Table II. Accumulation of [^{14}C]Carbaryl and XMC by Various Aquatic Organisms

	Carbaryl		XMC	
Concn in water, ppb	1.66 (1 day) 9.45 (22 days)		2.04 (1 day) 35.8 (34 days)	
Concn in soil, ppm	2.43 (start) 1.55 (22 days)		2.78 (start) 1.79 (34 days)	
Organisms	ppm	BAR[a]	ppm	BAR[a]
Algae	37.9	4000	109.0	3050
Duckweed	34.2	3600	81.0	2300
Snail	2.81	300	9.41	260
Catfish	1.33	140	19.8	550
Crayfish	2.48	260	3.73	104

[a] Bioaccumulation ratio calculated by dividing parts per million of dried tissue by parts per million in water at harvest.

1970; Warnick et al., 1966). It is speculated that algae and bacteria concentrate pesticides by absorption through membranes. However, whether this represents active transport or passive diffusion is not known.

In the hexane–ethyl acetate extract from soil, 9.6% of the recovered activity was 1-naphthol; the remaining 90% (or 16% of that originally added) was carbaryl. This ratio was obtained by eluting the corresponding spots from TLC plates and then counting. Unfortunately, ^{14}C-*N*-methyl-labeled XMC was used, so the ratio of 3,5-xylol to XMC was not easily obtainable. However, the parent XMC was identified by TLC from both soil and water. Unfortunately, the radioactivity was too low in the biomass to detect the parent insecticides or their metabolites.

Although carbamate insecticides have been assumed to degrade rapidly in the environment, our results suggest that some carbamates are relatively persistent in the aquatic environment. Bioaccumulation ratios, especially for algae and duckweed, were relatively high. Therefore, more detailed research on the effect of other carbamate insecticides on aquatic organisms, especially daphnids, at lower concentrations and for longer exposures is needed.

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Binding of 2,4-Dichloro- and 2,4,5-Trichlorophenoxyacetic Acids to Bovine Serum Albumin. A Proton Magnetic Resonance Study

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The binding of two important herbicides, 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acids (2,4-D and 2,4,5-T), with the protein bovine serum albumin has been studied. The use of the proton magnetic resonance technique to study protein–herbicide binding has been demonstrated. The line widths of methylene and ring protons of 2,4-D and 2,4,5-T showed an increase upon the addition of bovine serum albumin (BSA). These changes have been interpreted in terms of binding to BSA. These studies suggest that the methylene protons are closer to the binding site than the ring protons. The equilibrium constant for the binding process has been determined. The binding shows a decrease with increasing pH.

Once a chemical finds its way into a living system, it may interact with many biological polymers. The interactions will depend upon such factors as the water solubility, lipid/water partition coefficient, and the molecular structure of the chemical. Chlorinated hydrocarbons usually bind to phospholipids (Haque et al., 1973), whereas protein binding may be more important for chemicals possessing increased water solubility. The use of nuclear magnetic resonance technique to study pesticide binding has recently been introduced (Haque, 1974).

2,4-Dichloro- and 2,4,5-trichlorophenoxyacetic acids, commonly known as 2,4-D and 2,4,5-T are two well-known herbicides. These chemicals have been used for some time on a large scale. Although 2,4-D is considered relatively safe to the environment, 2,4,5-T has been linked with toxic dioxin impurities. The mechanism and the mode of action of these chemicals are not very well understood. A qualitative indication of the binding of phenoxy herbicides with proteins and nucleic acids has been reported by monolayer studies (Brian and Rideal, 1952) and equilibrium dialysis measurements (Venis, 1968). However, very little is known about the structural parameters involved in the binding of

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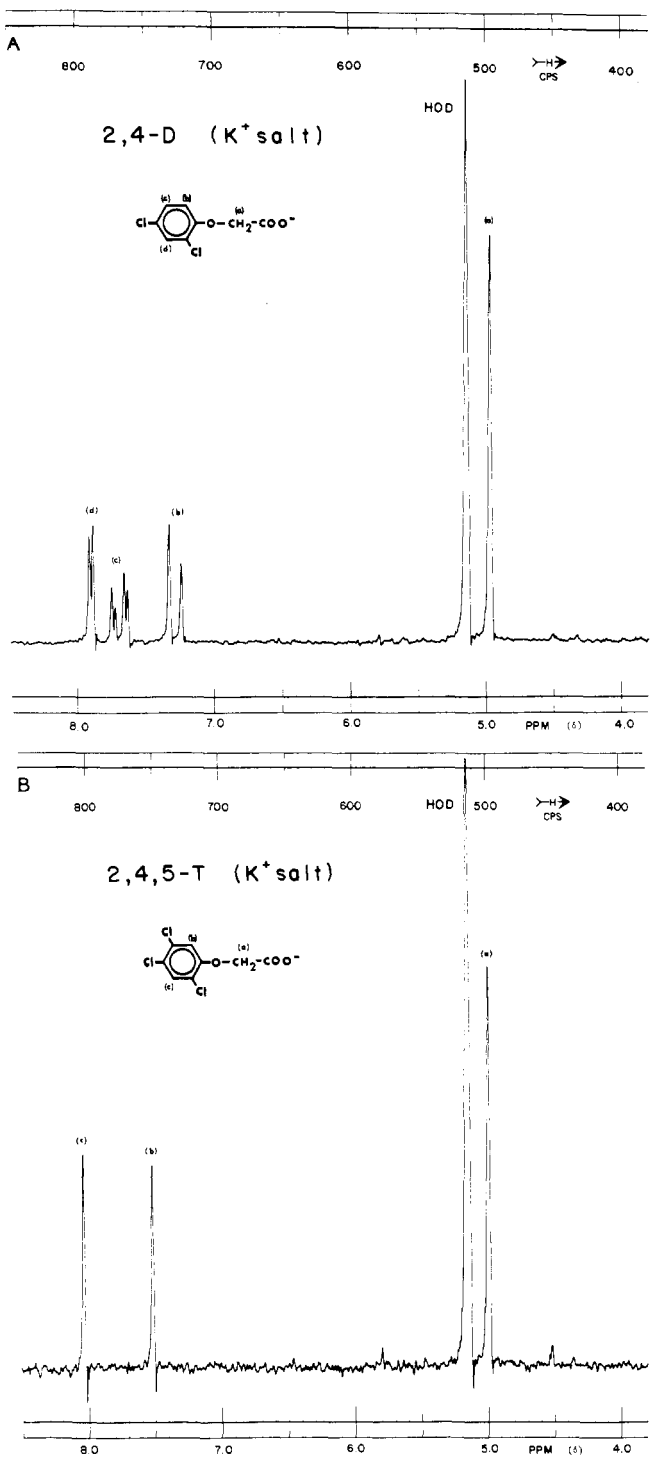

Figure 1. (A) ^{1}H NMR spectrum (100 MHz) of 2,4-D (K^+ salt) in D_2O at 29°. Me_4Si was used as the external reference. (B) ^{1}H NMR spectrum (100 MHz) of 2,4,5-T (K^+ salt) in D_2O at 29°. Me_4Si was used as the external reference.

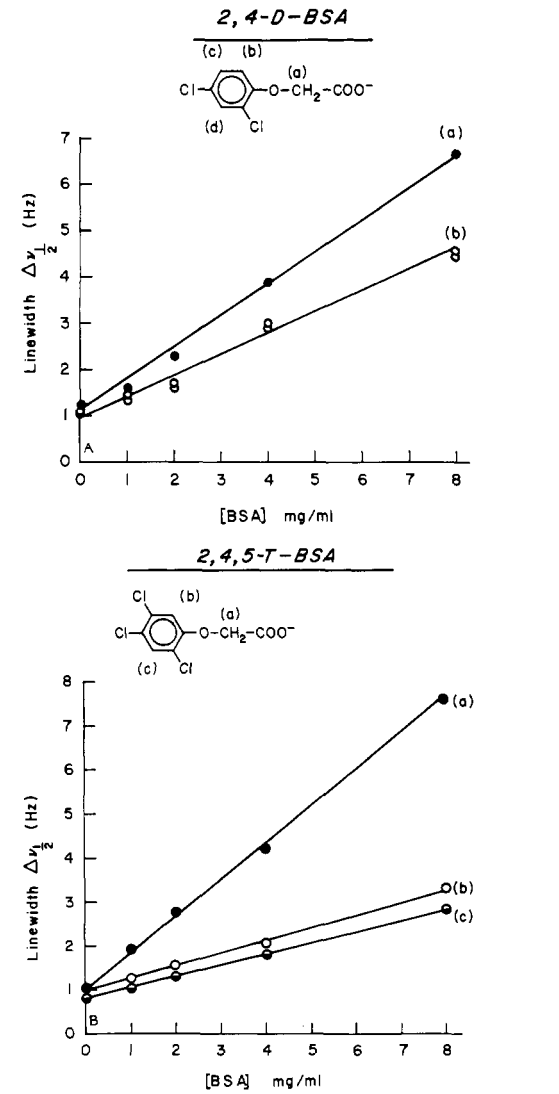

Figure 2. (A) Changes in line widths of 2,4-D protons in D_2O (pH 7.6), 29°, upon addition of BSA. (B) Changes in line widths of 2,4,5-T protons in D_2O (pH 7.6), 29°, upon addition of BSA.

2,4-D and 2,4,5-T to proteins. Such investigations may be helpful in understanding the molecular basis of the mode of action of phenoxy herbicides. In the present paper we present a proton magnetic resonance study of the interaction of 2,4-D and 2,4,5-T with the protein bovine serum albumin.

EXPERIMENTAL SECTION

Crystallized and lyophilized bovine serum albumin (BSA), essentially fatty acid free, was obtained from Sigma Chemical Co. A fresh protein solution was made for each series of runs by dissolving BSA in D_2O. Dialysis against D_2O removed exchangeable BSA protons from solution that would have greatly contributed to the HOD peak. Final protein concentration was determined by using $E_{280}^{1\%} = 6.6$. 2,4-D and 2,4,5-T were recrystallized and converted to the corresponding potassium salts. Fresh solutions were made by dissolving known amounts in D_2O. Dilute NaOD and DCl were used to bring all experimental solutions to pH 7.5–7.7.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian HA-100 high-resolution NMR spectrometer. Chemical shifts were measured against tetramethylsilane (Me_4Si) in a reference capillary. For maximum signal-to-noise ratio for the single ring proton linewidth measurements, H_2O was added (a concentration of 4%) for an internal lock on the HOD peak. All the measurements were carried out at a probe temperature of 29°.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of the potassium salts of 2,4-D and 2,4,5-T in D_2O are shown in Figures 1A and 1B. Both spectra are simple to analyze. The high field peak (δ 4.99) is due to the methylene protons. This peak appears just upfield of the HOD peak. The ring proton spectrum of 2,4-D shows doublets due to protons b (δ 7.31, $J_{b,c}$ = 8.8 Hz) and d (δ 7.94, $J_{c,d}$ = 2.4 Hz) and a quartet due to proton c (δ 7.74, $J_{b,c}$ = 8.8 Hz, $J_{c,d}$ = 2.4 Hz). The ring protons of 2,4,5-T

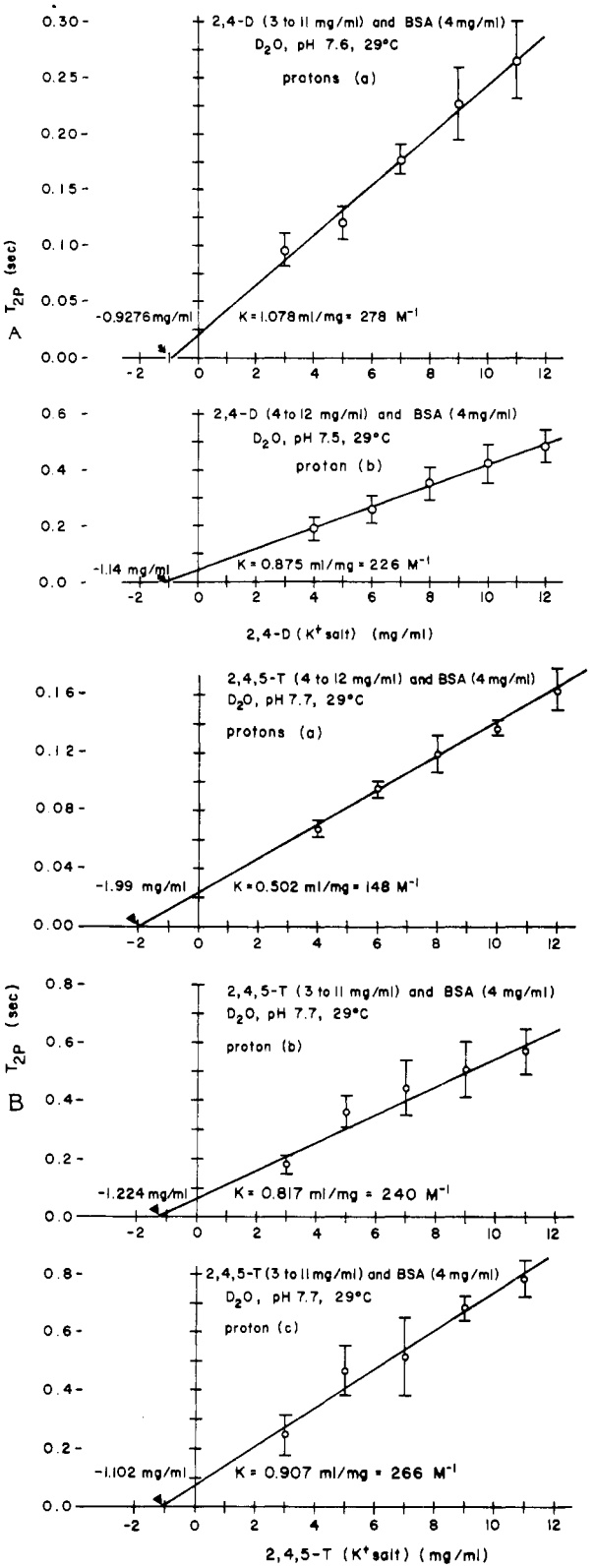

Figure 3. (A) T_{2p} as a function of 2,4-D concentration for the 2,4-D–BSA interaction. (B) T_{2p} as a function of 2,4,5-T concentration for the 2,4,5-T–BSA interaction.

show two sharp singlets corresponding to protons b (δ 7.54) and c (δ 8.05). The addition of BSA to a D_2O solution of 2,4-D or 2,4,5-T produced line-width changes in all the resonance peaks. In general, with increasing concentrations of BSA the line widths all broadened. However, the HOD peak showed negligible line broadening and the CH_2 peak was broadened more than the ring proton peaks (Figures 2A and 2B). The line-width plots for protons c and d for the 2,4-D–BSA interaction are not given in Figures 2A and 2B. This is because an accurate determination of line widths for these two proton resonance peaks was not possible. The addition of BSA to 2,4-D or 2,4,5-T solutions did not produce any changes in the chemical shifts of any of the resonance peaks.

Table I. K_p Values for the Herbicide–BSA Interactions as Calculated Using Different ^1H NMR Resonance Lines[a]

2,4-D		2,4,5-T	
Proton	K_p, M^{-1}	Proton	K_p, M^{-1}
a	278	a	148
b	226	b	240
		c	266

[a] The D_2O solutions are pH 7.5–7.7, at 29°.

The increase in the line widths of 2,4-D and 2,4,5-T peaks by the addition of BSA may be explained on the basis of binding of these herbicides to the protein. Different peaks show varied broadening, and since the HOD peak broadens negligibly, these changes are indeed due to binding and not due to some other effects. The exchange between the bound and free herbicide is rapid since an overall, broadened peak is observed. This also indicates that the binding is weak.

The equilibrium for the protein–herbicide system may be expressed by the following expression:

$$\text{herbicide} + \text{BSA} \rightleftharpoons \text{herbicide·BSA} \quad (1)$$

The line width $\Delta\nu_{1/2}$, and the relaxation time, T_2, are related as follows:

$$1/T_2 = \pi\Delta\nu_{1/2} \quad (2)$$

The change in the relaxation time, T_{2p}, of the herbicide protons due to BSA binding may be expressed as:

$$1/T_{2p} = (1/T_2) - (1/T_2^0) \quad (3)$$

where T_2 is the observed relaxation time in the presence of BSA and T_2^0 is the relaxation time in the absence of BSA due to inhomogeneity in the field. The relaxation time, T_{2p}, for solutions where [herbicide] $\gg$ [BSA] may be represented by the expression (Navon et al., 1970; Kan and Li, 1972):

$$1/T_{2p} = \{[\text{BSA}]/(K_p^{-1} + [\text{herbicide}])\}[1/(T_{2b} + \tau_b)] \quad (4)$$

In the above equation K_p^{-1} is the equilibrium constant defined as:

$$K_p = [\text{herbicide·BSA}]/[\text{herbicide}][\text{BSA}] \quad (5)$$

T_{2b} is the relaxation time of the herbicide proton and τ_b is the average time a herbicide molecule is bound to BSA before it exchanges. According to eq 4, a plot of T_{2p} vs. herbicide concentration at a constant protein concentration should result in a straight line, and the negative intercept on the x axis should give K_p^{-1}.

An increase in the herbicide concentration of a fixed BSA–herbicide solution produced a sharpening of the 2,4-D or 2,4,5-T resonance peaks. The line-width plots for T_{2p} vs. herbicide concentration are shown in Figures 3A and 3B. These plots produce straight lines in spite of the fact that the T_{2p} measurements are not very accurate.

The K_p values for the herbicide–BSA interactions are given in Table I. The K_p values for the 2,4-D–BSA interaction determined from the T_{2p} measurements of CH_2 and ring protons are in excellent agreement. The K_p values for the 2,4,5-T–BSA interaction agree to a lesser extent.

There are a few drawbacks in calculating K_p using this method. First, herbicide concentration is used rather than

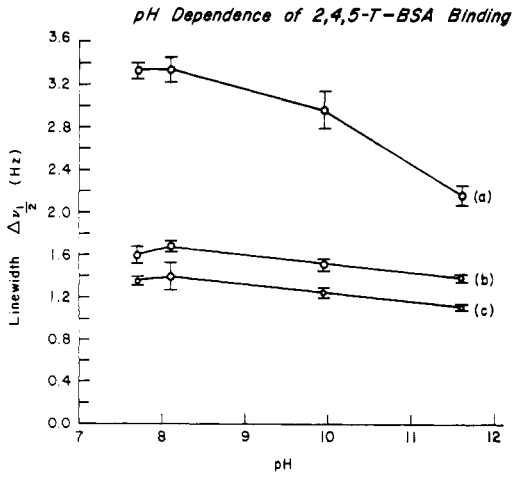

Figure 4. Effect of pH on binding of 2,4,5-T (10 mg/ml) to BSA (4 mg/ml) in D_2O at 29°.

activity. This may lead to some error when calculating K_p. Secondly, the potassium salt of the herbicide may produce some ion pairs. The calculation of T_2 using line width is not very accurate and thus T_2 measurements using the spin–echo method may be preferred.

The line-width changes in 2,4,5-T resonance peaks at a fixed BSA concentration in solutions of different pH are shown in Figure 4. The decrease in line width with increasing pH may be explained on the basis of a decrease in binding at higher pH values. Such a decrease may be due to denaturation of the protein at higher pH values.

The ^1H NMR results of herbicide–BSA binding indicate that the CH_2 protons are more affected by the binding than the ring protons, suggesting that they are closer to the binding site. This is in agreement with the earlier investigation of the binding of phenoxyacetic acid and proteins (Fischer and Jardetzky, 1965). It is interesting to note that this is in contrast with the binding of the chlorinated hydrocarbon bis(*p*-chlorophenyl)acetic acid with BSA, where the binding involves the benzene ring (Haque et al., 1974). Such behavior may be due to the large difference in the hydrophobic characteristics of chlorinated hydrocarbons and these herbicides.

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Differences in Germination Response of Spores of Several Species of Rust and Smut Fungi to Nonanal, 6-Methyl-5-hepten-2-one, and Related Compounds

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Uredospores of the following rust species were stimulated to germinate by nonanal or 1-nonanol and/or 6-methyl-5-hepten-2-one (methylheptenone): *Puccinia arachidis, P. coronata, P. hieracii, P. parca, P. pelargonii-zonalis, P. polysora, P. recondita, P. rubigo-vera, P. sorghi, Uromyces phaseoli, Coleosporium ipomoeae,* and *Melampsora abietis-canadensis.* Uredospores of *Puccinia helianthi* were not stimulated by these compounds, but were stimulated by cinnamaldehyde and β-ionone, as were some of the above. Aeciospores of *Puccinia podophylli* were stimulated by nonanol. Teleutospores of *Ustilago avenae, U. maydis,* and *U. tritici* were stimulated by nonanol. Striking differential responses to the five compounds were observed with certain species. The stimulatory action of nonanal and related compounds has been extended thus far to include members of four fungal families—the Pucciniaceae, Coleosporiaceae, Melampsoraceae, and Ustilaginaceae; five genera—*Puccinia, Uromyces, Coleosporium, Melampsora,* and *Ustilago;* and 19 species.

An endogenous spore germination stimulator from uredospores of *Puccinia graminis* Pers. f. *tritici* Eriks. & E. Henn. was first reported in 1957 (French et al.) and identified as *n*-nonanal (pelargonaldehyde) (French and Weintraub, 1957). More recently, nonanal was identified in volatiles collected directly from fresh uredospores, and in distillates of uredospores of *P. coronata* Cda., *P. recondita* Rob. ex Desm., *P. sorghi* Schw., *P. helianthi* Schw., *P. striiformis* West., and *Uromyces phaseoli* (Reben.) Wint. (Rines et al., 1974). In addition, 6-methyl-5-hepten-2-one (methylheptenone) was identified in *P. graminis* var. *tritici* as the stimulatory volatile that diffused from uredospores floated on water. Nonanal and more than 60 related compounds have been reported to stimulate germination of uredospores of *P. graminis* var. *tritici* (French, 1961, 1973; French and Gallimore, 1971). Chemical stimulation

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