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Minimised Bioconcentration Tests: A Useful Tool for Assessing Chemical Uptake into Terrestrial and Aquatic Invertebrates?

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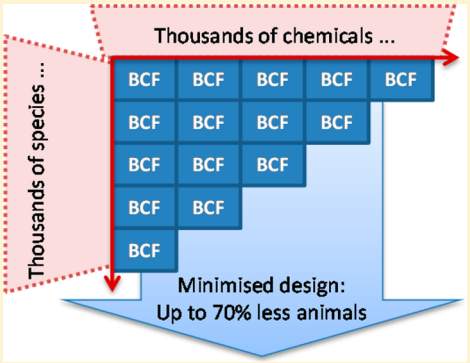
Supporting Information

ABSTRACT: Current guidelines for determining bioconcentration factors (BCF) and uptake and depuration rate constants require labor intensive studies with large numbers of organisms. A minimized approach has recently been proposed for fish BCF studies but its applicability to other taxonomic groups is unknown. In this study, we therefore evaluate the use of the minimized approach for estimating BCF and uptake and depuration rate constants for chemicals in aquatic and terrestrial invertebrates. Data from a range of previous BCF studies were resampled to calculate BCFs and rate constants using the minimized method. The resulting values were then compared to values obtained using full study designs. Results demonstrated a good correlation for uptake rate constants, a poor correlation for depuration rate constants and a very good correlation between the BCFs obtained using the traditional and minimized approach for a variety of organic compounds. The minimized approach therefore has merit in deriving bioconcentration factors and uptake rate constants but may not be appropriate for deriving depuration rate constants for use in, for example, toxico-kinetic toxico-dynamic modeling. The approach uses up to 70% fewer organisms, requires less labor and has lower analytical costs. The minimized design therefore could be a valuable approach for running large multifactorial studies to assess bioconcentration of the plethora of chemicals that occur in the environment into the many taxonomic groups that occur in the environment. The approach should therefore help in accelerating the development of our understanding of factors and processes affecting uptake of chemicals into organisms in the environment.



INTRODUCTION

Synthetic chemicals such as pesticides, pharmaceuticals, personal care products, industrial chemicals, and veterinary medicines can reach the environment and accumulate in biota.[1−3] It is important to study the uptake of these chemicals into nontarget organisms because toxic effects may be induced within the organism and there is the potential for them to be accumulated as they move up food chains.[4,5] A bioconcentration factor (BCF), which reflects the absorption of a chemical into an organism from the ambient environment through respiratory or dermal surfaces,[2,6] is typically used to describe the accumulation of chemicals within an organism. Information on the rates of uptake and depuration of chemicals into organisms is also being increasingly used to understand the impacts of chemicals on organisms e.g. in toxico-kinetic toxico-dynamic modeling.[15]

Concern about bioconcentration of synthetic chemicals in biota has led to the establishment of bioconcentration tests, guidelines and assessment criteria (e.g., OECD 305). For example environmental risk assessment regulations for pesticides, biocides, veterinary medicines, pharmaceuticals and industrial chemicals (e.g., REACH, EU legislation Regulation (EC No 1107/2009),[7] Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA),[8] China REACH[9]) require bioconcentration factors to be measured and these are typically compared to a threshold to determine whether there is a risk of bioaccumulation or not. Bioconcentration studies generally consist of an exposure phase, where test organisms are exposed to a chemical, followed by transfer of organisms to uncontaminated exposure media for a depuration phase. The concentration of the chemical in the organism at different time points during both phases is measured (Figure 1). BCFs can either be derived as the ratio of measured internal concentration and exposure medium concentration when steady state concentrations have been reached in the test organism, or, when steady state has not been achieved, can be estimated from the uptake and depuration rate constants. Generally, bioconcentration studies require a substantial amount of laboratory effort due to the degree of replication that is needed and the sampling frequency during the uptake and depuration phases. For example, fish bioconcentration test guidelines suggest that a minimum of four samples be taken at least five times during the uptake phase and four times during the depuration phase.[10] The rigor of the current guidelines means that large numbers of animals are required and that labor

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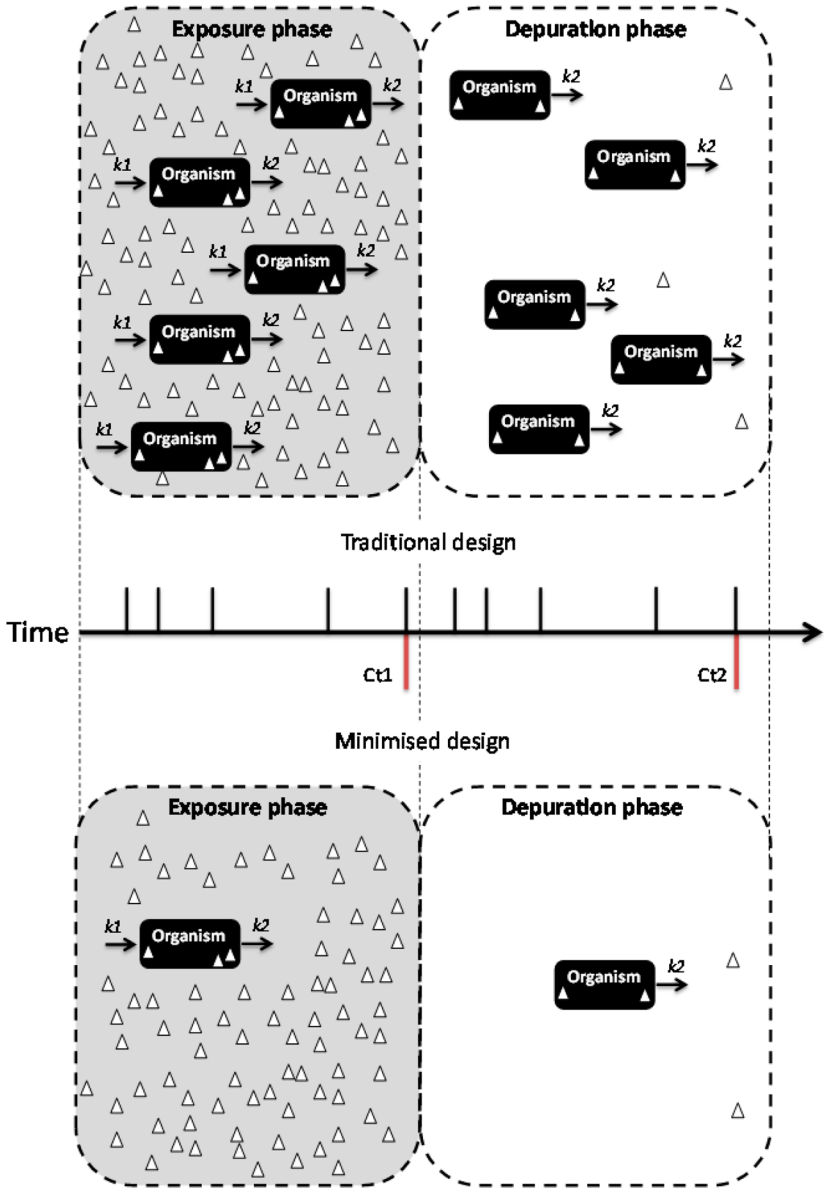

Figure 1. Schematic of an uptake and depuration experiment according to the traditional design (top) and the minimized design (bottom). An exposure phase is followed by a depuration phase. Uptake (k_1) and elimination (k_2) rates represent first order processes. The comparison of sampling dates between traditional design and minimized design illustrates when the organisms are sampled.

and analytical resources are costly. As it is almost physically impossible to perform large multifactor uptake studies using existing guidelines, this may be inhibiting our progress in understanding the factors and processes affecting the uptake of chemicals in the environment such as the effects of ionization of polar substances and species differences.

Recognizing the labor intensity of BCF studies for fish, Springer and colleagues[11] proposed a new minimized test design for the OECD 305[10] and U.S. EPA (850.1730) bioconcentration test guidelines for fish. This design aimed to estimate BCFs using a kinetic definition ($BCF_{minimized}$) which meant that steady state tissue concentrations did not need to be achieved. However, for the approach to work, both uptake and depuration must follow first order kinetics. The proposed design requires that test organisms are collected and analyzed only once at the end of the uptake phase/beginning of depuration (C_{t_1}) and once at the end of the depuration period (C_{t_2}). Water samples are also taken and analyzed on a regular basis throughout the uptake period (C_w/C_{pw}) to calculate an average exposure concentration in the test media. Using simple algebraic expressions (eq 1 and eq 2) uptake and depuration rate constants and BCFs can then be estimated.

$$k_2 = (\ln C_{t1} - \ln C_{t2})/t_d \quad (1)$$

Where $_{t_1}$ and $_{t_2}$ are the beginning and end of the depuration period, respectively. The uptake rate constant (k_1) is then calculated based on the depuration rate constant (k_2) generated from eq 1.

$$k_1 = k_2 \times C_{t_1}/C_{w/pw}(1 - e^{-k_2 t_2}) \quad (2)$$

Where k_2 is depuration rate constant, the mean concentration of the test substance in the medium during exposure phase is C_w (water) or C_{pw} (pore water) and t_u and t_d the length of uptake and depuration periods. A kinetic BCF from minimized design ($BCF_{minimized}$) can then be calculated by dividing the

uptake rate by the depuration rate (eq 3). See Table 1 for a full explanation of parameters used.

$$BCF_{minimized} = k_1/k_2 \quad (3)$$

Table 1. Parameters and Definitions for Minimised Design Equations

parameter	definition	units
k_1	uptake rate	L kg^{-1} d^{-1}
k_2	depuration rate	d^{-1}
C_{t1}	concentration in organism at end of uptake phase	mg kg^{-1}
C_{t2}	concentration in organism at end of depuration phase	mg kg^{-1}
C_w or C_{pw}	mean concentration in exposure medium during uptake phase (water or pore water)	mg L^{-1}
t_d	length of depuration phase	d
t_u	length of uptake phase	d
BCF	bioconcentration factor	L kg^{-1}

Springer et al.,[11] showed that this design uses significantly fewer animals and resources, yet still provides useful BCF estimates for fish. Since this publication a new approach has been adopted for the fish BCF test guideline (OECD 305)[12] which utilizes fewer fish for both cost and animal welfare reasons, similar to that proposed by Springer et al., in 2008. These changes indicate that regulatory agencies are keen to change experimental designs to reduce organism usage.

While the minimized approach has been shown to be valid for fish, to date no-one has explored its wider applicability to other taxonomic groups. Therefore, in this paper, we assess the applicability of the minimized design for estimating BCFs and corresponding uptake and depuration rate constants for chemicals in a range of terrestrial and aquatic invertebrates. We resample existing data sets on uptake and depuration of pesticides and pharmaceutical compounds in aquatic and terrestrial invertebrates to calculate BCFs and rate constants using the minimized method and compare these to the values obtained with the traditional method.

MATERIALS AND METHODS

Collation of Uptake and Depuration Data. Data sets from a number of BCF studies that the author group had participated in were collated. The studies included different periods of uptake and depuration and were performed on different classes of chemicals (Table 2; SI Table 1). Studies were chosen specifically to provide uptake data for a range of invertebrate species while also including a range of compounds with differing physicochemical properties and modes of toxic action and different test matrices (Table 2). For example, the log K_{ow} values of the chemicals in the data set ranged from

Table 2. Summary of Data Collated on Published BCFs (More Detailed Table Can Be Found in Supporting Information)

test species	chemicals tested	number of studies	log K_{ow} range[a]	uptake period (t_u) (days)	depuration period (t_d) (days)	BCF range (L kg^{-1})
Gammarus pulex	beta-blocker, anticancer, antiepileptic, sedative, antidepressant, insecticide, fungicide, herbicide, biocide, algaecide	25	(−0.81) - 5.31	<2	<6	1.64−185 900
Anax imperator	insecticide	1	4.96	2	5	100
Asellus aquaticus	insecticide	1	4.96	2	5	3242
Chaoborus obscuripes	insecticide	1	4.96	2	5	2428
Cloeon dipterum	insecticide	1	4.96	2	5	1782
Daphnia magna	insecticide	1	4.96	2	5	541
Molanna angustata	insecticide	1	4.96	2	5	5331
Neocaridina denticulata	insecticide	1	4.96	2	5	1291
Notonecta maculata	insecticide	1	4.96	2	5	407
paraponyx stratiotata	insecticide	1	4.96	2	5	1601
Plea minutissima	insecticide	1	4.96	2	5	654
Procambarus sp.	insecticide	2	4.96	2	5	280−1295
Ranatra lineariz	insecticide	1	4.96	2	5	392
Culex pipiens	insecticide	1	4.96	2	5	13 930
Sialis lutaria	insecticide	1	4.96	2	5	9625
Planorbarius corneus	beta-blocker	1	3.05	3	3	57.3
Notonecta glauca	beta-blocker, anticancer, antiepileptic, sedative, antidepressant	6	(−0.81)−4.65	2	2	0.13−1.60
Lumbriculus variegatus	antiepileptic, NSAID[b], antidepressant, stimulant, antimicrobial, antibiotic	17	(−0.02)−5.42	2	2	1−700 900
Eisenia fetida	antiepileptic, NSAID[b], antidepressant, weight loss aid	4	2.25−8.19	21	21	2.21−51.53

[a]log K_{ow} as reported in publications (specific log K_{ow} for chlorpyrifos not provided therefore Bowman and Sans (1983) reference used). [b]NSAID, Nonsteroidal anti-inflammatory drug.

−0.81 to 8.19 and the data set covered neutral compounds, weak acids and weak bases. Raw data from these previous studies were obtained; including measured internal concentrations and measured exposure medium concentrations for the duration of the experiment.

All of the studies had used a one compartment first-order toxicokinetic model to simulate the internal concentrations in the organisms using the measured concentrations of the test chemicals in the exposure medium as the driving variable. A more detailed description on how the uptake and depuration rates were calculated, together with the $BCF_{traditional}$ calculations can be found in Supporting Information. The aquatic studies consisted of a water only exposure and therefore the exposure medium was C_w. For the earthworms species, uptake was assumed to come from the pore water (C_{pw}). The estimated $BCF_{traditional}$ (based on the traditional approach) for the chemicals used in the studies ranged from 0.132 to 700 900 L kg^{-1}. A summary of studies used in this analysis is provided in Table 2.

Estimation of Rate Constants and BCFs Using the Minimized Approach. Measured internal concentrations of chemicals in organisms from the last day of uptake and last day of depuration, for each study, were taken from the data sets along with measured data on concentrations of the study compound in the test media during the uptake phase (water or pore water). These data were then used in eq 1 and eq 2 to re-estimate the uptake and depuration constants and then the $BCF_{minimized}$ values. BCFs generated from using this approach ($BCF_{minimized}$) were subsequently compared to those previously published in literature ($BCF_{traditional}$) to assess the applicability of the minimized design to estimate BCFs in a range of invertebrate species.

It should be noted in the Springer approach,[11] 28 days was used for t_u and t_d was 14 days. If the original study consisted of different time periods then the measurements were rescaled and interpolated from reported measurement to provide the 28 and 14 day measurements, respectively. For the purposes of recalculating BCFs in this study, the length of the uptake and depuration phases remained as they were in the original experiment (Table 2). This is an important difference, because it allowed us to test if the minimized design method is also applicable when much shorter experiments are used.

Statistical Analysis. The (log) $BCF_{traditional}$ and the (log) $BCF_{minimized}$ were plotted against each other in a correlation plot (Figure 2) and linear regression was performed. As both X (log $BCF_{minimized}$) and Y (log $BCF_{traditional}$) were subject to error, linear regression was fitted as a Deming (or Model II) regression. For the slope the null hypothesis (H_o) was that the slope is equal to zero while the alternative hypothesis (H_a) was that the slope is significantly different to zero. We also tested if the slope was significantly different from 1 (i.e., if confidence interval of slope includes 1), because a slope of 1 indicates perfect correlation between the two methods. For the intercept, the 95% confidence interval was used to test the hypothesis that the intercept equals zero. The hypothesis was accepted if the confidence interval for the slope contained the value zero whereas if the interval was significantly different from zero then the hypothesis was rejected. Separate correlations were also made between the uptake ($k_{1\ traditional}/\ k_{1\ minimized}$) and depuration rate ($k_{2\ traditional}/\ k_{2\ minimized}$) constants as well as individual data sets used in the analysis using Deming regression (Figure 2, SI Figure 1).

RESULTS

Uptake and Elimination Rates. Comparison of the rate constants obtained using the minimized (k_1 and $k_{2\ minimized}$) and traditional (k_1 and $k_{2\ traditional}$) approaches showed that generally there was a good correlation between uptake rate constants obtained using the minimized and traditional approaches, although the minimized approach appears to result in smaller predictions than the full approach where uptake rate constants are low (Figure 2a). Only a weak correlation was seen between the depuration rates derived using the minimized and full approaches (Figure 2b). For both the uptake and depuration rate correlations the regression line was significantly nonzero ($p < 0.0001$) however the slope was closer to 1 for the uptake rate (95% confidence interval: 0.8384−1.011; Figure 2a) than the depuration rate (95% confidence interval: 1.349−2.208; Figure 2b).

Only 41.7% of studies included in this analysis had reached steady state in the exposure phase duration. However, there is no relationship between the ratio of rate constants ($k_{1\ minimized}/k_{1\ traditional}$) and the percentage of steady state reached in each phase (SI Figure 2). As the percent steady state reached increases, the ratio remains variable around 1 and when 100% steady state had been achieved the greatest divergence around $k_{1\ minimized}/k_{1\ traditional}$ was noted. Thus, the minimized design yields similar rate constants to the traditional design when the duration of the experiment is less than required for steady state to be reached in the exposure and depuration phases, but also yields less reliable rate constants when study lengths approach steady state.

Bioconcentration Factors. BCFs could not be estimated using the minimized approach when the concentration in the organism at the end of depuration phase was greater than that measured at the end of the uptake phase. This occurred in a number of aquatic studies (SI Table 2). Some chemicals particularly where the $BCF_{traditional}$ was very high e.g. triclosan (Table 2) have very slow or nonexistent elimination of the chemical during the depuration phase. Due to the nature of these experiments there is the possibility of experimental variability between the replicates which may manifest itself as an increase in concentration in the organism when an average is used. In total, 60 BCF values could be used from the $BCF_{traditional}$ and compared to $BCF_{minimized}$ estimates.

Deming regression analysis demonstrated a statistically significant relationship between BCF values obtained using the traditional and minimized approaches (Figure 2). The slope of the regression line was significantly nonzero ($p < 0.0001$) and the hypothesis that the slope is equal to 1 was not rejected (slope: 0.99, 95% confidence interval: 0.915−1.075) suggesting there is a significant linear relationship between the two variables (Figure 2). The intercept of the regression is also close to zero (intercept: −0.14, 95% confidence interval: −0.337−0.055). Thus, the $BCF_{minimized}$ estimates are in agreement with the $BCF_{traditional}$ values and there are no systematic differences between the two methods. Specifically, 98% (97%; 65%) of the minimized design BCF values fall within a factor of 10 (factor 5; factor 2) of the $BCF_{traditional}$ values (Figure 2).

In comparison to the BCF regression there was a relatively weak correlation between the depuration rate constants generated by the minimized design and the original data (Figure 2b). The k_2 minimized values (Figure 2b) cover approximately 2 orders of magnitude in comparison to the k_1

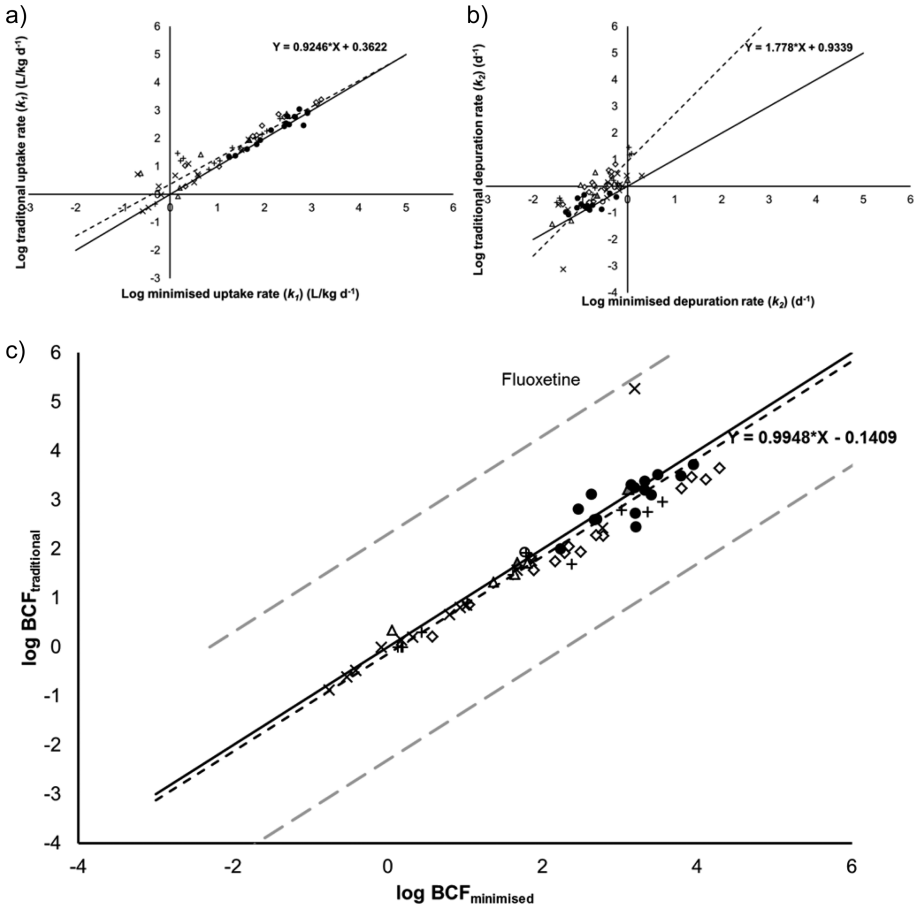

Figure 2. (a) Regression between uptake rate (k_1) from minimized design and k_1 provided in literature. (b) Regression between depuration rate (k_2) from minimized design and k_2 provided in literature. (c) Relationship between log BCF$_{minimized}$ estimates from the minimized design and log BCF$_{traditional}$ obtained from the literature. Aquatic data include Ashauer et al., 2006 (△); Ashauer et al., 2007 (○); Ashauer et al., 2010 (◇); Rubach et al., 2010 (●); Meredith−Williams et al., 2012 (×); Karlsson et al., 2013 (+) and terrestrial data Carter et al., (2014) (△). Deming regression line (black dash), with equation and 1:1 line (solid) with factor of 10 (gray dash) also provided.

minimized values (Figure 2a) which cover approximately 4 orders of magnitude. Therefore, even though the variation around the k_2 1:1 line appears greater than for k_1 and BCF (Figure 2a and c) the smaller range of the k_2 values appears to have minimal effect on the resulting BCF calculation (SI Table 3). The error in k_1 and k_2 parameter estimation covaries in the same direction and therefore cancels out in the BCF. This is demonstrated in SI Figure 3 where most of the data points that show a large difference between the traditional and minimized method fall in the top right quadrant which means that the minimized method generally underestimates both k_1 and k_2. Therefore, as BCF$_{minimized}$ is a ratio of k_1 and k_2, it appears the respective inaccuracies of the $k_{1\ minimized}$ and $k_{2\ minimized}$ compensate each other to calculate accurate, if not slightly overestimated, BCF$_{minimized}$ values.

It has been suggested that estimates of BCF$_{minimized}$ may not be accurate if the uptake and elimination kinetics differ greatly from first order.[11] Some data sets used in this analysis exhibited small but systematic deviations from first order toxicokinetics. However, the significant correlation between the BCF$_{traditional}$ and BCF$_{minimized}$ suggests that the BCF$_{minimized}$ results are in fact robust against slight deviations from first order toxicokinetics.

In a number of experiments the concentration of the chemical in the exposure media decreased (<72% of initial concentration), a result perhaps of dissipation from the test vessel or uptake into the organism. While in the depuration phase, it was not uncommon for the chemical to reappear in the exposure medium. Equation 2 assumes that the concentration in the exposure medium is constant over time, however in practice the minimized approach appears to be robust enough to changes in exposure medium concentration as the degree to which the exposure medium concentration changes does not affect the BCF very much (SI Figure 4). This is similar to the findings of Springer et al.,[11] where BCF$_{minimized}$ estimates were comparable to the BCFs obtained following the OECD guidelines[10] when the coefficient of varation for the mean water concentration was less than 20, which was the case for all the data analyzed in this study.

Therefore, it appears that there are no systematic errors in BCF calculation if changes in exposure medium concentration are observed, uptake is not entirely first order or if steady state has not been achieved in the test system. This is important because it demonstrates the robustness of the design. When steady state does not need to be reached this means that experiments can be shorter which reduces the costs and time of the tests.

Minimized Further? Further analysis explored whether the minimized design could be further reduced compared to the approach of Springer et al.[11] Instead of taking an average of several measurements of exposure concentrations during the exposure phase, which can be up to eight sampling points for one study, an average was calculated by only using

concentrations in the exposure media from the start and end of the exposure phase. Using an average of these two sampling points yielded comparable $BCF_{minimized}$ values to those when a full average was used (SI Figure 5). Deming regression demonstrates that there are no systematic differences between the two approaches (slope: 0.918, 95% confidence interval: 0.799−1.036; intercept: 0.091, 95% confidence interval: −0.199−0.380). These results suggest that it is possible to use an even further optimized test design to calculate accurate BCFs using considerably fewer materials. However, it is important to note that for the studies included in this analysis a single dose was added to the exposure medium at the beginning of the uptake phase. Reducing the exposure medium sampling points may not be appropriate for all test systems, e.g. when the ratio of organism to medium volume is very different or other dissipation processes dominate, and additional analysis is required to explore this.

Study Specific Analysis. Separating the analysis into study specific plots (SI Figure 1) provides further insight into the relationship between $BCF_{traditional}$ and $BCF_{minimized}$. In the $BCF_{traditional}$ data set, some of the smallest BCFs were obtained in the Meredith − Williams et al.,[16] study for *Notonecta glauca*, and while the minimized design predicted correspondingly low BCFs for *N. glauca*, 80% of these were overestimated. This fits with the overall trend as the minimized design generally overestimates BCFs in comparison to the original published BCFs (Figure 2). Comparatively some of the largest BCFs in the Meredith − Williams et al., study[16] study (<218 500 L kg^{-1}) were underestimated by up to 2 orders of magnitude (Figure 2).

The wide suite of chemicals analyzed in the Ashauer et al. study[13] demonstrates that the minimized design is a fairly robust way to estimate BCFs with limited laboratory effort as all of the data fit very well to the 1:1 line. Specifically for the Ashauer et al., 2010 data set the slope of the regression line was significantly different from zero (p = < 0.0001) and thus showed a significant relationship between the two methods for calculating BCFs (SI Figure 1). Collation of all *G. pulex* data from separate publications into one regression also demonstrates a significant relationship between the two methods specifically for this species (slope: 1.085, 95% confidence interval: 0.850−1.320; intercept: −0.383, 95% confidence interval: −1.001 to 0.235) (SI Figure 6).

Data from Carter et al., (2014)[17] also reveals that the minimized design accurately predicts BCFs for the earthworm species, *E. fetida* (SI Figure 1).This is interesting because the approach was originally designed for aquatic BCF calculation but results presented here demonstrate that it is also probably suitable for terrestrial BCF calculations. There is a substantial lack of earthworm studies on the toxicokinetics of pharmaceuticals in particular and the minimized design may be an attractive option to resolve this issue.

While there is a small amount of variation in data points around the 1:1 line and regression line for the Rubach et al., data can be attributable to the wider variety of test species which were used in this study.[18] Specifically differences in BCFs among 15 species of freshwater arthropods as well as between juvenile and adult species (*G. pulex* and *Procambarus* sp.) were observed in the original data set (SI Figure 1). Similarly variation in the Karlsson et al.,[19] data set is due to changing exposure medium pH affecting ionizable chemical uptake (SI Figure 1). The minimized approach seems to account well for assessing uptake of chemicals with different degrees of ionization and into different species as the variation in the BCF values is within the general noise of the whole data set. In view of the complete data set it is evident that the variation around the 1:1 line increases as the BCF value increases (Figure 2). It appears that larger BCFs are subject to greater error.

DISCUSSION

One of the most significant findings is that the minimized design appears to work well across a range of species, including both terrestrial and aquatic organisms, and media and test conditions. As the minimized design yields very good proxies for BCFs it offers an approach to calculate BCF values for regulatory purposes where risk assessments require BCFs to be reported within a range (Figure 3). A majority of the BCFs

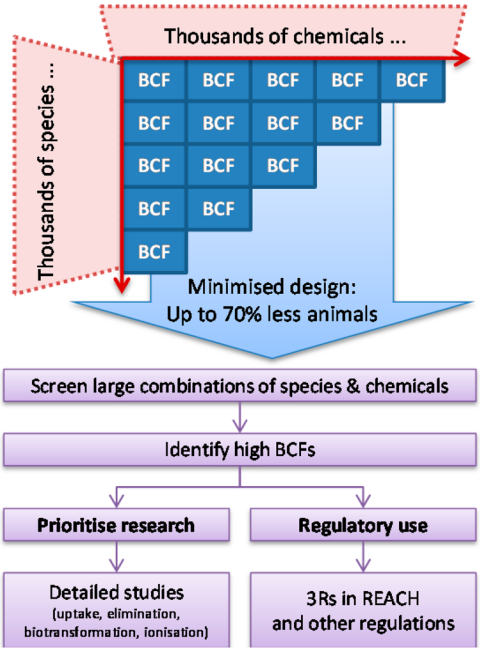

Figure 3. Schematic depicting potential applications of minimized design from both a regulatory and research perspective.

included in this analysis fall below the thresholds of 2000 L kg^{-1} for "bioaccumulative" or 5000 L kg^{-1} for "very bioaccumulative" in the Persistence Bioaccumulative and Toxic (PBT) classification. Therefore even with the differences between BCFs obtained using the two methods, the minimized design appears useful as a first screening step. For a single experiment, test organism usage would be reduced by ca 70% and there would also be a reduction in the experimental material and labor efforts required.

As predictions of depuration rate constants were less accurate in the minimized design, the use of the minimized approach for generating data for use in toxicokinetic-toxicodynamic modeling, which require data on rates, may not be appropriate. A lack of relationship between depuration rates is important because the minimized design uses k_2 to calculate the uptake rate constants (k_1). In this analysis, *G. pulex* exposure to 4-nitrobenzyl-chloride resulted in depuration rates of 3.16 d^{-1} and 0.043 d^{-1} which corresponds to uptake rate constants of 582 L kg^{-1} d^{-1} and 262.97 L kg^{-1} d^{-1} for the traditional and minimized calculations, respectively. Depuration rate costants are also important because they can influence the calculations of time to reach steady state. Using the 4-nitrobenzyl-chloride

example above depuration rates of 3.16 d^{-1} and 0.043 d^{-1} for the original and minimized calculations respectively corresponds to either 3 or 0.41 days to reach steady state within the organism. The minimized approach may therefore not generate data applicable in toxicokinetic-toxicodynamic modeling but would allow for identification of high BCFs and aid in prioritisation of further research. Care should also be taken when using the minimized design to calculate BCF values when compounds do not depurate from the organism. However, it is clear that the minimized design has potentially wide reaching impact as it offers a much more efficient approach for bioconcentration testing compared to current standard practice.

A major research challenge is that a huge number of species in the environment is exposed to thousands of synthetic chemicals in the environment. Additionally processes such as ionization (pH dependence),[19,20] biotransformation and species differences[16,18] further contribute to the factorial explosion in determining internal exposure to chemicals and subsequent risks. The minimized design allows us to address this combinatorial challenge as multifactorial bioconcentration studies now become feasible, especially when combined with multiresidue chemical analytics. As results would be cheaper and faster to generate the minimized design should allow us to better understand the uptake of the numerous chemicals that occur in the environment into the thousands of species that are exposed to these chemicals under a plethora of environmental conditions (Figure 3).

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and tables, including data removed from analysis, author specific Deming regression analysis and relationships between uptake rate and percentage of steady state reached in uptake and depuration phase for each experiment are provided. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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