ASSESSMENT OF DIOXIN-LIKE TOXICITY IN SOILS CONTAMINATED BY A CHLORALKALI PROCESS AND A LEBLANC FACTORY

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Abstract

Contaminated soils from a former chloralkali process and former Leblanc factory (with integrated chlorine production) with high levels of PCDD/PCDF and a wide range of other polychlorinated aromatic compounds were screened for their dioxin-like toxicity. As bioassays the permanent rat hepatoma cell line H4IIe and the permanent fish liver cell RTL-W1 obtained from Rainbow trout were used, and Bio-TEQ with and without oxidation and fractionation, respectively, were compared with chemical TEQ-values from PCDD/PCDDF instrumental analysis. Using this screening strategy it was revealed that the complex mixture of aromatic and polychlorinated aromatic compounds elicit significant agonistic and antagonistic effects. While for most samples the majority of the Bio-TEQ could be explained by PCDD/PCDF concentrations determined analytically, only approximately 10% of the Bio-TEQ could be explained by PCDD/PCDFs in some samples, even after oxidation and the routine fractionation for determining dioxin-like toxicity of the coplanar/dioxin fraction. The current study demonstrates the necessity of screening contaminated sites/processes where elemental chlorine has been produced. As evaluation tool, a combination of biological methods and a comprehensive instrumental screening is recommended.

Introduction:

PCDD/PCDFs are the most prominent unintentionally produced POPs (UPOPs) addressed globally by the Stockholm Convention. In addition to PCDD/PCDFs, also PCB and two chlorobenzenes (HexaCBz and PentaCBz) are listed as UPOPs (www.pops.int) under the Convention. However, there are a wide range of other dioxin-like and unintentionally produced persistent toxic substances which are formed and emitted as byproducts in the production of organohalogens, chlorine/halogen production/use and in other industrial and incineration processes.[1,2] One process with high historic total PCDD/PCDF release is the production of chlorine via the chloralkali process using graphite electrodes[2-5].and processes in Leblanc factories including synthesis of chlorine and production of bleaching powder (Calciumhypochloride)[6,7].For example, previous studies have identified PCDD/PCDF in kg TEQ scale in the soils near former chloralkali and Leblanc production sites[5-7]. PCDD/PCDF were formed in both processes by chlorination of tar[2,5,,6,7,15]. For the chloralkali process, tar was used as pitch binder in the graphite electrodes[2,15] and for the Leblanc process, tar was mainly used as insulation/protection material of walls/equipment[6,7] and chlorinated in the processes. Since dibenzofuran is only a minor aromatic compound in tar (about 1% of the PAHs).a wide range of other polychlorinated aromatic compounds were/are formed in these processes.[2,8,15] For most of these other polychlorinated aromatic compounds neither the amount, nor the dioxin-like potential or other toxic effects of single compounds is known. Therefore, we determined the dioxin-like toxicity with the H4IIE assay (rat hepatoma cells)[9] and the EROD RTL-W1 assay[10] in contaminated soil samples from both types of contaminated sites (chloralkali process and Leblanc factory) using whole extracts and after a simple fractionation procedure, and compared them with the TEQ values from GC/MS analysis.

Material and methods

Soil samples from the remediation projects of the former Leblanc-factory-area in Lampertheim/Germany[6,7] and a chloralkali electrolysis in Rheinfelden/Germany[5] were utilized for this study. Samples were freeze-dried, homogenized and subsequently stored in glass vials in the dark. For the bioanalytical investigations of the Ah Receptor mediated activites, the H4IIE assay with rat hepatoma cells[9] and the EROD RTL-W1 assay (Lee et al citation[10] were used. The samples were extracted using pressurized-liquid-extraction with n-hexan. Two soil aliquots were extracted. One aliquot extract was cleaned using a deactivated silica column (for details see Gustavsson et al., 2004[11]) and finally dissolved into DMSO (crude extract). The extract of the second aliquot was fractionated with a combination of multilayer-(including sulfuric acid/silica layer) column and a further aluminium- and carbon-fractionation (for details see Keiter et al. 2008[12]; Loos et al. 1997[13]). Multilayerfractions (ML-fraction) contained only the highly persistent compounds withstanding oxidation from sulfuric acid/silica
PCDD/PCDF were analysed in the soils by HRGC/HRMS in accordance to German standard.
TEQ were calculated from WHO (1998) TEF values and Chem-TEQs are calculated by multiplying compound concentrations and REP values that were applied as determined by Clemons et al. (1997)[14], specific for RTL-W1 and H4IIE cells. When cell-specific REPs were not available, WHO (1998) TEF were used.

Results and Discussion

To determine the dioxin-like activity, the soil extracts (crude-, multilayer-, PCB- and coplanar-fractions) were tested in the H4IIE- and RTL-W1 assays. The resulting biological toxicity equivalency concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (bio-TEQ) were compared to the chemical equivalency concentrations (chem-TEQ). Using this approach it is possible to estimate the part of the dioxin-like activity that can be explained by the detected PCDD/PCDF or other instrumentally identified compounds with known TEFs. Furthermore the biological-analytic of the different fractions allows a determination how they contribute to the total dioxin-like activity of the crude extracts/multilayer fraction and the comparison give a first insight into possible agonistic and/or antagonistic effects of the complex mixtures
Chem TEQs from instrumental analysis were high with values above 100,000 pg TEQ/g in hot spots at both contaminated sites (Figure 1).

H4IIE-assay crude extracts (H4IIE-bio-TEQs) The calculated Bio-TEQ-values for the H4IIE-assay of the crude extracts show high values between 34,415 to 268,335 pg/g. While for the samples of the contaminated site from chloralkali electrolysis the bio TEQ of the crude extracts and chemical TEQ were in close agreement, most of samples from the Leblanc site had a considerable higher bio TEQ compared to chemical TEQ.

Comparison of AhR-mediated activity

Luminescence-based measurement of activities with the EROD assay using RTL-W1 cells confirmed the high toxicities in the soil samples as determined by the mammalian cell line H4IIE. Regression analysis with all bio-TEQ values of crude extracts indicated a good correlation between RTL-W1 and H4IIE cell line (Pearson rank correlation coefficient r=0.87).

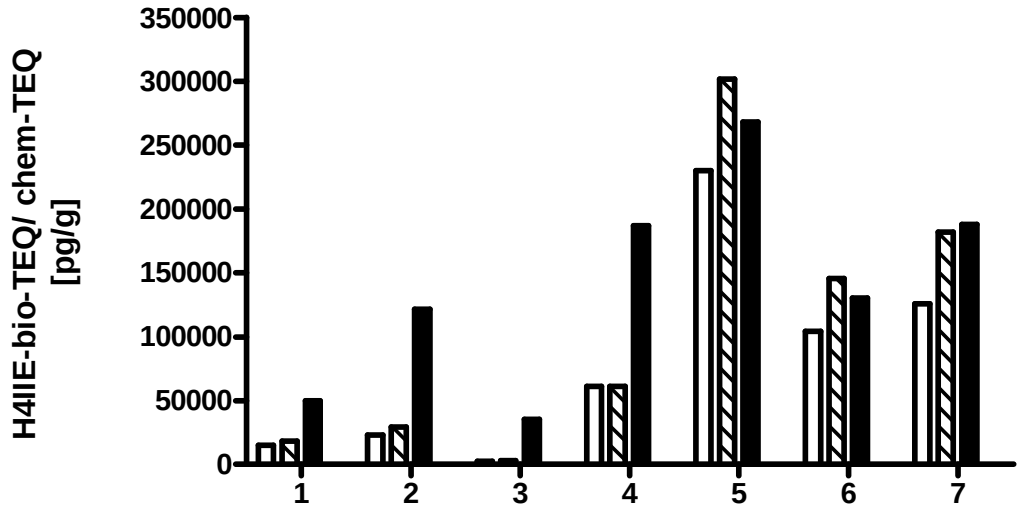

Fig. 1: Comparison of chem-TEQ ( ), H4IIE-chem-TEQ ( ) and H4IIE-bio-TEQ ( ) for the samples 1-5 (Lampertheim) and 6+7 (Rheinfelden).

H4IIE-bio-TEQs after oxidation step (multilayer column)

The bio TEQ from the extracts which was subjected to multilayer fractionation (after the oxidation step with sulfuric acid/silica) (MCE) were lower compared to the crude samples for both soils from the chloralkali site (Figure 2 sample 6 & 7). This indicates that some minor persistent agonistic compounds susceptible to oxidation by sulfuric acid treatment (e.g. PAHs) were removed by the treatment.

For the Leblanc site for sample 1 and 3, the same trend was observed indicating also oxidation of mainly agonistic compounds by the oxidation for these soils. However, for sample 2, 4 and 5 the bio-TEQ increased after the oxidation step. This indicates that for these samples the oxidation step removed mainly antagonistic compounds resulting in an increase in bio-TEQ compared to the crude extracts. Since the Leblanc site had at least 3 different processes leading to PCDD/F contaminated residues (the chlorine production, bleaching powder production and thermal soda process/HCl removal)[6,7] a high variability in chemical composition at the different locations of the area can be expected. However, for understanding details additional effect directed analysis approaches using HPLC and further instrumental analysis would be necessary.

Comparison of bio-TEQ after simple fractionation into PCB and a coplanar fraction

The extract resulting from the multilayer fractionation (the oxidation step) (MLE) was further subjected to a standard clean-up (combination of the aluminium- and carbon-column) to separate the complex samples into a fraction containing non dioxin-like PCBs (PCB-fraction) and an extract containing the so-called coplanar fraction (including PCDD/Fs, PCNs, coplanar PCBs and similar compounds.

The bio-TEQs of the PCB-fraction were relatively low (between 33-7659 pg/g and therefore 2% and lower) compared to the coplanar fraction bio TEQs (2106-402417 pg/g). The toxicity "ranking" of the seven samples for the PCB and the coplanar fractions, respectively, were almost identical.

For the contaminated soil samples from the chloralkali process, the dioxin-like toxicity of the coplanar fraction was higher compared to the extract of the multilayer column (Figure 2; samples 6&7). Therefore in the clean-up

step antagonistic compounds were separated from the coplanar fraction and ended up in the PCB fraction or remained on the columns.
A similar increase in dioxin-like toxicity was also observed for samples 1 and 2 of the Leblanc site. However, for two samples (4 and 5) the dioxin-like toxicity of the coplanar fraction was considerably lower compared to the extract. Therefore, agonistic compounds remained on the column or ended up in the PCB fraction and were masked with overlaying antagonistic effects of other compounds in this fraction or remained on the column.

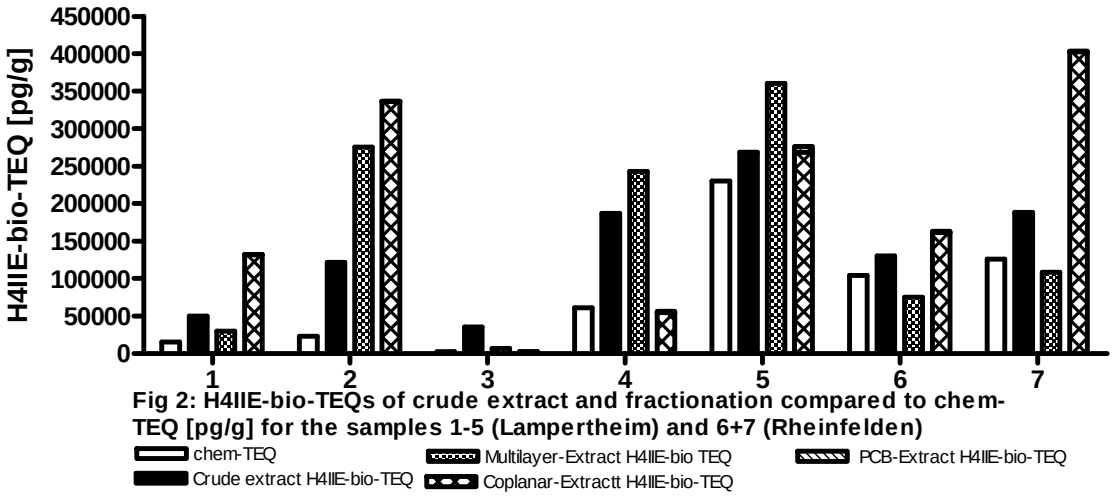

Fig 2: H4IIE-bio-TEQs of crude extract and fractionation compared to chem-TEQ [pg/g] for the samples 1-5 (Lampertheim) and 6+7 (Rheinfelden)

Compaison of chem. TEQ and bio-TEQ from the coplanar fraction (Figure 3)
Today, instrumental analysis is still used as the main tool for PCDD/PCDF compliance analysis. Further, in compliance or commercial routine analysis of dioxin-like toxicity with bio-assays (e.g. food and feed analysis or sediment) only the bio-TEQ of the coplanar fraction after oxidation with sulfuric acid/silica are typically measured and reported. These two datasets (instrumental TEQ and TEQ of coplanar fraction) are compared in Figure 3. For the soils contaminated from the chloralkali process, 78% and 45% of TEQ in the coplanar fraction could be explained by the PCDD/PCDF measured, indicating that for sample 7 more then 50% of dioxin-like compounds were not detected by the PCDD/PCDF routine analysis.
For the Leblanc site for three of the samples the bio-TEQ and the PCDD/PCDF analysis were in good agreement (sample 3-5), however, for sample 1 and 2 the PCDD/PCDF could only explain 11 and 7% of the bio-TEQ.

The HR-TOF-MS screening of soil from both sites revealed a wide range of polychlorinated aromatic and heteroaromatic compounds (including polychlorinated Phenanthrene/Anthracene, Pyrene/Fluoranthene, Benzo(a)anthracene/Chrysene, polychlorinated carbazoles etc.)[8] some in considerable higher concentration compared to PCDF and with unknown dioxin-like activity but all persistent, planar aromatic compounds in the appropriate molecular size for interacting with the Ah-receptor. This demonstrates in our opinion the shortcoming of only analyzing PCDD/PCDF in industrial processes where elemental chlorine resulted/results in chlorination of a wide range of polyaromatic compounds. This data and the current study demonstrate the necessity of more detailed assessments of contaminated sites and processes were elemental chlorine have chlorinated or are chlorinating a wide range of polyaromatic compounds.
The observed agonistic and antagonistic effects increasing or masking dioxin-like toxicity, need to be further evaluated. For further elucidating dioxin-like and toxic effects of these complex compound mixtures, a more comprehensive fractionation combined with a toxicity screening and instrumental analysis (effect directed analysis) seems most appropriate to adequately address these types of samples and contaminated sites.

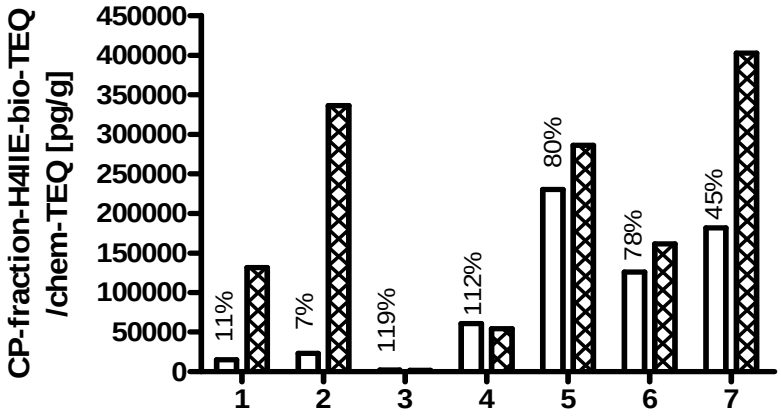

Fig 3: Comparison of chem-TEQ values (▭) and coplanar (CP)-fraction-H4IIE-bio-TEQ values (▩) for the samples 1-5 (Lampertheim) and 6+7 (Rheinfelden). Shown is the percentages of the chem-TEQ that accounts for the H4IIE-bio-TEQ.

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