Supporting information to

"Significance of xenobiotic metabolism for bioaccumulation kinetics of organic chemicals in *Gammarus pulex*"

*Roman Ashauer**†, Anita Hintermeister†, Isabel O'Connor†‡, Maline Elumelu†, Juliane Hollender°†, Beate I. Escher†§*

† Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

‡ Radboud University, Nijmegen, The Netherlands

§ The University of Queensland, National Research Centre for Environmental Toxicology (Entox), 39 Kessels Rd, Brisbane, Qld 4108, Australia
°Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Switzerland

* Corresponding author: phone: +41-587655233; fax: +41-587655311;
E-mail: roman.ashauer@eawag.ch

Table of contents

Text SI-1: Sources of ^{14}C-labeled material 2
Text SI-2: Details on metabolite screening tests 2
Text SI-3: Details on biotransformation kinetics experiments 4
Text SI-4: Quantification of aqueous concentrations 8
Text SI-5: Extraction method for HPLC with radio and UV detector 8
Text SI-6: Identification of metabolites in organisms 9
HPLC method with radiodetector and UV detector 9
Sample processing and extraction method for Orbitrap 9
Details of the HPLC-ESI-LTQ-Orbitrap MS method 10
Text SI-7: Contribution of dietary uptake 10
Quantification of amount adsorbed to leaf material 10
Calculation of dietary uptake 11
Text SI-8: Instability of ethylacrylate during extraction and analysis 13
Text SI-9: Comparison with study based on total ^{14}C internal concentrations (details to Figure 5) 14
Additional References in Supporting Information 15

Text SI-1: Sources of ^{14}C-labeled material

The ^{14}C-labelled chlorpyrifos, pentachlorophenol, carbaryl, malathion, aldicarb, carbofuran, imidacloprid were supplied by the Institute of Isotopes, Budapest, Hungary. 2,4-dichloroaniline, 2,4-dichlorophenol, 1,2,3-trichlorobenzene, 4,6-dinitro-o-cresol, 2,4,5-trichlorophenol, ethylacrylate, 4-nitrobenzyl-chloride were supplied by American Radiolabeled Chemicals, St. Louis, USA. Sea-nine (4,5-Dichloro-2-octyl-3-isothiazolone) was supplied by Amersham (GE Healthcare), UK.

Unlabelled material of these compounds was of analytical grade and purchased from Sigma-Aldrich, Buchs, Switzerland, except for Sea-Nine (>97% purity, Rohm and Haas), which was a gift of Christ Chemie AG, Rheinach, Switzerland.

Text SI-2: Details on metabolite screening tests

Limits of detection in aqueous samples were calculated as: LOD = mean counts of blanks + 3 × standard deviation of blanks. The minimum detectable amount (MDA) for concentrations in tissue samples of *Gammarus pulex* using HPLC analysis with radio-detector was calculated according to [2] for each peak. The MDA depends on the peak width and is approximately a factor of three lower for peaks of 0.5 min width compared to peaks of 1.5 min width. Here we give exemplary MDAs for peaks of 1 min width and calculate the corresponding limit of quantification using the average mass of *Gammarus pulex* material in the samples of the metabolite screening test.

Table S1: Limits of detection and quantification, number and mass of *Gammarus pulex* in samples of metabolite screening tests.

Test compound	Number of *Gammarus pulex* per sample	Mean sample wet weight	MDA in samples of *Gammarus pulex* in radio-HPLC	MDA in samples of *Gammarus pulex* in radio-HPLC	Recovery of extraction method (measured with LSC)	Overall recovery of spiked parent compound (measured with HPLC)	LOQ corrected for overall recovery	Total internal concentration expected after 24h [a)]	Factor of expected total $C_{internal}$ to LOQ	Average C_w (mean of 0h and 24h)
	#	mg	dpm	nmol / g $_{wet\ weight}$	%	%	nmol / g $_{wet\ weight}$	nmol / g $_{wet\ weight}$	-	nmol / mL
1,2,3-Trichlorobenzene	4	109.46	87.00	2.81	43	37	7.59	130.89	17	0.701
2,4,5-Trichlorophenol	4	146.41	74.60	0.50	102	95	0.53	296.13	563	0.185
2,4-Dichloroaniline	4	118.19	74.30	1.27	74	66	1.92	239.90	125	4.758
2,4-Dichlorophenol	4	106.84	89.50	0.82	94	93	0.88	1047.02	1187	1.251
4,6-Dinitro-o-cresol	4	130.91	72.70	2.23	108	106	2.10	8.62	4	0.350
4-Nitrobenzyl-chloride	4	144.48	70.90	5.17	96	84	6.15	695.79	113	3.943
Aldicarb	4	168.46	79.80	0.06	97	92	0.07	0.54	8	0.333
Carbaryl	4	207.31	69.80	0.05	101	88	0.06	1.31	23	0.064
Carbofuran	8	258.92	82.20	0.02	97	93	0.02	0.20	9	0.022
Chlorpyrifos	4	173.23	62.60	0.06	98	35	0.17	1.20	7	0.002
Ethylacrylate	4	125.15	70.50	1.49	2	3	49.67	874.94	18	13.444
Imidacloprid	4	144.94	77.30	0.07	104	107	0.07	0.59	9	0.341
Malathion	8	320.18	84.50	0.02	91	77	0.03	0.13	5	0.002
Pentachlorophenol	4	198.2	63.70	0.13	99	96	0.14	5.44	40	0.130
Sea-nine	4	147.56	81.90	0.01	79	45	0.02	12.84	578	0.011

a) Total expected internal concentration is calculated using the average concentration in water C_w: $C_{\text{internal, total}} = k_{in} / k_{out} \times C_w \times \left(1 - e^{-k_{out} \times t}\right)$ and the parameters k_{in} and k_{out} from toxicokinetic studies where total radioactivity was measured in *Gammarus pulex* for these compounds [3-5].

Text SI-3: Details on biotransformation kinetics experiments

The number of organisms per beaker, average weight and age, collection dates and acclimatization times for each experiment can be found in Table S2. Information on dosing and solvents can be found in Table S3 and the measured oxygen, pH and conductivity are listed in Table S4.

Table S2: Additional information on the test organisms in the biotransformation kinetics experiments

Experiment	Start date of experiment	Collection of *Gammarus pulex*	Acclimatization time between collection and experiment (days)	Average wet weight of sample (mg)	Standard deviation (mg)	Number of organisms per sample	Average wet weight of *Gammarus pulex* (mg)	Age [a] for average wet weight (days)	Number of organisms per beaker at start of experiment
2,4,5-Trichlorophenol	16-Jun-10	09-Jun-10	7	186.71	24.37	4	46.68	254	15
2,4-Dichloroaniline	16-Feb-10	05-Feb-10	11	176.70	31.57	4	44.18	241	10
2,4-Dichlorophenol	01-Jun-10	27-May-10	5	191.04	26.22	4	47.76	260	15
4-Nitrobenzyl-chloride	16-Feb-10	05-Feb-10	11	205.09	76.60	4	51.27	285	10
Aldicarb	16-Feb-10	05-Feb-10	11	187.44	46.76	4	46.86	255	10
Carbaryl	06-Jul-10	02-Jul-10	4	144.29	26.35	4	36.07	210	15
Carbofuran	16-Jun-10	09-Jun-10	7	313.62	39.34	8	39.20	221	20
Chlorpyrifos	06-Jul-10	02-Jul-10	4	294.09	57.90	8	36.76	212	20
Ethylacrylate	01-Jun-10	27-May-10	5	194.51	24.51	4	48.63	266	15
Malathion	16-Jun-10	09-Jun-10	7	335.84	47.91	8	41.98	232	20
Pentachlorophenol	06-Jul-10	02-Jul-10	4	152.99	30.27	4	38.25	217	15
Sea-nine	01-Jun-10	27-May-10	5	203.17	33.89	4	50.79	281	15

[a] Average age of *Gammarus pulex* calculated from the average wet weight after [6] under the assumption of equal proportions of males and females.

Table S3: Solvents and dosing in the biotransformation kinetics experiments.

Compound	Solvent	Percentage of solvent in test solution at start of experiment (v/v)
2,4,5-Trichlorophenol	methanol	0.024
2,4-Dichloroaniline	acetone	0.171
2,4-Dichlorophenol	acetone	0.105
4-Nitrobenzyl-chloride	acetone/methanol (40:60)	0.125
Aldicarb	acetone	0.140
Carbaryl	methanol	0.014
Carbofuran	acetone	0.007
Chlorpyrifos	methanol	0.003
Ethylacrylate	acetone/methanol (65:35)	0.290
Malathion	acetone	0.001
Pentachlorophenol	methanol	0.015
Sea-nine	methanol	0.005

Table S4: Oxygen, pH and conductivity in the biotransformation kinetics experiments.

Compound	O_2 [mg / L]	SD	pH	SD	conductivity [µS / cm]	SD	n
2,4,5-Trichlorophenol	3.11	1.55	6.89	0.15	644	9	8
2,4-Dichloroaniline	7.68	2.38	7.14	0.13	574	6	8
2,4-Dichlorophenol	3.90	0.83	6.96	0.12	592	14	7
4-Nitrobenzyl-chloride	7.35	2.76	7.13	0.23	577	6	8
Aldicarb	6.98	1.35	7.20	0.12	575	5	8
Carbaryl	4.16	0.54	7.03	0.12	588	19	7
Carbofuran	2.40	0.78	6.85	0.11	646	7	8
Chlorpyrifos	3.24	0.98	7.05	0.22	594	9	7
Ethylacrylate	4.05	0.57	7.03	0.25	592	8	7
Malathion	2.65	0.95	6.96	0.03	644	6	8
Pentachlorophenol	3.88	0.96	7.02	0.17	582	6	7
Sea-nine	3.90	0.65	7.01	0.20	600	6	7

Table S5: Initial concentrations in biotransformation kinetics experiments related to toxicity.

	C_{water} (at time 0)	24h-LC50 [a)]	Factor between initial C_{water} and 24h-LC50
	nmol/L	nmol/L	
2,4,5-Trichlorophenol	25.75	>2930	114
2,4-Dichloroaniline	5760	57896	10.1
2,4-Dichlorophenol	1652	22112	13.4
4-Nitrobenzyl-chloride	4831	> 30111	6.2
Aldicarb	424	3461	8.2
Carbaryl	66.33	144.12	2.2 [b)]
Carbofuran	21.03	82.44	3.9
Ethylacrylate	12893	> 70248	5.4
Chlorpyrifos	7.19	9.69	1.3 [b)]
Malathion	1.4	> 3.84	2.7
Pentachlorophenol	29.9	21026	703 [b)]
Sea-nine	15.5	122.9	7.9

a) From references[7-9].
b) Note that these are 48h-LC50 values, whereas exposure in the biotransformation kinetics experiment was for 24h only.

Text SI-4: Quantification of aqueous concentrations

Sample processing and quantification of radioactivity in aqueous samples is identical to previous studies [1,5]: Aqueous samples were analyzed immediately by adding 10 mL Ecoscint A scintillation cocktail (National Diagnostics, UK) and counting of radioactivity three times for 10 min on a Packard (Tri-Carb 2200CA, Packard, USA) scintillation counter (LSC). Counts were corrected for background activities using control samples. Color quenching and counting efficiency were corrected using the reverse spectrum transform method and the efficiency tracing technique as implemented in the Packard Tri-Carb 2200 CA based on a built-in external standard [2]. Counts were converted to moles using the known specific activities of the test compounds together with the mixing ratio in case of dosing with mixtures of unlabelled and labeled test compound.

Text SI-5: Extraction method for HPLC with radio and UV detector

The extraction method for analysis of *Gammarus pulex* samples using HPLC with a radio- and a UV-detector follows closely the method developed in [1], with slight modifications for each compound as detailed in tables S3 and S6.

Frozen samples of *Gammarus pulex* were ground with a glass rod after the addition of 1 mL methanol. Another 2.5 mL of methanol were used to rinse the glass rod and added to the sample material. Samples were sonicated in an ultrasonic bath for 3 min and the homogenate filtered (Minisart, 26 mm, pore size: 0.2 µm, hydrophilic, cellulose-, acetate- and surfactant-free membrane). Glass tubes, syringes and filters were rinsed twice with 2 mL methanol which was added to the samples. The sample filtrate was concentrated to about 1 mL at 60°C using a GeneVac (EZ-2 PLUS, Genevac, UK). In a final concentration step the samples were concentrated under nitrogen flow to 90 µL and 210 µL of distilled water were added to obtain a ratio of 30/70 (v/v) methanol to water.

For carbofuran and malathion the method was slightly modified. To achieve sufficient radioactivity two of the four samples from each sampling time were combined, tubes rinsed twice with 1 mL methanol and the combined samples concentrated again to about 1 mL. Hence the final two samples per sampling time comprised a total of eight *Gammarus pulex* per sample. Recovery and extraction efficiency for pooled samples of more than four organisms were insufficient; therefore the samples were extracted separately and combined during the concentration step.

Subsequently samples were split into two aliquots. 100 µL were analyzed by LSC after adding 10 mL Ecoscint A scintillation cocktail and another 100 µL were analyzed by HPLC (HP 1100, Agilent) with a radio-detector (500 TR, Packard) to quantify amounts of parent compound and metabolites (HPLC method in table S6).

Metabolites as well as the parent compound were identified by spiking unlabelled standard material of these to samples of control organisms during the grinding step

and identification of these peaks via UV-detection. Peaks with the same retention time in the chromatogram of the UV-detector and the radio-detector were assumed to originate from identical compounds.

In order to determine the recovery of the extraction method blank organism samples were spiked with a known amount of parent compound at the beginning of the extraction method. Comparison of the spiked radioactivity with the radioactivity measured by LSC yields the recovery of the extraction steps and comparison with the radioactivity measured on the HPLC yields the overall recovery (Table S1).

Text SI-6: Identification of metabolites in organisms

HPLC method with radiodetector and UV detector

Column: Nucleodur C18 Gravity (125x2x5)
Solvent A: Water with 0.1% acetic acid
Solvent B: Methanol with 0.1% acetic acid

Table S6: Details of HPLC method used in metabolite screening tests.

Test compound	Time	% solvent B
1,2,3-Trichlorobenzene	0-8-17-17.5-22.91	40-90-90-40-40
2,4,5-Trichlorophenol	0-9-17-17.5-22.91	30-90-90-30-30
2,4-Dichloroaniline	0-10-17-17.5-22.91	5-90-90-5-5
2,4-Dichlorophenol	0-10-17-17.5-22.91	20-90-90-20-20
4,6-Dinitro-o-cresol	0-10-17-17.5-22.91	30-90-90-30-30
4-Nitrobenzyl-chloride	0-11-17-17.5-22.91	20-90-90-20-20
Aldicarb	0-10-17-17.5-23	5-90-90-5-5
Carbaryl	0-11-17-17.5-22.91	10-90-90-10-10
Carbofuran	0-11-17-17.5-23.13	5-90-90-5-5
Chlorpyrifos	0-6.5-17-17.5-22.91	30-90-90-30-30
Ethylacrylate	0-11-17-17.5-22.91	5-70-70-5-5
Imidacloprid	0-11.5-17-17.5-22.91	5-55-90-5-5
Malathion	0-10-17-17.5-23.13	10-90-90-10-10
Pentachlorophenol	0-5.8-17-17.5-22.91	30-90-90-30-30
Sea-nine	0-7-17-17.5-22.91	40-90-90-40-40

Sample processing and extraction method for Orbitrap

In case the parent compound could not be detected in the pure or diluted *G. pulex* extract using HPLC-ESI-MS the extract was purified by solid phase extraction. Therefore the extract was diluted with acidified water (0.1 % acetic acid) to a methanol percentage of 5 % and passed through a preconditioned Isolute ENV+ SPE cartridge (Separtis GmbH, Germany). After washing with 2 mL 0.1 % acetic acid, the elution was carried out with 4 mL of methanol. The methanol extract was concentrated under nitrogen flow and diluted with distilled water to obtain a ratio of 30/70 (v/v) methanol to water prior HPLC analysis.

Details of the HPLC-ESI-LTQ-Orbitrap MS method

The HPLC system consisted of a quaternary pump of type Rheos 2200 from Flux Instruments (Basel, Switzerland) and a HTS PAL autosampler of CTC Analytics AG (Zwingen, Switzerland). Samples of 60 µL extract or diluted extract were injected into the HPLC system with the same column and the same HPLC gradient as for the method with radio-detection (see above). Detection with the LTQ-Orbitrap mass spectrometer (Thermo, Waltham, MA) was conducted after electrospray ionization in either positive or negative mode. Parameters adjusted for the ion source ESI were source voltage (4.5 kV) and capillary temperature (275°C). The mass spectrometry experiment consisted of a full-scan (resolution set to 60000) within the mass-to-charge range 115-1000 or 50 - 690 and MSMS experiments triggered when peaks were detected in the full-scan at the exact masses of the precursor ion for the parent compound, possible transformation products (two generations predicted by UMPPS, the University of Minnesota Pathway Prediction System, http://umbbd.msi.umn.edu/predict/aboutPPS.html) or the most intense ion. After transfer from the ion trap, fragmentation was achieved with higher energy collision dissociation (HCD) and fragments were detected in the Orbitrap with a resolution of 7500. External mass calibration was used to ensure a mass accuracy of < 5 ppm. Data were analyzed with Xcalibur (Thermo Scientific, USA) and parent compounds were identified with reference standards using retention time and exact masses as criteria.

Text SI-7: Contribution of dietary uptake

After the first 24h of exposure in the biotransformation kinetics experiments (end of exposure phase) leaf material was sampled, blotted dry, weighed and frozen at -20°C until analysis or analyzed immediately.

Quantification of amount adsorbed to leaf material

The amount of test chemicals adsorbed to the leaf material (food) was measured after the exposure phase by sequential extraction with 10 mL of Ecoscint A scintillation cocktail (National Diagnostics, UK) or Soluene-350 and Hionic-Fluor (Aldicarb and 4-Nitrobenzyl-chloride). The extractions were repeated until only a negligible amount remained on the leaf discs (< 5%). Radioactivity counts from all extractions were combined.

Radioactivity was counted three times for 10 min on a Packard (Tri-Carb 2200CA, Packard, USA) scintillation counter (LSC). Counts were corrected for background activities using control samples. Color quenching and counting efficiency were corrected using the reverse spectrum transform method and the efficiency tracing technique as implemented in the Packard Tri-Carb 2200 CA based on a built-in external standard 2. Counts were converted to moles using the known specific

activities of the test compounds together with the mixing ratio in case of dosing with mixtures of unlabelled and labeled test compound.

As it was not possible to "spike" leaf discs mimicking adsorption we could not quantify the recovery of our extraction and quantification method for compound concentrations on leaf material.

Calculation of dietary uptake

The mean concentration on the leaf material during the exposure phase was multiplied with an assimilation efficiency of 0.4 (approximated based on the review by Wang & Fisher[10]) and an average daily feeding rate[11] to estimate total uptake of the test compound via food. The uptake via food was then compared to the uptake via water during the same period and the relative contribution of dietary uptake to total uptake calculated for each compound (Table S7).

Table S7: Concentrations on leaf material in biotransformation kinetics experiments and approximate contribution of dietary uptake relative to total uptake.

	C_{leaf} (at 24h)	SD	n	Apparent [a] $K_{leaf\text{-}water}$	C_{water} (at time 0)	Extraction and scintillation cocktail	Dietary uptake [b]	Uptake from water [c]	Dietary uptake in % of total uptake
	pmol □ g_{leaf}^{-1}				nmol/L		pmol □ d^{-1}	pmol □ d^{-1}	%
2,4,5-Trichlorophenol	1082	260	8	42	25.75	EcoScint A	2.53	1670	0.15
2,4-Dichloroaniline	35474	13873	8	6	5760	EcoScint A	78.4	74562	0.10
2,4-Dichlorophenol	15518	1652	8	9	1652	EcoScint A	37.1	57044	0.06
4-Nitrobenzyl-chloride	17783	6031	8	4	4831	Soluene-350 and Hionic-Fluor	45.6	142667	0.03
Aldicarb	184.6	40.8	5	0.4	424	Soluene-350 and Hionic-Fluor	0.43	196	0.22
Carbaryl	2801	748	8	42	66.33	EcoScint A	5.05	56.0	8.28
Carbofuran	110.7	17.1	10	5	21.03	EcoScint A	0.22	4064	0.01
Chlorpyrifos	1191	350	10	166	7.19	EcoScint A	2.19	396	0.55
Ethylacrylate	14236	3470	8	1	12893	EcoScint A	34.6	75301	0.05
Malathion	24.6	10.3	10	18	1.4	EcoScint A	0.05	0.52	9.02
Pentachlorophenol	1224	372	8	41	29.9	EcoScint A	2.34	739	0.32
Sea-nine	321.7	66	8	21	15.5	EcoScint A [d]	0.82	272	0.30

a) $K_{leaf\text{-}water} = C_{leaf} / C_{water}$

b) Dietary uptake = $0.5 \times (C_{leaf,\,0h} + C_{leaf,\,24h}) \times AE \times FR \times Mass_{G.pulex}$,
where the Assimilation Efficiency: AE = 0.4, approximated based on the review by Wang & Fisher (1999)[10],
and the Feeding Rate: FR = 0.25 $g_{\,leaf}$ / (day × $g_{\,wet\ weight\ G.pulex}$) based on Maltby et al. (2002)[11],
and $C_{leaf,\,0h} = 0$.

c) Uptake from water = $C_{water} \times k_{in} \times Mass_{G.pulex}$, where k_{in} for ethylacrylate was taken from Ashauer et al. (2010)[5]

d) Deviation from experimental protocol: leaf material was not blotted dry before analysis.

Text SI-8: Instability of ethylacrylate during extraction and analysis

Recovery of ethylacrylate was very low, although the expected concentration (Table S1) was still 18 times higher than the LOD. However, the chromatograms of samples spiked with ethylacrylate exhibited three ill-defined peaks, which we attribute to reaction of ethylacrylate with methanol during the sample preparation (these peaks were not present in chromatograms of the parent stock solution). Thus abiotic transformation of ethylacrylate is so strong, that the additional biotransformation can not be quantified. It is possible that ethylacrylate is biotransformed in *G. pulex*, but biotransformation products and the products of abiotic hydrolysis are presumably very similar, preventing us from identifying and quantifying the biotransformation products of ethylacrylate.

Text SI-9: Comparison with study based on total ^{14}C internal concentrations (details to Figure 5)

The uptake rate constants based on the two different methods correlate within one order of magnitude variability (top left). For the uptake rate constants the regressions' slope (Deming type II, log transformed data) was not significantly different from 1 and intercept with the y-axis was not significantly different from 0 (Figure 5a, regression not plotted). This comparison also includes the uptake rate constant for diazinon[1] and excludes the uptake rate constants for carbaryl and 4-nitrobenzyl-chloride, because uptake rate constants for these two compounds were kept fixed to the previously measured, total ^{14}C-based values in this study.

Elimination rate constants from this study were those of parent compounds. For the elimination rate constants the regression's slope (Deming type II) was not significantly different from zero (i.e. no relationship). This comparison also includes data for diazinon[1] and excludes carbofuran and 2,4-dichlorophenol due to high uncertainty (Figure 5b, regression not plotted).

For BAF_{total} values from the two studies with the different methods the regressions' slope (Deming type II, log transformed data) was not significantly different from 1 and intercept with the y-axis was not significantly different from 0 (Figure 5c, regression not plotted).

The regression of BAF_{total} from the total ^{14}C-study vs. the BAF_{parent} from this study has an intercept with the y-axis of 0.8 (on log scale, Figure 5d, regression not plotted). Thus the BAF_{total} from the total ^{14}C-study and the BAF_{parent} from this study are correlated, but the BAF_{total} from the total ^{14}C-study is nearly one order of magnitude higher than the BAF_{parent} from this study (y-intercept = 0.8, slope = 1.0), because in the former the metabolites are also counted towards the total radioactivity.

Table S8: Comparison of bioaccumulation factors.

Parent compound	BAF_{total} total ^{14}C study	BAF_{parent} this study	$BAF_{parent} + \sum MEF_{Mi}$ this study
	[L/kg $_{wet\ weight}$]	[L/kg $_{wet\ weight}$]	[L/kg $_{wet\ weight}$]
Aldicarb	1.64	0.87	1.2
Carbaryl	86.67	4.163	157
Malathion	114.30	2.96	8
4-nitrobenzyl-chloride	184.56	4.84	157
2,4-dichloroaniline	55.73	29.32	109
Sea-nine	1732.14	271.96	660
Diazinon	82.07	12.76	81
2,4,5-trichlorophenol	2635.29	82.30	1645
Pentachlorophenol	50.57	202.18	520

Additional References in Supporting Information

(1) Ashauer, R.; Hintermeister, A.; Caravatti, I.; Kretschmann, A.; Escher, B. I., Toxicokinetic-toxicodynamic modeling explains carry-over toxicity from exposure to diazinon by slow organism recovery. *Environ. Sci. Technol.* **2010,** *44*, (10), 3963-3971.

(2) Packard, *Operation Manual. Tri-Carb Liquid Scintiallation Analyzers Models 2200 CA and 2250 CA.* Packard Instrument Company: Downers Grove, IL, USA, 1987.

(3) Ashauer, R.; Boxall, A.; Brown, C., Uptake and elimination of chlorpyrifos and pentachlorophenol into the freshwater amphipod *Gammarus pulex*. *Arch. Environ. Contam. Toxicol.* **2006,** *51*, (4), 542-548.

(4) Ashauer, R.; Boxall, A. B. A.; Brown, C. D., Simulating toxicity of carbaryl to *Gammarus pulex* after sequential pulsed exposure. *Environ. Sci. Technol.* **2007,** *41*, (15), 5528-5534.

(5) Ashauer, R.; Caravatti, I.; Hintermeister, A.; Escher , B. I., Bioaccumulation kinetics of organic xenobiotic pollutants in the freshwater invertebrate *Gammarus pulex* modeled with prediction intervals. *Environ. Toxicol. Chem.* **2010,** *29*, (7), 1625-1636.

(6) Sutcliffe, D. W.; Carrick, T. R.; Willoughby, L. G., Effects of diet, body size, age and temperature on growth rates in the amphipod *Gammarus pulex*. *Freshw. Biol.* **1981,** *11*, (2), 183-214.

(7) Ashauer, R.; Hintermeister, A.; Potthoff, E.; Escher, B. I., Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. *Aquat. Toxicol.* **2011,** *103*, 38-45.

(8) Ashauer, R.; Boxall, A. B. A.; Brown, C. D., New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environ. Sci. Technol.* **2007,** *41*, (4), 1480-1486.

(9) Bluzat, R.; Seuge, J., Effects of 3 Insecticides - Carbaryl, Fenthion and Lindane - Acute Toxicity in 4 Lymnaeid Invertebrates - Chronic Toxicity in *Pulmonate Lymnea*. *Environ. Pollut.* **1979,** *18*, (1), 51-70.

(10) Wang, W. X.; Fisher, N. S., Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. *Environ. Toxicol. Chem.* **1999,** *18*, (9), 2034-2045.

(11) Maltby, L.; Clayton, S. A.; Wood, R. M.; McLoughlin, N., Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: Robustness, responsiveness, and relevance. *Environ. Toxicol. Chem.* **2002,** *21*, (2), 361-368.