Comparative Biochemistry and Physiology, Part C 169 (2015) 46–54

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Transfer and effects of 1,2,3,5,7-pentachloronaphthalene in an experimental food chain

Tineke Slootweg [a,g,*], Helmut Segner [b], Philipp Mayer [c,1], Kilian Smith [c,2], Elizaveta Igumnova [d], Vladimir Nikiforov [d,e,3], Milena Dömötörová [f], Jörg Oehlmann [g], Markus Liebig [a,4]

[a] ECT Oekotoxikologie GmbH, Boettgerstrasse 2-14, 65439 Floersheim/Main, Germany
[b] Centre for Fish and Wildlife Health, University of Bern, Post Box 8466, 3001 Bern, Switzerland
[c] Aarhus University, Department of Environmental Science, Frederiksborgvej 399, DK-4000 Roskilde, Denmark
[d] Saint-Petersburg State University, Universitetskaya 7/9, 199034 St. Petersburg, Russia
[e] Institution of Russian Academy of Sciences Saint-Petersburg Scientific-Research Centre for Ecological Safety RAS (SRCES RAS), Korpusnaya ul., 18, 197110 St. Petersburg, Russia
[f] Slovak Medical University, Department of Toxic Organic Pollutants, Limbova 12, 833 03 Bratislava, Slovakia
[g] Goethe University Frankfurt am Main, Department Aquatic Ecotoxicology, Max-von-Laue-Str. 13, 60438 Frankfurt, Germany

ARTICLE INFO

Article history:
Received 11 November 2014
Received in revised form 9 January 2015
Accepted 11 January 2015
Available online 17 January 2015

Keywords:
Polychlorinated naphthalenes
Juvenile growth test
Dietary exposure
CYP1A1
ABCB1
Passive dosing
Rainbow trout

ABSTRACT

Polychlorinated naphthalenes are environmentally relevant compounds that are measured in biota at concentrations in the μg/kg lipid range. Despite their widespread occurrence, literature data on the accumulation and effects of these compounds in aquatic ecosystems are sparsely available. The goal of this study was to gain insights into the biomagnification and effects of 1,2,3,5,7-pentachloronaphthalene (PeCN52) in an experimental food chain consisting of benthic worms and juvenile rainbow trout. Worms were contaminated with PeCN52 by passive dosing from polydimethylsiloxane silicone. The contaminated worms were then used to feed the juvenile rainbow trout at 0.12, 0.25 or 0.50 μg/g fish wet weight/day, and the resulting internal whole-body concentrations of the individual fish were linked to biological responses. A possible involvement of the cellular detoxification system was explored by measuring PeCN52-induced expression of the phase I biotransformation enzyme gene *cyp1a1* and the ABC transporter gene *abcb1a*.

At the end of the 28-day study, biomagnification factors were similar for all dietary intake levels with values between 0.5 and 0.7 kg $lipid_{fish}$/kg $lipid_{worm}$. The average uptake efficiency of 60% indicated that a high amount of PeCN52 was transferred from the worms to the fish. Internal concentrations of up to 175 mg/kg fish lipid in the highest treatment level did not result in effects on survival, behavior, or growth of the juvenile trout, but were associated with the induction of phase I metabolism which was evident from the significant up-regulation of *cyp1a1* expression in the liver. In contrast, no changes were seen in *abcb1a* transcript levels.

1. Introduction

Polychlorinated naphthalenes (PCNs) are a family of two-ringed aromatic compounds with high chemical and thermal stability (Falandysz, 1998). They are widespread in the environment, and in 2011 the European Union proposed that PCNs should be included in the annexes of the "Stockholm Convention on Persistent Organic Pollutants". Currently they are under review by the Persistent Organic Pollutants Review Committee (POPRC).

The presence of PCNs in the environment is partly due to their use in a variety of industrial products such as wood preservatives, electronic equipment and cable insulation (Åkerblom et al., 2000). Although their production and use in the United States and Europe were discontinued in the 1980s (Jakobsson and Asplund, 2000), a major source of PCN in the environment remains a leakage from landfills containing PCN products and polychlorinated biphenyl (PCB) formulations in which PCNs are present as impurities (Yamashita et al., 2000). Another important source is via unintentional formation during industrial thermal processes (chloralkali industries, municipal waste incineration) (Liu et al., 2014). PCNs are also emitted during the domestic burning of coal and wood (Lee et al., 2005).

Although PCNs are measured in sediments and soils at concentrations in the μg/kg range (Marvin et al., 2002; Lundgren et al., 2003; Brack et al., 2008; Castells et al., 2008; Pan et al., 2008), and in the μg/kg lipid range in

* Corresponding author at: Het Waterlaboratorium, JW Lucasweg 2, 2031 BE Haarlem, The Netherlands. Tel.: +31 064 613 17 36; fax: +31 23 517 59 99.
E-mail address: tineke.slootweg@hetwaterlaboratorium.nl (T. Slootweg).
[1] Technical University of Denmark, Department of Environmental Engineering, 2800 Kgs. Lyngby, Denmark.
[2] Convergence Environment Team, Korea Institute of Science and Technology Europe, Campus E 7.1, Universitat des Saarlandes, Saarbrucken, Germany.
[3] Norwegian Institute for Air Research, Instituttveien 18, 2007 Kjeller, Norway.
[4] STADA Arzneimittel AG, Stadastrasse 2-18, 61118 Bad Vilbel, Germany.

http://dx.doi.org/10.1016/j.cbpc.2015.01.001
1532-0456/

biota (Falandysz, 1998; Corsolini et al., 2002; Domingo, 2004; Evenset et al., 2005; Llobet et al., 2007; Fernandes et al., 2011), literature data on the accumulation and effects of PCNs in aquatic ecosystems are sparsely available. Most studies investigating their toxicity have been performed with technical PCN-mixtures such as Halowax, with long-term effects of exposure to PCN-mixtures in rats and fish including liver damage, loss of focus or starvation (Holm et al., 1993; Norrgren et al., 1993; Åkerblom et al., 2000; Kilanowicz et al., 2009). Bioaccumulation and toxicity of PCNs in organisms are highly congener specific (UNEP, 2012). PCNs which do not have two adjacent carbon atoms unsubstituted with chlorine undergo little metabolic degradation and can accumulate in fish and fish-eating birds with biomagnification factors of up to 50 (Falandysz, 1998; UNEP, 2012).

The fate and effects of environmental contaminants in target species can in part be explained by the presence of cellular detoxification mechanisms (Schlenk et al., 2008). An important and well documented mechanism is the biotransformation of xenobiotics into more easily excreted compounds. Cellular biotransformation converts parent compounds by means of phase I enzymes into more hydrophilic metabolites through oxidation, reduction or hydrolysis reactions. The key enzymes in biotransformation during phase I are members of the cytochrome P450 family (CYP) which are regulated by the aryl hydrocarbon receptor (AhR) (Schlenk et al., 2008). In phase II the metabolites are conjugated to endogenous molecules such as glutathione to further increase the solubility of the compound. Besides the enzymatic processes of phase I and II metabolism, another process contributing to cellular detoxification involves the ATP-binding cassette (ABC) transport proteins. ABC transporters are ATP-dependent efflux pumps that can prevent the cellular accumulation of hydrophobic compounds (phase 0 metabolism) or extrude xenobiotics and their metabolites from cells (phase III metabolism) (Fischer et al., 2011; Luckenbach et al., 2014). In the liver, ABCB1, also known as P-glycoprotein, is a prominent ABC transporter that has been specifically linked with a xenobiotic transport and is located at the apical side (biliary membrane) of the hepatocytes (Sturm and Segner, 2005). Induction of ABCB1 expression is a protective response to xenobiotic uptake that has been found in several studies with fish (Albertus and Laine, 2001; Cárcamo et al., 2011; Diaz de Cerio et al., 2012).

This study focused on the aquatic food-chain transfer of an individual PCN congener and the resulting effects on the consumer species, including possible effects on cellular detoxification systems. The PCN congener 1,2,3,5,7-pentachloronaphthalene (PeCN52) was chosen as a model compound, because it is one of the most frequently found PCN in biota (especially in fish). In addition, it has been shown in in vitro assays that PeCN52 has a relatively high AhR-activating potency (Falandysz, 1998; Villeneuve et al., 2001). A controlled laboratory experiment was performed using a simplified experimental food chain in which juvenile rainbow trout were fed daily with live PeCN52-contaminated worms.

The specific aims were:

1) To determine the transfer of PeCN52 in an aquatic food chain. To this end the bioconcentration of PeCN52 from water to *Lumbriculus variegatus* worms was determined by exposing them to constant dissolved concentrations of PeCN52 using passive dosing. Next, the biomagnification of PeCN52 from worms to trout was measured. Here, fish were exposed individually to contaminated worms, and the biomagnification factors (BMF) were calculated for each fish based on the dietary intake from the PeCN52 contaminated worms and the measured internal whole-body concentrations.
2) To explore any possible involvement of detoxification processes, namely the ABCB1 transporter and the biotransformation enzyme CYP1A. For this, transcript levels of the genes encoding for ABCB1a, one of the isoforms of ABCB1 found in trout (Fischer et al., 2011), and CYP1A1 were measured in tissues of PeCN52 exposed trout.
3) To determine the toxicity effects in trout from dietary exposure to PeCN52. Here, the measured whole-body concentrations were linked to potential toxic effects including survival, growth, liver somatic index and behavior.

2. Materials and methods

2.1. Chemicals

PeCN52 (purity > 99%) was synthesized at Saint-Petersburg State University. Details of the synthesis are provided in the supplemental materials (Supplemental data, part 1). Briefly, preparation of PeCN52 was based upon conversion of 1-chloronaphthalene(I) to 1,1,2,3,4-pentachloro-1,2,3,4-tetrahydronaphthalene(II) via addition of chlorine in $CHCl_3$ solution, double dehydrochlorination of 1,1,2,3,4-pentachloro-1,2,3,4-tetrahydronaphthalene with NaOH in boiling ethanol, sulfonation of 1,2,3-trichloronaphthalene(III) followed by reaction with $SOCl_2$, and finally, reaction of 1,2,3-trichloronaphthalene-5,7-disulfochloride(IV) with hexachlorocyclopentadiene.

2.2. Medium

Medium for both culturing of the test organisms and use in the test systems was prepared by making a 1:1 dilution of reconstituted water, prepared according to the OECD test guideline 203 (OECD, 1992), using deionized water. For culturing and exposure of the trout, 0.1% of artificial seawater (28 g/L of Tropic MarinH sea salt, Dr. Biener) was additionally added to the above medium.

2.3. Culturing of the test organisms

Sediment dwelling worms (*L. variegatus*) were cultured in glass vessels containing aerated water and quartz sand. Cultures were kept at 20 ± 2 °C under a light regime of 16 h light and 8 h dark. Worms were fed twice a week with TetraMin®.

Juvenile rainbow trout *Oncorhynchus mykiss* (0.4–0.5 g body weight) were obtained from a commercial fish farm (Forellenzucht Trostadt GbR, Germany). Fish were held for 8 weeks in a 300 L aquarium at 15 ± 2 °C under a light regime of 12 h light and 12 h dark, and were fed twice a day with DAN-EX 1362 GR 1.0 mm pellets (DANA Feed A/S, Denmark). At the start of the test, fish were 2.5 months old.

The average dry weight (dw) percentages were 19.0% of wet weight (ww) for trout and 15.1% of ww for worms. These were used to calculate the lipid percentage based on dw, which were 10.4% for trout and 12.4% for worms.

2.4. PeCN52 contaminated diet

Worms were contaminated by exposing them for 168 h in medium saturated with PeCN52, with the concentrations in the medium kept constant by passive dosing from polydimethylsiloxane (PDMS) silicone cast into the bottom of the test jar as described in Mayer and Holmstrup (2008) and Engraff et al. (2011). The PDMS was first loaded to saturation with PeCN52, such that during the equilibrium passive dosing of the medium this was also saturated. To ensure that the PDMS was in fact saturated, an initial experiment was performed to determine the loading kinetics. More details on the preparation of the jars and the loading experiment are given in the supplemental materials (Supplemental data, part 2).

A volume of 150 mL medium was added to the passive dosing jars, together with some small pieces of paper tissues for the worms to attach to. The system was equilibrated for 24 h at 20 ± 2 °C, whereby the medium was mixed thoroughly through aeration via a Pasteur pipette. To prevent loss of PeCN52 by volatilization during the equilibration period, this air was pre-saturated with PeCN52 by passing through a cotton wool with 100 μL of PeCN52 saturated olive oil placed in the Pasteur