A COMPARATIVE LABORATORY BREEDING STUDY WITH EGGS FROM THE COMMON TERN (*STERNA HIRUNDO*) COLLECTED AT EIGHT DIFFERENT COLONIES IN THE NETHERLANDS AND BELGIUM

Bart A.T.C. Bosveld[1], Jeff Gradener[1], Meek van Kampen[2], Tinka A.J. Murk[3], Erik H.G. Evers[4] and Martin van den Berg[1].

[1] Research Institute Toxicology, University of Utrecht, P.O.Box 80176, 3508 TD Utrecht, The Netherlands.
[2] Department of Veterinary Physiology, University of Utrecht, Yalelaan 2, Utrecht, The Netherlands.
[3] Department of Toxicology, Agricultural University Wageningen, P.O. Box 8000, 6700 EA Wageningen, The Netherlands.
[4] Tidal Waters Division, Ministry of Transport and Public Works, P.O. Box 20907, 2500 EX The Hague, The Netherlands.

INTRODUCTION

Polychlorinated Biphenyls (PCBs), Dibenzo-p-dioxins (PCDDs) and Dibenzofurans (PCDFs) are widespread contaminants in abiotic and biotic material. Many environmental and toxicological studies focussed on the possible impact of PCCDs and PCDFs, but more recent the planar PCB congeners received more attention because of their relatively high toxicity and environmental abundance[1,2,3]. Numerous data support the accumulation of these pollutants in fish eating birds[4-7], but few data show clear dose effect relationship between these compounds and morphological, physiological or biochemical parameters[8,9]. In our laboratory such dose effect relationships were found for the Cormorant (*Phalacrocorax carbo*) indicating planar PCBs as major causal agents[8]. The aim of the present study, which is a part of an integrated field and laboratory study on the common tern (*Sterna hirundo*), was to determine if similar relationships could be found in this species and if so, the species could be used as a suitable biomarker in the future. In this paper only the results are presented concerning the PCB tissue levels, the cytochrome P-450 related liver activities, the morphological measurements and the embryonic metabolism. Results of the complete study will be published elsewhere.

EXPERIMENTAL

A detailed description of the experimental setup and the techniques used in this study will be published elsewhere. In May and June 1991 eggs were collected from seven breeding colonies in The Netherlands, located at Flevoland (F); Griend (G); Haringvliet (H); Prinsesseplaat, Oosterschelde (P); Saeftinghe, Westerschelde (S); Terneuzen (T); Westplaat, Northseacoast Oostvoorne (W) and from one colony in Belgium at Zeebrugge (Z). The colonies represented sites with different levels of aquatic contamination. In each colony 15 breeding pairs were selected from which the second egg was collected and transported to the laboratory. In the laboratory the eggs were weighed and breeded in an incubator at 37.5 °C. During the whole breeding period the O_2/CO_2 exchange of the developing embryo was measured daily. Within 12 hours

after hatching the young terns were weighed and sacrificed. Organs were removed, weighed and frozen (blood, liver, yolksac) or fixed in a buffered formalin solution (4%, pH=7.2, thymus, thyroid, bursa) for histopathological evaluation. Livermicrosomes were prepared to determine the ethoxyresorufin-O-deethylation (EROD) and pentoxyresorufin-O-depenthylation (PROD) activities and protein content as described earlier[10-11]. Results from hepatic thyroid hormone 5'-deiodenase activity, thyroid hormone and vitamin A levels will be published separately.

Yolksacs (200-1000mg) were extracted with 50 ml CH_2Cl_2 for 24 hours. 95% of this extract was used for PCDD/F and planar non ortho substituted PCB analysis, while the remaining 5% (5-20 mg lipid) was used for PCB analysis on GC-ECD . Results of the PCDD/F and planar PCB analysis will be published separately. Samples were cleaned up by alumina column and analyzed on a Carlo Erba Mega 536 GC-ECD (column J&W DB5, 60 m.). The mono ortho planar PCBs IUPAC no. #105, #118, #156, #157 and #167 wich are mixed inducers[12] and the non mono ortho PCBs IUPAC no. #28, #52, #101, #138, #153 and #180 which are non- or PB type inducers[12] were quantitated. Recovery percentages were between 78 and 100 % for the individual congeners.

ANOVA was used to test differences in parameters between colonies. Testing of dose effect relationships was done by linear regression analysis of log transformed data and t-test. The acceptance level was $p<0.05$.

RESULTS AND DISCUSSION

Significant differences in PCB residue levels between the different colonies were found. Yolksacs from the Flevoland colony were the least contaminated and showed significant lower concentration compared to all other colonies. The Haringvliet colony showed the highest total PCB levels. Eggs from this colony were significantly more contaminated with PCBs than eggs from all other colonies exept the Westplaat colony (fig.1).

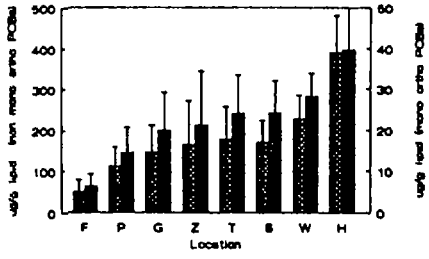

fig.1: PCB concentrations in Yolksac
total non mon ortho PCBs.
total mono ortho PCBs.
Averages (µg/g lipid) ± s.d. per colony.
F: n=7, G: n=7, S: n=3, P: n=5, W: n=10, T: n=6, Z: n=5, H: n=7.

Quantitatively PCB #153 was the most important PCB congener with the highest concentrations reached in the Haringvliet colony, 147 ± 37 µg/g lipid. The lowest levels were observed in the Flevoland colony, 19 ± 11 µg/g lipid. See figure 2a.

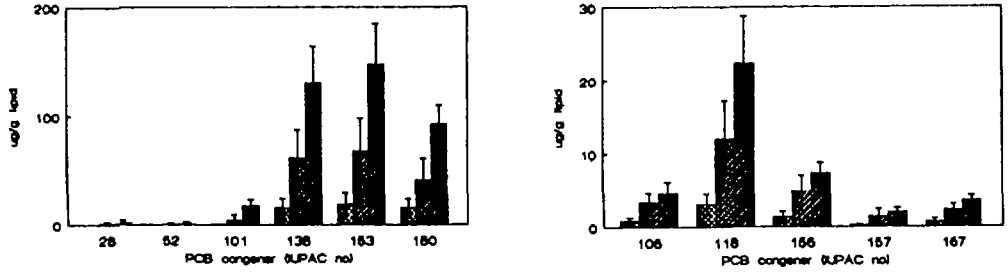

fig.2: Average non mono ortho PCB (left) and mono ortho PCB (right) congener concentrations (µg/g ± s.d) in yolksaclipid of the common tern in the lowest (Flevoland; n=7), an intermediate (Terneuzen; n=8) and the highest (Haringvliet; n=7) contaminated colony.

PCBs #138 and #180 were present in slightly lower concentrations, but in the same order of magnitude. PCB #101 was present in concentrations one order of magnitude lower than #153. PCBs #28 and #52 were present in concentrations two orders of magnitude lower than #153 (fig.2a). PCB #118 was the most abundant mono ortho congener in all colonies with highest levels in the Haringvliet colony, 22.3 ± 6.4 µg/g lipid and lowest values found in Flevoland, 3.1 ± 1.4 µg/g lipid. The PCBs #105, # 156 and #167 were present in 3 to 6 times lower concentrations than PCB #118. PCB #157 was present in concentrations one order of magnitude lower than PCB #118 (fig.2b).

When comparing the EROD activities for the different colonies (fig.3) a trend comparable with residue levels of PCBs in yolksac (fig.1) is observed. Hatchlings from the highest contaminated Haringvliet colony showed significant higher EROD activities than all other colonies studied exept the Westplaat colony (colony F, G and P: $p<0.005$; colony S, T and Z: $p<0.01$; colony W: n.s.) (fig.3).

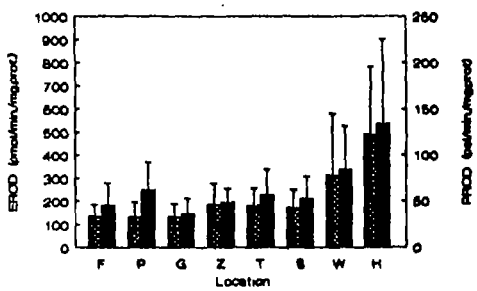

fig.3: EROD (■) and PROD (■) activity of liver microsomes.
Averages (pmol.min/mg.protein) ± s.d. per colony.
G: n=9, S: n=9, P: n=9, W: n=9, T: n=10, Z: n=10, H: n=9.

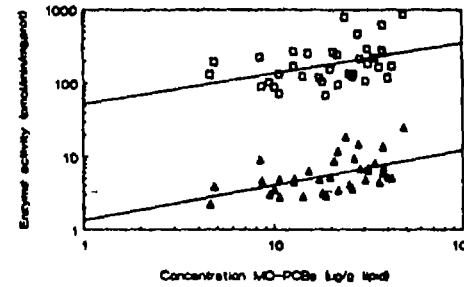

fig.4: Dose effect relationship between total mono ortho PCB concentrations in yolksaclipid (µg/g lipid) and EROD (□) or PROD (△) activities (pmol/min.mg.protein) in livermicrosomes of common tern hatchlings. Regression analysis EROD (a): $Log(y)=0.41*Log(x)+10^{1.3}$, r=0.4, n=36, p<0.025; PROD (b): $Log(y)=0.48*Log(x)+10^{2.9}$, n=36, r=0.5, p<0.005.

To establish the relationship between the presence of mono ortho PCBs in the yolksac and the hepatic EROD and PROD activities, data from all colonies were combined (fig.4). A significantly increasing dose effect relationship is visible for both EROD and PROD activity with the mono ortho PCB content of the yolksac (EROD: slope=0.41, $p<0.025$; PROD: slope=0.48, $p<0.005$).

In mammals the pentoxyresorufin O-depenthylation (PROD) is catalyzed highly specific by cytochrome P450 2B isoenzymes[10]. The dose related increase of PROD, parallel with EROD, may indicate either cytochrome P450 2B activity or a nonspecific depentylation of pentoxyresorufin by cytochrome P450 1A isoenzyme(s) in birds (fig.4). In addition the morphological parameters; eggweight, eggshell thick-ness, hatchlingweight, relative liverweight and relative yolkweight were tested against EROD activity or PCB concentrations for dose effect relationships. No statistical significant relationships between these parameters could be established. No relationship between O_2 consumption and CO_2 production with PCB concentrations in the yolk sac could be established either.

The results of this study indicate, that planar mono ortho PCBs and possibly related compounds alter hepatic cytochrome P450 1A levels in embryos of the common tern breeding in The Netherlands and Belgium. These alterations occur in a dose dependent way at levels of mono ortho PCBs in the yolksac ranging from 5 to 50 µg/g lipid. At present the relative contribution of 2,3,7,8- substituted PCDD/Fs and e.g. PCB #126 to the

elevation of EROD activity in this bird species can not fully be established, as part of the analysis are still in progress. Based on the results of this study so far, it is concluded that hepatic EROD activity is a sensitive biochemical indicator for early exposure to planar halogenated polyaromatics in the early life stage of fish eating bird species.

ACKNOWLEDGEMENTS

The financial support of this study by the Tidal Waters Division, Ministry of Transport and Public Works (Grant no. DG-292) is gratefully acknowledged.

This study is done in cooperation with Sjoerd Dirksen and Theo J. Boudewijn from Bureau Waardenburg, Culemborg and Tom Ysebaert and Geert Rossaert of the Laboratory of Ecology, University of Gent. They collected the eggs during their field study on the reproductive success of the common tern. Peter Meininger (Tidal Waters Division) and Patrick Meire (Institute of Nature Conservation, Belgium) did a lot of work on the coordination of the fieldwork.

Concerning the PCB analysis Frans Busser's experience with gas chromatography and data acquisition programming was indispensable. .

LITERATURE

1. Safe, S; Polychlorinated biphenyls (PCBs), Dibenzo-p-Dioxins (PCDDs), Dibenzofurans (PCDFs), and related compounds: Environmental and Mechanistic Considerations Which Support the Development of Toxic Equivalence Factors (TEFs), Crit. Rev. Toxicol. 21,1: 51-88 (1990).
2. Bosveld, A.T.C., M. van den Berg and R.M.C. Theelen; Assessment of the EROD Inducing Potency of Eleven 2378 Substituted PCDD/Fs and Three Coplanar PCBs in the Chick Embryo, Chem. 1992, in press.
3. Liem, A.K.D., R.M.C. Theelen, W. Slob and J.H. van Wijnen; Report no. 730501.034, National Institute of Public Health and Environmental Protection (1991)
4. Elliot, J.E., D.G. Noble and R.J. Norstrom; Organochlorine contaminants in Seabird Eggs from the Pacific Coast of Canada, 1971-1986, Environmental Monitoring and Assessment 12: 67-82 (1989).
5. Perry, A.S. and A. Zemach; Organochlorine Insecticide Residues in Birds and Bird Eggs in the Coastal Plain of Israel, Bull. Environ. Contam. Toxicol. 45: 523-530 (1990).
6. Smith, L.M., T.R. Scwartz, K. Feltz and T.J. Kubiak; Determination and occurance off AHH-active Polychlorinated Biphenyls, 2,3,7,8-TCDD and 2,3,7,8-TCDF in Lake Michigan Sediment and Biota The question of their relative toxicological significance; Cemosphere 21, 1063-1085 (1990).
7. Craane, B.L.H.J.,A. Brouwer, S. van Mourik, S. Dirksen, T.J. Boudewijn and M. van den Berg; A Comparative Laboratory Breeding Study with Cormorants *(Phalacrocorax carbo)* from Two Dutch Colonies with a Different Degree in Contamination; Organohalogen Compounds IV, DIOXIN'90, 207-210, 1990.
8. van den Berg, M., B.L.H.J. Craane, T. Sinnige, I.J. Lutke-Schipholt, Bert Spenkelink and A. Brouwer: The use of Biochemical Parameters in Comparative Toxicological Studies With the Cormorant *(Phalacrocorax carbo)* in The Netherlands; Chem. 1992, in press.
9. Hoffman, D.J., B.A. Rattner, C.M. Bunck and A. Krynitsky; Association between PCBs and Lower Embryonic Weight in Black-Crowned Night Herons in San Fransisco Bay, J. Toxicol. Environ. Health 19: 383-391, 1986.
10. Bradford, M.M.; A rapid sensative method for quantitation of microgram quantities of proein utilizing the principle of protein dye binding; Anal.Biochem. 72, 248-254, 1976.
11. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall; Protein measurement with the Folin Phenol Reagent, J.Biol.Chem. 193, 265, 1951.
12. McFarland V.A. and J.U. Clarke; Environmental Occurence, Abundance, and Potential Toxicity of PCB Congeners: Considerations for a Congener-Specific Analysis; Environ. Health Perspec. 81, 225-239, 1989.