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First Results on the Concentrations of Some Persistent Organochlorines in the Common Hamster *Cricetus cricetus* (L.) in Saxony-Anhalt

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The reasons for the decline of the common hamster (*Cricetus cricetus* [L.]) in Germany and western Europe, where it is now an protected and endangered species, are strongly linked with changes to agricultural practice (Backbier et al. 1998; Kayser and Stubbe 2000). Because of the habitat preference of hamster for the intensively used deep loess soils the common hamster come into contact with the wide range of applied pesticides, such as herbicides, fungicides, insecticides and rodenticides. For this reason the use of pesticides together with the widespread intensification of agricultural practice is often held responsible for the decline of this species (inter alia Wendt 1984). This is also discussed as a possible reason for the reduced reproduction, life span and vitality of common hamsters since there was found a distinct decrease in the birth-rate per year compared with previous studies (Backbier et al. 1998). In particular the release of accumulated pollutants through the consumption of stored fat depots during the hibernation period could be dangerous for the common hamster.

Effects of PCBs on reproduction can be shown in top predators e.g. through influences on the mortality of embryos, and therefore hatching success of birds of prey (Prinzinger and Prinzinger 1979); and in the American mink (*Mustela vison* Aulerich and Ringer 1977). Declining populations of otters (*Lutra lutra*) were found to be affected by higher concentrations of PCBs (Gutleb 1995; Smit et al. 1998). The aim of this study was to describe the concentration of some potentially important pollutants, like persistent organochlorine compounds, in the tissues of common hamsters from the state Saxony-Anhalt (Germany).

MATERIALS AND METHODS

Liver (n = 8) and fat samples (n = 2) of carcasses of common hamsters were collected between 1995 and 1998. The carcasses originated from the area around the Hakel wood in the north-eastern foot-hills of the Harz mountains (except for one animal found near Borne in Aschersleben-Staßfurt district). All hamster samples came directly from or adjacent to the landscape Magdeburger Börde (Saxony-Anhalt), one of the main distribution areas for this species in Germany. Most carcasses were found during an ecological study (see also Weidling and Stubbe 1997) and been

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killed by agricultural means, or by predation by raptors or by carnivores. Some were found dead on the road, killed by traffic. All carcasses were frozen and sent to the Institute of Zoology of the Martin-Luther-University Halle-Wittenberg where they were dissected and autopsies were undertaken.

Samples were analysed for the presence of organochlorines and nitro musk compounds in the laboratory of the state veterinary and food research department of Saxony-Anhalt office Stendal. This laboratory is accredited by the "Staatliche Akkreditierungsstelle Hannover" under the number AKS-P-11502-EU (notified accredication agency according to directive 93/99/EWG). All results are expressed in terms of mg/kg wet weight or lipid weight (ppm). Values below the detection limit are expressed as not quantifiable (n.q.). Results of chemical analyses are expressed as geometric means due to the distribution of the data in terms of few high but reliable outlying concentration values; this follows the advice of Sachs (1992). This removes disproportionate effects of outlying values. The arithmetic mean is close to the median but offers greater possibilities for statistical evaluation (Sachs 1992; Kruuk and Conroy 1996). No quantifiable values are set out as half of the detection limit for the logarithmical transformation.

Samples were analysed for a range of 40 organochlorines and nitro musk compounds, by methods which are normally used for the analysis of human food. Sample preparation and analysis by gas chromatography was carried out using the official collection of methods of analysis § 35 of the German food and commodity law (LMBG, method no. 00.00/37, 00.00/38 part 1-4 and 00.00/34). The detection limit was 0.001 mg/kg wet weight (liver) respectively fat. Analysed substances were HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, ε-HCH, op'-DDT, pp'-DDT, op'-DDE, pp'-DDE, op'-DDD, pp'-DDD, total DDT (sum of DDT, DDE and DDD), aldrin, dieldrin, isodrin, endrin, δ-ketoendrin, c-chlordane, t-chlordane, oxychlordane, heptachlor, c-heptachlorepoxid, t-heptachlorepoxid, α-endosulfan, β-endosulfan, endosulfansulfat, bromocyclen, methoxychlor, mirex, musk ambrette, musk xylene, musk mosken, musk tibeten, musk ketone, PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180. Out of the 209 different PCBs 6 congeners used in standard food control analysis were used as an indicator for total PCB contamination (Beyerbach et al. 1990).

The state veterinary and food research department of Saxony-Anhalt office Stendal had taken part successfully in three laboratory-comparison-analyses in 1999. In addition to the laboratory-comparison-analyses two reference-fats spiked with OC-pesticides and PCBs (HCB, α-HCH, γ-HCH, pp'-DDT, pp'-DDE, dieldrin, oxychlordane, c-heptachlorepoxid, t-heptachlorepoxid, bromocyclen, PCB 138, PCB 153, PCB 180; producer Dr. Ehrenstorfer GmbH, Augsburg) are determined in each charge of analysis (= 20 samples). The analysis-charge is correct, if the concentration of the known OC-pesticides and PCBs is found in the range of the mean ± two standard deviation. The recoveries varied between 90 and 110 %.

RESULTS AND DISCUSSION

None of the analysed liver samples of common hamsters was without any residues. Contamination with HCB, γ-HCH, pp-DDT, pp-DDE, pp-DDD, Dieldrin, PCB 101, PCB 138, PCB 153, PCB 180 was identified. Only γ-HCH and pp-DDE were present in all analysed liver samples. Others among the investigated organochlorines and nitro musk compounds were not found.

The lower chlorinated PCB congeners (PCB 28 and 52) were not detected, and PCB 101 was only found in two samples. PCB 180 was found only once, whereas the other higher chlorinated compounds 138 and 153 were located more often. The concentrations of all organochlorines found were very low, often at or below the detection limit. The geometric means of the concentrations and of the frequencies are given in fig. 1. In both fat samples α-HCH, op-DDE and PCB 28 were additionally found in concentrations near the edge of the detection limit. All other substances detected in the liver samples were also present in the fat samples but with distinctly higher values. There were no differences in the geometric means of organochlorines between juvenile and adult hamsters. Only the PCB concentration was insignificantly higher in the two juveniles. Because of the small sample size this can only be regarded as suggestive of a trend.

The use of hexachlorobenzene as a fungicide seed dressing has been banned in Germany since 1977; in the GDR it was allowed until 1984 (Heinisch 1992). After the prohibition, there was found to be a residue decline in bird eggs (Conrad 1981), human neonates (Lackmann et al. 1996) and human milk (Schade and Heinzow 1998). From 1974/75 to 1977/78, the HCB concentration in the storage fat of pine and beech marten (*Martes martes, Martes foina*) decreased significantly (Drescher-Kaden et al. 1979). Despite the agricultural ban there is remaining pollution from HCBs due to industrial input (Heinisch 1992). Chronically higher levels of HCBs can lead to damage to the reproductive organs. In mice and hamsters, tumours were observed (Falbe and Regitz 1995). Higher HCB concentrations levels in human milk were associated with lower birth weights (Schade and Heinzow 1998). The pollution of the investigated common hamsters with HCB is negligible; even in the body fat it is at or below the detection limit.

An isomer mixture of α-, β- and the true lindane γ-HCH was applied as an insecticide especially against soil pests as a seed dressing and was used as a wood preserver. The banning of the application of lindane in the FRG started in the years 1974-78 (Falbe and Regitz 1995). Then γ-HCH, the only isomers with an insecticide effect, was used. γ-HCH has a higher acute but lower chronic toxicity than the other isomers which are only by-products of the synthesis. Also after an exposure of many years there have been no proven health risks to humans from γ-HCH. But it is not so persistent as its isomers and most of the other higher chlorinated hydrocarbons (Falbe and Regitz 1995). On the other hand higher β-HCH levels in human milk were correlated with lower birth weight (Schade and Heinzow 1998).

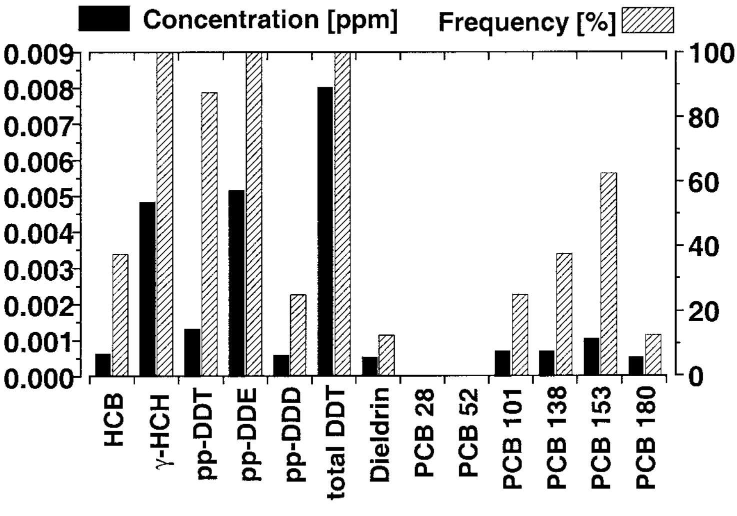

Figure 1. Concentration (geometric mean) and frequency of organochlorines found in the liver of the common hamster (n = 8)

These causes are at least partly responsible for the low pollution by HCH in the common hamster. A similar low concentration level was found in eggs of raptors from the same research area (Weber et al. 1998). In this study also a concentration decrease was recorded since 1990/91 when many agricultural changes took place.

The insecticide p,p'-DDT and its major metabolites p,p'-DDE and p,p'-DDD, which show no insecticide effects, have a long biological half-life and a low toxicity for homoiotherms (Falbe and Regitz 1995). o,p'-DDT is only a by-product of the DDT synthesis. For some decades DDT was the most important insecticide. At present it is banned in nearly all industrial states, in the FRG since 1972, because of its high persistence and its accumulation in the fat of mammals (Falbe and Regitz 1995). In the GDR it was applied with restrictions until the 1980's (Heinisch 1992). As a result of the high persistence of DDT and DDE in the soil and in animals there was only a slow decline of residues of DDT in the eggs of birds of prey after the ban on use (Conrad 1981). The median concentration of DDT and DDE in human milk has decreased distinctly in 1997 when compared to 1986 (Schade and Heinzow 1998). DDT and DDE have been present in samples taken from raptor eggs over long periods due to the long-term wide use (Weber et al. 1998). Even low doses of p,p'-DDT decreased the sexual behaviour in female rats (Uphouse and Williams 1989). DDT and DDE also act as endocrine-disrupting contaminants (Guilette and Guilette 1996). In our study, the ratio of DDT and DDE has shifted in favour of DDE. This reflects the former widespread application of DDT and the intensified catabolism in the body. Levels of DDT and its metabolites are very low in the common hamster. In most samples, the concentration is only at or below the detection limit and may therefore be a reflection of the ubiquitous distribution of DDT.

Because of its high persistence, the accumulation in fat tissue, and the high toxicity for homoiotherms, dieldrin is today not permitted as an agricultural pesticide in many countries. In studies of raptors, dieldrin is only present in older studies (e.g. Ratcliffe 1970). The evidence of dieldrin in only one hamster sample did not allow any further conclusions.

The nitro musk compounds are important as ingredients of cosmetics and washing detergents. In Germany their use was reduced due to the proved neurotoxical effects of musk ambrette, the hard suspicion of carcenogenic effects of musk xylene, the persistence of these substances, and its accumulation in fat tissue (Hahn 1996).

The polychlorinated biphenyls (PCB) are a class of isomers and homolog compounds with different levels of chlorination, which are used for a variety of industrial purposes. The application of PCBs was restricted e.g. to closed systems as a consequence of EC guideline from 1976 and the 10. decree of the Federal Immission Protection Law (26.07.1978) (Falbe and Regitz 1995). On account of their high persistence PCBs are global environmental contaminants. Besides their toxicological effects which depend on the chlorination level, PCBs are also carcenogenic and accumulate in the food chain, especially in the fat tissue (Falbe and Regitz 1995). PCBs have been shown to modify the sex determining mechanism of turtles in laboratory situations. Damages of the fertility of male Florida panthers (*Felis concolor coryi*) as well as grey and common seals in the wild have been attributed to pollution by PCBs and other organochlorines (summarised in: Harrison et al. 1997). The pollution of human neonates by the 6 marker PCB congeners had significantly decreased after the ban on use in the FRG (Lackmann et al. 1996) as had the concentrations in human milk (Schade and Hinzow 1998). Chronic PCB intakes reduced the fertility, the growth and the survival in *Peromyscus*, these effects were amplified through multi generational exposure (McCoy et al. 1995). Our results in the common hamster reflect the ubiquitous distribution of these substances.

The concentrations of organochlorines and nitro musk compounds have been compared with the legal tolerance limits for game and other food because there are no tolerable concentrations or limits for damage known for the common hamster, or other related species. The residues in our hamster samples are very low and quite distant of the limit of tolerance for human food (tab. 1). The tolerance limit for food was used as control value which is expected to show no negative effects on human health, even during long-term exposure, and high tolerance values. Altogether the residues of these substances in hamsters are extremely low, and represent more or less their ubiquitous distribution in the wider environment. Other organochlorines and nitro musk compounds as given in tab. 1 were not found in our samples.

The values of concentrations of organochlorines in common hamster fat and liver samples are much lower than those found in brown hares *Lepus europaeus* in the same study area by Stubbe and Stubbe (1997) and are considerably lower also than those in European otters (Gutleb 1995). Maybe this is at least partly due to the small sample size.

Table 1. Concentration values of organochlorines in common hamster samples from Saxony-Anhalt in comparison to the limit of tolerance for human food.

substance in mg/kg	common hamster liver n = 8	common hamster fat n = 2	limit of tolerance for human food* game meat	milk	fat
HCB	0.0006 (n.q. - 0.001)	(0.002 - 0.007)	0.20	0.25	0.20
α-HCH	n.q.	(0.001)	0.20	0.10	0.20
γ-HCH	0.0048 (n.q. - 0.012)	(0.006 - 0.021)	1.00	0.20	1.00
pp-DDT	0.0013 (n.q. - 0.006)	(0.002 - 0.009)			
op-DDE	n.q.	(n.q. - 0.001)			
pp-DDE	0.0052 (0.001 - 0.045)	(0.015 - 0.056)			
pp-DDD	0.0006 (n.q. - 0.001)	(0.001)			
total DDT	0.0080 (0.002 - 0.052)	(0.021 - 0.073)	1.00	1.00	1.00
dieldrin	0.0005 (n.q. - 0.001)	(n.q. - 0.002)	0.20	0.15	0.20
PCB 28	n.q.	(n.q. - 0.002)	0.08	0.04	0.08
PCB 101	0.0007 (n.q. - 0.002)	(n.q. - 0.003)	0.08	0.04	0.08
PCB 138	0.0007 (n.q. - 0.002)	(0.001 - 0.002)	0.10	0.05	0.10
PCB 153	0.0010 (n.q. - 0.003)	(0.002)	0.10	0.05	0.10
PCB 180	0.0005 (n.q. - 0.001)	(0.001)	0.08	0.04	0.08

Below the geometric mean minimum and maximum are shown. In fat samples no mean is given.
n.q. - not quantifiable - below the detection limit, *- tolerance limit pursuant to the German law (Pollutant Maximum Value Decree [Anonymous 1988] and Maximum Residues Decree [Anonymous 1999])

Organochlorine concentrations were extremely low compared with values published for American small mammals (Dimond and Sherburn 1969). For the well studied European otter a geometric mean of 30 mg/kg wet weight is seen as a limit for the total PCB concentration in liver samples for the reproductive success (Gutleb 1995). This limit value is often surpassed in residues of otter livers from many studies. The concentrations found were about 1000-2000 % higher than our values for the hamster. Nevertheless, even these high values of organochlorine pesticides were classified as not dangerous (survey in Gutleb 1995). Otter individuals in good conditions and in thriving populations were found with concentrations of total PCB of over 14 ppm wet weight (Kruuk and Conroy 1996). The general pollution with

PCB in the hamster is very low even when compared to the values attributed to birds of prey from the same area (Weber et al. 1998). Overall this leads to the conclusion that the organochlorine residues in the hamster are at present very low, often only at or below the detection limit, and could therefore be classified as not dangerous for the hamster.

Besides the investigated substances and compounds, other unanalysed pollutants as xenoestrogens and other endocrine-disrupting contaminants can have direct effects on the reproduction system, or long-term effects on the total reproductive output of this species as well as the fertility of following generations. For a number of chemicals these effects have been proven recently for other mammals, including humans, in the laboratory but rarely in the natural environment (inter alia Harrison et al. 1997). Until recently there are only a few chemicals whose effects on the reproductive system have been well documented e.g. the nematocide DBCP (Harrison et al. 1997). The organochlorine pesticide Dicofol together with DDT and its breakdown products caused harmful effects on the reproductive organs, the fertility of males, and of egg survival in an American alligator population in Florida (Guilette and Guilette 1996). In humans there are some indications, that the increased incidences of disorders of the reproductive health, and fecundity, as well as testicular and female breast cancer, could be possibly related to the effects of endocrine-disrupting contaminants, chemicals and heavy metals in the environment (Harrison et al. 1997). Concern has also been expressed regarding semen quality, cryptorchidism, hypospadias and polycyclic ovaries (Harrison et al. 1997). For clarification of the effects and risk evaluation of the endocrine disruptors on reproductive health and possible combination effects in common hamsters further investigations are necessary.

Due to the small sample size this data only provides a baseline, and should be substantiated and completed with further observations and studies of hamster samples from different parts of the area of the species‘ distribution. Because of the possible combined effects of different components of the variously used pesticides, and other environmental contamination, thus a lack of sufficient data regarding the effects of the application of pesticides, these cumulative effects should be studied in relation to the hamster. For precautionary hamster protection measures, the use of pesticides should be reduced to a minimum, this despite the low contamination of the hamster samples studied. This approach would also minimise the indirect effects of insecticides and herbicides on the food base of the omnivorous hamster (Backbier et al. 1998).

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