Adsorption of Zearalenone by Organophilic Montmorillonite Clay

Shawna L. Lemke, Patrick G. Grant, and Timothy D. Phillips*

Intercollegiate Faculty of Toxicology, College of Veterinary Medicine,
Texas A&M University, College Station, Texas 77843-4458

Acidic montmorillonite clay was chemically modified via exchange with various ammonium- and pyridinium-based organic cations. These organo clays were then shown to adsorb zearalenone (ZEN) with an efficacy related to the exchanged cation hydrophobicity, as gauged by estimated critical micelle concentration values (cmc) and length of the alkyl chain. ZEN adsorption was greatest for clay samples that had been modified by interlayer exchange with cations possessing 16-carbon alkyl chains: hexadecyltrimethylammonium (HDTMA) and cetylpyridinium (CP). Isothermal analysis of ZEN adsorption (at 37 °C, pH 6.5) indicated that the clay exchanged with CP at a level of less than or equivalent to the cation exchange capacity (CEC) binds ZEN primarily by a partition process. In contrast, clay exchanged at the 1.5 CEC level gave an S-shaped curve with at least two plateaus, suggesting additional mechanisms of adsorption. A capacity (Q_{max}) and distribution coefficient (K_d) for the first site were estimated. At acid pH, the adsorption capacity for 1.5 CEC CP clay decreased and the isotherm shape predicted a simple partition process. Conversely, the shape remained curved at alkaline pH, suggesting an adsorption site. These data imply an adsorption mechanism at neutral pH that involves hydrophobic attraction of ZEN into the interlayer in addition to multisite binding of the anionic form to hydroxyl groups on the edge of the clay via ion−dipole interactions and electrostatic attraction to excess exchanged surfactant cations.

Keywords: *Adsorption; cetylpyridinium; isotherm; mycotoxin; organo clay; zearalenone*

INTRODUCTION

Zearalenone (ZEN) is a hazardous mycotoxin that is elaborated by ubiquitous *Fusarium* molds on cereal crops such as corn, barley, oats, sorghum, wheat, and rye (Tanaka et al., 1988). The most dramatic effects of its presence in animal feed are associated with swine reproduction and include precocious sexual development in gilts, vulva enlargement, mammae, anestrus, pseudopregnancy, reduced litter size, and reduced viability of newborn pigs (Pier, 1981). ZEN also causes loss of embryos and retention of corpora lutea in affected pregnant gilts (Long and Diekman, 1986). In boars, ZEN can cause inflammation of the prepuce, decreased testes, epididymis, vesicular gland weights, cessation of spermatogenesis, and reduced libido. While the affects are not as dramatic, ZEN is also responsible for hyperestrogenism in cows (Diekman and Green, 1992).

Due to the economic and health costs of contamination of feedstuffs by ZEN and mycotoxins in general, considerable attention has been given to the alleviation of these problems. Prevention of mycotoxin production is one method of control. Strategies include the development of fungal resistant crops, diminishing insect damage, and controlling the humidity and temperature of grain storage. If prevention proves inadequate, physical removal of contaminated cereals or dilution with uncontaminated crops is often employed (Marasas, 1987).

* Address correspondence to this author at Professor & Chair Intercollegiate Faculty of Toxicology, Department of Veterinary Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4458 [fax (409) 862-4929; e-mail tphillips@cvm.tamu.edu].

Another approach to the remediation of animal feed is to reduce the bioavailability of the toxin via adsorption in the gastrointestinal tract. In recent years, aflatoxin-selective clay has been reported to bind and inactivate aflatoxin B_1 (AfB_1) in vitro and in vivo. In animal studies, inclusion of the clay in the diet at levels of 0.5% provided 38−100% protection from aflatoxins in young chickens, turkeys, swine, lambs, and rats (Phillips et al., 1995).

Reduction in the bioavailability of ZEN by adsorption has also been investigated, but the results have not been as dramatic as with AfB_1. The addition of fiber has been shown to have some efficacy in decreasing the toxicity of ZEN. When alfalfa was present at levels of at least 25% in the diet, it helped rats overcome the growth depression caused by ZEN (Stangroom and Smith, 1984). Use of HSCAS clay in the diets of mink fed ZEN helped to improve female reproductive performance but did not reduce the hyperestrogenic effects (Bursian et al., 1992). Cholestyramine, a quaternary ammonium anion exchange resin, has been shown to reduce the effects of ZEN in the prepuberal mouse uterine weight bioassay (Underhill et al., 1995). However, none of these methods have proven to be effective in the treatment of hyperestrogenism in swine.

An alternative approach to the adsorption of ZEN is the chemical modification of diverse phyllosilicate clays with organic cations. The predominant interlayer cations of montmorillonite clays can be very effectively exchanged with organic cations to produce a variety of substituted organo clays with altered morphological properties and binding capabilities.

Such modified clays have been the subject of a large volume of research in recent years. A variety of studies have been performed to characterize the adsorption and

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desorption of many surfactants to clays. It is generally thought that two mechanisms of adsorption predominate: first, a cation exchange of surfactant into the interlayer of the clay and, second, binding of surfactant in excess of the cation exchange capacity via hydrophobic interactions between the long tails of the surfactants (Xu and Boyd, 1995; Zhang et al., 1993). In addition, these clays have been utilized in a variety of studies aimed at environmental cleanup applications. For example, organo clays exchanged with cetylpyridinium (CP) and hexadecyltrimethylammonium (HDTMA) have been shown to bind pentachlorophenol, a common water pollutant at wood-preserving sites (Srinivasan and Fogler, 1990). This adsorption was further shown to be dependent upon pH and ionic strength of the contaminated water (Stapleton et al., 1994). HDTMA-exchanged clays were also shown to be able to adsorb benzene, nitrobenzene, chlorobenzene, trichloroethylene, and carbon tetrachloride (Sheng et al., 1996). A wide variety of surfactant molecules can be used to modify clay. These compounds range in number and size of alkyl chain groups and identity of the polar head. Smith et al. (1990) utilized 10 different quaternary ammonium cations to show their differing ability to adsorb tetrachloromethane from water onto clay. In short, the possibilities for applications of this technology are numerous.

The action of these modified clays is probably related to increased hydrophobicity of the clay surface, allowing partitioning of organic compounds onto the surface. Due to the hydrophobicity of ZEN, it is postulated that this mycotoxin may also be attracted and sequestered by some of these organo clays. It was our intent to develop and test the binding capabilities of several organo-substituted clays for ZEN adsorption from aqueous solution.

MATERIALS AND METHODS

Chemicals and Reagents. Zearalenone (ZEN) was purchased from Sigma Chemical Co. (St. Louis, MO). Low-pH montmorillonite clay (LPHM, a calcium−sodium montmorillonite that has had partial replacement of these cations with hydronium ion) was obtained from Engelhard Chemical Corp. (Cleveland, OH), batch SJ 106437. Cetylpyridinium chloride (CP), dodecylpyridinium chloride (DDP), dodecyltrimethylammonium bromide (DDTMA), benzyl triethylammonium chloride (BTEA), and tetradecyltrimethylammonium bromide (TDTMA) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Hexadecyltrimethylammonium chloride (HDTMA) and tetramethylammonium tribromide (TMA) were purchased from Fluka (St. Louis, MO). High-purity water (18 MΩ·cm) was used in all experiments. Polyplasdone XL (PVP) was purchased from ISP Technologies (Wayne, NJ). Several commercial clays were obtained: Sepiolita 100, product of Tolsa S.A. (Madrid, Spain); Condition-Ade, Agrisorbents Products Group (Oil-Dri, Chicago, IL); Antitox-Plus, Interpremix Corp. (St. Polten, Austria); Fix-A-Tox, Werfft-Chemie Corp. (Gesellschaft mbh, Austria); T-Bind, Biotech Development Co. (Atlanta, GA); and MilBond-TX, Milwhite, Inc. (Houston, TX). Dialysis tubing (MWCO 12000−14000) was obtained from Spectra/Por (Los Angeles, CA).

Water Solubility of ZEN. Two methods were utilized to estimate the solubility of ZEN: molecular modeling and an equilibration method based on dialysis.

The molecular modeling approach was based on a method reported by Meylan et al. (1996). Using a set of 817 compounds, they developed several equations that relate water solubility (S) to log K_{ow}, molecular weight (MW), melting point (T_m), and correction factors (CF) based on chemical structure. Three equations (eqs 1a−c) of varying complexity and high correlation of fit to the 817 standard set compounds were chosen and used to obtain estimates for the solubility of ZEN in water at neutral pH.

$$\log S = 0.686 - 1.123(\log K_{ow}) - 0.0099(T_m - 25) \quad (1a)$$

$$\log S = 0.978 - 0.935(\log K_{ow}) - 0.0082(T_m - 25) - 0.00468(\mathrm{MW}) \quad (1b)$$

$$\log S = 0.693 - 0.96(\log K_{ow}) - 0.0092(T_m - 25) - 0.00314(\mathrm{MW}) + \mathrm{CF} \quad (1c)$$

The octanol/water coefficient (K_{ow}) for ZEN was estimated according to methods recently reported in our laboratory (Grant and Phillips, 1998) using ISIS Draw 2.0 and HyperChem 4.5. The molecular structure was energy minimized using the semiempirical AM1 method (Dewar et al., 1985; HyperChem, 1994) and then imported into the ChemPlus module, which calculated the log K_{ow} value. This module utilizes previously derived atomic parameters to estimate the contribution made to the log K_{ow} by each individual atom (ChemPlus, 1993; Ghose et al., 1988; Vellarkad et al., 1989). The water solubility of ZEN was also estimated using a method developed by Etzweiler et al. (1995). Excess ZEN crystals were placed inside a borosilicate glass test tube (16 × 125 mm) containing 15 mL of distilled water. Dialysis tubing (Spectra/Por, MWCO 12000−144000, 1 cm width, ∼14 cm length) was filled with 3 mL of distilled water, and both ends were tied off. The tubing was placed in the test tube, capped to avoid pH changes due to dissolved CO_2, and left to equilibrate with stirring for 24 h at 25 °C. This procedure was performed at pH 2, 7, and 10. The water was pH adjusted with either 10% HCl or 1.0 N NaOH, and the pH was monitored throughout the equilibration time. An aliquot of the water inside the dialysis tubing was removed, and the concentration was verified with a Beckman DU-65 UV−vis spectrophotometer. The λ_{max} for the absorption of ZEN was 236 nm (Cole and Cox, 1981). The value for ϵ in water at this wavelength was experimentally determined by comparison with the ϵ in methanol [29700 1/(M·cm) (Cole and Cox, 1981)] and was found to be 24833 [1/(M·cm)].

Calculation of pK_a. Previous work has indicated a correlation between pK_a and electrostatic potential of the acidic proton of weak acids as a means for estimating the unknown pK_a of a compound of interest (Hehre and Nelson, 1997). This principle was applied to find the pK_a of the most acidic hydrogen of ZEN using HyperChem software. A set of 34 alcohols and monoprotic acids with a range of pK_a values were modeled and energy minimized using the semiempirical AM1 method. From the model of each compound, HyperChem software is able to calculate the charge on the acidic hydrogen. The electrostatic potential on the surface of the acidic hydrogen was calculated by placing a theoretical point charge at a distance equal to the standard van der Waals radius for hydrogen. The charge on the acidic hydrogen (q_1) and the standard van der Waals radius of hydrogen (1.2 Å) (r) were entered into eq 2 along with the values of q_2, a point charge (1.6×10^{-19} C), and ϵ_0, the vacuum permittivity constant (8.854×10^{-12} J^{-1} C^2 m^{-1}), to obtain a value for electrostatic force, F (Atkins, 1986).

$$F = (q_1 q_2 / 4\pi\epsilon_0)(1/r^2) \quad (2)$$

Theses values were plotted against the pK_a values of the acids, and linear regression was applied. From this relationship, an estimate for the pK_a of ZEN was obtained.

Clay Preparation. Exchanged clays were prepared by washing in high-purity water (500 mL/5 g of clay) for 120 h while shaking at 500 rpm. The clays were then spun down by centrifugation for 20 min at 2000 rpm. The supernatant was poured off, the clays were resuspended in water, and the exchanging organo cation was added in an amount based on the estimated cation exchange capacity (CEC or equivalents) for LPHM clay [i.e., 90 cmol$_c$/kg of clay, obtained from the

Table 1. Isotherm Equations Used To Fit Adsorption Data[a]

Freundlich model	$q = K(C_w)^n$
Langmuir model	$q = Q_{max}[K_d C_w/(1 + K_d C_w)]$
shifted Langmuir model	$q = Q_{max}[[K'(C_w - X)]/[1 + K'(C_w - X)]]$
summation factor for K_d	$K_d = (1/n)\sum_{i=1}^{i=n}[q_i/[(Q_{max} - q_i)C_i]]$

[a] q = ZEN adsorbed (mol/kg), Q_{max} = maximum capacity (mol/kg), K_d = distribution constant, C_w = equilibrium concentration of ZEN,

provider (Engelhard)]. In cases where more or less than the CEC was exchanged, the clays were referred to as the fraction of CEC, for example, 0.5 CEC. The clays were allowed to exchange for 24 h followed by centrifugation and disposal of the supernatant and subsequent washing with pure water for another 24 h period. Finally, the exchanged and washed clay was spun down, dried, ground, and sieved through a 325 mesh sieve to obtain particles that were <45 μm. The exchanged clays are hereafter referred to by the abbreviation for the exchanged cation described under Chemicals and Reagents.

Isotherm Studies. A stock solution of ZEN was prepared by dissolving the pure crystals in acetonitrile and then injecting an amount into purified water (pH 6.5 ± 0.2) to yield a solution concentration of ~4 mg/mL. The exact concentration of the stock solution was verified by UV−vis spectrophotometry. Each isotherm consisted of triplicate 5 mL aqueous samples of ZEN made from dilutions of the stock to yield approximate concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 μg/mL and placed in sterile 17 × 100 mm polypropylene centrifuge test tubes. A suspension of clay was prepared by weighing ~10 mg into a 25 mL Erlenmeyer flask and pipetting in the appropriate volume of water to make the clay concentration 1 mg/mL. A stir bar was added to the flask, and the suspension was mixed to keep the slurry homogeneous. From this suspension, 50 μL (containing 50 μg of clay) was pipetted out with an autopipet and added to each 5 mL test sample. Four controls were also prepared: a water control containing no toxin, toxin controls containing 0.5 and 4.0 μg/mL ZEN, and a control containing 5 mL water and 50 μg of clay. Samples were capped and shaken at 1000 rpm at 37 °C for 2 h (based on initial studies, data not shown). After shaking, the samples were centrifuged at 10000 rpm and 37° C for 15 min to separate the clay from the supernatant. A 2 mL aliquot of each sample was removed and placed in a clean borosilicate glass test tube. This aliquot was then acidified with 5 μL of 1.0 M HCl (to pH ~2.4) to ensure protonation of the two phenol groups and prevent alteration of UV absorption. The samples were then analyzed for absorbance at 236 nm using a UV−vis spectrometer (Grant and Phillips, 1998).

This technique has some advantages over the use of larger samples. For example, reduced sample volumes can facilitate the design of more extensive experiments with increased treatment replicates, especially for agents (such as the mycotoxins) that may be very hazardous and/or very expensive and/or available only in low levels. Using this technique, we have been able to consistently measure the adsorption of ZEN via the addition of small amounts of clay, as clearly demonstrated by the low standard deviations.

Data Calculations and Curve Fitting. From the UV−vis absorption data the amount of ZEN left in solution (C_w) and the amount adsorbed (q) were calculated and plotted. Running the samples in triplicate allowed calculation of standard deviation for each point, which was displayed as y-axis error bars in the plots. The data were transferred to Table Curve 2D v3 and fitted to isotherm equations input by the user. The curves were fit to the Freundlich equation (Table 1) to obtain a smoothed line fit and to provide K values as means of quantitative comparison. Data that displayed an S-shaped isotherm (Giles et al., 1960) and a multiplicity of sites could not be mathematically fit with a high correlation coefficient by conventional isotherm equations (i.e., Langmuir, Freundlich, generalized Freundlich, Langmuir−Freundlich, and Toth models). Therefore, a derived modification of the Langmuir, the shifted Langmuir, was utilized (Table 1) (Grant et al., 1998). Although this model gave a very good approximation for Q_{max}, the value for K_d was artificially inflated by the transformation (K'). Therefore, the K_d was calculated by averaging the summation of individual interpolated K_d values (n = 393), where K_d takes on the definition given by the original Langmuir model (eq 3). Calculation of K_d was performed by MathCad.

$$K_d = q/[(Q_{max} - q)C_w] \quad (3)$$

Molecular Models. CP was drawn in ISIS Draw 2.0 and then imported into HyperChem 4.5. The structures were energy-minimized using the semiempirical quantum mechanical AM1 method. LPHM was constructed using the unit cell coordinates of muscovite (Richardson and Richardson, 1982). These coordinates were then converted to orthogonal coordinates in an Excel spreadsheet that was constructed from a public domain C program (Kanters, 1993). The unit cells were replicated in three-dimensional space by applying the symmetry operations for a $C2/c$ space group (*International Tables for X-ray Crystallography*, 1952). The d_{001} spacing of the model was then set to the corresponding dimensions of CP-exchanged (1.5 CEC) calcium montmorillonite (21 Å) based on the report of Greenland and Quirk (1960). CP was inserted into the interlayer and on the external surface (Slade et al., 1978) to illustrate the proposed sites of ZEN adsorption (Phillips et al., 1995; Grant, 1998).

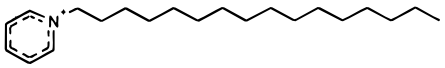
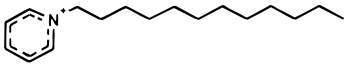
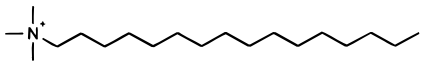
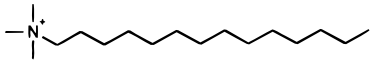
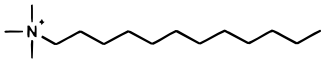
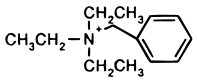
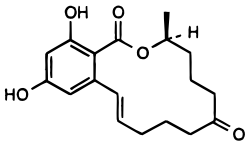
RESULTS

Physical Properties of ZEN. Calculation of log K_{ow} by HyperChem gave a value of 3.663 for ZEN. Entering this value plus the molecular weight (318.37 amu) and melting point (164−165 °C; Cole and Cox, 1981) for ZEN into the Meylan et al. equations (eqs 1a−c) gave estimates of water solubility at pH 7 of 2.62, 2.46, and 4.79 μg/mL, respectively. Solubility studies based on an equilibrium state between solid-state ZEN and ZEN in solution at 25 °C gave results of 4.51, 4.81, and 40.15 μg/mL for pH 2, 7, and 10, respectively. On the basis of these estimates and previous solubility studies (data not shown), it was determined that nuetral pH solutions ≤4 μg/mL at 37 °C were chemically stable and suitable for isotherm experiments.

From the molecular models of ZEN and the standard set of acidic compounds, the electrostatic potential (between the most acidic hydrogen and a point charge on the surface of the atom) was determined. Linear regression between pK_a and electrostatic potential for the standard set of compounds (Figure 1) showed a high correlation (r^2 = 0.9761). The most acidic hydrogen of ZEN is located at the hydroxyl on the carbon of the six-membered ring nearest the lactone (structure shown in Table 2) (Cole and Cox, 1981). The electrostatic potential for this atom was found to be 3.669×10^{-9} N. When this value was entered into the equation for the regression line for the standard set, an estimate of 7.62 was found for the pK_a of ZEN.

Adsorption of ZEN by Organo Clay. The seven exchanged cations used to modify the clay were chosen on the basis of their range of hydrophobicities as represented by their estimated critical micelle concentration (cmc) values (Mukerjee and Mysels, 1971). These values, along with the structural representation of each organic cation, are displayed in Table 2. Our results indicated that organophilic clay that was exchanged with 1 equiv of either CP, HDTMA, DDP, TDTMA, or DDTMA was more effective than unmodified base clay in adsorbing ZEN (Figure 2). Visual inspection of isotherm plots indicated notable differ-

Table 2. Structure and Critical Micelle Concentration[a] of Exchanged Surfactants

Clay Modifier	CMC (M)	
Cetylpyridinium (Chloride)	0.9×10^{-3}	
Dodecylpyridinium (Chloride)	14.7×10^{-3}	
Hexadecyltrimethylammonium (Chloride)	1.3×10^{-3}	
Tetradecyltrimethylammonium (Bromide)	3.51×10^{-3}	
Dodecyltrimethylammonium (Bromide)	14.5×10^{-3}	
Benzyltriethylammonium (Chloride)	-	
Tetramethylammonium (Tribromide)	-	
Adsorbate	log K_{ow}	
Zearalenone[b]	3.66	

[a] Mukerjee and Mysels (1971). When more than one value for cmc was available, the one ranked as "highest quality" was chosen. The anion is given in parentheses as this affects the cmc value. [b] 6-(10-Hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone.

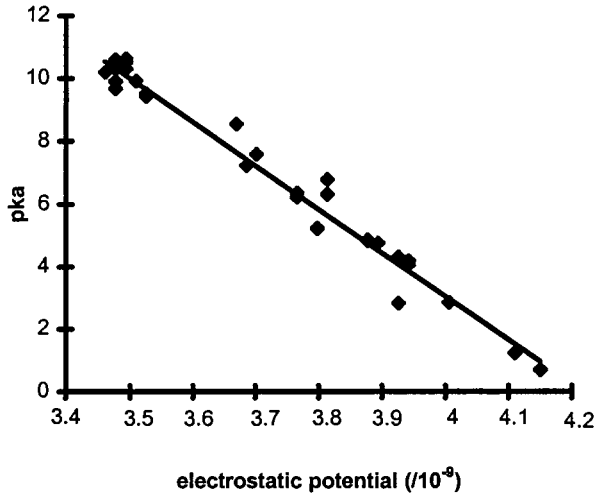


Figure 1. Correlation between pK_a and acidic hydrogen electrostatic potential. Data represent a standard set of 34 weak acids, which were modeled with HyperChem to determine the charge on the acidic hydrogen. This information was applied to an equation for electrostatic force, and the resulting value was related to pK_a through regression. This allows the prediction of pK_a for other weak acids, including ZEN.

ences in the adsorption of ZEN by the various clays. The data were fit to the Fruendlich isotherm to provide a means of quantitative comparison based on K values. These values are displayed in Table 3.

Binding with Cetylpyridinium Clay. On the basis of the binding performance of the 16-carbon chain surfactants, CP-exchanged clay was chosen to utilize in further studies to maximize and characterize this action. The adsorption of ZEN by base clay exchanged with various equivalents of CP (0.25, 0.5, 0.75, 1.0, and 1.5 CEC) was investigated. Representative isotherms are presented in Figure 3; Fruendlich K values are displayed in Table 3.

The 1.5 CEC CP-exchanged clay exhibited the best binding of ZEN as measured by K values, and further studies were based on this exchange level of clay alone. This isotherm was unique in that the shape appears to have one plateau and the rise of a second plateau. On the basis of Langmuir's isotherm development, plateaus are generally associated with specific adsorption sites. Therefore, an attempt was made to fit the data to obtain Q_{max} and K_d values for the first site. Due to the S shape of this curve (Giles et al., 1960), the data could not be fit to the Langmuir equation. However, the curve could be fit using a modified Langmuir equation (Figure 4), which makes use of a translation factor for the x-axis (Grant et al., 1998). Using this type of fit, the Q_{max} was estimated at 0.390 ± 0.057 mol/kg. The K_d was estimated using a summation calculation (Table 1) and found to be 3.11×10^5 for the first site. Due to the solubility limits of ZEN as discussed previously, the sample concentration range could not be extended to obtain a complete description of the second site. A fit was not attempted for this second site, because there were not enough data to accurately determine where the curve would break and plateau.

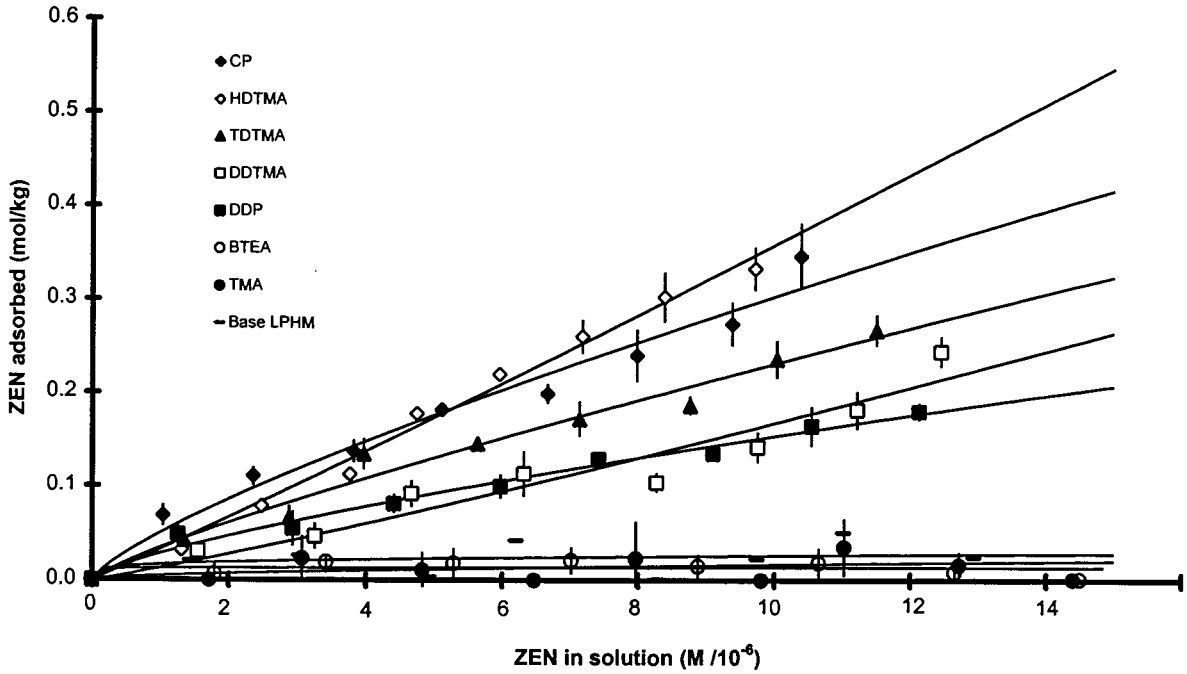


Figure 2. Adsorption isotherms for ZEN on LPHM clays exchanged with a variety of organophilic cations. Data represent the mean adsorption of ZEN to clay ± SD from three replicate experiments. Base clay (LPHM) was exchanged with either TMA, BTEA, DDP, DDTMA, TDTMA, HDTMA, or CP prior to analysis.

Table 3. Fruendlich Parameters for Isotherm Fits[a]

clay	K	n
CP	2.63×10^3	0.788
HDTMA	6.39×10^4	1.050
TDTMA	3.45×10^3	0.835
DDTMA	7.56×10^4	1.131
DDP	8.17×10^2	0.745
BTEA	2.34×10^{-2}	0.047
TMA	8.91×10^0	0.546
base	2.83×10^{-1}	0.207
CP 1.5 CEC	3.26×10^4	0.959
CP 1.0 CEC	2.62×10^3	0.788
CP 0.75 CEC	7.32×10^2	0.733
CP 0.5 CEC	1.05×10^1	0.427
CP 0.25 CEC	4.93×10^{-2}	0.046
CP 1.5 CEC pH 2	2.15×10^3	0.797
CP 1.5 CEC pH 6.5	3.26×10^4	0.959
CP 1.5 CEC pH 10	3.35×10^1	0.460
Antitox Plus	8.46×10^0	0.458
PVP	6.63×10^2	0.914
Sepiolite	1.25×10^{-1}	0.178

[a] Exchange rate is 1.0 CEC unless otherwise specified; base clay for all exchanged clays is LPHM.

Affect of pH on ZEN Binding to CP Clay. As a means of elucidating the mechanism of binding, isothermal adsorption of ZEN to CP clay at pH 2 and 10 was measured (Figure 5). The capacity for ZEN adsorption at pH 6.5 was greatly diminished by modification of pH to 2 and 10. At pH 2, the shape of the isotherm was linear. At pH 10 the isotherm shape was curvilinear.

Comparison with Other Adsorbents. In further studies, we examined the adsorption of ZEN to a variety of diverse mycotoxin-binding agents. Adsorption isotherms for Sepiolita 100, Antitox Plus, and PVP, like the base LPHM clay, showed no significant interaction with ZEN (Figure 6). Studies with other common mycotoxin-binding agents (i.e., Fix-A-Tox, Milbond-TX, Condition-Ade, and T-Bind) resulted in comparable findings. At the highest concentration of ZEN (4 μg/mL) and 10 μg/mL clay, all were shown to adsorb <6%.

DISCUSSION

The recent reports of effective adsorption of solvents (Sheng et al., 1996) and other organic compounds by hydrophobic montmorillonite clay led to the hypothesis that this technology could be useful in binding mycotoxins such as ZEN. The creation of a wide range of organo clays provides evidence in support of this hypothesis. Figure 2 shows a general trend of increasing adsorption with increasing hydrophobicity of the exchanged surfactant. Hydrophobicity was defined in this study by alkyl chain length and critical micelle concentration (cmc). Visual inspection of the data shows that the two cations (BTEA and TMA) which do not contain long alkyl chains and, therefore, do not form micelles, showed practically no adsorption of ZEN. Adsorption of ZEN steadily increases as the alkyl chain length of the exchanged surfactant increases (and the cmc decreases). Regression analysis was attempted to try to correlate these properties to the Fruendlich K parameter with little success ($r^2 = 0.0026$ for log K versus cmc and $r^2 = 0.0153$ for log K versus alkyl chain length). Despite the inability to ascribe a mathematical relationship to these results, it is clear that degree of hydrophobicity plays a role in ZEN adsorption. On the bais of these preliminary results, CP-exchanged organo clay was chosen for further study of ZEN adsorption.

In keeping with previous evidence, it was found that the greater the hydrophobicity of the clay, that is, the amount of CP exchanged by the clay, the higher the affinity for binding ZEN. The amount of binding as measured by log K was directly correlated to the level of organic cation substitution in the clay ($r^2 = 0.8853$). It is interesting that enhanced binding of ZEN for clay is seen beyond the addition of 1.0 CEC, because this is the theoretical limit of exchange. However, a study by Zhang et al. (1993) showed that quaternary amines can

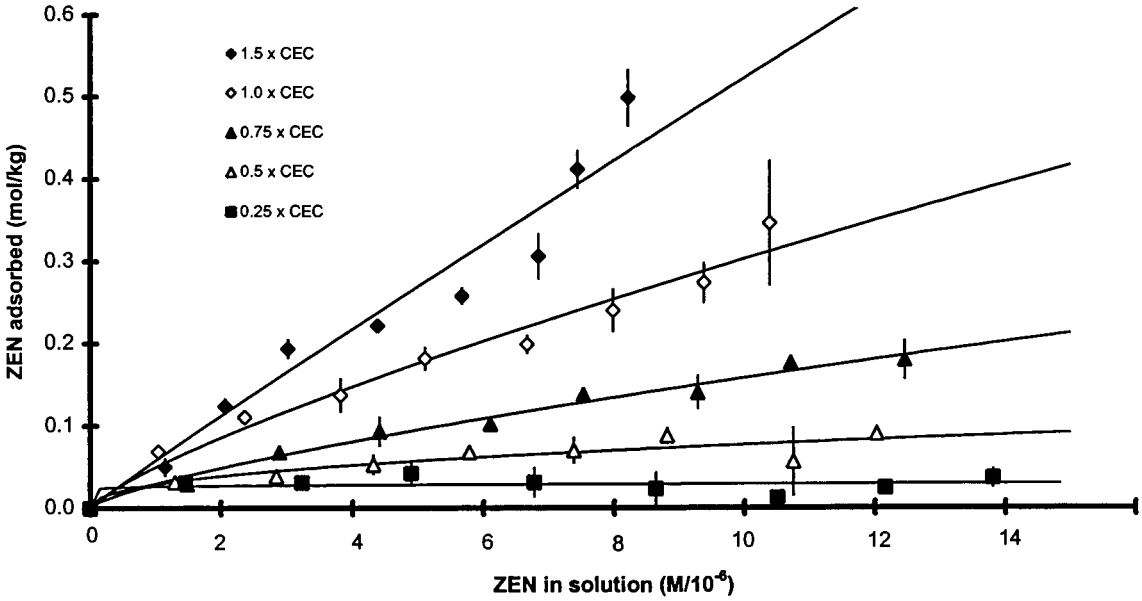


Figure 3. Adsorption isotherms for ZEN on LPHM clays with various levels of CP substitution. Data represent the mean adsorption of ZEN to clay ± SD from three replicate experiments. Base clay (LPHM) was exchanged with CP at rates between 0.25 and 1.5 CEC prior to analysis.

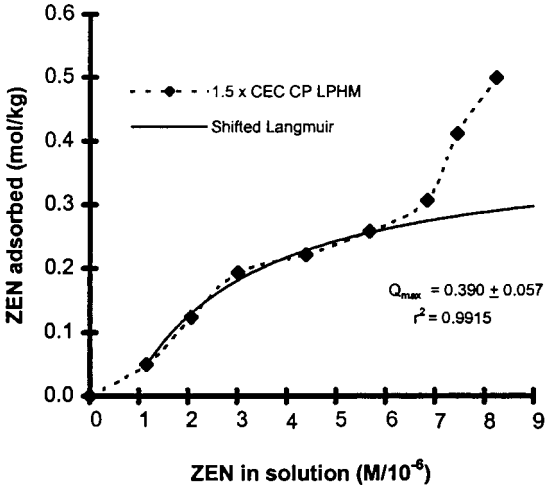


Figure 4. Use of a modified Langmuir equation for the estimation of Q_{max} for ZEN adsorption to clay. Data represent the mean adsorption of ZEN to CP LPHM clay (1.5 CEC exchange rate) and the curve obtained by fitting the data to the shifted Langmuir equation. This equation incorporates a translation along the *x*-axis to allow for a more accurate estimate of capacity for isotherms that display an S shape.

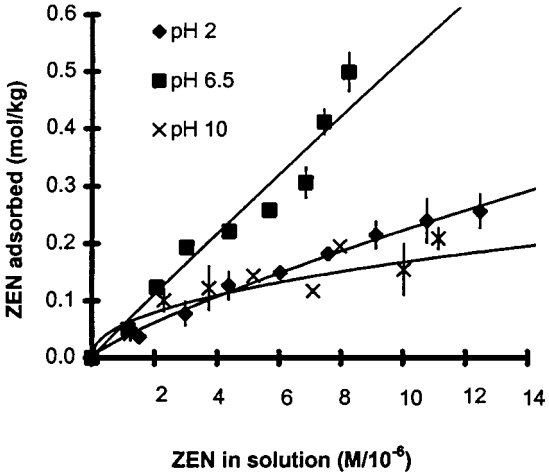


Figure 5. Adsorption isotherms for ZEN on CP-exchanged clay at acidic, neutral, and basic pH. Data represent the mean adsorption of ZEN to CP LPHM clay at three different pH levels ± SD from three replicate experiments. The diminished appearance of sites at low pH suggests that the anionic form of ZEN is important for adsorption.

be exchanged in excess of 2.0 CEC. They reported that this behavior was particularly characteristic of cations with alkyl chains of ≥12 and postulated that it was due to nonexchangeable sites involving interaction between the alkyl chains of the cations and between the cations and the clay surface. The same study showed that the longer alkyl chains also decreased the degree of desorption of exchanged surfactant. However, for cations with a 16-carbon alkyl chain (represented by HDTMA), this held true only for exchange rates up to 1.5 CEC, after which point the desorption rate increased rapidly. This property would be important to consider if the organo clay was to be used as a feed additive to adsorb harmful mycotoxins such as ZEN, with minimal dissociation of the surfactant in the gastrointestinal system of the animal.

Mechanistic evidence gained from the CEC and pH studies using CP clay led to the proposal that ZEN binding is due to a combination of hydrophobic association and charge. ZEN binding to CP clays at neutral pH and various exchange levels (CEC) showed a linear increase in adsorption with increase in solution concentration with all the clays exchanged at 1.0 CEC or less. This suggests that ZEN is partitioned onto the clay, most likely at the interlayer due to hydrophobic association with exchanged surfactant. At an exchange level of 1.5 CEC, the data took on an S shape containing one fully defined plateau and the beginning of a second rise. In this case, a second mechanism characterized as a site having a fixed capacity may be occurring. A likely possibility is an electrostatic charge association between the anion form of ZEN and the excess exchanged cation which may be residing on the external surface of the clay. ZEN is a diphenolic compound, with an estimated $pK_{a1} = 7.62$. This suggests that there is some amount of phenolate anion present in water near neutral pH and is further supported by the observed

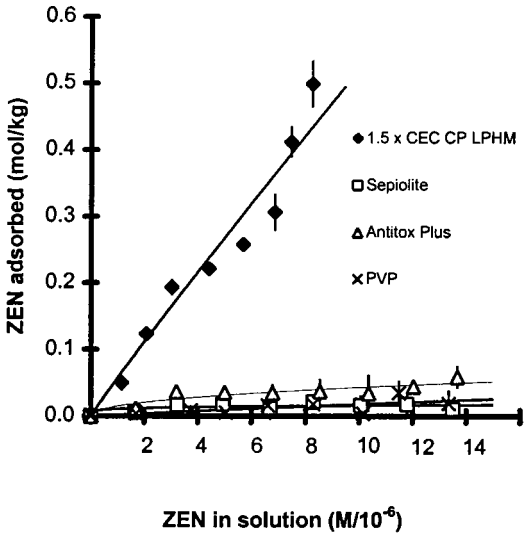


Figure 6. Comparison of the binding of ZEN by CP-exchanged LPHM, base LPHM, and commercial ZEN adsorbents. Data represent the mean adsorption of ZEN to several types of adsorbent materials ± SD from three replicate experiments. In comparison to the CP LPHM, the nonorgano adsorbents do not effectively bind ZEN.

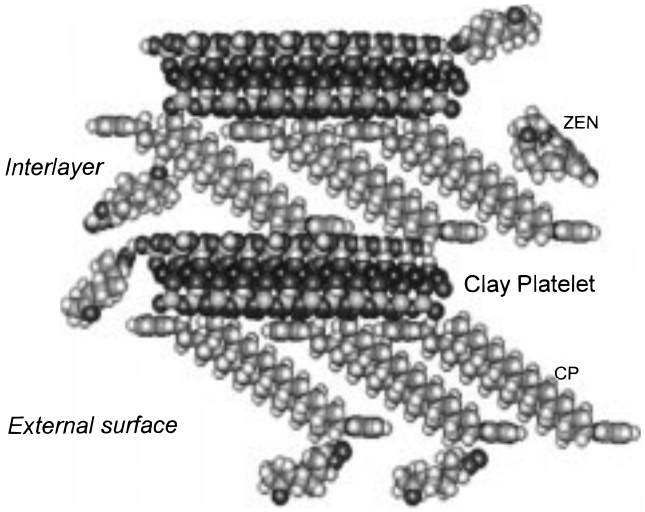


Figure 7. Molecular model illustrating the adsorption of ZEN to CP-exchanged LPHM clay as a HyperChem display of the proposed interaction of ZEN and CP inside the interlayer region and external surface of exchanged LPHM.

pH-dependent UV–vis absorption peaks. Another possible contribution to the site character seen in the isotherm is reaction of anionic ZEN to the exposed hydroxyl groups of edge sites through ion–dipole interactions.

When isotherm studies with 1.5 CEC CP clay were performed at pH 10, binding was significantly decreased. However, the shape still suggested that an adsorption site is present in addition to the partition mechanism. In contrast, the isotherm at pH 2 showed decreased binding and a linear curve suggesting only partition. These results confirm the importance of the negative charge associated with the phenolic anion in site specific binding. The decreased adsorption at alkaline pH compared to neutral pH is most likely due to the fact that the solubility of ZEN is greatly increased at alkaline pH due to the larger presence of the anionic form (40.15 versus 4.81 ppm at pH 10 and 7, respectively), thereby lessening its attraction to the exchanged cations and the edge hydroxyl groups of the clay. Figure 7 illustrates all of these proposed interactions using a molecular model of ZEN and a representative calcium montmorillonite clay exchanged with CP.

Previous papers have suggested the adsorption of ZEN by a variety of diverse mycotoxin-binding agents, including Sepiolite, crospovidone (PVP) (Ramos et al., 1996), Antitox Plus, T-Bind, MilBond-TX, and Tox-A-Fix. The discrepancy between these studies and our data may be related to the problem of solublizing high concentrations of ZEN in water. As discussed in results above, a level of 4 μg/mL ZEN in water at 37 °C was chosen because it was within the range of measured solubility. Using a level >10 μg/mL is questionable and may result in precipitation of toxin. Solubility can be enhanced through the use of cosolvents; however, this can unpredictably affect the clays and the outcome of the study, so this technique was avoided. Therefore, a caveat for ZEN studies is to avoid mistaking decreases in concentration for binding when it may be the result of precipitation. Because of this potential problem, all isotherms in this study were run at 37 °C with concentrations of ZEN between 0 and 4 μg/mL, a stable range for this temperature, to ensure that ZEN remained in solution.

Additionally, in binding studies, nonspecific adsorption of ZEN to the sample container could invalidate results. To ensure that this was not a problem in our study, preliminary experiments were performed in which ZEN was placed in polypropylene test tubes and shaken at 37 °C for 24 h to monitor the level of ZEN in the tube over that time period. No binding of ZEN to these tubes occurred (data not shown). Also, as a precaution, two separate controls were set up to monitor for any nonselective adsorption to the tubes, namely at the 0.5 and 4 ppm concentrations of ZEN that contained no clay. Therefore, nonselective adsorption is a parameter that needs to be addressed before adsorption to clay can be definitively determined; it did not present a problem with the polypropylene tubes selected for this study.

In this study we have characterized the ability of CP-exchanged organo clay to adsorb ZEN from aqueous solution. We have also shown that structurally diverse clay and zeolitic minerals are not all equal in their abilities to adsorb ZEN, and inclusion in animal diets as mycotoxin-binding agents may be ineffective. Potential mycotoxin-binding agents should be rigorously tested and thoroughly characterized in vitro and in vivo to provide an understanding of the fundamental chemical basis involved in ZEN adsorption and to expedite the identification and investigation of safe and effective clay products for the prevention of ZEN-induced disease in animals and ZEN residues in food of animal origin.

SAFETY

ZEN is an irritant that is destructive to mucous membrane tissues and the upper respiratory tract, causes reproductive disorders to laboratory animals, and is a suspected carcinogen. It is recommended that exposure be limited through the use of protective clothing and gloves and that all work be conducted in a fume hood. In addition, the use of stock solutions (rather than crystalline form) is suggested to avoid acute exposure to airborne toxic particulates.

ABBREVIATIONS USED

BTEA, benzyltriethylammonium; CEC, cation exchange capacity; CP, cetylpyridinium; DDTMA, dodecyltrimethylammonium; DDP, dodecylpyridinium; HDTMA, hexadecyltrimethylammonium; K, partition coefficient; K_{av}, average partition coefficient; K_d, distribution coefficient; LPHM, low pH montmorillonite clay; Q_{max}, maximum capacity of a sorption site; TDTMA, tetradecyltrimethylammonium; TMA, tetramethylammonium; ZEN, zearalenone.

LITERATURE CITED

Atkins, P. W. *Physical Chemistry*, 3rd ed.; Freeman: New York, 1986; p 253.

Bursian, S. J.; Aulerich, R. J.; Cameron, J. K.; Ames, N. K.; Steficek, B. A. Efficacy of Hydrated Sodium Calcium Aluminosilicate in Reducing the Toxicity of Dietary Zearalenone to Mink. *J. Appl. Toxicol.* **1992**, *12*, 85−90.

Cole, R. J.; Cox, R. H. Fusarium Toxins. In *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, 1981; pp 902−903.

Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

Diekman, M. A.; Green, M. L. Mycotoxins and Reproduction in Domestic Livestock. *J. Anim. Sci.* **1992**, *70*, 1615−1627.

Ertzweiler, F.; Senn, E.; Schmidt, H. W. H. Method for Measuring Aqueous Solubilities of Organic Compounds. *Anal. Chem.* **1995**, *67*, 655−658.

Ghose, A. K.; Pritchett, A.; Crippen, G. M. Atomic Physicochemical Parameters for Three-Dimensional Structure Directed Quantitative Structure−Activity Relationships III: Modeling Hydrophobic Interactions. *J. Comput. Chem.* **1988**, *9*, 80−90.

Giles, C. H.; MacEwan, T. H.; Nakhwa, S. N.; Smith, D. Studies in Adsorption. Part XI. A System of Classification of Solution Adsorption Isotherms, and its Use in Diagnosis of Adsorption Mechanisms and in Measurement of Specific Areas of Solids. *J. Chem. Soc.* **1960**, 3973−3993.

Grant, P. G. Investigation of the Mechanism of Aflatoxin B_1 Adsorption to Clays and Sorbents Through the use of Isothermal Analysis. Dissertation, Texas A&M University, May 1998.

Grant, P. G.; Phillips, T. D. Isothermal Adsorption of Aflatoxin B_1 on HSCAS Clay. *J. Agric. Food Chem.* **1998**, *46*, 599−605.

Grant, P. G.; Lemke, S. L.; Dwyer, M. R.; Phillips, T. D. Modified Langmuir Equation for S Shaped and Multi-Site Isotherm Plots. *Langmuir* **1998**, *14*, 4292−4299.

Greenland, D. J.; Quirk, J. P. Adsorption of 1-*n*-alkyl Pyridinium Bromides by Montmorillonite. *Clays Clay Miner.* **1960**, *9*, 484−499.

Hehre, W. J.; Nelson, J. E. *Introducing Molecular Modeling into the Undergraduate Chemistry Curriculum*; Wavefunctions: Irvine, CA, 1997; pp 78−84.

International Tables for X-ray Crystallography; Kynoch Press: Birmingham, England, 1952.

Kanters, R. P. F. "Fract2xyz.c", C-source code, Computational Chemistry Archives, Computational Chemistry List, Ohio Super Computer Facilities, Available at http://www.science.richmond.edu/~kanters/LOP.html.

Long, G. G.; Diekman, M. A. Characterization of Effects of Zearalenone in Swine During Early Pregnancy. *Am. J. Vet. Res.* **1986**, *47*, 184−187.

Marasas, W. F. O. Estrogenic Syndrome. In *Mycotoxicology: Introduction to the Mycology, Plant Pathology, Chemistry, Toxicology, and Pathology of Naturally Occurring Mycotoxicoses in Animals and Man*; Pennsylvania State University Press: Philadelphia, PA, 1987; pp 45−48.

Meylan, W. M.; Howard, P. H.; Boethling, R. S. Improved Method for Estimating Water Solubility from Octanol/Water Partition Coefficient. *Environ. Toxicol. Chem.* **1996**, *15*, 100−106.

Mukerjee, P.; Mysels, K. J. *Critical Micelle Concentrations of Aqueous Surfactant Systems*; National Standards Reference Data Series NSRDS-NBS 36: Washington, DC, 1971.

Phillips, T. D.; Sarr, A. B.; Grant, P. G. Selective Chemisorption and Detoxification of Aflatoxins by Phyllosilicate Clay. *Nat. Toxins* **1995**, *3*, 204−213.

Pier, A. C. Mycotoxins and Animal Health. *Adv. Vet. Sci. Comp. Med.* **1981**, *25*, 185−243.

Ramos, A. J.; Hernandez, E.; Pla-Delfina, J. M.; Merino, M. Intestinal Absorption of Zearalenone and *in Vitro* Study of Non-nutritive Sorbent Materials. *Int. J. Pharm.* **1996**, *128*, 129−137.

Richardson, S. M.; Richardson, J. M. J. Crystal Structure of a Pink Muscovite from Archer's Post, Kenya: Implications for Reverse Plechorism in Dioctahedral Micas. *Am. Miner.* **1982**, *67*, 69−75.

Sheng, G.; Xu, S.; Boyd, S. A. Mechanism(s) Controlling Sorption of Neutral Organic Contaminants by Surfactant-Derived and Natural Organic Matter. *Environ. Sci. Technol.* **1996**, *30*, 1553−1557.

Slade, P. G.; Raupach, M.; Emerson, W. W. The Ordering of Cetylpyridinium Bromide on Vermiculite. *Clays Clay Miner.* **1978**, *26*, 125−134.

Smith, J. A.; Jaffe, P. R.; Chiou, C. T. Effect of Ten Quaternary Ammonium Cations on Tetrachloromethane Sorption to Clay from Water. *Environ. Sci. Technol.* **1990**, *24*, 1167−1172.

Stangroom, K. E.; Smith, T. K. Effect of Whole and Fractionated Dietary Alfalfa Meal on Zearalenone Toxicosis and Metabolism in Rats and Swine. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1219−1224.

Stapleton, M. G.; Sparks, D. L.; Dentel, S. K. Sorption of Pentachlorophenol to HDTMA-Clay as a Function of Ionic Strength and pH. *Environ. Sci. Technol.* **1994**, *28*, 2330−2335.

Srinivasan, K. R.; Fogler, H. S. Use of Inorgano-Organo-Clays in the Removal of Priority Pollutants from Industrial Wastewaters: Adsorption of Benzo[*a*]pyrene and Chlorophenols from Aqueous Solutions. *Clays Clay Miner.* **1990**, *38*, 287−293.

Tanaka, T.; Hasegawa, A.; Yamamoto, S.; Lee, U. S.; Sugiura, Y.; Ueno, Y. Worldwide Contamination of Cereals by the *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone. *J. Agric. Food Chem.* **1988**, *36*, 979−983.

Underhill, K. L.; Rotter, B. A.; Thompson, B. K.; Prelusky, D. B.; Trenholm, H. L. Effectiveness of Cholestyramine in the Detoxification of Zearalenone as Determined in Mice. *Bull. Environ. Contam. Toxicol.* **1995**, *54*, 128−134.

Vellarkad, N.; Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. N.; Robins, R. K. Atomic Physicochemical Parameters for Three-Dimensional Structure Directed Quantitative Structure−Activity Relationships. 4. Additional Parameters for Hydrophobic and Dispersive Interactions and Their Application for an Automated Superposition of Certain Naturally Occurring Nucleoside Antibiotics. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163−172.

Xu, S.; Boyd, S. T. Cationic Surfactant Sorption to a Vermiculitic Subsoil via Hydrophobic Bonding. *Environ. Sci. Technol.* **1995**, *29*, 312−320.

Zhang, Z. Z.; Sparks, D. L.; Scrivner, N. C. Sorption and Desorption of Quaternary Amine Cations on Clays. *Environ. Sci. Technol.* **1993**, *27*, 1625−1631.

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