Environmental Pollution 198 (2015) 41–46

Contents lists available at ScienceDirect

Environmental Pollution

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

Short-chain chlorinated paraffins in terrestrial bird species inhabiting an e-waste recycling site in South China

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ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form
17 December 2014
Accepted 18 December 2014
Available online

Keywords:
SCCPs
Terrestrial birds
Homologue group profile
e-waste recycling area

ABSTRACT

Short-chain chlorinated paraffins (SCCPs) are under review by the Stockholm Convention on Persistent Organic Pollutants. Currently, limited data are available about SCCPs in terrestrial organisms. In the present study, SCCP concentration in the muscles of seven terrestrial bird species ($n = 38$) inhabiting an e-waste recycling area in South China was determined. This concentration varied from 620 to 17,000 ng/g lipid. Resident birds accumulated significantly higher SCCP concentrations than migratory birds ($p < 0.01$). Trophic magnification was observed for migratory bird species but not for resident, which was attributed to high heterogeneity of SCCP in e-waste area. Two different homologue group patterns were observed in avian samples. The first pattern was found in five bird species dominated by C_{10} and C_{11} congeners, while the second was found in the remains, which show rather equal abundance of homologue groups. This may be caused by two sources of SCCPs (local and e-waste) in the study area.

1. Introduction

Short-chain chlorinated paraffins (SCCPs), also known as polychlorinated *n*-alkanes having carbon chain lengths ranging from 10 to 13 and 1 to 13 chlorine atoms, are additives with high resistance and are used as cutting fluids, plasticizers, flame retardants, paint, polyesters, and polyolefins (Feo et al., 2009). In the last decade, SCCPs have attracted increasing attention as they represent a potential "new" category of persistent organic pollutants (POPs) (Poremski et al., 2001). Although they are one of the most challenging groups of compounds to quantify and analyze, they have been detected in various environmental matrices, biota, and humans, even in remote areas such as the Arctic (Bayen et al., 2006; Reth et al., 2006). SCCPs can bioaccumulate and are biomagnified in aquatic food webs (Houde et al., 2008; Zeng et al., 2011; Ma et al., 2014a). Although SCCPs have low acute toxicity, chronic toxicity and sub-lethal effects have been reported, including carcinogenicity (Ali and Legler, 2010). Because of their ubiquitous occurrence, bioaccumulation and biomagnification potential, toxicity to organisms, and high long-distance atmospheric transport potential (Tomy et al., 1999), the manufacture and use of SCCPs is banned in several countries, including the United States, Canada, and the Europe (Feo et al., 2009). Further, SCCPs are currently under evaluation to be included in the Stockholm Convention list of POPs (UNEP, 2010). However, since limited data are available on SCCPs in environmental samples, the Stockholm Convention decided to postpone decision-making and revise the risk profile with regard to SCCPs (Morales et al., 2012).

China is the largest producer of CPs in the world, producing up to 600,000 metric tons in 2007 (Fiedler, 2010). In recent years, there has been growing interest for CPs and their occurrence and effects in China. Higher SCCP concentrations have been reported in the sediments obtained from the Pearl River Delta of South China when compared to samples obtained from Europe and North America (Chen et al., 2011); higher concentrations were also observed in atmospheric samples from China compared to those from Japan and South Korea (Wang et al., 2013). Harada et al. (2011) also found that dietary SCCP exposure in Beijing was one order of magnitude higher than that observed in Japan and Seoul, South Korea. Additionally, intense e-waste recycling activities at various unspecified locations throughout China are a major source of CPs in the environment (Chen et al., 2011). It is, therefore, crucial to investigate the potential ecological and health risks of SCCPs in China.

Birds have been successfully used in POP biomonitoring in terrestrial ecosystems (Eens et al., 2013; Sun et al., 2012). Resident

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http://dx.doi.org/10.1016/j.envpol.2014.12.023
0269-7491/

bird species are especially suitable to study local contamination because of their small home ranges, territories, and foraging areas (Dauwe et al., 2006; Van den Steen et al., 2008), whereas migratory birds are useful for a wider geographical coverage (Hong et al., 2014; Evenset et al., 2007). Until date, few studies have reported SCCP pollution in aquatic organisms such as invertebrates, fish, and piscivorous birds (Ma et al., 2014b; Parera et al., 2013; Morales et al., 2012; Reth et al., 2006, 2005). However, no studies have been conducted on SCCPs in terrestrial organisms.

In the present study, seven bird species, five resident bird species and two migratory bird species, were collected from an e-waste recycling site in South China where elevated chlorinated paraffin concentrations were recorded in sediments (Chen et al., 2011). The present study aims to 1) examine the residual SCCP levels in bird species and 2) to investigate the congener-specific distribution of SCCPs in terrestrial birds. The corresponding results will aid in understanding SCCP contamination in the terrestrial ecosystem.

2. Materials and methods

2.1. Sampling

During the non-breeding season, a total of 38 birds, including seven white wagtails (*Motacilla alba*), six long-tailed shrikes (*Lanius schach*), four red-flanked bluetails (*Tarsiger cyanurus*), four grey-backed thrushes (*Turdus hortulorum*), nine great tits (*Parus major*), five oriental magpie-robins (*Copsychus saularis*), and three Gold-finches (*Carduelis sinica*), were collected between November 2011 and May 2012 from Longtang Town, Qingyuan County, Guangdong Province, where e-waste recycling activities have been conducted for the past two decades. Approximately 700, 000 t e-wastes are disposed each year within an area of 330 km^2 using crude recycling techniques. The birds were collected using mist netting. Of the seven bird species, the red-flanked bluetail and grey-backed thrush are two migratory bird species that breed in mixed coniferous forests with undergrowth in northeast China. They spend the winter mainly in South China (MacRinnon et al., 2000).

The permit for sample collection was obtained from the local Forestry Bureau. The birds were transported immediately to the laboratory and euthanized with nitrogen. The pectoral muscle was excised from each specimen and these samples were stored at −20 °C prior to chemical analysis.

2.2. Sample preparation and analysis

Approximately 2.0–4.0 g of muscle sample was homogenized after lyophilization, and Soxhlet extraction was performed with 200 mL acetone/hexane (1:1, v/v) for 24 h. The surrogate (1,1,1,3,10,11-hexachloroundecane) (20 ng) was spiked prior to extraction. The extract was concentrated to 10 mL by rotary evaporation. One aliquot (1 mL) was used for gravimetric determination of the lipid content. The other extract used for chemical analysis was purified using a gel permeation chromatographic column packed with 40 g SX-3 Bio-beads (Bio-Rad Laboratories, Hercules, CA) and eluted with dichloromethane/hexane (v/v = 1:1) for lipid removal. The elute from 90 to 280 mL containing the targets was collected and concentrated to 1 mL and further purified in a multilayer column (id., 1 cm) filled from bottom to top with 5 cm of Florisil, 2 cm of neutral silica, 5 cm of sulfuric acid impregnated silica (30%, w/w), and 4 cm of anhydrous sodium sulfate. The column was pre-rinsed with 50 mL of hexane, and the extract was eluted with 40 mL of hexane (first fraction), followed by 100 mL of hexane/dichloromethane (v/v = 1:1, second fraction). The first 40 mL of hexane was discarded, while the second 100 mL of 1:1 DCM/hexane was collected and finally concentrated to 200 μL. Finally, 20 ng of labeled ε-HCH was added as a recovery standard for GC/MS analysis.

2.3. Instrumental analysis

The SCCPs were quantified using Shimadzu model 2010 gas chromatography coupled with a model QP-2010 mass spectrometer (Shimadzu, Japan) with electron-capture negative ionization in the selective ion-monitoring mode and separated by a DB-5 HT capillary column (15 m × 0.25 mm × 0.10 μm; J&W Scientific). The temperature program was set as follows: 100 °C for 2 min, further increased to 280 °C at 40 °C/min. The temperature was then kept constant for 2 min, increased to 320 °C at 70 °C/min, and held for 6 min.

The injector temperature was set to 275 °C, while the transfer line temperature was set to 280 °C, and the ion source temperature was set to 200 °C. The mass-to-charge ratios used for quantification and identification were published elsewhere (Tomy et al., 1997). The most abundant isotope was used for quantification, and the second most abundant was used for the identification of possible interferences from CPs themselves or from other interfering compounds. To enhance instrument sensitivity, all monitored SCCPs ions were divided into four groups: $C_{10,}$ C_{11}, C_{12}, and C_{13}. Therefore, four individual injections were executed for each sample. A total number of 24 SCCP congeners with a chain length including 10–13 carbon atoms and 5–10 chlorine atoms were analyzed (Reth et al., 2005). The abundance profiles of the congener group were generated from the actual relative integrated signals corrected by isotopic abundance and response factors. The quantification of SCCPs has been detailed in our previous studies (Chen et al., 2011) and it also was provided in the Support information.

2.4. Stable nitrogen and carbon isotope analysis

The pectoral muscle isolated from the subsamples for nitrogen and carbon stable isotopic analysis were lyophilized and ground into ultra-fine powder. Approximately 1 mg of the ground samples were then weighed in tin capsules and analyzed using a flash EA 112 series elemental analyzer interfaced with a Finigan MAF ConFlo 111 isotope ratio mass spectrometer. Stable isotope ratios of samples were assessed against the reference standards ammonium sulfate and carbon black for $\delta^{15}N$ (0.4‰) and $\delta^{13}C$ (−30.9‰), respectively. The isotope ratio was expressed as δX (values [‰]), with $\delta X = ([R_{sample}/R_{standard} - 1] \times 1000)$, where X is ^{15}N or ^{13}C and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The precision for this technique is about 0.5‰ (2 SD) for $\delta^{15}N$ and 0.2‰ (2 SD) for $\delta^{13}C$.

2.5. QA/QC and data analysis

Quality assurance was performed by analyses of procedural blanks, spiked blanks, spiked matrixes, and sample duplicates. The procedural blanks ($n = 3$) were processed for each batch of samples. No CPs were detected in the procedural blanks. Three spiked blanks and three spiked matrixes were processed, in which 25 ng of commercial CP mixtures (CP52) and 1,1,1,3,10,11-hexachloroundecane were spiked in the blank solvent and pre-extracted bird muscle. The recoveries of SCCPs in the spiked blanks ranged from 82% to 97%, with a relative standard deviation of <15%. The recoveries of CPs in the spiked matrix ranged from 76% to 99%, and the relative standard deviation was <20% for all targets. The recoveries of surrogate standards (1,1,1,3,10,11-hexachloroundecane) in bird samples ranged from 76% to 105%. As for the sample duplicates ($n = 3$), the relative standard deviation was <15%.

Table 1
The data in muscle of bird collected from an e-waste recycling region (Longtang Town, Qingyuan Country) in South China.

	White wagtail	Long-tailed shrike	Red-flanked bluetail	Grey-backed thrush	Great tit	Oriental magpie-robin	Goldfinch
Number	7	6	4	4	9	5	3
Immigration habit	Resident	Resident	Migratory	Migratory	Resident	Resident	Resident
Feeding habit	Insectivorous	Insectivorous	Insectivorous	Insectivorous	Insectivorous	Omnivores	Granivorous
Lipid content (%)[a]	2.3 (1.7–2.9)	2.3 (1.9–2.7)	6.4 (3.9–8.4)	2.7 (1.9–4.3)	2.0 (1.3–2.4)	2.0 (1.1–3.8)	2.6 (1.9–3.5)
$\delta^{13}C$ (‰)	−21.9 (−23.7–20.4)	−22.4 (−24.1 −21.0)	−24.1 (−25.3 −22.1)	−22.0 (−22.7 −21.3)	−23.6 (−24.6–22.0)	−24.9 (−25.4–24.4)	−27.3 (−27.6–27.2)
$\delta^{15}N$ (‰)	8.0 (5.6–11.4)	7.3 (4.5–9.4)	6.9 (5.8–8.3)	5.6 (4.6–7.1)	7.2 (4.4–9.9)	9.4 (7.5–10.7)	4.2 (3.8–4.5)
SCCPs[b]	170 (120–260)	140 (110–190)	71 (66–75)	28 (19–37)	160 (140–200)	93 (64–150)	300 (220–340)
SCCPs[c]	7,600 (4700 −13,000)	6300 (4300–9900)	1200 (870–1700)	1200 (620–1900)	8200 (6400 −11,000)	4900 (4000–5400)	12,000 (9100 −17,000)

[a] All data presented in the table are mean and range.
[b] Wet weight normalized concentration.
[c] Lipid weight normalized concentration.

3. Result and discussions

3.1. Levels of SCCPs in bird species

All congeners were detected in samples except for Cl_{10} congeners in C_{10}, C_{11}, and C_{13} homologue and Cl_9 congeners in C_{10} and C_{11} homologues. SCCP concentrations are listed in Table 1. SCCPs in the seven bird species samples ranged from 620 (the minimum) to 17,000 ng/g lipid weight (lw) (the maximum). The levels of SCCPs in oriental magpie-robin (mean of 4900 ng/g lw) were comparable with previously detected levels of polybrominated biphenyl ethers (870–15,000 ng/g lw; median, 5200 ng/g lw) (Sun et al., 2012), but were one order of magnitude lower than the levels of PCBs (6100–190,000 ng/g lw; median, 48,000 ng/g lw, unpublished data) in the same bird species from the same area of the present study.

Until date, few studies have reported the levels of SCCPs in birds, especially in terrestrial bird species. Reth et al. (2006) reported SCCPs in two seabirds (little auk and kittiwake) from Bear Island. The SCCP levels in the muscles of these two seabirds ranged from 5 to 16 ng/g wet weight, which were lower than those observed in the present study (19–340 ng/g wet weight). The SCCP levels in gull eggs of two species (*Larus michahellis* and *Larus audouinii*) collected from the Ebro delta Natural Park, Spain, were 4.5 and 6.4 ng/g wet weight, respectively (Morales et al., 2012), which were one to two orders of magnitude lower than that observed in the present study. Since no data are available on the bird species in China, we were unable to draw a comparison between the bird species in China.

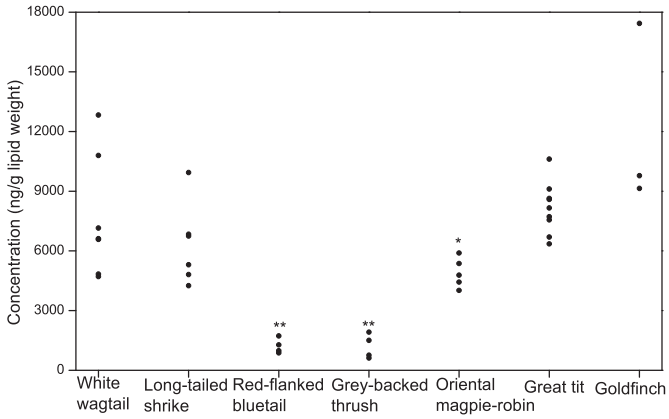

Fig. 1. SCCP concentration (ng/g lipid) in different bird species. The marker ** indicated SCCP concentrations were significant lower in both red-flanked bluetail and grey-backed thrush than other bird species, and the marker * indicated SCCP concentrations were significant lower in oriental magpie-robin than in great tit and goldfinch.

3.2. Species difference in SCCP levels and potential impact factors

SCCP levels varied among different bird species. The goldfinches exhibited the highest SCCP levels (mean, 12,000 ng/g lipid weight) and the red-flanked blue tail (mean, 1200 ng/g lw) and grey-backed thrush (mean, 1200 ng/g lw) showed the lowest level among seven bird species (Fig. 1). A one-way analysis of variance (ANOVA) with tukey's post hoc test indicates that the SCCP levels in both the red-flanked blue tail and grey-back thrush were significantly lower than those in other bird species ($p < 0.01$), whereas the levels of SCCPs in goldfinches and the great tits were significantly higher than those in the oriental magpie-robin ($p < 0.05$).

The migratory patterns can partially explain the observed interspecies variation. In the present study, the lowest SCCP concentrations were found in two migratory bird species: the red-flanked bluetail and grey-backed thrush. As mentioned above, these two bird species are migratory birds; they spend a considerable part of their life at their breeding ground in Northeast China and winter in South China. The lowest SCCP levels in these bird species reflected lower SCCP levels at their breeding grounds than at the study area and/or a short exposure time in the study area. This is reasonable considering the intensive e-waste recycling activities being conducted in the study area. Our previous study has revealed that the chlorinated paraffins in the environment were significantly higher

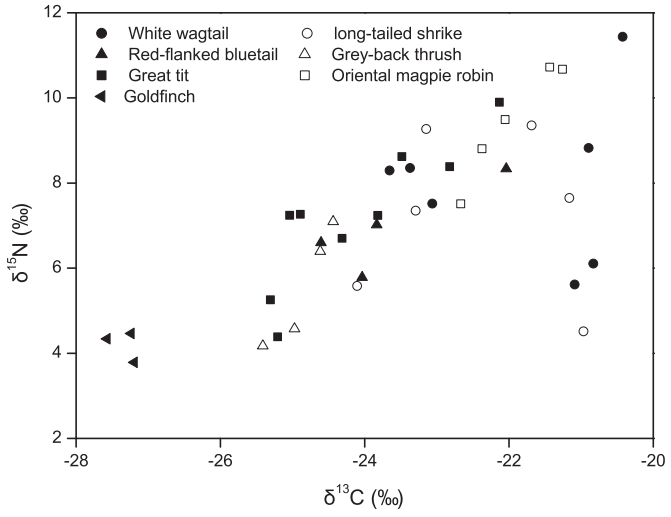

Fig. 2. Stable isotope ratio (‰) of nitrogen and carbon in the muscle tissues of bird species.

in the e-waste recycling region than in the non-e-waste recycling area (Chen et al., 2011). The biovector transport by birds is a third mode of long-distance POP transport because of their huge populations, obvious bio-magnification, and extensive migration (Choy et al., 2010; Evenset et al., 2007). The migratory behavior of the birds will transport the SCCPs from the study area to their breeding ground. This behavior should be studied further.

In previous studies, granivorous birds were found to generally have lower concentrations of halogenated organic contaminants than insectivores, omnivores, and piscivores (Hong et al., 2014; Luo et al., 2009; Naso et al., 2003). However, this was not the case in the present study. Goldfinch is a granivorous species that feeds on various tree seeds. The other bird species are all insectivorous birds that feed on insects, with the exception of the long-tailed shrike, which can consume a wide variety of animal prey and occasionally eats fish and small snakes (MacRinnon et al., 2000). However, the result of the present study should be treated with caution because of the small number of goldfinch ($n = 3$). In order to study the influence of diet and trophic level occupied by bird species on the body burden of SCCPs, stable carbon and nitrogen isotope analyses were performed. The stable isotope ratio of nitrogen and carbon increased with increasing trophic level. The increment in carbon isotope ratio was much smaller than that observed in case of nitrogen. Thus, the isotope ratio of carbon and nitrogen can be used to describe the carbon source and trophic level of organisms, respectively (Vander and Rasmussen, 2001). Goldfinches showed the lowest stable carbon and nitrogen isotopic ratios among seven bird species (Fig. 2), which is consistent with its herbivorous habits. For other bird species, a relative wide range of stable carbon or nitrogen isotopic ratios was found for each bird species. This suggested that a broad diet source and trophic levels were noted for an individual bird within a given species. Additionally, the difference in age among individual birds maybe another reason for this wild range of stable carbon or nitrogen isotopic ratios. A linear regression analysis between stable nitrogen isotopic ratio and log concentration of SCCPs revealed no significant relationship ($r = 0.18$, $p = 0.28$), which suggested that trophic level might not be a key factor affecting SCCP levels in birds in the study area. This is inconsistent with reports on aquatic organisms. Both Zeng et al. (2011) and Ma et al. (2014a) discovered biomagnification of SCCPs in the aquatic food web in freshwater and marine environments. Houde et al. (2008) also discovered biomagnification of SCCPs in food webs in Lake Michigan, but the trophic magnification factor in the food web in Lake Ontario was approximately equal to 1 (0.97).

Since not all bird species are indigenous species, the calculated trophic magnification factor can be disrupted if all samples were used. To remove the effects of migration pattern on the calculated trophic magnification factor, we divided the samples to two groups based on migratory pattern and performed correlation analysis between SCCP levels and nitrogen isotopic ratio on the two subgroups. The result indicated that the trophic magnification behavior of SCCPs is different between resident and migratory bird species. Trophic magnification ($r = 0.84$, $p < 0.01$) was observed for two migratory bird species. However, no trophic magnification ($r = -0.30$, $p = 0.11$) was found for resident bird species (Fig. 3). No trophic magnification of SCCPs observed in the resident bird species can be attributed to two reasons. Firstly, the pollution in the e-waste recycling area highly depend on the e-waste recycling activities. Thus, a high heterogeneity distribution of SCCPs in the environment in the study area was expected. This was confirmed by our investigation of polybrominated biphenyl ethers in chicken raised in the study area (Zheng et al., 2014). The body burden of SCCPs in the bird depends on a great extent on exposure to pollutants derived from e-waste, which is site-dependent. Higher levels of SCCPs can be found in site that near e-waste recycling workshops or e-waste dumping site but lower levels of SCCPs was in region that far away e-waste recycling activities. This resulted in the trophic level being no longer a key factor affecting SCCP levels in birds in the study area. Secondly, inter-species that metabolize SCCPs may also be responsible for the absence of biomagnification. Metabolism of chlorinated paraffins in organisms has been noted in several studies. For example, Fisk et al. (2000) and Vaughan and Hurd (2010) observed significant biotransformation of chlorinated alkanes in rainbow trout (*Oncorhynchus mykiss*). Houde et al. (2008) also found that lake trout metabolize SCCPs. Until date, limited data are available on the metabolism of chlorinate paraffins in birds. Birds have higher concentrations and activities of CYPs, higher rates of electron transport, and more active monooxygenases than lower organisms (e.g., fish), and therefore, have a higher capacity to detoxify and biotransform xenobiotic chemicals (Stegeman and Klopper-Sams, 1987). The effect of the metabolic rate of birds on the body burden of SCCPs is still unknown. As regarding migratory bird species, the effect of e-waste on the trophic magnification did not show due to short exposure period in the study area. Thus, trophic magnification still can be observed.

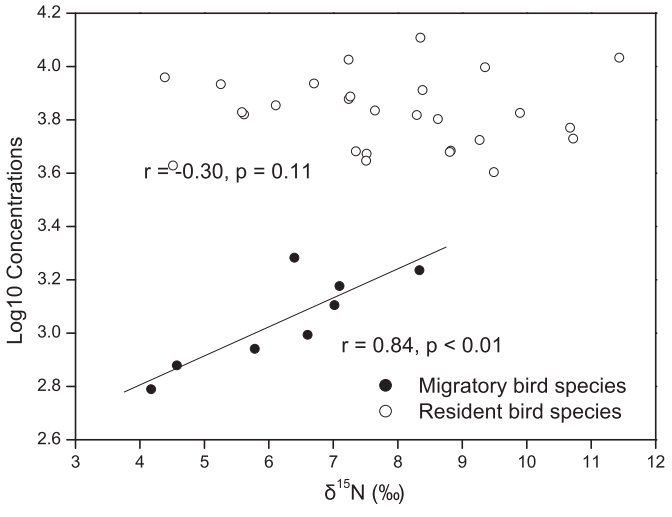

Fig. 3. Correlation between SCCP concentration and trophic level (represented by $\delta^{15}N$) in bird species.

3.3. Homologue group patterns

Congener group abundance profiles of SCCPs in the seven bird species are shown in Fig. 4. The congener groups C_{10} and C_{11} with six chlorine atoms were most abundant in all bird samples collected from the study area. When all congener groups within one chain length were summarized, two homologue group profiles were observed among the seven bird species (Fig. 4). For the great tit and oriental magpie-robin, an almost equal abundance of SCCP homologue groups was observed, which is consistent with the findings of a previous report on seabirds from the European Arctic (Reth et al., 2006). In the other five bird species, homologues with 10 and 11 carbon atoms dominated, and these were significantly more abundant than the C_{13} homologues (ANOVA with tukey's post hoc test, $p < 0.05$).

In our previous study on SCCPs in the sediment from the river, which run through the study area, two congener group patterns of SCCPs were found. The congener group patterns in the sediment from the lower reaches that were impacted by the e-waste were different from those in the regions not impacted by e-waste (Chen et al., 2011). High chlorinated congeners (Cl_{10} for C_{10} homologues

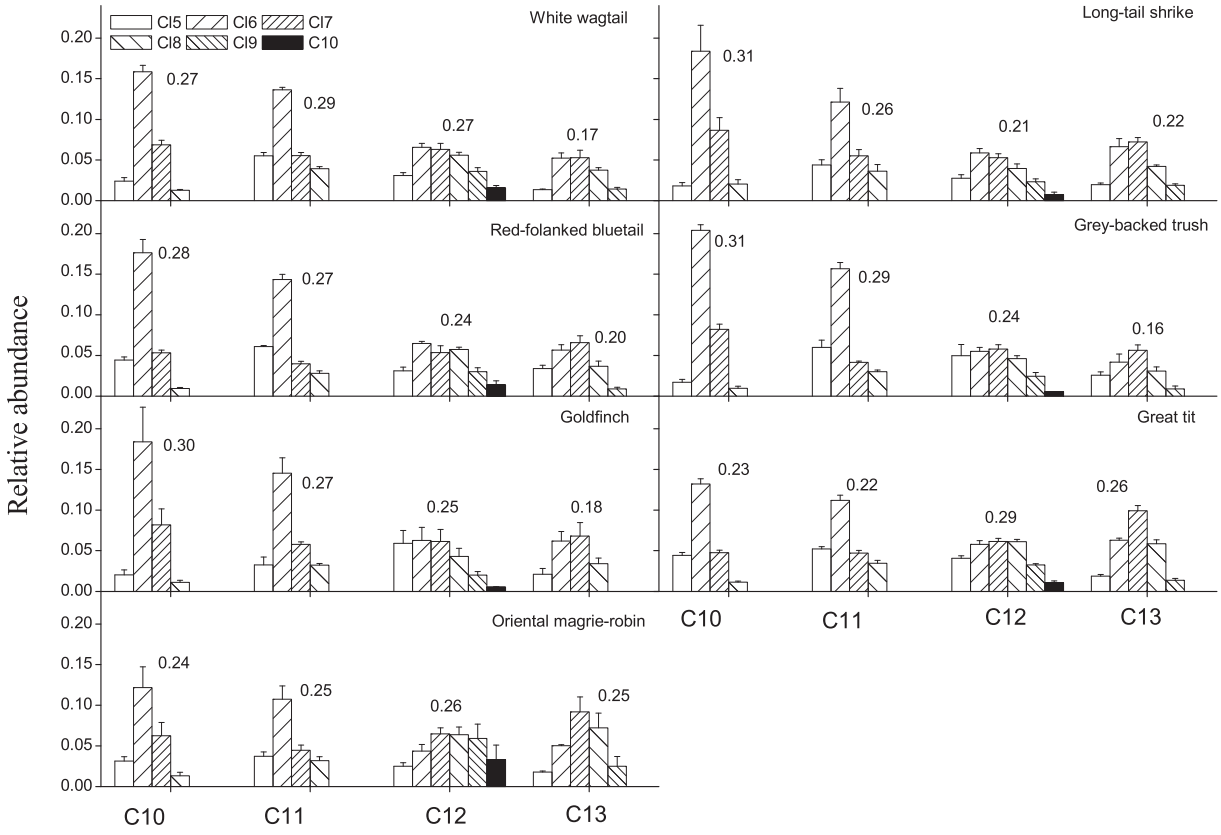

Fig. 4. Congener group pattern of SCCPs in avian muscles. Values are mean ± standard error.

and Cl_8 for the other three homologues) were the most abundant congeners in e-waste impacted area while relative low chlorinated congeners (Cl_7 for C_{10} homologue and Cl_6 for the other three homologues) was the most abundant congeners in non-e-waste impacted area (Chen et al., 2011). Therefore, we hypothesized that the different SCCP congener group patterns in the environment in the study area were responsible for the observed two homologue group profiles in the bird samples. The contributions of Cl_6 congeners to C_{10} (mean of 55% and 52%) and C_{11} homologues (44% and 47%) in the great tit and oriental magpie-robin were lower than those (mean of 59%–65% for C_{10} homologue and 47%–55% for C_{11} homologue) in the other bird species. Therefore, the contributions of e-waste related SCCPs to body burden of SCCPs were higher in great tit and oriental magpie-robin than in other bird species due to the relative low abundances of low chlorinated congeners in e-waste related SCCPs. The oriental magpie-robin and great tit are found in open woodland and cultivated areas often close to human habitations, which make them having more chance of exposure to e-waste related pollutants. Additionally, species-specific metabolism cannot be ruled out as another possible reason, although there is no evidence to support it.

The red-flanked bluetail and grey-backed thrush are winter migratory birds found in the study area. It was expected that the homologue group pattern of SCCPs in these two bird species would differ from those in the resident bird species. However, no remarkable differences were found in the SCCP homologue group profiles between the resident (mainly white wagtail, long-tailed shrike, and goldfinch) and migratory birds in the present study, although the contributions of Cl_6 congeners to C_{10} (mean of 62% and 65% for red-flanked bluetail and grey-backed thrush) and C_{11} homologues (55% and 53%) were the highest among all birds. The SCCP homologue patterns of two migratory bird species were more closed to that of the three resident bird species reinforced the great tit and oriental magpie-robin being more exposed to e-waste derived SCCPs. This result also suggested that the congener group profile of SCCPs derived from local source (not e-waste related emission) was similar to that noted at the breeding ground of two migratory bird species.

4. Conclusion

In summary, we studied SCCP contamination in several terrestrial bird species inhabiting an e-waste recycling area. SCCP levels in birds from the study area were higher than those observed in birds from other regions. The resident birds accumulated higher concentrations of SCCPs than migratory birds, which indicated high SCCP pollution in the study area. Granivorous birds exhibited higher SCCP concentrations than insectivores and omnivores, which is an unexpected finding. Trophic magnification was found in the migratory bird species but not in the resident bird species, which can be caused by the heterogeneity distribution of SCCPs in the environment in the study area. Two homologue group patterns of SCCPs were found in the seven bird species. The actual reasons for the different homologue patterns are unclear. Two different sources (local emission and e-waste recycling emission) existed in the study. This is the possible reason. The role of SCCP metabolism in the determination of SCCP concentration and homologue group patterns is not clear and warrants further study.

Acknowledgments

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB14020301), the National Nature Science Foundation of China (Nos. 41273118,

41473102, and 41230639), and the National Basic Research Program of China (2015CB453102). This is contribution No. IS-2000 from Guangzhou Institute of Geochemistry, Chinese Academy of Science.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.12.023.

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