Non-target screening of new compounds in Arctic apex predators

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Aim of the study

- The effects of Contaminants on polar bear energetics – using *in vitro* exposure studies on the peroxisome proliferator-activated receptors (PPARs)

  Binding ←→ Activation

- Extracts as well as synthetic mixtures was compared

- Screening of potential new pollutants

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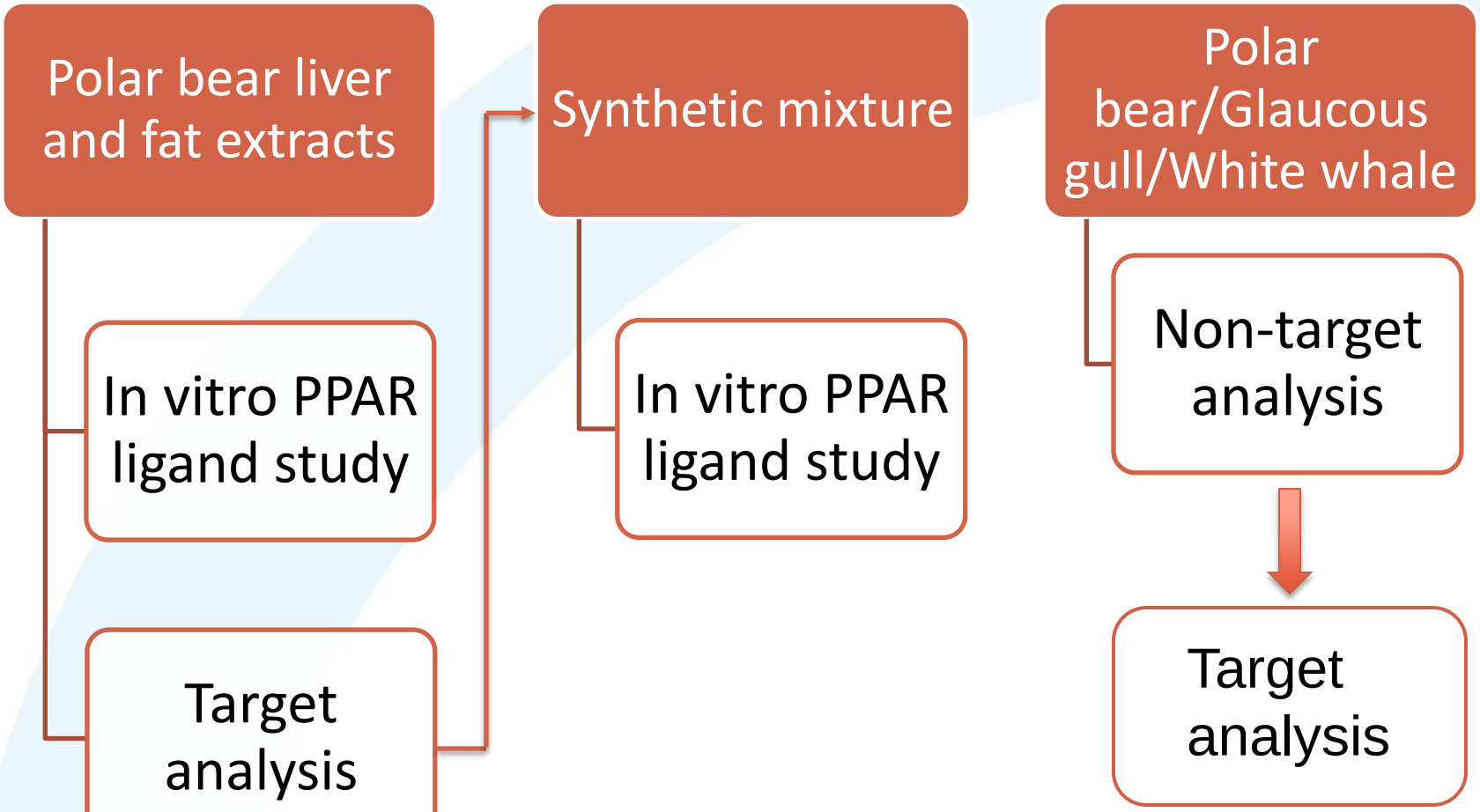
Project outline



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Extraction

Open-column Extraction

68g Polarbear liver
12g blubber
Control/blank + QC

Homogenization
Dry Na_2SO_4

Extracting
Lipids and POPs

Cleanup

Reverse Osmosis

Thin layflat polyethylene tubing
<10 Å pores

Removes:
88% lipids liver
>98% in blubber

GPC/SEC
Size exclusion
chromatography

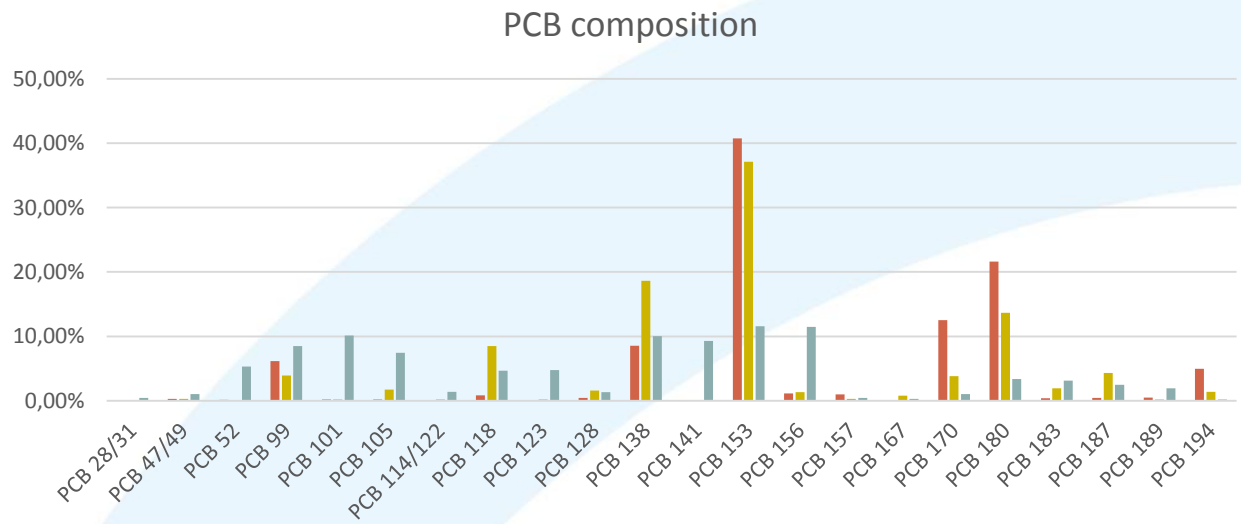
Chromatography
On Florisil

F1: Neutrals PCBs, PBDEs, pest etc
F2: $MeSO_2$-PCBs, $MeSO_2$-DDE
F3: OH-PCBs, PCP, OH-PBDEs

F1 F2 F3

Conc factor 100 to 1

Target screening



Biotransformation : Polar bear > Glaucous gull > White whale

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Non-target analyses

- Fat samples from 3 polar bears, 3 glaucous gulls, 3 White whales
- Svalbard 2013-14

- Extraction: Homogenized in Na_2SO_4 followed by extraction with acetone/cyclohexane
- Clean-up: semipermeable membrane, gel permeation chromatography and fractioning on Florisil to three according to polarity of the compounds

- Analyses: Leco Pegasus 4D, 2-D gas kromatografi coupled to time of flight mass spectrometry
- Data processing: Leco ChromaTOF software

Peak find → Peak deconvolution → matching to spectra database (NIST) → scripting

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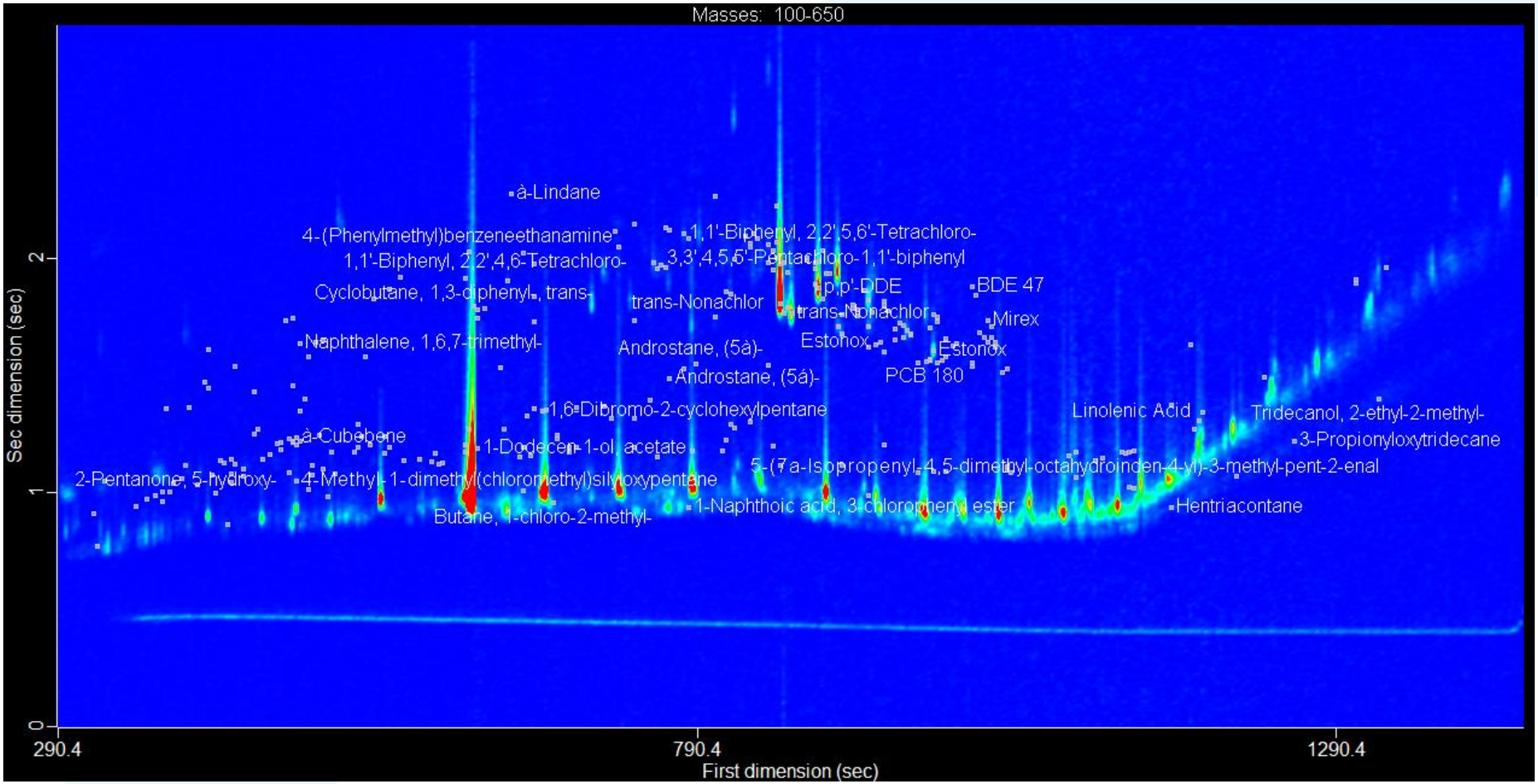
White whale


Masses: 100-650
Sec dimension (sec)
First dimension (sec)
290.4
790.4
1290.4
0
1
2
à-Lindane
4-(Phenylmethyl)benzeneethanamine
1,1'-Biphenyl, 2,2',4,6-Tetrachloro-
1,1'-Biphenyl, 2,2',5,6'-Tetrachloro-
3,3',4,5,6'-Pentachloro-1,1'-biphenyl
Cyclobutane, 1,3-diphenyl-, trans-
trans-Nonachlor
p,p'-DDE
BDE 47
trans-Nonachlor
Mirex
Naphthalene, 1,6,7-trimethyl-
Androstane, (5à)-
Estonox
Estonox
Androstane, (5à)-
PCB 180
1,6-Dibromo-2-cyclohexylpentane
Linolenic Acid
Tridecanol, 2-ethyl-2-methyl-
3-Propionyloxytridecane
à-Cubebene
1-Dodecen-1-ol, acetate
5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal
2-Pentanone, 5-hydroxy-
4-Methyl-1-dimethyl(chloromethyl)silyloxypentane
1-Naphthoic acid, 3-chlorophenyl ester
Hentriacontane
Butane, 1-chloro-2-methyl-

Results of new compounds by non-target analyses

Hit	CAS	Polar bear	Glaucous gull	White whale
Octachlorstyrene	29082-74-4	x	x	
Heptachlor-endo-epoxide (isomer A)	28044-83-9	x	x	
2,3-Dichlorobenzo[b]thiophene	5323-97-7	x	x	
1-Piperidinecarbodithioic acid, 2,3,5,6-tetrachloro-4-pyridinyl ester		x		
(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	131758-71-9	x		x
9-Allylanthracene	23707-65-5			x
l-Proline, n-pentafluoropropionyl-, heptyl ester				x
2-Chloro-1,1-bis(4-chlorophenyl)-ethene (DDMU)	1022-22-6		x	x
7-Methylbenz(a)anthracene	2541-69-7			x
Tris(3-chlorophenyl)phosphine	29949-85-7			x
6-Methoxy-2,2',4,4'-tetra (BC-3)	102739-99-1			x
Naphthalene, 1,2,3,4-tetrahydro-1-phenyl-				x
Bipyrrol Q1	428442-17-5			x
1,3-Cyclopentadiene, 1,2,3,4-tetrachloro-	695-77-2		x	
1-Silacyclopentadiene, 3-(diethylboryl)-4-ethyl-1,1-dimethyl-2-(3-methylbutyl)-5-trimethylstannyl-	153331-61-4		x	
Endosulfan	115-29-7		x	
2,3,4,5,6-Pentachlorophenyl acetat	1441-02-7		x	
2,7:3,6-Dimethanonaphth[2,3-b]oxiren-8-ol, 3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-, acetate, stereoisomer	55649-85-9		x	
1,4:5,8-Dimethanonaphthalene-2,3-diol, 5,6,7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-, diacetate, (1à,2à,3á,4à,4aá,5á,8á,8aá)-	34408-22-5		x	
Chlordecone	55570-85-9		x	

- **Qualitative**
- **No information of concentrat-ions**
- **Structures not confirmed by standards**

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Target analysis

- Fat samples from 10 polar bears, 8 glaucous gulls, 9 white whales
- Extraction and cleanup similar as non-target analysis (no semipermeable membrane)– full set of internal standards
- Analysis on GC-MS

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Target analyses of compounds found in non-target screening:

Concentrations (ng/g ww)	Polar bear	Glaucous gull	White whale
Octachlorstyrene	8.7	21.6	3.8
Heptachlor-endo-epoxide	< 0.9	< 0.9	< 0.9
2,3-Dichlorobenzo[b]thiophene	< 0.1	< 0.1	< 0.1
1-Piperidinecarbodithioic acid, 2,3,5,6-tetrachloro-4-pyridinyl ester			
(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-			
9-Allylanthracene			
l-Proline, n-pentafluoropropionyl-, heptyl ester			
2-Chloro-1,1-bis(4-chlorophenyl)-ethene (DDMU)	2.5	54.3	3.5
7-Methylbenz(a)anthracene	< 0.1	< 0.1	< 0.1
Tris(3-chlorophenyl)phosphine	< 0.3	< 0.3	< 0.3
6-Methoxy-2,2',4,4'-tetra (BC-3)			
Naphthalene, 1,2,3,4-tetrahydro-1-phenyl-	< 2.5	< 2.5	< 2.5
Bipyrrol Q1			
1,3-Cyclopentadiene, 1,2,3,4-tetrachloro-	< 0.3	< 0.3	< 0.3
1-Silacyclopentadiene, 3-(diethylboryl)-4-ethyl-1,1-dimethyl-2-(3-methylbutyl)-5-trimethylstannyl-			
Endosulfan	Will be analyzed		
2,3,4,5,6-Pentachlorophenyl acetat			
2,7:3,6-Dimethanonaphth[2,3-b]oxiren-8-ol, 3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-, acetate, stereoisomer			
1,4:5,8-Dimethanonaphthalene-2,3-diol, 5,6,7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-, diacetate, (1à,2à,3á,4à,4aá,5á,8á,8aá)-			
Chlordecone	< 0.1-2.5	24.7	1.7

- **New extractions**
- **Quantification using reference standards**
- **Retention time confirmed using authentic standards**
- **Quantitative results**

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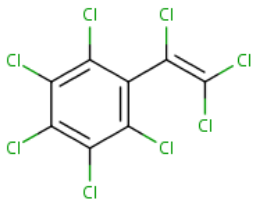
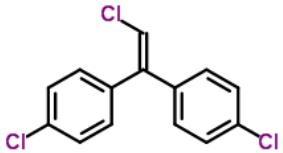
Target analyses of compounds found in non-target screening:

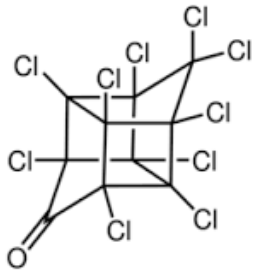
- Octachlorostyrene:
  - Industrial by-product, not regulated
  - Not new, reported in polar bears already in 2000
  - Highly toxic



- DDMU:
  - Metabolite of DDE
  - Toxic



- Chlordecone/Kepone
  - Pesticide, included in Stockholm Convention in 2009
  - Not measured in the Arctic (Muir & deWit, 2010)
  - Toxic



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The way forward

- Suspect screening, good alternative or complement to non-target screening
- Accurate high resolution MS for additional confirmation - standards are difficult and expensive to get
- Confirmation of compounds – Rentention time, accurate mass, mass fragmentation spectra, isotope ratios
- Negative and positive chemical ionization

→Selectivity and molecular fragments

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For more information, www.nilu.no

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