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PARTITIONING OF MODERATELY HYDROPHOBIC ENDOCRINE DISRUPTORS BETWEEN WATER AND SYNTHETIC MEMBRANE VESICLES

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Abstract—The partition coefficient between water and lipid membrane vesicles (K_{lipw}) has been used as an alternative to the 1-octanol–water partition coefficient (K_{ow}) between water and organic solvent, because it more closely represents actual biological membranes. Despite theoretical differences, log K_{lipw} correlates well with log K_{ow} for conventional nonpolar organic pollutants. In the present study, K_{lipw} values of 11 structurally diverse endocrine-disrupting chemicals (EDCs) were measured for three different types of lipid membrane vesicles from dipalmitoylphosphatidylcholine (DPPC), DPPC/cholesterol, and palmitoyloleoylphosphatidylcholine. Correlation analyses were conducted to evaluate the effects of hydrophobicity, molar liquid volume (MLV), and polar surface area (PSA) for 20 EDCs, including nine from a previous study. Correlations that include MLV and PSA reduce the predicted value of log K_{lipw}, suggesting that lipid membranes are less favorable than 1-octanol for a hydrophobic solute because of the higher molar volume and higher hydrogen-bonding potential. These results suggested that K_{ow} alone has limited potential for estimating K_{lipw} and that additional descriptors are required. In addition, K_{lipw} values vary by as much as two orders of magnitude because of the changes in membrane fluidity and the amount of cholesterol in the lipid bilayer. Therefore, lipid components should be chosen carefully to evaluate the bioconcentration of these compounds.

Keywords—Partitioning Lipid bilayer Endocrine disruptors Bioconcentration

INTRODUCTION

To assess possible harmful effects of organic micropollutants on both humans and wildlife, it is necessary to evaluate the fate and distribution of these pollutants. Understanding partitioning behavior in biological organic phases is of critical importance, because many pollutants are transported from the environment to an organism via passive diffusion and then stored in the organic phases within cells, such as plasma membranes. Because of the difficulty associated with measuring partitioning in biological samples, organic solvents, such as 1-octanol, have been widely used as a surrogate for biological organic phases (see, e.g., [1–3]). The 1-octanol–water partition coefficient (K_{ow}) has been used successfully to evaluate the bioconcentration of xenobiotics (see, e.g., [2,3]) and nonspecific toxicity, such as narcosis (see, e.g., [1]), through linear free-energy relationships. However, recent studies have shown that the thermodynamics of partitioning between water and 1-octanol are significantly different from those between water and fish [4] and between water and lipid membranes [5,6]. The highly organized structure of biological membranes is thought to be the main cause of disparities between partitioning coefficients measured using 1-octanol versus lipid membranes. For this reason, partition coefficients between water and synthetic membrane vesicles, called liposomes, often are used for evaluating bioconcentration or biological membrane permeation (see, e.g., [6–15]).

Although partitioning between water and 1-octanol is thermodynamically different from partitioning between water and synthetic membrane systems, many studies have shown linear relationships between the log K_{ow} and the logarithm of the lipid membrane–water partition coefficient (log K_{lipw}) for moderately hydrophobic organic chemicals of environmental concern having log K_{ow} values of between 1 and 5.5 [6,8,10–12]. For more hydrophobic chemicals, the linear relationship is not applicable [8,13]. Gobas et al. [8] observed that the linear correlation breaks down for chemicals with log K_{ow} values of greater than 5.5, corresponding to molar volumes of greater than 230 cm^3/mol using dimyristoylphosphatidylcholine (DMPC) membrane vesicles. They also observed that logarithms of the species' activity coefficients in 1-octanol are linearly related to their molar volume, whereas those in DMPC membranes exhibit a parabolic relationship with the molar volume of solutes. Similarly, Dulfer and Govers [13] found parabolic relationships between log K_{ow} and log K_{lipw} for highly hydrophobic PCBs ($5.0 < \log K_{ow} < 8.5$) using small, unilamellar vesicles of DMPC, dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine, and diarachidoylphosphatidylcholine. However, the effects of the molar volume of moderately hydrophobic chemicals could not be elucidated, because the molar volumes of the solutes were proportional to their log K_{ow} values in the aforementioned studies. Yamamoto and Liljestrand [14] showed that only moderate/weak linear relationships exist for structurally diverse endocrine-disrupting chemicals (EDCs) having log K_{ow} values between two and six using three different types of liposomes: DPPC, DPPC/cholesterol (60:40, w/v), and palmitoyloleoylphosphatidylcholine (POPC). The poor linear correlation likely results from the fact that the molar volumes of all the selected compounds were greater than the critical molar volume of 230 cm^3/mol proposed by Gobas et al. [8]. Unlike the work of Dulfer and Govers [13], the molar volumes were not linearly related with log K_{ow} values, so it should be possible to differentiate the effect of molar volume from log K_{ow}. In this

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regard, regressions developed using a combination of moderately hydrophobic EDCs and nonpolar hydrophobic organic molecules provide this range of behavior, because the log K_{ow} values typical of moderately hydrophobic EDCs are much smaller than those of nonpolar hydrophobic organics with equivalent molecular weight or molar volume. All the EDCs used in the present study contain polar functional groups, such as phenolic groups, that may contribute to log K_{lipw} because of hydrogen bonding and other polar interactions.

Several research groups also have shown that polar surface area (PSA) is an important contributor to partitioning correlations. Typically, PSA is negatively correlated with the permeability of drugs in humans (see, e.g., [16–18]), either by lowering the partition coefficient or by lowering the diffusivity in the lipid membranes. Thus, both molar volume and PSA should be evaluated for their impact on partitioning of EDCs.

In the present study, log K_{lipw} values were measured using the same three types of synthetic lipid membrane vesicles as used in Yamamoto and Liljestrand [14] for 11 additional EDCs to evaluate partitioning of EDCs into biological media. Relationships between log K_{lipw} and log K_{ow} were evaluated for all 20 EDCs, including those from the study by Yamamoto and Liljestrand [14]. Effects of the membrane fluidity and of cholesterol in the lipid membranes on log K_{lipw} were evaluated for the selected chemicals, because cholesterol content varies significantly in biological membranes. Effects of molar volume, PSA, and molecular geometry were examined. Relative contributions of the selected molecular descriptors were compared with respect to the characteristics of the lipid membranes.

MATERIALS AND METHODS

Chemicals

Three synthetic estrogens (diethylstilbestrol, hexestrol, and dienestrol), one natural hormone (progesterone), five industrial estrogenic chemicals (4,4′-dihydroxybenzophenone, benzyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, 4-phenylphenol, and diethylphthalate), and two breakdown products of alkylphenol ethoxylates (4-*sec*-butylphenol and 4-*tert*-amylphenol) were chosen. All are known to bind human estrogen receptor α [19]. All the chosen EDCs either have a phenolic group or are a phthalate ester. Diethylstilbestrol (purity, 99%), hexestrol (purity, 98%), dienestrol (purity, 98%), and diethylphthalate (purity, 99.5%) were purchased from Sigma Chemical (St. Louis, MO, USA). Progesterone (purity, 98%), 4-*sec*-butylphenol (purity, 98%), 4-*tert*-amylphenol (purity, 99%), 4,4′-dihydroxybenzophenone (purity, 99%), benzyl-4-hydroxybenzoate (purity, 99%), and cholesterol (purity, 99%) were obtained from Aldrich Chemical (Milwaukee, WI, USA). 4-Phenylphenol (purity, 98%) and butyl-4-hydroxybenzoate (purity, 99%) were purchased from Fluka Chemical (Milwaukee, WI, USA). Because palmitoyl (C16:0) and oleoyl (C18:1 9-*cis*) are the two most abundant components of fatty chains [20,21], DPPC (C16:0, C16:0) and POPC (C18:1, C16:0) were chosen as representative liposome components. Cholesterol was selected as a model sterol because of its ubiquitous presence. The model liposome with cholesterol consists of DPPC containing 40% cholesterol by mass or 56% by mole fraction. This high level of cholesterol may reflect plasma membranes having extremely low permeability, because typical plasma membranes contain approximately 30 to 50% cholesterol by mole fraction [20]. Chloroform solutions of model lipid components, DPPC and POPC, were purchased from Avanti Polar Lipids (Albaster, AL, USA).

Preparation of liposome suspensions

Large unilamellar vesicle suspensions of DPPC, DPPC/cholesterol (60:40, w/w), and POPC were prepared using the thin-film hydration technique [22] followed by rapid extrusion processes [23] as described previously [14]. In short, chloroform solution of the lipid was evaporated under a gentle nitrogen stream, and the thin film of the residue was dissolved in dilution water (buffered at pH 7.0 and ionic strength of 0.02 M using KH_2PO_4, NaOH, and NaCl) to make the liposome suspension. Then, the suspension was extruded several times through a polycarbonate membrane to reduce polydiversity of vesicles. The diameter of vesicles was mostly between 0.4 to 1.2 μm after extrusion.

Determination of K_{lipw}

Partition coefficients of the selected chemicals were determined by the equilibrium dialysis technique as described in detail by Escher and Schwarzenbach [9]. Two identical amber-glass vials, which served as donor and acceptor cells, were connected to each other by a Teflon® joint and were separated by a regenerated cellulose dialysis membrane (Por7 MWCO 10,000; Spectrum Scientific, Rancho Dominguez, CA, USA). Solution containing EDCs was used to fill the donor cells. Aqueous buffer and liposome suspensions were filled in the acceptor cells of reference and sample reactors, respectively. The reference reactors were used to monitor losses over time and to validate the initial concentration. The pH of dilution water (pH 7.0) was lower than the log of the ionization constant (pK_a) for all the selected chemicals, as shown in Figure 1. Thus, negligibly small amounts of the ionized form of each compound were present. Sodium azide (0.02%) was added to prevent microbial activity. The equilibrium dialysis reactors were mixed using a tumbler in the dark at room temperature (22°C) for 7 d, and at least four replicates were analyzed to determine the aqueous concentrations of reference cells and at least three sample reactors. Previous studies [14] and preliminary experiments showed that mass transfer is limited by permeation through the dialysis membrane and that apparent equilibrium is attained after 7 d. The aqueous concentration of the selected chemicals was measured using a Waters 2690 high-performance liquid chromatographic system equipped with a Waters 996 photodiode-array detector (Milford, MA, USA) for both sides of the reference reactors (C_{ref}, mg/L) and for the side without lipid vesicle suspension in the sample reactors (C_w, mg/L). The concentration of the lipid membrane vesicles (m, kg lipid/L) was calculated from the total organic carbon concentration, as measured using a Dohrmann Apollo 9000 (Tekmar-Dohrmann, Cincinnati, OH, USA) total organic carbon analyzer, multiplied by the stoichiometric ratio of each lipid component (g lipid/g C). The partition coefficients of the selected chemicals (K_{lipw}) were calculated as described by Escher and Schwarzenbach [9]:

$$K_{lipw}(\text{L/kg lipid}) = \frac{C_{ref} - C_w}{C_w m} \quad (1)$$

The initial concentration of the donor cell (C_o) was set between 0.7 and 5.0 mg/L for all the selected chemicals, considering aqueous solubilities and method detection limits. Mass recovery (R) calculated from Equation 2 was between 89

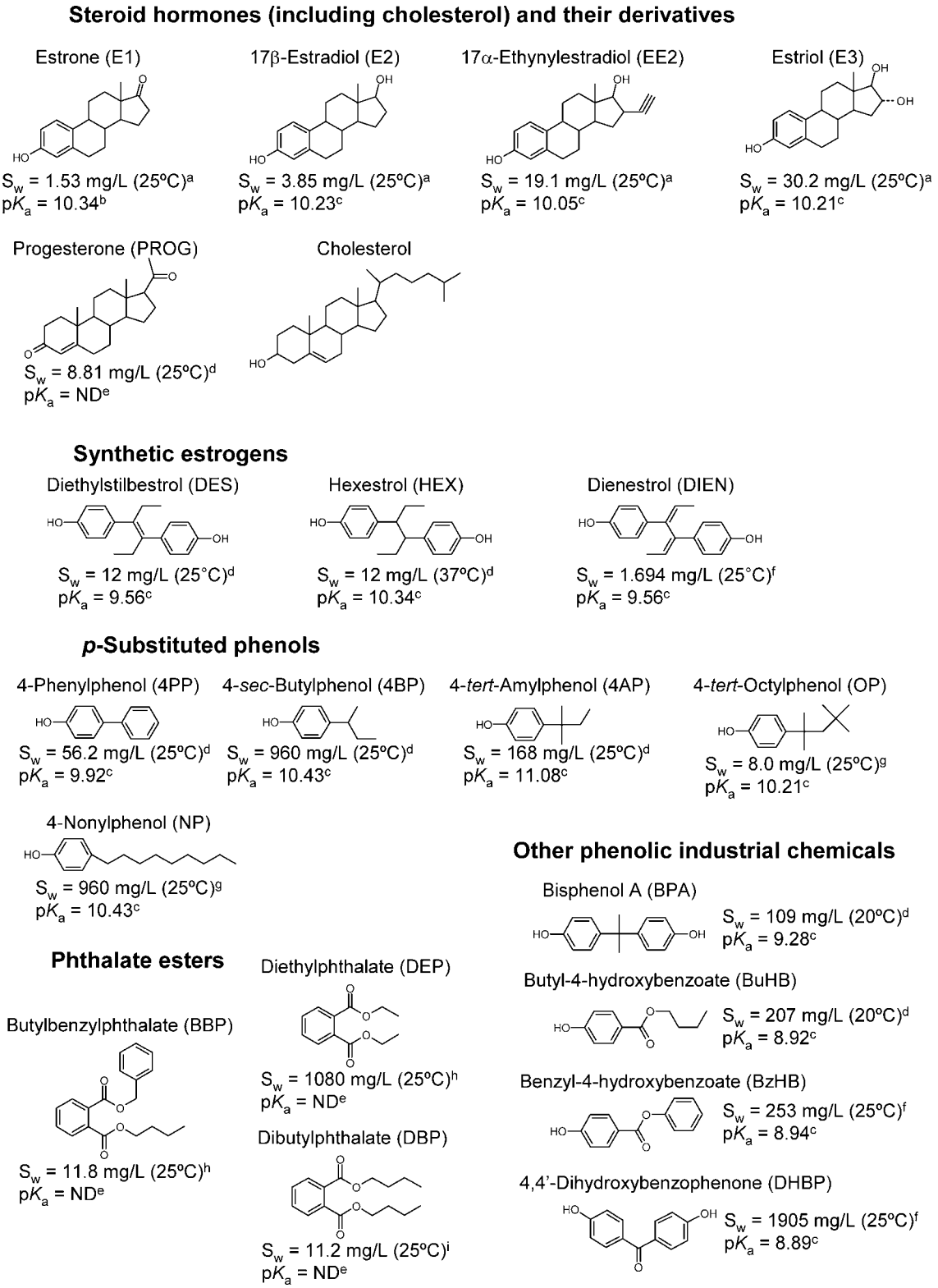

Fig. 1. Chemical structures, aqueous solubilities, and acid dissociation constants of the selected chemicals. [a]Aqueous solubility (S_w) from Yalkowski [41]; [b]log of the ionization constant (pK_a) from Perrin et al. [42]; [c]estimated from phenol using the fragmentation method [42]; [d]Yalkowski and Dannenfelser [43]; [e]not defined; [f]estimated from S_w, log K_{ow} using log S_w = 0.796 − 0.854 log K_{ow} − 0.00728 MW + 0.580 for phenols [39]; [g]Ahel and Giger [44]; [h]Ellington and Floyd [40]; [i]Howard et al. [45].

and 105% for all species, except for an 82.5% recovery calculated for dienestrol (see *Supplemental Data*; SETAC Supplemental Data Archive, Item ETC-25-08-001; http://etc.allenpress.com):

$$R = (C_o - C_{ref,donor} - C_{ref,acceptor})\cdot 100\ (\%) \quad (2)$$

The liposome concentrations ranged from 18 to 1,800 mg lipid/L and were based on a goal of achieving a C_w of ap-

Table 1. Octanol-water partition coefficients (K_{ow}), molar liquid volumes (MLV), polar surface areas (PSA), and lipid membrane-water partition coefficients (K_{lipw}) of selected endocrine disruptors[a]

Chemicals	Log K_{ow}	MLV[b] (cm^3/mol)	PSA[c] ($Å^2$)	$K_{lipw,DPPC}$	$K_{lipw,DPPC/cholesterol}$	$K_{lipw,POPC}$
17β-Estradiol[d]	4.01[e]	355.0	40.46	2.94 (±0.24) × 10^2	1.94 (±0.50) × 10^2	6.15 (±0.88) × 10^3
Estrone[d]	3.13[e]	347.6	37.30	4.13 (±0.32) × 10^2	2.82 (±2.31) × 10^2	8.36 (±0.24) × 10^3
Estriol[d]	2.45[e]	377.2	60.68	1.99 (±1.59) × 10	1.47 (±0.89) × 10	9.09 (±4.91) × 10
17α-Ethynylestradiol[d]	3.67[e]	384.6	40.46	5.62 (±1.31) × 10^2	1.65 (±0.69) × 10^2	1.57 (±0.03) × 10^4
4-Nonylphenol[d]	5.76[e]	303.2	20.23	6.87 (±0.92) × 10^4	6.88 (±3.91) × 10^3	3.18 (±0.62) × 10^5
4-*tert*-Octylphenol[d]	5.85[f]	281.0	20.23	3.48 (±1.29) × 10^5	1.38 (±0.39) × 10^5	4.04 (±0.35) × 10^5
Bisphenol A[d]	3.32[e]	266.0	40.46	1.79 (±0.99) × 10^3	1.78 (±1.23) × 10^2	2.91 (±0.30) × 10^4
Butylbenzylphthalate[d]	4.91[e]	365.4	52.61	5.74 (±1.18) × 10^2	1.25 (±0.94) × 10^2	4.75 (±1.06) × 10^4
Dibutylphthalate[d]	4.57[e]	347.2	52.61	9.29 (±4.05) × 10^3	1.03 (±0.45) × 10^2	1.55 (±0.43) × 10^4
Diethylstilbestrol	5.07[e]	325.5	40.46	1.66 (±0.15) × 10^4	1.67 (±0.83) × 10^2	9.61 (±1.18) × 10^4
Hexestrol	5.60[g]	332.6	40.46	9.23 (±2.38) × 10^3	1.28 (±0.48) × 10^2	3.82 (±0.49) × 10^4
Dienestrol	5.43[g]	317.8	40.46	1.63 (±0.41) × 10^4	1.45 (±0.12) × 10^2	2.84 (±0.40) × 10^5
4-*sec*-Butylphenol	3.08[e]	192.2	20.23	6.63 (±3.78) × 10	—	1.45 (±0.07) × 10^3
4-*tert*-Amylphenol	3.91[g]	214.4	20.23	3.04 (±0.56) × 10^2	3.32 (±1.66) × 10	4.01 (±0.33) × 10^3
4-Phenylphenol	3.20[e]	192.0	20.23	4.47 (±0.34) × 10^2	4.77 (±0.13) × 10^2	4.87 (±0.23) × 10^3
Butyl-4-hydroxybenzoate	3.57[e]	229.0	46.53	2.99 (±0.57) × 10^2	1.80 (±0.52) × 10^2	3.49 (±0.27) × 10^3
Benzyl-4-hydroxybenzoate	3.21[g]	214.0	46.53	4.67 (±0.53) × 10^2	2.63 (±0.40) × 10^2	6.93 (±0.86) × 10^3
4,4′-Dihydroxybenzophenone	2.19[g]	206.8	57.53	5.44 (±1.31) × 10	—	5.05 (±0.11) × 10^2
Diethylphthalate	2.42[h]	254.0	52.61	—	—	5.93 (±2.20) × 10
Progesterone	3.87[e]	406.6	34.14	2.53 (±1.19) × 10^2	1.19 (±0.75) × 10^2	1.89 (±0.49) = 10^3

[a] DPPC = dipalmitoylphosphatidylcholine; POPC = palmitoyloleoylphosphatidylcholine. Values in parentheses are ± standard error.
[b] Calculated using LeBas method [24].
[c] Calculated using the atomic contributors [25].
[d] Yamamoto and Liljestrand [14].
[e] Hansch et al. [37].
[f] Estimated from phenol using the fragment method [38].
[g] Estimated using KOWWIN software [39].
[h] Ellington and Floyd [40].

proximately 50% of C_{ref}. The C_w attained for all batches actually ranged between 10 and 95% of C_{ref}.

Correlation analyses and evaluation of the models

The molar liquid volume (MLV) of each chemical was calculated using the LeBas method [24], and PSA values were calculated by a simple fragmentation method proposed by Ertl et al. [25] (Table 1). To evaluate the model performance, Akaike information criterion (*AIC*) for small sample size was used (for details, see Burnham and Anderson [26]). The best model in this criterion is the one minimizing *AIC*. When errors are normally distributed, small-sample Akaike information criterion (AIC_c) for a least-square method is given as

$$AIC_c = n\left[\ln(2\pi) + \ln\left(\frac{RSS}{n}\right) + 1\right] + 2K + \frac{2K(K+1)}{n-K-1} \quad (3)$$

where n is the sample size, *RSS* is the sum of the squares of residuals in a least-square method, and K is the number of parameters. Least-square regression analyses were performed with SPSS for Windows (Ver 12.0; SPSS, Chicago, IL, USA).

RESULTS

Liposome–water partition coefficients

Table 1 summarizes the values of log K_{ow}, MLV, PSA, and the partition coefficients between water and synthetic lipid membrane vesicles (K_{lipw}) of all the selected chemicals determined using the three different lipid compositions—DPPC, DPPC/cholesterol, and POPC—in the present study as well as that of Yamamoto and Liljestrand [14]. The K_{lipw} values for diethylphthalate with DPPC liposomes and for 4-*sec*-butylphenol, 4,4′-dihydroxybenzophenone, and diethylphthalate with DPPC/cholesterol liposomes could not be determined, because little difference was found between the concentration in the reference reactor (C_{ref}) and the concentration in the sample reactor (C_w). For all the selected chemicals, K_{lipw} values increased in the order DPPC/cholesterol, DPPC, and POPC, except for 4-phenylphenol, for which $K_{lipw,DPPC/cholesterol}$ was only slightly greater than $K_{lipw,DPPC}$. The $K_{lipw,DPPC/cholesterol}$ values for moderately hydrophobic EDCs were within one order of magnitude, except for the most hydrophilic estriol and the two most hydrophobic alkylphenols, nonylphenol and *p-tert*-octylphenol. The log K_{lipw} values for three relatively hydrophilic chemicals (diethylphthalate, 4,4′-dihydroxybenzophenone, and 4-*sec*-butylphenol), for which log K_{lipw} values could not be determined because of the limitation in the experimental method, are thought to be 1.5 or less, considering the experimental limits.

Correlation between log K_{lipw} *and log* K_{ow}

As illustrated in Figure 2, log K_{lipw} values did not correlate well with reported log K_{ow} values. Correlation coefficients (r^2) for DPPC and POPC liposomes were approximately 0.78, whereas that for DPPC/cholesterol liposomes was only 0.37. These relationships are much weaker than those obtained in other studies using relatively smaller and structurally less diverse chemicals, for which r^2 values typically are 0.9 or greater [6,8,10–12]. The linear correlation is weakest for the liposomes containing a high amount of cholesterol (40% by mass or 56% by mole fraction). The slopes in Figure 2a and c were not statistically different, as determined by a t test, even at the 70% confidence interval. This indicates that selectivity in liposome–water systems is similar to that in octanol–water systems regardless of lipid saturation, whereas a higher affinity is shown for unsaturated versus saturated liposomes. Figure

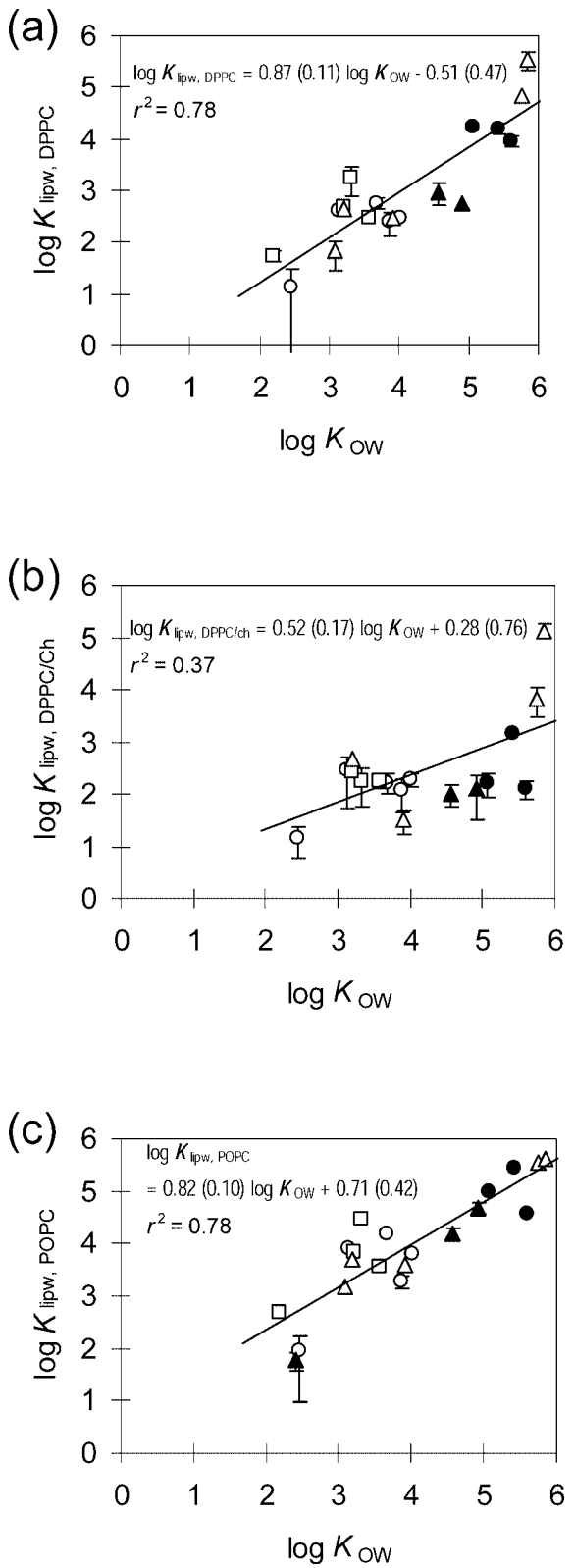

Fig. 2. Relationship between reported octanol–water partition coefficients (K_{ow}) and liposome–water partition coefficient (K_{lipw}) measured from (**a**) dipalmytoylphosphatidylcholine (DPPC), (**b**) DPPC/cholesterol (60:40, w/w), and (**c**) palmytoyloleoylphosphatidylcholine (POPC) with linear-regression lines and equations. Values in parentheses denote standard errors of the regression. Data are shown for steroid hormones and their derivatives (○), synthetic estrogens (●), *p*-substituted phenols (△), phthalate esters (▲), and other phenolic chemicals (□). Error bar denotes standard deviation and is not shown when it is smaller than the symbol.

2b shows that log $K_{lipw,DPPC/cholesterol}$ was increased only with increasing hydrophobicity when log K_{ow} was 3.0 or less and 5.5 or greater, implying an invariance to the increase in hydrophobicity for moderately hydrophobic chemicals.

Examination of the types of compounds that deviate from the trends shown in Figure 2 are revealing. The two most hydrophobic alkylphenols, 4-nonylphenol and 4-*tert*-octylphenol, fall above the regression lines for all types of liposomes, whereas the two hydrophobic phthalate esters, benzylbutylphthalate and dibutylphthalate, fall below the regression lines for all cases. In addition, variation in log K_{lipw} values for steroid hormones, except for the most hydrophilic estriol, was not as great as the variation in their hydrophobicity. If only the five *p*-substituted phenols (4-*sec*-butylphenol, 4-*tert*-amylphenol, 4-phenylphenol, 4-nonylphenol, and 4-*tert*-octylphenol) from Figure 2 are regressed, a much stronger linear relationship can be developed between log K_{ow} and log K_{lipw}, as shown in the literature using structurally similar and relatively small (MLV, <230 cm^3/mol) chemicals [6,8,10–12]:

$$\log K_{lipw,DPPC} = 1.15 \log K_{ow} - 1.57 \quad n = 5, \quad r^2 = 0.93 \text{ (at 22°C)} \quad (4)$$

$$\log K_{lipw,DPPC/cholesterol} = 0.94 \log K_{ow} - 1.12 \quad n = 4, \quad r^2 = 0.66 \text{ (at 22°C)} \quad (5)$$

$$\log K_{lipw,POPC} = 0.84 \log K_{ow} + 0.67 \quad n = 5, \quad r^2 = 0.96 \text{ (at 22°C)} \quad (6)$$

The 4-phenylphenol had higher log K_{lipw} values compared with those predicted by linear regression for all three lipid compositions, whereas 4-*sec*-butylphenol and 4-*tert*-amylphenol were slightly below the regression lines, suggesting that branching has a significant effect.

Effects of MLV and PSA

The free energy of solute transfer between an organic phase and water is determined by the free energy of cavity formation and solute–solvent interactions in the two phases. Gobas et al. [8] suggested that the free energy of cavity formation would be dominant when a series of chemicals are relatively large and have similar solute–solvent interactions. They derived the following equation when the free energy of cavity formation follows a parabolic relationship with respect to MLV in the lipid membranes and is linearly proportional to MLV in 1-octanol:

$$\log K_{lipw} = (a + b\text{MLV}) \log K_{ow} + c \quad (7)$$

where a and b are empirical constants related to the free energy of cavity formation in the lipid membranes and in 1-octanol and c is assumed to be a constant if differences in solute–solvent interactions among chemicals are negligible. Because of the relatively large molecular size of the selected EDCs, ranging from 190 to 400 cm^3/mol (Table 1), the free energy of cavity formation would be dominant if their solute–solvent interactions are not significantly different. Using Equation 10, best-fit parameters and correlation coefficients (r^2) were obtained from the values presented in Table 1:

$$\log K_{lipw,DPPC} = [1.23(\pm 0.21) - 0.00097(\pm 0.00049)\text{MLV}]\log K_{ow} - 0.77(\pm 0.46) \quad n = 19, \quad r^2 = 0.82 \text{ (at 22°C)} \quad (8)$$

$$\log K_{\text{lipw,DPPC/cholesterol}} = [0.94(\pm 0.30) - 0.00122(\pm 0.00073)\text{MLV}]\log K_{\text{ow}} - 0.57(\pm 0.44) \quad n = 17, \quad r^2 = 0.47 \text{ (at 22°C)} \quad (9)$$

$$\log K_{\text{lipw,POPC}} = [1.01(\pm 0.22) - 0.00051(\pm 0.00051)\text{MLV}]\log K_{\text{ow}} - 0.12(\pm 0.72) \quad n = 20, \quad r^2 = 0.79 \text{ (at 22°C)} \quad (10)$$

In all three equations, log K_{lipw} decreases with increasing MLV for compounds with the same hydrophobicity. This suggests that the additional free energy of cavity formation in the lipid membranes per unit volume increases with increasing cavity size, because the ($a + b$MLV) term represents the ratio of free energy required for the formation of cavities in two organic phases [8]. Correlation coefficients increased slightly by including the effects of MLV, as seen by comparing Equations 8 through 10 with the respective equations in Figure 2. The correlations including MLV effects also show increasing b values with decreasing membrane fluidity (i.e., in the order of POPC < DPPC < DPPC/cholesterol), although this general trend is not statistically significant.

Effects of PSA were evaluated by multiple linear regression using log K_{ow} and PSA as independent variables, and the results are as follows:

$$\log K_{\text{lipw,DPPC}} = 0.82(\pm 0.12)\log K_{\text{ow}} - 0.014(\pm 0.010)\text{PSA} + 0.22(\pm 0.70) \quad n = 19, \quad r^2 = 0.80 \text{ (at 22°C)} \quad (11)$$

$$\log K_{\text{lipw,DPPC/cholesterol}} = 0.40(\pm 0.16)\log K_{\text{ow}} - 0.032(\pm 0.014)\text{PSA} + 1.99(\pm 1.01) \quad n = 17, \quad r^2 = 0.54 \text{ (at 22°C)} \quad (12)$$

$$\log K_{\text{lipw,POPC}} = 0.78(\pm 0.11)\log K_{\text{ow}} - 0.009(\pm 0.010)\text{PSA} + 1.23(\pm 0.68) \quad n = 20, \quad r^2 = 0.79 \text{ (at 22°C)} \quad (13)$$

As shown in Equations 11 through 13, log K_{lipw} is negatively correlated with PSA, with a slight improvement in r^2 values as compared to those without including PSA. As was the case for MLV, the coefficient for PSA increases with decreasing membrane fluidity, but again, this trend is not statistically significant. This implies that transferring a molecule having hydrogen-bonding capacity into a more structurally ordered lipid membrane generally requires more free energy than transferring the same molecular into less structurally ordered membranes.

DISCUSSION

Relationship between log K_{lipw} *and log* K_{ow}

Weak/moderate relationships between log K_{lipw} and log K_{ow} for selected EDCs (Fig. 2) strengthen the suggestion that log K_{ow} has only limited potential for prediction of bioconcentration of endocrine disrupters [14]. Unlike dissolution of organic solutes in a solvent like octanol, which is a homogeneous distribution, the dissolution in liposomes distributed with respect to diameter and local sterol content is not a homogeneous distribution. Solute partitioning would be more favored in regions with more favorable cavity formation. Effects of molar volume become more apparent when the molar volume of a solute is not directly related with its hydrophobicity. Most of the previous studies on conventional water pollutants have found that molar volume of solutes was related linearly with their log K_{ow} [6,8,10–12]. Thus, steric effects could be masked from their regression analyses. Weakest correlation between log K_{lipw} and log K_{ow} occurred for liposomes containing a high amount of cholesterol (56% by mole fraction). The amount of cholesterol used in the present study would be an extreme case, because cholesterol content may vary from approximately 12% by mole fraction in the endoplasmic reticulum to between 30 and 50% by mole fraction in plasma membranes [20]. Whereas the compounds chosen do not reflect the full range of chemical structure for EDCs, further investigation is needed to determine the effects of cholesterol on the bioconcentration of moderately hydrophobic EDCs.

Effects of the membrane fluidity on K_{lipw}

Effects of lipid components on the partition coefficients can be interpreted by the fluidity of the membrane. Because of the carbon–carbon double bond in the unsaturated hydrophobic tails of POPC, the average distance between phospholipid molecules is greater than that of saturated lipid molecules, such as DPPC [20]. This less dense distribution of lipid molecules provides more cavities for sorption, lowering hydrophobic interaction and increasing membrane fluidity. The observed trend of higher K_{lipw} values for unsaturated lipid components compared with those for saturated lipids is in agreement with that in previous studies [14,15,27].

The presence of cholesterol in the lipid bilayer makes the lipid membrane more rigid [20] by filling the free spaces in the hydrophobic tail region (Fig. 3). Higher cholesterol contents in the lipid membranes also lower the partition coefficient between water and lipid membranes [14,15,27,28]. Moreover, the decrease in log K_{lipw} on addition of cholesterol to the lipid membrane generally was greater for more hydrophobic chemicals, as shown in Figure 4. The $\Delta\log K_{\text{lipw}}$ values, defined as the differences between log $K_{\text{lipw,DPPC}}$ and log $K_{\text{lipw,DPPC/cholesterol}}$, ranged from 0 (4-phenylphenol) to 2.0 (diethylstilbestrol). For 4-nonylphenol and 4-*tert*-octylphenol, the effects of cholesterol were not as great as those for other hydrophobic chemicals, such as diethylstilbestrol and hexestrol, which have $\Delta\log K_{\text{lipw}}$ values of 2.00 and 1.86, respectively. This tendency is consistent with the findings of Liu et al. [15] and Lagerquist et al. [28], who found that log K_{lipw} for hydrophobic drugs with lipid membranes containing 40% by mole fraction cholesterol were less than those from lipid membranes without cholesterol by 0.5 log units, whereas these drops were negligible for more hydrophilic drugs. As a result of this general tendency, log $K_{\text{lipw,DPPC/cholesterol}}$ did not correlate with log K_{ow}, especially for moderately hydrophobic EDCs, as shown in Figure 2. The binding locations of a solute into the lipid bilayer can be divided into three regions: Near the polar head, hydrophobic tails with a high conformational order, and center of the bilayer. In general, more hydrophobic chemicals are believed to partition closer to the bilayer center (see, e.g., [29]). A decrease in log K_{lipw} on the addition of cholesterol may be explained by two mechanisms. First, a solute competes for the same binding site with cholesterol, and second, cholesterol restricts the motion of fatty acyl chains by occupying free spaces in the outer parts of them and, thus, reducing the membrane fluidity. As described in Figure 3, the effects of cholesterol would be greatest for chemicals entering the ordered

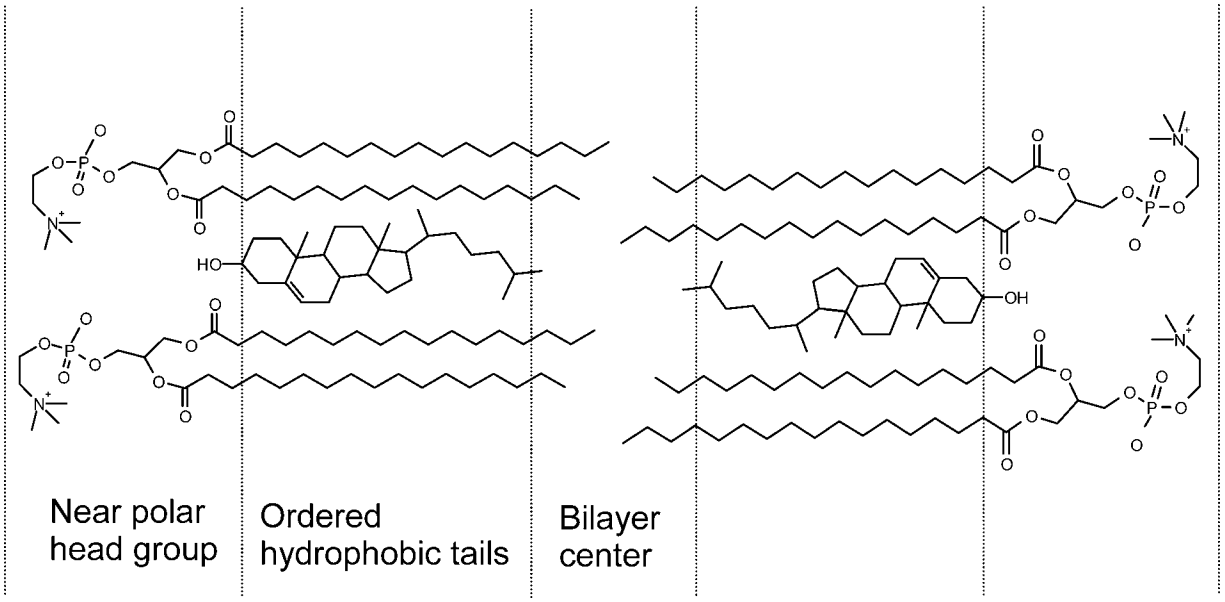

Fig. 3. Cholesterol molecule in the dipalmytoylphostatidylcholine (DPPC) lipid bilayer and three zones of the lipid bilayer.

hydrophobic tail region. Moderately hydrophobic EDCs with relatively high molar volume could be affected by both mechanisms, because they may partition into ordered hydrophobic tails and compete with cholesterol. Therefore, no increase in log $K_{\text{lipw,DPPC/cholesterol}}$ for these chemicals with increasing hydrophobicity could be explained by significant competition with cholesterol. For example, Jacobsohn et al. [30] and Golden et al. [31] have shown that the binding location of 17β-estradiol moves toward the outer part of the bilayer after the addition of cholesterol. Conversely, $\Delta\log K_{\text{lipw}}$ could be smaller for 4-nonylphenol and 4-*tert*-octylphenol if they do not compete with cholesterol.

Effects of MLV and PSA

Although it has been observed that linear correlations between log K_{lipw} and log K_{ow} break down for chemicals with higher molar volume [8,13], the effects of MLV could not be separated from the effects of hydrophobicity, because they were strongly correlated. However, Equations 8 through 10 showed independent contribution of MLV from hydrophobicity, because collinearity between MLV and log K_{ow} was very low for the chemicals selected in the present study.

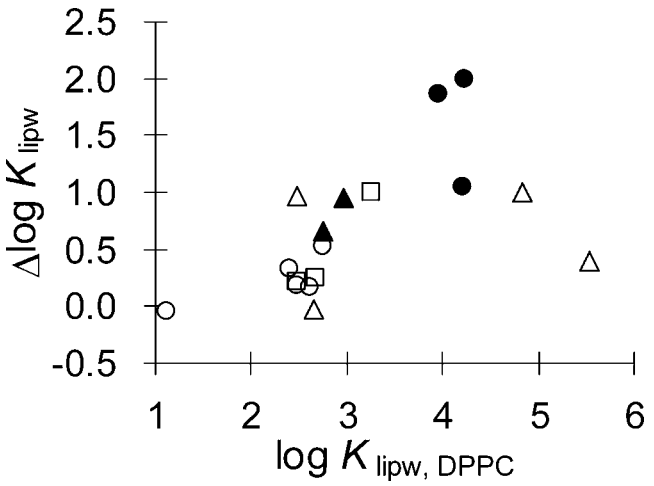

Fig. 4. Effects of cholesterol on the drop in log lipid membrane vesicles (K_{lipw}) from dipalmytoylphostatidylcholine (DPPC) liposomes. The $\Delta\log K_{\text{lipw}}$ is calculated from log $K_{\text{lipw,DPPC}}$ − log $K_{\text{lipw,DPPC/cholesterol}}$. Data are shown for steroid hormones and their derivatives (○), synthetic estrogens (●), *p*-substituted phenols (△), phthalate esters (▲), and other phenolic chemicals (□).

Studies have shown that human intestinal absorption of drugs (see, e.g., [17,18,32]) and blood–brain barrier partition coefficient (see, e.g., [33]) correlate negatively with PSA. Polar surface area, defined as the surface area contributed by oxygen or nitrogen atoms or hydrogen atoms attached to oxygen or nitrogen atoms, is related to the chemical's capacity to form hydrogen bonds [32]. Equations 11 through 13 suggest that more energy is required to transport a molecule having greater hydrogen-bonding capacity into the lipid membranes compared with into the bulk organic solvent 1-octanol. The negative contribution of PSA also is consistent with the findings of Vaes et al. [34] that log $K_{\text{lipw,DMPC}}$ correlated negatively with quantum mechanical descriptors representing greater hydrogen-bonding interaction. As for MLV, the coefficient for PSA increased with decreasing membrane fluidity, but again, this trend was not statistically significant. This implies that transferring a molecule having hydrogen-bonding capacity into a more structurally ordered lipid membrane requires more free energy than transferring into less structurally ordered membranes. However, there would be compensating effects of PSA if a molecule associates with polar head groups via specific polar interactions (see, e.g., [35]).

Effects of molecular geometry

In addition to hydrophobicity, molecular size, and polar interactions, molecular geometry may affect the relationship between log K_{lipw} and log K_{ow}. As shown in Figure 1, the chemical structures of the synthetic estrogens diethylstilbestrol, hexestrol, and dienestrol are very similar. Their differences in PSA and MLV are negligible. However, the measured log K_{lipw} values for hexestrol with each of the three different liposomes are significantly lower than those for diethylstilbestrol and dienestrol. The flat structures of diethylstilbestrol and dienestrol, resulting from the delocalization of π electrons, make these molecules easily accommodated in the structured

Table 2. Standardized regression coefficients from multiple linear regressions[a]

Variables in the model		Log $K_{lipw,DPPC}$	Log $K_{lipw,DPPC/cholesterol}$	Log $K_{lipw,POPC}$
Log K_{ow} and MLV, Equations 8 to 10	Log K_{ow}	1.232	1.198	1.015
	MLV × log K_{ow}	−0.411	−0.719	−0.153
Log K_{ow} and PSA, Equations 11 to 13	Log K_{ow}	0.831	0.472	0.840
	PSA	−0.162	−0.430	−0.117

[a] Regression coefficients on autoscaled variables, indicating the change in the standard units of the dependent variable for each increase of one standard unit in the independent variable, controlling for all other independent variables. DPPC = dipalmitoylphosphatidylcholine; MLV = molar liquid volume; POPC = palmitoyloleoylphosphatidylcholine; PSA = polar surface area.

hydrophobic tail region of the membrane bilayer. Conversely, conformational restriction is expected when hexestrol enters membranes, because rotation along the carbon–carbon single bond in the middle of the compound requires a large free volume. This also would be consistent with the observed log K_{lipw} for 4-phenylphenol being higher than that predicted by the regression line as a result of its flat structure. Although the carbon–carbon single bond contributes more in hydrophobicity to the log K_{ow} estimated by atomic fragmentation methods compared with a carbon–carbon double bond [36], a more careful approach is required when estimating log K_{lipw} from molecular structure.

Statistical evaluation of the models

The standardized regression coefficients were calculated to evaluate the importance of the two variables, log K_{ow} and MLV × log K_{ow} or log K_{ow} and PSA (Table 2). As can be seen in Equations 11 through 13, the importance of MLV was greatest for estimating log $K_{lipw,DPPC/cholesterol}$ and was comparable to the significance of log K_{ow}. Similarly, for Equations 11 through 13, the contribution of PSA was almost the same as that of log K_{ow} for predicting log $K_{lipw,DPPC/cholesterol}$, whereas PSA effects were very small for predicting log $K_{lipw,DPPC}$ and log $K_{lipw,POPC}$. Because improvements in correlation coefficients obtained by incorporating additional model parameters do not imply that a model is superior, a small-sample Akaike information criterion (AIC_c) was used to provide a standard criterion for model performance. The AIC_c values obtained using the MLV-containing model were lower for DPPC and DPPC/cholesterol liposomes compared with those obtained using solely log K_{ow}, suggesting that adding MLV to these systems is justified, especially for DPPC liposomes. The *AIC* analysis also showed that PSA effects were greatest for the most structurally rigid lipid membrane, DPPC/cholesterol liposomes. Adding either MLV or PSA effects did not improve the predictive model for the most flexible lipid membrane, POPC liposomes, in terms of AIC_c. As indicated above, the effects of an additional descriptor, MLV or PSA, were more significant for the more structurally organized lipid membranes.

Implication of the study

For certain EDCs in the present study, K_{lipw} varied almost two orders of magnitude because of the changes in lipid composition of the membrane vesicles. This may reflect the interspecies variation of lipid-normalized bioconcentration factor (LBCF) as well as differences in LBCFs among different organs. Effects of lipid components on LBCFs may be evaluated by further research. The equilibrium partition coefficient between aqueous buffer and the lipid compartment with a specific lipid composition could be a significant parameter determining internal distribution of hydrophobic pollutants in the body of aquatic organisms as well as other physiological processes. Potential improvement of empirical quantitative structure–activity relationships could be achieved by additional descriptors, such as MLV and PSA.

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