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Chapter 4

Optimizing Quantitative Structure-Activity Relationships for the toxicity of chlorinated compounds to soil organisms taking into account the dynamics of bioavailable concentrations

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Abstract

Recently, we developed quantitative structure-activity relationships (QSARs) for the toxicity to *Folsomia candida* of eight chlorobenzenes (CB) and four chloroanilines (CA). These QSARs used freely dissolved soil interstitial water concentrations determined by solid-phase microextraction (SPME), and corresponding with the EC_{10} and EC_{50} for effects on reproduction. Chemical concentrations however, may change over time and so may the available concentration, although not necessarily proportional to the change in the total concentration. QSARs relating toxicity to initial concentrations therefore may not be accurate. In this study, for the same chemicals SPME-extracted available concentrations in standardized natural LUFA2.2 soil were determined at three time points over the 28-day duration of the *F. candida* reproduction test. Toxicity based on actual available concentrations was negatively correlated with lipophilicity for the CB but showed no consistent trend for the CA. Over time the significance of the QSARs for the CB decreased due to differences in loss rate of the interstitial water concentrations of different compounds, which was highest for 1,3,5-trichlorobenzene (80%). 3,5-Dichloroaniline also showed a strong decrease (30%) of available concentrations. Interstitial water concentrations of compounds with high $\log K_{ow}$ were stable. For the CB, optimized QSAR models were developed based on the geometric mean of measured concentrations, representing actual exposure over the 28-day test period. This study shows that measured available concentrations over time can be used to develop and optimize QSARs and identify compounds with deviating exposure patterns and consequent deviating risk potentials.

Introduction

Quantitative structure-activity relationships (QSARs) developed for terrestrial ecotoxicology and risk assessments traditionally rely on the determination of the total soil contaminant concentration or are based on initial nominal concentrations (Van Gestel et al., 1991). From the total concentrations in the soil available concentrations in the interstitial water can be estimated (Van Gestel and Ma, 1988). However, factors determining the dynamics of compound concentrations over time, such as sorption, volatilization, and biodegradation, will determine the actual available concentration and therefore affect the outcome of toxicity tests. Studies on the time-dependence of bioavailability and effects of aging of organic pollutants indicate that (ir)reversible fate and losses occur with different chemical- and soil-specific kinetics in soils and therefore need careful attention for risk estimations (Alexander, 2000; Carmichael et al., 1997; Chamignon et al., 2008; Styrishave et al., 2008; Ten Hulscher et al., 1999). Measuring concentrations in the soil interstitial water at different time points automatically accounts for the various losses during the exposure of test organisms. In this way, the dynamics of the actual freely dissolved concentration may be determined.

The development of depletive and non-depletive techniques, successfully applied in soil, increased the potential for measuring available interstitial water concentrations of organic chemicals in soils and sediments with much greater accuracy and increased indicative value for bioavailability (Leslie et al., 2004a; Leslie et al., 2004b; Ouyang and Pawliszyn, 2006; Van der Wal et al., 2004a). Passive samplers such as solid-phase micro-extraction (SPME) fibers have been used for QSAR development (Giesen et al. 2012; Giesen et al. submitted) or to compare exposure concentrations in different soil types (Styrishave et al., 2010; Styrishave et al., 2008). The uptake of a chemical into the polymer of a passive sampler is governed by its fugacity in the water and the hydrophobic phase (soil organic matter) of the substrate matrix, and may be a reliable substitute for soil biota (Ter Laak et al., 2006a; van der Wal et al., 2004b). With the ongoing optimisation of thin polymer coated (<30 μm) SPME fibers, incubated in soil slurries to enhance equilibration, it is nowadays even possible to measure available concentrations of super-hydrophobic substances. Compound series of closely

related congeners can now be measured to the full range of their lipophilicities.

Many toxicity models are based on effect concentrations, e.g. concentrations causing 10 % (EC_{10}) or 50 % (EC_{50}) reduction of the reproduction of the test organism. The accumulation of organic compounds in membranes causes narcotic effects with the final disruption of the cell function (Leslie et al., 2004b). Effects occur when the internal concentration reaches a critical level and specific defence mechanisms have to be staged. In standard toxicity tests with soil organisms like earthworms, enchytraeids and collembolans, during typical test durations of 3-4 weeks effects on reproduction are assessed. But it is not clear from the measured effect on reproduction which life stage of the exposed organisms is affected as tests include exposure of different life stages. Tests usually start with late-stage juveniles or early-stage adults that produce eggs or cocoons, while the final endpoint is the number of hatchlings. All these life stages are exposed, but may react differently to the compounds. In addition, the exposure level may change during the test due to changes in total or available chemical concentrations. This makes models used to describe or predict toxicity, such as QSARs, prone to uncertainty due to changes in exposure, especially if compounds have different dissipation rates from the test system.

Arthropods like the springtail *Folsomia candida* are common terrestrial test organisms and have been used for soil QSAR development (Droge et al., 2006). These animals have a higher sensitivity to many organic pollutants than e.g. earthworms, which makes them an optimal indicator species for risk assessment. Furthermore, they do not ingest soil particles to a great extent and the interstitial water is the main exposure route.

In this study standardized natural LUFA2.2 soil, used in toxicity tests, was spiked with concentrations corresponding with the EC_{10} and EC_{50} for effects on the reproduction of *F. candida*, obtained from earlier toxicity tests (Giesen et al. 2012; Giesen et al. submitted). Concentrations in the soil interstitial water of two compounds series, eight chlorobenzenes and four chloroanilines, were measured using SPME at day zero, after 14 and 28 days covering the duration of a standard toxicity test. QSARs were developed based on the measured compound concentrations at the respective time points and their geometric mean with the main goal to optimize QSAR models for environmental risk assessment.

Material and Methods

Test compounds

Chlorobenzene test series: The selected chlorobenzene series included all three trichlorobenzene isomers (1,2,3-, 1,2,4-, and 1,3,5-TrCB), all three tetrachlorobenzene isomers (1,2,3,4-, 1,2,3,5-, and 1,2,4,5-TeCB), pentachlorobenzene (PeCB), and hexachlorobenzene (HCB). Compounds were purchased from Sigma-Aldrich, except for 1,2,4-trichlorobenzene, which was obtained from Acros Organics. The minimum purity was 98%.

Chloroaniline test series: Test compounds included 3,5-dichloroaniline (3,5-DCA) ordered from Aldrich, 2,3,4-trichloroaniline (2,3,4-TrCA) from TCI Europe, 2,3,5,6-tetrachloroaniline (2,3,5,6-TeCA) from Chemos and pentachloroaniline (PCA) from Pestanal.

All compounds had a purity of at least 97%.

Physico-chemical properties of test compounds are given in Table 1.

Test soil

Oven-dried (60°C for 24 hours) standardized natural LUFA2.2 soil (Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer, Germany), classified as a sandy loam (particle size distribution: 50-2,000 µm, 75.3%; 2-50 µm, 16.6% and < 2 µm, 8.1%) with 2.2±0.2 % organic carbon, was used. Soil pH-KCl was 5.5±0.4 and the maximum water holding capacity (WHC) was 44% (w/w).

Soil treatment and experimental design

LUFA2.2 soil was spiked up to the nominal EC_{10} and EC_{50} concentrations based on previous concentration-response analysis (Giesen et al. 2012; Giesen et al. submitted) using acetone as carrier solvent. A pre-spiking step was introduced in which ten percent of the total dry soil was spiked with 10 ml of the compound solution, shaken for one hour and stored over night in the dark. After mild air stream acetone evaporation, by opening the Teflon lids of the glass jars, the remaining soil was added, thoroughly mixed and moistened up to 50% of the WHC with demineralised water. To allow equilibration, jars were closed with methanol-cleaned screw caps and stored in the dark at 20 °C. Within 12 hours the first SPME measurements were started. Between single SPME measurements and at the end, after 28

days, each jar was aerated and controlled for water loss, which when necessary was replenished with demineralised water once a week.

Table 1. Selected test compounds and relevant properties: Chemical Abstract Service-number; molecular weight, organic carbon-water partition coefficient (log K_{oc}); octanol-water partition coefficient (log K_{ow}); and polyacrylate fiber-water partition coefficients ($logK_{fiber}$)

Chemical	CAS no.	MW	$logK_{oc}$	$logK_{ow}$	$logK_{fiber}$
Chlorobenzenes					
1,2,3-trichlorobenzene	87-61-6	181.45	3,29	4.13	3.91
1,2,4-trichlorobenzene	120-82-1	181.45	3,15	4.05	3.82
1,3,5-trichlorobenzene	108-70-3	181.45	2,85	4.18	3.83
1,2,3,4-tetrachlorobenzene	634-66-2	215.89	3,2	4.64	4.51
1,2,3,5-tetrachlorobenzene	634-90-2	215.89	3,59	4.65	4.39
1,2,4,5-tetrachlorobenzene	95-94-3	215.89	3,64	4.60	4.35
Pentachlorobenzene	608-93-5	250.34	3,5	5.18	4.96
Hexachlorobenzene	118-74-1	284.78	3,99	5.73	5.5
Chloroanilines					
3,5-dichloroaniline	626-43-7	162.02	2.49	2.90	3.25
2,3,4-trichloroaniline	634-67-3	196.46	3.03	3.68	3.82
2,3,5,6-tetrachloroaniline	3481-20-7	230.91	3.94	4.46	4.59
pentachloroaniline	527-20-8	265.35	4.62	5.08	5.09

SPME application

SPME measurements were performed in quadruplicate for each compound after 0, 14 and 28 days, by mixing 2 grams of soil and 6.5 ml demineralised water containing 0.01 calcium chloride and 25 mg/l sodium azide in 10 ml amber-coloured autosampler vials. After shaking the soil slurries, one 2 cm long and 30 μm thick polyacrylate-coated SPME fiber (Poly Micro Industries, Phoenix, USA) was introduced into each vial. Vials with fibers were incubated for 4 weeks for each time point on a "Rock-and-Roller" apparatus (Snijders Scientific) in the dark. Finally, fibers were removed, cleaned with millipore water wetted tissue and transferred into cyclohexane. An internal standard solution (10 mg/l PCB-31 in cyclohexane) was then added.

SPME-GC analysis

Concentrations in the fiber extracts were determined by GC-ECD (GC 8000 gas chromatograph; Fisons Instruments, Milan, Italy), equipped with a CombiPAL autosampler system (CTC Analytics, Zwingen, Switzerland), a Rtx-5 amine column (30 m length, coating thickness 25 μm, internal diameter 0.25 μm), a deactivated fused silica pre-column (length 2 m), and a ^{63}Ni electron capture detector. Chromatographic data were analyzed with Chromcard 1.21 (CE Instruments). Fiber concentrations (C_f) were used to calculate freely dissolved chemical concentrations in the soil interstitial water (C_w^{meas}), using polyacrylate fiber-water partition coefficients (K_{fiber}), published by Giesen et al. (2012) and Giesen et al. (submitted).

Data analyses

Measured interstitial water concentrations for the three exposure periods and their geometric mean were used for linear structure-activity relationship regression analysis. Derived QSARs for both effect concentrations were tested for significance with F-distribution analysis. Statistical analyses were performed in SPSS (Version 15.00; SPSS Chicago, US).

Results

SPME measurement

The measured soil interstitial water concentrations at the EC_{10} and EC_{50} spiking levels for both compound series are given in Table 2. The available concentrations of the lower chlorinated isomers decreased over time, while the higher chlorinated compounds were more or less stable in the water phase. Losses seemed to depend on the $\log K_{ow}$ of the compounds or isomers groups. In between the first and the second and the second and third measurement losses for individual highly chlorinated compounds (> TeCB for CB and > 2,3,4-TrCA for CA) occurred at comparable rates. Also the losses over the complete test period were relatively constant, e.g. around 20% for 1,2,3,5-TeCB for both effective concentrations and total and separate measurements. PeCB and HCB showed losses between 5% and 10%. For the lower chlorinated compounds no specific loss patterns could be identified. For 1,3,5-TrCB total losses over 28 days were consistently high with over 82% (EC_{10}) and 85% (EC_{50}) of the available concentration disappearing in 28 days. Its isomers showed losses between 20 and 60% for the EC_{10} and around 40% for the EC_{50}. The chloroanilines showed a similar behaviour, with the biggest loss occurring for 3,5-DiCA with 53% (EC_{10}) and 43% (EC_{50}). 2,3,5,6-TeCA concentrations remained constant over the 28-day incubation period, while losses for TriCA (EC_{10} 17%; EC_{50} 15%) and PCA (EC_{10} 8%; EC_{50} 5%) were comparably small.

Table 2. SPME measured concentrations in interstitial water of LUFA2.2 soil spiked with chlorobenzenes and chloroanilines at levels corresponding with the EC_{10} and EC_{50} for effects on the reproduction of *Folsomia candida.* Measurements after 0, 14, and 28 days of incubation. Concentrations are given in µgl/l with corresponding standard deviations (n=4).

	EC_{10}						**EC_{50}**					
	t=0		t=14 days		t=28 days		t=0		t=14 days		t=28 days	
Compounds	average	stdev	average	stdev	average	stdev	average	stdev	average	stdev	average	stdev
Chlorobenzenes												
1,2,3-TrCB	709.4 [a]	56.2	625.4	36.3	534.5	25.8	1426.9 [a]	47.1	1051.4	41.9	831.8	18.8
1,2,4-TrCB	394.6 [a]	24.4	195.5	10.1	149.4	9.4	724.1 [a]	34.0	630.5	50.5	435.8	17.7
1,3,5-TrCB	672.4 [a]	101.1	307.9	32.6	120.4	7.7	791.9 [a]	52.8	238.2	10.5	111.1	4.7
1,2,3,4-TeCB	290.0 [a]	7.7	262.2	7.6	226.4	2.3	358.7 [a]	18.6	306.5	13.0	267.3	10.8
1,2,3,5-TeCB	99.3 [a]	1.3	77.5	0.5	64.7	1.4	739.8 [a]	12.0	485.1	4.0	584.6	14.1
1,2,4,5-TeCB	160.1 [a]	10.9	139.4	2.6	123.8	8.2	237.6 [a]	8.4	223.0	3.4	205.6	2.7
PeCB	71.8 [a]	2.0	66.5	0.8	63.3	1.2	83.5 [a]	3.0	78.5	1.9	78.7	3.9
HCB	n.m.		n.m.		n.m.		6.8 [a]	0.1	6.7	0.2	6.6	0.2
Chloroanilines												
3,5-DCA	167.8 [b]	9.6	121.0	6.8	79.8	2.6	434.6 [b]	10.6	326.9	13.8	246.7	6.9
2,3,4-TCA	284.3 [b]	15.0	249.0	4.9	234.9	5.4	481.1 [b]	27.6	458.3	9.1	406.6	0.3
2,3,5,6-TeCA	60.2 [b]	2.3	62.4	3.6	60.3	4.8	187.2 [b]	7.6	203.6	4.2	201.9	4.0
PeCA	49.7 [b]	2.0	44.2	1.1	45.6	2.2	104.6 [b]	5.0	100.5	6.6	99.0	2.5

n.m. not measured

[a] From Giesen et al. (2012)

[b] From Giesen et al. (submitted)

QSAR development

QSAR regression equations for the chlorobenzenes, based on measured interstitial water concentrations at the three sampling times, are presented in Table 3. The QSARs show a strong decrease in reliability, with R^2 values decreasing from 0.82 at t=0 to 0.48 after 28 days. This can be attributed to the effect of the moderate concentration decreases in the isomer groups of tetra- and trichlorobenzenes together with the excessive loss of 1,3,5-TrCB, with coincidental stability of PeCB and, for the EC_{50}, also HCB (Table 3). For the chloroaniline series, QSARs were not developed due to the relatively small samples size.

Table 3. Equations for quantitative structure-activity relationships relating EC_{10} and EC_{50} levels for the effects of chlorobenzenes on the reproduction of the springtail *Folsomia candida*, based on SPME-measured interstitial water concentrations after 0, 14 and 28 days, with $\log K_{ow}$.

Regression Equation Chlorobenzenes	r^2	F	n	df
EC_{10} mmol/l (t0) = - 0.81 * $\log K_{ow}$ + 2.1 [a]	0.82	23.18	7	5
EC_{10} mmol/l (t14d) = - 0.84 * $\log K_{ow}$ - 1.93	0.66	9.86	7	5
EC_{10} mmol/l (t28d) = - 0.62 * $\log K_{ow}$ - 0.36	0.48	4.72	7	5
EC_{50} mmol/l (t0d) = - 1.36 * $\log K_{ow}$ + 3.44	0.91	60.68	8	6
EC_{50} mmol/l (t14d) = - 1.22 * $\log K_{ow}$ + 2.65	0.85	35.07	8	6
EC_{50} mmol/l (t28d) = - 1.09 * $\log K_{ow}$ + 1.96	0.76	19.20	8	6

[a] From Giesen et al. (2012)

QSAR optimization

The QSAR models shown in Figure 1 are based on the geometric mean of the chlorobenzene measurements at the three sampling times, representing a weighted average of the exposure over the entire 4-week test period.

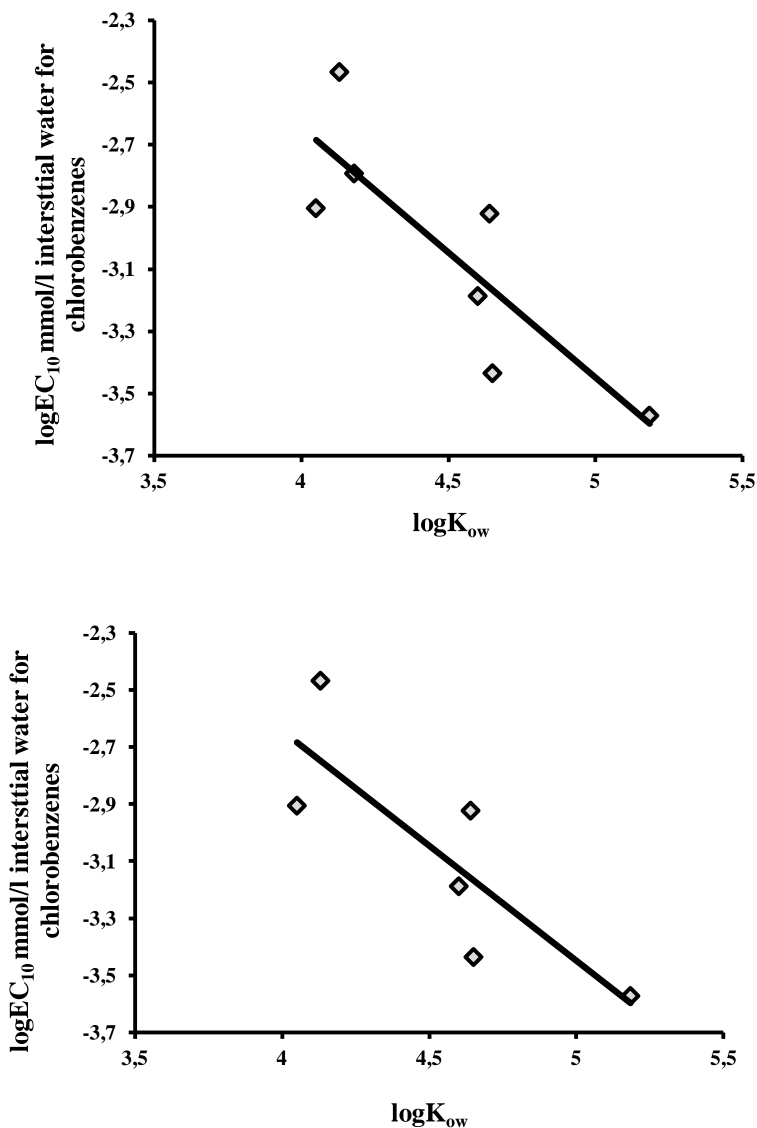

Figure 1. Regression of solid-phase microextraction measured interstitial water concentrations corresponding with the EC_{10} for effects of chlorobenzenes on the reproduction of *Folsomia candida* in Lufa 2.2 soil, averaged over the entire 28-day exposure period, and related to the octanol-water partition coefficients ($logK_{ow}$).

Further optimization of the EC_{50}-QSAR could be accomplished by excluding 1,3,5-TrCB based on its exceptional behaviour (Figure 2a), but exclusion had an opposite effect for the EC_{10} based model (Figure 2b).

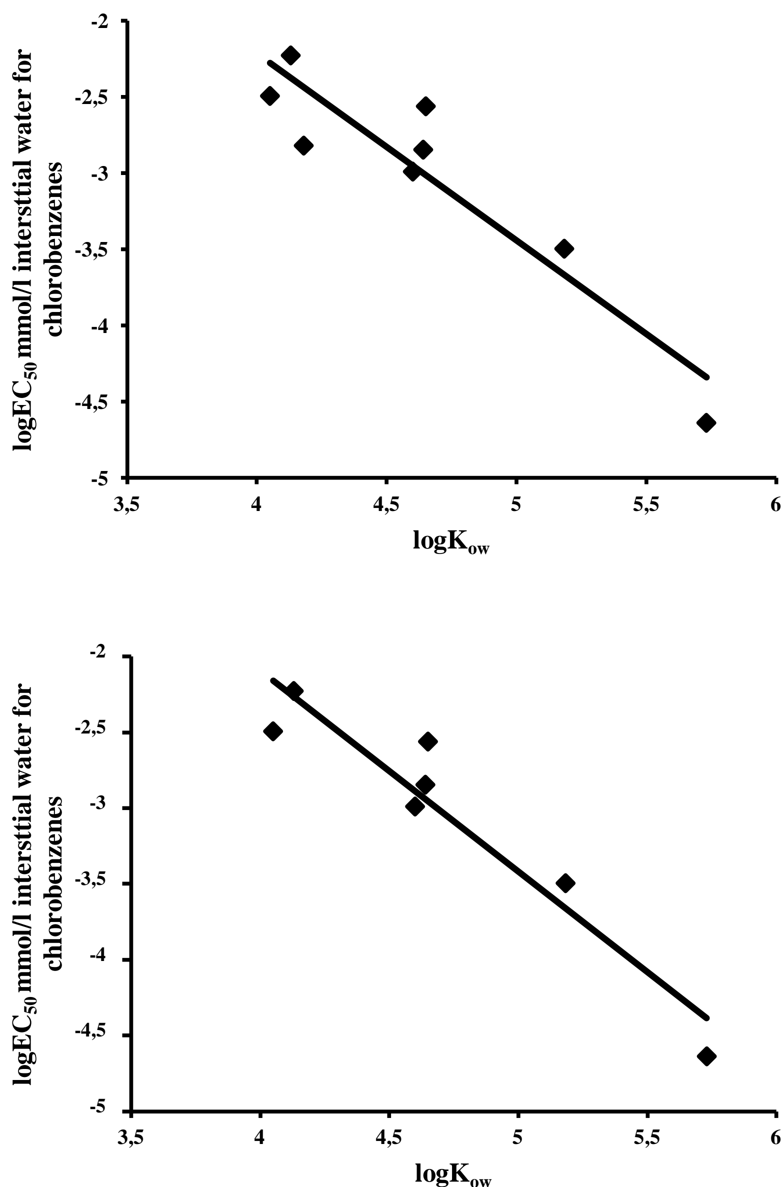

Figure 2. Regression of interstitial water concentrations corresponding the EC_{50} for effects of chlorobenzenes on the reproduction of *Folsomia candida* in Lufa 2.2 soil derived from solid-phase microextraction measurements, averaged over the entire 28-day exposure period, and related to the octanol-water partition coefficients ($logK_{ow}$). Shown are the geometric means of the EC_{50} values for a) all chlorobenzenes; b) with 1,3,5- trichlorobenzene excluded.

This was probably caused by the high concentration of 1,2,4-TrCB, which in this case dominated the isomer cluster instead of the substantial loss of 1,3,5-TrCB. The geometric means of the available chloroaniline concentrations over the 28-day exposure period are presented in Figure 3. No regression model was developed due to the small dataset and the high loss of 3,5-DiCA.

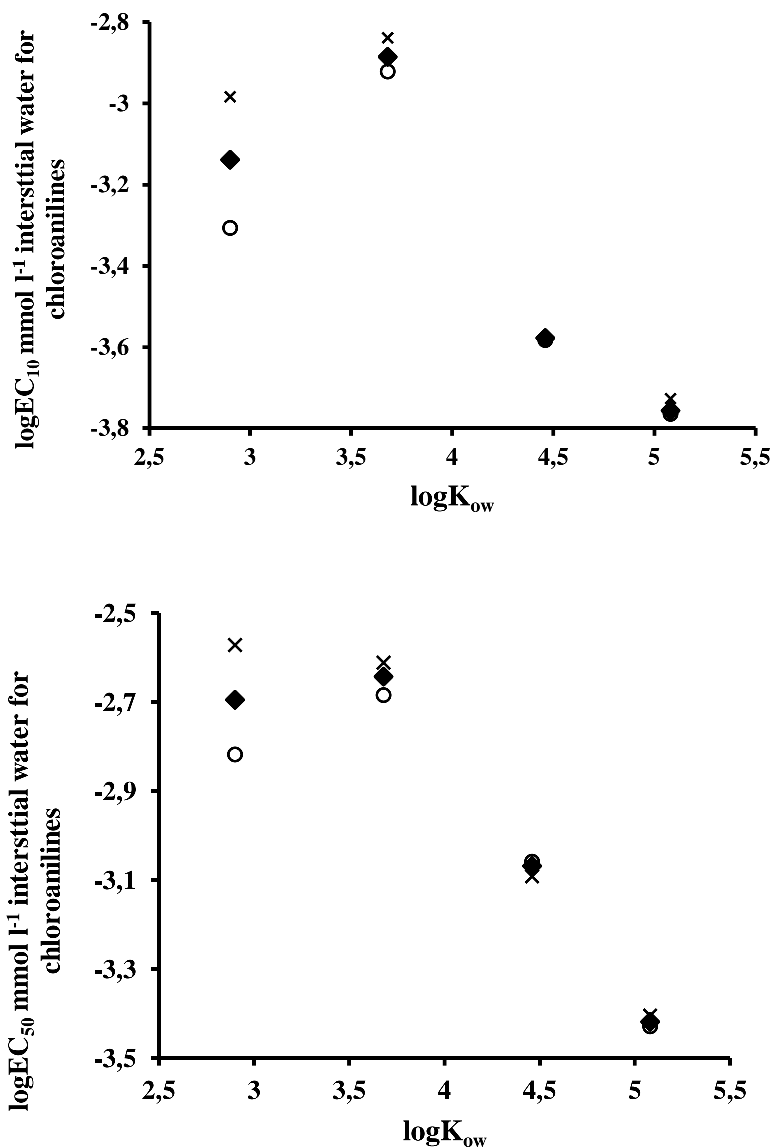

Figure 3. Solid-phase microextraction measured freely dissolved concentrations for a) EC_{10} values and a) EC_{50} values for effects on the reduction of reproduction of *Folsomia candida* of 3,5-dichloroaniline, 2,3,4-trichloroaniline, 2,3,5,6-tetrachloroaniline and pentachloroaniline at t=0 d (black x); at t=14 d (black rhombi) and t=28 d (white circles)

Discussion

Bioavailability in time

The results describe the SPME-estimated bioavailability of chlorinated benzenes and anilines as a time-dependent process. Although this was anticipated and has been reported before for other compounds (Arias-Estevez et al., 2008; Carmichael et al., 1997), the different behaviours of closely related congeners and even of some isomers is surprising. The variation in the physico-chemical properties (e.g. $\log K_{oc}$) within the chlorobenzene and chloroaniline compound series had a profound influence on the dynamics of their availability.

At the extremes within the two test series, 1,3,5-TrCB and 3,5-DiCA showed rapid losses while HCB and 2,3,5,6-TeCA had fairly constant available concentrations. Compound properties, e.g. the $\log K_{oc}$, and soil characteristics (organic matter quality / quantity, microbial community) can explain differences in the dynamics. Low chlorinated anilines have the ability to form covalent bonds with carboxyl groups of bio-organic polymers, such as humic substances (Park et al., 2000). Though most studies on dichloroanilines focused on 3,4-DiCA, the most common breakdown product of phenyl urea pesticides (Giacomazzi and Cochet, 2004), comparable reactions can be expected for 3,5-DiCA. Additional to this passive biotic sink, older studies revealed non-specific reactions of chloroanilines and chlorophenols catalysed by extracellular enzymes, for instance laccase of fungi and bacteria, followed by irreversible integration into the soil organic matter (Park et al., 1999). Chlorinated anilines can also be adsorbed to clay particles (Angioi et al., 2005; Polati et al., 2006) or oxidized on soil-born metal-oxide surfaces (Pizzigallo et al., 1998). It is however, more probable that the majority of the loss can be explained by moderate volatilization from the water phase into the porosphere and the headspace. A compound captured within soil pores may still have an effect on the organism's hydrophobic cuticle but has left the interstitial water and hence becomes undetectable for the SPME.

Based on the porewater hypothesis and the lack of knowledge of additional routes of exposure, however, the pore-born concentration is not bioavailable and therefore 'invisible' for the QSAR. The extreme losses of 1,3,5-TrCB on the other hand are probably the result of biodegradation that

has been described for trichlorobenzenes (Marinucci and Bartha, 1979; Oldenhuis et al., 1989). The vapour pressure for all three TrCB isomers is similar, so are their organic carbon sorption coefficients and any other physical-chemical characteristics that could explain a difference in measured concentrations. Comparative studies on de-halogenation or complete degradation with all three TrCB are rare (Adebusoye et al., 2007). This makes it hard to identify which of the isomers is generally the most conducive to microbial activity. Regardless, the measured loss of roughly 80% of the effective concentration is best explained by an efficient degradation by bacterial and/or fungal communities.

It is hard to determine if the decrease in the bioavailable fraction is due to lower degradability or reduced activity and therefore reduced sorption of the compounds to the soil (organic) matrix. Losses were minor, or did not occur at all, for the nearly or fully chlorinated congeners in both compound series. The marginal losses and fluctuations indicate an (pseudo) equilibrium in which concentrations to which the animals are exposed are constant. The unpredictability of the somewhat higher losses for lower chlorinated compounds on the other hand excludes equilibrium.

SPME measurements for QSAR

Keeping in mind that losses, caused by various factors (sorption, degradation and volatilisation), are the rule rather than the exception, measurement of available concentrations instead of total concentrations is a logical step. Using SPME over exhaustive or depleting extraction methods provides a more precise measure of freely dissolved concentrations that can be integrated into toxicity models. Over the last years this approach has gained credibility as an exquisite surrogate to determine exposure concentrations for a wide range of matrices and research questions (Jonker et al., 2007; Leslie et al., 2002b). Admittedly, QSARs are only rarely based on measured concentrations but rather make use of estimated concentrations. However, the factors influencing the dynamics and behaviour of compounds in a complex test substrate such as soil are notoriously hard to predict compared with, e.g. aquatic environment. With the applications of SPME, outliers can be identified which otherwise would have let to over-or underestimations with potential great impact on further analysis such as toxicity models.

QSAR

Toxicity models for organic compounds generally do not include the losses of organic compounds over time. While for acute tests, like gene-expression analysis, which usually is determined after a 2-day exposure period, the magnitude of losses might still be disregarded, it raises concern in case of chronic toxicity tests with durations of several weeks. QSARs were developed with the assumptions 1) that concentration at the start of the test causes the observable effect, and 2) that the test system is static with a constant exposure of the organisms. But as described earlier (Hurdzan and Lanno, 2009; Leslie et al., 2002a; Leslie et al., 2002b), uptake kinetics are an important factor and it can be argued that compounds with relatively high fugacity excite their effects at an earlier time point than others. Terrestrial tests, unlike aquatic tests, cannot be replenished with toxicants and any loss is irrevocable. Any replenishment of compounds to compensate for the constant loss or the transfer of animals to fresh spiked soils would give rise to pulse effects making the data incomparable to the remaining test series. Integrating the loss into QSAR development, by averaging the measured available concentrations, as done here for chlorobenzenes, compensates for violation of the constant exposure assumption and therefore may represent an optimal approach. Further improvement of the QSAR by excluding 1,3,5-TrCB with its outlier behaviour was only successful for the EC_{50} model, and failed for the QSAR based on EC_{10} values. In the EC_{50} QSAR, the vast decrease of the 1,3,5-TrCB interstitial water concentration determined the slope of the regression, while for the EC_{10} QSAR the high total interstitial water concentration of 1,2,3-TrCB was dominating the regression. Hence the omission has to be interpreted with due consideration not only of the fate but also of the origin of the concentration values. Because 1,2,3-TrCB has a steeper concentration-response curve, in comparison to its isomers, the EC_{10} concentration is relatively high compared to the EC_{50}. This had a stronger effect on the regression than the fast decline of the 1,3,5-TrCB concentrations with time.

Serious concerns regarding the reliability and predictive power of toxicity models arise from the behaviour observed for the chloroanilines. Aniline and monochloroaniline were excluded from the analysis for practical reasons, limiting the series to a minimum. Based on comparable physical-chemical properties, one might expect similar behaviours for aniline and the

lower chlorinated congeners. The result would be a flattened relationship of toxicity with $\log K_{ow}$ for compounds with lower hydrophobicity. In fact, toxicity would be expected to be independent from $\log K_{ow}$ for low chlorinated anilines, whereas for higher chlorinated compounds the QSAR still would be based on the uptake, driven by higher lipophilicity.

Consequences for environmental risk assessment

It is imperative to realize that toxicity of any compound is a process over time, and so is its risk. Yet results of standardized toxicity tests are generally based on initial (nominal) concentrations and do not include dynamics in the environment, hence, over– or underestimating effects. Processes that lead to a loss of bioavailability should be taken into account as they determine the long-term effects and risk assessment. Differences in the stability of interstitial water concentrations clearly need differential evaluation of compounds in the future. Compounds with a slow decrease in available concentrations present a chronic risk to soil biota on individual and population level. For less stabile and/or more volatile compounds the freely dissolved fraction in the soil interstitial water decreases faster. For these compounds toxic effects are mainly caused within the first days of a test and in the course of the test recovery of the test organisms is possible. Furthermore, it is interesting to question the cause of observed effects in standardized toxicity tests with labile compounds. In the *F. candida* test, compounds with a strong decline of bioavailability exert toxic effects only on the animals introduced upon commencement of the test. Stabile compounds on the other hand might exert toxicity also on the eggs or cocoons produced after a week or even on the hatching or developing juveniles which arise after 3-4 weeks. The biological dimension therefore requires attention in future studies, since for the final ecological risk assessment effects at the population or community level are the target. Also with this aim, currently trait-based approaches are receiving increasing interest and may become integral parts in environmental impact assessments. Such trait-based approaches however, cannot do without proper information on the sensitivity of different life stages and therefore require detailed information on exposure dynamics during toxicity tests.