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Marchand, J.F. Narbonne

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

BABUT M.^{a1}, MIEGE C.^b, VILLENEUVE B.^a, ABARNOU A.^c, DUCHEMIN J.^d, MARCHAND P.^e, NARBONNE J.F.^h

^a Cemagref, UR BELY, 3bis quai Chauveau – CP 220, F-69336 Lyon, France

^b Cemagref, UR QELY, F-69336 Lyon, France

^c IFREMER Département Biogéochimie et Ecotoxicologie, Centre de Brest, BP70, F 29280 Plouzané, France

^d Agence de l’Eau Seine-Normandie, DDD Eau et Santé, 21 rue l’Homme-de-Bois, F-14600 Honfleur, France

^e LABERCA, Ecole Nationale Vétérinaire, Atlanpôle La Chantrerie – BP 50707, F 44307 Nantes, France

^h LPTC, Bordeaux 1-CNRS, Bât A12, 351 cours de la Libération F-33405 Talence, France

¹ Corresponding author: marc.babut@cemagref.fr

19

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.

35

36 Keywords

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

38

38

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⁻¹ 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⁻¹. This applies to all fish species, except eel, which should not exceed 4
68 pg.g⁻¹ for the sum of dioxins and furans, but 12 pg.g⁻¹ 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°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 laboratory² 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 laboratory³ 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 pg.g^{-1} DW for PCBs
 132 and 0.004 to 0.6 pg.g^{-1} DW for PCDD-Fs. In fishes, limits of quantification range from
 133 0.02 to 8 pg.g^{-1} wet weight (WW) for PCBs and 0.002 to 0.01 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

² CARSO 321, avenue Jean Jaurès, Lyon, France

³ LABERCA Ecole Nationale Vétérinaire, Atlanpôle La Chantrerie -Nantes, France

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 online⁴.

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 (± 0.0166), slope= 1.0001 (± 0.0184), p<0.0001; normality test passed). Ideally, the
 187 slope should equal 1 while the intercept should be 0.

⁴ 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 ± 0.0328 , intercept 0.1558 ± 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 ± 0.0594 , intercept -0.0319 ± 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 ± 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 ± 0.0252 ,
 199 slope= 0.9018 ± 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 ± 0.056) seems also different from those in F1 and F2
 223 sets for the same species (1.03 ± 0.079 and 0.74 ± 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 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 ng.g^{-1} (DW), with a median at 4.5 ng.g^{-1} , whereas the sum of
 243 PCDD and PCDF concentrations laid between 0.0008 and 1.196 ng.g^{-1} (DW), with a
 244 median of 0.326 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 ng.g^{-1} (DW),
 254 with a median of 4.2 ng.g^{-1} . The sum of PCDD and PCDF concentrations laid between
 255 0.0565 and 9.2738 ng.g^{-1} (DW), with a median at 0.698 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 (± 0.0508), intercept = -0.7407
 258 (± 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 pg.g^{-1} (E.C., 2006a; b) as an example (Table 4).
 316 Measured TEQ in samples erroneously predicted above the threshold of 8 pg.g^{-1} (i.e. type
 317 I errors) range between 4.83 and 7.50 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 pg.g^{-1} , well above the regulatory limit.
 322 For the eel displays a TEQ at 9 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 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 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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Figure 1 - Relationship between predicted and measured DL-PCBs (logI-TEQ) in the F2 set

| 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 ≥ 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 ≥ 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

