

Aspects of the Adsorption of Azinphos-methyl by Smectites

M. Jesús Sánchez Martín and María Sánchez Camazano*

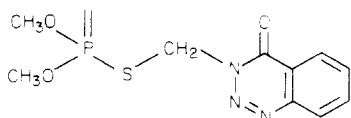
The adsorption and interaction mechanism of montmorillonite with the organophosphorus pesticide azinphos-methyl [*O,O*-dimethyl *S*-[[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl]methyl] phosphorothiolothionate] was studied. The compound is adsorbed, from a 0.3 M solution in 1,2-dichloroethane, into the interlayer space of the silicate, forming a defined interlayer complex of d_{001} basal spacing of 16.05 Å. IR results showed that, in agreement with the nature of the interlayer cation, the C=O and P=S groups in some cases and the -N=N- and P=S groups in others are involved in the bonding mechanism. A study was also made of the adsorption of azinphos-methyl adsorbed by calcium montmorillonite, copper montmorillonite, and calcium hectorite in an aqueous medium at 30 and 45 °C. The isosteric heats of adsorption were determined. The values obtained for such isosteric heats and the endo- or exothermic nature of the process are related to the nature of the interlayer cation and the layer charge of the silicate. These two factors also determine the kinetics of the adsorption process.

Study of interaction between soil clay minerals and pesticides is of great interest from two points of view: on one hand from the environmental aspect, since adsorption by soil colloids influences most processes determining the evolution of pesticides in the soil, and on the other from the point of view of pest control, when pesticides are active in the ground, because effectiveness and frequency of application, apart from depending on the tendency of the pesticide to degrade, also depend on adsorption.

Organophosphorus pesticides are widely used in agriculture, and according to Harris (1972) are those that are most strongly inactivated in the soil by adsorption. The bibliography relating to pesticide behavior in soil (Edwards, 1964; Helling et al., 1971; Russell and Adams, 1973) and to pesticide adsorption by clay minerals (Bailey and White, 1964, 1970; Cloos, 1972) show that clay-organophosphorus pesticide interactions have been studied only to a limited extent.

Accordingly, the Unit of Physical-Chemistry and Clay Mineralogy of the Centro de Edafología y Biología Aplicada in Salamanca has been working for some years now on the interaction between clay and organophosphorus pesticides. Preliminary studies dealt with the mechanisms of interaction between such compounds and montmorillonite. This silicate, of great adsorption capacity, is a common soil component. Studies were made of the interaction of this clay with a series of organophosphorus pesticides (phosphates, phosphorothionates, and phosphorothiolothionates) (Sánchez Camazano and Sánchez Martín, 1983b) with the aim of establishing relationships between adsorbate chemical structure and the mechanisms of adsorption by montmorillonite. Such studies are of interest in the manufacture of pesticides and for the preparation of formulations that are more bioactive and less hazardous in applications.

The present paper studies the interaction of azinphos-methyl with montmorillonite. Azinphos-methyl is the common name for *O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl]methyl phosphorothiolothionate having the structural formula



Studies were carried out in a montmorillonite-pesticide-organic solvent system with the aim of discovering the interaction mechanism and in a montmorillonite-pesticide-water system for the purposes of shedding light on pesticide behavior in actual soils.

MATERIALS AND METHODS

The <2- μ m fraction of Tidinit montmorillonite (Morocco), Wyoming montmorillonite, and Hector hectorite (California) were used in the study. Ca samples of the three clays and Na, K, Mg, Ba, Ni, and Cu samples of the Tidinit montmorillonite were prepared according to the method described by González García and González García (1953). The azinphos-methyl was supplied by Xpectrix International, Inc., Deerfield, IL. It is a solid crystalline substance with a melting point of 73 °C; its solubility in water is 29 mg/L and it is soluble in most organic solvents.

Azinphos-methyl is only slightly water soluble, and thus an organic solvent, 1,2-dichloroethane, was used in the study of the interaction mechanism. This solvent is not adsorbed into clay interlayer space (Sánchez Camazano et al., 1973). The azinphos-methyl concentration was 10% (0.3 M) and contact time was 4 days.

For study of the influence of hydration status on montmorillonite expansion and subsequent effects on adsorption, samples previously heated to 110 °C and samples previously exposed to atmospheres at different relative humidities (4-100%) were treated with azinphosmethyl.

X-ray powder diffraction studies were performed and diffractograms obtained with a Phillips instrument by using 114.7 mm diameter cameras and Cu K α radiation.

For IR studies, films were prepared of homoionic montmorillonite samples. Corresponding complexes were obtained by film immersion into the azinphos-methyl solution, after which these films were washed in 1,2-dichloroethane in order to remove excess pesticide. By means of X-ray diffraction of the complexes, before and after washing with dichloroethane, it was possible to observe first the formation of the complex and second that washing did not remove the azinphos-methyl adsorbed into the interlayer space. The vacuum used for complex dehydration was 0.1 mmHg in all cases. Spectra were registered on a double-beam Acculab-6 Beckman IR spectrophotometer.

A kinetic study was carried out by treating 5 mg of montmorillonite (0.004 mequiv of exchange cation) with 0.20 mg of azinphos-methyl in 10 mL of water (pH 6). The samples were shaken continuously in a thermostated camera at 30 °C. The samples were removed at different times and the azinphos-methyl concentration was mea-

*Centro de Edafología y Biología Aplicada de Salamanca del C.S.I.C., Salamanca, Spain.

Table I. Basal d_{001} Spacing of Calcium Montmorillonites Treated with Azinphos-methyl Being Maintained in Equilibrium in Atmospheres of Different Relative Humidities

relative humidity, %	Tidnit montmorillonite, d_{001}	Wyoming montmorillonite, d_{001}
0	15.38 diff ^a	13.10 diff
4.5	16.05	14.72 diff
16	16.05	16.05
35	16.05	16.05
75	16.05	16.05
100	16.05	16.05

^a diff, diffuse.**Table II. Basal d_{001} Spacings of Homoionic Montmorillonites Treated with 1,2-Dichloroethane-Azinphos-methyl Solution**

cation	d_{001} , Å	$\Delta\text{Å}$	cation	d_{001} , Å	$\Delta\text{Å}$
Na ⁺	14.72	5.12	Ba ²⁺	16.05	6.45
K ⁺	13.10	3.10	Ni ²⁺	16.05	6.45
Mg ²⁺	16.05	6.45	Cu ²⁺	16.05	6.45
Ca ²⁺	16.05	6.45			

sured by UV spectroscopy (absorption maximum, 228 nm). All determinations were performed twice.

To obtain adsorption isotherms for azinphos-methyl in aqueous medium, in each case, 5 mg of montmorillonite was treated with 10 mL of solutions at pH 6 containing 0.04, 0.08, 0.12, 0.16, and 0.20 mg of azinphos-methyl. The samples were shaken continuously in a thermostated camera at 30 and 45 °C for 48 h. Suspensions were centrifuged, and the equilibrium concentration was determined in the supernatant fluid by UV spectroscopy.

RESULTS AND DISCUSSION

Results of X-ray diffraction studies of Tidinit and Wyoming calcium montmorillonite treated with a 10% (0.3 M) solution of azinphos-methyl in 1,2-dichloroethane showed that the insecticide penetrates into the interlayer space of the silicate to form a complex of basal spacing d_{001} of 16.05 Å. The increase in spacing with respect to the value of 9.6 Å of dehydrated montmorillonite was 6.45 Å. The crystalline powder diagrams showed a regular sequence of reflections.

The spacing of the azinphos-methyl-montmorillonite complex was the same (16.05 Å) when the time of treatment of the montmorillonite was prolonged over a period of 4, 6, 8, or more days. It was also independent of azinphos-methyl concentration for solutions greater than 2.5%. The previous hydration status of montmorillonite, however, influenced complex formation as could be deduced from the results shown in Table I; a previous hydration status corresponding to a relative humidity of 4.5% for the Tidinit montmorillonite and of 16% for Wyoming montmorillonite was necessary for the complex formation. In dehydrated samples, there is greater proximity between the layers and cations with electrostatic attraction enhanced; these samples thus offered greater resistance to expansion by the organic compound. The layer charge of the Wyoming montmorillonite (0.320), higher than that of the Tidinit clay (0.264), gives rise to a greater electrostatic attraction between the layers and cations; this implies that Wyoming montmorillonite needs to be hydrated previously to a greater extent, in turn producing a decrease in the layer-cation electrostatic attraction that will permit the entry of the pesticide into the interlayer space.

Table II shows basal spacings of homoionic Tidinit montmorillonite samples treated with azinphos-methyl together with the corresponding increases relative to un-

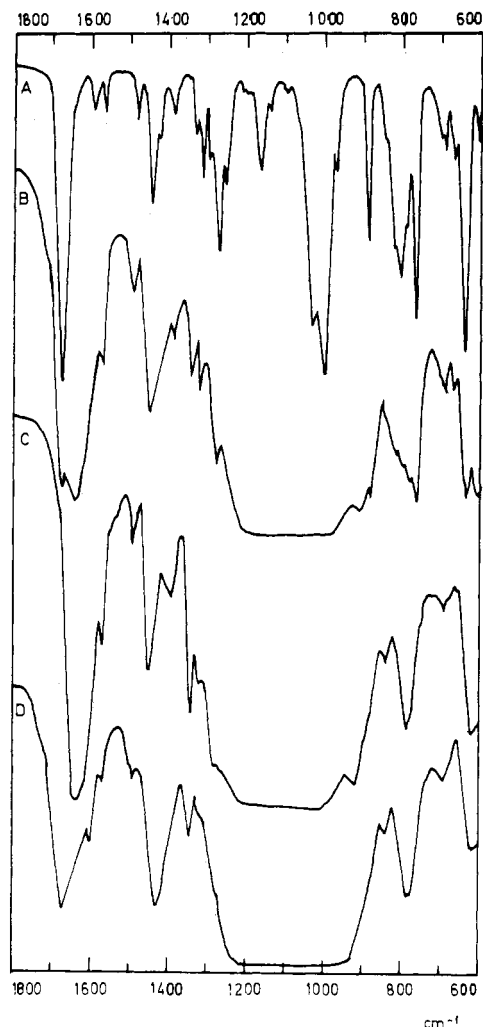


Figure 1. IR spectra (1800–600 cm^{-1}) of (A) azinphos-methyl, (B) the calcium montmorillonite-azinphos-methyl complex air-dried, (C) the calcium montmorillonite-azinphos-methyl complex dehydrated at 100 °C under vacuum, and (D) the copper montmorillonite-azinphos-methyl complex dehydrated at 100 °C under vacuum.

treated samples. As may be seen, samples saturated with divalent cations all gave basal spacings of 16.05 Å, while those saturated with monovalent cations gave lower and more diffuse spacings: 14.72 Å for sodium montmorillonite and 13.10 Å for potassium montmorillonite. The low basal spacings given by these latter two samples must correspond to partial solvation of the layers. Since interaction, as will be detailed below, takes place by ion-dipole interaction between the interlayer cations and certain functional groups of the pesticide and since the azinphos-methyl molecule is very large, it could impede the packing of these molecules into the available space per monovalent cation. As is known, the space available in the interlayer space per monovalent cation is half that corresponding to divalent cations.

The same spacing has been reported by us for complexes formed by other organophosphorus pesticides that also have two methyl radicals, such as dichlorvos, sumithion, monocrotophos, etc. (Sánchez Camazano and Sánchez Martín, 1983b). These results led to an initial idea that the arrangement and distance of the methyl radicals must be a fundamental factor in determining spacing. When the radicals are ethyl, spacing is greater, 17.66 Å, and is the same in all cases.

Figure 1 shows the IR spectra (1800–600 cm^{-1}) of azinphos-methyl, of the air-dried calcium montmorillonite-

Table III. Stretching Frequency (cm^{-1}) of the C=O, -N=N-, C-N, and P=S Groups of Free Azinphos-methyl and Azinphos-methyl Adsorbed by the Various Homoionic Samples

sample	C=O ^a	C=O ^b	-N=N- ^b	C-N ^b	P=S ^b
azin-phos-methyl	1680		1450	1325	645
Na	1680		1450	1335	630
	1670	1670			
K	1680		1450	1335	640
	1670	1670			
Mg	1680		1450	1340	625
	1640	1640			
Ca	1680		1450	1340	625
	1645	1645			
Ba	1680		1450	1335	628
	1650	1650			
Cu	1680	1680	1435	1345	622
Ni	1680	1680	1435	1345	620
	1640	1640			

^aComplex air-dried. ^bComplex dehydrated at 100 °C under vacuum.

azinphos-methyl complex, of the same complex dried under vacuum, and of the vacuum-dried copper montmorillonite-azinphos-methyl complex. From a detailed study of the complete spectra corresponding to all homoionic samples, it may be deduced that the P=S, C=O, -N=N-, and C-N stretching bands undergo modifications in the spectra of azinphos-methyl adsorbed by montmorillonite (Table III). The rest of the bands are not altered in any way.

In the spectrum of unadsorbed azinphos-methyl, the C=O stretching band appears at 1680 cm^{-1} and shifts partially to lower frequencies in the spectra of the air-dried complexes corresponding to the Na, K, Mg, Ca, and Ba samples. If samples are dehydrated in a 0.1-mmHg vacuum with simultaneous heating at 100 °C, the shift of the band is almost complete. The magnitude of the shift observed depends on the saturating cation; thus it is smaller for the K sample (10 cm^{-1}) and greater for the Mg sample (40 cm^{-1}). These results suggest that there is interaction between the interlayer cations and the C=O group. Such interaction in hydrated complexes is partly through the water molecules and partly direct and is only totally direct in the case of the dehydrated complexes. In the spectrum of the copper montmorillonite complex the C=O stretching band appears at the same frequency as in the case of free azinphos-methyl. However, a shift toward lower frequencies of the band at 1450 cm^{-1} may be observed; this band appears in the free azinphos-methyl at this frequency and shifts to 1435 cm^{-1} in the complex.

The band at 1450 cm^{-1} has been assigned to the -N=N- group, taking into account that the stretching band of this group in azo derivatives appears in the region 1450-1380 cm^{-1} , according to Morgan (1961). Furthermore, a band close to 1450 cm^{-1} (1410 cm^{-1}) has also been attributed to triazines by Lancaster et al. (1961).

In the complex of the Ni sample, an intermediate situation arises: the C=O stretching band frequency shifts partially toward low frequencies (Table III). These results show that Ni ions in the sample interact through the C=O group and that other ions interact through the N of the -N=N- group.

Parallel to the shift in these two bands toward low frequencies, a shift may be observed in the C-N stretching band at higher frequencies (Figure 1). It shifts partially when the shift of the C=O and/or -N=N- bands is incomplete and totally when the shift is complete. According to the structure of the molecule, the interaction through both groups may affect proximal C-N bonds.

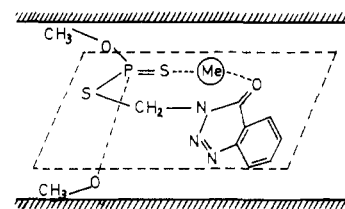


Figure 2. Proposed arrangement of azinphos-methyl molecules in the interlayer space of montmorillonite.

The stretching bands of the P=S group appears at 645 cm^{-1} (band I) and the frequency of the band coupled to this stretching (Thomas, 1974) at 815 cm^{-1} (band II). Both bands showed shifts in the dehydrated montmorillonite-azinphos-methyl complexes. The shift in band I ranged between 5 cm^{-1} for the K sample and 25 cm^{-1} for the Ni sample. The shift in band II could only be seen when it was very small, as in the case of the K sample; in the rest of the samples, the shift was greater and the band masked by a band of the clay present in that zone of the spectrum. In the hydrated complexes, these bands generally showed very small shifts toward lower frequencies. It may therefore be seen that interaction does exist between the P=S group and the interlayer cations, through the water molecules in hydrated complexes and directly in dehydrated complexes.

In previous studies carried out by the authors on the interaction of other organophosphorus pesticides with montmorillonite in organic medium (Sánchez Camazano and Sánchez Martín, 1983b), a description was also made of simultaneous interaction between the exchange cation and the P=O (or P=S) group and the C=O or -N= group of the side chains. The results from the present IR studies suggest that there is a simultaneous interaction between the oxygen atom of the C=O group (or the nitrogen of the -N=N- group) and the sulfur atom of the P=S group and that this is direct or through the water molecules, according to the hydration status of the complex.

The possible arrangement of the azinphos-methyl molecule in the interlayer space of montmorillonite was calculated according to (1) the molecular dimensions of the insecticide determined from the angles and distances of bonding, described by Rohrbaugh et al. (1976), (2) the increase in spacing, of 6.45 Å, (3) the atoms involved in the interaction as deduced from the IR studies, and (4) the fact that the molecule should be oriented with the positive groups toward the negative surface of the oxygen atoms. On considering the molecular dimensions of azinphos-methyl, the increase in spacing observed should correspond to that of a monolayer complex. The molecular arrangement compatible with this increase, together with what has been reported in the preceding paragraph, is shown schematically in Figure 2. The CH₃-CH₃ distance calculated is 6.43 Å. Each of the methyl groups is on a negative surface of oxygen atoms, and the P=S group and the oxobenzotriazinyl radical are oriented toward the interlayer space. The oxygen of the C=O group (or the nitrogen atom of the -N=N- group) and the sulfur atom of the P=S group interact simultaneously with the interlayer cation.

Kinetic Study on Adsorption. Studies on the adsorption of azinphos-methyl by montmorillonite in an aqueous medium were begun with a kinetic study of the adsorption of the pesticide by copper montmorillonite and calcium hectorite. Figure 3 shows the cumulative adsorption curves. In the hectorite curve, it may be seen that there is rapid initial adsorption followed by a slower,

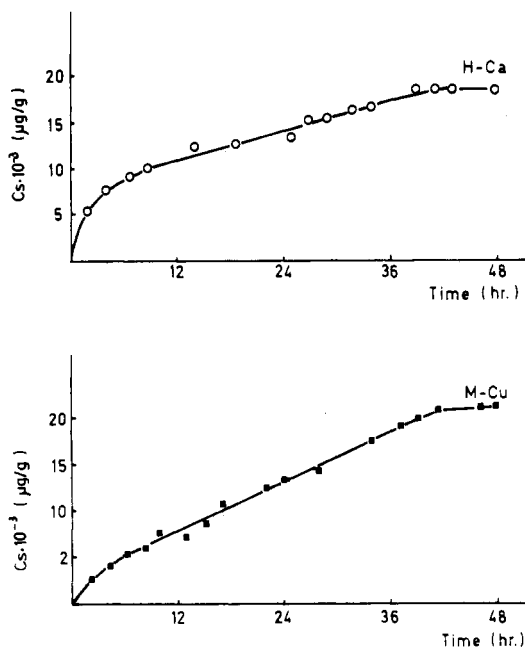


Figure 3. Adsorption of azinphos-methyl by calcium hectorite (H-Ca) and copper montmorillonite (M-Cu) as a function of time.

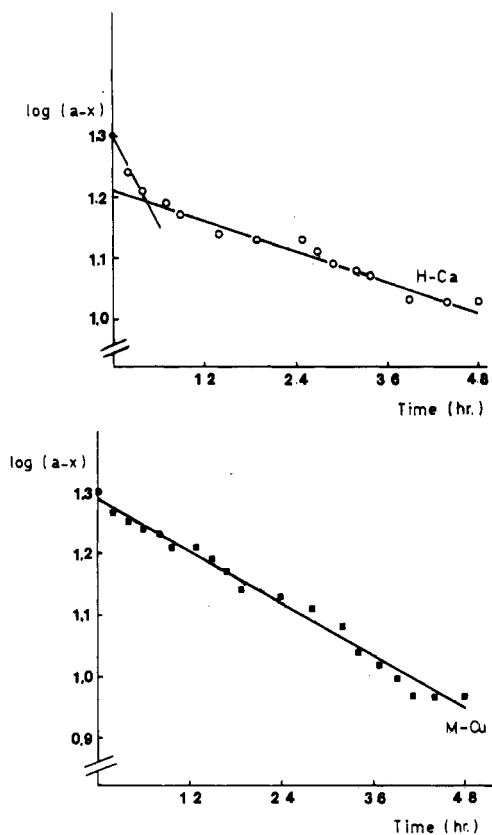


Figure 4. Adsorption kinetics of azinphos-methyl by calcium hectorite and copper montmorillonite (*a*, amount of azinphos-methyl at time 0; *x*, amount of azinphos-methyl adsorbed at time *t*).

prolonged adsorption that takes place until equilibrium is reached, at approximately 48 h. In the copper montmorillonite a gradual adsorption is observed from the beginning which later decreases; this also reaches equilibrium at 48 h. Both curves can be described by a first-order kinetic process; that is, adsorption is a function of adsorbate concentration.

The plot of $\log(a - x)$ vs. time (Figure 4) (*a*, initial amounts of azinphos-methyl; *x*, amount adsorbed at time

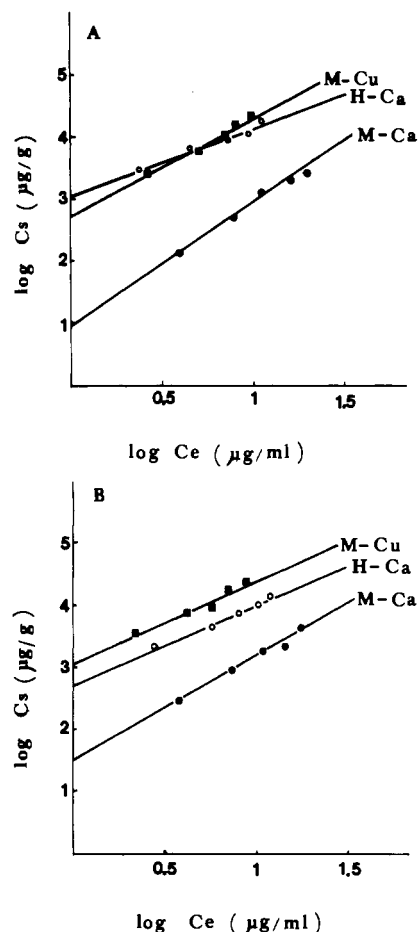


Figure 5. Freundlich isotherms for the adsorption of azinphos-methyl by the different samples: (A) 30 °C; (B) 45 °C.

t) for copper montmorillonite may be fitted to a straight line and for calcium hectorite to two intersecting straight lines. This shows that the kinetic process is of first order for the copper montmorillonite and that it takes place in two stages in the case of the calcium hectorite, each of them being of first order, though with different rate constants. The first stage has a high rate constant ($K_1 = 5.36 \times 10^{-2} \text{ h}^{-1}$) and short duration and the second a slower and more continuous rate ($K_2 = 0.97 \times 10^{-2} \text{ h}^{-1}$). The rate constant for the copper montmorillonite is greater than this latter, though lower than that of the first hectorite rate constant, $1.63 \times 10^{-2} \text{ h}^{-1}$. The difference between the initial adsorption rate for the two samples is due to the greater resistance to expansion of copper montmorillonite than that of the calcium hectorite, which, in turn, is due to the greater charge density of the montmorillonite and the smaller size of the Cu cation. This resistance will be observed below in the study of the adsorption isotherms and enthalpies.

Adsorption Isotherms: Influence of Temperature.

Adsorption isotherms of azinphos-methyl by montmorillonite in an aqueous medium were obtained at 30 and 45 °C. The adsorption of azinphos-methyl by the samples studied may in all cases be expressed by Freundlich's adsorption equation (Figure 5). The linear form of this equation is

$$\log C_s = \log K + 1/n \log C_e \quad (1)$$

where C_s is the amount of pesticide adsorbed ($\mu\text{g/g}$ of clay), C_e is the equilibrium concentration of the pesticide in solution ($\mu\text{g/mL}$), and K and n are constants that characterize the adsorption capacity for the pesticide. K is the amount adsorbed for an equilibrium concentration of 1

Table IV. Freundlich Constants (K and n) and Distribution Coefficient (K_d) for the Adsorption of Azinphos-methyl by the Various Samples

sample	temperature,		K , ^a $\mu\text{g/g}$	n	K_d ^b
	°C				
calcium montmorillonite	30		11.28	0.52	92.32
calcium montmorillonite	45		30.58	0.58	158.6
copper montmorillonite	30		519.7	0.62	2072
copper montmorillonite	45		1179	0.74	2598
calcium hectorite	30		1099	0.90	1418
calcium hectorite	45		519.8	0.77	1036

^a $C_e = 1 \mu\text{g/mL}$. ^b $C_e = 10 \mu\text{g/mL}$.

$\mu\text{g/mL}$ and therefore represents the adsorption to low levels of adsorbate concentration; n , which is the reciprocal of the slope of the straight line, is a measurement of the intensity of adsorption and reflects the degree to which adsorption is a function of concentration. The values of K and n of the isotherms are shown in Table IV. Also calculated is the distribution coefficient K_d , which measures the relationship between the concentration of the pesticide in the clay and in the equilibrium solution for a given equilibrium concentration ($C_e = 10 \mu\text{g/mL}$). As may be seen, the values of K and K_d vary considerably as a function of adsorbent characteristic and system temperature, while the constant n shows only small variations.

On comparing values for Freundlich constants at 30 °C, it may be seen that adsorption at the low level is lower for the calcium montmorillonite sample and increases considerably for the Cu sample (K is multiplied by 50) and that greater adsorption corresponds to the hectorite. However, on considering the distribution coefficients, it may be observed that at a higher equilibrium concentration, adsorption for the Cu sample is greater than that of the hectorite sample. This agrees with the lesser amount of energy necessary for the expansion of the hectorite sample and with the greater affinity of the Cu^{2+} ion for azinphos-methyl, compared with that of the Ca^{2+} ion, due to its greater polarizing power.

For the calcium and copper montmorillonite isotherms, values of K and K_d increase with temperature; however, for calcium hectorite, the opposite is true. This suggests that the adsorption process is endothermic for the calcium and copper montmorillonite samples and exothermic for the hectorite sample. These differences allow us to determine the isosteric adsorption heat, ΔH_{iso} . This heat does not represent the interaction heat of azinphos-methyl with the interlayer cations. Principal contributors to this adsorption energy are the heat absorbed in the process of interlayer expansion (endothermic) and the heat of interaction of the molecules adsorbed by the exchangeable cations (exothermic). The adsorption process can therefore be either endo- or exothermic, according to the values of these two terms. The literature makes frequent reference to positive or negative values according to adsorbent, solvent, etc. (Biggar and Cheung, 1973; Aly et al., 1980; Moreale and Van Bladel, 1979).

The change in enthalpy may be determined by applying van't Hoff's integrated equation to the adsorption curves:

$$\Delta H_{\text{iso}} = R \frac{\ln C_{e1} - \ln C_{e2}}{1/T_1 - 1/T_2} \quad (2)$$

C_{e1} and C_{e2} are the equilibrium concentrations of the isotherms at temperatures T_1 and T_2 , corresponding to an equivalent amount adsorbed C_s . The values obtained for the isosteric heat of adsorption, for a constant value of $C_s = 10^4 \mu\text{g/g}$, that is, for constant coverage and assuming that the solvent-solute exchange is the same in the two

Table V. Changes in Enthalpy (ΔH) in the Adsorption of Azinphos-methyl by the Various Samples

sample	ΔH , kcal/mol
calcium montmorillonite	1.71
copper montmorillonite	0.55
calcium hectorite	-2.87

systems, are shown in Table V. As may be seen, the adsorption process for azinphos-methyl by calcium hectorite is exothermic while for the Ca and Cu samples of Tidinit montmorillonite it is endothermic.

The expansion energy of montmorillonite, in agreement with the expression proposed by Norrish (1954) and which has been applied by several workers (Levy and Shamberg, 1972; Van Assche et al., 1973; Dios Cancela and González García, 1979), depends on the radius and charge of the exchange cation, on the layer charge of the sample, and also on the position of the charge. It is clear that as the three cations have the same valency, expansion energy must be less for hectorite, of smaller layer charge, than for montmorillonite. Thus, although in calcium hectorite and calcium montmorillonite interaction energy is the same, because the expansion energy of hectorite is smaller, the isosteric adsorption heat is negative for hectorite and positive for montmorillonite. For copper montmorillonite the adsorption process is also endothermic, though less so than in the case of calcium montmorillonite. From the above it may be deduced that the interaction energy of montmorillonite with azinphos-methyl in an aqueous medium is weak; this is in agreement with the smaller shifts in IR absorption bands of the hydrated complexes, as has been reported above.

Hydrolysis of Azinphos-methyl Catalyzed by Montmorillonite. In the azinphos-methyl adsorption kinetic study by copper montmorillonite in an aqueous medium at pH 6 and a temperature of 30 °C, it may be seen that after 30 h traces of pesticide hydrolysis begin to appear, yielding the same hydrolysis product as that obtained when the product is hydrolyzed in an alkaline medium (the same absorption maximum under UV light at 206 nm). It should be noted that under the same conditions of pH, temperature, and time, in an aqueous medium and in the absence of clay, the pesticide is not hydrolyzed. This process of catalytic hydrolysis of organophosphorus pesticides by smectites has already been reported by us (Sánchez Camazano and Sánchez Martín, 1983a) and was studied in depth in the case of phosmet, where the catalytic effect of montmorillonite was considerable. It was concluded that the interaction of pesticide with interlayer cations should increase the electrophilic nature of the phosphorus atom, thereby facilitating nucleophilic attack by the OH^- ions, producing fission of the P-S bond. This fission is the normal hydrolysis mechanism of these pesticides.

The data on the adsorption isotherms of copper montmorillonite referring to the UV spectra show that slight hydrolysis takes place at 30 and 45 °C. Thus, in the UV spectra of the corresponding equilibrium solutions it may be seen that there is a very small absorption at 206 nm. However, this is not the case in the calcium montmorillonite isotherms at either of the two temperatures and hydrolysis may only be observed at 45 °C in calcium hectorite. Hydrolysis is therefore linked to interaction energies, to temperature, and also to the endo- or exothermic nature of the process, and all these effects act jointly on the hydrolysis rate.

Accordingly, it may be deduced that the adsorption process of azinphos-methyl by montmorillonite (kinetics, enthalpy, the amount adsorbed, etc.) in an aqueous me-

dium depends on the system temperature and on silicate characteristics (exchange cations and layer charge). These factors also influence the catalytic hydrolysis of azinphos-methyl by the samples in an aqueous medium, under conditions of pH and temperature where the pesticide is normally stable.

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Registry No. Montmorillonite, 1318-93-0; azinphos-methyl, 86-50-0; hectorite, 12173-47-6.

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The Diphenyl Ether Herbicide Oxyfluorfen: Action of Antioxidants

Karl Josef Kunert* and Peter Böger

Measurement of ethane, as an index of in vivo lipid peroxidation, was done with *Scenedesmus acutus* and the diphenyl ether oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene] present at a concentration of 50 nM. In the light, lipid peroxidation is substantial in herbicide-treated cells, and peroxidation is directly related to pigment damage. Protection against peroxidation and pigment loss is only achieved by the antioxidants ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) and *dl*- α -tocopherol at a concentration of 0.1 mM or by exposure to the dark. Less effective protection is found with either of the antioxidants DPPD (*N,N'*-diphenyl-1,4-phenylenediamine), BHT (2,6-di-*tert*-butyl-*p*-cresol), ascorbic acid, or the hydroxyl radical scavenger mannitol. Cells exhibiting ethane formation after a foregoing treatment with oxyfluorfen cannot be effectively protected against pigment loss by subsequent addition of ethoxyquin.

In vivo peroxidation of polyunsaturated fatty acids has been identified as one of the deleterious reactions basic to the mechanism of membrane and cellular damage (Mead, 1976). It has been reported that, in light, certain diphenyl ether herbicides seemingly damage biomembranes by peroxidation of membrane lipids (Prendeville and Warren, 1977; Vanstone and Stobbe, 1977; Kunert and Böger, 1981; Orr and Hess, 1982a,b). The herbicide-induced peroxidation was either investigated by measurement of malondialdehyde formation (Orr and Hess, 1982a), degradation of sulfolipid (Sandmann and Böger, 1983a,b), or ethane production (Kunert and Böger, 1981; Lambert et al., 1983). The noninvasive method of measuring volatile hydrocarbons released during decomposition of unsaturated fatty acid hydroperoxides provides a useful index of lipid peroxidation (Dumelin and Tappel 1977; Tappel and Dillard, 1981).

However, little is known about the protection of algae and plants against the herbicide-induced peroxidation process. Orr and Hess (1982a) found that the antioxidant α -tocopherol inhibited membrane leakage in diphenyl ether treated cucumber cotyledons. Recently, we reported that in vivo lipid peroxidation seemed to be a function of the vitamin C concentration present in the plants after herbicide application (Kunert, 1983).

In this study, we describe some new aspects of the efficiency of antioxidants to decrease both diphenyl ether induced lipid peroxidation and pigment damage directly related to peroxidation. The sensitive measurement of ethane, a decomposition product of ω -3-unsaturated fatty acid hydroperoxides, was used as an index of in vivo lipid peroxidation. Experiments were carried out with the diphenyl ether oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene], and herbicidal activity was assayed with the unicellular alga *Scenedesmus acutus*. The use of microalgae has several advantages over higher plants, since absolute quantitative changes of cellular compounds can be measured when referred to a

Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-7750 Konstanz, West Germany.