Bull. Environ. Contam. Toxicol. (2003) 71:543–550

DOI: 10.1007/s00128-003-8912-z

Bulletin of Environmental Contamination and Toxicology

Chlorobenzenes in Snow Crab (*Chionoectes opilio*): Time-Series Monitoring Following an Accidental Release

T. L. King,[1] K. Lee,[1] P. Yeats,[2] R. Alexander[3]

[1] Center for Offshore Oil and Gas Environmental Research, Bedford Institute of Oceanography, Fisheries and Oceans Canada, Post Office Box 1006, Dartmouth, Nova Scotia, Canada, B2Y 4A2
[2] Marine Environmental Sciences Division, Bedford Institute of Oceanography, Fisheries and Oceans Canada, Post Office Box 1006, Dartmouth, Nova Scotia, Canada, B2Y 4A2
[3] Marine Environmental Sciences Division, Gulf Fisheries Centre, Fisheries and Oceans Canada, Post Office Box 5030 Moncton, New Brunswick, Canada, E1C 9B6

Received: 20 February 2003/Accepted: 12 May 2003

The *Irving Whale* barge, carrying 4200 tons of Bunker "*C*" heavy fuel oil, sank on September 7, 1970, in 67 meters of water in the Gulf of St. Lawrence, Canada. The barge was equipped with a closed loop heating system to maintain its Bunker "*C*" fuel oil cargo above its pour point so that it could be easily unloaded at its final destination (Gilbert and Walsh 1996). When the barge was built, in 1966, it was not unusual to incorporate PCBs as a heat transfer agent (HTA) in heating systems. The dielectric fluids were often blends of PCBs and polychlorinated benzenes (PCBzs) added to reduce viscosity. The HTA of the *Irving Whale* was identified as *Monsanto* MCS 295S comprised of 80% (7500 kg) Aroclor 1242 and 20% (1900 kg) CBzs. In response to public concern of a catastrophic leak due to corrosion, a precautionary approach was taken and the barge was raised from the seabed in July of 1996 and towed to Halifax, Nova Scotia. Unfortunately, only 2100 kg of the 7500 kg PCBs within the barge at the time of its sinking was recovered (Gilbert et al. 1998).

PCBzs are heavier than seawater (specific gravity of 1.57-1.85), have high octanol/water partition coefficients (log Kow 4.0-5.5) and are more water-soluble than PCBs (Lord et al. 1980 and Oliver 1987). While these compounds are typically removed by volatilisation and degradation more rapidly than PCBs under aerobic conditions (e.g. surface waters), they tend to persist and accumulate under anaerobic conditions in buried sediments and soils, and can biomagnify in the food chain (Oliver and Nicol 1982). CBzs may have acute and chronic effects on aquatic organisms Gilbert et al. 1998. In 1993, hexachlorobenzene (hexaCBz) was classified as toxic under Section 11 of the Canadian Environmental Protection Act due to emerging knowledge of detrimental reproduction of predatory species in birds and fish-eating mammals, and carcinogenic effects in humans (Government of Canada 1993a). Despite their widespread industrial use, there are few estimates for the amount of CBzs released into the marine environment and little information on their long-term environmental impacts. As part of a program to assess environmental impacts, following recovery of the barge the concentration of PCBs in snow crabs and sediment has been monitored annually from 1996 to 2000 at the site. Until now, little attention has been given to the 1900 kg of CBzs in the HTA. There is concern that this release of CBzs may have created a potential risk to the marine environment including the multi-

Correspondence to: T. L. King

million dollar snow crab fishery. This study is focused on monitoring CBzs in snow crabs from 1996 to 2000.

MATERIALS AND METHODS

Snow crabs were recovered from 13 locations at various distances (0-30 nautical miles) from the site of the sunken *Irving Whale* barge (Figure 1). Sites 12 and 13, 20 and 30 nautical miles distant with similar bottom topography and environmental conditions were identified as controls. The first samples were collected in October 1996, 2 months after the barge was raised. Subsequent samples were collected annually in May to June 1997-2000. To represent the catch of the commercial fishery only male snow crabs were collected. Snow crab (carapace width 108.6 ± 5.9 mm and claw width 26.6 ± 2.5 mm) digestive glands were removed, pooled (5 animals/site) and stored frozen prior to analysis.

The pooled digestive glands were homogenized. The homogenate (5.0 g) was weighed in a mixing cylinder and 30 mL of 3.6 *M* aqueous ethanolic potassium hydroxide (VWR/Canlab, Ontario Canada) was added. A blank and a duplicate sample were prepared with each batch of 12 samples. The samples were digested in a heated (70°C) ultrasonic bath (Branson 5510R-MT, Danbury, Connecticut) for 1.5 hr. After digestion, 30 mL of distilled water was added and mixed. The digest was extracted 3 times with 10.0 mL portions of hexane. Extracts were concentrated and exchanged into 1:1 v/v (dichloromethane:cyclohexane). The extracts were purified by gel-permeation chromatography (ABC autoprep 1001, Columbia, Missouri) using biobeads SX-3 select (ATS Scientific, Columbia, Missouri). The purified extracts were analyzed for CBzs by gas chromatography-selected ion monitoring (SIM) mass spectrometry (5890 Series II GC-5971A MS, Hewlett Packard, Toronto, Ontario). A minimum of 4 ions, one target (hexaCBz ion 284) and 3 qualifiers (282, 286, and 249) were used for each class of CBzs. CBzs were purchased from Ultra Scientific, Kingston, Rhode Island. All solvents used were distilled in glass (OmniSolv, VWR/Canlab, Toronto, Ontario).

Recoveries of 1,2,4-trichlorobenzene-d_3 and ^{13}C-hexaCBz (4 $ng \cdot g^{-1}$ wet weight) added to crab tissue, prior to digestion, averaged 71 ± 10% and 97 ± 5% respectively. A fortification study was performed at 1.0 $ng \cdot g^{-1}$ wet weight. Mean percent recoveries and relative standard deviations of the CBzs added to snow crab tissue were as follows: 86 ± 16% trichlorobenzenes (triCBzs), 96 ± 6% tetrachlorobenzenes (tetraCBzs), 101 ± 7% pentachlorobenzene (pentaCBz) and 94 ± 11% hexaCBz.

RESULTS AND DISSCUSSION

Sediments collected in 1994/1995 near the snow crab study sites were low in ΣCBzs (<0.0005 to 0.010 $\mu g \cdot g^{-1}$ dry weight) except adjacent to the barge (forward section) where higher concentrations of ΣCBz (65.2 $\mu g \cdot g^{-1}$ dry weight.) were found (Gilbert and Walsh 1996). Analysis of the HTA (Table 1) revealed that it contained 18% CBzs, consistent with the 20% indicated by *Monsanto* for MCS

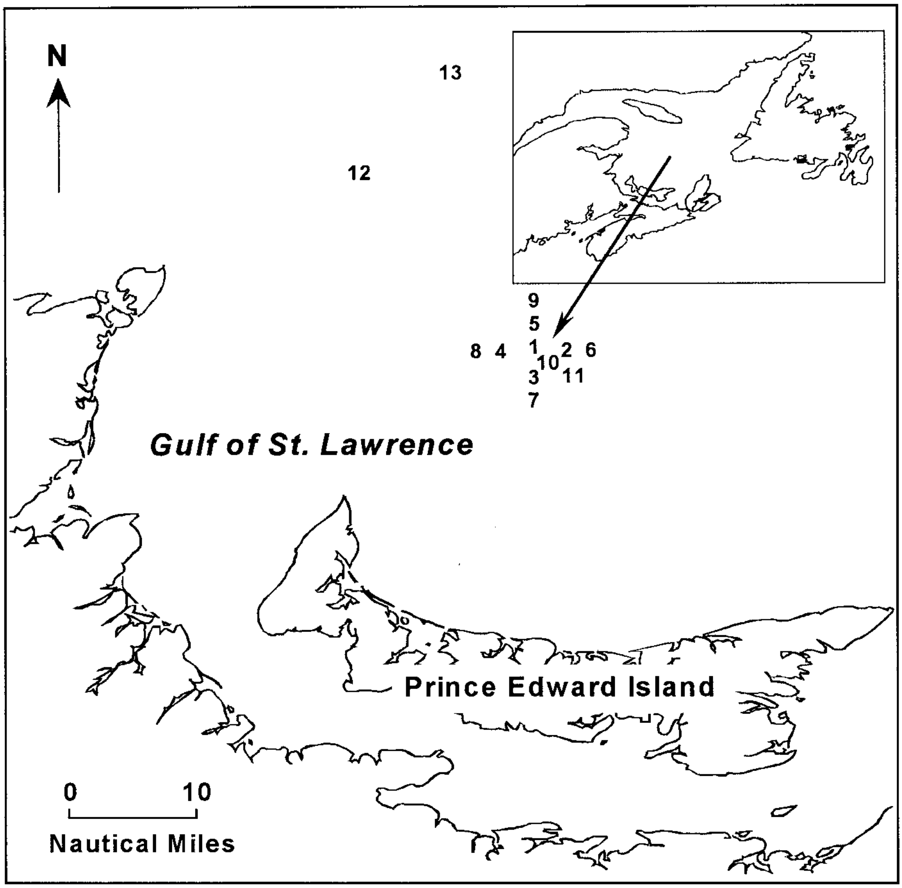

Figure 1. Snow crab sampling locations in the Gulf of St. Lawrence, Canada.

295S. The estimated 5400 kg of PCBs lost to the environment is 72 % of the 7500 kg that were present in the HTA. If we assume similar losses (72%) for CBzs (1900 kg in the HTA), then 1368 kg (1.4 tons) was released into the environment. Table 1 reports CBzs concentrations in the digestive glands of snow crabs collected annually from 1996 to 2000 from various locations within the 5 nautical mile^2 fishing exclusion zone area around the sunken barge (Figure 1). Sampling location 1 (barge site) contained the highest concentration of CBzs (1996-2000) in snow crab digestive glands. A plot of CBz concentrations in snow crabs at sampling location 1 (1996) against those in the HTA (Figure 2) showed a strong positive correlation (r^2 = 0.84, Pearson correlation r = 0.92, n = 8), indicating that the CBz in snow crab from location 1 were predominantly from the HTA.

The dominant congeners found in snow crab at site 1 (1996) and in sediments collected by the forward section of the barge (1994) were 1,2,4-triCBz and 1,2,3,4-tetraCBz. These same two were dominant in the HTA. Figure 3A compares multiple ion chromatograms for CBzs in snow crabs collected at location 1 in 1999 with an analytical standard (Figure 3B). The dominant peaks in the chromatogram arise from 1,2,4-TriCBz and hexaCBz. Not surprisingly, hexaCBz showed a strong presence in the chromatograms, as it is a persistent organochloride compound. In the HTA 1,2,4-TriCBz was dominant. Its signature in crabs collected in 2000 confirmed the persistence of this compound.

There was a rapid decline in tri- to pentaCBz concentrations in snow crabs from sampling location 1 between 1996 and 1998 (Figure 4). From 1998 to 2000 the CBz concentrations in snow crabs persisted at low levels. In 1996, CBz concentrations at locations 2 to 11 were much lower than at location 1, but showed no consistent decrease with time. By 1999 concentrations at all sites were similar. Snow crab from the control locations (12 and13) showed detectable but low levels of these CBzs in 1997 but none in 1996. Concentrations in 1996 decreased rapidly with distance away from the barge site. HexaCBz was different. It was the only one of the eight analytes that showed detectable concentrations at the control locations for all 5 years. The gradient for hexaCBz is much smaller than for the other compounds. The decreases in concentrations for hexaCBz with time, where they are evident at all, are much smaller. This suggests that there is an additional source of hexaCBz. At present, commercial use of hexaCBz in Canada is banned. HexCBz has been released and distributed throughout the Canadian environment through the application of certain pesticide formulations and long-range transport and deposition (Government of Canada 1993a, b). Other sources of hexaCBz include incineration, leachates from hazardous waste landfills, emissions from other industries, and effluents from municipal wastewater treatment plants.

CBzs are lipophilic and tend to bioconcentrate in food webs. Their bioaccumulation factors (BAFs) in water range from 100 to 10,000 depending on the chlorine content (Gilbert and Walsh 1996). For rainbow trout (*Oncorhynchus mykiss*), BAFs for 1,2,3- and 1,2,4-triCBzs are on the order of 1300 while those of 1,2,3,4- and 1,2,4,5-tetraCBzs are 5300 (Oliver and Niimi 1983). Our results were consistent with this trend. Higher concentrations in snow crabs relative to HTA were seen for tetra- and penta-CBzs than for tri-CBzs. CBzs are known to induce both acute and chronic effects on aquatic organisms. The LC_{50} of 1,2,3- triCBz and 1,2,4,5-tetraCBz for the amphipod, *Rhepoxiniusabronius*, are 9.3 and 29.5$\mu g \cdot g^{-1}$ respectively, for ten days of exposure (Gilbert and Walsh 1996). Sediments near the barge contained 65 $\mu g \cdot g^{-1}$ dry (ΣCBzs) and the highest level in snow crab (1996) was 1.12 $\mu g \cdot g^{-1}$ wet weight (ΣCBzs). As for chronic toxicity, health effects of CBzs include liver, thyroid, spleen, thymus, kidney, lymph node and lung pathological changes. These effects were induced at high concentrations and a no observed effect concentration (NOEC) of 300 $\mu g \cdot g^{-1}$ has been set by Environment Canada National Toxicity Division (Gilbert and Walsh 1996). HexaCBz, has been classified as a probable carcinogen in humans (Gómez-Catalán et al. 1995). It was a minor, but persistent, component of the HTA having a half-life of greater than six years in sediment (Mackay et al. 1992). It is an endocrine disrupter and has the potential to cause reproductive effects in

Table 1. Chlorobenzenes (ng·g^{-1} wet wt) in snow crabs and μg·g^{-1} in heat transfer agent.

		Sampling Location													
CBzs	**Year**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	HTA
1,3,5-	1996	0.9													130
triCBz	1997														
	1998														
	1999														
	2000														
1,2,4-	1996	330	11	7.2	9.1		4.6								91000
triCBz	1997	28	8.5	5.2	18	8.7	12	10	5.7	8.9	1.1	14		2.2	
	1998	6.7	3.3	3.4	5.2	7.8	5.4	10	6.8	2.5		8.6	3.3		
	1999	7.6	11		7.5	13	6.9	10	5.3	9.5	4.3	11	3.2		
	2000	11	8.0		11	6.5	10	10	8.3	8.9	5.9	5.7		5.4	
1,2,3-	1996	76	3.3	2.2	2.3		1.9								20000
triCBz	1997	23	2.9	1.9	6.4	3.5	4.0	3.9	2.0	2.9		5.3		1.4	
	1998	2.1				2.3	2.4	3.3				2.1			
	1999	2.5	3.9		2.3	4.1	2.3	3.6	1.9	3.2	1.2	3.5	1.1		
	2000	2.9	1.8		2.4	1.2	2.5	2.5	1.6	2.1	1.1				
1,2,3,5-	1996	0.9													700
tetraCBz	1997														
	1998														
	1999														
	2000														
1,2,4,5-	1996	42													4900
tetraCBz	1997	2.1													
	1998														
	1999														
	2000														
1,2,3,4-	1996	350	2.2	1.7											55000
tetraCBz	1997	160	1.9	0.9	4.3	1.4	2.5	1.2		1.3		2.1			
	1998	2.5													
	1999	4.5	2.4		2.3	2.4	2.6	2.8		1.0	1.0	3.3			
	2000	5.1					0.7								
pentaCBz	1996	150	2.6	2.1	1.7										11000
	1997	12	1.4		3.9	1.6	2.6	1.6		1.6		2.6		1.4	
	1998	3.6	2.5	2.3				2.2	2.2	2.3		2.6			
	1999	3.6	2.7		2.6	2.2	3.0	2.8		1.4	1.0	3.7			
	2000	3.1			1.4		2.1	1.1		1.0	1.4				
hexaCBz	1996	16	6.7	7.2	4.4		3.5							3.1	370
	1997	1.4	5.3	5.5	19	9.4	12	9.1	5.4	10	1.2	16		5.5	
	1998	9.8	9.8	9.7	6.0	6.5	5.4	8.4	6.8	10	3.6	9.8	13		
	1999	12	11	7.1	11	9.1	12	11	4.6	9.3	4.7	15	3.4		
	2000	12	5.0		8.3	4.2	9.6	7.1	5.3	6.6	6.8	2.9		1.5	

Blank space = not detected (<0.05 ng·g-1), grey = no sample taken.

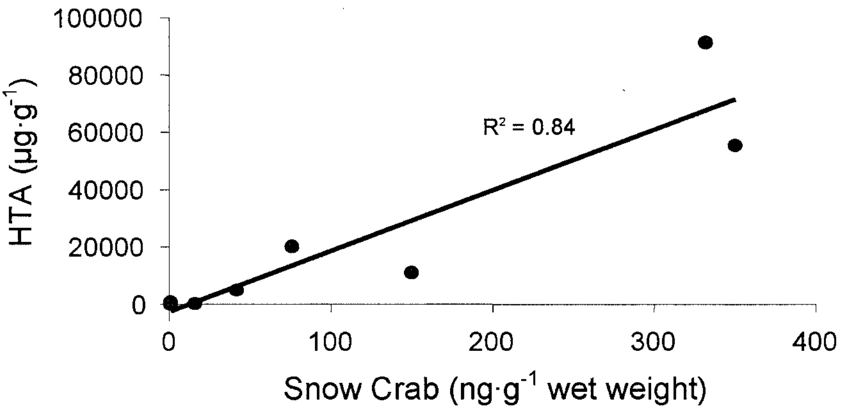

Figure 2. Correlation of chlorobenzenes in heat transfer agent vs. snow crab at sampling location 1 in 1996.

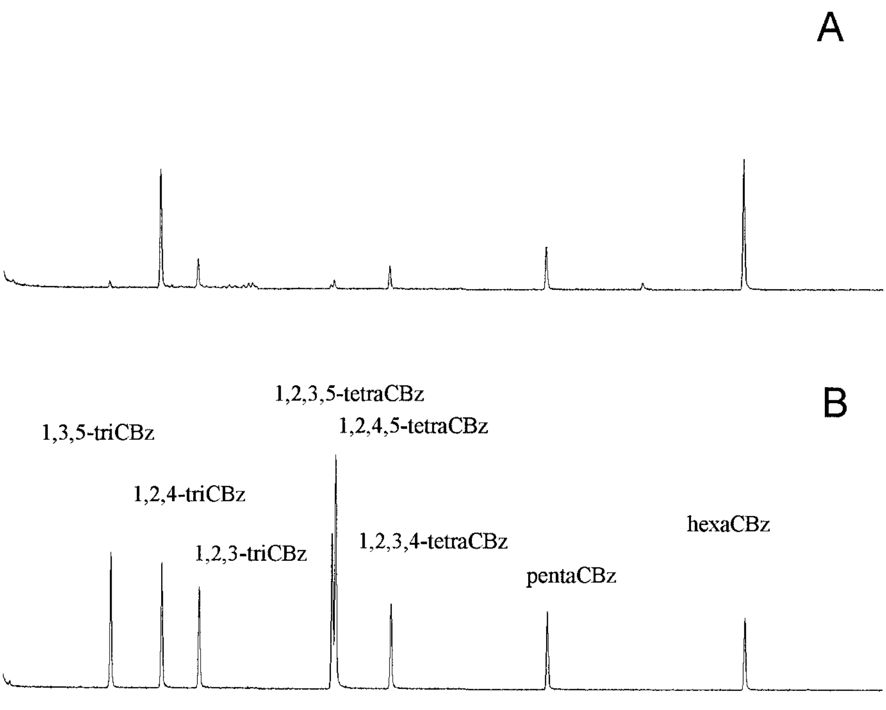

Figure 3. Multiple ion chromatograms of extract of snow crab digestive glands (A) and a CBz standard (B).

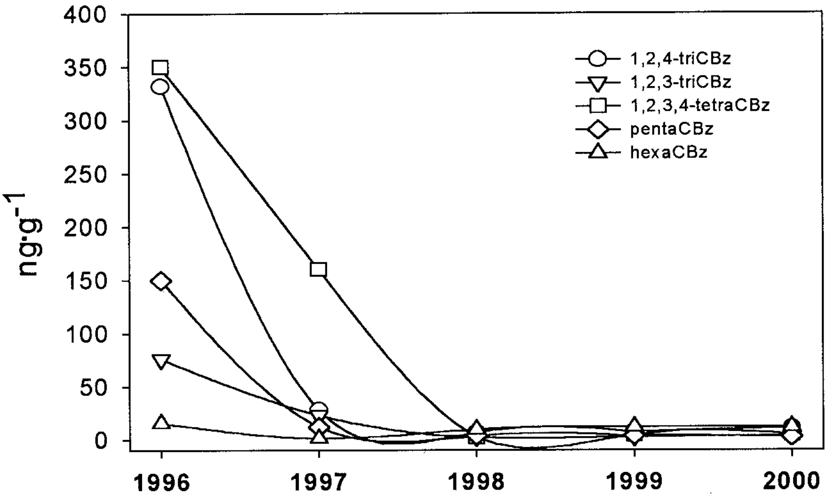

Figure 4. Chlorobenzenes in digestive glands of snow crab from sampling location 1 at the site of the *Irving Whale*.

predatory species of birds and fish-eating mammals. There is regulatory concern of potential risks to human health based on carcinogenic effects (Government of Canada 1993a). HexaCBz concentrations in snow crab ranged from 1.2 at location 10 (1997) to 19ng·g^{-1} wet weight at location 4 (1997). The concentrations were similar for all locations, but did not declined from 1996 to 2000. HexaCBz binds to the Ah receptor about 10,000 times less potency efficiency than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). If the relative potency for 2,3,7,8-TCDD was 1 (toxic equivalence factor) subsequently, for hexaCBz it would be 0.0001 (Van Birgelen 1998). If we apply this factor to the highest hexaCBz concentration of 19 ng·g^{-1} we get 1.9 ng·kg^{-1} wet weight expressed as 2,3,7,8-TCDD. This concentration does not exceed the Canadian tolerance of 20 ng·kg^{-1} wet weight for 2,3,7,8-TCDD (Health Canada 1993). However, hexaCBz is now targeted for elimination as part of the proposed Canada-United States Strategy for the virtual elimination of persistent toxic substances in the Great Lakes Basin (Government of Canada 1993a).

The results of this study have shown that the concentrations of CBzs, except hexaCBz, in snow crabs declined dramatically with distance from the barge site. Snow crabs accumulated high concentrations of CBzs in their digestive glands following exposure. The less chlorinated CBzs (3 to 4 chlorines) are more likely to degrade quickly under aerobic conditions, but the higher chlorinated CBz (5 to 6 chlorines) could bioaccumulate in biota and persist in buried sediments under anaerobic conditions. The highest CBz concentrations (1.12 μg·g^{-1} wet weight) in snow crabs were reported in 1996 just after the barge was raised. These levels in snow crabs, except hexaCBz, subsequently declined over time. It appears that CBz contamination from the *Irving Whale* barge no longer threaten the snow crab populations or fisheries in the Gulf of St. Lawrence.

Acknowledgments. We thank J. Arsenault, K. Pauley, D. Robertson and E. Battaller for collecting the snow crab samples and J. Dixon and Ginette Belbin for the GC-MS analysis completed in 2002.

REFERENCES

Gilbert M, Alexander R, Arsenault, Ernst WR, Fairchild W, Hennigar P, Julien G. King T, Lebeuf M, Lefaivre D (1998) PCB contamination of sediments and snow crab (*Chionoecetes opilio*) around the site of the sinking of the *Irving Whale* barge after its recovery. Canadian Tech Rep Fish Aquat Sci 2204:i-28

Gilbert M, Walsh G. (1996) Potential consequences of a PCB spill from the barge *Irving Whale* on the marine environment of the Gulf of St. Lawrence. Canadian Tech Rep Fish Aquat Sci 2113:i-59

Gómez-Catalán J, Lezaun M, To-Figueras J, Corbella J (1995) Organochlorine residues in the adipose tissue of the population of Navarra (Spain). Bull Environ Contam Toxicol 54:534-540

Government of Canada. (1993a) Hexachlorobenzene -Canadian Environmental Protection Act - Priority Substances List Assessment Report Ottawa, Ontario, Canada. ISBN 0-662-20291-0, i–52

Government of Canada (1993b) Hexachlorobenzene -Canadian Environmental Protection Act - Priority Substances List Supporting Document Ottawa, Ontario, Canada. i-248

Health Canada (1993) Departmental consolidation of the Food and Drugs Act and of the Food and Drug regulations with amendments to December 1993. Section B.01.047

Lord KA, Briggs GG, Neale MC, Manlove R (1980) Uptake of pesticides from water and soil by earthworms. Pestic Sci 11:401-408

Mackay, D, Shiu WY, Ma KC (1992) Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume I. Monoaromatic Hydrocarbons, Chlorobenzenes, and PCBs. Lewis Publishers, Chelsea, MI

Oliver BG (1987) Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ Sci Technol 21:785-790

Oliver BG, Nicol KD (1982) Chlorobenzenes in sediments, water, and selected fish from lakes Superior, Huron, Erie, and Ontario. Environ Sci Technol 16:532-536

Oliver BG, Niimi AJ (1983) Bioconcentration of chlorobenzenes from water by rainbow trout: correlations with partition coefficients and environment residues. Environ Sci Technol 17:287-291

Van Birgelen APJM (1998) Hexachlorobenzene as a possible major contributor to dioxin activity of human milk. Environ Hlth Perspect 106:683-688