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**The Ecological and Analytical Chemistry of Chlorinated Hydrocarbon Contaminants
and Methyl Sulfonyl-Containing Metabolites of PCBs and 4,4'-DDE in the
Polar Bear (*Ursus maritimus*) Food Chain**

by

Robert J. Letcher, B.Sc./M.Sc.

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of

Doctor of Philosophy

Department of Chemistry

Carleton University
Ottawa, Ontario
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and Methyl Sulfonyl-Containing Metabolites of PCBs and 4,4'-DDE in the
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submitted by **Robert J. Letcher, B.Sc./M.Sc.**

in partial fulfilment of the requirements for

the degree of Doctor of Philosophy

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Department

Dr. Ross J. Norstrom, Thesis Supervisor

Dr. Christopher Metcalfe, External Examiner

CARLETON UNIVERSITY

Date March 8, 1996

Abstract

Contamination of the Arctic ecosystem has resulted in exposure of polar bear, to lipophilic CHCs. In the present study, a simple column chromatography method was developed to determine MeSO₂-PCBs/-4,4'-DDEs and CHCs in biological matrices. Method application to tissues and lipid extracts spiked with MeSO₂-PCB/-4,4'-DDE and CHC standards showed that spike recoveries were > 75 %, precision of replicates was good, independent of analyte, substrate type and lipid weights (< 0.7 grams). Few residual interferences were present in GC/ECD and GC/ECNI-MS(TIC) chromatograms. GC/ECD and GC/ECNI-MS(TIC) detection had low picogram sensitivity and good response stability up to the nanogram level for MeSO₂-PCBs/-4,4'-DDEs. ECNI-MS fragmentation was influenced by MeSO₂-PCB structure, and useful for elucidation of unknown congener structures. Congeners possessing the 4-MeSO₂-2,5-dichloro moiety favoured (M)⁺ formation. From the PCB and MeSO₂-PCB patterns, ringed seal had low levels of CYP1A- and CYP2B-type activities. Polar bear had high levels of both CYP enzyme activities. Metabolite uptake from seal blubber, precursor PCB metabolism in bear and metabolite depuration contributed to MeSO₂-PCB/-4,4'-DDE bioaccumulation in bear. For example, MeSO₂-CB132 was completely bioaccumulated from seal. Metabolic pathways other than MeSO₂-metabolite formation are favoured for *meta-para*, chlorine-unsubstituted PCBs. All precursor PCBs had 2,5 or 2,5,6-chlorination on the MeSO₂-substituted ring. MeSO₂-PCB/-4,4'-DDE liver storage was preferred to testes, lung, fat and brain. The MeSO₂-PCB congener pattern was independent of geographic location. The ratio to residual PCBs decreased from west to east

from the Bering Sea to the Greenland Sea, reflecting the increasing input of higher chlorinated PCBs that are not major precursors of MeSO₂-PCBs. Immunoquantitated cytochrome P450 (CYP) 1A and CYP2B1 protein levels in polar bear liver microsomes correlated with toxic equivalents of CHCs. Correlations between CYP2B1 protein content and CHC residue levels in polar bear liver suggested that *ortho*-PCBs and chlordanes were the major contributors to CYP2B induction. Alkoxyresorufin-*O*-dealkylase activities increased with increasing CYP1A protein content up to an asymptotic level, suggesting that all these activities were primarily CYP1A-mediated. This was substantiated by antibody inhibition experiments. Immunoquantitated CYP1A and CYP2B1 isozymes are the most reliable measure of exposure to CHC inducers in polar bear.

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Glossary of Acronyms

ANOVA	analysis of variance
BMF	biomagnification factor
BROD	benzyloxyresorufin-<i>O</i>-debenzylase
C1Bz	chlorinated benzene
CB#	chlorobiphenyls, IUPAC numbering according to Ballschmiter and Zell^{1,2}
CHC	chlorinated hydrocarbon contaminant
CHLOR	polychlorinated dicyclopentadiene compounds
CV	coefficient of variation
CYP	cytochrome P450 monooxygenase enzyme or protein
4,4'-DDD	<i>bis</i>-2,2-(4-chlorophenyl)-1,1-dichloroethane
4,4'-DDE	<i>bis</i>-2,2-(4-chlorophenyl)-1,1-dichloroethene
4,4'-DDT	<i>bis</i>-2,2-(4-chlorophenyl)-1,1,1-trichloroethane
EROD	ethoxyresorufin-<i>O</i>-deethylase
ESQ method	external standard quantitation method
GC/FID	gas chromatography / flame ionization detection
GC/ECD	gas chromatography / electron capture detection
GC/ECNI-MS	gas chromatography / electron capture negative ion mass spectrometry
GC/EI-MS	gas chromatography / electron impact mass spectrometry

GC/MSD	gas chromatography / mass selective detector
GPC	gel permeation chromatography
GSH	glutathione
GST	glutathione-S-transferase
HCH	hexachlorocyclohexane
HLC	Henry's Law of Constant
HPLC	high-pressure liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
IDL	instrumental detection limit
IS	internal standard
MAP	mercapturic acid pathway
MDL	method detection limit
MeSO ₂ -PCB	methyl sulfonyl polychlorinated biphenyl
MFO	mixed-function oxidase
MO-PCB	mono- <i>ortho</i> polychlorinated biphenyl
MTBE	methyl- <i>t</i> -butyl ether
MRF	molar response factor
NO-PCB	non- <i>ortho</i> polychlorinated biphenyl
PAH	polynuclear aromatic hydrocarbon
PB	phenobarbital
PBQ method	polar bear quantitation method

PCB	polychlorinated biphenyl
PCC	polychlorinated camphene
PCDD	polychlorinated dibenzo-<i>p</i>-dioxin
PCDE	polychlorinated diphenyl ether
PCDF	polychlorinated dibenzofuran
PCN	polychlorinated naphthalene
PQL	practical quantitation limit
PROD	pentoxyresorufin-<i>O</i>-depentylase
RMRF	relative molar response factor
S/N	signal-to-noise ratio
SAR	structure-activity relationship
SD	standard deviation
SIM	selected ion monitoring
SV	significance of variance
TBB	total body burden
TCDD	2,3,7,8-tetrachlorodibenzo-<i>p</i>-dioxin
TCPMeOH	<i>tris</i>(4-chlorophenyl)methanol
TEF	2,3,7,8-TCDD toxic equivalency factor
TEQ	2,3,7,8-TCDD toxic equivalent concentration
TIC	total ion current

Chapter 1

Introduction

Polar regions of the Earth were once considered too remote to be recipients of chemical contamination resulting from human activity. The fallacy that these are pristine environments is demonstrated by the presence of pollutants in Arctic biotic and abiotic systems. A considerable influx of atmospheric anthropogenic substances occurs in the Arctic, including metals, acids, radionuclides and numerous synthetic organics ³.

Chlorinated hydrocarbon contaminants (CHCs), used as pesticides and for industrial applications, comprise a large part of the synthetic organic compounds known to enter the Arctic region (Table 1.1). The presence of CHCs in the environment is due to several factors including the massive amounts produced, wide spread applications, long-range atmospheric transport, and the favourable physicochemical properties necessary for long-term, environmental persistence. There are only a few significant CHC sources within the Arctic, therefore, anthropogenic contamination must be sourced elsewhere. The "Arctic haze" phenomenon exemplifies the long-range transport of hemispheric pollution ³.

The persistence of CHCs and their metabolites in the global ecosystem has been implicated as a causative factor for a number of biological and toxicological effects in living organisms, including man ^{4,9}. For example, the effects of CHCs have been linked to the occurrence of immunological, reproductive and teratogenic dysfunction in various higher animals.

Table 1.1. Arctic Environmental Contaminants of Most Concern[†]

1. chlorinated industrial organic compounds	chlorobenzenes (ClBzs) polychlorinated biphenyls (PCBs) polychlorinated dibenzo- <i>p</i> -dioxins/furans (PCDD/Fs)
2. organic pesticides	polychlorinated camphenes (PCCs, toxaphenes) hexachlorocyclohexanes (HCHs) polychlorinated dicyclopentadienes (CHLORs, chlordane) 4,4'-DDT/4,4'-DDE aldrin/dieldrin
3. polycyclic aromatic hydrocarbons	e.g., benzo[<i>a</i>]pyrene (B[<i>a</i>]P)

[†] Modified from Barrie *et al.* ³.

1.1 CHCs and the Arctic Atmosphere and Hydrosphere

1.1.1 Sources

The production of most CHCs has been controlled for some 20 years in the majority of industrialized nations of the northern hemisphere. However, CHCs, especially chlorinated pesticides, are known to be produced for use in countries in the southern hemisphere ¹⁰. PCBs were introduced in the 1930's, finding many applications as nonflammable, chemically inert fluids. Due to their chemical stability, the useful lifetime of PCBs can exceed 30 years or more. Common uses were as electric transformer and capacitor oils, hydraulic and heat exchange fluids, and lubricating and cutting oils ¹¹. The manufacture of PCBs ceased in most industrialized countries in the early 1970's due to their persistence in the environment, capacity to accumulate in tissue, and the connection between high tissue levels and reproductive and immunological abnormalities in mammals ^{5,8,9}.

The initial environmental release of PCBs occurs mostly by leakage and disposal of electric transformers and hydraulic equipment. The use of existing PCBs stocks has been limited to closed-systems, however, leakage from these systems continues to be a source of environmental contamination. Of the estimated total global production of 1.2 to 1.5 million tonnes, 31 % (370 000 tonnes) of PCBs were estimated to have been released into the environment as of 1988 ^{12,13}.

PCBs have the empirical formula $C_{12}H_{10-n}Cl_n$ ($n = 1$ to 10), allowing for 209 structurally different congeners, usually indicated by their IUPAC systematic number (Table 1.2, Figure 1.1a) ¹². Technical PCB mixtures have been marketed under several names including Aroclor, Kanechlor, Phenochlor, Clophen, Fenclor, Sovol and Delor. Approximately twenty of the congener structures (Table 1.2) are known to be absent from technical PCB mixtures.

High biomagnification of the lipophilic insecticide 4,4'-DDT (Figure 1.1b) in aquatic and terrestrial food chains, prompted discontinuation of its use in the 1970's in most nations, including Canada, the U.S. and Europe. However, 4,4'-DDT continues to be manufactured and used in more equatorial regions including southern Asia, Africa, Central America and South America, mostly for public health programs such as a mosquito larvicide to control malaria ³. In 1974, it was estimated that the total global production of 4,4'-DDT was 1.5 million tonnes. Production of 4,4'-DDT for agricultural use in India alone was estimated at 18,000 tonnes in the mid-1980's ¹⁴.

Table 1.2. Nomenclature, Number of Chlorines and Chlorine Content of Isomeric Groups of PCBs[†]

Empirical formula	PCB homolog	Number of isomers	IUPAC numbers [‡]	%Cl	Number of congeners in commercial mixtures
$C_{12}H_9Cl$	mono	3	1 - 3	18.79	3
$C_{12}H_8Cl_2$	di	12	4 - 15	31.77	12
$C_{12}H_7Cl_3$	tri	24	16 - 39	41.30	23
$C_{12}H_6Cl_4$	tetra	42	40 - 81	48.56	41
$C_{12}H_5Cl_5$	penta	46	82 - 127	54.30	39
$C_{12}H_4Cl_6$	hexa	42	128 - 169	58.93	31
$C_{12}H_3Cl_7$	hepta	24	170 - 193	62.77	18
$C_{12}H_2Cl_8$	octa	12	194 - 205	65.98	11
$C_{12}HCl_9$	nona	3	206 - 208	68.73	3
$C_{12}Cl_{10}$	deca	1	209	71.10	1

[†] Modified from de Voogt and Brinkman ¹³.

[‡] Based on the numbering system adopted by Ballschmiter and Zell ^{1,2}. See Figure 1.1a for PCB structure.

Figure 1.1. Chemical structure of PCBs and the reaction pathway and structures of 4,4'-DDTs. A) numbering scheme for PCB chlorine substitution and B) structures of 4,4'-DDT and its metabolites 4,4'-DDD and 4,4'-DDE.

1.1.2 Atmospheric Transport and Deposition to the Arctic

The presence of atmospherically transported CHCs in land, air and water media is a function of several physicochemical parameters including molecular mass, water solubility, vapour pressure, temperature, organic carbon partition coefficient and chemical reactivity in each medium. These relationships determine whether a CHC will exist primarily in surface waters and lands or in the troposphere ¹⁵. The atmospheric residence time of PCBs and DDTs have been estimated at 45 to 100 days ^{16,17}. In 1986, Tanabe and Tatsukawa ¹⁸ estimated that 20 % (230 000 tonnes) of the world production of PCBs were in the upper ocean layers and 790 tonnes were in the open ocean atmosphere.

There are three main factors which control the atmospheric residence time of a CHC, chemical reactivity, vapour pressure and water solubility ³. Many highly chlorinated CHCs are resistant to environmental degradation by hydrolysis, oxidation, reduction, free radicals and photolysis, and therefore tend to have long environmental half-lives ¹⁹. Some CHCs can be environmentally degraded, producing more chemically stable metabolites. For example, 4,4'-DDT is biologically degraded by a cytochrome P450 (CYP) enzyme-mediated, dehydrochlorination reaction to form 4,4'-DDE (Figure 1.1b). Minor amounts of 4,4'-DDE are known to be formed photochemically from 4,4'-DDT ²⁰. The 4,4'-DDE compound is more stable than 4,4'-DDT since the unsaturated ethene bond of 4,4'-DDE is conjugated with the benzene rings, resulting in a quinoid structure ²¹.

Despite the semi-volatility of PCBs and 4,4'-DDT, that is, moderate vapour pressures, the atmospheric flux is high when these compounds are spread over large surface areas such as soil or water. Substantial amounts of pesticides and herbicides enter the atmosphere from

spray drift, evaporation of spray droplets and volatilization from treated soil and plant surfaces. The major sources of PCBs in the global atmosphere are considered to be, i) evaporation from land fills, ii) incineration of hazardous wastes and iii) re-volatilization from environmental compartments²². The vapour pressure of CHCs is dependent on temperature²³. Repeated deposition and revolatilization of PCBs from land surfaces are known to be controlled by seasonal temperature variations^{16,24,25}.

The vapour-water partitioning of CHCs is related to Henry's Law²⁶. Henry's Law constants (HLCs) are the equilibrium ratios of the vapour pressure of a CHC in air relative to its concentration in water, or water solubility, and have units of $\text{pa.m}^3 / \text{mol}$. A unitless air-water partitioning coefficient (K_{aw}) is derived from the HLC for a CHC by the equation $K_{aw} = \text{HLC} / RT$, where R is the universal gas constant ($R = 8.314 \text{ pa.m}^3 / (\text{mol.K})$) and T is the temperature. Most CHCs, including 4,4'-DDT and PCBs, have HLCs (at 20°C) in the range of 1 to 20 $\text{pa.m}^3 / \text{mol}$, suggesting moderate volatility from water²⁶. HLCs for PCBs show large variations with temperature²⁷, and decline with decreasing temperature³. Therefore, HLCs change with latitude and seasonal climatic change.

The particle surface area available in the Arctic atmosphere for CHC absorption is huge, considering that even clean air masses have on the order of 10 $\mu\text{g}/\text{m}^3$ of permanently suspended particles²⁸. Snow and atmospherically suspended water droplets in the form of fog are also massive surfaces for CHC absorption, and especially important at the low temperatures encountered in the Arctic. In 1986/87, total (S-) DDT and S-PCB concentration ranges of 0.01 to 0.2 $\mu\text{g}/\text{m}^2$ and 0.01 to 0.3 $\mu\text{g}/\text{m}^2$, respectively, were measured in Canadian Arctic snow²⁴. Bidleman²³ showed a seven to twelve-fold increase in the proportion of

PCBs, DDT and CHLORs which were particulate-associated at 0°C than at 20°C. Particle washout and snow removal from the atmosphere are therefore major sources of CHCs available for uptake by marine organisms.

A "cold condensation" effect is believed to occur for CHCs causing migration from warmer to colder regions ^{29,30}, and is related to the decreasing HLCs with decreasing temperature. Wania and Mackay ^{30,31} argue that vapour pressure is the main determinant in CHC transport to the Arctic. A negative gradient exists for less volatile CHCs such as DDTs and highly chlorinated PCBs, with increasing latitude, indicating efficient removal close to sources at mid-latitudes. Iwata *et al.* ³² have shown, using flux calculations, that the net transfer of PCBs from air to the ocean surface increase with increasing latitude as the air and water become colder.

Atmospheric and oceanic circulation control the movement of CHCs into, through and out of the arctic region ³. Meteorological conditions in the Arctic and the relationship to pollutant transport have been well documented ^{33,34}. One of the major influencing factors of arctic atmospheric circulation is the circumpolar vortex, which is an extensive, cold-cored centre of low pressure in the middle to high troposphere and the lower stratosphere ^{33,34}. The circumpolar vortex is characterized by distinct summer and winter air flow patterns. For example, air movement into the Canadian Arctic is mainly from the Eurasian continent in the winter ³⁵, but the sources are more complex in summer. Further, these effects vary across the expansive Canadian Arctic region.

1.1.3 Uptake into the Arctic Marine Ecosystem

Bioconcentration of persistent, lipophilic CHCs from an aqueous medium to the lipid phase of an aquatic organism is also a function of a physical-chemical partitioning process³⁶. This is especially true of the Arctic because, CHC partitioning favours more hydrophobic media such as suspended organic particulates, microorganisms and fish gills, as water becomes colder³⁷. These partitioning equilibria are dependent on the degree of hydrophobicity of a CHC, since the lipophilic nature of a solute does not necessarily mean hydrophilic properties are lacking. CHCs are generally equally lipophilic, or lipid-soluble, but have large differences in hydrophobic character³⁷.

The bioconcentrating ability of a CHC compound is often modelled by octanol/water partition coefficients (K_{ow})³⁸. K_{ow} for a given CHC can be used to predict and quantify the environmental spreading or concentrating ability between an aqueous and a lipid phase. It has been concluded that unicellular organisms, such as marine phyto- and zooplankton, absorb lipophilic compounds on the outer cell surface³⁹. The compound then diffuses through the outer cell membrane. The arctic marine food web has been shown to be highly dependent on planktonic organisms and ice algae⁴⁰.

Bioaccumulation is the process of contaminant accumulation to predator via the ingestion of prey. Biomagnification is said to occur if:

$$[\text{contaminant}]_{\text{predator}} / [\text{contaminant}]_{\text{prey}} > 1 \quad (1)$$

After absorption from the gastrointestinal tract, lipophilic contaminants are distributed

among tissues by the circulatory system. Often, concentrations are dictated by the lipid content of the tissue, that is, the lipid pools are at equilibrium with each other ⁴¹. However, the tissue distribution may be disproportionate if the CHC is involved in a specific binding interaction. For example, methyl sulfonyl (MeSO_2 -)-containing metabolites of PCBs are preferentially retained in the liver of Baltic grey seal and laboratory mice ⁴².

The bioaccumulation and metabolism of CHCs are dependent on several factors. Uptake is a function of the level of CHC exposure, which is determined by energetic requirements and feeding ecology. CHC clearance from the organism is dependent on the CHC structure, which determines losses and the ability to induce CYP enzyme systems, the level to which the CYP system is induced, and the capacity of a CYP isozyme in an organism to metabolize a CHC ^{5,6,43-45}. The polar bear, for example, possesses unique metabolic capabilities relative to other arctic mammals, since the 4,4'-DDE biomagnification factor (BMF, defined in eq. 1) is less than one and many PCB congeners normally recalcitrant in mammals are metabolized ^{46,47}. The level of exposure and the manner in which PCBs and 4,4'-DDT and their metabolites interact with the CYP enzyme system have been implicated in reproductive and immunological abnormalities observed in some marine mammals ⁷.

1.2 The Polar Bear Food Chain

Arctic marine mammals are potentially vulnerable to lipophilic CHCs due to several factors, which include their longevity and the accumulation of large fat stores ^{3,45,48}. The

trophic level relationships in the arctic marine food web are central to understanding the bioconcentration and bioaccumulation of CHCs.

The arctic cod (*Boreogadus saida*) is resident throughout the circumpolar oceans and is an important component of arctic marine food webs ⁴⁹. Arctic cod consume mainly small invertebrates (i.e., planktonic copepods and amphipods), and thus transfer the energy, and the stored CHCs, to higher food chain creatures, mainly marine mammals and seabirds. Arctic cod reside mainly around the rough ice around pack ice pressure ridges. In the Barrow Strait in the central region of the Canadian high Arctic, cod are known to form large, pelagic schools when pack ice breaks-up in the summer months.

Arctic marine/maritime mammal biodiversity is restricted to a relatively small number of species including polar bear (*Ursus maritimus*) ⁵⁰. The ringed seal (*Phoca hispida*) is the most resident, abundant and widely distributed arctic pinniped ⁵¹. In the Barrow Strait area of the Canadian high Arctic, ringed seals are resident year round ⁵². Ringed seal is the only northern marine mammal adapted to living on land-fast ice ⁵¹.

Arctic cod are preyed on heavily by all Arctic resident marine mammals including ringed seal, beluga (*Delphinapterus leucus*) and narwhal (*Monodon monoceros*). Ringed seals are opportunistic feeders, eating mostly medium-sized crustaceans (mysids and amphipods) and arctic cod ⁵³. The proportion of each may vary, both seasonally and geographically. Ringed seals from the Barrow Strait area have been shown to ingest an annual average of 60 % cod and 35 % amphipods ⁵². The cod are taken by seal primarily in the summer and early autumn. The trophic level relationship for seal and cod has been determined by the enrichment of ¹³C and ¹⁵N stable isotopes ⁵². The low end value of the

trophic level range of 3.7 to 4.1 for ringed seal as compared to the cod value of 3.6, indicated that seal and cod occupy roughly the same trophic level at the time of sampling. This reflects the seasonal dietary input of invertebrates in the ringed seal diet.

The distribution of the terrestrially-evolved polar bear is circumpolar in the Arctic and Subarctic, and is closely tied to the pack ice⁵⁴⁻⁵⁶. The polar bear is the most carnivorous of the ursids and is the principal mammalian predator at the top of the arctic marine food chain^{55,57,58}. The polar bear diet consists mainly of ringed seal blubber and, to a lesser extent, bearded seal (*Erignathus barbatus*) blubber⁵⁶⁻⁵⁹. Polar bears enter a period of hyperphagia in the late spring and early summer during which time their body mass can more than triple⁶⁰. After this period, more than 50 % of the total body mass is fat. The polar bear is particularly vulnerable to CHC exposure as a consequence of its high lipid diet. Polar bears have been shown by $\delta^{13}\text{C}$ analysis to make little use of terrestrial food webs⁶¹.

Polar bears wander seasonally over large distances in search of ringed seal prey, however, they have territorial ranges. Therefore, distinct groups of bear populations are defined⁵⁶. It has been shown that there is little overlap between these population groups. Some bear populations, such as that of western Hudson Bay, migrate onto land when the sea ice melts during the summer⁵⁷. During the ice-free period, polar bears spend 70 to 90 % of their time resting. This period has been described as active hibernation⁶², during which time the bears fast⁶³. This period can last as long as 8 months for pregnant females and as little as 4 months for males. The fat reserves may be reduced to less than 10 % at the end of the fasting period⁶⁴. Moreover, the most energy costly portions of the polar bear reproductive cycle takes place while fasting, including gestation, parturition and early lactation^{60,63}. There

is a substantial mobilization of fat reserves for milk production. Polar bear milk has the highest fat content of any terrestrial mammal, in some cases exceeding 30 % on a wet weight basis ⁶⁵. These large seasonal changes have a significant influence on CHC dynamics in the polar bear ⁶⁶. A different effect may exist for more polar CHC metabolites such as MeSO₂-PCBs and -4,4'-DDE.

1.3 CHCs in Arctic Marine Biota

The presence of chlorinated pesticide (CHLORs, DDTs, HCHs, PCCs and ClBzs (Table 1.1) and PCB residues in fish, marine mammals, and birds of the Arctic have been documented since the early 1970's ^{46,47,66,67}. Greater detail on the identification and levels of individual CHCs was made possible with the advent of high-resolution, capillary GC coupled with mass spectrometry in the 1980's. Several comprehensive reviews on the levels and the temporal and geographical variation of CHCs in arctic marine mammals, including polar bear, have recently become available ^{45,48}. The present discussion will be restricted to some highlights of these findings.

In arctic seals, PCB concentrations are generally in the range of 1 to 10 ppm in lipid, whereas polar bear concentrations are ca.10 ppm in lipid ⁴⁵. Bioconcentration factors of CHCs from water in the arctic food web are as high as 10⁷ for fish and seals ⁶⁸. Biomagnification factors for PCBs from seal to polar bear can range from ca. 1 to 25 depending on the degree and the position of chlorination ⁴⁷.

The levels of total CHLORs, HCHs and ClBzs in arctic beluga whale are not

significantly different from the levels in beluga from the more southerly St. Lawrence river system ⁴⁸. It was suggested that the similar levels reflect the importance and homogeneity of atmospheric transport and deposition of these CHCs due to their volatility or higher HLC. Arctic beluga levels of the less volatile PCBs and DDTs were 25- and 32-fold lower, respectively, than St. Lawrence beluga. This indicated the importance of more local sources of CHC contamination for the St. Lawrence beluga, which supports the conjecture of Wania and MacKay ³¹ that higher chlorinated CHCs tend to remain closer to local sources.

Recently, the temporal and kinetic dynamics of CHCs have been reported in polar bear over a cycle of hyperphagia and fasting ⁶⁰. Preliminary results demonstrated that, as the fasting period progressed, concentrations of some CHCs, such as total PCBs, increased in fat and milk of bear mothers. The 4,4'-DDTs showed a poor relationship, likely a consequence of metabolism. The cubs, particularly cubs of the year, had the highest CHC levels of any life stage. This evidence was indicative of a significant CHC transfer from mother to cub. It was suggested that the CHC exposure via milk may be associated with chronic toxic effects, which influence aspects of cub physiology, such as growth. Developing young exposed to CHCs are known to suffer different health consequences than adults ⁶⁹. Once mating has occurred, polar bears show delayed implantation of the resulting blastocyst, until the females enter their birthing dens ⁶³. Species demonstrating delayed implantation appear to be susceptible to deleterious reproductive effects attributable to CHCs ⁷⁰. CHC-induced effects may explain the slow decline in reproductive performance observed in western Hudson Bay polar bears ⁶⁰.

Levels of DDTs, PCBs, CHLORs, HCHs and PCCs (Table 1.1) are generally lower

for females relative to males in arctic beluga, narwhal, ringed seal and fur seal ^{45,48}. This occurs because females have the additional ability to excrete lipids and their associated xenobiotics during lactation.

The relative composition of six major classes of CHCs (Table 1.1) in compartments of the arctic atmosphere, hydrosphere and ecosphere has been summarized (Figure 1.2). Changes in the CHC composition as the trophic level increases ⁵² are controlled by the capability of an organism to depurate a compound, with or without metabolism ⁴⁵. For example, the high proportion of CHLORs in polar bear (Figure 1.2) are due to the high contribution (57 %) of oxychlordan, a persistent metabolite of several CHLOR compounds⁴⁶⁻⁴⁸. The high metabolic capacity in polar bear was also shown by the greatly simplified PCB congener pattern relative to ringed seal and cod ⁴⁷. However, the identities, levels and the potential bioaccumulation of MeSO₂-PCB metabolites is unknown for the polar bear food chain, apart from two individual polar bears from the western Hudson Bay area ⁷¹.

In the few studies on arctic marine mammals, levels of the highly toxic coplanar PCBs (CB-77 (3,3',4,4'), CB-126 (3,3',4,4',5), CB-169 (3,3',4,4',5,5')), PCDDs and PCDFs were at least an order of magnitude lower than the total PCB levels ^{45,48,72,73}. Low bioaccumulation of PCBs without *meta-para* chlorine substitution and PCDD/Fs with *ortho* substitution was seen in some mammals. Individual differences in the accumulation/metabolism ability of non-*ortho* (NO-) PCBs were also observed in some arctic mammals ^{72,73}. Residual concentrations of NO-PCBs, PCDDs and PCDFs have often been expressed as toxic equivalents (TEQs) relative to 2,3,7,8-TCDD, to evaluate their toxic

Figure 1.2. The percent CHC composition in arctic air, snow, seawater and marine biota. Modified from Norstrom and Muir ⁴⁵, and the references therein.

potency ⁷⁴. In general, NO- and mono-*ortho* (MO-) PCBs contributed a larger share to the TEQs than PCDD/Fs in ringed seal, beluga, narwhal and polar bear from the Canadian and Norwegian (Svalbard) Arctic ^{45,72,75,76}.

1.4 Metabolism of PCBs and 4,4'-DDE in Mammals

1.4.1 Induction of CYPs

Exposure of marine mammals to persistent CHCs and their metabolites has been implicated as a causative factor in carcinogenicity, teratogenicity, sterility, growth retardation, immunologic dysfunction and reproductive abnormalities ^{5,7}. Swart *et al.* ⁴ speculated that pollution-induced immunosuppression had played a contributory role in the morbillivirus infection outbreak observed in harbour seals from the highly polluted Baltic Sea.

Many CHCs induce hepatic CYP enzymes, including members of the CYP1A and CYP2B subfamilies ⁷⁷. The CYP enzymes, also known as mixed-function oxidases (MFOs), are the major drug-metabolizing enzymes in liver. CYP enzymes function in the oxidative biotransformation of a wide variety of lipophilic xenobiotic and endogenous compounds ^{78,79}. Consequently, hepatic microsomal CYP enzymes promote the elimination of some hydrophobic organic pollutants by facilitating the introduction of polar functional groups for

subsequent excretion. Levin *et al.* ⁸⁰ reported the existence of at least 15 isozymes of CYP enzymes in the hepatic microsomes of mammals. Induction of CYP can significantly affect the metabolism, bioaccumulation and toxicokinetics of xenobiotics and therefore alter the toxicological susceptibility of exposed animals.

PCBs without *ortho* chlorine substitution (i.e., NO-PCBs) (Figure 1.1a) have the lowest energy barrier (i.e., 1.94 kcal mol⁻¹) necessary to achieve a coplanar configuration between the two phenyl rings ⁸¹. The coplanar configuration of NO- PCBs resembles the fused ring system of PCDDs and PCDFs. Induction of the CYP1A subfamily by PCDDs and PCDFs with halogens substituted in the 2,3,7,8 positions and 3,4,3',4'-halogen substituted PCBs, is mediated by a common mechanism of action that involves binding to the Ah- (aryl hydrocarbon) receptor ^{74,82,83}. CB-77 (3,3',4,4'), CB-126 (3,3',4,4',5) and CB-169 (3,3',4,4',5,5') are known to be the most potent CYP1A inducers of the NO-PCBs ^{74,84}. Several MO-PCBs identified in commercial PCB mixtures and environmental extracts, such as CB-105 (2,3,3',4,4') and CB-118 (2,3',4,4',5), have also been found to make a significant contribution to CYP1A induction ⁷⁴. MO-PCBs also have a relatively low energy barrier (i.e., 6.9 kcal mol⁻¹) necessary to achieve a coplanar configuration ⁸¹. *Ortho* chlorine substitution greater than one significantly reduces or abolishes Ah-receptor binding ⁸³.

The major phenobarbital (PB) inducible CYP enzymes in rat liver, CYP2B1 and CYP2B2, are also induced by halogenated CHCs which do not possess coplanar aromatic structures, including 4,4'-DDT, CHLORs and some *ortho*-chlorine substituted PCBs, by an as yet undefined mechanism of action ^{85,86}.

Induction of CYP1A has been recognized as a valid biomarker of exposure to Ah-

receptor active xenobiotics ⁶⁵⁷. Ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) bioassays are relatively specific markers of CYP1A-mediated activity and are used as a measure of CYP1A induction in free-ranging fish, birds and mammals. Hepatic microsomal alkoxy-*O*-dealkylase activities have yet to be determined for polar bear.

Polyclonal and monoclonal antibodies prepared against CYP of laboratory rodents and fish have been found to cross-react with the CYP isozymes of some wild marine mammals, in particular CYP1A ^{45,88-90}. Immunoquantitation of specific CYP isozymes for the purpose of monitoring environmental contamination in wild species has been limited to the hepatic CYP1A1 isozyme of arctic beluga whale (*Delphinapterus leucus*) ⁸⁸, a few species of fish eating birds ⁹¹ and fish ⁹². Recently, Bandiera *et al.* ⁹³ characterized the CYP1A and CYP2B1 isozyme proteins of polar bear. The results indicated that immunoquantitation of the proteins could be used to measure the degree of CHC exposure in polar bear.

1.4.2 CYP-Mediated Biotransformation

CHC metabolism is dependent on the structure of the compound and the capacity of the CYP enzyme system of an organism to be induced and/or metabolize the xenobiotic compound. For example, the ability of mammals to metabolize PCBs with *meta-para*, vicinal hydrogens (Figure 1.1) follows the order of terrestrial > seals > whales ^{5,6,44}.

By virtue of its size and high levels of detoxifying enzymes, the liver is the major site of biotransformation reactions of xenobiotics, however, many extrahepatic organs are capable of metabolizing xenobiotics, but at much lower rates ⁹⁴. In mammalian liver, CYPs are most abundant in the smooth endoplasmic reticulum. Adrenal cortical cells contain CYP

monooxygenases predominantly in the inner membrane of mitochondria.

PCBs and 4,4'-DDT are metabolized initially by three main mechanisms: oxidation, reduction and conjugation. Oxidation and reduction reactions are mediated by the microsomal CYP systems while conjugations can be mediated by glutathione-S-transferases (GST), sulfatases and glucuronidases.

In most cases, PCBs are initially biotransformed to epoxide intermediates by CYP enzymes at adjacent carbons which are not chlorine substituted. However, seal, mink, mice and rats, and possibly polar bears, can add oxygen to *meta* positions where there is one *para* chlorine substitution ^{95,96}. For example, CB-105 (2,3,3',4,4') is metabolized to 4-OH-2,3,3',4',5-Cl₅CB via a 1,2-shift of the chlorine in the 4-position, which is subsequently retained in the plasma. The type of epoxide formed is therefore a function of the chlorine substitution pattern of the PCB. Further metabolism of the PCB epoxide can occur by two major pathways. The epoxides are transformed to either hydroxylated metabolites or to sulfur-containing metabolites. In regards to MeSO₂-PCB formation, after epoxide formation and subsequent translocation to the cytosol, the metabolic pathway leading to sulfone formation (see Section 1.5.1) and the number of alternate metabolic pathways is complex.

The structural requirements for PCB metabolism in various marine mammals have become clearer in recent years from changes in the residual PCB congener patterns ^{6,43,44,47,97-104}. That is, the absence of certain congeners and the reduced or biomagnified levels of residual congeners relative to the pattern in the diet. In essence, the PCB patterns are the residuals after the enzyme-mediated biotransformations, and thus, define the CYP isozyme profile of a particular species.

MO-PCBs with *ortho-meta* vicinal hydrogens and a maximum of one *ortho* chlorine, can be metabolized by CYP1A isozymes ⁶⁴⁴. The capacity for the metabolism of PCBs with this configuration has been shown to increase in mammals with highly active CYP1A isozymes. Alternately, metabolism of PCBs with *meta-para* vicinal hydrogens, irrespective of the *ortho* chlorine substitution pattern, is favoured by CYP2B isozymes ⁶⁴⁴. The varying degree of CYP2B isozyme activity in whales, seals and porpoise has demonstrated the varying metabolic capacity toward PCBs with *meta-para* vicinal hydrogens ^{89,90,106}. For example, seals have higher CYP2B-type activity than whales ⁵⁴⁴, and as a consequence, have lower residual levels of PCBs with *meta-para* vicinal hydrogens, relative to recalcitrant PCBs such as CB-153. Brown ¹⁰¹ has recently attempted to summarize the structure-activity relationships for the metabolism of NO PCBs by CYP1A- and CYP2B-type enzymes in humans. The minimal requirement of a non-chlorinated *meta* position appeared to be necessary for PCB reactivity, suggesting that the initial CYP1A or CYP2B isozyme attack is at or near the 3-position. Further, reaction rates for CYP1A-mediated metabolism decreased with increasing *ortho* chlorine substitution whereas there was a minimal effect on the rates of CYP2B-mediated metabolism. The tendency for a PCB congener structure to be metabolized therefore has a direct bearing on its capacity to bioaccumulate. Similar metabolic factors probably influence the bioaccumulation of PCB metabolites. However, the number of factors are more complex since both PCB metabolites and the precursor PCBs may be metabolized.

1.5 Methyl Sulfone-Containing Metabolites

1.5.1 Metabolic Formation of MeSO_2 -PCBs and -4,4'-DDEs

One metabolic route for the biotransformation of PCBs involves the formation of sulfur-containing metabolites as a result of epoxide conjugation with the cytosolic protein, glutathione (GSH) ^{107,108}. Subsequent metabolism via the mercapturic acid pathway (MAP) produces a number of intermediates which are ultimately metabolized to thiol- (HS-) containing metabolites. PCBs, 4,4'-DDE, hexachlorobenzene, naphthalene and PCDDs are among more than fifty compounds now known to be further transformed *in vivo* in laboratory rats to thiol- (HS-), methylsulfide- (MeS-), methyl sulfoxide- (MeSO-), and methyl sulfone (MeSO_2 -) containing metabolites ^{109,110}. Many carcinogens are metabolized via arene oxides to MAP metabolites and may exert their toxic effects in the binding of reactive metabolites ^{111,112}.

PCBs possessing vicinal 3,4 hydrogens and 2,5 or 2,3,6 chlorines on one phenyl ring (Figure 1.1), are oxidized to the corresponding epoxides via microsomal, CYP2B isozymes in the liver (step I, product A, Figure 1.3) ^{113,114}. It is probable that 4,4'-DDE is metabolized by the same enzyme. These epoxide derivatives appear to be stable enough to resist hydrolysis to 1,2-diols by epoxide hydrolase, and migrate from the microsomes to the cytosol. Nucleophilic attack by the cysteinyl sulfur of cytosolic GSH on either the 3- or 4-epoxide carbon produces a dihydro-monohydroxy-PCB/GSH adduct (product B) ¹¹⁰. The rate of GSH-conjugate formation is enhanced by a glutathione-S-transferase (GST) isozyme, which is abundant in the liver cytosol ¹¹⁵. In mammals, liver is thought to be the major organ

Figure 1.3. Metabolic pathway for MeSO₂-PCB formation from PCBs with 3,4 vicinal hydrogens and 2,5 or 2,3,6 chlorine substitutions (Figure 1.1). Reaction flow diagram modified from several references^{107,116-118}.

of GSH conjugation, since up to 10 % of the cytosolic protein is GST ¹⁰⁷.

Dehydration of the dihydro-monohydroxy-PCB/GSH adduct results in the formation of product C (step II, Figure 1.3). The dehydrated GSH-adduct is further degraded via the MAP (step III) ¹¹⁶. Peptidase hydrolysis forms several MAP metabolites, all of which can undergo biliary excretion into the gastrointestinal tract ¹¹³. It is currently accepted that peptidase hydrolysis of GSH adducts does not occur until transported outside the cell in which they were formed ¹¹⁸. The S-cysteinyl conjugate undergoes a reversible N-methylation process in the liver to produce mercapturic acid (product D, Figure 1.3). Mercapturates are excreted in the bile and subsequently deacetylated back to the S-cysteinyl conjugate in the intestinal microflora ^{119,120}.

The dihydro-monohydroxy-PCB/GSH adduct may not necessarily have to dehydrate prior to derivitization by peptidase hydrolysis in the MAP, since thiophenol metabolites have been detected in laboratory rodents ¹¹¹. Once in the gastrointestinal tract, β -lyases cleave the C-S bond of MAP metabolites to produce a thiol intermediate (step IV metabolism). C-S β -lyase activity in the intestinal microflora shows no specificity for dehydrated or non-dehydrated MAP metabolites ^{121,122}. Similar C-S β -lyases have been found in mammalian liver, however, these isozymes exhibited specificity for S-cysteinyl conjugate ¹²³.

Thiol conjugates (product E, Figure 1.3) can be excreted as free thiol, methylated to methylsulfide groups, or conjugated with glucuronic acid to form S-glucuronides ¹²⁴. S-methylation (product F, step V, Figure 1.3) is presumed to be catalysed by S-methyltransferases using S-adenosylmethionine (SAM) as the methyl donor. Detoxication of thiol by methylation appears not to occur in the gastrointestinal tract ¹²³. Thiols remain in

the gastrointestinal tract until they reach the intestinal mucosal cells ^{116,121,125,126}. The importance of S-methyltransferases in the intestinal mucosa has been demonstrated with germ-free rats, which lack a viable microflora culture ^{121,124}. S-methyltransferase activity may also occur after reabsorption of the thiols, since these enzymes are present in most tissues ¹²⁷.

The methylsulfide derivatives are oxidized to MeSO- (product G) and MeSO₂- (product H, Figure 1.3) derivatives by two possible routes. Most of the oxidation of the methylsulfide group is believed to be mediated by CYP enzymes in the liver, thus, entero-reabsorption followed by transport to the liver facilitates the process of methylsulfide oxidation. However, oxidation can also be mediated by CYP enzymes in cells of the intestinal mucosa ¹²⁸, followed by reabsorption. If entero-absorption of MeSO₂-PCBs and -4,4'-DDEs present in ingested food also occurs, these metabolites may be bioaccumulated through the food chain. MeSO₂-PCBs generated in the liver or in the intestinal mucosa can be excreted into the intestinal lumen, and reabsorbed or excreted, or translocated to other lipid-containing tissues. In some cases, binding to proteins rather than dissolution in lipids occurs, such as in rat lung ^{111,129}. Studies done to date have not addressed the possibilities of bioaccumulation of MeSO₂-PCBs and -4,4'-DDE from the diet given the apparent recalcitrancy to further metabolism, however, further metabolism may occur.

1.5.2 Protein Binding

The ultimate fate of thiol and mercapturic acid conjugates of xenobiotics in the liver or in the intestinal lumen, may be directly or indirectly linked to toxicity. In this respect, the C-S β -lyase-mediated turnover of cysteine conjugates and methylsulfide groups of PCBs and

DDEs may be very important. The formation MeSO₂-PCB and -DDE metabolites may represent a process of re-toxicification, as is indicated in the following studies.

Studies on the tissue localization of [¹⁴C]-labelled MeSO₂-PCBs have shown that some of these compounds are selectively retained in tissues of laboratory rats and mice. Mice dosed with several [¹⁴C]-PCBs exhibited strong retention in the lung bronchial mucosa, due to reversibly bound 4-MeSO₂-PCB metabolites ¹²⁹. The 4,4'-bis-MeSO₂-2,2',5,5'-Cl₄CB metabolite of CB-52 (2,2',5,5') was bound with high affinity to a uteroglobin-like protein in the cytosol of nonciliated bronchiolar (Clara) cells of rat and mouse lung ¹³⁰⁻¹³². The protein complex is subsequently secreted into the bronchiolar lumen and apparently spread over the entire surface lining. Lung parenchyma of rats selectively takes up pentachloro-MeSO₂-PCBs with 2,5,6-trichloro-substitution on one phenyl ring (Figure 1.1) ¹²⁹. The 4-MeSO₂-2,2',5,5'-Cl₄CB metabolite was found to concentrate in the bronchi, trachea, larynx and nasal mucosa of mice ^{129,133}.

The affinity of MeSO₂-PCBs for lung is significant considering the respiratory symptoms observed in patients from the "Yusho" incidence in Japan in 1968, a mass food poisoning caused by the ingestion of rice oil contaminated with PCB ^{97,134-138}. These patients exhibited respiratory symptoms more than 10 years after the actual incident.

MeSO₂-PCB binding with carrier proteins has been observed. The 4,4'-bis-MeSO₂-2,2',5,5'-Cl₄CB metabolite forms a complex with, 1) α₂u-globin in kidney and urine in male rats (α₂u-globin is a major urinary protein in mouse) ¹³⁹, 2) liver fatty acid binding protein isolated from rat intestinal mucosa ¹⁴⁰ and 3) fatty acid binding protein isolated from the liver and intestinal mucosa of chicken ¹⁴¹. Carrier protein binding has also been observed for

MeSO₂-PCBs in rat liver ¹⁴². Fatty acid binding proteins in rat are thought to be involved in absorption, intracellular transport, compartmentalization and metabolism of free fatty acids¹⁴³.

MeSO₂-PCB metabolites of CB-31 (2,4',5) were shown to be enriched in the uterine fluid of pregnant mice ¹²⁶. Further, MeSO₂-PCBs bind to purified uteroglobin from rat lung¹⁴⁴. Uteroglobin is a steroid binding protein which interacts with progesterone. Moreover, uteroglobin serves as a model for binding of ligands to steroid hormone receptors. From ligand binding studies it was found that sulfone oxygens from MeSO₂-PCB, as well as the carbonyl of progesterone, may form hydrogen bonds with the hydroxyl group of tyrosine 21 of uteroglobin ¹⁴⁴. It was suggested that steroids and MeSO₂-PCBs may interact with the same protein, despite the structural dissimilarity of the two ligands. These factors may influence MeSO₂-PCB and -4,4'-DDE tissue distribution in polar bear. Studies on the possibility of preferential tissue retention in wild mammals have been limited to liver, muscle and blubber of grey seal, mink and otter from the Baltic Sea region ⁷¹.

1.5.3 Biological Effects and Toxicology

Several recent studies have shown that MeSO₂-PCB and -4,4'-DDE compounds present in tissue influence directly or indirectly the function of several biochemical processes. Moreover, it has been suggested that persistent xenobiotic intermediates derivatized by the MAP, including those with HS-, MeS- and MeSO₂-substituents, are all potential candidates for carcinogenic involvement ¹¹⁸. Three conditions are known to be conducive for the occurrence of chemical carcinogenesis, 1) proliferating cells, 2) initiation

of the cancer and 3) promotion of the cancerous growth ¹¹¹.

Mice injected with CB-52 and its 4-MeSO₂-metabolite were shown to have decreased drug-metabolizing CYP activity in the lung, whereas the activity was increased in liver ¹⁴⁵. It was suggested that the CYP induction in rat liver is due, in part, to MeSO₂-PCBs as well as the parent PCB. Very recently, 3-MeSO₂-PCBs have been shown to be potent inducers of microsomal CYP2B in rat liver ^{146,147}.

The 3- and 4-MeSO₂-3',4,4',5-Cl₄CB and 3-MeSO₂-3,3',4,5,5'-Cl₅CB metabolites were shown to reduce aryl hydrocarbon hydroxylase (AHH) activity in human lymphoblastoid cells ^{148,149} and mouse liver microsomes ^{150,151}. AHH is involved in the metabolism of various environmental carcinogens and it has been suggested that it is involved in the development of human cancers, especially respiratory cancers ¹⁵². A positive relationship between the inducibility of hepatic AHH and the susceptibility to chemically induced carcinogenesis has been reported ¹⁵³.

Female mink (*Mustela vison*) exposed to a mixture of fifteen MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE for one year, including one reproductive season, showed a decrease in the litter size of kits ¹⁵⁴. The results suggested that MeSO₂-metabolites contributed to the decreased kit survival, since the control litter was twenty kits whereas the MeSO₂-metabolite exposed groups had litters of only ten.

The metabolite 3-MeSO₂-4,4'-DDE has been shown to be bioactivated to a reactive metabolite that irreversibly binds to proteins in the adrenal cortex of birds, seals, mice, fish and humans ¹⁵⁵⁻¹⁶⁰. In some cases, steroid metabolism *in vitro* was affected, suggesting the reactive metabolites may disturb adrenal glucocorticoid production *in vivo*. The activating

enzyme was proposed to be CYP 11 β -hydroxylase, a CYP form known to be expressed specifically in the adrenal cortex and an important enzyme in glucocorticoid production.

A perturbation of steroid metabolism may underlie the toxicological effects observed in some animals exposed to high levels of CHCs known to be adrenocorticolytic toxicants. High body burdens of DDTs, MeSO₂-DDEs and MeSO₂-PCBs have been postulated as one possible explanation for a disease syndrome observed in Baltic seals, characterized in part by adrenocortical hyperplasia ^{7,161} and low and declining reproduction ¹⁶². It was proposed that a decreased formation of corticosterone/cortisol results in a compensatory synthesis of steroid-synthesizing enzymes and adrenocortical cells, and thus causes adrenocortical hyperplasia ¹⁵⁷.

1.5.4 Persistence in Biota

Biologically persistent MeSO₂-PCB and -DDE metabolites have received scant attention as environmental contaminants since their first discovery in the blubber of Baltic grey seals in 1976 ¹⁶³. Aryl methyl sulfone metabolites have escaped detection in standard analysis procedures as they are more polar relative to other common CHCs and quantitation standards have not been readily available.

The determination of levels of MeSO₂-PCBs and DDE in biota has been restricted mainly to mammals, including humans, in the Swedish and Japanese environments. MeSO₂-PCBs have been shown to be among the most persistent xenobiotics in the free-ranging mammals studied thus far. In Baltic grey seals (*Halichoerus grypus*), they were the third most abundant class of anthropogenic substances, often found at levels of 10 % to 20 % of

the total PCBs. Until the present study, MeSO₂-PCBs and -4,4'-DDE in the adipose and liver of two polar bears from western Hudson Bay was the only study of these metabolites in the Canadian Arctic ⁷¹. However, only twelve MeSO₂-PCB congeners out of the twenty-four detected were identified and quantitated.

Mink and otter (*Lutra lutra*) from the Swedish environment were also found to contain MeSO₂-containing metabolites. MeSO₂-PCBs were detected in the liver, lung and adipose tissue of Japanese patients with 'Yusho' PCB poisoning. The MeSO₂-PCB to PCB ratios ranged from 0.1 % to 8 %, increasing from adipose to lung to liver ^{134,136-138,164}. Ten MeSO₂-PCBs were detected in the egg homogenates of white-tailed sea eagles from the Baltic Sea, at levels three orders of magnitude lower than the total PCBs as compared to 10 % to 20 % of total PCBs in mammals from the same region ¹⁶⁵. A recent temporal (1972-1991) study on MeSO₂-PCBs and -4,4'-DDE in Swedish human milk reported that MeSO₂-PCBs and -4,4'-DDE decreased with the levels of PCB and 4,4'-DDE over the time period ^{166,167}.

Persistent MeSO₂-PCBs in biota are characterized by the structural features shown in Figure 1.4. Approximately thirty 3- and 4-MeSO₂-PCB congeners have been detected in grey seal, otter and wild mink from the Swedish environment, at total levels ranging from 0.1 to 21 µg/g extracted lipids ⁷¹. However, not all structures were identified. In a separate study, MeSO₂-PCB and -DDE levels in ringed, harbor and grey seal from different areas of the Swedish coastline and of differing age and sex, were between 0.7 µg/g (juveniles) and 110 µg/g (adult female grey seals with disease symptoms) ¹⁶⁸.

Figure 1.4. MeSO₂-PCB and -4,4'-DDE congener structures known to persist in biota. At least one *para* chlorine is required on the second phenyl ring to hinder oxidation to hydroxyl-PCB metabolites. The numbering scheme for the MeSO₂-PCB congeners follows the IUPAC numbering system for PCBs^{1,2}, with an additional prefix denoting the position of the MeSO₂-functional group (e.g., 4-MeSO₂-CB101) (see Table 2.2).

Recently, a comparative study on the tissue retention of MeSO₂-PCBs, mostly in liver and adipose tissues, in several wild mammals (grey seal, otter and mink) from the Baltic sea area and experimental (rat and mink) mammalian species, has been reported¹⁶⁹. Liver-specific retention of 3-MeSO₂-PCBs was observed in all species. Until the present study, no comprehensive study of MeSO₂-PCB and -4,4'-DDE tissue distribution has been done for any species.

The MeSO₂-PCBs patterns and levels varied between the mammals and birds studied so far. Species are known to be exposed to different PCB patterns and bioaccumulation is affected by differences in their CYP isozyme systems. This may also be true for MeSO₂-PCBs and -4,4'-DDE. MeSO₂-PCB bioaccumulation may be affected by; 1) different mechanisms of MeSO₂-PCB formation, 2) a kinetically or thermodynamically more favourable route of PCB metabolism, 3) variable capacities for further metabolic transformation to secondary metabolites, 4) variable rates of metabolite excretion and 5)

exposure to different levels of metabolites through the diet. At present, the toxicological significance of MeSO₂-PCBs and -4,4'-DDEs in wildlife is unknown. However, the MeSO₂-PCB levels in biota are high enough that further research is warranted to form a better understanding of these persistent metabolites in biota, especially the arctic ecosphere where virtually nothing is known.

1.5.5 Congener Identification and Quantitative Analysis

The hydrophobicities of MeSO₂-PCBs are similar to the parent PCBs since their octanol/water partition coefficients (log K_{ow}) are greater than three ^{38,170}. MeSO₂-PCBs are therefore lipophilic chemicals that fulfill the requirements for bioconcentration in fatty tissue. Aryl methyl sulfone compounds possess Lewis basicity, strong acid/base stability and high polarity which are useful properties for separation from co-extracting lipids and other CHCs in biological tissues. Analytical methodologies for aryl methyl sulfone cleanup and/or separation from CHCs have exploited these physicochemical characteristics by incorporation of liquid-liquid partitioning strategies ^{71,136,138,163,164,171,172}. Dimethyl sulfoxide, concentrated sulfuric acid, and acetonitrile partitioning from non-polar solvents have been common approaches. Lipid-destructive tissue extraction strategies such as saponification with alcoholic potassium hydroxide at elevated temperatures have also been used ¹⁷². However, these analytical methodologies have not been thoroughly validated for application as a routine method for MeSO₂-PCB and -4,4'-DDE determination. A reliable and simple method is required for routine analysis of MeSO₂-PCB and -4,4'-DDE metabolites in biological matrices.

The aryl methyl sulfone fractions obtained from tissue homogenates have been analyzed by GC/EI-MS, GC/ECD and more recently, GC/ECNI-MS to identify and quantitate MeSO₂-PCB and -4,4'-DDE compounds. ECNI-MS analysis of MeSO₂-PCBs was found to be 500 to 1000 times more sensitive than EI-MS¹⁷³. However, GC/ECNI-MS was used only for MeSO₂-PCB chlorinated isomer group and MeSO₂-4,4'-DDE characterization^{168,171,174,175}, not as a quantitative technique. The GC/ECNI-MS response characteristics of MeSO₂-PCBs and -4,4'-DDE need to be defined for application of this detection technique in the routine determination of the methyl sulfone-containing compounds.

GC/ECNI-MS has become popular for the detection of electrophilic CHC compounds since the introduction of buffering reaction gases such as methane^{176,177}. Buffering gases capture high energy electrons yielding de-energized, or, "thermalized" secondary electrons that are captured by analytes possessing energetically low-lying, vacant molecular orbitals^{176,178,179}. GC/EI-MS and GC/ECNI-MS have been useful for the MeSO₂-PCB and -4,4'-DDE identification, however, structural characterization of MeSO₂-PCBs from fragmentation patterns has been limited to the differentiation of *ortho*-MeSO₂-/*ortho*'-Cl-substituted MeSO₂-PCBs from other congeners^{173,180-182}. Therefore, the ECNI mass spectrometric properties of MeSO₂-PCBs are presently incomplete^{173,180-182}.

1.6 Objectives and Scope

The significance of CHCs in the Arctic, relative to more polluted environments in southern latitudes, is essentially unknown. Of greatest concern is the potential impact of

lipophilic CHCs on high trophic level mammals which possess large stores of body fat. The polar bear may be particularly vulnerable because of its exclusive diet of ringed seal blubber and unique metabolic capability. A full characterization of all CHC residues, their levels and potential effects is therefore necessary to assess risk.

Several aspects of the ecological and analytical chemistry of CHC and MeSO₂-PCB and -4,4'-DDE compounds are addressed in the present thesis. An integrated analytical method is presented, amenable to routine determination of MeSO₂-PCB/DDE metabolites and the parent PCB and 4,4'-DDE compounds. The method is validated using GC/ECD and GC/ECNI-MS, for several complex biological matrices. The response characteristics of MeSO₂-PCB and -4,4'-DDE compounds, among GC/ECD and GC/ECNI-MS in the SIM and TIC modes, are compared to determine which technique(s) are the best for precise and accurate quantitation. The sensitivity of the detection techniques for MeSO₂-PCBs and -4,4'-DDEs, isolated from tissue using the new analytical methodology, is evaluated by comparing the instrumental detection limits (IDLs) to the corresponding method detection limits (MDLs) and practical quantitation limits (PQLs). The stability of ECD, SIM and TIC molar response factors (MRFs) for the same MeSO₂-metabolites is also determined over a mass range from ca. 1 to 2 ng down to PQL levels. The constancy of the MRFs relative to an internal standard (RMRFs) among congeners is evaluated to determine if isomer-group RMRFs could be used for MeSO₂-PCB and -4,4'-DDE quantitation in environmental samples. A detailed examination of MeSO₂-PCB structure-fragmentation relationships in the GC/ECNI mass spectra is described to determine if additional MeSO₂-PCB structural information can be derived from fragmentation patterns. At the initiation of the thesis, certain

MeSO₂-PCB and -4,4'-DDE standards were available. As the thesis progressed, more standards became available. The results of the individual projects reflect the availability of authentic standards, and are noted accordingly in each chapter.

It has often been assumed that the parent compounds, rather than their metabolites, are of the greatest toxicological concern. MeSO₂-PCB and -4,4'-DDE metabolites are clearly persistent CHCs, however, little is known of their identities and levels in wild mammals and lesser still of their bioaccumulation potential and toxicity. In the present study, the ecodynamics, geographic distribution and biological effects of MeSO₂-PCBs and -4,4'-DDEs in the polar bear food chain are explored to determine the overall significance of these metabolites in this important element of the arctic ecosystem.

MeSO₂-PCB and -4,4'-DDE identities, levels, patterns and their relationship to PCBs and 4,4'-DDE within the polar bear-ringed seal-arctic cod food chain, are determined to evaluate the factors which effect the bioaccumulation of these metabolites. The metabolic capacity among species and therefore, the relative significance of metabolite formation, clearance and retention of MeSO₂-PCB and -4,4'-DDE is described. The metabolite tissue distribution in polar bear is also examined to reveal the possibility of preferential tissue localization, a potential indicator of biological activity through specific protein binding. The temporal dynamics and lactational transfer of MeSO₂-PCB and -4,4'-DDE metabolites are determined for the fat and milk of a polar bear mother and her cubs, over an annual regime of hyperphagia and fasting. Cubs may be more at risk due to exposure to high levels of potentially toxic MeSO₂-PCBs and -4,4'-DDE via lactational transfer from the mother.

The CYP1A and CYP2B isozyme protein levels are quantitated immunochemically

and related to CHC residue levels. Similarly, the alkoxy-*O*-dealkylase activities in polar bear hepatic microsomes are determined and related to both the immunoquantitated protein levels and the CHC residue levels. Further, antibody inhibition studies are carried out to determine which CYP isozymes are responsible for the observed alkoxy-*O*-dealkylase activities. Immunoquantitation of CYP isozymes may be a more reliable alternative to CYP enzyme activity as an indicator of CHC exposure.

An Integrated Analytical Method for Determination of Polychlorinated Aryl Methyl Sulfone Metabolites and Polychlorinated Hydrocarbon Contaminants in Biological Matrices[†]

2.1 Background

A simple column chromatography method was developed for the separation of CHCs and aryl methyl sulfonyl-containing metabolites from lipid-containing tissues. Existing analytical methodologies for aryl methyl sulfone clean-up and separation have exploited their Lewis basicity, strong acid/base stability and high polarity by incorporation of liquid-liquid partitioning strategies ^{71,134,138,163,168,171,172}. The present method takes advantage of aryl methyl sulfone polarity, which is higher than most other biologically persistent CHCs, such as PCBs and chlorinated insecticides (e.g., DDT and chlordanes), and the smaller molecular size of aryl methyl sulfones and CHCs than that of biogenic co-extracting compounds, mainly triglycerides.

GC/ECD was used to determine the percent recovery of: 1) standard mixtures of tetra- to heptachloro-, 3- and 4-MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE which are biologically significant ^{71,175} (see Chapters 4, 5 and 6) and MeSO₂-IS spiked to herring gull egg, whole

[†] Adapted from *Anal. Chem.*, 1995, 67:4155-4163.

fish (smelt), polar bear liver and polar bear adipose tissue homogenates; and 2) the same MeSO₂-PCBs, 3-MeSO₂-DDE, MeSO₂-IS, dichloro- to octachloro-PCBs and TCPMeOH spiked to contaminant-free lipid extracts of the same samples prepared by gel-permeation chromatography (GPC). The polarity of TCPMeOH is at the high end of routinely determined CHCs, such as dieldrin, and separation of TCPMeOH from MeSO₂-PCBs and -DDE is a good test of the method.

The aryl methyl sulfone fractions obtained from tissue homogenates were analyzed by GC/ECD as well as by GC/ECNI-MS to evaluate residual biogenic and xenobiotic interferences other than MeSO₂-PCBs and -4,4'-DDE. The response characteristics among GC/ECD and GC/ECNI-MS in the SIM and TIC modes were compared to determine which technique(s) were the best for precise and accurate MeSO₂-PCB and -4,4'-DDE quantitation. GC/ECNI-MS has been used for MeSO₂-PCB chlorinated isomer group and MeSO₂-4,4'-DDE characterization^{168,171,174,175} but not as a quantitative technique. Technique sensitivity was evaluated by comparing the instrumental detection limits (IDLs) of fifteen MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE to the corresponding method detection limits (MDLs) and practical quantitation limits (PQLs) determined from CHC-free lipid extracts of polar bear liver. The stability of ECD, SIM and TIC molar response factors (MRFs) for the same MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE were determined over a mass range from ca. 1 to 2 ng down to PQL levels. The constancy of the relative MRFs (RMRFs) to an internal standard among congeners was evaluated to determine if isomer-group RMRFs could be used for MeSO₂-PCB and -4,4'-DDE quantitation in environmental samples.

2.2 Experimental

2.2.1 Chemicals and Standards

Standards of MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE and the MeSO₂-IS were synthesized and provided courtesy of Dr. Å. Bergman (Wallenberg Laboratory, Stockholm University, Stockholm, Sweden) and Dr. K. Haraguchi (Daiichi Pharmaceutical College, Fukuoka, Japan) ¹⁷³. Each standard was received as an individual solution in 2,2,4-trimethylpentane. The MeSO₂-PCB labelling system (Table 2.1) was based on the IUPAC numbering system for PCBs ¹². The PCB standard mixtures, CLB-1-D and CLB-1-C, were obtained from the National Research Council (NRC) of Canada. TCPMeOH was from MTM Research Chemicals (Lancaster Synthesis Inc., Windham, NH, U.S.A.). All solvents were of analytical grade or better.

2.2.2 Verification of Standard Concentrations

Individual MeSO₂-PCB and -4,4'-DDE standards were available at various times over the course of this thesis research. At the time of the GC/FID study, twenty-two individual standards were available, whereas seventeen standards were available when the GC/EI-MS(TIC) study was performed (see Chapter 5). Both techniques were applied and compared to verify and/or correct the nominal concentration of the forementioned, individual MeSO₂-PCB/-4,4'-DDE standard solutions. The concentrations of the standards received after the present study, 4-MeSO₂-CB31, 3-MeSO₂-CB64, 3-MeSO₂-CB70, 4-MeSO₂-CB91 and 3-MeSO₂-CB95a (Table 2.1), were verified by comparison of their relative ECD response to

Table 2.1. MeSO₂-PCB, MeSO₂-4,4'-DDE and MeSO₂-IS Chemical Abbreviations

GC Peak Number* in Fig. 2.2 and 4.2	Substituted Biphenyl Structural Formula [†]	Compound Abbreviation [†]
1 ^σ	3-CH ₃ SO ₂ -2,4',5-Cl ₃ CB	3-MeSO₂-CB31
2	4-CH ₃ SO ₂ -2,4',5-Cl ₃ CB	4-MeSO₂-CB31
3	3-CH ₃ SO ₂ -2,2',5,5'-Cl ₄ CB	3-MeSO₂-CB52
4	3-CH ₃ SO ₂ -2,2',4',5-Cl ₄ CB	3-MeSO₂-CB49
5	4-CH ₃ SO ₂ -2,2',5,5'-Cl ₄ CB	4-MeSO₂-CB52
6	4-CH ₃ SO ₂ -2,2',4',5-Cl ₄ CB	4-MeSO₂-CB49
7	3-CH ₃ SO ₂ -2,4',5,6-Cl ₄ CB	3-MeSO₂-CB64
8	3-CH ₃ SO ₂ -2,2',5,5',6-Cl ₅ CB	3-MeSO₂-CB95a
9	4-CH ₃ SO ₂ -2,4',5,6-Cl ₄ CB	4-MeSO₂-CB64
10	3-CH ₃ SO ₂ -2,2',4',5,6-Cl ₅ CB	3-MeSO₂-CB91
11	4-CH ₃ SO ₂ -2,2',5,5',6-Cl ₅ CB	4-MeSO₂-CB95a
12	4-CH ₃ SO ₂ -2,2',4',5,6-Cl ₅ CB	4-MeSO₂-CB91
13	3-CH ₃ SO ₂ -2,3',4',5-Cl ₄ CB	3-MeSO₂-CB70
14	3-CH ₃ SO ₂ -2,2',4',5,5'-Cl ₅ CB	3-MeSO₂-CB101
15	4-CH ₃ SO ₂ -2,3',4',5-Cl ₄ CB	4-MeSO₂-CB70
16	4-CH ₃ SO ₂ -2,2',4',5,5'-Cl ₅ CB	4-MeSO₂-CB101
17	3-CH ₃ SO ₂ -4,4'-DDE	3-MeSO₂-4,4'-DDE
18	3-CH ₃ SO ₂ -2,2',3',4',5-Cl ₅ CB	3-MeSO₂-CB87
19	3-CH ₃ SO ₂ -2,3',4',5,6-Cl ₅ CB	3-MeSO₂-CB110
20	3-CH ₃ SO ₂ -2,2',4',5,5',6-Cl ₆ CB	3-MeSO₂-CB149
21	4-CH ₃ SO ₂ -2,3',4',5,6-Cl ₅ CB	4-MeSO₂-CB110
22	4-CH ₃ SO ₂ -2,2',3',4',5-Cl ₅ CB	4-MeSO₂-CB87
23	4-CH ₃ SO ₂ -2,2',4',5,5',6-Cl ₆ CB	4-MeSO₂-CB149
24	3-CH ₃ SO ₂ -2,2',3',4',5,6-Cl ₆ CB	3-MeSO₂-CB132
25	4-CH ₃ SO ₂ -2,2',3',4',5,6-Cl ₆ CB	4-MeSO₂-CB132
26	3-CH ₃ SO ₂ -2,2',3',4',5,5'-Cl ₆ CB	3-MeSO₂-CB141
27	4-CH ₃ SO ₂ -2,2',3',4',5,5'-Cl ₆ CB	4-MeSO₂-CB141
28	3-CH ₃ SO ₂ -2-CH ₃ -2',3',4',5,5'-Cl ₅ CB	MeSO₂-IS
29	3-CH ₃ SO ₂ -2,2',3',4',5,5',6-Cl ₆ CB	3-MeSO₂-CB174
30	4-CH ₃ SO ₂ -2,2',3',4',5,5',6-Cl ₆ CB	4-MeSO₂-CB174

* In order of elution from a DB-5 GC column.

[†] See Figure 1.4 for position of substitution on the biphenyl system.

[†] Chlorine substitution pattern of the precursor PCB congener defined by the IUPAC numbering system¹. The IUPAC numbering for the MeSO₂-CB95 metabolites are denoted by an, "a" to differentiate between the other possible MeSO₂-CB95 structure where the MeSO₂-functional group is on the 2,5-dichloro-phenyl ring. Standard concentrations for the MeSO₂-PCB congeners in bold were not verified by GC/FID (see Table 2.2). The ECD RRF of their isomer pair was used to verify the concentrations.

^σ An authentic standard for 3-MeSO₂-CB31 was not available.

the MeSO₂-IS (RRF), to their 3- or 4-MeSO₂-isomer pair.

For the GC/FID study, two standard mixtures (nominal concentration 60 ng/μl/congener) were prepared, one with fifteen MeSO₂-PCBs, MeSO₂-IS and eleven dichloro- to octachloro-PCBs (NRC-CLB-1-C) and the other with seven MeSO₂-PCBs, 3-MeSO₂-DDE, MeSO₂-IS and the NRC-CLB-1-C solution (Table 2.2). The PCB standards were included for comparison to the methyl sulfone standards since their concentrations were precisely known. Two standard mixtures were prepared to ensure complete GC resolution of the standards. The molar carbon response factors (MCRFs) were determined in triplicate. The injection technique for GC/FID was on-column whereas for GC/EI-MS(TIC) was splitless. GC/FID and GC/EI-MS(TIC) parameters are defined below.

The FID response of halogenated hydrocarbons, even those containing heteroatoms, has been shown to be relatively constant when adjusted for the fraction of the molecular weight (wt.) that is carbon ^{183,184}. This expression of FID response is often considered as the best approximation of the true relative concentrations among analytes in the same injected solution ¹⁸³. To express the nominal fraction molar carbon response factor for each congener (MCRF_i), the following equation was used;

$$\text{MCRF}_i = [(A_{\text{FID}})(\text{MW}(\text{c})_i)] / m_i \quad (2)$$

where m_i is the nominal MeSO₂-PCB/-4,4'-DDE congener mass injected (i.e. = nominal concentration, (g/μl) × volume injected (μl)), $\text{MW}(\text{c})_i$ is the congener molecular weight that is carbon, and A_{FID} is the FID chromatographic peak area of the congener. The mean MCRF

Table 2.2. Nominal GC/FID Molar Carbon Response Factors (MCRFs) and GC/EI-MS(TIC) Molar Response Factors (MRFs) for MeSO₂-PCB, 3-MeSO₂-4,4'-DDE and MeSO₂-IS

Congener	MCRF (FID)($\times 10^{14}$) ^a	MRF (EI-MS(TIC)) ($\times 10^{18}$) ^a
3-MeSO ₂ -CB49	6.16 $\pm$ 0.10	3.19 $\pm$ 0.12
4-MeSO ₂ -CB49	6.50 $\pm$ 0.11	3.33 $\pm$ 0.08
3-MeSO ₂ -CB52	6.33 $\pm$ 0.10	n.a.
4-MeSO ₂ -CB52*	9.10 $\pm$ 0.09	n.a.
4-MeSO ₂ -CB64	7.24 $\pm$ 0.11	3.27 $\pm$ 0.12
4-MeSO ₂ -CB70	4.64 $\pm$ 0.21	n.a.
3-MeSO ₂ -CB87	7.24 $\pm$ 0.18	2.91 $\pm$ 0.17
4-MeSO ₂ -CB87	6.56 $\pm$ 0.12	3.76 $\pm$ 0.10
3-MeSO ₂ -CB91*	1.35 $\pm$ 0.04	n.a.
4-MeSO ₂ -CB95*	2.74 $\pm$ 0.13	n.a.
3-MeSO ₂ -CB101	4.48 $\pm$ 0.08	2.71 $\pm$ 0.11
4-MeSO ₂ -CB101	4.14 $\pm$ 0.08	3.24 $\pm$ 0.16
3-MeSO ₂ -CB110	5.26 $\pm$ 0.19	n.a.
4-MeSO ₂ -CB110	5.79 $\pm$ 0.18	n.a.
3-MeSO ₂ -CB132	6.31 $\pm$ 0.14	4.26 $\pm$ 0.22
4-MeSO ₂ -CB132*	10.66 $\pm$ 0.15	7.15 $\pm$ 0.12
3-MeSO ₂ -CB141	4.16 $\pm$ 0.07	2.90 $\pm$ 0.15
4-MeSO ₂ -CB141	7.19 $\pm$ 0.17	3.46 $\pm$ 0.24
3-MeSO ₂ -CB149	6.61 $\pm$ 0.13	3.68 $\pm$ 0.10
4-MeSO ₂ -CB149*	3.02 $\pm$ 0.06	1.17 $\pm$ 0.18
3-MeSO ₂ -CB174	6.52 $\pm$ 0.11	3.48 $\pm$ 0.17
4-MeSO ₂ -CB174	5.10 $\pm$ 0.13	3.21 $\pm$ 0.23
3-MeSO ₂ -DDE*	3.69 $\pm$ 0.17	1.10 $\pm$ 0.12
MeSO ₂ -IS	4.51 $\pm$ 0.06	3.78 $\pm$ 0.13
Mean	5.57 $\pm$ 1.98	3.35 $\pm$ 1.32
Selected mean [†]	5.82 $\pm$ 1.05	3.42 $\pm$ 0.41
mean S-PCBs [†]	5.89 $\pm$ 1.15	3.40 $\pm$ 0.95

^a Mean MRF or MCRF $\pm$ SD of triplicate analyses for each congener, peak area counts $\times$ MW(c) / grams injected.

[†] The selected mean MCRF and MRF did not include congeners marked with an asterisk for FID or EI-MS(TIC), respectively.

[†] Mean MCRF (FID) or MRF (EI-MS(TIC)) of eleven PCBs in the CLB-1-C (NRC) standard mixture. n.a. = not analyzed.

(eq. 1) among the eleven PCB congeners in the primary standard was 5.89 ± 1.15 peak area counts $\times$ total carbon wt. of MW / grams injected. The mean MCRF of the twenty-two MeSO₂-PCB congeners, 3-MeSO₂-DDE, and MeSO₂-IS (Table 2.2) was 5.57 ± 1.98 peak area counts $\times$ total carbon wt. of MW / grams injected. MCRFs of 3-MeSO₂-DDE and five out of the twenty-two MeSO₂-PCB standards fell outside the mean $\pm$ SD range for PCBs. If these five MeSO₂-PCBs and 3-MeSO₂-DDE were excluded, the mean MCRF of the remaining seventeen MeSO₂-PCB and IS standards was 5.82 ± 1.05 , the same as that of the PCBs. Therefore, the nominal concentrations of the six outliers were adjusted by dividing by the ratio of their MCRFs to the average MCRF of the PCB and other MeSO₂-PCB standards.

An alternative MeSO₂-PCB and -4,4'-DDE standard concentration correction method using GC/EI-MS(TIC) was based on the results of Parker *et al.*¹⁸⁵. The relative GC/EI-MS(TIC) mass chromatographic response factors of mass, within and among the PCDD, PCDF, PCB, PCDE and PCN compound classes, were found to be fairly constant. This was also found to be true when using the peak area of the molecular ion cluster (MIC), however, a mass discrimination effect may exist if a quadrupole as opposed to a magnetic sector MS instrument is used.

GC/EI-MS(TIC) molar response factors (MRFs) for each MeSO₂-PCB and 4,4'-DDE using eq. 3 (Table 2.2), were determined from one standard mixture of seventeen MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE and MeSO₂-IS.

$$\text{MRF}_i = [(A_{\text{PIC}})(\text{MW}_i)] / m_i \quad (3)$$

where m_i is the nominal MeSO_2 -PCB and -4,4'-DDE congener mass injected (i.e. = nominal concentration, $(\text{g}/\mu\text{l}) \times \text{volume injected } (\mu\text{l})$) concentration in the mixture, MW_i is the congener molecular weight, and A_{TIC} is the area of the TIC mass chromatographic peak of the congener. The MeSO_2 -PCBs and 3- MeSO_2 -4,4'-DDE standards found to have outlying MCRF, in GC/FID, also had outlying MRF, in the GC/ECNI-MS(TIC) analysis. The mean MRF, of the MeSO_2 -compound standards in absence of the outliers (3.42 ± 0.41) were similar to the mean MRF, of the S-PCB standards (3.40 ± 0.95). The relative MRFs and MCRFs of TIC and FID, respectively, can both be used to approximate the concentrations of MeSO_2 -PCBs, 3- MeSO_2 -4,4'-DDE and MeSO_2 -IS standard solutions.

2.2.3 Column Chromatographic Materials and Apparatus

Florisil (magnesium silicate, BDH Chemical Inc., Toronto, ON, Canada, pesticide analysis grade, 60-100 mesh) was activated at 600°C overnight, cooled to 100°C , deactivated with 1.2 % (w/w) doubly distilled, n-hexane washed water and stored in a capped (teflon lined) glass bottle. Basic aluminum oxide (Fisher Scientific Inc., Ottawa, ON, Canada, activity grade I, 60-325 mesh) and silica gel (Bio-Rad Laboratories, Richmond, CA, U.S.A, Bio-sil A, 100-200 mesh) were activated at 300°C and 180°C , respectively, for 3 hours. The activated materials were stored at 100°C in an open top glass bottle for no more than 1 month before reactivation. The basic alumina was deactivated with 2.3 % (w/w) doubly distilled, n-hexane washed water 0.5 hour prior to use. Potassium hydroxide (KOH)/silica gel (33 % KOH (w/w)) was prepared by combining 1M KOH (Fisher Scientific Inc., Ottawa, ON, Canada, prepared with n-hexane washed, doubly distilled water) with the

appropriate amount of activated silica gel 0.5 hour prior to use. Sodium sulphate (BDH Chemical Inc., Toronto, ON, Canada, anhydrous, granular, analytical grade) was washed with 50:50 dichloromethane/n-hexane, air evaporated overnight, activated at 650°C overnight, cooled to room temperature and stored in a teflon lined, capped bottle. GPC was performed using an ABC Laboratories automated system. A 2.5 cm diameter column was packed with 60 g of SX-3 Envirobeads (ABC Laboratories, Columbia, MO, U.S.A, 200-400 mesh) after pre-soaking ~ 1 hour in 50:50 dichloromethane/n-hexane. Pyrex glass columns 1.0 cm in diameter were used for all liquid chromatography.

2.2.4 Tissue Sample Preparation, Standard Spiking and Extraction

Samples archived at -40°C in the specimen bank at the National Wildlife Research Centre in Hull, QU, Canada were used for method validation. Lake Ontario (1989) herring gull (*Larus argentatus*) egg and Lake Huron (1985) smelt (*Osmerus mordax*) samples were obtained from composites of 118 and 300 individual samples, respectively. Polar bear (*Ursus maritimus*) liver homogenate and minced adipose samples were from an adult male collected in 1993 from the Resolute Bay area of the Northwest Territories, Canada.

Homogenized or minced samples were dehydrated by grinding with sodium sulphate in a 1:5 weight ratio. Tissue substrates for method validation were spiked with one of three standard mixtures (Figure 2.1, position A) consisting of fifteen or twelve MeSO₂-PCBs, 3-MeSO₂-DDE and IS (Table 2.1). Spiked MeSO₂-PCB and -DDE levels (50 µl of 52 to 552 pg/µl) approximately matched native levels in 0.5 g wet weight of polar bear liver and adipose tissue to minimize error due to subtraction of GC/ECD peak areas. Native levels in

herring gull egg (5.0 g) and smelt (15.0 g) were low ng/g wet weight level and non-detectable, respectively, and therefore did not require spiking concentration adjustment. Herring gull egg and smelt homogenates were spiked with the same concentrations of MeSO₂-PCB, 3-MeSO₂-DDE and IS standards used for contaminant-free lipid extracts (see below).

Lipid extracts were obtained by column extraction of the tissue/Na₂SO₄ mixtures with 80 ml of 50:50 dichloromethane/n-hexane. The lipid containing dump cycle of the GPC (see next section) was free of CHCs that can be separated by gas chromatography. The GPC dump cycle was spiked with 50 µl of a CLB-1-D PCB (30 to 295 pg/µl) and TCPMeOH (500 pg/µl) standard mixture and 50 µl of a fifteen MeSO₂-PCB (211 to 604 pg/µl), 3-MeSO₂-DDE (595 pg/µl) and MeSO₂-IS (436 pg/µl) standard mixture (Table 2.1) prior to a second cycle of the GPC (Figure 2.1, position B). Contaminant-free lipid extract weights and MeSO₂-PCB and MeSO₂-DDE spiking levels were chosen to simulate co-extracted lipid loads and MeSO₂-metabolite levels encountered in the analysis of polar bear liver and adipose tissue ^{71,175}. PCBs were spiked only to contaminant-free lipid extracts because of relatively high natural PCB levels in the tissue substrates.

2.2.5 Contaminant Enrichment and Clean-up Procedure

The first step in enrichment involved removal of lipids by GPC ¹⁸⁶. After rotary evaporation, the lipid extract was diluted to 10 ml with 50:50 dichloromethane/n-hexane. An accurate 10 % volumetric portion was removed to determine lipid gravimetrically. The remaining volume was readjusted to 10 ml, vortexed and loaded onto the GPC. The first 140

Figure 2.1. Schematic diagram of CHC and MeSO₂-PCB and -4,4'-DDE column chromatography methodology. MeSO₂-PCB, 3-MeSO₂-4,4'-DDE and MeSO₂ IS standards were spiked at point A and MeSO₂-PCB, 3-MeSO₂-4,4'-DDE, MeSO₂-IS, PCB and TCPMeOH standards at point B (Table 2.1) for method validation. The part of the schematic in dashed lines was for method validation with contaminant-free lipid extracts only.

ml fraction containing the lipids was dumped or used for contaminant-free spiking. The second fraction (150 ml) containing CHCs and metabolites was collected, reduced in volume to ~ 2 ml by rotary evaporation and eluted through a 33 % KOH/silica gel column (1.5 g) with 50 ml of 50:50 dichloromethane/n-hexane. The extract volume was reduced to ~1 ml and chromatographed on Florisil (8.0 g). The first fraction (75 ml of 50:50 dichloromethane/n-hexane) contained all routinely-determined CHCs, including PCBs and TCPMeOH, substantially the same as the sum of the three-fractions in the method outlined in Norstrom *et al.*^{46,186}. The second fraction (80 ml of 7:93 methanol:dichloromethane) contained the aryl methyl sulfones. This fraction was rotary evaporated at 30°C under reduced pressure until just dry. The residue was re-dissolved in n-hexane and chromatographed on a 2.3 % H₂O deactivated basic alumina column (3.0 g). The first 10 ml 50:50 dichloromethane/n-hexane was discarded and the second 40 ml fraction containing aryl methyl sulfones was collected using the same solvent mixture. The aryl methyl sulfone and CHC fractions were adjusted to 1 to 2 ml by rotary evaporation and quantitatively transferred to 5 ml, acid-washed borosilicate glass vials with 3 × 0.5 ml n-hexane washes and ~50 µl of 2,2,4-trimethylpentane. The volume was reduced to ~100 µl by a gentle stream of N₂ gas (99.99 % purified) at ambient room temperature. The vial sides were washed down with ~100 µl of 2,2,4-trimethylpentane and the volume adjusted very slowly at ambient temperature by N₂-assisted solvent evaporation to 50 µl followed by brief vortexing and transfer to a GC injection vial.

2.2.6 Instrumental Analysis

GC/ECD. MeSO₂-PCBs were quantitated in environmental samples by MeSO₂-IS methods using GC/ECD and GC/ECNI-MS congener response factors relative to the MeSO₂-IS. The GC/ECNI-MS parameters are described below. Capillary GC/ECD was performed on a Hewlett-Packard (HP, Palo Alto, CA, U.S.A.) 5890 equipped with a ⁶³Ni ECD detector and HP 7673A automatic injector. The GC was fitted with a fused silica, Rtx-5 capillary column (Restek Corp., Bellefonte, PA, U.S.A., 30 m, 0.25 mm i.d., 0.1 µm film thickness, cross-linked 95 % dimethyl-5 % diphenyl polysiloxane). The carrier gas and ECD make-up gas were helium and 5 % methane-95 % argon, respectively. All injections were 2 µl in volume and made in the splitless mode. GC conditions and temperature ramping were optimized for MeSO₂-PCB and -4,4'-DDE separation. The injector and detector temperatures were set at 270°C and 330°C, respectively. The GC oven temperature program was as follows: initial temperature held at 100°C for 3 min, 20°C/min to 220°C and 3°C/min to 280°C. The injector and detector temperatures were 250°C and 320°C, respectively, for PCBs and TCPMeOH. The GC oven temperature program was as follows: initial temperature held at 70°C for 2 min, 5°C/min to 150°C and 3°C/min to 250°C.

GC/FID. The instrument and capillary column for validation of MeSO₂-PCB and -DDE standard concentrations were the same as for GC/ECD. The MeSO₂-PCB and -4,4'-DDE and MeSO₂-IS injection technique was cold on-column using the "four segment injection" approach¹⁸³: 0.5 µl pure solvent (2,2,4-trimethylpentane), 0.5 µl air, 0.6 µl sample and 0.8 µl air. On-column injection was used to prevent discrimination of these relatively nonvolatile compounds. The detector temperature was 350°C and the GC oven temperature

program was as follows: initial temperature held at 85°C for 3 min, 20°C/min to 200°C and 2.2°C/min to 265°C. The carrier gas was helium. The make-up gas had a total flow rate of 440 ml/min (390 ml/min compressed air, 30 ml/min helium and 30 ml/min hydrogen).

GC/ECNI-MS and GC/EI-MS. GC/ECNI-MS was used to confirm MeSO₂-PCB identities by total ion current (TIC) scanning from 83 to 550 amu. GC/EI-MS(TIC) was used to verify and/or correct the MeSO₂-PCB and -4,4'-DDE standard concentrations (Table 2.2) by scanning for ions over the same mass-to-charge (m/z) range as GC/ECNI-MS. For GC/ECNI-MS in the selected ion monitoring (SIM) mode, the (M)⁺ and (M+2)⁺ ions were scanned and the sum of their response was determined for each congener. The ions scanned in GC/ECNI-MS and the ion retention time windows for the trichloro- to heptachloro-MeSO₂-PCB isomer groups, MeSO₂-IS and MeSO₂-4,4'-DDEs, are listed in Table 2.3.

GC/ECNI-MS and GC/EI-MS were performed on separate HP 5985 mass spectrometers upgraded with a HP 5890 series II gas chromatograph coupled with a HP 5988 GC/MS direct interface. Both GCs were fitted with non-polar DB-5 columns. The DB-5 column was the same in every respect as the Rtx-5 capillary column used for GC/ECD, with the exception that the DB-5 has a lower temperature maximum and a shorter useable lifetime. All injections were performed in the splitless mode. The carrier and reagent gas for GC/ECNI-MS were helium and methane (0.5 mbar), respectively, whereas helium was the carrier gas for GC/EI-MS. The instruments were tuned with perfluorotributylamine (PFTBA), using ions of m/z 312, 414 and 464 for ECNI-MS and ions of m/z 69, 219 and 502 for EI-MS. The emission currents were 300 µA and the electron voltages were 130 eV. The injection port, transfer line and ion source temperatures were 270°C, 260°C and 140°C,

Table 2.3. GC/ECNI-MS Retention Time Windows and the Ions Monitored for Specific MeSO₂-PCB and -DDE Metabolites Determined by the MeSO₂-IS Method^{†*}

Time Range (min)	Compound	Target/Qualifier	Time Range (min)	Compound	Target/Qualifier
18.0-26.0	3-MeSO ₂ -CB31	334/336	26.0-30.0	3-MeSO ₂ -4,4'-DDE [‡]	396/394
	4-MeSO ₂ -CB31	334/336		3-MeSO ₂ -CB87	404/402
	3-MeSO ₂ -CB52	368/370		3-MeSO ₂ -CB110	404/402
	3-MeSO ₂ -CB49	368/370		3-MeSO ₂ -CB149	438/436
	4-MeSO ₂ -CB52	368/370		4-MeSO ₂ -CB110	404/402
	4-MeSO ₂ -CB49	368/370		4-MeSO ₂ -CB87	404/402
	3-MeSO ₂ -CB64	368/370		4-MeSO ₂ -CB149	438/436
	3-MeSO ₂ -CB95	404/402		3-MeSO ₂ -CB132	438/436
	4-MeSO ₂ -CB64	368/370	30.0-35.7	4-MeSO ₂ -CB132	438/436
	3-MeSO ₂ -CB91	404/402		3-MeSO ₂ -CB141	438/436
	4-MeSO ₂ -CB95	404/402		4-MeSO ₂ -CB141	438/436
	4-MeSO ₂ -CB91	404/402		MeSO ₂ -IS	420/418
	3-MeSO ₂ -CB70	368/370		3-MeSO ₂ -CB174	472/470
	3-MeSO ₂ -CB101	404/402		4-MeSO ₂ -CB174	472/470
	4-MeSO ₂ -CB70	368/370			
	4-MeSO ₂ -CB101	404/402			

[†] MeSO₂-PCBs and 4,4'-DDEs listed in order of retention time on a non-polar DB-5 or Rtx-5 GC column.

* Other MeSO₂-PCBs have been detected in seal samples (see Chapter 4), but were minor contributors.

[‡] The 2-MeSO₂-4,4'-DDE compound could be detected at low levels in some tissues.

respectively. The oven temperature programs was the same as that used for GC/ECD. A 2 μ l volume was used for all splitless injections.

2.2.7 Determination of Recoveries by GC/ECD

All analyses were done in triplicate. For the contaminant-free lipid extracts, herring gull egg and smelt substrates, areas of the MeSO₂-PCB, 3-MeSO₂-4,4'-DDE, PCB and TCPMeOH peaks were compared to those in an external standard to determine percent recoveries. The native MeSO₂-PCB and 3-MeSO₂-4,4'-DDE congener levels in polar bear liver and fat substrates were first determined by GC/ECD and twelve of the MeSO₂-PCB and 3-MeSO₂-4,4'-DDE standards were spiked to a second set of 0.5 g samples at matching levels. A spike of 100 μ l of 100 pg/ μ l MeSO₂-IS was also added (Figure 2.1, position A). The sample size was adjusted so that native MeSO₂-PCB and -DDE levels in the final fraction were in the 25-500 pg/ μ l range^{175,187}. The GC/ECD peak area contributions of the analytes of the unspiked replicates ($n = 3$) were subtracted from the spiked replicates ($n = 3$) for the tissue substrates. The difference in peak areas was compared to the original MeSO₂-PCB and -DDE standards to determine percent recoveries for method validation.

2.2.8 MeSO₂-PCB and MeSO₂-4,4'-DDE Response Parameters

A separate mixture of ~1 ng/ μ l per congener of the fifteen MeSO₂-PCB, 3-MeSO₂-4,4'-DDE and IS standards used for method validation (Table 2.1) was prepared and serially diluted down to ~0.01 pg/ μ l. The IDLs for ECD, SIM ((M)⁺ + (M+2)⁺) and TIC for each

congener were defined as the mass in picograms (pg) giving a signal-to-noise (S/N) ratio of three¹⁸⁸. Replicate ($n = 7$) 0.5 g wet weight equivalents of polar bear liver, contaminant-free lipid extract were spiked with the standard mixture at concentrations near the IDL for determination of the corresponding MDLs. The MDLs were defined as three times the significance of variation (SV) calculated using Student's t statistic. The SVs were calculated from the SDs ($n = 7$) using the appropriate Student t -values according to eq. 4;

$$SV = SD_{MDL} \times t_{(n-1, 95\%)} \quad (4)$$

where $t_{(n-1, 95\%)}$ was the t -distribution constant for $n-1$ degrees of freedom at the 95 % confidence level. The function of the SV calculation was to evaluate the variation in the MDL. The SV of the MDL can be used to judge the significance of a single measurement of a low level analyte in a future sample¹⁸⁹. The commonly accepted MDL is three times SV and the practical quantitation limit (PQL) is ten times the SV (i.e., 3.3 times the MDL)^{188,190}.

MRFs for ECD, SIM and TIC were determined in triplicate at several concentrations from ~ 1000-1700 pg down to the MDLs to determine the concentration range of linear response. The variance in the MRFs, IDLs and MDLs were assessed by a one-way analysis of variance (ANOVA) using Quattro Pro 6.0 (Novell Inc., Orem, Utah). The significance level of the TIC response variance was 95 % for $n = 3$ replicates at a given concentration and $n = 6$ concentrations. There were $n = 7$ different concentrations for ECD and SIM. The MRFs relative to the IS (RMRF) were also calculated.

2.3 Results

2.3.1 Recovery Studies

The fifteen MeSO₂-PCB standards chosen for method validation (Table 2.1) represented a majority of the twenty-two congeners identified so far in biota ^{71,168,171}. The mean percent recoveries obtained for the analyte standards spiked to contaminant-free lipid extracts and tissue substrates are shown in Tables 2.4 and 2.5, respectively. Percent recoveries were consistently greater than ca. 75 % regardless of tissue matrix or variation in sample weight. The SDs of the mean recoveries indicated there was no significant discrimination among MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE, MeSO₂-IS, PCBs and TCPMeOH, substrates or sample weights. High method precision was indicated by the low SD of percent recovery of individual congeners, which ranged from 0.3 % to 10 %, but was generally between 1 % to 6 %.

2.3.2 MeSO₂-PCB and -DDE Congener Identity and Method Clean-up Efficiency

GC/ECD chromatograms of the aryl methyl sulfone fractions of unspiked polar bear fat and liver samples are shown in Figures 2.2a and b and the GC/ECNI-MS(TIC) mass chromatogram of unspiked polar bear liver is shown in Figure 2.2c. Virtually all of the peaks were identified as MeSO₂-PCB congeners by comparison to authentic standards, or as isomeric MeSO₂-PCBs from the GC/ECNI-MS mass spectra. Native levels of total MeSO₂-PCBs in polar bear liver and fat samples were ca. 2000 ng/g and ca. 400 ng/g on a lipid

Table 2.4. Percent Recovery for MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE, MeSO₂-IS, PCBs and TCPMeOH from Contaminant-Free Lipid Extracts Determined by GC/ECD[†]

Lipid extract wt. (g)	Herring Gull Egg 0.18	Smelt 0.15	Polar Bear Liver 0.019	Polar Bear Adipose 0.65
3-MeSO ₂ -CB49	78 ± 4	75 ± 6	71 ± 1	79 ± 2
4-MeSO ₂ -CB49	75 ± 6	76 ± 7	74 ± 2	77 ± 2
4-MeSO ₂ -CB64	81 ± 6	76 ± 8	79 ± 3	77 ± 5
3-MeSO ₂ -CB101	70 ± 7	74 ± 2	72 ± 1	79 ± 1
4-MeSO ₂ -CB101	74 ± 6	78 ± 6	76 ± 4	89 ± 5
3-MeSO ₂ -CB87	74 ± 3	72 ± 3	71 ± 2	79 ± 4
4-MeSO ₂ -CB87	83 ± 4	75 ± 8	76 ± 4	79 ± 2
3-MeSO ₂ -CB149	82 ± 4	77 ± 8	79 ± 4	82 ± 3
4-MeSO ₂ -CB149	83 ± 7	78 ± 7	83 ± 8	87 ± 2
3-MeSO ₂ -CB132	80 ± 5	76 ± 9	77 ± 2	78 ± 1
4-MeSO ₂ -CB132	81 ± 6	78 ± 9	78 ± 3	79 ± 3
3-MeSO ₂ -CB141	77 ± 5	75 ± 9	72 ± 2	78 ± 3
4-MeSO ₂ -CB141	80 ± 7	75 ± 8	76 ± 3	79 ± 3
3-MeSO ₂ -CB174	67 ± 4	69 ± 4	77 ± 2	81 ± 4
4-MeSO ₂ -CB174	75 ± 2	75 ± 4	79 ± 4	82 ± 2
3-MeSO ₂ -4,4'-DDE	85 ± 5	78 ± 8	71 ± 1	78 ± 2
MeSO ₂ -IS	74 ± 2	72 ± 1	74 ± 2	79 ± 3
Mean % recovery	78 ± 5	75 ± 3	76 ± 4	80 ± 3
Relative mean % recovery [‡]	105 ± 7	105 ± 3	102 ± 5	102 ± 4
CB-18	71 ± 4	79 ± 3	75 ± 1	74 ± 1
CB-54	71 ± 6	76 ± 1	79 ± 1	77 ± 1
CB-31	71 ± 6	77 ± 3	84 ± 3	76 ± 4
CB-49	74 ± 7	78 ± 4	78 ± 3	78 ± 4
CB-44	69 ± 5	80 ± 4	77 ± 2	77 ± 2
CB-40	67 ± 3	79 ± 4	90 ± 7	77 ± 3
CB-121	67 ± 5	81 ± 2	90 ± 3	78 ± 3
CB-86	77 ± 2	84 ± 3	81 ± 5	82 ± 1
CB-87	69 ± 4	77 ± 4	89 ± 5	80 ± 1
CB-77	74 ± 4	78 ± 3	83 ± 2	81 ± 2
CB-153	86 ± 7	77 ± 2	90 ± 3	86 ± 2
CB-159	84 ± 5	84 ± 2	88 ± 9	80 ± 3
CB-156	81 ± 6	80 ± 0.4	82 ± 3	78 ± 2
CB-209	84 ± 7	81 ± 0.3	85 ± 4	82 ± 2
PCBs, mean % recovery	75 ± 7	79 ± 1	84 ± 5	79 ± 3
PCBs, relative mean % recovery [‡]	101 ± 9	110 ± 3	113 ± 7	100 ± 4
TCPMeOH, relative mean %	116 ± 2	110 ± 3	114 ± 2	99 ± 3

[†] Percent recovery ± SD (*n* = 3 replicate samples). There are fourteen dichloro- to octachloro-PCBs in the NRC-CLB-1-D solution.

[‡] Relative to the MeSO₂-IS.

Table 2.5. Percent Recovery for MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE and the MeSO₂-IS from Tissue Substrates Determined by GC/ECD[†]

	Herring Gull Egg	Smelt	Polar Bear Liver	Polar Bear Adipose
Wet wt. (g)	5.0	15.0	0.5	0.5
% Lipid	10.0	4.4	2.7	65.0
3-MeSO ₂ -CB49	74 ± 6	74 ± 2	85 ± 5	82 ± 6
4-MeSO ₂ -CB49	83 ± 5	77 ± 2	82 ± 5	84 ± 6
4-MeSO ₂ -CB64	81 ± 6	80 ± 1	82 ± 4	82 ± 10
3-MeSO ₂ -CB101	75 ± 6	83 ± 3	80 ± 5	84 ± 7
4-MeSO ₂ -CB101	85 ± 4	77 ± 5	84 ± 5	80 ± 6
3-MeSO ₂ -CB87	71 ± 3	73 ± 1	87 ± 0.5	77 ± 6
4-MeSO ₂ -CB87	80 ± 6	76 ± 2	n.s.	n.s.
3-MeSO ₂ -CB149	68 ± 4	80 ± 4	n.s.	n.s.
4-MeSO ₂ -CB149	72 ± 10	75 ± 2	87 ± 5	85 ± 10
3-MeSO ₂ -CB132	77 ± 4	81 ± 2	n.s.	n.s.
4-MeSO ₂ -CB132	72 ± 3	80 ± 2	75 ± 8	88 ± 4
3-MeSO ₂ -CB141	77 ± 3	86 ± 3	71 ± 7	86 ± 3
4-MeSO ₂ -CB141	n.s.	75 ± 1	81 ± 7	85 ± 2
3-MeSO ₂ -CB174	n.s.	78 ± 3	90 ± 9	83 ± 6
4-MeSO ₂ -CB174	n.s.	80 ± 1	88 ± 4	88 ± 6
3-MeSO ₂ -4,4'-DDE	74 ± 4	75 ± 1	88 ± 0.6	78 ± 5
MeSO ₂ -IS	86 ± 2	75 ± 2	77 ± 4	80 ± 3
Mean % recovery	77 ± 5	78 ± 4	83 ± 5	83 ± 3
Relative mean % recovery [‡]	88 ± 6	104 ± 5	108 ± 7	104 ± 4

[†] Percent recovery ± SD (*n* = 3 replicate spiked samples minus *n* = 3 replicate unspiked samples) among congeners. See Table 2.1 for congener structures in each matrix spike.

[‡] Relative to the MeSO₂-IS.

n.s. = no spike.

Figure 2.2. Chromatograms of the aryl methyl sulfone fraction obtained from the column chromatography methodology. A) GC/ECD of polar bear fat, B) GC/ECD of polar bear liver and, C) GC/ECNI-MS(TIC) of polar bear liver. Peak numbers refer to the MeSO₂-PCB and -4,4'-DDE structures in Table 2.1. The "Cl_x" denotes a MeSO₂-PCB isomer group identified using GC/ECNI-MS(TIC). The approximate GC elution window for MeSO₂-PCB isomer groups are denoted in (A). Connecting lines denote the 3- and 4-MeSO₂-PCB congener pairs. The shaded peaks were non-MeSO₂-PCB or -4,4'-DDE compounds.

Figure 2.3. Representative ECNI mass spectra for A) 3-MeSO₂-CB101, B) 4-MeSO₂-CB101, and C) 3-MeSO₂-4,4'-DDE selected from the mass chromatogram of the aryl methyl sulfone fraction from polar bear liver (Figure 2.2). The molecular and major fragment ions are labelled. A MeSO₂-Cl₅CB impurity co-eluted with 3-MeSO₂-4,4'-DDE and is labelled.

weight basis, respectively, consistent with the results presented in Chapters 4, 5 and 6. The MeSO₂-PCB and -DDE congener pattern in herring gull egg was similar to that in polar bear adipose and liver (not shown). Herring gull egg had relatively high native levels of total MeSO₂-PCBs, estimated to be approximately 150 ng/g on a lipid weight basis. Despite substantial levels of PCBs and other CHCs, aryl methyl sulfones were not detectable in smelt. The aryl methyl sulfone elution window for smelt and herring gull egg substrates was also essentially free of interferences for both GC/ECD and TIC. GC/ECNI-MS mass spectra of native 3- and 4-MeSO₂-CB101 and 3-MeSO₂-DDE (peaks 14, 16, and 17, respectively, c) in polar bear liver are shown in Figures 2.3a, b and c, respectively. All ions arose from molecular and fragment anions of MeSO₂-PCBs and -4,4'-DDE. Similarly clean mass spectra were found in the aryl methyl sulfone fraction of all four tissue substrates. The GC/ECNI mass spectra of MeSO₂-PCBs and -DDEs are discussed in more detail in Chapter 3.

2.3.3 MeSO₂-PCB and MeSO₂-4,4'-DDE Response Parameters

The mean MDL was generally sub-pg for SIM and in the low pg range for ECD and TIC (Table 2.6). The mean MDL to mean SV ratios were ca. 2.5 for the three techniques. A ratio of ca. 3 is acceptable for reliable, low mass quantitation. The ratio of mean MDL to mean IDL was seven for SIM, eleven for TIC to twenty-seven for ECD. Analysis of variance (ANOVA) of the MDL and IDL data was performed to determine the relative significance of among-congener and among-replicate variance (Table 2.6). The among-congener variance was larger than among-replicate variance, especially for TIC. Trends in MDL and IDL values with respect to MeSO₂-PCB and -4,4'-DDE structure, were not obvious for any technique.

Table 2.6. Mean Instrumental (IDL) and Method (MDL) Detection Limits, for MeSO₂-PCBs, 3-MeSO₂-4,4'-DDE and MeSO₂-IS

	GC/ECD	GC/ECNI-MS(SIM)	GC/ECNI-MS(TIC)
IDL (pg) ^{†,°}	0.23 (87) [24]	0.08 (47) [34]	0.99 (37) [14]
MDL (pg) ^{†,°}	6.15 (85) [19]	0.55 (34) [39]	11.25 (59) [31]
SV (pg) [*]	2.42	0.21	4.44

[†] IDLs of the sulfone standards used for method validation (Table 2.3 and 2.4), $n = 3$ replicates analysis for each congener.

[†] For MDLs, the same standards used for IDL determination were spiked at concentration levels near the IDLs, to n CHC-free polar bear liver lipid extracts ($n = 6$ for ECD and TIC, $n = 7$ for SIM).

[°] The % CV contributions of among-congener variance (parentheses) and among-replicate variance [brackets] of total variance were determined by a one-way ANOVA.

^{*} Mean SV (significance of variation (eq. 4) of MDL) of congener MDLs.

The mean PQLs among congeners, defined as 10 times the SV, were 24.2 ± 4.6 pg for ECD, 2.1 ± 0.9 pg for SIM and 44.4 ± 17.1 pg for TIC.

Mean MRFs were relatively undeviating among MeSO₂-PCB congeners for all three techniques, especially SIM (Table 2.7). There was less than a factor of 2 difference between the highest and lowest mean MRF for each technique. There was a slight tendency for mean MRFs to increase with increasing degree of chlorination from four to seven chlorines per molecule. No consistent pattern in MRF values between 3-MeSO₂-PCB and 4-MeSO₂-PCB pairs could be discerned because differences were similar to the SDs for each congener and within the expected accuracy of the standard concentrations in most cases. By far the largest difference occurred between 3- and 4-MeSO₂-CB101. The mean MRF for 3-MeSO₂-4,4'-DDE was similar to those of the MeSO₂-PCBs. The constancy of MRFs was similar for all MeSO₂-PCB congeners using the three detection techniques over a concentration range from low pg levels up to 1.7 ng. The exact threshold congener masses at which serious deviation from linearity began were not determined, but they were less than the PQL.

Analysis of variance (ANOVA) of the MRF data was performed to determine the relative significance of among-mass and among-replicate variance, which are given as % CV in Table 2.7. The mean *F*-ratios among congeners for ECD (18.68 ± 14.68) and TIC (55.67 ± 38.17) far exceeded the critical value (2.51)¹⁹¹ for the null hypotheses that among-mass variance was the same as among-replicate variance. Thus, the among-mass variance was significantly larger (e.g., the mean % CV for ECD = 7.6) than among-replicate variance (e.g., the mean % CV for ECD = 2.3) for these techniques (Table 2.7). The mean *F*-ratio among congeners (3.04 ± 2.24) and *F*-critical value (2.85)¹⁹¹ were very close for SIM,

Table 2.7. Mean Molar Response Factors (MRFs) and MRFs Relative to the MeSO₂-IS (RMRFs) for MeSO₂-PCB and 3-MeSO₂-4,4-DDE[†]

Congener	GC/ECD		GC/ECNI- MS(SIM) (M) + (M+2) [‡]		GC/ECNI- MS(TIC)	
	MRF [‡]	RMRF [°]	MRF [‡]	RMRF [°]	MRF [‡]	RMRF [°]
3-MeSO ₂ -CB49	0.46 (8.6) [2.4]	0.66 (4.5)	1.33 (21.5) [13.2]	1.12 (19.6)	0.82 (9.0) [2.2]	0.78 (8.9)
4-MeSO ₂ -CB49	0.38 (5.2) [2.2]	0.56 (5.4)	1.62 (19.4) [13.8]	1.35 (14.8)	0.81 (9.9) [4.1]	0.77 (10.4)
4-MeSO ₂ -CB64	0.44 (8.5) [2.2]	0.63 (4.8)	1.33 (29.7) [15.6]	1.11 (22.5)	0.75 (9.7) [3.0]	0.71 (9.8)
3-MeSO ₂ -CB101	0.39 (7.4) [2.0]	0.57 (1.8)	0.96 (27.2) [16.5]	0.81 (21.0)	0.65 (9.0) [2.0]	0.62 (8.1)
4-MeSO ₂ -CB101	0.56 (8.3) [2.2]	0.81 (2.5)	0.73 (17.0) [16.2]	0.61 (18.0)	0.86 (8.1) [2.7]	0.82 (9.8)
3-MeSO ₂ -CB87	0.62 (9.1) [2.2]	0.89 (4.5)	1.24 (28.0) [22.7]	1.04 (24.0)	0.96 (12.1) [2.3]	0.90 (10.0)
4-MeSO ₂ -CB87	0.66 (9.5) [2.2]	0.95 (4.2)	1.29 (18.7) [5.8]	1.08 (7.4)	1.18 (9.6) [3.4]	1.12 (10.7)
3-MeSO ₂ -CB149	0.63 (6.2) [2.1]	0.91 (3.3)	1.34 (21.5) [13.4]	1.12 (17.8)	1.06 (11.5) [2.5]	1.01 (8.9)
4-MeSO ₂ -CB149	0.57 (8.7) [2.6]	0.82 (4.9)	1.45 (21.5) [15.0]	1.22 (21.3)	0.99 (10.8) [3.0]	0.93 (6.4)
3-MeSO ₂ -CB132	0.67 (6.5) [2.4]	0.96 (1.0)	1.45 (26.1) [14.3]	1.22 (22.1)	1.11 (9.6) [1.7]	1.06 (9.4)
4-MeSO ₂ -CB132	0.58 (5.5) [2.2]	0.84 (2.4)	1.35 (29.0) [18.0]	1.13 (22.1)	0.95 (13.0) [2.0]	0.90 (12.2)
3-MeSO ₂ -CB141	0.65 (8.6) [2.4]	0.95 (2.1)	1.39 (29.0) [27.1]	1.17 (28.2)	1.16 (12.4) [3.9]	1.10 (7.3)
4-MeSO ₂ -CB141	0.64 (6.7) [2.3]	0.93 (1.1)	1.62 (28.4) [14.2]	1.36 (22.0)	1.15 (13.1) [2.0]	1.09 (9.2)
3-MeSO ₂ -CB174	0.75 (6.0) [2.4]	1.09 (2.8)	1.20 (25.6) [15.3]	1.01 (21.8)	1.14 (14.1) [3.6]	1.08 (8.3)
4-MeSO ₂ -CB174	0.59 (4.6) [2.6]	0.85 (2.4)	1.06 (16.0) [17.4]	0.89 (19.1)	0.96 (15.0) [2.8]	0.91 (5.5)
Mean % CV	(7.6) [2.3]		(23.9) [15.9]		(11.1) [2.7]	
Mean total %	(9.9)	(3.2)	(39.8)	(20.1)	(13.8)	(9.0)
CV						
3-MeSO ₂ -DDE	0.70 (9.4) [1.9]	1.02 (7.8)	1.51 (24.1) [16.5]	1.26 (20.6)	1.06 (12.6) [1.8]	1.02 (17.6)
IS	0.69 (5.6) [2.3]		1.20 (13.0) [5.4]		1.06 (13.4) [3.3]	

[†] MRF $\times 10^{18}$ (peak area counts/mole) for analysis at *I* mass levels from ca. 20-1700 pg for ECD and TIC and ca. 20-600 pg for SIM: *I* = 7 for GC/ECD and GC/ECNI-MS(SIM) and *I* = 6 for GC/ECNI-MS(TIC). At each *I* mass level, *n* = 3 replicates. Congeners partitioned into isomer groups (i.e., Cl₄ to Cl₇, in descending order) by the dashed lines.

[‡] The % CV contributions of among-mass variance (parentheses) and among-replicate variance [brackets] of total variance were determined.

[°] Total % CV.

indicating that among-replicate variance was closer to, but still not significantly higher than among- mass variance for this technique. Mean MRFs for SIM also had the greatest total variance. It therefore appears that SIM is an inherently less reliable technique than ECD and TIC.

The mean MRFs relative to the IS (RMRFs) ranged from 0.6 to 1.4 for all three techniques (Table 2.7). The total % CV of the mean RMRFs were less than the total % CV of the corresponding mean MRFs. The deviation from linearity of MRFs for ECD and TIC at low concentrations (Figures 2.4a and b), was improved slightly after conversion to RMRFs. In contrast, RMRFs for SIM declined more at low concentrations than did MRFs (Figure 2.4c). Fluctuations in the congener MRFs for SIM over the entire concentration range were not reduced after conversion to RMRFs.

2.4 Discussion

2.4.1 Extraction and Cleanup/Qualitative Analysis

The column chromatography-based extraction and cleanup method (Figure 2.1) is simple and non-destructive, and is applicable to determination of CHCs in a wide range of animal tissue substrates. Although the method was not validated for all CHC compound classes normally determined, the range in polarity (elution order from Florisil and alumina columns) was spanned by PCBs and TCPMeOH. The method was extended to the even more polar aryl methyl sulfones. This method is therefore suitable for multi-residue, routine determination of CHCs, including aryl methyl sulfones, in animal tissues. The method has

advantages over those previously used for aryl methyl sulfones, which included liquid-liquid partitioning steps ^{71,136,138,163,168,171}, or saponification ^{138,172}. There is a risk of analyte loss or fraction contamination using liquid-liquid partitioning. Strong acid and base conditions destroy some CHCs, rendering these methods unsuitable for multi-residue analysis. For example, higher chlorinated dibenzo-*p*-dioxins are base labile ¹⁹² and the oxygenated organochlorines, oxychlordane and dieldrin, are strong acid labile ¹⁹³.

Most co-extracting biogenic material was separated from CHCs by GPC. Under the same operating conditions, GPC has been shown to remove approximately 99 % of long chain lipids and about half of the carotenoid pigments from chicken egg yolk ¹⁸⁶. Residual acidic biogenic material remaining in the CHC fraction after GPC is also retained. In a separate experiment, it was possible to retain approximately half the lipid extract weight from polar bear adipose tissue on the 33 % (w/w) KOH impregnated silica gel column.

The aryl methyl sulfones were cleanly separated from other CHCs, including TCPMeOH, by chromatography on 1.2 % water deactivated Florisil. The aryl methyl sulfones require high polarity solvents for quantitative elution from Florisil. Neat methanol has been used for elution of MeSO₂-PCBs and -DDE with no further clean-up, although significant levels of residual interferences were observed ^{182,194}. The presence of long chain lipids was unlikely since lipid extracts had been subject to GPC prior to Florisil. However, residual interferences from polar compounds with smaller molecular diameters, such as retinols and sterols, may have co-eluted in the aryl methyl sulfone fraction. These compounds are sufficiently electrophilic as a result of conjugation or oxygenation to be ECD and ECNI-MS responsive ¹⁹⁵. In the present study, a 7:93 methanol/dichloromethane solvent mixture

eluted MeSO₂-PCBs and -DDE while retaining residual coloured biogenic co-extractants. Colour retention was especially obvious for polar bear liver. Some interferences were observed in the ECD and TIC chromatograms after Florisil but these mostly eluted prior to the aryl methyl sulfone elution window. Remaining interferences in the aryl methyl sulfone window were removed by the 2.3 % H₂O deactivated basic alumina column, giving clean chromatograms for all three techniques (Figure 2.2). Five or fewer minor peaks in the aryl methyl sulfone elution windows of polar bear adipose (Figure 2.2a) and polar bear liver (Figure 2.2b and c) were not MeSO₂-PCB or -DDE compounds. The lack of interfering ions in the ECNI mass spectra for MeSO₂-PCBs and -DDEs from polar bear liver (Figure 2.3) also emphasized the absence of non-arylsulfone compounds. Furthermore, the (M)⁺ / (M+2)⁺ ion abundance ratio of ca. 50:100 and 60:100 for 3- and 4-MeSO₂-CB101, respectively (Figure 2.3a and b), was comparable the ideal 61.5:100 ratio for pentachloro-MeSO₂-PCBs. The recently reported *bis*(4-chlorophenyl)sulfone (BCPS) in fish from the Baltic Sea¹⁹⁶ was found by ECNI-MS in the herring gull egg sample. BCPS eluted in the aryl methyl sulfone window among the hexachloro-MeSO₂-PCB congeners. It is therefore possible that the present method is suitable for determination of diaryl sulfones. *Bis*-methylsulfonyl-PCBs (*bis*-MeSO₂-PCBs) were detected in the aryl methyl sulfone fraction of polar bear liver (not shown).

2.4.2 ECD and ECNI-MS Quantitation

Internal standard correction improved accuracy of MeSO₂-PCB/DDE analysis, but over-compensated for PCB and TCPMeOH recovery in smelt and polar bear liver.

Approximately 10 % of the losses in the present study were subsequently shown to have occurred because of incomplete sample loading in the GPC step. Recoveries of all analytes from polar bear adipose and liver tissue were improved to ca. 90 % by optimizing GPC operating conditions. Good recoveries have also been obtained for standards spiked to other matrices and isolated by liquid-liquid partitioning-based methodologies. For example, ca. 90 % recovery was achieved for five dichloro- to hexachloro-substituted MeSO₂-PCBs spiked to bovine fat ¹⁷² and ¹⁴[C]-labelled MeSO₂-4,4'-DDE spiked to rat tissues ⁷¹. However, routine analysis using these methods has not been thoroughly demonstrated.

Precision of the whole analytical method was best demonstrated by the low variance in recoveries of MeSO₂-PCB and -DDE from spiked polar bear tissues. The recoveries were based on the difference between determination of native levels and spiked levels of the same compounds. The variance in the recoveries was therefore a compound variance of both spiked and unspiked determinations. In spite of this, the % CV of replicate analysis of individual compounds ranged from 2-10 %, with a mean of 6 ± 2 %, well within an acceptable range for environmental analyses. The best overall precision for MeSO₂-PCB and -4,4'-DDE determination was obtained in the analysis of smelt. The mean % CV of replicate determinations was 2.0 ± 1.1 %. Precision of determination of PCBs and TCPMeOH in contaminant free-lipid extracts was also excellent. Only one out of sixty triplicate recovery determinations for the PCBs and TCPMeOH had a CV > 7 %. The % CV of the mean recovery among MeSO₂-PCB and PCB congeners was < 9 %, indicating no discrimination among CHCs regardless of compound class or degree and position of chlorine substitution.

Figure 2.4. The concentration profile of the mean molar response factors (MRFs) of representative MeSO₂-PCBs. MRF units are peak area counts/mole of compound $\times 10^{18}$ ($n = 3$ per point). The concentration range examined was similar to the range commonly used for determination of CHCs^{71,168,171,175,197}. A) GC/ECD, B) GC/ECNI-MS(TIC) and, C) GC/ECNI-MS(SIM). $\square$ is 3-MeSO₂-CB49, ∇ is 3-MeSO₂-CB101, $\circ$ is 3-MeSO₂-CB149, and $\triangle$ is 3-MeSO₂-CB174. Error bars on individual points were not shown for clarity, see Table 2.7 for response variation contributions of the mean MRFs. The vertical line denotes the approximate low mass limit of MRF linearity.

The electron-capturing ability of PCBs ¹⁹⁸ is enhanced by the presence of the electro-negative MeSO₂-functional group ^{173,182}, therefore instrument detection limits were generally sub-pg for all three techniques (Table 2.6). These detection limits were lower by an order of magnitude relative to those previously reported for SIM and TIC, which ranged from 2-6 pg and 30-88 pg for SIM and TIC, respectively ¹⁷³. Sensitivity in the latter study may have been decreased by oxygen reactions in the ion source, since (M-Cl+O)⁺ fragment ions were abundant (see Chapter 3). Method detection limits for MeSO₂-PCB and -DDE quantitation increased in the order SIM < ECD ≤ TIC (Table 2.6). Therefore, if extremely high sensitivity is required, SIM is the best detection technique, capable of sub-pg detection. The low-pg detection limits of ECD and TIC were close enough that other criteria need to be considered to choose between them. In samples analyzed to date, the separation of aryl methyl sulfones from potential interferences has been so effective that ECD could be chosen because of significantly lower cost. However, if GC/ECNI-MS instrumentation is available, determination using TIC has the added benefit that the spectra can be checked for possible interferences.

Linearity of ECD response in determination of MeSO₂-PCBs has been assumed in previous studies ^{71,168,171}. Knowledge of the response stability over a practical concentration range is necessary for the most precise and accurate quantitation ¹⁸⁸. Therefore, in the present study, linearity was tested over the low pg to ng range for all three detection techniques (Figure 2.4). This covers the range of masses usually injected for GC/ECD quantitation of MeSO₂-PCBs and -4,4'-DDE in environmental samples studied to date ^{71,168,171} and for the polar bear, ringed seal and arctic cod samples analyzed in this study.

ECD was the most linear of the three techniques, as evidenced by minimal variation in molar response factors over the ca. 20-600 pg range (Figure 2.4a). Non-linearity in TIC response began below 100 pg and became significantly non-linear below 20 pg, indicating that a calibration curve would be required for accurate quantitative analysis (Figure 2.4b). SIM response did not fall precipitously until below 20 pg, as was the case for TIC, but response was highly variable among runs (Figure 2.4c).

The variability of response factors among replicates was higher for SIM than ECD and TIC (Table 2.7). Furthermore, the total variance in response factors for SIM among congeners and over the mass range tested was very high compared to ECD and TIC. SIM response variability may have been due in part to fluctuations in fragmentation between runs. The formation of hydrogen inclusion fragment ions (e.g., $(M-Cl+H)^+$ and $(M-SO_2CH_3+H)^+$) is known to occur via catalysis at the source walls¹⁷⁶, and was somewhat dependent on ion source temperature, but not ion source pressure (see Chapter 3). Among-replicate variations in these parameters or changes in source wall condition may result in variable abundance of fragment ions and thus variable molecular ion abundance. TIC may not be as affected. Thus, in spite of superior sensitivity, the more inherent variability in the molar response factors for SIM indicates that the technique is less suitable than ECD or TIC for quantitation of MeSO₂-PCBs and -DDEs.

MeSO₂-PCB response factors relative to a single IS are commonly used to quantitate MeSO₂-PCBs and -4,4'-DDEs^{71,168,171}. This approach has been applied to samples in the present study. The IS of choice has been a methylated-MeSO₂-PCB as it possesses physico-chemical properties similar to MeSO₂-PCBs. The conversion of the MRFs to RMRFs made

little difference to the linearity of ECD response (Figure 2.5), however, the linearity of TIC response was improved in the low pg range. The % CV of RMRFs was lower than that of MRFs for all three methods of detection, indicating that the IS compensated for a large amount of the overall response variation (Table 2.6). However, the RMRFs for SIM were actually more variable (% CV ca. 20 %) than MRFs, indicating that a single IS is probably not satisfactory for accurate quantitative analysis by SIM.

Variation in molar response factors among MeSO₂-PCB congeners was significantly lower than that found for PCBs ¹⁹⁹, probably because the proportionate contribution of the MeSO₂-group to electron-capturing outweighs the changes in electronegativity going from four and seven chlorines per molecule (Table 2.7). Variation in molar response for all three detection techniques was least among hexachloro-MeSO₂-PCBs, and little accuracy would be lost by using an average response factor for this group. However, the molar responses of pentachloro-MeSO₂-PCBs were more variable, driven largely by a low value for 3-MeSO₂-CB101. The RMRF of 3-MeSO₂-CB101 resembled those of the tetrachloro-MeSO₂-PCB congeners (Table 2.7; Figure 2.5). Most of the biologically relevant MeSO₂-PCB and -DDE standards are now available ¹⁷⁰, therefore individual standards should be used.

2.5 Conclusions

A simple column chromatography method was developed for separation and cleanup in the determination of chlorinated hydrocarbon contaminants and their methyl sulfonyl-metabolites in biological tissues. The method was validated for determination of eleven

Figure 2.5. The concentration profile of the mean molar response factors relative to the MeSO₂-IS (RMRF) of representative MeSO₂-PCBs ($n=3$ per point). RMRF values are unitless. A) GC/ECD, B) GC/ECNI-MS(TIC) and, C) GC/ECNI-MS(SIM). $\square$ is 3-MeSO₂-CB49, ∇ is 3-MeSO₂-CB101, $\circ$ is 3-MeSO₂-CB149, and Δ is 3-MeSO₂-CB174. Error bars on individual points were not shown for clarity, see Table 2.7 for overall RMRF variability of the mean RMRFs.

PCBs, fifteen tetra- to heptachloro 3- and 4-MeSO₂-PCBs, 3-MeSO₂-DDE, and *tris*(4-chlorophenyl)methanol spiked to herring gull egg, smelt, and polar bear liver and adipose tissue using gas chromatography with electron-capture detection (GC/ECD). The overall mean recovery relative to the internal standard was 103 % $\pm$ 8 %, independent of analyte, substrate type and lipid extract weights up to ca. 0.7 grams. Precision of replicate analyses of individual congeners was good. There were no significant residual biogenic or xenobiotic interferences in the aryl methyl sulfone fraction of any substrate. Sensitivity and linearity of molar response of MeSO₂-PCBs and MeSO₂-DDE was tested for ECD, and electron-capture negative ion mass spectrometry monitoring the total ion current (TIC) and the molecular ion (SIM). The mean practical quantitation limit among MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE was lowest for SIM (2.1 $\pm$ 0.9 pg), and similar for ECD and TIC (24.2 $\pm$ 4.6 pg and 44.4 $\pm$ 17.1 pg, respectively). Response factors were linear above the practical quantitation limit to at least the 1 ng level for all three techniques. In spite of superior sensitivity, there was more inherent variability in the response factors for SIM (ca. 18 % to 56 % CV) than for ECD (ca. 7 % to 12 % CV) or TIC (ca. 11 % to 18 % CV), therefore ECD or TIC are recommended for quantitative analysis.

The Influence of Congener Structure on the Electron-Capture/Negative Ion Mass Spectrometric Fragmentation Patterns of Methyl Sulfone-Substituted PCBs[†]

3.1 Background

Structural characterization of MeSO₂-PCBs by ECNI mass spectral fragmentation patterns has been limited to differences in 2-MeSO₂-/2'-Cl-substituted MeSO₂-PCBs from other MeSO₂-PCB structures^{173,182}. Further, previous methane GC/ECNI-MS analyses of MeSO₂-PCBs were complicated by ion/molecule reactions between radical anions of molecular (O₂^{•-}) or atomic (O^{•-}) oxygen and the MeSO₂-PCBs. The anionic oxygen species form in the ion source as a consequence of oxygen from either atmospheric air leakage and/or trace oxygen in the methane buffer gas¹⁷³. In the present study, a more detailed examination of MeSO₂-PCB structure-fragmentation relationships in oxygen-free GC/ECNI mass spectra are described to determine if additional MeSO₂-PCB structural information can be derived from fragmentation patterns. The effect of changes in ion source pressure and temperature on the mass spectra of MeSO₂-PCBs are also examined.

[†] To be submitted to *Mass Spectrom.*, 1996.

3.2 Experimental

3.2.1 Chemicals and Standards

The twenty-four individual MeSO₂-PCB standards used in this study were synthesized and provided courtesy of Dr. Åke Bergman (Wallenberg Laboratory, Stockholm University, Stockholm, Sweden) and Dr. K. Haraguchi (Daiichi Pharmaceutical College, Fukuoka, Japan)^{173,180}. The concentrations of the twenty-four MeSO₂-PCB standards, except 3-MeSO₂-CB70 and 4-MeSO₂-CB151, were verified and/or corrected by GC/FID (see Chapter 2). Two mixtures of MeSO₂-PCB standards, one containing sixteen and the other containing twelve congeners, were prepared in 2,2,4-trimethylpentane (BDH, analytical grade) at a concentration of ca. 250 pg/μl. Six of the MeSO₂-PCB congener standards were present in each mixture to ensure that the fragmentation patterns did not change between separate GC/ECNI-MS injections. Two separate mixtures were necessary to prevent co-elution of individual congeners, under the GC column and temperature ramping conditions used (see below).

3.2.2 GC/ECNI-MS Analysis

The MeSO₂-PCB standard mixtures were analyzed by GC/ECNI-MS(TIC), scanned from 83 to 550 amu to ensure detection of all molecular and predominant fragment anions. Ion source conditions were optimized for production of a plasma of thermalized secondary electrons generated from methane reaction gas¹⁷⁷ and based on the GC/ECNI-MS conditions for MeSO₂-PCB and -4,4'-DDE (see Chapter 2) determination in polar bear and

ringed seal in the Canadian Arctic (see Chapter 4) ^{175,197,200}.

GC/ECNI-MS was performed on a HP 5987B mass spectrometer upgraded with an HP 5890 series II gas chromatograph coupled with an HP 5988 GC/MS direct interface. The ECNI-MS system was fitted with stainless steel lines, fittings and other internal components, under the supervision of Dr. Michael Oehme (Norwegian Institute for Air Research, Lillestrøm, Norway), to minimize ion source contamination by reactive, synthetic components. Leakage of atmospheric gases, including oxygen, was also minimized. The carrier and reagent gas were helium (99.99 %) and methane (99.99 %), respectively.

The effect of temperature (100°C to 260°C) in increments of 20°C on the ECNI mass spectral fragmentation pattern of MeSO₂-PCBs was tested at two ion source pressures, 0.5 mbar and 0.05 mbar. An ion source temperature of 140°C and pressure of 0.5 mbar was chosen to compare the relative abundance of the fragment ions to the molecular ion. The emission current was 300 µA and the primary electron beam energy was 130 eV. The injection port and transfer line temperatures were 270°C and 260°C, respectively. A gas pressure of ca. 0.3 to 0.6 mbar, temperature of 140°C-200°C and primary electron beam energy of 35-150 eV in the ion source have been found to give optimal electron-capture enhancement of polyhalogenated aromatic hydrocarbons, including PCBs ¹⁷⁷. The instrument was tuned with perfluorotributylamine (PFTBA) using ion of *m/z* 312, 414 and 595. The GC conditions are described in detail in Chapter 2.

3.2.3 Statistical Analysis

The sum of the ion abundances in each isotopic cluster was used to determine the

relative ion abundances of the fragment ions to the molecular ion base peak (Table 3.1). The subtle differences in the relative abundances of the major fragment anions among the MeSO₂-PCB congeners warranted the use of a multivariate statistical approach to the analysis. Principal component analysis (PCA) was chosen using the Pirouette software package (version 1.2; Infometrix, Inc., Seattle, WA). PCA analysis was useful in simplifying the overall variation to more clearly observe MeSO₂-PCB structural factors that may be contributing to changes in the relative abundance of the ECNI-MS generated fragment anions. PCA transforms a complex data set into a new set of variables, known as eigenvectors or principal components (PC), that are linear combinations of the original variables in the data set. The magnitude of the PC determines how much of the data variation it contains. A favourable PCA analysis should explain a large proportion of the variation in the first two or three PCs. Score and loading plots for PC1 versus PC2 were generated to demonstrate the spatial relationships among MeSO₂-PCB congeners and how each fragment ion influenced the direction of the principal component, respectively.

3.3 Results and Discussion

3.3.1 MeSO₂-PCB Mass Spectra

The MeSO₂-PCB mass spectra in Figure 2.3 (Chapter 2) illustrated the absence of significant amounts of oxygen in the present GC/ECNI-MS system. The (M-Cl+O)⁻ fragment ion was either not measurable or < 1 % of the molecular ion base peak. Molecular oxygen captures electrons forming O₂⁻ and O⁻, so that even minute leakage of oxygen from

Surrounding air into the ECNI-MS ion source can result in reactions with halogenated analytes producing the $(M-X+O)^-$ species. For example, O_2^- and O^- reactions with PCBs^{201,202} and polycyclic chlorinated pesticides²⁰³ have been shown to produce $(M-Cl+O)^-$ ions. Moreover, in the GC-ECNI-MS analysis of ninety-four MeSO₂-PCBs by Haraguchi *et al.*¹⁷³, the $(M-Cl+O)^-$ ion was generally the most intense peak in the mass spectra, even though methane buffer gas used was > 99.95 % pure with < 100 ppm O₂. However, no effort was made to ensure that there was no air leakage into the GC/ECNI-MS system (Dr. Åke Bergman, personal communication). The presence of oxygen was enough to dramatically influence the MeSO₂-PCB fragmentation pattern, and obscure potential MeSO₂-PCB structure-fragmentation relationships.

The MeSO₂-PCB congeners used in the present study (Table 3.1) have been identified as persistent contaminants in biota (see Chapters 4, 5 and 6)^{71,138,168,175,187,197,200}. At an ion source temperature and pressure of 140°C and 0.5 mbar, respectively, the ECNI mass spectra of the twenty-four 3- and 4-MeSO₂-, tetrachloro- to heptachloro-MeSO₂-PCBs were dominated by the molecular ion $(M)^-$ (see Figure 2.3). The relative intensity of the fragment ions was < 25 % (Table 3.1), indicating that collisional stabilization of the vibrationally-excited molecular anion, or resonance electron-capture to form the $(M)^-$, was the favourable anion forming process. The alternative process is dissociative resonance electron-capture resulting in fragment anion formation. A vibrationally excited molecular ion is initially formed by electron capture of a "thermalized" secondary electron (i.e., 0 to 15 eV)¹⁷⁶. The secondary electron is ejected from energetically excited methane, which forms after absorbing high energy, bombarding electrons in the ion source.

Table 3.1. The Relative Percent Abundance of MeSO₂-PCB Fragment Ions to the Molecular Ion Base Peak Using GC/ECNI-MS^a

#	Congener	Cl subst. ¹	(M-CH ₃) (M-15)	(M-Cl+H) (M-34)	(M-CH ₂ -Cl+H) (M-49)	(M-Cl ₂ +2H) (M-68)	(M-Cl ₂) (M-70)	(M-SO ₂ CH ₃ +H) (M-78)
1	3-MeSO ₂ -CB49	2,2',4',5'	16	5	2	<1	<1	<1
2	4-MeSO ₂ -CB49	2,2',4',5'	8	7	1	<1	<1	<1
3	3-MeSO ₂ -CB76	2,3',4',5'	10	12	6	1	<1	<1
4	4-MeSO ₂ -CB70	2,3',4',5'	3	10	1	<1	1	<1
5	3-MeSO ₂ -CB52	2,2',5',5'	18	9	1	<1	<1	<1
6	4-MeSO ₂ -CB52	2,2',5',5'	5	6	<1	<1	<1	<1
7	4-MeSO ₂ -CB64	2,4',5',6'	21	8	<1	<1	<1	<1
8	3-MeSO ₂ -CB110	2,3',4',5',6'	16	13	1	1	1	<1
9	4-MeSO ₂ -CB110	2,3',4',5',6'	14	12	2	2	2	1
10	3-MeSO ₂ -CB101	2,2',4',5',5'	14	10	2	2	3	<1
11	4-MeSO ₂ -CB101	2,2',4',5',5'	4	8	2	2	2	1
12	3-MeSO ₂ -CB87	2,2',3',4',5'	18	14	1	1	2	1
13	4-MeSO ₂ -CB87	2,2',3',4',5'	5	13	3	2	3	2
14	3-MeSO ₂ -CB91	2,2',4',5',6'	22	14	2	2	2	<1
15	4-MeSO ₂ -CB95	2,2',5',5',6'	14	10	1	1	1	<1
16	3-MeSO ₂ -CB149	2,2',4',5',5',6'	19	16	3	3	3	2
17	4-MeSO ₂ -CB149	2,2',4',5',5',6'	15	24	2	7	6	2
18	3-MeSO ₂ -CB132	2,2',3',4',5',6'	16	14	2	3	3	1
19	4-MeSO ₂ -CB132	2,2',3',4',5',6'	14	15	2	4	3	1
20	3-MeSO ₂ -CB141	2,2',3',4',5',5'	14	18	3	4	4	1
21	4-MeSO ₂ -CB141	2,2',3',4',5',5'	3	14	<1	2	4	12
22	4-MeSO ₂ -CB151	2,2',3',5',5',6'	15	10	2	2	2	10
23	3-MeSO ₂ -CB174	2,2',3',4',5',5',6'	12	16	2	2	1	3
24	4-MeSO ₂ -CB174	2,2',3',4',5',5',6'	7	22	1	4	5	8
							3	9

^a The abundance of each ion is based on the cumulative response all the ions in the isotopic cluster. The molecular ion was consistently the base peak (ion source temperature and pressure of 140°C and 0.5 mbar, respectively) and given a relative abundance of 100 %.

¹ See Figure 1.4 for the positions of chlorine substitution.

* Refer to Figure 3.2a for congener numbering assignments.

2

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The dominance of the $(M)^{\cdot-}$ base peak was consistent with the oxygen-free GC/ECNI-MS spectra of twelve 3- and 4-MeSO₂-PCB congeners reported by Buser *et al.*¹⁸². However, the fragment ion abundance was generally lower in the present study. The difference in fragment ion abundance was probably not effected by ion source temperature since both analyses used ion source temperatures of 140°C. Buser *et al.*¹⁸² used a buffer gas of argon/10 % methane at a lower ion source pressure (1.0×10^{-4} mbar) compared to the pure methane and ion source pressure of 0.5 mbar used in the present study. Application of GC/ECNI-MS to polyhalogenated aromatic hydrocarbons and PCBs^{202,204} has demonstrated that higher ion source temperatures increase the abundance of fragment ions while lower temperatures increase the tendency for molecular anions to form. The effect of ion source temperature on the mass spectra of MeSO₂-PCBs demonstrated that regardless of the degree of chlorination, the abundance of the fragment ions relative to the molecular ion increased with increasing temperature (Figure 3.1). The largest effect was for the $(M-CH_3)^{\cdot-}$ ion. The greater abundance of the $(M-CH_3)^{\cdot-}$ fragment ion increased faster with temperature for the 3-MeSO₂-PCB relative to its 4-MeSO₂-PCB pair (Figure 3.1), and was true of all the 3- and 4-MeSO₂-PCB pairs studied (Table 3.1). This suggested that dissociative resonance electron capture of the vibrationally excited $(M)^{\cdot-}$ to form $(M-CH_3)^{\cdot-}$ was preferred over resonance electron capture to form $(M)^{\cdot-}$ with increasing ion source temperature, for the 3-MeSO₂-PCB isomer relative to the 4-MeSO₂-PCB pair.

The effect of changing the ion source temperature for the hydrogen-inclusion type ions (e.g., $(M-Cl+H)^{\cdot-}$) was minimal. The formation of $(M-Cl+H)^{\cdot-}$ ions has been shown to occur via a dissociative resonance electron-capture of an intermediate created by

Figure 3.1. The effect of ion source temperature on the ECNI mass spectral fragmentation of MeSO₂-PCBs. The ion source pressure was 0.5 mbar. Four representative MeSO₂-PCB are presented for simplicity, A) 3-MeSO₂-CB52, B) 4-MeSO₂-CB52, C) 3-MeSO₂-CB110 and D) 4-MeSO₂-CB110. The percent ion abundances are relative to the molecular ion, and based on the negative ion response of the whole isotopic ion cluster.

hydrogenation of the non-ionized analyte on the walls of the ion source, a process which appears to be facilitated by some form of heterogeneous catalysis ^{176,205}. The minimal temperature effect for (M-Cl+H)⁻ formation may be a function of a limiting rate of diffusion of MeSO₂-PCBs and/or the subsequent heterogeneous catalysis on the ion source wall.

At a lower ion source pressure of 0.05 mbar, the ECNI response sensitivity decreased by an order of magnitude for all MeSO₂-PCBs (not shown). However, the effect of changing the source temperature from 260°C down to 100°C by 20°C increments, had essentially the same effect on the relative abundance of the fragment ions as observed for an ion source pressure of 0.5 mbar (Figure 3.1). The pressure effect was consistent with the general finding for chlorinated aromatics, that is, increasing ion source pressure increases the negative ion response ¹⁷⁶, perhaps due to better thermalization of electrons.

The abundance of the (M-CH₃)⁻ and (M-Cl+H)⁻ fragment ions for 3- and 4-MeSO₂-PCBs was higher in the study by Buser *et al.* ¹⁸² compared to the present analyses. This may be explained by the different buffer gas used (10 % methane/argon) and the much lower ion source pressure (1.0×10^{-4} mbar) used by Buser *et al.*. It has been shown that, depending on the buffer gas used and the compound, molecular ion or fragment ion intensity may be enhanced as the ion source pressure is increased ¹⁷⁶. For example, the ECNI mass spectra of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin had greater abundance of molecular ion at lower ion source pressures when hydrogen and argon were used as buffer gases, whereas the fragmentation remained unchanged in response to decreasing pressure when helium was used as a buffer gas ²⁰⁶. Alternately, the differences in ECNI mass spectra may simply be due to the different instruments and their components, such as the ion source.

The presence of the major fragment ions, $(M-CH_3)^-$, $(M-Cl+H)^-$, $(M-CH_3-Cl+H)^-$, $(M-Cl_2)^-$, $(M-Cl_2+2H)^-$, and $(M-SO_2CH_3+H)^-$ (Table 3.1) were consistent with those described elsewhere^{173,182}. A detailed discussion on the assignment of the $MeSO_2$ -PCB fragment ions is therefore unnecessary, suffice to say that the $MeSO_2$ -PCB fragment ions were formed via a dissociative electron-capture process²⁰⁴. The fact that the most abundant $(M-CH_3)^-$ and $(M-Cl+H)^-$ fragment ions retained the SO_2^- and $MeSO_2$ -functional groups, respectively, suggested that the additional stabilization of negative charge by the energetically accessible d-orbitals of the sulfur heteroatom may have contributed to their greater abundance relative to other fragment ions. Resonance stabilization and electron sharing, which involves a non-bonding orbital on a heteroatom, are key factors determining the abundance of a product ion, since the presence of heteroatoms can influence fragment ion lifetime in the source²⁰⁷. The lower abundance of the $(M-CH_3-Cl+H)^-$, $(M-Cl_2)^-$ and $(M-Cl_2+2H)^-$ fragments, which also retain the SO_2 -functional group, may be due, in part, to multiple bond cleavage during the dissociative resonance electron capture process. It is still unclear what structural characteristics of $MeSO_2$ -PCBs are necessary for the formation of fragment ions where the $MeSO_2$ -functional group has been lost^{173,182}. It has been suggested that these unusual low-mass anions maybe useful in searching for $MeSO_2$ -PCBs in environmental samples¹⁸².

3.3.2 $MeSO_2$ -PCB Isotopic Cluster Abundance

Bergman *et al.*¹⁷³ and Buser *et al.*¹⁸² compared the relative abundances of molecular and fragment ions based on the monoisotopic ^{35}Cl ions in each isotopic cluster. The intensity of the isotopic ions for $MeSO_2$ -PCBs is dependent on the number of chlorines present.

Comparing abundances of a single isotopic ion among MeSO₂-PCB congeners and fragment ions is unacceptable because changes in the number of chlorines influences abundance. In the present study, determining the abundance of the whole isotopic cluster removed this variation.

3.3.3 Fragment Ion Abundance and MeSO₂-PCB Congener Structure

Recent research to determine possible correlations between MeSO₂-PCB structure and ECNI-MS fragmentation has been limited or inconclusive for two reasons; 1) the MeSO₂-PCBs products prepared by γ -irradiation of PCBs in dimethyl sulfide widely varied in structure, that is, there were few groupings of MeSO₂-PCBs that varied in only one structural attribute¹⁸² or 2) the presence of molecular oxygen in the ion source overwhelmingly influenced the course of the MeSO₂-PCB fragmentation pattern¹⁷³.

As mentioned, the GC/ECNI-MS system in the present study was devoid of significant O₂ interference. Further, determination of the potential relationships between MeSO₂-PCB structure and their fragmentation patterns was facilitated by the limited variation in the congener structures: 3- and 4-MeSO₂-substitution, tetrachloro- to heptachloro-substitution and 2,5-dichloro- or 2,5,6-trichloro-substitution on the MeSO₂-containing phenyl ring (Table 3.1). These are the only congeners identified in biota^{71,169,173}. The potential rules for the ECNI-MS fragmentation based on the congener structure may be useful for the identification of unknown MeSO₂-PCBs in environmental samples.

The 2-MeSO₂-/2'-Cl-substituted PCBs have been shown to give more complex fragmentation patterns than PCBs with 3,4 MeSO₂-substitution^{173,182}. For the former

MeSO₂-PCB structures, (M-CH₃)[·] and (M-Cl+H)[·] tended to dominate the mass spectra while (M)[·] was generally < 50 % of the fragment ion base peak ^{173,182}. Therefore, the low abundance of the (M)[·] ion in 2-MeSO₂-PCBs is presently the primary feature in ECNI mass spectra for structural differentiation among MeSO₂-PCBs. Since these congeners are not found in biota ^{71,169,173}, the information is of little practical use.

Further differentiation of 3- and 4-MeSO₂-PCB structure was indicated in this study by changes in the relative abundance of the fragment ions (Table 3.1). The relative abundance of the fragment ions tended to increase with increasing MeSO₂-PCB chlorination. This appeared to be especially true for (M-Cl+H)[·]. Tetrachloro-MeSO₂-PCBs generally had lower levels of the less abundant (M-CH₃-Cl+H)[·], (M-Cl₂+2H)[·], (M-Cl₂)[·] and (M-SO₂CH₃+H)[·] ions whereas hexachloro- and heptachloro-MeSO₂-PCBs had higher relative abundances of the same four fragment ions (Table 3.1). The 4-MeSO₂-PCBs tended to have less abundant (M-CH₃)[·] and (M-Cl+H)[·] ions, regardless of the degree of chlorination. Haraguchi *et al.* ¹⁷³ had previously observed a slightly greater abundance of (M-CH₃)[·] for 3-MeSO₂-, as opposed to 4-MeSO₂-PCBs, for MeSO₂-PCBs with > 5 chlorines. At the time, this observation was considered qualitative since the mass spectra were obscured by MeSO₂-PCB reactions with molecular O₂[·] or O[·] radical. Additional MeSO₂-PCB structure-fragmentation patterns were not obvious from the differences in relative abundances, however, the application of multivariant statistical analysis revealed further information.

PC1 and PC2 accounted for 88.6 % of the total variance among fragment ions and MeSO₂-PCB congeners. Figure 3.2a illustrates the scoreplot of PC1 versus PC2 for the twenty-four MeSO₂-PCB congeners. The PC1 versus PC2 loading plot (Figure 3.2b) shows

Figure 3.2. Principal component score (A) and loading (B) plots for the relative abundance of the major ECNI-MS fragment ions as a function of MeSO₂-PCB structure. See Table 3.1 for the MeSO₂-PCB numbering system and the major fragment ions. PC 1 and PC 2 contained 88.6 % of the total variance. See the Experimental section for a description of the multivariate analysis.

how the variation in the relative abundance of the fragment ions influenced the PC1 and PC2 eigenvectors. Ions with the largest influence on a given eigenvector, deviated the greatest from zero on the PCA loading plot (Figure 3.2b), in positive or negative direction.

Hexachloro- and heptachloro MeSO₂-PCBs had positive PC2 scores, with the exception of 4-MeSO₂-CB151, while less chlorinated congeners had negative scores (Figure 3.2a). Unlike the other hexachloro- and heptachloro-MeSO₂-PCBs, 4-MeSO₂-CB151 does not possess a 4'-chlorine. PC2 was driven primarily by the influence of (M-Cl+H)⁺ (Figure 3.2b), which tended to higher relative abundances than the lower chlorinated congeners (Table 3.1). The (M-SO₂CH₃+H)⁺ ion also had a high value along PC2 and higher relative abundances for hexachloro- and heptachloro-MeSO₂-PCBs as compared to lower chlorinated congeners. The most distinct clustering in the scoreplot was for MeSO₂-PCBs possessing the 4-MeSO₂-2,5-dichloro moiety at PC1 scores < -5. PC1 was driven almost entirely by (M-CH₃)⁺, since the PC1 values of the remaining ions were close to zero (Figure 3.2b), and therefore had little modelling power. The MeSO₂-PCBs containing the 4-MeSO₂-2,5-dichloro moiety all had exceptionally low relative abundances for (M-CH₃)⁺, indicating that this structural feature is important for charge stabilization of (M)⁺. Both (M-Cl+H)⁺ and (M-CH₃)⁺ were the most measurable fragment ions in the ECNI mass spectra (Table 3.1) and the most influenced by MeSO₂-PCB structure, and were therefore the most diagnostic fragment ions for aiding in the structural elucidation of MeSO₂-PCBs.

The ECNI mass spectrum of 2-MeSO₂-4,4'-DDE has been shown not to have (M)⁺, rather, a (M-2HCl)⁺ ion dominates the spectrum¹⁷³. In contrast, 3-MeSO₂-4,4'-DDE has been shown to have (M)⁺ as the base peak and a strong (M-CH₃)⁺ fragment ion. The

dominance of $(M)^-$ for 3-MeSO₂-4,4'-DDE, found on the present GC/ECNI-MS system, was consistent with this previous result, except that the $(M-CH_3)^-$ fragment ion was very minor (Figure 2.3c). The difference between 2- and 3-MeSO₂-4,4'-DDE mass spectra may be explained by the steric interaction of a 2-MeSO₂-functional group and chlorines on the ethene group. The mass spectra of 2,4'- (*o,p'*-) DDE has been found to have $(M-Cl_2)^-$ as the major ion, where the methane GC-ECNI-MS ion source temperature and pressure were 100°C and 0.5 mbar, respectively ²⁰⁸. It was suggested that the fragment ion resulted from the interaction of the chlorine in the 2-position of the phenyl ring with the substituents on the 2 carbon of the ethene molecule, resulting in the loss of Cl₂ and formation of an ion with a fused phenyl-ethylbutyl system. In contrast, 4,4'-DDE gave mostly $(M)^-$, as a result of the enhancement of negative charge stabilization by conjugation of the two phenyl rings via the ethene moiety. Further, chlorinated and brominated, *ortho* hydroxydiphenyl and diphenyl ethers with *ortho* chlorines have been shown to pyrolyze to dioxins and furans, respectively, at ion source temperatures ranging from 140 to 200°C ²⁰⁹.

3.4 Conclusions

Haraguchi *et al.* ¹⁷³ had previously concluded that ECNI-MS did not give extra structural information about MeSO₂-PCBs as compared to GC/EI-MS. ECNI-MS fragmentation patterns had been limited to differences between 2-MeSO₂-2'-Cl-substituted PCBs and 3- and 4-MeSO₂-substituted PCBs, that is, differentiation based on the base peak status of the $(M)^-$ ion. ECNI-MS results from the present study, using pure methane buffer gas and

an oxygen-free ion source, demonstrated that subtle congener structure-ECNI fragmentation patterns do exist among 3- and 4-MeSO₂-PCBs. Hexachloro- and heptachloro-MeSO₂-PCBs favour (M-Cl+H)⁻ and (M-SO₂CH₃+H)⁻ formation relative to lower chlorinated congeners. The most diagnostic ECNI mass spectral feature was the decrease in the relative abundance of the (M-CH₃)⁻ for congeners containing a 4-MeSO₂-2,5-dichloro moiety. The relative abundance of the other fragment ions were similarly low, suggesting that this feature is important for stabilization of negative charge on (M)⁻. The relative abundance for (M-CH₃)⁻ increased dramatically for ion source temperatures of 180°C or higher (Figure 3.1). It is therefore important to maintain ion source temperature when comparing the relative abundances of fragment ions. Under the present GC/ECNI-MS conditions, it was shown that previously unknown rules for MeSO₂-PCB fragmentation in relation to congener structure appear to exist, and maybe used as a diagnostic tool for the elucidation of unknown MeSO₂-PCB structures in environmental samples.

The Ecodynamics of Methyl Sulfone Metabolites of PCBs and DDEs in the Polar Bear Food Chain of the Canadian Arctic*

4.1 Background

Arctic marine/maritime biodiversity is limited to relatively few species, thus food chains are simple, with one or two major prey items at each trophic level. The polar bear sits atop the Arctic marine food web, eating mostly the blubber of ringed seal which in turn consumes largely arctic cod and amphipods.

CHC contamination of the Arctic and subsequent bioconcentration and bioaccumulation in the polar bear - ringed seal - arctic cod marine food chain has been well documented ^{46,47,48}. Patterns of residual CHCs in fatty tissue have been shown to vary among arctic species ⁴⁷. For example, the PCB pattern becomes increasingly more simplified moving up the food chain due to selective removal of certain congeners. CHC depuration is largely a function of metabolism, which occurs mainly in the liver ⁴¹. Polar bears in particular demonstrate a unique ability to metabolize normally recalcitrant PCB congeners and 4,4'-

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DDE ⁴⁶.

The pattern of residual PCBs in terrestrial and marine mammals can be used to demonstrate that CYP-mediated metabolic capacity varies among species and is dependent on the structure of the PCB congener ^{5,6,44,47,99-101}. For example, seals have a higher capacity than cetaceans to metabolize PCBs possessing *meta-para* vicinal hydrogens, regardless of *ortho*-chlorine substitution. In Chapter 2, it was demonstrated that 3- and 4-MeSO₂-PCBs with the same chlorine substitution patterns were present in polar bear liver and fat.

The ecodynamics of MeSO₂-PCB and -4,4'-DDE metabolites in the polar bear food chain are explored in the present study. The identities and levels of MeSO₂-PCB and -4,4'-DDE are determined in various lipid-containing tissues of polar bear, blubber of ringed seal and whole arctic cod. At present, there are very few studies on MeSO₂-PCB and -4,4'-DDE in the environment apart from, 1) some mammals and birds from the Baltic sea region, 2) a Swedish human milk study, 3) fat and liver of two polar bears from the Canadian Arctic, 4) beluga from the St. Lawrence estuary, 5) Japanese, "Yusho" patients exposed to high PCBs levels from ingestion of contaminated rice oil, and 6) one study on Japanese fish, blue whale and molluscs ^{71,138,166,168,210}. There have been no studies on bioaccumulation of MeSO₂-PCBs and -4,4'-DDE in food chains.

In the present study, the levels and patterns of the residual MeSO₂-PCB and -4,4'-DDE compounds and their precursors in polar bear food chain tissues are used to assess the relative significance of factors which may contribute to the bioaccumulation of these metabolites. These factors may include the metabolism of precursor PCBs and 4,4'-DDE ,

the retention of MeSO₂-PCBs and -4,4'-DDE, and the uptake of these metabolites from the diet. The distribution of MeSO₂-PCBs and -4,4'-DDEs is determined in adipose, liver, testes, muscle and brain of polar bear to determine the possible existence of preferential tissue retention of specific congeners and/or the methyl sulfonyl-containing metabolites in general, which may be an important signal of potential toxicity.

A study is presented on the temporal dynamics of MeSO₂-PCB and -4,4'-DDE metabolites in the adipose and milk of a polar bear family group, sampled over an annual cycle of hyperphagia and fasting. The potential for lactational transfer is compared to that of PCBs. The kinetics of MeSO₂-PCB and -4,4'-DDE are unknown for any mammal and may differ substantially from the parent PCB and 4,4'-DDE compounds. The mobilization of adipose reserves in polar bear mothers as a consequence of long-term fasting may increase the exposure of their fetuses and cubs, via placental transfer and milk, to potentially toxic MeSO₂-PCBs and -4,4'-DDEs. In a previous study, the effects of long-term fasting demonstrated that the levels of S-PCBs in the milk and adipose of adult females increased as the fasting period progressed⁴⁰. The levels of S-PCBs were highest in the cubs. Elevated PCB exposure may adversely influence the survival and growth of the cubs in the critically early phase of their development.

4.2 Experimental

4.2.1 Tissue Sampling

4.2.1.1 Polar Bear Food Chain and Tissue Distribution

The following tissues from adult male polar bears were obtained using a precise sampling protocol; six liver (distal portion of the right lobe), six adipose, three lung and testes, and one brain (*medulla oblongata*). Dr. Malcolm Ramsay obtained the samples from bears freshly-killed within three days of each other at the end of April, 1993 by Inuit hunters in the Resolute Bay area of the Canadian Arctic (Figure 5.1, Chapter 5). Adult male polar bears were chosen to minimize the effect of sex and age on tissue/trophic level comparisons.

Blubber and liver samples of eleven ringed seals and two pools of nine arctic cod each, were also collected from the Resolute Bay area by biologists for Dr. Derek Muir (Freshwater Institute, Winnipeg, Manitoba). The ringed seals were comprised of five males and six females. Seal ages were unknown, except for two juvenile females and adult status for 3 males. It was therefore not possible to take these factors into account in the CHC level comparisons.

4.2 1.2 Adipose Temporal Dynamics and Biotransfer from Polar Bear Mother to Cub

Adipose biopsy and milk samples were collected from a polar bear mother and her cubs in the vicinity of Churchill, Manitoba on the western side of Hudson Bay (Figure 5.1, Chapter 5) by Dr. Malcolm Ramsay and Susan Polischuk (Department of Biology, University of Saskatchewan, Saskatoon, SA). Opportunistic sample collection was carried out serially, over periods of hyperphagia and fasting. The initial sampling period started in 1992, from September 25 to October 9 and October 26 to November 9, and in 1993 from March 1 to March 17. A similar sampling regime was carried out in the fall 1993/spring 1994 period, depending on the recapture of the same individuals. All bears were immobilized with the

tranquillizing drug Telazol, from a helicopter using well-established methods²¹¹.

An adipose biopsy (0.6 cm × 1.0 cm, 200 mg) was obtained under anaesthesia from the subcutaneous depot at the base of the tail ²¹². Approximately 80 % of the adipose tissue of adult polar bears is superficial and thus readily accessible ⁶⁴. Samples were stored in pre-cleaned vials, sealed, and frozen at -20°C. The vials were pre-cleaned by rinsing three times each with acetone and n-hexane. The milk samples were obtained from lactating bears after administration of oxytocin intramuscularly ²¹³. The milk was expressed into pre-cleaned vials and frozen at -20°C for storage.

4.2.1.3 Polar Bear Body Composition

Body composition (i.e., water, lean dry and fat percentage) was determined by Dr. Malcolm Ramsay and Susan Polischuck. Briefly, polar bear body composition was determined by using a two-compartment model in which body mass is comprised of fat (TBF) and lean body mass. First, the bears were weighed using an electronic load cell (± 450 g) suspended from an aluminum tripod. Total body water (TBW) was determined *in vivo* by dilution of deuterated water (D₂O, Atomic Energy Commission of Canada) ²¹⁴. Immediately upon capture a blood sample was taken to determine the baseline isotopic ratio (D₂O/H₂O). A precise amount of saline buffered 99.85 % D₂O (i.e., 0.5 mg of D₂O per kg body weight) was then injected via an indwelling catheter placed in a vein underneath the tongue. Post-injection blood samples were collected after 2 to 3 hours for isotope equilibration analysis. Serum from blood samples collected before and after injection were vacuum sublimated and the deuterium concentration determined by infrared

spectrophotometry. An estimate of TBW derived from dilutions was expressed as a percentage of total body mass and applied to a regression equation which describes the inverse relationship between % TBW and % TBF (i.e., $\% \text{ TBF} = 96.85 - (1.33 \times \% \text{ TBW})$). The weight of the polar bear livers were determined using an electronic load balance.

The total weight of extractable lipid for adipose or liver was determined by multiplying the total body weight of the tissue by the fraction lipid. The total body burden (TBB) of a CHC was calculated by multiplying the total weight of extractable lipid for a given tissue by the concentration of a CHC on a lipid weight basis.

4.2.2 Standards, Chemicals and Other Materials

The solvents and chemicals necessary for tissue extraction and the chromatographic materials have been described in detail in Chapter 2.

For CHC determination, including *ortho*-chlorine substituted PCBs, the tissues were spiked with 20 μl of a fully ^{13}C -labelled standard mixture of tetra- (2.5 ng/ μl), penta- (2.5 ng/ μl), and hexa-CIBz (2.5 ng/ μl), and CB-28 (2.0 ng/ μl), CB-52 (2.0 ng/ μl), CB-118 (2.1 ng/ μl), CB-153 (2.1 ng/ μl), CB-180 (2.0 ng/ μl) and CB-194 (2.1 ng/ μl). Just prior to GC/EI-MS analysis, 5 μl of CB-154 (5.0 ng/ μl) normalization standard was added. All ^{13}C -labelled standards were purchased from Cambridge Isotope Laboratories (Andover, MA)

For MeSO_2 -PCB and -4,4'-DDE determination, tissues were also spiked with 100 μl of 100 pg/ μl of MeSO_2 -IS. As mentioned previously, the number of authentic standards available for MeSO_2 -PCB identification and quantitation (provided by of Dr. Åke Bergman, Stockholm University, Stockholm, Sweden), for the different phases of the thesis

work, varied depending on the time in which each sub-project was begun. Twenty-five MeSO₂-PCB and -4,4'-DDE standards (excluding 3-MeSO₂-CB31, -CB95 and 4-MeSO₂-CB91, Table 2.1) were available for the quantitative tissue analysis outlined in the present chapter. For 3-MeSO₂-CB31, -CB95 and 4-MeSO₂-CB91, the ECD RRF value of their isomer pair was used for quantitation. At the time of the polar bear tissue distribution study, seventeen of the twenty-five MeSO₂-PCB standards were available for definitive identification and quantitation of the congeners present in tissue. The eight congeners that were absent for this study were subsequently shown to account for ca. 8 % and ca. 28 % of S-MeSO₂-PCBs in polar bear liver and fat, respectively.

4.2.3 Sample Preparation and Lipid Extraction

One gram samples of polar bear fat, cut into small pieces, were quantitatively weighed into mortars and mixed with 5 grams (ca. five times the amount of sample) of Na₂SO₄ to remove excess moisture. One gram of liver and 10 grams each of brain, testes and lung were homogenized prior to Na₂SO₄ addition. The mixtures were transferred to an extraction column. The sample was then spiked with 20 µl of the ¹³C-labelled standard and 100 µl of 100 pg/µl MeSO₂-IS. Lipid extraction of the tissue proceeded using 60 ml 50:50 dichloromethane/hexane for fat and liver and 100 ml for testes, lung and brain.

Ringed seal blubber and liver samples were approximately 2 grams and 10 grams, respectively, anticipating lower CHC levels than polar bears, on a lipid weight basis. Arctic cod sample sizes were maximized in anticipation of even lower CHC levels⁴⁶. The 10 and 40 gram pool samples were the maximum size that could be obtained. Five times the weight

of Na_2SO_4 , relative to the sample, was used to remove excess moisture. After transfer to an extraction column, the samples were spiked with the ^{13}C -labelled standard mixture and the MeSO_2 -IS as before. Lipid extraction of the tissue/ Na_2SO_4 mixtures proceeded using 60 ml 50:50 dichloromethane/hexane for ringed seal blubber and liver and 200 ml for arctic cod.

Polar bear adipose biopsies required different treatment for preparation and extraction compared to larger samples as described above. The adipose biopsy (ca. 100 to 200 mg) was clipped from the surface skin and hair into a tared and chemically cleaned scintillation vial and weighed. One gram of Na_2SO_4 was added, ground with the biopsy using a stainless steel spatula, and allowed to stand 0.5 hours. The sample was then spiked with 20 μl of the ^{13}C -labelled standard mixture and 100 μl of the MeSO_2 -IS. The biopsy/ Na_2SO_4 mixture was extracted three times with 3 ml of 50:50 dichloromethane/n-hexane. The extracting solvent was drawn up with a disposable pipette for quantitative transfer of the solvent/lipid mixture. After the addition of each fresh extraction solvent, the mixture was allowed to stand ca. 0.5 hours to ensure complete lipid extraction. The extracts were combined (9 ml total volume). The vial was heated at 90°C for 0.5 hours to drive off the excess organic solvent, then re-weighed to determine the fraction lipid.

The two polar bear milk samples of ca. 3.0 grams were collected from the same female at different time periods, once in the early spring (March) and the other in the fall (October) of 1994. The milk samples were extracted according to a standard direct extraction procedure^{60,215,216}. The milk sample was weighed into a 50 ml screw-top centrifuge tube and spiked with 20 μl of the ^{13}C -labelled standard mixture and 100 μl of MeSO_2 -IS as before. A 0.60 M potassium oxalate ($\text{K}_2\text{C}_2\text{O}_4$) solution was prepared using doubly-distilled, n-

hexane washed H_2O . To the milk was added 3 ml of the $\text{K}_2\text{C}_2\text{O}_4$ solution, followed by a thorough vortexing. Milk contains high levels of Ca^{2+} which can interfere with the extraction process. $\text{K}_2\text{C}_2\text{O}_4$ dissociates in an aqueous solution such as milk to yield the $\text{C}_2\text{O}_4^{2-}$ anion which readily binds with available Ca^{2+} to form a water-insoluble salt. Methanol (3 ml) was added to the milk mixture, vortexed, and allowed to stand for 0.5 hours. After the addition of diethyl ether (6 ml), 6 ml of n-hexane was added followed by vortexing. After centrifuging for 2 minutes at ca. 2000 r.p.m., the organic and aqueous phases separated. The top organic layer was transferred to a clean flask. The extraction procedure was repeated twice more with 6 ml n-hexane. A small amount of Na_2SO_4 was added to absorb any residual moisture.

4.2.4 Sample Clean-up and Chemical Analysis

After solvent evaporation of the lipid extracts at reduced pressure to ca. 1 ml, the liquid residue was quantitatively transferred to a GPC tube, followed by three times 1 ml of n-hexane wash. The n-hexane level in the GPC tube was adjusted to 5 ml (i.e., by a pre-marked line on the tube). For all the samples except the adipose biopsies, a 0.5 ml volume (10 %) of the solution was transferred to a pre-weighed aluminum dish for lipid determination. The solvent was allowed to evaporate at ambient room temperature (i.e., ca. 0.5 hours). The aluminum dish was transferred to an oven pre-heated at 90°C to drive off the remaining solvent. The dish was re-weighed and the percent lipid was determined gravimetrically.

The volume in the GPC tube was re-adjusted to 5 ml with n-hexane (i.e., 90 % of

original sample) and 5 ml of dichloromethane was added. The lipid extracts from all sample types were cleaned up, and the contaminant fractions isolated, using the analytical procedure described in Chapter 2. The aryl MeSO₂-containing compound levels were determined using GC/ECD quantitation method and their identities confirmed using GC/ECNI-MS. See Chapter 2 for a full description of the quantitation method and the GC/ECD and GC/ECNI-MS instrumental parameters.

For the polar bear tissue fractions, the CHCs, including PCBs, were determined by a single fraction, single injection GC/MSD approach using a characterized polar bear adipose tissue extract as a secondary standard (i.e., the PBQ method). See Chapter 6 for a full description of the PBQ method including the GC/MSD instrumental parameters.

The CHCs in ringed seal and arctic cod tissues samples were determined by a single fraction, double injection GC/EI-MS approach using two external standard mixtures. The approach was titled as the external standard quantitation (ESQ) method. The method was different from PBQ, since the number of PCB congeners in cod and seal was anticipated to be higher than in polar bear^{47,48}. The CHC compounds monitored and the GC retention time windows defining the ions monitored by EI-MS for each of the two injections are listed in Tables 4.1 and 4.2. The GC/EI-MS parameters were the same as used for GC/MSD described in Chapter 6.

Chlorinated pesticides were determined in the first injection (ESQ1) and the PCBs in the second injection (ESQ2). For each compound monitored in the sample, there was a corresponding external standard of known concentration. External standard quantitation was used with CB-154 added to the standard and sample prior to GC injection to correct for

Table 4.1. GC/El-MS Retention Time Windows and the Ions Monitored for CHC Compounds Determined by the First Injection: ESQ1 Method^{†‡}

Time Range (min)	Compound	Target/Qualifier	Time Range (min)	Compound	Target/Qualifier
5.6-7.6	1,2,4,5-TeClBz	216	16.2-18.2	t-chlordane	373/375
	¹³ C-1,2,4,5-TeClBz	222		c-chlordane	373/375
	1,2,3,4-TeClBz	216		t-nonachlor	409
7.6-9.6	PnClBz	250	18.2-35.0	dieldrin	380
	¹³ C-PnClBz	256		¹³ C-CB-52	304
9.6-13.0	α-HCH	219/217		4,4'-DDE	246/318
	HxCIBz	284		4,4'-DDD	235
	¹³ C-HxCIBz	292		c-nonachlor	409
	β-HCH	219/217		¹³ C-CB-118	338
	γ-HCH	219/217		¹³ C-CB-153	372
13.0-16.2	¹³ C-CB-28	268		¹³ C-CB-180	406
	octachlorostyrene	380		¹³ C-CB-194	442
	heptachlor epoxide	353		4,4'-DDT	235
	oxychlordane	387		photomirex	272/274
				mirex	272/274
				TCPMeOH	251
				CB-154	360/362

[†] CHCs listed in order of retention time on a 30 m, DB-5 GC column.

[‡] The SIM dwell time was 50 milliseconds (ms) for retention time windows containing < 3 ions. For > 3 ions, the SIM dwell time was 100 ms.

Table 4.2. GC/EI-MS Retention Time Windows and the Ions Monitored for PCB Compounds Determined by the Second Injection: ESQ2 Method^{†‡}

Time Range (min)	Compound	Target/Qualifier	Time Range (min)	Compound	Target/Qualifier
11.0-17.0	CB-4/10	222/224	24.0-33.0	CB-97	326/328
	CB-7/9	222/224		CB-87	326/328
	CB-6	222/224		CB-85	326/328
	CB-5/8	222/224		CB-136	360/362
	CB-17/18	256/258		CB-110	326/328
	CB-24/27	256/258		CB-154	360/362
	CB-16/32	256/258		CB-135/151	360/362
	CB-28/31	256/258		CB-149	360/362
17.0-24.0	¹³ C-CB-28	268	33.0-37.5	CB-118	326/328
	CB-20/33	256/258		¹³ C-CB-118	338
	CB-22	256/258		CB-146	360/362
	CB-45	290/292		CB-153	360/362
	CB-52	290/292		¹³ C-CB-153	372
	¹³ C-CB-52	304		CB-105	326/328
	CB-49	290/292		CB-179	394/396
	CB-47/48	290/292		CB-141	360/362
	CB-44	290/292		CB-130	360/362
	CB-42	290/292		CB-138	360/362
	CB-37/39	256/258		CB-187	394/396
	CB-41	292/290		CB-183	394/396
	CB-64	290/292		CB-128	360/362
	CB-74	292/290		CB-174	394/396
	CB-70/76	290/292		CB-177	394/396
	CB-66	290/292		CB-180	394/396
	CB-95	326/328		¹³ C-CB-180	406
	CB-91	326/328		CB-170/190	430/428
	CB-56/60	290/292		CB-201	430/428
	CB-84	326/328		CB-196/203	430/428
	CB-101	326/328		CB-195	430/428
	CB-99	326/328		CB-194	430/428
				¹³ C-CB-194	442
				CB-206	464/466
				CB-209	498/500

[†] PCBs listed in order of retention time on a 30 m, DB-5 GC column.

[‡] The SIM dwell time was 100 ms for all ion retention time windows.

differences in the final volume and the volume injected.

The majority of the chlorinated pesticide levels (Table 4.1) and the PCB congener levels (Table 4.2) were recovery-corrected using the average recovery of the five higher chlorinated ^{13}C -labelled PCB standards (i.e., CB-52, CB-118, CB-153, CB-180 and CB-194, Table 4.2). The more volatile ClBz levels were recovery corrected using the percent recoveries of their corresponding ^{13}C -labelled ClBz standards (Table 4.1). A further correction of the ClBz levels was also necessary due to the overlap of the $(\text{M}+6)^+$ molecular ion peak of the ^{13}C -ClBz standard with the $(\text{M})^+$ molecular ion peak of the unlabelled contaminant. The ratio of the $(\text{M})^+$ to $(\text{M}+2)^+$ ions in the unlabelled ClBzs should be the same for both the sample and standard peaks. A ratio of these ratios was used to generate a correction factor to compensate for the deviation in the $(\text{M})^+$ to $(\text{M}+2)^+$ ratio in the unlabelled ClBzs due to the contribution of $(\text{M}+6)^+$ from the ^{13}C -labelled standard. The semi-volatile HCH compounds were recovery-corrected by interpolation, based on GC retention time, of the percent recovery of the ^{13}C -labelled ClBzs.

4.3 Results and Discussion

4.3.1 CHCs and Metabolites

Table 4.3 lists the total levels of the major CHCs determined in whole body arctic cod, ringed seal blubber, and polar bear fat and liver from the Resolute Bay area of the Canadian high Arctic. HCHs and ClBzs dominated the CHC profile in arctic cod, DDTs, PCBs, CHLORs and HCHs in ringed seal blubber, and PCBs and CHLORs in polar bear

liver and fat. The CHC patterns were similar to previous results for the same species from central Canadian Arctic locations, with some exceptions ^{45-48,99}. For example, S-ClBzs and S-CHLORs in whole body arctic cod were significantly higher than that found in arctic cod muscle from the same geographical area in 1984 ⁴⁷. Polychlorinated camphenes (PCCs, toxaphenes) have been shown to be the most significant CHC in cod ²¹⁷ and a major CHC in ringed seal blubber ⁴⁸, however, this contaminant class was not analyzed for in the present study.

Comparison with recent summaries of CHC levels in seals from areas of the Canadian Arctic, the Baltic and North Seas, the Dutch coast and the Lake Baikal, Russia, indicated that S-DDT and S-PCB levels in Canadian arctic seals (Table 4.3) were by far the lowest ^{48,218}. The S-DDT levels were roughly similar, and the S-PCB levels significantly lower (ca. 5 times), than seals from the Greenland Sea taken in 1991 ²¹⁹. Further east, ringed seals from Spitzbergen (Norway) sampled in 1986 had S-PCB and S-DDT levels that were significantly higher (ca. 2 and 3 times, respectively) than the present Canadian arctic ringed seal ²²⁰. Geographical area and temporal differences in tissue sampling were suggested as reasons for the varying S-DDT levels for seals from Spitzbergen and the Greenland Sea ^{48,219}.

The 4,4'-DDE was 66 % of S-DDT in ringed seal blubber (Table 4.3). In harbor seals from the North Sea area near the U.K. coast ²²¹, and seals from Spitzbergen²²⁰ and the Greenland Sea, 4,4'-DDE was ca. 65 % of S-DDT ²¹⁹, whereas in Baikal seals from Lake Baikal, Russia the value was ca. 56 % ²¹⁸. It was suggested that a lower percentage is indicative of a recent input of technical DDT into an ecosystem, whereas a higher percentage may indicate input at an earlier time ²¹⁸, however, it is doubtful that there is any statistically

Table 4.3. Total CHC and MeSO₂-PCB and -4,4'-DDE Levels (ng/g, lipid wt.) in the Polar Bear Food Chain from the Resolute Bay Area in the Canadian High Arctic*

species (year)	tissue [‡]	N	S-HCH	S-CIBz	S-CHLOR	S-DDT	4,4'- DDE	S-PCB [°]	S-MeSO ₂ - PCB	3-MeSO ₂ - 4,4'-DDE	S-MeSO ₂ - PCB/CB-153 Ratio [°]	3-MeSO ₂ - 4,4'-DDE/ CB-153 Ratio [°]
Arctic cod	whole body pools [†]	2 324/ 334	476/ 513	173/ 110	25.9/ 34.0	9.3/ 8.1	71.8/ 33.0	< 0.05/ < 0.05	< 0.05/ < 0.05	< 0.05/ < 0.05	N/A/ N/A	N/A/ N/A
ringed seal (1989)	blubber	11 450 ± 117	36.7 ± 8.9	567 ± 131	505 ± 143	335 ± 100	411 ± 88	13.6 ± 4.3	0.38 ± 0.16	0.22	0.0066	
polar bear (1993)	fat	6 725 ± 95	612 ± 53	5215 ± 279	301 ± 32	268 ± 27	6207 ± 948	458 ± 59	2.04 ± 0.68	0.14	0.0007	
	liver	6 717 ± 248	525 ± 249	34015 ± 26232	1251 ± 705	556 ± 282	23127 ± 4900	1830 ± 446	198 ± 84	0.28	0.30	

* The concentrations are the mean ± SD. MeSO₂-PCBs and -4,4'-DDEs in Arctic cod were below the detection limit.

† The percent lipid was 6.5/7.0 % in arctic cod, 90.1 ± 6.3 % in ringed seal blubber, 65.0 ± 3.2 % in polar bear fat and 6.6 ± 0.7 % in polar bear liver.

‡ There were 2 pools, containing 9 individual cod each. CHC levels for both pools are shown.

° 72 PCB congeners (Table 4.2) were determined in arctic cod and ringed seal, and 22 PCB congeners in polar bear (Table 6.1).
° Includes 2-MeSO₂-4,4'-DDE for polar bear liver.

significant difference in these percentages.

There was no significant difference between S-PCB levels for Canadian arctic ringed seal males versus females (not shown), however, this does not appear to be unusual for the Arctic. A similar result was reported for male and female ringed seals from Inukjuak (eastern Hudson Bay)⁹⁹. Other reported results have shown S-PCB levels in Resolute Bay ringed seal males to be significantly higher than females and to be age dependent^{47,222}. Generally, the lower CHC levels found in female relative to male seals^{48,218} are primarily due to CHC transfer from female to pup through gestation and lactation²²³. It was not possible to comment on differences in CHC levels in ringed seal due to age, as the data set was not large enough and some ages were not known.

Changes in the CHC composition during bioaccumulation in a food chain are determined primarily by the capability of a species to metabolically transform and subsequently excrete the compound⁴⁵. The biomagnification factor (BMF) is defined as the ratio of a CHC concentration in an organism relative to the concentration in its diet. The BMF for S-CIBz from cod to seal was 0.1 (Table 4.4), indicating excretion and/or metabolic clearance. The BMFs were greater than one for S-HCH, S-CHLOR, S-DDT and S-PCB, indicating biomagnification. The BMFs were especially high for S-DDT and S-PCB.

The BMF of 4,4'-DDE from cod to seal was about two times the BMF of S-DDT (Table 4.4), probably due to metabolic conversion of 4,4'-DDT to 4,4'-DDE. CB-153 is a highly recalcitrant PCB congener in most animal species⁶ (see Section 4.3.2), as indicated by the high BMF from cod to seal and seal to bear (Table 4.4). Using the ratios of DDTs to CB-153, the maximum metabolic conversion of 4,4'-DDT to 4,4'-DDE in seal (i.e.,

Table 4.4. Biomagnification Factors (BMFs) for the Polar Bear Food Chain[†]

compound	seal blubber/cod	bear fat/seal blubber
S-HCH	1.4	1.6
S-CIBz	0.1	16.7
S-CHLOR	4.0	9.2
S-DDT	17.1	0.6
4,4'-DDE	38.5	0.8
S-PCB	7.8	15.1
S-MeSO ₂ -PCB	N/A	29.7 (148) [‡]
S-MeSO ₂ -4,4'-DDE	N/A	5.4 (512) [‡]
CB-153	26.6	47.5

[†] Ratio of tissue concentration at one food chain level to those at the next lowest level (on a lipid weight basis).

[‡] Number in parentheses is the ratio of seal blubber to polar bear liver.

Table 4.5. Total Body Burden (TBB) of CHCs in Polar Bear from the Resolute Bay Area of the Canadian High Arctic[†]

compound*	liver [‡] (mg)	fat [‡] (mg)	% in liver relative to fat
S-HCH	0.12 ± 0.05	53.3 ± 7.0	0.2 ± 0.1
S-CIBz	0.10 ± 0.05	45.0 ± 3.9	0.2 ± 0.1
S-CHLOR	6.56 ± 5.06	383.7 ± 20.5	1.7 ± 0.5
S-DDT	0.24 ± 0.08	21.7 ± 2.4	1.1 ± 0.4
4,4'-DDE	0.11 ± 0.06	19.3 ± 29.7	0.6 ± 0.2
S-PCB	4.46 ± 0.94	430.3 ± 56.0	1.0 ± 0.2
S-MeSO ₂ -PCB	0.35 ± 0.10	33.7 ± 3.6	1.5 ± 0.3
S-MeSO ₂ -4,4'-DDE	0.07 ± 0.01	0.2 ± 0.1	38.5 ± 13.2

[†] The total body mass of liver and adipose were 6650 ± 2740 grams and 113180 ± 32570 grams, respectively (see Section 4.2.1.3 for method of calculation). The percent lipid in polar bear fat was 65.0 ± 3.2 % and in liver was 6.6 ± 0.7 %.

* See Table 4.3 for CHC level on a ng/g lipid weight basis.

[‡] The mean ± SD of liver and fat of *n* = 6 of individual male bears.

$(R^{153}_{DDE})_{seal(potential\ DDE)}$ plus the accumulation of 4,4'-DDE from cod, was used to calculate the maximum ratio of 4,4'-DDE to CB-153 in seal (eq. 5).

$$(R^{153}_{DDT})_{cod} - (R^{153}_{DDT})_{seal} = (R^{153}_{DDE})_{seal(potential\ DDE)}$$

then, $(R^{153}_{DDE})_{seal(potential\ DDE)} + (R^{153}_{DDE})_{cod} = (R^{153}_{DDE})_{seal(max.)}$ (5)

The ratio of the $(R^{153}_{DDE})_{seal(max.)}$ relative to the actual $(R^{153}_{DDE})_{seal}$ was 1.18, suggesting that the higher BMF of 4,4'-DDE relative to S-DDT was due, in large part, to conversion of 4,4'-DDT to 4,4'-DDE in seal. However, the value greater than one suggested that 4,4'-DDT was metabolized to metabolites other than 4,4'-DDE and/or 4,4'-DDE was itself further metabolized. Although the formation of $MeSO_2$ -4,4'-DDE was indicated (Table 4.3), 4,4'-DDE metabolism by the MAP pathway did not appear to be large, since $MeSO_2$ -4,4'-DDE was only ca. 3 % of 4,4'-DDE in adipose tissue.

CHC levels in polar bear fat were consistently greater than in seal (Table 4.3), with the exception of S-DDT and 4,4'-DDE, which had a BMF of less than one from seal to bear fat (Table 4.4). This was likely due to metabolism, but not conversion to $MeSO_2$ -4,4'-DDE, which had a TBB less than 2 % of 4,4'-DDE (Table 4.5). The largest contributor to S-CHLOR in polar bear fat and liver was the metabolite oxychlordanes (56.2 ± 6.6 % and 61.2 ± 5.6 %, respectively). The oxychlordanes contribution was ca. 40 % for ringed seal blubber and ca. 10 % for arctic cod, indicating the metabolic capacity was cod < seal < bear. Oxychlordanes contribution in cod was probably resulted from exposure to chlordanes metabolites already present in the environment rather than by metabolic formation in the fish.

Fish generally lack the CYP2B-type metabolic capacity necessary for chlordane degradation^{87,224}. S-CHLOR in arctic cod from Resolute Bay is known to be comprised of mostly of *cis*- and *trans*-chlordane and *trans*-nonachlor²²⁵. Further, the biotransformation of chlordane is known to be greater in higher trophic level organisms²¹⁸.

Overall, the BMFs from cod to seal and seal to bear for the CHC levels (Table 4.4), were similar to those generated by Muir *et al.*⁴⁷. These results were consistent with trophic level relationship of CHCs observed in the present study. Ringed seal and arctic cod in the high Arctic have been shown by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analysis to occupy roughly the same trophic levels at certain times of the year⁵², which was probably influenced by the presence of amphipods in the diet. The annual average food ingestion by ringed seals in Barrow Strait has been shown to be ca. 60 % cod and ca. 35 % amphipods^{49,226}. Pelagic cod feed largely on amphipods.

Levels of S-CHLORs and S-PCBs in polar bear were significantly higher in liver relative to fat (Table 4.3). In terms of the total body burden (TBB) (Table 4.5), fat contained > 98 % of the CHCs, except for S-MeSO₂-4,4'-DDE. Despite the small contribution of liver to the total tissue mass and lipid, 38.5 ± 13.2 % (Table 4.5) of the TBB of MeSO₂-4,4'-DDE was in the liver. In the early spring, polar bear adipose deposits are known to constitute approximately 45 % of the total body mass²¹², whereas liver is known to constitute 2.6 ± 0.3 % of total body mass, regardless of sex and the size of the adipose depots²²⁷.

4.3.2 Residual PCB Patterns and Capacity and Mode of Metabolism

Residual PCB congener levels in tissue have often been converted to ratios to the

major congeners CB-153 (2,2',4,4',5,5') or CB-180 (2,2',3,4,4',5,5') (i.e., $(R^{153}_p)_{\text{species}}$) to compare PCB patterns among organisms (eq. 6) ^{6,44,47,99,100,218}.

$$(R^{153}_p)_{\text{species}} = ([\text{PCB}_p] / [\text{CB-153}])_{\text{species}} \quad (6)$$

CB-153 and CB-180 are generally resistant to metabolic degradation by either CYP1A or CYP2B-type enzymes. Their biphenyl rings are non-coplanar as a result of *ortho*-chlorine substitution > 1, a lack of vicinal hydrogens, and *para* chlorine substitution which hinders *para*-hydroxylation ¹⁰². They are ideal reference PCBs for metabolic indices, to express the relative potential for PCB metabolism. CB-153 represented 44 % of S-PCBs in polar bear fat, 14 % in ringed seal and 4 % in whole cod. The percentage of CB-153 of the S-PCBs in cod and ringed seal blubber was slightly lower than the values of 20 % and 7 %, found previously ⁴⁷. Forty-four and thirty-eight PCB congener peaks for cod and seal, respectively, were above the detection limit in Muir *et al.* ⁴⁷, whereas forty-seven and fifty-three peaks were above the detection limit (0.05 ng/g lipid wt.) in the present study. Differences in the CB-153 recovery may be due to the recovery-correction of the PCB levels, unlike the levels reported in Muir *et al.* ⁴⁷. However, this would only be applicable to the less chlorinated congeners, which had lower percent recoveries than the higher chlorinated congeners.

The majority of R^{153}_p values decreased going from cod to seal to polar bear and revealed an increasingly simplified PCB pattern (Figure 4.1). The PCB pattern for cod was similar to that found previously ⁴⁷, where the PCB profile was dominated by CB-95, CB-101, CB-110, CB-118, CB-138, CB-149 and CB-153 ($R^{153}_p > 0.8$). CB-52 and CB-99 were

Figure 4.1. Ratios of PCB to CB-153 concentrations (R^{13}_p) in arctic cod, ringed seal blubber and polar bear fat from the Resolute Bay area of the Canadian high Arctic. The clear boxes on the baseline denote congeners that were below detection. Striped bars indicate PCB congeners known to be metabolized to persistent MeSO₂-PCBs. The most abundant PCBs in each species are labelled. The CB-153 reference peak is denoted by the dark gray bar.

Figure 4.2. Sum of the R^{133}_p by PCB isomer group for the polar bear food chain. Derived from Figure 4.1.

also significant contributors. There was little difference in the PCB pattern relative to the Aroclor standard (1242:1254:1260, 1:1:1) indicating minimal metabolic biotransformation activity as found previously ⁴⁷, where the apparent ratio of Aroclor mixtures (1242:1254:1260) was 0.6:3:1. Fish have little or no CYP1A- and CYP2B-type metabolic activity towards PCBs relative to higher trophic level species, as judged by residual PCB patterns relative to PCB technical mixtures ²²⁸. However, fish possess a high CYP1A-type metabolic capacity to metabolize petroleum hydrocarbons²²⁹. Arctic cod are critical species in energy, and therefore contaminant flow, in the arctic marine environment ⁵⁰. Patterns of bioavailable PCB congeners to higher trophic levels in the arctic marine environment are therefore represented by arctic cod ⁴⁷. In cod, PCBs of intermediate to higher chlorination were most significant (Figure 4.1). In air and water, lower chlorinated PCBs are known to be more significant than higher chlorinated PCBs ³. Selective clearance of lower chlorinated PCBs is known to occur at lower trophic levels, including fish ²³⁰.

The R^{153}_p values demonstrated that the tri- to penta-chlorinated PCB congeners were notably reduced in seal relative to cod (Figure 4.1). The decreased levels of the lower chlorinated congeners in seal are indicative of higher metabolic capacity relative to fish ²¹⁸. For ringed seal, the R^{153}_p values for lower chlorinated PCBs were disproportionately smaller than the higher chlorinated PCBs, relative to cod. The situation for the R^{153}_p in polar bear favoured the higher chlorinated PCBs even more. Figure 4.2 illustrates the increasing significance of the higher chlorinated PCBs moving up the polar bear food chain. It also demonstrates the relatively constant degree of PCB isomer group clearance in seal, since the PCB profiles in seal and cod were not so different, although > 50 % of PCBs were cleared

relative to CB-153. A combination of selective uptake of higher chlorinated PCBs and non-metabolic clearance of lower chlorinated PCBs may partially account for this trend. The reduction of certain PCB congeners relative to CB-153 in food chains is probably due to metabolism, mediated by increased activity of species-specific CYP enzymes ^{6,47,101}. For example, several studies by Tanabe and coworkers ^{5,44,218} have demonstrated that CYP2B-type activities increase from marine mammals to birds to terrestrial mammals. The level of CYP1A-type activities were found to be more random among species, however, higher CYP1A-type activities were linked to higher CHC body burdens.

PCBs in ringed seal blubber were dominated by CB-99, CB-101, CB-118, CB-138 and CB-153 ($R^{153}_p > 0.4$). These PCB congeners have been found to be more significant relative to other congeners in northern fur seals ²³¹, ringed seals from the Resolute Bay area of the Canadian Arctic ⁴⁷, harbor and ringed seals from the Netherlands ¹⁰⁰, ringed seals from the Inukjuak region (eastern Hudson Bay) ⁹⁹, and Baikal seals from Lake Baikal, Russia ²¹⁸. CB-52, CB-74, CB-110 and CB-105 were also significant in Canadian arctic ringed seals (Figure 4.1).

MO-CB-105 and MO-CB-118 are characterized by *ortho-meta* vicinal hydrogens and can achieve a coplanar configuration due to the low energy barrier of rotation between phenyl rings ⁸¹. Congeners with this structural feature are reduced in highly contaminated seals from other geographic locations, presumably because of metabolism by highly induced CYP1A isozymes ^{44,89,90,218}. Haraguchi *et al.* ¹⁶⁸ showed that Baltic grey seals with S-PCB ca. > 100 µg/g (lipid wt.) had non-detectable CB-118 levels whereas seals with S-PCB levels < 100 µg/g (lipid wt.) had detectable CB-118 levels. The presence of CB-105 and CB-118 in

Canadian arctic ringed seal (Figure 4.1) and a mean S-PCB level of $0.411 \pm 0.088 \mu\text{g/g}$ (lipid wt., Table 4.3) suggested a low level of CYP1A-type activity. Differences in the environmental input of CB-118 into the Canadian Arctic and Baltic regions is not expected to be very significant since CB-118 is a substantial component of all PCB technical mixtures.

Recently, Boon *et al.*¹⁰⁰ classed PCB structures in marine mammals into five groups, based on their metabolic clearance as a function of the residual PCB patterns relative to their prey (i.e., $R^{\text{rel}}_p = (R^{153}_p)_{\text{mammal}} / (R^{153}_p)_{\text{fish}}$). Whales and seals were both found to have $R^{\text{rel}}_p < 1$, for PCB with *ortho-meta* vicinal hydrogens and one *ortho* chlorine, indicating similar CYP1A capacities. However, the R^{rel}_p diverged for PCB with *meta-para* vicinal hydrogen, regardless of *ortho* chlorine substitution. The R^{rel}_p value is less than one for seal, but around unity for whales, indicating higher CYP2B capacity for seals. PCBs with *meta-para* vicinal hydrogens (e.g., CB-52, CB-49 and CB-101) are known to be metabolized by CYP2B-type enzymes^{78,84}. The R^{153}_p values (Figure 4.1) for PCBs with this structural characteristic were smaller in seal relative to cod (Figure 4.1), regardless of *ortho* chlorine substitution. A similar result was found for ringed seal from Foxe Basin and eastern Baffin Island in the Canadian Arctic⁹⁹.

From the R^{153}_p values (Figure 4.1), the R^{rel}_p demonstrated that all congeners were less significant in bear fat relative to seal with the exception of CB-180, CB-170/190 and CB-194, indicating that the largest degree of PCB biotransformation was in polar bear. CB-170/190 was most likely only CB-170, since the R^{153}_p value of 0.19 in bear was very close to the value of 0.22 found previously for CB-170⁴⁷. All PCBs with significant

bioaccumulation potential in polar bears have 2,2',4,4' chlorine substitution. However, unsubstituted *para* positions and increasing *ortho* substitution appear to enhance metabolic clearance in polar bear. For example, CB-183 possesses 2,2',4,4' substitution, but has another *ortho* chlorine (i.e., in the 6-position), yet is cleared to a much greater degree than CB-180, CB-194 and CB-170 in polar bear (Figure 4.1).

The severe reduction of mono-*ortho* CB-105 and CB-118, in contrast to the small change in CB-99 in polar bear (Figure 4.1) relative to seal indicated high CYP1A-type activity in bear. High levels of CYP1A proteins were found in these bears, which correlated with the NO- and MO-PCBs (i.e., including CB-105 and CB-118), expressed as TEQ concentrations (see Chapter 6). In the western hemispheric Arctic, this CYP1A-type activity appears to be unique to polar bear since CB-105 and CB-118 residues have been found in all arctic marine mammals studied so far⁴⁵. Some mammals with very high CHC body burdens, such as Baltic seals with S-PCB levels of ca. 100 ppm¹⁶⁸, did not have detectable levels of CB-118. However, Tanabe *et al.*⁵ has shown that killer whale and striped dolphin with S-PCB levels of ca. 100 ppm retained residual levels of both CB-105 and CB-118.

Normally recalcitrant PCBs in seal, such as CB-138, had somewhat reduced R^{rel} values in polar bear, suggesting that a planar PCB configuration was not necessary for metabolism of PCBs possessing *ortho-meta* vicinal hydrogens, however, this does not explain the relatively lower presence of CB-170. Perhaps, the increased metabolism of CB-170 is influenced by its higher chlorination, rendering it more compatible with the enzymatic cleft of the CYP1A-type enzyme in polar bear. Brown¹⁰¹ suggested that PCB metabolism in polar bear may arise from other CYP enzymes.

PCB congeners possessing *meta-para* vicinal hydrogens were present in seal blubber but notably absent in polar bear (Figure 4.1), indicating a high degree of CYP2B-type activity in bear. These PCBs included CB-49, CB-52, CB-64, CB-70, CB-91, CB-95, CB-101, CB-110, CB-141, CB-132, and CB-174. All of these PCB congeners form MeSO₂-PCBs (see Section 4.3.3). Marine mammals have been shown to have a lower capacity than terrestrial mammals to metabolize PCBs with *meta-para* vicinal hydrogens, probably mediated by CYP2B-type enzymes⁵⁴⁵. The rapid metabolism of *meta-para* unsubstituted PCBs in polar bear could be due to a combination of greater CYP2B induction as a consequence of higher CHC exposure due to more concentrated contaminant levels in the seal blubber diet, the induction of different CYP2B-type enzymes, or a higher metabolic capacity of CYP2B-type enzymes.

Parkinson *et al.*²³² have shown that chlorination of PCB in the *ortho* and *para* positions of both phenyl rings facilitates CYP2B-type induction in laboratory rodents, which is not altered by further *ortho*- or *meta*-chlorination. Highly induced levels of CYP2B1 proteins were found in these bears, which correlated with the higher *ortho* PCB residue levels (see Chapter 6).

The PCB structure-activity relationships (SARs) in bear and seal, as indicated by the residual PCB patterns, may have to do with the specific activity of their CYP1A- and CYP2B-type enzyme systems. Brown¹⁰¹ has speculated that the overall clearance rate for an individual PCB congener in a human was a function of the relative metabolic rate constants and activities of CYP1A- and CYP2B-type systems, as well as the non-metabolic PCB clearance rate.

4.3.3 MeSO₂-PCB and -4,4'-DDE Metabolites

4.3.3.1 Congener Identification and Structural Rules for Formation

Little is known of the identities and concentrations of MeSO₂-PCB and -4,4'-DDE metabolites in biota apart from the Baltic Sea environment (Table 4.6). The GC/ECNI-MS mass chromatograms for the aryl methyl sulfone fraction, isolated from adipose and liver of seal (Figure 4.3) and polar bear liver and fat (Figure 2.2) showed that the structures of the persistent MeSO₂-PCB congeners were, i) 3- and 4-MeSO₂-substituted and generally occurred in pairs derived from the same precursor PCB having *meta-para* vicinal hydrogens on at least one phenyl ring, ii) possessing trichloro- to heptachloro-substitution, iii) 2,5 or 2,5,6-chloro substituted on the MeSO₂-containing phenyl ring and iv) at least 4' (*para*) chlorine substituted on the non-MeSO₂-containing phenyl ring. This was consistent with the congeners detected so far in other biota, including humans (Figure 1.5) ^{71,136,138,164,168,171}.

The minimum requirement of 2,5 chlorine substitution of the persistent MeSO₂-PCBs in seal and bear may be due to the 3,4-epoxide stabilization afforded by the 2,5-dichloro substitution, and lack of an easily metabolized structure on the other ring. Epoxide formation is the primary metabolic step for PCB metabolism (see Chapter 1) ²³³. The epoxide is subject to attack by cytosolic GSH, the initial step in the mercapturic acid pathway eventually leading to methyl sulfone formation ¹¹⁴. However, this does not preclude the formation of hydroxylated PCB metabolites from the epoxide intermediate (see section 4.3.3.2). A minimum of one chlorine is also required at the 4'-position to hinder epoxidation at the 3',4'-position, which would lead to formation of readily conjugated and excreted mono- and di-hydroxylated metabolites ²³³. In addition, combinations of 2',5' and 2',3',6' chlorine

Table 4.6 A Summary of the Total Levels (lipid weight basis) and Number of Congeners Identified of Persistent MeSO₂-PCBs and -4,4'-DDE in Wildlife and Humans

species	location	age/sex	tissue	S-MeSO ₂ -PCB conc. (µg/g)	# of MeSO ₂ -PCB congeners detected	MeSO ₂ -DDE conc. (µg/g)	S-MeSO ₂ -PCB/S-MeSO ₂ -DDE Ratio	S-MeSO ₂ -DDE/DDE Ratio	Ref.
grey seal	Baltic Sea	juv. ¹	blubber	13.0	20	0.4	0.14	0.01	146
ringed seal	Baltic Sea	juv. ¹	blubber	4.4	--	0.1	0.17	0.012	146
harbour seal	Baltic Sea	juv. ¹	blubber	1.7	--	0.2	0.05	0.007	146
grey seal	Baltic Sea	juv.	blubber	2.7	33	0.1	0.10	0.006	71,173
		/female	liver	21.0	16	0.8	0.91	0.16	
otter	N. Sweden	male ¹	muscle	0.6	--	0.01	0.04	0.02	71
			liver	13.0	--	0.2	0.19	0.01	
polar bear	Hud. Bay, Canada	4 yr./male	blubber	0.6	24	n.d.	0.04	--	71
			liver	3.0	20	1.3	0.10	0.50	
beluga whale	St. Law.	25 yr.	blubber	0.4	17	n.d.	0.02	--	71
wild mink	R., Canada	/female	muscle	0.2	--	0.015	0.007	0.006	71
	N. Sweden	/1 male ¹	liver	1.5	--	0.06	0.07	0.04	
blue whale	Japan	unknown	blubber	0.0099	ca. 37	0.0003	--	N/A	210
rainbow trout	Japan	unknown	whole body	0.0043	ca. 28	n.d.	--	N/A	210
sardine	Japan	unknown	whole body	0.0009	ca. 50	n.d.	--	N/A	210
oyster	Japan	unknown	whole body	0.0005	ca. 35	n.d.	--	N/A	210
clam	Japan	unknown	whole body	0.0003	ca. 35	n.d.	--	N/A	210
human	Sweden	adult	milk	0.009-	--	0.005-	0.008	0.005	146
		/female	pool (10-20)	0.002	23	0.0004	--	--	
human	Japan	adult/..	liver*	3.4(3.2)	> 60	--	--	--	134-138
			lung*	15.9(2.1)	> 60	--	--	--	
			adipose*	10.3(5.7)	> 60	--	--	--	

¹ Homogenate of 10 individuals of both sexes.¹ Age unknown, average of 2 individuals. * Levels for a person afflicted with, "Yusho" PCB rice poisoning, value in parentheses was for a control individual. n.d. = not detected. N/A = not applicable.

substitution can lead the formation of *bis*-MeSO₂-PCBs as was observed in polar bear liver (Chapter 2). Applying these rules to commonly occurring PCBs in commercial mixtures¹, only 14 to 20 congeners are likely to be transformed into MeSO₂-PCBs. Seventeen and 14 PCBs with 2,5 or 2,3,6 chlorine substitution have been shown to be present in the technical PCB mixtures Clophen A50 and A60, respectively²³⁴. It is probable that the maximum of twenty-three MeSO₂-PCBs congeners identified so far (Table 4.6) represent the majority of the S-MeSO₂-PCBs likely to be formed in biota. The subject of PCB metabolism and MeSO₂-PCB depuration, and their effect on MeSO₂-PCB bioaccumulation in the polar bear foodchain is discussed further in Section 4.3.3.2.

The structures of 24 and 26 MeSO₂-PCBs were identified in bear fat and liver (Figure 2.2), respectively, and 29 congeners were identified in seal blubber and liver (Figure 4.3). Several minor peaks were identified in seal as trichloro- to hexachloro-MeSO₂-PCBs from their ECNI mass spectra. According to the rules for the metabolic formation of persistent MeSO₂-PCBs, there were no other PCB structures in cod with 2,5 or 2,3,6 chlorine substitution on the phenyl ring and at least a 4'-chlorine on the other ring. MeSO₂-PCBs with unblocked 4'-positions, such as MeSO₂-CB52 and -CB95, were further metabolized to *bis*-MeSO₂-PCBs (see Chapter 2). Fewer MeSO₂-PCBs additional to the major congeners were detected in Baltic seals, which are exposed to much higher CHC levels than Canadian arctic ringed seals^{71,166,173}. Perhaps the higher CYP2B-type activity of seals from the Baltic region relative to arctic ringed seals may explain the MeSO₂-PCB congener pattern differences.

(A) Ringed Seal Blubber

(B) Ringed Seal Liver

Figure 4.3. GC/ECNI-MS mass chromatograms (DB-5 column) of the aryl methyl sulfone fraction from, A) ringed seal blubber and B) ringed seal liver. Peak identification numbers correspond to the MeSO₂-PCB/-4,4'-DDE structures denoted in Table 2.1 (see Chapter 2). Peaks denoted by, "Cl_f," were detected as a MeSO₂-PCB chlorinated isomers from their ECNI mass spectra (see Figure 2.3).

Six of the MeSO₂-PCB congener structures identified in the seal and bear tissues, have yet to be observed in other biota ¹⁷³, that is, 3- and 4-MeSO₂-CB31, 3-MeSO₂-CB95a, 4-MeSO₂-CB91, 4-MeSO₂-CB52 and 3-MeSO₂-CB64. The number of MeSO₂-PCB congeners in polar bear was twice that identified previously in the liver and fat of two polar bears from the western Hudson Bay area ⁷¹. An additional 6 MeSO₂-PCB structures (i.e., 3- and 4-MeSO₂-CB31, 4-MeSO₂-CB52 and -CB95a, and 3- and 4-MeSO₂-CB64) were identified in Canadian arctic ringed seal on top of the 23 reported in Baltic grey seal blubber ¹⁷³. The number of MeSO₂-PCB congeners presently detected and structurally identified in wild mammals, humans, birds and fish is summarized in Table 4.6 along with S-MeSO₂-PCB and -4,4'-DDE levels.

The 4-MeSO₂-CB31, 3-MeSO₂-CB95a, 4-MeSO₂-CB91 and 4-MeSO₂-CB52 were identified in polar bear and ringed seal by GC retention time and ECNI mass spectral comparison to the authentic standards. The identification of the 3-MeSO₂-CB31 congener was based on the ca. 0.5 min separation between 3- and 4-MeSO₂-PCB pairs. The molecular ion and characteristic fragmentation of MeSO₂-PCBs in the ECNI mass spectra (Figure 2.3), indicated that the peak was a tetrachloro-MeSO₂-PCB. Further, the relative abundance of (M-CH₃)⁺ for 3-MeSO₂-PCBs tend to be higher than their 4-MeSO₂-PCB pair (see Table 3.1), and the 3- and 4-MeSO₂-CB31 were not exceptions to this rule. The assignment of the tetrachloro-MeSO₂-PCB peak identified as 3-MeSO₂-CB64 was tentative. No standard was available for comparison and the GC time separation from 4-MeSO₂-CB64 was less than 0.5 min. However, the peak coincided with a previously observed peak, under similar chromatographic conditions, in Baltic mink that had been dosed with the technical PCB

mixture, Clophen A50 ¹⁷¹. Also, the relative abundance of the (M-CH₃)⁺ ion for the apparent 3-MeSO₂-CB64, GC/ECNI-MS mass chromatogram peak was greater than the relative abundance of the (M-CH₃)⁺ in the peak identified as 4-MeSO₂-CB64 (not shown).

The GC retention times of MeSO₂-PCBs on a non-polar DB-5 GC column (e.g., Figures 2.2 and 4.3), indicated that, i) the 3-MeSO₂-PCB metabolites always eluted before their 4-MeSO₂-PCB pairs, ii) the GC time separation of 3- and 4-MeSO₂-PCB pairs was generally around 0.5 min under the conditions employed and iii) the peak separation appeared to increase slightly with increasing chlorination. This trend of GC elution has been observed on several different columns, with non-polar to slightly polar stationary phases ^{138,173,182}.

4.3.3.2 Bioaccumulation

Because there were no detectable MeSO₂-PCBs or -4,4'-DDE in arctic cod, all of these compounds in ringed seal must have been formed by the seal itself from the precursor PCBs and 4,4'-DDE. The apparent MeSO₂-4,4'-DDE biomagnification factors (BMFs) were 5.4 and 512 (Table 4.4) for seal blubber to bear fat and seal blubber to bear liver, respectively. The BMFs in bear fat were in the same order as that of other CHCs, suggesting that some of the metabolite may have been transferred from seal to bear. However, the BMF for 4,4'-DDE was less than one from seal to bear (Table 4.3), indicating that some proportion of 4,4'-DDE may have been biotransformed to MeSO₂-4,4'-DDE in bear. Applying equation 5, that is, assuming all metabolism of 4,4'-DDE in bear led to MeSO₂-4,4'-DDE, the ratio of $(R^{153}_{MeSO_2-4,4-DDE})_{bear(max.)}$ to $(R^{153}_{MeSO_2-4,4-DDE})_{bear(actual)}$ was 7656 and 202 for polar bear fat and

liver, respectively. Although the TBB of MeSO₂-4,4'-DDE favoured liver over fat (Table 4.5), both of the forementioned ratios vastly exceeded unity. This suggested that metabolism of 4,4'-DDE in bear favoured a route other than MeSO₂-4,4'-DDE formation and/or MeSO₂-4,4'-DDE was depurated by metabolic or non-metabolic pathways.

The apparent BMF for S-MeSO₂-PCB from seal to bear fat was the highest of any major CHC class (Table 4.4). The BMFs from seal blubber to bear fat and seal blubber to bear liver were 30 and 148, respectively. The former was still less than that of the recalcitrant CB-153⁶, therefore metabolite levels in bear may have been the result of biomagnification due to metabolite exposure from seal. However, metabolic formation from precursor PCBs was also possible. Depuration of the metabolites by excretion of unchanged compounds or further metabolism, may have also been factors in the bioaccumulative behaviour of individual MeSO₂-PCB congeners in bear.

Most methyl sulfone-forming PCB congeners with *meta-para* vicinal hydrogens were detected in seal (Figure 4.1), and thus, only a proportion were converted to 3- and 4-MeSO₂-PCBs and other metabolites by the seal. The only exception was CB-132, which was below the detection limit in seal. These results were consistent with changes in the residual PCB pattern from cod to seal (Figure 4.1), which indicated CYP2B-type activity existed in ringed seal from the Canadian Arctic, but was low relative to polar bear.

Polar bear liver and fat tissue contained MeSO₂-PCBs, but no detectable levels of precursor PCBs, with the exception of traces of CB-149. Residual levels of MeSO₂-PCB and -4,4'-DDE compounds were present in all the polar bear tissues studied, however, liver and fat were the most important storage sites (see Section 4.3.3.3). When viewed from a TBB

perspective, retention of S-MeSO₂-PCB in fat was 33.7 ± 3.6 mg as opposed to 0.35 ± 0.10 mg in liver (Table 4.5). The other organs were of little importance in TBB due to their smaller size relative to both the total adipose tissue depots and the liver.

The individual MeSO₂-PCBs in bear liver were ca. 1.0 % or less of TBB (Table 4.7). In regards to the bioaccumulation of MeSO₂-PCB and -4,4'-DDE metabolites in the polar bear food chain, fat was the most important storage tissue. This was consistent with the S-PCB and S-DDT distribution in adult marine mammals, where more than 90 % of the TBB has been shown to be present in the blubber ²³⁵.

The S-MeSO₂-PCB to CB-153 ratio in bear fat (0.14) was lower than in seal blubber (0.22) (Table 4.3). However, the patterns of MeSO₂-PCBs in bear and seal differ significantly, and therefore the relative importance of bioaccumulation versus formation in bear must be treated on a congener-specific basis.

The levels of the individual MeSO₂-PCBs were expressed as R^{153}_m (eq. 6) to assess the possible biotransfer, metabolic formation, and depuration factors affecting bioaccumulation from seal to bear ^{6,99,100}. The $(R^{153}_m)_{seal}$ was generally slightly greater for 4-MeSO₂-PCBs relative to their 3-MeSO₂-PCB pair (Figure 4.4). MeSO₂-PCB levels in the blubber of Baltic seals did not show a similar trend, rather, the 3-MeSO₂-PCB metabolites appeared to be more favoured, although the difference was not dramatic ^{71,168}.

Assuming that all the precursor PCBs accumulated from seal were converted to MeSO₂-PCBs in bear, and that all MeSO₂-PCBs were not further metabolized and retained in bear, we can predict the maximum MeSO₂-PCB metabolite concentration in bear, as was done for 4,4'-DDE (eq. 5).

Table 4.7. The Total Body Burden of MeSO₂-PCB and -4,4'-DDE Metabolites in Polar Bear Fat and Liver Tissues from the Resolute Bay Area of the Canadian High Arctic^{†‡}

MeSO ₂ -PCB congener	total body burden (µg)		% in liver relative to fat
	bear fat	bear liver	
3-MeSO ₂ -CB31	802 ± 66	5.6 ± 3.1	0.7 ± 0.2
4-MeSO ₂ -CB31	670 ± 110	7.9 ± 3.7	1.2 ± 0.6
3-MeSO ₂ -CB49	1501 ± 236	29.5 ± 17.9	2.0 ± 0.8
4-MeSO ₂ -CB49	1435 ± 280	33.2 ± 14.4	2.3 ± 0.9
3-MeSO ₂ -CB52	n.d.	n.d.	N/A
4-MeSO ₂ -CB52	n.d.	2.6 ± 0.8	N/A
3-MeSO ₂ -CB64	340 ± 80	2.6 ± 0.9	0.8 ± 0.3
4-MeSO ₂ -CB64	1163 ± 287	15.9 ± 5.9	1.4 ± 0.7
3-MeSO ₂ -CB70	3187 ± 795	3.4 ± 1.8	0.1 ± 0.1
4-MeSO ₂ -CB70	949 ± 162	4.2 ± 1.7	0.4 ± 0.1
3-MeSO ₂ -CB87	2024 ± 427	76.8 ± 30.5	3.8 ± 1.5
4-MeSO ₂ -CB87	3886 ± 773	28.6 ± 11.4	0.7 ± 0.4
3-MeSO ₂ -CB91	1160 ± 280	2.5 ± 0.8	0.2 ± 0.1
4-MeSO ₂ -CB91	4460 ± 600	5.0 ± 1.7	0.1 ± 0.1
3-MeSO ₂ -CB95	n.d.	n.d.	N/A
4-MeSO ₂ -CB95	n.d.	n.d.	N/A
3-MeSO ₂ -CB101	515 ± 103	22.6 ± 12.5	4.4 ± 2.5
4-MeSO ₂ -CB101	3408 ± 714	12.7 ± 4.2	0.4 ± 0.1
3-MeSO ₂ -CB110	10 ± 2	0.4 ± 0.2	4.0 ± 2.0
4-MeSO ₂ -CB110	684 ± 132	5.0 ± 1.9	0.7 ± 0.2
3-MeSO ₂ -CB149	500 ± 125	34.7 ± 14.1	6.9 ± 3.4
4-MeSO ₂ -CB149	4121 ± 221	21.8 ± 5.8	0.5 ± 0.3
3-MeSO ₂ -CB132	191 ± 29	10.6 ± 4.0	5.5 ± 1.6
4-MeSO ₂ -CB132	1178 ± 294	7.0 ± 2.5	0.6 ± 0.2
3-MeSO ₂ -CB141	493 ± 88	13.5 ± 6.4	2.7 ± 1.6
4-MeSO ₂ -CB141	618 ± 140	2.2 ± 0.7	0.4 ± 0.1
3-MeSO ₂ -CB174	74 ± 15	3.5 ± 1.2	4.7 ± 1.9
4-MeSO ₂ -CB174	338 ± 74	1.8 ± 0.4	0.5 ± 0.2

[†] The total body mass of liver and adipose were 6650 ± 2740 grams and 113180 ± 32570 grams, respectively. The percent lipid in polar bear fat was 65.0 ± 3.2 % and in liver was 6.9 ± 0.8 %.

[‡] Mean ± SD in liver and fat of *n* = 6 individual male polar bears.

n.d. = not detected. N/A = not applicable.

Figure 4.4. The maximum potential concentration ratios of a MeSO₂-PCB congener to CB-153 ($[(R^{153}_m)_{bear}]_{max.}$) in polar bear fat, compared to the actual R^{153}_m . The dark bars are the R^{153}_m of polar bear fat, the white bars are the R^{153}_m of seal blubber, and the checkered bars are the $R^{153}_{p(potential m)}$ for seal blubber.

$$[(R^{153}_m)_{\text{bear}}]_{\text{max.}} = (R^{153}_m)_{\text{seal}} + [(R^{153}_p)_{\text{seal}(\text{potential } m)} / 2] \quad (7)$$

Equation 7 assumes that the PCB precursors from seal are metabolized equally to 3- and 4-MeSO₂-PCB metabolites in bear. Preston *et al.*¹¹⁴ demonstrated that non-enzymatic reactions of the 3,4-epoxide of 2,2',5,5'-Cl₄CB reacted with methionine, N-acetylmethionine and cysteine producing a 1:1 ratio of 3- and 4-methylthio and 3- and 4-cysteinyl-2,2',5,5'-Cl₄CB, respectively.

The $(R^{153}_p)_{\text{seal}(\text{potential})}$ portion of the $[(R^{153}_m)_{\text{bear}}]_{\text{max.}}$ (Figure 4.4) suggested a high potential for MeSO₂-PCB formation in bear, particularly PCB congeners such as CB-52, CB-101 and CB-149. The actual R^{153}_m values for each MeSO₂-PCB in bear fat (Figure 4.4) were consistently less than $[(R^{153}_m)_{\text{bear}}]_{\text{max.}}$, especially for the higher chlorinated MeSO₂-PCBs. This shows that the clearance of PCBs with *meta-para* vicinal hydrogens in bear mainly occurred via an alternate metabolic pathway to MeSO₂-PCB formation. This appeared to be more true for the higher chlorinated PCBs, as the discrepancy between $[(R^{153}_m)_{\text{bear}}]_{\text{max.}}$ and $[(R^{153}_m)_{\text{bear}}]_{\text{actual}}$ was larger. For some congeners, $(R^{153}_m)_{\text{bear}}$ was less than $(R^{153}_m)_{\text{seal}}$, indicating that bioaccumulation alone could explain the levels in bears. In other cases $(R^{153}_m)_{\text{bear}}$ was greater than $(R^{153}_m)_{\text{seal}}$, indicating that metabolic formation in the bear was probable.

In order to better explain the bioaccumulative behaviour of MeSO₂-PCBs in bear, a depuration/retention/formation index was determined for each MeSO₂-PCB congener in bear relative to seal (DRF_m, eq. 8).

$$DRF_m = (R^{153}_m)_{\text{bear fat}} / (R^{153}_m)_{\text{seal blubber}} \quad (8)$$

Boon *et al.*^{6,100} used a similar approach for residual PCB patterns by generating $R_{\text{rel.}(CB-X)}$ (i.e., $R_{\text{rel.}(CB-X)} = \text{Ratio}^{153}(\text{CB-X in sample}) / \text{Ratio}^{153}(\text{CB-X in reference})$) to examine the parameters affecting the bioaccumulation of PCBs. The complexity of factors affecting the bioaccumulative behaviour of MeSO₂-PCBs is greater than that of PCBs. For example, secondary metabolism of MeSO₂-PCBs or alternate metabolic pathways for the precursor PCBs may occur in addition to bioaccumulation from the diet. Incomplete metabolism and/or excretion of precursor PCBs in bear was not a factor since their levels were below the detection limit of 0.05 ng/g, lipid weight (Table 4.3).

CB-132 was not detectable in seal blubber or bear fat and liver, indicating that this congener was completely metabolized by the seal. Therefore, the accumulation of 3- and 4-MeSO₂-CB132 in bear must have resulted entirely from ingestion of seal blubber. The average DRF_m value of 3- and 4-MeSO₂-CB132 (Figure 4.5) was therefore a good reference point to evaluate the potential for bioaccumulation of the other MeSO₂-PCB congeners.

For congeners with DRF_m values greater than the average DRF_m(CB132) value, a portion of the MeSO₂-PCB was likely formed metabolically in bear, depending on the relative rates of clearance to MeSO₂-CB132. For congeners that have DRF_m values close to that of MeSO₂-CB132, there is a strong possibility that bioaccumulation from seal is the main source in polar bear. This could be the case for MeSO₂-CB31, -CB49, -CB64, -CB101, -CB110 and -CB174, especially if the rate of clearance relative to MeSO₂-CB132 is faster for tetrachloro congeners and slower for heptachloro congeners. Although the DRF_m values

Figure 4.5. Depuration/retention/formation index (DRF_m) of MeSO₂-PCBs from ringed seal blubber to polar bear fat from the Resolute Bay area of the Canadian high Arctic. 3- and 4-MeSO₂-CB52 and 3- and 4-MeSO₂-CB95 were below detection in seal blubber. Bars with DRF_m above 1.0 are indicative of a contribution to metabolite level in bear by metabolism of precursor PCBs. DRF_m above the DRF_m(132) (light bars) of ca. 0.5 indicated that several factors could be influencing the MeSO₂-PCB level in the bear.

for 4-MeSO₂-CB70 and 3-MeSO₂-CB149 are also in the MeSO₂-CB132 range, the corresponding 3-MeSO₂-CB70 and 4-MeSO₂-CB149 DRF_m values are so much higher that formation of these metabolites by the bear is indicated. Nevertheless, there is insufficient information in the present study to rule out that both accumulation from ringed seal and formation by the bear, in combination with higher rates of clearance of certain MeSO₂-PCB congeners, could account for the similarity in DRF_m to that of MeSO₂-CB132.

For most of the MeSO₂-PCBs, secondary metabolism involving hydroxylation of the second phenyl ring was unlikely. Hydroxylation of PCBs is known to be favoured at *meta* or *para* positions^{101,102,236}. With the exception of MeSO₂-CB52 and -CB95, the *para* position of the non-MeSO₂-containing phenyl ring for all persistent MeSO₂-PCBs in seal and bear was chlorine substituted. MeSO₂-CB52 and MeSO₂-CB95a were below detection in bear fat and liver, except for very low residual levels of 4-MeSO₂-CB52 in liver (see Table 4.8). Secondary metabolism of these metabolites can occur, since MeSO₂-PCBs with a second pair of *meta-para* vicinal hydrogens may undergo a CYP2B-mediated biotransformation to hydroxy- or *bis*-MeSO₂-PCB metabolites^{129,130}. Several *bis*-MeSO₂-PCB metabolites were detected by GC/ECNI-MS in polar bear liver (see Chapter 2).

Another view of the potential for bioaccumulation versus metabolic formation of MeSO₂-PCBs in polar bears can be had directly from DRF_m values (Figure 4.5). No PCB or other CHC which is not formed metabolically, had a higher BMF than CB-153 in the polar bear (Table 4.4). One can assume that the rate of CB-153 depuration was very low, based on the recalcitrance of this PCB congener in other species^{56,44}. Therefore, MeSO₂-PCBs with DRF_m ≈ 1 may have been entirely bioaccumulated, if their rate of clearance was similar to

that of CB-153 (and less than that of MeSO₂-CB132), e.g., MeSO₂-CB87 and -CB174. Alternately, MeSO₂-PCBs with DRF_m = 1 may have clearance rates similar to MeSO₂-CB132, and be partially bioaccumulated and partially formed by the bear. MeSO₂-PCBs with DRF_m > 1 clearly must be formed at least partially by metabolism of precursor PCBs in the polar bear. The congeners which fall into this category are MeSO₂-CB70, -CB91, -CB149 and -CB141. At values of DRF_m < 1, it is impossible to determine with any certainty what combination of metabolic formation, bioaccumulation and clearance contributes to the MeSO₂-PCB levels found in bear.

4.3.3.3 Levels in the Polar Bear Food Chain and Tissue Distribution in Polar Bear

MeSO₂-PCBs were minor contaminants in the blubber of Canadian arctic ringed seal relative to other CHC classes at ca. 3 % of S-PCB (Table 4.3). In contrast, persistent MeSO₂-PCBs in Baltic seals were the third most abundant class of anthropogenic contaminants, and were ca. 10 % or more of S-PCBs ^{71,168}. MeSO₂-PCBs were also the third most abundant CHC class in Canadian arctic polar bear, exceeded only by S-CHLORs and S-PCBs (Table 4.3). MeSO₂-PCB and -4,4'-DDE metabolites were below the 0.05 ng/g detection limit in arctic cod. At present, no data exist indicating the formation of MeSO₂-PCB and -4,4'-DDE metabolites in fish, presumably because of their low capacity for metabolism mediated by CYP2B-type enzymes ^{87,224,237}. MeSO₂-PCBs have been detected in Japanese trout and sardine ²¹⁰, however this may be the result of MeSO₂-PCB uptake from their diet, rather than metabolic formation in the fish. MeSO₂-PCB uptake in arctic cod from their diet may also be possible, but at trace levels below the 0.05 ng/g detection limit.

The chromatograms from the liver and fat of both seal (Figure 4.3) and bear (Figure 2.2) illustrated that the relative significance of individual congeners differed among species and tissues. Seven MeSO₂-PCB congeners accounted for ca. 59 % of the S-MeSO₂-PCB in ringed seal blubber (Table 4.8). In seal liver, the congeners 3-MeSO₂-CB95 and -CB149 were dominant (Figure 4.3), along with the forementioned congeners that were most abundant in fat, that is, accounting for ca. 65 % of the S-MeSO₂-PCBs. The most abundant congeners in both tissues were somewhat different than observed in Baltic Sea seals ^{71,173}. In the Baltic Sea seals, the patterns were simpler with fewer congeners dominating the profile. In Baltic grey seal liver for example, there was some similarity to Canadian ringed seal in that 3-MeSO₂-CB95 and -CB149 were dominant congeners, however, these two congeners plus a congener identified as 3-MeSO₂-CB135 (2,2',3',5,5',6-Cl₆CB) accounted for > 90 % of the S-MeSO₂-PCBs in the Baltic seal. This may be the result of a combination of greater PCB exposure, a different dietary regime, the environmental input of PCBs and greater depuration of certain MeSO₂-PCB congeners. S-PCB and S-MeSO₂-PCB levels as high as 760,000 ng/g and 110,000 ng/g (lipid wt.), respectively, have been observed in Baltic grey seal ¹⁶⁸, whereas the levels in Canadian arctic ringed seal were 3 orders of magnitude lower, at 411 and 12.7 ng/g (lipid weight, Table 4.3), respectively. The presence of precursor PCBs in arctic ringed seal blubber at levels significantly higher than their MeSO₂-metabolites, except CB-132, indicated low CYP2B-type metabolic activity.

Levels of MeSO₂-PCBs in polar bear fat were dominated by five congeners which accounted for 57 % of the S-MeSO₂-PCBs (Table 4.8). In contrast, liver was dominated by seven congeners, which accounted for 70 %, however, 3-MeSO₂-CB87 alone accounted for

Table 4.8. Mean Levels of Precursor PCBs and MeSO₂-PCB Metabolites (ng/g, lipid weight) in Ringed Seal Blubber and Polar Bear Fat and Liver Tissues from the Resolute Bay Area of the Canadian High Arctic[‡]

parent PCB	level in seal blubber	MeSO ₂ -PCB [†]	level in [°] seal blubber	bear fat	bear liver
CB-28/31	10.4 ± 3.2	3-MeSO ₂ -CB31 (1)	0.61 ± 0.22	11 ± 1	29 ± 16
		4-MeSO ₂ -CB31 (2)	0.88 ± 0.33	9 ± 2	41 ± 20
CB-49	5.4 ± 1.1	3-MeSO ₂ -CB49 (4)	1.40 ± 0.54	20 ± 3	153 ± 93
		4-MeSO ₂ -CB49 (6)	1.62 ± 0.56	20 ± 4	172 ± 75
CB-52	20.6 ± 7.5	3-MeSO ₂ -CB52 (3)	0.12 ± 0.06	n.d.	n.d.
		4-MeSO ₂ -CB52 (5)	0.01 ± 0.01	n.d.	13 ± 4
CB-64	2.4 ± 1.4	3-MeSO ₂ -CB64 (7)	0.23 ± 0.07	5 ± 1	13 ± 4
		4-MeSO ₂ -CB64 (9)	0.83 ± 0.27	16 ± 4	83 ± 30
CB-70/76	13.4 ± 4.4	3-MeSO ₂ -CB70 (13)	0.40 ± 0.18	43 ± 11	18 ± 9
		4-MeSO ₂ -CB70 (15)	0.52 ± 0.21	13 ± 2	22 ± 9
CB-87	6.5 ± 1.6	3-MeSO ₂ -CB87 (18)	0.57 ± 0.22	28 ± 6	398 ± 158
		4-MeSO ₂ -CB87 (22)	0.90 ± 0.42	53 ± 10	148 ± 59
CB-91	2.4 ± 1.6	3-MeSO ₂ -CB91 (10)	0.22 ± 0.10	16 ± 4	13 ± 4
		4-MeSO ₂ -CB91 (12)	0.35 ± 0.13	61 ± 8	26 ± 9
CB-95a	2.8 ± 0.8	3-MeSO ₂ -CB95a (8)	0.02 ± 0.02	n.d.	n.d.
		4-MeSO ₂ -CB95a (11)	0.02 ± 0.01	n.d.	n.d.
CB-101	37.5 ± 9.2	3-MeSO ₂ -CB101 (14)	1.22 ± 0.51	7 ± 1	117 ± 65
		4-MeSO ₂ -CB101 (16)	1.24 ± 0.50	46 ± 10	66 ± 22
CB-110	7.1 ± 2.7	3-MeSO ₂ -CB110 (19)	0.01 ± 0.00	0.13 ± 0.03	2 ± 1
		4-MeSO ₂ -CB110 (21)	0.45 ± 0.17	9 ± 2	26 ± 10
CB-149*	9.1 ± 3.7	3-MeSO ₂ -CB149 (20)	0.28 ± 0.18	7 ± 2	180 ± 76
		4-MeSO ₂ -CB149 (23)	0.72 ± 0.40	56 ± 3	113 ± 30
CB-132	n.d.	3-MeSO ₂ -CB132 (24)	0.11 ± 0.06	3 ± 0.4	55 ± 21
		4-MeSO ₂ -CB132 (25)	0.52 ± 0.18	16 ± 4	36 ± 13
CB-141	3.2 ± 2.7	3-MeSO ₂ -CB141 (26)	0.08 ± 0.04	7 ± 1	70 ± 33
		4-MeSO ₂ -CB141 (27)	0.11 ± 0.04	8 ± 2	12 ± 4
CB-174	5.8 ± 2.1	3-MeSO ₂ -CB174 (29)	0.02 ± 0.02	1 ± 0.2	18 ± 6
		4-MeSO ₂ -CB174 (30)	0.12 ± 0.06	5 ± 1	9 ± 2

[‡] The mean ± SD of liver and fat for *n*=6 male bears, and *n*=11 male and female seals.

[†] Numbers in parentheses refer to the peak numbers in Figures 2.2 and 4.2.

[°] The most abundant MeSO₂-PCB congener levels are in bold.

* The only precursor PCB to be detected and quantitated in polar bear liver (85 ± 55 ng/g) and polar bear fat (1.8 ± 0.4 ng/g). n.d. = not detected.

22 %. The variation in metabolic capacity toward PCBs with *meta-para* vicinal hydrogens, and non-metabolic and/or metabolic depuration of the MeSO₂-PCB metabolites, explains the differences in individual MeSO₂-PCB congener levels among species (Table 4.8). MeSO₂-PCB and -4,4'-DDE bioaccumulation potential from seal to bear was discussed in the previous section.

The S-PCB distribution in several tissues of polar bear revealed that S-PCB levels were relatively constant at 3,000 to 6,000 ng/g on a lipid weight basis for testes, adipose and lung, whereas brain was as much as ca. 17 times lower and liver as much as ca. 4 times higher (Table 4.9). The 4,4'-DDE levels were highest in liver lipids (704 ng/g), whereas lipid in adipose and brain had approximately half this level and levels in testes and lung lipid were 8 to 15 times lower than in the liver.

CHCs frequently partition into tissue according to the triglyceride content. Overall, the mammalian brain has a very low triglyceride content²³⁸. However, the *medulla oblongata* of polar bear brain was high in neutral lipids (Table 4.9). It has been found that for polar bears and seals, the CHC levels in the brain were low relative to several other tissues, including liver, adipose, kidney and lung²³⁹. The low CHC levels in the brain of polar bear were not due to low lipid content, so difficulty in passing through the blood-brain barrier is probably the explanation.

A preference for S-PCB and S-DDT storage in the liver was observed in polar bear, grey seal and harp seal^{239,240}. A preference for S-PCB and S-DDT storage in adipose tissue relative to other tissues such as brain, liver and kidney, has been demonstrated for ringed seal and beluga whale²⁴¹. It was suggested that the discrepancy between higher levels in liver and

Table 4.9. Total MeSO₂-PCB and -4,4'-DDE Levels (ng/g, lipid wt.) and Ratios of the Congener Sums to Total PCBs and 4,4'-DDE in the Tissues of Polar Bears from the Resolute Bay Area of the Canadian High Arctic

	Liver [‡]	Fat [‡]	Testes [‡]	Lung [‡]	Brain [†]
% lipid	6.1 ± 0.5	64.5 ± 4.0	8.0 ± 8.7	2.6 ± 0.2	15.8
S-MeSO ₂ -PCBs	3059 ± 1290	351 ± 66	207 ± 31	143 ± 35	31
S-PCBs	28309 ± 9268	6210 ± 948	6387 ± 1940	3263 ± 354	377
S-MeSO ₂ -PCB/ S-PCB ratio	0.11 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.083
3-MeSO ₂ -4,4'-DDE	303 ± 85*	2.4 ± 1.0	2.7 ± 1.4	0.9 ± 0.1	n.d.
4,4'-DDE	704 ± 100	262 ± 32	89 ± 14	48 ± 9	224
3-MeSO ₂ -4,4'-DDE/ 4,4'-DDE ratio	0.46 ± 0.20	0.009 ± 0.004	0.03 ± 0.02	0.02 ± 0.003	N/A

[‡] The mean ± SD for *n* = 3 liver, fat, testes and lung.

[†] Only one sample.

* The sum of 2- and 3-MeSO₂-4,4'-DDE. The 2- to 3-MeSO₂-4,4'-DDE ratio was 0.48 ± 0.18.
n.d. = not detected. N/A = not applicable.

adipose tissue than other tissues such as brain and kidney, may be a function of deposition or mobilization of lipid and associated residues ²⁴¹.

The distribution of MeSO₂-PCB and -4,4'-DDE has been studied only in blubber, liver and muscle tissues of seals, otters and mink of the Baltic Sea region and liver and fat of two Canadian polar bears (Table 4.6). The present study therefore represents the most comprehensive examination of MeSO₂-PCBs and -4,4'-DDE tissue distribution in any wild animal species. The rank order of preference of storage for MeSO₂-PCBs in tissues was similar to that for PCBs, but there was a larger disparity among tissues (Table 4.9). Thus, S-MeSO₂-PCB levels were highest in liver at 11 % of S-PCBs, whereas fat, testes, lung and brain had 2, 9, 13 and 60 times lower levels than liver, on a lipid weight basis. Bergman *et al.* ⁷¹ found a similar preference for MeSO₂-PCB storage in liver relative to adipose or muscle tissue in Baltic Sea grey seal and Swedish mink and otter. In contrast, Japanese, "Yusho" patients were reported as having S-MeSO₂-PCB levels in the order of lung > adipose > liver ¹³⁸.

The S-MeSO₂-PCB to S-PCB ratios (Table 4.9) for testes, adipose, brain and lung averaged 0.05. The liver (0.11) ratio was higher, indicating that MeSO₂-PCBs are more selectively retained in liver than PCBs, relative to the other tissues. Moderately polar CHCs containing oxygen, such as oxychlordane, tend to accumulate preferably in polar bear liver, possibly because of the high density of lipid-containing Stellate (Ito) cells that are specialized in retinol and retinol ester (vitamin A) storage in adults ²⁴². Stellate cells are an adaptation in polar bears, due to the high levels vitamin A in ringed seal blubber, their primary dietary item. Vitamin A is a poison in excessive quantities.

The S-MeSO₂-PCB to S-PCB ratio for brain (*medulla oblongata*) was slightly higher than adipose, lung or testes (Table 4.9), indicating a marginal preference for storage of MeSO₂-PCBs in brain. It was suggested that more water soluble CHCs, such as α -HCH, easily pass the blood-brain barrier or have a specific affinity for a receptor protein in the brain ²³⁹.

In polar bear from Resolute Bay, the 2- and 3-MeSO₂-4,4'-DDE metabolites were detected at a total level of close to 200 ng/g in liver, 126 to 337 times higher than 3-MeSO₂-4,4'-DDE found in testes, adipose and lung. Prior to this study, MeSO₂-4,4'-DDE had not been detected in polar bear apart from the liver ⁷¹. Further, in a previous study, the S-MeSO₂-4,4'-DDE level was ca. 1000 ng/g (lipid weight) in liver, however, the two individuals were taken from the western Hudson Bay area. Polar bears from western Hudson Bay have been shown to have 4,4'-DDE levels ca. 2 times higher than those from the Resolute Bay area (see Chapter 5). Only the 3-MeSO₂-4,4'-DDE has been detected in other species, with the exception of false killer whale and Baltic grey seal blubber ⁷¹. Higher concentration of 3-MeSO₂-4,4'-DDE in the liver was demonstrated for Baltic grey seal and Swedish otter and mink (Table 4.6).

The 2- to 3-MeSO₂-4,4'-DDE ratio was 0.48 ± 0.18 for adult male polar bears from the Resolute Bay area. The ratio was similar to the liver of Baltic Sea grey seal, which had a value of ca. 0.5 ⁷¹. Only the 3-, and not the 2-MeSO₂-4,4'-DDE was detected in the liver of Canadian ringed seal in the present study. In Swedish human milk, the level of 3-MeSO₂-4,4'-DDE was also greater than that of 2-MeSO₂-4,4'-DDE ¹⁶⁶. A ratio of ca. 2.0 was found previously for both one adult male and one adult female polar bear from western

Hudson Bay ⁷¹. Interestingly, this was the exact opposite of the ratio found in the present bears. It was suggested that 2- to 3-MeSO₂-4,4'-DDE varies with species. However, the present results for polar bear, in conjunction with similar ratios found in grey seal liver and humans, suggested that the ratio is fairly well conserved in the species studied so far.

The preferential storage of MeSO₂-4,4'-DDEs in polar bear liver was emphasized by the S-MeSO₂-4,4'-DDE to 4,4'-DDE ratio of 0.46 as compared to the mean value of 0.04 for adipose, lung and testes. In brain, 4,4'-DDE was found at 224 ng/g whereas MeSO₂-4,4'-DDE was not detected. The highly asymmetric tissue distribution of S-MeSO₂-4,4'-DDE may be due to several factors. High levels in liver may result because this is the site of formation, or because of highly selective binding in hepatocytes or Stellate cells. Levels may be lower in other tissues because of competition with irreversible binding in specific organs. The 3-MeSO₂-4,4'-DDE metabolite has been shown to irreversibly bind in the adrenal cortex of mice, Baltic grey seal, mink and birds ¹⁵⁷⁻¹⁶⁰. The 3-MeSO₂-4,4'-DDE compound has been shown to be a potent adrenocorticolytic toxicant in mouse. Further, this compound was shown to bind, followed by bioactivation *in vitro*, in human adrenal gland ²⁴³.

Of the five major PCB congeners (i.e., CB-99, CB-138, CB-153, CB-170 and CB-180) found in the liver, adipose, muscle, teste, kidney and brain tissue of polar bear, Braune *et al.* ²³⁹ showed that there was no dramatic difference in the PCB congener pattern among tissues. In polar bear, the concentrations of individual MeSO₂-PCB congeners expressed as a fraction of the S-MeSO₂-PCB demonstrated some tissue dependence, especially in the case of liver (Figure 4.6).

Figure 4.6. MeSO₂-PCB congener patterns in various polar bear tissues, expressed as the congener fractions of the S-MeSO₂-PCBs. Individual congeners without bars were below the detection limit of 0.05 ng/g (see Table 2.4).

The congeners 3- and 4-MeSO₂-CB87 and 3- and 4-MeSO₂-CB101 dominated in all tissues with an average of ca. 50 % of S-MeSO₂-PCB in brain and adipose, 45 % in lung and liver and 55 % in testes. The liver MeSO₂-PCB congener pattern is the most dissimilar with 3- and 4-MeSO₂-CB87 accounting for ca. 33 % of the sum as opposed to ca. 22 % for the remaining tissues. Neither 3- or 4-MeSO₂-CB70 were retained to a significant degree in liver, possibly because of further metabolism of the non-MeSO₂-containing ring by CYP1A-type enzymes. MeSO₂-CB70 has 3',4' chlorine substitution similar to CB-105 and CB-118, which are metabolized by polar bears, as was demonstrated in Section 4.3.2. It has been shown that certain MeSO₂-PCB congeners bind to proteins in lung, kidney and intestinal mucosa of rats ^{129,140,244}. There was no evidence for selective retention in lung of the congeners found in polar bear. Among tissues, excluding liver, brain tended to have the highest proportion of tetrachloro- and pentachloro-MeSO₂ congeners, and the lowest proportion of hexachloro- and heptachloro-MeSO₂-PCB congeners, with relatively little specificity as to 3-MeSO₂- and 4-MeSO₂-PCB pairs. The 3-MeSO₂-CB87 and -CB149 congeners were retained in liver to a greater extent than their 4-MeSO₂-PCB isomers relative to other tissues (Figure 4.6). This selectivity did not occur below MeSO₂-PCB-CB87. Such congener-specific retention may be the result of binding to receptor or CYP proteins. The 3-MeSO₂-PCBs also were found to be preferentially retained in the liver relative to adipose or muscle in Baltic grey seal and Swedish mink and otter ^{71,168}. It was suggested that this preferential retention in liver was attributed to non-covalent binding to uteroglobin-type and fatty acid proteins. A similar explanation was suggested for preferential MeSO₂-PCB retention in rat

liver ^{138,140,144}. Recently, a higher proportion of 4-MeSO₂-PCBs relative to 3-MeSO₂-PCBs was found in human breast milk ¹⁴⁶.

4.3.3.4 Temporal Dynamics and Lactational Transfer

The temporal dynamics or lactational transfer of MeSO₂-PCB and -4,4'-DDE metabolites is unknown for any species. Further, MeSO₂-PCB and -4,4'-DDE metabolites in milk have yet to be determined in biota apart for humans from Swedish and Japanese environments ^{144,245,246}. The present study examined the effects of the feeding status, over a two year period, of a single polar bear female, on several aspects of the dynamics of MeSO₂-PCB and -4,4'-DDE residues in adipose and milk. These results were compared to the adipose levels of one yearling (YRLG) cub and two cubs-of-the-year (COYs). The following interpretation of the data must be considered with caution, since only one polar bear family group could be studied.

There was a marginal increase in S-MeSO₂-PCBs in the adipose tissue of the adult female over the two year time period (Figure 4.7). Over the first short fasting period (i.e., Oct./92 to Nov./92), the S-MeSO₂-PCB levels increased only slightly. Further, the ratio to S-PCB remained essentially constant. These results were probably not significant since there was only one month progression in the fasting period for one individual.

During the much longer, second fasting period of the pregnant female (i.e., Aug./93 to Mar./94; Figure 4.7), an increase in the adipose S-MeSO₂-PCB level was observed. Moreover, the S-MeSO₂-PCB to S-PCB ratio decreased, suggesting that MeSO₂-PCBs may have been transferred from adipose tissue to the fetus trans-placentally or through lactation

Figure 4.7. S-MeSO₂-PCB and 3-MeSO₂-4,4'-DDE levels and the ratios to the S-PCBs and 4,4'-DDE for a polar bear mother and her cubs from western Hudson Bay. Top to bottom, S-MeSO₂-PCB levels, the ratio to S-PCB, 3-MeSO₂-4,4'-DDE levels and the ratio to 4,4'-DDE.

more rapidly than PCBs as the fasting period progressed. It appeared that the opposite was true for 3-MeSO₂-4,4'-DDE relative to 4,4'-DDE (Figure 4.7). This may be explained by the continuing metabolism of 4,4'-DDE to 3-MeSO₂-4,4'-DDE and other metabolites and/or a low rate of excretion of 3-MeSO₂-4,4'-DDE during the fasting period. The results indicated that the mobilization of MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE from adipose tissue in polar bear was likely different from PCBs and 4,4'-DDE over the fasting period.

Regardless of feeding or reproductive status of the female, both the YRLG and the COYs have approximately three times higher S-MeSO₂-PCB levels than their mother's adipose tissue. Moreover, the ratio of S-MeSO₂-PCBs to S-PCBs was greater for the cubs. This is further indication that transfer of MeSO₂-PCBs in milk is faster than that of PCBs. The ratio of S-MeSO₂-PCB to S-PCB in the YRLG cub was approximately twice as high as the two COYs. This was equally due to a concentration effect since the YRLG cub was probably not feeding, and therefore using existing adipose reserves to survive. It was not possible to obtain milk samples from the female when she was with the YRLG as she was not lactating. Although the 3-MeSO₂-4,4'-DDE to 4,4'-DDE ratio was similar for the mother and the cubs, the very fact that 3-MeSO₂-4,4'-DDE was present in the cubs represents a substantial toxicological risk. The 3-MeSO₂-4,4'-DDE is known to be a adrenocorticolytic toxicant of varying potency in Baltic grey seal, birds, mink and mice ¹⁵⁷⁻¹⁶⁰. A reactive metabolite of 3-MeSO₂-4,4'-DDE has been shown to perturb 11 β -hydroxylase activity in these species.

The high MeSO₂-PCB levels in milk relative to female adipose tissue on a lipid weight basis, especially at the end of fasting in March 1994, explained why the S-MeSO₂-

PCB levels in the cubs were higher relative to the female adipose tissue. The results suggested that the lactational transfer of MeSO₂-PCBs to cubs is more significant than PCBs, and perhaps other CHCs, in polar bear⁶⁶. Moreover, the milk levels of S-MeSO₂-PCBs in the Mar./94 and Oct./94 sampling periods were 211 ng/g and 108 ng/g (lipid weight), respectively. These values were ca. 20-fold higher than was observed in Swedish human milk over a 10 year period from 1971 to 1992¹⁶⁶. Further, less than 1.0 ng/g of S-MeSO₂-PCBs were observed in the breast milk of a Japanese worker in a capacitor factory in the mid-1970's²⁴⁵. In contrast, the level of 3-MeSO₂-4,4-DDE in the milk of Swedish human subjects was 0.4 ng/g (lipid wt.) in 1992, as compared to the relatively constant value of 0.22 ng/g in the present polar bear samples (Figure 4.7).

The temporal change in the MeSO₂-PCB congener pattern of adipose tissue from the female was negligible (Figure 4.8). Further, the pattern in milk differed only slightly from adipose tissue, that is, there was a slight preference for lower chlorinated, tri- tetra- and penta-MeSO₂-PCB congeners in milk. The patterns for adipose tissue in both the YRLGs and COYs were also similar to both the milk and adipose of the female. Apparently, there was no congener preference either for mobilization for milk production or uptake by the cub.

Finally, the dominance of tetrachloro- and pentachloro-MeSO₂-PCBs in the congener pattern of polar bear milk from the Canadian Arctic (Figure 4.8) was in contrast to the dominance of pentachloro- and hexachloro-MeSO₂-PCBs observed in Swedish human milk¹⁶⁶. This may be due to several factors, including differences in dietary PCB patterns, metabolism of the precursor PCBs, and clearance of MeSO₂-PCBs between humans and polar bears.

Figure 4.8. The congener fractions of the S-MeSO₂-PCB and temporal effects for MeSO₂-PCBs in the milk and adipose tissue of a polar bear mother and the adipose tissue of her cubs. A) female adipose tissue (temporal mean $\pm$ SD) and milk and B) adipose tissue of the two COYs. The MeSO₂-PCB congener pattern for the adipose tissue of the YRLG was virtually identical to that of the COYs.

With the exception of MeSO₂-CB31, -CB101 and -CB174, the 4-MeSO₂-PCBs were more significant than their 3-MeSO₂-PCB pair (Figure 4.8). This was also observed in Swedish human milk ¹⁶⁶.

4.4 Conclusions

Several aspects of the ecodynamics of MeSO₂-PCBs and -4,4'-DDE in the polar bear food chain, relative to other CHCs, have been presented in this chapter. The residual anthropogenic compound patterns demonstrated that the polar bear has a high capacity to depurate, with or without metabolism, all CHC classes measured, relative to arctic ringed seal and cod. Further, polar bears are exposed to high levels and different MeSO₂-PCB and -4,4'-DDE patterns, indicating that the potential toxicological risks from chronic exposure may be different in polar bear relative to ringed seal.

The presence of PCBs with *meta-para* vicinal hydrogens in ringed seal indicated low CYP2B-type metabolism, however, some activity existed since MeSO₂-PCB metabolites were measured. CYP1A-type metabolism of PCBs is less in Canadian arctic ringed seal relative to highly induced Baltic seal populations, since residual levels of mono-*ortho* CB-105 and CB-118 were not selectively reduced. Both CYP2B- and CYP1A-type activity appear to be a function of the level of CHC exposure in Canadian arctic ringed seal.

Polar bear had the largest degree of PCB, 4,4'-DDT and 4,4'-DDE biotransformation. The bears possessed very low residue levels of CB-105 and CB-118 indicating high CYP1A-type activity. This type of CYP enzyme activity appears to be unique to the polar bear

relative to marine mammals in the arctic regions of the western hemisphere. This metabolism may be mediated by CYP1A- or CYP2B-type enzymes unique to the polar bear.

CYP2B-type activity was also high in polar bear as indicated by the virtual absence of PCBs with *meta-para* vicinal hydrogens and high levels of MeSO₂-PCBs, which was the third highest CHC residue class. MeSO₂-4,4-DDE metabolic formation in bear was also high, however, it appeared that a substantial amount of 4,4-DDE was metabolized by an alternate metabolic route and/or MeSO₂-4,4'-DDE was depurated in bear.

New MeSO₂-PCB structures were identified in seal (6 congeners) and polar bear (12 congeners) tissues, respectively. The MeSO₂-PCB pattern in Canadian arctic ringed seal was much more complex than observed in Baltic seals or in polar bears, that is, there were a greater number of equally dominant congeners. This may be due to several factors including, exposure to different PCB patterns, higher CYP2B-type activity, clearance by other CYP enzymes and/or differences in non-metabolic clearance ability.

MeSO₂-PCB and -4,4'-DDE metabolites were preferably retained in polar bear liver, which may be a consequence of Stellate cells, specialized in the retention of polar retinols and retinol esters, or binding to specific proteins. The MeSO₂-PCB congener pattern in liver was dissimilar from other lipid-containing tissues, favouring pentachloro-3- and 4-MeSO₂-CB87.

Until the present study, the parameters effecting MeSO₂-PCB and -4,4'-DDE bioaccumulation were not known for any species. The presence of precursor PCBs and 4,4'-DDE in seal indicated that only a portion of these congeners are converted to metabolites. For polar bear, 4,4'-DDE metabolism appears to favour an alternate pathway other than

MeSO₂-4,4'-DDE formation, and/or MeSO₂-4,4'-DDEs are non-metabolically or metabolically cleared.

The most intriguing aspect of the present study was the bioaccumulative behaviour of MeSO₂-PCBs in polar bear. In spite of the uncertainties in interpretation of the DRF_m values, it can be concluded that at least one congener, MeSO₂-CB132, is bioaccumulated entirely from ringed seal by polar bears, and has a BMF approximately one half that of CB-153, indicating slow depuration from the bear. It is probable that a number of other congeners are also bioaccumulated mainly from ringed seal. It can also be concluded that formation and/or slow clearance of MeSO₂-CB70, -CB91, -CB149 and -CB141 occurs in the bear. In no case, except MeSO₂-CB132, did the actual MeSO₂-PCB level exceed the maximum potential level due to metabolic formation from precursor PCBs in seals, indicating that metabolic pathways other than the MAP (see Chapter 1) were usually favoured. However, the MAP pathway appears highly favoured for CB-70 and CB-91, based on the $(R^{153}_m)_{\text{seal}} / [(R^{153}_m)_{\text{bear}}]_{\text{max}}$ ratios. It is clear that MeSO₂-PCB uptake from seal is an important source of MeSO₂-PCBs in bear. It appears that selective bioaccumulation, formation and retention of MeSO₂-PCBs all influence the resulting MeSO₂-PCBs congener levels and patterns in both seal and bear.

Finally, mobilization from adipose and lactational transfer of MeSO₂-PCBs from mother and cub appears to be more rapid than PCBs over the fasting period. Mobilization of 3-MeSO₂-4,4'-DDE also occurs. S-MeSO₂-PCB levels are higher, and 3-MeSO₂-4,4'-DDE levels similar, in the cubs relative to the mother, especially for YRLGs. Polar bear cubs may be at greater risk from the deleterious effects of MeSO₂-PCB and 4,4'-DDE metabolites than

adult bears. There appears to be no temporal influence on the MeSO₂-PCB pattern or any significant difference between the tissues of the polar bear mother and her cubs, regardless of feeding status of the female. Apparently, there is no significant congener preference either for adipose mobilization for milk production or uptake by the cub.

Geographical Distribution of Methyl Sulfone Metabolites of PCBs and 4,4'-DDE in Pooled Polar Bear (*Ursus Maritimus*) Adipose Tissue From Western Hemisphere Arctic and Subarctic[†]

5.1 Background

The Arctic is the recipient of atmospherically transported PCBs and DDTs sourced mostly at more southerly latitudes ¹⁰. The atmospheric residence time of PCBs has been estimated to be a few months. Moreover, CHCs including PCBs and DDTs, are transported and ultimately deposited at Arctic latitudes by a condensation effect as a consequence of decreasing temperature ^{3,28,29,31,32}. Oceanic circulation results in further distribution. Subsequent uptake of PCBs and DDTs into the Arctic marine food web renders these compounds susceptible to bioaccumulation and bioconcentration in tissues with high lipid content.

Mammals high on the food chain, such as polar bear, may roam over large distances in search of prey, however, these wanderings are restricted to distinct geographical zones ^{57,247}. The primary prey of polar bear, ringed seals, are more sedentary ⁵⁷. S-PCB and 4,4'-

[†] Adapted from *Sci. Total Environ.*, 1995, 160/161: 409-420.

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DDE levels in polar bear from the Canadian Arctic, have been shown to fluctuate depending on the level of exposure, resulting in part from changes in the diet, but largely from geographical location ⁴⁶. It was demonstrated in Chapter 4 that persistent MeSO₂-PCB and -DDE levels fluctuate among polar bear tissues collected from individuals in the Resolute Bay area of the Canadian Arctic. However, relatively constant ratios of S-MeSO₂-PCB to S-PCB and S-MeSO₂-4,4'-DDE to 4,4'-DDE in these tissues showed that fluctuations in the parent and the MeSO₂-metabolite levels were similar. The fraction of each MeSO₂-PCB congener as a function of the S-MeSO₂-PCBs also remained constant. There is a possibility that exposure to variable levels of PCBs and 4,4'-DDT, and different patterns of PCB congeners, as a consequence of geographic location in the arctic region influences MeSO₂-PCB and -DDE metabolite levels in polar bear. In this study, MeSO₂-PCB and -4,4'-DDE, PCB and 4,4'-DDE levels were determined for polar bear adipose tissue pools from twelve distinct areas spanning west to east from the Bering Sea to Scoresby Sound, east Greenland and south to north from southern Hudson Bay to Viscount Melville Sound. The fraction of individual MeSO₂-PCB congeners of the sum and ratios of S-MeSO₂-PCB and -4,4'-DDE to S-PCB and 4,4'-DDE, respectively, were compared among arctic geographical zones.

5.2 Experimental

5.2.1 Sampling

Male polar bear adipose samples were obtained by aboriginal hunters in Alaska, northern Canada and Greenland (see acknowledgements) at various times and locations over approximately a one year period between late 1989 and early 1991. The number labels on the map in Figure 5.1 illustrate the approximate geographic centre of the sampling locations for the individuals making up each pool.

Two gram fat samples, cut into small pieces, were quantitatively weighed into mortars and ground with 10g of Na_2SO_4 to remove excess moisture. The Na_2SO_4 /sample mixtures were extracted in a column with 80 ml 1:1 dichloromethane/n-hexane and concentrated to 5 ml by vacuum rotary evaporation at 30°C. Each sample was diluted to 10 ml with n-hexane, and 1 ml (equivalent to 0.2 g wet weight) was removed for % lipid determination.

Pools for each of the twelve areas shown in Figure 5.1 were prepared from the lipid extracts on an equal weight of fat basis from 3 to 27 individual adult male polar bears (Table 5.1). Lipid extract pools were stored in n-hexane at a lipid concentration of 0.1 g/ml. Adult males were chosen to minimize the effect of sex and age on geographical comparisons. It is known that adult males have higher PCB levels than adult females, but that there is minimal effect of age in adults of either sex (Canadian Wildlife Service, unpublished data).

5.2.2 Extraction and Chemical Analysis

Aliquots equivalent to 1 g and 0.5 g of lipid were taken separately from each pool for MeSO₂-PCB/4,4'-DDE and PCB/4,4'-DDE chemical analysis, respectively. PCB congeners and 4,4'-DDE were determined using the single fraction, PBQ method described in Chapter 6. Recoveries of the ¹³C₁₂-labelled PCB internal standards (see Table 6.1) were always greater than 85%. MeSO₂-PCB/4,4'-DDE metabolites were extracted and separated using the analytical method described in Chapter 2²⁰⁰. Each sample was spiked with 50 µl of 200 pg/µl of the MeSO₂-IS before extraction and chemical work-up. Recoveries of the MeSO₂-IS determined by GC/ECD were consistently 70 to 95 %.

The MeSO₂-PCBs/DDE were identified by retention times of authentic standards on GC/ECD and confirmed by GC/ECNI-MS. At the time of this study, seventeen MeSO₂-PCB standards were available (see Table 5.1). The nominal concentrations of these seventeen MeSO₂-PCBs and 3-MeSO₂-4,4'-DDE standards were verified and/or corrected by determining the molar carbon response (MCR_i) for each congener using GC/EI-MS(TIC) (see Chapter 2). The mean MCR_i for all MeSO₂-PCB standards were used to correct the concentrations considered to be outliers.

5.3 Results and Discussion

Polar bears feed mainly on ringed seal skin and blubber which simplifies the identification of sources of PCBs and 4,4'-DDE found in polar bear⁵⁷. The ability of polar bears to metabolize normally recalcitrant PCB congeners and 4,4'-DDEs accumulated

Figure 5.1. Western hemisphere Arctic and Subarctic geographic map showing distinct polar bear populations. Individual male polar bear samples were obtained from each region to prepare polar bear adipose pools (see Tables 5.1 and 5.2).

from ringed seal, their primary food, was demonstrated in Chapter 4. The dominant PCB congeners accumulating in polar bear fat were CB-153, -138, -180 and -170 as compared to CB-153, -138, -101 and -99 in ringed seal (see Chapter 4). In addition, PCB congeners with 2,5 or 2,3,6 chlorine substitution on one phenyl ring, which are the precursors to MeSO₂-PCBs, were below detection or noticeably reduced in polar bear fat compared to ringed seal blubber, except for trace amounts of CB-149 (see Chapter 4).

For polar bear adipose pools, the 3- and 4-MeSO₂-PCBs of CB-49, -64, -101, -70, -87, 149, -132, -141 and -174 consistently dominated S-MeSO₂-PCB, regardless of the region in the western hemisphere Arctic (Figure 5.1, Table 5.1). The 3-MeSO₂- and 4-MeSO₂-CB101 and 3-MeSO₂- and 4-MeSO₂-CB87 congeners accounted for an average of 27 % $\pm$ 4 % and 27 % $\pm$ 3 % of the total, respectively (Table 5.1).

S-MeSO₂-PCB and 3-MeSO₂-4,4'-DDE levels ranged from 145 to 633 ng/g lipid and 0.8 to 11 ng/g lipid, respectively, in polar bear fat pools (Table 5.2). These levels were generally higher in the south (southeast Hudson Bay and south Baffin Bay) and east (Scoresby Sound, east Greenland) (Figure 5.1) which reflects the PCB/DDT arctic input dictated by the movement of continental air masses from North American mid-latitudes to the northeast and north from Europe and Eurasia, respectively ^{3,31}.

The proportional change of S-MeSO₂-PCB levels among geographical areas was similar to variations observed in S-PCB levels (Table 5.2). S-PCB levels had previously been shown to be higher in Hudson Bay, east Greenland and Svalbard ^{48,248}. The proportion of higher chlorinated PCBs present in polar bear fat tended to increase from west to east

Table 5.1. MeSO₂-PCB and Selected PCB Congener Levels (ng/g lipid) in Polar Bear Adipose Pools from Arctic and Subarctic Regions

Geographical Area	Bering Sea	Beaufort Sea	V. Melv. Sound	Q. Maud Gulf	Barrow Strait	Gulf of Boothia	N. Baffin Bz.	S. Baffin Bay	Davis Strait	N.W. Hud. Bay	S.E. Hud. Bay	Scoresby Sound
Location in Fig. 5.1	1	2	3	4	5	6	7	8	9	10	11	12
# in pool	3	3	4	5	10	5	10	5	3	27	9	10
<i>MeSO₂-PCB</i>												
3-MeSO ₂ -CB49	6.9	11.5	25.2	15.2	11.8	5.6	17.3	19.8	14.6	13.1	19.2	36.7
4-MeSO ₂ -CB49	6.6	12.9	26.8	16.9	12.5	6.8	15.2	20.2	13.8	12.5	18.3	34.8
4-MeSO ₂ -CB64	4.2	9.8	17.3	13.4	10.1	4.6	10.0	10.2	7.3	5.8	11.9	15.8
3-MeSO ₂ -CB101	21.3	23.1	42.3	29.4	22.4	19.6	39.5	67.8	47.2	35.6	35.7	81.9
4-MeSO ₂ -CB101	27.1	30.2	59.8	40.9	28.9	23.5	38.2	65.3	39.0	37.0	44.0	78.1
3-MeSO ₂ -CB70	3.2	6.8	10.6	6.7	5.0	1.9	6.5	11.5	6.7	5.3	7.8	15.8
4-MeSO ₂ -CB70	4.9	8.4	14.7	11.5	8.0	2.5	7.4	10.1	7.7	6.0	9.8	15.8
3-MeSO ₂ -CB87	10.3	17.7	40.1	24.8	19.4	11.0	27.9	51.6	23.1	18.8	28.9	61.3
4-MeSO ₂ -CB87	23.2	54.0	89.2	58.8	41.8	29.7	47.9	86.1	47.2	37.8	60.9	115.9
3-MeSO ₂ -CB149	1.9	4.9	7.5	4.1	3.7	1.5	4.5	11.7	6.0	3.9	7.6	18.8
4-MeSO ₂ -CB149	11.1	34.0	56.4	34.7	26.3	12.0	29.6	47.4	34.6	20.2	41.9	69.4
3-MeSO ₂ -CB132	1.2	6.6	4.5	2.4	1.6	1.3	2.8	5.8	3.0	2.5	4.1	12.7
4-MeSO ₂ -CB132	7.2	12.1	23.0	12.8	10.5	11.3	17.9	20.3	16.8	6.8	14.3	33.0
3-MeSO ₂ -CB141	4.4	5.2	8.9	4.0	5.3	5.5	11.0	20.0	8.2	5.3	8.4	16.8
4-MeSO ₂ -CB141	3.6	6.8	13.3	6.0	5.8	5.9	7.4	14.4	6.9	5.3	9.1	17.4
3-MeSO ₂ -CB174	0.8	0.9	1.5	0.9	0.7	1.2	1.4	4.0	2.5	1.3	1.3	3.6
4-MeSO ₂ -CB174	0.4	2.0	3.8	1.6	1.5	0.6	2.4	4.1	1.9	1.1	2.7	5.1
<i>Selected PCB</i>												
CB-99	108.6	393.1	553.4	396.0	263.1	142.0	329.2	368.5	521.4	170.4	613.3	753.5
CB-180	292.7	1126.8	1540.0	790.3	683.7	513.1	1011.0	2185.4	1667.1	869.1	1176.3	3411.4
CB-149	0.0	1.6	3.3	1.9	1.9	0.0	2.0	2.2	3.3	2.1	3.5	3.1

Table 5.2. Total (S-) MeSO₂-PCB and -4,4'-DDE Levels (ng/g lipid) and Ratios to the S-PCB and 4,4'-DDE in Polar Bear Adipose Pools from Arctic and Subarctic Regions

Geographical Area	Bering Sea	Beaufort Sea	V. Melv. Sound	Q. Maud Gulf	Barrow Strait	Gulf of Boothia	N. Baffin Bay	S. Baffin Bay	Davis Strait	N.W. Hud. Bay	S.E. Hud. Bay	Scoresby Sound
Location in Fig. 5.1	1	2	3	4	5	6	7	8	9	10	11	12
(1) S-MeSO ₂ -PCBs	138	245	445	284	215	145	287	470	286	218	326	633
(2) S-PCBs	1737	5396	6181	4595	3361	2529	4720	8863	7637	4280	6690	13730
Ratio (1)/(2)	0.080	0.046	0.072	0.062	0.064	0.057	0.061	0.053	0.038	0.051	0.049	0.046
(1) 3-MeSO ₂ -DDE	0.8	1.8	2.7	1.1	1.9	0.6	3.6	4.4	3.2	2.1	2.8	11.0
(2) 4,4'-DDE	24.3	50.5	55.1	20.1	74.6	32.6	88.3	82.5	170.1	185.2	314.3	209.0
Ratio (1)/(2)	0.031	0.035	0.049	0.056	0.025	0.017	0.041	0.053	0.019	0.011	0.009	0.052

(except for Southeast Hudson Bay), demonstrated by a decreasing PCB-99 to PCB-180 ratio (Table 5.1, Figure 5.2). This can be viewed from a commercial PCB mixture perspective. CB-99 arises mainly from Aroclor 1254 (weight percent 3.60 %) rather than Aroclor 1260 (weight percent 0.11%), whereas CB-180 comes mainly from Aroclor 1260 (weight percent 7.12 %) rather than Aroclor 1254 (weight percent 0.38 %)²²³⁴. This implies that the pattern of PCB input is dominated by more highly chlorinated congeners related to Aroclor 1260 in the eastern part of the western hemisphere Arctic. Because more MeSO₂-PCB congeners originate from tetra- and penta-chlorinated PCB congeners which occur mainly in Aroclor 1254 and Aroclor 1242 (CB-49, CB-64, CB-70 and CB-87, Table 5.1), the S-MeSO₂-PCB to S-PCB ratio decreases from west to east (Table 5.2, Figure 5.3).

Despite the change in ratio of S-MeSO₂-PCB to S-PCB (Figure 5.3), the average MeSO₂-PCB congener composition among regions was very uniform (Figure 5.4). The geographic independence of the MeSO₂-PCB congener makeup suggests that the pattern of atmospheric PCB input and subsequent food chain biomagnification of their precursor PCBs is homogeneous in the western hemisphere. This may be a result of a relatively more uniform hemispheric distribution of less chlorinated PCBs^{31,32} and the long atmospheric lifetimes of PCBs¹⁶. The levels of 3-MeSO₂-4,4'-DDE in polar bear fat (0.8 to 11 ng/g lipid) were low compared to levels previously found in liver (see Chapter 4). The mean 3-MeSO₂-4,4'-DDE to 4,4'-DDE ratio in fat was 0.033 ± 0.016 (Table 5.2), ca. ten times lower than was found in liver. The mean S-MeSO₂-PCB to S-PCB ratio (0.056 ± 0.011) was ca. 3-fold lower than in liver. These results indicated less preferential storage of MeSO₂-PCB and MeSO₂-DDE in adipose tissue than liver, and imply that factors contributing to tissue

Figure 5.2. CB-99 to CB-180 ratios for geographically distinct polar bear adipose tissue pools, ordered west to east (see Figure 5.1).

Figure 5.3. S-MeSO₂-PCB to S-PCB ratios for geographically distinct polar bear adipose tissue pools, ordered west to east (see Figure 5.1).

Figure 5.4. Mean MeSO₂-PCB congener fractions of the S-MeSO₂-PCB for the twelve Arctic and Subarctic regions denoted in Figure 5.1. The I-beams denote the minimum and maximum fraction range for MeSO₂-PCB congeners from each region.

distribution and/or formation may be different from other CHCs.

The 3-MeSO₂-4,4'-DDE to 4,4'-DDE ratios were erratic going west to east due to the fluctuation of absolute 3-MeSO₂-4,4'-DDE levels, whereas absolute 4,4'-DDE levels gradually increased (Table 5.1, Figure 5.5). The irregular distribution of 3-MeSO₂-4,4'-DDE in polar bear fat was probably a consequence of preferential retention in the liver. Despite the small size of the liver relative to the fat depots in bear, 60.5 % of TBB of 2- and 3-MeSO₂-4,4'-DDE was shown to be in liver (Chapter 4, Table 4.7). Covalent binding in adrenals was also possible. The 3-MeSO₂-4,4'-DDE is a potent toxicant subsequent to metabolic activation in the adrenal cortex of mice, rats, seals and birds ^{154,159,160}. Polar bears in the eastern Canadian Arctic are subject to greater exposure to 3-MeSO₂-4,4'-DDE than those in the west.

There are other factors which may contribute to S-MeSO₂-PCB and 4,4'-DDE variation among geographical areas. Time of sampling varies within pools, which may be significant for pools with fewer individuals (Table 5.1) sampled over a narrow time range. The fat content of polar bears decreases during the ice-free period when the bears are forced on land and must utilize some of their fat reserves. This fasting period varies in time, length and place throughout the arctic and subarctic ²⁴⁹. In Hudson Bay, the ice-free period can last as long as four months from August to November during which time polar bears are not able to hunt for ringed seal ²⁴⁷. The polar bear diet may therefore vary over an annual period. In Chapter 4 it was shown that the S-MeSO₂-PCB concentration in female adipose tissue, on a lipid weight basis, increased linearly as the fasting period progressed, but at the same rate as PCBs. This seasonal difference was not dramatic. Therefore, the S-MeSO₂-PCB

Figure 5.5. The 4,4'-DDE and 3-MeSO₂-4,4'-DDE levels for geographically distinct polar bear adipose tissue pools, ordered west to east (see Figure 5.1). Dark bars are 4,4'-DDE levels and light bars are 3-MeSO₂-4,4'-DDE in ng/g (lipid weight).

level variation among Arctic and Subarctic regions was likely due to geographical effects, assuming males experience a minimal seasonal fluctuation in S-MeSO₂-PCB levels on lipid weight basis similar to females. The seasonal effect on 3-MeSO₂-4,4'-DDE levels in female adipose tissue was more erratic and may have had an effect on the geographical variation of 3-MeSO₂-4,4'-DDE levels presented in this chapter, in the adipose tissue pools of male polar bears.

5.4 Conclusions

The S-MeSO₂-PCB and 3-MeSO₂-4,4'-DDE levels were generally higher in the east (Greenland) and south (Hudson Bay). The ratio of S-MeSO₂-PCBs to accumulating PCBs decreased very gradually from west to east reflecting the proportion of higher chlorinated PCBs which are not major precursors of MeSO₂-PCBs. The health risk of MeSO₂-PCB exposure to polar bear in the east relative to west Arctic may therefore be different. In contrast, the 3-MeSO₂-4,4'-DDE to 4,4'-DDE ratio fluctuated among regions whereas the absolute 4,4'-DDE levels gradually increased. Irregular distribution of 3-MeSO₂-4,4'-DDE in polar bear fat was likely a consequence of the preferential storage of MeSO₂-4,4'-DDEs in liver and the possibility of covalent binding in adrenals. Temporal and feeding status effects may also have some influence. Increased 4,4'-DDE levels in the eastern Arctic represents a greater health risk to polar bear due to the greater exposure to MeSO₂-DDEs. Toxicological evidence has demonstrated that reactive metabolites of 3-MeSO₂-4,4'-DDE are produced in the adrenal mitochondria¹⁵⁵⁻¹⁶⁰. Cytotoxic intermediates decrease the activity

of enzymes involved in steroid metabolism, resulting in adrenal tissue damage.

The MeSO₂-PCB congener pattern represented as the congener fraction of the S-MeSO₂-PCB was essentially independent of geographic variation among the regions. For example, the congeners 3-MeSO₂- and 4-MeSO₂-CB101 and 3-MeSO₂- and 4-MeSO₂-CB87 consistently accounted for ca. 50 % of the S-MeSO₂-PCBs in polar bear fat, regardless of geographical location. The constancy of the MeSO₂-PCB congener pattern implied that the pattern of atmospheric input and subsequent biomagnification of precursor PCBs is homogeneous in the western hemisphere.

Immunoquantitation and Microsomal Monooxygenase Activities of Hepatic Cytochromes P450 1A and P450 2B and Chlorinated Hydrocarbon Contaminant Levels in Polar Bear (*Ursus maritimus*)[†]

6.1 Background

The sampling of polar bear tissue is a challenge for several reasons. The polar bear is a large, predatory mammal, requiring a considerable monetary and logistic effort for capture. The Arctic region is an immense, remote and often climatically hostile working environment. Finally, access to samples is limited since hunting is tightly controlled.

Recently, it was possible to obtain liver samples from several polar bears as part of the annual Inuit hunt in the Canadian Arctic. In conjunction with the hunt, a study was designed to determine the half-life of Telazol in polar bear. Telazol is a tranquillizing drug routinely used for the immobilization of polar bears ²¹¹. For the present study, bear liver samples were obtained in which the enzyme activity was preserved.

Immunochemical characterization of the polar bear hepatic cytochrome P450 (CYP)

[†] Adapted from *Toxicol. Appl. Pharmacol.*, **1995**, 137, in press.

Presented in part; 1) 14th International Symposium on Chlorinated Dioxins, PCBs and Related Compounds, Nov., 21-25, 1994, Kyoto, Japan and 2) 15th Annual Society of Environmental Toxicology and Chemistry Meeting, Oct., 30 - Nov., 3, 1994, Denver, Colorado, U.S.A..

system showed that CYP1A, CYP2B, CYP2C and CYP3A isozymes were cross-reactive with antibodies raised against the respective rat isozymes⁹³. CYP2B and CYP1A isozymes appeared to be induced, probably as a result of environmental contaminant exposure. The goal of the present study was to develop an immunological method for assessing the exposure of free-ranging polar bears to environmental contamination, specifically CHCs, and to examine the relationships among immunoreactive CYP1A and CYP2B protein levels, mixed function monooxygenase (MFO) activities and hepatic CHC concentrations.

CYP1A and CYP2B isozyme bands from the liver microsomes of sixteen adult male polar bears were quantitated by densitometric analysis of immunoblots and compared with ethoxyresorufin (EROD), pentoxyresorufin (PROD) and benzyloxyresorufin (BROD) *O*-dealkylase activities. Correlations were determined between CYP1A and CYP2B protein content and hepatic levels of residual PCDD/Fs, PCBs, pesticides such as chlorinated cyclodienes (chlordanes) and DDTs, zolazepam, a component of the drug Telazol, and CHC metabolites, including MeSO₂-PCBs. Telazol was used to tranquillize the bears at various times prior to death. Immunoinhibition studies of EROD and BROD activities were performed to determine the polar bear CYP isozymes responsible for specific MFO activities.

6.2 Experimental

6.2.1 Materials and Standards

Bovine serum albumin (BSA) (98 % fatty acid free) was purchased from ICN Biochemicals (St. Laurent, QU, Canada). Acrylamide, disodium hydrogen orthophosphate,

potassium dihydrogen orthophosphate, potassium chloride, sodium chloride, sodium dodecyl sulphate, disodium ethylenediaminetetraacetate (EDTA), magnesium chloride, glycine and ammonium persulfate were obtained from BDH Chemicals (Toronto, ON, Canada). N,N,N',N'-tetramethylethylenediamine (TEMED) was bought from Bio-Rad Laboratories (Mississauga, ON, Canada). HPLC grade methanol, *tris*(hydroxymethyl)aminoethane (Tris base), glycerol and 4-chloro-1-naphthol were purchased from Fisher Scientific (Vancouver, BC, Canada). Nicotinamide adenine dinucleotide phosphate (NADPH), tetrasodium salt, was obtained from Boehringer Mannheim (Laval, QU, Canada). Polyoxyethylene sorbitan monolaurate (Tween 20), N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) and dimethyl sulfoxide (DMSO) were bought from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Hydrogen peroxide (30 % w/v solution) was obtained from Anachemia (Montreal, QU., Canada) and resorufin was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Ethoxyresorufin, pentoxyresorufin and benzyloxyresorufin were bought from Molecular Probes Inc. (Eugene, OR, U.S.A.). Nitrocellulose membrane was obtained from Schleicher and Schuell (Keene, NH, U.S.A.). Horseradish peroxidase (HRP)-conjugated, affinity-isolated goat F(ab')₂ anti-rabbit immunoglobins (gamma and light-chain specific, human IgG absorbed) were purchased from TAGO Immunologicals Inc. (Inter-Medico, Markham, ON, Canada).

All solvents (BDH Chemicals) for chemical analysis were of analytical grade or better. The preparation of the column chromatography materials, 1.2 % water deactivated (w/w) Florisil (magnesium silicate, BDH chemicals, pesticide analysis grade, 60-100 mesh), 2.3 % water deactivated (w/w) basic aluminum oxide (Fisher Scientific, activity grade 1, 60-

325 mesh), 33 % potassium hydroxide (w/w) impregnated silica gel (Bio-Rad Laboratories, Bio-sil A, 100-200 mesh) and sodium sulphate (BDH chemicals, analytical grade) were described in Chapter 2. Tissues destined for non-*ortho* (NO)-PCB, PCDD and PCDF determination were spiked with the ^{13}C -labelled internal standards; CB-77 (3,3',4,4'), CB-126 (3,3',4,4',5), CB-169 (3,3',4,4',5,5'), 2,3,7,8-tetrachloro(Te)CDD, 1,2,3,7,8-pentachloro(Pn)CDD, 1,2,3,6,7,8-hexachloro(Hx)CDD, 1,2,3,4,6,7,8-heptachloro(Hp)CDD, and 1,2,3,4,6,7,8,9-octachloro(Oc)CDD, 2,3,7,8-TeCDF, 1,2,3,7,8-PnCDF, 1,2,3,6,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF. For CHC determination, including *ortho* PCBs, the liver samples were spiked with ^{13}C -labelled tetrachloro, pentachloro and hexachlorobenzene and CB-28, CB-52, CB-118, CB-153, CB-180 and CB-194. All ^{13}C -labelled standards were purchased from Cambridge Isotope Laboratories (Andover, MA). MeSO_2 -PCB and 3- MeSO_2 -2- CH_3 -5-2',3',4',5'- Cl_5CB (internal standard, MeSO_2 -IS) standards were provided by Dr. Åke Bergman (Wallenberg Laboratory, Stockholm University, Stockholm, Sweden) and synthesized according to known procedures ¹⁷³.

6.1.2 Polar Bear Liver Samples

Liver samples from the distal portion of the right lobe of 16 adult male polar bears, aged 5 to 22 years, were collected by Dr. Malcolm Ramsay (Department of Biology, University of Saskatchewan, SA, Saskatoon) in legally controlled hunts by Inuit in the Canadian Arctic. Bears A to D were collected in April 1992, bears E to M in April 1993, and bears N and O in April 1994 near Resolute Bay, Northwest Territories, near the end of a period of regular winter feeding and prior to the long summer fast ²¹². Bear X was collected

in November 1993 near Churchill, Manitoba, just after the fasting period. All bears had been exposed to the tranquillizing drug, Telazol ²¹¹, that is, a 1:1 mixture of tiletamine hydrochloride and zolazepam hydrochloride (A.H. Robins Co., Richmond, VA, U.S.A.), from 0.5 to 11 days prior to death. Liver samples for immunoassays and catalytic bioassays were removed within 10 to 15 min after death, cut into small pieces and frozen at -196°C in liquid nitrogen. Liver samples for chemical analysis were subsequently stored at -40°C, while liver samples for enzymatic and immunochemical analyses were stored at -80°C.

6.2.3 Microsome Preparation

Frozen liver samples of polar bear were thawed and hepatic microsomal fractions were prepared by differential centrifugation at 5°C as described by Thomas *et al.* ²⁵⁰. The microsomal pellets were suspended in 0.25 M sucrose. Aliquots of the suspension were stored at -80°C until needed. Hepatic microsomes had been prepared previously from pooled (i.e., at least 5 rats for each treatment group) adult male Long Evans rats that were either untreated or treated with sodium phenobarbital (75 mg/kg body weight/day ip for 4 days) or 3-methylcholanthrene (25 mg/kg body weight/day ip for 4 days) ⁹³.

6.2.4 Protein and Total Cytochrome P450 Determination

Protein concentration was determined by the method of Lowry *et al.* ²⁵¹. Total CYP content was determined from the dithionite difference spectra of carbon monoxide-treated microsomes using the method of Omura and Sato ²⁵². Hepatic microsome preparation and the

determination of protein and total CYP was carried out in conjunction with Dr. Stelvio Bandiera (Faculty of Pharmaceutical Sciences, University of British Columbia, BC, Vancouver).

6.2.5 Enzyme Assays

The *O*-dealkylation of ethoxyresorufin, pentoxyresorufin and benzyloxyresorufin were measured separately using the same spectrofluorometric assay as described by Burke *et al.*^{253,254}. In the assay, alkoxyresorufins are dealkylated to fluorescent resorufin by a CYP-mediated mechanism of action (see figure below).

Fresh frozen (-80°C) microsomes were thawed to room temperature, immediately packed on ice and diluted to 4 mg/ml with cold 0.25 M sucrose. Each microsomal sample was assayed directly in a fluorescence cuvette incubated at 37°C . The 2 ml reaction mixtures contained 0.1 M HEPES buffer with 5 mM magnesium chloride ($\text{pH} = 7.8$), 5 μM ethoxyresorufin, pentoxyresorufin or benzyloxyresorufin, and either 0.1 or 0.2 mg of polar bear hepatic microsomal protein. A blank reading was taken after a 5 min pre-incubation

period. Reactions were initiated with 10 μ l of 50 mM NADPH and a fluorescence intensity reading was taken after a 5 min incubation period. All measurements were made using a Shimadzu Model RF-540 fluorometer interfaced with a Shimadzu DR-3 data recorder. To measure resorufin, the excitation wavelength was set at 530 nm and the emission wavelength at 582 nm. The enzyme activity was determined from the resorufin content which was quantitated from a standard curve generated by measuring the fluorescence of resorufin standards with concentrations of 0, 200, 600, 1000 and 2000 pmol. There was a positive linear correlation ($r^2 = 0.997$, $p < 0.005$) between fluorescence intensity and the amount of resorufin (not shown).

The alkoxyresorufin *O*-dealkylase assay parameters were re-examined with polar bear hepatic microsomes to ensure that resorufin formation was optimized. The rate of resorufin formation was found to be linear with respect to incubation time for up to 10 min when 0.1 mg of microsomal protein was used. At an incubation time of 5 min, the rate of resorufin formation was linear for microsomal protein concentrations up to 0.25 mg/2 ml of reaction mixture. The substrate and NADPH concentrations used were saturating, although some substrate inhibition was evident at final substrate concentrations greater than 2.5 μ M.

6.2.6 Antibody Preparation

Polyclonal antibody against rat CYP2B1 and polyclonal antibody against rat CYP1A1 were generated in female New Zealand rabbits, immunized with electrophoretically homogeneous proteins. Immunoglobulin G (IgG) was purified from a pool of heat-inactivated

antisera. Polyclonal (polyspecific) antisera against rat CYP1A1, recognizing both 1A1 and 1A2 forms and a mixture of four monoclonal antibodies against rat CYP1A1 were provided by Dr. Paul Thomas (Rutgers - The State University of New Jersey, Piscataway, NJ, U.S.A.). The specificity of each antibody was determined by Dr. Steivio Bandiera (Faculty of Pharmaceutical Sciences, University of British Columbia, BC, Vancouver) and has been described in a preceding study ⁹³.

6.2.7 Antibody Inhibition of Catalytic Activities

The alkoxyresorufin-*O*-dealkylase assays were performed as described previously, except that polar bear hepatic microsomes were preincubated with increasing amounts of rabbit anti-rat CYP1A1 IgG, rabbit anti-rat CYP2B1 IgG or control rabbit IgG for 5 min prior to initiation of the reaction by the addition of NADPH. The antibodies and control IgG were used at concentrations of 0, 0.5, 1.0, 5.0 or 10 mg IgG per nanomole of total CYP and were added in a constant volume of phosphate-buffered saline, pH 7.4. Bears G and J were selected for this study as their EROD and BROD activities were relatively high. It was not feasible to test all sixteen bears as the experiments consume a large amount of antibody and antibody preparations were in limited supply.

6.2.8 Gel Electrophoresis and Immunoblotting

Immunoblotting was used to detect immunoreactive CYP proteins ²⁵⁰ in polar bear hepatic microsomes for subsequent densitometric quantitation. The electrophoretic conditions for separation of hepatic microsomal proteins were as follows. First, sodium

dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS-PAGE) was performed in a 7.5 % acrylamide separating gel that was 0.75 mm thick and 12.5 cm in length and width ²⁵⁵. Proteins (10 µg/well) resolved by SDS-PAGE were electrophoretically transferred to nitrocellulose and probed with the antibodies described previously. The nitrocellulose blots were incubated with primary antibody at concentrations specified in Figures 6.2 and 6.3 (see section 6.3 Results) for 2 hours at 37°C with constant but gentle rocking. Blots were then washed extensively and incubated with horseradish peroxidase (HRP)-conjugated, goat anti-rabbit F(ab')₂ IgG secondary antibody for a further 2 hours at 37°C. Immunoreactive protein bands were revealed colorimetrically by oxidation of 4-chloro-1-naphthol (0.018 %) with hydrogen peroxide (0.018 %) and catalyzed by HRP in a phosphate buffered saline (pH 7.4) solution.

6.2.9 Immunoquantitation

The band colour intensity was measured by computer image analysis using a VISAGE 110 Bio Image analyzer (Bio Image, Ann Arbor, MI) consisting of a high resolution camera and a Sun Microsystems workstation. The amount of immunoreactive protein present in each lane was determined from the integrated optical density of the stained band using the Whole Band Analysis software option. Microsomal samples were applied to each gel at a protein concentration of 10 µg/well.

Densitometry values were converted into picomole quantities using calibration curves to quantitate CYP1A and CYP2B isozyme protein levels. Calibration curves were generated by loading various concentrations of purified rat CYP1A1 or CYP2B1 on separate gels

followed by immunoblotting and densitometric analysis as described above. Figures 6.2 and 6.3 (see Section 6.3.3 Immunodetection of CYP) illustrate the immunoblots of rat CYP1A1 and CYP2B1 standards probed with monoclonal mouse anti-rat CYP1A1 IgG and polyclonal rabbit anti-rat CYP2B1 IgG, respectively. A single concentration of purified CYP standard and rat liver microsomes were routinely included on each blot as internal standards.

6.2.10 Chemical Analysis

CHCs and MeSO₂-PCB and -4,4'-DDE Metabolites. Homogenized polar bear liver samples of 0.5 g were used for the determination of CHCs including DDTs, polychlorocyclopentadiene pesticides (dieldrin, chlordanes and mirex), chlorinated benzenes (ClBzs), hexachlorocyclohexanes (HCHs), *ortho*-chlorine substituted PCBs and MeSO₂-PCB and -4,4'-DDE metabolites. After spiking each sample with the ¹³C-labelled PCB mixture (CB-28, CB-52, CB-118, CB-153, CB-180 and CB-194) and MeSO₂-IS, the extraction and separation of these CHCs was accomplished by the method outlined in Chapter 2²⁰⁰.

The fraction containing the *ortho*-PCBs, DDTs, chlordanes, ClBzs and HCHs was analyzed by a single fraction approach using GC/EI-MS and a characterized polar bear adipose extract as a secondary standard (i.e., PBQ method). The PBQ method was modified based on a similar method which has been described in some detail elsewhere (Norstrom *et al.*, in preparation). Table 6.1 lists the PCB congeners and CHCs that were analyzed for by the PBQ method. Ions scanned and their retention time windows are also given.

GC/EI-MS was performed using a HP 5987B GC/MSD (mass selective detector) in the EI mode at 70 eV using a DB-5 column (30 metre, 0.25 mm internal diameter (id), J&W

Table 6.1. GC/EI-MS Retention Time Windows and the Ions Monitored for PCB and CHC Compounds Determined by the PBQ Method[†]

Time Range (min)	Compound	Target/Qualifier	Time Range (min)	Compound	Target/Qualifier
5.6-7.6	1,2,4,5-TeClBz	216	17.2-18.35	4,4'-DDE	246/318
	¹³ C-1,2,4,5-TeClBz	222		CB-85	326/328
7.6-9.6	PnClBz	250		CB-154	360/362
	¹³ C-PnClBz	256		dieldrin	380
	U-3*	250	18.35-19.75	CB-149	360/362
9.6-11.6	α-HCH	219		U-2*	443
	HxCIBz	284		CB-118	326/328
	¹³ C-HxCIBz	290		¹³ C-CB-118	338
	β-HCH	219		4,4'-DDD	235
11.6-14.6	compound C	238		CB-146	360/362
	¹³ C-CB-28	268		CB-153	360/362
	¹³ C-CB-52	304		¹³ C-CB-153	372
	CB-47/48	292/290		CB-105	326/328
14.6-15.8	photoheptachlor	339	19.75-21.8	CB-137	326/328
	octachlorostyrene	380		CB-138	326/328
	U-4*	291		4,4'-DDT	235
	oxychlordane	387		CB-187	394/396
	heptachlor epoxide	353		CB-183	394/396
	CB-74	292/290	21.8-23.5	CB-156	360/362
15.8-16.45	C-3	339		CB-157	360/362
	CB-56/60	292/290		CB-180	394/396
	C-5	373/375		¹³ C-CB-180	406
	C-4	373/375	23.5-29.1	CB-170/190	394/396
16.45-17.2	CB-99	326/328		CB-195	430/428
	nonachlor-III	409/373		CB-194	430/428
	t-nonachlor	409/373		¹³ C-CB-194	442
				CB-206	464/466
				CB-209	498/500

[†] PCBs and CHCs listed in order of retention time on a 30 metre, DB-5 GC column.

* chlordane compounds ⁴⁷.

Scientific Inc.) under the following conditions: initial temperature held for 3 min at 100°C, 20°C/min to 180°C and 5°C/min to 320°C. The injector, transfer line and ion source temperatures were 250°C, 300°C and 200°C, respectively. Recoveries of the ^{13}C -labelled PCBs were always greater than 85 % and determined using CB-154 as a performance internal standard. The native PCB levels were corrected for the recoveries of the ^{13}C -labelled PCBs.

MeSO_2 -PCBs were quantitated by an IS method using GC/ECD congener response factors relative to the MeSO_2 -IS (RRF, see Chapter 2). For quantitation of individual congeners, all the MeSO_2 -PCB standard concentrations for RRF calculations were verified and/or corrected by GC/FID, except for 3- MeSO_2 -CB70 and 4- MeSO_2 -CB71 (see Table 2.2). In each of these cases, the RRF for the 3- or 4- MeSO_2 -PCB pair was used for quantitation. MeSO_2 -IS recoveries were 85 to 90 %. GC/ECNI-MS was used to confirm MeSO_2 -PCB identities by total ion current scanning from 83 to 550 amu (see Chapter 3). See Chapter 2 for mass spectrometry parameters for GC/ECNI-MS. The same GC conditions were used as for GC/ECD.

NO-PCBs, PCDDs and PCDFs. Extraction and separation of NO-PCBs (e.g., CB-77, CB-126 and CB-169), PCDDs and PCDFs was achieved using a modification of the GPC/carbon chromatography approach described in Norstrom and Simon ²⁵⁶ (Appendix A; Canadian Wildlife Service, unpublished method).

6.2.11 Statistical Analysis

The number of polar bear liver samples was limited to sixteen due to the limitations of the Telazol half-life study. The relationships between 1) specific CYP protein content and

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MFO activities, 2) the concentrations of CHC residues known to be good Ah-receptor ligands and CYP1A content, and 3) TEQ concentrations and CYP1A content were determined by simple linear regression. The significance was assessed using the one-tailed Student's *t* test at a confidence level of 95 %. Coefficients of determination (r^2) with a *p* value of < 0.05 , were considered statistically significant.

The relationship between CYP2B content and CHC residue concentrations (chlordanes, *ortho*-PCBs, DDTs, HCHs, ClBzs and MeSO₂-PCBs) as well as zolazepam were assessed by multiple linear regression using the Statistica™ software package (StatSoft Inc., 1994, Tulsa, OK, U.S.A.). A backward "stepwise" approach was used to assess the significance of *m* independent variables (residue levels) on the dependent variable (CYP2B content) to determine the best regression model. Independent variables not having a significant effect on the magnitude of the dependent variable, or having a *t*-value less than the *t*-critical at the 95 % confidence level, were omitted from the model. The remaining variables defined the regression model and multiple regression analysis was then performed.

6.3 Results

6.3.1 Total Hepatic CYP Content and Alkoxyresorufin-O-dealkylase Activities

Table 6.2 lists the total CYP content, EROD, PROD and BROD activities for hepatic microsomes prepared from sixteen adult male polar bears. The total CYP content was variable among bears and ranged between 1 to 2 nmol/mg microsomal protein. Different values of total CYP content for hepatic microsomes of bears A to D had been reported

Table 6.2. Total Cytochrome P450 Content and Alkoxyresorufin *O*-dealkylase Activities of Adult Male Polar Bear Hepatic Microsomes

Polar Bear	Total CYP [†]	EROD [‡]	PROD [‡]	BROD [‡]
A	1.98	1401 (147)	21(7)	65
B	1.01	192	n.d.	115
C	1.18	1266 (176)	24 (4)	238
D	1.09	1013 (95)	25 (8)	174
E	1.94	1459 (341)	24 (4)	239
F	1.35	794 (170)	18 (4)	206
G	1.82	1477 (177)	32 (4)	283
H	1.44	1596 (140)	28 (1)	259
I	1.38	1138 (272)	24 (4)	226
J	1.22	1571 (65)	24 (4)	150
K	1.34	1056 (253)	21 (1)	96
L	1.30	1680 (394)	28 (1)	199
M	1.26	863 (210)	18 (4)	166
N	1.73	692	17	161
O	1.08	518	5	198
X	1.19	1928	9	186

[†] Total CYP content is expressed as nmol/mg microsomal protein.

[‡] Two sets of two ($n = 4$) assays, approximately one month apart except for EROD and PROD of bears N, O, X and BROD of all bears (only one set of two, $n = 2$). Expressed as pmol resorufin formed/min/mg microsomal protein. Numbers in parentheses denote SD. n.d. = not detected, PROD activity less than basal fluorescence level.

previously⁹³. The CYP content of bear A was 2-fold higher than the earlier determined value whereas the values for bears B, C, and D were slightly lower. This difference may be explained by the fact that hepatic microsomes from bears A-D were prepared on two separate occasions from different pieces of liver in storage. The variability in total CYP content among bears must therefore be interpreted with caution, although the 1 to 2 nmol/mg range is likely valid. Moreover, the total CYP content may be underestimated due to the presence of a small amount of haemoglobin contamination represented by a near-ultraviolet spectral peak at 420 nm⁹³. The amount of haemoglobin contamination was similar among hepatic microsomes.

EROD measurements for bears A and C to M were the mean of two sets of two measurements, each determined almost one month apart. Despite this, the standard error of the mean EROD activities was approximately 10 % or less (Table 6.2). EROD activities varied ten-fold among individual bears (192 to 1928 pmol/min/mg protein) with a mean value of 1165 ± 454 pmol/min/mg protein. The mean PROD activity (21 ± 7 pmol/min/mg protein) was about 55 times lower than the mean EROD activity. The mean BROD activity was 8.8 times greater (185 ± 59 pmol/min/mg) than the mean PROD activity. When the catalytic activities were ranked in descending order, BROD activities did not exactly parallel PROD or EROD activities.

6.3.2 Electrophoretic Separation and Profile of CYP

Figure 6.1 illustrates a Coomassie-blue stained polyacrylamide gel electrophoretogram of polar bear liver microsomes. Untreated, PB-treated and 3-MC-treated

hepatic microsomes are included for comparison as well as the appropriate standard of a purified cytochrome P450 protein (i.e., CYP1A1 or CYP2B1). The electrophoretic pattern of protein-stained bands revealed multiple proteins with apparent molecular weights of 50 kilodaltons in the lanes containing polar bear hepatic microsomes, near the midway point of the gel. Numerous forms of cytochrome P450 appear to be expressed in polar bear liver in the electrophoretogram. However, the electrophoretic mobilities of the polar bear hepatic proteins were not the same as those from untreated, 3-MC-treated and PB-treated rats.

6.3.3 Immunodetection of CYP

After electrophoretic transfer to nitrocellulose, blots were probed with polyclonal antibody to rat CYP1A1 or polyclonal antibody to rat CYP2B1. Polyclonal anti-rat CYP1A1 sera recognized two proteins in the polar bear microsomes (Figure 6.2), that are immunochemically related to rat CYP1A1 and CYP1A2. The staining intensity of both bands was variable among the thirteen individual polar bear hepatic microsomes and reflected differences in hepatic levels of the CYP1A1 and CYP1A2 homologues among the bears. The distance between the purified rat hepatic CYP1A1 band and the two polar bear bands was measured and compared with an immunoblot probed with monoclonal antibody to rat CYP1A1 (Figure 6.2) in order to identify which band corresponded to CYP1A1 in the polar bear microsomes. The CYP1A1 isozyme in polar bear hepatic microsomes moved slightly faster than the isozymes in rat liver, indicating that the former had a slightly smaller molecular weight. Based on that comparison, the polar bear hepatic microsomal band with the faster electrophoretic migration in the immunoblot probed with polyclonal anti-rat

Figure 6.1. Polyacrylamide gel electrophoretogram of polar bear liver and rat liver microsomal samples. Microsomal samples were applied at a final concentration of 10 μ g of microsomal protein per lane. Proteins were stained with Coomassie blue R-250.

Figure 5.2. CYP1A1(2) immunoblots of adult male polar bear hepatic microsomes. Blots probed with A) monoclonal rabbit anti-rat CYP1A1 2 μ g IgG/ml and B) polyclonal rabbit anti-rat CYP1A1(2) sera at 1:500 dilution. Proteins (10 μ g/well for CYP1A1(2) and 20 μ g/well for CYP2B1) resolved by SDS-PAGE were electrophoretically transferred to nitrocellulose and probed with antibodies described previously. Purified rat CYP1A1 (i.e., (A) and (B)) or CYP2B1 (i.e., (C)) and untreated-, 3-MC- and PB-treated rat hepatic microsomes (lanes 14, 15, 16 and 17, 10 μ g/well) were included for comparison. The faster moving polar bear CYP band in (A) and (B) were labelled as CYP1A1 isozyme.

CYP1A1 sera, was identified as the CYP1A1 isozyme (i.e., the polar bear equivalent of rat) (Figure 6.2).

Polyclonal anti-rat CYP2B1 IgG recognized a single band of variable intensity in the polar bear microsomes (Figure 6.3). The staining intensity of the polar bear protein bands corresponding to CYP1A1, CYP1A2 and CYP2B was much greater than that of protein bands from untreated rats, therefore indicating induction relative to untreated rat. It was unlikely that anti-rat antibodies would react more strongly with the polar bear CYP isozymes than with rat CYP isozymes.

6.3.4 Immunoquantitation of CYP1A and CYP2B

Immunoquantitation of the protein bands corresponding to CYP1A1, CYP1A2 and CYP2B (Table 6.3) illustrated the relative variability in isozyme protein levels among individual bears. CYP2B content averaged 21.1 ± 11.2 pmol/mg protein and was significantly greater than the mean CYP1A (1A1 and 1A2) content of 4.9 ± 3.4 pmol/mg protein. However, caution is advised in comparing and interpreting the mole equivalent protein levels of CYP1A relative to CYP2B, which were determined using antibodies against rat CYP450 proteins. Immunoreactivity varies with the species and the antibody preparation used. Therefore, polar bear CYP1A and CYP2B isozyme content better represented the relative differences of one isozyme among bears rather than the absolute amounts. As CYP1A1 is generally the primary catalyst for EROD in mammals ^{6,253,254,257}, the relationship between EROD activity and CYP1A protein content in polar bear was examined. There was a significant linear correlation between increasing EROD activity and increasing

Figure 6.3. CYP2B isozyme immunoblots of adult male polar bear hepatic microsomes (lanes 1 to 13). Blots probed with polyclonal rabbit anti-rat CYP2B1 IgG at 3 μ g IgG/ml. Proteins (10 μ g/well for CYP1A1(2) and 20 μ g/well for CYP2B1) resolved by SDS-PAGE were electrophoretically transferred to nitrocellulose and probed with antibodies described above. Purified rat CYP2B1 and untreated-, 3-MC- and PB-treated rat hepatic microsomes (lanes 14, 15, 16 and 17, 10 μ g/well) were included for comparison.

Table 6.3. CYP1A1, CYP1A2 and CYP2B Isozyme Protein Content of the Hepatic Microsomes of Adult Male Polar Bear

Polar Bear	CYP1A1 (pmol/mg) [†]	CYP1A2 (pmol/mg) [‡]	CYP2B (pmol/mg) [†]
A	5.0 (0.5)	1.6 (0.1)	25 (4)
B	1.3 (0.2)	0.7 (0.1)	18 (7)
C	4.8 (0.5)	1.4 (0.2)	20 (5)
D	1.8 (0.2)	0.7 (0.1)	14 (4)
E	9.9 (3.7)	3.8 (0.7)	27 (8)
F	2.5 (0.8)	0.9 (0.1)	19 (5)
G	3.8 (0.8)	1.4 (0.3)	39 (12)
H	8.6 (2.0)	2.8 (0.7)	18 (7)
I	2.8 (1.0)	1.2 (0.4)	13 (8)
J	2.6 (0.7)	1.1 (0.3)	15 (4)
K	2.1 (0.5)	0.8	13 (6)
L	2.8 (0.7)	1.0 (0.2)	21 (2)
M	1.7 (0.8)	0.5 (0.2)	14 (3)
N	2.2 (1.7)	n.d.	13
O	1.0 (0.4)	n.d.	10
X	5.3 (0.3)	2.5	56

[†] $n = 4$ replicate blots (SD in parentheses), $n = 2$ replicate CYP2B blots for bears N, O, and X.

[‡] $n = 4$ replicate blots (SD in parentheses), $n = 2$ replicate blots for bear X, only one CYP1A2 blot band for bear K could be densitometrically quantitated.

n.d. = not detected, immunoreactive band too faint to be quantitated densitometrically.

CYP1A content up to approximately 5 pmol CYP1A/mg ($r^2 = 0.610$, $p < 0.004$) at which point the relationship appeared to become asymptotic (Figure 6.4a).

Relatively high CYP2B content was accompanied by very low PROD activity with a maximum observed activity of 32 ± 4 pmol/min/mg for bear G (Tables 6.2 and 6.3). PROD activity and CYP2B content were not correlated ($r^2 = 0.264$, $p < 0.002$). However, a good correlation between PROD activity and CYP1A content was observed up to 5 pmol/mg ($r^2 = 0.766$, $p < 0.0006$, Figure 6.4b). The correlation between CYP1A content and PROD activity was similar to the correlation between CYP1A content and EROD activity (Figure 6.4a). Moreover, PROD and EROD activities were strongly correlated (Figure 6.4c, $r^2 = 0.801$, $p < 0.0005$) if bear X was excluded.

The relationship between BROD activity and CYP1A content (Figure 6.5) bore some resemblance to that between EROD and PROD activities and CYP1A content (Figures 6.4a and b), but the scatter was greater. BROD activity did not correlate with either CYP1A content (Figure 6.5, $r^2 = 0.168$, $p < 0.11$) or CYP2B content (not shown, $r^2 = 0.056$, $p < 0.38$). Neither BROD versus EROD activity ($r^2 = 0.095$, $p < 0.24$) or BROD versus PROD activity ($r^2 = 0.102$, $p < 0.24$) were significantly correlated.

6.3.5 Antibody Inhibition of Catalytic Activities

Immunoinhibition of EROD and BROD activities by anti-rat CYP2B1 IgG and anti-rat CYP1A1 IgG was studied for two bears to identify the isozymes responsible for the catalytic activities. For bear G, EROD (Figure 6.6a) or BROD (Figure 6.6b) activities were not inhibited by purified control IgG at concentrations from 0.1 to 10 mg IgG/nmole CYP

Figure 6.4. Correlations between monooxygenase activity and CYP1A (1A1 + 1A2) content in polar bear hepatic microsomes. A) EROD activity versus CYP1A protein content and B) PROD activity versus CYP1A protein content and C) EROD activity versus PROD activity. CYP1A content was expressed as pmol of rat CYP1A1 equivalents per milligram microsomal protein. The dashed lines in (A) and (B) mark the beginning of an apparent asymptote in catalytic activity. The dotted lines in C denote confidence limits for 95 % significance of the correlation. Bear X was an outlier in PROD activity. See Experimental sections for CYP1A protein content calculation.

Figure 6.5. Relationship between BROD activity and CYP1A isozyme content in polar bear hepatic microsomes. CYP1A content (see section 6.2 Experimental) was expressed as pmol of rat CYP1A1 equivalents per milligram microsomal protein. Bear A was an outlier.

level. Anti-rat CYP1A1 and anti-rat CYP2B1 IgG inhibited EROD activity by approximately 70 % and 15 %, respectively, at the maximum antibody concentration of 10 mg IgG/nmole CYP. BROD activity was not inhibited after pre-incubation with control IgG. A decrease of about 60 % and 40 % of BROD activity was observed after incubation with 10 mg IgG/nmole P450 of anti-rat CYP1A1 and anti-rat CYP2B1 IgG, respectively. Similar results were found for bear J.

6.3.6 CHC Analysis

Hepatic PCDD, PCDF and PCB residue levels were expressed on a lipid weight basis (Table 6.4) and grouped according to their potential to induce CYP1A and CYP2B in laboratory rodents ^{74,84} and CYP1A isozymes in some marine mammals ⁶. The levels of S-PCBs were the second highest of all residues detected, exceeded only by S-chlordanes. Hepatic concentrations of both S-PCDDs and S-PCDFs were at the sub-ng/g level. Non-*ortho* CB-77, CB-126 and CB-169, which are classed as pure inducers of CYP1A, accounted for less than 1% of S-PCBs compared with about 25 % for PCB congeners that are mixed CYP1A/CYP2B-type inducers and about 71 % for CYP2B-type inducers. CB-99, CB-138, CB-153, CB-170 and CB-180 accounted for approximately 90 % of the S-PCBs and shared the structural similarity of 2,2',4,4'-chlorine substitution. CB-153 alone accounted for about 40 % of the S-PCBs. CB-153 and CB-180 also lack vicinal hydrogens on either phenyl rings. The average S-PCB to S-MeSO₂-PCB ratio of 10.09 ± 4.65 demonstrated the importance of MeSO₂-PCB metabolites as contaminants in polar bear liver. The S-3-MeSO₂-PCBs and S-4-MeSO₂-PCBs accounted for 57.0 ± 3.9 % and 43.0 ± 3.9 % of S-MeSO₂-PCBs, respectively.

Figure 6.6. Anti-rat CYP2B1 and CYP1A1 antibody inhibition of monooxygenase in the hepatic microsomes of polar bear. A) EROD activity and B) BROD activity in hepatic microsomes of polar bear G. The reactions were carried out in the presence of varying concentrations of control IgG, anti-rat CYP1A1 IgG and anti-rat CYP2B1 IgG. Control EROD and BROD activities were in the presence of 0, 0.5, 1.0, 5.0 and 10 mg control IgG per nmol total CYP.

Isomer-specific PCB analysis of residue levels in several species has shown CB-153 to be one of the most metabolically stable PCBs in biotic systems ⁶. For this reason, biologically recalcitrant CB-153 has frequently been used as the basis for a "metabolic index" for PCB congeners accumulated from the diet (see Chapter 4) ^{44,47,258}. The mean ratios of residue levels of PCBs classed as CYP1A-type inducers to CB-153 among polar bear livers are listed in Table 6.5. The ratios for mono-*ortho*-CB-105 and -CB-118 and NO-CB-169 were about ten times lower and CB-77 and CB-126 about one hundred times lower in polar bear liver than in ringed seal blubber from the Canadian Arctic ^{47,99}. CB-156 and CB-157 to CB-153 ratios in polar bear liver were within a factor of two lower than the ratios in ringed seal blubber.

6.3.7 TCDD-Toxic Equivalents (TEQs)

Total PCDD, PCDF and PCB residue concentrations were expressed as TCDD toxic equivalents (TEQs, Figure 6.7) using toxic equivalency factors (TEFs) proposed by Safe ^{74,236}. Safe ²³⁶ assigned CB-118 a TEF of 0.001 but recently revised the value to 0.0001 ⁷⁴. The mean total TEQs using a CB-118 TEF of 0.0001 and 0.001 were 0.377 ± 0.182 ng/g and 0.499 ± 0.209 ng/g, respectively, on a lipid weight basis. TEQ composition was determined based on both CB-118 TEF values for comparison (Figure 6.7). The TEQ contribution of CB-118 went from 4.0 ± 1.1 % (TEF 0.0001) to about 30 % (TEF 0.001), depending on the TEF value used. However, the rank order of TEQ contribution was the same regardless of the CB-118 TEF used, mono-*ortho*-PCBs >>> NO-PCBs > PCDDs MO-PCB (CB-118, CB-105, CB-156, CB-157 and CB-189) contribution to the TEQ was 79.6 ± 9.3 %

Table 6.4. Mean ($\pm$ SD) Levels of CYP1A-Type and CYP2B-Type Inducing PCBs, PCDDs, and PCDFs (Lipid Weight Basis) in Adult Male Polar Bears (Bears A to O, $n = 15$) Grouped According to Cytochrome P450 Induction Potential ⁷⁴

CYP1A (I)		CYP1A/2B (II)		CYP2B (III)		Other CYP2B-type CHCs	
Congener	ortho-Cl (ng/g)	Congener	ortho-Cl (ng/g)	Congener	ortho-Cl (ng/g)	Congener	(ng/g)
CB-77	0 0.025 (0.015)	CB-37 ^a	0 0.097 (0.037)	CB-99 ^a	2 1660 (694)	S-MeSO ₂ -PCB	1851 (692)
CB-126	0 0.244 (0.189)	CB-81 ^a	0 n.d.	CB-153 ^a	2 7469 (4062)	S-3-MeSO ₂ -PCB	1071 (452)
CB-169	0 0.404 (0.245)	CB-105 ^a	1 52 (23)	CB-154 ^a	3 7 (2)	S-4-MeSO ₂ -PCB	780 (251)
2378-TnCDD	0.019 (0.011)	CB-118 ^a	1 139 (42)	CB-180 ^a	2 3340 (2060)	S-chlordane	30770 (25354)
12378-PnCDD	0.005 (0.004)	CB-138 ^a	2 2331 (1677)	CB-183 ^a	3 159 (100)	S-DDT	951 (740)
123478-HxCDD	0.002 (0.003)	CB-156 ^a	1 130 (71)	CB-194 ^a	2 559 (481)	zolazepam	11 (9)
123678-HxCDD	0.002 (0.002)	CB-157 ^a	1 113 (61)				
12346789-OCDD	0.004 (0.003)	CB-189	1 0.110 (0.070)			S-PCB ^b	18680 (5053)
12478-PnCDF	0.002 (0.001)						
23478-PnCDF	0.010 (0.006)						

^a mean levels for bears A-M only ($n = 13$). SD in parentheses.

^b The total of all PCBs in the table and including detected PCB congeners classed as non-inducers or weak inducers (i.e. CB-47, CB-56, CB-85, CB-149, CB-146, CB-137, CB-195, CB-206, CB-209) ^{62a}.

n.d. = not detected.

Table 6.5. Ratios of Selected PCBs to CB-153 for Adult Male Polar Bear Liver and Ringed Seal Blubber

	Ringed Seal Blubber	Bear liver [†]
MO-PCBs[‡]		
CB-105	0.11	0.01 (0.01)
CB-118	0.32	0.03 (0.02)
CB-156	0.04	0.02 (0.01)
CB-157	0.01	0.02 (0.01)
NO-PCBs[°]		
CB-77	0.0004 (0.0003)	0.00004 (0.000003)
CB-126	0.001 (0.001)	0.00005 (0.00006)
CB-169	0.0002 (0.0003)	0.00007 (0.00005)

[†] Mean of bears A to M ($n = 13$, $\pm$ SD).

[‡] MO-PCBs/CB-153 ratios for ringed seal blubber ⁴⁷.

[°] Mean NO-PCBs/CB-153 ratios ($\pm$ SD) for ringed blubber for 7 females and 4 males, 1 to 14 years of age from the Inukjuak region of the Canadian Arctic ⁹⁹.

Figure 6.7. Mean contributions of NO- and MO-PCBs, PCDDs and PCDFs to the total TEQ composition of thirteen polar bear livers, when the TEF of CB-118 is A) 0.0001⁷⁴ and B) 0.001²³⁶.

and was comprised mostly of CB-156 ($32.4 \pm 6.9 \%$), CB-157 ($28.9 \pm 8.4 \%$) and CB-105 ($14.4 \pm 4.9 \%$). NO-PCBs (CB-37, CB-81, CB-77, CB-126 and CB-169) contributed an average of $12.3 \pm 4.6 \%$ to the S-TEQ and were comprised mostly of CB-126 ($6.9 \pm 3.8 \%$) and CB-169 ($5.4 \pm 1.5 \%$). PCDDs ($6.5 \pm 4.5 \%$) and PCDFs ($1.5 \pm 1.0 \%$) contributed the least to the mean TEQ. TEQs for di-*ortho*-PCBs were not included since there is insufficient information regarding the relative potencies of these compounds in mammals ⁷⁴.

6.4 Discussion

6.4.1 Monooxygenase Systems

Total CYP content has frequently been used as a crude measure of xenobiotic oxidative capacity of an animal. The total CYP content of polar bear liver was similar to that of laboratory rats ⁹³ whereas marine mammals studied so far have significantly lower levels ^{89,90,106}, perhaps due to lower CHC residue levels capable of CYP induction. For example, a total CYP content of about 0.3 nmol P450/mg and a concentration of approximately 500 ng/g lipid of PCBs, comprised of the NO-PCBs: CB-37, CB-81, CB-77, CB-126 and CB-169 and the MO-PCBs: CB-105, CB-114, CB-118 and CB-156, has been reported for arctic beluga whale liver ⁸⁸. These total PCB levels did not include several mixed CYP1A/CYP2B-type inducers such as CB-138 and CB-170 ⁷⁴, which are usually large contributors to total PCBs. In contrast, polar bear hepatic microsomes in the present study had a CYP content of 1 to 2

nmol/mg and a mean PCB concentration of approximately 4800 ng/g lipid for PCB congeners classed as CYP1A and mixed CYP1A/CYP2B inducers (Table 6.4).

The mean hepatic EROD activity in the polar bears was several orders of magnitude higher than observed in adult male seals studied to date, and about the same to two-fold higher than levels in adult whales. For example, hepatic EROD activities for hooded seal ⁹⁰, male arctic beluga ⁸⁸, killer whale ⁸⁹ and minke whale ¹⁰⁶ were 30 ± 7 , 1255 ± 651 , 612 ± 373 and 740 ± 170 pmol/min/mg microsomal protein, respectively. These EROD activities generally correlate with the level of PCB exposure ^{5,168}. Assuming that the inter-laboratory EROD activities are comparable, CYP1A-mediated activity was elevated in polar bears relative to marine mammals. However, the EROD activity differences in these species, especially the low value for seal, may have to do with differences in the Ah-receptor signalling of CYP1A1 isozymes ⁷⁷.

The correlation between EROD activity and CYP1A protein content up to the 5 pmol/mg level (Figure 6.4a) suggested CYP1A was the primary catalyst for EROD activity in polar bear. The immunoinhibition results verified that EROD activity (Figure 6.6a) was catalyzed predominantly by CYP1A in polar bear. A similar result has been observed with EROD activity in liver microsomes of short-finned pilot whales ⁸⁹. Several limiting factors may explain the apparent asymptotic EROD (Figure 6.4a and b) activity observed for bears with the highest CYP1A and CYP2B protein content (Table 6.2). Reduced CYP reductase activity has been observed for beluga whale liver microsomes that have high CYP1A content and low EROD activity ⁸⁸. Hepatic microsomal CYP reductase activity was not determined for the bears in the present study. Xenobiotic compounds are known to inhibit the enzyme

activity of specific CYP isozymes *in vitro*, by binding to active sites in either a covalent or non-covalent manner ²⁵⁹. Enzyme inhibition by contaminant substrates would not affect protein epitopic sites and therefore the immuno-reactive protein levels would remain measurable. Further *in vitro* studies are necessary to determine the possible inhibitory role of CHCs and metabolites on hepatic microsomal EROD activity in the polar bear.

PROD has been found to be catalyzed primarily, but not exclusively, by CYP2B isoforms in rat liver ²⁵⁴. The present results suggested that CYP1A, not CYP2B, was the primary catalyst for PROD in polar bear liver microsomes. Results of other studies support these findings. PROD activity was concluded to be a CYP1A-mediated activity in hepatic microsomes of arctic beluga whale on the basis of strong positive correlation between PROD activity and CYP1A content ⁸⁸. An inducible form of hepatic CYP2B isozyme has not been found in common terns from the Netherlands ²⁶⁰, however, PROD was measurable and correlated strongly with CYP1A content. They suggested that PROD was partially a CYP1A-mediated activity, but PROD activity may also have been due to an isozyme with CYP2B-like activity that does not cross-react with anti-rat CYP2B1 antibody. The anomalous PROD activity of bear X with respect to CYP1A content versus PROD activity (Figure 6.4b), may have been due to the time of sampling. Bear X was sampled after a lengthy fasting period, which may have affected PROD activities. However, the CYP1A-mediated EROD activity was not affected. Perhaps mediation of PROD activity by other CYPs existed but was influenced differently by fasting. The CYP isozyme responsible for PROD activity in polar bear may have been better defined by anti-rat CYP1A and/or CYP2B antibody immunoinhibition studies. These studies were not conducted because the level of PROD

activity approached background levels (Table 6.2), precluding accurate measurements of inhibition.

BROD activity is also selectively catalyzed by CYP2B forms in rat liver²⁵⁴. In the present study, no significant correlation was observed between BROD activity and CYP1A and CYP2B protein content in the polar bear liver microsomes. Results of immunoinhibition experiments with antibodies to rat CYP1A1 and CYP2B1 indicated that BROD activity (Figure 6.6b) was catalyzed mainly by CYP1A and, to a lesser extent, by CYP2B. Rattner *et al.*⁹¹ came to similar conclusions for hepatic BROD activity of PB- and 3-MC-treated black-crowned night heron embryos.

6.4.2 CYP Induction by CHCs

CYP1A. White *et al.*⁸⁸ recently concluded that high correlation between CYP1A content and NO- and MO-PCB levels (CB-37, -81, -77, -126, -169, -105, -114, -118 and -156) in arctic beluga whale liver indicated CYP1A induction by these compounds or compounds that co-vary with PCBs. In the present polar bear study, there was a strong positive correlation between hepatic concentrations of PCBs known to be CYP1A-type inducers or mixed CYP1A/2B-type inducers (Table 6.4) and CYP1A protein content ($r^2 = 0.875$, $p < 0.0001$). Di-*ortho* CB-138 and CB-170 accounted for greater than 90 % of PCBs known to be CYP1A or mixed CYP1A/CYP2B inducers (Table 6.4) but were not expected to be especially good ligands for the Ah-receptor⁷⁴. S-PCB levels (including di-*ortho*-PCBs) correlated strongly with levels of PCBs known to bind to the Ah-receptor ($r^2 = 0.953$, $p < 0.00005$). CYP1A protein content and S-PCDD/Fs levels were also well correlated (r^2

= 0.851, $p < 0.00004$). Because of the high degree of correlation among potential Ah-receptor ligands and non-ligating aromatic CHCs in polar bear, causal relationships cannot be established with certainty using these comparisons. However, levels of non-aromatic S chlordane levels were two- to four-fold higher than those PCBs known to be ligands for the Ah receptor, but exhibited no correlation to CYP1A content (Figure 6.8, $r^2 = 0.024$, $p < 0.6$) suggesting that the structure-activity rules for CYP1A induction in polar bears are similar to those in other mammals.

Concentrations of PCDD/Fs and the NO- and MO-PCBs involved in Ah-receptor mediated effects in biota were expressed as TEQs (see section 6.3 Results) to better evaluate the CYP1A-type induction potency. The mean total TEQ in polar bear was consistent with values found for terrestrial mammals, which are generally much lower than marine mammals⁵. CYP1A isozyme content and TEQs were strongly correlated ($r^2 = 0.857$, $p < 0.0004$, Figure 6.9) in polar bear liver. These results suggested CYP1A isozyme content was a good measure of exposure to xenobiotic compounds that are Ah-receptor ligands.

The TEQ contribution order of MO-PCBs >>> NO-PCBs > PCDDs > PCDFs in polar bear (Figure 6.7) was similar to the trends observed in wild birds²⁶⁰ and mammals⁹⁸, including humans²⁶¹. The dominant TEQ contributors in polar bear, mono-*ortho*-CB-156, CB-157 and CB-105, were considerably greater than CB-126. Mono-*ortho*-CB-118 and CB-105 generally dominate the TEQ in marine mammals and terrestrial mammals including humans, however, the TEQ contribution of non-*ortho*-CB-126 is usually more important⁵. For example, CB-126 has been shown to contribute 20 to 50 % to the TEQ in arctic narwhal, ringed seal and beluga⁷⁵ and 15 to 30 % progressing from terrestrial mammals (e.g., human

Figure 6.8. Relationship between hepatic microsomal CYP1A (1A1 + 1A2) content and total chlordanes levels (lipid weight) in polar bear liver. See section 6.2 Experimental for CYP1A protein content calculations.

Figure 6.9. Correlations between hepatic microsomal CYP1A (1A1 + 1A2) content and toxic equivalents (TEQ, lipid weight) in polar bear liver. Dotted lines denote confidence limits for 95 % significance of the correlation. See section 6.2 Experimental for CYP1A protein content calculations.

and dog) to fish-eating birds ^{5,98}. The TEQ contribution of CB-156 in marine and terrestrial mammals generally accounts for less than 20 % ⁵. Therefore, polar bears appear to have a greater capacity than most mammals to metabolize the majority of PCBs known to be Ah receptor ligands.

In polar bear, metabolism of PCBs known to be Ah receptor ligands is also demonstrated by the decrease in ratio of most of these compounds to CB-153 going from diet (ringed seal) to polar bear (Table 6.5). All compounds except CB-156, CB-157 and CB-169 decreased ten or more times relative to CB-153. The ratio for CB-157 actually increased slightly, and the mean ratio for CB-169 in bears was three times lower than in seals, however, the differences were not significant. The PCB congeners CB-156, CB-105, CB-118, CB-77 and CB-126 are all metabolized, probably by CYP1A. CB-156 shares a 3,4-dichloro ring with these congeners, but has four chlorines on the other ring, which may sterically hinder metabolism. Generally, only those PCBs possessing at least 2,2',4,4' chlorine substitution appear to escape metabolism in polar bears ⁴⁷. Thus, CB-99 (2,2',4,4',5'-pentachloro) accumulates readily, whereas CB-118 (2',3,4,4',5'-pentachloro) does not. It is noteworthy that CB-118 was not detected in ringed seals from the Baltic Sea, which are exposed to high levels of PCBs ¹⁶⁸, whereas CB-118 levels are "normal" in the cleaner arctic ringed seal ⁴⁷. A similar phenomenon exists in average versus high PCB-exposed human populations, and has been attributed to high-level CYP1A induction in the latter group ⁹⁷.

CYP2B. The structural diversity of CYP2B-type inducers known to activate expression of the CYP2B subfamily is much greater than the rigid, planar, TCDD-like structural requirement for CYP1A induction ⁷⁷. Several CHCs, such as *ortho*-PCBs and

chlordanes, known to be CYP2B-type inducers in laboratory animals ⁸⁵, were significant residues in polar bear liver (Table 6.4). High levels of 2,5- and 2,5,6-chloro-substituted and 3- and 4-MeSO₂-substituted PCB metabolites originating from 3,4-epoxides were also present (Table 6.4). The correlation between PCBs classed as pure CYP2B-type and mixed CYP1A/2B-type inducers (Table 6.4) and CYP2B content was moderate ($r^2 = 0.419$, $p < 0.03$), suggesting that PCBs were not the most important CYP2B-type inducers in polar bear. A moderate correlation was observed between S-MeSO₂-PCBs and CYP2B content ($r^2 = 0.326$, $p < 0.05$). The 3-MeSO₂-PCB congeners have been shown to induce hepatic microsomal CYP2B in rats, whereas 4-MeSO₂-PCBs had little effect ¹⁴⁷.

The CHCs with highest residue levels in polar bear liver were S-chlordanes, which correlated strongly with CYP2B content ($r^2 = 0.755$, $p < 0.0002$). Chlordanes have been shown to be CYP2B-type inducers in cultured hepatocytes of laboratory animals and humans ⁸⁵. S-DDTs are known to be CYP2B-type inducers ⁷⁷. S-DDT levels were half that of S-MeSO₂-PCBs (Table 6.4), and moderately correlated with CYP2B protein content ($r^2 = 0.372$, $p < 0.03$). Finally, hepatic levels of residual zolazepam, a component of the drug Telazol, were strongly correlated with CYP2B protein content ($r^2 = 0.810$, $p < 0.00004$). Preliminary research on the effect of Telazol on the rat hepatic CYP2B1 demonstrated an increase in BROD and testosterone 16 β -hydroxylase activities with increasing doses of Telazol ²⁶². Moderate to good correlations between CYP2B content and hepatic levels of various CHCs known to be capable of inducing CYP2B in laboratory animals, as well as zolazepam, suggested multi-residue CYP2B isozyme induction in polar bear.

S-Chlordane and S-*ortho*-PCBs (Table 6.4) were the only residues found to be

significant ($p < 0.05$) in the stepwise, backward multiple regression of CYP2B levels versus hepatic concentrations of the various residue classes (eq. 9, $r^2 = 0.784$, $p < 0.0005$).

$$\text{CYP2B}_{\text{predicted}} = 0.154 (\text{S-chlordane}) + 0.275 (\text{S-ortho-PCB}) + 9.424 \quad (9)$$

S-Chlordane and S-*ortho*-PCBs therefore are likely to be important contributors to CYP2B induction. This is to be expected, since both classes are known CYP2B inducers^{84,85}, and together constitute > 90 % of total CHC concentration in liver. Moreover, persistent chlordane residues have been related to neurobehavioural impairments in humans²⁶³. It is thought that chlordanes interact with the picrotoxin receptor in the nervous system²⁶⁴. In 1982, the National Research Council in the U.S. concluded that it could not determine a level of human exposure to any of the cyclodiene termiticides (including chlordanes) below which there would be no biological effect²⁶³.

A plot of the observed CYP2B levels versus predicted levels from eq. 9 is given in Figure 6.10. The relatively large residual CYP2B level unexplained by *ortho*-PCBs and chlordanes may be due to contributions from other CHC residues and zolazepam. Hepatic zolazepam levels and CYP2B were strongly correlated. Zolazepam and S-chlordane levels also strongly co-varied ($r^2 = 0.790$, $p < 0.0009$), therefore, zolazepam was probably dropped from the multiple linear regression because of collinearity. The contribution of this drug to CYP2B levels in polar bear remains unclear. However, rats dosed with zolazepam were found to have induced CYP2B1 protein levels, whereas tiletamine did not show a similar inductive effect (Bandiera *et al.*, 1996, unpublished results).

Interpretation of CYP2B induction in polar bears may be complicated by the relatively high abundance of hepatic stellate cells, which are lipid-rich cells specialized in storage of vitamin A ⁴². S-chlordane concentrations on a lipid weight basis in polar bear liver are up to fourteen-fold greater compared to adipose tissue, whereas S-PCB is more equally distributed in lipids ⁴⁶. Oxychlordane, a polar chlordane metabolite, is also a higher proportion of S-chlordane in liver than fat. It is possible that the disproportionate distribution of chlordane-related compounds is due to preferential storage in stellate cells, rather than hepatocytes where CYP2B induction occurs. Because zolazepam levels correlated well with S-chlordane, it may be that zolazepam is also preferentially stored in stellate cells.

6.5 Conclusions

Contamination of the Arctic ecosystem by anthropogenic compounds has resulted in exposure of polar bear (*Ursus maritimus*) to lipophilic CHCs accumulated through the marine food web. The levels of immunochemically-determined CYP1A content in polar bear liver were directly proportional to levels of Ah-receptor active xenobiotic chemicals, measured as 2,3,7,8-TCDD TEQs, whereas total chlordanes, the major residue present in liver, did not correlate. Therefore, the results for polar bears are consistent with the chemical agents and mechanism of CYP1A induction found for other mammals, birds and fish. TEQs

Figure 6.10. Correlations between CYP2B content (predicted, eq. 9) and CYP2B content (observed, Table 6.3) in polar bear liver. Chlordane and *ortho*-PCB residues were not determined for bears N,O, and X. Dotted lines denote confidence limits for 95 % significance of the correlation. See section 6.2 Experimental for CYP2B protein content calculations.

based on the TEF index of Safe ⁷⁴ were dominated by two MO-PCBs, CB-156 and CB-157, unlike other wild mammals and birds studied to date. Levels of CYP1A isozyme were significantly lower than CYP2B isozyme, possibly because of lower immunochemical cross-reactivity between rat and polar bear CYP2B. The multiple linear regression model based on a "backward" stepwise approach, demonstrated that total *ortho*-PCB and total chlordane compounds (mainly the metabolite oxychlordane) had the highest correlation with immunochemically determined CYP2B content. Polar bears in the Canadian Arctic may be suffering from subtle neurotoxic effects as a consequence of chlordane exposure, given that human exposure to chlordanes has been related to neurobehavioural impairment ²⁶³, including learning disorders in children ²⁶⁵. The drug zolazepam, to which the bears were exposed at various times prior to death, and other CHCs, may have contributed to CYP2B induction. Although zolazepam was not significant in the multiple regression modelling, it was highly correlated to CYP2B content on its own ($r^2 = 0.810$, $p < 0.0004$).

EROD activity correlated with CYP1A content up to approximately 5 pmol CYP1A/mg protein. EROD activity did not correlate with TEQs. Therefore, determination of EROD catalytic activity does not appear to be a viable method for determining degree of exposure of polar bears to CYP1A inducers or level of induction. Inhibition studies with anti-rat CYP1A and anti-rat CYP2B IgG showed that EROD was CYP1A-mediated. The lack of correlation of PROD activity with CYP2B content and the high correlation between EROD and PROD activities showed that PROD was a CYP1A-mediated activity. BROD activity appeared to be both CYP1A and CYP2B-mediated based on immunoinhibition studies. Quantitation of immunoblots of CYP1A and CYP2B is superior to catalytic assays in

assessing enzyme induction in polar bears, at least with the substrates tested to date. Moreover, immunological quantitation of CYP1A and CYP2B isozyme protein is more reliable because it is generally unaffected by physiological factors that can affect catalytic activity ²⁶⁶.

Conclusion and Future Directions

7.1 Conclusions

In this thesis the ecological and analytical chemistry of CHCs and MeSO₂-PCB and -4,4'-DDE metabolites in the polar bear food chain are elucidated. The conclusions presented in each chapter of this thesis can be summarized into a few important main points.

1) Cleanup based on gel permeation, Florisil and basic alumina chromatography was shown to give pure aryl-MeSO₂-compound fractions, and is recommended for the routine determination of CHCs and MeSO₂-PCB and -4,4'-DDEs in biological matrices.

2) GC/ECNI-MS(TIC) and GC/ECD can both be applied as accurate and precise quantitation techniques for unknown aryl-MeSO₂-compound determination. The sensitivity and response stability of both techniques was shown to be from low pg to ng levels, within the concentration range of MeSO₂-PCB and -4,4'-DDE metabolites determined in the polar bear and ringed seal samples analyzed in this thesis. GC/ECNI-MS(SIM) was found to be less accurate and precise, however, the technique would be ideal for detection of aryl-MeSO₂-compound at the sub-pg level.

3) The relative abundance of (M-CH₃)⁺ and (M-Cl+H)⁺ fragment ions for 3- and 4-MeSO₂-PCBs from GC/ECNI mass spectra can be used to distinguish some of the structural features of these congeners. Hexachloro- and heptachloro-MeSO₂-PCBs tend to have more

abundant $(M-Cl+H)^+$ ions whereas $MeSO_2$ -PCBs possessing the 4- $MeSO_2$ -2,5-dichloro moiety have a reduced abundance of the $(M-CH_3)^+$ ion. These rules would be useful for the elucidation of unknown structures in environmental samples. The relative abundance of the $(M-CH_3)^+$ increases with increasing ion source temperature, whereas Cl loss and H attachment fragments were relatively unaffected. Increasing ion source pressure increased ion abundance but did not affect fragmentation. Therefore, knowledge of the GC/ECNI-MS instrumental parameters is essential for interpreting $MeSO_2$ -PCB structure-fragmentation relationships with respect to the $MeSO_2$ -PCB fragmentation rules defined in this thesis.

4) The ecodynamics of $MeSO_2$ -PCBs and -4,4'-DDEs differ from the parent PCB and 4,4'-DDE classes in the polar bear food chain, which is a reflection of the type and the capacity of the CYP enzyme systems among species. Based on PCB accumulation patterns, Canadian arctic ringed seal have lower CYP2B- and CYP1A-type activity, relative to polar bear and Baltic Sea seals. Baltic seals have much higher CHC body burdens. The polar bear has a large capacity for PCB and 4,4'-DDE biotransformation. However, the major metabolic pathway for these compounds does not seem to be the MAP, which leads to the formation of $MeSO_2$ -PCB and -4,4'-DDE metabolites. Another possibility is that these metabolites are further metabolized or depurated non-metabolically. Uptake from seal, $MeSO_2$ -PCB formation in the bear and clearance of $MeSO_2$ -PCBs in bear all appear to contribute to the bioaccumulation of $MeSO_2$ -PCBs in bear. However, the relative influence of each of these factors vary depending on the $MeSO_2$ -PCB structure or that of the precursor PCB. $MeSO_2$ -CB132 in bear, for example, must be completely bioaccumulated from the seal since neither bear or seal contained detectable levels of the CB-132 precursor, yet both had measurable

levels of 3- and 4-MeSO₂-CB132. The tissue distribution of MeSO₂-PCBs and -4,4'-DDE in polar bear favours the liver. The presence of Stellate cells may account for this. Lower levels in other tissues may be due to irreversible protein binding, especially for 3-MeSO₂-4,4'-DDE. Selective retention of MeSO₂-PCB congeners occurred in the liver, that is, the pattern in liver was different from the other tissues studied (i.e., lung, adipose, testes and brain). Liver storage favoured the pentachloro- to heptachloro-MeSO₂-PCBs and the 3-MeSO₂-PCB isomer of the 3- and 4-MeSO₂-PCB pairs. MeSO₂-PCB and -4,4'-DDE mobilization from adipose into milk was different than PCBs and 4,4'-DDE over the fasting period. S-MeSO₂-PCB levels and S-MeSO₂-PCB to S-PCB ratios were higher in the polar bear cubs relative to their mother. Polar bear cubs in the western Hudson Bay area may be at a greater health risk to the potential deleterious effects of methyl sulfone metabolites than the parent PCBs and DDTs.

5) MeSO₂-PCB and -4,4'-DDE levels were higher in adipose tissue pools from male polar bears in the east and south of the western hemispheric Arctic. The S-MeSO₂-PCB to S-PCB ratios decreased from west to east reflecting the increasing proportion of higher chlorinated PCB which are not precursors to MeSO₂-PCBs, moving eastward. The MeSO₂-PCB congener pattern was geographically independent, indicating the homogeneous nature of the environmental input and food chain biomagnification of precursor PCBs.

6) The levels of immunochemically-determined CYP1A protein content in polar bear liver were directly proportional to levels of Ah-receptor active xenobiotic chemicals, measured as 2,3,7,8-TCDD TEQs. Levels of CYP1A isozyme were significantly lower than CYP2B isozyme, possibly because of lower immunochemical cross-reactivity between rat

and polar bear CYP2B. A multiple linear regression model demonstrated that total *ortho*-PCB and total chlordane compounds (mainly the metabolite oxychlordane) had the highest correlation with immunochemically determined CYP2B content. Polar bears in the Canadian Arctic may be suffering from subtle neurotoxic effects as a consequence of chlordane exposure, via its effect on CYP2B induction as shown in this thesis. The drug zolazepam, to which the bears were exposed at various times prior to death, and other CHCs, such as MeSO₂-PCB and -4,4'-DDE metabolites, may have contributed to CYP2B induction. It was not possible to deduce whether MeSO₂-PCB and -4,4'-DDE had an effect on CYP2B induction, since chlordanes and *ortho* PCBs were the most significant CYP2B inducers in the mixture of CHC residues present in polar bear liver. EROD activity correlated with CYP1A content up to approximately 5 pmol CYP1A/mg protein, but did not correlate with TEQs. The determination of EROD catalytic activity does not appear to be a viable method for determining degree of exposure of polar bears to CYP1A inducers or level of induction. Inhibition studies with anti-rat CYP1A and anti-rat CYP2B IgG showed that EROD and PROD were CYP1A-mediated. BROD activity appeared to be both CYP1A and CYP2B-mediated based on immunoinhibition studies. Quantitation of immunoblots of CYP1A and CYP2B is superior to catalytic assays in assessing enzyme induction in polar bears, at least with the substrates tested to date.

7.2 Future Directions

Several possible research directions emerged from the research presented in this thesis. The

following represent a few of the potential studies which would be of interest.

1) 3-MeSO₂-4,4'-DDE as an Adrenocorticolytic Toxicant in Polar Bear

A toxicological effect has yet to be proven in any wild species as a consequence of MeSO₂-PCB or -4,4'-DDE exposure. The DDT metabolites, 4,4'-DDD, 2,4'-DDD, 4,4'-DDE and 3-MeSO₂-4,4'-DDE have been shown to be bioactivated to adrenocorticolytic metabolites that irreversibly bind to proteins in the adrenal cortex of birds and several mammalian species, including man. In some cases, steroid metabolism *in vitro* was affected, suggesting the reactive metabolites may disturb adrenal glucocorticoid production *in vivo*. The activating enzyme was proposed to be cytochrome P450 11 β -hydroxylase, a P450 form known to be expressed specifically in the adrenal cortex and an important enzyme in glucocorticoid production.

It would be of interest to see if, 1) adrenal mitochondrial CYP-mediated bioactivation *in vitro* of [¹⁴C]-labelled 4,4'-DDT, 2,4'-DDD, 4,4'-DDD, 4,4'-DDE and 3-MeSO₂-4,4'-DDE occurs in polar bear, 2) the resulting reactive metabolites covalently bind to protein and 3) the reactive metabolites bind to and inhibit the activity of 11 β -hydroxylase in adrenal mitochondria, an enzyme important for steroid metabolism. The results from such a study would confirm that a toxicological effect results from the presence of MeSO₂-containing metabolites in polar bear.

2) Persistent MeSO₂-PCB and -4,4'-DDE Metabolites in Other Arctic Species and Fish-Eating Birds in the Great Lakes

MeSO₂-PCB and -4,4'-DDE metabolites persist at levels comparable to other CHCs in high trophic level mammals such as polar bear and ringed seals. Further, the potential toxicity of MeSO₂-PCBs and -4,4'-DDE remains unproven in Arctic biota. These factors warrant further research to determine the identities and levels of methyl sulfone metabolites in other marine mammals of the arctic ecosystem, such as walrus and narwhal. The comparative levels of congener-specific PCBs and DDTs to MeSO₂-PCBs and -4,4'-DDEs among species would be a good indicator of the type and capacity of their CYP enzyme systems.

The herring gull is found throughout the Great Lakes and sampled for CHC analysis as part of a long-term temporal study on contaminants in the Great Lakes system. As shown in the present thesis, MeSO₂-PCBs were detected in egg homogenates of Great Lakes herring gull. The presence of sulfone metabolites in herring gull egg demonstrates that *in ovo* biotransfer from parent to offspring does occur. The temporal dynamics of this potential biotransfer of metabolites levels, and in relation to their parent compounds, in whole body herring gulls and their eggs would be worth further investigation. Knowledge of sulfone metabolites in herring gull is also important since very little data exist on MeSO₂-PCB and/-4,4'-DDE metabolites in avian species. Further, the determination of sulfones in other species is necessary to better understand the metabolic pathways which lead to and/or prevent MeSO₂-PCB/-4,4'-DDE metabolite formation.

3) The stability of PCBs with Meta-Para Epoxides

Meta-para epoxides of PCBs must initially be formed for subsequent MeSO₂-PCB

metabolite formation. These epoxides are formed in the liver microsomes by the action of CYP enzymes. However, for nucleophilic attack by glutathione to occur, these epoxides must migrate out of the microsomes and into the hepatocyte cytosol. The stability of the PCBs with *meta-para* epoxides, which are precursors to MeSO₂-PCBs, to attack by epoxide hydroxylase in the microsomes, may be the key factor for MeSO₂-PCB formation and formation of other metabolites. Further, the stability may vary depending on the structure of the PCB epoxide. If this is the case, the epoxide may migrate into a medium containing a trapping agent, perhaps glutathione itself in the presence of glutathione-S-transferase. A large pool of hepatic microsomes from fresh polar bear liver could be collected and stored in liquid nitrogen for this purpose. However, it is not clear whether the microsomes would retain epoxide hydroxylase activity. *In vitro* metabolism of [¹⁴C]-labelled CB-101, CB-105 and CB-153 could be used for this study. HPLC could be used to separate the potential glutathione-epoxide conjugates which may form. Their presence could be measured by radioactivity.

4) The Influence of Alternate Metabolic Pathways on the Meta-Para Epoxide PCB to Sulfone Metabolite Biotransformation

The differences in MeSO₂-PCB congener patterns and the levels of MeSO₂-PCBs/-4,4'-DDEs among species are likely dependent on the variation of influence of metabolic processes subsequent to the formation of the *meta-para* epoxide PCB. Further investigation is required to probe species differences in the relative importance of glutathione, glucuronide and/or sulfate conjugations. Variations in the transformations mediated by gut microflora and

entero-absorption may also contribute to species differences in MeSO₂-PCB/-4,4'-DDE levels and congener patterns.

5) Hydroxylated PCB Metabolites in Polar Bear

In the present thesis, a large portion of the precursor PCBs to MeSO₂-PCBs appeared to be metabolized by alternate metabolic routes, rather than the MAP. In a preliminary study at the conception of this thesis work, several hydroxylated (HO-) PCB metabolites were detected in polar bear plasma. HO-PCBs probably bind to a transport protein in rat plasma, which is important to thyroid function. HO-PCBs in plasma may be a causative factor in similar toxicological effect in polar bear.

Literature Cited

1. Ballschmiter K, Mennel A, Butyen J, (1993). Long chain alkyl-polysiloxanes as non-polar stationary phases in capillary gas chromatography. *Fresenius J. Anal. Chem.* 346:396-402.
2. Ballschmiter K, Zell M, (1980). Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Composition of technical Aroclor- and Clophen-PCB mixtures. *Fresenius Z. Anal. Chem.* 302:20-31.
3. Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, Tracey B, Bidleman T, (1992). Arctic contaminants: sources, occurrence and pathways. *Sci. Total Environ.* 122:1-74.
4. Swart RDL, Ross PS, Vedder LJ, Timmerman HH, Heisterkamp S, Van Loveren H, Vos JG, Reijnders PJH, Osterhaus ADME, (1994). Impairment of immune function in harbour seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio* 23:155-159.
5. Tanabe S, Iwata H, Tatsukawa R, (1994). Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Sci. Total Environ.* 154:163-178.
6. Boon JP, Van Arnhem E, Jansen S, Kannan N, Petrick G, Schulz D, Duinker JC, Reijnders PJH, Goksøyr A, (1992). The toxicokinetics of PCBs in marine mammals with special reference to possible interactions of individual congeners with the cytochrome P450-dependent monooxygenase system: an overview. In: *Persistent Pollutants in Marine Ecosystems*. Walker CH, Livingstone DR, editors. Pergamon Press, Oxford, 6, pp.119-160.
7. Olsson M, Karlsson B, Ahnland E, (1994). Diseases and environmental contaminants in seals from the Baltic and the Swedish west coast. *Sci. Total Environ.* 154:217-227.
8. Martineau D, De Guise S, Fournier M, Shugart L, Girard C, Lagacé A, Béland P, (1994). Pathology and toxicology of beluga whales from the St. Lawrence estuary, Quebec, Canada. Past, present and future. *Sci. Total Environ.* 154:201-215.
9. Reijnders PJH, (1994). Toxicokinetics of chlorobiphenyls and associated physiological responses in marine mammals, with particular reference to their potential for ecotoxicological risk assessment. *Sci. Total Environ.* 154:229-236.

10. Goldberg ED, (1991). Halogenated hydrocarbons: past, present and near-future. *Sci. Total Environ.* 100:17-28.
11. Verschueren K, (1983). *Handbook of Environmental Data on Organic Chemicals*. Van Norstrand Reinhold Co., New York, p.1310.
12. Tanabe S, (1988). PCB problems in the future: foresight from current knowledge. *Environ. Pollut.* 50:5-28.
13. de Voogt P, Brinkman UATh, (1989). Production, properties and usage of polychlorinated biphenyls. In: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. 2nd: Kimbrough RD, Jensen AA, editors. Elsevier Science Publishers, Amsterdam, The Netherlands, 1, pp.3-45.
14. Agarwal HC, Kaushik CP, Pillai MKK, (1987). Organochlorine insecticide residues in the rainwater in Delhi, India. *Water Air Soil Pollut.* 32:293-300.
15. Murphy T, (1984). Atmospheric input of chlorinated hydrocarbons to the Great Lakes. In: *Toxic Contaminants in the Great Lakes*. 1st: Nriagu JO, editors. Wiley, New York, NY, 3, pp.53-79.
16. Manchester-Neesvig JB, Andren AW, (1989). Seasonal variation in the atmospheric concentration of polychlorinated biphenyl congeners. *Environ. Sci. Technol.* 23:1138-1148.
17. Bidleman TF, Christensen EJ, Billings WN, Leonard R, (1981). Atmospheric transport of organochlorines in the North Atlantic gyre. *J. Mar. Res.* 39:443-464.
18. Tanabe S, Tatsukawa R, (1986). Distribution, behaviour and load of PCBs in the oceans. In: *PCBs and the Environment*. 1st: Waid JS, editors. CRC Press, Boca Raton, FL, 8, pp.143-162.
19. Ballschmiter K, (1991). Global distribution of organic compounds. *Environ. Carcin. Ecotox. Rev.* C9:1-46.
20. Göthe R, Wachtmeister CA, Åbermark B, Baeckström P, Jansson B, Jensen S, (1976). Photo-isomerization and photo-degradation of DDE. *Tetrahedron Lett.* 49:4501-4504.
21. Abou-Donia MB, (1975). Ultraviolet spectroscopic studies of DDT-type compounds. *Appl. Spectroscopy.* 29:261-264.

22. Standley LJ, Hites RA, (1991). Chlorinated organic contaminants in the atmosphere. In: *Organic Contaminants in the Environment: Environmental Pathways and Effects*. Jones KC, editors. Elsevier Applied Science, London, UK, 1, pp.1-33.
23. Bidleman TF, Wideqvist U, Jansson B, Soederlund R, (1987). Organopesticides and PCBs in the atmosphere of southern Sweden. *Atmos. Environ.* 21:641-654.
24. Gregor DJ, Gummer WD, (1989). Evidence of atmospheric transport and deposition of organochlorine pesticides and polychlorinated biphenyls in Canadian Arctic snow. *Environ. Sci. Technol.* 23:561-565.
25. Gregor DJ, (1990). Deposition and accumulation of selected agricultural pesticides in Canadian Arctic snow. In: *Long Range Transport of Pesticides*. 1st: Kurtz DA, editor. Lewis Publishers, Chelsea, pp.373-386.
26. Mackay D, Shiu WY, (1981). A critical review of Henry's law constants for chemicals of environmental interest. *J. Chem. Phys. Ref. Data.* 10:1175-1199.
27. Burkhard LP, Armstrong DE, Andren A, (1985). Henry's law constraints for the polychlorinated biphenyls. *Environ. Sci. Technol.* 19:590-596.
28. Gregor DJ., (1991). Trace organic chemicals in the Arctic environment: atmospheric transport and deposition. In: *Pollution of the Arctic Atmosphere*. Sturges WT, editor. Elsevier Applied Science, 8, pp.217-254.
29. Ottar B, (1981). The transfer of airborne pollutants to the Arctic region. *Atmos. Environ.* 15:1439-1445.
30. Mackay D, Wania F, (1995). Transport of contaminants to the Arctic: partitioning, processes and models. *Sci. Total Environ.* 160/161:25-38.
31. Wania F, Mackay D, (1993). Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. *Ambio* 22:10-18.
32. Iwata H, Tanabe S, Sakai N, Tatsukawa R, (1993). Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. *Environ. Sci. Technol.* 27:1080-1098.
33. Rahn KA, (1981). Relative importances of North America and Eurasia as sources of Arctic aerosol. *Atmos. Environ.* 15:1447-1455.

34. Raatz WE, (1995). The climatology and meteorology of Arctic air pollution. In: *Pollution of the Arctic Atmosphere*. Sturges WT, editor. Elsevier Applied Science, London, UK, 2, pp.13-42.
35. O Connor JF, (1961). Mean circulation patterns based on 12 years of recent northern hemisphere data. *Mon. Wea. Rev.* 84:211-227.
36. Gobas FAPC, Opperhuizen A, Hutzinger O, (1986). Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation. *Environ. Toxicol. Chem.* 5:637-642.
37. Mackay D, Clark KE, (1991). Predicting the environmental partitioning of organic contaminants and their transfer to biota. In: *Organic Contaminants in the Environment*. Jones KC, editor. Elsevier Applied Science, London, UK, 5, pp.159-188.
38. Hawker DH, Connell DW, (1988). Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol.* 22:382-387.
39. Baughman GL, Paris DF, (1981). Microbial bioconcentration of organic pollutants from aquatic systems - a critical review. *CRC Crit. Rev. Microbiol.* Jan.205-225.
40. Alexander V, (1995). The influence of the structure and function of the marine food web on the dynamics of contaminants in Arctic ocean ecosystems. *Sci. Total Environ.* 160/161:593-603.
41. Walker CH, (1987). Kinetic models for predicting bioaccumulation of pollutants in ecosystems. *Environ. Pollut.* 44:227-240.
42. Bergman Å, Haraguchi K, Athanasiadou M, Larsson C, (1993). Selective retention of PCB methyl sulphones in liver of mammals. *Organohalogen Compounds* 12:199-201.
43. Boon JP, Eijgenraam F, Everaarts JM, (1989). A structure-activity relationship (SAR) approach towards metabolism of PCBs in marine animals from different trophic levels. *Mar. Environ. Res.* 27:159-176.
44. Tanabe S, Watanabe S, Kan H, Tatsukawa R, (1988). Capacity and mode of PCB metabolism in small cetaceans. *Mar. Mam. Sci.* 4:103-124.
45. Norstrom RJ, Muir DCG, (1994). Chlorinated hydrocarbon contaminants in arctic marine mammals. *Sci. Total Environ.* 154:107-128.

46. Norstrom RJ, Simon M, Muir CG, Schweinsburg RE, (1988). Organochlorine contaminants in arctic marine food chains: identification, geographical distribution, and temporal trends in polar bears. *Environ. Sci. Technol.* 22:1063-1071.
47. Muir DG, Norstrom RJ, Simon M, (1988). Organochlorine contaminants in arctic marine food chains: accumulation of specific PCB congeners and chlordane-related compounds. *Environ. Sci. Technol.* 22:1071-1079.
48. Muir DCG, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ, (1992). Arctic Marine Ecosystem Contamination. *Sci. Total Environ.* 122:75-134.
49. Welch HE, Crawford RE, Hop H, (1993). Occurance of arctic cod (*Boreogadus saida*) schools and their vulnerability to predation in the Canadian high Arctic. *Arctic.* 46:331-339.
50. Bradstreet MSW, Cross WE, (1982). Trophic relationships at high arctic ice edges. *Arctic.* 35:1-12.
51. Smith TG, Hammill MO, (1981). Ecology of the ringed seal, *Phoca hispida*, in its fast ice breeding habitat. *Can. J. Zool.* 59:966-981.
52. Hobson KA, Welch HE, (1992). Determination of trophic relationships within a high Arctic marine food web using δ -¹³C and δ -¹⁵N analysis. *Mar. Ecol. Prog. Ser.* 84:9-18.
53. Weslawski JM, Ryg M, Smith TG, Oritsland NA, (1994). Diet of ringed seals (*Phoca hispida*) in a fjord of west Svalbard. *Arctic.* 47:109-114.
54. Stirling I, Calvert W, (1983). Environmental threats to marine mammals in the Canadian Arctic. *Polar Record.* 21:433-449.
55. Derocher AE, Andriashek D, Stirling I, (1993). Terrestrial foraging by polar bears during the ice-free period in western Hudson Bay. *Arctic.* 46:251-254.
56. Derocher AE, Stirling I, (1990). Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. *Can. J. Zool.* 68:1395-1403.
57. Stirling I, Archibald WR, (1977). Aspects of predation of seals by polar bears. *J. Fish. Res. Board of Canada.* 34:1126-1129.
58. Stirling I, McEwan EH, (1975). The caloric value of whole ringed seals (*Phoca hispida*) in relation to polar bear (*Ursus maritimus*) ecology and hunting behavior. *Can. J. Zool.* 53:1021-1027.

59. Best RC, (1985). Digestibility of ringed seals by the polar bear. *Can. J. Zool.* 63:1033-1036.
60. Polischuck SC, Letcher RJ, Norstrom RJ, Ramsay MA, (1995). Preliminary results on the kinetics of organochlorines in western Hudson Bay polar bear (*Ursus maritimus*). *Sci. Total Environ.* 160/161:465-472.
61. Ramsay MA, Hobson KA, (1991). Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. *Oecologia.* 86:598-600.
62. Lunn NJ, Stirling I, (1985). The significance of supplemental food to polar bears during the ice-free period of Hudson Bay. *Can. J. Zool.* 63:2291-2297.
63. Ramsay MA, Stirling I, (1988). Reproductive biology and ecology of female polar bears, *Ursus maritimus*. *J. Zool., Lond. (A).* 214:601-634.
64. Pond CM, Mattacks CA, Colby RH, Ramsay MA, (1992). The anatomy, chemical composition and metabolism of adipose tissue in wild polar bears (*Ursus maritimus*). *Can. J. Zool.* 70:326-341.
65. Gittleman JL, Oftedal OT, (1987). Comparative growth and lactation energetics in carnivores. *Symp. Zool. Soc. Lond.* 57:41-77.
66. Bowes GW, Jonkel CJ, (1975). Presence and distribution of polychlorinated biphenyls (PCB) in Arctic and Subarctic marine food chains. *J. Fish. Res. Board Can.* 32:2111-2123.
67. Holden AV, (1972). Monitoring organochlorine contamination of the marine environment by the analysis of residues in seals. In: *Marine Pollution in Sea Life*, FAO. Ruivo M, editor. Fishing News Books, Surrey, UK, pp.266-272.
68. Hargrave BT, Harding GC, Vass WP, Erickson PE, Fowler BR, Scott V, (1992). Organochlorine pesticides and polychlorinated biphenyls in the arctic ocean food web. *Arch. Environ. Contam. Toxicol.* 22:41-54.
69. Colborn T, von Saal FS, Soto AM, (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.* 101:378-384.
70. Sandell M, (1990). The evolution of seasonal delayed implantation. *Q. Rev. Biol.* 65:23-42.

71. Bergman Å, Norstrom RJ, Haraguchi K, Kuroki H, Béland P, (1994). PCB and DDE methyl sulfones in mammals from Canada and Sweden. *Environ. Toxicol. Chem.* 13:121-128.
72. Oehme M, Biseth A, Schlapach M, Wiig Ø, (1995). Concentrations of polychlorinated dibenzo-*p*-dioxin and furans and non-*ortho* polychlorinated biphenyls in polar bear milk from Svalbard (Norway). *Environ. Pollut.* 90:401-407.
73. Norstrom RJ, Simon M, Muir DCG, (1990). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in marine mammals in the Canadian North. *Environ. Pollut.* 66:1-19.
74. Safe SH, (1994). Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* 24:87-149.
75. Ford CA, Muir DCG, Norstrom RJ, Simon M, Mulvihill MJ, (1993). Development of a semi-automated method for non-*ortho* PCBs: Application to Canadian arctic marine mammal tissues. *Chemosphere.* 26:1981-1991.
76. Daelemans FF, Mehlum F, Lydersen C, Schepens PJC, (1993). Mono-*ortho* and non-*ortho* substituted PCBs in Arctic ringed seal (*Phoca hispida*) from the Svalbard area: analysis and determination of their toxic threat. *Chemosphere.* 27:429-437.
77. Okey AB, (1990). Enzyme induction in the cytochrome P-450 system. *Pharmacol. Ther.* 45:241-298.
78. Guengerich FP, (1993). Cytochrome P450 enzymes. *American Scientist.* 81:440-447.
79. Guengerich EP, (1991). Reactions and significance of cytochrome P-450 enzymes. *J. Biol. Chem.* 266:10019-10022.
80. Levin W, Thomas PE, Reik LM, Ryan DE, Bandiera S, Haniu M, Shively JE, (1985). Immunochemical and structural characterization of rat hepatic microsomes P-450. In: *Microsomes and Drug Oxidations*. Boobis AR, Caldwell J, De Matteis S, Elcombe CR, editors. Taylor and Francis, London, UK, pp.13-22.
81. Cullen JM, Kaiser KLE, (1996). An examination of the role of rotational barriers in the toxicology of PCBs. In: *QSAR in Environmental Toxicology*. Kaiser KLE, editor. Reidel, Dordrecht, pp.39-
82. Poland A, Knutson JC, (1982). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22:517-554.

83. Borlakoglu JT, Haegele KD, (1991). Comparative aspects on the bioaccumulation, metabolism and toxicity with PCBs. *Comp. Biochem. Physiol.* 100C:327-338.
84. McFarland VA, Clarke JU, (1989). Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ. Health Perspect.* 81:225-239.
85. Waxman DJ, Azaroff L, (1992). Phenobarbital induction of cytochrome P-450 gene expression. *Biochem. J.* 281:577-592.
86. Lewis DFW, Ioannides C, Parke DV, (1986). Molecular dimensions of the substrate binding site of cytochrome P-448. *Biochem. Pharmacol.* 35:2179-2185.
87. Stegeman JJ, Brouwer M, Di Giulio R, Förlin L, Fowler BA, Sanders BM, van Veld PA, (1992). Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress*. 1st: Huggett RJ, Kimerle RA, Mehrle PMJ, Bergman HL, editors. Lewis Publishers, Chelsea, MI, 6, pp.235-335.
88. White RD, Hahn ME, Lockhart WL, Stegeman JJ, (1994). Catalytic and immunochemical characterization of hepatic microsomal cytochromes P450 in beluga whale (*Delphinapterus leucas*). *Toxicol. Appl. Pharmacol.* 126:45-57.
89. Watanabe S, Shimada T, Nakamura S, Nishiyama N, Yamashita N, Tanabe S, Tatsukawa R, (1989). Specific profile of liver microsomal cytochrome P-450 in dolphin and whales. *Mar. Environ. Res.* 27:51-65.
90. Goksøyr A, Beyer J, Larsen HE, Andersson T, Förlin L, (1992). Cytochrome P450 in seals: monooxygenase activities, immunochemical cross-reactions and response to phenobarbital treatment. *Mar. Environ. Res.* 34:113-116.
91. Rattner BA, Melancon MJ, Custer TW, Hothem RL, King KA, LeCaptain LJ, Spann JW, Woodin BR, Stegeman JJ, (1993). Biomonitoring environmental contamination with pipping black-crowned night heron embryos: induction of cytochrome P450. *Environ. Toxicol. Chem.* 12:1719-1732.
92. Collier TK, Anulacion BF, Stein JE, Goksøyr A, Varanasi U, (1995). A field evaluation of cytochrome P4501A as a biomarker on contaminant exposure in three species of flatfish. *Environ. Toxicol. Chem.* 14:143-153.
93. Bandiera SM, Torok SM, Lin S, Ramsay MA, Norstrom RJ, (1995). Catalytic and immunologic characterization of hepatic and lung cytochromes P450 in the polar bear. *Biochem. Pharmacol.* 49:1135-1146.

94. Hietanen E, Bartsch H, Ahotupa M, Park SS, Gelboin HV, (1985). Tissue specificity of extrahepatic monooxygenases in the metabolism of xenobiotics. In: *Drug Metabolism: Molecular Approaches and Pharmacological Implications*. Siest G, editor. Pergamon Press, Exeter, UK, pp.141-146.
95. Klasson-Wehler E, Lindberg L, Jönsson C-J, Bergman Å, (1993). Tissue retention and metabolism of 2,3,4,3',4'-pentachlorobiphenyl in mink and mouse. *Chemosphere*. 27:2397-2412.
96. Bergman Å, Klasson-Wehler E, Kuroki H, (1994). Selective retention of hydroxylated PCB metabolites in blood. *Environ. Health Perspect.* 102:2-6.
97. Brown JF, Lawton RW, Ross M, (1989). Persistence of PCB congeners in capacitor workers and Yusho patients. *Chemosphere*. 19:829-834.
98. Kannan N, Tanabe S, Ono M, Tatsukawa R, (1989). Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-ortho and mono-ortho coplanar polychlorinated biphenyls from land to ocean. *Arch. Environ. Contam. Toxicol.* 18:850-857.
99. Muir DCG, Segstro MD, Hobson KA, Ford CA, Stewart REA, Olpinski S, (1995). Can seal eating explain elevated levels of PCBs and organochlorine pesticides in walrus blubber from eastern Hudson Bay (Canada)? *Environ. Pollut.* 90:335-348.
100. Boon JP, Oostingh I, van der Meer J, Hillebrand MTJ, (1994). A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. *Eur. J. Pharmacol.* 270:237-251.
101. Brown JF Jr, (1994). Determination of PCB metabolic, excretion, and accumulation rates for use as indicators of biological response and relative risk. *Environ. Sci. Technol.* 28:2295-2305.
102. Borlakoglu JT, Walker CH, (1989). Comparative aspects of congener specific PCB metabolism. *Euro. J. Drug Metab. Pharmacokin.* 14:127-131.
103. Boon JP, Duinker JC, (1985). Processes determining the kinetics of PCB congeners in marine organisms: a comparison between laboratory and environmental studies. *Mar. Environ. Res.* 17:301-305.
104. Boon JP, Eijgenraam F, (1988). The possible role of metabolism in determining patterns of PCB congeners in species from the Dutch Wadden Sea. *Mar. Environ. Res.* 24:3-8.

105. Kuroki H, Koga N, Yoshimura H, Masuda Y, (1994). *In vivo* metabolism of 3,3',5,5'-tetrachlorobiphenyl in rats. *Organohalogen Compounds* 20:505-507.
106. Goksøyr A, Tarlebø J, Solbakken JE, Klungsøyr J, (1985). Characteristics of the hepatic microsomal cytochrome P-450 system of the minke whale (*Balaenoptera acutorostrata*). *Mar. Environ. Res.* 17:113-116.
107. Bakke JE, (1989). Metabolites derived from glutathione conjugation. In: *Intermediary Xenobiotic Metabolism in Animals*. Hutson DH, Caldwell J, Paulson GD, editors. Taylor & Francis, London, UK, pp.205-224.
108. Siegers C-P, (1989). Glutathione and GSH-dependent enzymes. In: *Intestinal Metabolism of Xenobiotics*. 1st: Koster AS, Pichter E, Lauterbach F, Hartmann F, editors. Gustav Fischer Verlag, New York, NY, 2.5, pp.171-180.
109. Bergman Å, Brandt I, Larsson Y, Wachtmeister CA, (1980). Cysteine and methionine-precursors of methyl sulphone metabolites of 2,4',5-trichlorobiphenyl in mice. *Chem. -Biol. Interact.* 31:65-71.
110. Bakke J, Bergman Å, Larsen G, (1995). Methylsulfone formation and PCB metabolism. *Organohalogen Compounds*. 25:413-417.
111. Bakke J, Gustafsson J-Å, (1984). Mercapturic acid pathway metabolites of xenobiotics: generation of potentially toxic metabolites during enterohepatic circulation. *Trends Pharmac. Sci.* 5:517-521.
112. Mannervik B, (1982). *Metabolic Basis of Detoxication Metabolism of Functional Groups*. 1st: Jakoby WB, Bend JR, and Caldwell J, editors. Academic Press, New York, p.185.
113. Klasson-Wehler E, Bergman Å, Kowalski B, Brandt I, (1987). Metabolism of 2,3,4',6-tetrachlorobiphenyl: formation and tissue localization of mercapturic acid pathway metabolites in mice. *Xenobiotica*. 17:477-486.
114. Preston BD, Miller JA, Miller EC, (1984). Reactions of 2,2',5,5'-tetrachlorobiphenyl 3,4-oxide with methionine, cysteine and glutathione in relation to the formation of methylthio-metabolites of 2,2',5,5'-tetrachlorobiphenyl in the rat and mouse. *Chem.-Biol. Interact.* 50:289-312.
115. Jakoby WB, Habig WH, (1980). Glutathione transferases. In: *Enzymatic Basis of Detoxication*. 1st: Jakoby WB, editor. Academic Press, New York, NY, pp.63-94.

116. Bakke JE, Bergman ÅL, Larsen GL, (1982). Metabolism of 2,4',5-trichlorobiphenyl by the mercapturic acid pathway. *Science*. 217:645-647.
117. Bakke JE, Huwe JK, Mulford DJ, Bergman Å, (1992). Metabolism of 1,2,4-trichlorobenzene in rats: examination of thiol formation. *Xenobiotica*. 22:199-210.
118. Bakke JE, (1990). Biochemical and physiological dispositions of glutathione conjugates. *Drug Metab. Rev.* 22:637-647.
119. Bakke JE, Bergman ÅL, Brandt I, Darnerud P, Struble C, (1983). Metabolism of the mercapturic acid of 2,4',5-trichlorobiphenyl in rats and mice. *Xenobiotica*. 13:597-605.
120. Bakke JE, Feil VJ, Bergman Å, (1983). Metabolites of 2,4',5-trichlorobiphenyl in rats. *Xenobiotica*. 13:555-564.
121. Brandt I, Klasson-Wehler E, Rafter J, Bergman Å, (1982). Metabolism of 2,4',5-trichlorobiphenyl: tissue concentrations of methylsulphonyl-2,4',5-trichlorobiphenyl in germfree and conventional mice. *Toxicol. Lett.* 12:273-280.
122. Tateishi M, Suzuki S, Shimizu H, (1978). Cysteine β -lyase in rat liver - A novel enzyme catalyzing formation of thiol-containing metabolites of drugs. *J. Biol. Chem.* 253:8854-8860.
123. Larsen GL, (1985). Distribution of cysteine conjugate β -lyase in gastrointestinal bacteria and in the environment. *Xenobiotica*. 15:199-209.
124. Bakke JE, Rafter JJ, Lindeskog P, Feil VJ, Gustafsson J-Å, Gustafsson BE, (1981). Metabolism of 2-acetamido-4-(chloromethyl)thiazole in germfree and conventional rats. *Biochem. Pharmacol.* 30:1839-1844.
125. Mizutani T, (1978). Identification of sulfur-containing metabolites of 2,4,2',4'-tetrachlorobiphenyl in mice. *Bull. Environ. Contam. Toxicol.* 20:219-226.
126. Brandt I, Bergman Å, Darnerud PO, Larsson Y, (1982). Metabolism of 2,4',5-trichlorobiphenyl: enrichment of hydroxylated and methyl sulphone metabolites in the uterine luminal fluid of pregnant mice. *Chem.-Biol. Interact.* 40:45-56.
127. Pinkus LM, Ketley JN, Jakoby WB, (1977). The glutathione S-transferases as a possible detoxification system of rat intestinal epithelium. *Biochem. Pharmacol.* 26:2359-2363.
128. Hänninen O, Lindström-Seppä P, Pelkonen K, (1987). Role of gut in xenobiotic metabolism. *Arch. Toxicol.* 60:34-36.

129. Brandt I, Bergman Å, (1981). Bronchial mucosal and kidney cortex affinity of 4- and 4,4'- substituted sulphur-containing derivatives of 2,2'-5,5'-tetrachlorobiphenyl in mice. *Chem.-Biol. Interact.* 34:47-55.
130. Lund J, Brandt I, Poellinger L, Bergman Å, Klasson-Wehler E, Gustafsson J-Å, (1985). Target cells for the polychlorinated biphenyl metabolite 4,4'-bis(methylsulfonyl)-2,2',5,5'- tetrachlorobiphenyl characterization of high affinity binding in rat and mouse lung cytosol. *Molecular Pharmacol.* 27:314-323.
131. Brandt I, Lund J, Bergman Å, Klasson-Wehler E, Poellinger L, Gustafsson J-Å, (1985). Target cells for the polychlorinated biphenyl metabolite 4,4'-bis(methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl in lung and kidney. *Drug Metab. Dispos.* 13:490-496.
132. Lund J, Nordland L, Devereux T, Glaumann H, Gustafsson J-Å, (1987). Physiological and immunochemical characterization of a binding-protein for PCB methyl sulfones. *Chemosphere* 16:1677-1680.
133. Bergman Å, Brandt I, Darnerud PO, Wachtmeister CA, (1982). Metabolism of 2,2',5,5'-tetrachlorobiphenyl: formation of mono- and bis-methyl sulphone metabolites with a selective affinity for the lung and kidney tissues in mice. *Xenobiotica* 12:1-7.
134. Haraguchi K, Kuroki H, Masuda Y, (1989). Polychlorinated biphenyl methylsulfone congeners in human tissues: Identification of methylsulfonyl dichlorobiphenyls. *Chemosphere* 18:477-484.
135. Haraguchi K, Kuroki H, Masuda Y, (1987). Determination of hydroxy methylsulfone metabolites of PCB in Yusho patients. *Chemosphere* 16:2033-2038.
136. Haraguchi H, Kuroki H, Masuda Y, Shigematsu N, (1984). Determination of methylthio and methylsulphone polychlorinated biphenyls in tissues of patients with 'Yusho'. *Fd. Chem. Toxic.* 22:283-288.
137. Haraguchi K, Kuroki H, Masuda Y, (1986). Determination of PCB-methylsulfone congeners in Yusho and control patients. *Chemosphere* 15:2027-2030.
138. Haraguchi K, Kuroki H, Masuda Y, (1986). Capillary gas chromatographic analysis of methylsulfone metabolites of polychlorinated biphenyls retained in human tissues. *J. Chromatog.* 361:239-252.
139. Larsen GL, Bergman Å, Klasson-Wehler E, (1990). A methylsulfonyl metabolite of a polychlorinated biphenyl can serve as a ligand for $\alpha 2u$ -globin in rat and major urinary protein in mice. *Xenobiotica* 20:1343-1352.

140. Larsen GL, Bergman Å, Klasson-Wehler E, Bass NM, (1991). A methylsulfonyl metabolite of a polychlorinated biphenyl can serve as a ligand for liver fatty acid binding protein in rat intestinal mucosa. *Chem.-Biol. Interact.* 77:315-323.
141. Larsen GL, Huwe JK, Bergman Å, Klasson-Wehler E, Hargis P, (1992). Methylsulfonyl metabolites of xenobiotics can serve as ligands for fatty acid binding proteins in chicken liver and intestinal mucosa. *Chemosphere* 25:1189-1194.
142. Larsen GL, Bergman Å, (1994). Interaction of methylsulfonyl-containing PCB with mammalian carrier proteins. *Organohalogen Compounds* 20:451-454.
143. Bass NM, (1985). Function and regulation of hepatic and intestinal fatty acid binding proteins. *Chem. Phys. Lipids.* 38:95-114.
144. Gillner M, Lund J, Cambillau C, Alexandersson M, Hurtig U, Bergman Å, Klasson-Wehler E, Gustafsson J-Å, (1988). The binding of methylsulfonyl-polychloro-biphenyls to uteroglobin. *J. Steroid Biochem.* 31:27-33.
145. Lund B-O, Bergman Å, Brandt I, (1986). Decreased pulmonary drug metabolism in mice treated with the PCB metabolite 4-methylsulphonyl-2,2',5,5'-tetrachlorobiphenyl. *Toxicol. Lett.* 32:261-267.
146. Haraguchi K, Kawashima M, Yamada S, Isogai M, Masuda Y, Kimura R, (1995). Characterization of hepatic microsomal cytochrome P-450 from rats treated with methyl sulfonyl metabolites of polychlorinated biphenyl congeners. *Chem.-Biol. Interact.* 95:269-278.
147. Kato Y, Haraguchi K, Kawashima M, Yamada S, Masuda Y, Kimura R, (1995). Induction of hepatic microsomal drug-metabolizing enzymes by methyl sulfonyl metabolites of polychlorinated biphenyl congeners in rats. *Chem.-Biol. Interact.* 95:257-268.
148. Nagayama J, Kiyohara C, Mohri N, Hirohata T, Haraguchi K, Masuda Y, (1989). Inhibitory effect of methylsulphonyl polychlorinated biphenyls on aryl hydrocarbon hydroxylase activity. *Chemosphere* 18:701-708.
149. Kiyohara C, Mohri N, Hirohata T, Haraguchi K, Masuda Y, (1990). *In vitro* effects of methylsulfonyl polychlorinated biphenyls and 7,8-benzoflavone on aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells. *Pharmacol. Toxicol.* 66:273-276.
150. Kiyohara C, Hirohata T, (1994). Effects of vitamin C and PCB metabolites on the inhibition of aryl hydrocarbon hydroxylase and suppression of mutagenicity of benzo[a]pyrene. *Toxic. in Vitro.* 8:1185-1189.

151. Kiyohara C, Hirohata T, Mohri N, Masuda Y, (1990). Effects of 3-methylsulfonyl-4,5,3',4'-tetrachlorobiphenyl and 7, 8-benzoflavone on mouse liver aryl hydrocarbon hydroxylase activity *in vitro*. *Toxic. in Vitro*. 4:103-107.
152. Kellerman G, Shaw CR, Luyten-Kellerman M, (1973). Arylhydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *New Eng. J. Med.* 286:934-937.
153. Hosomi J, Nemoto, Kuroki T, (1983). Genetic variation in metabolism of benzo[a]pyrene in cultured epidermal and dermal cells of responsive and non-responsive mice. *Cancer Res.* 43:3643-3648.
154. Brunström B, Bergman Å, Backlin B-M, Lund B-O, Orberg J, (1994). Effects of long-term exposure to PCB and PCB methylsulfones on reproduction in the mink. *Organohalogen Compounds* 20:471-473.
155. Brandt I, Jönsson C-J, Lund B-O, Bergman Å, (1990). 3-Methylsulfonyl-DDE: A potent toxicant following metabolic activation in the adrenal *Zona fasciculata* in mice. In: *Dioxin '90 EPRI Seminar Short Papers, Volume 1*. Hutzinger O, Fiedler H, editors. Eco-Informa Press, Bayreuth, F.R.G. pp.63-66.
156. Lund B-O, Bergman Å, Brandt I, (1988). Metabolic activation and toxicity of a DDT-metabolite, 3-methylsulphonyl-DDE, in the adrenal *Zona Fasciculata* in mice. *Chem.-Biol. Interact.* 65:25-40.
157. Brandt I, Jönsson C-J, Lund B-O, (1992). Comparative studies on adrenocorticolytic DDT-metabolites. *Ambio*. 21:602-605.
158. Jönsson C-J, Lund B-O, Brunström B, Brandt I, (1994). Toxicity and irreversible binding of two DDT metabolites - 3- methylsulphonyl-DDE and *o,p'*-DDD - in adrenal interrenal cells in birds. *Environ. Toxicol. Chem.* 13:1303-1310.
159. Lund B-O, (1994). In vitro adrenal bioactivation and effects on steroid metabolism of DDT, PCBs and their metabolites in the gray seal (*Halichoerus grypus*). *Environ. Toxicol. Chem.* 13:911-917.
160. Jönsson C-J, Lund B-O, Brandt I, (1993). Adrenocorticolytic DDT-metabolites: studies in mink, *Mustela vison* and otter, *Lutra lutra*. *Ecotoxicol.* 2:41-53.
161. Bergman Å, Olsson M, (1986). Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? *Finn. Game Res.* 44:47-62.

162. Bergman Å, Wachtmeister CA, (1977). Synthesis of methanesulfonyl derivatives of 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), present in seal from the Baltic. *Acta Chemica Scandinavia* 31:90-91.
163. Jensen S, Jannsson B, (1976). Methyl sulphone metabolites of PCB and DDE. *Ambio*. 257-260.
164. Haraguchi K, Masuda Y, Bergman Å, Olsson M, (1991). PCB methyl sulphone: comparison of tissue levels in Baltic grey seals and a Yusho patient. *Fukuoka Acta Medica*. 82:269-273.
165. Olsson A, Asplund L, Helander B, Bergman Å, Kylin H, (1993). Isomer specific analysis of PCB and PCB methyl sulphones in eggs from white tailed sea eagle. *Organohalogen Compounds* 14:113-116.
166. Norén K, Lunden A, Bergman Å, (1994). Methyl sulfone metabolites of polychlorinated biphenyls and *p,p'*-DDE in Swedish human milk. *Organohalogen Compounds* 20:509-512.
167. Norén K, Lundén Å, Pettersson E, Bergman Å, (1996). Methylsulphonyl metabolites of polychlorinated biphenyl (PCB) and DDE in Swedish human milk, 1972-1992. *in preparation*.
168. Haraguchi K, Athanasiadou M, Bergman Å, Hovander L, Jensen S, (1992). PCB and PCB methyl sulfones in selected groups of seals from Swedish waters. *Ambio* 21:546-549.
169. Haraguchi K, Bergman Å, Masuda Y, (1994). Comparative study on tissue retention of PCB methyl sulfone metabolites in different mammalian species. *Organohalogen Compounds* 20:501-504.
170. Bergman Å, Haraguchi K, (1994). Analysis of PCB and DDE methyl sulfones - persistent metabolites of PCB and DDT in biota. *The Standard: Cambridge Isotope Laboratories* 1:1-8.
171. Bergman Å, Athanasiadou M, Bergek S, Haraguchi K, Jensen S, Klasson-Wehler E, (1992). PCB and PCB methyl sulfones in mink treated with PCB and various PCB fractions. *Ambio* 21:570-576.
172. Haraguchi K, Kuroki H, Masuda Y, (1984). Analytical method for minute amounts of polychlorinated biphenyl methylsulfones from fatty tissue. *J. Anal. Toxicol.* 8:177-181.

173. Haraguchi K, Bergman Å, Jakobsson E, Masuda Y, (1993). Negative ion chemical ionization mass spectrometry in the analysis of polychlorinated biphenyl methyl sulfones. *Fresenius J. Anal. Chem.* 347:441-449.
174. Kuroki H, Hattori R, Haraguchi K, Masuda Y, (1989). Metabolism of 2,8-dichlorodibenzofuran in rats. *Chemosphere* 19:803-808.
175. Letcher RJ, Norstrom RJ, Bergman Å, (1995). Geographical distribution and identification of methyl sulphone PCB and DDE metabolites in pooled polar bear (*Ursus maritimus*) adipose tissue from western hemisphere Arctic and Subarctic regions. *Sci. Total Environ.* 160/161:409-420.
176. Stemmler EA, Hites RA, Arbogast B, Budde WL, Deinzer ML, Dougherty RC, Eichelberger JW, Foltz RL, Grimm C, Grimsrud EP, (1988). Interlaboratory comparison of methane electron capture negative ion mass spectra. *Anal. Chem.* 60:781-787.
177. Oehme M, (1983). Determination of isomeric polycyclic aromatic hydrocarbons in air particulate matter by high-resolution gas chromatography/negative ion chemical ionization mass spectrometry. *Anal. Chem.* 55:2290-2295.
178. Dougherty RC, (1981). Negative chemical ionization mass spectrometry. *Anal. Chem.* 53:625A-636A.
179. Oehme M, Stockl D, Knoppel H, (1986). Comparison of the reproducibility of negative ion chemical ionization mass spectra obtained by different reagent gases on two commercial quadrupole instruments. *Anal. Chem.* 58:554-558.
180. Haraguchi K, Kuroki H, Masuda Y, (1987). Synthesis and Characterization of Tissue-Retainable Methyl sulfonyl Polychlorinated Biphenyl Isomers. *J. Agric. Fd. Chem.* 35:178-182.
181. Bergman A, Jansson B, Bamford I, (1980). Methylthio- and methylsulfonylpolychlorobiphenyls: synthesis and studies of correlations between structure and fragmentation pattern on electron impact. *Biomed. Mass Spectrom.* 7:20-27.
182. Buser H-R, Zook DR, Rappe C, (1992). Determination of methyl sulfone-substituted polychlorobiphenyls by mass spectrometric techniques with application to environmental samples. *Anal. Chem.* 64:1176-1183.
183. Tong HY, Karasek FW, (1984). Flame ionization detector response factors for compound classes in quantitative analysis of complex organic mixtures. *Anal. Chem.* 56:2124-2128.

184. Yieru H, Qingyu O, Weile Y, (1990). Characteristics of flame ionization detection for the quantitative analysis of complex organic mixtures. *Anal. Chem.* 62:2063-2064.
185. Parker CE, Albro PW, Robenrieth M-J, Cochran TW, (1983). Quantitation of halogenated aromatic compounds by gas chromatography-mass spectrometry. *J. Chromatog.* 278:1-11.
186. Norstrom RJ, Simon M, Muirvihi MJ. (1986). A gel-permeation/column chromatography cleanup method for the determination of CDDs in animal tissues. *Inter. J. Environ. Anal. Chem.* 23:267-287.
187. Letcher R, Norstrom RJ, Bergman Å, Muir D, (1992). Methylsulfonyl-PCB and -DDE metabolites in polar bears - comparison to parent compounds in the diet. *Org. Polychlorinated Compounds* 8:357-360.
188. MacDougall D, Lal J, Amore FJ, Langer RR, Cox GV, McCelland NI, Crosby DG, Phillips WF, Freeman DH, Pojasek RP, (1980). Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Anal. Chem.* 52:2242-2249.
189. Glaser JA, Foerst DL, McKee GD, Quave SA, Budde WL, (1981). Trace analysis for wastewaters. *Environ. Sci. Technol.* 15:1426-1432.
190. Chang RR, Jarman WM, Henning JA, (1993). Sample cleanup by solid-phase extraction for the ultratrace determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in biological samples. *Anal. Chem.* 65:2420-2427.
191. Pearson ES, Hartley HO, (1958). *Biometrika Tables for Statisticians*. 2nd:Cambridge University Press, Cambridge, UK.
192. Lamparski LL, Nestrick TJ, Stehl RH, (1979). Determination of part-per-trillion concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in fish. *Anal. Chem.* 51:1453-1458.
193. Smith LM, Stalling DL, Johnson JL, (1984). Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. *Anal. Chem.* 56:1830-1842.
194. Zook DR, Buser H-R, Bergqvist P-A, Rappe C, Olsson M, (1992). Detection of tris(4-chlorophenyl) methane and tris(4-chlorophenyl) methanol in ringed seal (*Phoca hispida*) from the Baltic Sea. *Ambio* 21:557-560.
195. Gurr MI, James AT, (1980). *Lipid Biochemistry: An Introduction*. 3rd:Chapman and Hall Ltd.London, UK, pp.1-127.

196. Olsson A, Bergman Å, (1995). A new persistent contaminant detected in Baltic wildlife: bis(4-chlorophenyl)sulfone. *Ambio* 24:119-123.
197. Letcher RJ, Norstrom RJ, Lin S, Ramsay MA, Bandiera SM, (1996). Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P450 1A and P450 2B and chlorinated hydrocarbon contaminant levels in polar bear (*Ursus maritimus*). *Toxicol. Appl. Pharmacol.* 137:in press.
198. Arbogast B, Budde WL, Deinzer M, Dougherty RC, Eichelberger J, Foltz RD, Grimm CC, Hites RA, Sakashita C, Stemmler E, (1990). Interlaboratory comparison of limits of detection in negative chemical ionization mass spectrometry. *Org. Mass Spectrom.* 25:191-196.
199. Mullin MD, Pochini CM, McCrindle S, Romkes M, Safe SH, Safe LM, (1984). High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners. *Environ. Sci. Technol.* 18:468-476.
200. Letcher RJ, Norstrom RJ, Bergman Å, (1995). An integrated analytical method for determination of polychlorinated aryl methyl sulfone metabolites and polychlorinated hydrocarbon contaminants in biological matrices. *Anal. Chem.* 67:4155-4163.
201. Guevremont R, Yost RA, Jamieson WD, (1987). Identification of PCB congeners by collision-induced dissociation of [M-Cl+O]⁻ from oxygen-enhanced negative chemical ionization mass spectrometry. *Biomed. Environ. Mass Spectrom.* 14:435-441.
202. Crow FW, Bjorseth A, Knapp KT, Bennett R, (1981). Determination of polyhalogenated hydrocarbons by glass capillary gas chromatography-negative ion chemical ionization mass spectrometry. *Anal. Chem.* 53:619-625.
203. Stemmler EA, Hites RA, (1985). Methane enhanced negative ion mass spectra of hexachlorocyclopentadiene derivatives. *Anal. Chem.* 57:684-692.
204. Stemmler EA, Hites RA, Arbogast B, Budde WL, Deinzer ML, Dougherty RC, Eichelberger JW, Foltz RL, Grimm C, Grimsrud EP, (1988). Interlaboratory comparison of methane electron-capture negative ion mass spectra. *Anal. Chem.* 60:781-787.
205. Erhardt-Zabik S, Watson JT, Zabik MJ, (1990). Selective sensitivity of highly chlorinated species in negative ion mass spectrometry: implications for complex mixture analysis. *Biomed. Environ. Mass Spectrom.* 19:101-108.
206. Laramee JA, Arbogast BC, Deinzer ML, (1986). Electron capture negative ion chemical ionization mass spectrometry of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin. *Anal. Chem.* 58:2907-2912.

207. McLafferty FW, (1980). *Interpretation of Mass Spectra*. 3rd: University Science Books, Mill Valley, CA, 4: pp.45-74.
208. Stemmler EA, Hites RA, (1988). Electron capture negative ion mass spectra of halogenated diphenylethane derivatives. *Anal. Chem.* 60:787-792.
209. Buser H-R, (1986). Selective detection of brominated aromatic compounds using gas chromatography/negative chemical ionization mass spectrometry. *Anal. Chem.* 58:2913-2919.
210. Haraguchi K, Kuroki H, Masuda Y, (1989). Occurrence and distribution of chlorinated aromatic methylsulfones and sulfoxides in biological samples. *Chemosphere* 19:487-492.
211. Stirling I, Spencer C, Andriashek D, (1989). Immobilization of polar bears (*Ursus maritimus*) with Telazol in the Canadian Arctic. *J. Wild. Diseases.* 25:159-168.
212. Ramsay MA, Mattacks CA, Pond CM, (1992). Seasonal and sex differences in the structure and chemical composition of adipose tissue in wild polar bears (*Ursus maritimus*). *J. Zool. Lond.* 228:1-12.
213. Derocher AE, Andriashek D, Arnould JPY, (1993). Aspects of milk composition and lactation in polar bears. *Can. J. Zool.* 71:561-567.
214. Reilly JJ, Fedak MA, (1990). Measurement of body composition of living gray seals by hydrogen isotopic dilution. *J. Appl. Physiol.* 69:885-891.
215. AOAC, (1990). *Official methods of analysis*. 15th: Helrich K, editor. AOAC, Arlington, VA, p.821.
216. Mes J, Davies DJ, Lau P-Y, (1980). The effect of extraction technique on the fat content, polychlorinated biphenyl level and tri- to octa-biphenyl distribution in human milk. *Chemosphere* 9:763-769.
217. Bidleman TF, Patton GW, Walla MD, Hargrave BT, Vass WP, Erickson B, Fowler P, Scott V, Gregor DJ, (1989). Toxaphene and other organochlorines in Arctic ocean fauna: evidence for atmospheric delivery. *Arctic.* 42:307-313.
218. Nakata H, Tanabe S, Tatsukawa R, Amano M, Miyazaki N, Petrov EA, (1995). Persistent organochlorine residues and their accumulation kinetics in Baikal Seal (*Phoca sibirica*) from Lake Baikal, Russia. *Environ. Sci. Technol.* 29:2877-2885.

219. Oehme M, Schlabach M, Hummert K, Luckas B, Nordøy ES, (1995). Determination of levels of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls and pesticides in harp seals from the Greenland sea. *Sci. Total Environ.* 162:75-91.
220. Oehme M, Furst P, Kruger C, Meemken HA, Groebel W, (1988). Presence of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and pesticides in arctic seal from Spitsbergen. *Chemosphere* 17:1291-1300.
221. Mitchell SH, Kennedy S, (1992). Tissue concentrations of organochlorine compounds in common seals from the coast of Northern Ireland. *Sci. Total Environ.* 115:163-177.
222. Addison RF, Zinck ME, Smith TG, (1986). PCBs have declined more than DDT-group residues in Arctic ringed seals (*Phoca hispida*) between 1972 and 1981. *Environ. Sci. Technol.* 20:253-256.
223. Addison RF, Smith TG, (1974). Organochlorine residue levels in arctic ringed seals: variation with age and sex. *Oikos.* 25:335-337.
224. Stegeman JJ, (1989). Cytochrome P-450 in fish: catalytic, immunological and sequence similarity. *Xenobiotica.* 19:1093-1110.
225. Muir DCG, Wageman R, Lockhart WL, Grift NP, Billeck B, Metner D, (1987). Heavy metal and organic contaminants in arctic marine fish. Indian and Northern Affairs Canada, Environmental Studies 42, Ottawa, 64 pp. .
226. Welch HE, Bergman MA, Siferd TD, Martin KA, Curtis MF, Crawford RE, Conover RJ, Hop H, (1992). Energy flow through the marine ecosystem of the Lancaster Sound region, Arctic, Canada. *Arctic.* 45:343-357.
227. Cattet M, (1995). Polar bear liver: percent of the total body weight, personal; communication.
228. Brown JF Jr, (1992). Metabolic alterations of PCB residues in aquatic fauna: distributions of cytochrome P4501A- and P4502B-like activities. *Mar. Environ. Res.* 34:261-266.
229. Porte C, Albaiges J, (1993). Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans and fishes. *Arch. Environ. Contam. Toxicol.* 26:273-281.
230. Kalmaz EV, Kalmaz GD, (1979). Transport, distribution and toxic effects of polychlorinated biphenyls in ecosystems: review. *Ecol. Model.* 6:223-230.

231. Tanabe S, Sung J-K, Choi D-Y, Baba N, Kiyota M, Toshida K, Tatsukawa R, (1994). Persistent organochlorine residues in northern fur seal from the Pacific coast of Japan since 1971. *Environ. Pollut.* 85:305-314.
232. Parkinson A, Safe SH, Robertson LW, Thomas PE, Ryan DE, Reik LM, Levin W, (1983). Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. *J. Biol. Chem.* 258:5967-5976.
233. Safe SH, (1989). Polychlorinated aromatics: uptake, disposition and metabolism. In: *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. 2nd: Kimborough RD, Jensen AA, editor. Elsevier, Amsterdam, The Netherlands, 5, pp.131-159.
234. Schultz DE, Petrick G, Duinker JC, (1989). Complete characterization of polychlorinated biphenyl congeners in commercial Arochlor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environ. Sci. Technol.* 23:852-859.
235. Tanabe S, Tatsukawa R, Tanaka H, Maruyama K, Miyazaki N, Funiyama T, (1981). Distribution and total burdens of chlorinated hydrocarbons in bodies of striped dolphins (*Stenella coeruleoalba*). *Agric. Biol. Chem.* 45:2569-2578.
236. Safe SH, (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* 21:51-88.
237. Bergman Å, (1994). PCB and DDE methyl sulphones - persistent environmental contaminants. *Organohalogen Compounds* 20:431-436.
238. Kawai S, Fukushima M, Miyazaki N, Tatsukawa R, (1988). Relationship between lipid composition and organochlorine levels in the tissues of striped dolphin. *Mar. Pollut. Bull.* 19:129-133.
239. Braune BM, Norstrom RJ, Leighton FA, Cattet M, (1990). Tissue distribution and effect of age on bioaccumulation of organochlorines in polar bears. unpublished data.
240. Ronald K, Frank RJ, Dougan JL, Frank R, Braun HE, (1984). Pollutants in harp seals (*Phoca groenlandica*). *Sci. Total Environ.* 38:133-152.

241. Martineau D, Béland P, Desjardins C, Lagacé A, (1987). Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*). *Arch. Environ. Contam. Toxicol.* 16:137-147.
242. Leighton FA, Cattet M, Norstrom RJ, Trudeau S, (1988). A cellular basis for high levels of vitamin A in livers of polar bears (*Ursus maritimus*): the Ito cell. *Can. J. Zool.* 66:480-482.
243. Jönsson C-J, Lund B-O, (1994). *In vitro* bioactivation of the environmental pollutant 3-methylsulphonyl-2,2-bis(4-chlorophenyl)-1,1-dichloroethene in human adrenal gland. *Toxicol. Lett.* 71:169-175.
244. Bergman Å, Brandt I, Jansson B, (1979). Accumulation of methylsulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. *Toxicol. Appl. Pharmacol.* 48:213-220.
245. Yoshida S, Nakamura A, (1979). Residual status after parturition of methylsulfone metabolites of polychlorinated biphenyls in the breast milk of a former employee in a capacitor factory. *Bull. Environ. Contam. Toxicol.* 21:111-115.
246. Yoshida S, Nakamura A, (1977). Studies on a metabolite of PCB (methylsulfone PCB) in mother milk. *J. Food Hyg. Soc.* 18:387-388.
247. Stirling I, Jonkel C, Smith P, Robertson R, Cross D, (1977). The ecology of the polar bear (*Ursus maritimus*) along the western coast of Hudson Bay. *Canadian Wildlife Service Occasional Paper.* 33.
248. Norheim G, Skaare JU, Wiig Ø, (1992). Some heavy metals, essential elements, and chlorinated hydrocarbons in polar bear (*Ursus maritimus*) at Svalbard. *Environ. Pollut.* 77:51-57.
249. Stirling I, (1988). *Polar Bears*. The University of Michigan Press, Ann Arbor, Michigan, pp.61-65.
250. Thomas PE, Reik LM, Ryan DE, Levin W, (1983). Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. *J. Biol. Chem.* 258:4590-4598.
251. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
252. Omura T, Sato R, (1964). The carbon monoxide binding pigment of liver microsomes. I. Evidence for its' heme protein nature. *J. Biol. Chem.* 239:2370-2378.

253. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T, Mayer RT, (1985). Ethoxy-, pentoxy and benzyloxyphenones and homologues: a series of substrates to distinguish between different induced cytochromes P450. *Biochem. Pharmacol.* 34:3337-3345.
254. Burke MD, Thompson S, Weaver RJ, Roland-Wolf C, Mayer RT, (1994). Cytochrome P450 specificities of alkoxyresorufin *O*-dealkylase in human and rat liver. *Biochem. Pharmacol.* 48:923-936.
255. Laemmli UK, (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 207:680-685.
256. Norstrom RJ, Simon M, (1991). Method 5: determination of specific polychlorinated dibenzo-*p*-dioxins and dibenzofurans in biological matrices by gel-permeation-carbon chromatography and gas chromatography-mass spectrometry. In: *Environmental Carcinogens Methods of Analysis and Exposure Measurement. Polychlorinated Dioxins and Dibenzofurans*. Rappe C, Buser HR, Dodet B, O'Neill IK, editors. World Health Organization, International Agency for Research on Cancer, IARC Scientific Publications No. 108, Lyon, pp.281-297.
257. Burke MD, Mayer RT, (1974). Ethoxyresorufin: direct fluorometric assay of a microsomal-*O*-dealkylation which is preferably inducible by 3-methylchloanthrene. *Drug Metab. Dispos.* 2:583-588.
258. Norstrom RJ, Muir DCG, Ford CA, Simon M, Macdonald CR, Béland P, (1992). Indications of P450 monooxygenase activities in beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) from patterns of PCB, PCDD and PCDF accumulation. *Mar. Environ. Res.* 34:267-272.
259. Walsh CT, (1984). Suicide substrates, mechanism-based enzyme inactivators: recent developments. *Annu. Rev. Biochem.* 53:493-535.
260. Bosveld ATC, Gradener J, Murk AJ, Brouwer A, van Kampen M, Evers EHG, van den Berg M, (1995). Effects of PCDDs, PCDFs, and PCBs in common tern (*Sterna hirundo*) breeding in estuarine and coastal colonies in the Netherlands and Belgium. *Environ. Toxicol. Chem.* 14:99-116.
261. Duarte-Davidson R, Harrad SJ, Allen S, Sewart AS, Jones KC, (1993). The relative contribution of individual polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs) to toxic equivalent values derived for bulked human adipose tissue samples from Wales, United Kingdom. *Arch. Environ. Contam. Toxicol.* 24:100-107.

262. Bandiera SM, Torok SM, Letcher RJ, Norstrom RJ, (1995). Immunoquantitation of cytochromes P450 1A and 2B and comparison with chlorinated hydrocarbon levels in archived polar bear liver samples. *Organohalogen Compounds* 25:303-308.
263. Kilburn KH, Thornton JC, (1995). Protracted neurotoxicity from chlordane sprayed to kill termites. *Environ. Health Perspect.* 103:690-694.
264. Matsumura F, Tanaka K, (1984). The molecular basis of neurotoxicity actions of cyclodiene-type insecticides. In: *Cellular and Molecular Neurotoxicology*. Anonymous Raven Press, New York, NY.
265. Angle CR, McIntire MS, Meile RL, (1968). Neurologic sequelae of poisoning in children. *J. Pediatr.* 73:531-539.
266. Goksøyr A, (1991). A semi-quantitative cytochrome P450IA1 Elisa: a simple method for studying the monooxygenase induction response in environmental monitoring and ecotoxicological testing of fish. *Sci. Total Environ.* 101:255-262.

Appendices

Appendix A. CWS unpublished method for the determination of NO-PCBs, PCDDs and PCDFs in Polar Bear Liver

A.1 Sample Extraction

A polar bear liver sample of 5.0 g was homogenized with Na_2SO_4 (1:5 weight ratio) and spiked with ^{13}C -labelled NO-PCBs (CB-77, CB-126 and CB-169), PCDDs (five, tetrachloro- to octachloro-substituted) and PCDFs (four, tetrachloro- to heptachloro-substituted). The amount of internal standard added was dependent on the sample size and hence, the native levels of NO-PCBs, PCDDs and PCDFs. For the ^{13}C -labelled PCDD and PCDF standards, 100 pg were spiked to every 1.0 gram of sample, except for 1,2,3,4,6,7,8,9-OCDD which was 200 pg. The NO-PCB standards, 200 pg of ^{13}C -labelled CB-77, CB-126 and CB-169, were spiked to every 1.0 gram of sample. The spiked sample was transferred to an extraction column and extracted with 100 ml of 50:50 dichloromethane/n-hexane.

A.2 Isolation of NO-PCBs, PCDDs and PCDFs

The volume of the lipid extract was reduced to ca. 1 ml and transferred to a 10 ml graduated vial, followed by 3×1 ml of n-hexane wash. The combined sample volume was adjusted to 5 ml with n-hexane. An accurate volume of 0.5 ml (10 % of the total volume) was removed for lipid determination (see Chapters 4,5 and 6). The volume of the remaining sample was readjusted to 5 ml, and 5 ml of dichloromethane was added for a total of 10 ml.

The lipid extract was then subjected to GPC (see Chapter 2) followed by basic alumina chromatography (10.0 g, eluted with 80 ml of 50:50 dichloromethane/n-hexane). Chlorinated compounds containing a planar structure (eg. NO-PCBs, PCDDs and PCDFs) were retained on a carbon/glass fibre column and back-eluted with 150 ml of toluene. A 1.2% H₂O Florisil column (8.0 g) fractionated NO-PCBs (40 ml, 5:95 dichloromethane/n-hexane) and PCDD/Fs (150 ml, 50:50 dichloromethane/n-hexane).

After isolation of the two separate fractions containing the NO-PCBs and PCDD/Fs and volume reduction; performance standards were added. To the NO-PCB fraction, 200 pg of CB-112 per 1.0 gram of sample was added and the final volume was adjusted to 10 µl. For the PCDD/F fraction, 100 pg per 1.0 gram of sample of ¹³C-1,2,3,4-TeCDD and ¹³C-1,2,3,7,8,9-HxCDD was added and the final volume adjusted to 10 µl.

A.3 Determination of NO-PCBs, PCDDs and PCDFs

The levels of NO-PCBs and PCDD/Fs in each of the fractions were determined by high-resolution GC/MS (HRGC/HRMS) in the EI mode at 70 eV using a VG Autospec double-focusing mass spectrometer (Fisons Instruments, Manchester, UK) coupled with a HP 5890 Series II gas chromatograph. The transfer line and ion source temperatures were both 280°C. GC was performed using a 30 m DB-5MS column (0.25 mm id, J&W Scientific Inc.). The GC conditions were as follows: initial temperature held for 3 min at 100°C, 20°C/min to 180°C and 5°C/min to 325°C. The injector temperature was 260°C. The HRGC/HRMS was operated in the SIM mode. The two largest isotopic ions in the molecular ion cluster were monitored for each NO-PCB, PCDD and PCDF. The peak area response in

the resulting mass chromatogram was the additive response of these two isotopic ions of the molecular ion cluster.

Two external standard solutions were prepared for quantitation of NO-PCBs and PCDD/Fs. The first solution contained the same concentration of ^{13}C -labelled NO-PCB internal standards and CB-112 performance standard that was spiked to the sample. In addition, 200 pg per 1.0 g of sample of ^{12}C -unlabelled CB-77, CB126 and CB-169 was added. The second solution was prepared similarly, except 100 pg per 1.0 gram of sample of the ^{12}C -unlabelled PCDD and PCDF standards were added instead of the NO-PCBs and 100 pg per 1.0 gram of sample of ^{13}C -1,2,3,4-TeCDD and ^{13}C -1,2,3,7,8,9-HxCDD were added instead of CB-112. The amount of standards added to this solution was scaled so that the final concentrations were similar to the standard concentrations in the sample. Any small differences in standard concentrations between the external standard solution and the sample was corrected for by the normalization standard. The mass chromatogram response ratio between the ^{13}C -labelled and ^{12}C -unlabelled standard compounds in the external standard solution were determined and compared to the same ratios in the sample fraction to quantitate the unknown NO-PCB, PCDD and PCDF concentrations. The ^{13}C -labelled and ^{12}C -unlabelled response ratios are verified daily and just prior to a sequence if the number of samples is large. The ratios of the two strongest ions in the molecular ion cluster were also monitored on a daily basis to confirm the chlorine substitution of a given NO-PCB, PCDD or PCDF and thus, the compound identity. Peaks identified as PCDDs or PCDFs and without a ^{13}C -labelled surrogate were quantitated using the peak ratio of the most closely eluting PCDD or PCDF for which a ^{13}C -labelled surrogate was added.

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