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Chlorinated hydrocarbon contaminants in feces of river otters from the southern Pacific coast of Canada, 1998–2004

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ABSTRACT

Chlorinated hydrocarbon contaminants in coastal river otters (*Lontra canadensis*) were evaluated by sampling feces (scats) collected on the south coast of British Columbia, Canada. A broad survey of industrialized areas of the Strait of Georgia region was conducted in 1998, and a subsequent survey of working harbours in 2004. Samples from 1998 were analyzed for polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides, and polychlorinated dioxins (PCDDs) and furans (PCDFs), while in 2004, chemistry was confined to ∑PCBs and OC pesticides. Concentrations of OC pesticides were low in both years, with only dichlorodiphenyldichloroethylene (DDE; range: 0.01–2.12 mg/kg lw) and hexachlorocyclobenzene (HCB; range: 0.003–0.25 mg/kg lw) detected in all samples. In 1998, octachlorodibenzo-*p*-dioxin (OCDD) and other higher chlorinated PCDD/Fs were found in most samples, with OCDD ranging from 120 ng/kg lw in Clayoquot Sound to 19,100 ng/kg lw in a pooled sample from two latrines in Nanaimo. PCBs were present in all samples. In 1998 geometric mean concentrations of the sum of 59 PCB congeners ranged from 0.49 mg/kg lw in Nanaimo to 12.3 mg/kg lw in Victoria Harbour. Six years later, mean ∑PCBs remained elevated (geometric mean 9.5 mg/kg lw) in Victoria Harbour. Geometric mean concentrations of ∑PCBs from Victoria Harbour in 1998 and 2004 were >9 mg/kg lw, a published adverse effect level for reproduction. At some latrines in both Victoria and Esquimalt Harbours, concentrations of TCDD-toxic equivalents exceeded 1500 ng/kg lw, a value for health effects in otters that we derived using published information. As shown in previous studies, analysis of scats provides an efficient and non-intrusive approach to assessing contaminant threats to otter populations, and to documenting spatial trends in residues.

1. Introduction

The Georgia Basin–Puget Sound trans-boundary region is a highly productive, temperate zone ecosystem located in southwest British Columbia, Canada and northwest Washington, USA. Monitoring studies have documented contamination of the region by a plethora of legacy contaminants including organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), dioxins (PCDDs), furans (PCDFs), polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Golder Associates, 2003). Research has linked those contaminants to deformities and carcinomas in fish (Malins et al., 1984), as well as reproductive and physiological effects in birds (Elliott et al., 1989, 1996a; Sanderson et al., 1994; Gill and Elliott, 2003) and marine mammals (Ross et al., 2000, 2004; Simms et al., 2000).

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The North American river otter (*Lontra canadensis*) inhabits coastal regions of the Georgia Basin–Puget Sound (Melquist et al., 2003) and has life history characteristics that make it an intriguing candidate as an ecosystem sentinel species. As sedentary, high trophic level piscivores (Larsen, 1984; Stenson et al., 1984; Ben-David et al., 2005) of the marine intertidal and subtidal zones, river otters accumulate persistent contaminants in local environments and may be sensitive to dioxin-like chemicals, based on extrapolation from the acute sensitivity of another aquatic mustelid, the mink (*Mustela vison*, Jensen et al., 1977; Bleavins et al., 1980). Although the cryptic and elusive nature of the river otter makes it a difficult species to study directly in the wild, employing indirect techniques that take advantage of their predictable behavioral traits can be an efficient means of population monitoring. River otters communicate via scent deposition at specific locations along the land–sea interface, known as 'latrines' (Bowyer et al., 1995). Coastal river otters deposit feces (scat), anal gland secretions, and urine at latrines, thus facilitating the transfer of marine derived nutrients to the terrestrial ecosystems (Ben-David et al., 1998, 2005). The conspicuousness of latrines and their habitual use by otters provide opportunities to study populations using indirect methods. In fact, the analysis of otter scat has proven useful to measure physiological variables such as reproductive steroid hormones (Kalz et al., 2006), as a source of genomic DNA for individual identification (Dallas et al., 2003; Hung et al., 2004), to evaluate and monitor the incidence of disease (Gaydos et al., 2007), and to describe food habits (Larsen, 1984; Stenson et al., 1984; Ben-David et al., 2005). Scat samples have also been used effectively as a way to monitor exposure of Eurasian otters (*Lutra lutra*) to chlorinated hydrocarbon contamination for a number of years (e.g. Smit et al., 1994; Van den Brink and Jansman, 2006). These methods offer advantages over conventional techniques in that they are logistically simple and non-intrusive to wildlife populations.

In this study, we examined the exposure of river otters to environmental contaminants in the Georgia Basin using non-intrusive scat sampling. Our objectives were to: (1) determine chlorinated hydrocarbon concentrations in coastal river otter scat from urban and industrial areas of the region; (2) relate findings to potential sources of contamination; and (3) compare data to published criteria on critical concentrations in otter scat and other tissues.

2. Methods

2.1. Study area

In 1998, sampling areas were selected on the basis of proximity to industrial pollutant sources: Victoria Harbour, an industrial/urban region with known PCB contamination in the inner harbour (City of Victoria, 2001); Esquimalt Harbour, an industrial/urban area and the home port for the Canadian Pacific naval fleet; Nanaimo, an urban area with a concentration of forest industries; Cowichan Bay, Crofton, and Powell River, relatively rural areas but with concentrated forest industries (e.g. large pulp mills at Crofton and Powell River, and concentrated wood storage and processing in Cowichan Bay); and Clayoquot Sound, a mainly wilderness reference site on the west coast of Vancouver Island (Fig. 1).

In 2004, sample collection was limited to three working harbours: Victoria Harbour was re-sampled; Vancouver Harbour, the largest industrial/urban center and main shipping port for western Canada; and Comox Harbour, a relatively rural reference site with a naval port, some agricultural runoff, and some forest product processing.

2.2. Sample collection

Fresh scats were collected from May to August in 1998 and 2004. Potential latrine sites were identified from marine charts. The shoreline was surveyed by small watercraft and/or by foot, and the GPS location of each latrine was recorded. Active sites were identified by the presence of at least ten aged scat remains and/or the presence of fresh scats, as well as having well-established river otter trails, scrapes, and bedding sites. All fresh scats discovered at each latrine site were colleted by hand using a latex glove or a metal spoon, combined in acetone/hexane cleaned 50 ml jars, and stored at −20 °C. Where mucus deposits (anal jellies) were present, they were collected with the scats. Samples were shipped to the National Wildlife Research Centre (NWRC), Ottawa, ON where they were stored at −40 °C prior to processing.

Because different sample types were collected during the two sampling periods (scat, anal jelly, and mixtures of both), we chose to examine the relationship between chlorinated hydrocarbon levels in the two fractions. In 2006, five otter scats with a distinct anal jelly portion disassociated from the fecal material were collected from Victoria Harbour latrines. We assumed that each sample was derived from an individual animal based on its location from other excrements in the field. The two fractions were carefully separated in the field, resulting in a total of ten samples. Anal jelly and fecal portions were collected in 50 mL acetone/hexane cleaned glass jars. Samples were shipped to the National Wildlife Research Centre (NWRC), Ottawa, ON where they were stored at −40 °C prior to processing.

2.3. Chemical analysis

In 1998, individual fresh scats were prepared as composite samples by latrine site, varying in volume from approximately 20 to 200 g. In 2004, some individual scats were analyzed along with composite samples from the same latrines; composite volumes varied according to number of fresh scats available. Scats were placed in chemically cleaned stainless steel trays and stirred by hand with a cleaned stainless steel rod until appearing homogenized. Sub-samples of approximately 12 g were removed and placed into glass jars. Split samples from 2006 were frozen in liquid nitrogen and then homogenized to a powder in a cryogenic Teflon ball mill (RETSCH MM301, Newtown, PA, USA).

Concentrations of OC pesticides and PCB congeners were measured in scat samples using a multi-residue gas chromatography (GC)/mass selective detector (MSD) technique. Sub-samples of 6.5 g were mixed with anhydrous Na_2SO_4 and let stand for at least three hours to dry the sample. Dehydrated scat samples were submitted to neutral lipid extraction (1:1 DCM:hexane), gel permeation chromatography, and Florisil column chromatography as clean-up steps prior to chemical quantification (Norstrom et al., 1988). In a variation from standard procedures, during extraction, the sample-Na_2SO_4

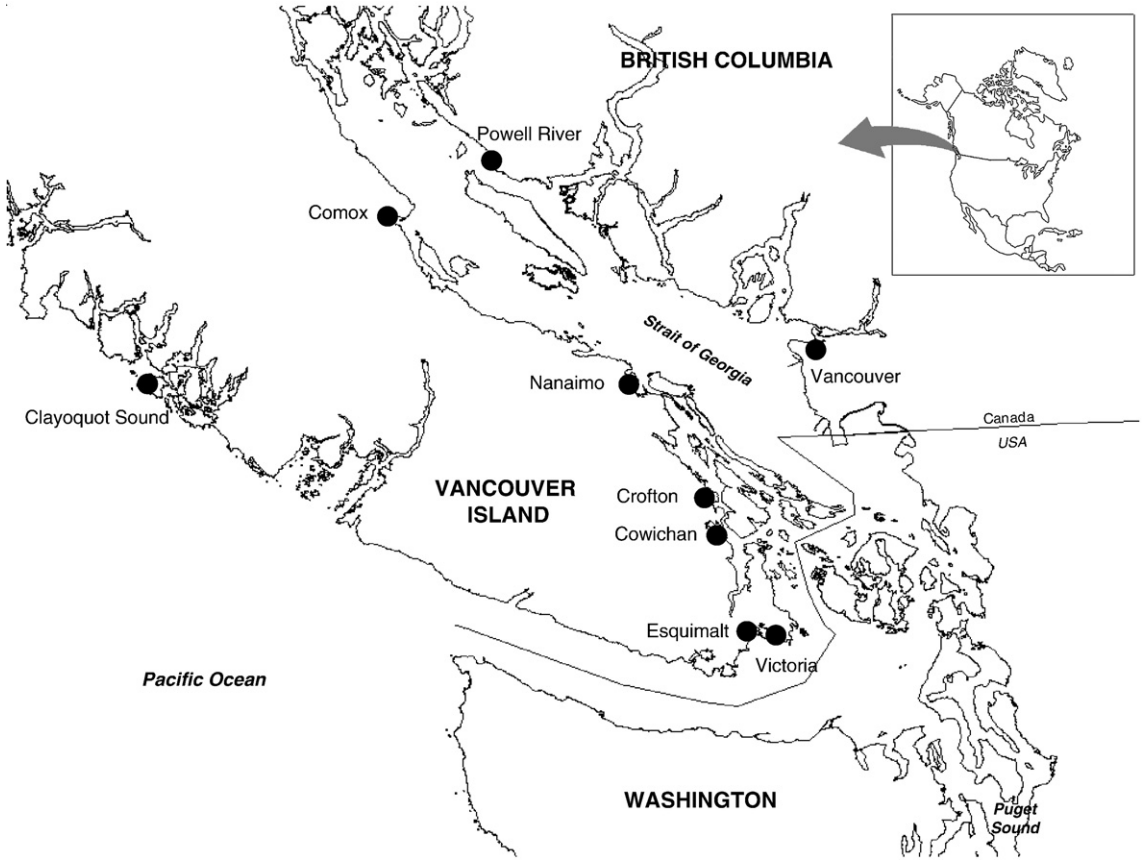

Fig. 1 – General locations of latrine sites on the south coast of British Columbia, Canada where river otter scats were collected for environmental contaminant concentrations (1998, 2004, and 2006).

mixture was loaded onto a glass column and soaked with 50 mL of 1:1 DCM/Hexane overnight, then eluted with another 250 mL of 1:1 DCM/Hexane. Sample extracts were injected twice onto a HP5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a HP5971 mass selective detector (operated in selected ion monitoring mode) and a 30 m DB-5 fused silica column (0.25 mm i.d., 0.25 μm film thickness). The first injection was used to measure OC pesticides by comparison with 21 OC standards, while the second injection was used to measure PCB congeners against an Aroclor 1242:1254:1260 (1:1:1) standard mixture.

OC pesticides routinely quantified using the above method were 1,2,4,5- and 1,2,3,4-tetrachlorobenzene (TCB), pentachlorobenzene, hexachlorobenzene (HCB), octachlorostyrene (OCS), *trans*- and *cis*-nonachlor, *trans*- and *cis*-chlordane, oxychlordane, heptachlor epoxide (HE), *p,p'*-dichlorodiphenyldichloroethane (-DDD), *p,p'*-dichlorodiphenyldichloroethylene (-DDE), *p,p'*-dichlorodiphenyltrichloroethane (-DDT), photo-mirex, mirex, α-, β-, and γ-hexachlorocyclohexane (-HCH) and dieldrin. Fifty-nine PCB congener peaks were quantified with the above method. A 1989 diluted herring gull egg was analyzed with each run as a reference (Turle and Collins, 1992). Results were initially expressed on a wet weight basis uncorrected for percent recoveries, and later converted to a lipid basis. Minimum limits of detection varied from 0.001 to 0.0001 μg/g.

Concentrations of 18 PCDDs, 23 PCDFs and 6 non-*ortho* PCBs were measured in scat samples using a high-resolution gas chromatography/mass spectrometry (GC/MS) procedure. The preparatory procedures (neutral extraction, gel permeation chromatography, alumina column clean-up, Florisil column chromatography) have been described by Letcher et al. (1996). Quantification was achieved using a VG AutoSpec double-focusing high-resolution MS (Waters Corp, Milford, MA, USA) linked to a Hewlett-Packard 5890 Series II high-resolution GC with a 30-m DB-5 fused silica column.

Isotopically-labelled ($^{13}C_{12}$) internal standards were used for all PCDD, PCDF and non-*ortho* PCB congeners measured, and corrections for percent recovery of each individual congener were made. Herring gull egg reference samples were used to check analytical accuracy as described above for OC and PCB analyses. PCB congeners are described in the text using International Union of Pure and Applied Chemistry (IUPAC) numbers. Minimum detection limits were assessed for each sample and are reported in the results where relevant. Lipid and moisture content were determined using gravimetric methods.

2.4. *Statistical analysis*

Samples collected in 1998 were combined as composites by latrine site. In 2004, some individual scats were analyzed along with composites. In both cases we treated the latrine as the primary sampling unit. As there was significant variation in lipid content within samples from a site, but not between sites, data were lipid normalized as recommended by Hebert and Keenleyside (1995). Statistical analyses were performed using SPSS version 15.0 (Chicago, IL, USA), or by JMP version 4.0.2 (SAS Institute, Cary, NC, USA). Chlorinated hydrocarbon

data were converted to natural logarithms to approximate a normal distribution. Geometric means were calculated by site and comparisons among sites were performed using analysis of variance (ANOVA). When an ANOVA was significant, multiple comparisons were made using the Tukey HSD test. The pattern of PCBs among sites was compared by selecting the most prevalent congeners and determining the ratio to ∑PCBs as a percentage. Percentage values were arcsine — transformed before testing with ANOVA as described previously. Statistical significance was set at $P<0.05$.

Separated anal jelly and fecal samples collected from 2006 were analyzed individually. Tests for normality (Kolmogorov–Smirmov) were conducted and it was determined that data transformations were unnecessary. Linear regression was used to examine the relationship between chlorinated hydrocarbon concentrations in the fecal and anal jelly portions of individual scat samples. Linear regression analyses were performed using program R version 2.6.0 (Vienna, Austria).

We used the same approach as Mason et al. (1992) and Smit et al. (1994, 1996) to derive safe and critical concentrations for TEQs in scat. Their values were used for the variables: assimilation efficiency, conversion factor from fresh to lipid weight, food ration, TEQ concentration in food, excretion constant and accumulation time period. We recognize that those values are approximate given that TEQs are based on PCDD and PCCF as well as PCB compounds, and potential variation exists between *Lutra lutra* and *Lontra canadensis*, but considered they were likely adequate to derive a tentative TEQ criteria for scat.

3. Results

3.1. Sample type

A linear regression was performed on individual scat and anal jelly data to determine if there was a significant relationship between contaminant concentrations in the two fractions (Fig. 2). A significant relation was found between scat and anal jelly fractions for ∑PCBs ($R^2=0.679$, $P<0.05$, Pearson's). The slope of the line of best-fit was not statistically different from the ideal line with a slope of one (regression slope=0.676, SE=0.269, *t*-test of regression slope, $P>0.05$) and the intercept was not statistically different from zero (regression *y*-intercept=1.44, SE=1.06, *t*-test of regression *y*-intercept, $P>0.05$).

There was also a significant relationship for total OC pesticide contaminant concentrations in fecal material and anal jelly portions ($R^2=0.647$, $P=0.05$, Pearson's). The slope of the line of best-fit was not statistically different from one (regression slope=1.21, SE=0.517, *t*-test of regression slope, $P>0.05$) and the intercept was not statistically different from zero (regression *y*-intercept=−0.013, SE=0.102, *t*-test of regression *y*-intercept, $P>0.05$).

3.2. OC pesticides

In 1998, geometric mean total OC pesticide concentrations were highest in samples from Victoria (0.70 mg/kg lw), which were significantly higher than mean concentrations from Nanaimo, Powell River, and Clayoquot Sound ($P<0.05$, Table 1). In 2004, highest mean concentrations of total OC pesticides were

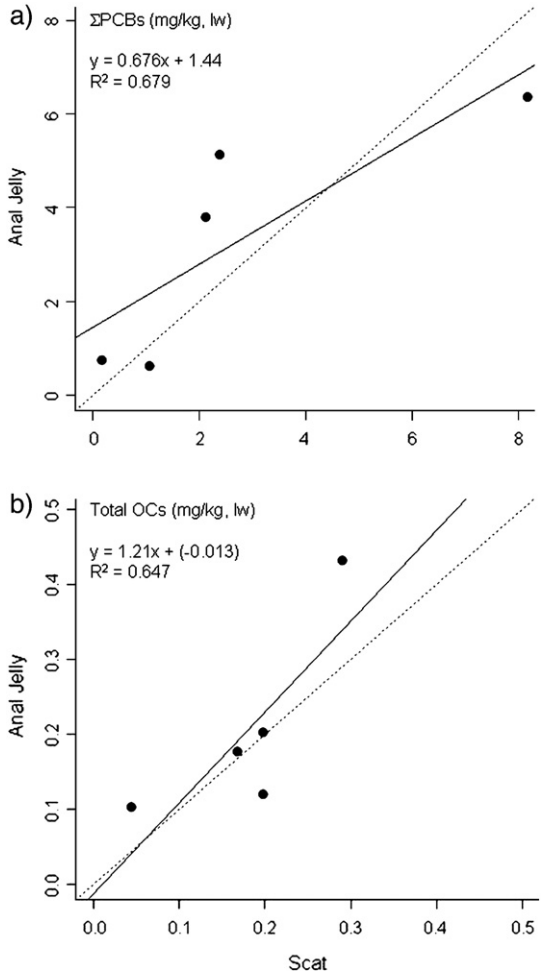

Fig. 2 – Concentrations of a) ∑PCBs and b) total OC pesticides in fecal material versus jelly portions from individual river otter scat samples collected on the south coast of British Columbia, Canada, 2006. The solid line is the best-fit line of the linear regression between variables. A theoretical 1:1 relationship is shown as a dotted line (slope=1.0, y-intercept=0.0). In both cases, the slope of linear regression is not statistically different from one, and the y-intercept is not statistically different from zero.

measured in samples from Comox with similar concentrations from Victoria, but significantly lower in samples from Vancouver ($P<0.05$). DDE and HCB were the only individual OC compounds quantified in the majority of samples. As major contributors to the total OCs, those individual compounds followed the same spatial pattern; for example, in 1998, geometric mean concentrations of HCB and DDE in samples from Victoria Harbour were significantly greater than samples from Nanaimo only ($P<0.05$, Table 1). In 2004, there were no significant differences among sites for any of the individual OC pesticide compounds.

3.3. PCBs

In 1998, the highest geometric mean concentrations of ∑PCBs were measured in samples from Victoria Harbour (12.3 mg/kg lw, Table 1). ∑PCB concentrations in scats collected from Victoria were significantly greater than concentrations at all sites other

Table 1 – Mean ± standard error (and range) of total lipids and geometric mean (and range) of selected chlorinated hydrocarbons measured in river otter scats collected from sites on the south coast of British Columbia, Canada, 1998 and 2004

			Residue levels (mg/kg, lw)			
Year	Location	Percent lipid	HCB	*p,p'*-DDE	Total OCs	∑PCBs
1998	Victoria ($n=4$)	2.00±0.76 (0.17–3.76)	0.05^{a} (0.03–0.12)	0.29^{a} (0.11–2.12)	0.70^{a} (0.34–3.88)	12.3^{a} (3.63–108.4)
	Esquimalt ($n=4$)	2.54±0.36 (1.56–3.17)	0.03^{ab} (0.01–0.05)	0.07^{ab} (0.02–0.23)	0.18^{ab} (0.13–0.37)	2.11^{ab} (1.17–2.79)
	Nanaimo ($n=5$)	1.0±0.18 (0.51–1.61)	0.01^{b} (0.005–0.04)	0.03^{b} (0.01–0.13)	0.07^{b} (0.04–0.20)	0.49^{b} (0.23–1.42)
	Powell R. ($n=5$)	0.79±0.18 (0.37–1.28)	0.03^{ab} (0.02–0.05)	0.05^{ab} (0.01–0.13)	0.13^{b} (0.05–0.24)	0.73^{b} (0.31–1.74)
	Cowichan ($n=2$)	1.88±0.94 (0.94–2.82)	0.02^{ab} (0.01–0.02)	0.08^{ab} (0.07–0.08)	0.14^{ab} (0.13–0.16)	0.79^{b} (0.69–0.89)
	Clayoquot ($n=5$)	1.88±0.23 (0.45–1.78)	0.03^{ab} (0.02–0.04)	0.07^{ab} (0.04–0.21)	0.14^{b} (0.10–0.43)	0.66^{b} (0.34–1.28)
2004	Victoria ($n=7$)	0.91±0.07 (0.66–1.14)	0.04^{x} (0.01–0.08)	0.17^{x} (0.04–0.56)	0.46^{x} (0.13–1.72)	9.48^{x} (2.60–67.6)
	Vancouver ($n=7$)	0.91±0.08 (0.59–1.15)	0.03^{x} (0.003–0.05)	0.07^{x} (0.006–0.17)	0.17^{y} (0.04–0.34)	3.78^{xy} (2.41–5.12)
	Comox ($n=7$)	0.85±0.15 (0.29–1.42)	0.07^{x} (0.03–0.25)	0.26^{x} (0.06–0.59)	0.54^{x} (0.17–0.82)	1.58^{y} (0.43–4.46)

Differences between sites were analyzed by year.
One way ANOVA, Tukey HSD, $P=0.05$.
Locations in each year sharing the same letters in each category are not significantly different.

than Esquimalt ($F=9.71$, $P<0.001$; Table 1, Fig. 3). One composite sample collected from a Victoria latrine had a concentration of 108 mg/kg lw. In 2004, the highest geometric mean concentrations of ∑PCBs were again measured in samples from Victoria (9.48 mg/kg lw). ∑PCB concentrations in Victoria scat samples were significantly higher than Comox, but not Vancouver Harbour (Fig. 3). Samples from two Victoria Harbour latrines had elevated concentrations of 37.6 and 67.6 mg/kg lw.

An examination of PCB congeners (Table 2) shows that concentrations of non-*ortho*-PCBs were highest in samples collected from Victoria and Esquimalt Harbours, specifically PCB-126 and PCB -77. Those concentrations were significantly greater ($P<0.05$) than concentrations measured in samples collected from Crofton or Clayoquot Sound. No other significant differences were observed between sampling locations.

The pattern of 33 individual PCB congeners was expressed as a percentage of ∑PCBs (Fig. 4). The overall patterns are very similar among sites and years, and there were no significant differences among sites for any of the PCB congeners in both 1998 and 2004 ($F=0.37$, $P>0.05$; $F=0.05$, $P>0.05$ respectively). Samples from Victoria Harbour appear to have lower relative amounts of the higher chlorinated congeners, particularly CBs-153, -180, and -170/190. In contrast, in 2004, there appears to be relatively more of the lower chlorinated congeners, CBs-66, -87 and -101/90, in Victoria compared to Comox Harbour samples.

3.4. *PCDDs and PCDFs*

Concentrations of this group of chemicals were marked by extreme variability within and among sites. Generally, the

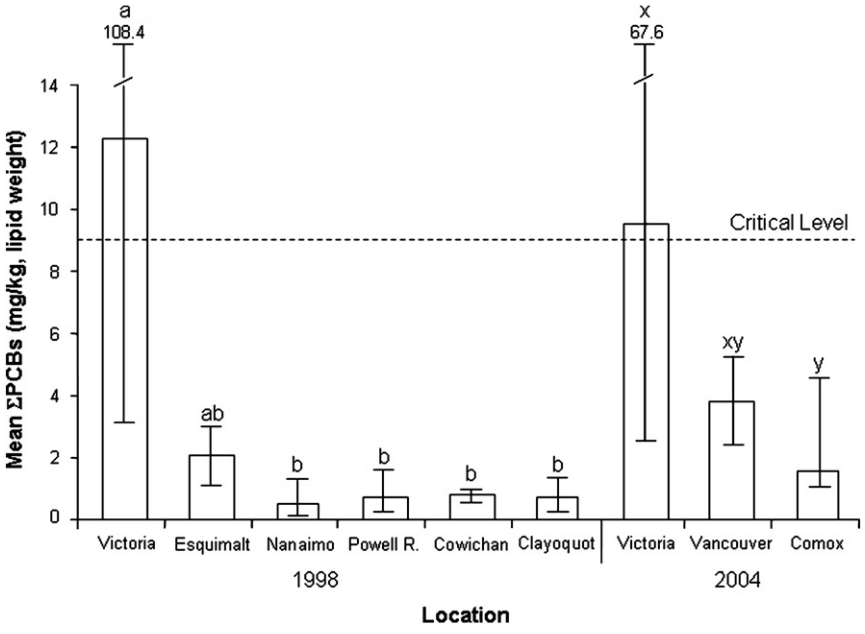

Fig. 3 – Geometric mean concentrations (and range) of ∑PCBs in river otter scats (expressed on a lipid basis) collected at sites on the south coast of British Columbia, Canada and compared to published criteria for reproductive effect in otter species (Smit et al., 1994). Sampling locations that have different subscripted letters are significantly different ($P<0.05$).

Table 2 – Geometric mean (and range) of selected polychlorinated biphenyl (PCB) congeners (lw) in river otter scats collected from sites on the south coast of British Columbia, Canada, 1998

Location	PCB congener							
	Non-*ortho*-PCBs (pg/g)				Mono-*ortho*-PCBs (ng/g)			
	CB-81	CB-77	CB-126	CB-169	CB-118	CB-105	CB-156	CB-157
Victoria ($n=4$)	116[a] (44–713)	2510[a] (493–16700)	934[a] (355–4530)	87.3[a] (59.5–376)	0.550[a] (0.136–7.24)	0.296[a] (0.077–3.18)	0.193[a] (0.10–2.32)	0.107[a] (0.05–0.880)
Esquimalt ($n=4$)	52.8[a] (39–64)	1270[a] (1090–1590)	495[a] (386–607)	45.5[a] (39–55.8)	0.076[a] (0.047–0.120)	0.047[a] (0.027–0.073)	0.037[a] (0.023–0.048)	0.017[a] (0.006–0.028)
Nanaimo ($n=3$)	11.0[a] (4.2–21)	273[ab] (158–410)	104[ab] (48.5–175)	7.0[a] (2.0–57.5)	0.022[a] (0.012–0.40)	0.014[a] (0.006–0.040)	0.012[a] (0.007–0.113)	0.006[a] (0.004–0.010)
Powell R. ($n=5$)	11.1[a] (6.0–23.0)	291.2[ab] (186–481)	92.1[ab] (58.6–160)	43.6[a] (39.1–52.1)	0.022[a] (0.012–0.080)	0.016[a] (0.006–0.054)	0.017[a] (0.009–0.034)	0.010[a] (0.006–0.034)
Crofton ($n=2$)	4.2[a] (3.0–6.0)	68.4[b] (31.6–148)	59.0[b] 32.8–106)	28.0[a] (17.8–44.0)	NA	NA	NA	NA
Cowichan ($n=2$)	37.5[a] (27.0–52.0)	733[ab] (357–1510)	329[ab] (255–425)	45.2[a] (42.7–47.9)	0.032[a] (0.032–0.032)	0.021[a] (0.021–0.021)	0.05[a] (0.015–0.016)	0.011[a] (0.007–0.018)
Clayoquot ($n=5$)	9.4[a] (8.0–11.0)	198[b] (164–238)	54.9[b] (48.2–62.5)	37.6[a] (25.0–56.7)	0.023[a] (0.018–0.029)	0.014[a] (0.009–0.018)	0.013[a] (0.009–0.018)	0.010[a] (0.009–0.011)

One way ANOVA, Tukey HSD, $P=0.05$.
Locations sharing the same letters in each category are not significantly different.
NA – not analyzed.

pattern in otter scats was: OCDD > TCDF > HxCDD > HpCDF > TCDD (Fig. 5). Concentrations of OCDD > 15,000 pg/g lw were measured in two samples; one from Victoria Harbour and the other from the Nanaimo area. Highest geometric mean concentrations of TCDD (21.5 pg/g lw) and TCDF (411 pg/g lw) were measured in samples from Esquimalt Harbour, although differences were not significant. Relatively high concentrations of PCDFs also were found in otter scats, with the concentration of HpCDF at one latrine in Victoria Harbour measuring 1140 pg/g lw.

The presence of elevated 2,3,7,8-TCDF in biota from the Georgia Basin has been related primarily to bleached-kraft pulp mill sources (Elliott et al., 2001). The highest individual latrine value of 2,3,7,8-TCDF (111 pg/g lw) was measured from a sample taken near the pulp mill at Powell River. However, high geometric mean values were found at sites in Victoria, Esquimalt and Cowichan Bay. This chemical was not detected in Clayoquot Sound samples.

The ratios of specific PCDFs considered as marker compounds for chemical sources were examined in more detail. Three compounds; 12469-PnCDF, 124678-HxCDF, and 124689-HxCDF, considered indicative of chlorophenolic sources (Hagenmaier and Brunner, 1987), one compound; 234678-HxCDF, considered indicative of combustion sources, and two compounds; 12478- and 23478-PnCDF, important in Aroclors (Hagenmaier and Brunner, 1987; Wakimoto et al., 1988) were assessed for their spatial patterns and relative concentrations in otter scats.

Concentrations of the combustion marker, 234678-HxCDF, were remarkably consistent among samples. Scat samples from Clayoquot Sound had a geometric mean concentration of 15.2 pg/g lw, compared to 10.5 pg/g lw in Victoria, and 12.6 pg/g lw in Esquimalt Harbours. Elevated concentrations of the chlorophenol indicators 12468-PnCDF and 124678-HxCDF were also detected at some sampling locations. For example, in scat samples collected from Victoria Harbour, the geometric mean concentration of 12468-PnCDF was 80.8 pg/g lw and the geometric mean of 12468-HxCDF was 82.8 pg/g lw. Concentrations of those congeners were also elevated in samples from Nanaimo (e.g. 124689-HxCDF, geometric mean 98.2 pg/g lw). These chemicals were not detected in Clayoquot Sound.

The Aroclor tracer 23478-PnCDF was more uniformly present in scat samples, with a geometric mean of 16.7 pg/g lw measured in samples from Clayoquot Sound compared to 13.9 pg/g lw at Nanaimo. The geometric mean concentration of 23478-PnCDF, however, was markedly higher in Victoria Harbour (56.4 pg/g lw) samples than all other sites. The other Aroclor tracer, 12478-PnCDF, was present at elevated concentrations in Victoria Harbour (geometric mean 51.8 pg/g lw), but non-detectable at many latrines elsewhere, including Clayoquot Sound.

3.5. *TCDD-toxic equivalents (TEQs)*

The greatest geometric mean concentrations of TCDD-toxic equivalents were measured in the samples from Esquimalt, followed by Victoria Harbour and Cowichan Bay, all of which were significantly greater than Clayoquot Sound, but not other Georgia Basin sites ($F=4.79$, $P<0.01$; Fig. 6). In terms of relative contribution to total TEQs, PCDFs were major at all sites, with

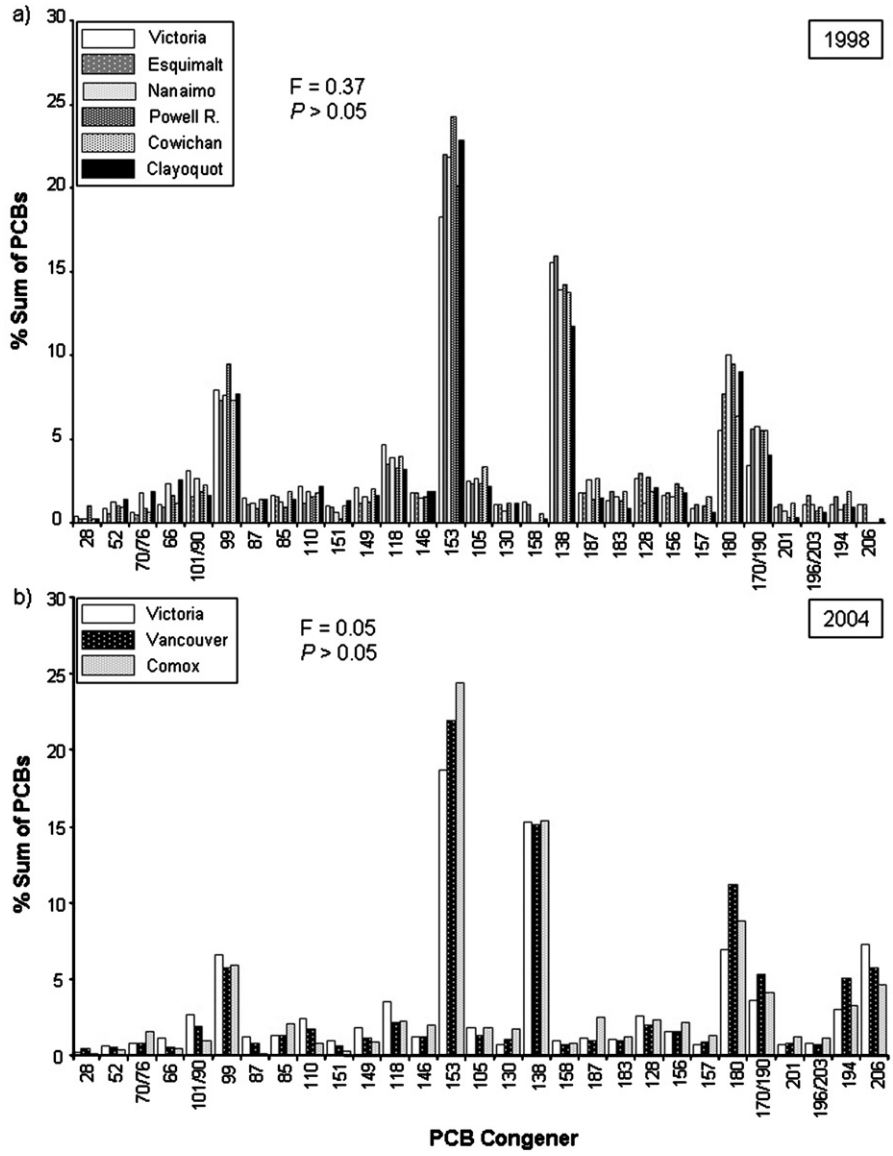

Fig. 4 – Pattern of selected PCB congeners in river otter scats collected from latrine sites on the south coast of British Columbia, Canada in a) 1998 and b) 2004. Data expressed as a percent of ∑PCBs and compared among sites using ANOVA.

non-*ortho* PCBs or PCDDs being the other key contributors, depending on the location.

3.6. *Comparison to published literature and toxicological criteria*

Published data on PCB concentrations in scat samples from Europe and elsewhere are summarized in Table 3, and examined below. Our data on concentrations of ∑PCBs are compared to published effect levels by sampling location in Fig. 3, and by individual latrine site in Fig. 7.

To derive TEQ criteria for scats, we used the tissue values of 2000 ng/kg lw as the safe level and 5000 ng/kg lw as the critical value as described in Smit et al. (1996, pp. 87), which are based on the depletion of Vitamin A. Therefore, we determined corresponding criteria in scats of 600 ng/kg lw for the safe value and 1500 ng/kg lw for the critical value. TEQ concentrations at individual latrine sites are compared to the effect levels in Fig. 8.

4. Discussion

In 1998, coastal river otters inhabiting all sampled areas of the Georgia Basin were exposed to a variety of chlorinated hydrocarbon contaminants, based on residues measured in scat samples. Samples from 1998 and 2004 collected from Victoria Harbours had ∑PCB and TEQ concentrations which exceeded the criteria for reproductive effects in otter.

4.1. *Sample type*

We differentiated two main types of samples; scats consisting mainly of fecal material, and mucus deposits or "anal jellies." Often confused with secretions from the anal gland located at the base of the rectum, the mucus deposit referred to here is formed in the intestine and expelled through the rectum like a scat (Kruuk, 2006). It remains unclear whether the function of

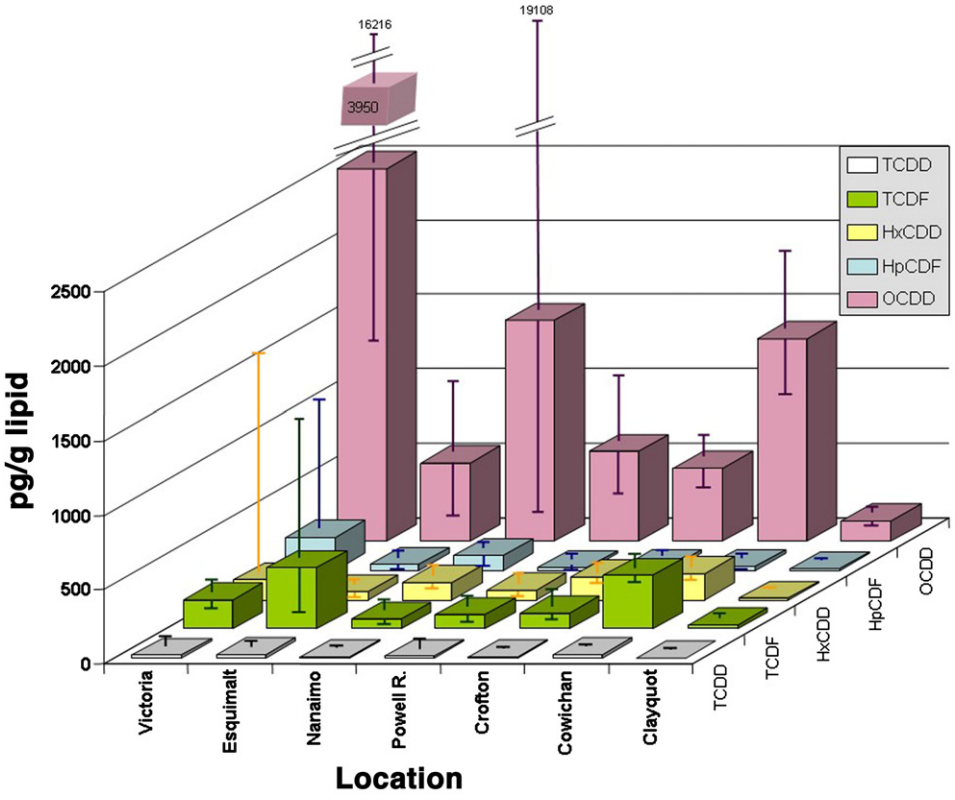

Fig. 5 – Geometric mean concentrations and range of selected PCDDs and PCDFs in river otter scat collected from latrines on the south coast of British Columbia, Canada, 1998.

the anal jelly is a response to undigested irritants in the digestive tract such as bones and scales and/or a mechanism of olfactory communication. Nevertheless, the contaminant concentrations could vary considerably within the two fractions due to differences in chemical make-up. Van den Brink and Jansman (2006) used PCB patterns to examine relationships between 'spraint' (scat) and internal fat samples from individual otters. They reported a significant linear relationship between scat and fat samples in cases where PCB 138 and 153 accounted for at least 42.5% of ∑PCBs, and referred to those as 'otter

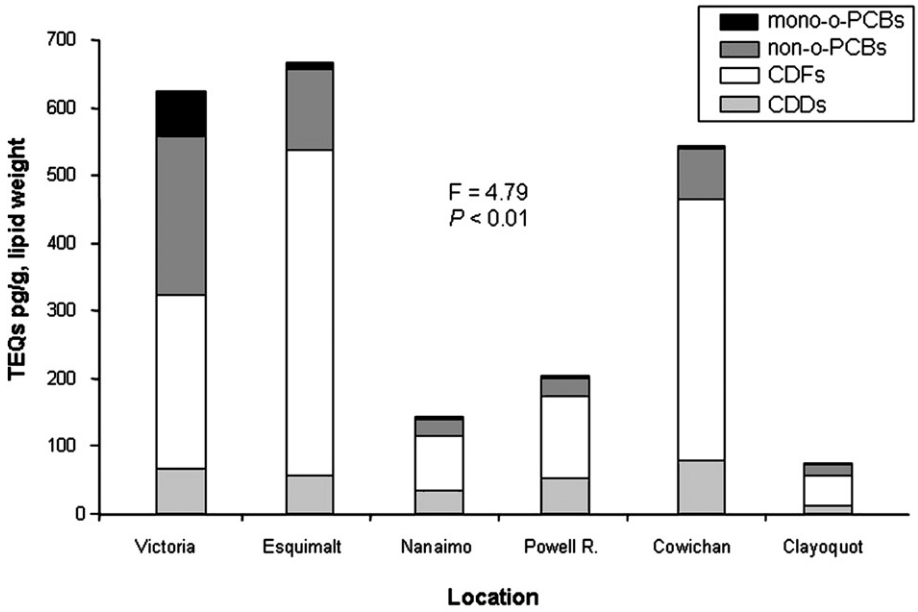

Fig. 6 – Concentrations of TCDD Toxic Equivalents (TEQs), and contribution of key components in river otter scats collected in 1998 at latrine sites on the south coast of British Columbia, Canada. Data compared among sites using ANOVA.

Table 3 – Arithmetic mean (except where noted) of ∑PCBs concentrations reported in otter scat (mg/kg, lw) from various locations

Location (year)	*n*	Mean	Range	Source
West England/Wales (1984–87)				Macdonald and Mason (1988)
Teme	15	5.61	<DL – 29.4	
Severn	30	0.91	<DL – 29.3	
Wissey	24	15.98	<DL – 32.6	
Wales (1986)				Delibes et al. (1991)
Severn	19	NA	2.73 (max)	
West England/Wales (1989–91)				Mason and Macdonald (1993b)
Severn, upper	89	3.18	0.03–62.68	
Severn, lower	47	8.47	0.33–44.68	
Teme, upper	83	2.28	0.04–15.47	
Teme, lower	50	5.76	0.06–40.81	
Clun	55	2.35	0.05–11.94	
Lugg, upper	11	1.44	0.04–5.93	
Lugg, lower	6	8.39	0.20–67.23	
Arrow	57	1.77	0.02–7.47	
East England (1989–91)				Mason and Macdonald (1993a)
Little Ouse	96	11.31	0.09–103.9	
Thet	76	7.77	0.37–37.93	
Black Bourn	75	8.71	0.20–38.06	
Wissey	22	8.07	0.56–21.15	
Wensum	23	13.97	0.79–107.48	
Northern England (1991–92)				Mason (1993b)
Northumberland	15	7.88	0.17–34.42	
Cumbria	6	8.81	4.20–13.72	
Esk	8	4.51	0.43–8.31	
Annan	21	2.34	0.07–4.79	
Galloway	8	0.58	0.12–1.52	
Southwest England (1989–91)				Mason and Macdonald (1994)
North Levels	25	5.11	0.23–40.16	
South Levels	17	9.44	1.20–46.55	
East Devon	14	3.70	0.51–7.90	
South Devon	15	6.04	0.86–25.58	
Taw	23	5.71	1.27–19.59	
Torridge	21	2.51	0.54–6.07	
Tamar	21	2.42	0.18–14.31	
East Cornwall	16	4.15	0.76–30.46	
West Cornwall	15	5.52	0.93–14.41	
Scotland (1990–91)				Mason et al. (1992)
Upper River Clyde	15	2.06[a]	0.17–4.63	
Lower River Clyde	11	19.49[a]	5.84–180.48	
Ayrshire rivers	11	5.38[a]	2.78–11.91	
Inner Clyde estuary	19	9.66[a]	0.83–53.55	
Outer Clyde estuary	28	8.45[a]	0.37–74.35	
West coast	29	3.34[a]	0.27–28.21	
Southern Ireland (1991)				O'Sullivan et al. (1993)
Bantry Bay	11	2.38	0.37–6.88	
Dunmanus Bay	13	0.70	0.04–4.40	
R. Gergus, Co. Clare	5	0.97	0.14–3.95	
Cork Harbour	65	1.62	0.03–6.69	
Cork City	15	2.71	0.54–7.29	
Upper R. Lee	11	2.14	0.90–3.63	
R. Blackwater	62	0.96	0.02–4.30	
East Cork	25	1.28	0.23–8.26	
Northern Ireland (1992)				Mason (1993a)
River Bann	7	2.54	0.33–6.82	
River Foyle	10	10.86	0.45–28.90	
Belfast/Larne Loughs	7	11.23	2.54–43.14	
Southwest Spain (1986)				Delibes et al. (1991)
Guadalquivir	17	NA	1.69 (max)	
Netherlands (NA)	7	3.5[a]	1.6–6.8	Smit et al. (1994)
Northeast Slovenia (1992–93)	2	0.38	0.223–0.634	Gutleb (1994)
Germany (1992)				Reuther and Mason (1992)
Schleswig-Holstein	3	2.93	1.54–5.71	

Table 3 (*continued*)

Location (year)	*n*	Mean	Range	Source
Mecklenburg-Vorpommem	38	5.76	0.56–25.80	
Brandenburg	40	8.43	0.35–8.43	
Niedersachsen	14	23.75	0.99–88.42	
Bayem	10	4.75	0.32–16.47	
Sachsen	28	3.54	0.15–18.53	
Niedersachsen (1991/92)	9	35.6	0.99–88.42	
Niedersachsen (before 1991)	5	2.43	1.35–4.80	
Hungary (NA)	4	5.48[a]	3.42–7.78	Mason (1987)
Austria/Czech Rep. (1990–91)	25	4.0[a]	0.42–41.0	Gutleb and Kranz (1998)
Denmark (1990)	19	2.23[a]	0.36–45.95	Mason and Madsen (1993)
Denmark (1991–94)				Elmeros and Leonards (1994)
Limfjorden	10	<9.0	NR	
Skals	10	<9.0	NR	
Karup	10	>9.0	NR	
Central France (2004–05)				Lemarchand et al. (2007)
upper Sioule River	17	5.06[a]	NR	
upper Allier River	8	7.01[a]	NR	
middle Allier River	24	7.88[a]	NR	
lower Allier River	22	13.58[a]	NR	
South Africa (1990) (*Lutra maculicollis*)	4	0.12[a]	0.05–0.24	Mason and Rowe-Rowe (1992)

Samples obtained from *Lutra lutra* unless specified otherwise.
[a]geometric mean.
<DL – less than detection limit.
NR – not reported.

spraints' that reflect the internal PCB concentrations of otters, as opposed to 'fish spraints' that reflect concentrations in otter prey. For the purposes of the analysis here, we have not undertaken a detailed statistical analysis of PCB or other contaminant patterns in scat and anal jellies. We have opted to rely on our direct comparisons of chemical concentrations between anal jelly and fecal portions of individual scat samples. Although sample size was low, regression analyses indicates that PCB and OC pesticide concentrations in fecal material were quantitatively similar to those in anal jellies; therefore, we have treated all sample types as equivalent and comparable.

4.2. *OC pesticide exposure*

Although there were significant differences between sampling sites, OC pesticide concentrations measured in river otter scats were generally low throughout the Georgia Basin in both 1998 and 2004, and were similar to those reported at relatively clean sites in Britain for example (Mason 1993b; Mason and Macdonald 1994). This is consistent with other studies measuring OC pesticide concentrations in liver samples obtained from river otter and mink inhabiting the lower Fraser River basin in British Columbia (Elliott et al., 1999; Harding et al., 1999). In contrast, concentrations of PCBs pose a greater threat to river otter populations throughout the region.

4.3. *PCB exposure and significance*

Conclusions about the negative impacts of PCBs on Eurasian otter populations have been based on the assumption that otters are as sensitive to PCB exposure as mink, and that 50 mg/kg lw in fat tissue was a critical level above which mink reproduction could be severely affected (Mason and Macdonald, 1994). To estimate tissue concentrations in otters, Mason et al. (1992) applied a single compartment model, originally developed by de Vries (1989, cited in Mason et al., 1992). Target values to assess the significance of PCB levels in otter scat were determined based on a back-calculation of the model, assuming that the contaminants measured in scats are derived entirely from unassimilated chemicals in the diet. Model parameters were derived from experiments with mink and adapted for the otter when possible. Target values were further assessed by Smit et al. (1994), and they recommended use of a no effect level (NOEL) of 4 mg/kg lw ∑PCBs, a range of concern from 9–16 mg/kg lw, and a critical value of 16 mg/kg lw ∑PCBs in scat. This model has been used in a variety of studies in Europe, and estimates of low otter population density were correlated with areas where PCB levels in otter scats exceeded the model's level of concern (Mason and Macdonald, 1993a,b, 1994). Despite potential differences in the physiology and behaviour of *Lutra lutra* and *Lontra canadensis*, those criteria provide a basis to assess the toxicological significance of PCB levels in North American river otter scat. We have opted to use the critical value of 9 mg/kg lw for potential PCB effects on otter reproduction as suggested by de Vries (1989, cited in Smit et al., 1994), based on the geometric mean tissue concentration of 30 mg/kg lw in a Swedish otter population (with an arithmetic mean of 50 mg/kg lw). A study of Swedish otter populations in relation to chlorinated hydrocarbon exposure appears to validate the use of that lower threshold value (Roos et al., 2001). It should be recognized, however, that the value of 30 mg/kg lw ∑PCBs may not be particularly conservative, given that tissue concentrations of approximately 12 mg/kg lw in adult female mink were associated with reproductive effects in a more recent chronic dosing study (Brunstrom et al., 2001).

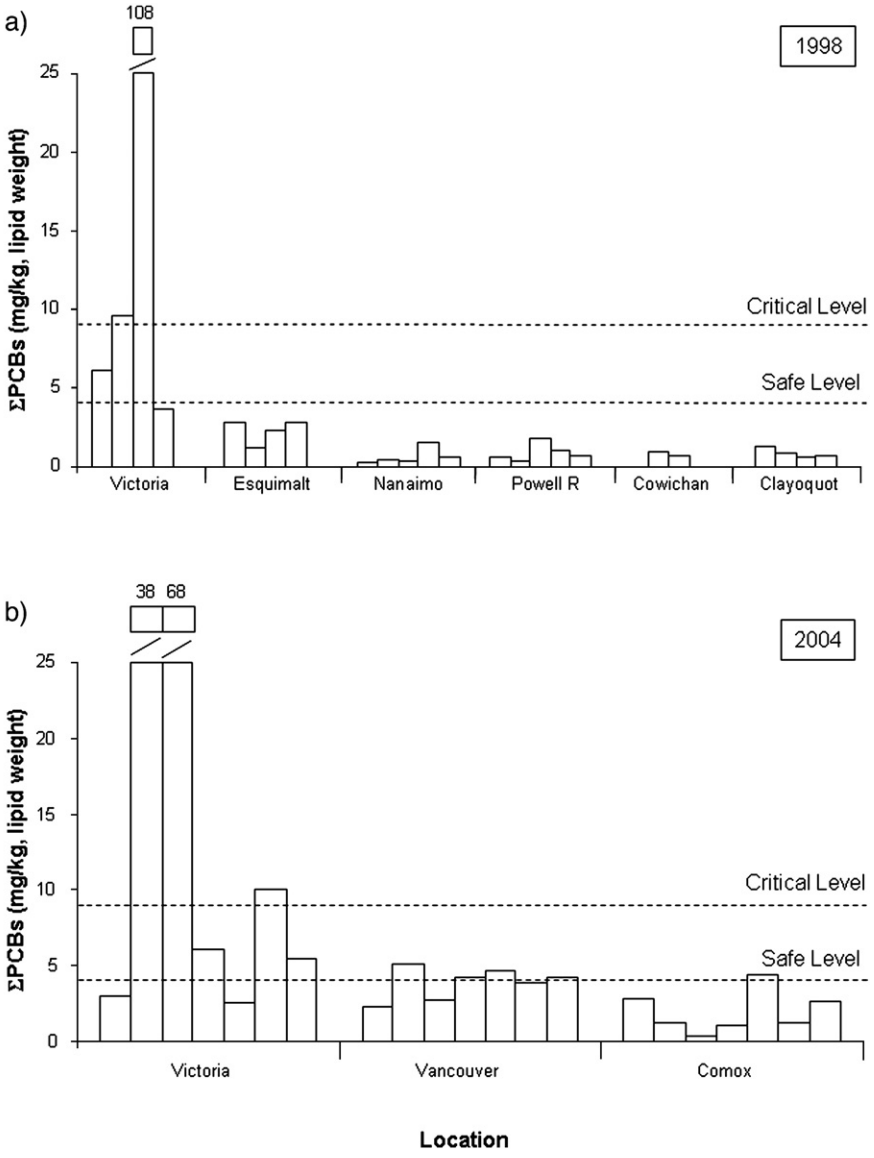

Fig. 7 – Concentrations of ∑PCBs in separate scat samples collected from individual river otter latrines sites on the south coast of British Columbia, Canada in a) 1998 and b) 2004 compared to published criteria for reproductive effects in otter species.

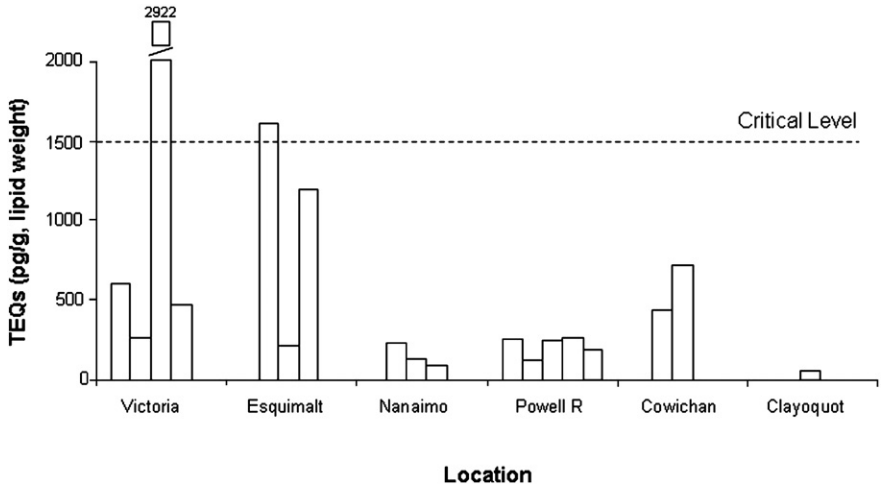

Fig. 8 – Concentrations of TEQs in scats collected from individual river otter latrine sites on the south coast of British Columbia, Canada (1998), compared to criteria for reproductive effects in otter species.

A study of Swedish otter populations provided some support for that lower threshold value (Roos et al., 2001).

∑PCB concentrations in scats from most sampled locations, including Vancouver Harbour, were relatively low and below the putative safe level. Comparisons with published data should be made with caution, however, given variation in factors such as sampling and pooling, extraction, instrumentation, and statistical analysis. Nevertheless, we have summarized the literature values for comparative purposes (Table 3).

Scat samples collected from coastal southwestern British Columbia, with the exception of Victoria Harbour, had ∑PCB concentrations similar to concentrations measured in Eurasian otter scats collected from areas of low human density in Wales and southwest Spain (Delibes et al., 1991), and from otter population strongholds in Slovenia, Austria, Czech Republic (Gutleb, 1994; Gutleb and Kranz, 1998), Denmark (Mason and Madsen, 1993; Elmeros and Leonards, 1994), and coastal southern Ireland (O'Sullivan et al., 1993).

While the majority of scat samples in both surveys had low concentrations of ∑PCBs, geometric mean concentrations of ∑PCBs measured in samples from Victoria in 1998 (12.3 mg/kg lw) and 2004 (9.48 mg/kg lw) exceeded the level of concern (9–16 mg/kg lw) and our suggested critical level (9 mg/kg lw) for the health or reproductive ability of otters, based on ∑PCBs alone (Fig. 3). In fact, out of all sites sampled, only samples collected from latrines in Victoria Harbour in both 1998 ($n=2$ latrines) and 2004 ($n=3$ latrines) exceeded this level (Fig. 7). Those values are slightly greater than the maximum allowable concentration if combined with the total OCs as suggested by Mason and Macdonald (1993b). The elevated concentrations measured in scats from Victoria Harbour are comparable to those from aquatic systems throughout Europe that were considered too contaminated to support and sustain viable populations of Eurasian otters (Mason et al., 1992; Reuther and Mason, 1992; Mason, 1993a,b).

In contrast, concentrations of PCBs in livers from otters from the sparsely inhabited Shetland Isles of Scotland were comparable to those from more contaminated sites in coastal British Columbia, and yet the Shetland populations were described as thriving (Kruuk and Conroy, 1996) with high fecundity and survival attributed to overall quality of habitat, food availability, and the relative lack of other anthropogenic stressors in the system. Otters inhabiting large urban harbours such as Victoria, however, are subject to many stressful and resource-limiting conditions associated with degraded habitat quality, including a modified prey base, human and pet disturbance, auto, boat, and seaplane traffic, pollution by domestic sewage, parasites, and other chemical contaminants such as PAHs and heavy metals (City of Victoria, 2001). Such factors may combine to create biological sinks for river otter populations. Further examination of the health and population status of river otter populations of the British Columbia coast is required to determine the cumulative effects of co-exposure to contaminants and other stressors. In particular, further study of river otters inhabiting the waters of Victoria Harbour is necessary to understand the extent to which those animals are impacted by PCBs and other contaminants. An investigation is underway using fecal DNA to determine the number of otters potentially impacted by elevated contaminant levels, and the relevant aspects of their population dynamics (Guertin, unpublished data). The goal is to determine whether the population is entirely or partially resident or transient, the degree to which animals are related, and whether there is evidence of successful local reproduction, critical to determining whether the harbour is a sink for otters moving in from neighboring less polluted environments.

4.4. *PCDD, PCDF and TEQ exposure*

In addition to PCB and OC pesticide contamination, river otters were exposed to substantial concentrations of chlorinated dioxins and furans at some locations in the Georgia Basin, at least in 1998. The pattern of PCDDs and PCDFs observed in this study, particularly the domination of OCDD, is similar to that reported in river otter liver tissue obtained from trappers along the Columbia River downstream of Portland, OR, USA (Elliott et al., 1999). Chlorophenolic wood preservatives were considered the source of the PCDD/F contaminants in the lower Columbia River system. In addition, the presence of 12468-PnCDF, 124678-HxCDF, and 124689-HxCDF (indicative of chlorophenolic wood preservatives), and relatively lower amounts of 234678-HxCDF (indicative of combustion, Hagenmaier and Brunner, 1987; Wakimoto et al., 1988) in otter scats from Victoria and Esquimalt Harbours is consistent with chlorophenol sources, as determined previously for ospreys (*Pandion haliaetus*) from the Columbia River (Elliott et al., 1998) and beluga whales (*Delphinapterus leucas*) from the St. Lawrence River estuary (Muir et al., 1996). The presence of elevated concentrations of the two Aroclor tracers (*ibid.*) 12478- and 23478-PnCDF in scats from Victoria Harbour is indicative of Aroclor pollution in the harbour. This is also clearly illustrated by the high PCB concentrations measured.

In contrast to the data from the early 1990s in resident marine-foraging bird populations (Elliott et al., 1996b, 2001; Harris et al., 2003), there was minimal evidence of elevated TCDD or TCDF in scat samples collected in the vicinity of the major bleached-kraft pulp mills at Crofton, Nanaimo, or Powell River. A latrine site at Powell River had the highest individual TCDF contamination, demonstrating some residual contamination. TCDF was also found at relatively high concentrations in scats from both Esquimalt and Cowichan. The source is likely associated with the substantial chlorophenolic contamination indicated by the other PCDD and PCDF contaminants present at high concentrations in otter scat. That TCDF was present in scat samples contrasts with the previous analyses, where no TCDF was detected in the livers of otters collected in the same general area (Elliott et al., 1999). TCDF is readily metabolized (Van den Berg et al., 1994), likely accounting for its absence from liver samples, while its presence in scat is probably due to unassimilated prey material, particularly crab or fish. TCDD, at 11 pg/g wet weight was reported previously in one otter liver collected close to the pulp mill at Castlegar, British Columbia.

In 1998, TEQ concentrations at some latrine sites in Victoria and Esquimalt Harbours exceeded our calculated threshold of 1500 ng/g lw, providing further support for potential toxicological impacts on otters using the harbours. Non- and mono-*ortho*-PCBs contributed substantially to TEQs at both sites,

and data from 2004 show ongoing PCB contamination, and therefore TEQ contamination in Victoria Harbour.

5. Summary

Scat samples collected around the Strait of Georgia region of southwest British Columbia show that coastal river otters were contaminated with a variety of chlorinated hydrocarbons, particularly PCBs, PCDDs and PCDFs, and that exposure was significantly higher than samples from Clayoquot Sound on the west coast of Vancouver Island. Mean concentrations of ∑PCBs in scat samples from Victoria Harbour and TEQ concentrations in samples from individual latrines in Victoria and Esquimalt Harbours exceeded criteria for reproductive effects developed for the Eurasian otter. Further work is needed to determine if exposure to those toxic and endocrine-disrupting substances is impacting the health of river otter populations in Victoria Harbour or elsewhere in the Georgia Basin–Puget Sound region.

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