Pesticide and PCB contamination in a small population of the Hoopoe (*Upupa epops*) in Switzerland and toxicological implications

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Abstract **4**

1. Introduction **5**
1.1. Decline of the Hoopoe populations in Europe **5**
1.2. Development in Valais **5**
1.3. Ecotoxicology **6**
1.4. Aims of the study **7**

2. Materials and methods **8**
2.1. Determination of pesticide and PCB contamination in carcasses and eggs **8**
2.1.1. Sample collection 8
2.1.2. Sample selection 8
2.1.2.1. *Juvenile carcasses* 8
2.1.2.2. *Eggs* 10
2.1.3. Analysis 10
2.1.3.1. *Autopsy* 10
2.1.3.2. *Extraction* 11
2.1.3.3. *Purification* 11
2.1.3.4. *Gas chromatography* 12
2.1.3.5. *Data interpretation* 14
2.1.3.6. *Lipid determination* 14
2.1.3.7. *Purity standards, reproducibility, and accuracy* 15
2.1.3.8. *Spiking and limit of detection and quantification* 15
2.1.3.9. *Statistical analyses* 16
2.2. Investigation of chlorpyrifos uptake in Valais **16**
2.2.1. Experimental area 16
2.2.2. Experimental set-up 17
2.2.2.1. *Pesticide-free pre-treatment period* 18
2.2.2.2. *Application of chlorpyrifos* 18
2.2.2.3. *Sample collection* 18
2.2.3. Blood analysis 19

2.2.3.1. *Reaction* 19
2.2.3.2. *Preparation of samples* 19
2.2.3.3. *Photospectrometry* 20
2.2.4. Weather conditions 20
2.2.5. Statistical analyses 21
2.3. Theoretical approach of chlorpyrifos uptake in Valais 21

3. Results 22
3.1. Pesticide and PCB contamination in carcasses and eggs 22
3.1.1. Biometric data and characteristics 22
3.1.2. Concentrations 23
3.1.3. Differences of contamination levels in juveniles and eggs 25
3.1.4. Correlations 27
3.1.5. Contamination patterns 28
3.2. Chlorpyrifos uptake in Valais 29
3.2.1. General conditions of juveniles 29
3.2.2. Pre-Exposure butyrylcholinesterase activity 29
3.2.3. Inhibition 30
3.3. Theoretical Approach 31

4. Discussion 32
4.1. Concentrations of organochlorine contaminants 32
4.2. Contamination patterns and origin of pollutants 34
4.3. CPF contamination 36

5. Conclusion 38

Acknowledgements 39

References 39

Abstract

Since the beginning of the 20th century, many Hoopoe populations have declined or have disappeared in Europe. In Switzerland, a last population remained in the upper Rhone Valley (Valais). As part of a conservation project of the Swiss Ornithological Institute of Sempach and the University of Berne, a laboratory and a field study were carried out to investigate the contamination with organochlorines and chlorpyrifos, an organophosphate, which is applied in Valais to combat Mole crickets, the Hoopoe's main food.

Whole body homogenates of 12 freshly conserved carcasses and 11 eggs that died in the nests were analysed by gas chromatography (GC) to determine the levels of 18 organochlorine pesticides, 12 polychlorinated biphenyl congeners (PCBs), and chlorpyrifos (CPF). Whereas most organochlorines (OCs) were only sporadically present in small amounts or completely absent, the levels of pp'-DDE and total PCBs were clearly elevated in both sample types. Two eggs were burdened with PCB concentrations of 1.8 and 1.2 µg/g wet weight (ww), and three different eggs experienced pp'-DDE levels ranging from 1.4 to 1.7 µg/g ww. These quantities approached the thresholds usually associated with negative impacts on reproductive outputs. The concentrations of most OCs did not differ significantly between eggs and juveniles, except heptachlor epoxide B, which had higher concentrations in eggs. The contamination is believed to occur in the wintering habitats, such as the Sahel. DDT was present in very low quantities, suggesting no recent application on the wintering grounds. The higher contamination levels of endosulfan 1 and CPF in juveniles reflect the fact that these pesticides are actually used in Switzerland.

A field experiment was performed to investigate a possible contamination by CPF due to its application as a granular formulation directed at Mole crickets, the main prey of the Hoopoe in Valais. For this purpose the inhibition of butyrylcholinesterase (BChE), a non-destructive biomarker, was measured in 17 juveniles of three clutches after the simultaneous experimental pesticide treatment of 38 out of 166 private vegetable gardens, which should represent a realistic worst-case scenario. BChE activity was by 22% significantly lower in post-treatment samples. The results suggest that application of CPF in vegetable gardens may potentially lead to contamination of Hoopoe nestlings. BChE activity is very high in this species. Since it can act as a buffer for acetylcholinesterase, higher burdens of CPF might be tolerated. The juveniles showed no directly visible effects of CPF contamination during the field experiment, and all fledged.

1. Introduction

1.1. Decline of the Hoopoe populations in Europe

Since the beginning of the 20th century, the populations of the Hoopoe have declined continuously in Middle Europe. Only in the fifties a simultaneous recovery was observed in many countries, perhaps due to outstanding weather conditions (Glutz 1980). Yet, in the late fifties, most populations declined again and many of them went extinct afterwards. Whereas climatic changes towards colder and wetter conditions were possibly the major reason for the drop of the Hoopoe populations in the first decades of the 20th century, the loss of appropriate breeding habitats seems to be the most probable factor since the fifties (Bauer 1997). Today, this bird is one of the most endangered species in Central and Western Europe (Hustings 1997).
In contrast to Europe, the conditions prevailing in the Sahel, which is supposed to be the main wintering habitat of European populations (Glutz 1980), do not seem to have worsened in the last decades. There, Hoopoe populations are widely and thinly distributed over most savannah habitats, which makes them less susceptible to anthropogenic influences in Africa.

1.2. Development in Valais

In Switzerland, the Hoopoe is classified as an endangered species (Keller *et al.* 2001). The Swiss population had similar fluctuations as those in most countries in Middle Europe during the last two centuries. Whilst this bird was widely distributed in the Swiss lowlands in the fifties, it is now mainly restricted to the canton of Valais (Schmid *et al.* 1998). Since 1979 this last population has been monitored intensively (Arlettaz 1984; Arlettaz *et al.* 1998; Arlettaz *et al.* 2000a; Arlettaz *et al.* 2000b; Schaad 2002).
In order to conserve this species, a long-term program was started in 1999 by the Swiss Ornithological Institute of Sempach. Despite improvements in habitat quality through the installation of nestboxes on the plain and so as to reduce the distance between nesting sites and feeding grounds (Fournier and Arlettaz 2001), the reproductive output has been fluctuating a lot, mainly because the annual breeding success is strongly influenced by weather conditions (Schaad 2002). Because pesticide contamination might further affect reproduction we investigated the toxicological burden of the pollutants taken up in the Sahel and in Switzerland.

1.3. Ecotoxicology

Among all pesticides organochlorines (OCs) and organophosphates (OPs) have the widest impact on non-target organisms, since they act on basic biochemical pathways. Both pesticide classes are lipophilic but because of their fast biotic and abiotic degradation organophosphates show only acute toxicity whereas organochlorine pesticides, which are much more persistent, can cause chronic effects, too. The latter can thus accumulate in the food chain and reach highly toxic concentrations. Similarly, polychlorinated biphenyls (PCBs) have very long half-lives and biomagnification to dangerous levels have often been reported. Many recent studies have shown elevated levels of these persistent substances (OCs) in terrestrial bird species (Provini 1999; Kunisue *et al.* 2002; Pain *et al.* 1999; Minh *et al.* 2002; Rattner *et al.* 2000). The toxicity of OPs has been studied extensively over the past 30 years and recent studies still show the negative influence of these substances on the fitness of various terrestrial bird species, mainly because of the continual marketing of new products (Busby D.G. *et al.*, 1990; Yawetz A. *et al.*, 1993; Mullié W.C., Keith J.O., 1993; Fossi C.M. *et al.*, 1994).

In Europe organochlorine pesticides and PCBs were banned during the seventies except for endosulfan I, which is still used in arboriculture and vineyards. The current use of organochlorines (OCs) in the Sahel is rather limited, although until recently a number of compounds were still widely used, such as heptachlor in seed treatments. Currently the only organochlorines being legally applied in that region are endosulfan, lindane, dieldrine, and dicofol, a DDT derivate, all in very low quantities, while PCBs are still in use in industry.

A wide range of OPs belong to the authorized pesticides in the Sahel, including chlorpyrifos (CPF), dichlorvos, and fenitrothion. They are regularly used in grasshopper control in savannah habitat and fallow land (Mullié, pers. comm.). The use of OPs in Europe is much more restricted and in Valais only phosalone, CPF, diazinone, dimetholate, and thiocyclam are used in arboriculture (Office cantonal de la protection des plantes, 2002). There, CPF, which is used in control of Mole crickets, is expected to be the only relevant OP affecting the Hoopoe population.

1.4. Aims and hypotheses

The primary aim of this study was to quantify the levels of a broad mixture of organochlorine pesticides (DDTs, HCHs (hexachlorocyclohexanes), CHLs (chlordanes), HCB (hexachlorbenzene), heptachlor and its derivates, dieldrine, and endosulfan 1), PCBs, and CPF in

juvenile carcasses and unhatched eggs by gas chromatography. We expected that juveniles show equal or lower concentrations of OCs than eggs, since we assume that these substances mainly originate from the winter habitats, such as the Sahel. Furthermore, contamination patterns could provide information about the actual use of these contaminants, both in Africa and in Switzerland. Because of progressive decomposition we expected higher ratios of native pesticides to their degradation products in eggs than in juveniles. In view of the fact that organochlorine pesticides are prohibited in Europe (except endosulfan) and that they have been largely replaced by other pesticide classes, such as OPs, in most fields of application in the Sahel, we expected only low contamination levels of OCs. An exception may be DDE, the degradation product of DDT, since in the nineties DDT containing pesticide mixes were still delivered from Europe to Africa (Nentwig 1995).

Because of their relatively fast biotic and abiotic degradation (Abdel-Rahman *et al.* 2002; Hayes and Laws 1991), OPs originating from the Sahel cannot be detected by gas chromatography when analysing Swiss samples. Among the OPs used in Valais, CPF appears to be the only direct threat to the Swiss Hoopoe population, because it is directed as a granular formulation (cortilan® Maag) at its major prey, the Mole cricket (*Gryllotalpa gryllotalpa*). The other OPs applied in arboriculture are directed at shoot-mining insects, which are not preyed by Hoopoes. Therefore, they were not included in analysis. A field experiment was carried out to investigate whether application of may lead to a CPF contamination in Hoopoes. The extent of contamination was estimated by measuring the activity of blood butyrylcholinesterase (BChE), a non-destructive biomarker, after application in private vegetable gardens (PVGs) on the Hoopoe's feeding grounds. Because the PVGs covered only a small portion of the total feeding area, we expected only low or moderate inhibition, with severe contamination being only local and temporary. Moreover, we hypothesized that the intensity of inhibition is in proportion to the distance between broods and sites of CPF application. In addition, a model of CPF uptake was developed to assess the probability of an accumulation of this OP up to lethal amounts.

2. Materials and methods

2.2. Determination of pesticide and PCB contamination in carcasses and eggs

2.1.1. Sample collection

From 1998, nestboxes were installed on the plain of the upper Rhone valley, which allowed regular controls of Hoopoes breeding in that region. From 1999 to 2002, unhatched eggs (n = 129) and carcasses of adults (n = 7) and juveniles (n = 90) found in these nestboxes were collected and immediately frozen at –18 °C.

Because controls did not take place daily, the carcasses were found in different stages of autolysis. Some eggs were collected after the nestboxes were left or when the breeding period was over. This resulted in a strong variation of post-mortem exposition to environmental conditions. Asynchronous egg laying of one egg per day resulted in further variation. Therefore, an egg could be between 30 to 50 days old before collection. 35 days were the maximal time span between death and freezing.

2.1.2. Sample selection

2.1.2.1. Juvenile carcasses

Selection criteria

Four selection parameters were applied to sample a homogenous group of juveniles for analysis. Since pesticides and PCBs are degraded and eliminated during chick development, individuals with the same age (degradation) (see table 4) and similar feather development (elimination) were selected. Furthermore, the level of autolysis and injuries were considered as important factors affecting pollutant concentrations. Injuries can lead to selective removal of tissue and lead to a better accessibility of the pollutants to degrading microorganisms.

Estimation of the degree of autolysis

To estimate the degree of decomposition six steps of autolysis were determined in collaboration with Dr. Nadia Robert from the Pathological Institute, University of Berne. The scale mainly

used the colour of the skin around stomach and breast. The criteria were drawn from a sample with carcasses of different stages of autolysis.

Fresh carcasses have pink skin, which becomes green during the advancing decay, because the inner organs begin to autolyse and the liquid of the gall bladder invades the interorganic spaces of the stomach. Later, the whole skin becomes darker because of its own decay. Additional information such as odour and fixation of feathers were also taken into account.

Table 1. Stages of autolysis. The stages 0 and 1, from which the carcasses were taken for analysis, correspond to a degradation of up to one day at room temperature under laboratory conditions

Stage	Decomposition	Description
0	fresh carcass	The skin has a pink colour.
1	low	The colour is still pink with some greenish parts in the region of the stomach where the gall bladder is situated (a single dark green spot is also possible if the gall bladder is near the surface).
2	moderate	The whole stomach has become greenish while the skin being slightly darker in general.
3	advanced	The colour of the whole skin has become dark purple with a dark green stomach. Feathers are easy to remove.
4	strong	The whole skin is dark and feathers begin to decompose. The mass of the body has strongly declined and the skin is very easy to remove.
5	complete	Soft body parts have disappeared.

Age determination

The age was estimated by a method based on the measurements of Glutz (1980) using the width of the beak basis and the beak length (from the upper beak basis to the peak). The juveniles were classified into four groups according to the criteria shown in table 2.

Table 2. Age classes of juvenile Hoopoes. Whereas the width of the beak basis mainly served to estimate the age of juveniles of the stages one to three, the beak length served to separate fledglings of stage four from adults.

Stage	Age of juvenile	Widht of lower beak	Width of upper beak	Beak lenght
1	1-6 days	4-6 mm	4-6 mm	
2	6-9 days	15-18 mm	12-14 mm	22-27 mm
3	10-20 days	<17 mm	<12 mm	27-40 mm
4	>20 days	7-9 mm	7-9 mm	<45 mm

2.1.2.2. Eggs

Selection criteria

The selection characters for eggs were and intact shell and an embryo-age of less than 5 days. Openings in the shell enable microorganisms to enter and degrade the pollutants. Hydroxyl radicals are another external factor affecting degradation. They are buffered by the lipids of the yolk, which are targets, too. Because of unknown parameters, such as the sequence of egg laying, post-mortem exposure was not taken into account.

Determination of pre-death age

The approximate age of the embryos could first be roughly estimated by candling (shining a bright light through the egg), which was used for pre-selection.

Estimation of age was done by calculating the ratio of egg- and embryo size, which was then compared to a developmental table of chicken embryos of known age and size. With a given ratio a corresponding embryo age could be determined in the chicken table (I). Age was corrected for different development times in the two species (chicken 21 days, Hoopoe 17 days) (II):

$$\text{(I) } E_1/S_1 = E_2/S_2 \rightarrow y \qquad \text{(II) } x = y*17/21$$

E_1 and S_1: Size of Hoopoe embryo and egg, respectively

x: Estimated age of Hoopoe embryo.

E_2 and S_2: Size of chicken embryo and egg, respectively

y: Age of chicken embryo

2.1.3. Analysis

2.1.3.1. Autopsy

To avoid pseudo-replication, only one egg or carcass per brood were used. The carcasses were first checked for external and internal injuries, then they were displumed and the degree of autolysis and age were determined. To eliminate contamination through undigested insects (mainly Mole crickets), the stomach was opened and contents were removed and stored at –20 °C for subsequent analyses. An aliquot of the tissues and feathers were stored in Eppendorf tubes for future sex determination.

Eggs showing no sings of damages were weighted and measured. Then the eggshells were removed and the content was examined. The size of the embryo, if present, was measured and its stage of development was determined by the method outlined above. Again, an aliquot of the tissue was stored in Eppendorf tubes for future sex determination.

2.1.3.2. Extraction

The displumed carcasses were mixed with anhydrous sodium sulphate (Na_2SO_4) (99% purity; Merck RgaG, Darmstadt, Germany), which resulted in a fine powder of whole body homogenates. The eggs were homogenized without the shells. The sodium sulphate was added to absorb the water molecules of the samples. This enabled the organic solvents to solubilise the pollutants.
Extraction was done by distillation with 150 ml of a 3:1 mixture of n-hexane and acetone (sps quality, Romil ltd., UK) in a soxhlet (IKA, HPM6 basic) at 50% heat during 18 hours, which resulted in about 40 repetitions of the extraction cycle. Insert porosity was 0.5.

2.1.3.3. Purification

The extracts of the carcasses had to be centrifuged at 2300 g for ten minutes because of the high amount of particles that passed into the solvent during distillation. The supernatant was taken for further purification. Because the eggs did not contain impurities in form of particles, they skipped this step. After lipid determination (*2.1.3.5.*), the solutions were concentrated to two millilitres at 330 mbar and 40 °C by evaporation (rotavapor R-114; vacuum controller B-720; waterbath B-480; Büchi Labortechnik GmbH, Flawil, Switzerland) and separated by size with gel permeation chromatography (GPC) (100g Bio Beads SX3, Bio-Rad Laboratories, Switzerland flow rate 5 ml/min controlled by a Shimadzu LC-9A pump, Shimadzu Deutschland GmbH; column type 721,775-1, Sigma Aldrich, Switzerland) to exclude bigger molecules such as long fatty acids and proteins. As solvent a 1:1 mixture of Dichloromethane (sps quality; Romil ltd., United Kingdom) and n-Hexane (measured by volume) was used, which was exposed to ultrasound (ELGA BCL14, Biel, Switzerland) at 38 kHz for five minutes to eliminate air bubbles, which otherwise could have disturbed proper separation. The fraction leaving the column between 35 and 80 minutes containing the pesticides and PCBs was concentrated to 2 ml.
To eliminate remaining impurities, the solution obtained by GPC underwent a second step of purification on a florisil column (100-200 mesh; Fluka Chemie GmbH, Switzerland), which was

freshly prepared as follows: for each column, 10 g of florisil powder was dried at 650 °C for three hours in a sole-tyne 6004 oven and was then activated with 2% ultra pure water and shaken for 20 minutes. One hour later, after equilibration, the powder was mixed in n-hexane, which was then filled into a column of 1 cm interior diameter. After the florisil reached a stable height, about 1 g of Na_2SO_4 was added, which served to absorb eventually occurring traces of water in the sample.
The extracts were purified with 40 ml n-hexane and then with 40 ml of a 9:1 mixture of n-hexane and dimethylether (quality sps; Romil ltd., UK) (measured by volume) as mobile phases. This step of purification eliminated remaining impurities and separated pesticides from PCBs by polarity. PCBs, DDE, hexachlorbenzene, and endosulfan 1 left the column in the first fraction, which was unpolar. The slightly less hydrophobic pesticides were obtained by the second fraction. Separation into two fractions served to eliminate interferences of PCBs with some pesticides in the gas chromatographer.

2.1.3.4. Gas chromatography

The fractions were carefully evaporated down to about 1 ml, then filled into 2 ml vials. After the flasks were washed twice with Isooctane (2,2,4-Trimethylpentane) (sps purity; Romil ltd. UK), the solvent used for gas chromatography, the volume of the solutions was reduced to 0.5 ml by evaporation with gaseous nitrogen, measured by weight with a Mettler PR503 Delta Range precision balance. Because n-Hexane and Dimethylether are more volatile than Isooctane, this resulted in a change of solvent.
Quantification was performed by injecting the extracts into two GC-ECDs (HP 6890 and Varian CP3800 – MS1200L) with different column polarities (DB5 and Rtx-35), which resulted in different chromatogram positions of the pollutants. The oven temperature was programmed from 80 °C (0.5 min hold) to 150 °C (1 min hold) at a rate of 50 °C/min and then to 285 °C (30 min hold) at a rate of 2.5 °C/min. Initial injector temperature was 85 °C (0.2 min hold) and increased to 250 °C (58 min hold) at a rate of 70 °C/min. Detector temperature was kept 300 °C. Helium was used as carrier gas. The double measurement served to identify the peaks properly, which could be affected in two ways: first, a peak could be caused by a substance other than the pesticide identified, and second, a pesticide peak could be enlarged by an impurity leaving the column at the same time.
Identification was done on a personal computer using the Star Chromatography Workstation Version 5.3. (Varian inc., Palo Alto, USA) by comparing the sample peaks to those of standards of known concentrations and chromatogram positions. Organochlorine pesticides were compared

to an internal standard (99% purity; BGB analytik AG, Switzerland) and to an alternative mixture of 17 OC pesticides (99% purity; Ehrenstorfer GmbH, Augsburg, Germany). To identify PCBs, a mixture of twelve congeners was used (PCB 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180; the PCB congeners are referred using their IUPAC number throughout the manuscript) (>99% purity; LGC Promochem GmbH, Germany). Whereas the internal standard was purchased as a mixture solubilised in isooctane, chlorpyrifos (99,2% purity; Dr. Ehrenstorfer GmbH, Augsburg, Germany), the PCBs, and the pesticides of the alternative OC standard were purchased separately as pure crystals and solubilised in isooctane. For each chromatography sequence, the standards were measured at the beginning and then after 4 to 6 samples each because the correlation between pollutant concentration and resulting peak height evolved during the measurements. To calculate the concentrations of the pollutants in the samples, the average heights of the standard peaks were taken.

In most cases the peak height does not increase linearly with rising pollutant concentrations. Therefore the sample units containing pesticides or PCBs with much higher concentration than the standards were diluted and measured again. The concentrations of the pollutants were calculated as follows:

$$\text{(III) } ng/g\ (ww) = (P_{SA}/P_{ST})*C*(V/W)*S_{DIL}*100/R$$

$$\text{(IV) } ng/g\ (lw) = (ng/g\ (ww))*100/p$$

P_{SA}: Height of sample peak

P_{ST}: Height of standard peak

W: Wet Weight of measured sample fraction

R: Recovery rate

C: Concentration of standard (ng/ml)

V: Volume of sample

S_{DIL}: Dilution factor of sample

p: Percentage of lipid content of sample

ww: Wet weight

lw: Lipid weight

Only twelve out of 209 PCB congeners were analyzed throughout all samples. To obtain the entire PCB burden, randomly chosen sample units were additionally compared to a 1:1:1 mixture of Arochlor 1242, 1254, and 1260, whose peaks and corresponding concentrations were identified by Schulz *et al.* (1989). The twelve PCB congeners analysed in the present study represent 39.9% (± 6.3) of the total PCB concentration. The values of total PCB were corrected for this factor.

2.1.3.5. Data interpretation

Based on the data of two independent chromatographs (columns), the concentrations of the pollutants were determined for each column separately, resulting in no, one, or two values per substance and sample unit. Those pollutants that were confirmed in both chromatographs in amounts differing less than 40% from each other were rated as real. The threshold level was increased when the absence of a pollutant was highly improbable, which was the case when

1. the majority of the samples contained it or
2. when the substance was the only possible precursor of another substance found in the sample (for example pp'-DDT and pp'-DDE) or
3. when the presence of a pollutant was typically linked with another substance being confirmed in the sample (for example PCB153 and PCB138).

To take into account the effect of impurities, only the lower of the two values of each pollutant was taken for final results.

2.1.3.6. Determination of lipid weight

All pollutants analysed in this study were lipophilic. Therefore, lipid weight was determined because the level of contamination strongly depends on the lipid content. After extraction and concentration to 10 ml, an aliquot of 1 ml was taken from the resulting solution. Because the extracts of the carcasses contained too many particles, they were first centrifuged, and then the aliquot was taken from the supernatant. In the next step, it was placed into a dried aluminium jar, which was then put into a dessicator overnight for evaporation. The weight was measured with a Mettler H35 precision balance (± 0.0001 g). The advantage of this method was that it allowed a fast determination of lipid weight with a relatively small portion of the samples. On the other hand, its disadvantage was that impurities, which interfered with the measurements, could not be eliminated completely. Therefore, as a control, a carcass and a chicken egg were also measured by a more precise method using chloroform (sps quality, Romil ltd., UK) as solvent (Boer *et al*. 1988). Since this method needs at least 3 g of sample it could not be applied to Hoopoe eggs, which weighted in most cases less. The deviation of lipid content between the two methods was about 10%. Because of the heterogeneity of the amount of particles interfering with lipid determination within the samples, the values were not corrected for this deviation.

2.1.3.7. Purity standards, reproducibility, and accuracy

To avoid cross contamination, all materials that came in touch with the samples were alternately washed three times with bidistilled acetone and n-hexane. The last fraction of n-hexane was controlled for impurities by gas chromatography. The whole equipment used for extraction and purification was two times tested for impurities. In order to increase accuracy, all measurements to determine the volume of the sample solutions were performed by weight, except for the fraction of lipid weight determination, which was achieved with a 1 ml pipette. In order to test the reproducibility of the method, the full content of a nestbox (A-47, June 2002), which consisted of five juveniles, was measured three times independently. The deviations of the quantifiable pollutants lied in the range the range of the error resulting from Gas Chromatography.

2.1.3.8. Spiking and limit of detection and quantification

Detection- and quantification limits were defined as the three- and the tenfold of the background noise, respectively. The corresponding pollutant concentrations were calculated using the minimal sample weight.

Table 3. Detection and quantification limits expressed in ng/g lipid weight (wt).

Product	Detection limits lipid wt (ng/g)	Quantification limits lipid wt (ng/g)	Product	Detection limits lipid wt (ng/g)	Quantification limits lipid wt (ng/g)
alpha HCH	1.26	4.41	Trans-nonachlore	1.69	5.93
beta HCH	2.89	10.12	Hexachlorbenzene	1.35	4.72
gamma HCH	1.38	4.85	Endosulfan I	1.85	6.47
delta HCH	1.69	5.93	PCB 28	2.97	10.38
Chlorpyrifos	2.11	7.38	PCB 52	3.93	13.75
Heptachlor	2.58	9.03	PCB 101	3.11	10.89
Heptchlor epoxide B	1.75	6.12	PCB 149	2.98	10.43
Heptachlor epoxide A	1.57	5.50	PCB 118	2.46	8.61
Oxy Chlordane	1.64	5.74	PCB 153	2.73	9.56
alpha Chlordane	1.69	5.90	PCB 105	1.82	6.37
gamma Chlordane	1.67	5.85	PCB 138	2.17	7.59
Dieldrine	1.73	6.06	PCB 128	1.71	5.98
pp'-DDT	2.30	8.04	PCB 156	1.68	5.89
op'-DDT	1.93	6.77	PCB 180	0.92	3.21
pp'-DDD	2.30	8.04	PCB 170	3.63	12.70
pp'-DDE	1.73	6.04			

To assess the recovery rate of the method, a group of carcasses of the same clutch and a chicken egg were spiked with target compounds according to the guidance of the Institute for Reference of Materials and Measurements (IRMM). Briefly, the samples were spiked with different levels

of each contaminant covering a range from 50% to 200% of the previously detected amount. The standards were solubilised in acetone, which facilitated absorption. After a period of 24 h, witch allowed the added concentrations to penetrate the matrix, the spiked sample units were extracted and purified as described above. Recovery rates of carcasses were as follows. Hexachlorbenzene: 75.8% (± 5.1), chlorpyrifos: 92.7% (± 8.1), pp'-DDE: 72.3% (± 15.5), pp'-DDT: 89.4% (± 8.3), PCBs: 95.1% (± 11.7). The percentage of recovery in eggs was: Hexachlorbenzene: 73.9% (± 2.2), pp'-DDE: 75.0% (± 5.4), PCBs: 101.9% (± 4.0). Values with recovery rates below 100% were corrected. Because of losses in the second fraction of egg spiking, the recovery rates of chlorpyrifos and pp'-DDT could not be determined properly. The other pollutants were below the limit of quantification and/or occurred only sporadically and were therefore not analysed for recovery rates.

2.1.3.9. Statistical analyses

Analyses were based upon concentrations by lipid weight. Values below the limit of quantification were included, whereas those below the limit of detection were counted as zero. Because residue data were non-normal, a Wilcoxon Rank test was applied to test for differences of pollutant concentrations between carcasses and unhatched eggs and for differences between the ratios of native pollutants and their degradation products. In the investigation of contamination patterns a Spearman Rank Correlation test was performed to look at correlations between pollutants in eggs and juveniles. Data are shown as mean ± SD.

2.2. Investigation of chlorpyrifos uptake in Valais

2.2.1. Study area

The only region in Valais where high Hoopoe density and sufficient amount of private vegetable gardens (PVGs), the only relevant source of chlorpyrifos (CPF) in that region, occurred, was within orchards west of St-Léonard. The area had the shape of a triangle with 2.2 km length and 1 km width. 166 PVGs were distributed over this region, with most of them on the eastern half. Because Hoopoes cover distances of up to 1 km in search for food, a safety border of one kilometre width was included, containing 30 more PVGs. To the north, the area was limited by a

rocky slope; to the west, it was flanked by meadows; St-Léonard and Uvrier set the limit on the eastern side and Pontafontana, a nature reserve mainly consisting of swamp, was on the south.

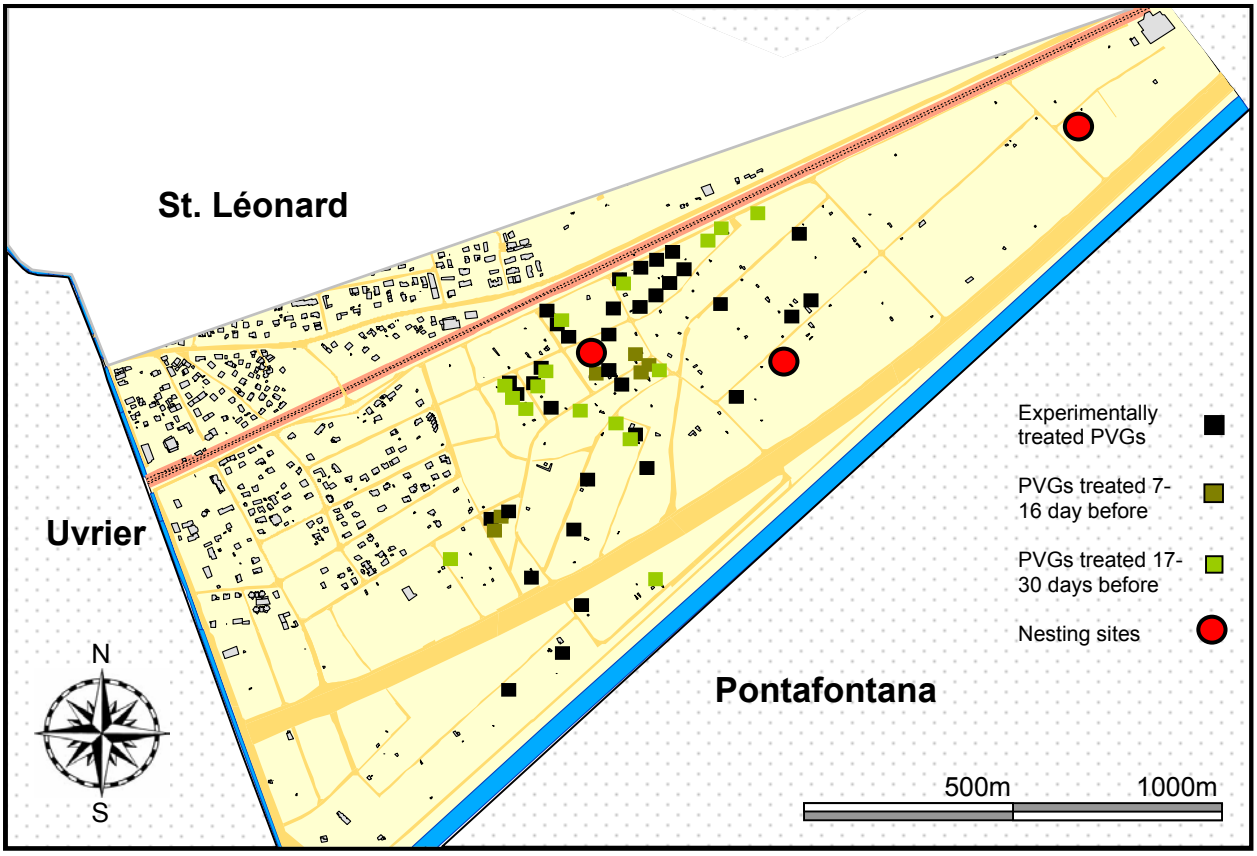

Fig. 1. Study area on the plain of the Rhône. Circles depict nesting sites of the measured broods. Black rectangles represent treated private vegetable gardens (PVGs) (n = 38) that took part to the experiment. Dark and light grey rectangles are PVGs treated by their owners 7 to 16 days, and 17 to 30 days before the experiment, respectively. The safety border is not shown.

2.2.2. Experimental set-up

The aim of the field study was to quantify the amount of CPF taken up by juveniles just after a single massive treatment representing a realistic worst-case scenario. The CPF treatment took place after a pesticide-free period of about one month. Blood samples were taken before and after CPF application in PVGs to measure the activity of plasma Butyrylcholinesterase (BChE), a non-destructive biomarker, which is inhibited by organophosphorus pesticides. BChE activities in pre- and post-treatment samples were compared to estimate the contamination levels.

According to literature the values of day-to-day variation range from 5.0% in red-legged partridges (Johnston *et al.* 1994) to 30% in eastern bluebirds. Intermediate percentages were

found in European starlings (23%) (Gard 1993). We considered that a variation of ≤ 30% was indicating inhibition.

2.2.2.1. Pesticide-free pre-treatment period

The only possible source of CPF in the region of St-Léonard during the period of the experiment (April and May) was cortilan®, a bran bait containing 1,5% chlorpyrifos-ethyl, which is used in PVGs to control Mole crickets, the main prey of the Hoopoe in Valais.
In the end of July, chlorpyrifos-methyl is sprayed to control *Eupoecilia ambiguella* and *Cydia pomonella* (Lepidoptera) in vineyards, and apple orchards, respectively. Because of the fast decomposition of these two substances in the environment by hydroxyl radicals, microorganisms and UV light (Hayes *et al.* 1991), neither those sprayings nor applications of cortilan® in the preceding year are expected to affect Mole crickets in spring.
In order to ensure that pre- and post-treatment samples were not influenced by uncontrolled CPF-application, all local PVG owners were contacted one month before sampling, at the beginning of March 2003, when cortilan® was not yet used. From 166 PVGs at the study plot 72 have been treated regularly with CPF during the past years. 49 of the 72 PVG owners agreed not to interfere during the experiment, whereas 17 and 6 farmers scattered between 17 - 30 and 7 - 16 days prior to the experiment, respectively. The gardens within the safety border were not treated during the experiment.

2.2.2.2. Application of chlorpyrifos

On the evening of the day of the first blood sampling, cortilan® was scattered in 38 PVGs. The gardens were treated with quantities determined by the owners. They ranged from 2 - 15 kg/ha. Totally 17.5 kg were applied, which corresponds to 263 g CPF.

2.2.2.3. Sample collection

At three broods, blood samples were taken from 11 and 17 nestlings prior to and three days after application of chlorpyrifos, respectively. To avoid the influence of diurnal changes in activity, the samples were collected between 10:30 and 11:30 am. Six birds could not be sampled for control measurements because they were too young, too weak, or too old (expected to having left the

nests before the second blood sampling). 20 to 30 µl of blood were obtained from the brachial vein and were frozen at –80 °C on dry ice in 150 ml Eppendorf tubes.

2.2.3. Blood analysis

Blood analysis was carried out at the Pathological Institute of the University of Bern using the method of Ellman *et al.* (1961) modified by Thompson (1999). All chemicals were purchased at Dr. Grogg Chemie AG, Switzerland. To equilibrate storage times, pre-exposure samples were measured first (in random order) and post-exposure samples were analysed three days later (again in random order). All samples were measured in triples. In the case of strong deviation of one of the three replicas resulting from errors of pipetting, only the average of the two others was taken to calculate the activity of BChE.

2.2.3.1. Reaction

BChE from the blood samples hydrolyses Butyrylthiocholine Iodide (BTCI) into its thiocholine and acetic acid compounds. This is accomplished by the continuous reaction of the thiol with DTNB (5,5-dithiobis-2-nitrobenzoic acid) to produce the yellow anion 5-thio-2-nitro-benzoic acid.

2.2.3.2. Preparation of samples

The blood samples were thawed and centrifuged at 11'000 g for 10 min in 1,5ml Eppendorf tubes (Thompson 1991) by a refrigerated J2 MC Beckman centrifuge (4 ºC) to separate the serum from the erythrocytes. The supernatant (serum) was put into 1.5ml Eppendorf tubes and kept on ice. To reduce the activity of BChE in the measured sample 5µl serum was diluted in 95 µl 0,1M Tris HCL pH 8 and mixed well. At this pH, the activity of BChE is maximal (Thompson 1999). 285 µl DTNB (0,1%) in 0,1M Tris HCL buffer and 5µl diluted serum were pipetted into wells of a microtiter plate, which was then incubated for 5 minutes inside a Victor Reader (Perkin Elmer, Germany) at 42 °C to ensure complete equilibration. Then, 10 µl 0.02 M BTCI in high-purity water were added to the sample wells and mixed by pipette. Our manipulations showed that this concentration has turned out to be optimal, whereas higher concentrations caused non-linear absorption-slopes in the spectrograms.

Blanks, which were used to determine non-enzymatic conversion of DTNB, consisted of 285 µl DTNB, 5 µl diluted serum, and 10 µl high-purity water.

2.2.3.3. Photospectrometry

Absorbance was measured immediately at 405 nm and in 30 sec intervals during three minutes resulting in a spectrogram with twelve linearly ordered data points. To be as close as possible to the *in vivo* conversion rates, the assay temperature was kept constant at 42 °C.
The steeper the slope of the spectrogram, the more BChE was hydrolysed and the higher was the activity of the BChE in the samples. At zero activity the straight line was horizontal. The average steepness of the slopes of the three replicates was used to calculate the conversion rate of BChE using formula (V).

$$(V)\ \mu mol/(ml*min)=((dA/min)*E*Av*(Sv*L)*Dil)-B$$

dA/min: difference in absorbance per minute
Av: Assay volume: 0.3 ml
Dil: Dilution factor of sample in buffer: 20x
B: Activity of blank
E: Extinction coefficient: 1/13.6 (for DTNB)
Sv: Sample volume in assay: 0.005 ml
L: Height of solution in wells: 0.75 cm

2.2.4. Weather conditions

The amount of Mole crickets provisioned by parent Hoopoes to juveniles strongly depends on the weather conditions (Schaad 2002). The colder and wetter the weather the less Mole crickets are provided. Weather data for St-Léonard were supplied by the Federal Office of Meteorology and Climatology in Zurich (Switzerland). Pre- and post-treatment measurements took place on the 27th and 30th May, respectively. Average daily temperature was 18,5 ± 3.5 °C (n = 5) on average. A short and weak rain shower occurred on the first day after pre-treatment measurements. Average relative humidity ranged from 56.3% (30th May) to 73,6% (27th May). The days from the 20th to the 27th were a little colder but had otherwise similar weather conditions.

2.2.5. Statistical analyses

Six birds from two clutches (D-7 and D-38) could not be sampled during pre-treatment measurements. In view of the fact that there was only a small difference of pre-treatment BChE activity between juveniles of the same clutch (see 3.2.1.), the lowest measured value of each of the two nests was allocated to these juveniles. Further reasons were that all juveniles experienced the same general conditions, and had similar weight and age (see 3.2.1. and 3.2.2.), factors that have been shown to affect activity of serum BChE (Rattner and Fairbrother 1999; Thompson 1993; Fairbrother and Bennett 1989; Gard 1993; Bennett and Bennett 1991).

Restricted maximum likelihood estimation (REML) was used to test for differences in activity before and after application of CPF. Because within nests the juveniles were not independent from each other, the samples were blocked, where each nest represented a block. The treatment was considered as fixed factor, and nests and individuals were considered as random factors. The residuals were tested for normality. Data are shown as mean ± SD.

2.3. Theoretical approach of CPF uptake in Valais

A theoretical model was developed as additional tool to estimate the levels of CPF contamination following the ingestion of contaminated Mole crickets, since experimental do not exist. The following data and assumptions have been used to set up the model.

1. Uptake of Mole crickets: Schaad (2002) has shown that, on average, juveniles between eleven and twenty days of age receive each ten Mole crickets per day whereas those between twenty and twenty-five days receive only eight. The mean wet weight is about 1 g (Arlettaz and Fournier 2001).
2. Time span between CPF uptake and death: Mole crickets that have consumed a bran bait (mean weight 0.025 g, 1.5% CPF) will die within hours without taking up other baits (Locher, pers. comm.). This is mainly due to their feeding behaviour (Mole crickets take food items into their subterraneous galleries) and because of the side effects of CPF, such as anorexia, tremors, physical weakness, etc. (Richardson 1995).
3. Percentage of absorption: Since CPF is a lipophilic substance the calculations include a absorption rate of 80%. CPF is assumed to be homogeneously distributed in Mole crickets.
4. Degradation of CPF: CPF is enzymatically Degraded by A-esterases and cytochrom P-450 oxidases to 3,5,6-trichloro-2-pyridinol (TCP) and CPF-oxon (Richardson 1995). The maximal

time span between CPF resorption and death is estimated to be 10 hours. In rats this period corresponds to a degradation of about 35% ($T^{1/2}$ = 18 h) in blood plasma (Abdel-Rahman *et al.* 2002). In view of the fact that in insects decomposition rates of this substance might be higher, we assumed 50% degradation in Mole crickets. In contrast, the rates might be inferior in birds because they have lower activity of degrading enzymes, such as A-esterases (Grue *et al.* 1997). In order to design a prudent model the degradation rate in rats was applied to the Hoopoes without modifications.

3. Results

3.1. Pesticide and PCB contamination in carcasses and eggs

3.1.1. Biometric data and characteristics

From 90 juveniles, 12 fulfilled the selection criteria and were chosen to form a homogeneous group (Table 4). The stomachs of most birds were empty. This suggests that before death they did not receive food items for several hours, since otherwise at least fragments would have remained. These chicks were probably starving. All birds were completely feathered except juvenile number five, where the feathers were just beginning to fan out. Its featherweight accounted for 16.43% of the whole body mass (wet weight) whereas the average lied at 20.92% (± 4.70).

Table 4. Biometric data and characteristics of juveniles retained for analyses

Carcass #	Date	Location (nestbox code)	Weight (g)	lipid content in %	Age (days)	Stage of decomposition	Content of stomach
1	16.08.1999	Bramois	31,88	2.6	6-9	0	none
2	26.06.2000	Fully	35,1	9.7	6-9	0	insect fragments
3	26.06.2000	Gd Blettay	37,28	2.9	10-20	1	none
4	15.07.2000	Ardon	29,00	8.9	6-9	0	none
5	27.07.2000	Mazembroz (A-53)	31,10	2.0	6-9	1	none
6	14.06.2001	Fully (A-18)	38,20	12.7	6-9	0	1 small annelid
7	17.06.2001	Uvrier (C-37)	29,90	2.3	6-9	0	none[a]
8	26.07.2001	Leytron	29,94	3.1	6-9	0	none
9	June 2002	Fully (A-47)	28,80[b]	3.4	6-9	1	none[c]
10	24.06.2002	Ardon (B-11)	19,30	3.8	6-9	0	none
11	25.06.2002	St. Léonard (D-5)	24,93	2.6	6-9	1	none
12	01.07.2002	Fully (A-42)	23,96	2.4	6-9	1	none

[a]Two Molecrickets in beak [b]Average of five juveniles [c]Eight Molecrickets in stomach

Table 5. Biometric data and characteristics of eggs chosen for analyses

Egg #	Date	Location (nestbox number)	Age (days)	Fertilised?	Weight of content (g)	Lipid content in %	# of dead eggs in clutch
1	18.05.2000	Fully	<1	?	2.50	5.9	3
2	22.05.2000	Ardon	<5	y	2.35	10.1	1
3	27.05.2000	St. Pillet	0	n	3.60	7.2	2
4	31.05.2000	Fully	<4	y	2.86	6.4	3
5	13.06.2000	Fully	<1	?	1.22	8.7	10
6	25.06.2000	Ardon	<3	y	4.10	6.4	2
7	31.05.2001	Ardon (B-8)	<1	?	2.14	10.1	1
8	31.05.2001	Fully (A-18)	0	n	2.20	8.0	2
9	11.06.2001	Fully (A-15)	<3	y	2.91	9.4	2
10	26.06.2001	Ardon (B-15)	<3	y	2.58	10.3	1
11	14.06.2002	Chalais (D-F)	<4	y	2.10	7.1	11

From 129 eggs, 11 were chosen for analysis (Table 5). Because the yolk membranes of the eggs 1, 5, and 7 were bursted, it could not be determined whether they were fertilised. However, they were at most one day of age, since there was no embryo visible. All of the chosen eggs lacked blood vessel networks, which is an additional indicator for a low developmental stage. The mean percentual lipid content was higher in eggs than in carcasses. Also, variability was much higher in juveniles (4.70% ± 3.59) than in eggs (8.10% ± 1.66).

3.1.2. Concentrations

The concentrations of OCs and CPF in juvenile carcasses and eggs are presented in Table 6. PCBs and pp'-DDE were the dominant compounds among all OCs analysed. The residue pattern in both samples followed the order of pp'-DDE > PCBs > pp'-DDT > HCB > HCHs. Heptachlor, heptachlor epoxide A, α- and δ-HCH, pp'-DDD, op'-DDT, endrin and aldrin were below the limit of detection. The quantities of β- and γ-HCH, CHLs, dieldrine, and endosulfan 1 were very small. In one carcass, there was an exceptionally high amount of CPF of 6.04 µg/g wet weight or 248.12 µg/g when expressed as lipid weight. The high value of lipid weight was due to the small amount of adipose tissue in this bird.

The highly chlorinated PCB congeners 153, 138, 180, 170, and 118 were most abundant in both sample types. In eggs they followed the order of 153 > 138 > 180 > 170 > 118, whereas in juveniles, the levels of PCB118 were higher than those of PCB180 and PCB170. In both sample types, the congeners 52, 28, 101, and 149 had the lowest concentration, and as a matter of fact PCB52 was completely absent from all juveniles.

Within the group of HCHs, β-HCH was most abundant (n = 4 juveniles, n = 8 eggs) with mean lipid weight of 7.20 ng/g (± 6.62) lipid weight in eggs, and 35.17 ng/g (± 32.73) in juveniles. In the latter γ-HCH was below the limit of quantification (n = 2) and the mean concentration in eggs was 5.09 ng/g (± 2.09) lipid weight. Similar to the group of HCHs, there was only a slight contamination of chlordanes. *Trans*-nonachlore, oxychlordane, and *trans*- and *cis*-chlordane were only sporadically present in carcasses (n = 1, for each), and in eggs only low quantities of *trans*-nonachlore were found (n = 6).

Table 6. Levels of PCBs, organochlorine pesticides, and CPF in juvenile carcasses and unhatched eggs (lipid weight). PCBs = total PCBs; HCHs = α + β + γ isomers; CHLs = oxychlordane + *trans*-chlordane + *cis*-chlordane + *trans*-nonachlore; HCB = hexachlorbenzene; H.e.B = heptachlor epoxide B; CPF = chlorpyrifos.

Sample	PCBs	pp'-DDT	pp'-DDE	Dieldrine	HCHs	CHLs	HCB	H.e.B	Endosulf. 1	CPF
Juveniles										
1	4471.4	10.4	2235.9	nd[a]	7.8	nq[b]	39.3	10.0	nd	32.0
2	964.6	1.9	1594.9	nd	nd	nd	10.1	nd	nd	13.5
3	5923.0	45.5	18952.8	nd	7.2	nq	nq	nd	nq	255.9
4	2065.1	nd	799.3	nd	nq	nd	nq	nd	nd	76.4
5	4872.4	52.9	12412.9	nd	nd	14.0	75.6	nd	nd	nd
6	326.4	1.8	1367.6	nd	nd	nd	nq	nd	nd	nd
7	2306.4	4.6	3457.8	nd	22.9	nd	14.5	24.5	nd	852.1
8	8023.9	7.6	5462.0	nd	nd	nd	26.1	nd	nd	16.8
9	3975.7	32.3	7577.6	12.9	nq	7.2	137.4	nd	27.4	97.3
10	1328.5	22.8	13748.8	nq	nd	nd	nd	nq	nd	1676.7
11	3267.9	nd	1967.1	nd	nd	nd	24.4	nd	nq	nd
12	2464.7	5.6	1148.9	nd	nq	nd	nd	nd	nd	248116.3
Eggs										
1	1762.8	nd	3461.7	nd	nd	16.9	nd	17.9	nd	nd
2	1131.3	21.0	1715.7	nq	94.1	nd	17.6	nq	nd	nd
3	997.6	33.4	6431.3	nd	37.3	nd	19.5	6.8	nd	nd
4	6130.0	53.7	8582.0	100.0	42.3	18.1	66.9	48.3	nd	nq
5	1396.5	nd	3809.5	nd	nd	14.0	16.0	12.1	nd	12.5
6	19308.8	12.7	3838.0	nd	5.9	nd	70.8	nq	nd	nq
7	1024.8	6.9	1656.7	nd	40.0	nd	15.0	nq	nd	19.6
8	1982.5	103.5	18126.6	230.4	nd	11.6	19.0	18.1	nd	11.3
9	20101.3	118.5	19090.5	39.2	6.3	6.7	29.2	16.1	nd	nd
10	3356.2	15.4	14458.9	nd	4.4	nd	25.5	nd	nd	nq
11	2164.6	47.6	6006.6	nd	75.7	nd	29.9	1036.5	nd	7.9

All values expressed as ng/g lipid weight; [a]not detected; [b]not quantified

The pp'-DDT/pp'-DDE ratio was significantly higher in eggs than in juveniles (Wilcoxon Rank Sum test, $z = 2.408$, $p = 0.016$) (Fig. 2a). This implies that there is a relevant degradation of pp'-

DDT during this short period of development. Because HCHs were only sporadically present in small amounts, the ratios could not be calculated. Similarly, it was not possible to calculate the ratio between heptachlor and its degradation products, since heptachlor was completely missing in both sample types.

There was a strong variability of pesticide concentrations within the samples. In eggs, PCB concentrations ranged from 1 to 20 µg/g lipid weight. Likewise, the highest concentration of pp'-DDE and pp'-DDT was 11 and 17 times higher than the lowest, respectively.

Table 7. Mean, median and SD of PCBs, organochlorine pesticides, and CPF in juvenile carcasses and unhatched eggs expressed as wet weight. PCBs = total PCBs; HCHs = α + β + γ isomers; CHLs = oxychlordane + *trans*-chlordane + *cis*-chlordane + *trans*-nonachlore; HCB = hexachlorbenzene; H.e.B = heptachlor epoxide B; CPF = chlorpyrifos.

	PCBs	pp'-DDT	pp'-DDE	Dieldrine	HCHs	CHLs	HCB	H.e.B.	Endosulf. 1	CPF
Juveniles										
Mean	113.2	0.5	273.7	0.0	0.1	0.1	0.9	0.1	0.1	551.9
Median	97.2	0.2	222.1	0.3	0.0	0.0	0.5	0.3	0.0	2.6
SD	63.8	0.5	242.8	0.2	0.2	0.1	1.3	0.2	0.3	1877.0
Eggs										
Mean	428.6	3.8	886.8	7.2	2.4	0.9	2.2	8.3		0.7
Median	154.3	2.4	570.8	5.1	0.6	1.0	1.9	1.1		0.6
SD	591.1	3.6	808.5	7.8	3.2	0.3	1.3	23.0		0.6

All values expressed as ng/g wet weight

3.1.3. Differences of contamination levels in juveniles and eggs

The concentrations of CPF and endosulfan 1 were significantly higher in carcasses (n = 12) (Wilcoxon Rank Sum test, z = -2.278, p = 0.023, and z= -2.002, p = 0.045, respectively) (Fig 2c and g) than in eggs (n = 11). On the other hand, contamination of HCHs and heptachlorepoxide B was higher in eggs (z = 1.760, p = 0.078, and z = 2.826, p = 0.005, respectively) (Fig 2b and 2h). There was no significant difference of the concentrations of the other pesticides and ∑PCBs between the two samples (Fig 2a, d, e, and f). Within the PCBs, only congener 101 was represented in significantly higher amounts (z = 2.577, p = 0.01) in eggs.

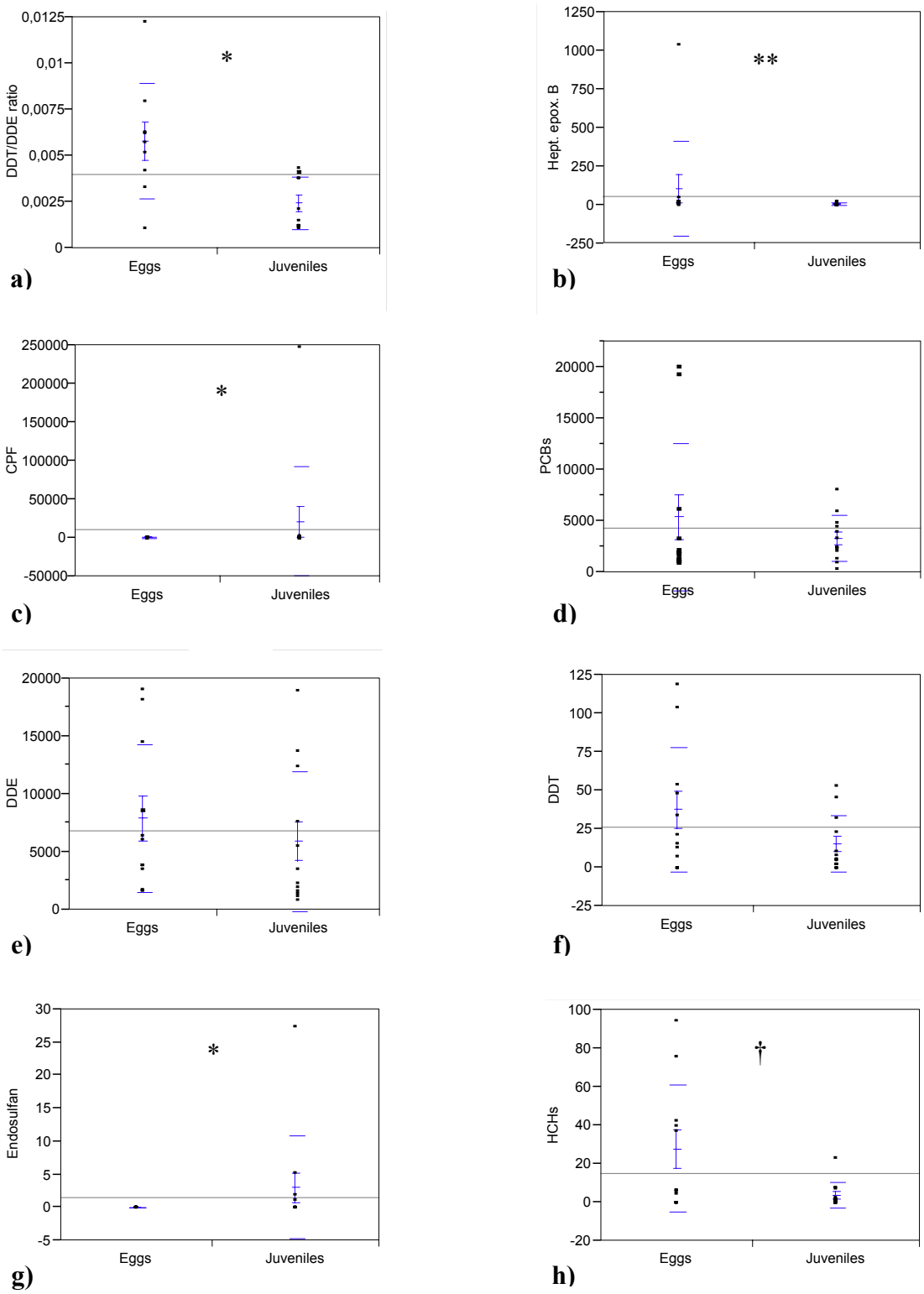

Fig. 2. (a) Difference of pp'-DDT/pp'-DDE ratio between juveniles and eggs. **(b) – (h)** Concentrations of organochlorine pesticide and PCB in juveniles and eggs (ng/g lipid weight). Mean ± SD and 95% confidence interval are indicated. † = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$.

Table 8. Mean pollutant concentrations in juveniles and eggs and significance of the difference. The distributions of most pollutants were skewed. Concentrations expressed as lipid weight.

	Juvenile carcasses (n=12)			Unhatched eggs (n=11)			
Organochlorine Contaminant	**Arithmetic mean**	**Standard deviation**	**Number detected**	**Arithmetic mean**	**Standard deviation**	**Number detected**	**Significance of difference**
PCBs	1333.0	893.4	12	2002.9	2924.4	11	
pp'-DDT	15.4	18.6	10	37.5	40.5	10	
pp'-DDE	5893.8	6029.7	12	7925.2	6396.8	11	
Dieldrine	1.4	3.8	2	34.0	72.1	4	
HCHs	3.7	6.6	7	27.8	33.1	8	†
CHLs	2.1	4.3	4	6.5	7.4	6	
Hexachlorbenzene	28.1	40.8	10	28.1	21.7	10	
Heptachlor epoxide B	3.1	7.3	2	105.9	308.9	10	**
Endosulfane I	3.0	7.8	4	0.0	0.0	0	*
Chlorpyrifos	20928.1	71547.5	9	6.1	6.4	7	*

† $p < 0.1$; * $p < 0.05$; ** $p < 0.01$

3.1.4. Correlations

In both sample types, the distribution of most pollutants was strongly skewed due to outliers, which in most cases did not overlap in single sample units. Thus, the extremes were distributed over the whole sample. Nevertheless, there were some correlations between pollutants (Spearman Rank test), which are presented in the correlation matrix in table 9.

In eggs (n = 11), strong correlations were found between total PCBs and HCB ($r_s = 0.72$, $p < 0.05$), pp'-DDT and pp'-DDE ($r_s = 0.77$, $p < 0.02$), trans-nonachlore and heptachlor epoxide B ($r_s = 0.76$, $p < 0.02$), and pp'-DDT and dieldrine ($r_s = 0.75$, $p < 0.02$). The only strong correlation in carcasses (n = 12) was between pp'-DDT and pp'-DDE ($r_s = 0.83$, $p < 0.01$). There were modest correlations between ∑PCB and HCB ($r_s = 0.61$, $p < 0.05$), and pp'-DDT ($r_s = 0.60$, $p < 0.05$).

There were strong to very strong correlations between PCB congeners 180, 170, 153, 156, 138, 118, and total PCBs ($r_s = 0.82$-1.0, $p < 0.01$) in both samples. In addition, there was a modest but not significant correlation between egg weight and total PCBs ($r_s = 0.40$, $p > 0.1$) and between egg weight and HCB ($r_s = 0.54$, $p < 0.1$).

Table 9. Correlation matrix (Spearman Rank test) for a selection of contaminants in juvenile carcasses and unhatched eggs

	pp'-DDE	pp'-DDT	HCB	Endosulf 1	β-HCH	Dieldrine	H.e.B	*t* -nonachl.
Juvenile Carcasses								
pp'-DDT	0.834 **							
HCB	0.340	0.361						
Endosulfane 1	0.179	0.046	0.238					
β-HCH	0.036	0.236	0.109	0.121				
PCBs	0.539†	0.599 *	0.613 *	0.266	0.283			
Unhatche Eggs								
pp'-DDT	0,770 *							
HCB	0.555 †	0.556 †						
Endosulfane 1								
β-HCH	-0.220	0.331	0.321					
Dieldrine	0.516 †	0.749 *	0.200		0.106			
Heptachlor epox. B	0.336	0.456	0.245		0.000	0.400		
trans -nonachlore	0.219	0.110	-0.086		-0.346	0.409	0.763 *	
PCBs	0.609 †	0.424	0.727 *		-0.101	0.326	0.291	0.267

† $p < 0.1$; * $p < 0.05$; ** $p < 0.01$

3.1.5. Contamination patterns

The contamination patterns of PCBs in eggs were very similar to those in juvenile carcasses. Although there were differences in the total PCB concentrations between sample units, the relations of the single congeners to each other were very consistent throughout both sample types. Since the quantities of total PCBs between juveniles and eggs did not differ significantly, a Wilcoxon Rank test was performed to test for correlations between single congeners when both samples were combined (n = 23). As in the separated tests, strong to very strong correlations between PCB congeners 180, 170, 153, 156, 138, 118, and total PCBs (r_s = 0.88-0.99, $p < 0.01$) were found. Hence, in the Swiss Hoopoe population these congeners have a very consistent relation to each other, which is independent of the total PCB concentration of the sample units.

Unlike PCBs, the pesticides did not show any clearly visible contamination pattern, apart from relatively constantly occurring contaminations of pp'-DDE, pp'-DDT, and HCB throughout all samples. Dieldrine, chlordanes, heptachlorepoxide B, and β-HCH occurred more frequently in eggs than in juveniles. In contrast, occurrence of CPF in eggs was low, and endosulfan 1 was completely missing.

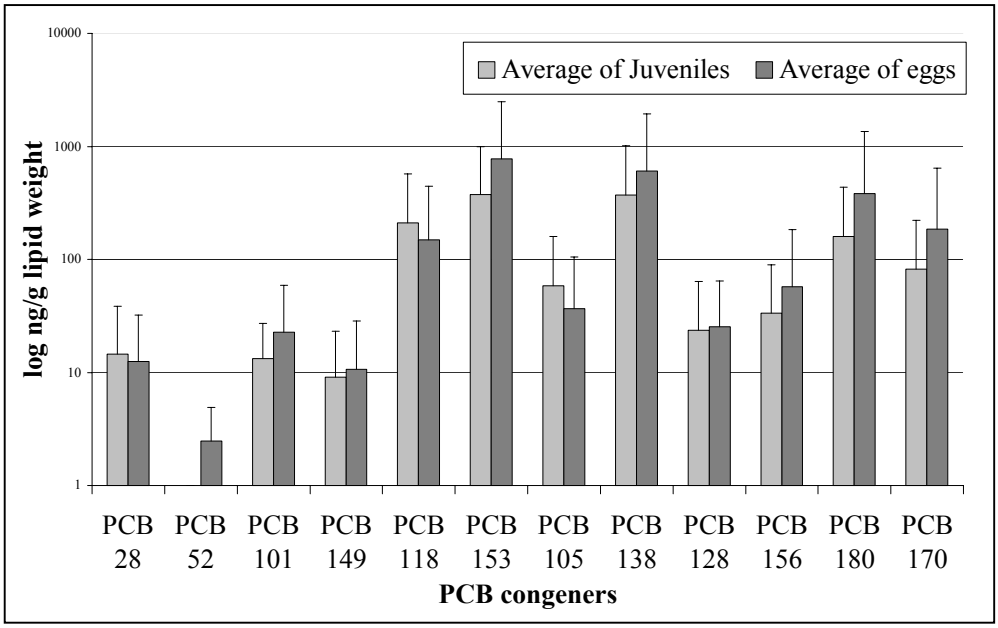

Fig. 3. Levels of the twelve PCB congeners with SD on a log scale. The congeners 153, 138, 179, and 180 had proportionally always the highest contamination levels throughout both samples.

3.2. Chlorpyrifos uptake in Valais

3.2.1. General condition of juveniles

None of the birds showed any clearly visible sign of toxication after CPF treatment, such as tremors, lethargy, wing droop, or difficulties of muscular coordination (Bennett 1989). The juveniles were between 10 and 25 days of age, with many of them close to fledging at the second measurement. Average chicken body mass in the three broods was 70,12 (± 2.14) g, 85.33 (± 11.02) g, and 67.75 (± 2.02) g, respectively.

3.2.2. Pre-exposure BChE activity

Pre-exposure samples had a mean BChE activity [expressed as µmol/(min*ml)] of 3,97 (± 0.59), 3.79 (± 0.28), and 5.47 (± 0.42) in the three broods, respectively. There was little variation within nests. Mean activity of all pre-exposure sample units (n = 17) was 4.41 (± 0.92).

3.2.3. Inhibition

The inhibition patterns in post-treatment measurements showed a higher variability than in pre-treatment measurements (mean post-treatment activity expressed as µmol/(min*ml) in the three broods were 2,51 (± 1.68), 3,77 (± 0.55), and 4.19 (± 1.14), respectively). This is most likely due to an unequal uptake of contaminated Mole crickets by the juveniles.

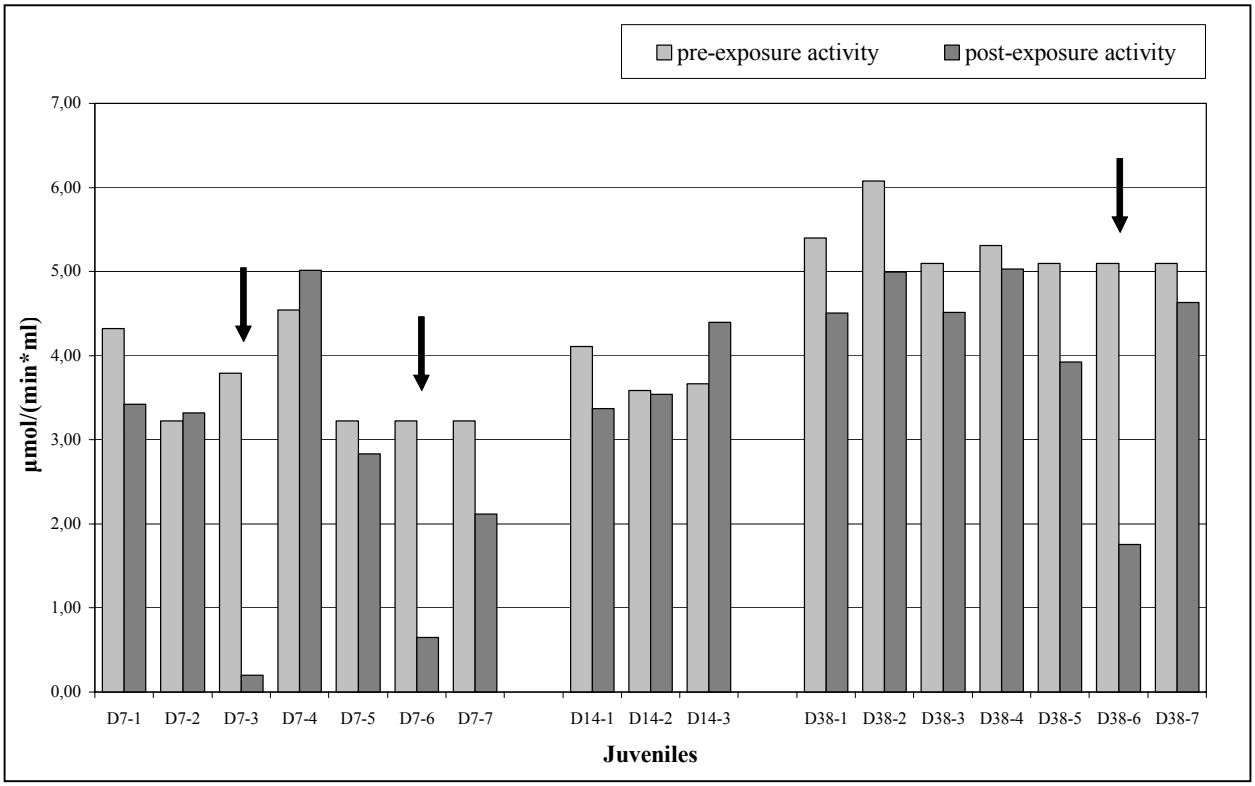

Fig. 4. Serum BChE activity before and after chlorpyrifos exposure. Activity is expressed as µmol of butyrylthiocholine iodide (BTCI) converted per minute and millilitre of serum. Arrows indicate juveniles whose post-exposure activity of BChE decreased to levels above the threshold of natural daily variation (see Methods for more details).

Three juveniles experienced a decrease of BChE activity that was far above the threshold of natural daily variation of 30% (94.8%, 79.9%, and 65.6%, see Fig. 4). Mean Inhibition was highest in clutch D7 with 32.7% (± 40.4), followed by 21.3% (± 20.4) in D38. The effect in clutch D14 was balanced with a mean inhibition of -0.2% (± 18.9), which means that the activity was almost identical in both treatments.

The overall post-treatment activity of all 17 juveniles was by 22.2% significantly lower than pre-exposure activity (Table 10). As expected, the factor nest had a significant effect on the levels of inhibition. The residuals were normally distributed (Shapiro-Wilk W Test, $W = 0.972$, $p = 0.598$).

Table 10. Effect of the factors treatment, nest, and individual nested with nest on the activity of serum BChE. A restricted maximum likelihood estimation (REML) was performed. The factors nest and treatment had significant effects.

Source of Variation	N parameters	df	Sum of squares	F Ratio	Probability
Treatment (fixed)	1	1	7,4449	10,2057	0,0056
Nest (random)	3	2	9,8494	6,7509	0,0089
Individual[nest] (random)	17	14	7,7778	0,7616	0,6927
Error		16	11,6718		
C. Total		33	56,7523		

3.3. Theoretical approach

We calculate the accumulation profiles of CPF over five days, assuming degradation of 65% within 24 hours ($T^{1/2}$=18h) (Abdel-Rahman *et al.* 2002). In the case of multiple daily uptakes of contaminated Mole crickets, the model included a gap of 6 hours between each ingestion. This period results in a degradation of 20%.

A bait of average weight contains 0.38 mg CPF. This leads to a contamination of 380 µg/g in Mole crickets weighting one gram, the average biomass of this prey fed to Hoopoe nestlings. Chicks of 30 g (the average biomass of the carcasses analysed in this study) consuming one contaminated Mole cricket might then experience CPF levels of 5 µg/g after absorption. The younger and therefore the lighter a nestling, the higher the contamination levels. Thus, in small chicks weighting 10 g the uptake of the same Mole cricket might result in 15 µg/g. The amounts may rise to dramatic levels when two or more contaminated items are consumed on the same day. The accumulation profiles for five days show that due to the daily degradation of 65% the concentrations increase faster in the first days whereas later on the steps become marginal. Nonetheless, under extreme conditions, where a juvenile of a weight of ten gram receives on average three Mole crickets per day, the concentrations might approach levels of 80 µg/g.

Since digestion and absorption of Mole crickets takes several hours, the actual levels of CPF within the Hoopoes blood serum and tissues may actually be lower than predicted by the model. The reason is that CPF is degraded as soon it enters the bloodstream; thus as the last portion is digested, a fraction of the previously absorbed CPF molecules is already metabolised.

Table 11. Theoretical uptake of CPF in µg/g wet weight of juveniles. As half-life of CPF in Hoopoes, the value of Abdel-Rahman *et al.* (2002) for rats was taken ($T^{1/2}$ = 18h), which resulted in 65% degradation within 24 hours. The model included a gap of six hours between uptakes of contaminated Mole crickets within the same day.

	Bird Weight (g)			
Days	**10**	**20**	**30**	**40**
1 contaminated Mole cricket per day				
1	15.00	7.50	5.00	3.75
2	20.25	10.13	6.75	5.06
3	22.09	11.04	7.36	5.52
4	22.73	11.37	7.58	5.68
5	22.96	11.48	7.65	5.74
2 contaminated Mole crickets per day				
1	27.00	13.50	9.00	6.75
2	40.50	20.25	13.50	10.13
3	47.25	23.63	15.75	11.81
4	50.63	25.31	16.88	12.66
5	52.31	26.16	17.44	13.08
3 contaminated Mole crickets per day				
1	36.60	18.30	12.20	9.15
2	58.56	29.28	19.52	14.64
3	71.74	35.87	23.91	17.93
4	79.64	39.82	26.55	19.91
5	84.38	42.19	28.13	21.10

4. Discussion

4.1. Concentrations of OC contaminants

A collection of dead individuals rarely represents a random sample of a target population. However, the concentrations of organochlorines (OCs) and other contaminants have been found to vary far more among than within broods (Newton and Bogan 1978, Newton *et al.* 1989). Because most individuals stemmed from otherwise successful broods, our sample probably

mirrors the concentrations present in alive relatives of the dead individuals. We therefore believe that the deviation of our sample from the population mean was negligible.

Feathers were removed prior to the analysis of whole body homogenates. Since feathers account for less than four percent of the total body burden (Tanabe *et al.* 1998), their elimination from analysis did not have a significant effect on OC concentrations. Consequently, omitting feathers did not affect the comparison between carcasses and unhatched eggs.

The contamination levels of HCHs, CHLs, HCB, heptachlorepoxide B, endosulfan 1, and dieldrine were very low between 0 and 0.1 µg/g. It is therefore very unlikely that their individual and potentially additive or synergistic effects may have been strong enough to display toxicity. Moreover, some of these pesticides may even exert antagonistic effects, such as β- and γ-HCH, with β-HCH counteracting the effect of the gamma isomer at equal or higher doses (Hayes *et al.* 1982). Trans-nonachlore and heptachlorepoxide B were more often detected in eggs than in juveniles, which might reflect the biotransformation of these pesticides during egg and chick development.

The levels of pp'-DDE and total PCBs were clearly elevated. In eggs, there were two outliers with PCB concentrations of 1.8 and 1.2 µg/g wet weight. These quantities are within the range of levels associated with detrimental reproductive effects in field and laboratory studies in great blue herons (1.5 µg/g) (Elliott 1989) and with decreased hatching success in chicken (1-5 µg/g) (reviewed in Hoffman *et al.* 1996). At sublethal concentrations, PCBs may provoke various chronic effects on the endocrine system (Colborn *et al.* 1993). Endocrine disruption may actually affect reproductive behaviour because the breeding cycle is hormonally controlled. Effects of OCs on bird behaviour include decreased parental attentiveness, impaired courtship behaviour, and subtle neurological effects such as impaired avoidance behaviour (Farner and Wingfield 1980). PCB concentrations in eggs appear to approximate or be less than maternal concentrations when normalized to lipid content (Lincer and Peakall 1973; Tanabe *et al.* 1986). Therefore, the PCB burden in breeding females probably reflected the levels in their eggs. In a comparative field study in glaucous gulls, a positive relationship was found between absence from the nest site and PCB burden. Nest site attentiveness was thus affected by exposure to PCBs. Concentration of eight PCB congers in blood samples generally ranged from 0.05 to 1 µg/g wet weight (Bustnes *et al.* 2001). In the eggs of our study the levels of these eight congeners fall within that range (0.03 to 0.7 µg/g wet weight). However, it should be noted that contamination levels in eggs only approximate the burden of adults when expressed in lipid weight. Since adults usually have a lower lipid content than eggs, their burden appeared smaller when expressed in wet weight. On the other hand, the average body mass of foraging birds can decrease for about 20% from pre-

breeding to late chick-rearing. During this period, the mean concentration of PCBs in brain tissue may rise to several times that of the average body burden. This increase can be attributed to a redistribution of PCBs from utilized fat reserves to metabolising organs (Henriksen *et al.* 1996). Therefore, although the general body burden in adult Hoopoes might be below thresholds associated with behavioural effects starvation during chick rearing may raise the brain levels to toxic concentrations. Several other studies have observed altered behaviour provoked by increased PCB and pp'-DDE burden (Risebrough 1994; Fyfe et al. 1976) but did not quantify residue levels.

The three highest concentrations of pp'-DDE in eggs (1.75, 1.45, and 1.42 µg/g wet weight) approached concentrations known to cause shell thinning, but were probably low enough to have no effects on egg-breakage or on the number of young birds per clutch (Newton and Wyllie 1992; White *et al.* 1973; Fyfe *et al.* 1976). The highest levels found in juvenile carcasses were 0.54 and 0.51 µg/g wet weight. Lethal brain concentrations of pp'-DDE measured in four bird species ranged from 305 to 694 µg/g in dead birds, whereas the concentrations were independent of the species (Stickel *et al.* 1984). Hence, the levels found in juvenile carcasses and estimated for adults were several orders of magnitude lower than those associated with lethality.

Problems may possibly take place during migration, since fat reserves are used as a source of energy during migration, with contaminants stirred in fat deposits being mobilized to other body tissues and blood (Tanabe *et al.* 1997; Bogan and Newton 1977; Henriksen *et al.* 1996). As a result, PCBs and pp'-DDE tend to accumulate in the adipose tissue of the brain, thus potentially reaching toxic concentrations during migration and chick rearing. In addition, PCBs are believed to increase the effect of pp'-DDE (Newton and Bogan 1974; Dirksen *et al.* 1995), although this has never been properly quantified.

4.2. Contamination patterns and origin of pollutants

The results show that most OC concentrations are equal or stronger in eggs than in chick carcasses. Therefore, exposure to these contaminants most likely occurs in the winter habitat, such as the Sahel. The low DDT/DDE ratio in juveniles and eggs suggests that there was no recent application of pp'-DDT in the place of origin. The higher levels of pp'-DDE are probably due to the massive application of DDT in the winter habitats in the seventies and eighties and by the longevity ($T^{1/2} \leq 20$ years) of this contaminant. The same might be true for most other contaminants (HCHs, endosulfan, CHLs, HCB, heptachlorepoxide), since all had very low levels.

This reflects the fact that the use of OCs is very restricted in the Sahel (Mullié, pers. comm.). There, the only OCs being legally used are endosulfan, lindane and dicofol in very low quantities. However, occasionally stocks of obsolete dieldrine seem to subsist (Mullié, pers. comm.). The traces of heptachlorepoxide B in most eggs and some juveniles might result from the application of heptachlor, the parent compound, in the Sahel until recently. At present, concentrations of DDTs and HCHs in Indian and Asian birds (Tanabe *et al.* 1997; Minh 2002) seem to be higher than those in European birds.

The correlations between pollutants within samples probably reflect a heterogeneous and clustered distribution of these chemicals. The clustering may be on a regional scale within a contamination site or on a global scale, where the different winter habitats vary in contamination levels. Alternatively, clustering may occur due to regionally different prey items of dissimilar trophic levels. However, the correlations between PCBs, DDT, and HCB and between DDT and dieldrine suggest a clustering of these substances in certain regions. In some cases this may lead to a stronger exposure of these birds to high doses of pesticide and PCB combinations. On the other hand, it is known that the Hoopoe populations are widely but thinly distributed in the Sahel, the main winter habitat. Therefore, clusters of high pollutant concentrations should affect only a small fraction of these populations and may therefore not be relevant for fitness.

There were strong outliers of pp'-DDE and PCBs in both samples. Because the Swiss Hoopoe population may have several winter habitats, which remain unknown, it is not possible to localize the source of contaminations. Also, the variability of contamination levels could indicate a great heterogeneity of wintering grounds. In Africa, illegal dumping of transformers from electricity plants may be a major source of PCB contamination (Mullié, pers. comm.). In Europe, escape of PCBs into the environment from products of earlier years (mainly transformers, hydraulic systems, and printer's inks) is expected to continue for some years, and exposure of birds to PCBs will continue (Pain *et al.* 1999).

The patterns of PCB contamination were similar both in eggs and juvenile carcasses. PCBs 153, 138, 180, 170, and 118 accounted for 90% of the twelve measured congeners, their relative proportions were constant throughout. The consistence of the patterns between juveniles and eggs provides no support to the general assumption that lower chlorinated congeners have a faster metabolism than those of higher chlorination. Only PCB 101 showed significantly higher concentrations in eggs than in juveniles.

Endosulfan 1 and chlorpyrifos had significantly higher concentrations in juveniles than in eggs, which suggests recent application in Europe. However, endosulfan 1 in carcasses occurred only

sporadically and at very low levels. Hence, this pesticide surely does not represent a threat to our Hoopoe population.

4.3. CPF contamination

The concentrations of CPF in eggs and carcasses hardly reflect the actual contamination pattern, since in organisms there is a considerable degradation of OP compounds. Whereas post-mortem exposure ranged from 15 to 35 days in eggs, it was ranged from only several hours to roughly one day in chick carcasses. Yet it should be noted that eggshell and yolk may protect the pesticides from bacteria and hydroxyl radicals.

7 out of 11 eggs contained traces of CPF. Since adult Hoopoes may stay up to one month in the breeding grounds prior to egg laying, it seems unlikely that these concentrations originate from contamination of abroad. 8 out of 12 juvenile carcasses contained very low levels of CPF, which may be interpreted as remaining concentrations of an earlier contamination. It seems unlikely that these birds died because of lethal CPF contamination, since rather high concentrations are needed to reach sufficient toxicity. Consequently, there would still be elevated quantities in freshly collected carcasses. However, sublethal concentrations may have led to physical weakness and reduced food consumption (Grue C.E. *et al.*, 1997), which then could have led to a poorer ability to compete for food against other nestlings.

Our field experiment represented a worst-case scenario for unravelling whether there is a transfer of CPF from Mole crickets to Hoopoes after application of cortilan®. In 38 gardens we applied quantities that are generally used by their owners (2 to 17kg/ha), which were up to three times higher than recommended by Maag AG (2 to 5 kg/ha), the producer this pesticide formulation. Synchronized treatment was considered as realistic, at least for a first application in the season, because Mole crickets tend to appear more or less at the same time everywhere. Later in the season CPF applications become more asynchronous. However, since the baits persist up to two weeks after application (Fischer *et al.* 1995; Locher, pers. comm.) and treatments occur frequently throughout the whole season, total field concentrations under normal circumstances are expected to approximate our worst-case levels.

The results of our experiment suggest that there is a transfer of CPF via Mole crickets to the nestlings. There was no measurable relation among the distance between nests and treated gardens and the strength of inhibition, which might be explained by the small number of broods.

The brood in the core zone of CPF treatment experienced the strongest butyrylcholinesterase (BChE) inhibition (32%) with a very strong inhibition in two nestlings (94.8% and 79.9%).

Astonishingly, pre- and post-treatment BChE activity of a second brood at the border of that zone did not differ, probably due to a preference for an uncontaminated area. The mean BChE activity of the third brood at the far end of the study area was the second highest (20%). All nestlings had consistently lower post-treatment level with a notable inhibition in one bird (65.6%). The overall picture shows that there is a general tendency to lower post-treatment levels with three cases of strong inhibition. Since the initial recovery of cholinesterase activity is very rapid, with a recovery of up to 50% within 24 hours (Fleming 1981; Thompson *et al.* 1991; Fossi *et al.* 1994), the nestlings with low inhibition might already have partly recovered from a possible contamination initiated by the experiment.

None of the chicks showed sings of alteration in condition and behaviour between pre- and post-treatment sampling, which suggests that Hoopoe nestlings can support high BChE inhibition. This could be due to a potential buffer effect of BChE and other type "B" esterases in blood serum, with a consequent slowing and decrease of the inhibitory effect on acetylcholinesterases in the brain (Russel and Overstreet 1987; Thompson 1993). The mean pre-exposure activity of BChE was with 4.4 µmol/(ml*min) very high and lied in the upper midfield when compared to other species (Thompson 1993).

According to our calculations, Mole crickets consuming one bait experience a body burden of approximately 375 µg/g wet weight. When transferred to a juvenile weighting 30 g, this might result in a contamination level of about 5 µg/g wet weight. Our model suggests that consecutive uptake of one contaminated Mole cricket per day does not lead to strong additive effects, since CPF is continually metabolised. In mature male hybrid red-legged partridges oral administration of 9 µg/g CPF produced serum BChE inhibition of 69, 81, and 56% at one, four, and 24 hours after dosing, respectively (Johnston *et al.* 1994). Similarly, in adult chicken hens that were dosed daily for 20 days (10 µg/g CPF/day in 2 ml corn oil) plasma BChE activities were inhibited by 49-80% when compared to contemporaneous controls (Richardson 1993). Similar results were obtained by Lari *et al.* (1981) when feeding azinphos-methyl to japanese quail. Applying these dose-response profiles to the Hoopoe (9-12 µg/g CPF), the inhibition of approximately 95% in one juvenile could be caused by the ingestion of one or two contaminated Mole crickets.

Younger chicks receive less Mole crickets than old ones (Schaad 2002). Therefore, their contamination risk should be lower. On the other hand, smaller nestlings experience stronger CPF burdens after a single ingestion. Repeated uptakes might then potentially lead to death, since younger animals tend to be more sensitive to OPs than adults (Brimijoin *et al.* 1999). Mallards are most sensitive when exposed to CPF on day seven after hatching, after which their sensitivity decreased to 1.7- and 2.8-fold less on the days 30 and 180 (Hudson *et al.* 1972). Similar relations

have been observed in red-winged blackbirds and European starlings (Meyers *et al.* 1992; Wolfe *et al.* 1998).

Continuous sporadic uptake of CPF may provoke sublethal effects in Hoopoes. Toxic effects at higher sublethal concentrations are reduction in egg production, eggshell thickness, egg weight, diminished clutch size, higher incubation times, hatchling deformities, hypothermia, and reduction in body weight (Gile *et al.* 1986; Grue *et al.* 1997; Martin 1990). Grue and Shipley (1984) observed a 50% decrease in singing and displaying activity by captive male European starlings within 4 hours after being given 2,5 µg/g dicrotophos, an organophosphate. Neurological findings suggest, that even low CPF burdens may cause severe effects such as depressed DNA synthesis throughout the brain (Whitney *et al.* 1994) and net loss of neurons in the forebrain (Campbell *et al*. 1997) at doses of 1 and 2 µg/g, respectively. Furthermore, CPF may be able to disturb multiple neurotransmitter pathways in the developing central nervous system (Song *et al.* 1997). However, these neurological data are drawn from experiments with rats and therefore must not hold true for bird species.

It has been reported that birds living in nearly permanently contaminated areas show habituation to OPs (McInnes *et al.* 1996). This suggests that bird species might be able to compensate for a large extent the effect of pesticides at sublethal levels. In addition, several studies have shown that birds are able to distinguish between contaminated and normal food items (Bennett 1989; Conover 1985; Kononen *et al.* 1986). Hoopoes could thus have the potential to adapt to continuous exposure to CPF. Further investigations are needed to assess the actual risks faced by the Hoopoe population in Valais. These should include sequential measurements of BChE activity in adults and juveniles after treatment of several representative regions. Analysis of the brain AChE and serum BChE activity of fresh carcasses would reveal the significance of BChE inhibition. Moreover, residue analysis in Mole crickets collected from nestlings should give information about actual CPF burdens in Hoopoes.

5. Conclusions

Considerable amounts of pp'-DDE and PCBs occurred in some eggs and carcasses. PCB levels found in two eggs were close to thresholds inducing reproductive effects, whereas the quantities of pp'-DDE exhibited in three eggs approached concentrations known to cause shell-thinning in raptors (Newton and Wyllie 1992).

Our field experiment suggests that Hoopoes living in treated areas may occasionally consume contaminated Mole crickets, potentially leading to critical body concentrations theoretically reaching up to 15 µg/g. Yet, given that the degradation of CPF is rather fast, an accumulation bearing lethal consequences might be rare and presumably limited to young chicks. In the end, negative effects provoked by sublethal concentrations might be compensated by behavioural and/or physiological adaptations.

The fact that the local population has one of the greatest reproductive successes in Europe (Vivaldi 1999; Oeschlaeger 2002; Arlettaz 2002a; Arlettaz 2002b; Steiner 2003) suggests that Valais Hoopoes probably do not suffer from direct lethal effects of pesticides and PCBs, although chronic stress might occur.

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