Using TEF Concept for Assessing Toxic Potency of Polycyclic Aromatic Hydrocarbons in Industrial Samples

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Introduction

The EROD bioassay has been developed as a rapid and cost effective method for screening 2,3,7,8-TCDD equivalents (TEQ) in environmental and industrial samples [1-4]. In contrast to chemical instrumental analysis, this bioassay determines the induction of CYP 1A1 which is a sum parameter for the toxic potential of *all* persistent polyhalogenated aromatic hydrocarbons (HAH). Not only these halogenated compounds, but polycyclic aromatic hydrocarbons (PAH) as well are known to be Ah receptor agonists [5, 6]. However, in biological systems PAH cause low induction of CYP 1A1 resulting in low toxic equivalency factors (TEF). This phenomenon is usually explained by rapid metabolism of PAH compounds [5].
In native samples PAH levels are often found to be much higher then HAH levels. This leads to overestimating TEQ in standard bioassays [3, 4]. To avoid interference with HAH, PAH are removed from sample extracts in cleanup procedures and the inducing potency of persistent HAH is determined only [1, 3].
In this paper our focus of investigation was a different one. Our experiments were particularly designed to measure the inducing potency of PAH. For this, sample extracts were taken as raw extracts without further cleanup and tested on CYP 1A1 induction in H4IIE rat hepatoma cells. Equally important, the standard procedure of the micro-EROD assay was altered and the exposure time shortened from 72 to 24h. First, TEF values for each of the 16 PAH according to EPA method 610 [7] were determined. Second, the CYP 1A1 inducing potency of a PAH standard mixture was investigated for additivity of the individual compounds in the mixture. Third, filter dust and emission samples were analysed both on biological induction and PAH, PCDD/F and PCB concentrations respectively. Finally the results of bioassay and chemical analysis were compared and discussed.

Materials and Methods

Tissue filter dust and emission samples were taken from a secondary aluminium plant. The sampling method is described elsewhere [8].
A rat H4IIEC3/T (H4IIE) hepatoma cell line was cultured and for experiments exposed to the raw sample extracts in dimethyl sulfoxide (DMSO). After 24h exposition the induction of CYP1A1 was detected as EROD (pmol resorufin formed/mg protein/min). Details about the treatment of cells and the enzyme assay have been already described in an earlier publication [3]. Biological TEQ values were determined by comparing the induction of EROD activity caused by PAH standard solutions and environmental sample extracts with those of a concentration series of 2,3,7,8-TCDD standards [9].

PAH levels were analysed by HPLC with fluorescence detection. The diluted DMSO sample extracts of the bioassay were injected without further cleanup. For a description of equipment and measurement parameters see elsewhere [10].
PCDD/F and PCB concentrations were determined by standard chemical analysis. The method has recently been reported elsewhere [11]. For instrumental, chemical analysis, TEQ-values for PCDD/F and PCB were calculated according to NATO/CCMS [12] and WHO/IPCS [13] respectively.

Results and Discussion

CYP1A1 induction potency of 16 PAH according to EPA method 610

Benzo(*b*)fluoranthene, benzo(*a*)pyrene and benzo(*k*)fluoranthene were found to be the most potent compounds of the 16 PAH tested in 24h micro-EROD assay (see Tab. 1). Their induction potency of CYP1A1 enzymes is in the same order of magnitude as coplanar PCB-77 (3,3',4,4'-tetrachloro-biphenyl), whereas indeno(*1,2,3-cd*)pyrene, dibenz(*a,h*)anthracene and benz(*a*)anthracene were 4-10 times less potent. The remaining 10 PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene and benzo(*g,h,i*)perylene) did not induce EROD activity at all, even at high concentrations. As a result most of the toxicological relevant (i.e. carcinogenic) PAH showed EROD-inducing potency in our rat hepatoma cell system. Thus, this bioassay turns out to be a powerful test system for assessing toxic, i.e. carcinogenic potency of PAH.
However in this paper, the order of EROD induction potency and the absolute TEF-values in relation to 2,3,7,8-TCDD differed from results in other publications [5, 6]. Different kinetics of metabolism in various bioassay systems are supposed to be the reason for this [6].

Tab. 1: EC_{50}- and TEF-values of 6 PAH in relation to 2,3,7,8-TCDD (24h micro-EROD assay)

Compound	EC_{50} (nM)	EROD-TEF
Benzo(*b*)fluoranthene	0.071	$3.8 \cdot 10^{-4}$
Benzo(*a*)pyrene	0.090	$3.0 \cdot 10^{-4}$
Benzo(*k*)fluoranthene	0.093	$2.9 \cdot 10^{-4}$
Indeno(*1,2,3-cd*)pyrene	0.305	$8.6 \cdot 10^{-5}$
Dibenz(*a,h*)anthracene	0.327	$7.8 \cdot 10^{-5}$
Benz(*a*)anthracene	0.715	$2.7 \cdot 10^{-5}$

Additivity of individual compounds in a PAH mixture

The TEF concept can be used as an approach to assess effects of different Ah receptor agonists in mixtures. In this concept, concentration additivity of the Ah receptor agonists is presupposed. Therefore, we calculated a theoretical TEQ (TEQ_{theor}). TEQ_{theor} is defined as the sum of the concentrations of the individual PAH times their respective 24h EROD-TEF values (Tab. 1):

$$TEQ_{theor} = \sum_{i=1}^{n} c_i \cdot TEF_{i\,(EROD)}$$

Then, the EROD-inducing potency of a well defined PAH mixture (TEQ_{EROD}) was compared with the theoretical TEQ (TEQ_{theor}). Interestingly enough, the PAH mixture of 16 equally concentrated PAH (EPA method 610) gave an TEQ_{EROD} of 5.3 pg, whereas the calculation of TEQ_{theor} resulted

in 3.8 pg. This means a weak synergistic effect on EROD induction by PAH in mixtures. Similar results have been reported by other authors [14].

Comparing results from micro-EROD assay and chemical analysis

For comparing chemical instrumental analysis with biological TEQ-values, two tissue filter dust samples and three emission samples were investigated. The filter dust samples were highly contaminated by PAH and showed similar PAH patterns (Tab. 2). TEQ_{PAH} is defined as the sum of the concentrations of the individual PAH times their respective 24h EROD-TEF values (Tab. 1). In both cases $TEQ_{PCDD/F}$ and TEQ_{PCB} values were several times lower than TEQ_{PAH} values (Tab. 3). Furthermore the sum of TEQ-values for PCDD/F, PCB and PAH calculated by chemical analysis, accounted for about 70% of the TEQ in the 24h micro-EROD assay. In terms of experimental practice, this means almost equal results for biological and instrumental analysis if the synergistic effects of PAH mixtures on EROD induction were taken into account.

Tab. 2: PAH concentrations of various industrial samples (chemical analysis)

Compound	Tissue filter dust 2 (mg/kg)	Tissue filter dust 3 (mg/kg)	Emission sample 1 (µg/m^3)	Emission sample 15 (µg/m^3)	Emission sample 16 (µg/m^3)
Naphthalene	170	114	225	66453	21.7
Acenaphthylene	n.n.	75.9	n.n.	22884	n.n.
Acenaphthene	5.8	4.7	n.n.	1011	n.n.
Fluorene	21.6	30.5	19.0	4568	1.8
Phenanthrene	180	220	50.7	15873	2.5
Anthracene	45.3	44.3	12.2	4397	0.27
Fluoranthene	79.2	90.7	22.6	6012	1.8
Pyrene	98.6	116	14.3	6831	1.0
Benz(*a*)anthracene	27.6	30.5	8.0	2426	n.n.
Chrysene	30.7	36.0	7.1	2334	1.0
Benzo(*b*)fluoranthene	24.3	28.4	9.1	2039	0.52
Benzo(*k*)fluoranthene	12.8	14.4	4.9	891	0.31
Benzo(*a*)pyrene	35.9	32.7	7.6	2326	n.n.
Dibenz(*a,h*)anthracene	6.3	5.5	n.n.	647	n.n.
Benzo(*g,h,i*)perylene	25.9	20.8	n.n.	1458	n.n.
Indeno(*1,2,3-cd*)pyren	19.0	20.6	n.n.	1558	n.n.
Sum of PAH	783	885	381	141708	30.9

The three emission samples differed extremely in their PAH concentrations. In emission sample 15 TEQ_{PAH} contributed with more than 99% of the sum of TEQ-values for PCDD/F, PCB and PAH (Tab. 3). As expected from the high ratio of PAH to HAH, TEQ were almost equal in both chemical and biological analysis. Again, synergistic effects of PAH on EROD activity were taken into account. Contrasting with this, in emission sample 1 and 16 TEQ_{PAH} accounted only for approximately 50% and 1% of the bioassay TEQ respectively. These results could not be

explained merely by synergistic effects of PAH. Probably the latter samples contained other compounds which also induced EROD activity.

Tab 3: Comparison of TEQ-values of instrumental and bioassay analysis in various industrial samples

TEQ	Tissue filter dust 2 (ng/kg)	Tissue filter dust 3 (ng/kg)	Emission sample 1 (ng/m^3)	Emission sample 15 (ng/m^3)	Emission sample 16 (ng/m^3)
PCB (WHO)	33.0	54.3	0.2	0.4	0.01
PCDD/F (NATO/CCMS)	250	362	11.9	4.5	1.1
PAH ($\sum c_i \cdot TEF_{i\,(EROD)}$)	33293	35214	37.2	1947	0.03
$\sum_{PCB+PCDD/F+PAH}$	33577	35630	49.3	1952	1.1
Bioassay (24h)	45640	53000	73	2850	3.5

To summarise, 24h micro-EROD bioassay turns out to be a powerful test system for assessing toxic, i.e. carcinogenic potency of PAH in industrial samples, as shown in various emission and filter dust samples.

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