Electronic Supplementary Information

Monitoring of the ecotoxicological hazard potential by polar organic micropollutants in sewage treatment plants and surface waters using a mode-of-action based test battery

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More details on the validation of extraction and comparison to earlier extraction studies

As is discussed in the main manuscript, the focus of this study is on evaluating the toxicity of micropollutants of moderate hydrophobicity covering a window of octanol-water partitioning coefficients $\log K_{ow}$ in the range of 0 to 5. Accordingly, the following more polar and hydrophilic solid phase materials were evaluated: (i) 100 mg LiChrolut® EN plus 250 mg LiChrolut® RP-C18, for extraction procedure see above. (ii) Empore™ SDB-RPS disks (Infochroma AG, Zug, Switzerland), and (iii) Empore™ C18 disks (Infochroma AG, Zug, Switzerland). In earlier work we also tested the following additional phases using a cocktail of various pharmaceuticals and urine:[1] (iv) 200 or 250 mg Carbopack (ENVI-Carb 120/400 mesh, Supelco, Bellafonte, U.S.A.), (v) 500 mg Isolute C18 (Separtis, Grellingen, Switzerland), (vi) 200 mg Isolute Env+ (polystyrene-divinylbenzene copolymer) (Separtis, Grellingen, Switzerland), (vii) 200 mg Oasis HLB (N-vinylpyrrolidone- divinylbenzene copolymer) (Waters, Bergen op Zoom, Netherlands), (viii) 200 mg Chromabond EASY (Macherey Nagel, Oensingen, Switzerland) (ix) Extrelute NT 3 for 3mL sample (Merck, VWR, Dietikon, Switzerland). For comparison, a selection of the results from this earlier study[1] is also presented below.

As the previous work has demonstrated, the recovery of the SPE (defined as the ratio of EC_{50} of the cocktail prior to SPE to EC_{50} of cocktail after SPE) with all particulate SPE materials in cartridges was comparable and lay between 99% and 159% for Lichrolut EN/C18, Carbopack, Isolute C18, Isolute Env+ and Oasis HLB, and was <90% only for Chromabond and Extrelut.[1] Since the matrix effect in urine (defined as the ratio of EC_{50} cocktail in urine after SPE to EC_{50} cocktail in water after SPE) was best, i.e. close to one, for Lichrolut EN/C18, this phase was used in an earlier study with urine.[1,2] Leusch et al. found similarly good results for various SPE phases including Oasis HLB, C18 and Isolute.[3]

Here, we additionally tested the two Empore™ disks SDB-RPS (in the following abbreviated by SDB) and C18 because the Empore™ disks can also be applied for passive sampling, as a recent study in an Australian STP has demonstrated.[4]

In Figure ESI1, the baseline-TEQ in the bioluminescence inhibition test with *V. fischeri* (Fig. ESI1A), the DEQ from the 24 h IPAM endpoint (Fig. ESI1B) and the PTEQ from the AChE inhibition test (Fig. ESI1CC) are depicted for Lichrolut EN/C18 and the two Empore™ disks, SDB and C18 in all investigated wastewater and surface water samples with and without added cocktail. The results for the YES are given in the main manuscript (Figure 2).

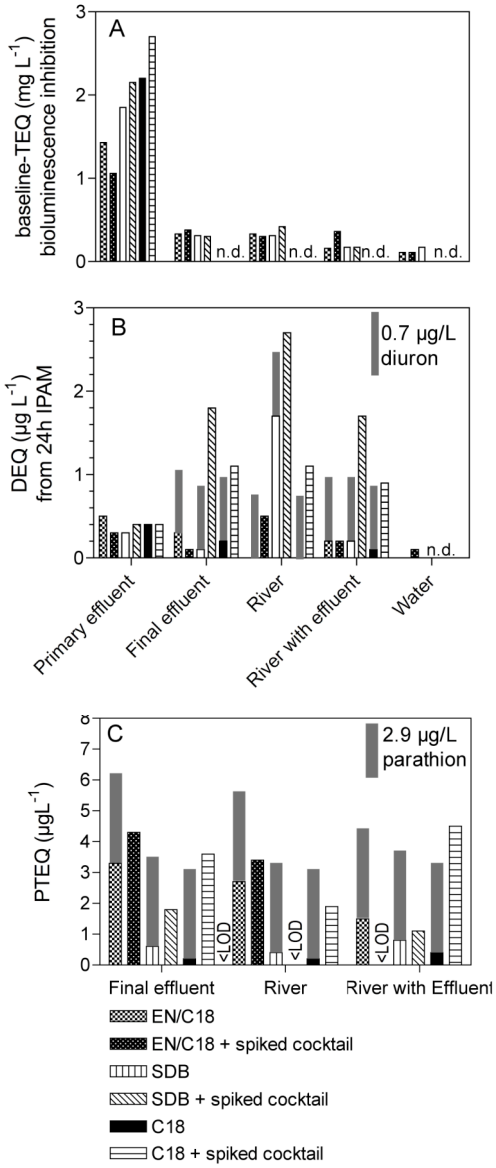

Fig. ESI1 Extraction efficiency of the three SPE methods using Lichrolut EN/C18 and the two Empore™ disks SDB and C18 as solid phase. (A) Baseline-TEQ determined with the bioluminescence inhibition test with *V. fischeri*, (B) diuron equivalent concentrations DEQ determined for the 24 h IPAM endpoint with the combined algae test, (C) parathion equivalent concentration PTEQ determined with the AChE inhibition test.

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In the bioluminescence inhibition test, the added cocktail did not dominate the overall effect. Separate extraction experiments confirmed that the volatile 3,5-dichlorophenol was evaporated during the SPE extraction procedure. Therefore it was not possible to do a full mass balance of the experiment. Because of this observation, only the first sample series in 2006 was evaporated to dryness after SPE, subsequent samples were only reduced to 500 µL of eluate and then made up to exactly 1 ml of extract. Nevertheless it was possible to compare the different extraction methods. The baseline-TEQ were comparable for a given sample (Fig. ESI1A), indicating that the recovery is similar with all three extraction methods. In fact, the secondary and final effluent and surface water samples were very uniform while the primary effluent sample varied by a factor of two, indicating some variability due to large matrix effects.

The DEQ derived from the 24 h IPAM endpoint of the combined algae test are a mixed indicator of specific inhibition of photosystem II and baseline toxicity, as is discussed in the accompanying paper.[5] Again, there was no significant difference between the SPE extraction methods using Lichrolut EN/C18 and the two Empore™ disks, SBD and C18. The DEQ were uniform for the primary effluent samples and they were well below the spike of 0.7 µg L^{-1} diuron indicating that the extraction efficiency was not perfect for this sample, as was similar for the baseline-TEQ, presumably due to the high load of toxicant and matrix effects. In effluent and river water, the samples with spiked cocktail, which contained 0.7 µg L^{-1} diuron, had correspondingly higher DEQ than the samples. This is indicated in Figure ESI1B by the grey bars. Thus, extraction efficiency must have been close to 100% despite the matrix components.

In the AChE inhibition test, only effluent, river and mixtures thereof were tested (Fig. ESI1C). With Lichrolut EN/C18 and SDB the samples plus computationally added 2.9 µg L^{-1} PTEQ gave always slightly higher PTEQ than when 2.9 µg L^{-1} parathion was added prior to the extraction, while the PTEQ were similar when C18 was used as SPE material.

In the umuC test, all samples spiked with 1.5 µM 2-aminoanthracene were positive at REF of 20 and above and did not exhibit any genotoxicity without spiked 2-aminoanthracene up to an REF of 20 (primary effluent) to 100 (river).

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Table ESI1 Detailed overview of results of the June 2007 sampling campaign.

	Primary effluent			Secondary effluent			Final effluent			River upstream of STP		River downstream of STP		Procedural blank
Sample	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**1**	**2**	
Inhibition of bacterial luminescence														
TEQ (mg L^{-1})	5.73	3.20	8.80	0.45	0.43	0.47	0.31	0.31	0.29	0.45	0.46	0.35	0.51	0.01
standard deviation[a]	1.01	0.93	3.07	0.08	0.05	0.03	0.07	0.03	0.05	0.10	0.06	0.05	0.10	0.00
EC10 (REF)	0.24	0.45	0.17	3.08	3.20	2.88	4.51	4.45	4.76	3.06	2.99	3.93	2.72	241
standard deviation	0.05	0.13	0.06	0.57	0.41	0.17	0.95	0.36	0.85	0.69	0.42	0.49	0.56	43
Inhibition of algal growth														
TEQ (mg L^{-1})	4.01	3.16	6.79	0.70	0.66	0.59	0.43	0.65	0.68	0.57	0.52	0.64	0.70	0.08
standard deviation	1.76	1.71	2.61	0.05	0.07	0.19	0.26	0.16	0.12	0.01	0.12	0.49	0.24	0.04
EC10 (REF)	1.05	1.16	0.51	1.70	1.25	1.09	1.47	1.00	0.89	1.59	1.64	2.33	1.61	25
standard deviation	0.08	0.40	0.02	0.04	0.25	0.10	0.13	0.21	0.21	0.53	0.08	1.12	0.60	3
Inhibition of photosynthesis (2 h IPAM)														
DEQ (µg L^{-1})	0.26	0.25	0.33	0.14	0.15	0.18	0.18	0.19	0.19	0.16	0.31	0.18	0.20	0.0018
standard deviation	0.06	0.09	0.10	0.05	0.05	0.07	0.06	0.06	0.09	0.10	0.09	0.04	0.12	0.0005
EC10 (REF)	1.93	3.18	2.59	2.64	2.54	3.55	3.51	3.20	3.35	4.78	1.98	3.34	3.47	342
standard deviation	0.31	0.20	0.16	0.69	0.37	0.97	1.48	0.90	1.55	3.14	0.56	0.73	2.00	89
Inhibition of acetylcholine esterase														
PTEQ (µg L^{-1})	2.63	2.47	3.78	0.57	0.48	0.46	0.51	0.32	0.41	0.36	0.18	0.18	0.23	0.02
standard deviation	0.83	0.61	0.44	0.11	0.29	0.15	0.05	0.02	0.11	0.25	0.09	0.05	0.03	0.01
EC10 (REF)	1.02	1.07	0.67	4.48	6.42	5.88	4.94	7.83	6.58	9.23	16.17	14.30	11.03	186
standard deviation	0.30	0.31	0.08	0.87	2.89	1.89	0.51	0.46	2.18	6.43	7.11	3.86	1.61	121
Yeast Estrogen Screen (YES)														
EEQ (ng L^{-1})	83.14	66.63	123.39	1.08	1.03	0.57	1.18	0.71	0.54	0.00	0.63	0.67	0.44	
standard deviation	12.12	11.50	54.08	1.32	0.96	0.68	1.40	1.23	0.76	0.00	0.89	0.95	0.76	
Genotoxicity umuC test														
LOEC (REF)	na	30	na	74	74	74	74	74	74		148		148	
NOEC (REF)		14.81		37	37	37	37	37	37	148	74	148	74	148

[a] Errors denote the standard deviation of the average of the three independent replicates (three times SPE followed by at least duplicate bioassays) of each sampling event (sample 1, sample 2, sample 3).

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Table ESI2 Overview of results of the July 2006 sampling campaign.

	Primary effluent	Secondary effluent	Final effluent	River upstream of STP	River downstream of STP	Procedural blank	Treatment efficiency
Inhibition of bacterial luminescence							
TEQ (mg L^{-1})	6.92±2.46[a]	n.d.	0.43±0.43	2.681.11	0.87±0.29	0.07	93.8±3.0%
EC_{10} (REF)	0.21±0.06		2.850.10	0.560.19	1.73±0.72	19.3	
Inhibition of algal growth							
TEQ (mg L^{-1})	4.48±3.41	n.d.	1.16±0.76	1.79±0.85	2.33±2.05	0.04	74.1±80.6%
EC_{10} (REF)	0.71±0.53		2.30±1.52	1.33±0.54	1.46±1.05	48.5	
Inhibition of photosynthesis (2 h IPAM)							
DEQ (µg L^{-1})	0.29±0.20	n.d.	0.10±0.01	0.21±0.09	0.19±0.01	0.002	33.8±44.0%
EC_{10} (REF)	0.70±0.23		3.22±0.47	8.43±2.64	0.43±0.14	709	
Inhibition of acetylcholine esterase							
PT-EQ (µg L^{-1})	24.2±161	n.d.	5.92±1.65	n.d.	n.d.		75.5±12.5%
Yeast Estrogen Screen (YES)							
EEQ (ng L^{-1})	43.4±9.0	n.d.	2.70±0.59	3.53±2.84	2.93±1.26	0.23	93.8%
Genotoxicity umuC test							
No activity							

[a] Errors denote the standard deviation of the average of the three independent consecutive sampling events (sample 1, sample 2, sample 3).

Table ESI3 Overview of results of the November 2006 sampling campaign.

	Primary effluent	Secondary effluent	Final effluent	River upstream of STP	River downstream of STP	Procedural blank	Treatment efficiency
Inhibition of bacterial luminescence							
TEQ (mg L^{-1})	3.57±0.67[a]	0.13±0.02	0.15±0.03	0.25±0.15	0.25±0.08	0.02	95.9±5.6%
EC_{10} (REF)	0.39±0.08	10.7±1.5	9.53±2.31	7.56±5.59	5.71±1.88	87	
Inhibition of algal growth							
TEQ (mg L^{-1})	0.64±0.15	0.73±0.35	0.89±0.79	0.16±0.10	0.57±0.18	0.02	No decrease
EC_{10} (REF)	3.39±0.90	3.55±2.26	4.35±3.93	20.0±17.9	3.83±1.18	128	
Inhibition of photosynthesis (2 h IPAM)							
DEQ (µg L^{-1})	0.07±0.03	0.43±0.28	0.16±0.10	0.34±0.24	0.34±0.12		No decrease
EC_{10} (REF)	7.81±2.71	1.65±1.20	4.51±3.31	2.08±1.38	1.58±0.54		
Inhibition of acetylcholine esterase							
PT-EQ (µg L^{-1})	2.94±1.72	0.16±0.05	0.14±0.04	0.04±0.01	0.19±0.08		95.3±1.4%
Yeast Estrogen Screen (YES)							
EEQ (ng L^{-1})	43.0±11.0	1.03±0.49	0.82±0.14	0.66±0.21	0.92±0.07		98.1±0.7%
Genotoxicity umuC test							
No activity							

[a] Errors denote the standard deviation of the average of the three independent consecutive sampling events (sample 1, sample 2, sample 3).

Notes and references

1. B. I. Escher, N. Bramaz, M. Maurer, M. Richter, D. Sutter, C. von Känel and M. Zschokke, *Environ. Toxicol. Chem./SETAC*, 2005, **24**, 750-758.
2. B. I. Escher, W. Pronk, M. J.-F. Suter and M. Maurer, *Environ. Sci. Technol.*, 2006, **40**, 5095-5101.
3. F. D. L. Leusch, M. R. van den Heuvel, H. F. Chapman, S. R. Gooneratne, A. M. E. Eriksson and L. A. Tremblay, *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, 2006, **143**, 117-126.
4. R. Muller, J. Tang, R. Thier and J. F. Mueller, *J. Env. Monitor.*, 2007, **9**, 104-109.
5. B. I. Escher, N. Bramaz, J. F. Mueller, P. Quayle and S. Rutishauser, *J. Env. Monitor.*, 2008.