Occurrence of non-ortho, mono-ortho and di-ortho substituted PCB congeners in polecats, stone martens and badgers from the state of Baden-Württemberg, Germany

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1. Introduction

Although polecats (*Mustela putorius*) have declined in Europe since the middle of this century[1], other members of the mustelid family such as badgers (*Meles meles*) and stone martens (*Martes foina*) could expand their populations. Apart from the habitat destruction, exposure to chlorinated aromatic compounds, in particular to polychlorinated biphenyls (PCB), may have an impact on populations of some species of the mustelid family[2,3,4]. PCB are ubiquitous pollutants derived from former technical use and formed *de novo* in combustion processes. Predators such as the polecats acquire PCB from biomagnification with each trophic level in a food web[5]. Earlier studies from the Netherlands[2-4] and from Switzerland[1] suggest that PCB residues in polecat might interfere with the reproduction. We present the first data of PCB levels in polecats in Germany. We studied whether there are differences in the levels of PCB residues in the tissues of above mentioned species. Additionally PCB levels in the prey of polecats were determined.

2. Objective

The aim of this study was to compare the total PCB concentrations and the levels of di-ortho-, mono-ortho- and non-ortho-PCB congeners in three different tissues (adipose tissue, liver and brain) of polecats, stone martens and badgers from selected areas in the state of Baden-Württemberg in the south-west of Germany.

This approach is recommended by the BgVV (Federal Institute of Human and Environmental Health). It is suggested that for routine analytic purposes the six indicator congeners PCB 28, 52, 101, 138, 153, and 180 and the total PCB concentrations should be analyzed as a base for toxic assessment[6]. The TEQ approach should only be used in special cases. The reason is that effects of PCB on behavior and on reproduction rather than other toxic effects linked to the EROD activity might be relevant. Synergistic and antagonistic effects are not considered in the 2,3,7,8-TCDD-TEF concept for PCBs. Therefore both the total PCB concentration as the homologues sums and the TEQ approach are applied.PCB levels of potential polecat prey i.e. amphibs[7] were analyzed and compared with the experimentally determined NOAEL for the reproduction[8] of minks (*Mustela vison*), a closely related species.

3. Material and Methods

Non-ortho (IUPAC # 77, 126, 169), mono-ortho (IUPAC # 105, 114, Σ 118/123, 156, 157, 167, 169) and di-ortho substituted PCB congeners (IUPAC # 28, 52, 101, 138, 153, 180, Σ Octa-chlorinated-PCB, Σ Nona-chlorinated PCB, 209) were determined in tissues of polecats (n=16), stone martens (n=5) and badgers (n=3).

All animals were adult and provided by local hunters (1 *M.p.*/4 *M.f.*/1 *M.m.*), by the Wildforschungsstelle Baden-Württemberg (14/0/1) or were road victims (1/1/1). They were collected in the period from 1992-1994 in areas near the cities of Freiburg, Karlsruhe, Stuttgart and Tübingen. Adipose tissue could not be obtained in all animals. In one specimen liver could not be collected due to mechanical destruction. Amphibs (6 toads, 6 frogs, 1 salamander) were collected in the area of Tübingen in spring 1995. All samples were stored at -40°C prior to extraction.

Samples were weighed, homogenized and dried with anhydrous sodium sulfate in a mortar (Na_2SO_4:tissue, 4:1 w/w). They were later transferred to precleaned Soxhlet extraction thimbles (4h-Soxhlett extraction with n-heptane). Internal PCB-standard (IUPAC #3, 15, 28, 52, 101, 105, 118, 126, 156, 169, 180, 202, 209, all $^{13}C_{12}$-labeled) were added. Soxhlet extraction was executed with toluene (50-400 ml, depending on sample weight, ranging from 10 to 98,6 g). The solvent was evaporated to dryness and the lipid content was determined gravimetrically.

For sensitive samples i.e. brain (5-7 g) we used the analytical method described earlier by Hagenmaier et al.[9].

Liver-, adipose tissue-, and prey-samples: The extract was redissolved in n-heptane and lipids were digested by concentrated sulfuric acid. After addition of 4 x 50 ml n-heptane the combined heptane fractions were treated with 20-40 g silica gel/conc. sulfuric acid 44%. After four times extracting with 40-50 ml n-heptane the combined heptane fractions were evaporated under a stream of nitrogen to about 200-500 µl.

Clean up was carried out by chromatography on a column with 0,6 g alumina B Super 1 (ICN Biomedicals) and 0,3 g silica gel/conc. sulfuric acid 44%. After pre-elution with 4 ml pentane, PCB were collected by elution with 6 ml heptane/dichloromethane (98:2), followed by eluting the non-ortho chlorinated PCBs with 1,8 ml benzene and the PCDD/F-fraction with 6 ml heptane/dichloromethane (1:1).

Analysis was performed by HRGC/LRMS using a 30 m DB5 or a 15 m DBXLB (both J&W). The DBXLB column has the advantage of separating the pairs of PCB 28/31, PCB 118/123, and PCB 156/157. The mass spectrometer was run in the SIM (Selected Ion Monitoring) mode. Total PCB was calculated from the GC/MS determination of total tri- to decachlorinated Biphenyls.

4. Results and Discussion

Fig. 1 illustrates the origin and the PCB levels in liver and adipose tissue of each individual polecat from Baden-Württemberg. Total PCB concentrations vary roughly within one order of magnitude.

Our results (median: 1,3 mg/kg lipids), when compared with previous studies from the Netherlands and Switzerland1-4, indicate a decrease in PCBs from 1982 to 1994 in polecats from central Europe (Fig.2).

Our comparison is based on estimations. The Swiss study quantified PCBs in polecat kidneys against a Aroclor 1260 standard. We applied a conversion factor of 2.5 to calculate kidney concentrations (which we did not analyze) from adipose tissue concentrations (median: 31,3 mg/kg lipids)1. In the Dutch study 29 congeners were determined. Thus the total PCB concentrations might be underestimated (median: 10,2 mg/kg lipids). Still, PCB levels differ enough to recognize a decreasing trend. This corresponds with the PCB decrease in human and environmental samples (i.e. sediment core[10,11,12] and air[13]).over the last decade.

Fig.1: Map of Baden-Württemberg with origin of the polecats and total PCB concentrations in adipose tissue ▦ and liver ▩

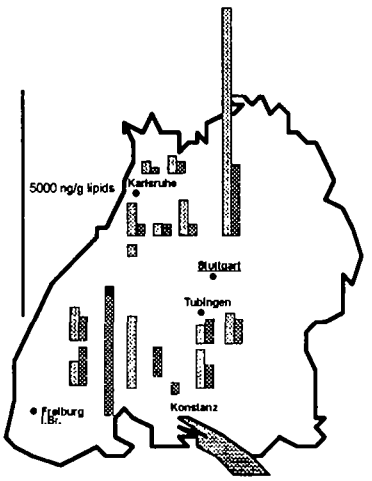
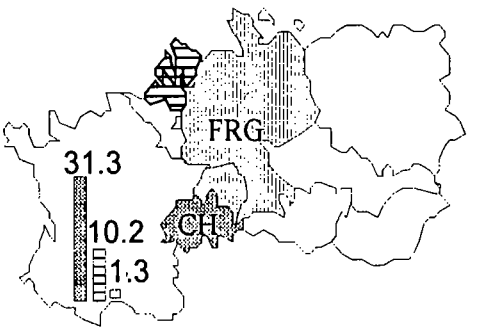
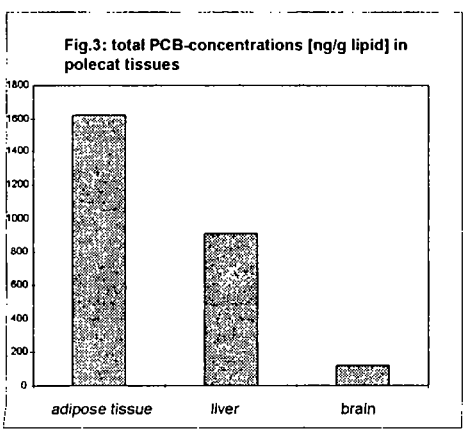
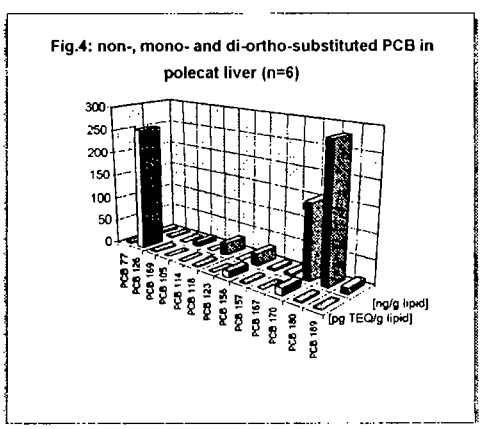


Fig.2: PCB levels [µg/g lipid] in polecats from Switzerland CH (1983-1985), the Netherlands NL (1985-1990) and Germany FRG (1992-1994)

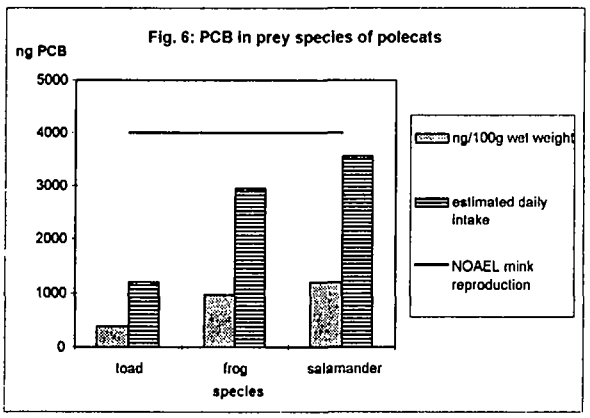


As indicated in Fig. 3, the mean total PCB concentration in polecat brain is 123 ng/g lipids. This compares with 1621 and 911 ng/g lipids respectively in corresponding adipose tissue and liver samples. The concentrations of non-ortho, mono-ortho and di-ortho substituted PCB found in polecat liver samples (n=6) and the calculated TEQ values are compared in Fig. 4. In accordance to the study of Leonards et al.[2], PCB # 126 accounts for most of the toxic equivalents (68-93 %) , while #77 and #169 contribute 0,7% (0,2-1,1%) and 1,0 % (0,6-1,3%). More important in our study are the PCB congeners #170 (average 7,3%; range: 1,5-13,1 %) , PCB #156 (5,8%; 1,6-10%), PCB #180 (1,3%; 0,3-2,3%) and PCB #118 (1,1%; 0,5-1,7%).





In Fig. 5 congener profiles of median concentrations mono-ortho and di-ortho substituted PCB congeners in liver and adipose tissue of polecats, stone martens and badgers are shown. There is not much difference in PCB concentrations between polecats and stone martens. This findings do not support the hypothesis that PCB account for the dwindling polecat populations. However, the mean concentrations of total PCBs in adipose tissue and liver are different for the three species [ng/g lipid] 1290/900 (polecat), 968/886 (badger) and 581/488 (stone marten). In the omnivorous badgers median PCB concentrations are lower than in the carnivorous polecats and stone martens. But in one old starved badger rather high concentrations were found. PCB-congeners 138, 153, 170 and 180 occur in highest concentrations.



The main source for PCB intake is food[15]. The total PCB concentrations in amphibs were determined (Fig. 6). It is estimated that a polecat has to ingest 300 g of amphibs per kg body weight to cover the daily nutritional needs. The NOAEL and LOAEL for the reproduction (litter size, kid survival) of the mink is 4 and 160 µg total PCBs or 0.27 and 4.23 ng TEQ/kg body weight/day respectively. Feral minks were fed with a PCB contaminated fish diet. In our study area the PCB intake (average: 3410 ng; 0,37 ng TEQ) of polecats feeding solely on amphibs would be below a critical level.

5. Conclusions

We can not with certainty answer the question, whether PCBs have an effect on reproduction and behavior in polecats:

Contra

- The exposure of polecats to PCBs has decreased in the last decade.
- Median congener concentrations are comparable in the three mustelid species.These contradicts the hypothesis that the decline in polecat populations is due to the PCB contamination Because badgers and stone martens obviously reproduce sufficiently.
- Estimated dietary intake make it unlikely that PCB contamination (only) can be responsible for a further decline of polecat populations.

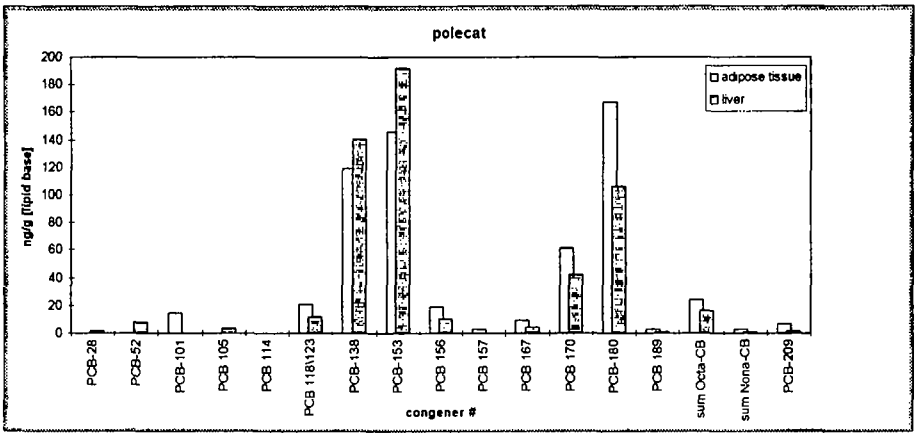
Pro

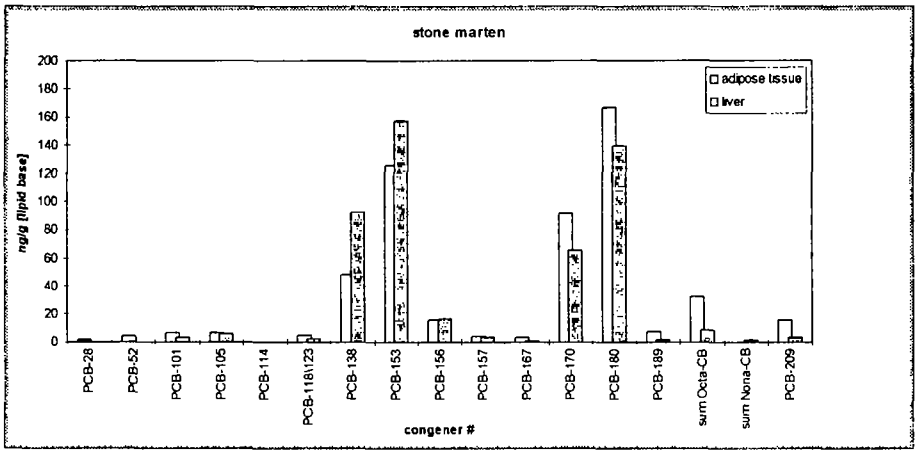
-Some polecats and badgers have high PCB concentrations deviating considerably from the mean PCB concentrations of this two species.

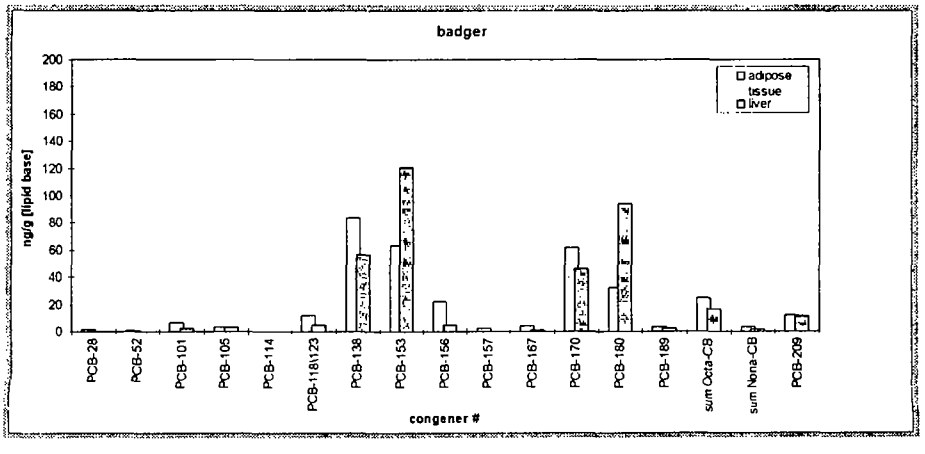
- Synergistic effects with other compounds can not be excluded.

Other environmental factors, such as habitat destruction, have most likely a much greater impact on polecat populations than PCBs.

Fig 5: PCB patterns in polecats, stone martens and badgers







6. References

[1]) Mason C.F.and Weber D. (1990): Organochlorine residues and heavy metals in kidneys of polecats (Mustela putorius) from Switzerland. Bulletin of Environmental Contamination and Toxicology 45, 689-696.

[2]) Leonards P.E.G., van Hattum B., Cofino W.P. and Brinkman U.A.Th. (1994): Occurrence of non-ortho-, mono-ortho- and di-ortho-substituted PCB congeners in different organs and tissues of Polecats (Mustela putorius L.) from the Netherlands. Environmental Toxicology and Chemistry 13, 129-142.

[3]) De Voogt P., van Velzen M.J.M. and Leonards P.E.G. (1993): Planar PCB levels and patterns in aquatic and terrestrials mustelids in relation to food sources. Organohalogen Compounds 14, 101-104.

[4]) De Voogt P., van Velzen M.J.M. and Leonards P.E.G. (1993): PCB metabolites, chlorinated dioxins and furans in tissues of mustelids from the Netherlands. Organohalogen Compounds 14, 105-108.

[5]) de Witt C.A., Järnberg U.G., Asplund L.T., Jansson B., Olsson M., Lindstedt I.L., Bergek S., Hjelt M., Rappe C. and Andersen Ö. (1993): Organohalogen Compounds 12, 325-328.

[6]) Beck H., Heinrich-Hirsch B., Koss O.G., Neubert D., Roßkamp E., Schrerk D., Schuster J., Wölfle D. and Wuthe J. (1996): Anwendbarkeit von 2,3,7,8-TCDD-TEF für PCB für Risikobewertungen. Bundesgesundheitsblatt 4/96, 141-147.

[7]) Weber D. (1989): The diet of polecats (Mustela putorius) in Switzerland. Zeitschrift für Säugetierkunde 54, 157-171.

[8]) Heaton S.N., Bursian S.J., Giesey J.P., Tillitt D.E., Render J.A., Jones P.D., Verbrugge D.A., Kubiak T.J. and Aulerich R.J. (1995): Dietry exposure of mink to carp from Saginaw Bay, Michigan. 1. Effects on reproduction and survival, and the potential risk to wild mink populations. Archives of Environmental Contamination and Toxicology 28, 334-343.

[9]) Hanf V., Behnisch P. Körner W., Sonntag C.-P., Tinneberg H.-R. and Hagenmaier H (1995): Concentrations and Congener Profiles of PCB in Human Preovulatory Cervical Mucus and Effects on Sperm Motility in vitro. Organohalogen Compounds 26, 155-161.

[10]) Hagenmaier H. et al. , Dioxin 96

[11]) Sanders G., Eisenreich S.J. and Jones K.C. (1994): The rise and fall of PCBs: Time trend data from temperate industrialised countries. Chemosphere 29(9-11), 2201-2208.

[12]) Kjeller L-O and Rappe C. (1995): Time trends in levels, patterns and profiles for polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in a sediment core from the baltic proper. Environmental Science and Technology 29, 346-355.

[13]) Jones K. C., Duarte-Davidson R. and Cawse P.A. (1995): Changes in the PCB concentrations of United Kingdom air between 1972 and 1992. Environmental Science and Technology 29, 272-275.

[14]) Ahlborg U.G., Becking G.C., Birnbaum L.S., Brouwer A., Derks H.J.G.M., Feeley M., Golor G., Hanberg A., Larsen J.C., Liem A.K.D., Safe S.H., Schlatter C., Waern F., Younes M. and Yrjänheikki E. (1994): Toxic equivalent factors for dioxin-like PCBs. Chemosphere 28(6), 1049-1067.

[15]) Roßkamp E. (1991): Polychlorierte Biphenyle im Innenraum. In: Leidel J. (Ed.): Asbest, PCB in Gebäuden: Kalkulierbare Risiken? Verlag TÜV Rheinland, 126.