Evaluation of potential bioaccummulative compounds exerting endocrine-disrupting activities in wild animals using *in vitro* bioassays and chemical fractionation

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Introduction

Environmental pollution by anthropogenic chemicals is one of the most pressing global problems, which have international attention as environmental concern and it is closely related with quality of life of human beings and wild animals. About fifty million chemicals have been produced industrially, and it is estimated that a huge number of artificial chemicals is released to the environment and will be accumulated in wild animals via food the web. If accumulated compounds in wild animals exert any toxicity such as endocrine-disruption, such bioaccumulative toxic chemicals should be determined and monitored. However, it is difficult for monitoring with only instrumental analysis to target on important compounds selected from the huge number of chemicals due to lack of toxicity information for all compounds. Therefore, our research has been focused on using *in vitro* bioassay together with chemical fractionation in an attempt to determine the existence and activity profile of potential bioaccumulative pollutants in wild animals for future monitoring studies.

In this study, accumulated compounds in blubbers and/or livers of Baikal seal, Common cormorant, Raccoon dog and Finless porpoise were extracted and subjected to chemical fractionation for subsequent *in vitro* bioassays. As *in vitro* bioassays, a panel of rat and human cell-based CALUX reporter gene bioassays was utilized to evaluate steroidal hormone-disrupting potency (androgen receptor (AR), estrogen receptor alpha (ERa), glucocorticoid receptor (GR), and progesterone receptor (PR)-mediated activities), dioxin-like toxicity (aryl hydrocarbon receptor (AhR) -mediated activity) and lipid metabolism-disrupting potency (peroxisome proliferator-activated receptor gamma (PPARg) -mediated activity) in fractionated extracts.

Materials and Method

Applied wildlife samples

Baikal seal
(*Phoca sibirica*)

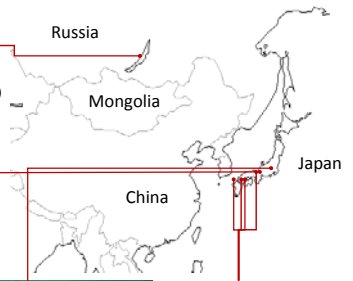
Tissue: Liver (*n*=6: 1992, *n*=10: 2005), Blubber (*n*=10: 1992, *n*=10: 2005)
Sampling year: 1992, 2005
Location: Lake Baikal, Russia
Characteristics: Fish eater, top predator

Common cormorant
(*Phalacrocorax carbo*)

Tissue: Liver (*n*=10)
Sampling year: 2002
Location: Lake Biwa, Japan
Characteristics: Fish eater, top predator

Raccoon dog
(*Nyctereutes procyonoides*)

Tissue: Liver (*n*=10)
Sampling year: 2001
Location: Kanagawa, Japan
Characteristics: Omnivorous feeder
High-order predator

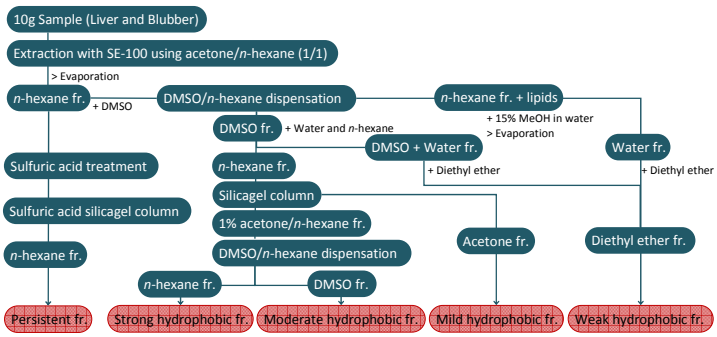


Finless porpoise
(*Neophocaena Phocaenoides*)

Tissue: Liver (*n*=10)
Sampling year: 2005 to 2007
Location: Hyogo, Ehime, Oita, Nagasaki, Japan
Characteristics: Fish eater, top predator

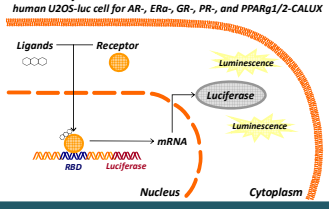
Extraction and chemical fractionation

Using 25 chemicals including brominated flame retardants (BFRs), polyaromatic hydrocarbons (PAHs) and hormonal agents, an extraction and chemical fractionation scheme was established according to fractionation characteristics of applied pure chemicals, , and applied to above-mentioned wild animal samples.



Screening of potential bioaccumulative compounds exerting endocrine-disrupting activities using CALUX assays

Agonistic and antagonistic responses of all wild animal extracts were evaluated with human U2OS-*luc* cell line for AR-, ERa-, GR-, PR-, PPARg1-, and PPARg2-mediated responses and a DR-CALUX cell line for AhR-mediated response. The CALUX assay procedures have been described for U2OS-*luc* (van der Linden et al. 2008) and DR-CALUX (Suzuki et al. 2007).



Conclusion

This study clearly indicates that potential bioaccumulative compounds exerting various endocrine-disrupting activities such as the steroidal hormone-disruption potency, dioxin-like toxicity and lipid metabolism-disruption potency exist in the investigated wild animals.

Results and Discussion

In this study, accumulated compounds exerting endocrine-disrupting activities in blubber and/or liver of wild animals such as Baikal seal, Common cormorant, Raccoon dog, and Finless porpoise were evaluated with several CALUX assays. As a result, agonistic and/or antagonistic (synergistic) responses were observed in some extracts prepared from wild animals as follows.

	Agonistic activity					Antagonistic activity				
AR-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	NA	7.1.E-03	1.1.E-03	1.1.E-03	4.3.E-04	1.1.E-03	7.1.E-03	1.1.E-03	1.1.E-03
Baikal seal (1992)-Blubber	4.5.E-04	NA	2.3.E-03	3.4.E-04	1.0.E-03	4.2.E-04	2.8.E-04	2.3.E-03	3.4.E-04	1.0.E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-03	7.3.E-03	5.8.E-03	5.0.E-03	1.5.E-02	1.5.E-03	7.3.E-03
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	2.8.E-03	1.2.E-03	1.6.E-03	1.2.E-03	9.3.E-03	2.8.E-03	1.2.E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	1.2.E-02	1.9.E-03	1.2.E-03	9.3.E-03	2.8.E-04	1.2.E-02
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	2.8.E-03	1.2.E-02	1.9.E-03	1.2.E-02	9.3.E-03	8.3.E-04	1.2.E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02	1.9.E-03	4.1.E-04	9.3.E-03	8.3.E-04	1.2.E-02
ERa-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	1.1E-02	7.1.E-03	NA	1.1E-03	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	2.6E-03	2.3.E-03	NA	1.0E-03	4.5E-04	2.6E-03	7.7E-03	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	4.9.E-03	7.3.E-03	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	1.2E-02	NA	NA	1.2.E-03	1.9E-03	1.2E-02	3.1E-03	2.8E-04	1.2E-03
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	3.4E-03	1.9E-03	1.2E-03	9.3E-03	2.8E-04	NA
Raccoon dog-Liver	1.9.E-03	NA	9.3.E-04	NA	2.2E-03	1.9E-03	1.2E-02	9.3E-03	2.8E-04	NA
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	4.1E-03	1.9E-03	4.1E-04	9.3E-03	2.8E-04	NA
GR-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	1.1E-02	7.1.E-03	1.1.E-03	1.1.E-03	4.5E-04	1.1E-02	2.1E-02	1.1E-03	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	2.6E-03	2.3.E-03	3.4.E-04	1.0.E-03	4.5E-04	2.6E-03	2.3E-02	1.0E-03	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	1.5E-02	1.5E-02	4.9.E-03	7.3.E-03	1.8E-03	1.5E-02	1.5E-02	1.5E-03	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	4.1E-03	NA	2.8.E-03	NA	1.9E-03	4.1E-03	3.1E-03	8.3E-04	4.1E-04
Common cormorant-Liver	1.9.E-03	NA	3.1.E-03	2.8.E-03	1.2.E-02	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9.E-03	4.1E-03	NA	2.8.E-03	1.2.E-02	1.9E-03	4.1E-03	9.3E-03	8.3E-04	1.2E-02
Finless porpoise-Liver	1.9.E-03	NA	3.1.E-03	8.3.E-04	1.2.E-02	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
PR-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	NA	7.1.E-03	NA	1.1E-03	4.5E-04	1.1E-02	2.1E-02	3.6E-04	1.1E-03
Baikal seal (1992)-Blubber	4.5.E-04	NA	2.3.E-03	NA	NA	4.5E-04	7.7E-04	2.3E-02	3.4E-04	1.0E-03
Baikal seal (2005)-Liver	1.8.E-03	NA	NA	NA	NA	1.8E-03	5.0E-03	1.5E-02	4.9E-04	2.2E-03
Baikal seal (1992)-Liver	1.9.E-03	NA	NA	NA	NA	1.9E-03	1.2E-03	3.1E-03	2.8E-04	1.2E-04
Common cormorant-Liver	1.9.E-03	NA	NA	2.8.E-03	NA	1.9E-03	1.2E-03	9.3E-03	8.3E-04	1.2E-02
Raccoon dog-Liver	1.9.E-03	NA	NA	NA	1.2E-02	1.9E-03	4.1E-03	3.1E-03	2.8E-04	1.2E-02
Finless porpoise-Liver	1.9.E-03	NA	NA	NA	1.2E-02	1.9E-03	4.1E-04	9.3E-03	2.8E-04	1.2E-02
DR-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	1.5.E-05	1.1.E-04	NA	NA	3.6E-05	NA	NA	2.1.E-02	3.6.E-04	NA
Baikal seal (1992)-Blubber	1.4.E-05	2.6.E-05	NA	NA	1.0E-03	NA	NA	7.7.E-03	3.4.E-04	NA
Baikal seal (2005)-Liver	6.1.E-05	1.5.E-04	5.0.E-03	NA	2.2E-03	NA	NA	NA	4.9.E-04	NA
Baikal seal (1992)-Liver	1.9.E-05	4.1.E-05	NA	NA	1.2E-04	NA	NA	3.1.E-03	2.8.E-04	NA
Common cormorant-Liver	1.9.E-05	4.1.E-05	NA	NA	NA	NA	NA	9.3.E-03	2.8.E-04	1.2.E-02
Raccoon dog-Liver	6.4.E-05	1.2.E-04	NA	NA	NA	NA	NA	3.1.E-03	8.3.E-05	1.2.E-02
Finless porpoise-Liver	1.9.E-04	NA	9.3.E-04	8.3.E-05	4.1E-03	NA	1.2.E-04	NA	NA	NA
PPARg1-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	1.1.E-03	7.1.E-03	1.1.E-04	3.6.E-04	4.5.E-04	NA	NA	NA	NA
Baikal seal (1992)-Blubber	4.5.E-04	2.6.E-04	2.3.E-03	1.0.E-04	3.4.E-04	4.5.E-04	NA	NA	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	1.5.E-03	1.5.E-02	4.9.E-04	2.2.E-04	1.8.E-03	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	4.1.E-04	3.1.E-03	2.8.E-04	1.2.E-04	1.9.E-03	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	1.2.E-04	9.3.E-04	8.3.E-05	4.1.E-03	1.9.E-03	NA	NA	NA	NA
Raccoon dog-Liver	1.9.E-03	1.2.E-03	3.1.E-03	8.3.E-05	1.2.E-02	1.9.E-03	NA	NA	NA	NA
Finless porpoise-Liver	1.9.E-03	1.2.E-04	3.1.E-03	8.3.E-05	1.2.E-03	1.9.E-03	NA	NA	NA	NA
PPARg2-CALUX	Persistent fraction	Strong	Moderate	Mild	Weak	Persistent fraction	Strong	Moderate	Mild	Weak
		Crude hydrophobic fraction				Crude hydrophobic fraction				
Baikal seal (2005)-Blubber	4.5.E-04	1.1.E-03	7.1.E-03	3.6.E-05	3.6.E-05	NA	NA	NA	NA	NA
Baikal seal (1992)-Blubber	4.5.E-04	7.7.E-05	7.7.E-04	3.4.E-05	1.0.E-04	NA	NA	NA	NA	NA
Baikal seal (2005)-Liver	1.8.E-03	1.5.E-03	5.0.E-03	4.9.E-05	7.3.E-05	NA	NA	NA	NA	NA
Baikal seal (1992)-Liver	1.9.E-03	1.2.E-04	9.3.E-04	2.8.E-05	4.1.E-05	NA	NA	NA	NA	NA
Common cormorant-Liver	1.9.E-03	1.2.E-04	3.1.E-04	2.8.E-05	1.2.E-03	NA	NA	NA	NA	NA
Raccoon dog-Liver	1.9.E-03	4.1.E-04	3.1.E-04	8.3.E-06	4.1.E-03	NA	NA	NA	NA	NA
Finless porpoise-Liver	1.9.E-03	1.2.E-04	3.1.E-04	8.3.E-06	4.1.E-04	NA	NA	NA	NA	NA

NA: Not analyzed due to ago/antagonistic response	Response at **more than 1.0E-02** g-wet/well
Not detected at indicated dose	Response at **1.0E-2 to 1.0E-03** g-wet/well
Cytotoxicity at indicated dose	Response at **1.0E-3 to 3.0E-04** g-wet/well
Synergistic response at indicated dose	Response at **less than 3.0E-04** g-wet/well

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