.

[Cu(Hglyp)(H_2O)]·0.4CuO. Glyphosate (1.69 g, 0.010 mol) was dissolved in a solution containing 0.8 g of NaOH (0.02 mol) in 25 mL of H_2O. This was mixed with a solution of 2.6 g of $Cu(ClO_4)_2$ (0.010 mol) in 25 mL of H_2O. The solution was allowed to stand at room temperature, whereupon a blue crystalline product formed. It was insoluble in water, alcohols, CH_3CN, and dimethylformamide (DMF). After the solid was washed with water and air-dried, the yield was 1.2 g. Anal. Calcd for $Cu(C_3H_6NPO_5)(H_2O)\cdot 0.4CuO$: C, 12.85; H, 2.88; N, 4.99; P, 11.05; Cu, 31.72. Found: C, 12.85; H, 2.76; N, 4.91; P, 11.60; Cu, 30.99.

Na[Ni(glyp)(H_2O)$_2$]·0.5H_2O. $Ni(ClO_4)_2$ (2.6 g, 0.010 mol) was dissolved in 25 mL of H_2O, and 3.38 g of glyphosate (0.020 mol) and 1.6 g (0.040 mol) of NaOH were dissolved in 25 mL of water. The two solutions were combined. The resulting solution was allowed to evaporate slowly. Upon addition of EtOH a green precipitate was obtained, which was soluble in water. It was purified by recrystallizing from a 50% ethanol solution. Anal. Calcd for $NaNi(C_3H_5NPO_5)(H_2O)_{2.5}$: C, 12.31; H, 3.44; N, 4.78; Ni, 20.05. Found: C, 12.06; H, 3.67; N, 4.89; Ni, 20.13. Visible absorption spectrum: λ_{max} 392 nm (ϵ = 9.1), 654 (ϵ = 4.5), 750 (sh).

[Fe(glyp)(H_2O)$_2$]·1.25H_2O. The procedure for the nickel complex was followed, using 3.5 g of $Fe(ClO_4)_3$ (0.010 mol). A pale yellow crystalline precipitate was obtained, which was filtered and washed with water. It was insoluble in water, alcohols, and DMF. Anal. Calcd for $Fe(C_3H_5NPO_5)(H_2O)_{3.25}$: C, 12.85; H, 4.13; N, 4.99; P, 11.04; Fe, 19.91. Found: C, 12.87; H, 4.40; N, 5.21; P, 11.04; Fe, 19.94.

Ca(Hglyp)·2.25H_2O. The procedure for the copper complex was used, starting with 1.1 g of $CaCl_2$ (0.010 mol). A white crystalline product was obtained that was insoluble in water and organic solvents. Anal. Calcd for $Ca(C_3H_6NPO_5)\cdot 2.25H_2O$: C, 14.55; H, 4.27; N, 5.66; P, 12.51; Ca, 16.18. Found: C, 14.62; H, 4.29; N, 5.76; P, 12.56; Ca, 16.29.

Mg(Hglyp)·2.25H_2O. The procedure for the copper complex was used, starting with 1.0 g of $MgCl_2$. The white crystalline product was insoluble in water and organic solvents. Anal. Calcd for $Mg(C_3H_6NPO_5)\cdot 2.25H_2O$: C, 15.54; H, 4.56; N, 6.04; P, 13.35; Mg, 10.48. Found: C, 15.49; H, 4.49; N, 6.10; P, 13.33; Mg, 10.88.

Instrumentation. X-ray powder diffraction spectra (Philips APD-3600) were recorded to confirm the crystallinity of the complexes. Infrared spectra were measured on a Mattson Cygnus 25 FTIR spectrometer and UV-visible spectra on a Hewlett-Packard 8451A diode array spectrometer.

RESULTS AND DISCUSSION

All the complexes obtained were crystalline substances, as confirmed by the presence of well-defined peaks in the X-ray powder diffraction spectra. In each of the synthetic procedures above, two of the three ionizable glyphosate hydrogens were neutralized, leaving the solution at a near-neutral pH. With both iron(III) and nickel(II) the third proton was removed upon coordination. Since the third ionization involves the second phosphonate proton, this indicates that glyphosate can coordinate as a tetradentate ligand and that some metal ions can compete successfully against protons for the fourth (second phosphonate) site.

Infrared data can be used to show that glyphosate is coordinated through the amine nitrogen, the carboxylate oxygen, and the phosphonate oxygen(s), confirming at the same time that the neutral compounds prepared are not just ionic salts. Precipitation of a noncoordinating ionic salt would be quite unusual for the transition-metal ions but is certainly possible for the alkaline-earth ions.

Carboxylate coordination is indicated by the presence of a strong symmetric $-CO_2$ stretching frequency around 1640 cm^{-1}. It is shifted downward from the protonated uncoordinated $-CO_2H$ frequency around 1700 cm^{-1} and upward from the free, deprotonated $-CO_2^-$ frequency between 1560 and 1580 cm^{-1} (Nakamoto, 1978). A strong band at 1640 cm^{-1} was present in the IR spectra of all the glyphosate complexes, including those with Ca^{2+} and Mg^{2+}, indicating carboxylate coordination in each case.

Amine coordination can be established by the shift of the $\delta(NH_2)$ band to higher frequencies from ca. 1500 cm^{-1} in free amino acids (Nakamoto, 1978). The bands most diagnostic for phosphonate coordination are the PO_3 symmetric and asymmetric stretching vibrations, at 980 and 1100 cm^{-1}, respectively, in phosphonic acids (Tsuboi, 1957). In free glyphosate the 1100-cm^{-1} band is split. These bands may be shifted up to 35 cm^{-1} in either direction upon coordination to metal ions. Table I sum-

Table I. Selected Infrared Frequencies (cm^{-1}) for Metal–Glyphosate Complexes (G = $glyp^{3-}$)

mode	H_3G	$[Cu(HG)H_2O]$	$Na[NiG]$	$[FeG]$	CaHG	MgHG
$\nu_{as}(CO_2)$	1734	1634	1600	1621	1651	1640
$\delta(NH_2)$	1563	*a*	*a*	*a*	1601	*a*
$\nu_s(PO_3)$	981	1002	975	994	991	1014
$\nu_{as}(PO_3)$	1094 1159	1106	1129	1136	1100 1124	1104 1120

[a] Obscured by intense carboxylate stretching band.

marizes the infrared data in these regions, and the change in the 600–2000-cm^{-1} portion of the spectrum upon complexation by Fe(III) can be seen in Figure 1.

Each of the neutral complexes formed was highly insoluble. The copper complex was perhaps the most unusual because of the cocrystallization of CuO. This was verified by matching the X-ray powder diffraction peaks for pure CuO (Smith, 1967). Molecular models indicate that the four coordination sites of the glyphosate molecule cannot conform to a tetrahedral or square-planar geometry around a metal ion but can occupy four of the six positions of an octahedron. Thus, to a large extent, whether glyphosate is three- or four-coordinate will depend on the overall geometric preference of the metal ion. Since the Fe(III) and Ni(II) complexes are with tetradentate $glyp^{3-}$, they can be expected to incorporate two additional water molecules into an octahedral coordination sphere. The pale yellow of the iron complex and the absorption spectrum of the nickel are consistent with this. The molar conductance of a 0.01 M solution of the Ni(II) complex in water was 65 M^{-1} cm^{-1} Ω^{-1}, which is indicative of a 1:1 salt and further confirmation that $glyp^{3-}$ is the coordinating species.

Cu(II) normally adopts a square-planar geometry. The tridentate $Hglyp^{2-}$ will thus be accompanied in the coordination sphere by one water molecule. Ca^{2+} and Mg^{2+} do not have strongly defined preferences for coordination number and geometry. The stoichiometry of the compounds formed leads us to speculate that they are four-coordinate, with one water molecule coordinated.

Spectrophotometrically, a concentration of 10^{-5} M of either Fe(glyp) or Cu(Hglyp) would be detectable. Since we have found no trace of either complex in solution, K_{sp} for each of these complexes is less than 10^{-10}. Glyphosate will be a dianion in moderately buffered soils and water systems when the pH is higher than about 6.5. This work suggests that under such conditions the glyphosate will be complexed strongly by free metal ions and precipitated by many of them.

Groundwater analyses indicate that calcium, magnesium, and iron are all to be found at high concentrations (Harkness and Haffield, 1982). Since these form insoluble complexes, precipitation appears to be the likely fate of glyphosate reaching the groundwater. In rivers and streams the extent of precipitation would depend on the metal ion content, which can be quite variable (Harkness and Haffield, 1982). However, glyphosate is such a strong complexing agent that there is some question that it could even pass through the soil without complexing with metal ions, even when they are completely tied up in mineral form. This, in fact, seems a likely explanation for the observed inactivation as a herbicide, of glyphosate once it passes into the soil (Hensley et al., 1978).

Iron may be present in soil or groundwater as either Fe(II) or Fe(III). This may make little difference. In our experiments, solutions of glyphosate and Fe(II) were rapidly oxidized, yielding the same iron(III) complex. The low solubility of this compound doubtless contributes a large part of the driving force for the oxidation process.

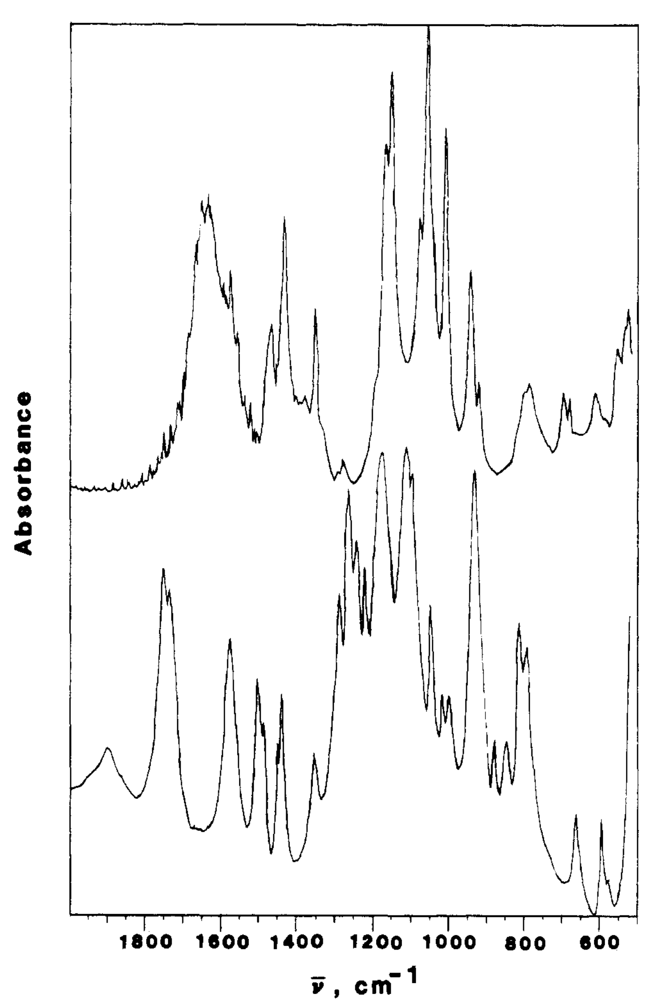

Figure 1. Infrared spectra of glyphosate: H_3glyp (bottom) and the iron(III)–glyphosate complex, $[Fe(glyp)(H_2O)_2]\cdot1.25H_2O$ (top).

We emphasize once again that metal ion complexation and precipitation are the exception, not the rule for herbicides. Glyphosate is in a unique class of strongly chelating herbicides.

ACKNOWLEDGMENT

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Registry No. H_3glyp, 1071-83-6; $Fe(glyp)(H_2O)_2$, 116211-79-1; $Cu(Hglyp)(H_2O)$, 116211-82-6; Ca(Hglyp), 39600-55-0; Mg(Hglyp), 116211-81-5; $Na[Ni(glyp)(H_2O)_2]$, 116211-80-4.

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Processing and Quality Assessment of Solubles Prepared from Dogfish Processing Wastes

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Solubles were produced through the digestion and concentration of ground raw dogfish processing wastes under acidic and heated conditions. The optimum conditions for the digestion were pH 3.5 and 50 °C. Proximate analysis of the soluble product indicated, on a dry-weight basis, 67.4% crude protein, 26.9% lipid, and 7.6% ash. The nonprotein nitrogen constituents contained free amino acids, urea, trimethylamine, volatile base nitrogen, and ammonia in the amounts 41.3, 3.2, 1.5, 1.0, and 1.3%, respectively. Free amino acids ranged between 65 and 85% of the protein nitrogen present. Fatty acid analysis indicated the predominance of unsaturated fatty acids.

Spiny dogfish (*Squalus acanthias*) is an abundant yet underutilized resource available to the seafood industry on the eastern coast of the United States (Seymour, 1982). Obstacles to the development of food processing for this species include high proportion of processing waste, rapid deterioration of flesh quality (Southcott et al., 1960; Boyd et al., 1967; Bilinski et al., 1985), and the unpleasant name. Although there is no market in the United States at present, dogfish in Europe has long been consumed both fresh and smoked (Kizevetter, 1971). Dogfish flesh has a high lipid content (Boyd et al., 1967), which makes it suitable for smoking (Shiau and Chai, 1985). Oily fish has also been receiving positive publicity due to recent research findings of the possible health benefits of diets rich in ω^3 fatty acids (Kromhout et al., 1985; Phillipson et al., 1985).

The greatest obstacle to the development of dogfish processing is the problem of waste disposal (Jackson, 1987). Dogfish processing wastes consist of the head, viscera, skin, and small fins. Due to their large proportion, high water content, and high degree of perishability which readily produces a large quantity of ammonia, it is not practical to heat-dry these processing wastes to meal as is done with more conventional species. These wastes are also extremely difficult to process due to the abrasive nature of the skin and the tendency of the wastes to clog grinding equipment. Therefore, the wastes must be disposed of, and this process can be expensive. Current methods of disposal include landfill burial and dumping at sea, both of which have been associated with pollution problems (Jackson, 1987). Since dogfish processing produces such a great amount of waste, the development of an economical and useful waste process would benefit all other aspects of seafood industry.

The objective of this study was to develop an alternative economical process and quality evaluation of a useful, soluble product prepared from dogfish processing wastes.

MATERIALS AND METHODS

Dogfish Processing. Iced, freshly harvested female dogfish were transported to the laboratory where they were promptly decapitated and dressed. Male dogfish, which are small in size, were not harvested and hence not included in this study. Each sample consisted of 250–550 lb of female dogfish. The skinned carcasses were set aside for human consumption, the livers were allocated for oil production, and the skin and fins were frozen for other uses such as a possible replacement for traditional blue crab baits. The remaining head, viscera, embryos and pups, blood, and all debris were collected for soluble production.

Solubles Production. The dogfish processing wastes were ground in a Hobart food mixer with a meat grinding attachment with a $^1/_8$-in. pore size head. The ground wastes were transferred to a steam-jacketed kettle outfitted with a variable speed agitator. The pH and temperature were adjusted, and the mixture was agitated for 4 h under these conditions. After the digestion was complete, the temperature was increased to 80 °C to drive off moisture, concentrating the product to about 70% total solids. The solubles were packed in sanitized 5-gal plastic buckets, sealed, and stored at 23 °C for further use and testing.

Optimum Process Determination. The primary environmental factors influencing the rate of digestion were pH and temperature. The optimum pH was determined by adjusting four equal portions of the ground processing wastes to pH 3.5, 4.0, 4.5, and 5.0 with concentrated hy-

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