LEVEL OF PCDD/PCDF IN MARINE MAMMAL FAT FROM THE CHUKOTSKIY PENNINSULA, SIBIRIA, FROM CA. 600-700 AD

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Introduction

During the past 25 years a discussion has been on-going if PCDD/PCDF levels in the environment are mainly or exclusively man-made. The investigation of natural sources for PCDD/PCDF such as forest fires as well as the analysis of historical samples can help to answer this question. However, there is always the risk that the expected low levels in historical samples were altered by contamination on site or during storage. So far, the information obtained from historical sample is controversial. Compared with present levels, soil samples from the middle of the 19th century contained lower but still measurable concentrations of PCDD/PCDF[1]. Human tissue from about 2800 years old Chilean mummies showed levels equal to the blank or the detection limits for all 2,3,7,8-chlorine substituted congeners (0.3 to 5.5 pg/g)[2]. Lung and liver tissue from Eskimo women frozen in the Arctic ice and being 100 to 400 years old had concentrations comparable to the blank for tetra- to hexachloro congeners (0.3 to 5 pg/g) but measurable quantities of HpCDD (13 pg/g) and OCDD (30 pg/g)[3].

So far, no data are available for animals from top of the food webs such as marine mammals known to have high burdens of PCDD/PCDF at present. The reason is simply the availability of suitable sample material. During an excavation at Ekven on the Chukotskiy Penninsula, permafrost soil was detected containing a high content of fat from marine mammals which was released from the blubber by heating[4]. Levels of PCDD/PCDF in such a fat/soil sample are given in this work and compared with concentrations present in contemporary samples. Furthermore, the concentration profile of main fatty acids was determined and compared to that of fresh fish, whale and seal fat in order to obtain information about the origin of the fat and its alteration by oxidation.

Methods and Materials

Sample collection

Ekven (ca. 66° N, 170° W) is situated at the shore of the Chukotskiy Penninsula vis-à-vis the Diomede Islands in the Bering Street[4]. Fat containing soil samples of ca. 10-50 g originating from the upper layer (0-5 cm) of working areas in front of house 18 sector H (assumed to be from 600-700 AD) were collected during the 1998 expedition by scientists from the Universities of Tübingen. In addition, soil samples were obtained from the University of Neuchatel from the area around houses from 100-1000 AD at the erosion front. The samples were delivered in February 1999 wrapped in aluminium foil. They were not frozen between sampling and delivery.

Extraction and analysis

A preliminary fat determination was carried out by column extraction of 1g of soil with a mixture of n-hexane/CH_2Cl_2 1+1. The extracts were used for lipid class analysis. The fat content varied between

2-14 % and the colour from whitish to brownish. 12.1 g of a sample from house 18 was selected for the determination of PCDD/PCDF (14.3 % of whitish fat). The soil sample with the lowest lipid content (erosion front, 0.33 %) was used as a control. Dioxin analysis was carried out after a blank determination. A standard procedure was employed based on homogenisation of the sample with sea sand and sodium sulphate, addition of 17 ^{13}C-isotope labelled 2,3,7,8-chlorine substituted isomers followed by column extraction with 250 ml of n-hexane/CH_2Cl_2 1+1, matrix removal with first a column packed with H_2SO_4-coated SiO_2, then one containing basic, acidic and neutral SiO_2, one filled with neutral Al_2O_3 and finally a column with Carbopak/cellite[5]. Quantification was carried out by high resolution gas chromatography combined with high resolution mass spectrometry[5].

Lipid analysis

The extracted material was separated into lipid classes by high-performance thin-layer chromatography (HPTLC) using SiO_2 60 pre-coated plates (Merck, Germany) and heptane/diethyl ether/acetic acid (80+20+1 v/v) as the developing solvent system. After charring with copper acetate the separated lipid classes were quantified by a Camag TLC scanner 3 (Muttenz, Switzerland) at a wave length of 350 nm. Methyl esters were prepared from the lipid sample by acid-catalysed transmethylation[6] for 16 h at 60 °C. Prior to analyses the samples were cleaned-up by solid phase extraction using aminopropyl Bond Elut columns (Varian, USA). The purified methyl esters were analysed using a Fison Instruments HRGC 8000 series gas chromatograph equipped with a DB-23 capillary column (60m, 0.32 mm i.d., 0.25 mm film thickness, J&W Scientific, USA). Helium was used as carrier gas and samples (1 ml) were on-column injected. The temperature program was as follows: 100 °C, 2 min, then 20 °C/min to 200 °C, then 2 °C/min to 225 °C and 5 °C/min to 240 °C, 3 min. The instrument was equipped with a flame ionization detector, and separated fatty acids were identified by comparison of retention time with reference standards.

Multivariate data analysis

Relative ratios of saturated (14:0, 16:0 and 18:0) and mono unsaturated (16:1n-7, 18:1n-9, 20:1n-9 and 22:1n-9) fatty acids were compared with those found in samples from lipid rich Arctic species and with literature data[7,8]. Principal component analysis (PCA) was performed with the software Sirius 6.0 (Pattern Recognition Systems, Norway)[9].

Results and Discussion

PCDD/PCDF levels

The fat content in the soil sample selected for PCDD/PCDF analysis was much higher (>14 %) than the amount of naturally occurring lipids (<0.5 %). Furthermore, the extracted fat at contents >4 % was sometimes still whitish and had a line-seed like smell. As can be seen from Table 1, the blank for tetra- and pentachloro 2,3,7,8-chlorine substituted congeners was much lower than the levels found in contemporary seal blubber samples from the Arctic and often equal to the detection limit. Only for hepta- and octachloro congeners the blank was comparable to found concentrations. High blanks for these compounds have also been reported for other studies of historical samples and are caused by the ubiquitous presence of these congeners in the environment and the limited amount of extracted fat (1.73 g) available for this study. Nevertheless, the lipid based PCDD/PCDF level of 0.7 pg/g I-TEQ in the fat/soil sample from house 18 (see Table 1) is much lower than for any Arctic and even Antarctic seal blubber. This is also valid for concentrations of single congeners such as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and some hexachloro congeners. Except for TCDF, the total soil contamination is extremely low as well (0.10 pg/g I-TEQ, ΣPCDD/PCDF 9.8 pg/g, the lowest level in historical soils from south-east England (1893 sample) was 0.5 pg/g I-TEQ, ΣPCDD/PCDF 31 pg/g)[1].

Table 1. Lipid-based levels of PCDD/PCDF in the historical fat/soil sample compared to concentration ranges in selected contemporary seal blubber samples from the Arctic and Antarctic. Levels in pg/g soil are given in parentheses for sample "house 18".

Congener	Concentrations on lipid base [pg/g]				
	Sample house 18	Ringed seal blubber[10]	Harp seal blubber[11]	Fur seal blubber[12]	Blank
2,3,7,8-TCDD	0.1 (0.01)	<2.0-8.2	0.69-1.26	0.22-0.46	<0.1
ΣTCDD	3.0 (0.42)				<0.1
1,2,3,7,8-PeCDD	0.1 (0.01)	2.6-25	1.7-3.3	0.73-1.9	<0.1
ΣPeCDD	2.7 (0.38)				<0.1
1,2,3,4,7,8-HxCDD	0.7 (0.11)	0.2-2.0	0.31-1.2	0.15-0.46	n.a.
1,2,3,6,7,8-HxCDD	0.1 (0.02)	0.8-7.8	1.4-9.3	0.68-1.7	n.a.
1,2,3,7,8,9-HxCDD	0.1 (0.02)	0.3-3.4	0.30-0.81	0.25-0.64	n.a.
ΣHxCDD	5.4 (0.77)				n.a
1,2,3,4,6,7,8-HpCDD	1.4 (0.19)	0.7-1.8	0.44-1.0	0.80-11	1.0
ΣHpCDD	2.6 (0.37)				1.8
OCDD	6.8 (0.97)	18-37	1.2-6.6	3.6-71	9.4
2,3,7,8-TCDF	2.5 (0.35)	11-21	3.7-10	0.84-1.5	0.7
ΣTCDF	30.0 (4.3)				5.5
1,2,3,4/7,8-PeCDF	0.3 (0.05)	0.6-6.6	0.41-0.85	0.23-1.1	0.1
2,3,4,7,8-PeCDF	0.2 (0.03)	2.4-11	2.1-5.1	0.64-1.4	0.1
ΣPeCDF	8.8 (1.3)				0.2
1,2,3,4,7,8/9-HxCDF	0.3 (0.04)	<0.2-<0.3	0.56-2.9	0.16-2.5	n.a.
1,2,3,6,7,8-HxCDF	0.1 (0.02)	<0.2-<0.3	0.50-1.8	0.13-1.9	n.a.
1,2,3,7,8,9-HxCDF	<0.1 (0.01)	<0.2-<0.3	<0.1-<0.25	<0.05-<0.12	n.a.
2,3,4,6,7,8-HxCDF	0.2 (0.03)	<0.2-<0.3	0.34-2.5	0.10-0.27	n.a.
ΣHxCDF	1.7 (0.24)				n.a.
1,2,3,4,6,7,8-HpCDF	2.0 (0.29)	<0.2-0.5	0.29-0.92	0.31-2.0	1.1
1,2,3,4,7,8,9-HpCDF	0.5 (0.07)	<0.1-<0.2	<0.04-0.13	<0.04-0.11	0.5
ΣHpCDF	3.6 (0.51)				3.2
OCDF	4.3 (0.62)	0.7-3.0	0.21-1.3	0.77-6.9	4.2
I-TEQ (PCDD/PCDF)	0.73 (0.10)	5.9-29	3.4-9.6	1.4-2.7	0.17

The assumption that the found PCDD/PCDF may origin from the soil itself was supported by the presence of some non-2,3,7,8-chlorine substituted congeners and by comparing isomer patterns and levels with a soil of very low lipid content (0.33 %) from the erosion front. Nearly the same patterns and concentrations were found for SPCDD/PCDF (9.6 pg/g) and I-TEQ (0.10 pg/g) on a total sample weight basis. This would imply that the contribution from the 14 % fat in the sample "house 18" is negligible.

Investigation of fat origin

Lipid class analysis of the extracted fat by HPTLC showed substantial amounts of polar lipid material as well as the presence of free fatty acids. Only minor amounts of intact triacylglycerols were

found. The increased content of polar lipids is due to lipid oxidation[13], while free fatty acids are formed by hydrolysis in wet soil[14]. Also the fatty acid profile supported this degradation by a dominance of the saturated (14:0, 16:0 and 18:0) and mono unsaturated (16:1, 18:1, 20:1 and 22:1) fatty acids. No polyunsaturated fatty acids typical for marine lipids could be identified, due to substantial oxidation and possible polymerisation. It is also known that lipids under wet conditions are converted into adipocere, a solid waxy material consisting largely of saturated fatty acids containing two carbon atoms less than the original fatty acids[14]. The presence of monounsaturated fatty acids indicates that the conversion to adipocere had taken place only partially, possibly due to the permafrost conditions. A delayed transformation has also been reported for other lipid rich archaeological samples from the Arctic[14,15].

The presence of the monounsaturated fatty acids 22:1 and 20:1 indicates as origin marine fat[14]. Source identification is difficult due to the limited number of preserved fatty acids. A principal component analysis of the distribution of the saturated and mono unsaturated fatty acids of the samples and possible sources shows four distinct groups the archaeological samples, marine mammals (seals and whales), marine fish and freshwater fish. Although not conclusive, is it possible that the source of the lipids found in the soild is blubber from marine mammals.

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