The role of the peregrine falcon (*Falco peregrinus*) as a bioindicator for the accumulation of organohalogen compounds and other persistent organic pollutants has been established in field studies. Biometric indices for damage caused by pollutants such as the shell thickness and the shell index were determined and the egg contents were analyzed for various pollutants by gas chromatography/mass spectrometry. A wide range of chemically activated luciferase expression (CALUX*) bioassays were performed on subsamples of the eggs.

The following organohalogen compounds were found in the eggs of 2009: dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenylchloroethane, heptachlor epoxide, hexachlorobenzene, dieldrin, hexachlorocyclohexane, polychlorobiphenyls and polychlorodibenzodioxins and polychlorodibenzofurans, polybromodiphenyl ethers, hexabromocyclododecane, tetrabromobisphenyl A, perfluoro compounds, and mercury. The DDT metabolite, dichlorodiphenyldichloroethene (DDE) (11,800 ng/g dry matter), was found to be the most highly concentrated egg contaminant followed by 2,2′,4,4′,5,5′-hexachlorobiphenyl (3800 ng/g). After a past general drop in pollution level, most egg contaminants presently plateau at levels that may still exceed limit values in foods of animal origin (DDE) or even toxicological thresholds (polychlorobiphenyls + polychlorodibenzodioxins, polybromodiphenyl ethers, perfluorooctanesulfonate, methylmercury).

Accumulation of DDE could be shown in peregrine falcon eggs from the uplands of Southwest Germany with elevations up to 1500 m, presumably due to its global distribution and its cold condensation in higher altitudes. In contrast, the concentration of polychlorobiphenyls in falcon eggs decreases with elevation, indicating that these pollutants originate mainly from conurbations and local industrial sites.
Significant negative correlations were found between both shell index and thickness and the concentration of Hg. A deleterious effect is also evident from a no-observed-adverse-effect level of 120 ng MeHg per gram egg determined by other authors in chronic feeding studies with ibises, which resulted in decreased egg productivity and male homosexual nesting and courtship behavior. The average Hg concentration in the peregrine falcon eggs from 2009 is almost four times higher than this level. MeHg accounted for 82.5% of the Hg present in the eggs of 2009 and 2010. The cell test DR CALUX® for screening of dioxin-like activities can be used to detect not only the 29 regulated dioxin-like substances but also many other persistent organic pollutants with dioxin-like potencies, such as mixed halogenated dioxins/biphenyls. In our case, the results of bioanalytical screening methods showed no additional effect of other compounds with dioxin-like activity. Ninety-three out of 177 analytes sought could be detected in the eggs. Chlorinated paraffins, organotin compounds, some pesticides that are still in use, and phthalic esters with the exception of traces of diethylhexyl phthalate could be excluded. All pollutants found in the eggs belong to substance classes banned by the Stockholm and Minamata Conventions.

1. Introduction

As serious environmental pollutants, organohalogen compounds (OHC) have been banned by an international treaty, the Stockholm Convention (EU 2010; SCS 2014). Yet these lipophilic and persistent pollutants remain ecotoxicologically relevant for wildlife (Bernanke and Köhler 2009; Köhler and Triebskorn 2013) and accumulate in bird eggs as indicators of environmental pollution. The level of OHC contamination correlates with egg shell deficiencies such as a decrease in shell thickness (ST) and a reduction of the shell weight as a result of greater pore size, and a consequent increase in fragility. The United Nations Environment Program has established bird eggs as suitable bioindicators for monitoring persistent organic pollutants (POPs) in the terrestrial ecosystem. Earlier studies (Schmidt and von der Trenck 2006; von der Trenck et al. 2005, 2006, 2008a, 2008b) had shown that OHC accumulate in the terrestrial avian food chain in the order: peregrine falcon > eagle owl > barn owl > little owl ≈ jackdaw > great tit ≈ blue tit > coal tit. Weber, Schmidt, and Hädrich (2003) confirmed the peregrine falcon as most highly OHC-polluted species among a group comprising the osprey, the sparrow hawk, the red kite, and the common tern. With respect to perfluorinated compounds (PFCs), only the cormorant is known to accumulate higher levels in its eggs (Rüdel et al. 2011). These results underscore the peregrine falcon’s prominent position at the top of the food web and its outstanding suitability as a bioindicator species (VDI 2009; Baum and Hädrich 1995; Wegner 2000; Schilling and Wegner 2001; Lindberg et al. 2004; Wegner et al. 2005; von der Trenck et al. 2005, 2006; von der Trenck, Schilling, and Schmidt 2007; Malisch and Baum 2007; Wegner and Fürst 2008; Guerra et al. 2010a, 2010b; Holmström et al. 2010; Park et al. 2010; Borg et al. 2013).

Data from peregrine falcon eggs that failed to hatch are available in the German State of Baden-Württemberg (BW) from 1970 to 2009 (Schilling and Wegner 2001; von der
Trenck et al. 2005, 2006, 2010; Wegner et al. 2005) for the organochlorine pesticides (OCPs), dichlorodiphenyltrichloroethane (DDT) and metabolites, hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), heptachlor epoxide (HCEP), and the industrial chemicals and byproducts known as polychlorinated biphenyls (PCBs). More recent results are only partly covered by this report. Dichlorodiphenyldichloroethene (DDE) and dichlorodiphenyldichloroethane (DDD) are degradation products of the “obsolete” pesticide DDT. Introduced worldwide into the environment on a megaton scale from the 1940s to the 1970s (Li and Macdonald 2005), DDT and its metabolites (and other OCP) were identified as endocrine disruptors responsible for the almost complete extinction of raptor populations, especially the peregrine falcon (Ratcliffe 1958, 1967, 1970, 1980). Eggshell deficiencies are characterized by a decrease in thickness and a reduction of shell weight, as a result of greater pore size, and a consequent increase in fragility. Critical parameters for damage caused by DDT/DDE contamination are, according to Ratcliffe (1958, 1967, 1970, 1980), the ST and the shell index (SI); Schilling and Wegner 2001; Wegner et al. 2005).

During the past 40 years, the organochlorine (OC) pollution of peregrine falcon eggs has shown a general decrease which is paralleled by an encouraging increase in the number of breeding pairs in BW (from less than 30 to about 300; von der Trenck et al. 2006; von der Trenck, Schilling, and Schmidt 2007). The investigations described here draw upon the dedicated efforts of a group of amateur naturalists (primarily ornithologists), the “Working Group for the Protection of the Peregrine Falcon” (AGW), to conserve an endangered indigenous raptor species (Hepp et al. 1995; Schilling and Wegner 2001). They were not initially intended as a long-term biomonitoring program, and caution is appropriate in comparing recent results with earlier data, especially in the case of PCBs, because of the differing laboratories and analytical methods. Nevertheless, the steadily decreasing time trends of all five OC compounds in combination with the recovery of the falcon population confirm the general reliability of the analytical results, even during the earlier phases of the project (von der Trenck et al. 2005, 2006; von der Trenck, Schilling, and Schmidt 2007).

Since 1999, the “Institute for the Environment, Measurements, and Nature Protection of the German State of Baden-Württemberg” (LUBW) has been funding the determination of OHC in failed eggs collected by AGW members in the course of nest monitoring and ringing of juveniles. This cooperation constitutes the only long-term terrestrial monitoring program for POPs conducted by the State of BW. In 2009, the analytical program was expanded to screen for new POPs (von der Trenck 2012). The main objectives of the project are

- to monitor the POPs banned by the Stockholm Convention;
- to check the effectiveness of the convention within BW; and conversely,
- to explore the suitability of peregrine falcon eggs as bioindicators and the range of chemicals accumulated.

After their use was banned, the concentrations of DDE (the most stable DDT metabolite), HCEP, HCB, HCH, and PCB decreased considerably from their levels in the 1970s. Thus, the DDT ban (in 1972 in Western Germany) in conjunction with unremitting conservation efforts of the AGW throughout the past four decades has been instrumental for the survival of the peregrine falcon as a species in southwestern Germany (Hepp et al. 1995; Schilling and Wegner 2001; von der Trenck, Schilling, and Schmidt 2007).
The drop in pollution levels during the 1980s is illustrated by the example of DDE (Figure 1; von der Trenck 2012). A concomitant recovery of the population from metabolic disorders was also noted. For example, \( p,p' \)-DDE interferes with the birds’ calcium metabolism, leading to eggshell thinning and unsuccessful breeding (Lundholm 1997; Wegner et al. 2005). The peregrine falcon had become extinct in most of Germany with only individual breeding pairs being reported in BW. Although its population has meanwhile recovered in this federal state, at 10 \( \mu \)g/g of dry matter (dm) DDE levels in the eggs of this species have nevertheless been exceeding the limit value for chicken eggs by a factor of 100 for the past 20 years and continue to do so (Figure 1). The temporal trend of DDE content depicted in Figure 1 is indicative of a general phenomenon that has also been shown for other OC compounds (von der Trenck et al. 2006). Doubtless, the decline in peregrine falcon eggs of OCP and PCB is due to the bans issued in the 1970s and 1980s, and nowadays, the concentration of all OCPs other than DDE amounts to less than 3% (Figure 2) of their DDE content (Schilling, Schmidt, and von der Trenck 2007).

Dittmann and Becker (2012) also observed the levels of PCBs, DDT, HCB, HCH, and Hg in common tern and oystercatcher eggs from the coasts of the North Sea from 1980 to 2010, and concluded that the POP contamination has generally decreased from pre-1990 peaks and now has reached a more or less constant level. However, ecological quality objectives (EcoQOs) were exceeded at most measuring sites. EcoQOs are based on achievable background concentrations and approximate the expected status after a complete

![Figure 1. Decade mean contents of DDE in peregrine falcon eggs collected in Baden-Württemberg between 1971 and 2009 and standard deviation. In parentheses: \( n; \Delta = \) shell index; residue limit value in food: 0.1 \( \mu \)g/g (RHmV 2008).](image-url)
stop of any further input of anthropogenic pollutants (Dittmann et al. 2011, 2012; Heslenfeld and Enserink 2008).

2. Materials and methods

2.1. Sample collection

In the process of caring for breeding wild peregrine falcon (Falco peregrinus) pairs, private ornithologists of the AGW collected failed eggs for chemical and biological analyses and sent them to the NABU (Naturschutzbund — Nature Conservation Society) bird protection center for further processing (VDI 2009). The eggs were opened and the contents were transferred into 100-mL screw-cap vessels.

2.2. Measurement of eggshell parameters

Ratcliffe’s index (SI = shell mass/(egg length · egg diameter) as described by Wegner et al. [2005]) and the ST were determined by Schwarz (2010). These biometric data were correlated with the content of egg pollutants. The shell parameters were measured for 338 eggs, collected between 2000 and 2009 (Schwarz 2010). The egg shells collected between 2007 and 2009 were obtained from the “NABU-Vogelschutzzentrum” (Bird Protection Center), Mössingen, Germany, and the other egg shells from the “Staaitliches Museum für Naturkunde” (State Museum for Natural History), Stuttgart, Germany. All samples had originally been collected by the members of the AGW. Prior to the measurements, the egg contents were removed and the shell was dried in air at room temperature for several months. Eggshell length and width were measured using a digital gauge (Electronic Digital Caliper 0–150 mm, d = 0.01 mm). The weight of the dry eggshell was determined using

Figure 2. OCPs in peregrine falcon eggs collected in 2009, mean, and range; p,p'-DDE, cis-HCEP, HCB, and β-HCH were detected in all 10 eggs analyzed, dieldrin in 8 eggs, p,p'-DDD in 7 eggs, p,p'-DDT in 6 eggs, and γ-HCH in 1 egg.
an analytical balance (AE 240, Mettler (Gießen, Germany), \( d = 0.1 \) mg). The SI was corrected for hole size and eccentricity (Nygard 1999). Measurement of ST included the shell membranes and was performed using a dial indicator (FMD 50 T, measuring range 50 mm, measuring force 1.25–2.70 N, \( d = 0.001 \) mm (Käfer, Villingen-Schwenningen, Germany)). Ten separate measurements were taken at the equator region of each individual sample. Their arithmetic means served as reliable estimates of the overall ST of each egg at its equator region.

2.3. Chemical analysis

Weighing about 40 g, peregrine falcon eggs are almost as large as hen’s eggs. The analytical work was contracted by LUBW (formerly LfU, Karlsruhe, Germany) from 1999 onwards. Extraction and determination of the analytes were described earlier (von der Trenck et al. 2006; von der Trenck, Schilling, and Schmidt 2007). Analysis of the polybrominated diphenyl ethers (PBDEs), mercury (Hg), and OC compounds with the exception of polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) was performed by Berghof, Tübingen, Germany. Tetrabromobisphenol A (TBBA) and hexabromocyclododecane (HBCDD) were added to the program in 2006 and analyzed by Ökometric, Bayreuth, Germany. PCDD/F determination of selected falcon eggs was carried out by the Chemical and Veterinary Investigation Office Freiburg, Germany (CVUA-FR). The extended program of analytes determined in the years 2009 through 2014 was contracted out to Eurofins GfA (Münster/Hamburg, Germany) and methylmercury (MeHg) determinations were performed by the Chemical and Veterinary Investigation Office Karlsruhe, Germany (CVUA-KA).

The egg contents were dried at max. 50 °C (before 1993) or freeze dried (since 1993) and homogenized. Subsequent aliquots were weighed into extraction thimbles (organic compounds) or digestion vessels (Hg). The concentrations of the measured substances refer to the dm of the egg content. Alternatively, concentrations relative to the wet weight (ww) or the lipid content of the egg may be reported. The best reference for our study, however, is dm because the eggs were collected at widely differing ages after laying and the moisture content changes with storage time. The development of the embryo may also change the water and lipid contents of the egg (Baum and Hädrich 1995; Wegner 2000). This problem does not exist with fresh egg samples. The conversion from fresh tissue concentration to dm or lipid-based concentration was calculated for every egg individually. For the comparison of mean values with limit values or with other authors’ results, rounded values of 20% dm and 5% lipid content were used. Thus, general factors of 1/5 or 4 may be used to convert dm-based into ww-based or lipid-based concentrations. We report dm-based concentrations unless stated otherwise.

The egg contaminants were measured by gas chromatography (GC) initially coupled with electron capture (EC), later with mass spectrometric (MS) detection (Table 1). The samples were analyzed for OCP and PCB in some cases by CVUA-FR, or by the Freiburg Institute for Animal Hygiene as one of its parent institutes (Baum and Hädrich 1995), or contracted out to commercial laboratories (Wegner 2000). The respective laboratories are listed by Schilling and Wegner (2001) in their monograph. The eggs from 2001 till 2008 were analyzed by Institute Berghof in Tübingen according to the German Foodstuffs and Commodities Act (LMBG L00.00-12; Table 1). This institute checked the analyte recovery through the whole procedure by spiking the samples with \( ^{13} \)C-PCB-101. Basis for the
Table 1. Substances determined in bird eggs.

| Substances                          | Time period | LOQ[^1] | Laboratory                  |
|------------------------------------|-------------|---------|-----------------------------|
| OCP                                | 2001–2008   | 2       | Berghof                     |
| PCBs + mono-CB up to nona-CB       | 2001–2008   | 5       | Berghof                     |
| d,l-PCB                            | 2001–2008   | 0.5     | Berghof                     |
| OCP                                | 2009–2011   | 0.3     | CVUA-FR                     |
| PCBs + mono-PCB ortho-PCB          | 2009–2011   | 0.3     | CVUA-FR                     |
| Non-ortho-PCB                      | 2009–2011   | 0.03    | CVUA-FR                     |
| PCDD/F                             | 2000–2011   | 0.0003  | CVUA-FR                     |
| PCDD/F                             | 2007        | 0.005   | Ökometric                   |
| PBDE                               | 2003–2008   |         | Berghof                     |
| BDE-28, -47, -49, -99, -100, -153  | 2009–2010   | 2       | Berghof                     |
| BDE-154, -183                      |             | 50      | Berghof                     |
| BDE-209                            |             | 500     | Berghof                     |
| PBBE[^2]                            | 2008–2011   | 0.01–0.6| GfA                         |
| TBBA (tetrabromobisphenol A)        | 2006–2008   | 2       | Ökometric                   |
| HBCDD                              | 2006–2008   | 500     | Ökometric                   |
| TBBA                               | 2009–2011   | 1       | GfA                         |
| HBCDD                              | 2009–2011   | 1       | GfA                         |
| Pentachlorobenzene                 | 2003–2008   | 2       | Berghof                     |
| Octachlorostyrene                  | 2003–2008   | 2       | Berghof                     |
| Octachlorostyrene                  | 2009–2010   | 2       | GfA                         |
| Musk xylene                        | 2009–2010   | 20      | GfA                         |
| Bisphenol A                        | 2009–2010   | 5       | GfA                         |
| Pesticides (Aclonifen, Bifenthrin, Brodifacoum, Esfenvalerate, Difenacoum, Lufenuron, Metalflumizone, Pendimethalin, Quinoxifen) | 2009–2010 | 5, 2, 2, 5, 10, 2 | GfA |
| PFC                                | 2008–2011   | 0.5     | GfA                         |
| Phthalate esters (DMP, DEP, DBP, BBP, DEHP, D-n-OP) | 2009–2010 | 1000 | GfA |
| Short chain chlorinated paraffinsC_{10–C_{13}} | 2009–2010 | 2–20 | GfA |
| Mercury (Hg)                       | 2003–2008   | 30      | Berghof                     |
| Methylmercury (MeHg)               | 2009–2011   | 5       | GfA                         |
| Mono-, di-, tri-, tetrabutyltin    | 2008–2011   | 150     | CVUA-KA                     |
| Mono-, diocytlditinin              | 2009–2010   | 10      | GfA                         |
| tricyclohexylditin, triphenyltin   | 2009–2010   | 3–10    | GfA                         |
| Various POPs                       | 2010–2011   | 2.5     | CVUA-FR                     |
| Ah-receptor activation             | 2004–2011   | 5 pg TEQ/g dm | BDS |
| ER activation                      | 2004–2011   | 80 pg EEQ/g dm | BDS |
| AR inhibition                      | 2004–2011   | 10 µg FEQ/g dm | BDS |
| GR activation                      | 2008        | 30 ng DEQ/g dm | BDS |
| TR activation                      | 2008        | 20 ng T3EQ/g dm | BDS |
| PPARy-activation                   | 2012–2013   | 80 pg REQ/g dm | BDS |

[^1]: Approximate limit of quantitation [ng/g dm]  
[^2]: BDE-17, -28, -47, -49, -66, -71, -77, -85, -99, -100, -119, -126, -138, -153, -154, -156, -183, 184, -191, 196, 197, -206, -207, -209 + tri-nonaBDE-isomers

Ah: aryl hydrocarbon  
AR: androgen receptor  
BDS: BioDetection Systems BV, Science Park 406, NL-1098 XH Amsterdam  
BEQ: Bioanalytical equivalents in the DR CALUX® assay  
Berghof: Berghof Analytik + Umweltengineering GmbH & Co KG, Ob dem Himmelreich 9, D-72074 Tübingen  
CALUX®: Chemically activated luciferase expression  
CVUA-FR: Chemisches u. Veterinär-Untersuchungsamt, Bissierstr. 5, D-79114 Freiburg  
CVUA-KA: Chemisches u. Veterinär-Untersuchungsamt, Weißenburger Str. 3, D-76187 Karlsruhe  
dl-PCB: dioxin like polychlorinated biphenyls  
dm: dry matter  
DEQ: dexamethasone equivalents  
DR: dioxin responsive  
EEQ: 17ß-estradiol equivalents  
ER: estrogen receptor  
FEQ: fluoxetine (androgen inhibitor) equivalents  
GfA: Eurofins GfA Lab Service GmbH, Otto-Hahn-Str. 22, D-48161 Münster  
GR: Glucocorticoid receptor  
HBCDD: hexabromocyclododecane, all isomers  
OCP: organochlorine pesticides  
Ökometric: Ökometric GmbH, Bemeckerstr. 17-21, D-95448 Bayreuth  
PDB: the six ndl-polychlorinated biphenyls (IUPAC-no. 28, 52, 101, 138, 153, and 180) are used as indicator congeners up to the present day (LfU BW 1995)  
PFC: perfluorinated compounds (PFBS, PFHxS, PFOS, PFOA, PFOA, PFNA, PFDA, PFOA, PFDoA; full names in legend to Figure 10)  
Phthalate esters: dimethylphthalate (DMP), diethylphthalate (DEP), dibutylphthalate (DBP), butylbenzylphthalate (BBP), diethylhexylphthalate (DEHP), di-n-octylphthalate (D-n-OP)  
PPARγ: peroxisome proliferator-activated receptor γ  
REQ: rosiglitazone equivalents  
TEF: toxic dioxin equivalence factor, potency compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)  
TEQ: toxic dioxin equivalents  
TR: thyroid hormone receptor  
T3EQ: T3 equivalents
accreditation of this laboratory according to DIN EN ISO/IEC 17025 is round robin tests involving the media water, wastewater, soil, and sludge. Some complementary analyses of egg samples were carried out for dioxins and dioxin-like PCBs (dl-PCB) by CVUA-FR. This laboratory was certified in 1998 (DIN EN ISO/IEC 17025) and accredited as reference laboratory for dioxins for the European Union in 2006. The identification and quantification of the analyzed substances have been described in detail by Baum and Hädrich (1995), Wegner (2000), and Malisch and Baum (2007).

2.3.1. Mercury
Microwave-pressurized digestion (closed system) with nitric acid and hydrogen peroxide was followed by cold vapor atomic absorption spectrophotometry for the determination of total Hg. Analyses including Hg in eggs from 2003 to 2008 were carried out by Berghof (Tübingen, Germany). Eggs from 2009 onwards were analyzed by Eurofins GfA (Münster/Hamburg, Germany).

MeHg was determined by CVUA-KA according to the published analytical method (Kuballa et al. 2009). The method consisted of an alkaline sample digestion in methanolic potassium hydroxide solution, derivatization with sodium tetraethylborate, and extraction with n-hexane. The ethylated mercury species were analyzed using GC, combined with atomic emission detection. Working with a sample of 0.1 g yielded limits of detection of 35 or quantitation of 120 ng MeHg/g, respectively.

2.3.2. Organic substances
The concentrations of the following substances were measured in the egg samples:

OCP:
Aldrin, dieldrin, chlordan (cis- and trans-), sum DDT consisting of DDT and its metabolites DDD and DDE (the op'- and pp'-isomers, respectively), endosulfan (α- and β-), endosulfan sulfate, endrin, heptachlor and its epoxide (HCEP, cis- und trans-), HCB, and different HCH conformers (α-, β-, γ-, and δ-HCH; γ-HCH = Lindane).

PCB and structurally related molecules:
From 2001 on, 26 PCB-congeners (IUPAC-numbers 28, 52, 101, 138, 153, 180 (PCB₆) plus the coplanar congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189 (dl-PCB) plus the congeners 47, 49, 170, 187, 194, 199, 203, and 209) plus all other isomers from monochlorobiphenyl up to nonachlorobiphenyl (PCB-screening) were measured. Before 2001, the congeners 138, 153, and 180 (PCB₃) were measured and extrapolated to the sum of all PCB according to the LUFA Augustenberg formula (Baum and Hädrich 1995).
Seventeen 2,3,7,8-substituted congeners of the PCDD/F were determined in a few of the eggs from 2000 on.
Nine congeners of the PBDE were measured from 2003 on, namely the IUPAC numbers 28, 47, 49, 99, 100, 153, 154, 183, and 209.
Twenty-three peregrine falcon eggs, three eagle owl eggs, and one osprey egg collected in 2010 and 2011 were tested by CVUA-FR for various POPs, which are routinely analyzed in food of animal origin, such as aldrin, BDE-28, -47, -99, -100, -153, -154, bromocyclen, chlordan (cis- and trans-), DDT, DDD, and DDE (the op'- and pp'-isomers, respectively), dieldrin, HCB, α-, β-, γ-, and δ-HCH, endosulfan (α- and β-), endosulfan sulfate, endrin and δ-keto-endrin, heptachlor and HCEP (cis- und trans-), iodofenphos, methoxychlor, musk ketone and musk xylene, nitrofen, nonachlor (trans-),
octachlorodipropylether (S 421), oxychlordan, pendimethalin, 2,4,6-tribromo anisole and 2,4,6-tribromo aniline, and triclosan-methyl.

All substances and receptor binding measured in bird eggs in the course of this project are listed in Table 1.

### 2.4. Bioanalytical method

#### 2.4.1. Dioxin-like compounds (DR CALUX®)

From the years 2004 to 2011, 61 peregrine eggs were selected and analyzed via the dioxin responsive chemically activated luciferase expression bioassay (DR CALUX®) using H4IIE rat liver cells for the persistent dioxin-like compounds (such as PCDD/Fs and dl-PCBs) and their aryl hydrocarbon receptor (AhR) activities (Behnisch, Hosoe, and Sakai 2001, 2003; van Vugt-Lussenburg et al. 2013).

As chemical analysis by GC/HRMS (high-resolution mass spectrometry) gives results for the 29 dioxin-like compounds (17 3,4,7,8-chlorinated PCDD/Fs and 12 dl-PCBs), analysis by DR CALUX® was also included in the monitoring program in order to detect further compounds with dioxin-like activity such as halogenated, mixed halogenated (bromine, chlorine, fluorine), methylated, or otherwise substituted PXDD/PXDF/PXBs (X = Br, Cl, F, J), as well as other sulfuric acid stable POPs (such as PBDEs, N-dioxins; Behnisch, Hosoe, and Sakai 2001, 2003).

#### 2.4.2. Endocrine disrupting chemicals and obesogens (ER-, antiAR-, GR-, TR-, and PPAR-CALUX®)

Several peregrine eggs were selected from 2004 to 2013, and analyzed by ER- (for estrogenic compounds such as bisphenol A, phthalates, DDE, DDT), anti-AR- (for anti-androgenic compounds such as bisphenol A or DDE), GR- (for glucocorticoids such as dexamethasone), TR- (for thyroids such as T3, T4, hydroxy-BDEs and hydroxy-PCBs), and PPAR-CALUX (for peroxisome proliferator-activated receptor-γ agonists such as tributyltin (TBT) or perfluorooctanoic acid (PFOA)/perfluorooctane sulfonic acid (PFOS) assays for endocrine disrupting chemicals and obesogens using stable transfected U2OS human osteosarcoma cells (van der Burg et al. 2013).

### 2.5. Assessment of PCBs and dioxins

The term “dioxins” is used for two classes of different chlorinated compounds. Theoretically,

- 75 polychlorinated dibenzo-p-dioxins (PCDDs) and
- 135 polychlorinated dibenzofurans (PCDFs)

are possible. The 17 “dioxin” (PCDD/F)-congeners that are chlorinated in the 2,3,7,8-positions are especially toxic and persistent, and accumulate in the fatty tissues of animals and humans. These congeners exert their toxicity by binding to the cytosolic AhR in different cell types and inducing polycyclic hydrocarbon hydroxylation activities (cytochrome P-448) and phase II enzymes of xenobiotic metabolism (Sipes and Gandolfi 1986).
2.5.1. *dl-PCBs*

The group of PCBs comprises 209 substances with different (toxicological) properties. Dioxins and PCBs degrade only very slowly and accumulate via the food chain. Among the PCBs, a distinction is to be made between dioxin-like and non-dioxin-like actions (*dl-PCBs* and *ndl-PCBs*). This distinction is justified because the *dl-PCBs* interact in the organism with the same bonding site as dioxins (the AhR) causing a chain of events that can lead to a metabolic disruption of the hormonal balance. On chronic exposure, *dl-PCBs* cause damage to the skin, are hepatotoxic, immunotoxic, embryotoxic, and promote tumor growth. Acute effects in humans have been described after industrial accidents, high work place exposure, and intentional poisoning. These effects include elevated concentrations of triglycerides, cholesterol, and transaminases in the blood and characteristic inflammatory skin alterations termed chloracne (BfR 2012). The differing potency of the individual congeners after chronic exposure is expressed in toxic equivalent factors (TEFs) comparing the potency of a compound relative to the most toxic congener (2,3,7,8-TCDD), which is assigned a TEF of 1. After multiplying the concentrations by the respective TEFs, all PCDD/F- and *dl-PCB*-congeners can be added to yield the potency of the whole mixture in toxic equivalents (TEQ). Their tolerable intake via food and feed, in which dioxins and PCBs are ubiquitously present, has been regulated by setting a range from 1 to 4 pg daily (tolerable daily intake (TDI)) or 14 pg weekly (tolerable weekly intake (TWI)) or 70 pg monthly (provisional tolerable monthly intake (PTMI)), respectively, of PCDD/F + PCB TEQ/kg body mass (BfR 2012). The European Commission regulates dioxins and *dl-PCBs* in meat, milk and dairy products, and eggs and egg products by setting maximum levels. The maximum level for hen eggs is 5 pg TEQ/g fat (EU 2011a; = 1.25 pg TEQ/g dm in peregrine falcon eggs). Dioxins and *dl-PCBs* in different food items add up to the total intake. The current uptake via food in Germany amounts to 1–2 pg TEQ/(kg-d) on average. Since the tolerable intake of dioxins is based not primarily on toxicological considerations but on background levels, no immediate health risk will result from briefly exceeding the TDI in moderation. Nevertheless, as a matter of principle, food items exceeding the maximum levels have to be taken from the market (BfR 2012). The assessment of dioxin-like toxicity is based on TEQs originally published by the WHO in 1998 (van den Berg, et al. 1998). A reassessment in 2005 resulted in a new set of TEFs (WHO 2006). The reassessment of *dl-PCB* is not based on new data but on modified statistical considerations. The German Federal Institute for Risk Assessment, therefore, discouraged the use of the new TEFs (BfR 2007; Rauchfuß 2007), which result in a reduced calculated toxicity (10%–25% less toxic). With Regulation 1259/2011, the TEF05 values have become mandatory in the EU (2011a).

2.5.2. *ndl-PCBs*

In soil and water samples, the sum of all PCB-congeners has been determined for decades via the *ndl-PCB* and subsequently multiplied by 5 (Σ PCB = 5⋅PCB; DFG 2012) according to the German waste oil ordinance (DIN 51527) in order to prevent underestimating the total concentration in samples with unknown congener pattern (Table 1, footnotes; BMU 1989; Wegner 2000). In aqueous samples, for example, a limit of quantitation (LOQ) of 0.05 μg/L thus results from LOQs of 0.01 μg/L, multiplied by a factor of 5, for the single congeners (von der Trenck et al. 1999, 2006). In case of a known congener pattern, the total concentration is calculated by simple summation (DIN 38407-F3). In food
and human samples, frequently only the higher chlorinated congener nos. 138, 153, and 180 (PCB3) are determined. These congeners are strongly accumulated in the food chain (Table 2) because of their pronounced persistence and can therefore be regarded as guide congeners. Their concentration is extrapolated to the total concentration by an empirical formula derived by the Agricultural Research Institute (LUFA) Augustenberg, Germany: total PCB = (8.7-PCB-138 + 10.2-PCB-153 + 14.3-PCB-180)/3 (Baum and Hädrich 1995; Wegner 2000; Schilling and Wegner 2001; Wegner et al. 2005). The extrapolations according to LUFA or DIN overestimated 2.3-fold or 3.3-fold the true content in peregrine falcon eggs determined by simple summation (von der Trenck et al. 2006).

ndl-PCBs are regulated by the German contaminant ordinance (KmV 2010). This regulation contains a limit value in eggs of 20 ng/g ww for each of the six congeners (≥ 100 ng congener/g dm corresponding to 600 ng PCB6/g dm in falcon eggs). PCB6 amounts to approximately 50% of the Σ PCB in foodstuffs and is regulated by the European Commission (EU 2011a). The new European maximum level in foodstuffs of 40 ng PCB6/g fat (≥ 10 ng PCB6/g dm in falcon eggs; EU 2011a) offers a greater degree of protection than the German contaminant ordinance. In many cases, the analysis is even restricted to the PCB3, which, in peregrine falcon eggs, account for about 50% of Σ PCB (PCB3 ≈ PCB6; von der Trenck, Schilling, and Schmidt 2007). Owing to the almost constant 10% of dl-PCB in the total PCB content (Σ PCB) in peregrine falcon eggs, it has been suggested that the more commonly analyzed ndl-PCB should be used for screening the more relevant dl-PCB (Figures 3 and 4).

Some of the ndl-PCBs have a known estrogen receptor affinity and exert a direct stimulating or inhibitory effect on the complex action of the female sexual hormone. This latter activity also seems to apply to PBDEs (von der Trenck, Schilling, and Schmidt 2007). Recently, ndl-PCBs in high concentrations (ca. 1 μM) were reported to antagonize the dl-PCB action (specifically of PCB-126) at the AhR (Brenerova et al. 2015).

### 2.6. Statistical analysis

Statistical analysis was carried out using SAS-JMP 8.0.1 software. Samples for which the eggshell parameters could not be reliably measured were excluded from the analysis. Comparison of ST and SI over the years was made using a Kruskall–Wallis test with α = 0.05. The relationship between the eggshell parameters and the concentrations of p,p'-DDE and mercury in the egg content was investigated using a Spearman rank correlation test with α = 0.025. Data for all examined years were pooled to gain a sufficient amount of data points for analysis. In order to gain independent data, clutches were analyzed instead of single eggs. The unusually low concentration measured for p,p'-DDE in the year 2000 was apparently an artifact due to a systematic error and therefore excluded from the analysis.

The different environmental pathways of DDE and PCB3 can be deduced by measuring their concentrations in peregrine falcon eggs and correlating them with the mean elevation of the hunting range of the respective breeding pair (Figure 8). We performed our calculations with the eggs from 2001 to 2009 assuming a standard hunting range for peregrine falcons to have a radius of 5 km around the aerie. The average altitude of the hunting range was specified as the mean of its highest and lowest elevations. A list of aeries is included in the ‘Supplemental Material’ (online Table 1A). Confounders were removed from our calculations wherever possible. Because the height correlation of the general
Box
PCB Distribution in the Environment

Single PCB congeners accumulate to varying degrees in different environmental compartments (Kalberlah et al. 2002). In the atmosphere, the balance lies on the side of the more volatile, lower chlorinated congeners but gradually shifts toward the higher chlorinated, hydrophobic PCBs through deposition, sedimentation, uptake, and accumulation in biota (Table 2). The heptachlorobiphenyl congener 180 is an obvious exception to this rule, probably because of its molecular size inhibiting passage through biological membranes (Braun 2011).

Jonker and van der Heijden (2007), however, refer to the cut-off observed in the relation between BCF and hydrophobicity above $\lg K_{ow} \approx 6$ as not finally resolved. In peregrine falcon eggs, the sum of the congeners 138, 153, and 180 (PCB3) amounts to nearly 100% of PCB6 with negligible contribution of the lower chlorinated congeners 28, 52, and 101.

The degree of chlorination also determines whether PCBs are deposited in the deep sea or evaporated and degraded in the air (Wania and Daly 2002). Hydrophobic PCBs (represented by the ndl-PCB3 and the dl-congeners 156, 157, 167, 169, 189) are particle bound and transferred into the deep sea. Incidentally, this pathway cannot be considered as a sink, since these substances may still act as toxicants towards deep sea organisms. Lower chlorinated, more water soluble, and more volatile PCBs (represented by the ndl-congeners 28 and 52 and the dl-congeners 77 and 81) are deposited to a much lesser extent but rather degraded in the troposphere. The pentachloro-biphenyls occupy an intermediate position (represented by the ndl-PCB 101 and the dl-PCBs 105, 114, 118, 123, and 126). For these congeners, both pathways are about equally important. Peregrine falcon eggs are a compartment of extreme accumulating power as can be seen from the almost exclusive presence of the higher chlorinated PCB3 in Figure 3 and Table 2. The lack of accumulation in peregrine eggs of PCB-28, PCB-52, and PCB-101 resembles the situation in livestock. Therefore only the hydrophobic, non-volatile, and persistent PCB3 are regulated in fattening bulls (VDI 1995).

Table 2. Indicator—PCB profiles of different environmental compartments (Knetsch 2011).

| PCB con-gen | $\lg K_{ow}$ | Atm. Concentration | Atm. Deposition Total | Atm. Grass | Curly Spruce | Sediment | Brown Trout*** | Soil | Peregrine falcon |
|-------------|--------------|---------------------|-----------------------|------------|--------------|----------|---------------|------|-----------------|
| 28          | 5.5          | 22%                 | 10%                   | 20%        | 13%          | 8%       | 10%           | 5%   | 2%              | 2%   |
| 52          | 5.9          | 19%                 | 12%                   | 11%        | 11%          | 10%      | 10%           | 6%   | 6%              | 3%   |
| 101         | 6.3          | 18%                 | 19%                   | 12%        | 19%          | 19%      | 15%           | 14%  | 12%             | 11%  |
| 138         | 6.7          | 15%                 | 23%                   | 20%        | 17%          | 19%      | 18%           | 24%  | 29%             | 32%  |
| 153         | 6.9**        | 19%                 | 24%                   | 22%        | 28%          | 34%      | 37%           | 25%  | 28%             | 33%  |
| 180         | 7.1          | 7%                  | 5%                    | 13%        | 12%          | 11%      | 10%           | 13%  | 19%             | 15%  |

*Data from Erickson (2001); **from Fiedler et al. (1995); ***adapted from Braun (2011).
immission load was to be tested, aeries located in urban centers or influenced by a known source of contamination (primary emissions) were excluded from the correlation (online Table 1B). In case of two or more eggs from one clutch, the mean concentration of the clutch was used in order to avoid pseudo-replication.

3. Results and discussion

3.1. Organochlorine pesticides (OCPs)

Figure 2 shows the OCPs found in the eggs collected during 2009. The single most concentrated compound $p,p'-$DDE (mean concentration: 11,790 ng/g) constitutes over 97% of all OCP residues detected.

3.2. PCBs and PCDD/Fs

The dm concentrations of DDE and the indicator PCBs are shown in Figure 3. The six non-dioxin-like indicator PCBs (PCB$_6$) in peregrine falcon eggs include three major (138, 153, and 180 = PCB$_3$) and three minor congeners (28, 52, and 101). Their sum (7990 ng/g dm) exceeds the limit value of the German contaminant regulation by a factor of 13 (KmV 2010: limits PCB$_6$ to 100 ng/g dm for each of the six congeners). In the eggs of 2009, the sum of the higher chlorinated PCB$_3$ dominates the sum of PCB$_6$ with 99.8%. Although the lower chlorinated indicator PCBs (28, 52, and 101) constitute the majority in the atmosphere (Table 2, box), they are apparently not accumulated in peregrine falcon eggs. The lack of lower chlorinated PCBs in peregrine falcon eggs as well as in livestock (VDI 1995) is due to the general phenomenon of accumulation of persistent, hydrophobic, non-volatile compounds in cold and lipophilic compartments at the top of the food chain (Table 2, box).

![Figure 3. DDE (mean, minimum, and maximum of 10 eggs) and ndl-PCB$_6$ (mean, minimum, and maximum of 13 eggs).](image-url)
3.2.1. PCB concentration and potency

In 2009, the sum of the indicator and dioxin-like PCBs in peregrine falcon eggs amounted to $8930 \pm 6120$ ng/g ($\Sigma$ PCB$_6$ + dl-PCB, mean ± standard deviation), the dl-PCB contributing $10.6% \pm 1.5%$ (mean ± standard deviation) to this sum. Figure 4 displays the minor ndl- and the dl-PCBs ordered according to their concentration in the falcon eggs. The mean and range of 13 eggs collected in the year 2009 are shown. The dl-PCBs are dominated by congener 118, and the six most prevalent congeners (118, 156, 167, 105, 189, and 157) contribute more than 97%. The minor dl-congeners 126, 169, 77, and 81 are hardly visible in the diagram. However, the ranking according to toxic potency results in a very different outcome: The non-ortho-congener 126 alone contributes 46% of the TEQs and combined with the mono-ortho-PCBs 156, 118, and 157 over 90% of the dl-PCB-TEQ of 374 pg/g dm (human TEF; van den Berg et al. 1998; WHO 1998). The changed order of significance demonstrates the importance of measuring the biological activity of pollutants rather than their mass. The prevalence of dl-PCBs in peregrine falcon eggs corresponds to their occurrence in humans (Silverstone et al. 2012). The congener patterns are typical for the last link of the food chain after accumulation and metabolic degradation (Supplemental Material, Figures 1B and 2B). It is, therefore, difficult to draw any conclusions regarding specific sources.

Since the TEF$_{05}$ has been criticized as underestimating the true potency by 10%—25% (Bfr 2007; Rauchfuß 2007), the dioxin-like toxicity is expressed in TEQ$_{98}$, TEQ$_{05}$, and TEQ$_{birds}$ for comparison. Applying the new TEF$_{05}$ (EU 2011a) as opposed to TEF$_{98}$ reduces the resulting PCB toxicity alone to 60%, from 374 to 226 pg PCB-TEQ/g dm. Even this lower number amounts to 180 times the EU maximum level for hen eggs. Also, the use of TEF$_{birds}$ (WHO 1997) yields a mean PCB potency for birds of 264 pg PCB-TEQ/g dm (92—637 pg PCB-TEQ range), which approaches and in most cases exceeds the no-observed-effect level (NOEL; Figure 6) of 200 pg TEQ/g (Elliott et al. 2001). The single congener PCB-126 contributes only 0.2% mass (1.7 ng/g) to the sum dl-PCB but...
77% to the dl-PCB potency measured in TEQ05. This places a considerable weight on the sensitivity and accuracy of the analytical method\(^3\).

The contribution of PCB-126 to the total TEQ in peregrine falcon eggs ranges between 43% and 87% with an average of 67% (WHO 2006). The direct comparison of the PCB-126 TEQ with the (PCDD/F+PCB)-TEQ showed an excellent correlation with an \(R^2\) of 0.99 and a slope of 0.79 (Figure 5). The concentration of PCB-126 could, therefore, be used as a good indication for the total TEQ. The dominance of PCB-126 potency in spite of its negligible concentration precludes the use of the ndl-PCB\(_6\) as an indicator of dioxin-like potency, which might be deemed possible. But \(\sum\) PCB\(_6\) and total dioxin-like activity are correlated only moderately (correlation coefficient \(R^2 = 0.71\); “Supplemental Material,” Figure 3B).

### 3.2.2. Total dioxin-like potency

Despite the general decrease of the alarmingly high pollution levels of the eggs in the 1960s and 1970s (Figure 1), today’s monitoring results continue to give cause for concern. Peregrine falcon eggs still contain highly toxic PCBs and PCDD/Fs in hazardous amounts. Figure 6 displays the total dioxin-like activity for single eggs collected in 2009, again calculated as TEQ\(_{98}\), TEQ\(_{05}\), and TEQ\(_{\text{birds}}\). Expression in TEQ\(_{05}\) yields a toxic potency reduced to 62% on average, whereas TEQ\(_{\text{birds}}\) amount to 78% of the human TEQ\(_{98}\). But regardless of the assessment scheme employed, all eggs reach the NOEL (Elliott et al. 2001) and the most polluted ones significantly exceed this threshold.

The mean of the eggs of 2009 (409 pg PCDD/F+PCB-TEQ\(_{98}\)/g dm) exceeds the NOEL for dioxin-like compounds by a factor of 2, the European limit for foodstuffs (5 pg TEQ/g lipid; EU 2011a) by a factor of 327. Such an “action threshold” has also been determined for chicken eggs: Falcon eggs are contaminated with dioxin-like compounds to a degree at which breeding success with broody hens was found to drop dramatically (Malisch and Baum 2007; von der Trenck et al. 2006; von der Trenck, Schilling, and Schmidt 2007).

PCBs contribute an average of 91.5% to the total dioxin-like activity (TEQ\(_{98}\)) in peregrine falcon eggs (89% in TEQ\(_{05}\) and 82% in TEQ\(_{\text{birds}}\); Figure 6). This is much more than...
in the eggs of the chicken or the little owl with a contribution of about 70% PCBs to the total dioxin-TEQ. This ratio underscores the continuing environmental significance of the PCBs, although the use of this class of industrial chemicals was abandoned 30 years ago. Even after adding the PCDD/F-TEQ, the importance of PCB-126 remains overwhelming. This congener alone contributes 67% (see also Figure 5) of the total dioxin-like activity (PCDD/F + PCB TEQ98) in falcon eggs (52% in PCDD/F + PCB TEQbirds and 42% in PCDD/F + PCB TEQ98).

3.3. Bioanalytical methods for dioxin-, hormone-, and PPAR-like activity

Altogether, 59 peregrine eggs collected in BW between 2004 and 2011 were analyzed for PCDD/F and dl-PCB (TEQs) by quantitative chemical analysis (GC/HRMS) and for bioanalytical equivalents (BEQs) by the semi-quantitative cell and receptor-based reporter gene assay (DR CALUX®) using stable transfected H4IIE rat liver cells. Activities were expressed in pg TEQ or BEQ per g fat (Figure 7) rather than dm to compare with maximum levels regulated by the EU (2011a) in hen eggs: 5 pg PCDD/F + dl-PCB TEQ/g fat.
The linear correlation coefficient for 59 peregrine eggs was $R^2 = 0.86$, the slope of the correlation was 0.73, and the ratio of the mean value of the BEQs to TEQs was 0.75.

This ratio of 0.75 (rather than 1.0) can be explained by the fact that PCB-126 partially acts as AhR antagonist. TCDD and PCB-126 may bind to the same AhR but act at different sites and induce different conformations of the AhR complex which exhibit different activities as nuclear transcription factors (Li et al. 1999a, 1999b). As the dominating contributor (77%) of the dl-PCB TEQs (Figure 4), PCB-126 lowers the ratio of the REP (relative equivalent potency for the DR CALUX® method based on the EC50; cf. Supplemental Material, Figure 4B) and chemical TEF values to 0.75. Therefore, we would obtain more identical outcomes for the toxicity of the sum of dioxin-like compounds, by using the DR CALUX® bioassay REP value of 0.075 for PCB-126 (Behnisch, Hosoe, and Sakai 2001) instead of the WHO-TEF of 0.1 for calculating the PCDD/F + dl-PCB TEQs of the chemical GC/HRMS analysis. Alternatively, the lower activity could be explained by the AhR antagonistic action of ndl-PCBs (Brenerová et al. 2015).

By including two peregrine falcon eggs from the German North Sea coast [Jade Bight (BEQ = 4012; TEQ = 3752 pg/g fat) and Norderney (BEQ = 955; TEQ = 3183 pg/g fat), data not shown], the correlation coefficient dropped to $R^2 = 0.80$. Regional differences in the prey species and their contamination spectra may be causing the North Sea outliers.

Even though the relationship between BEQ and TEQ is dominated by the most contaminated eggs [Nellingen (BEQ = 4123; TEQ = 4997 pg/g fat) and Mannheim (BEQ = 2470; TEQ = 3800 pg/g fat), shown in Figure 7], by omitting these two we are still left with a clearly positive correlation and a linear correlation coefficient $R^2 = 0.58$ for the remaining 57 eggs.

The bioassay results also demonstrate that there is very little or no additional effect of dioxin/PCB-like compounds other than those analyzed by GC/HRMS, i.e. the 29 PCDD/F + dl-PCB congeners. PCB-126 appears to be the dominating dioxin-like compound (Figure 5; cf. Supplemental Material, Figures 1B and 2B), while other mixed halogenated dioxin/PCB-like compounds are of little relevance compared to this congener. Such high

Figure 7. Sum of PCDD/F and dl-PCB levels in peregrine falcon eggs from BW measured by the semi-quantitative (DR CALUX) and quantitative (GC/HRMS) methods. Scale: pg (PCDD/F + dl-PCB-TEQ) or (BEQ)/g fat. Samples from 2004 to 2011 ($n = 59$).
PCB-126 levels (compared to all other persistent dioxin-like compounds) are frequently measured in top predators of wildlife (Behnisch et al. 1997) and to a lesser extent in eggs from commercial production, apparent from many cases of PCB contamination in hen eggs all over Europe since the Commission Regulation for dl-PCBs in 2006 (EU 2011a). Similarly, Levy et al. (2011) concluded from measurements in alpine soil that there was no significant contribution of unknown AhR inducers because of equal bioassay derived and analytically determined PCDD/F + dl-PCB TEQs.

Similar bioassays were performed with estrogen and androgen receptor responsive cells (ER CALUX® and AR CALUX®). The results are reported as 17β-estradiol (EEQ) or flutamide equivalents (FEQ) in the egg samples (von der Trenck et al. 2008a). Between 12 and 766 pg EEQ/g dm (mean: 159 pg EEQ/g, chicken egg: 99 pg EEQ/g) and between 8 and 491 μg FEQ/g dm (mean: 103 μg/g, chicken egg: 3 μg/g) were found (cf. Supplemental Material, Figure 5B: typical distribution of dioxin-, estrogen-, and anti-androgen-like activities for 18 different locations in BW measured by DR-, ER-, and anti-AR CALUX).

The results show that the CALUX® panel can successfully be applied to complex samples such as bird egg extracts. This correlation of bioassay results with chemical analyses served to establish the cell tests for screening bird eggs for POPs. No glucocorticoid (GR-CALUX) or thyroid (TR-CALUX) hormone-like activity was detected in three selected samples or in a chicken egg serving as reference. Likewise, no activity of obesogenic compounds tested by PPARγ-CALUX was detected in a peregrine falcon egg from the North Sea coast or in a chicken egg. TBT and PFC, like PFOA and PFOS, are known active compounds in the PPARγ-CALUX assay (Behnisch et al. 2012).

Further work correlating bioassay results with chemical analyses is planned with the aim of evaluating the applicability of the cell-based receptor methods as a viable screening method for POPs and endocrine disrupting chemicals. It is also necessary to develop reliable assessment criteria for human health and ecological risk caused by environmental OHC such as PCBs, DDE, and PBDE, and their metabolites. Despite the decline in OHC concentrations in the past decades, the peregrine population of BW is still at risk from OHC and mercury (cf. Figure 9) in their environment. Therefore, egg monitoring should continue, and measures to reduce contamination levels should be considered where appropriate.

3.4. Higher pollution levels in conurbations and at higher altitudes

DDE correlated positively and PCB3 negatively with elevation above sea level (Figure 8). Both correlations were statistically significant. A positive correlation with elevation (DDE) indicates long-range atmospheric transport and condensation at higher altitudes (general load, Figure 8; von der Trenck et al. 2006; Wegner and Fürst 2008). Table 3 (box) illustrates the migratory path of ∑ DDT from (sub)tropical application areas to arctic sinks. In contrast, a negative correlation (PCB3) points toward nearby pollution sources at lower altitudes (primary emissions; Weber et al. 2014). Similar negative correlations were found for the single PCB congeners (153: \( \rho = -0.19, p = 0.028 \); 180: \( \rho = -0.19, p = 0.031 \); 138: \( \rho = -0.16, p = 0.063 \); each \( n = 131 \)).

Previous results already indicated that peregrine falcon eggs from the northwest region and the uplands of BW contained higher levels of DDE, in the former case owing to the high population density and in the latter owing to the higher altitudes (von der Trenck...
et al. 2005, 2006; von der Trenck 2012). A similar observation was made by Wiemeyer, Bunck, and Krynitsky (1988), who report the highest DDE concentrations in osprey eggs from Montana (500 m elevation and higher) and the lowest from Virginia (Atlantic coast). The highest PCB concentrations, however, were found in the Virginian osprey

**Box**

**Atmospheric DDT concentrations**

$p,p'$-DDE is a potent androgen receptor antagonist (Ritter et al. 2011). Through the Stockholm convention its use was restricted to vector control. Diminished agricultural use, however, still continues in India, North Korea, and some Central Asian countries. In tropical countries, DDT is still in use to combat malaria. In 2006, the WHO revoked its policy against DDT and recommended its use as an indoor pesticide. Indoor spraying for malaria control leads to concentrations of the order of 1–10 μg DDT/m$^3$, which is roughly 1000 times higher than typical concentrations measured in tropical continental air (5 ng/m$^3$) and about 1 million times higher than typical concentrations measured in outdoor air in the north (5 pg/m$^3$ according to Ritter et al. 2011 (Table 3).

| Compartment                        | Σ DDT concentration (ng/m$^3$) |
|------------------------------------|-------------------------------|
| Tropical indoor air after spraying | 1000–10,000                   |
| Tropical continental air          | 5                             |
| Northern atmosphere                | 0.005                         |

The order of magnitude of atmospheric DDT concentrations is confirmed by Qiu et al. (2004) who report 8.7 pg/m$^3$ at Green Bay, Lake Michigan, and 8.3 pg/m$^3$ at Lake Baikal, Russia.
eggs. The mercury concentrations found in this study were similar to ours (300—400 μg/g dm, recalculated), in six counties. Only two counties in New Jersey (high population density) exceeded this range.

3.5. Minor contaminants

Some contaminants added to the program starting in 2003 are depicted in Figure 9.

3.5.1. Mercury

There seems to be no change in the average mercury (Hg) concentration from 2003 to 2011. The yearly means fluctuate around 500 ng/g with a standard deviation of about 250 ng/g. Similar Hg concentrations were found in osprey eggs. An osprey egg collected in 2011 in Eastern Bavaria contained 480 ng Hg/g dm (this study); the geometric mean of 40 osprey eggs collected in 2004 along the Columbia River (Canada, USA) amounted to 450 ng Hg/g dm (Henny, Grove, and Kaiser 2008). A new NOAEL of 120 ng/g for decreased egg productivity and manifestation of male homosexual nesting and courtship

Figure 9. Mercury, total PBDE, HBCDD, and TBBA in peregrine falcon eggs from BW (mean and standard deviation). Scale: μg/g dm. Samples from 2003 to 2011, in parentheses: number of samples per year. NOAEL\textsubscript{MeHg} = 0.12 μg/g.
behavior in ibises can be derived from controlled chronic MeHg feeding studies (Frederick and Jayasena 2011; cf. Section 3.7). This NOAEL is not far from the German residue limit value of 50 ng/g on a dm basis (RHmV 2008: 10 ng/g ww = maximum Hg level in eggs), which regulates mercury in foodstuffs. Throughout the monitoring period in BW (2003—2011), the mean mercury concentrations in the falcon eggs considerably exceeded the German limit value 8-fold to 15-fold. But the mercury concentration in falcon eggs also exceeds (threefold to sixfold) the newly established threshold with relevance for population decline (NOAEL = 120 ng/g), indicating that peregrine falcons are still under acute threat.

### 3.5.2. Polybrominated diphenyl ethers

Although PBDEs are currently only in limited use as flame retardants (banned by the Stockholm Convention; SCS 2014), significant inputs into the environment come from flame-proofed appliances still in use or from waste disposal sites. The LUBW monitoring program for PBDE in the peregrine falcon has been in place since 2003 and reliable measurements on peregrine eggs were obtained from 2004 onwards. The results are shown in Figure 9. Since 2005, the values seem to have been leveling off from about 3000 ng/g to the present value of about 400 ng/g dm (1600 ng/g lipid). Leung et al. (2010) report more than 10 times lower values of around 120 ng/g lipid in the milk of women living in the neighborhood of an electronic waste recycling site. Even these lower human milk levels correspond to an estimated intake of 500—2200 ng/(kg·d) for a breast-fed baby of age six months and partially exceed the toxicity threshold (RfD) for penta-BDE of 2000 ng/(kg·d).

### 3.5.3. Other flame retardants

Another flame retardant, HBCDD, was analyzed during the period from 2006 to 2011 and showed no temporal trend but a very heterogeneous distribution ranging from <3 ng/g dm (LOQ) to almost 1000 ng/g dm. The HBCDD statewide mean and standard deviation during six years are shown in Figure 9. HBCDD is mainly used in insulating boards in buildings. It has been classified by the EU as substance of very high concern because of its persistent, bioaccumulative, and toxic properties. The sixth meeting of the Conference of the Parties of the Stockholm Convention held in Geneva during the period between 28 April and 10 May 2013 decided to list HBCDD in Annex A (worldwide ban) with specific exemptions for production and use in expanded polystyrene and extruded polystyrene in buildings.

TBBA generally occurred in much lower concentrations of a few ng/g dm (Figure 9). In 2009 and 2010, this compound was not detected in any egg. In 2011, one positive sample (2.0 ng/g) was found in eight eggs, whereas no residue above 1.0 ng/g (LOD) could be detected in the other seven eggs. The fact that TBBA covalently binds to its matrix may explain its lower concentrations in the environment.

### 3.5.4. Perfluoro compounds (PFC)

All four perfluorosulfonates analyzed (C₄, C₆, C₈, and C₁₀) were detected, PFOS contributing by far the greatest share (Figure 10). Concentrations of PFOS in the eggs reach the effect threshold in toxicity studies (NOAEL = 500 ng/g dm; Molina et al. 2006; O’Brien
et al. 2009). This result is corroborated by the studies of Borg and Håkansson (2012) and Borg et al. (2013), who assessed the risk of a number of perfluoroalkylated and polyfluoroalkylated substances to the human population as well as to seals, otters, and peregrine falcons in Sweden. In our study, the perfluorocarboxylic acids increased with the increasing chain length from C8 to C12 (Neugebauer, Dreyer, and von der Trenck 2012). Neither the perfluorohexanoic and perfluoroheptanoic acids nor the perfluorooctane sulfonamide (PFOSA) were found (LOQ D 0.5 ng/g dm). The low levels of PFDS may indicate a lesser contamination of the environment with this sulfonate rather than a lower accumulation potential. Perfluorocarboxylic acids with carbon chains of more than eight atoms are also accumulated (Staude and Vierke 2013) in the livers of bream (Abramis brama), grey seal (Halichoerus grypus), and polar bear (Ursus maritimus). In peregrine falcon eggs, none of the compounds displayed a clear temporal trend during the observation period (2008–2011). This finding is contrary to what one would expect from studies of human dietary PFC intake, which has been shown to decrease from 1999 on (Perfood Study, Vester gren et al. 2012). Two eggs from the German North Sea coast were clearly more contaminated with five of the PFC (PFOS, PFHxS, PFOA, PFNA, PFDA). Unexpectedly, an osprey egg from Bavaria did not show the same high level of contamination with the sulfonates and PFOA, PFNA, and PFDoA.

**Figure 10.** Mean and range of 11 PFC (ng/g dm) in peregrine falcon (PF) eggs collected from 2008 to 2011 in BW (number in parentheses): perfluorobutanesulfonate (PFBS, C4), perfluorohexanesulfonate (PFHxS, C6), perfluorooctanesulfonate (PFOS, C8), perfluorodecanesulfonate (PFDS, C10), perfluorooctanesulfonamide (PFOSA, C8), perfluorohexanoate (PFHxA, C7), perfluorohexanoate (PFHpA, C8), perfluorooctanoate (PFOA, C8), perfluorononanoate (PFNA, C10), perfluorodecanoate (PFDA, C11), and perfluorododecanoate (PFDoA, C13). Two peregrine falcon eggs from the German North Sea coast and one osprey egg from Bavaria are shown for comparison. LOQ/2 = 0.25 ng/g; NOAELPFOS = 500 ng/g.
3.6. Biometric measurements

SIs could be calculated for 215 samples. Their values were normally distributed with an arithmetic mean of 1.775 and a standard deviation of 0.157 mg/mm². ST was measured reliably for 219 samples, the obtained values showed a normal distribution with an arithmetic mean of 0.327 and a standard deviation of 0.025 mm (Schwarz 2010). Statistical analysis did not reveal any significant changes of SI and ST during the investigated time period (SI: Kruskall–Wallis test, $\chi^2 = 13.961, df = 9, P = 0.124$; ST: Kruskall–Wallis test, $\chi^2 = 8.034, df = 9, P = 0.531$). Results are shown in Figure 11.

Neither SI nor ST was significantly correlated with the concentration of $p,p'$-DDE in the samples (SI: Spearman, $n = 114, \rho = -0.174, P = 0.064$; ST: Spearman, $n = 107, \rho = -0.107, P = 0.274$). Significant negative correlations were found between both SI and ST, and the concentration of mercury (SI: Spearman, $n = 81, \rho = -0.253, P = 0.024$; ST: Spearman, $n = 73, \rho = -0.397, P = 0.0003$). The results are shown in Figure 12. The closest correlation was found between the mass of the eggshells, and above all their thickness, and the Hg content of the eggs (Schwarz 2010). This result was unexpected because the average Hg content corresponded to just 20% of the action threshold known up to 2010 (2.5 ng/g; Wegner et al. 2005; Henny, Grove, and Kaiser 2008), but plausible because Hg is known to lower the calcium content of the blood. The correlation with DDE is somewhat less clear, although DDT and DDE have long been known to reduce the mass, thickness, and calcium content of the eggshell. This is due to inhibition of calcium transport in the shell gland or uterus, where the shell composed largely of calcium carbonate is formed in female birds. Shells that are too thin can be crushed during brooding.

No correlation was observed between the SI and PCB₃ (from 2000 to 2009; Schwarz 2010) nor the sum of the PBDE congeners 49, 99, 100, 153, 154, 183, and 209 (from 2003 to 2009; Schwarz 2010; data not shown). The lack of association between PCB
concentrations and eggshell thinning was also reported in a review on the osprey as a sentinel species for monitoring environmental contamination (Grove, Henny, and Kaiser 2009).

The mean eggshell index (1.775 mg/mm²) observed in this study accords with the value of 1.788 mg/mm² recorded for the time period between 1990 and 2000 in BW (Schilling and Wegner 2001). According to these data, no changes in SI and ST worthy of note have occurred during the last decade. Nevertheless, the recorded values for index (1.775 mg/mm²) and thickness (0.327 mm) are still slightly lower than the reference values of 1.826 mg/mm² and 0.341 mm, respectively, reported for the time period between 1850 and 1936, prior to the use of DDT (Wegner et al. 2005). These slight differences could be due to the persistent presence of residues of DDE and other harmful chemicals such as Hg in birds’ tissues and eggs.

Both the SI and ST decreased with the increasing DDE concentration. While this correlation was only vague in the case of the ST, it almost reached significance in the case of the SI.

Figure 12. SI and ST vs. concentrations of \( p,p' \)-DDE and mercury. The 95%-density ellipse is marked with a dashed line, the 50%-density ellipse with a continuous line. There was no significant correlation between the eggshell parameters and the concentration of \( p,p' \)-DDE. The concentration of Hg showed a significant correlation with the SI and a strong correlation with the ST.
SI ($P = 0.064$). The apparent lack of a significant relationship between the concentration of DDE and the examined shell parameters may be due to the lower level of DDE contamination in the last two decades. Earlier studies covering a wider range of DDE concentrations revealed a clear decrease of eggshell index and thickness with increasing DDE concentration in many different bird species (Cade, Lincer, and White 1971; Lundholm 1997; Wegner et al. 2005; von der Trenck, Schilling, and Schmidt 2007; Kellner and Lage 2009; see also Figure 1).

### 3.7. Mercury effect threshold

Studies on the influence of mercury on the eggshell thickness in birds are rare and equivocal. Ingestion of high MeHg doses has been shown to decrease the eggshell index in domestic fowl (Lundholm 1995), but environmentally relevant concentrations of mercury have not yet been proven to decrease the reproductive output by eggshell thinning (Cooke 1973; Peakall and Lincer 1972). Henny, Grove, and Kaiser (2008) report a lowest adverse effect level (LOAEL) of 2.5 μg/g egg for adverse reproductive effects of Hg. Wegner et al. (2005) calculate LOAELs of 2.5–15.5 μg/g in eggs from Japanese quail and pheasant from the relevant literature and consider falcon egg concentrations between 0.09 and 1.25 μg/g as innocuous. This notion must now be revised, because a recent study reports MeHg effects on pairing and courtship behavior as the cause of decreased reproductive success in ibises (Frederick and Jayasena 2011). These authors established 0.05 μg MeHg/g ww (0.14 μg/g dm) in the diet of ibises (Eudocimus albus) as consistent LOAEL for homosexual nesting and courtship behavior and decreased egg productivity.

Albers et al. (2007) explored the relationship between MeHg in the diet and in the eggs of American kestrels (Falco sparverius), which can serve as a model for the peregrine falcon. According to these authors’ data, 0.14 μg/g dm in the diet corresponds to 1.2 μg/g dm in the egg. This mercury concentration in the egg (as MeHg) is the LOAEL established so far. Extrapolating by a factor of 10, an NOAEL can be assumed at 0.12 μg/g dm in the egg. There is not much tolerance between this NOAEL and the German residue limit value of 0.01 μg/g ww (RHmV 2008), which regulates mercury in foodstuffs and corresponds to 0.05 μg/g dm in falcon eggs.

Since the onset of our measurements in 2003, the mean mercury burden of peregrine falcon eggs from BW ranging from 0.38 to 0.80 μg/g (Figure 9) has regularly exceeded threefold to sevenfold the NOAEL. The significant correlation of this mercury burden with the eggshell index and thickness (Figure 12) confirms the validity of the effect threshold derived from ibis and kestrel data for the peregrine falcon.

More recently, a low mercury effect threshold for reduced reproductive success was confirmed in other bird species such as the Carolina wren (Thryothorus ludovicianus), a territorial passerine bird (LOAEL = EC25 = 0.25 μg/g egg ww = 1.2 μg/g egg dm; Jackson et al. 2011) and the common loon (LOAEL = 1.0 μg/g egg ww = 5.0 μg/g egg dm; Evers et al. 2014).

#### 3.7.1. Ex-post MeHg determination

Although not initially established, the presence of mercury, mainly as MeHg, in the eggs of peregrine falcons nevertheless seemed likely because mercury is known to be methylated by microbes in the environment and subsequently accumulated in methylated form
in the food chain (Wolfe, Schwarzbach, and Sulaiman 1998; Marquardt and Schäfer 2004; Hohenblum et al. 2011). This sequence describes a well-known entry port of mercury into biota that was first established in the aquatic environment (see e.g. Buhler, Claeys, and Shanks 1973; Buhler, Claeys, and Mate 1975; Kösters, Rüdel, and Schröter-Kermani 2009). Wolfe, Schwarzbach, and Sulaiman (1998) state that “ingested Hg [ ] is usually in the form of MeHg in higher trophic level feeders.” This observation was confirmed for the peregrine falcon in particular by reanalysis of the eggs of 2009 and 2010. Eighteen eggs were examined (five of these in duplicate); on average, 82.5% of the mercury was found to be present as MeHg (standard deviation: 7.5%). Incidentally, the difference in intrinsic toxicity between elemental Hg and MeHg is debatable and observations of lower effective doses of the latter might only be due to its lipophilic nature which enables it to cross biological membranes such as are found in the placenta and the blood–brain barrier (Schweisngberg 2011).

One explanation for the recent discovery of the relationship between Hg accumulation and eggshell thinning could be that the effect of Hg was masked in earlier studies by the stronger effect of some 10-fold higher DDE concentrations as well as the action of other OC compounds. The effect of Hg may have just come to light with decreasing concentrations of these other chemicals. Further research in this area is, therefore, recommended. It is planned to investigate the influence of long-range atmospheric transport and cold condensation on Hg in peregrine falcon eggs as well.

3.8. Priority of contaminants

3.8.1. Raptor priority

The good negative correlation found with Hg and SI and especially ST was unexpected (Figure 12). There was a somewhat less clear correlation with the DDT degradation product, DDE. DDT and its metabolites were identified as being responsible for the almost complete extinction of the raptor population, especially the peregrine falcon. DDT and DDE inhibit the calcium transport in egg formation (cf. Section 3.6). Our results show that Hg has a similar effect (Figure 12). This means that the present level of contamination with anthropogenic pollutants, although significantly lower than in the past, can have serious consequences of egg fragility and population decline for free-living raptors even though the population of peregrine falcons has meanwhile recovered.

A significant shell-thickness-reducing effect of the two principal pollutants PCB and DDE could no longer be determined in eggs collected between 2001 and 2009. However, such an effect was found for MeHg, which consequently now heads the negative list of environmental pollutants in spite of its relatively low concentration of around 0.4 μg/g (Figures 9 and 12; Schwarz 2010). In addition to possessing neurotoxic and embryotoxic activities, MeHg interferes with hormonal regulation, and in controlled feeding experiments led to homosexual mating behavior and reduced breeding success among ibises at an action threshold of 0.12 μg/g in eggs (cf. Section 3.7). This concentration was exceeded in all peregrine falcon eggs examined, in some cases up to 25-fold.

An interim assessment of the Stockholm POP Convention was undertaken at a workshop held at Brno, Slovakia. Attention was also directed to the heavy metal Hg, for which the United Nations have been planning a dedicated convention, because it undergoes biogenic methylation in the environment and as MeHg shows a global distribution.
resembling that of the persistent lipophilic POPs (AMAP/UNEP 2008; Lammel 2011).
There are several reasons why Hg has been mentioned in a similar context as POPs (K. Magulova, Programme Officer, Stockholm Convention Secretariat, UNEP, 11/13 Chemin des Anémones, CH-1219 Châtelaine, personal communication, 10 October 2011) and our finding them both in falcon eggs supports this notion:

- Hg behaves similarly in the environment: it is persistent, toxic, bioaccumulates, and undergoes long-range transport, accumulating in the cold polar regions.
- Global action is needed to tackle the problem.
- The model of the Stockholm Convention was used for the Hg negotiations. Although it was originally intended to add Hg to the Stockholm Convention under a special protocol, a separate convention was ultimately concluded (UNEP 2013).
- Both conventions were initiated under UNEP Chemicals.

### 3.8.2. Human priority

Human uptake of PCBs, PCDD/Fs, and Hg via food in Germany has been assessed by the German Federal Institute for Risk Assessment (BfR 2010). This survey showed that in humans PCBs (including PCDD/Fs) ranked higher than MeHg and fully accounted for or exceeded (90%–121%) the tolerable weekly intake (TWI) of 14 pg TEQ/(kg-week), whereas MeHg accounted for only 11% of the provisional TWI of 1.6 μg/(kg-week).

For dioxins and dl-PCBs, this theoretical estimation was confirmed in practice by Neu-gebauer et al. (2014) who found a disturbed attentiveness in children aged 8.5 years correlating with increasing maternal blood levels of dioxin TEQ_{05} during pregnancy (geometric mean: 19.9 pg TEQ_{05}/g lipid, 31.8% dl-PCB).

Our results in conjunction with the new, lower mercury effect threshold as an endocrine disruptor found in the ibis study (Frederick and Jayasena 2011; Albers et al. 2007) raise the question of a possible as yet undiscovered hormonal action of low MeHg doses in humans and other mammalian species analogous to that discovered in birds.

### 3.9. Other substances

The search for pollutants in the eggs collected in 2009–2011 was extended to include 177 individual substances belonging to 15 classes and five bioassays (von der Trenck et al. 2008b, 2010). The selection of substances was based partly on the fundamental considerations of the European legislation (REACH) regarding the properties, production quantities, and uses of so-called existing substances, i.e. substances listed in the European Inventory of Existing Commercial Chemical Substances. The set goal is to increase our knowledge about their distribution and their toxic action in organisms as well as the recognition of critical pollutants and trends. Although a number of prime suspects could be ruled out, representatives of all Stockholm Convention substance classes were also found in peregrine falcon eggs, viz.

- OCPs.
- PCBs and PCDD/Fs.
- Polybrominated flame retardants such as PBDEs and TBBA, as well as HBCDD.
- The only new group to appear in 2009 was that of the PFC with PFOS as the principal representative. It was included in the POP ban in the same year (SCS 2014).
• Hg (of which an average of 82.5% occurs as MeHg; von der Trenck 2012). Hg is not subject to the restrictions of the Stockholm Convention and a separate convention has, therefore, been adopted (Minamata Convention; UNEP 2013; BMU 2013, 2014; EU 2011b; Lammel 2011). Hg compounds and a number of POPs are counted among the approximately 200 well-studied neurotoxins and endocrine disruptors.

The following substances were not found:
• short-chain chloroparaffins (<3–<20 ng/g),
• organotin compounds (<2–<12 ng/g),
• phthalic esters (<1 μg/g), except for traces of DEHP,
• musk ketone and musk xylene (<0.1 ng/g) and bisphenol A (<5 ng/g), and
• non-chlorinated pesticides still in use (<2–<10 ng/g) such as herbicides (acronifen, pendimethalin), fungicides (lufenuron, quinoxyfen), insecticides (bifenthrin, esfenvalerate, lufenuron, metaflumizone), and rodenticides (brodifacoum, difenacoum).

4. Conclusions

As a result of these studies, mercury, mostly in the form of MeHg, has gained unexpected significance. Whereas in former years dl-PCBs and DDE were the pollutants of greatest concern and mercury was regarded as an also-ran (von der Trenck, Schilling, and Schmidt 2007; von der Trenck et al. 2008b), this metal is now emerging as a substance sharing the top rank with dl-PCBs and therefore deserves much more attention (von der Trenck 2012). In fact, nowadays MeHg and dl-PCB are the two substances that significantly exceed toxicological thresholds in peregrine falcon eggs.

Significant differences in estrogenic and anti-androgenic activity in different locations in BW have been observed by ER and anti-AR CALUX®. The semi-quantitative bioanalytical method DR CALUX® for screening of dioxin-like activities yielded results similar to the quantitative chemical GC/HRMS analysis and can therefore be used as a cost-efficient and easier method to detect not only the 29 regulated PCDD/PCDF/dl-PCB congeners, but also many other POPs with dioxin-like potencies such as mixed halogenated dioxins/biphenyls. In peregrine falcon eggs, however, an average of 66% of the total dioxin-like activity (PCDD/F+PCB TEQ05) is contributed by PCB-126 alone.

The German Council of Experts for Environmental Problems criticized the inadequate knowledge about chemical pollution of terrestrial ecosystems, especially by pesticides and industrial chemicals (SRU 2012). As the only terrestrial POPs biomonitoring program carried out in the State of Baden-Württemberg, the bird eggs monitoring should, therefore, be continued and extended over a greater set of potential chemical pollutants.

Substances to be added to the monitoring program in the future include
• brominated flame retardants [decabromodiphenyl ethane (Ricklund, Kierkegaard, and McLachlan 2010; Mo et al. 2012; Wei et al. 2012), 1,2-bis(2,4,6-tribromophenoxy) ethane (Mo et al. 2012; Wei et al. 2012), 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (de Wit, Herzke, and Vorkamp 2010; Marteinson et al. 2012), bis-(2-ethylhexyl)-tetrabromophthalate (Springer et al. 2012)];
• biocides [alachlor, chlorfenapyr, chlorothalonil, chlorothal-methyl, cybutryn (Irgarol), cyfluthrin, dacthal, difethialon, dimethyltetrachlorophthalate, epoxiconazole, fenpropimorph, flufenoxuron, flocoumafen, hexaflumuron, methyltriclosan, phenothrin, spinosad, terbuthylazine, triclosan (Fraunhofer IME 2012; Grove, Henny, and
Kaiser 2009; Lemarchand, Rosoux, and Berny 2011; as recommended by Dr Stefanie Jäger, Umweltbundesamt IV 1.2 - Biozide, Dessau, Germany, 2012);

- polycyclic aromatic hydrocarbons (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]-anthracene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene), and
- heavy metals [cadmium and lead, although the transferability of Pb into eggs is supposed to be limited (Grove, Henny, and Kaiser 2009)].

5. Outlook

Peregrine falcon eggs are good bioindicators for monitoring POPs as required by the Stockholm Convention.

The results obtained underscore the need to continue monitoring the current pollution situation although the peregrine falcon is no longer threatened in its existence by POPs, as its population has recovered significantly since 1965.

Correlation of differences in egg pollution levels and the respective breeding success of the parent animals should allow determination of causative actions and ultimately lead to the elimination of local polluters.

Research into environmental impact should be intensified in order to gain more certainty in the assessment and prioritization of individual pollutants. Reporter gene assays in stable cell culture systems can help to save costs and also to detect the interaction of unexpected substances with their biological targets. However, the results have to be confirmed by instrumental chemical analysis (effect-guided analysis) since alleviating measures can only be applied to individual chemically defined substances.

Avoidance of environmental pollution by persistent organic substances must be vigorously pursued. To quote Scheringer (2012): "Risk assessment for chemicals is a structural problem and there are few incentives to search for truly different solutions. Therefore, the guiding principles of sustainable chemistry should be incorporated into the development of new chemical products considerably stronger than up to now."

Notes

1. Quotation marks, because WHO has readmitted DDT for indoor use to fight the Anopheles vector in areas where Malaria is endemic (→ box "Atmospheric DDT concentrations").
2. Rauchfuß, K. 2007: “Reevaluierung der Toxizitätäquivalenzfaktoren für Dioxine und dioxinähnliche Substanzen: WHO 2005 — Auswirkungen auf den Immissionsschutz.” Expertenmeinung aus dem Landesamt für Natur, Umwelt, und Verbraucherschutz Nordrhein-Westfalen (LANUV NRW), Essen. Advisory opinion from the State Agency for Environment, Nature, and Consumer Protection of North Rhine Westphalia, Essen: LANUV NRW, unpublished.
3. PCBs 126, 169, 77, and 81 are readily quantifiable with concentrations at least 60-fold above their LOQ (Table 1). The issue of how to treat values below the LOQ (upper bound vs. lower bound; Malisch, Kotz, and Wahl 2012) is therefore not relevant in this context.
4. PFOS proved embryotoxic and caused inflammation of the portal vein, bile duct hyperplasia, and cellular necrosis in the liver of domestic chicken embryos and also increased the expression of genes regulated via the PPARα, and playing a role in obesity. The PFOS concentrations in glaucous gull (Larus hyperboreus) eggs from a colony of the Norwegian Arctic were similar to the liver levels of adult gulls from the same region (Molina et al. 2006; O’Brien et al. 2009).
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No potential conflict of interest was reported by the authors.

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