# Immunoquantitation of Cytochromes P450 1A and 2B and Comparison with Chlorinated Hydrocarbon Levels in Archived Polar Bear Liver Samples

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## 1. Introduction

The Arctic Ocean is subject to considerable influx of atmospherically transported anthropogenic pollutants including chlorinated biphenyls (PCBs) and organochlorine pesticides. Polar bears (*Ursus maritimus*), as obligate carnivores, are at the top rung of the arctic marine food chain and subject to bioaccumulation of lipophilic halogenated hydrocarbons that have been linked to reproductive failures in other marine mammals[1]. Polar bears are widely distributed throughout the arctic circumpolar regions with individual bears ranging over large expanses of frozen ocean in search of food. In addition, tissue specimens can be obtained from the regular Inuit hunt without the need to kill bears for sampling purposes. For these reasons the polar bear is an excellent species for monitoring arctic marine ecosystem health. Moreover, because the Inuit also eat marine mammals and their diet is high in fat, the polar bear is ideally suited as a surrogate for human exposure.

A common and characteristic biochemical response to halogenated hydrocarbon exposure that accompanies and often precedes toxicity in all animals examined thus far is the induction of hepatic microsomal cytochromes P450[2]. Because cytochrome P450 enzymes play a critical role in xenobiotic metabolism and therefore, in the bioacumulation and potential toxicity of halogenated hydrocarbons found in the food chain, the amounts of individual isozymes present in liver are important determinants of susceptibility to environmental contaminant exposure. Measurement of cytochrome P450 enzyme induction in wild animal populations has been suggested as a sensitive biochemical marker of contaminant exposure, and by inference, of ecosystem health[3].

The goal of our research is to determine if measurement of cytochrome P450 levels in free-ranging polar bears is useful as an indicator of environmental contamination. Polar bear liver samples are routinely available through aboriginal hunters, but it is difficult to obtain tissue specimens in a manner that preserves enzyme activity. However, tissues stored at normal freezer temperatures are relatively easy to obtain. Moreover, antibodies offer an alternative means to measure the levels of specific cytochrome P450 isozymes in tissues that have not been collected and stored under favourable conditions. The purpose of the present study was to immunoquantitate cytochromes P450 1A and 2B in archived liver tissue and to compare levels of the proteins with hepatic concentrations of PCBs, chlorinated benzenes (CBz) and organochlorine pesticides that had been measured previously[4].

## 2. Methods

Liver Samples: Liver samples from 44 female and male polar bears, aged 3 to 29 years, were obtained from the Canadian Wildlife Service Specimen Bank. The samples had been obtained by Inuit hunters in the 1982-1984 hunting seasons from various regions of the Canadian arctic archipelago, as previously described[5]. The samples had been subjected to a variety of freezings and thawings and were stored at -40°C for 9-10 years before they were transferred to the University of British Columbia where they were kept at -80°C. Hepatic microsomal fractions were prepared by differential centrifugation. The microsomal pellets were suspended in 0.25 M sucrose and aliquots of the suspension were stored at -80°C until used.

Determination of cytochrome P450 and protein: Total cytochrome P450 content was determined from the carbon monoxide difference spectrum using the method of Omura and Sato[5]. Protein concentration was measured by the method of Lowry *et al.*[6].

Preparation of antibodies: Polyclonal antibody against rat cytochrome P450 2B1 was raised in female New Zealand rabbits immunized with the eletrophoretically homogeneous protein. Polyclonal antisera against rat cytochrome P450 1A1 was provided by Dr. Paul Thomas, Rutgers University (Piscataway, NJ).

Immunoblots and densitometric quantitation: Polyacrylamide gel electrophoresis (PAGE) was performed essentially as described by Laemmli[7]. Microsomal proteins resolved on SDS-PAGE were transferred electrophoretically to nitrocellulose and probed with antibodies as described by Towbin *et al.*[8]. Blots were incubated with antisera to cytochrome P450 1A1 at a dilution of 1:1000 or anti-cytochrome P450 2B1 IgG at a concentration of 3 μg IgG/ml. Bound primary antibody was located using horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody. Immunoreactive proteins were subsequently detected by reaction of peroxidase with a substrate solution of 4-chloro-1-naphthol (0.018%) and hydrogen peroxide (0.018%) in phosphate-buffered saline, pH 7.4. The staining intensities of the bands were quantitated using a Visage 110 Bio Image Aalyzer (Bio Image, Ann Arbor, MI) consisting of a high resolution camera

and a computer workstation and normalized with a rat hepatic microsomal sample that was included on every gel.

Chemical analysis: Extraction and separation of organochlorines from homogenized liver samples, and the identification and quantitation of the various compounds was described previously[4].

Statistical analysis: Data are presented as the mean±standard error of the mean of values determined from 44 individual animals. Correlations between hepatic chlorinated hydrocarbon concentrations and cytochrome P450 1A or P450 2B levels were analyzed by simple linear regression. Coefficients of variation ($r^2$) with a $p$ value $<0.05$ were considered statistically significant.

### 3. Results

Table 1 lists the mean values of the microsomal protein yield and cytochrome P450 content for microsomes prepared from 44 archived polar bear liver samples. The total cytochrome P450 content was highly variable among the bears and in all samples a large peak at 420 nm was detected and measured. Carbon monoxide difference spectra obtained in the absence of sodium dithionite indicated the presence of variable amounts of contaminating hemoglobin in many of the preparations, precluding the use of cytochrome P450 content as a reliable basis for comparison among the animals.

Table1. Total Cytochrome P450 Content, P420 Content, and Cytochrome P450 1A and Cytochrome P450 2B Levels in Archived Polar Bear Liver Samples

| Parameters | Mean | SEM | Range |
|---|---|---|---|
| Age (year) | 7.39 | 0.88 | 3-29 |
| Microsomal protein yield (mg/g liver) | 4.75 | 0.39 | 1.76-14.68 |
| Total cytochrome P450 content (nmol/mg protein) | 0.18 | 0.05 | 0-1.69 |
| Total P420 content (nmol/mg protein) | 1.04 | 0.08 | 0.31-2.54 |
| Amount of immunoreactive cytochrome P450 1A[a] | 1.40 | 0.36 | 0-13.21 |
| Amount of immunoreactive cytochrome P450 2B[a] | 6.06 | 1.26 | 0-32.95 |

[a] Values were determined by densitometric analysis of immunoblots probed with antibody against rat cytochrome P450 1A or against rat cytochrome P450 2B and expressed as the integal of the optical density of the band per milligram protein.

As shown in Table 1, the mean level of cytochrome P450 2B was significantly greater than the mean level of cytochrome P450 1A. Although antibody to rat cytochrome P450 1A1 detected two immunoreactive protein bands in a few of the polar bear microsomal preparations, only the band with the faster electrophoretic migration, identified previously[9] as cytochrome P450 1A1, was stained sufficiently strongly to allow densitometric quantitation in the majority of samples.

Hepatic organochlorine residue levels expressed on a lipid weight basis are listed in Table 2. The predominant compounds found in polar bear liver were chlordanes followed by PCBs. Four di-*ortho*-chlorinated congeners namely, CB-138, CB-153, CB-170 and CB-

170 comprised approximately 81%, while CB-153 alone accounted for 42%, of the total PCBs present.

Table 2. Mean Concentrations of Organochlorines in Archived Polar Bear Liver Samples

| Compound | Mean | SEM | Range |
|---|---|---|---|
| s-PCB (ng/g lipid) | 17459 | 1831 | 2285-63612 |
| CB-138 (ng/g lipid) | 3579 | 318 | 860-9237 |
| CB-153 (ng/g lipid) | 7460 | 715 | 1500-23720 |
| CB-170 (ng/g lipid) | 2279 | 345 | 240-13558 |
| CB-180 (ng/g lipid) | 4219 | 555 | 785-22399 |
| s-CBz (ng/g lipid) | 344 | 40 | 94-1465 |
| s-DDT (ng/g lipid) | 5078 | 848 | 305-32151 |
| s-chlordane (ng/g lipid) | 60031 | 5562 | 10111-135771 |
| Dieldrin (ng/g lipid) | 6027 | 569 | 256-17302 |

The relationship between cytochrome P450 1A or cytochrome P450 2B levels and hepatic concentrations of various organochlorines was examined. As shown in Figure 1, a significant positive correlation was found between the amount of immunoreactive cytochrome P450 1A and the s-PCB levels ($r^2=0.37$, $p<0.05$). The best correlation between the amount of immunoreactive cytochrome P450 2B and organochlorine concentrations was observed with s-chlordane ($r^2=0.13$, $p<0.05$) and CB-153 ($r^2=0.11$, $p<0.05$) (Figure 2).

Figure 1. Correlation Between P450 1A1 and s-PCB Levels In Archived Polar Bear Liver Microsomes

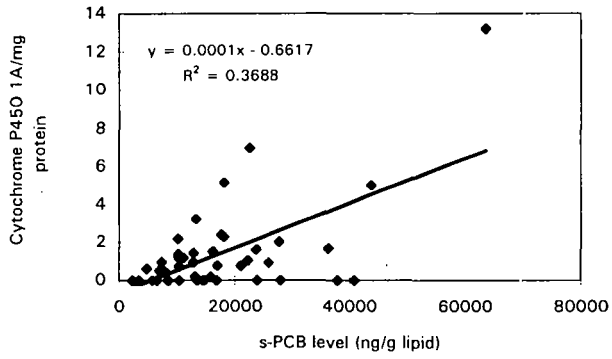
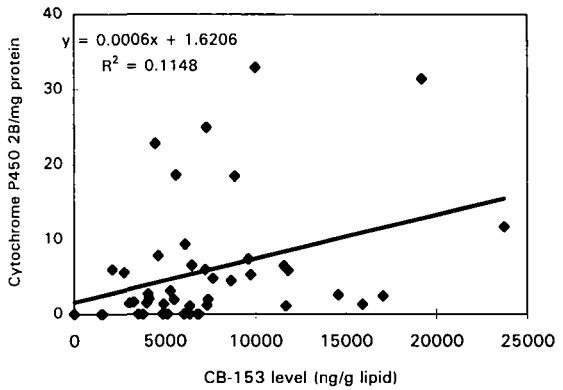


Figure 2. Correlation Between P450 2B and CB-253 Levels in Archived Polar Bear Liver Microsomes




## 4.Discussion

Using tissue samples from freshly killed polar bears, we previously characterized the polar bear hepatic cytochrome P450 system and showed that rat cytochrome P450 1A, 2B, 2C and 3A isozymes were immunochemically cross-reactive with polar bear forms of cytochrome P450[10]. More recently, we demonstrated that hepatic levels of cytochrome P450 1A correlated strongly with tissue concentrations of Ah-receptor active PCBs, PCDDs and PCDFs and their corresponding toxic equivalents, while cytochrome P450 2B content correlated with hepatic levels of chlordanes and methyl-sulphone PCB metabolites[9]. The present study, using polar bear liver samples collected and archived at less than favourable conditions, demonstrated a similar relationship between cytochrome P450 1A content and s-PCB levels, as well as between cytochrome P450 2B content and s-chlordane and CB-153 levels. However, the correlations obtained were much weaker than those determined previously[9]. Although levels of s-PCDDs, s-PCDFs and PCB congeners that are strong inducers of cytochrome P450 1A were not available for the polar bears analyzed herein, tissue concentrations for these compounds are probably relatively low (<1ng/g lipid), as reported for the 16 bears analyzed by Letcher *et al.*[9]. Moreover, because the contribution of s-PCDDs and s-PCDFs to induction of cytochrome P450 1A was less than 10% in that study[9], s-PCBs is an adequate measure of toxic equivalents. On the other hand, it was somewhat surprising that cytochrome P450 2B content did not correlate more strongly with tissue concentrations of s-chlordane and CB-153, a PCB congener classed as a pure cytochrome P450 2B inducer. Mean cytochrome P450 1A and cytochrome P450 2B levels in polar bear liver samples obtained and stored at optimal conditions that preserve enzyme actitivty (*i.e.* -80°C) were 3.5 times higher than mean levels in the present study despite similar

organochlorine levels[9], suggesting that nonspecific loss of cytochromes P450 by proteolytic digestion occurred during collection and storage of the samples in this study. In conclusion, the results indicate that liver homogenates stored at -40°C, or higher temperatures, cannot be used reliably for determination of cytochrome P450 levels.

**5. References**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