RELATIONSHIP BETWEEN ORGAN AND FEATHER PERFLUOROOCTANE SULFONIC ACID LEVELS TOGETHER WITH BIOCHEMICAL EFFECTS IN EXPOSED ZEBRA FINCHES (*Taeniopygia guttata*)

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Introduction

Although perfluorooctane sulfonic acid (PFOS) has been produced and used for more than 50 years, the effects and exposure to wildlife has only recently been investigated. However, most of these studies have focused on mammals. Only a few studies have reported the levels of exposure of birds in the wild.[1,2,3] These studies mainly focused on top predators in the food chain, for example sea eagles and cormorants. Studies dealing with the effects of PFOS on birds have only been performed on Northern Bobwhites and Mallards[4]. The only study describing both the exposure and effects of PFOS passerines was the study done on Blue and Great Tit nestlings near a fluorochemical plant in Antwerp, Belgium[5]. This study showed that the levels of PFOS in the smaller passerines, which are lower on the food web, were higher than those found in the larger birds higher in the food web. Therefore more data should be found on both the effects and exposure to PFOS in the smaller birds. The data already found in the wild birds should also be corroborated with controlled laboratory experiments.

Feathers have successfully been used in the biomonitoring of pollutants, for example metals and some organic pollutants.[6,7] There are several advantages to using feathers as indicators of environmental pollution. The most important is that feathers are non-invasive and can thus even be collected from more scarce or vulnerable species. The use of contaminant levels in feathers have been demonstrated as good non-destructive indicators of exposure, since they can be collected routinely without much stress to the birds. Currently no information is available on the use of feathers as reliable indicators of PFOS exposure.

The first aim of this study is to determine the relationship between the levels of PFOS in the liver and feathers of a small passerine, the Zebra Finch (*Taeniopygia guttata*). This will be done by exposing the birds in the laboratory to various concentrations of PFOS, through both food and water. The second aim of this study is to determine several biochemical endpoints of PFOS on the Zebra Finch. This will further our knowledge on the effects of PFOS on passerines.

Materials and Methods

Thirty adult Zebra Finches purchased from Aquaservice (Berchem, Belgium) were exposed to various levels of PFOS. The birds were housed in wooden breeding cages at 25 ºC for 21 days before the start of the experiment. The birds were fed commercial bird feed and water *ad libitum*. The birds were divided into 6 groups of 5 individuals each, one group being the control group. Two groups were exposed to 1 µg/g (F1) and 2 µg/g (F2) PFOS in their food. Three groups were respectively exposed to 1 mg/L (W1), 2mg/L (W2) and 5 mg/L (W5) PFOS through drinking water. The birds were exposed to these concentrations for 31 days. These concentrations were calculated using the LC_{50} value for Northern Bobwhites (*Colinus virginianus*) which is 220 ppm.[4] The LC_{50} value was divided by 10 as the Northern Bobwhites weighs about 10 times more than Zebra Finches. This value was then divided by 10 again to get the highest exposure concentration. The food was prepared according to the method of Seacat et al, with modifications.[8] At the start of the exposure the two most inner primary wing feathers of each wing was removed. After the exposure the birds were killed by an

overdose of ethyldiether and then decapitated. The birds were weighed and the tarsus length was measured to determine the body condition index.[9] The liver was dissected out and weighed. The two regrown inner most primary wing feathers of each wing were pulled out to be used for the PFOS determination.

PFOS extraction was done according to the Powley method.[10] The measurement of PFOS concentrations in the liver and feathers was done using high performance liquid chromatography coupled to high-resolution tandem mass spectrometry according to Berger et al, with some modifications.[11]

The serum alanine aminotransferase activity was determined by the spectrophotometric method of Bergmeyer et al.[12] The cholesterol concentration was measured according to Allain et al and the triglyceride concentration according to the method of Spayd et al.[13,14] The serum protein content will be determined with the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany). For the determination of the haematocrit, the relative red blood cell volume will be determined after centrifugation of heparinised blood in sealed glass capillaries (2000 rpm, 5 min).

Results and Discussion

The mean hepatic PFOS concentrations for the various exposure groups were 65.5 ± 11.3 ng/g for the control and 1037.8 ± 304.7 (W1), 6884.5 ± 1568.9 (W2), 13985.2 ± 4275.5 (W5), 2558.4 ± 882.7 (F1) and 6403.3 ± 789 (F2) ng/g wet weight (mean ± SEM) for the respective exposure groups. The hepatic PFOS values in the control and W1 and F1 exposures were in the same range than those found in tits living in the vicinity of the Belgian fluorochemical plant, while those of the W2, W5 and F2 were higher.[5] The values were also much higher than those found in the liver of several wild water birds in North America and Asia.[1,3] The mean feather PFOS concentrations for the various exposure groups were 1.1 ± 0.3 ng/mg for the control and 2.5 ± 0.4 (W1), 2.6 ± 0.9 (W2), 9.2 ± 3 (W5), 2.09 ± 0.3 (F1) and 3.1 ± 0.7 (F2) ng/mg (mean ± SEM) for the respective exposure groups. There was an increase in the feather PFOS concentrations with an increase the hepatic PFOS concentrations. This shows that feathers might be used as bio-indicators of PFOS contamination.

The serum cholesterol concentrations decreased as the PFOS concentrations increased (Table 1). This corroborates the findings where similar effects were observed in exposed tits near a fluorochemical plant[5]. The serum triglyceride concentrations were lower in the exposed groups than in the control group (Table 1). This was also seen in tits observed by Hoff et al.[5] There was no visible trend for the response of haematocrit and serum alanine aminotransferase activity (Table 1). Hoff et al. did find a correlation between serum alanine aminotransferase activity and hepatic PFOS concentrations, but only between hepatic PFOS concentrations and haematocrit in one species of tit[5]. Most of the values of the biochemical endpoints were in the same range as values found for tits living in the vicinity of the Belgian fluorochemical plant[5].

Table 1. The results of the various biochemical endpoints and the haematocrit values for the various exposure groups. Values are given as mean ± SEM.

	Serum alanine aminotransferase activity (U/g protein)	Serum Triglyceride concentrations (mg/dL)	Serum cholesterol concentration (mg/dL)	Haematocrit (%)
Control	3.6 ± 0.2	396.8 ± 42.3	118 ± 8.5	47.7 ± 2.2
W1	1.8 ± 0.6	402.8 ± 70.3	104.5 ± 0.5	48.9 ± 5.1
W2	3.9 ± 2.2	396.8 ± 48.2	105 ± 20.4	49.6 ± 1.8
W5	4.2 ± 0.8	260.8 ± 36.2	108.8 ± 11.7	49 ± 2
F1	6.6 ± 1.1	221.2 ± 63.3	104.2 ± 14.4	42.3 ± 2
F2	2.4 ± 0.8	353.8 ± 21	87 ± 10.7	50.4 ± 2.6

The body condition index for the Control was 0.195 and 0.31 (W1), 0.526 (W2), 0.919 (W5), 0.354 (F1) and 0.752 (F2) for the various exposure groups. For both the two types of exposure groups the body condition index increased with the increase in PFOS exposure. This indicates that the general body condition of the birds became worse with an increase in exposure to PFOS. This was not found for tits in Belgium near a fluorochemical plant[5].

Due to this study further data on the impact of PFOS on bird health have been generated. Similar effects were seen under controlled laboratory conditions, compared to previous field studies. The extraction methods for feathers need further optimisation. The relationship between PFOS levels in feathers and liver should be quantified in further detail. In conclusion, our initial results show that feathers could become non-destructive bio-indicator of PFOS exposure.

References

1. Giesy JP, Kannan K. *Environmental Science and Technology* 2001;35:1339
2. Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP. *Environmental Science and Technology* 2001;35:3065
3. Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP. *Environmental Science and Technology* 2002;36:3210
4. Gallagher SP, Casey CS, Beavers JB, Van Hoven RL. *PFOS: A Dietary LC50 Study with the Northern Bobwhite.* Wildlife International Ltd. Project No. 454-103, 2004. Wildlife International, Minnesota, USA
5. Hoff P, Van de Vijver K, Dauwe T, Covaci A, Maervoet J, Eens M, Blust R, De Coen, W. *Chemosphere* 2005;61:1558
6. Burger J. *Revue of Environmental Toxicology* 1993;5:203
7. Dauwe T, Jaspers V, Covaci A, Schepens P, Eens M. *Environmental Toxicology and Chemistry* 2005;24:442
8. Seacat AM, Thomford PJ, Hansen KJ, Clemen LA, Elridge SR, Elcombe CR, Butenhoff JL. *Toxicology* 2003;183:117
9. Merila J, Przybylo R, Sheldon BC. *Genetic Research* 1997;73:165
10. Powley CR. *Poster presentation 25th Annual Meeting SETAC World, Portland Oregon, USA, 2004*
11. Berger U, Haukås M. *Journal of Chromatography A* 2005;1081:210
12. Bergmeyer HU, Hørder M, Rej R. *Journal of Clinical Chemistry and Clinical Biochemistry* 1986;24:481
13. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. *Clinical Chemistry* 1974;20:470
14. Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, Figueras J, Goodhue CT, LaRossa DD, Nelson RW, Rand RN, Wu TW. *Journal of Clinical Chemistry* 1978;24 :1343