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# Polychlorinated dioxins, furans and dl-PCBs in ringed seals (*Pusa hispida botnica*) from the Baltic Sea 1978-2014

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1978–2014

**Summary**
Blubber tissue samples from in total 40 juvenile ringed seals from the Baltic Sea collected between 1978 and 2014 were analyzed for ”dioxins”; polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/PCDF) and dioxin-like PCBs (dl-PCB). Thirty-eight of the seals were bycaught in fishing gear and two were shot by mistake, taken for grey seals.

Concentrations of PCDDs, PCDFs and dl-PCBs have decreased over the study period in ringed seals with only a few exceptions. The decrease was primarily seen until year 2000 while the concentrations have been stable the last 15 years with a few exceptions. Two dioxins (1,2,3,4,6,7,8-HpCDD and OCDD) and one furan (1,2,3,7,8-PeCDF) demonstrate increased levels the last 15 year of the study period while one furan decreases (1,2,3,7,8,9-HxCDF). OCDD demonstrates the highest increase rate with 15 % yearly increase the last 15 years.

Concerning the dioxin-like PCBs, the four non-ortho, dl-PCBs demonstrate no trend the last 15 years. Four of the mono-ortho dl-PCBs continue to decrease until 2014 while the other four mono-ortho congeners show no statistically significant trends the last 15 years.

It should however be stated that a limited number of seals have been analyzed with an average of one seal/year and more analyses are demanded in order to achieve more significant time trend results.

Ringed seals show a low increase in population, approximately half of what is natural and reproductive impairment is still noted among ringed seals. If this has a connection to the concentrations of PCDD/F and dl-PCBs cannot be ruled out.

# 1. Aim

Previously, 30 ringed seals collected between 1978 and 2006 were analyzed for dioxins, furans and non- and mono-ortho-PCBs. The aim of this study was to extend the time series to 2014.

# 2. Introduction

The ringed seal is a circumpolar species, found in Arctic waters. It was landlocked in the Baltic Sea after the last ice age, in Lake Saima in Finland and Lake Ladoga in Russia, resulting in several subspecies (*Pusa hispida botnica, P.h. saimensis and P.h. ladogensis*). In the Baltic, ringed seals (*P. h. botnica)* are mostly found in the northern parts (Bottenhavet, Bottenviken, Norra Kvarken), but also in the Bay of Finland and the Bay of Riga.

The population of ringed seals in the Baltic Sea was estimated at around 190 000-220 000 individuals in 1900 and decreased to approximate 5000 in the late 1970s [1]. This decrease was more dramatic than that seen for the grey seal (*Halichoerus grypus*), which decreased from approximately 88 000-100 000 in the beginning of 1900s to approximately 4000 in the late 1970s [1]. Both species decreased in numbers first because of intense hunting but also, after the 1970s, due to problems with reproduction. A disease complex among ringed and grey seals was identified, including sterility among females, and elevated concentrations of organochlorines were believed to be the cause for this sterility [2]. Now the population of ringed seals is recovering. Surveys on ringed seals starting in 1988 indicate a 4.5% yearly increase. This increasing rate is approximately 50% lower than what could be expected from a healthy population and occluded uteri are still found among ringed seals, though not in grey seals. [3]. The ringed seal populations in The Bay of Finland and in the Bay of Riga are decreasing.

Dioxins and furans, here referred as PCDD/Fs, are unintentionally released to the environment. They are by-products in several industrial processes and from combustion processes as well as in natural forest fires. Several of them are extremely toxic and harmful to animal health. PCDD/Fs can cause a variety of biological and toxicological effects in animals and humans, such as developmental toxicity, carcinogenity and immunotoxicity. Most toxic effects are due to the binding of PCDD/Fs to the aryl hydrocarbon (Ah) receptor.

In this study, we elucidate the concentrations of polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) and dioxin like CBs (dl-PCB) in juvenile ringed seals from the Baltic Sea over a time limit of 4 decades. 2,3,7,8-TCDD is the most toxic congener and is used as a reference for all other related chemicals. Each of the 29 relevant and most toxic congeners has been assigned a toxic equivalency factor (TEF), where 2,3,7,8-TCDD equals 1. The dioxins, furans and dl-PCB concentrations are reported individually as well as in TCDD-equivalents (TEQ), which is the sum of the individual congener concentrations multiplied with its specific TEF. Two different TEF values were calculated, one set from 1998 [4] and the other from a new revised publication from 2006 [5]. Only a few of the TEFs differed between the two publications and it was mostly the PCBs that were assigned with new TEF values.

# 3. Material and Methods

## 3.1 Ringed seals

Blubber (6-10 gram) from 40 juvenile ringed seals from the Baltic Sea collected between 1978 and 2014 were analyzed for polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) and dioxin like PCBs (dl-PCB). Thirty-eight of the seals were bycaught in fishing gear and two were shot by mistake (taken for grey seal). They were all juvenile (between 0-1 years old), 18 males and 22 females between 85 and 114 cm total length and 26-58 kg. They were necropsied at the Swedish Museum of Natural History and samples from them were stored in the Environmental Specimen Bank at the museum, and subsampled from there for this study.

## 3.2 Analytical methods

The analyses of PCDD/F and dl-PCBs were carried out at the Department of Chemistry, Umeå University. The extraction method is described by Wiberg *et al.* [6], the clean-up method by Danielsson *et al.* [7], and the instrumental analysis (GC-HRMS) by Liljelind *et al.* [8]. The laboratory participates in the annual FOOD intercalibration rounds, including laboratory reference material (salmon tissue) with each set of samples.

## 3.3 Statistics

Concentrations of contaminants were logged before statistical analysis, in order to approach normal distribution. Simple regression analysis was performed on each compound.

$P<0,05$ is used as a limit for statistical significance. In the diagrams, statistically significant regressions are shown as a black regression lines.

# 5. Results

The results from this study must be treated with caution, since the sample size is very small.

All data is presented in pg/g lipid weight, with the exception of the mono-ortho CBs which are presented in ng/g lipid weight, since the concentrations of these compounds are so much higher than the non-ortho CBs, dioxins and furans.

The blubber had a lipid content of 90-100%.

## 5.1 Polychlorinated dibenzo-p-dioxin and dibenzofurans

The concentrations of 1234789-HpCDF were most often under detection limit (<0,3 pg/g, in 33 out of 40 samples), so no statistical analyses were performed on that compound. The concentrations of the other compounds are shown in Table 1. The concentrations of OCDF were below the limit of quantification in ten samples (the most recent years), so the limit of quantification (0,5) was divided

with square root of 2, in order to use all the data. OCDF showed a decreasing trend, that was almost statistically significant (p<0,051).

| | min (pg/g lw) | max (pg/g lw) | median (pg/g lw) | SD | Yearly change (%) | p-value | Yearly change last 15 years | p-value |
|---|---|---|---|---|---|---|---|---|
| 2378-TeCDD | 3,8 | 45 | 11 | 12 | -5,6 | p<0,001 | No change | |
| 12378-PeCDD | 14 | 129 | 30 | 27 | -4 | p<0,001 | No change | |
| 123478-HxCDD | 1,5 | 7,0 | 2,9 | 1,7 | -3,1 | p<0,001 | No change | |
| 123678-HxCDD | 10 | 125 | 30 | 31 | -4,1 | p<0,001 | No change | |
| 123789-HxCDD | 0,5 | 6,4 | 1,3 | 1,4 | -4,5 | p<0,001 | No change | |
| 1234678-HpCDD | 0,3 | 2,2 | 0,7 | 0,4 | | ns | +9,2 | p<0,001 |
| OCDD | 0,3 | 8,9 | 0,8 | 1,5 | +3,1 | p<0,01 | +15 | p<0,001 |
| 2378-TeCDF | 9,1 | 58 | 21 | 9,5 | -1,3 | p<0,01 | No change | |
| 12378-PeCDF | 1,4 | 13 | 3,6 | 2,3 | -2,2 | p<0,001 | +3,1 | p<0,05 |
| 23478-PeCDF | 19 | 119 | 46 | 25 | -2,7 | p<0,001 | No change | |
| 123478-HxCDF | 0,3 | 2,9 | 1,3 | 0,7 | -2,5 | p<0,001 | No change | |
| 123678-HxCDF | 0,5 | 3,4 | 1,3 | 0,7 | -2,6 | p<0,001 | No change | |
| 234678-HxCDF | 0,3 | 2,0 | 0,9 | 0,4 | -2,3 | p<0,01 | (+2,7) | p<0,08 |
| 123789-HxCDF | 0,3 | 4,5 | 1,0 | 1,1 | -3,1 | p<0,01 | -11 | p<0,001 |
| 1234678-HpCDF | 0,2 | 1,0 | 0,4 | 0,2 | -1,5 | p<0,01 | No change | |
| 1234789-HpCDF | <0,3 | 0,23 | <0,3 | | | | No change | |
| OCDF | 0,1 | 4,0 | 0,4 | 0,7 | *(-1,6)* | p<0,051 | No change | |

*Table 1. Concentrations of polychlorinated dibenzo-p-dioxin and dibenzofuran (PCDD/F) in juvenile ringed seals (pg/g lw in blubber). Yearly changes are shown in %. ns= not significant, and values in parenthesis are trends, almost significant.*

Generally, the concentrations of dioxins and furans decreased in ringed seals during the study period, with annual rates between 1,5-5,6% over the time period (See Appendix Figure 1-2 for individual compounds). However, there were two exceptions: concentrations of octachlorodibenzodioxin, (OCDD) showed a positive trend (with 3,1% yearly increase), and 1234678-HpCDD did not show any trend. OCDD has a very low TEF value (0,0003) but the TEF value for 1234678-HpCDD is set higher (0,01) [5]. They were the two dioxins with the lowest concentrations in ringed seals and did not add much to the TEQ-values. The two dominant dioxins in ringed seals were the most toxic ones (2,3,7,8-TCDD and 1,2,3,7,8-PeCDD), accounting for more than 50% of the dioxins.

As can be seen in Figure 1 and in the Appendix Figures 1 and 2, the decrease in concentrations generally took place before 2000; after that concentrations stabilized. There were some exceptions though; two dioxins (1234678-HpCDD and OCDD) and one furan (12378-PeCDF) increased in concentrations significantly over the last 15 years of the time series (see Table 1) and 123789-HxCDF decreased significantly (-11%). Also, 234678-HxCDF showed a tendency to increase in concentrations over the last 15 years, but this was not statistically significant (p<0,08).

Using the WHO2005 TEF values, the rate of decrease was higher for dioxins compared to furans: 4,5% annual decrease for dioxins and only 2,5% annual decrease for the furans (see Figure 1 for the total TCDD equivalent of dioxins and furans).

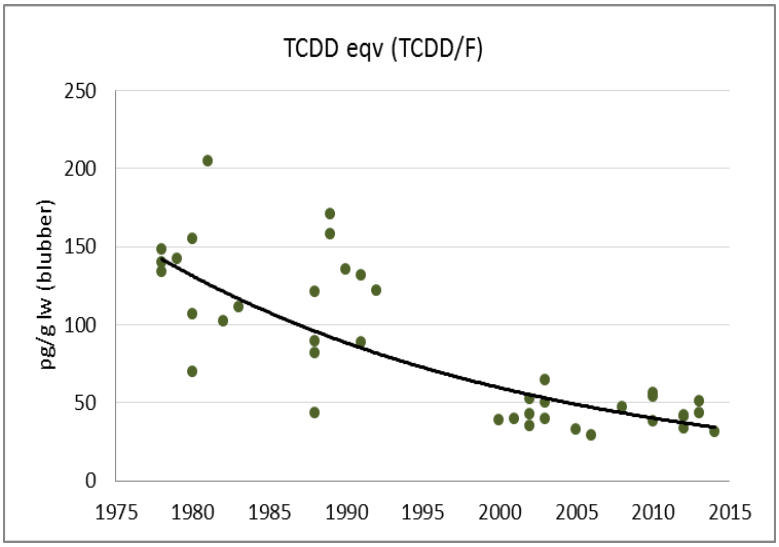


*Figure 1. TCDD equivalent of dioxins and furans in juvenile ringed seals from the Baltic Sea (pg/g lipid weight in blubber). Concentrations decrease with 3,9% annually for the whole period using the WHO 2005 TEF values. No trend is seen after 2002.*

## 5.2 Non-ortho CBs

Four non-ortho-CBs were analyzed in the present study: CB 77, 81, 126 and 169 (Table 2). They all decreased over the study period, by approximately 4% annually, with the exception of CB-169 which had a rate of decrease of only 1% ($p<0,05$), Figure 2. For the last 15 years there were no significant decreasing trends for any of the compounds. (See Appendix Figure 3 for the individual congeners).

The concentrations of the four dl-like CBs are approximately five times as the sum of dioxins and furans (PCDD/F).

| | range (pg/g lw) | mean (pg/g lw) | median (pg/g lw) | SD | Yearly change (%) | p-value | Yearly change last 15 years |
|---|---|---|---|---|---|---|---|
| CB-77 | 26-217 | 79 | 63 | 49 | -4,0 | p<0,001 | No change |
| CB-81 | 7,0-135 | 30 | 23 | 25 | -4,2 | p<0,001 | No change |
| CB-126 | 282-1897 | 831 | 737 | 429 | -3,8 | p<0,001 | No change |
| CB-169 | 24-104 | 55 | 51 | 20 | -1,0 | p<0,05 | No change |

*Table 2. Range, mean and median values and standard deviation in ringed seal blubber (pg/g lw) for the four non-ortho–substituted CBs included in the study.*

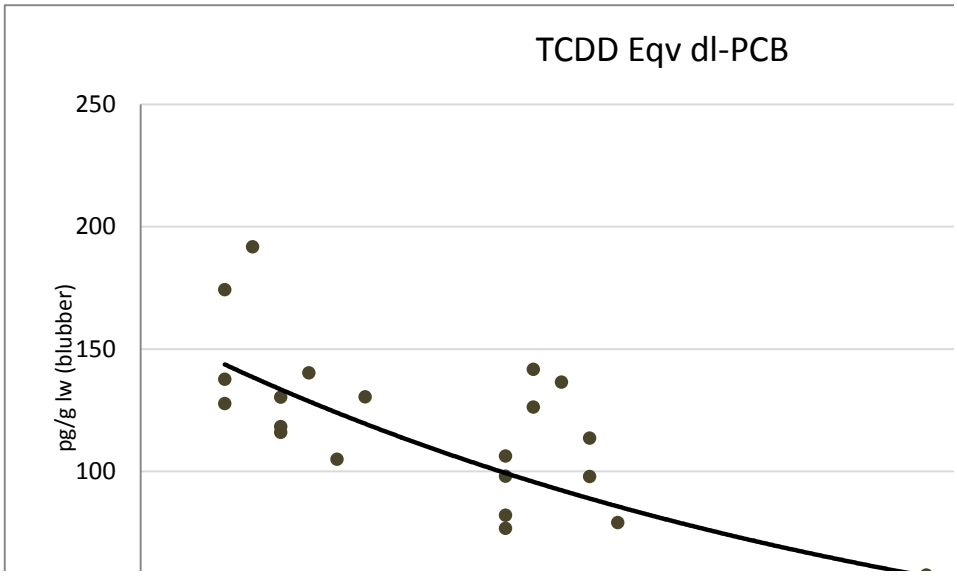


*Figure 2. TCDD equivalent of sum of non-ortho dl-PCBs (CB-77, 81,126 and 169). Yearly rate of decrease for the whole period is 3,7% but, as for all individual congeners, there is no trend after 2002.*

## 5.3 Mono-ortho CBs

All mono-ortho CBs analysed had decreased during the study period, with 2,0-8,7% annually. CB-123 showed the strongest rate of decrease, particularly over the last 15 years. The concentrations of CB-105, 156 and 157 had not changed over the last 15 years, but CB-114, 118, 123 and 167 showed higher rates of decrease the last 15 years (see Table 3). The sum of all mono-ortho CB decreased with 3,9% annually (p<0,001) and is shown in Figure 3. See Appendix Figure 4 for the individual congeners.

| | range (ng/g lw) | mean (ng/g lw) | median (ng/g lw) | SD | Yearly change (%) | p-value | Yearly change the last 15 years | p-value |
|---|---|---|---|---|---|---|---|---|
| CB-105 | 42-368 | 156 | 146 | 84 | -3,4 | p<0,001 | No change | |
| CB-114 | 2,9-23 | 22 | 22 | 6,0 | -3,7 | p<0,001 | -5,1 | p<0,01 |
| CB-118 | 38-542 | 244 | 238 | 147 | -5,3 | p<0,001 | -11 | p<0,001 |
| CB-123 | 0,2-8,7 | 2,9 | 1,8 | 2,5 | -8,7 | p<0,001 | -11 | p<0,001 |
| CB-156 | 35-256 | 118 | 99 | 62 | -2,4 | p<0,001 | No change | |
| CB-157 | 6,8-65 | 30 | 28 | 16 | -2,9 | p<0,001 | No change | |
| CB-167 | 2,9-59 | 20 | 16 | 15 | -5,7 | p<0,001 | -12 | p<0,001 |
| CB-189 | 3,4-21 | 10 | 8 | 5,1 | -2,0 | p<0,01 | (-3,9) | P<0,09 |

*Table 3. Range, mean and median values and standard deviation in ringed seal blubber (ng/g lw) for the eight mono-ortho–substituted PCBs included in the study. The yearly change and p-value is shown for the whole period as well as for the lastt 15 years. Values in parenthesis are close to significant.*

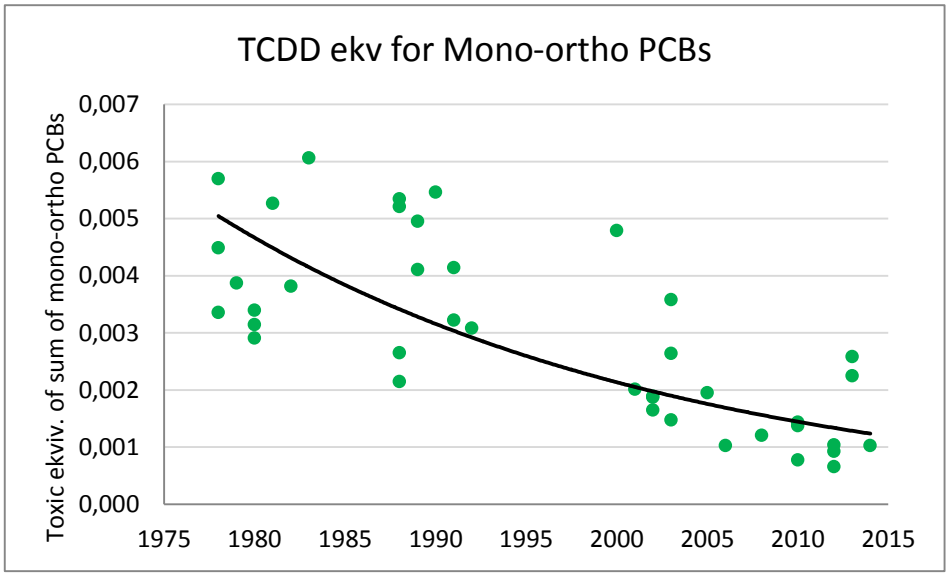


*Figure 3. Sum of toxic evivalent of eight mono-ortho-PCBs (PCB-105+ 114+ 118+ 123+ 156+ 157+ 167+ 189). Yearly rate of decrease for the whole period is 3,9% but, as for the individual congeners, there is no trend after 2002.*

## 5. Concluding remarks

Generally concentrations of dioxins, furans and dl-PCBs decreased over the study period but there were a few exceptions. However, the rate of decrease stabilized, and after 2000 no trends were seen with some exceptions. Three dioxins (1234679-HpCDD, OCDD and 12378-PeCDF) and one furan (12378-PeCDF) demonstrated increasing trends over the last 15 years of the study period. And one furan, 123789-HxCDF, showed an even stronger decreasing rate the last 15 years.

Ringed seals in the Baltic Sea show a low increase in population, approximately half of what is natural, and reproductive impairment is still noted among ringed seals. A connection between the concentrations of PCDD/F and dl-PCBs and reproductive impairment in ringed seals cannot be ruled out.

## 6. Acknowledgement

Many people have helped collecting seals to Swedish Museum of Natural History (SMNH). Fishermen have provided the seals. Anders Bergman and Britt-Marie Bäcklin have performed necropsy and Charlotta Moraeus and Malin Stenström and others have helped with sampling. The Swedish Environmental Protection Agency has given economic aid for 9 analyses, and SMNH for the reminding 31.

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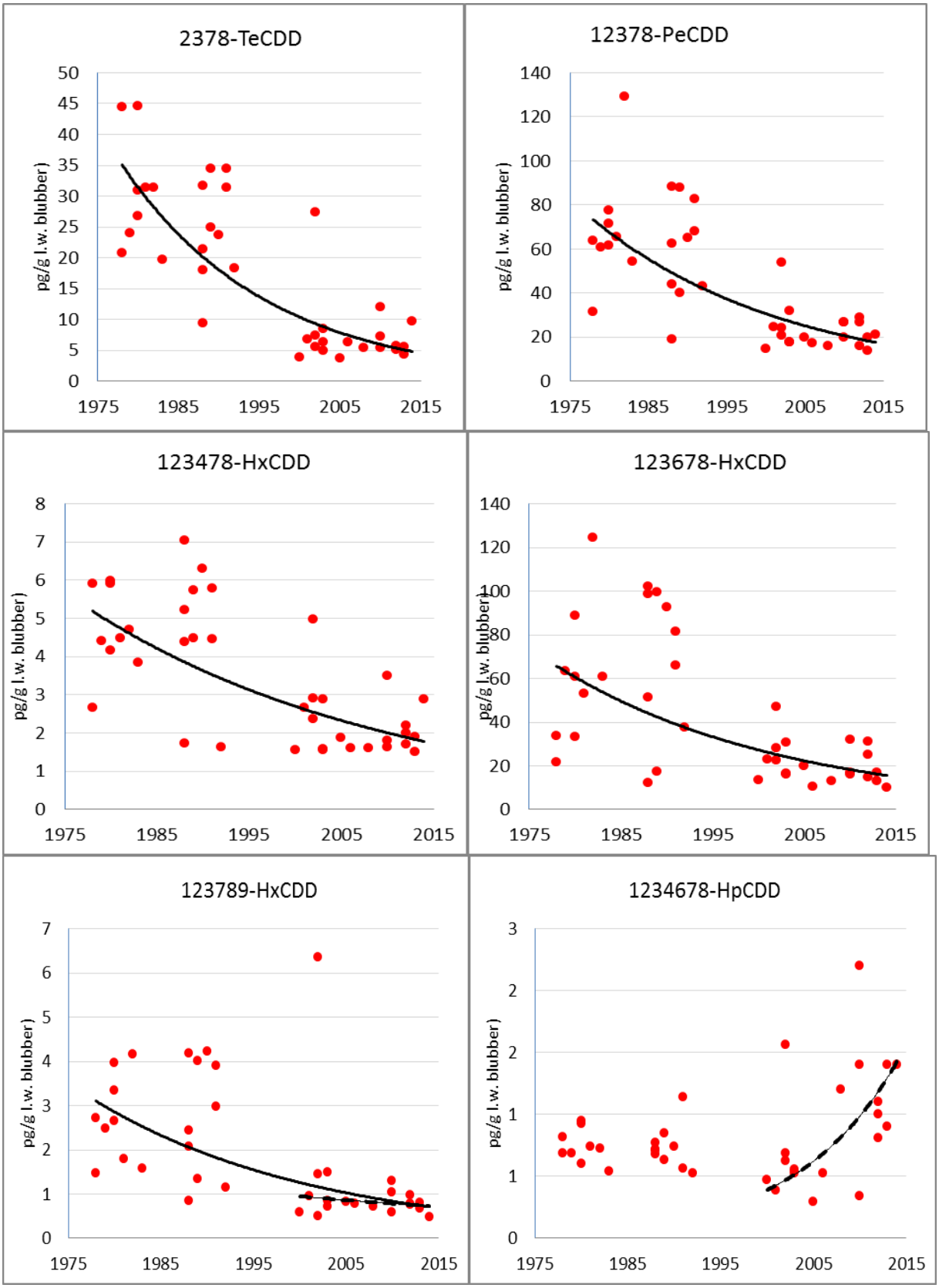
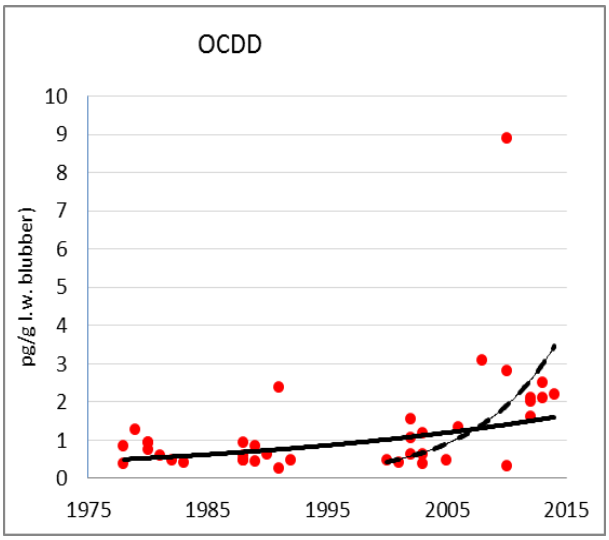
# 7. Appendix

**Appendix Figure 1. Dioxins**

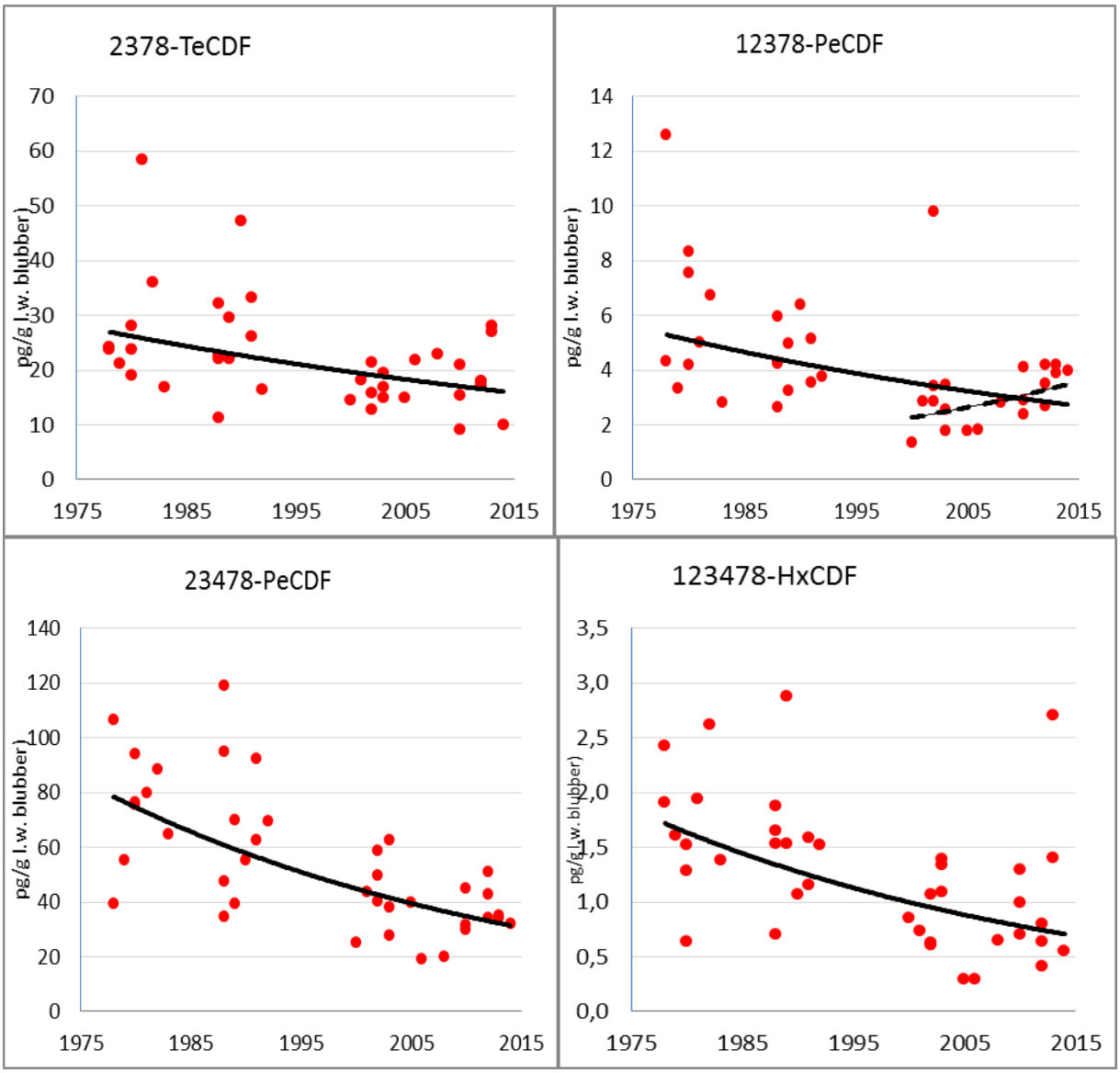
*Dioxins in juvenile ringed seals from the Baltic Sea (pg/g lw in blubber). All but OCDD showed a decreasing rate for the whole period (1978-2014), and 1234678-HpCDD showed no significant trend.*

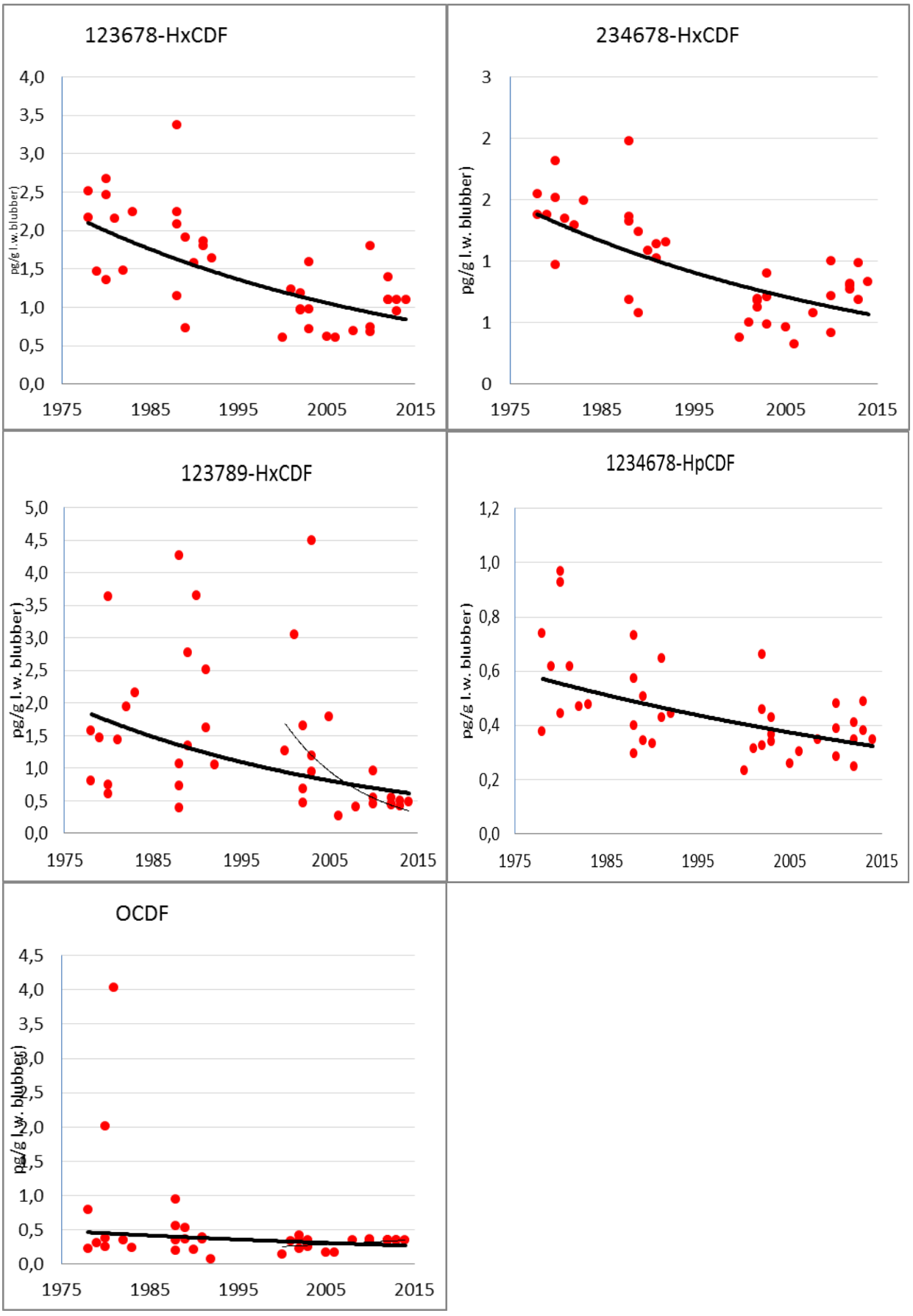



OCDD
pg/g l.w. blubber)
10
9
8
7
6
5
4
3
2
1
0
1975
1985
1995
2005
2015

**Appendix Figure 2. Furans**

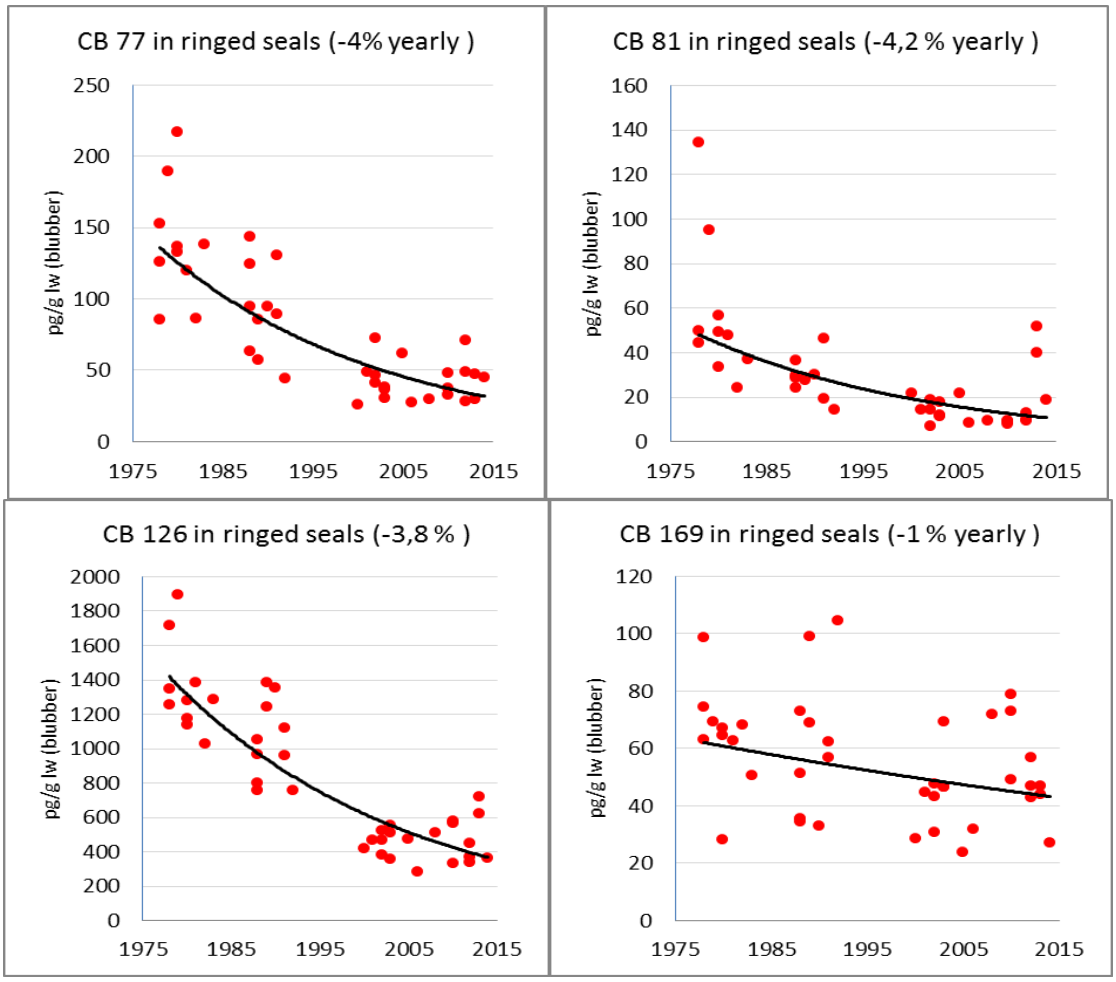
*Furans in juvenile ringed seals from the Baltic Sea (pg/g lw in blubber). All congeners showed a decreasing rate for the whole period (1978-2014).*




123678-HxCDF
pg/g l.w.blubber)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1975
1985
1995
2005
2015
234678-HxCDF
pg/g l.w. blubber)
3
2
2
1
1
0
1975
1985
1995
2005
2015
123789-HxCDF
pg/g l.w. blubber)
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1975
1985
1995
2005
2015
1234678-HpCDF
pg/g l.w. blubber)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1975
1985
1995
2005
2015
OCDF
pg/g l.w. blubber)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1975
1985
1995
2005
2015

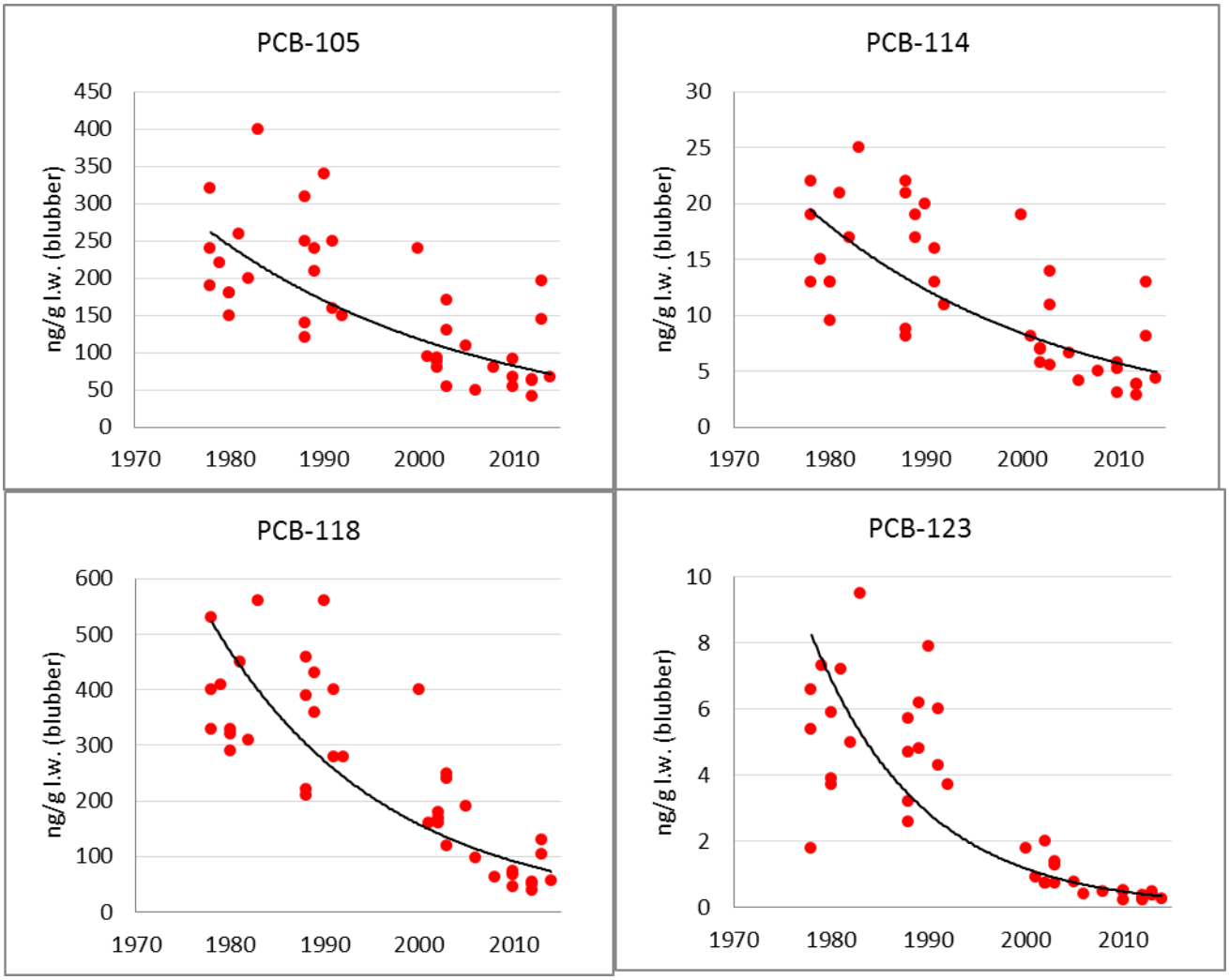
**Appendix, Figure 3. *Non-ortho* PCBs**

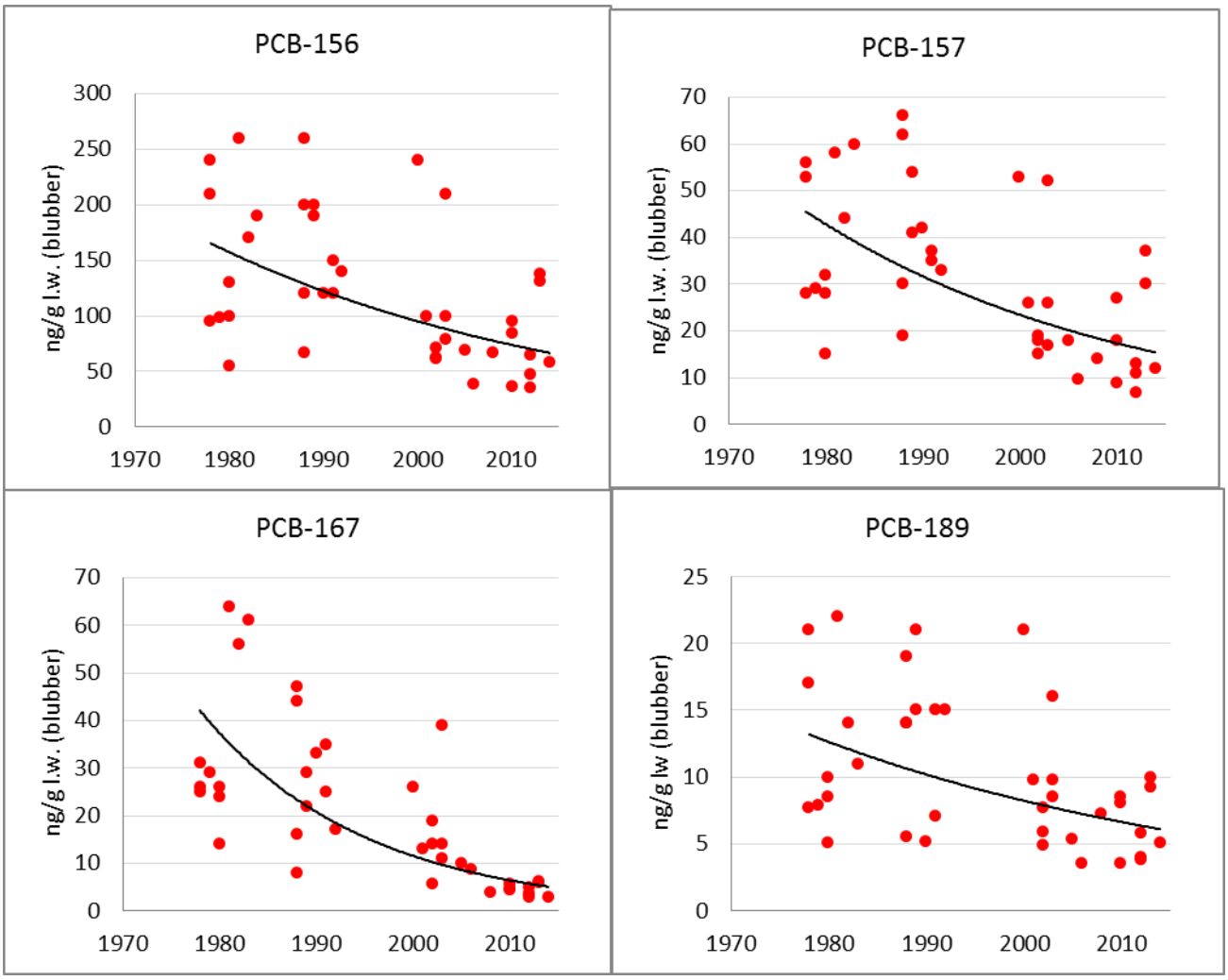
*Non-ortho dl-PCBs in juvenile ringed seals from the Baltic Sea (pg/g lw in blubber). All congeners decreased in concentrations over the time period (1978-2014).*



**Appendix, Figure 4. *Mono-ortho*-CBs**

*Mono-ortho-CBs in juvenile ringed seals from the Baltic Sea (ng/g lw in blubber). All congeners decreased in concentrations over the time period (1978-2014).*




PCB-156
ng/g l.w. (blubber)
300
250
200
150
100
50
0
1970
1980
1990
2000
2010
PCB-157
ng/g l.w. (blubber)
70
60
50
40
30
20
10
0
1970
1980
1990
2000
2010
PCB-167
ng/g l.w. (blubber)
70
60
50
40
30
20
10
0
1970
1980
1990
2000
2010
PCB-189
ng/g lw (blubber)
25
20
15
10
5
0
1970
1980
1990
2000
2010