SUPPORTING INFORMATION

Contamination and Effects of Perfluorochemicals in Baikal Seal (*Pusa sibirica*) II: Molecular Characterization, Expression Level, and Transcriptional Activation of Peroxisome Proliferator-Activated Receptor α

Hiroshi Ishibashi [1], Hisato Iwata [1,*], Eun-Young Kim [1], Lin Tao [2], Kurunthachalam Kannan [2], Shinsuke Tanabe [1],Valeriy B. Batoev [3], and Evgeny A. Petrov [4]

[1]Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama 790-8577, Japan

[2]Wadsworth Center, State University of New York, Albany, New York 12201-0509, USA

[3]Baikal Institute of Nature Management, Siberian Branch of Russian Academy of Sciences, Ulan-Ude, Buryatia 670047, Russia

[4]The Eastern-Siberian Scientific and Production Fisheries Center, "VOSTSIBRYBCENTR," Ulan-Ude, Buryatia 670034, Russia

Microsomal Preparation and Immunoblot Analysis. Microsomal preparation of 16 seal livers was performed according to the method of Iwata et al. *(27)* and Hirakawa et al. *(13)*. Briefly, approximately 5 g of frozen liver tissue were homogenized in 5 volumes of cold buffer (50 mM Tris-HCl, 0.15 M KCl, adjusted to pH 7.4-7.5 at 25˚C) with a Teflon-glass homogenizer, and were centrifuged at 750 x *g* for 10 min at 4˚C. The nuclear pellets were removed, and the supernatant was then centrifuged at 12,000 x *g* for 10 min at 4˚C; and the supernatant was further centrifuged at 105,000 x *g* for 1.5 h at 4˚C. Following centrifugation, the supernatant (cytosol fraction) was removed, and the microsomal pellet was resuspended in an equivalent volume of resuspension buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 20% (v/v) glycerol, pH 7.4-7.5). Protein content in the microsomal fraction was determined by the bicinchoninic acid method, using bovine serum albumin as a standard *(28)*.

Immunoblot analysis for determination of hepatic CYP4A-like protein was performed as previously described *(13, 27)* with some modifications. Protein (20 µg per lane) in the microsomal fraction was resolved by electrophoresis on a sodium dodecyl sulfate polyacrylamide gel (5-20% concentration gradient; ATTO Co., Tokyo, Japan), and was electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was reacted with anti-human CYP4A11 polyclonal antibody (Fitzgerald Industries International, Inc. MA, USA), and then conjugated with a secondary antibody, anti-rabbit IgG-horseradish peroxidase (HRP). Detection of the protein cross-reacted with antibody was performed using highly sensitive enhanced chemiluminescence (Amersham Biosciences). The signal intensity of each band was measured using a

ChemiDoc system (Bio-Rad Laboratories, Hercules, CA). The expression level of CYP4A-like protein in each sample was expressed as a value relative to staining intensity from the antibody cross-reactive protein in a given sample.

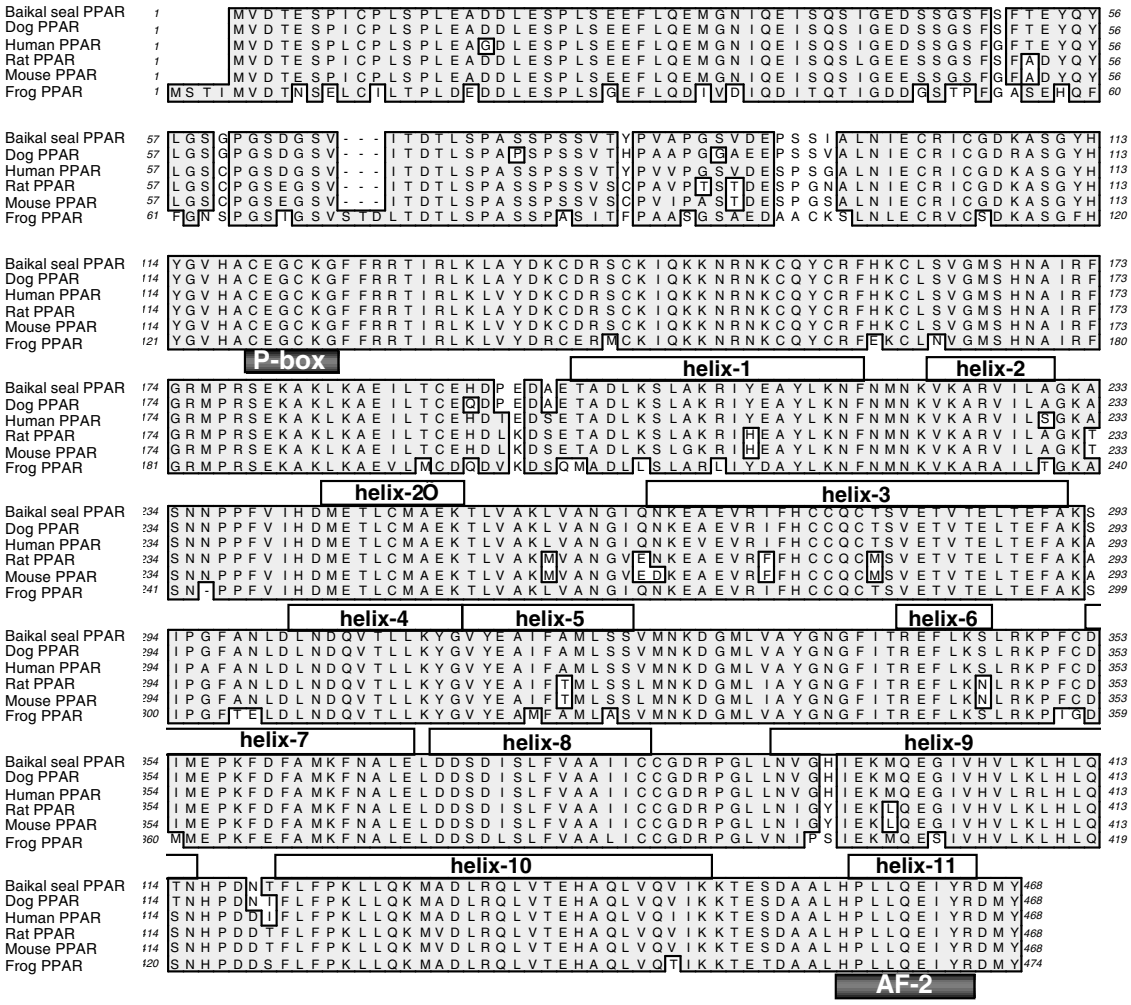

Figure S1. Alignment of amino acid sequence deduced from Baikal seal, dog, human, rat, mouse and frog PPARα cDNAs. Accession numbers used are: dog (AF350327), human (L02932), rat (M88592), mouse (AK035676) and frog (M84161).

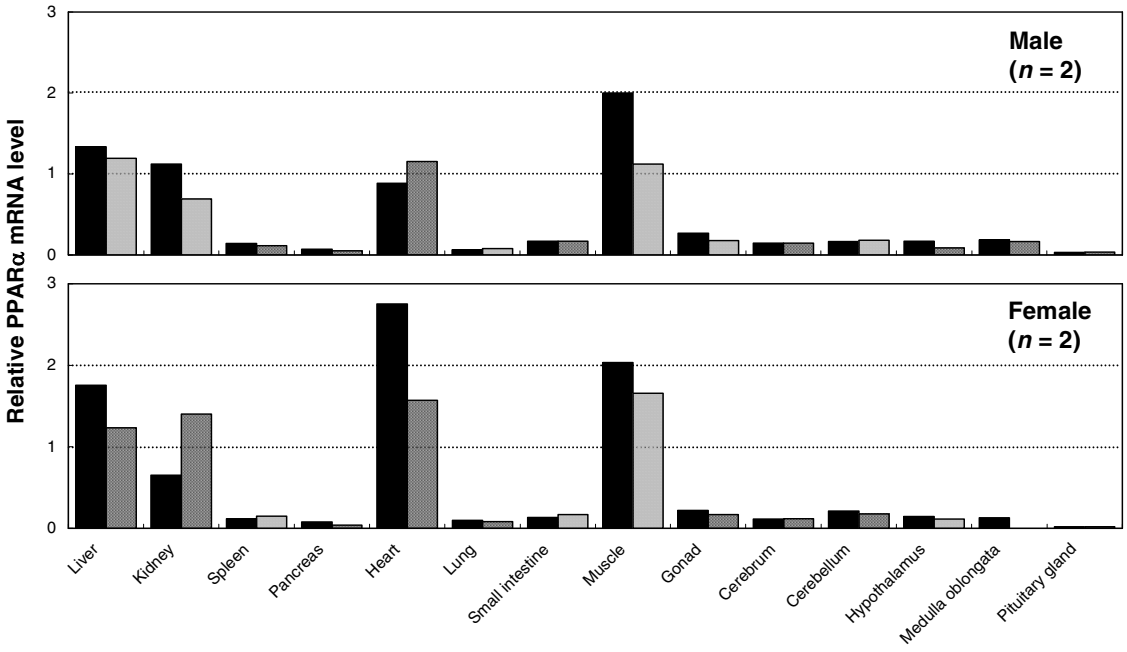

Figure S2. Tissue expression profiles of Baikal seal PPARα mRNA. Expression levels of mRNAs were quantified using a real-time RT-PCR method. The relative PPARα mRNA levels were normalized to 18S ribosomal RNA content. The results are expressed as the individual mRNA expression level of two female and two male seals.

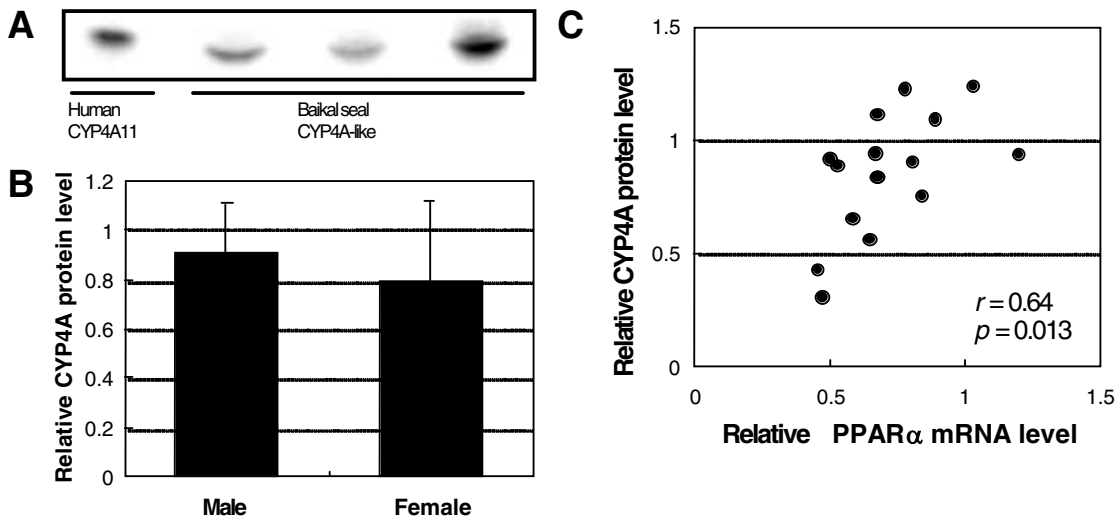

Figure S3. Band image of CYP4A-like protein expressions in the hepatic microsomes of Baikal seals using anti-human CYP4A11 antibody (A), gender difference in the CYP expression levels (n = 16) (B), and relationship between the expression levels of hepatic PPARα mRNA and CYP4A-like protein by Spearman's rank correlation test (n = 16) (C).