



Maisons-Alfort, 27 November 2009

OPINION

of the French Food Safety Agency regarding the determination of threshold values for NDL-PCBs as a tool for assessing the risk of PCB contamination in freshwater and marine fish

1- BACKGROUND

Dioxins (i.e. dioxins [PCDDs] and furans [PCDFs])¹ and dioxin-like polychlorinated biphenyls (DL-PCBs) in marketed fish species are currently regulated at the European level (Regulation (EC) no. 1881/2006 of 19 December 2006 amended setting the maximum levels for some contaminants in foodstuffs). AFSSA's opinion regarding a national sampling plan to monitor PCBs in freshwater fish (Request no. 2008-SA-0019) evoked the possibility of screening only non-dioxin-like PCBs (NDL-PCBs) to assess contamination in rivers so as to limit the number of the more expensive assays of dioxins and DL-PCBs.

In this latter opinion, AFSSA stated that as soon as enough data became available, it would assess a methodology based on screening only NDL-PCBs for analysing the risk of dioxins contamination through the correlation of NDL-PCB levels with dioxins and DL-PCB levels, thereby precluding the necessity for dioxins and DL-PCB assays in a sizeable number of fish samples.

Given the quantity of data currently available on dioxins and PCBs (DL-PCBs and NDL-PCBs) in freshwater and marine fish matrices, AFSSA issued an internal request to determine the NDL-PCB threshold levels that correspond to the regulatory limits of dioxins and DL-PCBs and that could be used as a tool for assessing the risk of PCB contamination.

AFSSA notes that the determined NDL-PCB threshold values (sum of the six congeners PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180) are not public health thresholds but thresholds for determining whether freshwater and marine fish meet EU regulation requirements in regard to levels of dioxins and DL-PCBs (total TEQ_{WHO98}) with a specified error rate.

After analysing available data at AFSSA's Quantitative physico-chemical risk assessment unit and consulting the Emergency collective expert assessment group on 'Assessing risks of PCBs in food and feed' on 24 June and 14 September 2009, AFSSA is issuing the following opinion.

¹ Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) make up a group of halogenated polycyclic compounds that are commonly referred to under the generic name of dioxins (PCDD/Fs). For more information, consult AFSSA's opinion of 9 January 2006 regarding the assessment of exposure of the French population to dioxins, furans and DL-PCBs as well as the accompanying report of November 2005, both available on www.afssa.fr.

2- DATA AND METHODOLOGY

2.1 Data

Data on the contamination of freshwater and marine fish come from the following sources:

- the targeted monitoring plan carried out by the Directorate General for Food (DGAL) in 2008, representing 362 assays;
- specific complementary plans set up by the DGAL, representing 912 assays;
- the national sampling plan for freshwater fish carried out in 2008 by the French National Agency for Water and Aquatic Environments (ONEMA), representing 912 assays;
- surveillance plans carried out by the DGAL from 2004 to 2006, representing 420 assays on marine fish.

The sample of freshwater fish analysed in this opinion involves 2024 assays (379 assays on eels and 1645 assays on other species), and the marine fish sample is based on 542 assays.

There are different regulatory limits for different species:

- eels (12 pg TEQ_{WHO98}/g fresh weight);
- other species (8 pg TEQ_{WHO98}/g fresh weight).

Eels and other fish species were analysed separately.

The results of the analyses carried out in this opinion were obtained using gas chromatography-high resolution mass spectrometry (GC/HRMS), the method of reference for assaying dioxins compounds and DL-PCBs.

2.2 Methodology

To determine, with a low error rate, the NDL-PCB thresholds that correspond to the regulatory limits of the sum of dioxins and DL-PCBs (dioxins+DL-PCBs), several decision criteria based on the calculation of false positives or false negatives were applied (see Appendix 1).

- Estimation of a false negative (FN) rate lower than 1% is absolutely essential because it guarantees that, below the determined NDL-PCB threshold, 99% of the analysed samples have a level of dioxins+DL-PCBs lower than the regulatory limit. A low error rate is one of the basic requirements for acceptance of analytical procedures for screening methods as described in the Commission Regulation (EC) No. 1883/2006.
- Determination of an upper NDL-PCB threshold that guarantees, with an acceptable false positive (FP) rate, that dioxins+DL-PCB levels are greater than the regulatory limit is also important. The acceptable false positive rate is not stipulated in the EC Regulation. Given the absence of a maximum false positive rate, the following three scenarios for risk management were proposed:
 - 1st scenario: a 1% false negative rate and a 1% false positive rate;
 - 2nd scenario: a 1% false negative rate and a 5% false positive rate;
 - 3rd scenario: definition of a single threshold, based on a model, that makes it possible to declare compliance/non-compliance with 1% false negative rate and with an estimation of the corresponding false positive rate.
- Regarding the uncertainty of laboratory measurements: the predictability of NDL-PCB thresholds is defined with respect to the maximum limit of 12 pg TEQ_{WHO98}/g fresh weight for eels and 8 pg TEQ_{WHO98}/g fresh weight for other species. However, measurement uncertainty of analytical results produced by testing laboratories — in general of the order of 20% — was

also taken into account for determining these thresholds insofar as risk managers are required to use these values to assess the compliance of the assayed food. The regulatory limit for the sum of TEQ_{WHO98} is thus bounded by 10 and 15 $pg\ TEQ_{WHO98}/g$ fresh weight for eels and 6 and 10 $pg\ TEQ_{WHO98}/g$ fresh weight for other species. The threshold levels of NDL-PCBs were therefore determined based on the regulatory limit and on the confidence interval of this limit.

3- RESULTS

Analysis of the data shows that dioxins+DL-PCB levels and NDL-PCB levels were significantly correlated ($p < 0.0001$) for all three species groups. Appendix 2 gives the individual contributions of DL-PCB and NDL-PCB congeners as well as the correlations between dioxins+DL-PCBs, calculated based on WHO 1998 TEFs, and NDL-PCBs. Appendix 3 gives the congener contributions and the correlations based on WHO TEFs established in 2005.

The tables and figures in Appendix 4 summarise the determined NDL-PCB thresholds that account for a 20% measurement uncertainty.

The NDL-PCB thresholds corresponding to those set for dioxins+DL-PCBs based on 2005 TEFs are given in Appendix 5.

1. For eels (regulatory limit for dioxins+DL-PCBs: 12 pg TEQ_{WHO98}/g fresh weigh), the determined thresholds for NDL-PCBs were as follows:
 - With a **1% false negative rate and a 1% false positive rate**, the determined indicator thresholds for PCB-NDLs were respectively <180 ng/g for compliance and >350 ng/g for non-compliance. Based only on the assay of NDL-PCBs, 82% of the analysed eel samples could be declared compliant or non-compliant. For the remaining 18% of samples, additional screening targeting dioxins and DL-PCBs would be necessary.
 - With a **1% false negative rate and a 5% false positive rate**, the determined indicator thresholds for PCB-NDLs were respectively <180 ng/g for compliance and >200 ng/g for non-compliance. Based only on the assay of NDL-PCBs, 96% of the analysed eel samples could be declared compliant or non-compliant. For the remaining 4% of samples, additional screening that targets dioxins and DL-PCBs would be necessary.
 - Finally, with a **1% false negative rate and a 6% false positive rate**, using only one NDL-PCB threshold level (180 ng/g), 100% of eel samples could be declared compliant or non-compliant without any additional screening for dioxins and DL-PCBs.

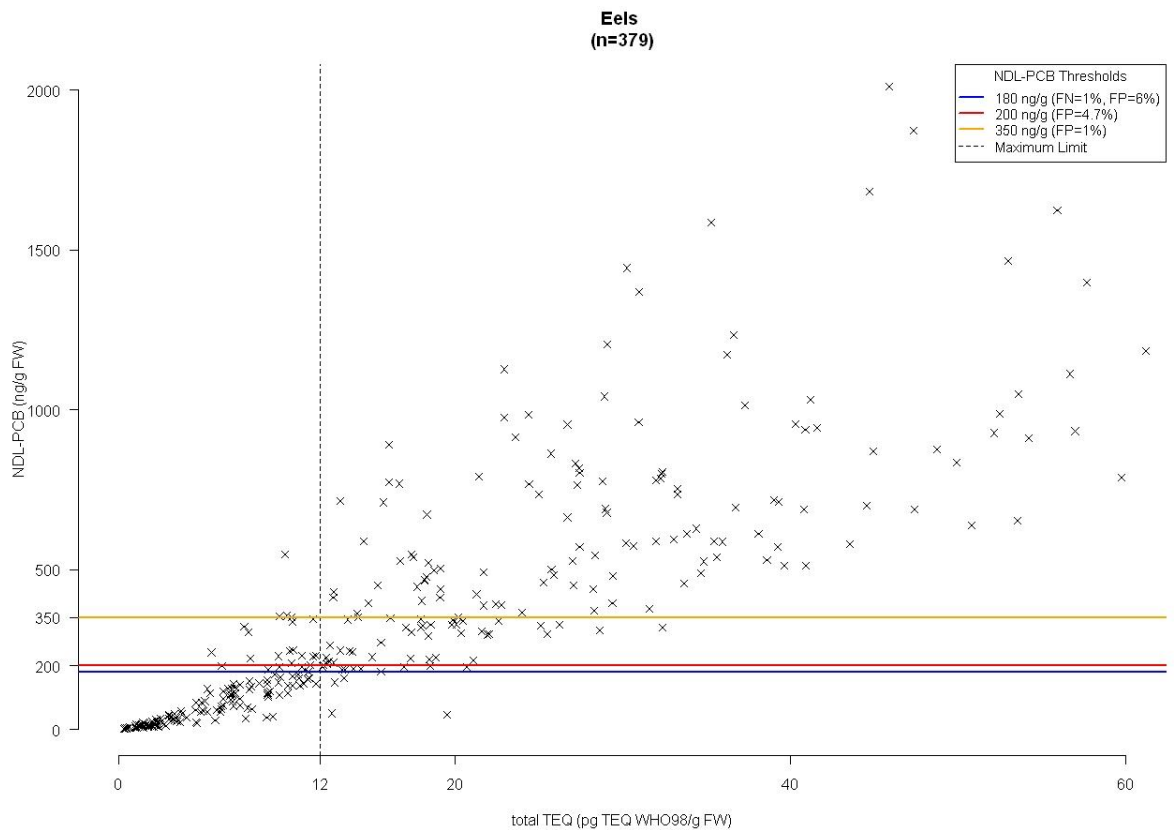


Figure 1. NDL-PCB thresholds defined for eels

2. For other freshwater fish (regulatory limit for dioxins+DL-PCBs: 8 pg TEQ_{WHO98}/g fresh weight), the determined NDL-PCB thresholds were as follows:
- With a **1% false negative rate and a 1% false positive rate**, the determined indicator NDL-PCB thresholds were <110 ng/g for compliance and >220 ng/g for non-compliance, respectively. Based only on the assay of NDL-PCBs, 79% of the analysed freshwater fish samples could be declared compliant or non-compliant. For the remaining 21% of samples, additional screening targeting dioxins and DL-PCBs would be necessary.
 - With a **1% false negative rate and a 5% false positive rate**, the determined indicator NDL-PCB thresholds were <110 ng/g for compliance and >160 ng/g for non-compliance, respectively. Based on the assay of only NDL-PCBs, 89% of the analysed freshwater fish samples could be declared compliant or non-compliant. For the remaining 11% of samples, additional screening targeting dioxins and DL-PCBs would be necessary.
 - Finally, with a **1% false negative rate and a 13% false positive rate**, using only one NDL-PCB threshold level (110 ng/g), 100% of freshwater fish samples could be declared compliant or non-compliant without any additional screening for dioxins and DL-PCBs.

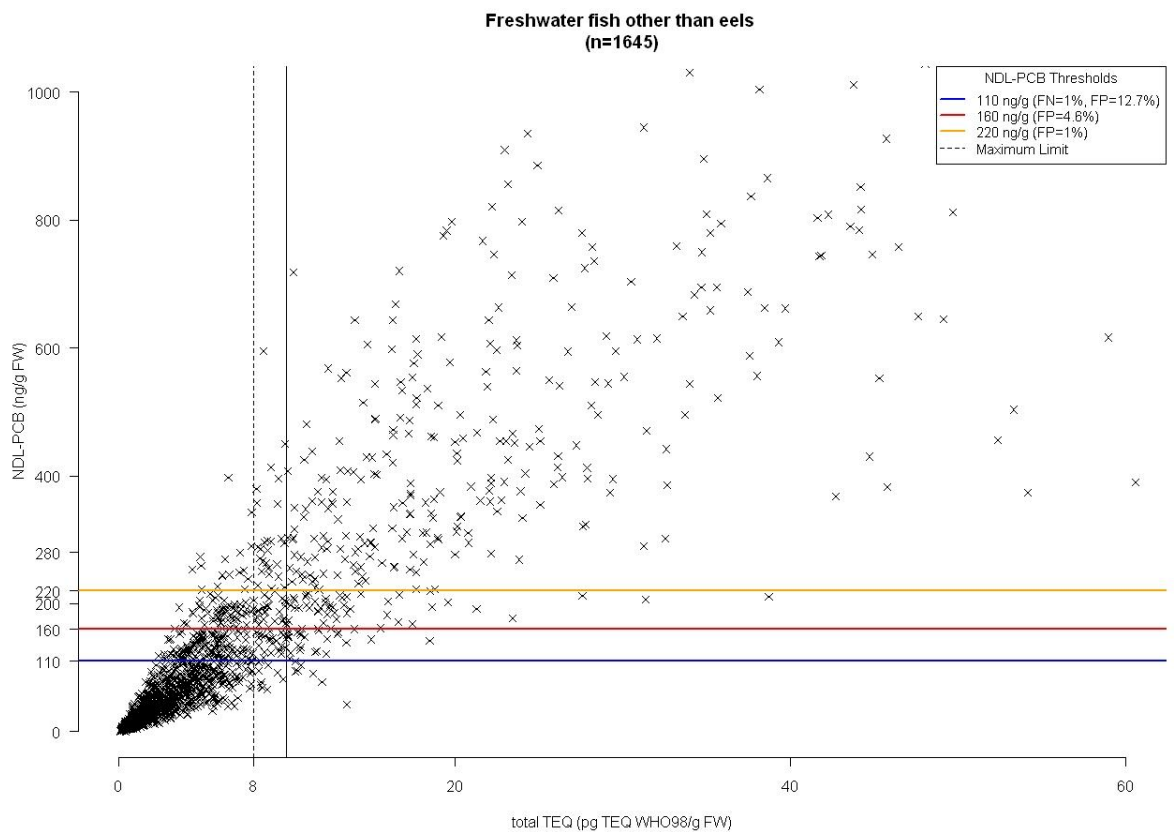


Figure 2. NDL-PCB thresholds defined for freshwater fish other than eels

3. For marine fish (regulatory limit for the sum of dioxins and DL-PCBs: 8 pg TEQ_{WHO98}/g fresh weight), the determined NDL-PCB thresholds were as follows:
 - For a **1% false negative rate and a 1% false positive rate**, the indicator thresholds determined for NDL-PCBs were <80 ng/g for declaring a sample compliant and >160 ng/g for declaring a sample non-compliant. Based only on the assay of NDL-PCBs, 96% of the analysed samples of marine fish could be declared compliant or non-compliant. For the remaining 4% of samples, additional screening targeting dioxins and DL-PCBs would be necessary.
 - With a **1% false negative rate and a 3% false positive rate**, using only one threshold level (75-80 ng/g) determined for NDL-PCBs, 100% of freshwater fish samples could be declared compliant or non-compliant without any additional screening for dioxins and DL-PCBs.

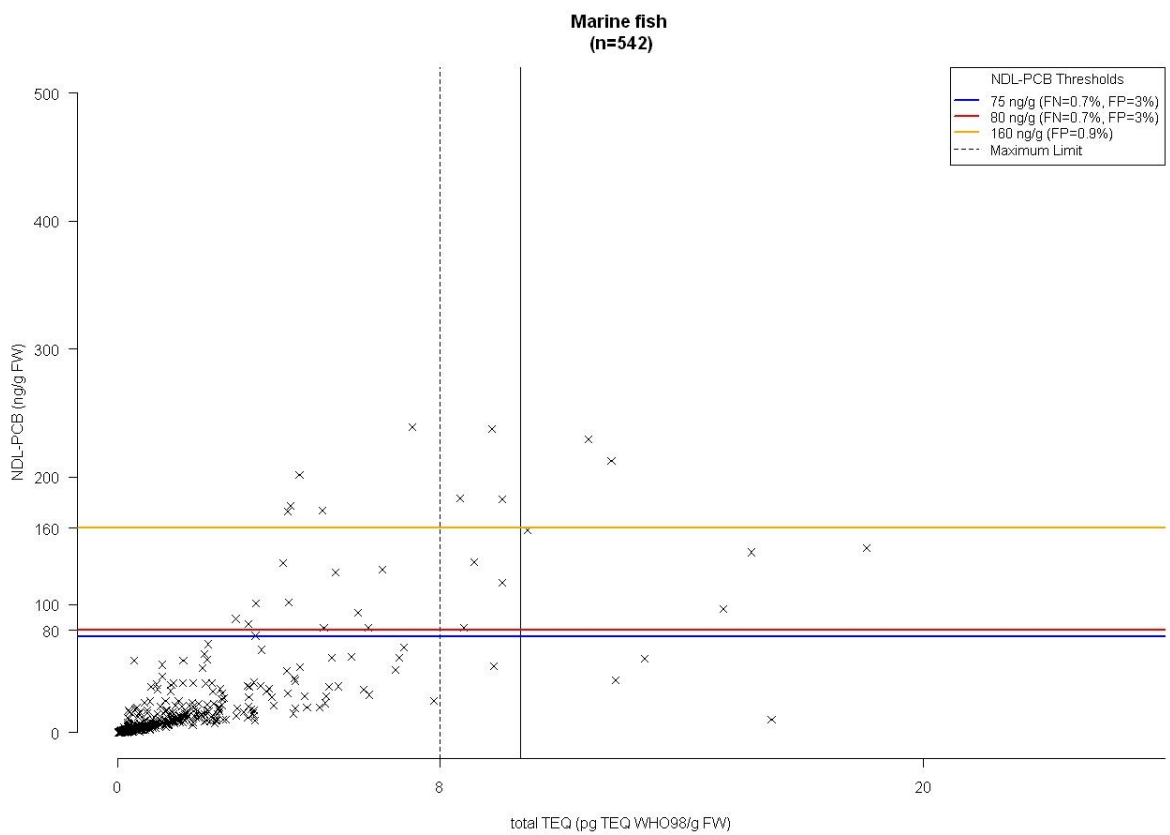


Figure 3. NDL-PCB thresholds defined for marine fish

4- CONCLUSION

In conclusion, this analysis shows that it is possible to establish correlations between NDL-PCB levels and dioxins+DL-PCBs levels. As a result, NDL-PCB thresholds could be determined with specific error rate to declare the compliance and non-compliance of freshwater and marine fish with regards to dioxins and DL-PCB levels. These correlations did not include any data from accidental contamination events.

This approach provides a practical method for routine analyses (excluding accidents) of the PCB risk contamination: the sole determination of NDL-PCB levels indicates the compliance or non-compliance, regarding to the regulatory limit for dioxins and DL-PCB levels, with high precision (1% of false negatives), in 80 to 100% of the fishery (marine and freshwater) product samples, depending on the false positive rate selected by risk managers.

The Director General

Marc Mortureux

5. Main References:

Afssa - Agence française de sécurité sanitaire des aliments, 2009. Avis du 13 mai relatif à l'interprétation des données du plan national PCB 2008 dans les poissons de rivière et à la proposition du plan d'échantillonnage 2009 (ref : 2009-SA-0118).

Afssa - Agence française de sécurité sanitaire des aliments, 2008. Avis du 5 février relatif au plan d'échantillonnage national des PCB dans les poissons de rivière : proposition de méthodologie (ref : 2008-SA-0019).

6. KEYWORDS:

NDL-PCBs, Dioxins, DL-PCBs, correlation, thresholds

APPENDIX 1

Definition of false negatives (FN), true negatives (TN), false positives (FP) and true positives (TP)

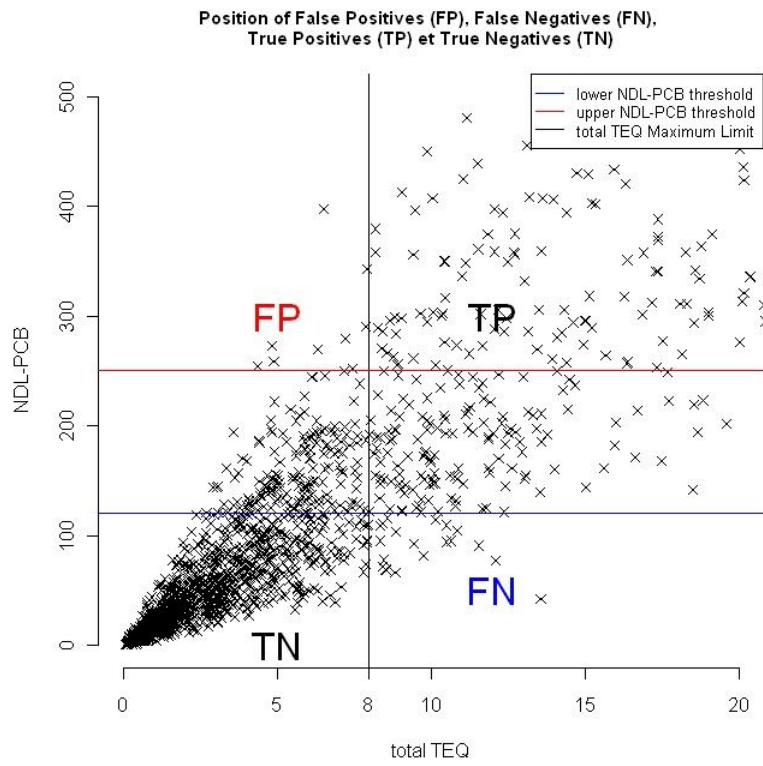
A false negative corresponds to an analysis whose result indicates that the NDL-PCB concentration is lower than the determined threshold level although the sum of dioxins and DL-PCBs (dioxins+DL-PCB) concentration is higher than the regulatory limit.

A true negative corresponds to an analysis whose result indicates that the NDL-PCB concentration is lower than the determined threshold level and the dioxins+DL-PCB concentration is lower than the regulatory limit.

A false positive corresponds to an analysis whose result indicates that the NDL-PCB concentration is higher than the determined threshold level although the dioxins+DL-PCB concentration is lower than the regulatory limit.

A true positive corresponds to an analysis whose result indicates that the NDL-PCB concentration is higher than the determined threshold level and the dioxins+DL-PCB concentration is higher than the regulatory limit.

	Exceeds the regulatory limit of total TEQ _{WHO98}	Does not exceed the regulatory limit of total TEQ _{WHO98}
Exceeds the NDL-PCB threshold	True positive	False positive
Does not exceed the NDL-PCB threshold	False negative	True negative



The percentages are calculated with respect to the whole data set; thus, when data points are found between the two NDL-PCB thresholds, the sum (FP+FN+TP+TN) is lower than 100.

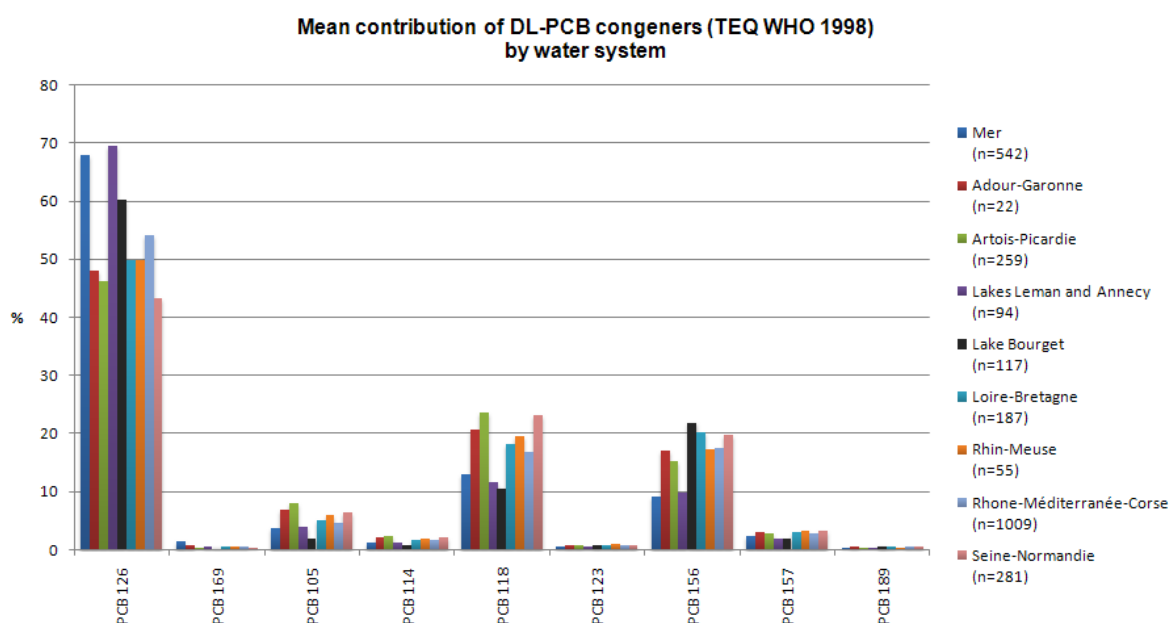
APPENDIX 2

Contributions of DL-PCB and NDL-PCB congeners and correlation of dioxins+DL-PCBs with NDL-PCBs based on 1998 TEFs

Congener profiles were established for NDL-PCBs and DL-PCBs for each water system, with Alpine lakes (Lakes Lemman and Annecy, Lake Bourget) treated separately.

The following three figures show the congener profiles for DL-PCBs (based on 1998 TEFs).

- All species taken together:

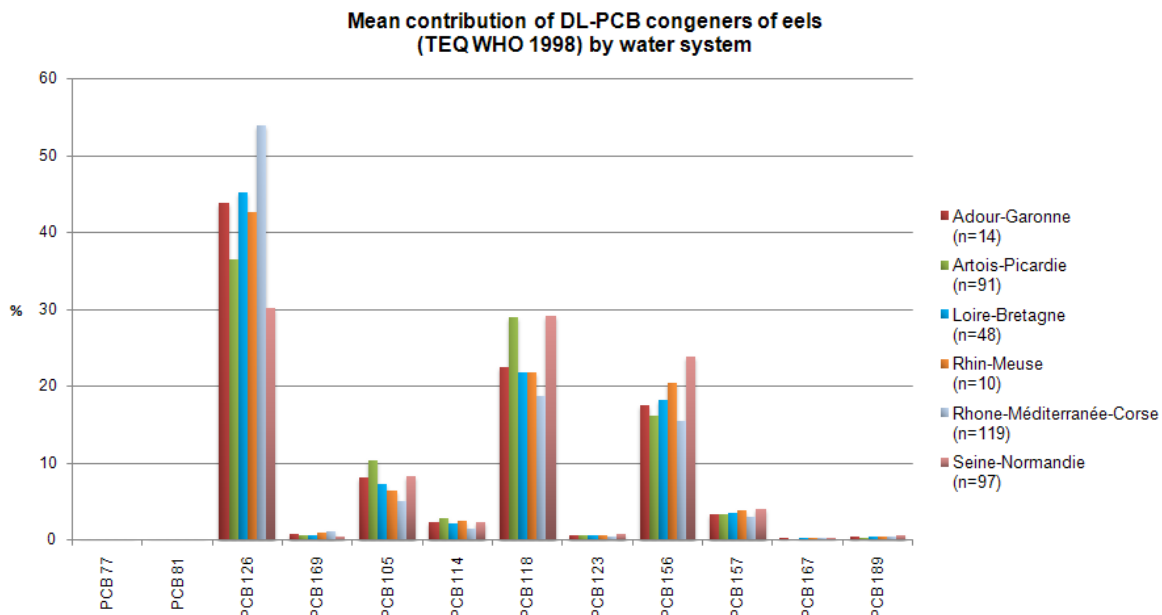


PCB 77, 81 and 167 are not shown because they contributed only negligible amounts to the TEQ.

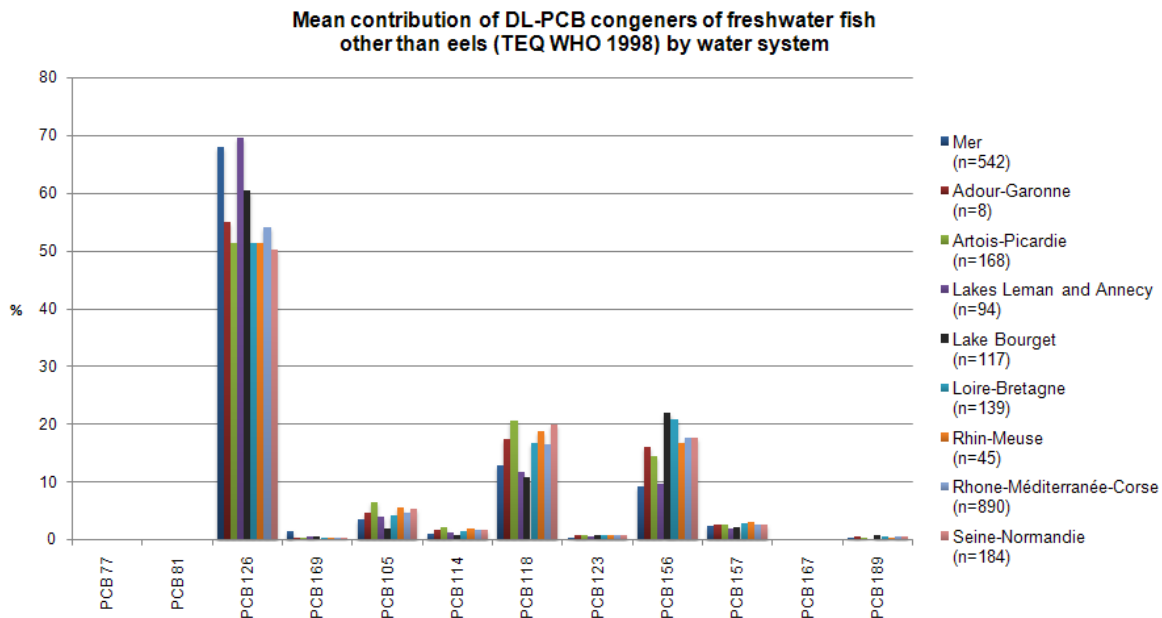
The congener that contributed the most to DL-PCBs (around 50%) is PCB 126.

All congeners have the same profile in the various water systems, except in marine fish and in those that come from Alpine lakes (Lakes Lemman and Annecy) for which PCB 126 and 118 are the dominant DL-PCB congeners and have lower and higher relative contributions, respectively, in the other basins.

- Eels:

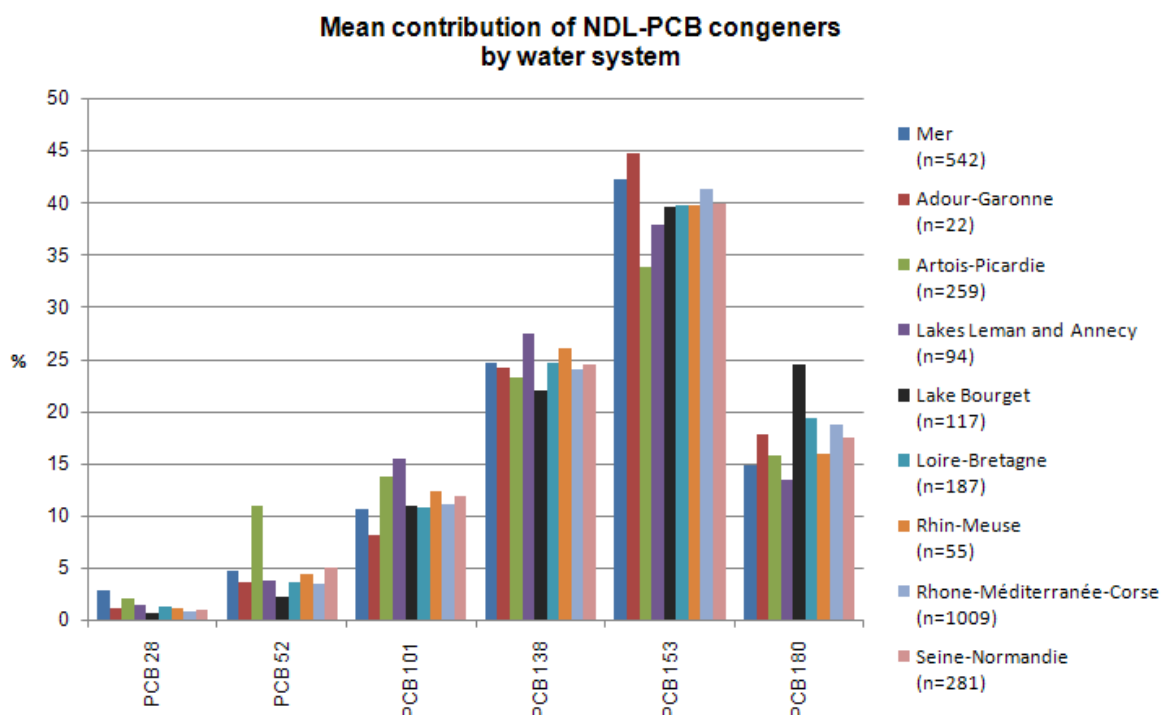


- Freshwater fish other than eels:



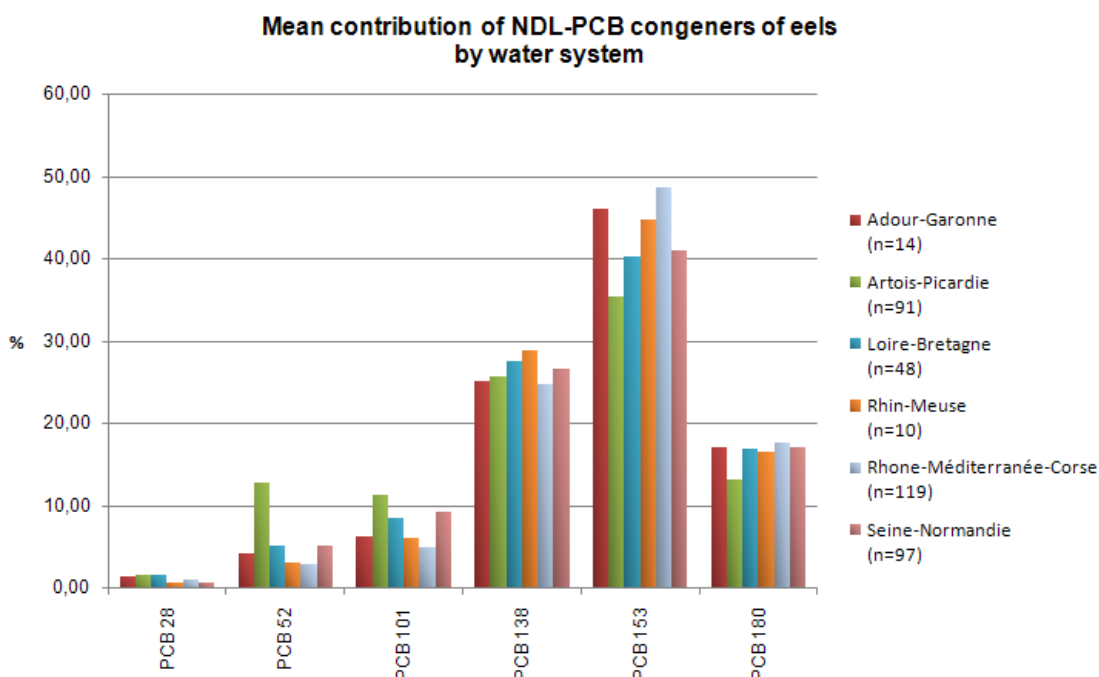
The following three figures show the NDL-PCB congener profiles.

- All species taken together:



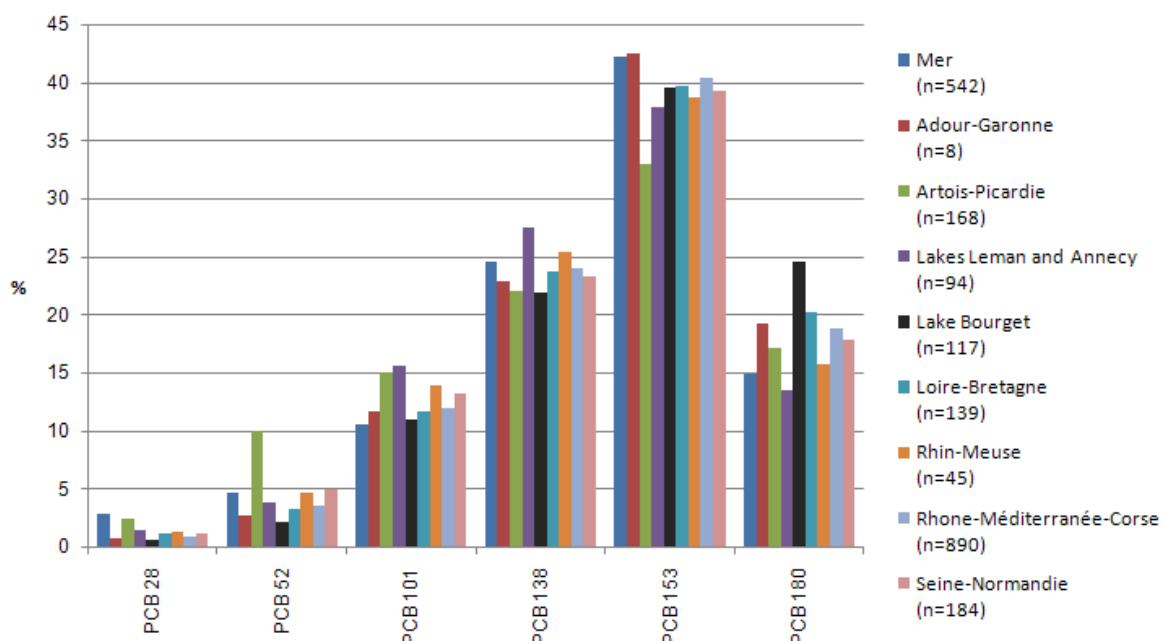
The congener that contributes the most to NDL-PCBs (around 40%) is PCB 153.

- Eels:



- Freshwater fish other than eels:

Mean contribution of NDL-PCB congeners of freshwater fish other than eels by water system



The strong correlations of dioxins+DL-PCBs with NDL-PCBs are shown in the following table:

Water system	<i>all species</i>			<i>eels</i>			<i>freshwater fish other than eels</i>		
	n	correlation total TEQ and NDL-PCBs	P-value	n	correlation total TEQ and NDL-PCBs	P-value	n	correlation total TEQ and NDL-PCBs	P-value
Adour-Garonne	22	0.94	<0.0001	14	0.91	<0.0001	8	0.98	<0.0001
Artois-Picardie	259	0.97	<0.0001	91	0.97	<0.0001	168	0.96	<0.0001
Lakes Lemman and Annecy	94	0.96	<0.0001	-	-	-	94	0.96	<0.0001
Lake Bourget	117	0.98	<0.0001	-	-	-	117	0.98	<0.0001
Loire-Bretagne	187	0.91	<0.0001	48	0.89	<0.0001	139	0.93	<0.0001
Rhine-Meuse	55	0.94	<0.0001	10	0.95	<0.0001	45	0.88	<0.0001
Rhone-Méditerranée-Corse	1009	0.91	<0.0001	119	0.96	<0.0001	890	0.90	<0.0001
Seine-Normandie	281	0.84	<0.0001	97	0.79	<0.0001	184	0.94	<0.0001
freshwater fish	2024	0.89	<0.0001	379	0.85	<0.0001	1645	0.90	<0.0001
marine fish	542	0.86	<0.0001						

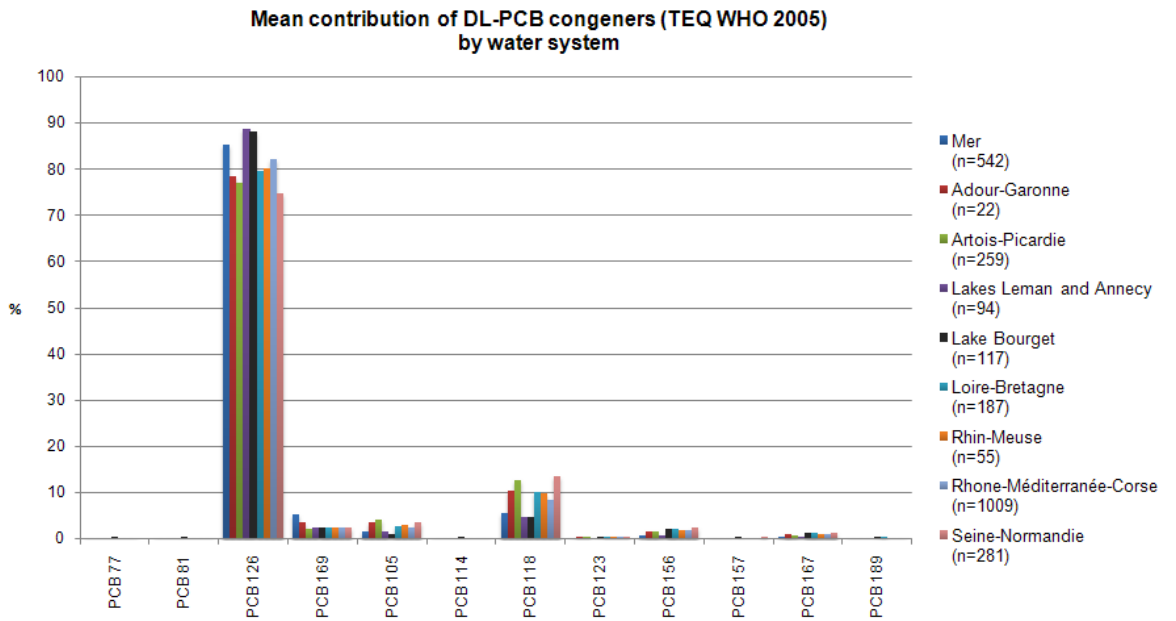
The DL-PCB congener profile and the correlations of dioxins+DL-PCBs with NDL-PCBs for all species and for eels and freshwater fish other than eels, based on 2005 TEFs are given in Appendix 3.

APPENDIX 3

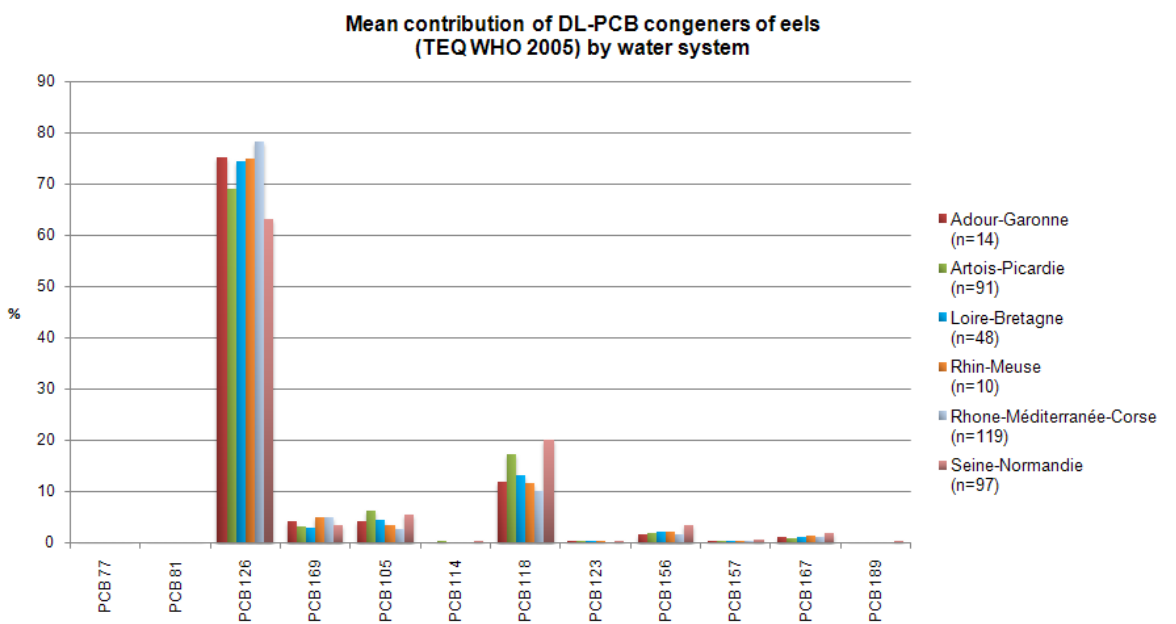
Contributions of DL-PCB and NDL-PCB congeners and correlation of dioxins+DL-PCBs with NDL-PCBs based on 2005 TEFs

The following three figures show the congener profiles for DL-PCBs (based on 2005 TEFs).

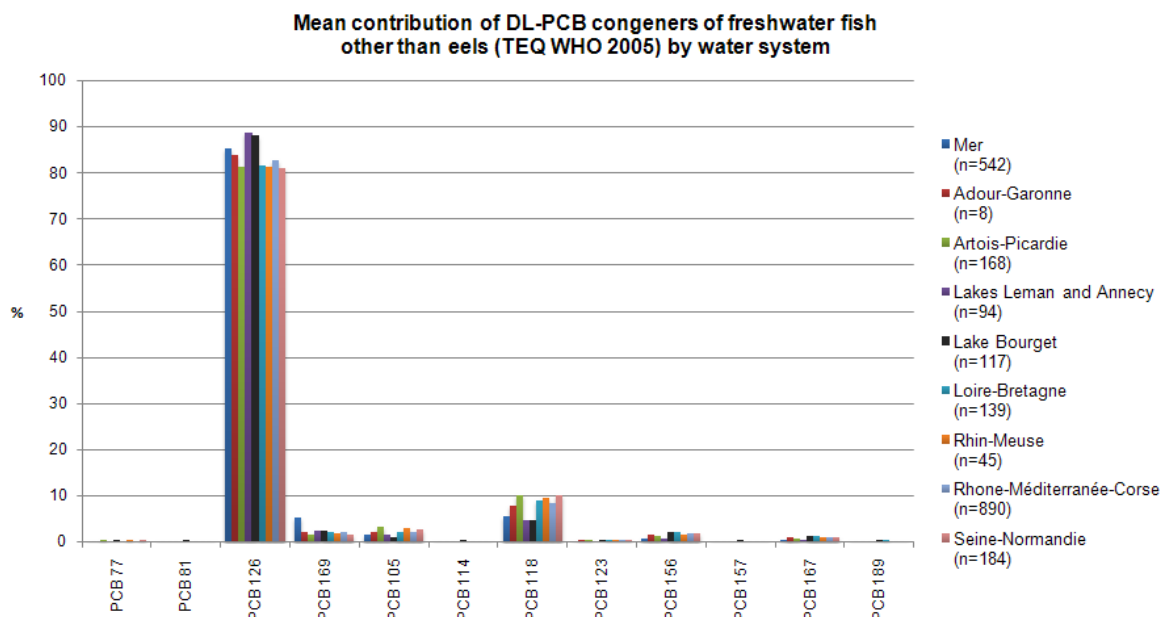
- All species taken together:



- Eels:



- Freshwater fish other than eels:



The strong correlations of dioxins+DL-PCBs with NDL-PCBs are shown in the following table:

Water system	<i>all species</i>			<i>eels</i>			<i>freshwater fish other than eels</i>		
	n	correlation total TEQ and NDL-PCBs	P-value	n	correlation total TEQ and NDL-PCBs	P-value	n	correlation total TEQ and NDL-PCBs	P-value
Adour-Garonne	22	0.94	<0.0001	14	0.91	<0.0001	8	0.96	0.0002
Artois-Picardie	259	0.96	<0.0001	91	0.96	<0.0001	168	0.93	<0.0001
Lakes Leman and Annecy	94	0.97	<0.0001	-	-	-	94	0.97	<0.0001
Lake Bourget	117	0.93	<0.0001	-	-	-	117	0.93	<0.0001
Loire-Bretagne	187	0.83	<0.0001	48	0.77	<0.0001	139	0.84	<0.0001
Rhine-Meuse	55	0.91	<0.0001	10	0.93	<0.0001	45	0.83	<0.0001
Rhone-Méditerranée-Corse	1009	0.87	<0.0001	119	0.93	<0.0001	890	0.87	<0.0001
Seine-Normandie	281	0.78	<0.0001	97	0.73	<0.0001	184	0.90	<0.0001
freshwater fish	2024	0.83	<0.0001	379	0.81	<0.0001	1645	0.87	<0.0001
marine fish	542	0.82	<0.0001						

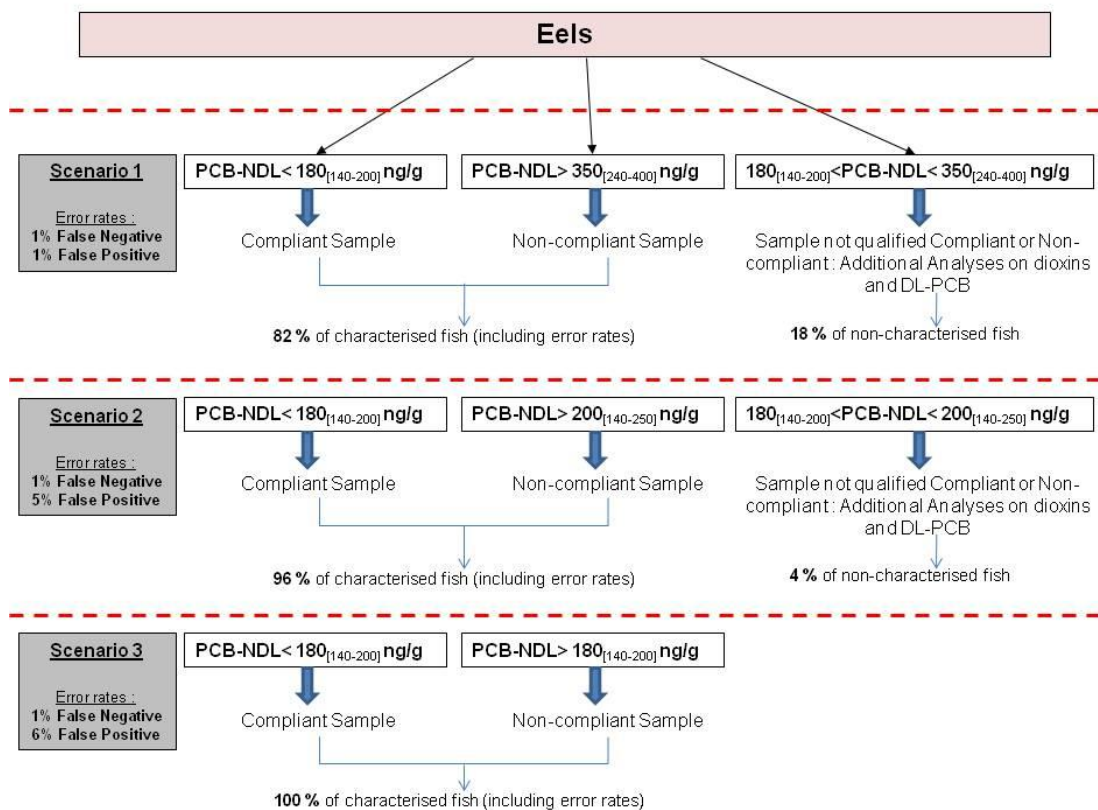
APPENDIX 4

Threshold bounds of compliance and non-compliance of dioxins+DL-PCBs (calculated on 1998 TEFs) based on the assay of NDL-PCBs

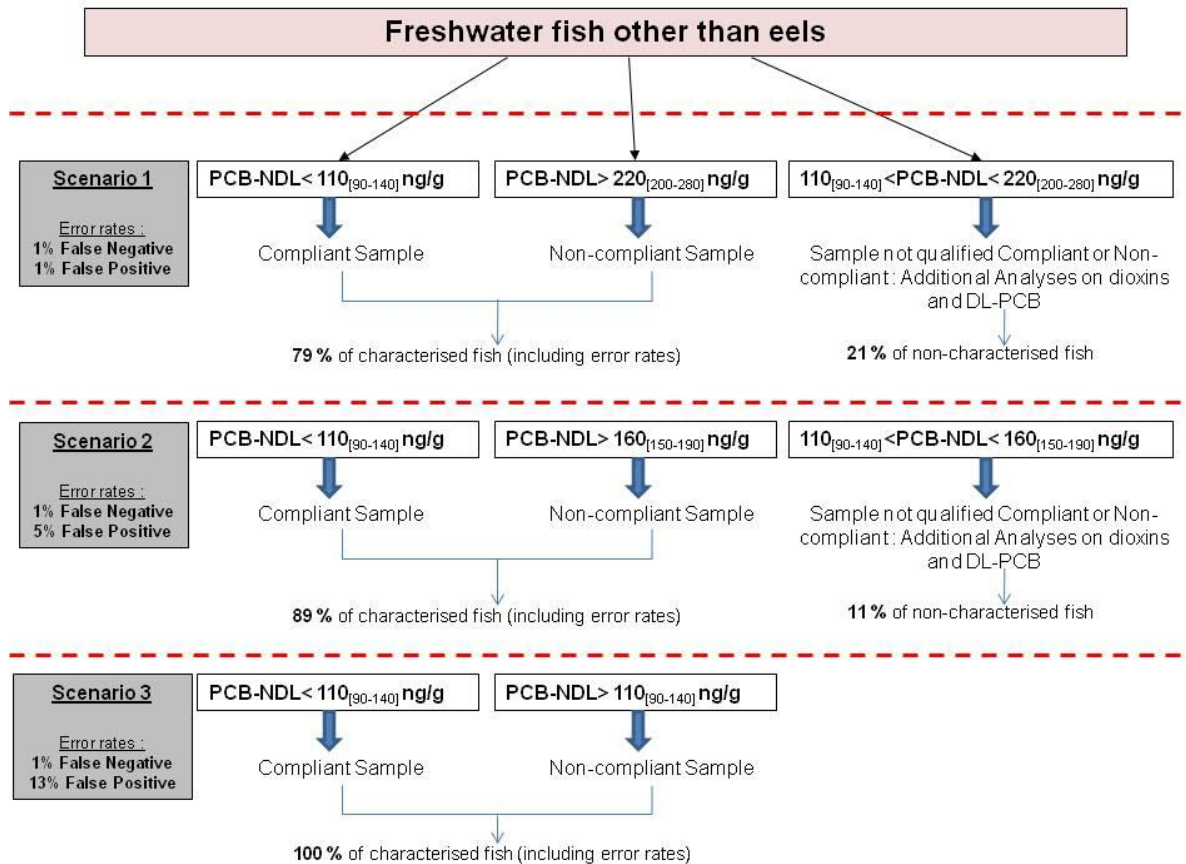
The table below summarises the NDL-PCB indicator thresholds determined for the different groups of species studied — eels, freshwater fish other than eels and marine fish — according to the desired precision (%FN and FP) in the three scenarios elucidated and accounting for a 20% measurement uncertainty of laboratory results.

Group of species	Scenario	Error rate		Determined thresholds and confidence intervals (in ng/g fresh weight)	% fish characterised (including error rates)	% non-characterised fish
		% False negative	% False positive			
eels	1	1	1	180 [140 - 200] and 350 [240 - 400]	82	18
	2	1	5	180 [140 - 200] and 200 [140 - 250]	96	4
	3	1	6	180 [140 - 200]	100	0
freshwater fish other than eels	1	1	1	110 [90 - 140] and 220 [200 - 280]	79	21
	2	1	5	110 [90 - 140] and 160 [150 - 190]	89	11
	3	1	13	110 [90 - 140]	100	0
marine fish	1	1	1	80 [60 - 100] and 160 [130 - 180]	96	4
	2 and 3	1	3	75-80 [60 - 100]	100	0

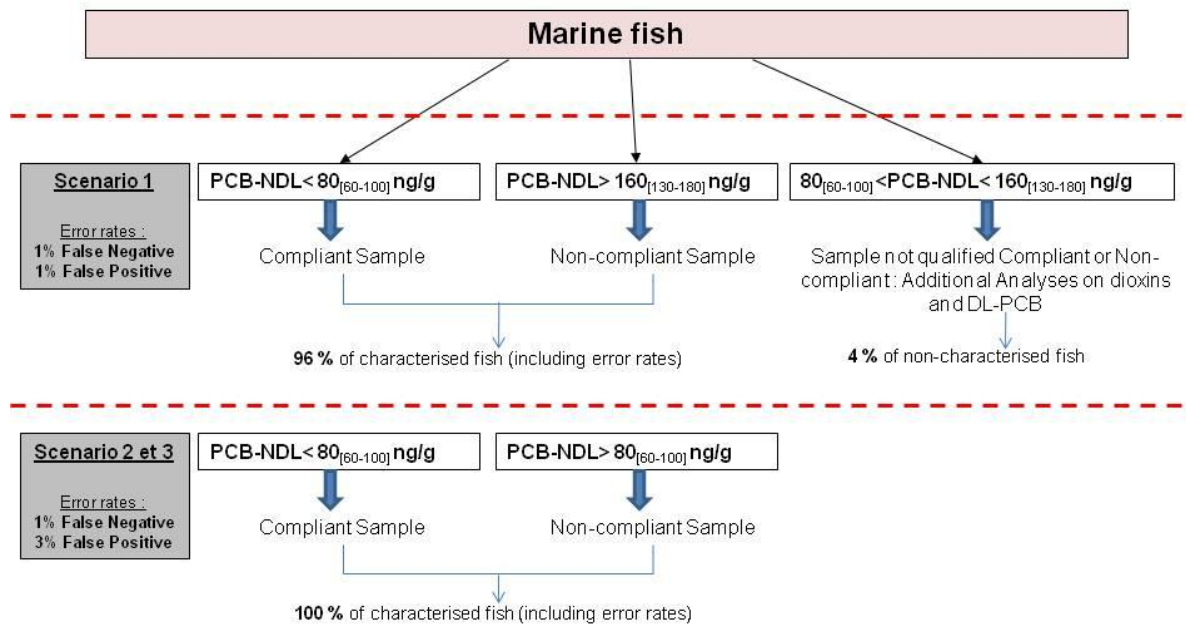
Decision chart for eels:



Decision chart for freshwater fish other than eels:



Decision chart for marine fish:

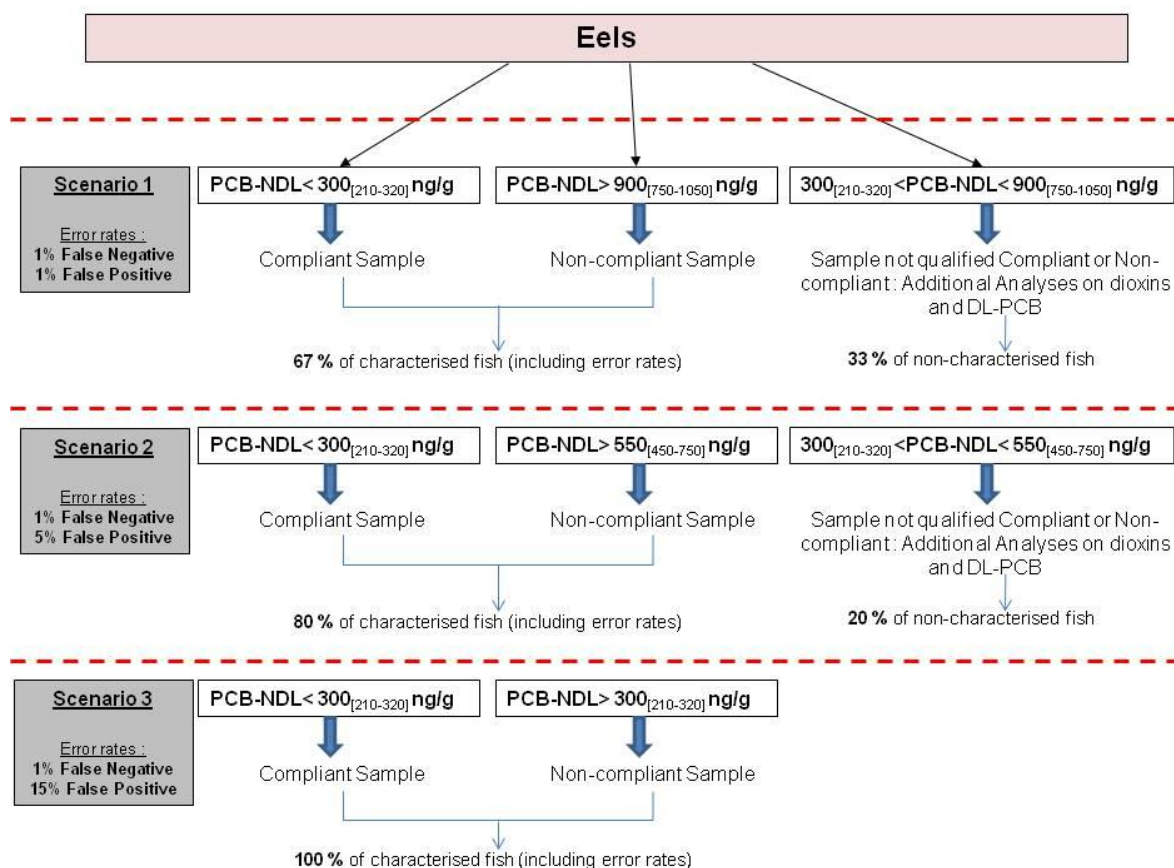


APPENDIX 5

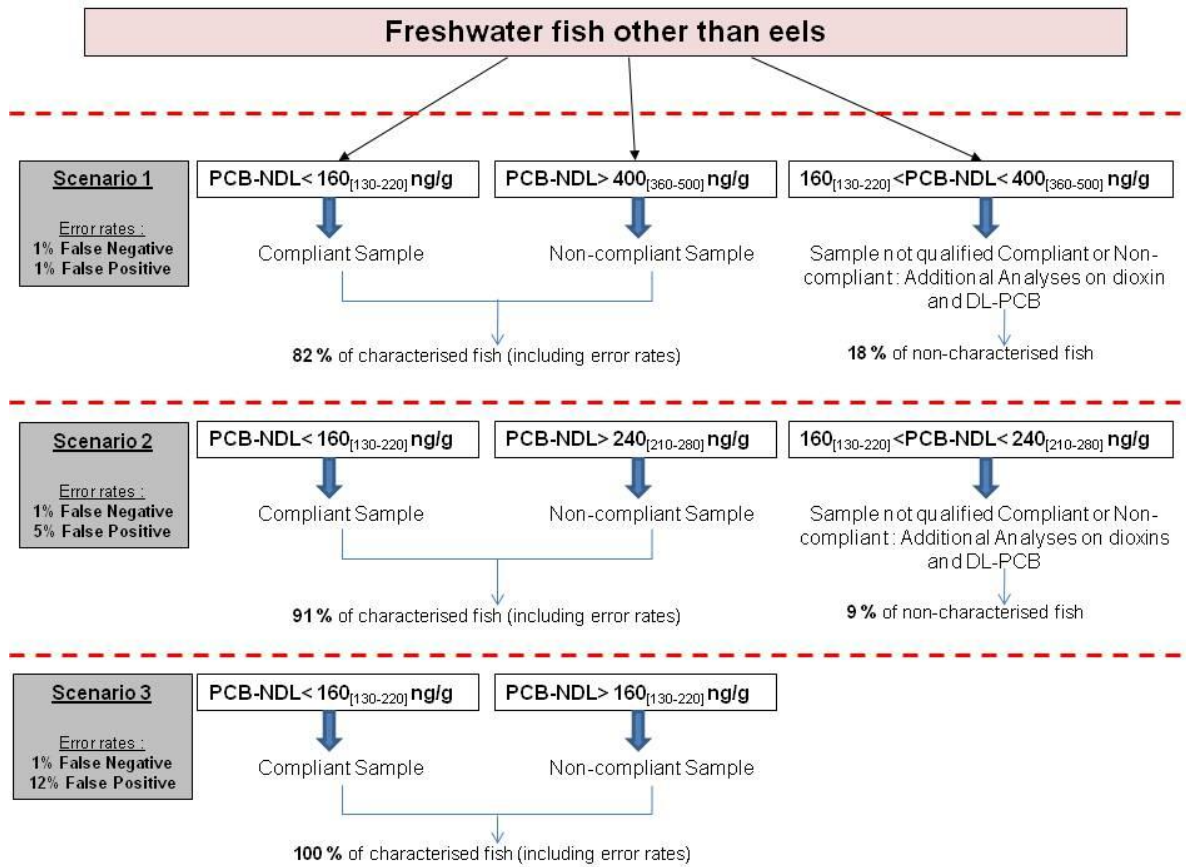
Thresholds and confidence intervals of compliance and non-compliance with regard to dioxins+DL-PCBs (calculated on 2005 TEFs) based on the assay of NDL-PCBs

Group of species	Scenario	Error rate		Determined thresholds and confidence intervals (in ng/g fresh weight)	% fish characterised (including error rates)	% non-characterised fish
		% False negative	% False positive			
eels	1	1	1	300 [210 - 320] and 900 [750 - 1050]	67	33
	2	1	4	300 [210 - 320] and 550 [450 - 750]	80	20
	3	1	15	300 [210 - 320]	100	0
freshwater fish other than eels	1	1	1	160 [130 - 220] and 400 [360 - 500]	82	18
	2	1	5	160 [130 - 220] and 240 [210 - 280]	91	9
	3	1	12	160 [130 - 220]	100	0
marine fish	1,2 and 3	1	2	130 [110 - 150]	100	0

Decision chart for eels:



Decision chart for freshwater fish other than eels:



Decision chart for marine fish:

