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RESEARCH ARTICLE

Bioanalytical and instrumental screening of the uptake of sediment-borne, dioxin-like compounds in roach (*Rutilus rutilus*)

Kathrin Eichbaum[1] · Markus Brinkmann[1] · Leonie Nuesser[1] · Sebastian Buchinger[2] · Georg Reifferscheid[2] · Garry Codling[3] · Paul Jones[3] · John P. Giesy[3,4,5,6,7] · Markus Hecker[3] · Henner Hollert[1,8,9,10]

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Abstract To examine the uptake of dioxin-like compounds (DLCs), common roaches (*Rutilus rutilus*) were exposed for 28 days to differently contaminated sediments from two major European rivers in a purpose-built facility. Dietary transfer of DLCs was investigated by exposing fish to sediments inoculated or non-inoculated with black worms (*Lumbriculus variegatus*). Dioxin-like polychlorinated biphenyls (DL-PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), measured via high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) in sediments and whole fish, were used to calculate toxicity equivalent quotients (TEQs). TEQs were compared with biological toxicity equivalent quotients (BEQs) determined via the 7-ethoxyresorufin-*O*-deethylase (EROD) assay, performed with mammalian (H4IIE) and fish (RTL-W1) liver cell lines. TEQs and BEQs indicated an uptake of sediment-borne DLCs by roach, which was independent of sediment contamination levels, but rather reflected sediment-specific characteristics. For most sediment treatments, DLC uptake did not increase with time. Highest congener-specific uptake (DL-PCB 123) was 10-fold compared to control. Exposure to worm-inoculated sediment of highest overall DLC contamination caused a 2-fold (TEQ and H4IIE BEQ) greater uptake of DLCs by fish compared to the respective non-inoculated treatment. H4IIE cells showed the greatest sensitivity (0.37 ± 0.25 pM TCDD) and the strongest correlation with TEQs ($r^2 = 0.79$), hence, they seem to be best suited for DLC screening of sediments and biota, amended by compound-specific instrumental analysis if required.

Keywords Dioxin · EROD · Micro EROD · BEQ

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✉ Henner Hollert
henner.hollert@bio5.rwth-aachen.de

[1] Department of Ecosystem Analysis, Institute for Environmental Research, ABBt—Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany

[2] Department G3: Biochemistry, Ecotoxicology, Federal Institute of Hydrology (BfG), Am Mainzer Tor 1, 56068 Koblenz, Germany

[3] School of the Environment and Sustainability and Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK S7N 5B3, Canada

[4] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

[5] School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China

[6] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

[7] Department of Biology, Hong Kong Baptist University, Hong Kong, SAR, People's Republic of China

[8] Key Laboratory of Yangtze River Environment of Education Ministry of China, College of Environmental Science and Engineering, Tongji University, Shanghai, China

[9] College of Resources and Environmental Science, Chongqing University, Chongqing, China

[10] School of Environment, Nanjing University, Nanjing, China

Introduction

Sediments are well known but poorly understood sources of pollutants for aquatic environments. Their interfaces constitute areas of intense recycling of organic carbon and persistent organic pollutants (POPs) (Berglund et al. 2001), and their characteristics can influence bioavailability and accessibility of POPs (Eggleton and Thomas 2004).

Among the POPs, which are all capable of being persistent, toxic and bioaccumulative, the so-called dioxin-like compounds (DLCs) are of particular concern (Hilscherova et al. 2000). DLCs share similarities in structure and bind to the aryl hydrocarbon receptor (AhR). Although this group comprises a large variety of contaminants, many of which are still unknown, the term "DLCs" in the present work exclusively refers to 12 dioxin-like polychlorinated biphenyls (DL-PCBs), the 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), and the 10 dibenzofurans (PCDFs) with 2,3,7,8-chlorosubstitution, when considering results of instrumental analyses.

While PCBs were produced for various applications such as pesticide additives, fluids in capacitors, and transformers as well as lubricants in cutting oils, PCDD/Fs are undesired industrial byproducts, which, among others, can be formed during incineration, chemical processes involving chlorine, and paper bleaching processes (Aarts et al. 1995; Safe 1994; Weber et al. 2008). Despite being banned for several decades (Stockholm Convention; Yoder 2003), PCBs are continuously emitted into the environment through leakages from old capacitors, elastic sealants, and other building materials, whereas the emission of PCDD/Fs decreased in recent years (Besselink et al. 2004) through banning of critical chlorine chemicals and emission control measures (Lee et al. 2007). DL-PCBs and PCDD/Fs are persistent and toxic organic compounds, differing in number and position of chlorine atoms bound to their basic aromatic structures. Because so much research has been done on PCBs, they are useful reference chemical for use in studies. Due to their physical chemical properties, these contaminants are globally distributed and can be found in almost every matrix including sediments, soils, wildlife, human tissue, blood, and milk. Their potential to bioaccumulate and biomagnify along the food chain endangers wildlife, the environment, and also human beings (Fent 2007). Exposure to DLCs, which exceeds a hazardous level, can cause wasting syndrome, reduced fecundity, hepatic damage, dermal disorders, thymic atrophy, immunotoxicity, endocrine disruption, and reproductive toxicity (Safe 1986; Whyte et al. 2000).

All DLCs, including 2,3,7,8-tetrachloro dibenzo-*p*-dioxin, which is considered the most toxic congener (Safe 1990, 1994), bind to the AhR. The AhR is a ligand-activated transcription factor in the Per-Amt-Sim (PAS) family of proteins that mediates the pleiotropic expression of a suite of genes and is believed to regulate most, if not all, adverse effects associated with exposure to DLCs (Lindebro et al. 1995).

Among the downstream effects of the AhR induction is expression of enzymes involved with xenobiotic metabolism (Okey 2007). One prototypic biomarker for the activation of the AhR by DLCs in vertebrates is induction of the phase I biotransformation enzyme cytochrome P4501A (CYP1A). Within this group, member CYP1A1 7-ethoxy-resorufin-*O*-deethylase (EROD) can be found. EROD develops in vertebrate cells that have been exposed to environmental sample extracts and can be quantified by determining both the amount of EROD-catalyzed fluorescent reaction product resorufin (built following addition of the artificial substrate 7-ethoxyresorufin) and the amount of protein present at the moment of reaction. EROD constitutes the endpoint of both the RTL-W1 EROD and the H4IIE micro-EROD assay used in this study.

These assays possess a certain predictive ability and might therefore serve as screening tools for the detection of DLCs in various environmental matrices (2012/252/EU 2012; Eichbaum et al. 2014). They represent supporting bioanalytical methods for classical, instrumental analysis of individual DLC congeners. One advantage of bioanalytical characterization of DLCs in complex mixtures such as sediment or tissue samples is that they provide a more realistic, ecotoxicological relevant exposure assessment and allow for both the integration of all interactions among DLC congeners and the detection of inducers not monitored in compound-specific instrumental analyses (Giesy et al. 1997; Wernersson et al. 2015). Bioanalytical and instrumental results can be compared by using the approach of toxicity equivalent quotients (TEQs) and biological equivalent quotients (BEQs) (Safe 1998).

When considering exposure of aquatic organisms to sediment-borne DLCs, different exposure pathways include aqueous (particle, sediment, and/or water contact via integument and gills) and dietary exposure. Rates of accumulation are dependent on the species, its developmental stage, behavior, and sexual condition as well as season, environment, and climatic conditions (as reviewed by Eggleton and Thomas 2004) but are also influenced by physical and chemical properties of compounds. For example, the octanol/water partitioning coefficient (log K_{ow}) can be used to determine the affinity of a compound to biotic tissue or fat. For aqueous exposure pathways, a linear relationship between log K_{ow} and bioavailability of a chemical up to log K_{ow} values <7 has been found. Log K_{ow} values >7 result in strong binding of chemicals to, e.g., sediments, and thus, their bioavailability decreases (Engwall et al. 1998; Hollert et al. 2002). Concerning the accumulation of DLCs, foodstuff of animal origin is one of the main sources of POPs (van Leeuwen et al. 2000) and that feeding, movement, and burrow formation can increase contaminant release from sediments. Moreover, sediment ingestion might be primary routes for congeners exhibiting high log K_{ow} values (Eggleton and Thomas 2004).

In the present study, a cyprinid fish, the common roach (*Rutilus rutilus*) was studied. This species is abundant in

Eurasian lakes and rivers (Jamet and Desmolles 1994) and thus, a species of high ecotoxicological relevance. To determine the extent to which DLC uptake in roach depends on the initial sediment contamination and/or sediment-specific characteristics, individuals were exposed to sediments differently contaminated with DLCs (Feiler et al. 2013; Höss et al. 2010). Exposure of roach to sediments to which oligochaetes had been added aimed in determining whether contaminated diet increases uptake of DLCs compared to uptake via the water phase alone. All exposure scenarios were applied to create more realistic exposure pathways of DLCs in aquatic systems. Whether an uptake of DLCs by roach is detectable by means of bioanalytical methods was investigated by analyzing homogenates of fish and sediments by means of two in vitro bioassays and by verifying those results via results obtained by chemical instrumental analysis.

Materials and methods

Study design

In a 28-day exposure experiment, juvenile common roach were exposed to three sediments and a 1:10 mixture of two of these sediments, which differed in characteristics and level of DLC pollution. The dietary uptake of DLCs in fish was investigated by comparing the uptake following the exposure of fish to either worm-inoculated or non-inoculated sediment treatments. Finally, sediment and biota samples were extracted, subjected to clean-up and bioanalytically investigated using the EROD with RTL-W1 cells and the micro-EROD assay with H4IIE cells. Concentrations of 29 DLCs were determined using capillary gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

Sediments

Sediments were collected by the German Federal Institute of Hydrology (BfG, Koblenz) during a sampling campaign in April 2012. Sediment of the river Rhine originated from the harbor Ehrenbreitstein (EBR) near Koblenz, Germany. This location has been used as site of low to moderate contamination in former studies (Heise et al. 2008; Höss et al. 2010). Two locations were chosen from the river Elbe. Sediments of location Prossen/Schmilka (PR), a harbor located close to the Czech border at river kilometer 13.2 and Zollelbe (ZE), a cut-off meander in the city of Magdeburg, Germany. Finally, one dry mass (dm) part of ZE was diluted with nine dm parts of EBR.

Further details of the three sampling locations EBR, PR, and ZE as well as the mixture (EBR/ZE) are listed in Table 1. Sediments were filled in polyethylene buckets, immediately transferred to RWTH Aachen University and stored at 4 °C until further use. Prior to the start of the experiments,

Table 1 Sampling details, locations, instrumental (HRGC/HRMS), and bioanalytical (RTL-W1 EROD; H4IIE micro-EROD) determined concentrations of DL-PCB and PCDD/F fractions as well as physical and chemical characteristics of sediments Ehrenbreitstein, Prossen, Zollelbe, and a laboratorial manufactured sediment mixture

	Ehrenbreitstein Harbor	10/1 mixture Ehrenbreitstein/Zollelbe	Prossen Schmilka	Zollelbe Magdeburg
Sample acronyms	EBR	EBR/ZE	PR	ZE
River system Unit km	Rhine main stream 591.4	–	Elbe main stream 13.2	Elbe cut-off meander 0.1
Longitude	7.60792	–	14.11631	11.65087
Latitude	50.35400	–	50.92776	52.13.256
Sampling date	12.04.2012	–	11.04.2012	10.04.2012
Grab (max. sampling depth)	Van-Veen grab (15 cm)	–	Van-Veen grab (15 cm)	Van-Veen grab (15 cm)
Concentration of 12 WHO-PCB (ng/g dm)	4.38	4.18	5.22	9.72
Concentration of 17 WHO-PCDD/F (ng/g dm)	1.06	1.22	0.24	3.70
$EROD_{EC25}$ TEQ of PCB fraction (pg/g dm)	36.0 ± 14.7	38.4 ± 0.9	50.6 ± 9.1	192.5 ± 26.4
$EROD_{EC25}$ TEQ of PCDD/F fraction (pg/g dm)	270.6 ± 77.0	180.1 ± 58.5	488.0 ± 279.8	955.8 ± 551.9
Micro-$EROD_{EC25}$ TEQ of PCB fraction (pg/g dm)	16.7 ± 7.1	18.6 ± 4.6	18.4 ± 6.6	76.4 ± 18.8
Micro-$EROD_{EC25}$ TEQ of PCDD/F fraction (pg/g dm)	63.7 ± 8.8	60.5 ± 13.9	73.9 ± 2.9	159.0 ± 32.1
TOC (g/kg)	49.6	n.a.	63.1	64.3
Percentage of sand/silt/clay (%)	4/79/17	n.a.	19/68/13	24/69/7
Loss on ignition (%)	10.6 ± 0.4	n.a.	13.1 ± 0.2	14.1 ± 0.3
Percentage of dry matter (%)	36.1 ± 0.1	n.a.	33.4 ± 0.6	34.0 ± 0.2

Coordinates according to the international terrestrial reference system 1998 (ITRS 98)

sediments were thoroughly homogenized using an electric stirrer. The mixture EBR/ZE consisted of nine parts dm EBR and one part dm ZE. Exposure experiments to sediments ZE and EBR/ZE were conducted in summer 2012 (07 June 2012–14 July 2012), those of sediments PR and EBR in autumn 2012 (24 October 2012–30 November 2012).

Fish

Juvenile common roach were obtained from a pond aquaculture of a local supplier (Inquadro, Aachen, Germany), transported to RWTH Aachen University and transferred to aerated 1000-L tanks. Fish were maintained under flow-through conditions in tap water (approx. 15 °C; pH 7.8 ± 0.2; $NH_3 < 0.1$ mg/L) with a water exchange rate of 0.5–1/day. Light and dark phases were 12 h each. Fish were fed ad libitum with frozen chironomids (Aquahobby, Peine, Germany) and allowed to acclimatize for at least 1 month. A total of 156 fish were used and distributed among the exposure units in similar dimensions, on average, 118 ± 5 mm length and 36 ± 6 g wet body mass (wm). Fish were used in accordance to the Animal Welfare Act and with permission of the federal and local authorities, registration no. 84-02.04.2011.A368.

Experimental conditions

Exposures of fish were conducted in accordance with OECD test guideline 305 with the most important deviations including the lack of true flow-through conditions and depuration time as well as the exposure to sediment (Ahlf et al. 2002). Experiments were conducted in a purpose-built exposure facility in an air-conditioned room at the Institute for Environmental Research, RWTH Aachen University. The exposure facility, which enabled simultaneous testing of three sediments, consisted of six independent exposure systems, each consisting of five 100-L aquariums. Four aquaria were used as exposure tanks and one as a sump. The latter enabled continuously pumping and recirculation of water to the individual aquariums at a rate of about 10 L/min. Water was aerated (approx. 20 L/min) and temperature was maintained at 17.0 ± 0.6 °C by use of stainless steel heat exchangers connected to a recirculating chiller via the sumps. Light/dark phases were 12 h each.

Experimental setup

Exposure scenarios

Individual roach were exposed to sediments under two different exposure scenarios: (1) sediments EBR, EBR/ZE, PR, and ZE that were inoculated with 100 g wm black worms (*Lumbriculus variegatus*, Fauna topics GmbH, Marbach, Germany) and which are referred as (+) approaches, and (2) non-inoculated sediments ZE and PR, which are referred to as (−) approaches. Here, fish were daily fed with uncontaminated black worms at a rate of 1 % collective body mass (body mass per aquarium). Feeding was only performed on populated aquariums.

Conduct of exposure experiments

Each homogenized sediment was tested in four pseudo-replicate tanks connected via a sump. Sediment (8 kg wet mass (wm)/replicate) was covered with tap water (approx. 75 L) avoiding resuspension. Sediments were allowed to consolidate over 10 days. In case of (+) scenarios, sediments were inoculated with 10 g wm of living black worms (*L. variegatus*) per replicate before consolidation. Following this period, six individuals of *R. rutilus* from the maintenance tank were transferred to each replicate tank (except the sump), and in the case of (−) scenarios were fed daily with 1 g wm of living black worms. Following an exposure period of 10 days, fish were transferred to new tanks that had been subjected to the same 10-day consolidation period as described above, to maintain stable sediment contaminant concentrations. This procedure was repeated until an exposure period of 28 days was reached.

Daily measurement of limnochemical parameters

During both consolidation and exposure periods, limnochemical parameters were daily measured in four pseudo-replicates and the sump of each test system, respectively. Parameters that were consistent among replicate tanks of a system until the first fish transfer were thereafter only monitored in the sump. Daily measured limnochemical parameters included *temperature* and *conductivity* (conductivity electrode LF91 KLE 1/T, WTW, Weilheim, Germany), *dissolved oxygen* (DO meter HI 9146, Hanna Instruments, Kehl, Germany), *pH* (pH meter, Mettler Toledo AG, Schwerzenbach, Switzerland), *redox potential* (ORP 15, VWR international, Darmstadt, Germany), and *turbidity* (turbidimeter, Ratio/XR, HACH company, Loveland, Colorado, USA). The *concentration of alkaline earth metals* (i.e., total hardness) was determined in 100-mL water filtrates (0.7 μm glass-fiber filters, Macherey und Nagel GmbH and Co. KG, Düren, Germany) containing both an indicator buffer tablet (Merck, Darmstadt Germany) and 1 ml of ammonia (32 %, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Samples were titrated (25 ± 0.075 ml, 20 °C, Brand, Germany) with Titriplex solution B (Merck) until color change.

Sediment and biota sampling

Aliquots of the three sediments and the mixture were taken prior to the exposure experiments.

Fish samples were taken on days 4, 7, 14, and 28. Specifically, all fish from one tank (in case no mortality occurred: six individuals) were removed (starting with the first tank on day 4 and ending up with the fourth tank on day 28), anesthetized using benzocaine and killed by exsanguination. Standard lengths and mass of fish were determined. Fish were wrapped in aluminum foil and stored at −80 °C until further use. Six randomly chosen animals from the maintenance tank were treated the same way and used as "summer" and "autumn" controls.

Condition factor (K) of each fish was determined according to the equation (Iannuzzi et al. 1995):

$$K = 10^{N} * W * L^{-3} \quad (1)$$

where N represents the numerical factor 5, L is standard length measured in millimeters, and W is mass measured in grams.

Sample preparation, extraction, and clean-up

All steps described in this section were conducted at the RWTH Aachen University, Aachen, Germany. Homogenates of whole fish consisting of six frozen animals from one treatment (refer to the Sediment and biota sampling section) were prepared by mixing in a Philips blender (2096/00, Aschaffenburg, Germany) for 10 min under addition of deionized water. Both sediments and fish homogenates were freeze-dried for 72 h (Alpha 1–4 LD plus, Martin Christ GmbH, Osterode, Germany), sieved <2 mm, and homogenized by using a mortar and a pestle. Masses of fractions >2 mm of fish homogenates (mostly containing bones and scales) were determined gravimetrically and— under the assumption that wet mass (wm) equates dry mass (dm) in this fraction—allowed for recalculation of the initial homogenate fresh masses.

Until extraction, samples were stored in brown glass vials at 4 °C. Sediment and biota samples were extracted for 48 h according to the methodology used in the joint research project Elbe Hochwasser 2002 (Umlauf et al. 2004) using Soxhlet extraction (Behr Labor Technik, Düsseldorf, Germany) and a solvent mixture of n-hexane/acetone (352/48; *v/v*). For sediment and biota samples, 20 and 3 g dm, respectively, were weighed and mixed with an amount of 5 g of previously muffled sodium sulfate (99 % anhydrous powder, Sigma Aldrich, Germany). Three process control samples were extracted at the beginning, middle, and end of the whole extraction run, only containing 5 g of sodium sulfate.

Samples for chemical analysis were spiked with $^{13}C_{12}$-labeled PCDD/F standards (EPA 1613 LCS, Wellington Laboratories, Campro Scientific GmbH, Germany) and $^{13}C_{12}$-labeled PCB standards (EPA 68C LCS, Wellington Laboratories, Germany). The clean-up included the following chronological steps: gravimetrical fat content determination (in fish extracts), desulfurization with activated copper (24 h), sulfuric acid treatment (24 h), multilayer silica column clean-up, activated carbon column clean-up. Each step was performed according to method 8290 of the US EPA (EPA 1994) with the following modifications: multilayer silica columns were equipped in the following order from bottom to top: glass wool, 1 g of activated silica gel, 2 g of basic silica gel (30 g of sodium hydroxide dissolved in 750 ml methanol, put together with 100 g of silica gel and rotary evaporated until dryness for approximately 90 min in a 55 °C water bath), 1 g of activated silica gel, 4 g of acidic silica gel, 1 g of activated silica gel, and 1 g of sodium sulfate. Supelclean™ ENVI-Carb™ (Sigma Aldrich) was chosen as a carbon adsorbent in the activated carbon columns.

To obtain DL-PCB and PCDD/F extract fractions for bioassay and HRGC/HRMS purposes, extracts were volumetrically divided into aliquots. Aliquots were stored in 4-ml vials (amber glass, 45 × 14.7 mm with butyl/PTFE septum and screw cap, VWR International) until further use. For bioassay purposes, extracts were reduced close to dryness under a gentle stream of nitrogen and redissolved in dimethyl sulfoxide (DMSO ≥ 99.5 % p.a., Carl ROTH). The respective volume of DMSO depended on both matrix and fraction.

Bioanalytical and instrumental sample analysis

EROD assay

Permanent fish liver cell line RTL-W1 (*Oncorhynchus mykiss*, rainbow trout liver—Waterloo 1) (Lee et al. 1993), donated by Dr. Niels C. Bols, University of Waterloo, Canada (Bols et al. 1999), were subcultivated weekly in Leibowitz (L15) medium, supplemented with 9 % fetal bovine serum (FBS, Biochrom AG, Berlin, Germany) and a 1 % penicillin streptomycin solution (Sigma Aldrich) and maintained at 20 °C in darkness. CYP1A1 induction measurement was performed according to Wölz et al. (Wölz et al. 2009). Briefly, cells of passage numbers 73 to 76 were seeded in 96-well plates (TPP, Trasadingen, Switzerland) and incubated 72 h until confluence. Thereafter, medium was removed and cells were exposed to serial diluted concentrations of extracts (two samples/plate) and positive control 2,3,7,8-TCDD (3.1 M to 100.0 pM; Promochem, Wesel, Germany), which were put on plates in triplicates and duplicates, respectively. Thereby, DMSO concentrations in all wells did not exceed 0.5 %.

Following a 72-h incubation time, exposure medium was removed and cells were lysed by freezing them at −80 °C for at least 1 h. First, an enzyme-substrate complex consisting of EROD present in the cells and added substrate 7-ethoxyresorufin was allowed to develop within a reaction time

of 10 min. Thereafter, addition of reduction equivalent NADPH caused the demethylation of the substrate, which was stopped after further 10 min through the addition of fluorescamine dissolved in acetonitrile. After 15 min, specific EROD activity was determined by measuring the fluorescence of reaction product resorufin (extinction 544 nm, emission 590 nm) and the absorbance of fluorescamine-amine complexes (extinction of 360 nm, emission of 460 nm, according to a method of Lorenzen et al. (1997) with a multiwall-plate reader (Tecan infinite M200).

Micro-EROD assay

H4IIE cells (passage number 29 to 49), provided by the Lower Saxony State Office for Consumer Protection and Food Safety (LaVes), were cultivated using Dulbecco's modified eagle medium with phenol red (DMEM; low glucose, Life Technologies GmbH, Schwerte, Germany) supplemented with 10 % FBS (Biochrom AG, Berlin, Germany) and 2 % L-glutamine (2 mM, GIBCO® GlutaMAX™, Life Technologies GmbH). The assay was performed according to a protocol provided by LaVes (O'Connor and Paul 2000). Briefly, cells were grown to confluence, trypsinized and 50 μl cell suspension (200,000 cells/ml DMEM without phenol red) was seeded into a 96-well plate (96-Well, Growing surface, Sarststaedt) and incubated for 2 h in a humidified 95:5 air/CO_2 atmosphere at 37 °C in darkness. Then, cells were exposed to serial (1:2) prediluted concentrations of extracts and the positive control 2,3,7,8-TCDD (0.58 to 18.64 pM; Promochem, Wesel, Germany) in triplicates. Concentrations of DMSO in all wells did not exceed 0.5 %.

Following a 72-h incubation time, medium was removed and 100 μl of an 8 μM ethoxyresorufin (ETX) solution containing 10 μM dicumarol was added to all cell-containing wells. After 30 min, the reaction was stopped by adding 75 μl of methanol (p.a.; ROTH). Plates were horizontally shaken for 10 min (300 rpm) and resorufin production was determined fluorometrically (excitation 530 nm, emission 590 nm) by using a multiwell plate reader (Tecan infinite M200; Tecan Deutschland GmbH, Crailsheim, Germany). For determination of specific EROD activity, protein was determined by using a bicinchoninic acid (BCA) protein assay kit (Sigma Aldrich). A protein standard curve was prepared in the remaining ETX solution and put on the plate (100 μl/well). Absorption measurement at an extinction of 550 nm was performed following the addition of 100 μl/well BCA solution and an incubation time of 20 min at 50 °C (Tecan infinite M200).

Chemical analysis by means of HRGC/HRMS

The HRGC/HRMS analyses of sediment and biota extracts prepared by the RWTH Aachen University were performed by münster analytical solutions GmbH (mas GmbH, Münster, Germany). For instrumental analysis, a capillary gas chromatograph coupled to a high resolution mass spectrometry was used (Thermo Scientific Trace Ultra GC with Thermos scientific DFS HRMS, Thermo Fisher Scientific, Bremen, Germany). The GC was equipped with a 60-m DB-5MS capillary column of 0.25-mm inner diameter and 0.25-μm film thickness (Agilent J&W, Santa Clara, CA, USA). The capillary column was used for both PCDD/F and PCB analysis. Separate HRGC/HRMS runs at different instrumental conditions were applied for the analysis of the two compound classes.

Since the precleaned extracts, provided by the RWTH Aachen, partly showed insufficient separation of the PCDD/Fs and the DL-PCBs in the matrix fish and insufficient extract purification in case of the sediments, the PCDD/F and PCB fractions of the initial clean-up were recombined and reprocessed for chemical analysis by mas. The HRGC/HRMS analyses also revealed that the hepta-, octa-, and partly hexa-CDD/Fs were retained within the initial clean-up. Hence, quantified congeners encompassed *2,3,7,8-tetraCDD/F*; *1,2,3,7,8-pentaCDD/F*; *2,3,4,7,8-pentaCDF*; *and* most *2,3,7,8-hexaCDD/Fs*, as well as the 12 WHO-DL-PCBs, comprising the non-*ortho* PCBs *77*, *81*, *126*, and *169* and the mono-*ortho* PCBs *105*, *114*, *118*, *123*, *156*, *157*, *167*, and *189*. However, we could show that the comparably low TEF values of these retained congeners make them negligible for TEQ calculation.

Quantification of PCDD/Fs and PCBs was performed via isotope dilution and method of the internal standard, based on the labeled PCDD/F and PCB standards added by the RWTH Aachen prior to the initial clean-up. Overall recoveries of the internal standards through both clean-up procedures were determined by means of labeled recovery standards added prior to the instrumental analysis. Based on blanks and sample dry masses, LOQs for PCDD/Fs and PCBs were below 2 and 1 pg WHO^{2005}TEQ/g for fish and sediment samples, respectively.

Recoveries of the $^{13}C_{12}$-labeled tetra- through hexa-CDD/F quantification standards were in the range of 15–106 and 3–128 % for sediment and biota samples, respectively. Recoveries of the DL-PCBs ranged from 4 to 120 and from 27 to135 % for sediment and biota samples, respectively. Recoveries of PCDD/Fs and DL-PCBs in the three process controls ranged from 11 to 102 and from 52 to 108 %, respectively.

Calculation of BEQs and TEQs

Data were processed via Microsoft Office Excel 2003 and concentration-response curves were plotted via GraphPad Prism 5 (GraphPad Prism 5 Software Inc., La Jolla, CA, USA) using a non-linear regression (dose response stimulation; log agonist vs. response). Due to the partially very small

efficacy of the samples, effect concentrations at 10 % (EC_{10}) were used for biological toxicity equivalent (BEQ) calculation according to the following equation:

$$BEQ\ [pg/g] = \frac{TCDD\ EC_{10}\ [pg/ml]}{extract\ EC_{10}\ [g/ml]} \quad (2)$$

Instrumental derived TEQs were determined on basis of DLC congener concentrations measured via HRCG/HRMS and mammalian WHO-TEFs from 2005 (Van den Berg et al. 2006) and did not include congeners below the analytical detection limit:

$$\text{TEQ}\ [pg/g] = \sum(conc_i * TEF_x) \quad (3)$$

Data analysis and presentation

All plots and linear correlation analyses (Pearson correlation; $p < 0.05$) were conducted in GraphPad Prism 5. Statistical analyses were conducted by use of the software Sigma Stat 3. Normally distributed (Kolmogorov-Smirnov test, $p < 0.05$) data sets with equal variances (Levene's test, $p < 0.05$) were analyzed by use of parametric one-way ANOVA (Dunnett's test; $p < 0.01$). Data sets that were not normally distributed were analyzed with a Kruskal-Wallis ANOVA on ranks ($p < 0.01$) and was performed with Dunn's test as post hoc test ($p < 0.01$). A Student's *t* test ($p < 0.05$) was used to statistically analyze the impact of contaminated feed.

Results and discussion

The following sections will address sediment characterization, experimental conditions during the exposure experiments and, based on this, fish condition and mortality. In particular, instrumentally and bioanalytically derived results will be discussed with respect to method comparability (BEQs and TEQs) as well as congener-specific and dietary uptake behavior of DLCs in fish.

Characterization of sediments

Despite the missing natural origin of the lab-prepared 1:10 mixture EBR/ZE, this mixture will also be termed "*sediment*" to simplify matters.

The Elbe sediments PR and ZE clearly differ from Rhine sediment EBR in terms of composition. Generally, Elbe sediments are characterized by a greater amount of sand and thus, lesser proportions of silt and clay. Despite this smaller fraction of fine particulate matter, these sediments had greater concentrations of TOC, which was confirmed by their higher loss on ignition (Table 1). This in turn indicated a higher number of possible DLC binding sites of Elbe sediments, in principle allowing higher concentrations of contaminants.

Generally, HRGC-HRMS measurements found that sediments PR, EBR, and the mixture EBR/ZE were equally contaminated with 12 DL-PCBs and 17 PCDD/Fs, whereas sediment ZE showed an approximately 2 and 4-fold higher contamination, respectively (Table 1).

In contrast to chemically determined concentrations, RTL-W1 EROD and H4IIE micro-EROD BEQs determined for DL-PCB and PCDD/F fractions were magnitudes higher and signal strengths for PCDD/Fs were higher than those for DL-PCB fractions. However, they showed the above-described trend of sediment ZE to distinctly differ from the remaining sediments and the mixture (Table 1).

Limnochemical parameters

Limnochemical parameters provide important information on the environmental conditions during animal experiments, and moreover, form the basis for the availability of xenobiotics and reflect sediment dynamics.

Sediments differed greatly with respect to their percentage of fine particulate matter (refer to the "Characterization of sediments" section). This characteristic influenced turbidity during the exposure experiments and, hence, the amount of particulate matter present in the water phase and most likely the amount of particle-bound DLCs. *S*uspended matter concentrations of sediments EBR and EBR/ZE (consisting of nine parts EBR) were approximately twice of those observed for the remaining scenarios (Fig. 1). Moreover, concentrations were dependent on fish behavior: In contrast to the non-inoculated (−) approaches, fish in the respective (+) approaches began to feed on the worms after their transfer, and in turn, caused higher resuspension of sediment (Fig. 1). Because suspended matter concentrations are known to influence DLC uptake kinetics from water (Ahlf et al. 2002), comparably higher concentrations in EBR containing scenarios and all (+) scenarios most likely have led to a higher DLC uptake in fish through the water phase.

Average temperature and dissolved oxygen concentrations during exposure met criteria required by OECD 305 with temperature changes <2 °C (17 ± 0.6 °C) and dissolved oxygen concentrations >60 % saturation (8.3 ± 1.4 mg/L) along the six treatments (OECD 2011). Redox potential and conductivity averaged to 175.5 ± 17.0 mV and 342.2 ± 17.8 μS cm^{-3}, respectively, and pH stayed neutral to slightly alkaline among the treatments with an average unit of 7.83 ± 0.42. Total water hardness averaged to 78.5 ± 6.2, which equates 1.4 mmol calcium oxide/mL and thus can be classified as soft (Breitung and Keller 2010). Hence, all limnochemical parameters were in the ranges of tolerance of common roach.

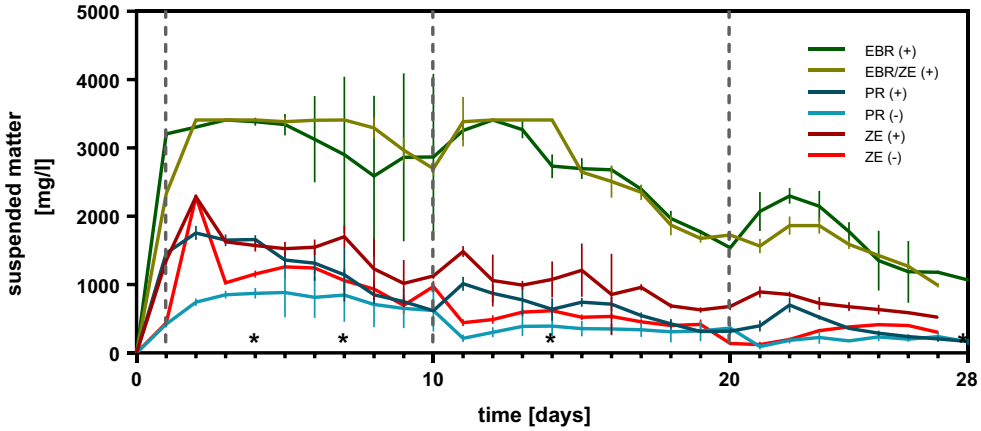

Fig. 1 Course of the suspended particulate matter concentration during a 28-day experiment with common roach (*Rutilus rutilus*) exposed to six sediment approaches: *EBR* Ehrenbreitstein, *PR* Prossen, *ZE* Zollelbe, *EBR/ZE* mixture consisting of nine dry mass (dm) parts EBR and one dm parts ZE. Approaches marked with *negative sign* in contrast to the remaining ones were not inoculated with black worm. Graphs represent the concentrations measured in four aquariums of one system (one approach) and *error bars* show standard deviations thereof. *Asterisks* mark dates of fish sampling, each performed on one of the four aquariums of the respective sediment approaches. *Dashed lines* mark dates of a fish transfer to freshly consolidated sediment

Mortality and condition of test animals

Mortality was observed in all treatment groups, particularly at the end of the 28-day exposure period, reaching values between 4 % (EBR, PR, PR (−)) and 25 % (ZE). Highest mortalities of 21 % (EBR/ZE, ZE (−)) and 25 % were observed for fish exposed to sediments containing ZE sediment. Whether mortality was caused by xenobiotics released from the different sediments is unclear, but it can be excluded that they were due to limnochemical conditions, which were comparable among treatment groups and were in the range of tolerance (*refer to* the "Limnochemical parameters" section). Health effects during the exposure experiments have not been observed, partly because high turbidity complicated such observations. Control fish originating from the tap water-filled maintenance tank did not show mortality or any effects.

The index of condition K gives information on the fish's fitness and nutritional status. It assumes that the heavier the fish in relation to its standard length, the better its condition (Kortet et al. 2003). Fish in the summer and autumn run exhibited K values of 1.8 ± 0.1 and 1.9 ± 0.2, thereby corresponded to former K values measured for common roach (Jamet and Desmolles 1994; Kortet et al. 2003). A Student's *t* test between condition indices of fish prior to and at the end of each experimental period as well as between fish of different feeding strategies showed no significant changes. These findings and the fact that K constantly was in the range of *excellent*, *good*, and *fair* (K = 1.6; 1.4; and 1.2, respectively) (Iannuzzi et al. 1995) shows that test animals did not suffer from any stress caused by experimental or environmental conditions.

Bioanalytical and HRGC/HRMS results

Bioanalytically based quality criteria

Overall, 78 measurements were performed with extracts of fish in both assays. Thereby, limits of detection (LOD) and quantification (LOQ) indicated the H4IIE micro-EROD to be the most sensitive assay. LOD and LOQ exhibited values of 0.37 ± 0.25 and 0.57 ± 0.27 pM TCDD in the H4IIE micro-EROD, respectively, and 1.20 ± 0.71 and 2.03 ± 1.24 pM TCDD in the RTL-W1 EROD assay, respectively. The use of EC_{10} values for BEQ calculation is appropriate as long as these values are well above assay-specific LODs and LOQs. While this was the case for the H4IIE micro-EROD assay, an overlap between LOQ and EC_{10} TCDD became obvious for RTL-W1 cells, thus RTL-W1 BEQs have to be evaluated with care. While the maximum EROD induction strengths of *sediment* fractions averaged to 70 ± 18 and 80 ± 9 % in the H4IIE micro-EROD and RTL-W1 EROD assay, respectively, induction strengths of *fish homogenate* extracts only averaged to 54 ± 18 and 59 ± 13 % in the H4IIE micro-EROD and RTL-W1 EROD assay, respectively. This result shows that equal efficacy of sample and standard (Villeneuve et al. 2000) was not reached for fish extracts, reflecting difficult initial test conditions of this matrix.

BEQs and TEQs of whole fish extracts depending on the sediment type

When interpreting BEQs derived in this study, it has to be considered that the insufficient initial clean-up possibly has led to impurity-related false-positive effects in extracts of fish

and sediment. Due to the missing PCB and dioxin separation in fish extracts, these substance classes cannot be interpreted separately (see the "Chemical analysis by means of HRGC/HRMS" section). However, according to Hasegawa et al. (2007) and our own experience, concentrations of BEQs accounting for the sum of DL-PCBs and PCDD/Fs correlate better with respective concentrations of TEQs as compared to concentrations of BEQs and TEQs of the single fractions. An explanation for that is that dioxins are much more potent in activating the AhR as compared to DL-PCBs. Hence, fish homogenate extracts solely allowed for evaluating the overall dioxin-like potential of all DLCs present in these samples.

The following section aims of exploring the question whether different DLC contaminations of sediments influenced uptake kinetics of those compounds into biota. Concentrations of BEQs and TEQs, reported on a lipid mass basis, in fish exposed to the four sediments to which *L. variegate* had been added (referred as (+)) are given in Fig. 2.

BEQs in fish exposed to EBR (+) exhibited temporal dependency, which was significant compared to the control in both the RTL-W1 EROD ($r^2 = 0.808$) and H4IIE micro-EROD ($r^2 = 0.864$) assay. BEQs measured in fish exposed to the remaining sediments exhibited a similar trend: a marked uptake (day 4) was followed by a slight decrease in concentration of BEQ (day 7) followed by an increase in concentration of BEQ (day 14), which in turn was followed by another decrease in concentrations of BEQ (day 28). Several other studies revealed that fine-grained bottom sediments such as EBR can act as reservoirs by reducing toxicity potential to aquatic organisms. Due to their sorptive nature, they accumulate contaminants more effectively (as reviewed by Eggleton and Thomas 2004). But once resuspended (Fig. 1), this reservoir can become a source, which in the case of fish exposed to EBR (+), could have led to temporal increasing concentrations of BEQs as measured in both assays. Trends observed for the remaining sediments could be explained by feeding behavior. As described in the "Mortality and condition of test animals" section, fish began to feed on the worms as soon as they were transferred to sediments containing the worms. This transfer took place on days 0, 10, and 20. Sampling days 4 and 14, which are located closest in time to these transfer activities, exhibited the overall highest concentrations of BEQs (Fig. 2).

Concentrations of BEQs in fish on sediment EBR (+) were significantly higher compared to the control from days 7 (H4IIE) and 14 (RTL-W1). However, there was no clear tendency observed for fish exposed to EBR/ZE (+). Concentrations of BEQs in fish at day 14 were the only ones significantly different from the control in both assays. In contrast to concentrations of BEQs determined via the RTL-W1 EROD assay, H4IIE BEQs in fish exposed to PR (+) on all sampling dates were significantly different from the control (ANOVA, Dunnett's test; $p < 0.01$). The patterns of BEQs determined for fish exposed to ZE (+) and that of EBR/ZE (+) were similar for both assays, suggesting that the single dm part ZE present in the sediment mixture substantially influenced

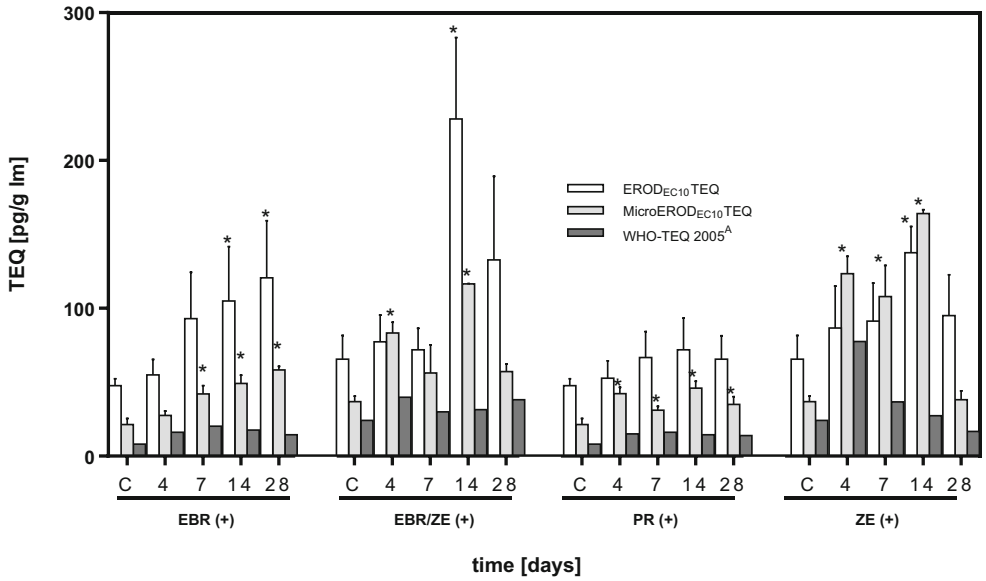

Fig. 2 Lipid mass (*lm*) normalized, instrumentally (HRGC/HRMS; WHO_{2005}TEQs; A = congeners below the detection limit were excluded) and bioanalytically (RTL-W1 EROD; H4IIE micro-EROD) determined toxicity equivalents (*TEQs*) of whole fish homogenates of common roach (*R. rutilus*), exposed to four different worm-inoculated sediments for 28 days. *EBR* Ehrenbreitstein, *PR* Prossen, *ZE* Zollelbe, *EBR/ZE* mixture consisting of nine dry mass (dm) parts EBR and one dm parts ZE. *White* (RTL-W1 EROD assay) and *light grey* (H4IIE Micro EROD assay) *bars* represent TEQs of three independent biological replicates, calculated on EC_{10} basis. *Error bars* show standard deviations thereof. *Asterisks* mark results significantly different to C = control (parametric one-way analysis of variance, ANOVA, Dunnett's test; $p < 0.01$ with Kolmogorov-Smirnov and Levene's test as pretests)

the DLC uptake in fish on sediment EBR/ZE (+). But in this circumstance, it should be mentioned that lipid mass-based DL-PCB and PCDD/F concentrations on average were 4.6-fold higher in autumn than in summer control fish, although the absolute lipid mass for both groups of fish were similar. Hence, similarities of DLC uptake in fish exposed to ZE (+) and mixture EBR/ZE (+) could also reflect seasonal differences. However, concentrations of BEQs in fish exposed to ZE (+) and EBR/ZE (+) were significantly different from the control on days 4 and 7 for H4IIE cells and day 14 for RTL-W1 cells (Fig. 2).

The comparability of BEQs determined by use of both assays was inversely proportional to sediment DLC concentrations. While linear correlation between the two assays was not significant ($r^2 = 0.11$) when fish exposed to ZE (+) were included, correlation was significant ($r^2 = 0.32$) when fish exposed to ZE (+) were excluded. Possible inhibitory effects on expression of the reporter gene in RTL-W1 cannot be excluded entirely (Behnisch et al. 2001; Lorenzen et al. 1997). BEQs determined via both assays and respective WHO_{2005}TEQs showed comparable trends (Fig. 2). Looking at the results of all three methods, concentrations of TEQs and BEQs in the roaches increased from sediments EBR (+) and PR (+) over EBR/ZE (+) to ZE (+).

In total, 29 ± 18 % of the RTL-W1 and 44 ± 15 % of the H4IIE-based BEQs could be explained through the WHO-TEQs, showing that by using the fish cell line, a higher percentage of the BEQ remains unexplained. The fact that BEQs obtained by using both cell lines were consistently higher than the respective TEQs could be due to synergism and/or the presence of compounds in the extracts not targeted by chemical analysis (Zacharewski et al. 1989). The correlation between H4IIE BEQs and TEQs ($r^2 = 0.62$) was distinctly higher than that of RTL-W1 BEQs and TEQs ($r^2 = 0.25$). The correlation of H4IIE BEQs and TEQs was higher than previously determined for whole fish samples from Saginaw Bay, Michigan, USA, where the correlation was poor ($r^2 = 0.44$) and the unexplained percentage in BEQs amounted for 75 % (Giesy et al. 1997).

Taking all these results into account and the fact that for the cell line, RTL-W1 LOQs partly overlapped with EC_{10} values, the H4IIE micro-EROD is the appropriate assay to be compared to TEQs calculated from instrumental quantification of individual congeners.

Classification of DLC uptake on an environmental quality standards basis

In the 2013 regulation, 2013/39/EU entered into force (2013/39/EU 2013), which established an environmental quality standard (EQS) of 6.5 pg TEQ/g fm for DLCs in biota. To compare the present data with this, EQS lipid mass (lm)-based data underlying Fig. 2 were normalized to fresh mass (fm) (data not shown). BEQs ranged from 1.8 to 4.9 and 1.0 to 5.5 pg BEQ/g fm in the RTL-W1 EROD and H4IIE micro-EROD assays, respectively. Chemical-based TEQs ranged from 0.7 to 3.6 pg TEQ/g fm for the sum of WHO-PCDD/Fs and DL-PCBs. Hence, all methods indicated internal DLC concentrations to be less than the threshold EQS, although the uptake of DLCs by roach was most likely promoted by the contaminated feed present in all sediments.

DLC uptake as a function of ingestion

In order to examine the hypothesis that ingestion of contaminated feed is a relevant route of exposure for fish, different feeding scenarios (see section the "Exposure scenarios" section) were applied. So far, only fish exposed to sediments containing worms, which equates another route of uptake, has been discussed. Those results (assigned by (+)) were compared with fish exposed to sediments that did not contain worms (assigned by (−)). Uptake of sediment by *L. variegatus* could be observed through the skin a few hours following their transfer on the sediments. Hence, it might be assumed that DLCs could have been passed to fish through the worms' guts. However, this matrix was not chemically investigated, so it is not proven that DLCs were accumulated in the worm.

The general assumptions for uptake of DLCs were that DLC uptake in fish (1) exposed to (+) treatments was higher than for fish on (−) treatments, (2) has a temporal dependency, and (3) increases with the DLC contamination of the sediment. For a better comparison between bioanalytically and instrumentally derived results, BEQs and TEQs in fish of each sampling date were normalized to the respective BEQs and TEQs determined in the control fish, resulting in so-called *uptake factors*.

While a Student's *t* test proved that fish on sediment PR did not meet assumption 1, differences between ZE (−) and ZE (+) fish data were significant ($p < 0.05$), at least for H4IIE micro-EROD data and, except day 28, corresponded to assumption (1).

Concerning assumption 2, instrumentally derived uptake factors for ZE (−) showed a temporal trend, which was opposite to that of ZE (+) uptake factors, demonstrating that fish on sediment, which did not include worms, constantly accumulated DLCs over time, while fish that fed on worms had a higher initial uptake of DLCs followed by a distinct decrease. The high initial uptake of DLCs most likely caused a higher degradation of DLCs through xenobiotic enzymes, which in turn could have led to the aforementioned decrease of uptake factors. By comparing these observations with H4IIE micro-EROD data, ZE (−) data correlated well with instrumental ZE (−) data ($r^2 = 0.99$; $n = 4$), and except day 14, the trend of a high initial uptake and subsequent decrease has been shown.

On a relative basis, uptake factors aligned both seasonal (summer; autumn) and methodological (BEQs; TEQs) differences among fish exposed to the different scenarios. Uptake factors of fish exposed to sediment PR predominantly

indicated an uptake of DLCs (factors > 1). The general pattern of uptake factors determined for fish on PR (Fig. 3a) and ZE (Fig. 3b) distinctly differed from one another, which points toward but does not verify assumption 3. According to Rubinstein and coworkers (Rubinstein et al. 1984), who chemically investigated the dietary uptake of PCBs by spot croaker, sediments serve as a source of PCBs. They moreover demonstrated that fish exposed to PCB-contaminated sediments and daily fed with polychaetes from the same sediment accumulates more than twice as much whole-body concentrations of PCBs after 20 days than fish exposed to similar conditions but fed with uncontaminated polychaetes (Rubinstein et al. 1984). This corresponds to our results for fish exposed to sediment ZE, where the uptake of DLCs in the presence of worms in the sediment was 2.1 (TEQs) and 1.8-fold (BEQs) higher than in the absence of worms. Despite the fact that sediment ZE had the highest concentration of DLCs, uptake factors of fish on average did not significantly differ from those calculated for fish on PR ((−) 1.8; (+) 2.0), but species with a higher body fat content, such as rainbow trout, most likely would have exhibited higher uptakes of DLCs.

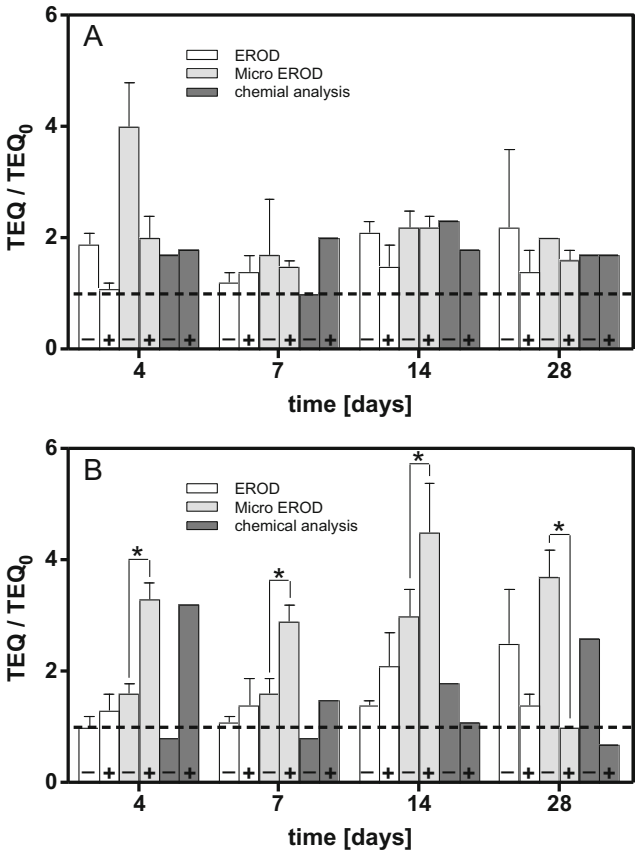

Fig. 3 **a**, **b** Comparison between instrumentally (HRGC/HRMS, WHO-TEQ2005) and biochemically (RTL-W1 EROD, H4IIE micro-EROD) determined factors (TEQ/TEQ control) of fish exposed to sediment Prossen (**a**) and Zollelbe (**b**) under consideration of presence (+) and absence (−) of contaminated feed. *Dashed line* marks the level above which uptake took place. *White* (RTL-W1 EROD assay) and *light grey* (H4IIE Micro EROD assay) *bars* represent TEQs of three independent biological replicates, calculated on an EC_{10} basis. *Error bars* show standard deviations thereof. *Asterisks* mark significant differences between (-) and (+) approaches analyzed by using a Student's *t* test ($p < 0.05$)

Internal DLC concentrations in context to former studies

BEQs in fish exposed to four (+) sediments averaged to 1.8 to 4.9 and 1.0 to 5.5 pg/g fm based on the RTL-W1 EROD and H4IIE micro-EROD assay, respectively, and with this were highly comparable to BEQs determined in whole fish and seafood samples (0.1 to 4.5 pg/g fm) using DR-eco screen cells (Kojima et al. 2011). Moreover, minimum concentrations of congeners 105 and 118 found in fillets of roach from the Baltic Sea (Burreau et al. 2004) corresponded to control fish concentrations determined in this study. Both examples imply that concentrations of DLCs in roach during this study might correspond to background contamination. The percentage of individual DL-PCBs and PCDD/Fs found in the present study was highly comparable to those observed in previous studies, where PCB 77 was the most abundant congener among the non-*ortho*-substituted PCBs and PCB 118, 105, and 156 being the most abundant congeners among the mono-*ortho*-substituted PCBs (Hasegawa et al. 2007), whereas 2,3,7,8-TCDF was the most abundant congener among the dioxins (Hasegawa et al. 2007; Zacharewski et al. 1989). The relative abundance of the 12 DL-PCBs in this study furthermore highly corresponded to previous analyses of PCB patterns in fillets of three cyprinid species from the river Po (Viganò et al. 2000).

Congener-specific uptake following 28 days of exposure

This section focuses on concentrations of individual DLCs and their uptake by roach following 28 days of exposure to four (+) sediments. DLC congeners, their octanol-water portioning coefficient (log K_{ow}), water solubility, and initial sediment concentrations are given in Table 2, accompanied by their respective uptake factors in roach.

DL-PCB uptake factors ranged between 0.5 (ZE (+)) and 4.8 (EBR/ZE (+)). In total, only four of the factors were < 1, showing that mainly uptake of DLCs took place during the exposure. This corresponds to previous findings of Blanco et al. (2007), who found that all DL-PCB except PCB 169 accumulated in fish. The non-*ortho*-substituted PCBs 77, 81, 126, and 169 are more potent DLCs than are their mono-*ortho* analogs and thus, of greater concern. However, some studies have found that planar PCBs 77, 126, and 169 accumulate to a lesser extent than might be expected based on their structure (Engwall 1995; Van Bavel et al. 1996). PCB 81 and 169 occurred at small concentrations or were not detected in sediments and in turn were not detected in roach. Hence, these two congeners, although present in sediments and relatively potent, were not of toxicological concern for common roach.

Table 2 Log K_{ow} values, water solubilities, as well as uptake factors (in fish after 28 days of exposure to four sediments and normalized to concentration of fish control) and initial concentrations (in sediments) of non-*ortho* (77, 81,126, 169) and mono-*ortho* (105, 114, 118, 123, 156, 157, 167, 189) PCBs and several PCDD/Fs

			Uptake factors (c/c_0) in fish exposed to sediment				Sediment congener concentration (pg/g dm)			
	Log K_{ow}[a]	Water solubility at 25 °C[a,c]	EBR	EBR/ZE	PR	ZE	EBR	EBR/ZE	PR	ZE
PCB 77	6.63[b]	0.0298	2.5	1.6	2.1	1.7	297	422	357	948
PCB 81	6.34	0.0532	n.d.	n.d.	n.d.	n.d.	n.d.	7	12	28
PCB 126	6.98	0.0094	1.5	2.2	1.0	1.0	25	35	24	50
PCB 169	7.41[b]	0.0025	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PCB 105	6.79[b]	0.0136[b]	1.6	1.5	1.3	1.4	446	453	391	939
PCB 114	6.98	0.0094	1.5	1.4	1.2	1.4	23	32	29	62
PCB 118	7.12[b]	0.0071	1.5	1.4	1.2	1.4	1600	1970	2330	5300
PCB 123	6.98	0.0094	3.8	4.8	3.1	2.9	21	34	23	81
PCB 156	7.60[b]	0.0017	1.6	1.9	1.4	0.9	533	607	1030	1170
PCB 157	7.62	0.0016	1.5	2.0	1.1	0.9	102	121	223	162
PCB 167	7.50[b]	0.0021	1.4	1.9	1.2	0.7	339	369	518	706
PCB 198	8.27	0.0003	1.4	1.9	1.3	0.5	112	132	279	270
12378-penta-CDD	6.64[b]	0.0009	1.4	n.a.	1.2	n.a.	1.1	2.8	0.7	4.4
2378-tetra-CDF	6.63	0.0019	2.3	2.6	1.7	1.8	0.6	5.8	0.5	3.0
12378-penta-CDF	7.27	0.0003	n.a.	4.3	n.a.	4.1	2.2	6.6	3.7	67.7
23478-penta-CDF	7.27	0.0003	2.6	3.8	1.8	2.8	3.1	7.4	4.5	81.1

EBR/ZE mixture consisting of nine dry mass (dm) parts EBR and one dm parts ZE. Uptake factors were calculated on a dry mass (dm) basis

EBR Ehrenbreitstein, *PR* Prossen, *ZE* Zollelbe, *n.d.* not detectable, *n.a.* not analyzable (in case congener was not detectable in the control group)

[a] Estimated values (unless highlighted with [b]) from the US-EPA data base "EPISuite"

[b] Experimentally determined value from the EPI database

[c] Estimated from the respective log K_{ow}

The remaining non-*ortho* congeners were present in sediments at relatively small (PCB 126) or high (PCB 77) concentrations, but exhibited equivalent uptake factors in roach. RTL-W1 EROD and H4IIE micro-EROD relative potencies (REPs) for PCB 77 and 126 correspond to 60 and 1170 times the average REP of all mono-*ortho* DL-PCBs, respectively (Behnisch et al. 2002; Clemons et al. 1997).

Assuming that these in vitro REPs are comparable to potentials which occur in vivo, PCB 126 and 77 would be expected to pose the greatest potential risk to fish from all 12 WHO-DL-PCBs. For mono-*ortho* PCBs, most of the uptake factors were independent of initial concentrations in the sediments. For instance, the concentration of PCB 118 among the four sediments was, on average, 75 times the concentrations of PCB 114. Nevertheless, uptake factors calculated for fish were equivalent. Hence, it might be assumed that an uptake of DLCs from sediment into biota cannot reliably be deduced from the sediments' initial contamination level of DLC, which in turn complicates the determination of environmental quality standards.

The largest uptake factors among all chosen congeners were observed for PCB 123. Despite small initial concentrations of this congener in all sediments, uptake factors of as much as 10-fold higher compared to control were observed (ZE (+) day 4, Fig. 4). These findings are supported by a study which revealed considerable concentrations of PCB 123 in liver and muscle tissue of eels (*Anguilla anguilla*) from the Camargue Nature Reserve, France, compared to the remaining mono-*ortho* DL-

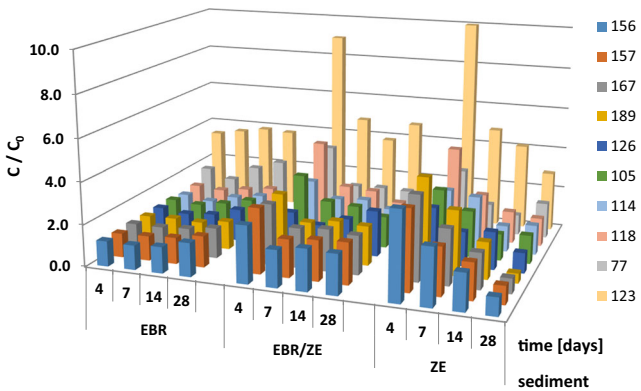

Fig. 4 Temporal course of the control normalized uptake of 12 WHO-DL-PCB congeners (except PCB 169) in common roach (*R. rutilus*) exposed to sediments *EBR* Ehrenbreitstein, *ZE* Zollelbe, and a 9:1 dry mass mixture thereof (EBR/ZE) for 28 days. Congeners (*legend*) were determined instrumentally (HRGC/HRMS)

PCBs (Oliveira Ribeiro et al. 2008). Uptake factors observed might be due to the small log K_{ow} value and relatively high water solubility of PCB 123, compared to the other mono-*ortho* PCB congeners. Moreover, more chlorinated PCBs such as PCB 123 are known to have a higher potential to biomagnify (Porte and Albaigés 1994). Hence, besides the non-*ortho* relates 77 and 126, PCB 123 is predicted to have the highest potential to adversely affect the aquatic fauna.

Although scientists have revealed DL-PCBs to bioaccumulate more efficiently than PCDD/Fs (Blanco et al. 2007; Isosaari et al. 2002), uptake factors of PCDD/Fs in roach were comparable to those determined for DL-PCBs and exhibited values between 1.2 and 4.3, which slightly correlated ($r^2 = 0.44$) with their initial concentrations in the sediments. This might demonstrate that the uptake kinetics of PCDD/Fs and DL-PCBs follow different principles and that the uptake of dioxin is a function of how much sediment is ingested. Thus, it can be concluded that in addition to log K_{ow}, water solubility, and initial concentration of DLCs in the sediments, other factors influence the uptake of DLCs (Table 2).

Temporal congener-specific uptake depending on sediment characteristics

The temporal patterns of uptake of DL-PCB by roach exposed to sediment mixture EBR/ZE (+) were compared with those obtained for each sediment EBR (+) or ZE (+) alone (Fig. 4). The mixture consisted of nine dm parts EBR and one dm part ZE. Both redox potential and pH can accelerate desorption, partitioning, and bacterial degradation, thereby increasing bioavailability of organic chemicals (Eggleton and Thomas 2004). Hence, sediments of different origins can differentially transform contaminants into more bioavailable or toxic forms. Predicted concentrations of the 12 DL-PCBs in sediment EBR/ZE, deduced from the 1:10 mixing ratio, on average only differed by 12 % from the actual concentrations, demonstrating that uptake factors measure in fish on differing sediments, are the result of sediment-specific characteristics and not a result of varying distributions of congeners.

While, in fish exposed to EBR (+), most of the congeners exhibited a slight increase in uptake factors over time, fish exposed to ZE (+) showed a high initial uptake, followed by a decrease. The high initial uptake has been observed previously in fish exposed to sediments containing PCBs (Rubinstein et al. 1984).

For fish exposed to the 1:10 mixture of sediment, it was expected that uptake factors would be similar to those determined for fish exposed to EBR (+), since there was a higher proportion of EBR (+) present in the mixture. But, in contrast, fish exhibited the same high initial uptake as observed for fish exposed to ZE sediment. These uptake factors moreover showed comparable values ranging from 1.7 to 9.0 and 2.7 to 10.0 for EBR/ZE (+) and ZE (+), respectively. The high initial uptake by fish exposed to the mixture was followed by a constant baseline, which stayed unaffected by the decrease of uptake factors as it was observed for fish on ZE (+). The baseline was consistently higher, which was expected due to the nine dm parts EBR present in the mixture.

Results of this study indicate that (1) the initial uptake of PCBs by fish is controlled by sediment ZE (+), the sediment with higher concentrations of DL-PCBs, and (2) characteristics of sediment EBR promote the uptake of DL-PCBs originating from sediment ZE (+). Hence, the uptake of DLCs by roach after 28 days of exposure is dependent on sediment-specific characteristics rather than the initial congener concentration.

Conclusion

HRGC/HRMS and bioassay-derived uptake factors predominantly indicated an uptake (factors > 1) of sediment-borne DLCs by common roach. Calculation of factors eliminated seasonal (summer; autumn) and methodological (HRGC/HRMS; bioassay) differences observed for TEQs and BEQs. BEQs in fish exposed to sediment EBR (+) increased with time, whereas BEQs of fish exposed to the remaining sediments reflected suspended matter concentrations caused by fish transfer and feeding activities. In contrast to fish exposed to sediment PR, contaminated feed (+) on average caused a 2.1 (TEQs) and 1.8-fold (H4IIE BEQs) higher DLC uptake in fish exposed to sediment ZE compared to feeding with uncontaminated worms (−). This indicates that contaminated feed only promotes the uptake of DLCs by roach exposed to highly contaminated sediment.

Results based on both TEQs and BEQs revealed that the uptake of DLCs was largely independent of the initial concentrations of DLCs in sediments. This was further confirmed by a comparison of the pattern of uptake of DL-PCBs by fish exposed to lesser contaminated sediment from EBR (+), the more contaminated sediment from ZE (+), and a 1:10 mixture (+) thereof. This demonstrated that uptake of DL-PCBs by roach after 28 days of exposure depends rather on sediment-specific characteristics than on initial concentrations of DLCs in the sediment. Congener-specific considerations of the uptake of DL-PCBs by roach indicated PCB congeners 123, 77, and 126 among all of the DL-PCBs to pose the highest risk to roach.

All bioassay and HRGC/HRMS-derived equivalents were less than the EQS of 6.5 pg TEQ/g fm for DLCs in biota. Organic extracts from fish turned out to be a complicated matrix for BEQ calculations due to low induction strengths. However, especially H4IIE, BEQs showed a high sensitivity (LOD = 0.37 ± 0.25 pM TCDD) and a high correlation with TEQs ($r^2 = 0.62$). The authors therefore suggest using the H4IIE micro-EROD as bioanalytical alternative or amendment for congener-specific instrumental sediment and biota screening analysis.

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