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ORAL BIOAVAILABILITY AND TOXICOKINETICS OF 3,3′,4,4′,5-PENTACHLOROBIPHENYL IN NORTHERN LEOPARD FROGS, *RANA PIPIENS*

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Abstract—This study is the first report on oral bioavailability, whole-body elimination, and distribution of a specific polychlorinated biphenyl (PCB) congener on an amphibian species, northern leopard frogs. Each frog was orally dosed once with either 0.35 or 5.0 mg/kg PCB 126 (based on frog wet wt), including tracer ^{14}C-PCB 126 (3′,4′,5′-phenyl-ring-^{14}C) by force feeding it a cricket injected with the PCB. We found no statistical difference ($t = 0.917$, $df = 5$, $p = 0.401$) in the average 48-h oral bioavailabilities of 0.35- and 5.0-mg/kg dosage groups, which were 84.6 ± 5.8% (mean ± SE, $n = 4$) and 90.9 ± 1.5% ($n = 3$), respectively. Statistical analysis indicated that time was the only independent variable affecting the retention of whole-body ^{14}C content. Kinetics were apparently first order because elimination rate was independent of dose. Assuming a single pool and one elimination rate, the $t_{1/2}$ value for whole-body elimination of PCB-derived ^{14}C was 763 d. Liver, fat bodies (corpora adiposa), carcass (head, bone, cartilage materials, and residues of other tissues), skin, and muscle were the major organs for PCB 126 retention in both dosage groups. The concentrations of ^{14}C residue in fat bodies were relatively constant throughout the experiment. However, total residues in fat bodies declined throughout the experiment in both dosage groups in correlation with declining masses of fat bodies. Gonad, kidney, stomach, intestine, and a tissue pool including esophagus, lung, spleen, heart, and cloacal materials each accumulated <1% of the initial total ^{14}C residue. The egg follicles in 19 females contained 1 to 23% of the initial total ^{14}C residue, with an average of 10.0 ± 9.2% (mean ± SE, $n = 19$).

Keywords—Toxicokinetics Oral bioavailability 3,3′,4,4′,5-pentachlorobiphenyl Northern leopard frogs *Rana pipiens*

INTRODUCTION

Polychlorinated hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) are ubiquitously present in the environment [1–4]. Due to their lipophilicity, they tend to biomagnify in the food chain [5,6]. One example contaminated area is Green Bay and the lower Fox River, Wisconsin, United States. This ecosystem has 13 pulp and paper plants, the largest such aggregate in the world, alongside the Fox River, which drains into the Great Lakes basin. Farming, municipal discharge, and barnyard runoffs created a hypereutrophic ecosystem [7]. Consequently, thousands of kilograms of waste discharges such as polychlorinated hydrocarbons, ammonia compounds, carbamates, resin acids, metals, and dyes are present in the water body and sediment [8].

Environmental risk assessment requires determination of the level of exposure and measuring of adverse effects. Estimates of toxicant exposure can be obtained by measuring the levels of toxicants in feral animals, the use of physiological markers of exposure, and toxicokinetic approaches that provide information on uptake, distribution, and elimination rates of the toxicants of interest. There have been many studies on bioaccumulation of planar chlorinated hydrocarbons in fish, birds, and mammals [9–11] using these three methodologies. We have explored exposure of PCBs in frogs using the first two methodologies [12,13]. To our knowledge, no studies have been conducted on oral bioavailability, distribution, and elimination of PCBs in adult frogs.

Therefore, in the present study, we conducted a toxicokinetic study to determine oral bioavailability, half-life, and tissue distribution of a specific PCB congener, 3,3′4,4′,5-pentachlorobiphenyl. In a previous study [13], we found that frogs in the Green Bay ecosystem were exposed to PCBs. They could obtain PCBs via ingesting food and/or contacting the water. In the present study, we force fed each northern leopard frog with one PCB 126-laden cricket. Frogs were killed at designated times for calculating oral bioavailability, whole-body elimination rate, and distribution of PCB 126 in various organs. Two doses of PCB were chosen on the basis of results from a dose–response study [12]. In that study, the low dose did not cause the induction of detoxifying enzymes (i.e., cytochrome P450-associated monooxygenases; hereafter, P450 enzymes), whereas the high dose induced P450 enzymes to a maximal level.

MATERIALS AND METHODS

Chemicals

Radiolabeled 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126, 3′,4′,5′-phenyl-ring-^{14}C) was custom synthesized by Sigma Chemical (St. Louis, MO, USA). Its purity was 98.5%, and specific activity was 12.5 mCi/mmol, according to manufacturer's high performance liquid chromatography analyses. Unlabeled 3,3′,4,4′,5-pentachlorobiphenyl was purchased from AccuStandard (New Haven, CT, USA), and its purity was

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>99%, according to manufacturer's gas chromatography/flame ionization detector and gas chromatography/mass spectrometry analyses. Soluene®-350 and scintillation cocktail (Hionic-fluor™) were purchased from Packard Instruments (Meriden, CT, USA). Stabilized hydrogen peroxide (30%) was purchased from Sigma Chemical.

Animal source and care before experimentation

Forty male and 40 female northern leopard frogs were purchased from Nasco Biological Supply (Fort Atkinson, WI, USA) during early spring 1996. They were randomly assigned to four plastic aquaria (210 × 56 × 58 cm) with running dechlorinated water at 20°C. The aquaria were tilted so that frogs could swim in the water pool or sit on dry substrate. Each frog was fed every other day two mealworms (*Tenebrio molitor*) and one cricket (*Acheta domestica*) sprayed with multivitamins (Bio-Vite, Ocean Nutrition, San Diego, CA, USA). The room temperature was set at 20°C under a 12-h-light/12-h-dark photoperiod. All procedures for animal housing, handling, and dissection adhered to guidelines provided by the Animal Use Committee of Research Animal Resources Center and the Office of Biological Safety of the University of Wisconsin–Madison.

Experimental procedures

Preparation of PCB-loaded crickets. Two hundred microcuries ^{14}C-PCB 126 (5.184 mg PCB 126) was dissolved in 10 ml *p*-dioxane to obtain a labeled stock solution containing 20 μCi/ml ^{14}C and 0.52 mg/ml PCB 126. An unlabeled stock solution was prepared with 15.0582 mg unlabeled PCB 126 dissolved in 3.76 ml *p*-dioxane to yield 4.005 mg/ml PCB 126. Appropriate amounts of these two solutions were mixed, dried down, and reconstituted in corn oil to acquire injection solutions for the two treatment groups. For the 0.35 mg/kg treatment group, each milliliter of corn oil contained a total of 0.35 mg PCB 126 and 0.5 μCi. For the 5.0 mg/kg treatment group, each milliliter of corn oil contained a total of 5.0 mg PCB 126 and 1.0 μCi.

Based on each frog's body mass, a cricket was injected with an adequate amount of injection solution so that a radiolabeled dose of 0.35 or 5 mg/kg would be delivered when the frog was force fed the PCB-loaded cricket. The injection solution (approximately 19–40 μl) was injected into the abdomen of a cricket using a 22-gauge Hamilton microliter syringe needle (Hamilton Supplies, Reno, NV, USA). After the needle was withdrawn from the cricket, any leakage out of the injection point was absorbed with a piece of Kimwipe™ (Kimberly-Clark Corp., Roswell, GA, USA) and subjected to radioactivity measurement by liquid scintillation counting. The average leak of injection was 0.31 ± 0.07% (n = 40) of the intended amount of PCB 126 solution. The final dose in each cricket was calculated as the amount injected minus the amount of leakage observed by scintillation counting.

Dosing regime and animal care during experiment. Two weeks before the experiment was conducted, frogs were acclimatized individually in Rubbermaid® tubs (Rubbermaid, Winchester, VA, USA; 58.4 × 42.5 × 22.9 cm) lined with heavy density polyethylene plastic bags. The room temperature was set at 20°C under a 12-h-light/12-h-dark photoperiod. The frogs were fed one mealworm each day, which permitted maintenance of body mass or growth. To ensure adequate moisture for frogs, dechlorinated water was supplied in a polystyrene petri dish (100 × 15 mm, diameter × height) and replenished at least daily. Each frog was fasted 24 h, fed a PCB-laden cricket, then returned to a Rubbermaid container. Each treatment group consisted of 33 frogs. Frogs were fed mealworms ad libitum from day 1 to day 22. Starting day 23, we adopted a new feeding plan. Frogs of each treatment group were further divided with one subgroup fed ad libitum and the other group fed one mealworm per frog per day. Each of four subgroups (two dosage groups × 2 feeding levels) consisted of eight individuals. The purpose of this design was to determine whether we could manipulate frogs' body mass to cause differential elimination rates of PCB 126. Frogs were weighed every 5 d starting at day 23.

In both holding regimes before and during the experiment, frogs ate normally and no skin diseases were observed.

Tissue sampling and radioactivity determination. Frogs were decapitated at days 2, 4, 7, 9, 37, 76, 111, 149, 186, and 226 after dosage. Blood was obtained by cardiac puncture. Originally we had 66 frogs. Several frogs at 5.0 mg/kg dosage were excluded from analysis due to possible unrecorded injection volume, no presence of fat bodies, or red leg disease. According to our observation, most of the blood in the heart was removed after cardiac puncture. Gross tissue abnormality and peritoneal edema were inspected at killing. A liver was recorded as abnormal when it appeared paler or darker than normal and/or with grey or white lumps. A swollen kidney was considered abnormal. Liver, fat bodies (corpora adiposa, located anterior to the gonads), stomach, stomach contents, intestine, intestine contents, egg follicles, kidneys, gonads, skin, muscle, cloacal material, carcass, and tissue pool were collected and weighed. Egg follicles were a combination of previtellogenic follicles, growing vitellogenic follicles, and fully grown postvitellogenic ova. The tissue pool consisted of esophagus, lung, spleen, and heart. These tissues were pooled because, in a pilot study, we found very low to undetectable levels of ^{14}C in these tissues. Carcasses included entire head, bone, cartilage materials, and remains of other tissues. Whole skin was used to estimate the ^{14}C residue because we analyzed ^{14}C residues in head, leg, back, and abdominal areas of skin and found that ^{14}C residues were not evenly distributed in skin.

For counting and analysis by liquid scintillation, a quench curve with counting efficiency ranging from 53 to 60% was prepared using liver as quenching agent. Whole blood, skin, kidney, egg follicles, cloacal materials, and the tissue pool were bleached with adequate amounts of 30% stabilized hydrogen peroxide so that quenching values of these samples would be within the quenching range of the quench curve. All tissues were completely digested in Soluene-350 at 45 to 50°C and allowed to cool to room temperature before the addition of Hionic-fluor liquid scintillation counting cocktail. The amounts of Soluene-350 and Hionic-fluor added to each kind of tissues were determined according to the guidelines of the manufacturer. Radioactivities in the vials were determined with a Tracor Analytic Mark III Scintillation Counter (Tracor Analytic, Elk Grove Village, IL, USA). Only values above 10× background dpm were considered in statistical analyses; trace levels (2–4× background dpm) were not used for statistical analyses.

Statistical analyses. ^{14}C residues in tissues are given as mean ± one SE. All statistical analyses were performed using SYSTAT® for Windows®, Version 5 (SYSTAT, Evanston, IL, USA). Statistical significance in all analyses was set at α = 0.05. The qualitative data from gross tissue examination were analyzed for effects of time, dose, and time × dose using the

binary logit analysis. Appropriate models were selected based on the Akaike information criterion [14,15]. Body mass measured between days 23 and 147 ($n = 17$) was used for the repeated-measures analysis of variance (ANOVA) testing dose, gender, feeding, dose × gender, dose × feeding, and gender × feeding as independent variables. The ANOVA models were followed by backward elimination analyses to obtain appropriate reduced models. In the course of model derivation by ANOVA, data and plots of residuals were inspected. We decided that data would be considered outliers and removed if values for Cook's distance, a measure of the influence of each sample observation on the coefficient estimates, approached or exceeded one [16]. We removed no data from our analysis.

The 48-h oral bioavailability was calculated based on the PCB activity in the force-fed crickets and summed activity in all tissues at 48-h postdosing as follows:

$$\left[\sum (\text{dpm in tissues/dpm } ^{14}\text{C-PCB 126 in oral dose})\right] \times 100\%$$

We used this time point because, in a pilot study, the experimental animals had no PCB-laden cricket in their gastrointestinal tract 48 h after consumption of a PCB-laden cricket. The oral bioavailability of both treatment groups was compared using a *t* test.

In the statistical model relating ^{14}C-PCB 126 retention (percentage of the initial dose in the whole body) in frogs to dose, time, and time × feeding, backward elimination followed by multivariate analysis was used to obtain appropriate models. The $t_{1/2}$ values for whole-body elimination of ^{14}C-PCB 126 during the elimination period were calculated assuming first-order elimination as

$$t_{1/2} = (\ln 2)/(K_{el})$$

where K_{el} is the slope of the least-squares regression line of ln[^{14}C-PCB 126] content in frogs versus time.

RESULTS

Gross liver and kidney appearance and body mass

Throughout the study, frogs exhibited no anorexia, skin or organ lesions, or mortality caused by ingested PCB 126. Although this study was not designed to be a toxicity study (e.g., there is no zero-dosage control group), we did test whether the incidence of peritoneal edema or liver or kidney abnormalities varied with dose or time. The occurrences of liver abnormality were not affected by dose ($p = 0.123$, $n = 60$) but significantly increased over time ($p = 0.0002$, $n = 60$). No dose or time effects in the occurrences of peritoneal edema or kidney abnormality were found (all p's > 0.15, $n = 60$). Backward elimination analyses following repeated-measures ANOVA indicated that body mass increased significantly ($p < 0.001$) over time in these adults exposed semichronically to PCB 126, with slight variation depending on dose and feeding level ($F_{1,15} = 5.512$, $p = 0.033$, $n = 17$). Frogs feeding ad libitum appeared to increase in mass faster initially than those fed 1 mealworm/d, and the increase was fastest in the higher dose group. But after 5 weeks, the increase was similar in all the groups. The body mass of experimental frogs in the low- and high-dose groups increased about 15.6% (from 33.2 ± 4.0 g to 38.3 ± 3.2 g [mean ± SE], $n = 32$) and 16.4% (from 31.0 ± 4.5 g to 36.1 ± 3.7 g, $n = 28$) of initial mass in 5 weeks, respectively.

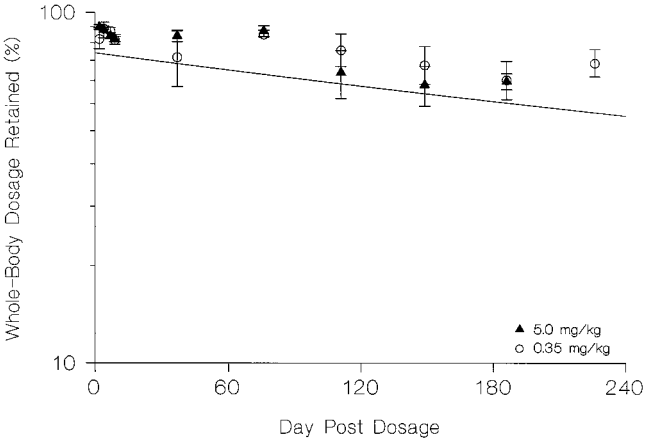

Fig. 1. Percentage of ^{14}C (administered as PCB 126) as a function of time. Values are mean ± SE ($n = 2$–4). There were no significant dose effects on the retention of whole-body ^{14}C content. The solid line is the fit of all the data to the equation ln(% of initial dose) = 4.48327 − (0.00091)·(day postdosage) (see Results: *Oral bioavailability*).

Oral bioavailability and elimination of PCB 126 in northern leopard frogs

The 48-h oral bioavailabilities of ^{14}C-PCB 126 in the 0.35- and 5.0-mg/kg dosage groups were 84.6 ± 5.8% ($n = 4$) and 90.9 ± 1.5% ($n = 3$), respectively, and they were not significantly different ($t = 0.917$, $df = 5$, $p = 0.401$). This was a minimum estimate based on residues in the body. The calculated bioavailability would be higher (91.5–96.4%) had we calculated it as the difference between dose and the material excreted in the first 48 h. The ^{14}C residues of cloacal materials at 48 h in the low- and high-dosage groups were 3.6 ± 0.5% ($n = 3$) and 8.5 ± 5.4% ($n = 4$), respectively. In both dosage groups, the average ^{14}C radioactivities of cloacal materials after 48 h and throughout the experiment ranged from 0.11 ± 0.05% to 0.59 ± 0.16% of the initial total ^{14}C residue.

^{14}C residue in frogs decreased with time ($F_{9,47} = 4.855$, $p < 0.001$, $n = 59$), with no significant difference in rate according to dose ($F_{1,47} = 0.531$, $p = 0.47$, $n = 59$) or feeding level ($F_{1,47} = 0.169$, $p = 0.683$, $n = 59$) (Fig. 1). Assuming a single pool and one elimination rate ($K_{el} = -0.00090803 \pm 0.00014398$, mean ± SE), the $t_{1/2}$ value for whole-body elimination of PCB-derived ^{14}C was 763 d.

Distribution of PCB 126 in northern leopard frogs

We present data on concentrations of PCB 126 residues in different organs, as well as total amounts that are the product of concentration and organ mass. Liver, fat bodies, carcass (including head, bone, cartilage material, and residues of other tissues), skin, and muscle were the major organs for PCB 126 retention in both dosage groups (Figs. 2 and 3). The concentrations of PCB residue were initially very high in liver and small intestine [17] but then declined, presumably due to distribution following absorption. Concentrations in fat bodies of both dosage groups were relatively constant throughout the experiment (Fig. 4). However, total residues in fat bodies declined over time in correlation with declining masses of fat bodies in both the high-dosage group (Fig. 5) and the low-dosage group (data not shown). Total PCB 126 residues were highest in organs with high PCB concentration and/or high tissue mass, such as carcass, fat bodies, skin, and egg follicles (Figs. 6 and 7).

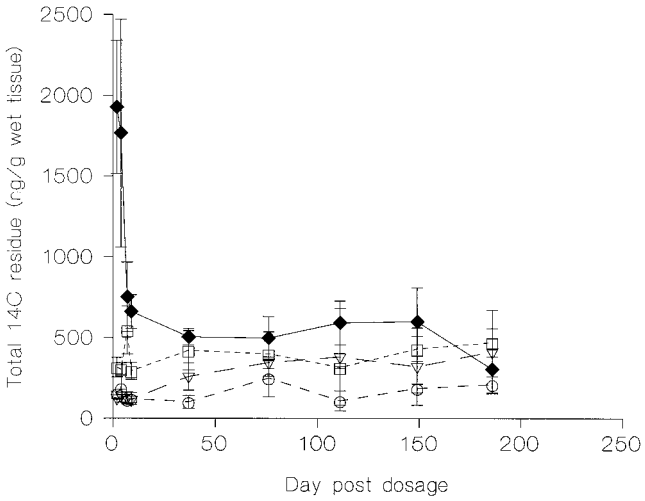

Fig. 2. Concentrations of ^{14}C (administered as PCB 126) as a function of time in four major tissues of the 5.0 mg/kg-dosage group (mean ± SE, n = 2–4). ----- = carcass; – – – = muscle; — — = skin; ——— = liver.

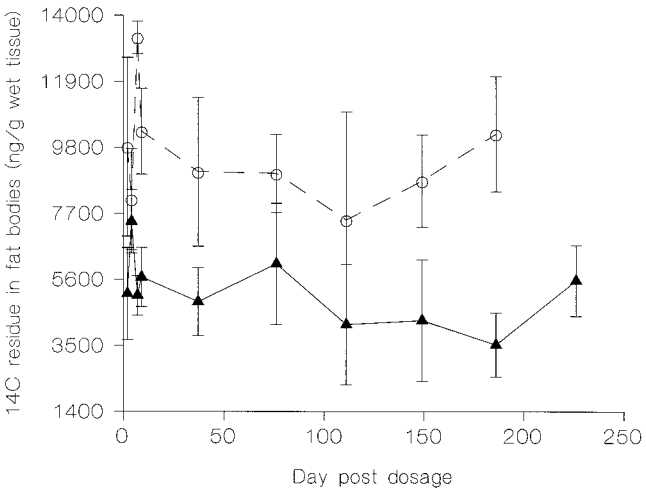

Fig. 4. Concentrations of ^{14}C (administered as PCB 126) as a function of time in fat bodies (corpora adiposa) in the low- and high-dosage groups (mean ± SE, n = 2–4). ○ = 5 mg/kg; ▲ = 0.35 mg/kg.

The concentrations of PCB residues were relatively low (e.g., lower than for muscle shown in Figs. 2 and 3) in kidney and the tissue pool [17]. Testes had concentrations of PCB residues similar to those in liver (see Figs. 2 and 3) and higher concentrations than those in oviducts [17]. Nineteen females were found to carry egg follicles. The concentrations of ^{14}C-derived residues in egg follicles were between 300 and 1,000 ng/g, somewhat higher than the concentrations in oviducts.

DISCUSSION

High oral bioavailability and slow elimination of PCB 126 absorbed through a cricket

Experimental frogs absorbed ≥85% of PCB 126 ingested. In the field, we would expect northern leopard frogs to have a similar, high oral bioavailability of PCB 126 because they consume similar types of food as we used in this study. However, morphology of the anuran gastrointestinal tract can vary seasonally [18,19], which could have effects on digestion. Therefore, endogenous (e.g., seasonal rhythm) and exogenous (e.g., stress, type, and amount of food) factors might influence, to some extent, northern leopard frogs' absorption efficiencies of PCBs and other lipophilic toxicants.

Direct measurements of dietary assimilation in other vertebrates have shown that efficiencies of 40 to 70% are typical for many halogenated hydrophobic organics [20]. Absorption efficiencies for young rainbow trout (*Oncorhynchus mykiss*) were about 50% for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [9] and 41 to 44% for 2,3,4,7,8-pentachlorodibenzofuran [21]. Pheasant (*Phasianus colchicus*) absorbed 30 to 58% of 2,3,7,8-TCDD via ingestion, depending on the types of food [10]. European starlings (*Sturnus vulgaris*) absorbed 37 to 44% of 2,3,7,8-TCDD from diet (J. Martin et al., unpublished data). Collectively, these results suggest that there are species- and chemical-dependent differences in the absorption efficiency of lipophilic toxicants. The variation of absorption efficiency of toxicants might result from variation in retention time in the gastrointestinal tract, food items used for toxicant administration, animal body size, and stress.

Northern leopard frogs had a slow elimination rate of PCB 126 ($t_{1/2}$ = 763 d), though it might be shorter or longer de-

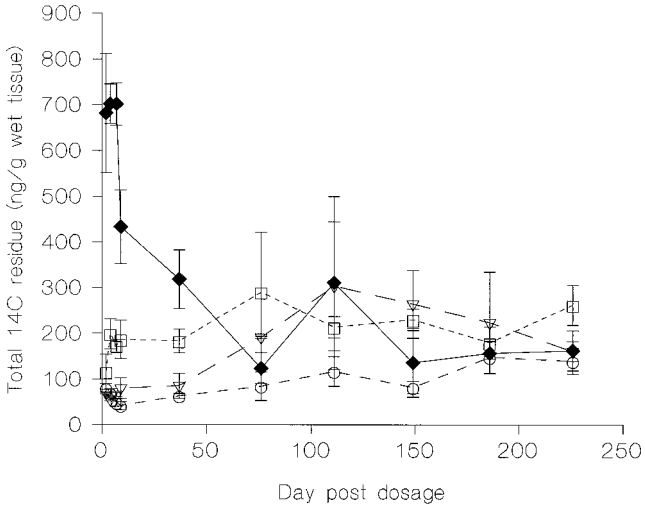

Fig. 3. Concentrations of ^{14}C (administered as PCB 126) as a function of time in four major tissues of the 0.35 mg/kg-dosage group (mean ± SE, n = 2–4). ----- = carcass; – – – = muscle; — — = skin; ——— = liver.

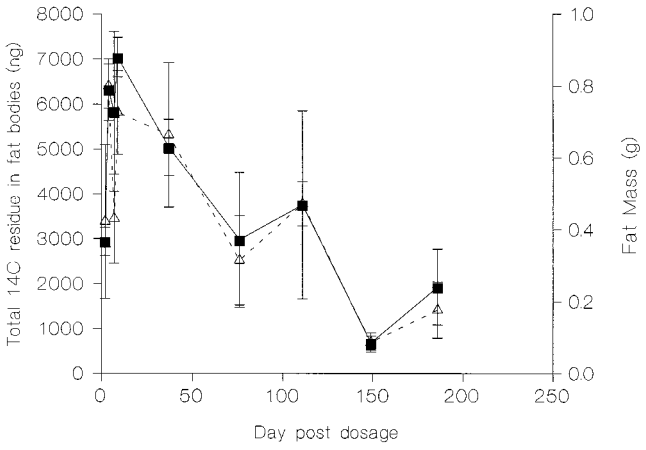

Fig. 5. Total ^{14}C-PCB 126 residues in fat bodies and mass of fat bodies as a function of time in the 5.0 mg/kg-dosage group. Solid line represents ^{14}C quantity; dashed line represents the mass of fat bodies.

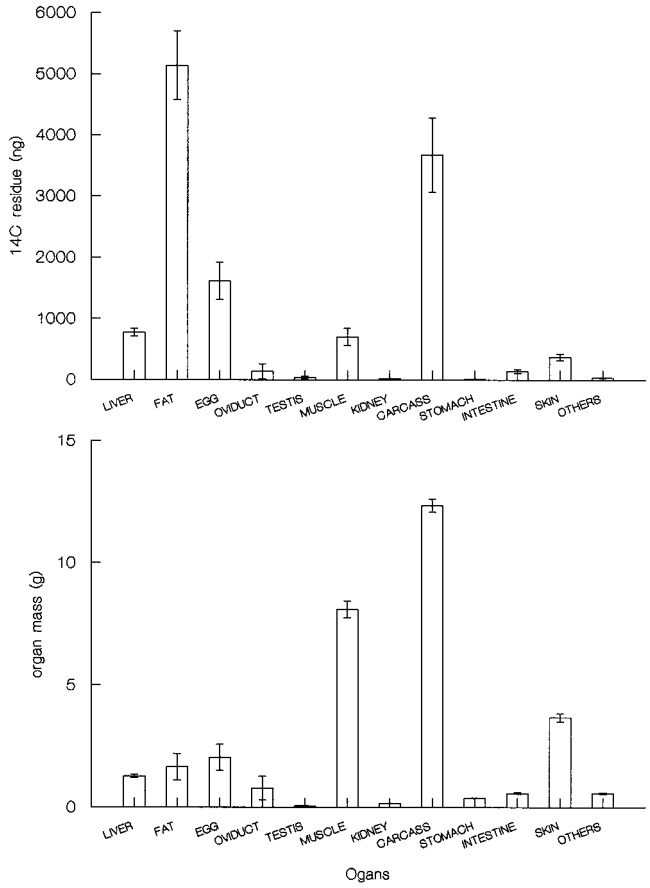

Fig. 6. Total ^{14}C-PCB 126 residues in various organs (upper panel) and organ masses (lower panel) between days 7 and 9. Except for egg follicles (n = 3), oviducts (n = 6), testes (n = 10), and stomach (n = 14), the sample size for each organ is 16. Data are expressed as mean ± SE.

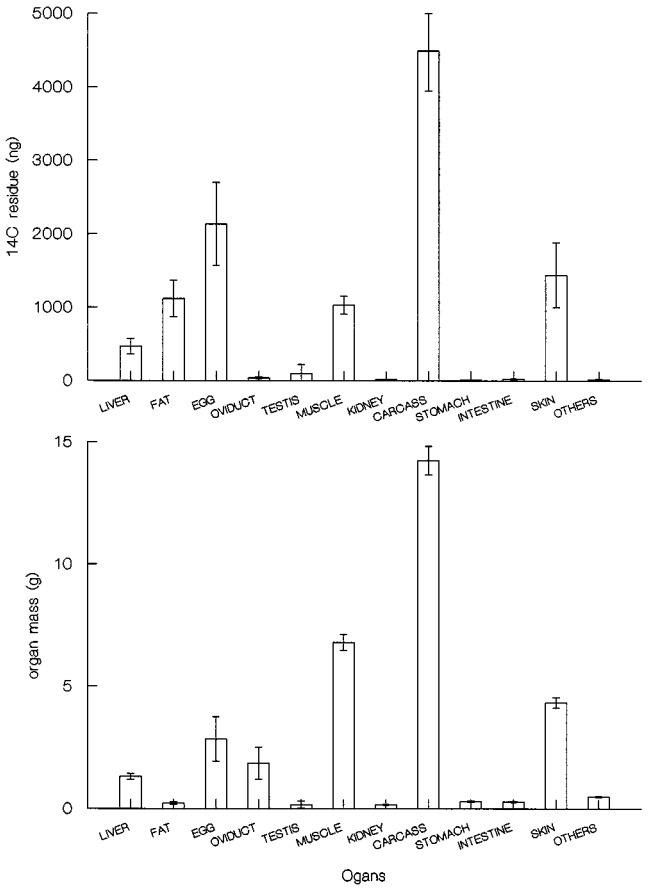

Fig. 7. Total ^{14}C-PCB 126 residues in various organs (upper panels) and organ masses (lower panel) between days 149 and 186. Except for liver (n = 7), egg follicles and testes (n = 5), and oviducts (n = 7), the sample size for each organ is 12. Radioactivities in five livers were below 10× background dpm. These livers were thus excluded from the calculation. Data are expressed as mean ± SE.

pending on average body temperature. The $t_{1/2}$ for rainbow trout treated with 2,2′,5,5′-tetrachloro[^{14}C]biphenyl via static water exposure for 36 h was 642 d in females and 522 d in males [22]. The half-life values for elimination of 2,3,7,8-TCDD in hamster, rat, guinea pig, herring gull, rainbow trout, and monkey were 11, 31, 94, 100, 105, and 365 d, respectively [9,23–26]. Ectothermic frogs and fish have relatively slower metabolic rates than those of endotherms. Therefore, in general, we would expect a tendency of relatively slower elimination rates for the same compound in amphibians than in endotherms.

In the present study, we did not attempt to characterize whether the radioactivity in the tissue was associated with native PCB 126 and/or its metabolites. Presumably, the low dose (0.35 mg/kg) would not cause the induction of P450 enzymes, while the high dose (5.0 mg/kg) induced P450 enzymes to a maximal level [12]. In the event that PCB 126 was metabolized by detoxifying enzymes faster in the high-dosage group than in the low-dosage group, the metabolism of PCB 126 in this study apparently was not a rate-limiting step in elimination of PCB 126 in frogs because we found a dose-independent elimination.

Distribution of PCB 126 in various organs

Among the organs examined, fat bodies accumulated the highest concentration of PCB 126. An important finding in this study was that total amount of PCB 126 in fat bodies decreased in correlation with declining mass of fat bodies over time. The decline of PCB content in fat bodies might be due to redistribution to other organs, such as carcass and muscle, and to elimination. In the field, frogs utilize their fat, mainly in fat bodies, for survival during the early stage of emergence from hibernation (e.g., early to late March in Wisconsin) or during vitellogenesis in the breeding period [27]. Whether or not there were acute toxicities due to PCB redistribution was unknown and needs to be studied.

The liver retained <10% of initial total ^{14}C residue or 300 to 600 ng/g wet tissue. The liver was considered a minor site for TCDD accumulation in rainbow trout [9]. In contrast, as much as 50% of a dose of TCDD may be distributed to rat liver, with most of it sequestered in hepatic microsomal protein [28]. Liver residues constituted from 2.5 to 4.8% of body burden of PCDFs in rainbow trout [29]. The differences in retention in the liver for the same lipophilic compound among species might be due to the content of fat in the livers and/or the sequestering of toxicants by hepatic microsomal protein [30–35].

Testis, oviduct, kidney, stomach, intestine, and the tissue pool containing esophagus, lung, spleen, and heart retained <1% of the initial total ^{14}C residues. Although the PCB 126 levels in testis, kidney, stomach, intestine, lung, and heart were low, a variety of tissues in these organs have been shown to induce P450 enzymes at and above 2.3 mg/kg of PCB 126

[12]. Therefore, these organs might play a role in processing PCB 126 and might be target sites for aryl hydrocarbon receptor-related toxicity.

Deposition of PCB 126 into the egg follicles

Though we found that female egg follicles could retain up to 23% of ^{14}C-derived radioactivity of the initial dose, the concentrations in egg follicles were not high. The PCBs perhaps entered preformed egg follicles mainly by diffusion. The egg follicles in females were likely produced before they were caught because female leopard frogs are generally unable to produce egg follicles in captivity (L. Northy and C. Richards, personal communication). The high percentage of the initial doses in egg follicles could have ecological implications. Frog ovaries containing complements of fully grown vitellogenic oocytes may constitute a substantial fraction of the body mass, as high as 30% or more [36]. In our study, females with egg follicles of 14 to 17% of their body masses retained 19 to 23% of the initial dose in egg follicles. Thus, we could expect that the egg follicles would retain $\geq$23% of the initial dose of PCB in the event of egg follicles constituting $\geq$17% of their body masses. The impacts of PCBs and other lipophilic compounds on elimination rates of contaminated adult females, hatchability, metamorphosis timing, growth rate, and survival rate of tadpoles need to be further investigated. On the other hand, the elimination of PCB 126 in testes via reproductive activity in males might be less important because males retained $<$1% of initial total PCB 126 in gonads. However, when expressed as concentration of PCB in gonads, the concentrations of PCB 126 in testes were much higher than in oviducts. Whether toxicity occurs in testes due to the high PCB concentration is our future research interest.

Excretion routes

Ingested PCB could be excreted via the integument or bile and urine in the digestive tract of frogs. Forty-eight hours after frogs ingested PCB-laden crickets, about 3.5 to 8.5% of initial ^{14}C residue was found in cloacal materials. Unless there was an acute elimination, the ^{14}C-PCB residue in cloacal materials should be unabsorbed from food. After 48 h, the ^{14}C-PCB residues in cloacal materials dropped to $<$0.7%, and they were likely to be from bile and/or urinary excretion. Although the ^{14}C radioactivities in most of the bile samples collected at dissection were at trace levels (twofold to threefold above background dpm), ^{14}C-derived radioactivity in nine bile samples ranged from 0.04 to 0.1% of total initial ^{14}C residue, with an average of 0.061% $\pm$ 0.017%.

Frogs shed the skin constantly at a regular time interval. The concentrations and percentages of the initial total ^{14}C residues in skin increased over time. The reasons for the increase of the ^{14}C residue in skin were unknown and need to be investigated. On a few occasions, we pooled shed skin over 4 to 7 d as a sample because ^{14}C-PCB 126 in shed skin was undetectable in a single daily collection. On two occasions, the ^{14}C in pooled, shed skin was detectable (approx. 10$\times$ above background dpm), indicating that frogs eliminated PCB through shedding their skin. In a study of immunohistolocalization of P450 enzymes [17], P450 enzymes were shown to be present in the epithelial cells of mucous glands and dermal vascular endothelium. Thus, skin was a site for both PCB 126 accumulation and elimination.

Toxicity of PCB 126

There was no mortality, and no skin or organ lesions were found in this study. This was consistent with results from our previous studies [13,17] that no mortality or skin or organ lesions were found following the injection or ingestion of PCB 126, even at doses as high as 7.8-mg/kg dosage. Assuming the toxic equivalency factor for PCB 126 is one tenth that of 2,3,7,8-TCDD, as in fish [37], the LD50 of experimental frogs would be at least 10 times higher than those of aquatic or terrestrial vertebrate species examined [38–40]. The incidences of peritoneal edema or abnormal liver or kidney were not significantly different between the two treatment groups. Because there were no control animals, we cannot conclude whether PCB 126 caused adverse effects on liver, kidney, or peritoneal edema. The increase of liver abnormality over time might be due to long-term animal housing or other reasons. In this study, the frequency of kidney abnormality was not significantly different in either dosage group. However, in the dose–response and time-course studies [12], we encountered significantly more transparent or yellow kidneys (but found no swollen kidneys) in the 7.8-mg/kg PCB group (7 of 20 individuals) than in the control group (2 of 22 individuals). More detailed histological examination on PCB-related toxicity in amphibian species needs to be done in the future.

The TCDD-related wasting syndrome has been reported in fish [40] and mammals [39]. In this study, frogs did not lose body mass following ingestion of PCB 126-laden crickets. Instead, frogs in both dosage groups gained approximately 16% of their initial body masses. The increase of their body mass might be attributed to the constant feeding and their sitting inert in the tub over a long period of time. Because there were no control frogs for comparison of body mass change with treatment groups, we could not conclude whether PCB had effects on the growth of animals. In the wild frogs collected from the Green Bay ecosystem, the total PCB residues ranged from 3 to 152 μg/kg [13]. In the present study, frogs gained weight at and above 0.35-mg/kg PCB 126. Thus, it seems unlikely that the growth or weight maintenance of postmetamorphosis frogs inhabiting the Green Bay ecosystem would be limited by their PCB accumulation.

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