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2 Correlations between dioxin-like and indicators PCBs: potential consequences for  
3 environmental studies involving fish or sediment

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20 Abstract

21 Among the numerous PCB congeners, most of the dioxin-like PCBs (DL-PCBs) need to be  
22 characterized by hyphenated techniques. It has been shown in several instances that  
23 these congeners are well related to the total PCB content in fish. We examined datasets  
24 collected mainly in France, on freshwater and marine fish and sediments. A statistical  
25 model linking DL- and indicator PCBs was developed for a dataset composed of  
26 freshwater fishes, and proved to predict well DL-PCBs from indicator PCBs in all other fish  
27 sets, including marine ones.. Type II error rates remained low in almost all fish sets. A  
28 similar correlation was observed in sediments. Non dioxin-like PCBs elicit various adverse  
29 effects and represent 95% of the total PCBs. A European guideline for them is needed;  
30 the correlation between DL- and indicator PCBs could help develop this standard in the  
31 future.

32

33 Capsule

34 Dioxin-like PCBs in fish and maybe sediments are rather well predicted by indicator PCBs.

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36 Keywords

37 Dioxin-like PCB, indicator PCB, correlations, fish, sediment

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## 39 Introduction

40 Among the numerous PCB congeners, most of the so-called dioxin-like PCBs (DL-PCBs)  
41 need to be analysed separately with sophisticated and expensive techniques. It was  
42 shown recently that these congeners are fairly related to the total PCB content in fish  
43 (Bhavsar et al., 2007a, b). This finding might open the way to simplifying analytical  
44 approaches for analysing and assessing the environmental risks of PCBs, provided this  
45 relationship was proven to be general. In Europe, a set of 7 congeners, called "indicator  
46 PCBs" (iPCBs) are currently used rather than Aroclor ® or similar PCB technical mixtures  
47 to estimate "total PCBs". Thus, the relationship between this set of congeners and DL-  
48 PCBs has to be confirmed.

49 Most of the toxicological properties of DL-PCBs are related to their affinity to the Ah  
50 receptor (Safe and Phil, 1990; Safe, 1994), a characteristic these substances share with  
51 polychloro-dibenzodioxins (PCDDs) and polychloro-dibenzofurans (PCDFs). This common  
52 mode of action lead to the adoption of Toxicity Equivalent Factors (TEF) for each  
53 congener, so as to estimate the overall toxicity of PCDDs and similar substances for  
54 human beings on the basis of the toxicity of the 2,3,7,8 tetrachlorodibenzo-dioxin (Van  
55 Den Berg et al., 1998). Non dioxin-like PCBs, on the other hand, tend to link to other  
56 biological receptors. As a consequence, they display other toxicological characteristics.  
57 These include neuro-behaviorial alterations (Faroon et al., 2000) and a range of endocrine  
58 effects related to reproduction (Monosson, 1999; Faroon et al., 2001). To date no  
59 common toxicological metrics could be adopted for non-dioxin-like effects of PCBs. Non  
60 dioxin-like PCBs are not regulated in foodstuffs in Europe, while dioxins and related  
61 compounds are.

62 Dioxin-like compounds in foodstuffs are a significant concern for European authorities,  
63 which issued regulations in order to limit human exposure to these chemicals in 2001-  
64 2002, updated it in 2006 (E.C., 2006a; b) and plan further revisions in 2008-2009. The  
65 current regulation states that fish meat should not exceed a level of 8 pg.g<sup>-1</sup> for the sum  
66 of dioxins, furans and dioxin-like PCBs (WHO-TEQ 1998); dioxins and furans alone should  
67 not exceed 4 pg.g<sup>-1</sup>. This applies to all fish species, except eel, which should not exceed 4  
68 pg.g<sup>-1</sup> for the sum of dioxins and furans, but 12 pg.g<sup>-1</sup> when DL-PCBs are accounted for  
69 (E.C., 2006a; b).

70 In this study, we examined various datasets collected mainly in France in order to study  
71 the relationships between DL-PCBs and iPCBs. Following Bhavsar et al. (2007b), our  
72 purpose is to examine further whether systematic analysis of DL-PCBs in environmental  
73 matrices is justified or not.

## 74 Materials and Methods

75 *Freshwater fish collection:*

76 Depending of the location, 3 sets of freshwater fishes (F1 to F3, Table 1) were captured  
77 along the Rhone river with nets by professional fishermen or technicians from fish  
78 management authorities. The set F1 is made of fishes collected in the Rhone river around  
79 Lyon (France), between Lucey and Vernaison, from Sep. 2005 till Nov., 2006, while in  
80 the set F2 fishes were caught in the Rhone further downstream between March and June,  
81 2007. The F3 set is composed of 79 individual fishes caught in the Rhone river in  
82 autumn, 2007. The prospected area lies between the French-Swiss border and Lucey, the  
83 upstream station in the F1 set. Thus, F3 fishes are not subjected to the same local PCBs  
84 sources as most F1 fishes. PCB sources for F3 fishes include essentially unknown  
85 historical local sources and atmospheric inputs. A selection was made among the fishes  
86 captured, focussing on those living in contact to sediments as well as on piscivorous  
87 species. Whole fishes were kept individually at 4°C and brought to the laboratories, then  
88 freeze dried immediately after reception. Fillet cuttings of a minimum of about 130 g  
89 (fresh weight) per fish were taken and the skin removed, according to European  
90 guidelines (E.C., 2006c).

91 *Marine fish collection:*

92 The F4 fish samples set is made of 22 composite samples of sea bass, plaice and flounder  
93 pooled by size collected along the French coast in Normandy, mainly in the Seine  
94 estuary, a known PCB-contaminated area (Abarnou, 2008). Another set (F5) was  
95 obtained by sampling various species as composite samples pooled by size in commercial  
96 fisheries or imported in France, either along the French coast or from the North-Eastern  
97 Atlantic Ocean. These 73 samples include various species: sole, sea bass, plaice, salmon,  
98 sardine, red mullet, blue whiting, mackerel, sea bream, tuna, herring, anchovy etc.  
99 Samples were kept in the same conditions as above until analysis.

100 *Sediment collection:*

101 A first set (S1) of surface sediments from 15 locations was sampled with a grab operated  
102 from a boat in autumn 2006 in the Rhone river and in a tributary, the Bourbre, in the  
103 same area as the F1 fish subset. 15 core samples were also collected in the Rhone river  
104 during the same period. A second set (S2) composed exclusively of surface sediments  
105 was obtained in fall 2007. The samples were gathered with a grab operated either from  
106 boat or from the river bank, in sedimentation areas, along an upstream-downstream  
107 gradient covering the whole course of the Rhone in France.

108 Sediments were sieved at 2 mm, stored at  $-18^{\circ}\text{C}$  and sent to the laboratories.

109 *PCB, DL-PCBs, PCDD-F analysis*

110 Two different laboratories performed the analyses. Sediments and fishes were  
111 homogenized and freeze dried after reception by these laboratories. Quantities of 50 g of  
112 dried sediments or 50-100 g of dried fishes were used.

113 The first laboratory<sup>2</sup> analysed both fish and sediments. Soxhlet extractions were  
114 performed with a mixture of toluene/ethanol (30/70). USEPA standard 1613 for PCDDs  
115 and PCDFs analysis and 1668 for PCB were applied. Analyses were achieved by gas  
116 chromatography (Agilent 6890) coupled with high resolution mass spectrometry  
117 (Micromass Ultima Waters). Chromatographic separation was achieved with a DB5ms  
118 column for PCDDs and PCDFs and with a HT8 column for PCBs.

119 The second laboratory<sup>3</sup> analysed only fishes, following Directive 2002/69/EC guidelines  
120 for the official control of dioxins and the determination of DL-PCBs in food (E.C., 2002).  
121 The extraction was performed in a Dionex ASE 300 device with toluene/acetone, 70:30  
122 (v/v) mixture. Purification and fractionation encompassed three successive steps, using  
123 silica, Florisil and celite/carbon columns. Separation of coplanar (non-ortho) PCBs from  
124 non-planar PCBs was achieved on an activated mixture of Florisil/ Carbopack C/Celite  
125 545. Analysis were performed by gas chromatography coupled with high resolution mass  
126 spectrometry (HP 6890 GC coupled with JMS 700D, Jeol). Chromatographic separations  
127 were achieved on a DB-5MS column.

128 Concerning quality insurance, both laboratories used surrogates (whose 13C12-1,2,3,4-  
129 TCDD for the PCDD/Fs, 13C12-PCB111 for PCBs, ... ) to check for analytical recoveries.  
130 Uncertainties on concentration results for PCB and PCDD-F analysis are evaluated at  
131 20%. Limits of quantification in sediments range from 0.06 to 12.00  $\text{pg.g}^{-1}$  DW for PCBs  
132 and 0.004 to 0.6  $\text{pg.g}^{-1}$  DW for PCDD-Fs. In fishes, limits of quantification range from  
133 0.02 to 8  $\text{pg.g}^{-1}$  wet weight (WW) for PCBs and 0.002 to 0.01  $\text{pg.g}^{-1}$  WW for PCDD-Fs.

134 *TEF, Toxic Equivalent (TEQ) calculation*

135 Though they share the same mode of action, PCDDs, PCDFs and co-planar PCBs do not  
136 display the same toxic potency (Van den Berg et al., 2006). The overall toxicity of a  
137 mixture of these compounds is commonly expressed as a single number, the Toxic  
138 Equivalent (TEQ), obtained by summing individual compounds concentrations weighed by  
139 Toxic Equivalent Factors. A first set of TEFs was initially applied by the North Atlantic  
140 Treaty Organisation (Kutz et al., 1990; Van Den Berg et al., 1998). Though the World

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141 Health Organisation (WHO) suggested recently another approach to deriving TEFs,  
 142 resulting in different TEF values for most congeners (Van den Berg et al., 2006), the  
 143 European regulation is still based upon 1998 TEFs (E.C., 2006b). The TEQ values used in  
 144 the present study are calculated on the basis of 1998 TEF.

#### 145 *Statistics*

146 Our starting hypothesis was that DL-PCBs and iPCBs are correlated. In order to test this  
 147 hypothesis for fish samples, a two-step approach was applied: (1) determine a statistical  
 148 model describing the relationship between DL-PCBs' TEQ and iPCBs in a first fish set (i.e.  
 149 F1), (2) use this model to calculate TEQ of DL-PCBs from iPCBs in the other sets, and  
 150 then compare predicted and measured TEQ values. For sediment samples, due to the  
 151 limited number of samples, we applied only linear regression.

152 Linear regression was used to correlate Log transformed DL-PCBs (expressed as TEQ)  
 153 and iPCBs. We used Analysis of Covariance (ANCOVA) using XLSTAT 2008 (Addinsoft) to  
 154 detect differences in slope among species for linear relation between Log transformed  
 155 DL-PCBs and iPCBs. The ANCOVA method belongs to a larger family of models called GLM  
 156 (Generalized Linear Models) as do the linear regression and the analysis of variance  
 157 (ANOVA). The specificity of ANCOVA is that it mixes qualitative and quantitative  
 158 explanatory variables. In a first step, the ANCOVA tests the assumption of parallelism of  
 159 slopes of X on Y for all the groups. In a second step, ANCOVA tests the homogeneity of Y  
 160 intercepts for all the groups.

#### 161 Results

162 Detailed results are provided as supplementary information. For the Rhone river, the fish  
 163 contamination data presented here were produced in the context of a large diagnosis of  
 164 fish contamination in the Rhone catchment. The complete database is also available  
 165 online<sup>4</sup>.

#### 166 *Fish*

167 The sums of iPCBs follow either log-normal distributions (sets F3, F5), or no specific  
 168 distribution pattern (F1, F2, F4). DL-PCBs (expressed as TEQ) as well as total-TEQ values  
 169 generally follow log-normal distributions, except for both variables in the F2 subset  
 170 (Table 1). DL-PCBs represent on average 90.2% (65-99%, F2) to 94% (84-99.7%, F3) of  
 171 total TEQs in freshwater fishes. Marine fishes display lower rates, namely an average of  
 172 83% (71-97%) for F4 and 72.4% (18-98.5%) for F5 fishes, allowing the exploration of  
 173 the relationships between these variables by regression techniques.

174 Log transformed DL-PCBs (expressed as TEQ) and iPCBs are linearly correlated in the F1  
 175 series after removing 4 outliers, namely specimens caught immediately downstream a  
 176 known source of PCBs, displaying very high concentrations of either iPCBs or DL-PCBs  
 177 (N=128, R=0.96, p<0.0001; Eq. 1). Note that in this series DL-PCBs generally represent  
 178 the main component of the total-TEQ, and that iPCB and the total TEQ content are also  
 179 strongly correlated (R = 0.96, p<0.0001; normality test passed; Eq. 2).

$$180 \log TEQ_{DL-PCB} = -1.167(\pm 0.051) + 0.932(\pm 0.022) \times \log \sum iPCB$$

#### 181 **Eq. 1**

$$182 \log TEQ_{tot} = -1.128(\pm 0.053) + 0.929(\pm 0.023) \times \log \sum iPCB$$

#### 183 **Eq. 2**

184 The Eq. 1 model was applied to predict DL-PCBs' TEQ for F2-fishes. Measured and  
 185 predicted DL-PCBs are linearly correlated (Figure 1; N=143; R=0.98, intercept = 0.1346  
 186 ( $\pm 0.0166$ ), slope= 1.0001 ( $\pm 0.0184$ ), p<0.0001; normality test passed). Ideally, the  
 187 slope should equal 1 while the intercept should be 0.

<sup>4</sup> [http://www.rhone-mediterranee.eaufrance.fr/milieux-continentaux/pollution\\_PCB/index.php#donnees](http://www.rhone-mediterranee.eaufrance.fr/milieux-continentaux/pollution_PCB/index.php#donnees)

188 DL-PCBs in F3 are well predicted from iPCB according to the model in Eq. 1 above  
 189 (R=0.96,  $p < 0.0001$ ; slope  $0.9812 \pm 0.0328$ , intercept  $0.1558 \pm 0.0137$ ). Again, slope  
 190 and intercept are close to ideal values and the normality test passed.

191 The model in Eq. 1 provided also a good prediction of DL-PCBs from iPCBs for F4 samples  
 192 (N=22; R=0.96,  $p < 0.0001$ ; slope  $1.0125 \pm 0.0594$ , intercept  $-0.0319 \pm 0.0459$ ;  
 193 normality test passed). Total TEQ prediction from iPCBs was also quite as good as TEQ  
 194 PCB-DL prediction (R=0.96,  $p < 0.0001$ ; slope  $1.0469 \pm 0.0679$ , intercept  $-0.0901 \pm$   
 195  $0.0521$ ; normality test passed).

196 For F5 fishes, the model in Eq. 1 provided again a good prediction of the toxic  
 197 equivalency on the basis of iPCBs; the predicted DL-PCB values were strongly correlated  
 198 to the measured values (N=73; R=0.96,  $p < 0.0001$ , intercept= $-0.2298 \pm 0.0252$ ,  
 199 slope= $0.9018 \pm 0.0309$ , normality test passed).

200 The same arises with TEQ prediction from iPCBs (Eq. 2) in all sets F2, F3, F4 and F5:  
 201 regression between predicted and measured TEQ yielded R values of 0.97, 0.97, 0.96  
 202 and 0.94 respectively,  $p < 0.0001$  in all cases. Slopes ranged between 0.90 (F5) and 1.04  
 203 (F4) and intercepts between  $-0.33$  and  $0.16$ .

204 As the congener 118 was primarily included both in the set of indicators and in the DL-  
 205 PCBs series, the model predicting DL-PCBs from iPCBs (Eq. 1) was adjusted to 6  
 206 congeners, as follows:

$$207 \log TEQ_{DL-PCB} = -1.062(\pm 0.059) + 0.896(\pm 0.026) \times \log \sum iPCB_6$$

### 208 Eq. 3

209 With  $\log \sum iPCB_6$  the sum of congeners 28, 52, 101, 138, 153 and 180 concentrations.  
 210 Because the overall TEQ content is mostly determined by DL-PCBs, a similar model can  
 211 be established for the total TEQ (Eq. 4)

$$212 \log TEQ_{tot} = -1.025(\pm 0.060) + 0.894(\pm 0.027) \times \log \sum iPCB_6$$

### 213 Eq. 4

214 Eq. 1 and Eq. 2 models (or Eq. 3 and Eq. 4) are general because they are based on a  
 215 dataset encompassing several species. Therefore, their eventual applicability to particular  
 216 species raises question about slope differences among species, due to e.g. their feeding  
 217 regime, lipid content etc.. Assuming that inter-site differences within a given set are not  
 218 significant, three ANCOVA analyses were performed on F1 to F3 samples subsets in order  
 219 to test for regression slope differences among species. Subsets were composed of  
 220 species with more than 10 individuals. Results are reported in Table 2: the ANCOVA  
 221 models indicate that there was no difference among slopes, except in the F3 set. Barbel's  
 222 regression slope in this set ( $0.69 \pm 0.056$ ) seems also different from those in F1 and F2  
 223 sets for the same species ( $1.03 \pm 0.079$  and  $0.74 \pm 0.095$  respectively). There is no  
 224 difference among intercepts, except in the F1 set where the slope for the pike perch is  
 225 lower than the slopes for other species. This could be due to the fact that all the pike  
 226 perch fishes were youngsters, and displayed low fat contents, whereas individuals from  
 227 other species were older and generally fatter.

228 Type I error ("false positives") correspond to samples predicted to exceed the standard  
 229 which actually do not, whereas Type II ("false negatives") correspond to samples  
 230 predicted below the standard and actually exceeding it. Type I and type II error rates on  
 231 predictions of total TEQ, i.e. based on Eq. 2, were determined in fish data series F2, F3,  
 232 F4 and F5 (Table 3). Because of the reduced sample size, the rates for F4 are only  
 233 indicative. The type I error rate were 26.2% and 100% in the F2 and F3 sets  
 234 respectively, but the number of samples predicted to exceed  $8 \text{ pg.g}^{-1}$  in F3 was rather  
 235 low (8 samples), therefore this error rate would be meaningless. Both types of error  
 236 rates in the F2 series are calculated on higher numbers of predictions, and are therefore  
 237 more significant. The type II error rate in the F3 subset remains also very low.

238

239 *Sediments*

240 The sum of iPCBs concentrations for S1 sediment samples ranged from 0.25 to 131.5  
 241  $\mu\text{g.kg}^{-1}$  (dry weight, DW), with a median at 22.6  $\mu\text{g.kg}^{-1}$ . DL-PCBs were comprised  
 242 between 0.054 to 30.5  $\text{ng.g}^{-1}$  (DW), with a median at 4.5  $\text{ng.g}^{-1}$ , whereas the sum of  
 243 PCDD and PCDF concentrations laid between 0.0008 and 1.196  $\text{ng.g}^{-1}$  (DW), with a  
 244 median of 0.326  $\text{ng.g}^{-1}$ .

245 DL-PCBs and iPCBs were linearly correlated without transformation; nevertheless, the  
 246 normality test failed, suggesting that the values should be log-transformed. Log-  
 247 transformed concentrations of DL-PCBs and iPCBs were also correlated (Eq. 5; DL-PCBs  
 248 expressed as concentrations ;  $R=0.96$ ,  $p<0.0001$ ). iPCBs were also correlated with the  
 249 sum of PCDDs and PCDFs concentrations.

$$250 \log \sum DL - PCBs = -0.685(\pm 0.072) + 1.022(\pm 0.052) * \log \sum iPCBs$$

251 **Eq. 5**

252 The sum of iPCBs concentrations in S2 sediments ranged from 2.1 to 73  $\mu\text{g.kg}^{-1}$  (DW),  
 253 with a median at 29  $\mu\text{g.kg}^{-1}$ . DL-PCBs were comprised between 0.47 to 12.1  $\text{ng.g}^{-1}$  (DW),  
 254 with a median of 4.2  $\text{ng.g}^{-1}$ . The sum of PCDD and PCDF concentrations laid between  
 255 0.0565 and 9.2738  $\text{ng.g}^{-1}$  (DW), with a median at 0.698  $\text{ng.g}^{-1}$ .

256 A relationship between iPCBs and DL-PCBs very similar to the one in S1 can be observed  
 257 in this series ( $N=21$ ,  $R=0.95$ ;  $p<0.0001$ , slope = 0.7127 ( $\pm 0.0508$ ), intercept = -0.7407  
 258 ( $\pm 0.0730$ ).

## 259 Discussion

260 *Correlations in fish*

261 The major contribution of DL-PCBs to the total TEQ content in wild fish have already been  
 262 observed in Europe, for instance in the Netherlands (de Boer et al., 1993; van Leeuwen  
 263 et al., 2007). A similar feature was also demonstrated in farmed trout throughout France,  
 264 at concentrations well below the authorized residue level (Marchand et al., 2004). These  
 265 authors also showed a good correlation between iPCBs and DL-PCBs expressed as TEQ.  
 266 In a study published in 2007, the French Agency in charge of risk assessment in  
 267 alimentation (AFSSA) noticed a strong correlation between iPCBs and DL-PCBs, not only  
 268 in fish ( $r>0.948$ ) but also in other foodstuffs: eggs, milk, poultry (AFSSA, 2007). Some  
 269 years before, a correlation between specific congeners, in particular the congener 153  
 270 and DL-PCB, was evidenced in marine and freshwater fish in the Netherlands (de Boer et  
 271 al., 1993), and more recently in eels, bream and chub in the Elbe and some tributaries in  
 272 Germany (Stachel et al., 2007). A large study on fish from the North American Great  
 273 Lakes, and extended to other datasets, recently reached the same conclusions (Bhavsar  
 274 et al., 2007b). Thus, this strong correlation between parameters summarising PCB and  
 275 dioxin/dioxin-like compounds content appears to be a rather common feature, at least in  
 276 fish. There is less evidence in other biota. Kay et al. (2005) found only a poor correlation  
 277 in insects at the Kalamazoo Superfund site. Oh et al. (2003) found a correlation between  
 278 total PCBs and TEQ of DL-PCBs in oysters and mussels along the South Korean coast,  
 279 with outliers at local point sources. Moreover the PCBs' contribution to the total TEQ was  
 280 variable, owing to local sources of PCDDs and PCDFs. In a large Mediterranean study,  
 281 Storelli et al. (2006) could not test such a relationship in cephalopod molluscs, due to  
 282 DL-PCBs below the quantification limit.

283 The above statistics on relationships between iPCBs and either DL-PCB or total TEQ in  
 284 fish may appear somewhat biased, in the sense that congener 118, a non ortho  
 285 congener, is common to both tested variables. These correlations nevertheless can make  
 286 sense because the purpose is to predict DL-PCB toxic equivalency, or total TEQ, from less  
 287 time and resource consuming measurements. Moreover, congener 118 on average  
 288 represents about 10% of the total iPCBs (Table 4). A study of the relationships between  
 289 6 and 7 iPCBs (with and without this congener) in a large array of food items (fish, milk,

290 eggs, poultry, beef meat) in France also showed that both variables were strongly  
 291 correlated, suggesting that congener 118 is not critical in the evaluation of biota  
 292 contamination by PCBs (AFSSA, 2007). The average contribution of congener 118 to the  
 293 TEQ content of DL-PCBs in F1 to F5 subsets (Table 4) falls between around 10-15%,  
 294 except in F5 (24.6%). This appears consistent with other fish datasets, as discussed by  
 295 (Bhavsar et al., 2008). Furthermore the sum of concentrations of 6 iPCBs, i.e. without  
 296 congener 118, is strongly correlated to DL-PCBs expressed as TEQ in F1 samples (log-  
 297 transformed values,  $R=0.95$ ,  $p<0.0001$ ). So it can be inferred that the congener 118 is  
 298 not essential to the evaluation of the overall iPCB content, and that accounting for it in  
 299 both variables had not significantly biased the relationships.

300 Testing for eventual differences in regression slopes among species aimed to examine  
 301 whether the relationship between iPCBs and DL-PCBs is general or not. In the sets F1 to  
 302 F3, only one species in one set displays a significant difference. There is no obvious nor  
 303 simple explanation for this. We note first that the barbel displays this difference in the F3  
 304 set, but not in the F1 and F2 ones. F1 and F2 present much wider ranges of  
 305 concentrations than F3, including for the barbel. Moreover, F3-barbels predicted TEQ  
 306 values are systematically higher than measured ones. Nevertheless, the hypothesis of an  
 307 analytical bias should be discarded, as the barbel samples were randomly placed in the  
 308 analytical series, and no bias appeared for the other species. The model in Eq. 1 seems  
 309 therefore to correctly predict TEQ values for DL-PCBs from iPCBs in most cases. The  
 310 unexplained bias observed for one species in one area suggests to use this model with  
 311 several fish species and extended concentration ranges. The same is true for its variation  
 312 based on 6 iPCBs.

### 313 *Predictions accuracy*

314 The prediction accuracy in fish sets F2, F3, F4 and F5 was tested against the current  
 315 European management threshold of  $8 \text{ pg.g}^{-1}$  (E.C., 2006a; b) as an example (Table 4).  
 316 Measured TEQ in samples erroneously predicted above the threshold of  $8 \text{ pg.g}^{-1}$  (i.e. type  
 317 I errors) range between  $4.83$  and  $7.50 \text{ pg.g}^{-1}$ . Four species are concerned in F2, and 3 in  
 318 F3. According to the small number of type I samples, no distinct pattern could be  
 319 distinguished in terms of species or other fish characteristics.

320 The rate of type II error in the F2 subset corresponds to 2 samples out of 71, one eel and  
 321 one barbel. The barbel displays a TEQ value of  $12 \text{ pg.g}^{-1}$ , well above the regulatory limit.  
 322 For the eel displays a TEQ at  $9 \text{ pg.g}^{-1}$  and the lower bound of the measurement  
 323 confidence interval falls below the regulatory limit. In the meantime, iPCBs sums of  
 324 concentrations are  $54$  and  $129 \text{ }\mu\text{g.kg}^{-1}$  respectively, which is, according to Eq. 2, in the  
 325 confidence interval of the prediction for the eel, but not for the barbel. Unless an  
 326 undetected analytical error for the barbel, there is no explanation for this gap. Both "false  
 327 negative" samples in the set F4 are sea basses, with lipid contents of 9 and 17% (DW)  
 328 respectively, indicating the fishes were rather old; they were caught in the vicinity of a  
 329 harbour, suggesting a possible specific PCB source. The only false negative sample in the  
 330 F5 set is a sardine, with again a measured iPCBs load ( $92 \text{ }\mu\text{g.kg}^{-1}$ ) well below the value  
 331 corresponding to the measured DL-PCBs. Thus, apart from undetected analytical errors,  
 332 most of the type II errors in the 4 sets remain unexplained. Type II error rates are rather  
 333 low, especially in the large size sets F2, F3 and F5. Nevertheless, they are still above the  
 334 limit recommended by the European Commission for screening methods, i.e. 1% (E.C.  
 335 2006c). Similar error rates were obtained with the model based on 6 iPCBs (Eq. 4). Both  
 336 types of errors do not have the same consequences in terms of public health or  
 337 environmental protection. Indeed, a low type II error rate, if the models reported in Eq. 2  
 338 or Eq. 4 were used instead of DL-PCBs and PCDDs – PCDFs measurements, would be a  
 339 strong requirement in terms of environmental or consumers' health protection, as  
 340 investigations would stop at this stage. Conversely, a high type I error would not have  
 341 any consequence in terms of environmental or public health protection. Nevertheless, a  
 342 type I error rate such as that observed in F2 samples suggests to confirm the prediction  
 343 by specific measurements of DL-PCBs, PCDDs and PCDFs in case of exceedance of a  
 344 given threshold, for instance the i-PCBs sum corresponding to  $8 \text{ pg.g}^{-1}$  TEQ. This  
 345 corresponds to a value of  $154 \text{ }\mu\text{g.kg}^{-1}$  (WW; confidence interval  $120\text{-}200 \text{ }\mu\text{g.kg}^{-1}$ ) for

346 the sum of 7 iPCBs on the basis of Eq. 2, or  $143 \mu\text{g.kg}^{-1}$  (WW; confidence interval 124-  
347  $165 \mu\text{g.kg}^{-1}$ ) for the sum of 6 iPCBs on the basis of Eq. 4. In a monitoring perspective  
348 accordingly, concentrations above e.g.  $105 \mu\text{g.kg}^{-1}$  for the sum of 6 iPCBs could be  
349 considered as close to the regulatory limit for dioxins and related compounds, and  
350 potentially exceeding it, and DL-PCBs, PCDDs and PCDFs concentrations could be  
351 measured accurately.

### 352 *Correlations in sediments*

353 Though the observed concentrations of both iPCBs and DL-PCBs are generally lower in  
354 surface sediments as compared to core samples in the S1 series, the relationship  
355 between both groups obviously does not differ according to their depth. Furthermore, the  
356 number of samples was too small to attempt the same approach as for fish, i.e. to  
357 predict and evaluate the predictive ability of Eq. 5.

358 We find it inappropriate to attempt to calculate TEQ levels in sediments and relate them  
359 to iPCBs contamination, for two reasons. First, as a summary of the dioxin-like toxicity, a  
360 sediment-TEQ would be relevant if either benthic invertebrates or fishes were concerned.  
361 Invertebrates do not have Ah receptors and thus are rather insensitive to dioxin effects  
362 (summary in EC, 2001). Furthermore, the congeners present in sediments are not evenly  
363 transferred to fish through the food chain, as discussed below. Sediment-TEQ values  
364 would therefore neither predict risk for benthic invertebrates nor for fishes.

365 Differences in PCB congener profiles between sediment and biota have already been  
366 shown, e.g. (Ankley, 1992). Kay et al. (2005) showed that the dioxin-like toxic potency  
367 differed among trophic levels in the Kalamazoo aquatic food webs, primarily because  
368 more chlorinated congeners were enriched in higher trophic levels. To a certain extent,  
369 these differences can be explained by considerations of availability. Nevertheless,  
370 another explanation holds for the degree of chlorination and the spatial conformation of  
371 congeners, the higher chlorine substituted and non planar PCBs showing less chemical  
372 and biological availability due to their stronger sorption to sediment, compared to the  
373 lower chlorinated and planar PCBs (You et al., 2007) or less ability to metabolize (Froese  
374 1998, Metcalfe 1997 in Kay et al., 2005). For these reasons, it does not seem  
375 appropriate to compare the slopes among matrixes, e.g. sediment and fish. Specific  
376 models have to be developed and tested for each matrix.

### 377 *Adverse effects PCBs in a management perspective*

378 Given that non dioxin-like PCBs represent different modes of action, DL-PCBs as such are  
379 not sufficient alone to assess the whole risk to human health generated by the PCBs  
380 associated with food (AFSSA, 2007). PCBs have different modes of action. The first mode  
381 to have been accurately described involves the Ah receptor. Coplanar congeners, which  
382 bind to this receptor as do PCDDs and PCDFs represent about 5% of the total load of  
383 PCBs. Their relative toxicity has been recently reviewed and the respective toxic  
384 equivalent factors (TEF) decreased (Van den Berg et al., 2006). Non dioxin-like PCBs  
385 bind to several other receptors and may have various adverse effects, including neuro-  
386 toxicity on embryos (Ribas-Fito et al., 2001). For these congeners, early symptoms  
387 appear in foetus exposed in utero, resulting in audiometry impairment. Other primary  
388 effects on health are related to sugars and lipids metabolisms, involved in the etiology of  
389 diabetes (Codru et al., 2007). Adverse effects on reproduction involve modifications of  
390 the hypothalamus-hypophysis-gonads-liver axis which are caused by both dioxin-like and  
391 non dioxin-like congeners (Monosson, 1999).

392 Therefore, a rigorous assessment of the potential impacts of fish or other food items  
393 contaminated with PCBs mixtures should involve both non dioxin-like and DL-PCBs. Since  
394 (1) DL-PCBs analysis is more difficult and costly than iPCBs analysis, (2) DL-PCBs are  
395 well predicted by iPCBs, and (3) non-dioxin like PCBs also induce important toxic effects,  
396 DL-PCBs do not need to be measured systematically but could be introduced at the  
397 second stage of a tiered approach.

398 In this perspective, appropriate threshold values for indicator PCBs in fish or other  
399 matrices are needed, at least in Europe.

## 400 Conclusion

401 Indicator PCBs and DL-PCBs concentrations are well correlated in freshwater fishes from  
402 the Rhone river. As DL-PCBs represent the bulk of TEQ in these fishes, iPCBs are also  
403 well correlated to the TEQ content in Rhone fishes. The statistical models derived from  
404 these fishes proved to be appropriate for describing the correlation in marine fishes, and  
405 appear thus very promising, and possibly general. This finding is consistent with other  
406 studies performed with different variables, which led nevertheless to similar conclusions  
407 (Bhavsar et al., 2007a; Bhavsar et al., 2007b; AFSSA, 2007).

408 Similar relationships are likely to exist in other biota and should therefore be explored.

409 Moreover, iPCBs and DL-PCBs are also correlated in bottom sediments in the Rhone river.  
410 It is not possible yet to establish whether this relationship is general or not.

411 iPCBs concentrations remained correlated with TEQ contents after congener 118 removal  
412 from the former variable. Moreover, the resulting model (Eq. 4) yielded close proportions  
413 of type II errors (false negatives) in most of the tested fish series.

414 Either the statistical model based on 7 indicator PCBs or its variation based on 6  
415 congeners were shown to have a good predictive ability when used to predict exceedence  
416 of the current guideline for dioxin-like compounds ( $8 \text{ pg.g}^{-1}$ ), with low type II errors  
417 rates. Type I error rates could not be assessed accurately in all sets, due to low sample  
418 sizes. When the type I error rate was determined, it ranged between 14 and 26 %,  
419 depending of the statistical model used. Nevertheless, this type of error appears less  
420 important from the perspective of consumers' health protection, i.e. if these models were  
421 used to predict guideline exceedence.

422 Non-dioxin like PCBs elicit various adverse effects, including neuro-toxicity on embryos,  
423 and effects on reproduction. Therefore, assessing health effects of PCBs cannot rely  
424 solely on DL-PCBs. A specific guideline for total PCBs is therefore strongly needed. The  
425 correlation between DL- and iPCBs, could help develop this standard and monitor its  
426 implementation in the future.

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## 435 References

436 Abarnou A., 2008. Distribution et devenir de contaminants persistants dans les  
437 écosystèmes littoraux. Comparaison Manche Ouest-Manche Est. Agence de l'Eau  
438 Seine Normandie - IFREMER, Plouzané, p. 118.

439 AFSSA, 2007. Avis relatif à l'établissement de teneurs maximales pertinentes en  
440 polychlorobiphényles qui ne sont pas de type dioxine (PCB "non dioxin-like", PCB-  
441 NDL) dans divers aliments. Agence Française de Sécurité Sanitaire des Aliments,  
442 Maisons-Alfort, p. 28.

443 Ankley G.T., 1992. Bioaccumulation of PCBs from sediments by oligochaetes and fishes:  
444 comparison of laboratory and field studies. Canadian Journal of Fisheries & Aquatic  
445 Sciences 49, 2080.

446 Bhavsar S.P., Fletcher, R., Hayton, A., Reiner, E.J., Jackson, D.A., 2007a. Composition of  
447 Dioxin-like PCBs in Fish: An Application for Risk Assessment. Environmental  
448 Science and Technology, 3096-3102.

- 449 Bhavsar S.P., Hayton, A., Reiner, E.J., Jackson, D.A., 2007b. Estimating Dioxin-like  
450 polychlorinated biphenyl Toxic Equivalents from total polychlorinated biphenyl  
451 measurements in fish. *Environmental Toxicology and Chemistry* 26, 1622-1628.
- 452 Bhavsar S.P., Hayton, A., Jackson, D.A., 2008. Uncertainty analysis of dioxin-like  
453 polychlorinated biphenyls-related Toxic Equivalents in fish. *Environmental*  
454 *Toxicology and Chemistry* 27, 997-1005.
- 455 Codru N., Schymura, M.J., Negoita, S., Rej, R., Carpenter, D.O., 2007. Diabetes in  
456 relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in  
457 adult native Americans. *Environmental Health Perspectives* 115, 1442-1447.
- 458 de Boer J., Stronck C.J.N., Traag W.A., van der Meer J., 1993. Non-ortho and mono-  
459 ortho substituted chlorobiphenyls and chlorinated dibenzo-p-dioxins and  
460 dibenzofurans in marine and freshwater fish and shellfish from the Netherlands.  
461 *Chemosphere* 26, 1823.
- 462 E.C., 2002. Commission Directive 2002/69/EC laying down the sampling methods and  
463 the methods of analysis for the official control of dioxins and the determination of  
464 dioxin-like PCBs in foodstuffs, Official Journal of the European Union, pp. 209/5-  
465 209/14
- 466 E.C., 2006a. Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I  
467 and II to Directive 2002/32/EC of the European Parliament and of the Council on  
468 undesirable substances in animal feed as regards dioxins and dioxin-like PCBs.  
469 Official Journal of the European Union, pp. 32/44-32/53.
- 470 E.C., 2006b. Commission Regulation (EC) n° 199/2006 of 3 February 2006 amending  
471 Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in  
472 foodstuffs as regards dioxins and dioxin-like PCBs. Official Journal of the European  
473 Union, pp. 32/34-32/38.
- 474 E.C., 2006c. Commission Regulation (EC) n° 1883/2006 of 19 December 2006 laying  
475 down methods of sampling and analysis for the official control of levels of dioxins  
476 and dioxin-like PCBs in certain foodstuffs, 20/12/2006 ed. Official Journal of the  
477 European Union, pp. L 364/332 - 364/343
- 478 EC, 2001. Canadian Sediment Quality Guidelines for the protection of aquatic life and  
479 Canadian Tissue Residues Guidelines for the protection of wildlife consumers of  
480 aquatic biota - Polychlorinated Dibenzop-dioxins and Polychlorinated Dibenzop-  
481 furans (PCDD/Fs) - Technical supporting document - Volume I: guideline  
482 derivation. Environment Canada, Environmental Quality Branch, , Ottawa, p. 190.
- 483 Faroon O., Jones D., De Rosa C., 2000. Effects of polychlorinated biphenyls on the  
484 nervous system. *Toxicology and Industrial Health* 16, 305-333.
- 485 Faroon O.M., Keith S., Jones D., De Rosa C., 2001. Effects of polychlorinated biphenyls  
486 on development and reproduction. *Toxicology and Industrial Health* 17, 63-93.
- 487 Kay D.P., Blankenship A.L., Coady K.K., Neigh A.M., Zwiernik M.J., Millsap S.D., Strause  
488 K., Park C., Bradley P., Newsted J.L., Jones P.D., Giesy J.P., 2005. Differential  
489 accumulation of polychlorinated biphenyl congeners in the aquatic food web at the  
490 Kalamazoo River superfund site, Michigan. *Environmental Science and Technology*  
491 39, 5964-5974.
- 492 Kutz F.W., Barnes D.G., Bottimore D.P., Greim H., Bretthauer E.W., 1990. The  
493 international toxicity equivalency factor (I-TEF) method of risk assessment for  
494 complex mixtures of dioxins and related compounds. *Chemosphere* 20, 751.
- 495 Marchand P., Matayron G., Gade C., Le Bizec B., André F., 2004. PCDD/F, dioxin-like and  
496 markers PCBs in trouts from french aquaculture. *Organohalogen Compounds* 66,  
497 1932-1939.
- 498 Monosson E., 1999. Reproductive and developmental effects of PCBs in fish: A synthesis  
499 of laboratory and field studies. *Reviews in Toxicology* 3, 25-75.
- 500 Oh J.R., Ikonomou M.G., Fernandez M.P., Hong S.H., 2003. PCB and PCDD/F totals,  
501 TEQS, and congener patterns in Korean coastal marine environments, 1987,

- 502 1988, 1990, and 1996-1999. Archives of Environmental Contamination and  
503 Toxicology 44, 224-236.
- 504 Ribas-Fito N., Sala M., Kogevinas M., Sunyer J., 2001. Polychlorinated biphenyls (PCBs)  
505 and neurological development in children: A systematic review. Journal of  
506 Epidemiology and Community Health 55, 537-546.
- 507 Safe S., Phil D., 1990. Polychlorinated biphenyls (PCBs) dibenzo-p-Dioxins (PCDDs),  
508 dibenzofurans (PCDFs), and related compounds: environmental and mechanistic  
509 considerations which support the development of toxic equivalency factors (TEFs).  
510 CRC Critical Reviews in toxicology 21, 51-87.
- 511 Safe S.H., 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical  
512 and toxic responses, and implications for risk assessment. Critical Reviews in  
513 Toxicology 24, 87.
- 514 Stachel B., Christoph E.H., Götz R., Herrmann T., Krüger F., Kühn T., Lay J., Löffler J.,  
515 Pöpke O., Reincke H., Schröter-Kermani C., Schwartz R., Steeg E., Stehr D., Uhlig  
516 S., Umlauf G., 2007. Dioxins and dioxin-like PCBs in different fish from the river  
517 Elbe and its tributaries, Germany. Journal of Hazardous Materials 148, 199.
- 518 Storelli M.M., Barone G., D'Addabbo R., Marcotrigiano G.O., 2006. Concentrations and  
519 composition of organochlorine contaminants in different species of cephalopod  
520 molluscs from the Italian waters (Adriatic Sea). Chemosphere 64, 129-134.
- 521 Van Den Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy  
522 J.P., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., Larsen J.C., Van  
523 Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S.,  
524 Schrenk D., Tillitt D., Tysklind M., Younes M., Wærn F., Zacharewski T., 1998.  
525 Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife.  
526 Environmental Health Perspectives 106, 775.
- 527 Van den Berg M., Birnbaum L.S., Denison M., De Vito M., Farland W., Feeley M., Fiedler  
528 H., Hakansson H., Hanberg A., Haws L., Rose M., Safe S., Schrenk D., Tohyama  
529 C., Tritscher A., Tuomisto J., Tysklind M., Walker N., Peterson R.E., 2006. The  
530 2005 World Health Organization reevaluation of human and mammalian toxic  
531 equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences  
532 93, 223.
- 533 van Leeuwen S.P.J., Leonards P.E.G., Traag W.A., Hoogenboom L.A.P., De Boer J., 2007.  
534 Polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in fish from the  
535 Netherlands: concentrations, profiles and comparison with DR CALUX® bioassay  
536 results. Analytical & Bioanalytical Chemistry 389, 321-333.
- 537 You J., Landrum P.F., Trimble T.A., Lydy M.J., 2007. Availability of Polychlorinated  
538 Biphenyls in field-contaminated sediment. Environmental Toxicology and  
539 Chemistry 26, 1940-1948.

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Set	Sample size	Number of species	Sample type	<i>i</i> -PCBs ( $\mu\text{g}\cdot\text{kg}^{-1}$ WW)		DL-PCBs ( $\text{pg}\cdot\text{g}^{-1}$ I-TEQ, WW)		PCDD-PCDF ( $\text{pg}\cdot\text{g}^{-1}$ I-TEQ, WW)	
				median	standard deviation	median	standard deviation	median	standard deviation
F1	128	9	I	183	345	9.00	17.70	0.46	1.59
F2	143	17	I	117	613	4.50	17.60	0.27	1.44
F3	79	9	I	30	73	1.40	1.72	0.07	0.11
F4	22	3	P	27	129	1.63	5.53	0.46	0.44
F5	73	33	P	6	102	0.68	7.04	0.21	0.64

**Table 1 - Summary of fish and molluscs PCB and PCDD-PCDF contamination levels**

I= individuals ; P=pooled, by size class

Series	Subset size	Number of species	Slopes different?	Intercepts different?
F1	109	6	No	No
F2	69	5	No	No
F3	55	3	Yes	Yes

**Table 2- ANCOVA results in F1 to F3 subsets**

Series	Number of samples predicted $\geq 8$ $\mu\text{g}\cdot\text{g}^{-1}$	Number of these samples actually $< 8$ $\mu\text{g}\cdot\text{g}^{-1}$	Type I error rate	Number of samples predicted $< 8$ $\mu\text{g}\cdot\text{g}^{-1}$	Number of samples actually $\geq 8$ $\mu\text{g}\cdot\text{g}^{-1}$	Type II error rate
a- model based on 7 indicator PCBs (Eq. 2)						
F2	61	16	26.2	82	2	2.4
F3	8	8	-	71	0	0
F4	6	2	-	16	2	12.5
F5	2	0	-	71	1	1.4
b- model based on 6 indicator PCBs (Eq. 4)						
F2	62	9	14.5	81	2	2.5
F3	8	8	-	71	0	0
F4	6	2	-	16	2	12.5
F5	1	0	-	72	2	2.7

**Table 3 - Error rates in TEQ predictions for fishes**

		F1	F2	F3	F4	F5
iPCB	minimum	0.8%	2.6%	2.1%	11.1%	0.3%
	median	5.1%	5.9%	5.8%	11.9%	10.0%
	maximum	10.1%	21.0%	13.9%	13.6%	24.7%
DL-PCB (TEQ)	minimum	2.8%	8.4%	9.7%	8.5%	0.5%
	median	10.8%	15.5%	15.4%	24.6%	10.6%
	maximum	18.7%	48.7%	23.7%	59.3%	36.0%

**Table 4 - Contribution of congener 118 to iPCBs sum of concentrations and DL-PCBs TEQ**

**Figure 1 - Relationship between predicted and measured DL-PCBs (logI-TEQ) in the F2 set**

