Bull Environ Contam Toxicol (2007) 79:315–318
DOI 10.1007/s00128-007-9228-1

Polychlorinated Biphenyls and Organochlorine Pesticides Bioaccumulated in Green Frogs, *Rana clamitans*, from the Lower Fraser Valley, British Columbia, Canada

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Received: 29 November 2006 / Accepted: 15 June 2007 / Published online: 18 July 2007

Abstract Seven adult green frogs (*Rana clamitans*) were collected from three sites adjacent to intensive agriculture in the lower Fraser River valley, BC Canada. The highest mean concentrations of chemicals were *pp′*DDE at 0.313 μg/g lipid wt. and Aroclor 1254/1260 at 2.12 μg/g lipid wt. On a lipid weight basis, both *pp′*DDE and PCB concentrations varied by almost an order of magnitude among sites. Only ortho-substituted PCB congeners were detected. The concentrations of organochlorine pesticides and PCBs measured in these frogs from British Columbia are unlikely to elicit negative effects in frogs.

Keywords Amphibians · Pesticides · Polychlorinated · Biphenyls

In amphibians, lethal and sublethal effects of organochlorine pesticides and polychlorinated biphenyls (PCBs) are one of many causal factors considered to be important in worldwide declines in the size and number of amphibian populations (Cooke 1972; Hayes 2000). However, there are relatively few studies that have measured OC pesticide contaminant concentrations in amphibians from areas where bio-effects at the individual and/or population level are occurring.

In the Sumas Prairie and Elk Creek watersheds in the lower Fraser Valley, British Columbia (BC), Canada, significant effects on survivorship and biochemical indicators of stress were observed in red-legged frogs (*Rana aurora aurora*) and northwestern salamander (*Ambystoma gracile*) early life stages (de Solla et al. 2002a, b; Loveridge 2002). OC pesticides were applied in the Fraser Valley in the 1970s (Finizio et al. 1998), and today organophosphate (OP) pesticide use in the area is widespread (Wan et al. 1994). The objective of this study was to quantify organochlorine pesticide and PCB concentrations in adult amphibians from the site in the Sumas Prairie studied by deSolla et al. (2002a) and the nearby Elk Creek watershed which has not been previously sampled to measure contaminants in wildlife. Green frogs (*Rana clamitans*) were sampled because they are a very common introduced species in the area, and are in the same genus, *Rana*, as two native species in the lower Fraser valley in BC: the Red-legged frog (*Rana aurora aurora*) is designated special conservation concern in Canada (COSEWIC 2004) and the Oregon spotted frog (*Rana pretiosa*) is an endangered species in Canada (COSEWIC 2000). It was a further objective to directly compare our green frog results to another location in Canada and analyzed using similar methods (Russell et al. 1997).

Materials and Methods

Seven adult green frogs were collected from three sites in the lower Fraser River valley, BC. Two sampling sites

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were in the Elk Creek watershed (Nevins Road/49.147918 N, 121.896527 W and Banford Road/49.147918 N, 121.896527 W) and the third sampling site was in the Sumas watershed (49.80375 N, 122.183408 W). All three sites were ditches adjacent to intensive agriculture potentially receiving agricultural effluent.

Elk Creek, a tributary of the Fraser River, originates in the Skagit Mountain range before flowing east into the lower Fraser Valley. Intensive agricultural activity such as dairy and poultry farming, field crops, greenhouses, and nurseries dominates land-use in the lowlands of this watershed (Loveridge 2002).

Sumas Prairie is located approximately 30 km west of the Elk Creek watershed. Primary agricultural activities include livestock, market crops, and sod production (de Solla et al. 2002b).

Frog tissues were stored in hexane-rinsed aluminium foil at –20°C until transportation to the laboratory. Samples were pooled from each site for analyses.

The samples were thawed to room temperature and extracted with dichloromethane (DCM):hexane (1:1 v/v) after the samples were dehydrated with anhydrous Na_2SO_4. The quantitative analysis of organochlorine compounds was performed using capillary gas chromatography, coupled with a mass selective detector operated in selected ion monitoring mode. Each cleaned sample was injected twice. The first injection was designed to determine the organochlorine pesticide compounds by using 21 organochlorine pesticide standards. The second injection was to determine PCBs by using Aroclors 1242/1254/1260, at a 1:1:1 quantisation standard mixture. The samples were analysed using the HP 5890 GC #1 and HP Mass Selective Detector HP 5971 (Hewlett-Packard, Wilmington, DE, USA). The detection limit was 0.1 ng/g wet wt. The concentration of trace levels was between 0.1 and 0.9 ng/g wet wt. The total PCBs reported is the sum of 59 non-coplanar PCB congeners. The pesticides measured were hexachlorobenzene (HCB), pentachlorobenzene (PnCB), α-, *β*-, and *γ*-hexachlorocyclohexane (HCH), octachlorostyrene (OCS), heptachlor epoxide (HE), oxychlordane, *trans*- and *cis*-chlordane, *trans*- and *cis*-nonachlor, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, dieldrin, photomirex, and mirex. The percent recovery of $^{13}C_{12}$ labelled internal standard PCBs ranged from 85 to 94.5%, with a mean percent recovery efficiency of 90.5%. The percent recovery of $^{13}C_{12}$ labelled internal standard of tetra-, penta-, and hexa-chlorobenzene ranged from 82.8 to 88.7% with a mean percent recovery of 85.1%. The reported concentrations of pesticides and PCBs were not corrected for the percent recoveries. Lipids were determined by homogenizing 1–2 g of sample with 4 ml of hexane, and the mixture was centrifuged to isolate the hexane later. The hexane was then dehydrated with anhydrous Na_2SO_4. This process was repeated twice, and the hexane extracts were evaporated and the lipids weighed.

Results and Discussion

On a lipid weight basis, PCB concentrations were almost an order of magnitude higher in green frogs from the Sumas Prairie when compared to the Elk Creek sites (Table 1). For *pp′*DDE, concentrations at Sumas Prairie and Banford Road, Elk Creek were similar and an order of magnitude higher than from Nevin Road, Elk Creek. Most organochlorine pesticides (1,2,4,5-TClBz, 1,2,3,4-TClBz, α-HCH, *β*-HCH, *γ*-HCH, OCS, H.E., oxychlordane, trans-chlordane, *cis*-chlordane, dieldrin, photomirex, and mirex) were not detected in frog tissues. The percent lipid in frogs (whole-body) was very low, and ranged from 0.29 to 0.75% of body weight (Table 1). Concentrations in northwestern salamander eggs compared to green frogs in the Sumas Prairie site (de Solla et al. 2002a) had similar concentrations of all compounds except Aroclor 1254/1260 which was about twice as high in the green frog eggs. The OC pesticide levels and sum of PCB concentrations were similar in green frogs from the Elk Creek and Sumas Prairie watershed in comparison to green frogs from southern Ontario (Table 1; Russell et al. 1997).

Only ortho-substituted PCB congeners were detected, and the same general pattern is evident at both watersheds (Fig. 1). The congener profile for green frogs in southern Ontario (Russell et al. 1997) is very similar to the profile for green frogs in the Sumas Prairie (Fig. 1).

Organochlorine concentrations in these frogs probably represent ambient levels in the Pacific northwest given that PCB concentrations in green frogs sampled here were lower than in whole-body tadpoles of Pacific treefrogs (*Pseudacris regilla)* from the Sierra Nevada mountains (Angermann et al. 2002) in 1996–1997. Detectable concentrations of organic pollutants are being found in amphibians in agricultural areas and pristine sites throughout North America (Zabik and Seiber 1993). However, as evidence of the distinct local variation possible when sampling amphibians, PCB deposition was higher in the Sumas Prairie versus the Elk Creek watershed despite the close proximity of these areas and their similar agricultural history.

The concentrations of organochlorine pesticides and PCBs measured in adult green frogs from British Columbia are unlikely to elicit negative health effects in these amphibians. Endocrine disruption of red-legged frog metamorphosis by agricultural effluent, as measured by concentrations of plasma thyroid hormones, was not observed in Elk Creek (Loveridge 2002). The lowest reported

Table 1 Concentrations of organochlorine pesticides and PCBs in green frogs and in northwestern salamander eggs from Elk Creek and Sumas Prairie, British Columbia and southern Ontario, Canada (μg/g lipid wt.; μg/g wet wt. italicized and in parentheses), % moisture, and % lipid)

	Elk Creek watershed		Sumas Prairie		Southern Ontario (from Russell et al. 1997; means of seven sites)
	Nevin Road (n = 2 green frogs pooled for analyses)	Banford Road (n = 2 green frogs pooled for analyses)	n = 3 green frogs pooled for analyses	Northwestern salamander eggs (from deSolla et al. 2002a)	
Moisture %	79.98	78.06	79.22	96.33	
Lipid %	0.750	0.516	0.288	0.39	
Analyte					
Hexachlorobenzene	0.0133 (*0.0002*)	0.0388 (*0.0001*)	0.0694 (*0.0002*)	0.0769	(*0.000329*)
p,p′-DDE	0.0267 (*0.0009*)	0.116 (*0.0002*)	0.313 (*0.0006*)	0.385	(*0.000269*)
Sum of PCB congeners	0.0533 (*0.0028*)	0.0969 (*0.0004*)	0.972 (*0.0005*)	1.231	(*0.007514* [a])
Aroclor (1254/1260), (1:1)	0.213 (*0.0028*)	0.310 (*0.0016*)	2.12 (*0.0016*)	0.949	
Aroclor 1260	0.200 (*0.0045*)	0.388 (*0.0015*)	1.56 (*0.0020*)	0.692	

[a] Sum of PCB congeners 99, 101, 105, 118, 138, 146, 153, 171, 180, 183, 194, 201, and 203

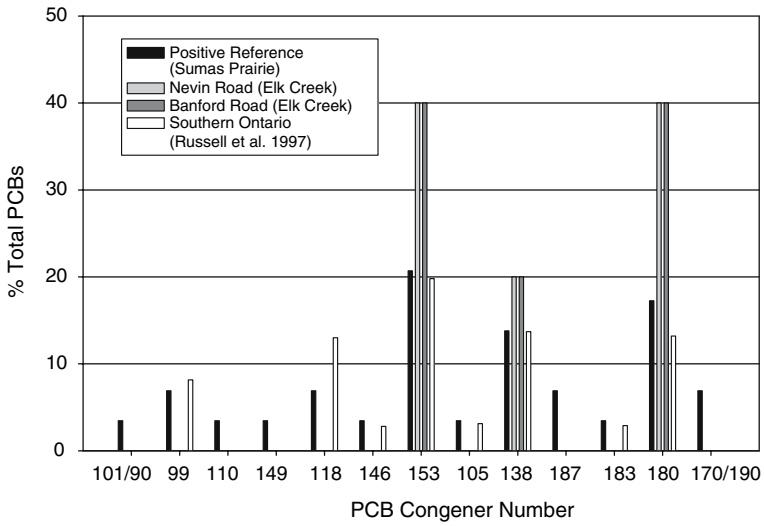

Fig. 1 Detected PCB congeners in green frogs (*Rana clamitans*) from two sites in the Elk Creek Watershed and the Sumas Prairie, BC, Canada (2000)

concentration of DDT that has a toxic effect on amphibians was 2.4 μg/g wet wt. in common frog (*R. temporaria*) tadpoles (Cooke 1972). Whereas, for PCBs, a whole body concentration of 0.55 μg/g dry wt. caused organ damage in tiger salamanders (*A. tigrinum*) (Johnson et al. 1999). Savage et al. (2002) observed decreased activity levels in *R. sylvatica* tadpoles at tissue concentrations of 5.966 μg/g wet wt. total PCBs but mortality was not significantly different from controls at this concentration. The control tadpoles, which probably received PCBs in the laboratory diet, still had body burdens of 0.033 μg/g wet wt. total PCBs indicating that even at low ambient exposures amphibians can bioaccumulate persistent organic contaminants.

Acknowledgments The National Wildlife Research Centre in Hull, Quebec, performed OC pesticide and PCB analysis. This study was funded by the Georgia Basin Ecosystem Intiative of Environment Canada and by an NSERC grant to CJK. The Simon Fraser University Animal Care Facility permit for this study was 550 B.

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