PCDD/Fs, DIOXIN-LIKE PCBs AND MARKER PCBs IN EGGS OF PEREGRINE FALCONS FROM GERMANY

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Introduction

Adverse effects of persistent organochlorine pesticides (POPs) on wildlife have been widely documented in the literature. For birds, the reproductive cycle is negatively influenced. Therefore, bird's eggs are frequently used to monitor the contamination of the environment with xenobiotic substances. A high content of PCBs and p,p'-DDE (as main metabolite of p,p'-DDT) was found in eggs of peregrine falcons collected between 1988 and 1993 in the German "Bundesland" Baden-Württemberg (i). Many other publications presented results for organochlorine pesticides, indicator PCBs or organobromine compounds in various bird's eggs. PCDD/Fs and dioxin-like PCBs were determined in eggs of California peregrine falcons (ii), of cormorants in Japan (iii,iv), of predatory birds in Spain (v), of common terns in Michigan, USA (vi), of peregrine falcons in Spain (vii) and of different sorts of hawks in Germany (viii).

The Stockholm Convention is a global treaty signed now by 55 parties to take action against certain POPs, among them PCBs, PCDDs and PCDFs (ix). After ratification by France as the 50th Party, the Convention entered into force on 17 May 2004. The effectiveness should be evaluated four years after the date of entry into force and periodically thereafter at intervals. Therefore, a Global POPs Monitoring Programme was developed. United Nations Environment Programme (UNEP) organized a workshop to provide a scientific basis for this programme. One of the conclusions was to select the following matrices: air; bivalves; wildlife species (fish, bird's eggs, marine mammals) and human milk (x). The main reason for inclusion wildlife including bird's eggs was to gain information on temporal trends on, at the least, a regional basis, in animals, which represent either top predators or important species within aquatic or terrestrial food chains. For falcons, a high accumulation of POPs was observed. Regarding the migration habits it is known that older peregrine falcons stay in their breeding grounds; therefore, their eggs are suitable bioindicators for a relatively small area.

Materials and Methods

Eggs of peregrine falcons (Falco peregrinus) were collected by members of organizations for protection of birds in different regions of Germany. All eggs were non hatched and some of them deserted.

The eggs were stored at –20 °C in glassware.

After freeze-drying of the whole sample, fat and contaminants of interest are extracted in a hot extraction device ("Twisselmann extractor") with cyclohexane/toluene (50/50) for 8 hrs. After evaporation of the solvent, an aliquot of fat is spiked with ^{13}C-labeled internal standards (17 PCDD/Fs, 5 non-ortho PCBs [37, 77, 81, 126, 169], 6 mono-ortho PCBs [28, 60, 105, 118, 156, 189] and 7 di-ortho PCBs [52, 101, 153, 138, 180, 194 and 209]). Gel permeation chromatography on Bio Beads S-X3 removes fat. A silica column impregnated with sulfuric acid removes remaining oxidizable substances. A florisil column separates PCDD/F from PCBs. The PCDD/F-fraction is purified on a Carbopack C-column. After addition of 1,2,3,4-$^{13}C_{12}$-TCDD, determination is performed by HRGC/HRMS (Fisons Autospec; resolution 10,000; DB5-MS).

The PCBs are separated on a Carbopack B-column into three fractions of first di-ortho PCBs (elution with hexane), then mono-ortho PCBs (elution with hexane/toluene; 92.5/7.5) and finally non-ortho PCBs (reversed elution with toluene). After addition of $^{13}C_{12}$-PCB 80, the different PCB groups are determined by HRGC/HRMS (Fisons Autospec; resolution 10,000; DB5-MS) in three separate runs. Marker PCBs are PCB 28, 52, 101, 138, 153 and 180.

TEQs were calculated using the WHO toxic equivalency factors (TEFs) for humans (xi).

The method was successfully applied in 15 interlaboratory studies with altogether 72 samples to be analysed in the fields "food and feedingstuffs" for the determination of PCDD/F and/or dioxin-like PCBs, including a special study with eggs (xii). The analytical performance was demonstrated for analysis of breast milk samples as reference laboratory for the third round of WHO-coordinated exposure studies (xiii).

Results and Discussion

The 31 eggs samples of peregrine falcons have high levels of PCDD/Fs (median 257, range 79 to 844 pg WHO-PCDD/F-TEQ/g fat) and even higher levels of PCBs (median 1,191, range 309 to 10,143 pg WHO-PCB-TEQ/g fat) (table 1 and 2). The relative contribution of PCBs to the total TEQ is on average 84 % (range 71 to 95 %). Within the chosen regions, a wide range of contamination can be found: Even in the same cities (Stuttgart or Ludwigshafen), results of eggs collected in the same year (2003) varied by a factor of about 5 to 10. These findings show the high variation of biological matrices. Such variation is also found in other matrices, e.g. in breast milk as another example for a member of the food chain being on top of this chain: The frequency distribution of 271 breast milk samples from Germany collected in 1995-1998 showed a variation between 4.7 and 39.0 pg I-TEQ/g fat with a mean of about 15 pg I-TEQ/g fat (xiv). Therefore, for comparison of regions or time trends, a considerable number of samples has to be analysed. For comparison of results of bird's eggs, also the species has to be taken into account due to different consumption habits.

As mentioned above, TEQs were calculated using the WHO TEFs for humans. There are differences for some congeners in comparison to TEFs for birds. This would cause higher WHO-PCDD/F-TEQ results (mainly due to higher TEFs for TCDF and PeCDF). However, this would not effect the WHO-PCB-TEQ result to a great extent. As orientation, the Rottweil sample with 258 pg WHO-PCDD/F-TEQ/g fat (applying TEFs for humans) would give 376 pg WHO-PCDD/F-TEQ/g fat (applying TEFs for birds). The PCB result would not be changed significantly (804 pg WHO-PCB-TEQ applying TEFs for humans corresponding to 881 pg WHO-PCB-TEQ applying TEFs for birds). The sum would be increased from 1061 to 1257 pg sum WHO-TEQ (about 20 %).

sampling year	Region / City	PCDD/F-WHO-TeQ pg/g fat	PCB-WHO-TEQ pg/g fat	Sum WHO-TeQ pg/g fat	% contrib of PCB to sum TEQ	sum marker PCBs mg/kg fat	Dry Matter (%)	Fat (of (%)
	B-W, region Stuttgart							
2001	Reutlingen	98	958	1056	91	22.6	21.0	5.4
2003	Esslingen	123	935	1058	88	33.6	21.3	6.7
2003	Stuttgart	79	1148	1227	94	17.7	22.8	6.0
2003	Heilbronn	156	1418	1574	90	37.9	22.2	6.5
2003	Stuttgart	458	6233	6691	93	139.4	14.5	2.1
	B-W, region Karlsruhe							
2003	Ludwigshafen	168	729	897	81	25.5	24.0	6.8
2001	Mannheim	147	964	1111	87	36.1	18.8	3.7
2003	Neckargmünd	267	1081	1348	80	27.5	15.9	2.1
2000	Karlsruhe	148	2098	2247	93	65.9	23.5	6.7
2001	Karlsruhe	244	3750	3994	94	82.4	20.1	4.8
2003	Ludwigshafen	844	10143	10988	92	259.1	21.7	6.0
2003	Mannheim	393	n.a.	n.a.	n.a.	71.9	19.7	5.4
	B-W, region Freiburg							
2003	Rottweil	258	804	1061	76	29.5	20.2	5.2
2003	Haigerach/OG	167	1345	1512	89	35.6	22.1	6.6
2001	Breitnau-Freiburg	214	1378	1592	87	39.2	20.7	5.2
2000	Breitnau-Freiburg	392	2289	2681	85	54.0	21.9	5.2
	B-W, region Tübingen							
2001	Neufra, Wolfental	100	544	643	84	16.8	18.6	4.3
2000	Deggenh./Bodensee	93	564	657	86	19.5	17.5	4.7
2003	Immendingen	144	2922	3066	95	76.9	20.8	5.1

Table 1: PCDD/F and PCB in eggs samples of peregrine falcons from Germany, Bundesland “Baden-Württemberg” (here abbreviated B-W) (in pg WHO-PCDD/F-TEQ/g fat, pg WHO-PCB-TEQ/g fat and pg sum WHO-TEQ/g fat; marker PCBs as sum of PCB 28, 52, 101, 138, 153 and 180 in mg/kg fat)

sampling year	Region / City	PCDD/F-WHO-TeQ pg/g fat	PCB-WHO-TEQ pg/g fat	Sum WHO-TeQ pg/g fat	% contrib of PCB to sum TEQ	sum marker PCBs mg/kg fat	Dry Matter (%)	Fat (of (%)
1990	former GDR	*97*	*309*	*406*	*76*	*7.0*	*99.1*	*51,1*
1990	former GDR	160	493	653	76	13.0	31.3	10.1
1990	former GDR	269	671	940	71	14.9	22.0	5.9
1990	former GDR	257	785	1042	75	21.2	23.9	7.0
1990	former GDR	313	835	1148	73	27.7	17.8	7.3
1990	former GDR	305	1091	1396	78	27.4	23.8	5.8
1990	former GDR	428	1234	1662	74	29.1	28.4	5.4
1990	former GDR	364	1567	1930	81	32.6	20.0	7.0
1990	former GDR	380	1620	2000	81	32.8	21.9	6.4
1990	former GDR	278	2011	2289	88	52.4	22.7	10.4
	Northern Germany							
2003	Brunsbüttelkoog	608	1736	2343	74	29.8	25.3	6.1
2003	Brunsbüttelkoog	518	2234	2752	81	39.8	19.2	5.8

Table 2: PCDD/F and PCB in eggs samples of peregrine falcons from Northern Germany and East Germany (former GDR) (in pg WHO-PCDD/F-TEQ/g fat, pg WHO-PCB-TEQ/g fat and pg sum WHO-TEQ/g fat; marker PCBs as sum of PCB 28, 52, 101, 138, 153 and 180 in mg/kg fat)

The changes in TEF factors have to be kept in mind when data of samples analysed before 1998 (when the WHO-TEFs valid at present were derived) should be compared. As an example, a result of ”120 ng total TEQ/kg” as found in eggs of peregrine falcons in California includes only PCDD/F and non-ortho PCB (no mono-ortho PCBs), was calculated with different TEFs, and is on product basis (see lit. 2). Eggs of cormorants from Japan had 925 pg WHO-PCDD/F-TEQ/g fat (applying TEFs for birds) (see lit. 3). PCDD/Fs in eggs of predatory birds in Spain were found between 14.8 to 22.8 pg/g fresh weight (f.w.), however it is unclear whether these are TEQ-figures (see lit. 5). In eggs of common terns from Michigan, USA, total TEQs ranged from 810 to 1900 pg WHO-TEQ/g fat (applying TEFs for birds). In eggs of peregrine falcons from Spain, results ranged between 12.1 and 14.4 pg WHO-TEQ/g f.w. (applying TEFs for birds) (see lit. 7). Eggs of hobbies from Germany contained a mean of 478 pg WHO-PCDD/F-TEQ/g fat and 551 pg WHO-PCB-TEQ/g fat (applying TEFs for birds), eggs of sparrowhawks 424 respectively 1278 and goshawks 211 respectively 935 pg/g fat (see lit. 8).

For the purpose of comparison of data from different regions and years it should be considered to present data on dry matter basis, as well. PCDD/F and PCB data can be calculated on fresh weight basis, if the eggs are fresh; otherwise, results could be quite erroneous. For matrices of animal origin it is quite common to present data on fat basis. However, over time and depending on the conditions of storage, fat can be metabolised. Thus, for old eggs samples (partly dried; rotten; begun to sit on), data on dry matter are considered to provide best comparability. It is recommended to report dry matter and fat content generally to allow recalculations for the required comparison.

As a different point of view, hen eggs intended for human consumption have far lower PCDD/F and PCB contamination: The average background contamination of eggs of caged chicken is below 1 pg WHO-PCDD/F-TEQ/g fat (applying TEFs for humans). Eggs of free-range chicken are usually below 3 pg WHO-PCDD/F-TEQ/g fat. EU has set a tolerance of 3 pg WHO-PCDD/F-TEQ/g fat for eggs which will be applied also for free-range chicken from 2005 (xv). Only eggs from free-range chicken in highly contaminated areas were found to have a dioxin contamination in the range of 100 to 300 pg WHO-PCDD/F-TEQ/g fat. In comparison to the data for the usual background contamination of hen eggs for human consumption, eggs of birds of prey are extremely high contaminated with PCDD/Fs and PCBs.

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