'Dioxinlike' Components in Incinerator Fly Ashes: A Comparison between Chemical Analysis Data and Results from a Cell Culture Bioassay

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Abstract

Potent PCDD, PCDF, and 'dioxinlike' PCB are among the most relevant toxic emissions from incinerators. Induction of cytochrome P4501A1-catalyzed 7-ethoxyresorufin O-deethylase (EROD) activity in mammalian cell culture is thought to be a selective and sensitive parameter used for the quantification of 'dioxinlike' compounds. Fly ash extracts from municipal waste incinerators (MWI), a crematorium, wood combusters (WCB), and a noble metal recycling facility (NMRF) were analyzed in the bioassay using rat hepatocytes in primary culture. 2,3,7,8-Substituted PCDD/F, 'dioxinlike' PCB, and sixteen major polycyclic aromatic hydrocarbons (PAH) were analyzed by GC/MS. It was found that, with MWI samples, the bioassay resulted in a 2-5fold higher estimate of TCDD equivalents (TEQ) than the chemical analysis of PCDD/F and PCB. However, the outcome of both methods was significantly correlated making the bioassay useful as a rough estimate for the sum of potent PCDD/F and 'dioxinlike' PCB in extracts from MWI fly ash samples. In NMRF and WCB samples, higher amounts of PAH were found contributing to more pronounced differences between the results of both methods. The remaining 'unexplained' inducing potency in fly ash samples probably results from additional 'dioxinlike' components, not analyzed in this study. The hypothesis that the levels of hitherto unidentified 'dioxinlike' compounds in MWI fly ashes are by orders of magnitude higher than those of potent PCDD/F and 'dioxinlike' PCB could not be confirmed.

Introduction

The incineration of municipal waste, wood, and other organic material is a major source of PCDD, PCDF, and probably also of PCB in the environment [1,2]. Since PCDD and PCDF can differ in their toxic potency by several orders of magnitude, and most if not all toxic effects of TCDD and related compounds are thought to be mediated *via* a common receptor, the Ah receptor (AHR) [3,4], the use of equivalency factors for the risk estimation of mixtures of these compounds has been widely accepted in toxicology for PCDD, PCDF [5-7], and for a number of 'dioxinlike' PCB congeners [8]. In various countries, the emissions of incinerators are regulated on the basis of TEQ per gas volume emitted from the plant as calculated from the chemical analysis of 2,3,7,8-substituted PCDD/F.

Induction of cytochrome P450 (CYP)1A1 is one of the best understood and most sensitive biochemical effects mediated *via* the ligand-activated AHR [3,4]. A very good correlation was found

between the rank order of PCDF congeners relating their *in vivo* toxicity in rats to their CYP1A1-inducing potency [5]. These findings and the common biochemical mode of action of 'dioxinlike' compounds led to the establishment of bioassays based on AHR activation. Among these, induction of CYP1A1 in mammalian cell culture, is the most widely used [9-14]. In particular, additive or almost additive interaction was found for the induction of CYP1A1-catalyzed 7-ethoxyresorufin O-deethylase (EROD) activity in rat hepatocytes in primary culture, in rat H4IIE hepatoma cells or in human HepG2 hepatoma cells using complex PCDD or PCB mixtures [11-14] or extracts from environmental samples[15]. Moreover, EROD-TEF values of PCDD [11] and PCB [13] in the bioassay were in very good agreement with I-TEFs or WHO-TEFs, respectively.
In the present investigation, we analyzed a variety of fly ashes from municipal waste incinerators (MWI), a crematorium, wood combusters (WCB), and a noble metal recycling facility (NMRF) for potent PCDD/F, and for 'dioxinlike' PCB. Furthermore, sixteen major PAHs were analyzed. In parallel experiments using the EROD bioassay in rat hepatocyte culture, it was found that the EROD-TEQ values in many instances were about two to five times higher than the TEQ calculated from the chemical analysis.

Experimental Methods

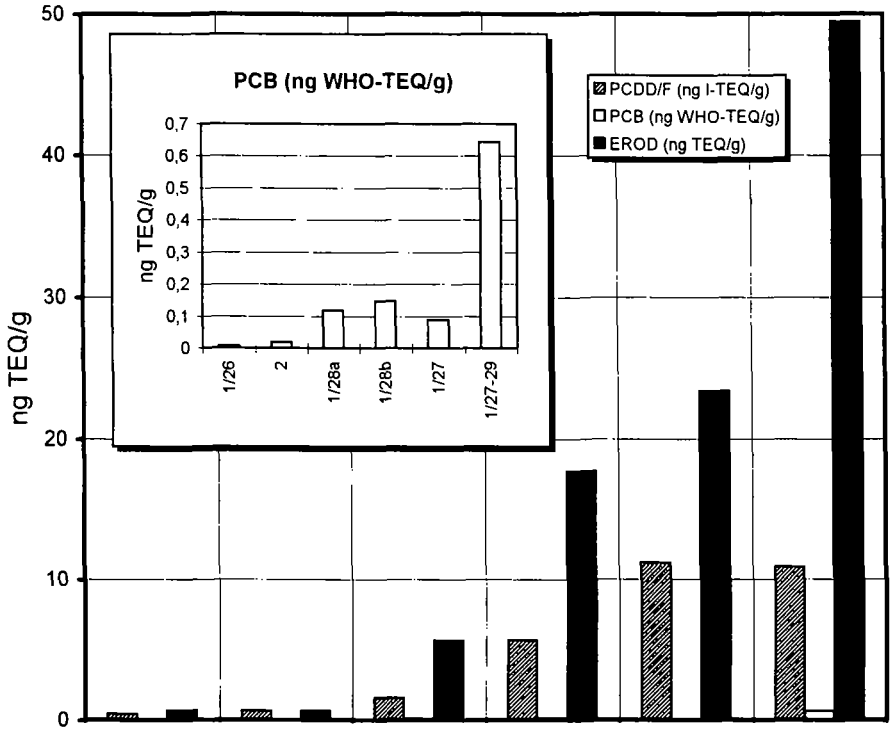
Sample Extraction and Chemical Analysis
Electric filter dust samples were from the municipal waste incinerator in Stuttgart-Münster (boiler 27-29), fly ash samples from a noble metal recycling facility in Pforzheim from the dioxin disengager before (NMRF 1a and 1b) and after (NMRF 1c) Sorbalit (mixture of lime and acvtivated carbon) addition into the fluegas, fly ash samples from the crematorium at the Prag-graveyard in Stuttgart, and from the municipal waste incinerator in Ulm-Weißenhorn after Sorbalit injection into the fluegas (cloth filter ash). The fly ashes from the wood combusters are from wood processing industries in Stuttgart. All samples were collected between 1994 and 1996.
The methods of sample preparation, extraction and analysis used for PCDD, PCDF, PAH and PCB included spiking with isotopically labelled (PCB, PCDD/F) or deuterated (PAH) internal standards, Soxhlet extraction using toluene, chromatographic clean up and separation of PCDD, PCDF, PAH and PCB using a HP 5970 A mass selective detector interfaced with a HP 5890 A gaschromatograph fitted with a DBXLB column for the PCB and PCDD/F, and a HP 5890/HP 5972-GC/MS fitted with a DB 5 column for the analysis of PAH. The following PAH were analyzed: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*g,h,i*]perylene, and indeno[1,2,3,*c,d*]pyrene.
Extracts were prepared as follows: 50-100 g fly ash were stirred for one h with 300 ml 1 n hydrochloric acid and dried at room temperature for 2-3 days. Then the acid-treated fly ash was extracted with toluene overnight in a Soxhlet extractor.
A quarter of these extracts was used for the EROD bioassay, and another quarter for chemical analysis. It was spiked with internal PCB standards (IUPAC Nr. 3, 15, 28, 52, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180, 202, 209, all $^{13}C_{12}$-labelled) and PCDD/F standard mixture. The solvent was evaporated to dryness, the extract redissolved in about 100-200 ml n-heptane, and stirred in an ultrasonic bath. After addition of 20-50 g silica gel (ICN silica 63-300 µm, active)/44% (w/w) conc. sulfuric acid (95-97 %, for analyses), the extract was rotated 10 min at 70°C in a water bath. The supernatant was taken and if necessary this procedure was repeated. The supernatants were combined, the solvent was evaporated to about 0.5-3 ml, and the clean-up was carried out by chromatography on a column (2 x 30 cm) with 25 g basic Alumina B Super I (ICN

Biomedicals)/3 g silica gel/44 % conc. sulfuric acid, and eluted with 350 ml n-heptane/dichloromethane (1:1). After the evaporation of the solvent to about 50-100 µl, the further clean-up was continued with a micro-column with 0.8 g Alumina B Super I and 0.3 g silica gel/44% conc. sulfuric acid. After pre-elution with 4 ml n-pentane, PCB were collected by elution with 6 ml n-heptane/dichloromethane (98:2), followed by eluting the non-ortho-chlorinated PCB with 1.8 ml benzene, and the PCDD/F fraction with 6 ml n-heptane/dichloromethane (1:1).
Analysis was performed by high resolution GC/low resolution MS using a 15 m DBXLB or a 30 m DB 5 column for the PCB and the Cl_7/Cl_8-PCDD/F, and a CP-Sil 88 column for the Cl_4 - Cl_6-PCDD/F. The DBXLB column separated the PCB-pairs of IUPAC-Nr. 118/124, 156/157, and 28/31. The mass spectrometer was run in the SIM (Selected Ion Monitoring) mode. For analysis of PAH, extract from 0.08 - 0.3 g sample was applied to a column (0.7 x 14 cm) filled with 2.5 g basic Alumina B Super I (mixed with 10 % water), PAH were eluted with 10 ml toluene/cyclohexane (1:1), and analyzed with GC/MS.

Cell Culture Bioassay
Hepatocytes were isolated from adult male Wistar rats, and were plated at a density of 100,000 cells/cm^2 on collagen-coated petri dishes (9 cm diameter) in DMEM supplemented with 10 % calf serum, 10 % fetal calf serum, 0.1 µM dexamethasone, 100 units penicillin per ml, and 100 µg streptomycin per ml as described [11]. After 2 h, medium was replaced by fresh medium, fly ash extracts or TCDD were added in DMSO, and cells were harvested after 48 h. EROD activity in cell homogenates was determined using the method of Burke and Mayer [16]. Dose-response curves were calculated using a computerized log-probit procedure (SAS Institute, Cary, USA; Technical report P-179) which also allows calculation of EC_{50}-values and 95 % confidence intervals.

Results and Discussion

CYP1A1-catalyzed EROD activity in rat hepatocyte cultures was inducible with extracts from all fly ash samples investigated. Fitting of a log-probit function to the mean values from four independent experiments led to sigmoidal concentration-response curves as were found previously for PCDD or PCB mixtures [11,13]. Using TCDD as reference inducer (not shown), the EROD-TEQ per g fly ash were calculated based on comparison of EC_{50} values. Chemical analysis of the extracts from MWI fly ash samples showed that the content of PCDD/F and PCB varied considerably between samples ranging from 0.44 - 11.2 ng TEQ/g fly ash for PCDD/F, and from 0.007 - 0.64 ng TEQ/g fly ash for 'dioxinlike' PCB (Fig. 1).The dioxin-like PCB accounted for ca. 1 % of total calculated TEQ. In most instances, EROD-TEQ values determined in the bioassay were higher by a factor of 2 - 5 than expected from the chemical analysis of PCDD/F and PCB.
Further chemical analysis revealed that PAH were also present in MWI samples and in all other samples analyzed. The gap between EROD-TEQ and chemical TEQ (ΔEROD-TEQ/chem.TEQ) was found to be roughly correlated to the 'PAH content' (sum of sixteen PAH) analyzed in MWI samples. A more detailed analysis revealed, however, no clearcut correlation between the PAH pattern and ΔEROD-TEQ/chem. TEQ (not shown).
In a fly ash sample from a crematorium, PCDD/F, 'dioxinlike' PCB, and PAH could be identified. Total TEQ were in the same range as in the MWI sample with the lowest contamination. An about 3fold higher TEQ-EROD was found than expected from the chemical analysis of PCDD/F and 'dioxinlike' PCB (Tab. 1). In fly ash samples from wood combusters chemical TEQ contents also were in the range of MWI fly ash samples with lower contamination. The contribution of PCB to the total chemical TEQ value was clearly lower than in the case of MWI samples (< 0.5 %). In



MWI sample	1/26	2	1/28a	1/28b	1/27	1/27-29
PCDD/F (ng I-TEQ/g)	0,44	0,67	1,61	5,69	11,20	10,90
PCB (ng WHO-TEQ/g)	0,01	0,02	0,12	0,15	0,09	0,64
EROD (ng TEQ/g)	0,72	0,66	5,66	17,74	23,38	49,47
PAH-content (µg/g)	0,05	0,19	0,52	0,99	0,30	0,86
Δ EROD-chem. TEQ	0,28	-0,03	3,94	11,91	12,09	37,93

Figure 1. Levels of potent PCDD/F (in ng I-TEQ/g), and 'dioxinlike' PCB (in ng WHO-TEQ/g) in comparison to EROD-TEQ, and the sum of sixteen major PAH (in µg/g) in extracts of fly ash samples from municipal waste incinerators (MWI).

contrast, the relative content of PAH was higher than in MWI samples. In the three WCB samples no correlation was found, however, between total PAH content and ΔEROD-TEQ/chemical TEQ. TEQ-values similar to those in strongly contaminated MWI samples were found in NMRF fly ash samples. With 1.6 - 3 %, PCB accounted for a higher percentage of total TEQ than in other sample types. Furthermore, relatively high PAH contents led to a larger difference between EROD-TEQ and chemical TEQ. Detailed analysis of PAH showed a relative abundance of highly condensed

Table 1. Levels of potent PCDD/F, and 'dioxinlike' PCB in comparison to EROD-TEQ , and the sum of sixteen major PAH in extracts of fly ash samples from a crematorium, wood combusters (WCB) and a noble metal recycling facility (NMRF).

Sample	PCDD/F (ng I-TEQ/g)	PCB (ng WHO-TEQ/g)	EROD (ng TEQ/g)	ΔEROD/ chem. TEQ (ng/g)	PAH (μg/g)
crematorium	0.47	0.01	1.12	0.64	2.85
WCB 1	0.45	0.02	10.37	9.9	7.40
WCB 2	0.25	0.03	10.93	10.62	59.64
WCB 3	1.74	0.12	23.31	21.45	1.91
NMRF 1 a	24.9	0.74	57.09	31.45	301.9
NMRF 1 b	25.2	0.79	279.22	253.23	536.4
NMRF 1 c	12.8	0.21	34.63	21.62	0.7

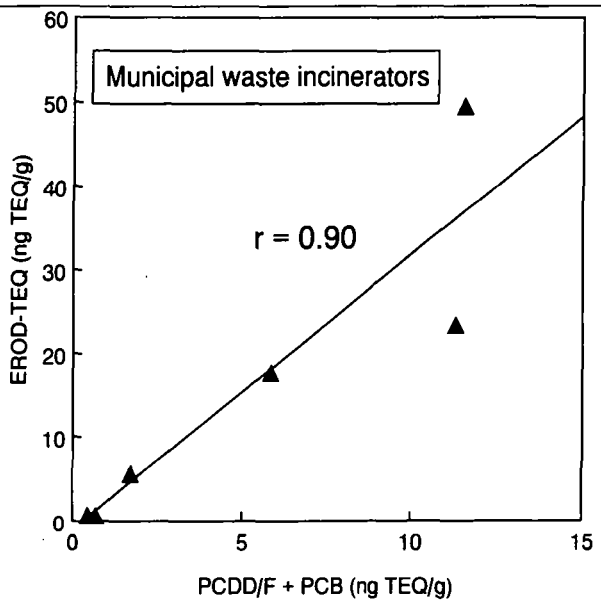


Figure 2. Linear correlation analysis between chemical TEQ for potent PCDD/F, and 'dioxinlike' PCB, and EROD-TEQ for extracts of MWI fly ash samples.

PAH such as benzo[*g,h,i*]perylene and indeno[1,2,3,*c,d*]pyrene in two samples and benzo[*k*]-fluoranthene in a third sample (NMRI 1c).

The EROD-TEQ data were in reasonable correlation with the sum of PCDD/F and 'dioxinlike' PCB levels, showing a correlation coefficient of 0.75 (not shown). When the same type of analysis was performed for the six MWI samples (Fig. 2), an even better correlation was found (r = 0.90). Analysis of the influence of total PAH on the difference between chemical TEQ and EROD-TEQ suggested only a rough correlation between both parameters (not shown).

In conclusion, it is shown that samples from different types of incinerators but also from the same type of incinerator, and even from the same incinerator plant, can differ widely in the TEQ level of

PCDD/F and 'dioxinlike' PCB and in the concentration of PAH in fly ashes. The EROD bioassay was found to be suitable as an estimate for the level of PCDD/F (and 'dioxinlike' PCB) in fly ash samples from MWI or from a crematorium containing relatively low levels of PAH. EROD-TEQ of MWI fly ash extracts were about equal or 2-5fold higher than those calculated from chemical analysis, whereas a stronger overestimation was found for fly ashes from wood burning or NMRF. These differences are partially due to PAH present in these samples in very different patterns and concentrations. However, the presence of additional 'dioxinlike' constituents leading to a measurable EROD-induction appears likely. The contribution of these compounds including PAH in MWI fly ashes did not exceed a factor of five. A dramatically higher presence of hitherto unidentified toxic compounds including 'dioxinlike' compounds in MWI fly ashes (and emissions) than determined in routine chemical analysis could not be found in our study. Further identification and toxicological characterization of unidentified 'dioxinlike' compounds in incinerator emissions will help to decide if the calculation of TEQ based on PCDD/F and 'dioxinlike' PCB is sufficient for risk assessment and regulatory measurements.

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