ORGANOHALOGEN COMPOUNDS IN THE EGGS OF PEREGRINE FALCONS AND OTHER WILD BIRD SPECIES IN BADEN-WÜRTTEMBERG – PRESENT STATE AND TIME TREND

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Introduction

Organohalogen compounds (OHC) and endocrine disrupting chemicals are known to seriously pollute the environment and to cause considerable damage to living organisms including humans. These lipophilic and persistent pollutants accumulate in birds' eggs and can thus be detected in the environment. The United Nations Environmental Programme[1] has established birds' eggs as suitable bioindicators to monitor persistent pollutants (POPs) in the terrestrial ecosystem. Therefore, the concentrations of some OHC have been measured in eggs of Peregrine falcons, owls, jackdaws and tits from the German state of Baden-Württemberg.

Materials and Methods

Sample Collection and Chemical Analysis

In the process of caring for breeding wild falcon pairs, private ornithologists of the 'Working Group for the Protection of Peregrine Falcons' (AGW) collected failed eggs for chemical and biological analysis and thus established this species as bio-indicator. The analytical work was contracted by the LUBW (formerly LfU) from 1999 onwards. Extraction of the egg samples and determination of the analytes were described earlier[2, 3]. Analysis of the polybrominated diphenyl ethers (PBDE) and OC compounds with the exception of PCDD/F was performed by Berghof, Tübingen. Tetrabromobisphenol A (TBBA) and hexabromocyclododecane (HBCD) were added to the program in 2006 and analysed by Ökometric, Bayreuth. PCDD/F-determination of selected falcon eggs was carried out by the Chemical and Veterinary Investigating Office (CVUA), Freiburg.

CALUX® Reporter Gene Assays for Dioxins (DR) and Hormones (ER, AR, GR, and TR)

Approximately one gram of bird egg was extracted by means of shake solvent extraction (hexane:diethylether, 97:3 for the DR CALUX® and methyltertiarybutylether for the hormone responsive CALUX®). The cleaned extracts were dissolved in 25 µL DMSO. CALUX® reporter gene assays were performed as described elsewhere[4]. In short, cells were seeded into 96-well plates in medium supplemented with hormone-stripped serum. The next day, the medium was replaced with medium containing the egg extracts (0.1 % DMSO). After 24 hours exposure, the medium was removed and the cells were lysed. The substrate luciferin was added to the wells to quantify the amount of luciferase produced by the cells by measuring the amount of light using a luminometer. For reference equivalents calculation, a dose-response curve of the reference compound was included in the analysis. The reference compounds used for the different bioassays were: 2,3,7,8-TCDD (DR), 17β-estradiol (ERα), dihydrotestosterone and flutamide (anti-AR), dexamethasone (GR), and triiodothyronine (TRβ).

Results and Discussion

OHC Accumulation

Earlier studies[2] have shown that OHC are accumulated in the terrestrial avian food chain in the order: Peregrine falcon > eagle owl > barn owl > little owl ≈ jackdaw > great tit ≈ blue tit > coal tit (Fig. 1). This demonstrates the Peregrine falcon's exposed position in the food web and its outstanding suitability as a bioindicator.

DDE Temporal Trend

As a result of bans of their use, the concentrations of DDT/DDE, heptachloroepoxide (HCEP), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH) and polychlorinated biphenyls (PCB) have decreased considerably from their levels in the 1970s. The DDE-decline (Fig. 2) was accompanied by an increase in eggshell thickness to normal values. DDT/DDE are known to disrupt the calcium metabolism causing the

thinning of eggshells and thereby a diminished breeding success. Thus, the banning of DDT (in 1972 in Western Germany) in conjunction with unremitting efforts of private ornithologists (Working Group for the Protection of Peregrine Falcons, AGW[2, 3]) throughout the past four decades were instrumental for the survival of the Peregrine falcon as a species in Southwestern Germany.

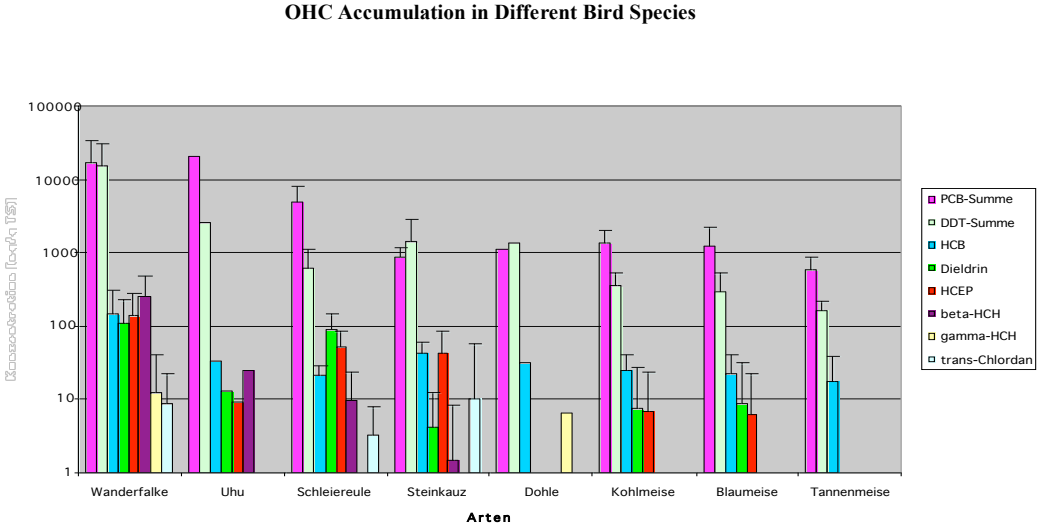

Figure 1: The organochlorine pollution [ng/g dry weight] of eggs (2001) decreases in the order: Peregrine falcon > eagle owl > barn owl > little owl ≈ jackdaw > great tit ≈ blue tit > coal tit.

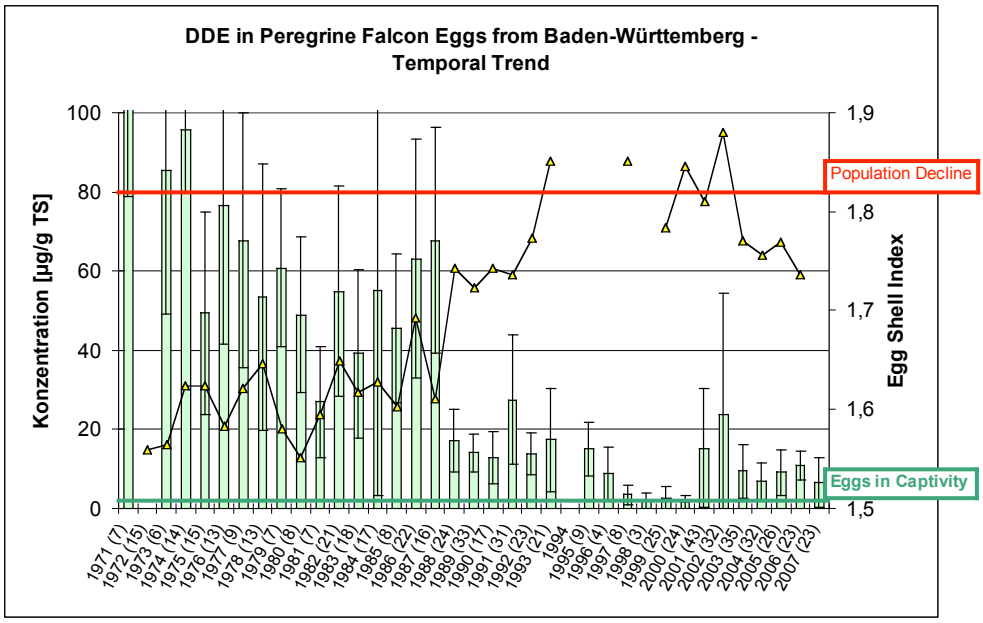

Figure 2: Time trend (parentheses: number of eggs analysed per year) of DDE [µg/g dry matter with standard deviation], left scale, in eggs of Peregrine falcons from Baden-Württemberg. The egg shell index (Δ right scale) is related to shell thickness. DDE concentrations of eggs laid in captivity in 2007 (1.9 µg/g dm) and DDE threshold of population decline (80 µg/g dm) are indicated.

OHC Found in Peregrine Falcon Eggs in 2007

Of 88 analytes searched for, 51 could be detected in last year's eggs (Fig. 3). PCBs and total DDT, which consists of the DDT-metabolite DDE to >99%, were found as the two main pollutants in the Peregrine eggs.

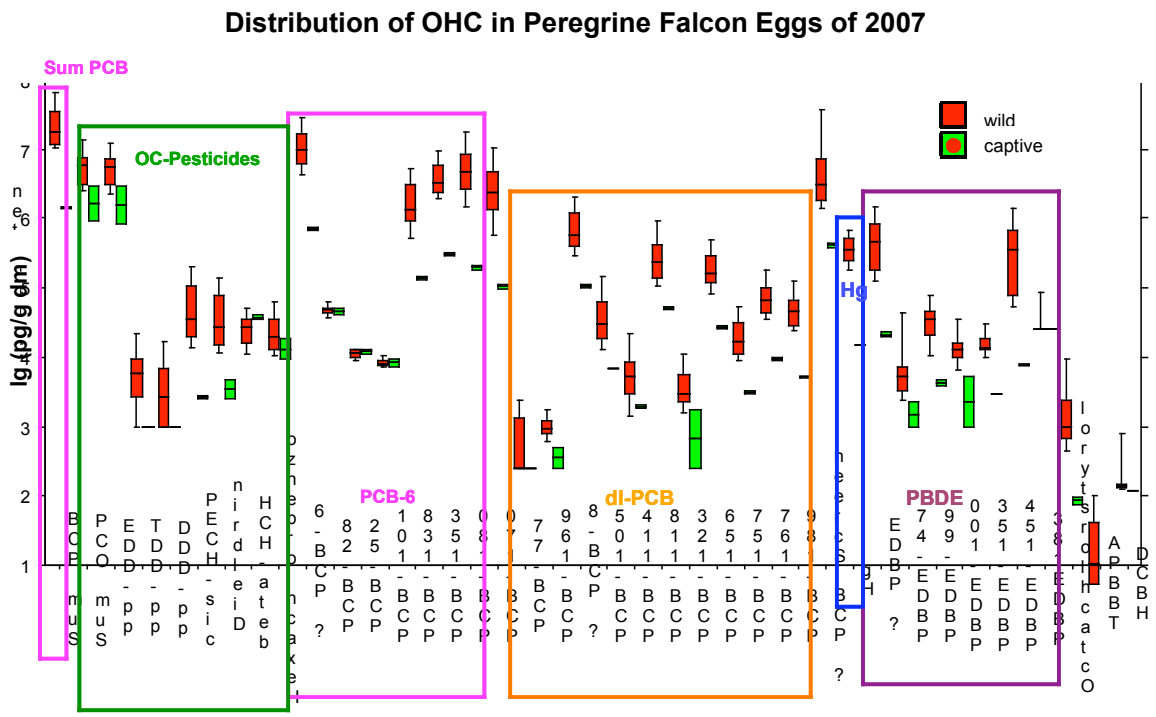

Figure 3: Box plot presentation of the main analytes. Red boxes: 25. – 75. percentile of distribution in wild eggs; green boxes: 25. – 75. percentile of distribution in eggs from falcons kept in captivity.

PBDE Temporal Trend

Polybrominated diphenylethers (PBDE) have been measured in Peregrine eggs since 2003 (Fig. 4). After an initial sharp rise by a factor of 14, their levels seem to be declining in the years after 2005.

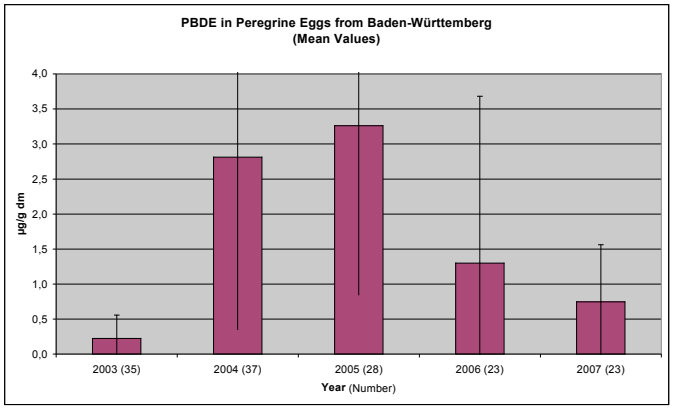

Figure 4: PBDE in Peregrine falcon eggs from Baden-Württemberg (mean ± std. dev.)

Toxic Effects

Several OHC are still found in falcon eggs in very high concentrations and exert a potential health risk. Limit values for foodstuffs are exceeded up to 1000fold in the eggs. PCB were identified as most critical, since they are the only class of compounds exceeding the threshold of dioxin-like effects in birds of prey like the osprey, and in chickens (200 pg WHO TEQ/g dry matter[2]). Like polychlorinated dibenzodioxins and –furans (PCDD/F), the **coplanar** PCB bind to the Ah-receptor and induce cytochrome P450 isozymes of the steroid- and xenobiotic-metabolism. Dioxin-like effects include a metabolic disruption of the hormonal balance,

immunotoxicity, liver-toxicity, embryo-toxicity and tumor promotion. **Non-coplanar** PCB are known for their affinity to the estrogen-receptor eliciting agonistic (the lower chlorinated congeners) or antagonistic effects (the higher chlorinated congeners such as 138, 153, 170, 180, 194, 199 and 203 as well as some important PCB-metabolites). This rule of thumb seems to hold true for the polybrominated diphenyl ethers (PBDE) as well[3].

Dioxin- and Hormone-Like Bioanalysis by a Series of CALUX® BioDetectors (DR, ER, AR, GR, TR)
Eighteen Peregrine eggs were selected from the years 2004 to 2007 and analyzed via the dioxin responsive chemically activated luciferase expression bioassay (DR CALUX®). The bio-assay results (ranging from 44 to 2055 with a mean of 364 pg dioxin TEQ/g dm, Fig. 6) confirm the earlier chemical analyses (19 eggs from the years 2000 to 2003 ranging from 149 to 954 with a mean of 397 pg dioxin TEQ/g dm).

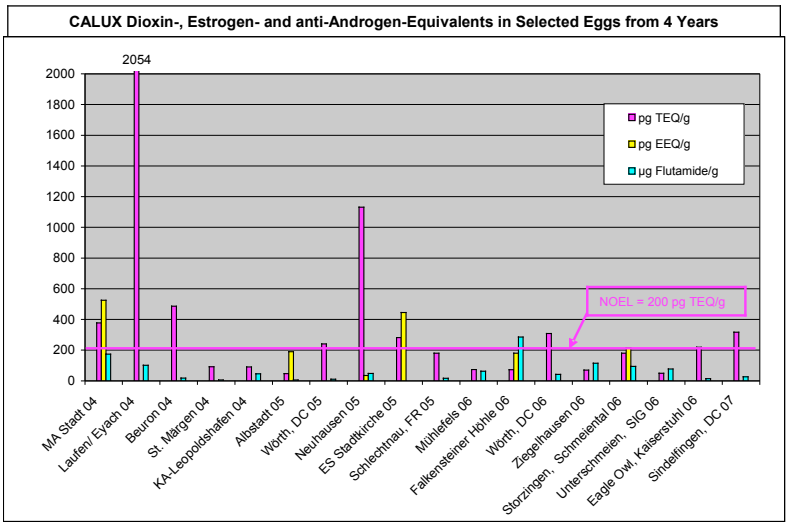

Figure 6: CALUX® dioxin-, estrogen- and anti-androgen equivalents in selected eggs from 4 years

Similar bioassays were performed with estrogen and androgen receptor responsive cells, respectively (ER CALUX® and AR CALUX®). The results are shown as 17β-estradiol or flutamide equivalents in the egg samples (Fig. 6). Glucocorticoid (GR CALUX®). and thyroid hormone-like activity (TR CALUX®) were neither detected in a randomly selected subsample of three falcon eggs and one eagle owl egg nor in a chicken egg serving as reference. The results show that the CALUX® panel can successfully be applied to complex samples as birds' eggs extracts. Further work correlating bioassay results with chemical analyses is planned with the aim to establish the cell tests as a screening method.

Further research is necessary to develop adequate criteria for a reliable assessment of the human health and ecological risk caused by environmental OHC such as PCB, DDE, and PBDE, and their metabolites. The preliminary bioassay results are to be repeated and to be correlated with the chemical analyses in order to establish the CALUX® cell tests for screening. Despite the decline in OHC concentrations in the past decades, the Peregrine population of Baden-Württemberg is still at risk through OHC in their environment. Therefore, the egg monitoring should continue, and measures to reduce the contamination should be considered where appropriate.

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References: [1)]UNEP workshop to develop a global monitoring programme to support the effectiveness evaluation of the Stockholm Convention, United Nations Environmental Programme, Geneva, Switzerland, 24-27 March 2003 [2)]von der Trenck KT, Baum F, Hartwig H, Malisch R, Schilling F, Straub H-P, Zimmermann RD (2006): UWSF – Z Umweltchem Ökotox 18(4), 228-241 [3)]von der Trenck KT, Schilling F, Schmidt D (2007): UWSF – Z Umweltchem Ökotox 19(2), 75-82 [4)]Sonneveld E, Jansen HJ, Riteco JAC, Brouwer A, Van der Burg B (2005): *Toxicol Sci* 83, 136ff [5)]Wania F (2004): GAIA 13 (3), 176-185