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Experimental Determination of Octanol–Water Partition Coefficients of Selected Natural Toxins

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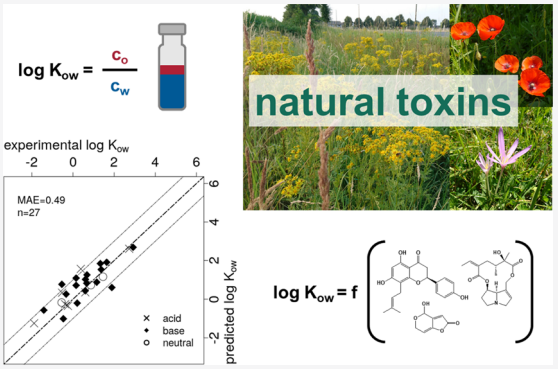
ABSTRACT: Natural toxins are widely occurring, highly diverse organic compounds produced by, for example, plants or fungi. In predictive environmental fate and risk assessment of organic chemicals for regulatory purposes, the octanol–water partition coefficient (K_{ow}) remains one of the key parameters. However, experimental data for natural toxins are largely missing, and the current estimation models for K_{ow} show limited applicability for multifunctional, ionizable compounds. Thus, log K_{ow} data were first experimentally derived for a diverse set of 45 largely ionizable natural toxins and then compared with the predicted values from three different models (KOWWIN, ACD/Percepta, and Chemicalize). Both approaches were critically evaluated with regard to their applicability for multifunctional, ionizable compounds. The miniaturized shake-flask approach allowed reliable quantification of pH-dependent partitioning behavior for neutral, acidic, and basic ionizable natural toxins. All the analyzed toxins are rather polar with an average log K_{ow} < 1 and an observed maximum log K_{ow} of 2.7. Furthermore, the comparison of experimental data for the neutral form of ionizable toxins with those of commonly used prediction models showed that the latter match the former with only slightly increased errors. The Chemicalize tool gave the best overall predictions for the dataset generated here, with a mean absolute error of 0.49.



INTRODUCTION

The number of investigations on the environmental behavior of synthetic chemicals such as pesticides or pharmaceuticals is continuously increasing.[1,2] In contrast, a huge knowledge gap exists when it comes to the occurrence and distribution of naturally toxic compounds. Studies on natural toxins are limited to just a few compounds found in water resources.[3−7] Mycotoxins and plant secondary metabolites (phytotoxins) are two of the largest subgroups of natural toxins. For their producers, they act as advantageous protection or defense molecules against herbivores, microbes, viruses, or other plants.[8,9] As such, they can be seen as nature's own pesticides and may pose a threat to livestock and human health.[10−12] Their molecular diversity is immense, spanning from alkaloids to steroids, flavonoids, terpenoids, and many others.[13]

Once released into the environment, natural toxins, like anthropogenic pollutants, are subject to different fate processes that are largely dependent on the compounds' physicochemical properties. For the purpose of environmental risk assessment and as a basis for predictive modeling and remediation strategies, those properties affecting a compound's distribution have to be described in a systematic and quantitative manner.[14] The octanol–water partition coefficient (K_{ow}) remains one of the key parameters in environmental fate and risk assessment studies of organic chemicals. Many single-parameter quantitative structure-property relationships (QSPRs) and quantitative structure–activity relationships (QSARs) rely on K_{ow} as the main input parameter.[15−18] Those models are based on the mathematical relationship between the structural features of a set of molecules and their experimentally derived properties. Once validated, they can also be applied to predict the properties of compounds that have not been experimentally assessed so far.[19] In bioconcentration and toxicity estimation, in particular, K_{ow} continues to be an important estimate that allows one to derive a tendency on the hydrophobicity or lipophilicity of a compound.[16,20] The K_{ow} is valid only for a neutral chemical, whereas the partitioning of ionizable chemicals is described by the respective distribution coefficient (D_{ow}), which accounts for both neutral and charged species of the chemical. In the environmentally relevant pH range 4–9, D_{ow} is considered more relevant for the description of environmental behavior of ionizable compounds.[15]

Different well-established methods are available for the experimental determination of K_{ow}/D_{ow} (e.g., OECD 107, 117).[21,22] The indirect analysis of the partitioning behavior based on column chromatography (i.e., OECD 117)[22] allows

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Table 1. List of Analyzed Neutral, Charged, Basic, and Acidic Ionizable Natural Toxins, Including Their First Basic or Acidic pK_a, as Well as Experimentally Derived log D_{ow} Values and Standard Deviations at Different pH values and Ionization States[a]

compound	pK_a[b]	fraction of charged species [%] at pH 4.0/pH 7.3/pH 10.2	log D_{ow} (pH 4.0)	log D_{ow} (pH 7.3)	log D_{ow} (pH 10.2)
Neutral					
caffeine		neutral	*−0.12 ± 0.07 (2)*	*−0.08 ± 0.03 (6)*	*−0.32 ± 0.06 (9)*
colchicine		neutral	*1.11 ± 0.02 (3)*	*1.14 ± 0.03 (9)*	*1.23 ± 0.05 (9)*
strophanthidin		neutral		*0.69 ± 0.06 (3)*	*0.77 ± 0.05 (3)*
Charged					
berberine		cationic	−0.42 ± 0.23 (3)	−1.03 ± 0.18 (3)	−0.09 ± 0.07 (3)
Acids					
3-acetyl-deoxynivalenol	11.80	-/0/-		*0.33 ± 0.13 (3)*	
ailanthone	11.85	0/-/-	*−0.34 ± 0.23 (3)*		
cucurbitacin E	8.51	-/-/99			2.60 ± 0.06 (3)
10-deacetylbaccatin III	11.50	0/-/-	*0.36 ± 0.31 (2)*		
daidzein	7.01	0/71/100	*2.61 ± 0.03 (6)*	2.22 ± 0.05 (3)	−0.26 ± 0.13 (6)
diacetoxyscirpenol	13.40	-/0/-		*1.57 ± 0.48 (3)*	
deoxynivalenol	11.90	-/2/-		−0.42 ± 0.19 (3)	
nivalenol	11.80	-/0/-		*−1.25 ± 0.24 (3)*	
patulin	12.10	-/0/-		*−0.26 ± 0.09 (3)*	
8-prenylnaringenin	7.70	-/-/100			2.03 ± 0.07 (3)
sterigmatocystin	6.90	-/76/-		1.45 ± 0.07 (2)	
β-zearalenol	7.60	-/39/-		1.90 ± 0.04 (3)	
zearalenone	7.60	-/39/-		2.07 ± 0.12 (3)	
Bases[c]					
7-acetyl lycopsamine	7.85	-/74/0		−0.43 ± 0.33 (3)	*1.08 ± 0.14 (4)*
aconitine	5.15	-/0/-		*0.76 ± 0.35 (3)*	
(+)-bicuculline	6.71	100/17/-	−0.49 ± 0.04 (3)	1.86 ± 0.01 (3)	
cytisine	10.50	-/-/66			−0.45 ± 0.01 (2)
echimidine	7.36	100/48/0	−0.93 ± 0.03 (2)	0.64 ± 0.15 (3)	*1.26 ± 0.14 (3)*
erucifoline	5.92	99/3/0	−0.95 ± 0.12 (3)	−0.17 ± 0.05 (3)	*0.44 ± 0.21 (3)*
erucifoline *N*-oxide	4.65	82/-/-	−0.35 ± 0.31 (3)		
europine	8.49	-/-/2			0.96 ± 0.06 (3)
galantamine	7.92	-/-/0			*0.88 ± 0.04 (15)*
gramine	9.47	100/-/15	−1.32 ± 0.40 (4)		1.94 ± 0.02 (3)
huperzine A	9.01	-/98/6		0.74 ± 0.01 (2)	1.70 ± 0.02 (3)
(+)-isocorydine	6.77	-/-/0			*2.68 ± 0.06 (3)*
jacobine	5.86	-/3/0		−0.32 ± 0.38 (3)	*0.83 ± 0.24 (3)*
jacobine *N*-oxide	4.63	81/-/0	−0.87 ± 0.84 (2)		*−1.01 ± 0.40 (3)*
lasiocarpine	7.35	100/47/0	−0.42 ± 0.35 (3)	1.22 ± 0.29 (3)	*1.84 ± 0.11 (6)*
lasiocarpine *N*-oxide	3.87	43/0/-	−0.50 ± 0.49 (3)	*−0.21 ± 0.24 (6)*	
lycopsamine *N*-oxide	4.26	65/-/0	−1.00 ± 0.43 (3)		*−0.57 ± 0.43 (3)*
lycorine	6.34	-/8/0		−0.30 ± 0.31 (3)	*0.71 ± 0.05 (3)*
monocrotaline	5.90	-/-/0			*0.27 ± 0.33 (6)*
papaverine	6.32	100/-/-	0.30 ± 0.02 (3)		
protopine	7.86	100/-/-	−0.67 ± 0.43 (4)		
reserpine	7.25	100/-/-	−0.16 ± 0.63 (2)		
retrorsine	5.79	98/2/0	−1.87 ± 0.09 (2)	−0.11 ± 0.09 (3)	*1.03 ± 0.08 (6)*
senkirkine	6.51	100/2/0	−0.78 ± 0.01 (2)	−0.48 ± 0.11 (3)	*0.61 ± 0.11 (3)*
senecionine	5.86	99/-/0	−0.63 ± 0.57 (3)		*1.90 ± 0.10 (6)*
seneciphylline	5.87	99/3/0	−0.91 ± 0.17 (2)	0.58 ± 0.25 (3)	*1.53 ± 0.02 (3)*
tetrahydropalmatine	6.53	100/-/-	−0.15 ± 0.21 (3)		
vincamine	7.82	100/-/-	−0.91 ± 0.13 (3)		

[a]Number in parentheses depicts the number of independent measurements performed. When log D_{ow} is given in italics, log D_{ow} = log K_{ow} as the compound was evaluated in its neutral state, defined by ionization <1%. [b]ACD/Percepta-predicted data. [c]pK_a is valid for the corresponding cation. Empty cells: no data generated.

simple and fast analysis of larger groups of compounds by pooled injection.[23−26] However, it was shown that data reproducibility is rather poor,[15] and charge as well as steric effects may outcompete hydrophobic effects in retention analysis.[27] Thus, the shake-flask technique (OECD 107)[21] is currently preferred for the reliable determination of K_{ow}/D_{ow}, particularly, when focusing on ionizable compounds with an expected log K_{ow}/D_{ow} < 4.5.[15,28] This method can be applied to ionizable compounds by performing experiments based on pH-dependence. Miniaturization can speed up the total measurement time and considerably minimizes the required amount of analytes and laboratory consumables. This was

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demonstrated by Rothwell et al. for dietary flavonoids.[20] In combination with a modified in situ approach developed for application in drug analysis,[29,30] the approach can be seen as a potential high-throughput alternative for the analysis of ionizable organics.[31,32]

Experimental K_{ow}/D_{ow} data for natural toxins are extremely scarce. Hence, the current risk assessment often has to resort to in silico prediction tools.[33,34] However, for compounds with physicochemical and structural complexity due to large numbers of ionizable functional groups, the current estimation models for phase distribution coefficients show limited applicability.[2,35−38] Thus, reliable experimental data are required to improve the understanding of natural toxin behavior in the environment.[33] In this study, we provide experimentally derived log K_{ow}/D_{ow} values for a set of 45 mainly ionizable natural toxins from different compound classes prioritized as potential aquatic micropollutants.[34] In addition, we critically evaluate the applicability of both experimental (miniaturized shake-flask approach[21,31]) and predictive methods (EPISuite,[39] ACD/Percepta,[40] and Chemicalize[41]) for K_{ow}/D_{ow} determination.

MATERIALS AND METHODS

Materials. The chemicals used in this study were purchased from the following companies: high-performance liquid chromatography (HPLC) grade *n*-octanol and methanol, and sodium salts used for the preparation of buffer solutions from Sigma-Aldrich (Buchs, Switzerland); the investigated natural toxins from PhytoLab (Vestenbergsgreuth, Germany), Sigma-Aldrich (Buchs, Switzerland), or Fermentek (Jerusalem, Israel). Water was deionized using a Milli-Q system (Merck Millipore, Darmstadt, Germany). All natural toxin stock solutions were prepared in methanol. Details on analytes (CAS, formula, molecular structure), suppliers, and stock solution concentrations are given in the Supporting Information (Tables S1 and S4; Figure S1). The chosen set of 45 natural toxins (37 phytotoxins and 8 mycotoxins, Table 1) covered a molecular weight range of 150−650 g mol^{-1} and contained representatives of neutral, charged, acidic, and basic ionizable compounds.

Experimental Approach. Octanol−water partition coefficients were quantified using a miniaturized shake-flask approach based on OECD 107 (Figure S2a).[20,21,31] To investigate the analytes in either fully ionized or neutral state as well as their pH-dependent partitioning behavior, measurements were performed using sodium-based buffer solutions at a constant ionic strength of 0.1 M covering three different pH values: pH 4.0 (citrate buffer), pH 7.3 (phosphate buffer), and pH 10.2 (carbonate buffer). The stability of pH was regularly checked throughout the experiment. All buffer solutions were saturated with octanol prior to analysis and vice versa. Stock solutions of natural toxins were first diluted 1:100 in defined volumes of buffer solution and added to standard 1.5 mL HPLC vials. Second, octanol was added to all but one of the dilutions, which served as a reference stock dilution. Varying ratios of octanol (0.01−1 mL) and aqueous buffer solutions (0.5−1.5 mL) were chosen based on the predicted K_{ow}, with the goal that between 30 and 80% of the toxin would remain in the aqueous phase after partitioning. Thus, the theoretical operational range of the method was set from −1.0 to 2.5 log D_{ow}, and it was limited by: (1) detection limits of the detector and (2) minimum aqueous phase volumes of 0.5 mL required for phase separation and HPLC injection. Further details on the preparation of buffer solutions and octanol−water phase ratios for individual natural toxins are presented in Section II of the Supporting Information (Tables S3 and S4).

All octanol−water mixtures were first vortexed for 1 min and then shaken for 2 h (125 rpm) at room temperature (25 ± 3 °C) to reach equilibrium and phase distribution. Kinetic measurements were performed to confirm the equilibrium conditions in the setup (Figure S2b). After equilibration and phase separation, the aqueous phase of all samples was analyzed on an Agilent HPLC 1260 with diode array detection (at λ = 210, 228, or 248 nm, analyte dependent, details are given in Table S4). Reference dilutions were analyzed twice, before and after equilibration, to assess recovery and test for complete mass recovery. Applying the water plug technique,[42] 50 μL of the aqueous phase was injected for all analyses. In brief, first 5 μL of blank buffer solution was aspirated, followed by the aqueous phase of the respective sample. To avoid any carryover of the analyte-containing octanol phase, the outside of the syringe was subsequently washed in methanol before injection. Analysis was done with a Macherey-Nagel Nucleoshell RP 18plus column (length 50 mm, i.d. 2 mm, and particle size 2.7 μm) and without any column for comparison. Runs were performed at 40 °C in isocratic mode using a methanol/water mixture (40/60, v/v) at a flow rate of 0.33 mL min^{-1}. As the resultant partition coefficients did not differ significantly between analyses performed with and without a column, the final results were obtained from measurements without a column. This led to a substantial decrease in the analysis time and prevented potential memory effects due to accumulation of octanol traces on the stationary phase of the column. However, the obtained peak areas of analytes had to be corrected with peak areas of the aqueous (octanol-saturated) buffer solution blanks at the respective measurement wavelengths.

Data Evaluation. Distribution coefficients for all analytes were derived from the difference in the blank corrected peak areas of the toxin in both the reference stock dilution ($area_{Std}$) and the aqueous phase of the partition samples containing octanol ($area_w$) and multiplying with the volumetric ratio of water (V_{water}) and octanol ($V_{octanol}$) of the respective sample (given in Table S4), as represented by eq 1.

$$\log D_{ow}\,[-] = \log\left(\frac{area_{Std}}{area_w} - 1\right) \times \left(\frac{V_{water}\,[mL]}{V_{octanol}[mL]}\right) \quad (1)$$

Overall method applicability was tested using previously investigated natural toxins for which experimental log K_{ow} were available (caffeine, colchicine, and daidzein; Table S2) as reference compounds.

For comparing the data with commonly used prediction models, only experimentally derived partitioning data for compounds examined in their neutral state were considered. In this case, log D_{ow} = log K_{ow}, and no errors due to partial partitioning of ionized species are introduced. To calculate the ionization state of a given analyte under experimental conditions, the ionized fraction was obtained based on the compound's pK_a (ACD/Percepta,[40] Table 1) and average measurement pH (Table S3). The calculation is based on the relationships presented in eq 2 (basic ionizable analytes) and eq 3 (acidic ionizable analytes). If the ionized fraction was lower than 1%, a compound was assumed to be in its neutral state.

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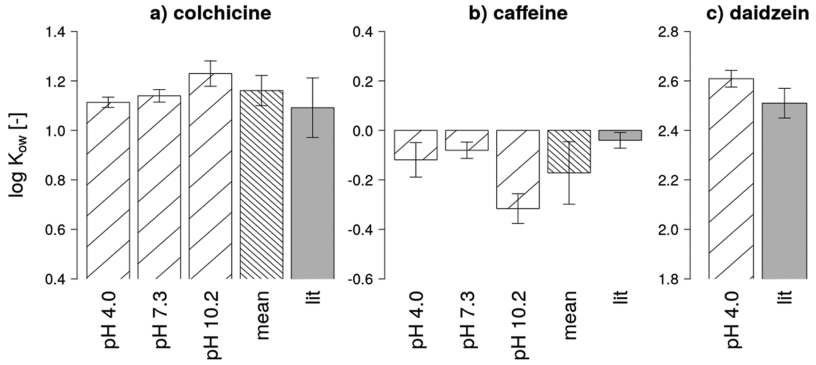

Figure 1. Measured log K_{ow} values for the analyzed reference compounds (a: colchicine, b: caffeine, and c: daidzein) at different pH values (pH 4.0, 7.3, and 10.2) and the average of all measurements (mean). For the acidic ionizable reference daidzein (c), experimental results from measurements at pH 4.0, where the compound is in its neutral state, are shown. Including measurement errors, no statistically significant difference ($p > 0.05$) can be observed when comparing the experimental values from this study and the literature data (lit).[20,31]

$$\text{Ionized fraction, base [\%]} = \left(\frac{1}{\left(\frac{10^{-pK_a}}{10^{-pH}} \right) + 1} \right) \times 100\% \quad (2)$$

$$\text{Ionized fraction, acid [\%]} = \left(1 - \left(\frac{1}{\left(\frac{10^{-pK_a}}{10^{-pH}} \right) + 1} \right) \right) \times 100\% \quad (3)$$

The commonly used QSPR models evaluated with regard to the predictability of natural toxin log K_{ow} were the free of charge KOWWIN, integrated with the Estimation Program Interface (EPI) Suite from the U.S. EPA[39] and the commercially available ACD/Percepta[40] and Chemicalize[41] tools. The predicted log K_{ow} data for all compounds and models are given in the Supporting Information (Table S2). The evaluation was based on regression analysis as well as the mean absolute error (MAE) of the models, according to eq 4

$$\text{MAE} = \frac{1}{n} \times \sum | \log K_{ow,exp} - \log K_{ow,model} | \quad (4)$$

where n is the number of observations and $K_{ow,exp}$ and $K_{ow,model}$ are the experimentally derived and QSPR-predicted partition coefficients, respectively.

RESULTS AND DISCUSSION

Octanol–Water Partition Coefficients and Applicability of the Experimental Approach. Distribution coefficients (log D_{ow}) for all the investigated natural toxins are given in Table 1, sorted first by the type of the toxins' first ionizable group and second by alphabet. It is often neglected that in many cases, the ionic species also partitions into the organic phase when extrapolating log K_{ow} from log D_{ow}.[43] Deriving log K_{ow} from measurements of a partially ionized compound could thus lead to increased errors.[44] Therefore, log K_{ow} values are presented here only for those compounds analyzed in their neutral state (Table 1, in italics).

Overall, the results range from a minimum of log D_{ow} = −1.87 to a maximum of log D_{ow} = 2.68 with highly variable experimental errors (Table 1). This range is larger than the theoretical one (see above) because of deviations of actually measured D_{ow} values from predicted ones, discussed in more detail below. The general error variability, to some extent, can be enhanced by slight instability of experimental conditions, such as pH and temperature, or by potential sorption of cationic analytes onto glass surfaces. The ionic strength and the type of counterions in solution are known to affect partitioning as well.[44] All buffers were based on sodium salts, and the variation of ionic strength was kept as narrow as possible (Table S3). However, the counterions varied and may have increased variations of K_{ow} obtained at different pH values, as seen for the neutral reference compounds colchicine and caffeine (Figure 1a,b). Experimental errors obtained by the analysis of replicates are larger for the analytes with lower affinity for octanol (Table 1, Figure S3). Of all the results, the average relative standard deviation for analytes with log D_{ow} < 0 is 0.6, and thus is more than three times as high as for compounds with log D_{ow} > 0. A small log D_{ow} results in only little changes in the aqueous phase concentration that are not as easily detected as for the analytes with high log D_{ow} values. No clear tendency toward higher experimental errors with increasing degree of compound ionization is observed though (Figure S3). Overall, the errors observed here are comparable with those described in the literature for similar experimental approaches and structurally related analytes.[20,31]

Nevertheless, the obtained log K_{ow} values for the reference compounds are consistent (i.e., within 0.1 log units) with literature values and show good reproducibility (maximum standard deviation of mean < 0.15, Figure 1). Thus, the miniaturized shake-flask approach provides robust data for natural toxin K_{ow}/D_{ow}. In the following, the data are further evaluated regarding the influence of pH changes on partitioning and serve as a basis for the critical assessment of the predictive power of different QSPR models.

Influence of pH on Octanol–Water Partitioning. As a consequence of proton transfer reactions and formation of the corresponding charged species, partitioning of ionizable compounds is pH dependent. For seven of the ionizable toxins, data for both the neutral and fully ionized species were acquired. Additional 16 compounds were measured only as neutral and nine as fully ionized species. All the other compounds were evaluated independent of their speciation (Table 1). In the environmentally relevant range of pH 4–9, they would also never occur in their fully ionized or neutral form. The medium pH would need to be more than one unit above (basic ionizable) or below (acidic ionizable) the compound's pK_a to achieve a system with only one species

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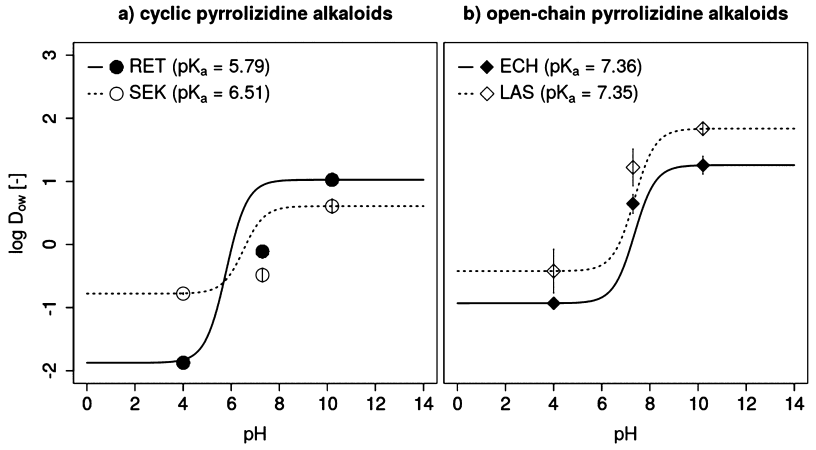

Figure 2. Observed log D_{ow} in the range from pH 4.0 to pH 10.2 for exemplary basic ionizable compounds (a: RET, retrorsine and SEK, senkirkine; b: ECH, echimidine and LAS, lasiocarpine). For visualization purposes only, trend lines are fitted to show the pH-dependence of log D_{ow} over the whole pH range from 0 to 14 considering the compound's ionization, as obtained by eq 2.

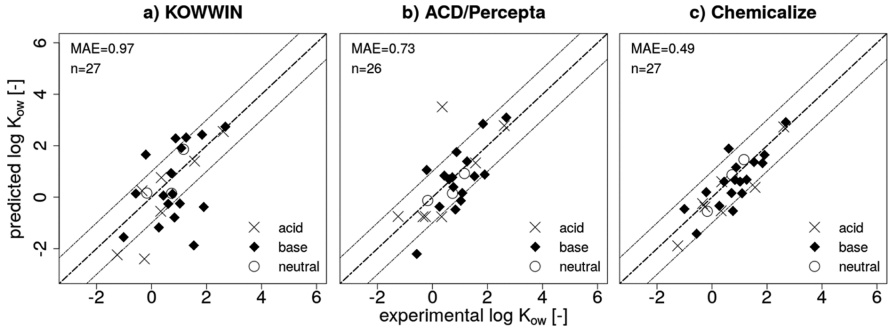

Figure 3. Results of three different QSPR prediction models (a: KOWWIN, b: ACD/Percepta, and c: Chemicalize) plotted against experimentally derived log K_{ow} values grouped according to different ionizability of analytes. Only compounds measured in their neutral state are considered (see Table 1). The inner dashed line represents the 1:1 line of agreement between predictions and experiments, while the outer lines indicate an error range of ± 1 log unit. MAE is the mean absolute error between all the experimental (eq 4) and predicted values and n is the number of values considered in evaluation.

of the compound present.[15,21] Additionally, measurement limitations such as limited variability of octanol−water ratios and high pH values (pH > 10) that are not advisable for common HPLC systems did not allow deriving those values in this study.

Generally, neutral species showed higher affinity for the octanol phase than their respective (partly) ionized counterparts (Table 1). However, in the case of acidic ionizable isoprenoids (daidzein, 8-prenylnaringenin, cucurbitacin E) as well as some mycotoxins (β-zearalenol, sterigmatocystin, zearalenone), relatively high partitioning (log D_{ow} > 1.4) was observed, although a substantial fraction of the analyte was ionized (> 39%). This illustrates that partitioning of the ionized species cannot always be neglected.

The data generated for those toxins where both single species systems (fully ionized or neutral) were evaluated can be used for modeling the partitioning behavior over the whole pH range for ionizable compounds (Figure 2). Such calculations are based on the fact that a compound's log D_{ow} is independent of pH as long as only one species exists in the system. A compound's speciation at any pH can be derived by applying eqs 2 and 3. It is not possible to calculate a compound's log D_{ow} over the whole pH range when only one species is considered though, as the total difference between log K_{ow} of the neutral and log D_{ow} of the fully ionized species varies from compound to compound.[44] An example is given by the two cyclic pyrrolizidine alkaloids retrorsine (RET) and senkirkine (SEK) for which an absolute log D_{ow} shift of 2.9 (RET) compared to 1.4 (SEK) is observed when considering the change from a fully ionized to neutral form (Figure 2a). For the two open-chain pyrrolizidine alkaloids echimidine (ECH) and lasiocarpine (LAS), the absolute difference is comparable at log $D_{ow} \approx 2.2$ (Figure 2b). Both those compounds have highly similar molecular structures; the higher affinity of LAS toward octanol can be explained by the difference in one specific molecular substituent; one hydroxy group (−OH) in ECH is replaced by the less-polar methoxy group (−O−CH_3) in LAS. According to the observed pH trends for the examples displayed in Figure 2, the experimental data suggest that pK_a values for the cyclic pyrrolizidine alkaloids (Figure 2a) are most likely underpredicted by ACD/Percepta, as the experimental data do not fall onto the fitted curves, but would if the pK_a would be increased by about one log unit. In a recent review, a typical pK_a range of pK_a 9−10 was suggested for amines, further reduced by additional functional groups such as esters.[45] As for both types of pyrrolizidine alkaloids (cyclic and open-chain), the functional groups attached to the amine base structure are comparable, their pK_a should be within a similar range as well.

Assessment of Prediction Model Applicability. The predicted log K_{ow} values for those natural toxins analyzed in their neutral state are displayed in relation to the obtained

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experimental values in Figure 3. Analytes are subdivided into acidic ionizable, basic ionizable, and neutral compounds. To evaluate the overall model applicability and predictive power for different subgroups, the MAE between predicted and experimental data as well as the slope and intercept of regression lines are considered (Figures 3, S4−S6). Combining all analytes into one large set for each of the prediction models, data are only evenly distributed around the line of agreement between the predicted and experimental data (1:1 line) for the Chemicalize model (Figure 3c). However, slopes of regression lines are close to one for all models (0.92−0.99, Figures S4−S6), and intercepts indicate only a slight underprediction of partitioning into octanol (intercept −0.26 to −0.10, Figures S4 to S6). In KOWWIN and ACD/Percepta, about two-thirds of all compounds fall within the range of variation of ± one log unit (KOWWIN 67%, ACD/Percepta 69%), while in the case of Chemicalize, 89% are well predicted (Figure 3).

As only three true neutral compounds were assessed in this study, no reliable conclusions can be drawn about the predictive power of different models. However, Chemicalize predicts both ionizable subgroups equally well with slopes close to one and the lowest overall MAE (Figures 3c; S6). For the other QSPR models, tendencies toward limited applicability for ionizable compounds can be observed. In KOWWIN, both acidic (slope = 1.24) and basic (slope = 0.66) ionizable compounds seem poorly predicted, while in ACD/Percepta, a similar trend can be seen only for basic ionizable compounds (slope = 1.14) (Figures 3a,b; S4 and S5). It has been previously shown that multifunctional molecules with several ionizable groups are not well predicted by those two models.[46] However, as measurement errors can be rather large (maximum SD = 0.48 for a compound measured in the neutral form, Table 1), and some compounds considered for comparison may not be in their neutral state because their predicted pK_a is erroneous, this observation should only be seen as a tendency.

In summary, the absolute predictive power of the investigated QSPR models increases from EPISuite's KOWWIN to ACD/Percepta and Chemicalize. Similar observations were made in previous studies comparing different prediction models and experimental data based on larger compound sets.[46,47] Most methods assume a neutral state of the compounds for calculation, thus differences in the predicted values may be a result of how charges are generally handled. Additionally, the slightly better performance of ACD/Percepta, in comparison with KOWWIN, may be due to the larger number of fragments and correction factors considered in ACD/Percepta.[15,47] All models under investigation are based on atom/fragment contribution methods introducing correction factors among others and account for interactions between individual fragments. Only three commonly used, easily accessible, QSPR models are considered here, while the number of publicly available models is constantly increasing.[47] Thus, to evaluate the general performance of individual models, those with other underlying mechanisms should be included. Property-based methods such as linear solvation energy relationships or methods relying on 3D structures of molecules (e.g., COSMO-RS) may show increased predictive power, particularly, for ionizable, multifunctional analytes as those assessed in this study.[47]

For the evaluation of environmental mobility and sorption behavior of polar, ionizable compounds, K_{ow}/D_{ow} has been criticized several times and other intrinsic compound properties such as the organic carbon−water partition coefficient (K_{oc}) were suggested to be better and more reliable indicators of aquatic mobility.[48,49] First, K_{ow} is not always proportional to, for example, K_{oc} for polar molecules, particularly for those that show additional specific (polar) and/or nonspecific (apolar) interactions.[50] Second, as a proxy for sorption behavior, it largely neglects ionic interactions of ionizable analytes both with ions in solution and ionizable surface functional groups.[48] However, K_{ow}/D_{ow} gives reliable indications on a compound's polarity and thus can be used to further explain the observed interactions in sorption studies or as input for bioaccumulation and toxicity models.

CONCLUSIONS

Overall, the experimental data are of great value for describing the partitioning behavior of ionizable natural toxins. The miniaturized shake-flask approach as such can be used as a reliable method for the determination of log D_{ow} values for polar compounds. The theoretical operation range of $-1.0 < \log D_{ow} < 2.5$ could be extended here to include natural toxins with a log D_{ow} as low as −1.87. Experimental errors are on average smaller than those introduced by commonly used QSPR models. However, the differences between errors of experiments and predictions are only minor. Thus, in cases where an average error of around ± one log unit is acceptable, prediction models can complement the experimental assessment of compound properties. Using QSPR models for prioritization purposes, for example, could narrow down the number of analytes by screening large diverse sets of compounds for those with properties most relevant for further evaluation. Applying models saves a substantial amount of time and resources in comparison to the experimental evaluation of partitioning behavior. However, experiments allow obtaining more detailed insights into the partitioning behavior and would particularly be of added value when the focus is on one specific group of compounds only.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jced.9b01129.

Details on the chemical properties (e.g., CAS number, molecular structure, supplier, etc.) and experimental setup (e.g., λ of measurements, octanol−water ratios, and details on the preparation of buffer solutions); literature and predicted log K_{ow} data; results of error analysis; results of regression analysis of each individual QSPR model (PDF)

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Notes

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