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Persistent organochlorines in raccoon dogs (*Nyctereutes procyonoides*) from Japan: Hepatic sequestration of oxychlordane

Tatsuya Kunisue [a], Nozomi Takayanagi [a], Toshio Tsubota [b], Shinsuke Tanabe [a,*]

[a] *Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*
[b] *Laboratory of Zoo and Wildlife Medicine, Faculty of Applied Biological Sciences, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*

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Abstract

The present study determined the accumulation features of persistent organochlorines (OCs) such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), and chlordane compounds (CHLs) in wild raccoon dogs (RDs; *Nyctereutes procyonoides*) collected from Kanagawa prefecture in Japan during 2001. In livers of RDs, CHLs were remarkably dominant (20 times higher than PCBs) followed by PCBs > DDTs > HCHs > HCB, whereas the chemicals in muscles were in the order of CHLs > PCBs > HCHs > DDTs > HCB. The accumulation pattern of OCs in RDs was different from those in Japanese humans and avian species reported previously, which generally accumulate higher levels of DDTs and PCBs than CHLs. This result indicates that RDs have been exposed to relatively high levels of CHLs and have high metabolic and elimination capacity for DDTs. In fact, CHL levels in RDs were higher than those in humans and some avian species, while DDT levels in RDs were much lower than other animals. In particular, extremely high accumulation levels of oxychlordane, which is a metabolite from chlordanes and nonachlors, were observed in RD livers. The higher toxic potency of oxychlordane than parent compounds may suggest that RDs are at high risk by this metabolite. On lipid weight basis, PCBs, HCHs and HCB levels were almost similar in livers and muscles, suggesting that the tissue distribution of these compounds principally followed the lipid-dependent accumulation. However, accumulation levels of oxychlordane and *p*,*p*′-DDD in livers were significantly higher than those in muscles, and concentration ratios of liver to muscle (L/M ratios) of these compounds were greater than 1.0 in all the specimens. This phenomenon was similar to PCDD/DF congener accumulation patterns observed previously in RDs. When relationships between hepatic TEQs and L/M ratios were examined for oxychlordane and *p*,*p*′-DDD, L/M ratios for these compounds significantly increased with hepatic TEQ levels, suggesting their hepatic sequestration in TEQs-dependent manner.

Keywords: Organochlorines; Oxychlordane; Hepatic sequestration; Raccoon dog; Japan

1. Introduction

Persistent organochlorines (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), and chlordane compounds (CHLs), have been found in humans and various wildlife because of their persistency in the environment and highly bioaccumulative nature (Tanabe, 2002). Especially, OC contaminations in marine mammals and predatory birds are still prominent and hence their toxic impacts to high trophic wildlife are of great concern (Tanabe, 2002). At the same time, it is found that levels of OCs in humans from developed countries have decreased in recent decades (Schade and Heinzow, 1998; Norén and Meironyté, 2000).

In Japan, enormous amounts of technical PCB and organochlorine insecticides such as DDTs and HCHs were used mainly during 1960s. Thus relatively high levels of these contaminants have been detected in humans and

* Corresponding author. Tel./fax: +81 89 927 8171.
E-mail address: shinsuke@agr.ehime-u.ac.jp (S. Tanabe).

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avian species even in recent years (Minh et al., 2001; Kunisue et al., 2003). But it was reported that concentrations of PCBs, DDTs, and HCHs in human breast milk have been gradually decreasing since the ban on production and use of these contaminants during 1970s (Konishi et al., 2001). On the other hand, levels of CHLs in Japanese milk have not been decreasing since 1986, when CHLs usage was banned (Konishi et al., 2001). In Japan, approximately 15000 tons of CHLs were used mainly under floor of timber houses, where it is open to outdoor-air circulation, as a termiticide. It was reported that humans living in houses treated with CHLs have been exposed to higher levels of CHLs via contaminated foods, which had been stored at the position close to bottom house soil, than those living in untreated houses, indicating food contamination by volatilization and deposition of CHLs (Konishi et al., 1990). Recently, we demonstrated that pet dogs, which had been kept outdoors, accumulated relatively higher levels of CHLs compared with other OCs (Kunisue et al., 2005). This implies that pollution sources of CHLs are still present near CHLs treated houses and wild terrestrial mammals inhabiting the vicinity may be exposed to these contaminants. However, very few data is available on contamination status of OCs including CHLs in Japanese wild terrestrial mammals.

In our previous studies on OCs in humans and aquatic mammals, it was found that the body distribution of these contaminants is lipid-dependent because of their highly lipophilic nature (Tanabe et al., 1981; Watanabe et al., 1999; Minh et al., 2001). However, our recent study on tissue distribution of OCs showed hepatic retention of CHLs in pet dogs, which may be recently exposed to CHLs as described above (Kunisue et al., 2005). So, it is suspected that kinetic behaviors of OCs in animal body vary by species and exposure profiles of contaminants. More recently, we demonstrated that extremely high levels of dioxins were accumulated in livers of wild raccoon dogs (RDs; *Nyctereutes procyonoides*), and the liver-adipose distribution was congener-specific and liver retention of certain congeners were strongly dependent on the hepatic TEQs (Kunisue et al., 2006). RDs generally inhabit the forest areas near rural houses and prey on not only natural foods such as small animals and fruits but also man-made agricultural crops. Hence it is likely that raccoon dogs have been continuously exposed to dioxins derived from contaminated soil by pentachlorophenol (PCP) and chloronitrophen (CNP) sprayed for agricultural field in the past and they showed the specific tissue-distribution, hepatic sequestration, which is associated with CYP1A enzyme(s) induced by these contaminants (Kunisue et al., 2006). Considering these observations, it is anticipated that wild terrestrial mammals such as RDs inhabiting the environment close to human activity have been continuously exposed to dioxins and CHLs and may exhibit the species-specific tissue-distribution of these contaminants. To our knowledge, however, no information on tissue-distribution of CHLs in such wild terrestrial mammals is available.

In this study, we attempted to elucidate the contamination status and accumulation features of OCs in RDs collected from Kanagawa prefecture, Japan. In addition, the present study also addresses the toxicokinetic behavior of CHLs in association with distribution of these contaminants in livers and muscle tissues.

2. Materials and methods

2.1. Samples

Liver and muscle tissues of raccoon dogs (RDs; *N. procyonoides*) ($n = 10$; male = 5, female = 5) were obtained from the specimens died from traffic accidents in Kanagawa prefecture, adjacent to Tokyo, during 2001 (Fig. 1). All the RDs obtained were mature, which was diagnosed by veterinarians of Gifu University, Japan. These liver and muscle samples were stored at −20 °C until analysis. We already reported the contamination status and accumulation features of dioxins and related compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (Co-PCBs) in livers and adipose tissues of the same specimens (Kunisue et al., 2006). According to Mammalian Invasive Species Database of National Institute for Environmental Studies (2005), wild RD inhabits whole of Japan and feeds on small animals, insects, fruits, and crops. The life span of RD is about 7–8 years and its area of activity is within 100 ha, indicating that RD reflects distinctive regional contamination of OCs.

2.2. Chemical analysis

PCBs, DDTs, HCHs, HCB, and CHLs were analyzed following the method described previously (Minh et al., 2001) with some modification. Briefly, liver and muscle samples (5–10 g) were ground with anhydrous sodium sulfate and extracted in a Soxhlet apparatus with a mixture of hexane and diethyl ether. After concentration of the extract, an aliquot was dried at 80 °C to determine lipid content. Lipid in the remaining extract was removed by gel permeation chromatography (GPC) packed with Bio-Bead S-X 3 (Bio-Rad Laboratories, USA). The lipid-removed extract was passed through activated Florisil (Florisil PR: Wako chemicals USA, Inc., USA) packed in a glass column. The first fraction eluted with hexane contained PCBs, HCB, *p*,*p*′-DDE and *trans*-nonachlor, and the second fraction eluted with 20% dichloromethane in hexane contained *p*,*p*′-DDT, *p*,*p*′-DDD, HCH isomers (α-, β-, and γ-), *cis*-nonachlor, *trans*-nonachlor, *cis*-chlordane, *trans*-chlordane, oxychlordane. Total PCBs, DDTs (*p*,*p*′-DDE, *p*,*p*′-DDT and *p*,*p*′-DDD), HCHs (α-, β- and γ-isomers), CHLs (*trans*-nonachlor, *cis*-nonachlor, *trans*-chlordane, *cis*-chlordane, oxychlordane) and HCB were quantified using a gas chromatograph (GC; Agilent 6890 series) equipped with ECD (electron capture detector) and an automatic injector (Agilent 7683 series). The con-