Chemosphere 137 (2015) 52–58

Contents lists available at ScienceDirect

Chemosphere

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

Organochlorine pesticides and polychlorinated biphenyl congeners in wild terrestrial mammals from Croatia: Interspecies comparison of residue levels and compositions

Snježana Herceg Romanić [a], Darija Klinčić [a], Zorana Kljaković-Gašpić [b,*], Josip Kusak [c], Slaven Reljić [c], Đuro Huber [c]

[a] Biochemistry and Organic Analytical Chemistry Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia
[b] Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia
[c] Department of Biology, Veterinary Faculty, University of Zagreb, Zagreb, Croatia

HIGHLIGHTS

- PCBs and OCPs were determined in adipose tissue of wild bear and wolf from Croatia.
- PCBs were dominant accounting for 72–81% of total analyzed POPs.
- Distribution of PCBs was species dependent.
- The results were discussed in the light of different food preferences of species.
- Contaminant levels were low and normal for large terrestrial carnivores.

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 29 April 2015
Accepted 7 May 2015
Available online 22 May 2015

Keywords:
OCPs
PCBs
Brown bear (*Ursus arctos*)
Grey wolf (*Canis lupus*)
Terrestrial wildlife
Croatia

ABSTRACT

In this pilot study, we investigated levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in the adipose tissues of two free-ranging terrestrial carnivores from Croatia sampled in 2010 and 2011: the brown bear (*Ursus arctos*; $N = 32$) and the grey wolf (*Canis lupus*; $N = 29$). Concentrations of $\sum$OCPs and ΣPCBs ranged from 0.45 to 4.09 ng g^{-1} lipid mass (lm) and from 0.93 to 8.52 ng g^{-1} lm in brown bear, and from 1.18 to 5.67 ng g^{-1} lm and 2.68 to 48.9 ng g^{-1} lm in grey wolf adipose tissues, respectively. PCBs were dominant accounting for over 72% of total analyzed persistent organic pollutants (POPs). The sum of six indicator non-dioxin-like PCBs (Σ6IndNDL PCBs) made up 60–93% and 58–85% of the total congener concentrations in brown bears and wolves, respectively. Although the levels of the measured parameters were significantly higher in grey wolves than in bears, the contaminant profiles of the two species were similar, with γ-HCH, HCB, β-HCH and DDE as major OCP contaminants, and PCB-153 > PCB-180 $\approx$ PCB-170 > PCB-138 as the dominant congeners. The sum of hexachlorocyclohexanes (ΣHCHs) and 8 toxicologically relevant dioxin-like PCBs (Σ8ToxDL PCBs) was higher in the males than in the females of the brown bear. Concentrations of ΣDDTs, HCB, ΣOCP, ΣPCBs, Σ6IndNDL PCBs, and toxicologically relevant non-dioxin-like PCBs (ΣToxNDL PCBs) were significantly positively correlated with lipid content in the grey wolf. Concentrations of OCPs and PCBs in brown bears and wolves from Croatia were low and normal for large terrestrial mammals.

1. Introduction

Global contamination by environmentally persistent organic compounds (POPs) has been extensively documented (Ritter et al., 1995; ATSDR, 2002; AMAP, 2004). Due to their physical and chemical properties, PCBs and OCPs are resistant to physical, chemical and biological breakdown mechanisms. As they are semi-volatile, they can be globally distributed through the atmosphere to remote regions where they have never even been used (Muir et al., 1992; Ritter et al., 1995). Due to their persistence and lipophilic properties, they bio-accumulate in the adipose tissues of living organisms and pose a threat to wildlife and humans (Ritter et al., 1995). Exposure to POPs has been associated with

* Corresponding author at: Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, P.O. Box 291, HR-10001 Zagreb, Croatia.
E-mail address: zorana.kljakovic-gaspic@imi.hr (Z. Kljaković-Gašpić).

http://dx.doi.org/10.1016/j.chemosphere.2015.05.026
0045-6535/

cancer, neurotoxic, behavioral and reproductive effects (Ritter et al., 1995; Fisk et al., 2005; Letcher et al., 2010). Due to their accumulation in fat they are likely to be passed on to succeeding generations *in utero* and/or through breast milk (Bernhoft et al., 1997). Despite the global actions undertaken to reduce and eliminate the production, use and releases of these substances (Stockholm Convention; UNEP, 2001), they are still found in the environment at levels that may cause negative effects to the health of individual animals and have negative impact on entire animal populations.

The accumulation of POPs in terrestrial food webs has been studied far less (Carril González-Barros et al., 2000; Shore et al., 2001; Christensen et al., 2005; Polder et al., 2009; Herceg Romanić et al., 2012; Mateo et al., 2012) than in aquatic environments (AMAP, 2004; Dietz et al., 2004; Bentzen et al., 2008a,b; Letcher et al., 2010; Dietz et al., 2013). This is mainly because the levels of POPs in terrestrial food webs tend to be lower. As a result, little is known about the fate, distribution and effects of POPs in the terrestrial environment. To the best of our knowledge, so far there have been no studies investigating PCBs and OCPs concentrations in terrestrial carnivores in Croatia. Data about PCBs and OCPs presence in terrestrial wildlife are also generally scarce, and exist only for herbivores (Herceg Romanić et al., 2012). In the region, the only study on PCBs and OCPs concentrations in the brown bear was conducted in the neighboring Slovenia (Bolta et al, 2006).

To address this gap in knowledge, we evaluated the concentrations of 17 PCBs and 7 OCPs in the adipose tissues of two top terrestrial carnivores, the grey wolf (*Canis lupus*) and the brown bear (*Ursus arctos*), from Croatia. The objective of this study was also to provide for the first time information on a wide range of legacy organic pollutants in the wolf and bear in general. In addition we explored the differences between POPs burden in relation to the age, sex and food preferences of a species.

2. Material and methods

2.1. The investigated species, their habits and diets

The present study included two carnivorous species: brown bear (*U. arctos*) and grey wolf (*C. lupus*).

The brown bears that live in Croatia and neighboring Slovenia are a part of the Dinara–Pindos population, the third largest population in Europe. It is estimated that around 1000 individuals of this population live in Croatia alone (Kaczensky et al., 2012). With Croatia's EU accession in 2013, it became a strictly protected species (OG, 2013). The average body mass of Croatian adult brown bear females is 120 kg and males 210 kg; however, some individuals can attain a mass of more than 300 kg. In the course of a year, the mass of the individual is largest before denning in the late autumn, and lowest at the beginning of summer or at the end of mating season. Bears usually become sexually mature at the age of 3–4, and females may have 1–4 cubs, but most frequently 2. The average age of the Croatian brown bear population is around 5 years, although they can survive in the wilderness up to the age of 10 to 20 years. In Croatia, bears hibernate for 2.5–4.5 months per year. According to its feeding habits, the brown bear is an omnivore, usually deriving up to 95% of dietary needs from green vegetation (plants, soft fruits, berries, beech nuts, pine cones, mushrooms), the rest being small insects and carcasses of big mammals. The plant foods in spring and summer are mostly green plants and grasses, supplemented in the summer with soft fruits, and in the autumn with beechnuts – the main food source for the accumulation of winter subcutaneous fat. The population number is monitored and regulated by prescribed annually quota hunting (Huber et al., 2008; Cicnjak et al., 1987).

The grey wolf is also a strictly protected species in Croatia (OG, 2013). According to a map of wolf habitat distribution in 2009, Croatian wolves are present in the areas of Gorski kotar, Lika and Dalmacija, and occasionally appear in the Dinaric border areas in the north and south (Kusak, 2002). For wolves, the main source of food is meat (Kusak, 2002). The average body mass of adult grey wolf males is 35.7 kg and females 28.8 kg. The differences in the content of a wolf's prey depend on what food sources are available locally; in uninhabited areas wolves consume even-toed (roe deer, red deer, wild boar) and smaller mammals, while in areas with developed extensive livestock breeding, domestic animals account for 84% of a wolf's diet.

2.2. Sampling

Adipose tissues of the grey wolf and brown bear were collected from animals legally hunted or accidentally killed (on roads and railways) in cooperation with local hunters and conservation officers. No bears and wolves were killed or otherwise sampled for the purpose of this study. The study was conducted in the Croatian regions of Gorski kotar, Lika and Dalmacija during 2010 (from March to October) and 2011 (from March to December) (Supplementary materials; Fig. S1, Table S1).

Adipose tissues were collected from 32 brown bears (23 males, 9 females), ranging in age from 6 months to 15 years and 29 grey wolves (15 males, 14 females) ranging in age from 6 months to 6 years (Supplementary materials; Table S1). Each fat tissue sample was packed separately in polypropylene BD falcon tubes, frozen and sent to the Faculty of Veterinary Medicine, Zagreb, Croatia, accompanied with the matching first premolar tooth and information about each animal (sex, estimated age, body measures, location coordinates). The samples were stored in a freezer (−20 °C) until analysis in the Biochemistry and Organic Analytical Chemistry Unit of the Institute for Medical Research and Occupational Health (IMROH). Age determination in bears was performed using counts of cementum layers surrounding the root of the first premolar tooth (Stoneberg and Jonkel, 1966), while the age of the wolves was estimated by tooth growth and wear method (Gipson et al., 2000).

2.3. Chemical analysis

In all of the samples, 17 PCB congeners (IUPAC numbers: 28, 52, 60, 74, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 189) and 7 OCPs (hexachlorobenzene (HCB), hexachlorocyclohexane isomers (α-, β-, and γ-HCH), 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-di(4-chlorophenyl)ethane (DDD) and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT)) were analyzed. For an easier comparison with other studies, we calculated the summary data for several major groups of contaminants (Table 1). ΣHCHs is the sum of the α-, β- and γ-hexachloro cyclohexanes. ΣDDTs is the sum of p,p-DDT, p,p-DDD and p,p-DDE. ΣPCBs is the sum of concentrations of all of the analyzed 17 PCBs. Σ6IndNDL PCBs includes six indicator non-dioxin-like PCBs (PCB-28, -52, -101, -138, -153 and -180), whose selection was based on their dominant presence in technical mixtures, environment, and animal and human tissues. Their sum comprises about half of the amount of total non-dioxin-like PCBs present in food and feed (EFSA, 2012). Σ8ToxDL PCBs represents more toxic fraction of PCBs in the sample and includes 8 mono-*ortho* substituted dioxin-like PCBs (PCB-105, -114, -118, -123, -156, -157, -167, and -189). ΣToxNDL PCBs represents the sum of 3 toxicologically relevant non-dioxin-like PCBs (PCB-60, -74, -170).