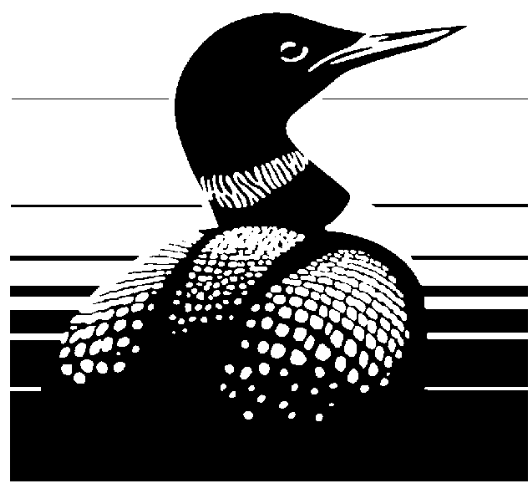
ASSESSMENT OF CHLORINATED HYDROCARBON CONTAMINANTS IN MAMMALS FROM AGRICULTURAL AREAS OF DELTA, BRITISH COLUMBIA

Christy A. Morrissey, R.W. Elner and J.E. Elliot

Pacific and Yukon Region 2004
Canadian Wildlife Service
Environmental Conservation Branch



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Abstract

Pesticide residues from agricultural operations and particulate emissions from wood fired boilers of greenhouses are of growing concern for their potential impacts to wildlife, livestock and humans. In 1998, a pilot study was conducted in Delta, British Columbia, to assess levels of organic contaminants in Townsend's voles (*Microtus townsendii*) and a domestic horse. In particular, concentrations of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and several organochlorine pesticides (OCs) were determined in two pools of vole livers and in one horse plasma sample. Samples were collected from a farm adjacent to a large greenhouse heated by wood fuel and natural gas boilers in an agriculturally dominated area, and at a protected national wildlife area of Delta. Reference voles showed higher levels of TCDD toxic equivalents (TEQs) and total PCBs compared to the greenhouse site. However, samples from both locations had consistently low levels of organic contaminants that were generally below reported toxicity thresholds. While organochlorine residues were frequently below detection for most compounds, concentrations of *p,p'*- dichlorodiphenlydichloroethylene (DDE), heptachlor epoxide, dieldrin and hexachlorobenzene (HCB) were detectable in both sample pools and the equine plasma. Endosulphan sulphate was also detected in voles from the exposed site. We discuss the results and implications of this study in light of limitations of the methodology.
Keywords: dioxin, PCB, pesticides, greenhouse, *Microtus townsendii*, wildlife

Résumé

On s’inquiète de plus en plus des impacts que les résidus de pesticides des activités agricoles et les émissions de particules des chaudières à bois utilisées dans les serres pourraient avoir sur les espèces sauvages, le bétail et l’homme. En 1998, une étude pilote a été menée à Delta, en Colombie-Britannique, pour évaluer les niveaux de contaminants organiques dans des campagnols de Townsend (*Microtus townsendii*) et un cheval domestique. En particulier, on a déterminé les concentrations de dibenzodioxines polychlorées (PCDD), de dibenzofuranes polychlorés (PCDF), de biphényles polychlorés (PCB) et de plusieurs pesticides organochlorés (OC) dans deux ensembles d’échantillons de foies de campagnols et dans un échantillon de plasma de cheval. Les échantillons ont été recueillis dans une ferme adjacente à une grande serre chauffée par des chaudières au bois et au gaz naturel, dans une région à prédominance agricole, et dans une réserve nationale de la faune de Delta. Les campagnols de l’échantillon de référence montraient des niveaux plus élevés d’équivalents toxiques (ET) de TCDD et de PCB totaux que ceux du site de la serre. Cependant, les échantillons des deux endroits présentaient des niveaux régulièrement bas de contaminants organiques, qui étaient généralement inférieurs aux seuils de toxicité signalés. Bien que les résidus d’organochlorés aient souvent été inférieurs au seuil de détection pour la plupart des composés, les concentrations de *p,p’*-dichlorodiphényldichloroéthylène (DDE), d’heptachlor époxyde, de dieldrine et d’hexachlorobenzène (HCB) étaient détectables tant dans les ensembles d’échantillons que dans le plasma équin. On a aussi détecté du sulfate d’endosulfane dans les campagnols du site exposé. Nous discutons les résultats et implications de cette étude à la lumière des limites de la méthodologie.

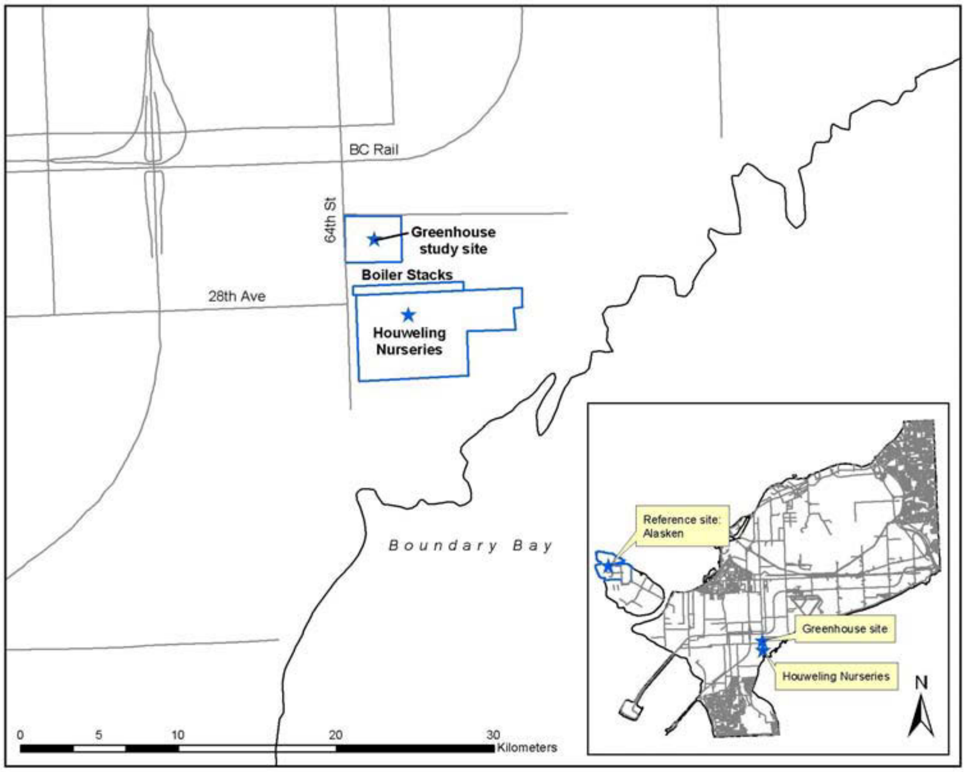
Mots-clés : dioxine, PCB, pesticides, serre, *Microtus townsendii*, espèces sauvages

Introduction

Expanding greenhouse operations in Delta, British Columbia are reducing the area for open-soil agriculture and concomitantly depleting habitat suitable to wildlife. A primary concern for wildlife managers has been the amount of open-soil farmland lost to glass coverage and subsequent wildlife impacts. Less attention has been paid to potential impacts from wood fuel emissions from greenhouse operations, which in combination with current agricultural pesticide applications, can further contribute to persistent chlorinated hydrocarbons bioaccumulating in non-target species (Elliott *et al*. 1989, 1998).

Houweling Nurseries is the oldest and largest greenhouse operation in Delta, B.C. covering over 40 acres of agricultural land abutting an internationally significant migratory stopover site at Boundary Bay (Figure 1). During the study period (1998), the operation generated heat through a combination of two wood fired boilers and eight natural gas boilers. Logs that are transported across the Strait of Georgia or boomed in the Fraser River estuary, take up chlorine from the water and the wood waste from these logs are used as fuel for boilers. Burning salt-laden wood is known to release polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) as a byproduct of combustion (Van Oostdam and Ward 1995). In British Columbia, the burning of salt-laden wood results in an annual release of 8.6 g TEQ/ year or 4.3 % of the national total of dioxin and furan atmospheric emissions documented (CCME 2001). In general, dioxin and furan byproducts are extremely persistent in the environment with half-lives of months to years and have been shown to be harmful to mammals (Fanelli *et al.* 1980, Norris 1981). Some congeners of dioxins and furans, in particular 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8- tetrachlorodibenzofuran (TCDF), are known to be extremely toxic to other wildlife, especially fish and birds (Merhle *et al*. 1988, Elliott *et al*. 1989, Gilbertson *et al.* 1991). Dioxins and furans can then bioaccumulate in wildlife food chains in habitats where combustion of chlorinated organics is occurring (Phaneuf *et al.* 1995, Champoux 1996, Alcock and Jones 1996, Meharg *et al*. 1997).

Figure 1: Map of study sites in Delta, British Columbia showing the locations of Townsend's vole collections: a) the greenhouse site adjacent to Houweling Nurseries boiler stacks and b) the reference site at the Alaksen National Wildlife Area on Westham Island.



Investigations of emissions from the Houweling greenhouse indicated that the particulate matter deposition around the greenhouse generally remained below the Greater Vancouver Regional District (GVRD) air quality limits of 1.7-2.9 mg/dec^2/day during a seven week study (Bovar 1996). The boiler stacks exceeded dustfall limits only during one sampling period when winds were from the northwest, a situation which Bovar attributed to sources other than Houweling Nurseries. Dispersion models also predicted concentrations of nitrous oxide (NO_x) to be below GVRD guidelines under current operating conditions (Bovar 1996). No measurements were made of organic contaminants produced by the boiler stacks.

Boundary Bay is one of the most important migratory bird stopover sites along the Pacific Flyway and is a common wintering ground for many species of waterfowl, shorebirds and raptors in coastal British Columbia (Butler and Cannings 1989). Organic contaminants from agricultural and industrial sources are of concern because of the potential adverse effects on indigenous wildlife populations, livestock and humans (Hoffman *et al.* 1986, Nosek *et al.* 1993, Elliott and Martin 1998). Prior to this study, no local sampling of terrestrial mammals had been conducted to assess exposure and associated risks from organic contaminants.

Townsend's voles (*Microtus townsendii*) were chosen as an indicator species to assess wildlife exposure to contaminants from agricultural practices and greenhouse emissions. Previous researchers have successfully used small mammals to quantify effects from exposure to various persistent environmental contaminants (Sullivan and Sullivan 1981, Lindzey and Grant 1994). Small mammals are considered good biomonitors because they are abundant, easily caught, have small home ranges, a widespread distribution and generalized food habits (Talmage and Walton 1991). Additionally, they are an important food source for raptors and other mammalian predators. Property owners adjacent to the greenhouse had also expressed concern over the health of their domestic livestock. For this reason, we collected a blood sample from a domestic horse to assess potential risks to livestock. Therefore, we designed this study as a pilot assessment of chlorinated hydrocarbon contaminants in mammals inhabiting an area impacted by agricultural and greenhouse emissions.

Materials and methods

Two study sites were chosen for collection of small mammals: a large hay field adjacent to the Houweling greenhouse site on 64th Street, Delta, and an uncultivated reference field remote from industrial activity on the Alaksen National Wildlife Area in Delta (Figure 1). Small mammal trapping was conducted from June to August, 1998, and involved collecting Townsend's voles *(Microtus towsendii*), the most abundant species of small mammal in the area, for the purpose of tissue residue analysis. Fifteen voles were trapped using baited Longworth live mammal traps and euthanized using CO_2 asphyxiation. An additional 6 voles from the greenhouse site were collected immediately after being killed by a hay cutter on July 10, 1998. All voles were weighed with an electronic balance, measured (nose to tail), and assessed for general body condition and parasites. Each individual was dissected to remove its liver within 1 hour after death.

While we were primarily interested in assessing contaminants from small mammals, property owners adjacent to the greenhouse had expressed concern over the health of their domestic livestock. Therefore, a licensed veterinarian collected 60 mL of blood from a 12-year old horse living adjacent to the greenhouse.

Liver and plasma residue analysis were performed by Axys Analytical Services Ltd. in Sidney, B.C. In total, each group of thirteen exposed livers and 8 reference livers were pooled and homogenized. Organochlorine pesticides were analyzed using high resolution gas chromatography with low resolution mass spectrometric detection (HRGC/LRMS) after sample extraction and clean-up. A few polar chlorinated pesticides were analyzed by gas chromatography and electron capture detection (GC/ECD). Organochlorine analyses included DDT metabolites (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'* -DDD and *o,p'*-DDD), chlorobenzenes (tetra-, penta- and hexa-chlorobenzene), chlordane related compounds (oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, heptachlor and heptachlor epoxide), hexachlorocyclohexanes (α-, β-, γ- and δ-HCH), mirex, photomirex, aldrin, dieldrin and endrin. Polychlorinated biphenyl (PCB) congeners were analyzed by HRGC/LRMS and total PCBs were calculated by summing the concentrations of 87 congener peaks. PCDDs (tetra-, penta-, hexa-, hepta-, and octa-chlorodioxin) and PCDFs (tetra-, penta-, hexa-, hepta-, and octa-chlorofuran) were analyzed by high resolution gas chromatography with high resolution mass spectrometric detection (HRGC/HRMS). 2,3,7,8-TCDD toxic equivalents (TEQs) were calculated using toxic equivalency factors (TEF) established by the World Health Organization (Van den Berg *et al*. 1998) which estimate the combined toxicity of all dioxin and furan congeners. Sample detection limits varied by tissue type based on available wet masses (horse plasma 50.5 g, reference vole livers 2.52 g, and exposed vole livers 5.02 g). All analyses were done with adherence to quality control protocols including the use of procedural blanks and spiked matrices with each batch. All samples were also spiked with ^{13}C surrogate standards prior to analysis to assess recovery. Surrogate recoveries were in the range

of 79 % – 140 % for OCs, 59 % - 80 % for individual PCBs, and 24 % - 77 % for dioxins and furans. The results presented are not blank or recovery corrected.
Comparisons of contaminant residues between the exposed and reference sites are simply qualitative. Statistical tests were not possible because liver samples were pooled by site.

Results

In total, 13 voles were collected from the agricultural field near the greenhouse site and a further 8 voles were collected from a reference field at Alaksen's National Wildlife Area. Details of the Townsend's voles trapped at each of the two sites in Delta included basic measurements and body condition (Table 1). Voles at the greenhouse site (mean mass 46.9 g ± 13.1; length 16.6 ± 2.1) were significantly larger than those collected from the reference site (mean mass 30.3 g ± 5.5; length 15.0 ± 0.8) ($t_{18} = -3.37$, $p = 0.003$). Half of the animals (n = 4) collected from the reference site had been parasitized by botfly larvae (*Cuterebra spp.)* and one vole (CWS-07) was noticeably emaciated. All the voles from the greenhouse site appeared normal. We have no information on the age and sex of individual Townsend's voles.

Results from the liver residue analyses showed that although dioxin and furan concentrations were extremely low; the reference voles had higher total toxic equivalents (1.24 pg TEQ/g) than those collected near the greenhouse (0.31 pg TEQ/g) (Table 2). The more heavily chlorinated octachlorodioxin congener (O8CDD) (56.9 % of total) was most prevalent in the mixture of dioxins and furans at both the exposed and reference sites, but these have the lowest toxic equivalency factors (0.0001). The highest concentration of furans in the greenhouse samples was from the tetrachlorofuran congener (T4CDF) (83.3 % of total) and for the reference samples, it was the heptachlorofuran isomer (H7CDF) (33.3 % of total). The horse plasma had the lowest total TEQs (0.16 pg TEQ/g) containing a mixture of all the dioxin and furan isomers, with the H7CDD and H7CDF isomers predominating (45.5 % and 70.0 % of the totals respectively).

Table 1: Summary of Townsend's vole capture data for the two sampling locations in Delta: Alaksen National Wildlife Area (reference site) and a hay field adjacent to Houweling greenhouse.

Site Location	ID Number	Collection date	Weight (g)	Length (cm)	Cause of death	Notes
Alaksen	CWS-01	10-Jun-98	28.82	14.6	euthanasia	
Alaksen	CWS-02	17-Jul-98	21.62	14.0	euthanasia	
Alaksen	CWS-03	18-Jul-98	32.00	15.5	euthanasia	botfly larvae
Alaksen	CWS-04	18-Jul-98	36.94	16.4	euthanasia	botfly larvae
Alaksen	CWS-05	21-Jul-98	33.66	15.4	euthanasia	
Alaksen	CWS-06	24-Jul-98	32.90	14.9	euthanasia	botfly larvae
Alaksen	CWS-07	27-Jul-98	22.77	14.0	euthanasia	emaciated
Alaksen	CWS-08	4-Aug-98	33.60	15.5	euthanasia	botfly larvae
Greenhouse	GH-01	26-Jun-98	63.12	19.8	euthanasia	
Greenhouse	GH-02	26-Jun-98	57.21	17.8	euthanasia	
Greenhouse	GH-03	30-Jun-98	47.40	16.5	euthanasia	
Greenhouse	GH-04	30-Jun-98	50.87	17.2	euthanasia	
Greenhouse	GH-05	10-Jul-98	unknown	unknown	lacerations	
Greenhouse	GH-06	10-Jul-98	25.32	13.0	lacerations	
Greenhouse	GH-07	10-Jul-98	51.69	16.8	lacerations	
Greenhouse	GH-08	10-Jul-98	53.81	16.2	head injury	
Greenhouse	GH-09	10-Jul-98	42.84	16.1	internal injuries	
Greenhouse	GH-10	10-Jul-98	48.88	17.4	lacerations	
Greenhouse	GH-11	12-Jul-98	33.29	15.1	euthanasia	
Greenhouse	GH-12	13-Jul-98	24.85	13.6	euthanasia	
Greenhouse	GH-13	13-Jul-98	63.58	19.9	euthanasia	

Table 2: Total polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs) and total toxic equivalents (TEQs) in pooled samples of Townsend vole livers and horse plasma collected from a reference and greenhouse site in Delta, B.C., 1998.

Dioxin/ Furan (totals)	**Concentration (pg/g ww)**		
	Reference Vole Livers	**Greenhouse Vole Livers**	**Horse Plasma**
T4CDD	0.8	0.2	0.02
P5CDD	ND[a]	0.1	0.04
H6CDD	1.1	ND	0.5
H7CDD	2.5	0.5	0.8
O8CDD	5.8	1.4	0.4
T4CDF	0.8	0.5	0.02
P5CDF	0.6	ND	0.1
H6CDF	1.6	ND	0.4
H7CDF	2.5	0.1	1.4
O8CDF	2	ND	0.08
Total TEQ (pg/g)[b]	**1.24**	**0.31**	**0.16**

[a]ND = not detected
[b]Total TEQs were calculated by multiplying the concentration of each isomer detected in the sample by its toxic equivalency factor (TEF) from Van den Berg *et al.* (1998) and summing all toxic equivalents.

Forty-one of 87 PCB congeners identified represented 96.9% and 91.3% of the total PCB residues found in each of the reference and greenhouse liver samples respectively, with congeners CB-153, 138, 118 and 180 predominating at both locations (Table 3). Although PCB concentrations were generally low, the reference voles had higher total PCBs (648.2 pg/g) than the greenhouse voles (352.6 pg/g). Total PCBs in the horse plasma collected at the greenhouse site was 74.0 pg/g.

The majority of organochlorine pesticide residues were below detection limits for both pools of vole livers as well as the horse plasma. Low concentrations of *p,p'*-DDE, heptachlor epoxide, dieldrin, and hexachlorobenzene, were detected in both liver pools and the horse plasma (Table 4). Detectable concentrations of endosulphan sulphate (7000 pg/g) and α-HCH (30 pg/g) were also found in the greenhouse vole livers. In addition, the horse plasma had 25 000 pg/g of dieldrin, which was more than 1000 fold higher than any other OC pesticides detected in that sample.

Table 3: Summary of PCB congeners detected and total PCBs in pooled samples of Townsend's vole livers from a reference and greenhouse site and horse plasma collected from the greenhouse site in Delta, B.C. 1998. Congeners analyzed but not detected in any of the 3 samples are not shown.

PCB Congener	Concentration (pg/g ww)		
	Reference Vole Livers	Greenhouse Vole Livers	Horse Plasma
19	NDR[a]	2.3	0.24
18	17.0	9.1	1
17	NDR	3.3	0.34
24,27	ND[b]	NDR	0.13
16,32	6.6	NDR	0.49
26	4.7	NDR	0.14
31,28	20.0	12.0	1.5
33	5.3	4.0	0.48
22	33.0	1.7	0.18
52	40.0	20.0	2.1
49	11.0	6.6	0.68
47,48	NDR	4.0	0.58
44	11.0	NDR	0.77
42	ND	ND	0.2
41,71,64	8.7	5.7	0.75
74	24.0	15.0	2.9
70,76	32.0	10.0	1.7
66	20.0	11.0	2.4
56,60	10.0	3.5	1.2
95	13.0	8.6	0.86
91	ND	ND	0.15
84,92	6.0	3.6	0.44
89,90,101	21.0	10.0	1.2
99	24.0	15.0	3.3
97	6.3	NDR	0.37
87	8.7	5.7	0.77
85	9.9	5.0	1.2
110	23.0	11.0	1.6
107	ND	1.7	ND
118	45.0	26.0	6.5
114	ND	NDR	0.35
105	16.0	9.8	2.5
136	2.3	0.9	0.22
151	NDR	1.1	0.47
144,135	2.8	1.6	0.24
149	16.0	5.4	1.9
146	3.6	3.2	0.18

PCB Congener	Concentration (pg/g ww)		
	Reference Vole Livers	Greenhouse Vole Livers	Horse Plasma
153	51.0	35.0	9.3
141	2.9	2.2	0.21
137	NDR	2.0	0.51
138,163,164	42.0	30.0	8.3
158	6.1	3.2	1.2
128	1.5	3.4	1.1
156	2.8	3.5	0.83
157	ND	NDR	0.15
179	1.5	0.8	0.24
178	NDR	NDR	0.1
187,182	NDR	7.4	0.59
183	6.2	4.0	0.79
174	2.4	2.7	0.51
177	5.0	3.1	0.25
171	2.0	NDR	0.24
172	1.5	ND	ND
180	25.0	15.0	4.1
193	NDR	0.9	0.16
191	ND	ND	0.08
170,190	8.8	5.4	2.1
189	ND	ND	0.08
201	12.0	ND	0.63
199	4.9	3.6	0.23
196,203	5.6	4.2	0.81
194	4.5	3.0	0.87
207	NDR	1.5	ND
206	ND	1.9	NDR
209	17.0	2.0	0.3
PCB Coplanar			
77	4.1	0.8	0.2
126	0.5	0.3	0.069
169	NDR	NDR	0.022
Total PCBs	**648.2**	**352.6**	**74.0**

NDR = value did not meet quantification criteria
ND= not detected

Table 4: Summary of organochlorine pesticides analyzed in pooled samples of Townsend's vole livers collected from a reference and greenhouse site and horse plasma collected from the greenhouse site in Delta, B.C., 1998.

Pesticide	**Concentration (pg/g ww)**		
	Reference Vole Livers	**Greenhouse Vole Livers**	**Horse Plasma**
Hexachlorobenzene	20	40	20
alpha HCH	ND[a]	30	ND
beta HCH	ND	ND	ND
gamma HCH	ND	ND	ND
delta HCH	ND	ND	20
Heptachlor	ND	ND	ND
Aldrin	ND	ND	ND
Oxychlordane	ND	NDR[b]	ND
trans-Chlordane	ND	ND	ND
cis-Chlordane	ND	ND	ND
o,p'-DDE	ND	ND	ND
p,p'-DDE	130	380	10
trans-Nonachlor	ND	NDR	ND
cis-Nonachlor	ND	ND	ND
o,p'-DDD	ND0	ND	ND
p,p'-DDD	ND	ND	NDR
o,p'-DDT	ND	ND	ND
o,p'-DDT	ND	ND	10
Mirex	ND	ND	ND
Heptachlor Epoxide	60	370	10
alpha-Endosulphan (I)	ND	ND	ND
Dieldrin	60	110	25000
Endrin	ND	ND	ND
beta-Endosulphan (II)	ND	ND	ND
Endosulphan Sulphate	ND	7000	ND

[a]ND= not detected
[b]NDR = value did not meet quantification criteria

Discussion

Small mammals have been shown to be effective for monitoring environmental contaminants. In general, there is a relationship between concentrations of organic contaminants in the soil or food and concentrations in target tissues of several small mammal species (Talmage and Walton 1991). Voles captured in the vicinity of the greenhouse were living in the fly ash zone and were assumed to be suitable biomonitors. Townsend's voles occupy small home ranges and are herbivorous (Johnson and Johnson 1982). Therefore, exposure would be through deposition of organic contaminants onto the surface of the plants within the animals' home range that are subsequently ingested. However, studies show a relationship exists between contaminant concentrations and trophic levels. Talmage and Walton (1991) conducted a review of small mammals as biomonitors and found that insectivores had the highest levels of contaminants and were the most useful species for determining exposure to organic and inorganic contaminants. Next were omnivores followed by herbivores, which had much lower body burdens or residues in target tissues. Since we only collected one species (Townsend's voles), which is an herbivore, our samples may only represent the lesser body burdens of a lower trophic level mammal.

Information on age and sex of the voles collected was not available and such factors may have also influenced our results. Since the animals were collected during summer, it is possible that some of our pooled samples may have been juveniles which could have further biased the residue levels to be lower. Juveniles have a narrow window of exposure for accumulating contaminants and can thus have lower body burdens than adults. We found that voles collected from the greenhouse site were larger on average than those at the reference site. Therefore, the reference pool likely contained a larger proportion of juveniles which may have produced lower overall contaminant residues for this site. Future sampling should be conducted during the late winter or very early spring to obtain a more assured sample of adults.

All samples showed consistently low levels of dioxins, furans and PCBs. However, in comparing our two sites, there was an enigma; the reference voles had higher total TCDD toxic equivalents (TEQs) and total PCBs than the exposed voles. Our reference area, the Alaksen Wildlife Reserve, is located on Westham Island and is remote from industrial and commercial pollution sources. Although the total residue levels are low, the reference voles had nearly 2x higher PCBs and 3x greater dioxin TEQs as those at the greenhouse site. For wildlife that prey on Townsend's voles, liver residues at both sites were below the published dietary guideline of 4.75 pg TEQ/g diet ww for avian predators but the reference site marginally exceeded the mammalian guideline of 0.71 pg TEQ/g diet, assuming liver residues are representative of whole body burdens (CCME 2002).

Dioxin levels found in the vole samples are below known toxicity levels for small mammals. Thalken and Young (1983) investigating rodent populations subjected to long-term exposure to TCDD concluded that soil concentrations in the range of 0.1-1.5 ng/g had no adverse effect on the small mammal species indigenous to their study area. Furthermore, no significant histopathological lesions were observed in field and laboratory contaminated beach mice with liver concentrations of TCDD in the range of 960-1300 pg/g (Cockerham and Young 1983). Therefore, the total TCDD levels (0.2 and 0.8 pg/g) observed in the voles we collected were not likely sufficient to cause toxicity, even combined with other congeners. However, typical bioconcentration factors for TCDD in the liver of herbivorous rodents relative to soil are approximately 6 to 1 in females and 18 to 1 in males (Rose *et al*. 1976). Therefore, rodents and particularly males, are capable of bioaccumulating dioxins in the liver during long term exposure.

Although few studies exist on PCBs in small mammals, population effects and reproductive impairment in the white footed mouse (*Peromyscus leucopus*) have been observed in contaminated PCB sites (Batty 1990 *et al*., Linzey and Grant 1994, Shore and Douben 1994). However, those contaminated sites had levels of PCBs several orders of magnitude higher than our sites near either the greenhouse or the wildlife reserve. PCB levels in our vole samples were most likely too low to cause similar toxic effects.

Horses are common in our study area and consideration for the levels of exposure in domestic livestock was included. In an example from the literature, TCDD caused accidental poisoning of horses from contaminated riding arenas (Carter *et al*. 1975). Improper disposal of contaminated waste oils has also caused acute PCB poisoning in cattle (Robens and Anthony 1980). The concern for livestock living near the greenhouse prompted us to include a blood sample from a domestic horse living adjacent to the greenhouse boiler stacks. The horse had spent its whole life on the same farm feeding on grass and hay that was hypothesized to have been subjected to fly ash and agricultural pesticide deposition. As with the vole livers, the equine plasma similarly showed low levels of dioxins, furans and PCBs. Since organic contaminants in plasma reflect more immediate exposure as compared to long term bioaccumulation in target organs such as the liver, we expected and determined these values to be lower than the vole liver samples.

Additional data were collected on organochlorine pesticides in both the vole liver and the equine plasma samples. While these have no direct relationship to greenhouse emissions, they are useful in determining levels of contamination from past and current agricultural practices. In general, the samples contained low levels of only a few organochlorine compounds, with the reference site being lower than the greenhouse site. We detected *p,p'*-DDE, hepatochlor epoxide, dieldrin and hexachlorobenzene in both liver sample pools and in the equine plasma. These organochlorine pesticides are no longer in use but are not readily biodegradable (Shore and Douben 1994). Dieldrin was highest in the horse plasma sample (25 000 pg/g), likely indicating exposure from historical agricultural applications in the area. Dieldrin, a cyclodiene insecticide, accumulates in the fatty tissues of mammals and can lead to liver and kidney damage (Scott *et al*. 1959), genetic resistance, and reduced juvenile survival (Allen and Otis 1998). Also, we found elevated levels of endosulphan sulphate in the greenhouse vole livers (7000 pg/g). Endosulphan sulphate is a breakdown product of endosulphan, an acutely toxic insecticide that is still currently in use in the Delta area. Overall, past reports of avian mortality, including predatory raptors, from exposure to organochlorine insecticides in B.C. were rare compared to poisonings from organophosphates and carbamate insecticides (Wilson *et al.* 1995).

In conclusion, the levels of dioxins, furans, PCBs and organochlorines detected in samples from the two sites in Delta, B.C. appear below levels of concern for toxicity to small mammals and their avian predators. Although the pooled samples from the greenhouse were lower than those at the reference site, the results are not conclusive because of potential biases in the target species' diet and life history, differences in mass, lack of data on age and sex ratios, and the time of year for collections. Therefore, these results should be considered as a preliminary assessment only. Further sampling is recommended to obtain more conclusive results on the degree of exposure and effects of the agricultural pesticides and greenhouse emissions to mammals in the area.

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Appendix 1: Summary and critique of protocol used for sampling Townsend's voles with recommendations for future sampling.

Method	Protocol Used	Critique	Ideal Protocol
Sampling species	Townsend's voles	Voles are herbivourous; insectivorous species are more suitable as biomonitors.	Insectivorous species (e.g. shrews)
Sex of animals	Not known	Animals were not sexed at time of collection.	Sex the animal during dissection
Age of animals	Not known	Juveniles abundant at time of collection and generally have lower contaminant body burdens than adults.	Trap earlier in the year (pre-breeding)
Collection time of year	June-August	Summer months are more difficult for trapping due to abundance of food.	January-March
Collection method 1	Longworth live-mammal traps	Satisfactory method.	Longworth live mammal traps
Collection method 2	Trauma from hay cutter	Unsatisfactory method due to exposure of body cavity from wounds prior to dissection.	Same as above

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