

Endocrine disruptors in wildlife



Dead birth



Deformity



Deformity

Relation with contaminants?



Mass mortality



Deformity



Cancer

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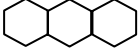
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Introduction

- Based on outcomes of monitoring research for environmental pollutants, the persistent organic pollutants (POPs) have been banned in the Stockholm Convention which took effect in 2004.
- Priority compounds based on toxicological point of view?

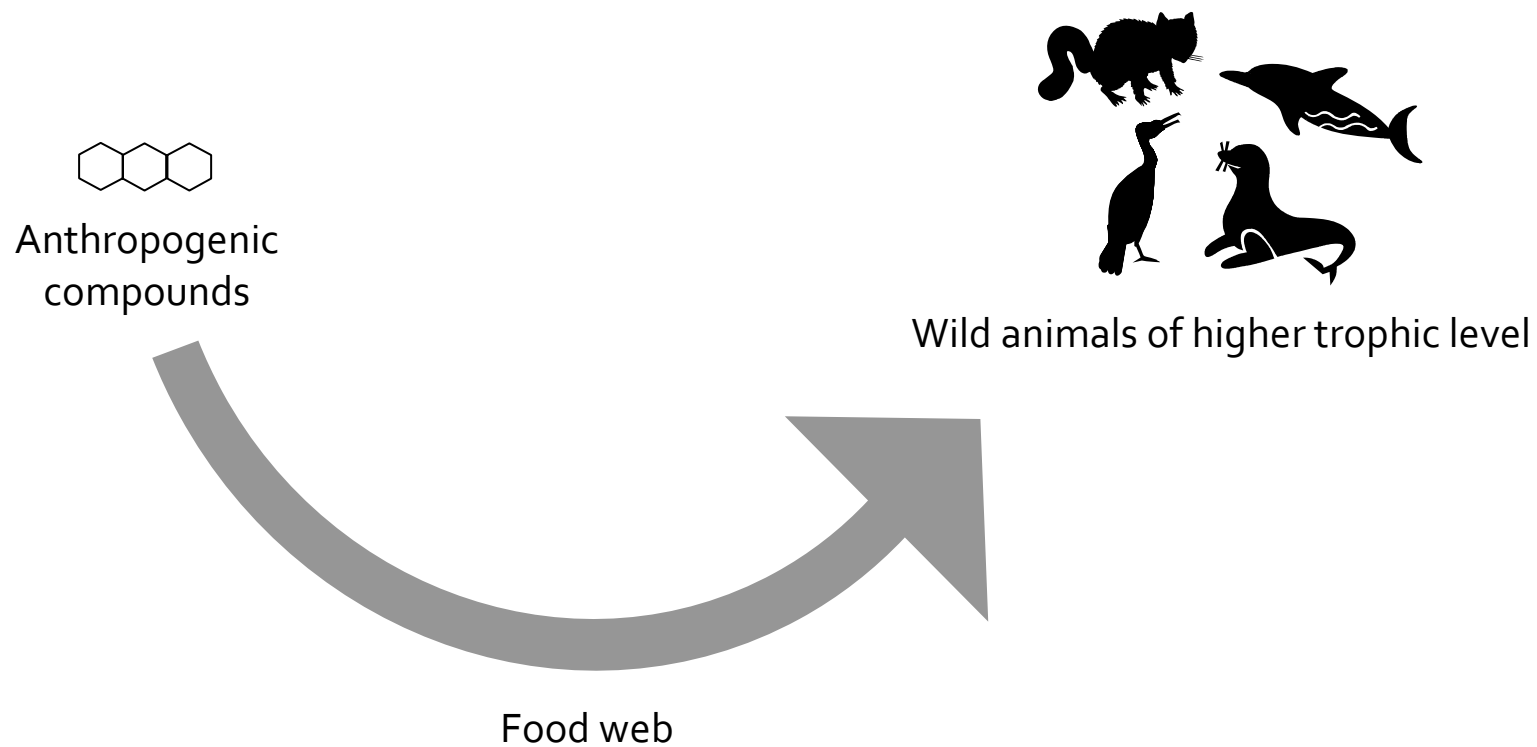

Artificial
compounds

- > Impurity?
- > By-products?
- > Toxic metabolites?


Environment
Wild animals of higher trophic level

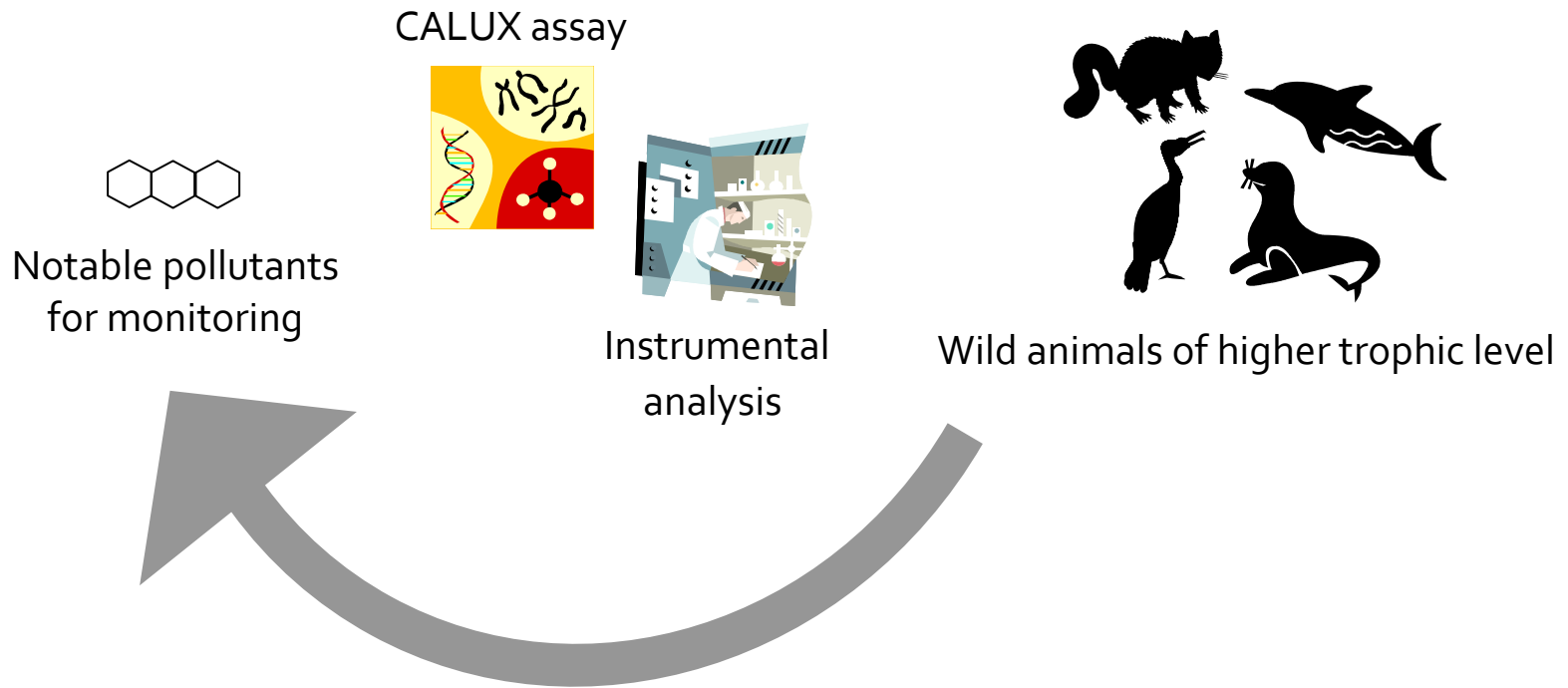
Food web

Our target is all kinds of accumulated compounds



Purpose of this study

- Final goal of this study is to reveal existence and profile of notable pollutants accumulated in wild animals of higher trophic level using CALUX assay and instrumental analysis.



Experimental section

Experimental Section: Applied animal samples

Russia

Sampling point

Mongolia

China

Japan

Mass mortality of Baikal seal
Year 1987/88
 $n=8,000$ (10% of population)

Baikal seal (*Phoca sibirica*)



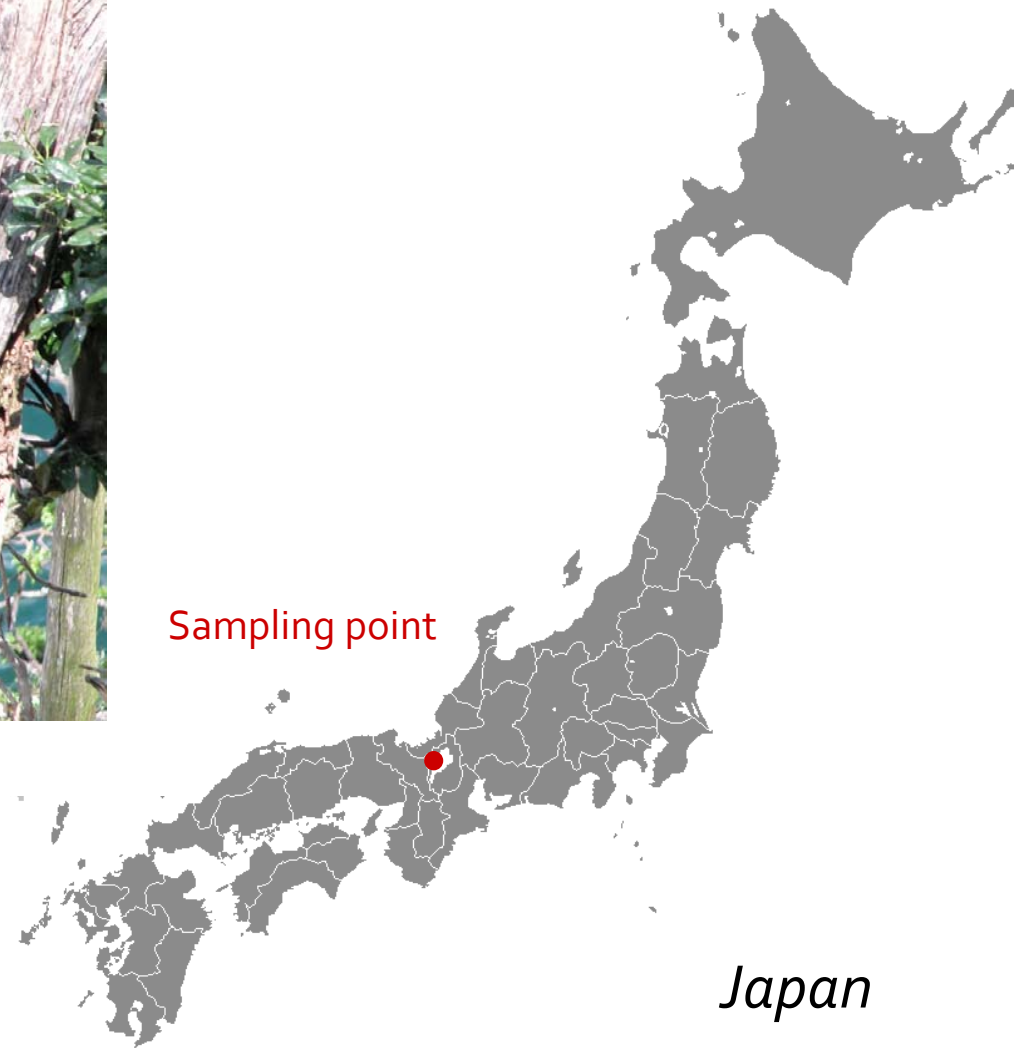
Tissue:	Liver ($n=6$: 1992, $n=10$: 2005), Blubber ($n=10$: 1992, $n=10$: 2005)
Sampling year:	1992, 2005
Location:	Lake Baikal, Russia
Characteristics:	Fish eater Top predator

Experimental Section: Applied animal samples

Common cormorant (*Phalacrocorax carbo*)



Tissue:	Liver (n=10)
Sampling year:	2002
Location:	Lake Biwa, Japan
Characteristics:	Fish-eating bird Top predator



Sampling point

Japan

Experimental Section: Applied animal samples

Raccoon dog (*Nyctereutes procyonoides*)



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

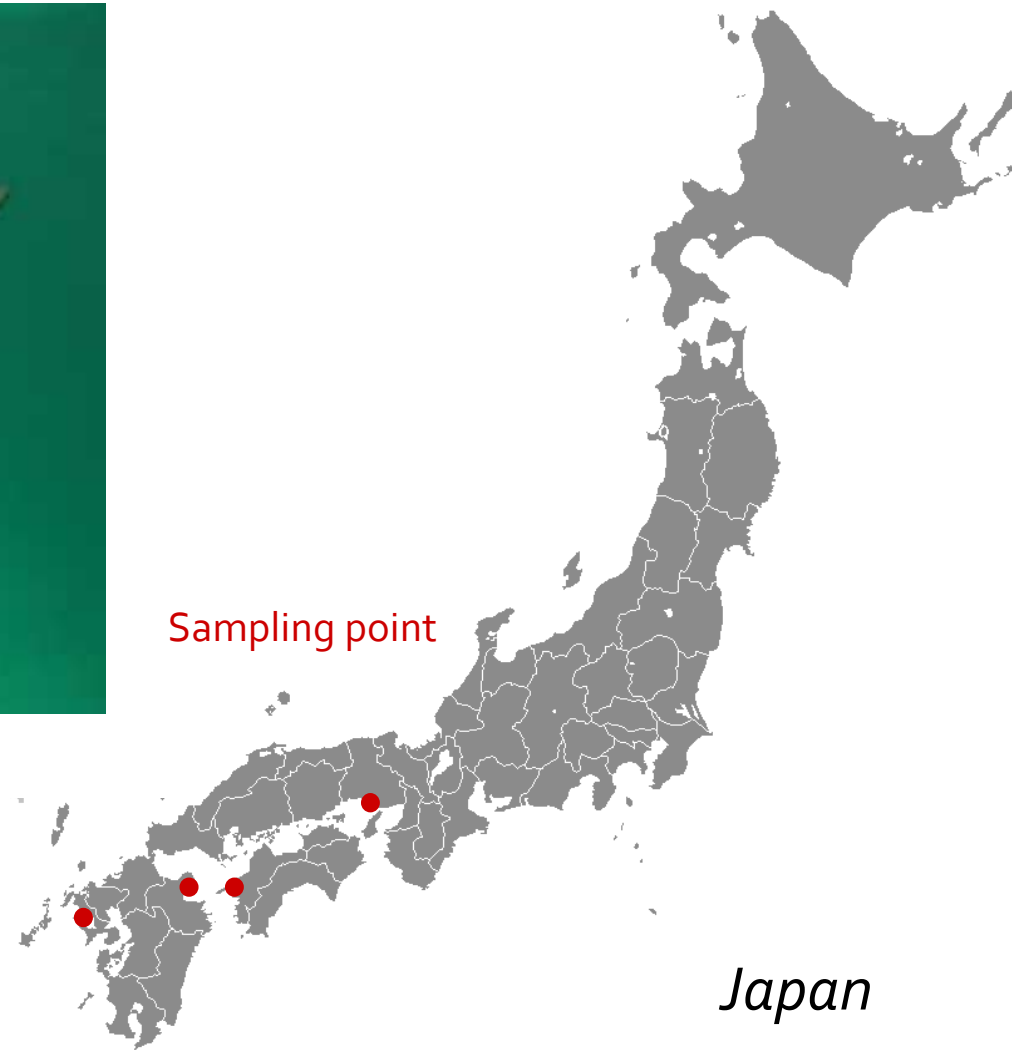


Experimental Section: Applied animal samples

Finless porpoise (*Neophocaena phocaenoides*)



Tissue:	Liver ($n=10$)
Sampling year:	2005~2007
Location:	Kanagawa, Japan
Characteristics:	Fish eater Top predator



Sampling point

Japan

Experimental Section: Applied animal samples



Blubber (2005): 96~540 pg WHO-TEQ/g lipid (n=10)
Blubber (1992): 120~840 pg WHO-TEQ/g lipid (n=10)
Liver (2005): 310~3,400 pg WHO-TEQ/g lipid (n=10)



Liver (2002): 360~23,000 pg WHO-TEQ/g lipid (n=28)

They are highly contaminated by anthropogenic compounds such as dioxins!

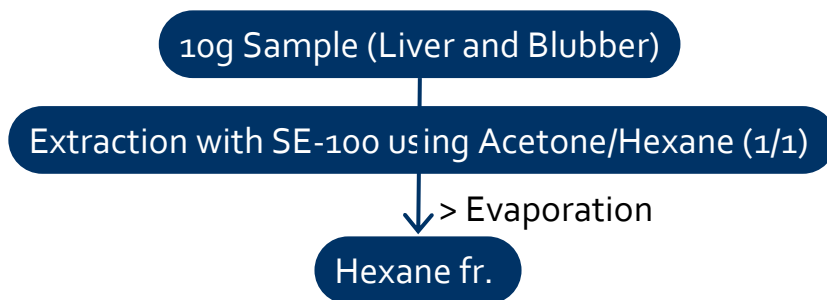


Liver (2001): 250~3,300 pg WHO-TEQ/g lipid (n=9)



Liver (2001): 4.7~460 pg WHO-TEQ/g lipid (n=18)

Experimental Section: Applied extraction and fractionation



SE-100 High speed solvent extractor



Condition

Solvent: Acetone:hexane (1:1)

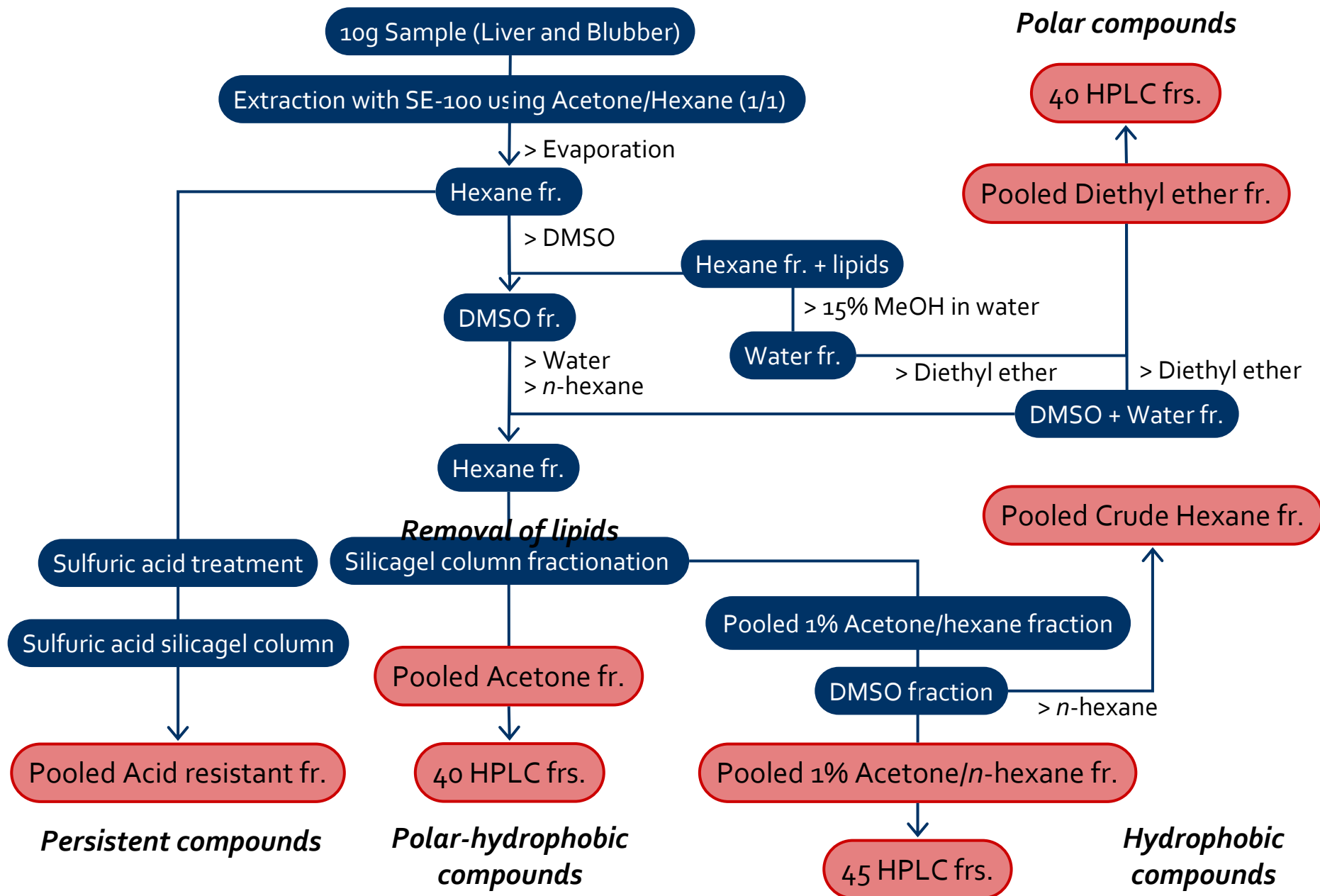
Column temp: 35 °C

Heat-up time: 10 min

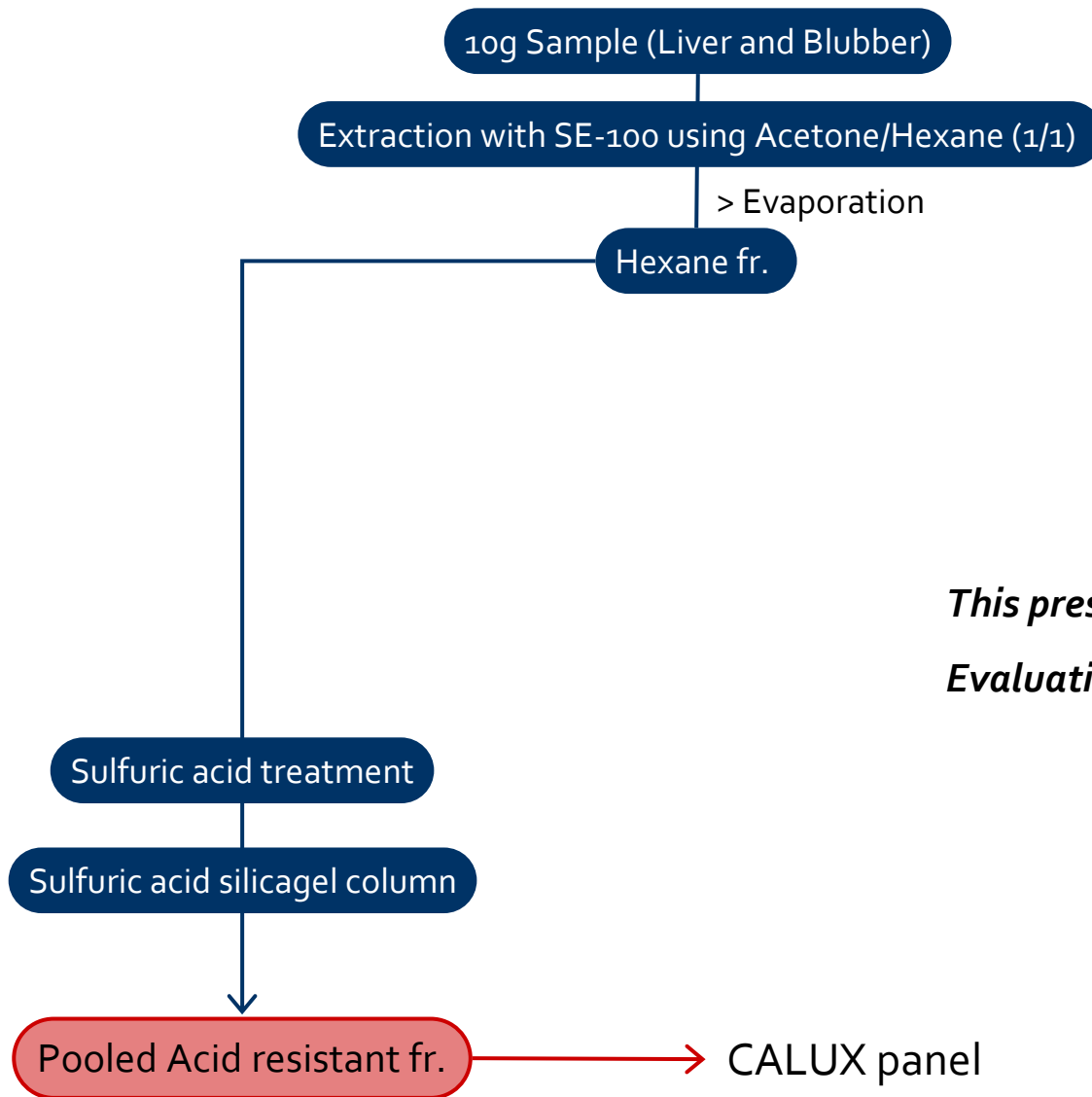
Extraction time: 30 min

Flow rate: 10ml/min

Experimental Section: Applied extraction and fractionation

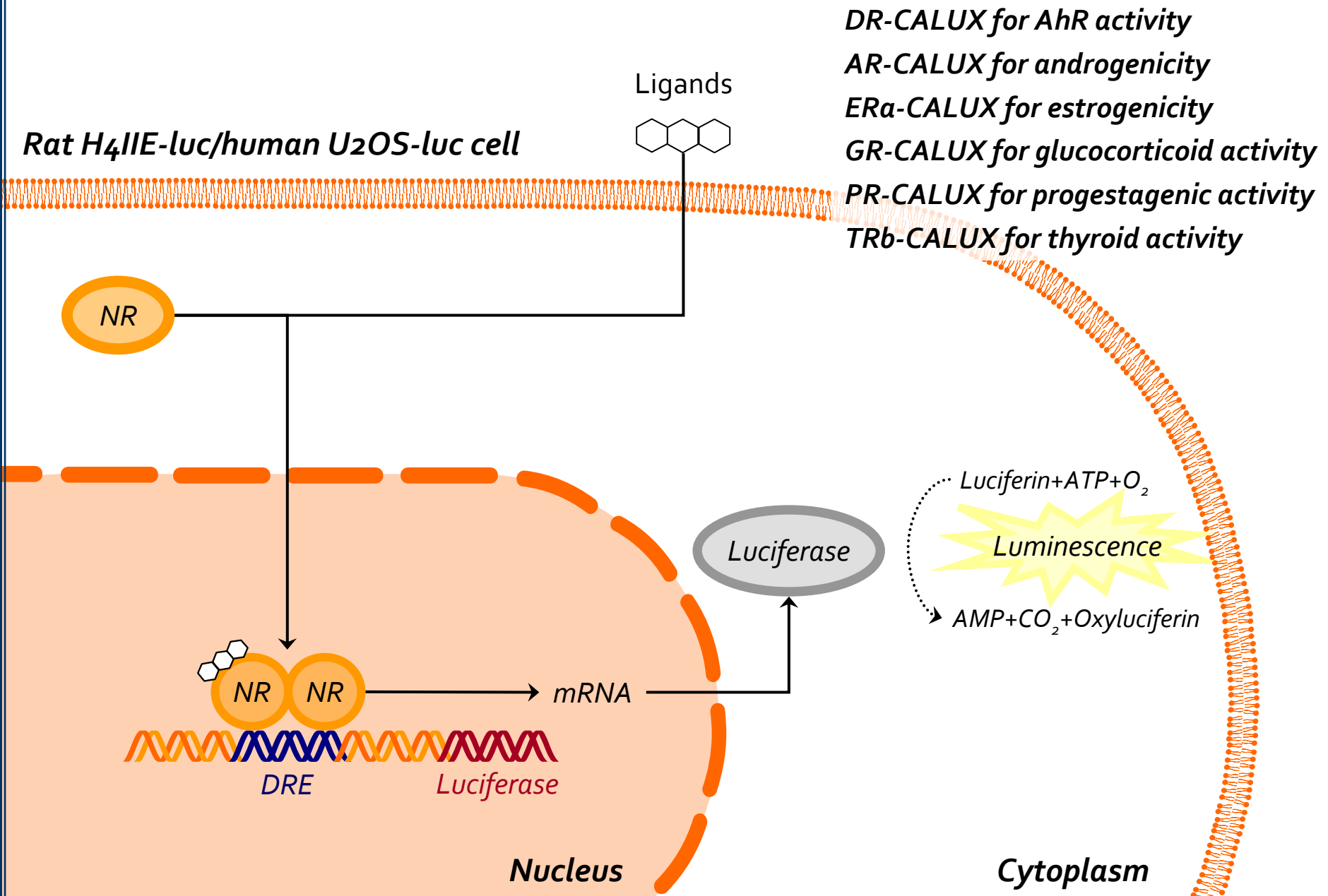


Experimental Section: Applied extraction and fractionation

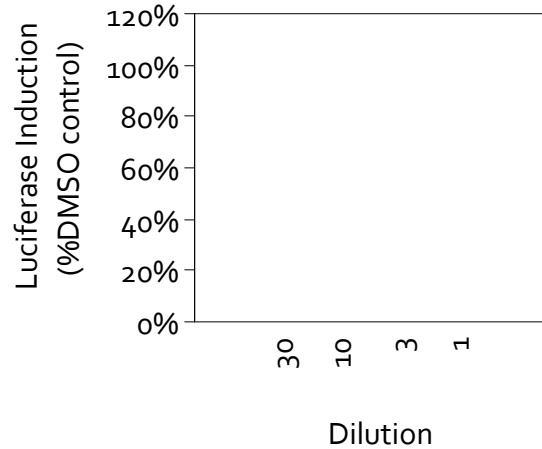


This presentation topic
Evaluation of persistent compounds

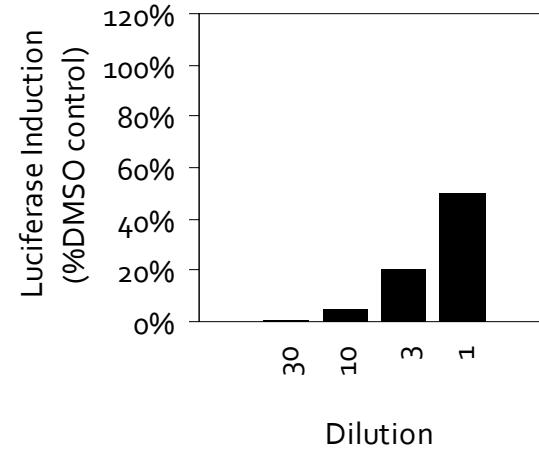
Experimental Section: CALUX panel



Experimental Section: Analysis for agonistic response



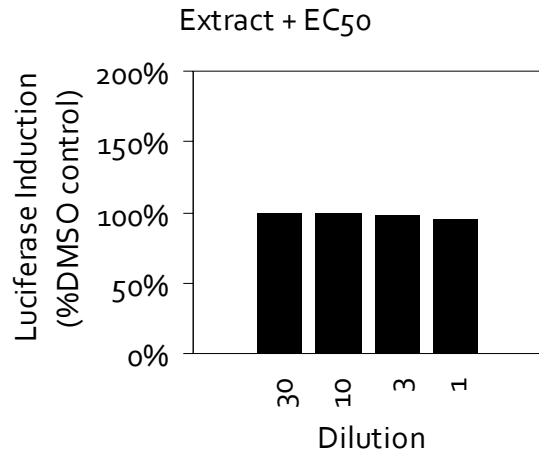
No agonistic response



Agonistic response

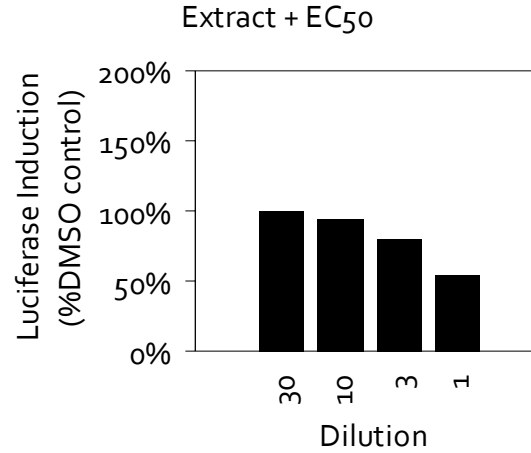
LOD is more than 1% luciferase induction.

Experimental Section: Analysis for antagonistic response



↓

No antagonistic/synergistic response?



↓

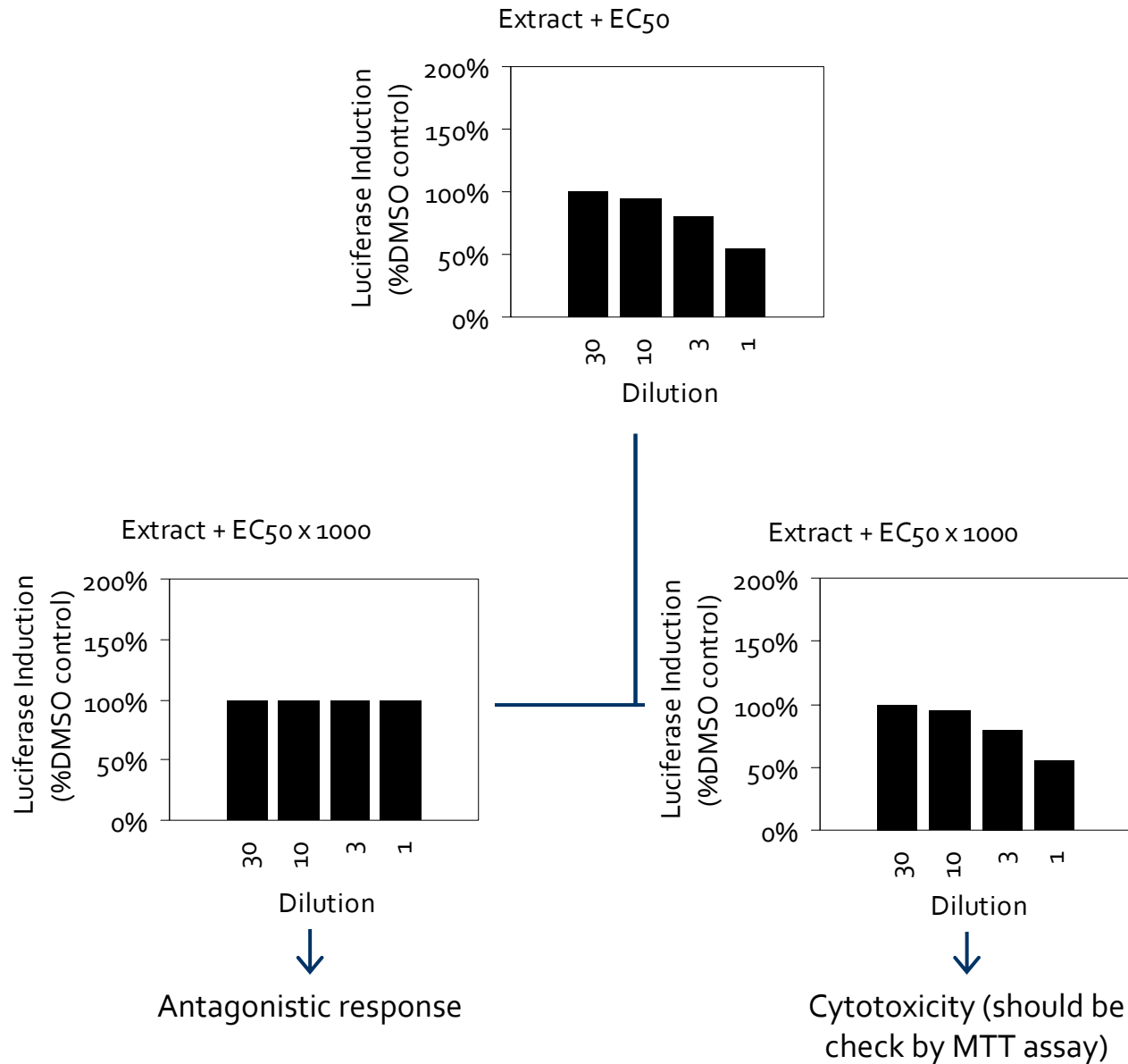
Antagonistic response?

↓

Evaluation with Extract and EC₅₀ x 1000

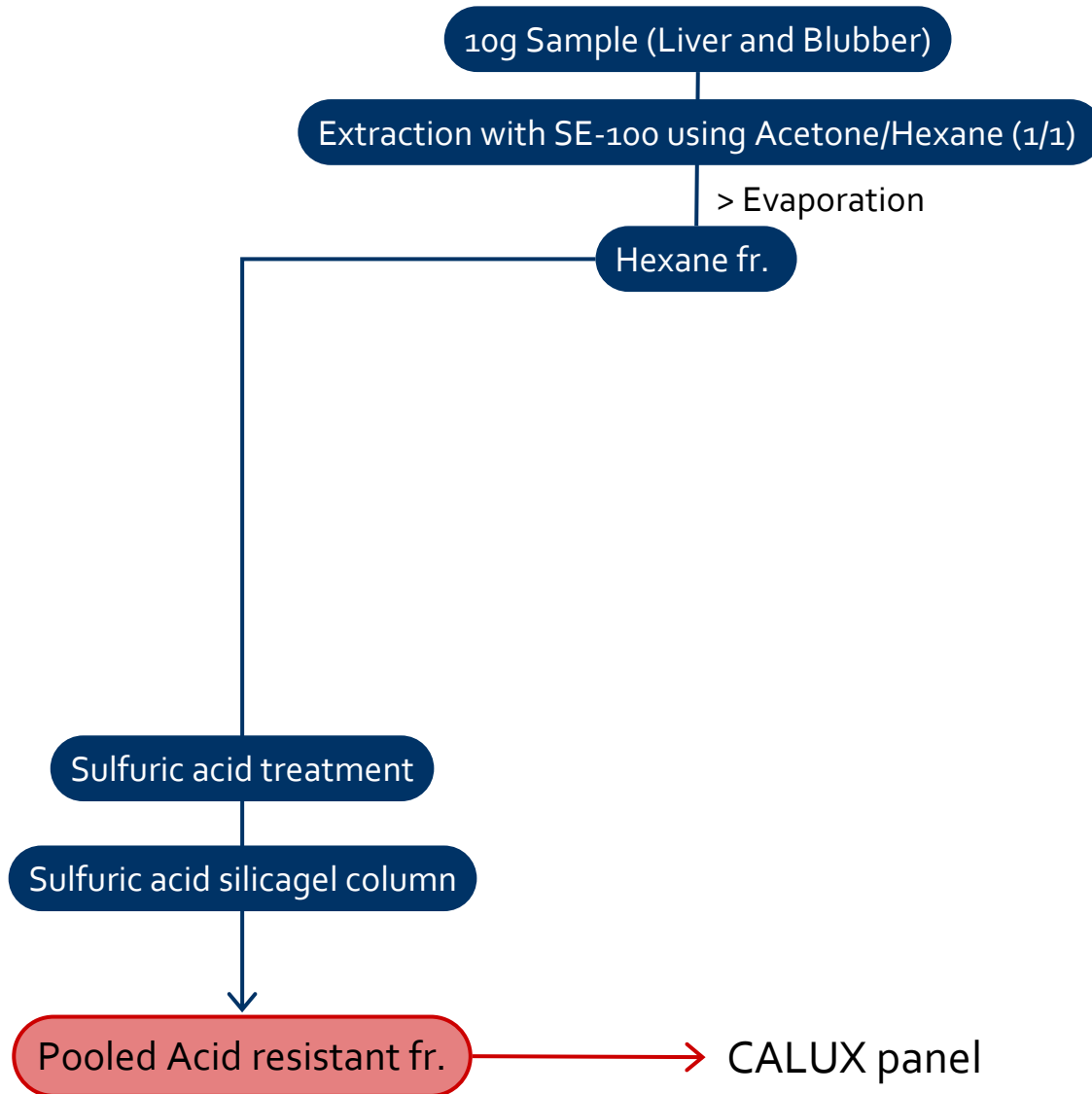
For antagonistic effect, LOD is less than 80% luciferase induction.

Experimental Section: Analysis for antagonistic response



Results and discussion

Results and discussion: CALUX panel for pooled acid-resistant fraction



Results and discussion: CALUX panel for pooled acid-resistant fraction

Agonistic response

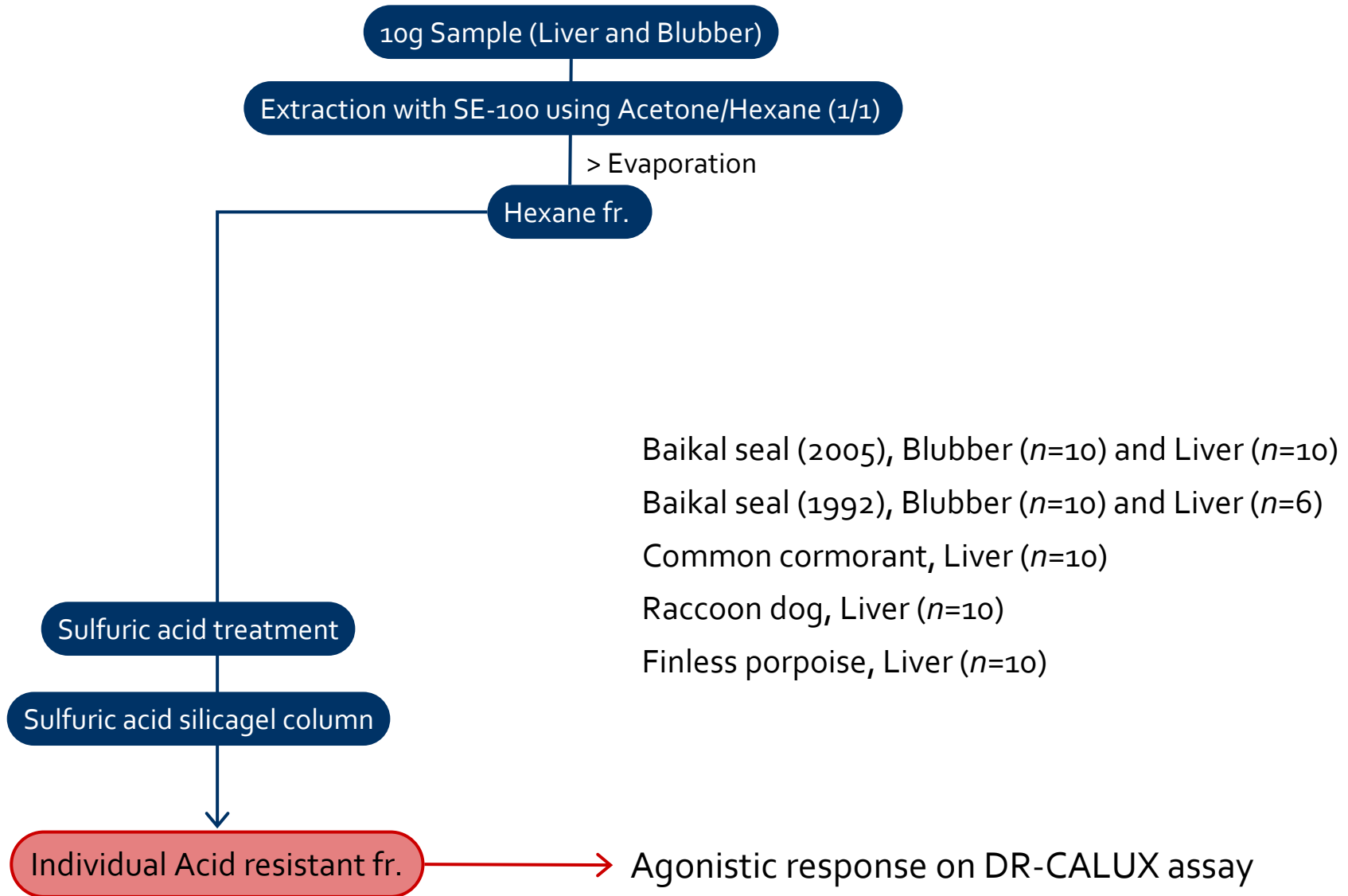
Sample	Sample amount (mg-wet/well)	AhR	AR	ER α	GR	PR	TRb
<i>Baikal seal (2005), Blubber</i>	0.45	Positive	Negative	Negative	Negative	Negative	Negative
<i>Baikal seal (1992), Blubber</i>	0.42	Positive	Negative	Negative	Negative	Negative	Negative
<i>Baikal seal (2005), Liver</i>	1.8	Positive	Negative	Negative	Negative	Negative	Negative
<i>Baikal seal (1992), Liver</i>	1.9	Positive	Negative	Negative	Negative	Negative	Negative
<i>Common cormorant, Liver</i>	1.7	Positive	Negative	Negative	Negative	Negative	Negative
<i>Raccoon dog, Liver</i>	1.8	Positive	Negative	Negative	Negative	Negative	Negative
<i>Finless porpoise, Liver</i>	1.9	Positive	Negative	Negative	Negative	Negative	Negative

Positive: more than 1% luciferase induction

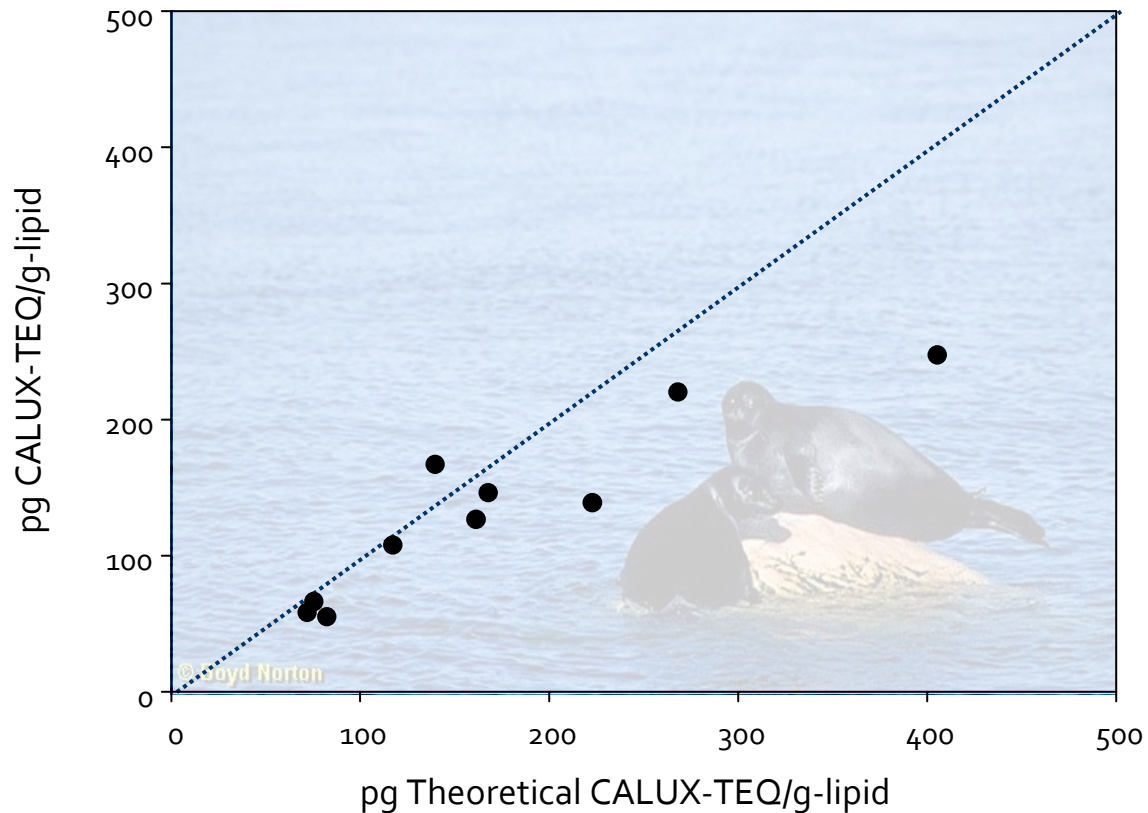
Negative: Less than 1% luciferase induction

> Evaluation for individual samples with DR-CALUX

Results and discussion: Evaluation of individual Acid resistant fr. with DR-CALUX



Baikal seal (2005), Blubber (n=10)

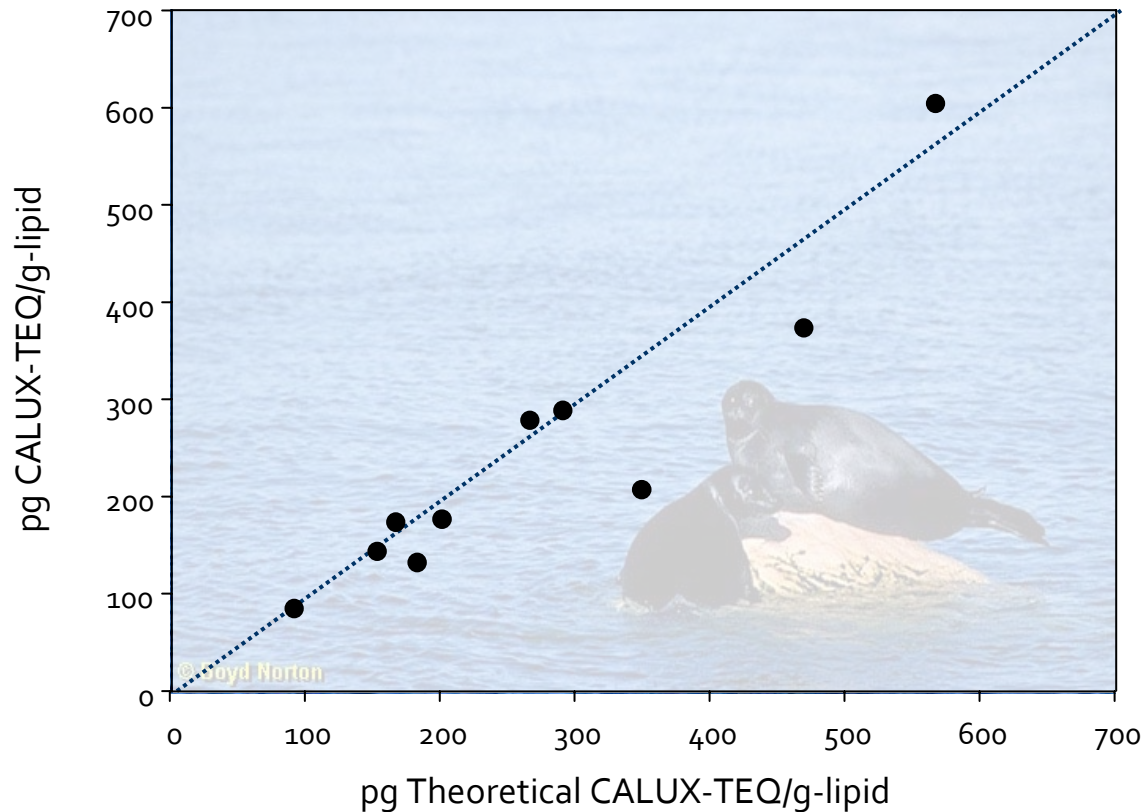


*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

Chemical values were quoted from Imaeda et al. 2009, Kunisue et al. 2006, and Kubota et al. 2006 for Baikal seal, Raccoon dog, and Common cormorant. CALUX-REPs were cited from Behnisch et al. 2001.

Results and discussion: CALUX-TEQ vs Theoretical CALUX-TEQ

Baikal seal (1992), Blubber (n=10)

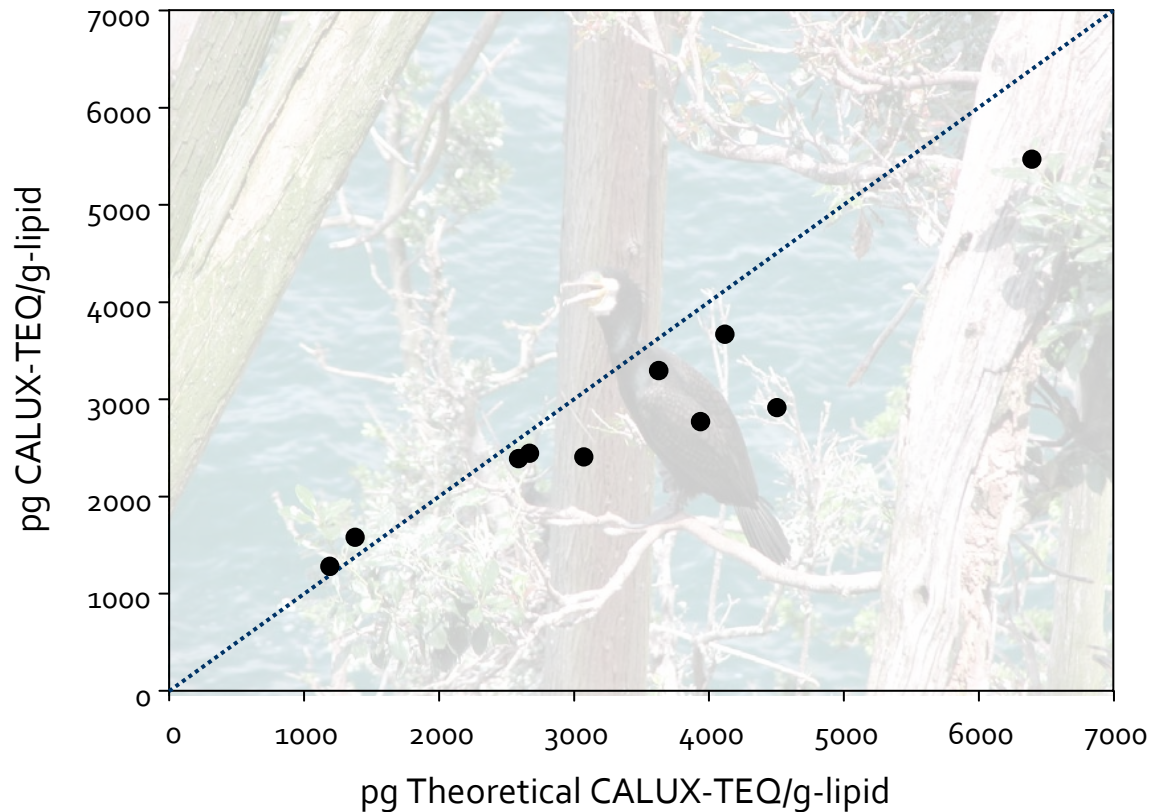


*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

Chemical values were quoted from Imaeda et al. 2009, Kunisue et al. 2006, and Kubota et al. 2006 for Baikal seal, Raccoon dog, and Common cormorant. CALUX-REPs were cited from Behnisch et al. 2001.

Results and discussion: CALUX-TEQ vs Theoretical CALUX-TEQ

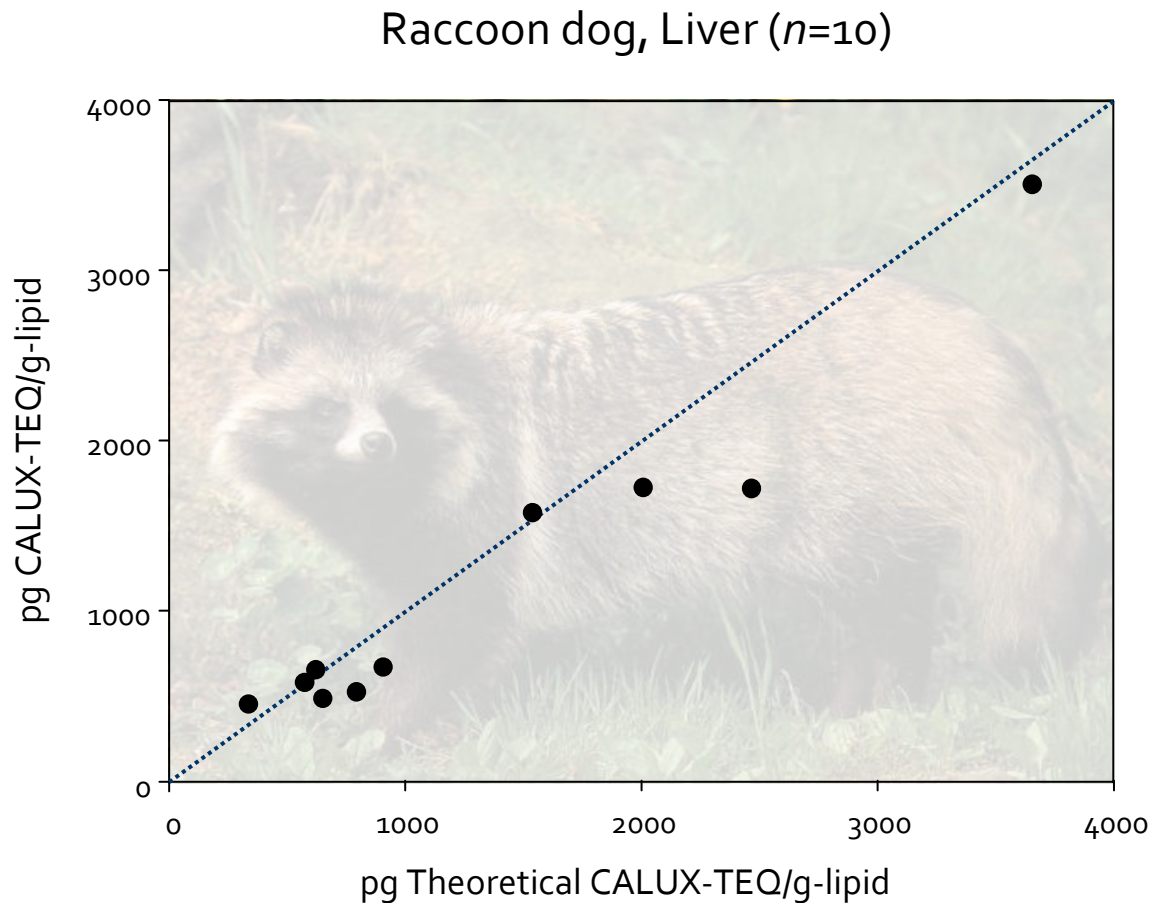
Common cormorant, Liver (n=10)



*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

Chemical values were quoted from Imaeda et al. 2009, Kunisue et al. 2006, and Kubota et al. 2006 for Baikal seal, Raccoon dog, and Common cormorant. CALUX-REPs were cited from Behnisch et al. 2001.

Results and discussion: CALUX-TEQ vs Theoretical CALUX-TEQ

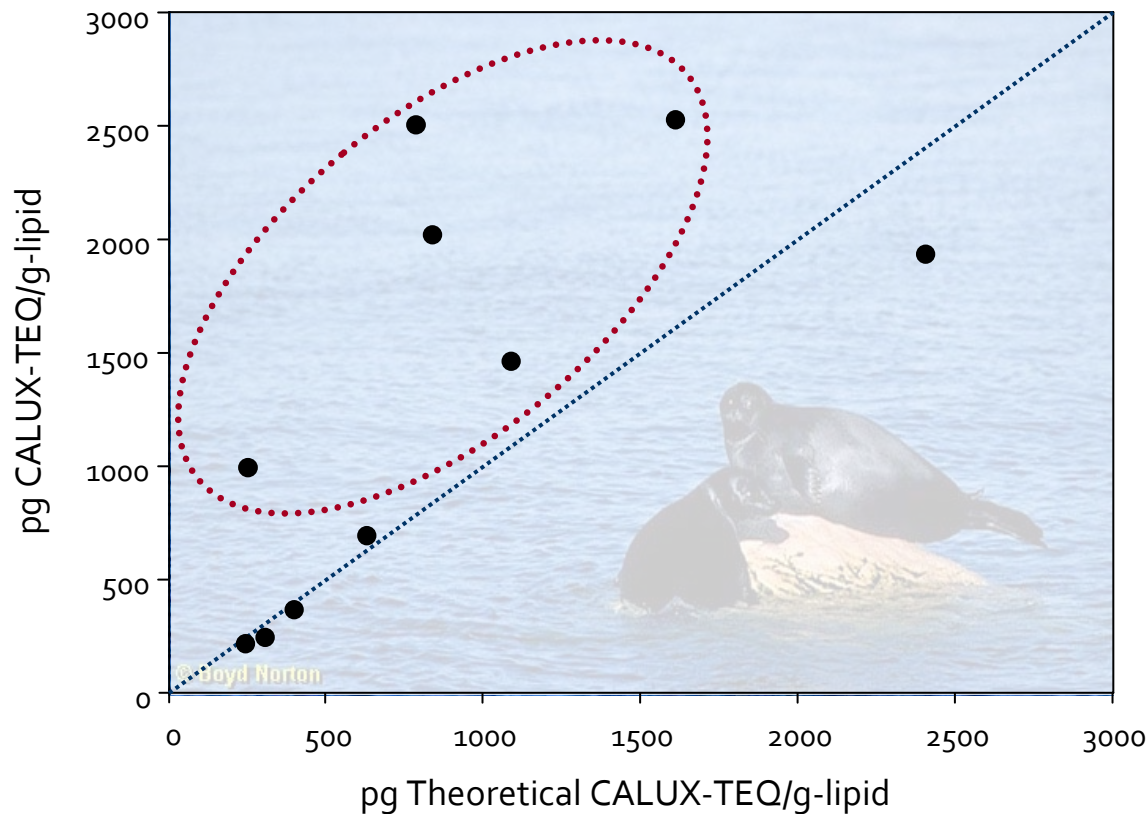


*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

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Results and discussion: CALUX-TEQ vs Theoretical CALUX-TEQ

Baikal seal (2005), Liver (n=10)

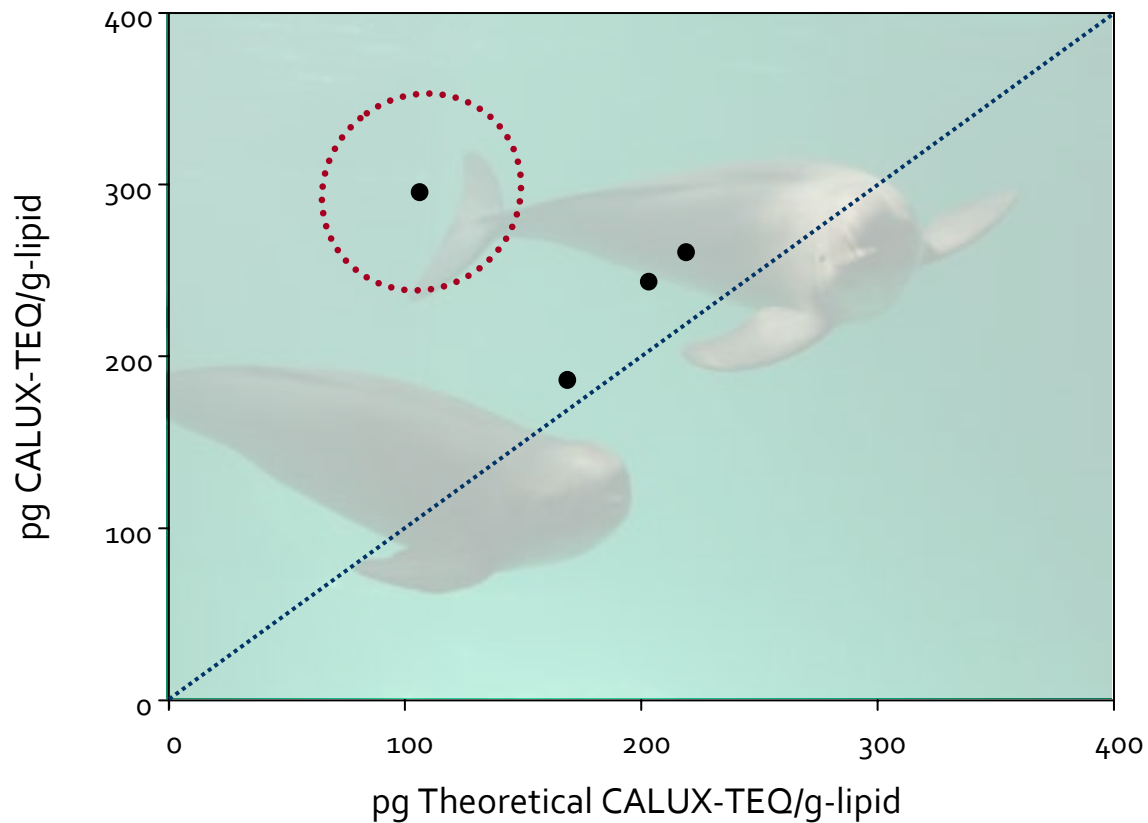


*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

Chemical values were quoted from Imaeda et al. 2009, Kunisue et al. 2006, and Kubota et al. 2006 for Baikal seal, Raccoon dog, and Common cormorant. CALUX-REPs were cited from Behnisch et al. 2001.

Results and discussion: CALUX-TEQ vs Theoretical CALUX-TEQ

Finless porpoise, Liver (n=4)



*Theoretical CALUX-TEQ = EC_{50} -based CALUX-REP x Concentration

Chemical values were quoted from Imaeda et al. 2009, Kunisue et al. 2006, and Kubota et al. 2006 for Baikal seal, Raccoon dog, and Common cormorant. CALUX-REPs were cited from Behnisch et al. 2001.

Conclusion

- Regarding agonistic activity, dioxin-like activity, but not others, are detected in acid-resistant extract from all tested samples.
- Dioxin-like activities in blubber of Baikal seals, liver of Common cormorant and liver of Raccoon dog are comparable to values calculated with instrumental values of chlorinated dioxins and their REPs on DR-CALUX.
- On the other hand, CALUX-TEQs in some extracts from liver of Baikal seal and Finless porpoise are much higher than values derived from instrumental analysis for chlorinated dioxins.
- This study suggests that not only chlorinated dioxins but also other dioxin-like compounds contribute to dioxin-like toxicity in liver of Baikal seal and Finless porpoise.