Bioassay-derived 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Equivalents and Mono-*ortho* Polychlorinated Biphenyl Concentrations in Liver of Glaucous Gulls, *Larus hyperboreus*, from Svalbard

Espen O. Henriksen[1], Björn Brunström[2], Janneche Utne Skaare[3,4] and Geir Wing Gabrielsen[1]

[1] Norwegian Polar Institute, N-9005 TROMSØ, Norway
[2] Department of Environmental Toxicology, Uppsala University, Norbyvägen 18 A, S-752 36 Uppsala, Sweden
[3] Norwegian College of Veterinary Medicine, PO Box 8146 Dep., N-0033 OSLO, Norway
[4] National Veterinary Institute, PO Box 8156, N-0033 OSLO, Norway

Introduction

Extractable blood and tissue lipids from glaucous gulls, *Larus hyperboreus*, caught in the Norwegian Arctic (Svalbard) have been found to contain polychlorinated biphenyls (PCBs) in the 10 - 1 000 ppm range [1-3]. Non-*ortho* and mono-*ortho* PCBs are among the halogenated aromatic hydrocarbons (HAHs) which exert a range of toxic effects through binding to the cytosolic Ah-receptor [4]. For Ah-receptor mediated effects, toxic equivalent factors (TEFs) can be used to express the toxic potencies of HAHs relative to the compound with highest affinity to the Ah-recptor, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [5]. In complex mixtures of HAHs, TCDD-equivalent (TEQ) concentrations can be defined as the sum of the concentration of the individual HAHs times their respective TEF-values, assuming that the effects of the different compounds are additive [5,6]. An alternative approach for assessment of the dioxin-like potencies of complex HAH mixtures is the use of bioassays, in which an Ah-receptor mediated biochemical response integrates the effects of the individual compounds and their interactions [7,8].

The bioassay used in the present study determines TEQs based on 7-ethoxyresorufin *O*-deethylase (EROD) induction in cultured chick embryo livers [8]. We compared the bioassay derived TEQs (Bio-TEQs) in glaucous gull liver extracts with the concentrations of mono-*ortho* PCBs in the same samples. We also assessed the approximate contribution of non-*ortho* PCBs to the Bio-TEQs, based on published concentrations of the individual non-ortho PCBs in glaucous gulls from Svalbard [9]. The purpose was to investigate to which extent the Bio-TEQs in the glaucous gull liver extracts could be attributed to the presence of mono-*ortho* and non-*ortho* PCBs.

Material and Methods

Fifteen adult glaucous gulls were captured near Ny-Ålesund, Svalbard in June 1995. The birds were held captive and fed polar cod, *Boreogadus saida*, for 24-41 days before they were killed [3]. In addition, three glaucous gulls found dead in the same area were included in the present study. Organochlorines were quantified by GC-ECD as described previously [3]. A subsample of the extractable liver lipids was dissolved in *n*-hexane and cleaned on a silica gel column. After evaporation of the hexane, the extract was redissolved in DMSO. The dioxin-like potency of the extract was then assayed in cultured chick embryo livers, using a modified version of the method of Brunström et al. [8]. We assumed that approximate mean concentrations of the non-*ortho* PCBs could be estimated from the mean concentration of one of the quantified mono-*ortho* PCBs, given adequate data from comparable birds (see [10] and [11]). Thus, we assumed that the non-*ortho* PCBs -77, -126 and -169 were present in roughly the same proportions relative to PCB-118 as found in liver samples of 13 glaucous gulls from Svalbard analysed by Daelemans *et al.* [9]. Accordingly, mean concentrations were estimated as illustrated for PCB-77:

$$Mean\,PCB{-}77 \approx \frac{Mean\,PCB{-}77\,(Daelemans)}{Mean\,PCB{-}118\,(Daelemans)} \times Mean\,PCB{-}118\,(this\,study)$$

TEQs were calculated from the concentrations of the individual PCBs, based on TEFs chosen to reflect the potency of each congener in the bioassay. The dioxin-like potencies in the bioassay have been determined for PCB-77, PCB-105 and PCB-126 (Table 1). PCB-118 is about ten times less potent than PCB-105 as an EROD inducer *in ovo*, while PCBs -156 and -157 are about as potent as PCB-105 [12]. PCB-169 is about 100 times less potent than PCB-126 as an EROD inducer *in ovo* [13]. For PCBs -114 and -189, the avian TEFs suggested by a WHO group were used [14].

Results and Discussion

The Bio-TEQs ranged from 5 to 254 ng/g lipid (extractable lipids 3.33 - 5.83 %; wet weight Bio-TEQs 205 - 8 450 ppt), with the two highest concentrations in birds found dead (Tables 1 & 2). Similar levels of TCDD or TCDD-equivalents in eggs have been associated with embryotoxicity in other bird species [15]. In terms of concentrations of individual congeners, PCB-118 was the major mono-*ortho* PCB (Tables 1 & 2). Based on TEQs, however, PCB-156 and PCB-105 were more important (Tables 1 & 2). The concentrations of mono-*ortho* PCBs in the gulls found dead were from three to thirteen times higher than the mean concentration in the gulls that were kept in captivity (Tables 1 & 2). Assuming that the induction effects of the individual PCB-congeners are additive, the mean TEQ concentration associated with mono-*ortho* PCBs in the captive gulls was 2.8 ng/g lipid, which is 11 % of the mean Bio-TEQs (Table 1). The estimated concentrations of non-*ortho* PCBs could account for the major part of the Bio-TEQs, almost entirely due to PCB-126 (Table 1). Using TEFs based on aryl hydrocarbon hydrolase (AHH) induction in an hepatoma cell line, Daelemans *et al.* found that PCB-126 contributed with about 99% of the dioxin-like toxicity in their glaucous gull samples, while mono-*ortho* PCBs accounted for less than 1% [9]. The difference in contribution to total TEQs by different congeners in our study and in the study by Daelemans and co-workers [9] is largely due to the use of different TEF values.

Table 1. Concentrations of mono-*ortho* PCBs (MO-PCBs), estimated concentrations of non-*ortho* PCBs (NO-PCBs)*, and bioassay derived TCDD-equivalents (Bio-TEQs) in livers from glaucous gulls caught on Svalbard. Values are mean and standard deviation, N=15. TEFs were as far as possible chosen to reflect the potency of the individual congeners in the chick embryo liver bioassay (see methods).

Congener	Conc. (µg/g lipid)	TEF	TEQs (ng/g lipid)	$\frac{Mean\ TEQs}{Mean\ Bio-TEQs} \times 100\%$
105	3.25 (2.68)	$3 \cdot 10^{-4}$	0.98 (0.81)	3.8 %
114	0.46 (0.49)	$1 \cdot 10^{-4}$	0.05 (0.05)	0.2 %
118	14.83 (13.60)	$3 \cdot 10^{-5}$	0.44 (0.41)	1.8 %
156	3.42 (3.41)	$3 \cdot 10^{-4}$	1.02 (1.02)	4.0 %
157	0.95 (0.88)	$3 \cdot 10^{-4}$	0.29 (0.26)	1.2 %
189	0.30 (0.33)	$1 \cdot 10^{-5}$	0.003 (0.003)	0.01 %
ΣMO-PCBs	**23.2 (21.31)**	-	**2.78 (2.54)**	**10.9 %**
77	*0.021**	$5 \cdot 10^{-4}$	*0.01*	*0.04 %*
126	*0.158**	0.11	*17.4*	*68.4 %*
169	*0.079**	0.001	*0.08*	*0.3 %*
ΣNO-PCBs	***0.258****	-	***16.3***	***68.7 %***
Bio-TEQs	**0.0254 (0.0269)**	**1**	**25.4 (26.9)**	**100 %**

*Concentrations of non-*ortho* PCBs are estimated based on ratio of the reported mean concentrations of the individual non-ortho PCBs relative to the mean concentration of PCB-118 in 13 glaucous gulls from Svalbard [9].

Table 2. Concentrations of mono-*ortho* PCBs and bioassay derived TCDD-equivalents (Bio-TEQs) in livers from three glaucous gulls found dead on Svalbard.

Congener	Conc. (µg/g lipid)			Mean TEQ (ng/g lipid)
	#1	#2	#3	
105	8.8	21.6	31.5	6.2
114	1.2	8.0	10.9	0.7
118	45.1	139.6	202.3	3.9
156	11.8	28.1	57.3	9.7
157	2.8	8.0	11.3	2.2
189	2.4	0.8	6.2	0.03
ΣMO-PCBs	**70.5**	**207.6**	**319.5**	**22.7**
Bio-TEQs	**0.0483**	**0.254**	**0.143**	148.4

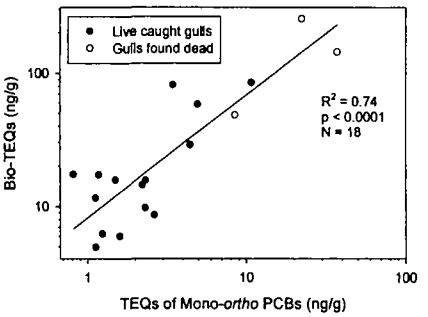

Figure 1. Relationship between bioassay derived TCDD-equivalents (Bio-TEQs) and TEQs calculated from mono-*ortho* PCBs in livers from glaucous gulls from Svalbard.

Although the estimation of the mean non-*ortho* PCB concentrations involves uncertainty, the comparison between the PCB congener levels and the Bio-TEQs indicates that a major part of the total dioxin-like potency in glaucous gull liver extracts may be attributed to the presence of non-*ortho* and mono-*ortho* PCBs. It cannot be excluded that other compounds contributed to the dioxin-like activity measured in the present study, but they probably were of minor importance compared to the non-*ortho* PCBs. Daelemans *et al.* [9] found the dioxin levels in their glaucous gull samples to be below their detection limit. In yolk samples from common terns, *Sterna hirundo*, breeding in the Netherlands, non- and mono-*ortho* PCBs accounted for more than 90 % of the bioassay-derived TEQs, while the quantified polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) were equivalent to 5% of the bioassay-TEQs [10]. In contrast, PCBs and PCDD/Fs could only account for approximately one third of the bioassay-derived TEQs in bird tissues from the contaminated Green Bay, Wisconsin, USA [16].

For all the 18 gulls combined, the mono-*ortho* PCBs could explain 74% of the variation in Bio-TEQs (based on Log_{10}-transformed values, see Fig. 1). Thus, even if only a minor part of the Bio-TEQs can be attributed to the mono-*ortho* PCBs, total TEQs can nevertheless be reasonably estimated from the concentrations of the mono-*ortho* PCBs and vice versa. Due to the high intercorrelation between the PCBs, it is also possible to use the concentration of the most abundant congener, PCB-153, as an index of the general PCB and TEQ burden. In the present study, the coefficient of determination (r^2) for the correlation between Log(Bio-TEQs) and Log(PCB-153) was 0.76. In spite of the high Bio-TEQ levels in the liver extracts, hepatic EROD activities in the same gull individuals were low (< 70 pmol min^{-1} mg $protein^{-1}$) [17]. The hepatic EROD activities were not correlated with the Bio-TEQs found in the present study.

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