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AN ABSTRACT OF THE THESIS OF Andreas Blochel for the
Master of Science in Physics presented June 8, 1992.

Title: Adsorption of Halogenated Phenolate Ions to Egg-
Phosphatidylcholine Vesicles.

APPROVED BY THE MEMBERS OF THE COMMITTEE:

Pavel Smejtek, Chair

Erik Bodegom

Arnold Pickar

Carl Wamser

In this study, parameters for the adsorption of several halogenated phenolate ions to egg-phosphatidylcholine vesicles have been determined by microelectrophoresis. The compounds under investigation were 2,6-Dichlorophenol (2,6-DCP), 3,5-Dichlorophenol (3,5-DCP), 2,4,6-Trichlorophenol (2,4,6-TrCP), 2,4,5-Trichlorophenol (2,4,5-TrCP), 2,3,5,6-Tetrachlorophenol (2,3,5,6-TCP), Pentachlorophenol (PCP), Pentafluorophenol

(PFP) and Pentabromophenol (PBP). Vesicle mobilities were measured at a temperature of 25°C as a function of phenolate concentration using the Doppler frequency shift of light scattered from the vesicles moving in an electric field. ζ -potential isotherms were determined from mobilities by means of the Helmholtz-Smoluchowski equation. Association constant K and adsorption site area P_s were obtained by a best fit of the Langmuir-Stern-Grahame adsorption model to the ζ -potential isotherms. The linear partition coefficient β of halogenated phenolate ions between membrane and water was computed from these two adsorption parameters. For chlorophenolates β increased with the number of chlorine substituents and was generally higher for isomers with substituents in the 3rd, 4th and 5th position on the benzene ring. In the pentahalogenated series (PFP, PCP, PBP) β increased with the size of the substituting halogen. A strong correlation ($r=0.96$) was found between $\log \beta$ of phenolate ions and $\log$ of P_{ow} , the octanol-water partition coefficient of the neutral phenol molecules. The correlation improved slightly when permanent dipole moment ($r=0.98$) or polarizability ($r=0.99$) were included. In addition, the correlations of $\log P_{ow}$ and $\log \beta$ with toxicity data for bacteria and yeast were compared. For yeast the correlation between toxicity and $\log \beta$ or $\log P_{ow}$ was about the same for ($r=0.93$), while for bacteria $\log P_{ow}$ yielded a better result than $\log \beta$ ($r=0.87$ versus 0.80). Determination of parameters for the adsorption of halogenated phenolate ions to

phosphatidylcholine contributed to our understanding of the structure-toxicity relationship of environmental pollutants.

ADSORPTION OF HALOGENATED PHENOLATE IONS
TO EGG-PHOSPHATIDYLCHOLINE VESICLES

by

ANDREAS BLOCHEL

A thesis submitted in partial fulfillment of the
requirements for the degree of

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in
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1992

TO THE OFFICE OF GRADUATE STUDIES:

The members of the committee approve the thesis of
Andreas Blochel presented June 8, 1992.

Pavel Smejtek, Chair

Erik Bodegom

Arnold Pickar

Carl Wamser

APPROVED:

Erik Bodegom, Acting Chair, Department of Physics

C. William Savery, Vice Provost for Graduate Studies and
Research

Für

Timo, Andi und Stephanie

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CHAPTER I

INTRODUCTION

All processes occurring within cells and their organelles require the existence of very specific conditions. The task of membranes in every organism is to maintain these conditions by controlling the concentrations of various molecules and ions within the cell or subcellular unit against osmotic pressure.

The function of membranes depends on the action of specific proteins and enzymes attached to or inserted into the membrane as well as on the membrane's passive role as a permeability barrier. This barrier function is achieved by lipids and their ability to self-assemble into membranes.

Certain molecules can disrupt the proper function of membranes by disrupting the permeability barrier (Flewelling and Hubbell, 1989a). Lipophilic molecules can penetrate the membrane (Verkman and Solomon, 1982) and act as carriers for other molecules or ions (Benz et al., 1979) or they can adsorb to the membrane and alter the distribution of the electrostatic potential across the membrane, which affects the action of membrane-bound proteins (Krämer 1989). Adsorption of lipophilic molecules can also change the membrane structure (Maher and Singer, 1984) and properties such as the temperature of phase transition of the lipid components (Smejtek et al., 1989).

Molecular adsorption to membranes also results in various toxic effects. The halogenated phenols investigated in this study belong to the class of protonophoric uncouplers of oxidative phosphorylation.

Synthesis of ATP by phosphorylation of ADP occurs in mitochondria largely due to the action of membrane-bound F_0F_1 -ATPase according to

The phosphorylation of ADP requires energy which, according to Mitchell's chemiosmotic theory, is supplied by protons driven into the inner mitochondrial matrix via ATPase by a negative gradient in their chemical potential $\Delta\mu_{H^+}$. $\Delta\mu_{H^+}$ originates from a proton concentration gradient and an electrostatic potential difference across the inner mitochondrial membrane. Ultimately, the energy required to maintain $\Delta\mu_{H^+}$ is, at least in animals, a result of the oxidation of NADH, a product of the citric acid cycle. The overall reaction maintaining $\Delta\mu_{H^+}$ is

In plants, the energy is provided by the photosynthetic electron transport chain (Terada 1990). Equation (2) describes the net result of an elaborate, stepwise process of electron transfer to oxygen - the respiration. During this process the energy of oxidation is used by several enzymes to pump protons

across the membrane. In this way the respiratory chain provides the proton gradient essential for the production of ATP. The action of the respiratory chain is controlled by this gradient and thus, under normal conditions, ATP is produced. If $\Delta\mu_{H^+}$ is reduced by a process other than phosphorylation of ATP, oxidation proceeds but is uncoupled from ATP synthesis. When $\Delta\mu_{H^+}$ drops below a critical value ATP production ceases (and ATP is actually hydrolyzed by the reverse action of the enzyme ATPase, Nicholls 1982) and respiration increases, both processes working in the exothermic direction.

Protonophoric uncouplers cause lower $\Delta\mu_{H^+}$ and uncoupling of ATP synthesis by carrying protons across the inner mitochondrial membrane. Two mechanisms are known by which $\Delta\mu_{H^+}$ dissipation occurs. Protons are carried across the membrane by neutral uncoupler molecules (AH) which readily permeate the membrane. Protons are released at the more basic matrix side of the membrane and the ionized form of the uncoupler (A^-) diffuses back to pick up another proton at the more acidic cytoplasmic side (Terada 1990). The other alternative to back diffusion of A^- is the return flow of AHA^- dimers (Smejtek et al., 1976), a mechanism which has been shown to explain the toxicity of pentachlorophenol to algae (Jayaweera et al., 1982).

Two properties are most important for protonophoric activity: (1) Lipophilicity is a requirement for adsorption and permeation of the membrane. (2) The uncoupler has to be

weakly acidic ($4.5 < pK_a < 6.5$) to allow for effective proton release and uptake on the respective side of the membrane and/or for enough ions for dimer formation to be present under physiological conditions.

Uncouplers of eminent environmental importance are chlorinated phenols. An extensive review of risks posed by chlorinated phenols can be found in Ahlborg and Thunberg (1980). Penta-, tetra- and trichlorophenols have been used for several purposes since the early 1930s. Pentachlorophenol has been used for wood preservation and as a herbicide in sugar cane, rice and pineapple plantations. Tetrachlorophenols were also used as insecticides and fungicides for wood treatment. 2,4,5-Trichlorophenol is utilized for the production of hexachlorophene and 2,4,5-trichlorophenoxyacetic acid and is a by-product in paper and pulp mills. Furthermore it is used for leather processing and as a disinfectant. The worldwide production of chlorophenols in 1980 was 200 000 tons (Ahlborg and Thunberg, 1980).

Environmental problems are caused by burning of chlorophenol-treated wood which causes the formation chlorinated dioxins as well as by contamination of the aquatic environment due to release of contaminated waste-water from paper and pulp mills. Although chlorophenols are degradable by bacteria (Suflita and Miller, 1985), high concentrations were still found in fish and water 6 months and in sediment 2 years after an accidental contamination. In Sweden the use of Cps in pulp

mills and for wood preservation was prohibited for these reasons in the 1970s. Chlorinated phenols occur also as metabolic products of other pesticides such as lindane.

Accumulation of pentachlorophenol in humans ranges from 0.003 ppm to 10 ppm in heavily exposed workers. Acute toxicity is associated with the effects of uncoupling of oxidative phosphorylation. The concern about chlorophenols in the environment is largely related to their strong accumulation in an aquatic environment and to the formation of hazardous metabolic and degradation products (Ahlborg and Thunberg, 1980).

Many toxicity studies have been conducted with series of chlorinated or halogenated phenols. Most of the recent ones apply a systematical approach to establish quantitative structure-activity relationships (QSAR). In QSAR studies, values characteristic for toxicity are correlated with molecular parameters, most often the ones proposed by Hansch and Leo (1979). These parameters express steric, electronic and hydrophobic properties of the toxic compound and were introduced with the aim to obtain methods for a rough estimate of toxicity without the need for elaborate tests (Hansch et al., 1989).

The parameter which proves to be the most useful in the toxicity studies is the parameter for hydrophobicity (P_{ow}), the octanol/water partition coefficient (Xie and Dyrssen, 1984, Xie et al., 1984). The correlation of toxicity data with

parameters expressing hydrophobicity was found to be best in studies on bacteria (Gombar and Enslein, 1989, Pill et al., 1991), green algae (Shigeoka 1988), yeast (Arnold 1988), goldfish cells (Saito et al., 1991), fish (Koenemann and Musch, 1981), bovine spermatozoa (Seibert et al., 1989), rat liver mitochondria (Shannon et al., 1991), earthworms (van Gestel and Ma, 1990) and fungi (Cohen et al., 1988). In two other studies on mitochondria (Tollenaere et al., 1976, Stockdale and Selwyn, 1971) the best correlations were obtained when the parameter which is expressing lipophilicity is combined with σ , a parameter which describes the ability of substituents to remove electrons from the benzene ring. A combination of $\log P_{ow}$ and Pk_a yielded the best correlation for toxicity and accumulation in earthworms (van Gestel and Ma, 1988). The only studies available which did not include $\log P_{ow}$ or a related parameter in the best correlation equation were the ones by Ravanel and coworkers on uncoupling and respiration inhibition in Acer cells (1989) and plant mitochondria (1985).

Several of the studies mentioned above included not only chlorophenols but a variety of substituted phenols, further suggesting that lipophilicity provides in general a good estimate for relative toxicities among a certain class of molecules. In the case of uncoupling this approach is especially reasonable since the protonophoric effect of halogenated phenols is not site-specific and thus can be related to

adsorption of phenols to the membrane.

To address this question directly, the adsorption characteristics of the halogenated phenols have to be determined and to be related to the chemical model- P_{ow} or directly to toxicity. Since biological membranes are rather complex and often do not allow for accurate determination of adsorption characteristics (see Chapter II), studies of adsorption to membranes are often done with model membranes formed from lipid bilayers. We selected egg-phosphatidylcholine, a neutral phospholipid, as the model membrane.

As mentioned above, dissipation of $\Delta\mu_{H^+}$ depends in part on the availability of the ionized form as the proton carrier within the membrane. It is therefore necessary to determine the adsorption properties of the ionized form of chlorinated and halogenated phenols.

CHAPTER II

LIPID MEMBRANES AND ADSORPTION OF ORGANIC IONS

This chapter introduces three major elements of this study: (1) egg-phosphatidylcholine and multilayered egg-PC vesicles as a model system for the lipid portion of biological membranes, (2) the adsorption model including the adsorption parameters needed to relate the molecular structure of the adsorbing species to their adsorption properties and toxicity, and (3) a description of the microelectrophoretic mobility method.

PHOSPHATIDYLCHOLINE

Membranes of plant and animal cells and subcellular organelles are composed of three classes of lipids: sterols, glycolipids and phospholipids (Rouser et al., 1968). All these lipids are amphipathic due to a polar headgroup which is linked to a lipophilic tail consisting typically of two chains of 16 to 20 $-\text{CH}_2-$ units each. In our study we have used phosphatidylcholine (PC), one of the major components of membranes. The molecular structure of PC is given in Figure 1. The headgroup of PC is polar due to the fact that the phosphoryl group, $-\text{PO}_4-$, is more electronegative than the choline

group $-N(CH_3)_3$. As opposed to most other phospholipids, the PC molecule is electrically neutral (Cullis and Hope, 1985).

Due to the amphipathic nature of PC, the lowest energy aggregation of lipids in water is the bilayer structure with the hydrophobic tails shielded from the aqueous phase by the polar headgroups (see Fig.2). The thickness of these bilayers is about 4-6 nm, depending on the length of the hydrocarbon chains (Flewelling and Hubbell, 1986b). In biological membranes the distribution of lipids among the two leaflets of the bilayer is asymmetric, whereas in our system this asymmetry is absent. In biological membranes the lipids are in the fluid state.

Due to their importance for biological systems, model membranes made of phosphatidylcholine are widely used in adsorption studies (Smejtek et al., 1987, Tatulian 1987, McLaughlin et al., 1978, among others). Often used is egg-PC, a mixture of several phosphatidylcholines (differing by their hydrocarbon tails) which is extracted from hen egg yolk (Bangham et al., 1974).

Several types of interactions between the adsorbing species and the membrane determine the adsorption properties. Flewelling and Hubbell (1986b) developed a model in which four terms contribute to the free energy of transfer of an ion from the aqueous into the membrane phase: (1) The Born energy represents the energy necessary to charge a molecule in a dielectric. In transition from water to a less polarizable

dielectric this energy has to be provided to the ion. (2) The image charge term is due to the existence of a dielectric boundary which changes the potential energy of the ion inside the dielectric, close to the dielectric/water interface. (3) Then there is a neutral energy term which does not involve electrostatic interactions but accounts for hydrophobic interaction. (4) Finally, there exists below the membrane surface a dipole surface layer which gives rise to a positive potential of about 240 mV within the bilayer. The origin of this potential is not exactly known but it could be attributed to the ester linkages of the hydrocarbon chain (Flewelling and Hubbell, 1986b). This dipole layer attracts anions into the membrane interior and hence explains why lipid membranes are more permeable to negatively charged ions compared to cations. We assume that these ion potential energy wells are the origin of adsorption sites of halogenated phenolate ions.

THE ADSORPTION MODEL

The model used in this study to describe the adsorption of charged molecules to model lipid membranes combines the Gouy-Chapman theory of the diffuse double-layer and the Langmuir adsorption model. Both elements will be described separately in this section.

The Gouy-Chapman Theory

The Gouy-Chapman model describes the electrostatic potential in an electrolyte adjacent to a charged surface. The potential arises from the surface charge and the inhomogeneous distribution of ions in the electrolyte which is a result of their electrostatic interaction with the membrane surface charge (attractive or repulsive, depending on the sign of the ion charge) and diffusion towards a region of low concentration (McLaughlin 1977). The equilibrium between these effects is expressed by the Boltzmann relation

$$n(x) = n(\infty) \exp\left(-\frac{q\psi(x)}{kT}\right) \quad (3)$$

where $n(x)$ is the volume density of the ion and $\psi(x)$ is the electrostatic potential at a given distance x from the membrane, q the charge of the ion, k Boltzmann's constant and T the temperature of the electrolyte. $n(\infty)$ is the ion density at infinite distance from the membrane.

The Boltzmann relation together with the appropriate boundary conditions can now be invoked to solve the Poisson equation

$$\frac{d^2\psi(x)}{dx^2} = -\frac{1}{\epsilon_w\epsilon_0} \rho(x) \quad (4)$$

ϵ_w and ϵ_0 are the dielectric constant of the solution and the permittivity of free space and ρ is the volume charge density which is given by

$$\rho(x) = \sum z_i e n_i(x) \quad (5)$$

where e is the proton charge and the valence z_i is assumed to have the sign of the ion charge to which it refers. The boundary conditions require that the potential and its slope be zero at infinity and that ψ_0 , the potential at the surface, be specified.

It has to be mentioned that in this approach the surface charge on the membrane is assumed to be "smeared" uniformly over the surface rather than undergoing local changes due to discrete charges at certain positions. The combination of equations 3 to 5 is called the Poisson-Boltzmann relation.

The expression for $\psi(x)$ resulting from integration of the Poisson-Boltzmann relation is rather complicated but may be linearized if $q\psi_0 < 2kT$. Then the potential shows exponential decrease with distance from the surface (McLaughlin 1977):

$$\psi(x) = \psi_0 \exp(-\kappa x) \quad (6)$$

$1/\kappa$ is the Debye length, given by

$$\kappa = \sqrt{\frac{2}{\epsilon_w \epsilon_0 kT} \sum (z_i e)^2 C_i} \quad (7)$$

where C_i is the bulk concentration of ions. Equation 6 is also valid when the distance x is small compared to $1/\kappa$.

The ultimate purpose of this approach is to relate surface charge to surface potential. This can be achieved by taking into account that electroneutrality holds for membrane

and electrolyte combined. Thus the surface charge is obtained by integrating the electrolyte charge density over all space. The result is Grahame's equation (McLaughlin 1977)

$$\sigma = \sqrt{(2\epsilon_w\epsilon_0 kT) \sum C_i \left[\exp\left(\frac{-z_i e\psi_0}{kT}\right) - 1 \right]} \quad (8)$$

Assumptions have to be made for the derivation of Grahame's equation. Uniform surface charge distribution has already been mentioned. Furthermore, the equation implies that the ions are point charges, the dielectric constant of the solution does not change up to the membrane surface and that image charge effects due to the existence of a dielectric boundary can be neglected.

The accumulation of counterions in the region close to the membrane/water interface expressed by the Boltzmann relation results in the fact that close to a negatively charged membrane the proton concentration will be higher than in the bulk while the OH⁻ concentration will decrease. This results in a local pH change close to a charged surface according to

$$(pH)_m = (pH)_0 + \frac{\psi_0}{0.059} \quad (9)$$

where ψ_0 is the surface potential in mV.

The Langmuir-Stern-Grahame Adsorption Model

The Langmuir adsorption model assumes that the membrane surface density of adsorbed ions, $(A)_m$, is proportional to

their concentration of the molecule in the aqueous phase at the membrane-solution interface, $[A^-]_m$, and to the surface density of free adsorption sites on the membrane. These assumptions are reflected in the equation for the Langmuir adsorption isotherm

$$(A^-)_m = K[A^-]_m \left(\frac{1}{P_s} - (A^-)_m \right) \quad (10)$$

P_s is the area of one adsorption site and the association constant K describes the strength of the interaction between the adsorbing species and the membrane (Smejtek and Wang, 1990).

To determine $[A^-]_m$, the Boltzmann relation has to be invoked which relates the bulk concentration $[A^-]_0$ to the concentration at the membrane-water interface $[A^-]_m$

$$[A^-]_m = [A^-]_0 \exp\left(\frac{e\psi_0}{kT}\right) \quad (11)$$

If the concentration of lipids is high, the mass balance for A^- has to be considered. The mass balance takes into account the reduction of the initial concentration of ions in the aqueous phase ($[A^-]_{aq}$, the parameter controlled by the experimentalist) by adsorption to the membrane:

$$[A^-]_{aq} = [A^-]_0 + (A^-)_m [L] N_{AV} P_l \quad (12)$$

$[L]$ is the molar lipid concentration, N_{AV} is Avogadro's number and P_l the area per lipid.

As described in Chapter III, the adsorption parameters K

and P_s can be computed from the membrane surface potential ψ_0 by combining the equation for the Langmuir isotherm, equation 11 and Grahame's equation. In applying Grahame's equation it has to be taken into account that the total surface charge density σ on the membrane is the sum of an initial charge density σ_m and the surface charge density due to adsorption of ions $\sigma_{ads} = -e(A^-)_m$. In the limit of low membrane surface coverage with A^- ($1/P_s \gg (A^-)_m$) the linear partition coefficient β can be obtained according to

$$\beta = \frac{K}{P_s} \quad (= \frac{(A^-)_m}{[A^-]_m}) \quad (13)$$

Tests of the Langmuir-Stern-Grahame adsorption model usually involve the separate determination of surface or ζ -potential (often by electrophoresis) and surface charge (by probe methods). Several tests (McLaughlin et al., 1976 and 1978, Puskin and Coene, 1980, Lau et al., 1981, among others) have shown the applicability of this model to adsorption of ions.

Limitations of the theory resulting from the discrete-charge effect have been investigated by Lau and coworkers (1981). He showed that the surface potential of phosphatidyl-glycerol at the adsorption site of a NMR probe is larger than the surface potential derived from ζ -potential measurements. This indicates that for the region close to the membrane surface the assumption of an average potential due to homoge-

neous surface charge density does not always hold. The discrete-charge effect is largest for low surface charge density and high ionic strength (McLaughlin 1977).

MICROELECTROPHORESIS

Several methods have been developed to measure membrane surface potentials with the objective to determine the adsorption characteristics of charged molecules to membranes. Some of them, such as NMR and fluorescence probe methods, are indirect in the sense that the surface potential is derived from the response of probe molecules which themselves are adsorbed to the membrane, thus potentially perturbing the system under investigation (Lelkes and Miller, 1980). By microelectrophoresis, on the other hand, the potential close to the membrane surface is determined without the need for probes.

When an electric field E is applied to a charged particle suspended in an electrolyte, a balance between electrostatic and viscous forces causes the particle to move with a constant velocity v . The electrophoretic mobility μ of the particle is defined by

$$\mu = \frac{v}{E} \quad (14)$$

Since the particle is suspended in an electrolyte, it will attract counterions. The first layer of counterions is considered to stick rigidly to the particle, which is a

consequence of the assumption that the electrolyte is a Newtonian fluid. This layer of counterions thus increases the effective radius of the particle and information on the potential which we get from electrophoretic measurements consequently refers to this so-called "surface of shear" or "shear plane". The potential at this surface is called " ζ -potential".

The assumption of a Newtonian fluid (meaning that the viscous forces on the electrolyte dominate inertial forces as it flows past the particle) and the restriction to fields smaller than the ratio of ζ and the Debye length $1/\kappa$ result in the fact that the mobility does not depend on shape, properties and electrostatic boundary conditions on the particle but only on the properties of the electrolyte and the ζ -potential (O'Brien and White, 1978). If in addition the particle radius is large compared to the Debye length, the relation between μ and ζ is linear, independent of size and shape, and is given by the Helmholtz-Smoluchowski equation (Cafiso et al., 1989)

$$\zeta = \frac{\eta}{\epsilon_w \epsilon_0} \mu \quad (15)$$

where η is the viscosity of the fluid.

Microelectrophoresis is often used in combination with the Gouy-Chapman theory and applied to model membranes (Tatulian, McLaughlin, Smejtek). In this case the relation between the surface potential and the ζ -potential is given by

$$\zeta = V_m \exp(-s\kappa) \quad (16)$$

where s is the shear plane distance. Equation 16, however, holds only if $s \ll \kappa$. The location of the shear plane as a function of ionic strength has been determined by Smejtek et al. (1987) by measuring the electrophoretic mobility of lipid vesicles of known surface charge. A. McLaughlin et al. (1983) determined the shear plane distance by directly relating ζ -potential (from electrophoresis) and surface potential (from measurements of membrane conductance).

For some biological particles the electrophoretic mobility method is not that powerful (Cafiso et al., 1989). Woodle et al. (1992) mention that results for the electrophoretic mobility of red blood cells are not in agreement with the Gouy-Chapman theory and attributes this fact to the presence of sugars which protrude from the surface, causing additional hydrodynamic drag. In addition the mobility depends on the location of charged groups with respect to the shear surface. These complications do not apply to phospholipid vesicles used in this study.

STRUCTURE OF HALOGENATED PHENOLS

The general structure of a halogenated phenol is shown in Figure 3. The central unit is a benzene ring consisting of 6 carbon atoms with three delocalized double-bonds. The positions on the ring are numbered (1-6). A phenol has a hydroxy-group bound to the first carbon and hydrogen atoms bound to

all the other carbon atoms. When the phenol is halogenated, the hydrogen atoms in the positions given in the name of the phenolic compound are replaced by halogen atoms. 2,6-Dichlorophenol for example has chlorines in positions 2 and 6 and hydrogens in positions 3,4 and 5.

Figure 1. Phosphatidylcholine.

Figure 2. A lipid bilayer vesicle.

Figure 3. Structure of a substituted phenol.

CHAPTER III

MATERIALS AND METHODS

CHEMICALS

2,6-Dichlorophenol (2,6-DCP, 99% purity), 3,5-DCP (97%), 2,4,5-trichlorophenol (2,4,5-TrCP, 99%), 2,4,6-TrCP (98%), 2,3,4,5-tetrachlorophenol (2,3,4,5-TCP, 98%), 2,3,5,6-TCP, pentachlorophenol (PCP, 99%), pentabromophenol (PBP, 96%) and pentafluorophenol (PFP, 99%) were all bought from Aldrich Chemical Company, Milwaukee, WI. A solution of 20 mg egg-PC per ml in chloroform was from Avanti Polar Lipids, Birmingham, AL and was 99% pure. Potassium phosphate dibasic trihydrate, boric acid (Mallickrodt Chemicals, St. Louis, MO), potassium citrate monohydrate (EM Science, Cherry Hill, NJ), chloroform (American Burdick and Jackson, Muskegon, MI), potassium hydroxide (Matheson, Coleman & Bell Manufacturing Chemists, Norwood, OH) and potassium chloride (J.T. Baker Chemical Co., Phillipsburg, NJ) were of reagent grade or better. All chemicals were used without further purification. Deionized water was used for the preparation of all the solutions.

SAMPLE PREPARATION

Egg-PC from the stock solution was added to chloroform in a 25-ml round bottom flask. The chloroform was evaporated under rotation in a flash-evaporator (Buchler Instruments, Fort Lee, NJ). Ice was used to reduce the vapor pressure in the evaporator. This procedure resulted in a thin film of egg-PC deposited in the flask. The flask was then transferred to a mechanical pump and the evacuation continued for at least two more hours at a pressure of ca. 0.1 torr. A buffer solution (PH 10) containing 0.002 M phosphate, 0.002 M citrate, 0.0005 M borate and 0.03 M KCl was added so that a concentration of 0.16 mg/ml of PC was obtained. The flask was then shaken gently to allow for the formation of PC vesicles (Bangham et al., 1974) with a diameter of 1-3 μm . The particle size was determined by light scattering (see below).

The halogenated phenols were dissolved in 0.02 M KOH and 0.03 M KCl. The concentration of phenols was as high as could be achieved by stirring for several hours. The final solutions were prepared by serial dilution with KCl and buffer concentrations set to 0.03 M (KCl) and 0.002 M:0.002 M:0.0005 M (phosphate:citrate:borate). The pH was adjusted to 10.00 ± 0.05 by titration with KOH and HCl. This ensures more than 95% ionization of all the compounds under investigation even when taken into account that the local pH drops close to the negatively charged membrane surface (see Chapter II). The pH measurements were made with a Chemtrix model pH meter (Chem-

trix, Inc., Hillsboro, OR) equipped with an Orion (Boston, MA) Ag/AgCl glass pH electrode. The filling solution was a 4 M KCl solution saturated with AgCl (Corning, Medfield, MA). The pH meter was calibrated daily with pH 7.00 and 10.00 standard buffer solutions (VWR Scientific, MEDIA, PA and American Scientific & Chemical, Portland, OR). After pH adjustment, 1 ml of the PC/buffer stock suspension was added. The electrophoretic measurements were performed within 15 min after sample preparation. The pH proved to be stable over this period of time. Only PC/buffer suspension not older than 2 days was used.

ELECTROPHORETIC MEASUREMENTS

The electrophoretic mobility of the PC vesicles at varying concentrations of halogenated phenols was determined using DELSA (Doppler Electrophoretic Light Scattering Analyzer) model 440 by Coulter Electronics, Inc. (Hialeah, FL), an instrument utilizing the Doppler shift of laser light scattered by the vesicles to determine their velocity in an external electric field. The sample suspension is placed into the electrophoretic cell which is equipped with two electrodes and a thermistor. The central region of the cell is a block of quartz glass with a channel bored into it. The suspension within the channel is exposed to the laser beam and the scattered light is analyzed.

Light Scattering

A monochromatic, horizontally polarized light ray is generated by a 5 mW Helium-Neon laser (wavelength $\lambda \approx 600$ nm). Its intensity is manually adjustable. The ray is split into five different rays by means of a diffraction grating. The main beam carries about 80% of the intensity. The remaining 20% is in the four reference beams which all have the same, but lower intensity. There is a beam blocker for each beam. A converging lens makes all five rays intersect within the sample chamber. The beam intersection can be viewed through a 24x microscope and its position is manually adjustable. The reference beams are directed towards detectors at angles with the main beam of 7.5° , 15° , 22.5° and 30° , respectively. Light from the main beam scattered by the particles within the volume of the intersection is collinear with the respective reference beam when it hits the detector. Due to the movement of the particles, the scattered beam undergoes a frequency change of

$$\Delta\nu = 2nV\cos(\alpha)\sin(\theta/2)/\lambda \quad (17)$$

where n is the index of refraction of the medium, V the velocity of the particle with respect to the detector, θ the scattering angle, α the angle between scattering vector and particle velocity and λ the wavelength in vacuum (see Fig. 4). The scattering vector $\mathbf{K}$ is given by

$$K = k_1 - k_0 = 4\pi n \sin(\theta/2)/\lambda \quad (18)$$

where $\mathbf{k}_1$ and $\mathbf{k}_0$ are the wave vectors after and before the scattering event.

Signal Processing

The interference of the scattered main beam and the unscattered reference beam at the photodetector results in a signal whose amplitude is varying sinusoidally with a frequency equal to $\Delta\nu = \nu_1 - \nu_0$. This intensity fluctuation translates into a detector current

$$I(t) = \sum A_i \sin(2\pi \Delta\nu_i t) \quad (19)$$

where i runs over the different scattering species.

This intensity distribution is now Fourier transformed to a power spectrum (one for each angle)

$$S(\nu) = \sum a_i [\delta(\nu - \Delta\nu_i) + \delta(\nu + \Delta\nu_i)] \quad (20)$$

Since this spectrum does not discriminate between positive and negative $\Delta\nu$, the sign of the particle motion is not known. Therefore, DELSA is equipped with a frequency shifter for the main beam. Different signs of velocity now result in a different value for the Doppler shift. The shift frequency is one-half of the selected frequency range (see below).

The power spectrum is computed on a IBM Personal System/2 Model 50 Z computer. It is converted into either a frequency, a mobility or a zeta-potential spectrum. Due to the particles being subject to Brownian motion, so-called diffusion broaden-

ing of the peaks occurs. This broadening is inversely proportional to the particle radius. Measurements without application of an electric field thus allow for determination of the particle radius. The angular dependence of the Doppler shift also enables the operator to separate peaks due to true particle motion from artifacts of the instrument. The latter are not expected to be proportional to the detector angle as is true for real peaks (approximation for small $\theta/2$).

The conversion of the spectra from frequency to mobility utilizes the defining relation between mobility, particle velocity and applied field, equation 14. V is known from the Doppler shift and the applied field E (or the current $I=\sigma E$) is selected by the operator. σ , the specific conductance, is measured by the instrument. The relation between μ and ζ is given by the Helmholtz-Smoluchowski equation, equation 15.

In these studies, the dielectric constant ϵ and viscosity η of water at 25°C were used to convert mobility into ζ -potential. The effect of the electrolyte was neglected. In this case, a ζ -potential of 12.8 mV corresponds to a mobility of 1 $\mu\text{m}/\text{Vs}$. Tatulian (1988) reports a value of 14 mV for low CaCl_2 concentrations ($< 10 \text{ mM}$) at 20°C, and Seaman (1975) cites 13.3 mV at 0.15 M NaCl and 25°C.

The analysis of the data is performed by the software. Peak position and mean value of mobility (over the selected region) as well as standard deviation are determined separately for each peak. Another feature of the instrument is the

capability to determine the particle size. For this purpose, the scattering measurements have to be performed without an electric field applied to allow for measurement of the Brownian motion alone.

The Electrophoretic Cell

The electrophoretic cell (see Fig.5) consists of two silver electrodes with a glass insert. The cell is filled through plastic fill tubes which are connected with the hemispherical cavities within each electrode. The surfaces of these cavities act as electrodes. The glass insert (thickness 5 mm) is provided with an antireflective coating and contains a channel which is 1 mm high and connects the two hemispheres. A heat conducting block in contact with the cell is used to assure that no temperature gradient is established along the cell.

During operation of the instrument the cell is placed on a stage such that the laser beams pass through the channel. The vertical position of the stage is adjustable so that measurements can be performed at any height within the channel. Originally, the instrument was equipped with an analog gauge which we changed to a digital gauge (Mitutoyo Digimatic Indicator IDC 543 by Mitutoyo Corp., Tokyo, Japan) allowing for accuracy in cell positioning of 1 μm . Furthermore, it prevents reading errors due to parallaxes. The reproducibility of the positioning is of the same order of magnitude, as determined during determination of the channel height (see

section "Measurements" in this chapter).

Flow Profile

In general, the walls of the channel are at a potential different from that of the sample suspension. This will cause a non-homogeneous ion distribution within the channel, since a net charge on the channel wall will attract counterions. This effect is known as electroosmosis. Due to the counterion distribution, the electrostatic potential changes as a function of depth in the channel and the velocity of the electrolyte is position-dependent. For a channel of rectangular cross section the resulting flow profile is parabolic (see Fig.6). Sedimentation of charged particles may change the flow profile but it did not occur during the periods of the measurements presented here (about 30 min).

The cell is a closed system and therefore the net flow has to be zero across every cross section of the channel. As a consequence, there exist two positions, symmetric with respect to the center of the channel, at which the velocity of the electrolyte is zero. These positions are called "stationary layers". Since DELSA measures the superposition of particle and liquid velocity, it is important to measure the velocity of particles at a stationary layer if true mobilities and zeta-potentials are to be determined. The upper stationary layer is generally preferred since any sedimentation of charged particles affects its position less than that of the lower layer.

Adjustable Parameters

Following parameters have to be provided to DELSA prior to a measurement: Temperature, viscosity, refractive index and dielectric constant of the medium, cell constant, frequency range, "field on" time, "field off" time, run time and electric field strength or current.

In these experiments, the temperature was kept at 25 °C, viscosity (0.00890 poise), refractive index (1.3330) and dielectric constant (78.360) were set to values for water given in the Handbook of Chemistry and Physics. The error introduced by salt is small enough to be neglected.

The cell constant is a parameter which assures calibration of the electric field. It was adjusted such that the conductivity of a standard solution ($1000 \pm 5 \mu\text{S/cm}$, Yellow Springs Instruments Co., Inc., Yellow Springs, OH) was measured correctly by the instrument.

The frequency range was determined by the desired resolution and the mobility of the sample. The instrument is equipped with a 256 channel analyzer and the frequency range can be chosen in six steps between 50 and 1000 Hz.

The resolution is then given by the frequency range divided by 256. Most of the measurements presented here were done at a frequency range of 250 Hz, but for zeta-potentials more negative than -50 mV the noise at the edge of the spectrum interfered with the peaks of interest, so that the 500 Hz range was chosen for these samples.

The measurements were done by applying the electric field for a period called the "field on" time, then switched off for the "field off" time to allow the sample to recover. This was followed by reversing the field polarity and repeating the cycle. This reversal of the field prevents electrode polarization artifacts. The run time is the total period in which measurements are made. Since the frequency resolution depends inversely on the "field on" time, care was taken so that the resolution was not affected by short "field on" time intervals. Our conditions were "field on" time = 2.0 s, "field off" time = 0.5 s and 120 s run time, which was in accord with the frequency range used.

Two modes of selection of the electric field strength were used: (1) constant current and (2) constant voltage. Operating in the "constant current mode" has the advantage that effects of temperature changes due to Joule heating are minimized: Energy dissipation is proportional to σE^2 or I^2/σ , where σ is the conductivity of the sample. An increase in temperature causes an increase in σ since the viscosity decreases. If the field is kept constant, the heating will increase. If the current is kept constant the energy dissipation artifacts are smaller. We always obtained good results when the instrument was operated at a current value (in mA) equal to the square root of the suspension conductivity (in mS). Problems with convection due to heating did not occur and the instrument worked in the linear range as could be seen

from the frequency spectra (frequency shift proportional to the detector angle). Conductivities were in the range from 5 to 8 mS, depending on total ion concentration.

Determination of the Stationary Layer

Although the DELSA manual gives a formula for the calculation of the stationary layer based on the parabolic shape of the flow profile, it is obviously better to obtain this position by measuring the mobility of a standard suspension. Liposomes prepared from egg-PC have been found to have no surface potential over a wide pH range (Bangham et al., 1974) and have been used as a standard (Woodle et al., 1992, Cafiso et al., 1989). The manufacturer of the instrument also provides a mobility standard, but the standard value ($4.3 \pm 0.4 \mu\text{mcm/Vs}$) shows a very high error margin, corresponding to ca. 5 mV, and thus is not really suitable for our purpose. In addition to that, even when literature values for PC mobility had been achieved in this lab earlier at a position close to that given in the manual (which is 160 μm from the top edge for the upper stationary layer), the value for the mobility standard was out of the specified range. Since a potential for egg-PC vesicles of around -10 mV was obtained consistently in the studies presented here (see Chapter IV), which was not in agreement with the data given by Bangham et al., we have defined the stationary layer at the position 150 μm below the top edge of the channel. At this position a mobility value of 3.6 $\mu\text{mcm/Vs}$ for the Coulter standard was obtained, which was

in agreement with the conventional value for the mobility standard. To estimate the error arising from an inaccurate determination of the stationary layer, we have determined that a difference of 10 μm in the cell position resulted in a difference of about 1.0-1.5 mV in zeta-potential for egg-PC liposomes.

Measurements

Before the measurements could be taken, the beam intersection (see above) and the output level of the laser had to be checked. To ensure correct operation, the main beam intensity should be greater by a factor of 2 to 30 than that of the reference beams.

After the sample had been filled into the cell and the cell placed into the instrument, the system was allowed to equilibrate thermally, which took about ten minutes. Then the top edge of the channel was determined by positioning the cell up or down until the two minima in detector current could be found. The minima occur because light is scattered very strongly at the channel edge. Subsequently, the laser beam was positioned at the stationary layer, the run parameters were set and data were taken. Two measurement runs were executed each time, then the channel edge and position of the stationary layer were checked again (reproducibility was always within 2 μm) and two more runs performed. The cell was taken out of the instrument, rinsed several times with deionized water, dried by flushing with air for five minutes and

refilled with another sample. Twelve concentration points, starting with a sample of pure PC, were taken for each isotherm, evenly distributed (on a log scale) over the concentration range accessible under solubility limits. The data analysis was performed by selecting a symmetric region of the spectrum around the peaks, assuring that neither noise nor peaks caused by particles sticking to the channel wall were included in the data analysis. If peaks at different angles occurred at positions differing for more than 1 mV, the analysis was performed separately for these peaks. The mean values were recorded for each angle and averaged. The data for one concentration point are thus the average results of four runs at four angles each, and the standard deviation is the result of this averaging process.

NUMERICAL METHODS

To extract the adsorption parameters for each molecule, the adsorption isotherms now had to be fitted to the Gouy-Chapman model described above. For this purpose a FORTRAN 77 routine had already been developed in this lab. It utilizes the Marquardt algorithm for the fit of experimental data to a nonlinear function as described by Bevington (1969).

Once Debye length κ , shear plane distance s and initial surface charge σ_m of the PC vesicles have been determined according to the model (see Chapter II), the two basic equations, the Grahame equation (8) and the Langmuir adsorption

isotherm (equation 10) are fitted to the experimental data by adjusting the association constant K and the adsorption site area P_s . Then the linear partition coefficient $\beta=K/P_s$ is computed.

The Marquardt algorithm minimizes

$$\chi^2 = \sum \frac{(y_{i,fit} - y_{i,exp})^2}{\sigma_i^2} \quad (21)$$

by first approaching the location of the minimum in parameter space in a direction given by the negative gradient of $\chi^2(K, P_s)$. Close to the actual minimum the computation of the gradient becomes too inaccurate and the minimum is found by expanding the fitting function up to second order in K and P_s and computing the location of the minimum analytically.

The fit of $\log \beta$ to molecular parameters and toxicity data was performed by means of a FORTRAN 77 routine written along the lines given by Bevington (1969) for a weighted fit to a multilinear function.

In both cases uncertainties for the regression coefficients can be obtained by weighing the uncertainties of every single data point with its importance for the computation of the particular coefficient. This is given by the partial derivative of the regression coefficient, a_j , with respect to the data point y_i :

$$\sigma_{a_j} = \sqrt{\sum [\sigma_i^2 (\frac{\partial a_j}{\partial y_i})^2]} \quad (22)$$

Statistical parameters used for the evaluation of correlations are the variance s , defined by

$$s^2 = \frac{N}{(N-1)} \frac{\chi^2}{\sum \frac{1}{\sigma_i^2}}, \quad (23)$$

which describes the deviation of the experimental data from the fit values, linear correlation coefficients which describe the extent to which single parameters in the correlation equation are independent from each other, and the multiple correlation coefficient r which describes the degree of correlation of the dependent variable with the dependent parameters to which it is fitted.

Figure 4. The scattering vector.

Figure 5. The electrophoretic cell.

Figure 6. Flow profile due to electroosmosis.

CHAPTER IV

RESULTS AND DISCUSSION

ADSORPTION ISOTHERMS

Two adsorption isotherms were measured for each compound and the adsorption parameters determined separately for each isotherm. If the deviation in the linear partition coefficient, β , was more than 15% a new stock solution of the compound was prepared and a third isotherm measured. We found that parameter β derived from the second isotherm was always lower, indicating that either degradation or precipitation had occurred. Precipitation can be a consequence of a drop in pH over the course of time, since the stock solutions were always prepared close to the solubility limit and the nonionized form of the compound is less soluble in water. A third isotherm was taken for 2,3,5,6-TCP, 2,4,6-TrCP and 2,6-DCP. In the case of 2,6-DCP the third isotherm was measured because the adsorption model yielded large uncertainties in the adsorption parameters. We found that new stock solution, when used within two days after preparation, always yielded sufficiently similar adsorption parameters.

The final parameters were obtained by the best fit of the adsorption model to two isotherms at once. This yielded common, representative parameters for a given compound.

A surprising result, but consistently obtained, was the mobility of PC vesicles of ca. $0.8 \mu\text{mcm/Vs}$ (corresponding to a ζ -potential of about -10 mV) in the absence of halogenated phenols. This value is in disagreement not only with Bangham et al., (1974) who showed that egg-PC is electrically neutral over the pH range from 3 to at least 9.5, but also with results obtained in this lab (Smejtek et al., 1987) under the same conditions. Most recently zero mobility has been obtained by measurements with DELSA 440 (Woodle et al., 1992). We tested several hypotheses for the origin of this mobility:

The buffer solution was changed to glycyl-glycine without any effect. Adsorption of anions from the solution cannot account for such a large potential since the change of the buffer did not affect the mobility and the association constant of Cl^- , the only other anion present, is very small (0.2 M^{-1} on egg-PC, Tatulian 1988).

In glycyl-glycine solution several experiments were done which showed that the mobility is pH dependent and that it is due to surface charge on the vesicles since increasing salt concentration resulted in a decrease of mobility. This result is understandable in terms of the screening of the electric surface charge by ions present in the solution.

We have verified that the anomalous mobility of PC vesicles is not caused by the distortion of the flow profile due to a pH dependent surface charge on the channel wall. Since the position of the stationary layer is merely a

function of channel geometry this distortion would influence the particle velocity only if measurements were not done at the stationary layer and should increase with the distance from this position. We measured vesicle mobility versus position of the laser beam within the channel at two different pH values and found that the mobility curves were merely shifted with respect to each other. A shift, however, cannot be caused by a change in electrolyte flow since it would contradict the requirement for zero net flow through the cross section of the channel.

We decided to treat the mobility of PC vesicles in the absence of organic anions as due to an initial surface charge which was taken into account in the data analysis separately for each isotherm. Under our experimental conditions (0.03 M KCl, pH 10, phosphate/citrate/borate buffer (0.002:0.002:0.0005 M)) this surface charge amounts to about $-6.0 \cdot 10^{-3} \text{ C/m}^2$ or 1 charge per 38 lipids if an area of $70 \text{ \AA}^2$ per lipid is assumed.

Adsorption parameters were also calculated for data with the initial ζ -potential subtracted from each concentration point. A comparison of β s for both sets of data is given in table I. As expected, β decreases by a factor of 1.5 to 2 when the initial ζ -potential is subtracted.

The validity of the Helmholtz-Smoluchowski equation (12) is assured by the fact that the particle diameter ($1\text{--}3 \text{ }\mu\text{m}$) is much larger than the Debye length (1.38 nm for the suspending

solution). The fitting routine adjusts the Debye length according to the total ionic strength at each concentration point but since the phenolate ion concentration never exceeds the concentration of buffer and salt ions combined, the Debye length remains of the same order of magnitude for the whole study.

The values for the shear plane distance at varying ionic strength were taken from Smejtek et al. (1987). The initial shear plane distance was 2.5 Å and hence small compared to the Debye length. This justifies the assumption of exponentially decreasing potential between the particle surface and the shear plane which was assumed for computing the surface potential from the ζ -potential data (equation 13).

Relationship between Adsorption Characteristics and Molecular Structure of Adsorbing Species

The affinity of adsorbing species to PC membranes can be measured by the linear partition coefficient β relating the surface density of adsorbed ions $(A^-)_m$ and the bulk aqueous concentration $[A^-]_{aq}$,

$$\beta = (A^-)_m / [A^-]_{aq} \quad (24)$$

The other adsorption parameters, the association constant, K , and the area of adsorption site, P_s , are determined from the fit of the adsorption model to the measured ζ -potential isotherms.

Chlorinated Phenols

The measured and theoretical ζ -potential isotherms are given in tables VI - XII which also contain the sets of best adsorption parameters. Their graphical presentation is given in Figures 7 - 13 and 16. The main feature of the adsorption characteristics is that with the decreasing number of chlorine atoms in the phenolate ion the association constant decreases from 75570 liter/mole for pentachlorophenol to 23.4 liter/mole for 2,6-dichlorophenol. This decrease in the number of chlorine atoms in the phenolates is also reflected in the magnitude of the linear partition coefficient β , from $\log \beta = -4.44$ for PCP to $\log \beta = -7.39$ for 2,6-DCP (see Fig.18).

The other tendency that can be observed is, that adsorption is weaker when the chlorine substituents are located in the ortho positions close to the oxygen-group. The strength of this steric effect increases with the number of chlorines in the phenol. The difference in $\log \beta$ is 0.65 for the dichlorophenols under investigation and 0.23 for the trichlorophenols. For the tetrachlorophenols it is not detectable within the error margin. The physical reason for this steric effect is that phenolate ions adsorb to the membrane with the oxygen facing towards the aqueous solution and the benzene ring inserted into the membrane in the region of the hydrocarbon chain. Chlorine substituents enhance adsorption when they are located in meta (3,5) or para (4) positions.

The results for PCP ($K=76000$, $P_s=4.9$ lipids/adsorption

site, $\beta=3.6 \cdot 10^{-5}$) show fairly good agreement with the data obtained by Smejtek (1987) ($K=55000$, $P_s=4.3$ lipids/site, $\beta=3.0 \cdot 10^{-5}$). The data in Table VIII show, however, that saturation of the adsorption sites had not been reached in the earlier study indicating that the values of the association constant K and the adsorption site area P_s should be regarded more reliable in the study presented here.

Since the requirement for saturation could not be fulfilled for the less chlorinated phenols a discussion of the results for K and P_s will be limited to phenols with three or more chlorines. A feature of these results is that the adsorption site area decreases with decreasing partition coefficient (with the exception of 2,4,6-TrCP). This suggests that the adsorption site for weakly adsorbing phenolate ions is situated closer to the membrane-water interface, as Smejtek and Wang, 1990, show. The reason for this is that the potential energy of repulsion between two adsorbed ions decreases with the adsorption depth l according to

$$U(r, l, \epsilon_m) = \frac{q_1 q_2}{4 \pi \epsilon \epsilon_m} \left[\frac{1}{r} - \frac{\omega}{\sqrt{(r^2 + 4 l^2)}} \right] \quad (25)$$

where ϵ_m is the dielectric constant of the membrane (≈ 10), $\omega = (\epsilon_{aq} - \epsilon_m) / (\epsilon_{aq} + \epsilon_m)$, r the separation between adsorbed ions. q_1 and q_2 are ion charges. The two ions can approach each other up to the distance R_c , the "exclusion radius", where $U=kT$. The adsorption site area is then $P_s = \pi R_c^2$. For decreasing adsorption

depth the repulsive potential energy U decreases and consequently the ions can approach each other closer, yielding a smaller R_c and a smaller adsorption site area.

Pentahalogenated Phenols

The data for the series of phenolate ions with five halogen substituents are given in Tables VIII-X with graphical representation in Figures 13-15 and 17. This series shows an increase in adsorption as the size of the substituents increases. This can be explained in terms of a decrease in the Born energy required for transferring an ion from a medium with a high dielectric constant to a less polarizable one. The Born energy for the transfer of an ion with charge q and radius r from an aqueous solution to the membrane is given by

$$W_B = \frac{1}{4\pi\epsilon_0} \frac{q^2}{2r} \left(\frac{1}{\epsilon_m} - \frac{1}{\epsilon} \right) \quad (26)$$

(Flewelling and Hubbell, 1986b)

More interesting relations can be found when the adsorption parameters are correlated with other molecular parameters. This will be done in the following section.

CORRELATION OF LOG β WITH MOLECULAR PARAMETERS

The principal aim of this study was to investigate whether there exists a significant relationship between the linear partition coefficient β for the phenolate ions A^- and the water-octanol partition coefficient for HA , the neutral

species. Octanol-water partition coefficients for the ionized form cannot be determined because the partitioning of A^- into the octanol phase depends upon the redistribution of cations. Westall (1985) studied the partitioning of PCP^- and 2,3,4,5- TCP^- and found that, although the partitioning depends strongly on salt concentration, the order of lipophilicity for PCP and TCP is maintained. Since the octanol/water partition coefficient also increases with the number of chlorine atoms in the phenol a correlation of $\log P_{ow}$ (for HA) with $\log \beta$ (for A^-) can be expected. In the analysis the values for β were weighted by their standard deviations and linear fits to up to two parameters have been obtained. The other parameters for which fits were obtained are the molecular dipole moment p and the molecular polarizability α , calculated values of which are quoted by Xie et al. (1984), and Gombar (1989), respectively. Xie et al. (1984), also determined the $\log P_{ow}$ for all the chlorophenols used in this study by two different methods. We use the values determined by High Performance Liquid Chromatography because this method has been found to be especially accurate for the determination of $\log P_{ow}$ (Terada 1986). A compilation of the molecular and adsorption parameters is given in Table XI. Since $\log P_{ow}$ values could not be found for PBP this molecule had to be omitted from the study. Also, data on dipole moment and polarizability of PFP were not available. The data for the various fits are given in Table XII and Figure 19.

Tables XIII and XIV show that the correlation of $\log \beta$ and $\log P_{ow}$ alone is already highly significant as expressed by the multiple correlation coefficient r , which is essentially a measure for the goodness of the fit.

Introducing the dipole moment p does improve the correlation and leads to a much smaller variation of the data. It is interesting that the effect of the dipole moment is to decrease adsorption to the membrane since the regression coefficient for p is negative. This result can be rationalized by the fact that the dipole moments of the phenolate ions when adsorbed to the membrane are in the same direction as the dipole moments associated with the glycerol region of the lipids. In terms of dipole-dipole interaction an antiparallel orientation of the phenolate dipoles with respect to the dipole layer in the glycerol region would be favored. The effect of including molecular polarizability in the correlation with $\log P_{ow}$ is not understood. The regression coefficient for α is positive which means that polarizability enhances adsorption and the correlation parameters are even slightly better than those for the correlation with dipole moments. Polarizability exhibits two competing effects on adsorption. On one hand, strongly polarizable ions are more hydrated and thus stabilized in the aqueous medium. On the other hand, the water molecules surrounding the molecule are ordered by hydration, thus decreasing the entropy of the aqueous phase which favors partitioning into the lipid membrane. A conclu-

sion as to whether the positive correlation of α with β bears a physical meaning could not be reached.

CORRELATION OF LOG β WITH TOXICITY DATA

Parameters for lipophilicity have been found to be related to toxicity in numerous studies on halogenated phenols. This suggests that adsorption of the toxic molecules to cellular and subcellular membranes plays an important role in the mechanisms of toxicity. We addressed the question whether the linear partition coefficient for halogenated phenolate ions correlates better with toxicity data than the log P_{ow} which is used most frequently. Two studies could be found which included enough phenols to obtain meaningful correlation.

Gombar and Enslein (1989) quote data for toxicity of (among others) chlorophenols to *Photobacterium phosphoreum* obtained by Bulich and Isenberg (1981). They measured the concentration EC_{50} of chlorophenol required to inhibit light emission by the bacterium by 50 %. The values used for correlation in this study are the pEC_{50} values, the negative logarithms of EC_{50} (in M). The correlation, including all the chlorophenols investigated in this study, is not very good and is worse for log β than for log P_{ow} (see Table XV).

The other reference on toxicity data, Arnold et al. (1988), examines the electro-optic behavior of yeast cells

(rotation of plane of polarization in an external electric field). Weakening of anti-field rotation and enhancement of co-field rotation, according to Arnold, is closely related to toxic effects. The correlation was performed for two data sets. Correlation of $\log \beta$ and $\log P_{ow}$ with the negative logarithm of the concentration required to increase co-field rotation by 20 and 50 % (pCR_{20} and pCR_{50} , respectively).

The toxicity data for a set of halogenated phenols including PCP, PFP, 2,3,4,5-TCP, both TrCPs and 2,6-DCP were correlated with both partition coefficients. Since the $\log P_{ow}$ was not available for PBP, another correlation with $\log \beta$ was computed which included pentabromophenolate.

The correlation for yeast was better than that for the bacteria. Coefficients for both $\log \beta$ and $\log P_{ow}$ yielded equally good correlation coefficients.

More detailed insight into the significance of membrane partition coefficients depends on the knowledge of mechanisms of toxic effects.

CONCLUSIONS

In this study, adsorption of halogenated phenolate ions to egg-PC vesicles was investigated by means of microelectrophoresis. Experimental adsorption isotherms were fitted to the Gouy-Chapman-Stern adsorption model from which association constants, adsorption site areas and linear partition coefficients were determined. 7 chlorinated phenols were examined,

including 2,6- and 3,5-dichlorophenol, 2,4,6- and 2,4,5-trichlorophenol, 2,3,5,6- and 2,3,4,5-tetrachlorophenol and pentachlorophenol. Two other pentahalogenated phenols, pentabromo- and pentafluorophenol, were also investigated.

The most important results are:

- 1) Adsorption of chlorinated phenolate ions to lipid membranes increases with the number of chlorine substituents on the benzene ring and, for a given isomer, with the number of chlorines in 3,4,5 - positions.
- 2) Adsorption of pentahalogenated phenolate ions increases with size of halogen substituents.
- 3) The linear partition coefficient β of phenolate ions and egg-PC membranes correlates very well with the water/octanol partition coefficient of the neutral halogenated phenol.
- 4) The correlation between the linear partition coefficient β and the water/octanol partition coefficient is improved when dipole moments of the undissociated phenols are included.

TABLE I

 β FOR ORIGINAL DATA (1) AND WITH ζ -POTENTIAL SUBTRACTED (2)

compound	1		2	
	β (m)	$\log \beta$	β (m)	$\log \beta$
PCP	$(3.63 \pm 0.17) \cdot 10^{-5}$	-4.440 ± 0.020	$(1.92 \pm 0.09) \cdot 10^{-5}$	-4.718 ± 0.020
2345-TCP	$(5.57 \pm 0.20) \cdot 10^{-6}$	-5.246 ± 0.015	$(3.22 \pm 0.11) \cdot 10^{-6}$	-5.493 ± 0.015
2356-TCP	$(5.74 \pm 0.20) \cdot 10^{-6}$	-5.241 ± 0.023	$(3.54 \pm 0.19) \cdot 10^{-6}$	-5.451 ± 0.022
245-TrCP	$(1.10 \pm 0.08) \cdot 10^{-6}$	-5.957 ± 0.030	$(5.37 \pm 0.17) \cdot 10^{-7}$	-6.270 ± 0.013
246-TrCP	$(6.55 \pm 0.33) \cdot 10^{-7}$	-6.184 ± 0.022	$(4.15 \pm 0.21) \cdot 10^{-7}$	-6.382 ± 0.021
35-DCP	$(1.81 \pm 0.13) \cdot 10^{-7}$	-6.741 ± 0.030	$(1.13 \pm 0.08) \cdot 10^{-7}$	-6.946 ± 0.028
26-DCP	$(4.07 \pm 2.93) \cdot 10^{-8}$	-7.390 ± 0.235	$(4.07 \pm 2.93) \cdot 10^{-8}$	-7.620 ± 0.163
PBP	$(1.44 \pm 0.14) \cdot 10^{-4}$	-3.841 ± 0.040	$(9.87 \pm 0.68) \cdot 10^{-5}$	-4.005 ± 0.029
PFP	$(1.26 \pm 0.34) \cdot 10^{-7}$	-6.900 ± 0.104	$(8.35 \pm 2.12) \cdot 10^{-8}$	-7.078 ± 0.098

TABLE II

ADSORPTION ISOTHERM FOR 2,6 - DICHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	-10.15	0.80	-10.15	-10.91	1.60	-10.91
50	-10.18	0.77	-10.35	-10.91	1.33	-11.10
100	-10.97	0.72	-10.54	-10.89	1.91	-11.29
200	-10.54	0.70	-10.91	-10.51	1.26	-11.65
500	-12.38	0.89	-11.94	-11.08	1.17	-12.63
1000	-13.17	0.75	-13.42	-14.50	1.13	-14.06
2000	-14.28	0.76	-15.76	-16.70	0.97	-16.33
4000	-19.41	0.65	-19.08	-19.06	1.02	-19.56
8000	-23.00	0.63	-23.21	-24.12	1.05	-23.59
12000	-26.37	0.71	-25.86	-27.12	0.86	-26.19
20000	-29.44	0.84	-29.29	-29.13	1.20	-29.55
30000	-31.35	0.83	-31.99	---	---	---

$$K = 23.44 \pm 16.85 \text{ l/mole}$$

$$P_s = 1.365 \pm 0.898 \text{ lipids/site}$$

$$\beta = (4.074 \pm 2.930) \cdot 10^{-8} \text{ m}$$

2,6 - DICHLOROPHENOL

Figure 7. Adsorption isotherm for 2,6-Dichlorophenolate.

TABLE III

ADSORPTION ISOTHERM FOR 3,5 - DICHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	- 9.71	0.92	-9.71	-10.08	0.51	-10.08
25	- 8.35	0.71	-10.17	---	---	---
50	- 9.54	0.99	-10.61	-11.67	0.78	-10.96
100	- 9.23	1.12	-11.42	-13.2	0.86	-11.76
200	---	---	---	-14.27	1.28	-13.17
250	-11.53	0.68	-13.49	---	---	---
500	-14.29	0.69	-16.14	-16.71	0.68	-16.41
1000	-20.15	0.66	-19.85	-19.75	0.82	-20.09
2000	---	---	---	-25.32	0.73	-24.69
2500	-26.09	1.04	-26.12	---	---	---
5000	-32.11	0.57	-31.38	-32.01	0.57	-31.54
10000	-35.79	0.98	-36.42	-36.58	0.54	-36.56
20000	---	---	---	-40.85	0.43	-40.66
25000	-40.29	0.59	-41.54	---	---	---
30000	---	---	---	-42.60	0.52	-42.30
40000	-42.67	0.63	-42.89	-43.20	0.46	-42.99

$$K = 262.8 \pm 18.8 \text{ l/mole}$$

$$P_s = 3.437 \pm 0.138 \text{ lipids/site}$$

$$\beta = (1.814 \pm 0.130) \cdot 10^{-7} \text{ m}$$

3,5 - DICHLOROPHENOL

Figure 8. Adsorption isotherm for 3,5-Dichlorophenolate.

TABLE IV

ADSORPTION ISOTHERM FOR 2,4,6 - TRICHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	- 9.25	0.77	- 9.25	-12.78	1.03	-12.78
20	-11.18	0.48	-10.55	-13.44	1.54	-13.88
50	-11.41	0.41	-12.20	-14.27	1.42	-15.31
100	-13.97	0.55	-14.42	-17.10	1.15	-17.52
200	-17.17	0.44	-17.69	-20.14	1.35	-20.21
500	-23.20	0.62	-23.61	-26.99	0.98	-25.70
1000	-28.97	0.45	-28.98	-30.74	1.31	-30.78
2000	-36.06	0.52	-34.69	-36.99	1.08	-36.24
4000	-41.69	0.71	-40.21	-41.70	1.35	-41.58
8000	-43.68	0.34	-44.87	-47.16	1.35	-46.10
15000	-48.65	0.49	-47.58	-50.57	1.35	-48.74
27300	-47.79	0.49	-48.05	---	---	---

$$K = 1158 \pm 59 \text{ l/mole}$$

$$P_s = 4.192 \pm 0.104 \text{ lipids/site}$$

$$\beta = (6.553 \pm 0.332) \cdot 10^{-7} \text{ m}$$

2,4,6 - TRICHLOROPHENOL

Figure 9. Adsorption isotherm for 2,4,6-Trichlorophenolate.

TABLE V

ADSORPTION ISOTHERM FOR 2,4,5 - TRICHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	-10.49	0.86	-10.49
20	-12.56	0.64	-12.48
50	-15.11	0.50	-14.82
100	-18.28	0.52	-17.76
200	-21.49	0.86	-21.82
500	-27.89	0.67	-28.68
1000	-34.77	0.82	-34.59
2000	-40.29	0.84	-44.69
4000	-45.56	0.70	-46.46
8000	-52.66	0.61	-51.26
13000	-53.51	0.51	-53.55
20000	-54.01	0.65	-54.50

$$K = 1651 \pm 120 \text{ l/mole}$$

$$P_s = 3.551 \pm 0.125 \text{ lipids/site}$$

$$\beta = (1.103 \pm 0.080) \cdot 10^{-6} \text{ m}$$

Figure 10. Adsorption isotherm for 2,4,5-Trichlorophenolate.

TABLE VI

ADSORPTION ISOTHERM FOR 2,3,5,6 - TETRACHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	-10.96	1.19	-10.96	-10.19	1.43	-10.19
5	-13.95	0.65	-13.39	-14.47	1.59	-12.70
10	-17.00	0.68	-15.30	-16.28	1.23	-14.66
20	-18.92	0.83	-18.21	-19.27	1.00	-17.64
50	-24.85	0.53	-23.72	-24.85	1.30	-23.25
100	-29.32	0.56	-28.95	-28.09	1.07	-28.54
200	---	---	---	-33.78	1.17	-34.39
250	-35.61	0.72	-36.68	---	---	---
500	-41.39	0.76	-42.70	-42.08	1.25	-42.39
1000	-47.03	0.90	-48.44	-47.52	1.14	-48.16
2000	-51.32	0.75	-53.45	-53.26	1.12	-53.18
5000	-58.01	1.04	-57.90	-58.13	0.87	-57.63
9000	---	---	---	-61.70	1.26	-58.40
10000	-59.60	1.12	-58.59	---	---	---

$$K = 10570 \pm 572 \text{ l/mole}$$

$$P_s = 4.367 \pm 0.111 \text{ lipids/site}$$

$$\beta = (5.742 \pm 0.311) \cdot 10^{-6} \text{ m}$$

2,3,5,6 - TETRACHLOROPHENOL

Figure 11. Adsorption isotherm for 2,3,5,6-Trichlorophenolate.

TABLE VII

ADSORPTION ISOTHERM FOR 2,3,4,5 - TETRACHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	-12.29	0.45	-12.29	-10.96	1.19	-10.96
5	-14.46	0.60	-14.52	-11.68	0.50	-13.33
10	-15.41	0.47	-16.29	-14.26	0.44	-15.19
20	-20.48	0.63	-19.04	-17.86	0.56	-18.05
50	-24.58	0.44	-24.31	-25.24	0.68	-23.48
100	-29.55	0.41	-29.35	-30.90	0.78	-28.63
250	-35.97	0.40	-36.88	-39.56	1.00	-36.27
500	-42.65	0.66	-42.76	-45.20	0.78	-42.22
1000	-47.04	0.62	-48.39	-49.81	0.95	-47.88
2000	-51.52	0.32	-53.28	-55.14	0.54	-52.80
5000	-56.07	0.68	-57.56	-58.30	0.95	-57.09
10000	-58.59	0.51	-58.14	-61.08	0.74	-57.66

$$K = 10600 \pm 375 \text{ l/mole}$$

$$P_s = 4.516 \pm 0.070 \text{ lipids/site}$$

$$\beta = (5.570 \pm 0.197) \cdot 10^{-6} \text{ m}$$

2,3,4,5 - TETRACHLOROPHENOL

Figure 12. Adsorption isotherm for 2,3,4,5-Tetrachlorophenolate.

TABLE VIII

ADSORPTION ISOTHERM FOR PENTACHLOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	-13.47	0.68	-13.47	-13.03	0.76	-13.03
2	-15.9	0.62	-17.97	-17.52	0.67	-17.61
5	-22.33	0.68	-22.15	-20.88	0.76	-21.84
10	-27.71	0.76	-26.52	-25.63	1.17	-26.25
20	-32.34	0.91	-31.70	-32.68	0.99	-31.47
50	-40.05	0.86	-39.22	-38.97	1.06	-39.02
100	-46.74	0.93	-44.95	-44.66	0.93	-44.77
200	-48.43	1.25	-50.36	-48.89	1.16	-50.18
500	-57.86	1.2	-56.40	-55.28	1.63	-56.23
1000	-59.42	1.15	-59.71	-58.70	0.54	-59.54
2500	-61.53	0.54	-61.89	---	---	---
5000	-63.64	0.85	-61.57	---	---	---

$$K = 75570 \pm 3560 \text{ l/mole}$$

$$P_s = 4.944 \pm 0.074 \text{ lipids/site}$$

$$\beta = (3.627 \pm 0.171) \cdot 10^{-5} \text{ m}$$

PENTACHLOROPHENOL

Figure 13. Adsorption isotherm for pentachlorophenolate.

TABLE IX

ADSORPTION ISOTHERM FOR PENTABROMOPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0.00	-11.88	1.17	-11.88	- 9.60	1.90	- 9.60
0.42	-14.90	1.33	-15.46	-12.59	1.30	-13.50
1.05	-20.00	1.18	-19.28	-18.46	1.74	-17.60
2.10	-25.62	1.90	-23.61	-23.37	1.21	-22.18
4.20	-29.89	1.41	-29.00	-27.89	1.37	-27.81
10.5	-38.28	1.59	-37.10	-38.01	1.58	-36.17
21.0	-43.66	1.74	-43.46	-43.10	1.39	-42.66
42.0	-48.81	1.41	-49.64	-48.63	2.05	-48.92
105	-55.55	1.40	-57.00	-53.99	1.21	-56.34
350	-62.73	1.20	-64.29	-62.43	1.38	-63.64
1050	-68.19	1.53	-67.82	-68.23	1.71	-67.15
3150	-70.05	1.50	-68.10	-68.78	1.48	-67.41

$$K = 255900 \pm 24990 \text{ l/mole}$$

$$P_s = 4.208 \text{ lipids/site}$$

$$\beta = (1.443 \pm 0.141) \cdot 10^{-4} \text{ m}$$

Figure 14. Adsorption isotherm for pentabromophenolate.

TABLE X
ADSORPTION ISOTHERM FOR PENTAFLUOROPHENOLATE

conc. (μM)	$V_{\zeta,\text{exp}}$ (mV)	stddev (mV)	$V_{\zeta,\text{fit}}$ (mV)
0	- 9.59	1.57	- 9.59
25	-10.30	0.99	- 9.91
50	- 9.02	1.22	-10.22
100	-11.30	0.63	-10.80
250	-11.91	0.87	-12.32
500	-15.43	1.03	-14.33
1000	-16.83	1.10	-17.23
2000	-20.57	1.53	-20.92
3000	-22.85	1.19	-23.31
5000	-27.50	1.36	-26.36
10000	-29.46	0.74	-30.05
15000	-32.29	0.99	-31.64

$$K = 342.7 \pm 92.9 \text{ l/mole}$$

$$P_s = 6.454 \pm 1.034 \text{ lipids/site}$$

$$\beta = (1.260 \pm 0.342) \cdot 10^{-7}$$

Figure 15. Adsorption isotherm for pentafluorophenolate.

CHLOROPHENOLS

Figure 16. Adsorption isotherms for chlorophenolates.

PENTAHALOGENATED PHENOLS

Figure 17. Adsorption isotherms for pentahalogenized phenolates.

LOG β VERSUS NUMBER OF HALOGEN SUBSTITUENTS

Figure 18. Log β versus number of halogen substituents.

TABLE XI

MOLECULAR AND ADSORPTION PARAMETERS FOR HALOGENATED PHENOLS

compound	K(1/M)	P _s , l/s	log β	lgP _{ow}	p(D) ¹	α^3	pK _a
PCP	75570 ± 3560	4.944 ±0.074	-4.440 ±0.020	5.08 ¹	2.22	5.75	4.8 ⁴
2345-TCP	10600 ± 375	4.516 ±0.070	-5.246 ±0.015	4.68 ¹	3.17	5.13	5.2 ⁵
2356-TCP	10570 ± 572	4.367 ±0.111	-5.241 ±0.023	4.47 ¹	1.55	5.76	5.6 ⁵
245-TrCP	1651 ± 120	3.551 ±0.125	-5.957 ±0.030	3.96 ¹	2.22	4.87	7.1 ⁴
246-TrCP	1158 ± 56	4.192 ±0.104	-6.184 ±0.022	3.69 ¹	1.55	5.25	6.5 ⁴
35-DCP	263.2 ± 18.8	3.437 ±0.138	-6.741 ±0.030	3.68 ¹	2.22	4.56	8.3 ⁶
26-DCP	23.4 ± 16.9	1.365 ±0.898	-7.390 ±0.235	2.92 ¹	2.22	5.38	7.1 ⁴
PBP	255900 ±24990	4.208 ±0.136	-3.841 ±0.040	---	---	---	3.7 ⁴
PFP	342.7 ±92.9	6.454 ±1.034	-6.900 ±0.104	3.23 ²	---	---	5.7 ⁴

¹ Xie and Dyrssen 1984, ² Hansch and Leo, 1979,³ Gombar and Enslein, 1989, ⁴ Arnold et al., 1988,⁵ Cohen et al., 1988, ⁶ Xie et al., 1984

TABLE XII
 LINEAR CORRELATION LOG β / LOG P_{ow}

correlation of log β with:			log P_{ow}	p, P_{ow}	α , P_{ow}
compound	log β_{exp}	stddev	log β_{fit}	log β_{fit}	log β_{fit}
PCP	-4.440	0.020	-4.574	-4.445	-4.485
2345-TCP	-5.246	0.015	-5.094	-5.260	-5.231
2356-TCP	-5.241	0.023	-5.366	-5.165	-5.139
245-TrCP	-5.957	0.030	-6.029	-6.071	-6.138
246-TrCP	-6.184	0.022	-6.380	-6.297	-6.239
35-DCP	-6.741	0.030	-6.393	-6.477	-6.598
26-DCP	-7.390	0.235	-7.381	-7.581	-7.004
PFP	-6.900	0.104	-6.978	---	---

CORRELATION OF LOG β WITH MOLECULAR PARAMETERS

Figure 19. Correlation of $\log \beta$ with molecular parameters.

TABLE XIII
INTERCORRELATION MATRIX FOR CHLOROPHENOLS

parameter	$\log \beta$	$\log P_{ow}$	p	α
$\log \beta$	1.0000	0.9670*	0.2696	0.7560
$\log P_{ow}$		1.0000	0.4693	0.5971
p			1.0000	-0.3756

*0.9680 with PFP included

TABLE XIV
CORRELATION EQUATIONS FOR $\log \beta$ WITH MOLECULAR PARAMETERS

	$\log \beta =$	n	s	r
1	$-11.18 + 1.30 \log P_{ow}$ (± 0.08) (± 0.02)	8	0.1717	0.9680
2	$-11.27 + 1.45 \log P_{ow} - 0.25 p$ (± 0.08) (± 0.02) (± 0.02)	7	0.0879	0.9893
3	$-12.88 + 1.08 \log P_{ow} + 0.60 \alpha$ (± 0.12) (± 0.02) (± 0.03)	7	0.0744	0.9923

TABLE XV
CORRELATION OF TOXICITY DATA WITH $\log \beta$ AND $\log P_{ow}$

correlation (for details see text)	s	r
$pEC_{50} / \log P_{ow}$ (Gombar), 7 CPs	0.3275	0.8859
$pEC_{50} / \log \beta$ (Gombar), 7 CPs	0.4004	0.8237
$pCR_{20} / \log P_{ow}$ (Arnold), 5 CPs, PFP	0.2969	0.9307
$pCR_{20} / \log \beta$ (Arnold), 5 CPs, PFP	0.2925	0.9328
$pCR_{20} / \log \beta$ (Arnold), 5 CPs, PFP, PBP	0.4360	0.9390
$pCR_{50} / \log P_{ow}$ (Arnold), 5 CPs, PFP	0.3052	0.9157
$pCR_{50} / \log \beta$ (Arnold), 5 CPs, PFP	0.3236	0.9046
$pCR_{50} / \log \beta$ (Arnold), 5 CPs, PFP, PBP	0.4439	0.9269

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