Measuring TCDD Equivalents (TEQ) in Emission Samples from a Plant, Utilising Secondary Aluminium and Environmental Samples with a Bioassay

[1]A. M. Hofmaier, [2]A. Markmann, [3]R. Lehnardt [3], K.-W. Schramm,[1]A. Kaune,[1,3]A. Kettrup

[1]Technische Universität München, Lehrstuhl für Ökologische Chemie und Umweltanalytik, D-85350 Freising; GSF-National Research Center for Environment and Health, [2]Institute of Toxikology, [3]Institute of Ecological Chemistry, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany

Introduction

Polyhalogenated aromatic hydrocarbons (HAH) like 2,3,7,8-Tetrachloro-*p*-dibenzodioxin (TCDD) elicit a multitude of toxic and biological effects. A common trait of these substances is their affinity for a cytosolic receptor protein and the subsequent induction of the synthesis of several gene products, including cytochrome P450 1A1 (CYP 1A1)[1,2]. In this publication we present the application of a bioassay to determine the induction of CYP 1A1 as a sum parameter for the total toxic potential of critical halogenated compounds in complex environmental matrices and emission samples from a secondary aluminium process plant. Compounds like polycyclic aromatic hydrocarbons (PAH) and naturally occurring substances which might interfere with the bioassay and lead to false-positive rates were removed by a simple cleanup procedure [3].

Materials and Methods

The emission samples were collected at a plant, utilising secondary aluminium. The sampling method is described elsewhere [4]. Soil samples one, two and three were collected near a motorway parking area (Germany); soil sample four was derived from a toxic waste dump (Hungary).

Sample preparation and extraction

Analytical procedures have been described in an earlier publication[5].

Cleanup and quantification using capillary HRGC/HRMS were carried out as published elsewhere [6].Scanning were conducted using a HP 5890 Series II, (DB-5, 30 m, 0.32 mm ID,

0.25 µm film thickness, 90 °C, 1.5 min, 21 °C/min, 300° C, 20 min; carrier gas: helium, pre-pressure: 29 psi; ionisation: EI, 70 eV, 150 °C, manifold 70 °C) coupled to a massspetrometer SSQ 7000 (Finnigan).
Cleanup for bioassay, cell culture and EROD-assay on 96-well-plates were described elsewhere [3].

Calculation of biological TEQ

The biological TEQ values were determined according to Hanberg et al.[7] comparing the induction of EROD activity by environmental sample extracts with those of a concentration series of TCDD standards (0-20 pg TCDD/25 µl standard solution). The TCDD-standard series was fitted to a four-parameter function. The determination limit for emission samples is approximately 1 pg/m³, for solid samples approximately 1 ng/kg TEQ.

Results and Discussion

Biological TEQ values (Tab. 1 and 2) of analysed samples are higher than values derived from chemical analyses. The apparent discrepancy (factor of mean deviation for emission samples in average 4.7, for soil samples 3.7 excluding soil sample 4) may be partly due to the fact that chemical analysis is restricted to only seventeen PCDD/F. All emission samples determining until now, never passed a ratio of 10 between EROD-TEQ values and values derived from chemical analysis.

Table 1: TEQ-values of emission samples: comparison of Micro-EROD-assay and chemical analysis

sample	micro-EROD-assay	chemical analysis	bioassay/chemical analysis
	[ng/m³]		
emission sample 1	21 ±3 [a]	6,53 (6,4; 0,13) [b]	**3,2**
emission sample 2	14 ±2	1,7 [c]	**8,2**
emission sample 3	12 ±1,5	2,6 (2,3;0,3)	**4,6**
emission sample 4	2,5 ±0,4	1,48 (1,39;0,09)	**1,7**
emission sample 5	100 ±12	14,68 (14,04; 0,64)	**6,8**
emission sample 6	35 ±7	12,13 (11,9; 0,23)	**2,9**
emission sample 7	1,6 ±0,3	0,32 (0,3; 0,015)	**5,1**
emission sample 8	2,1 ±0,3	0,43 (0,4;0,03)	**4,9**

[a] standard deviation of quadruplicate
[b] TEQ = PCDD/F (according to NATO/CCMS)
[c] TEQ = PCDD/F (according to NATO/CCMS) + PCB (according to WHO)

The bioassay results comprise the biological response to compounds like polyhalogenated azo- and azoxy compounds, biphenylethers, naphthalene, dibenzothiophene [8] and alkylated, brominated or mixed halogenated dibenzodioxin/furans, which also bind to the Ah receptor and thereby induce CYP 1A1. However, these substances which are also thought to induce teratogenesis, immunotoxicity and tumor promotion [9,10,11] are not detected routinely by chemical analysis. Moreover, TEF values for such compounds are at present not entirely determined.

Table 2: TEQ-values of soil samples: comparison of Micro-EROD-assay and chemical analysis

sample	micro-EROD-assay	chemical analysis	bioassay/chemical analysis
	[ng/kg]		
soil 1	7951 ±350	1290 [b]	**6,1**
soil 2	459 ±74	133	**3,5**
soil 3	6500 ±250	4478	**1,5**
soil 4	1022 ±275	3,3	**310**

[a] standard deviation of quadruplicate
[b] TEQ = PCDD/F (according to NATO/CCMS)

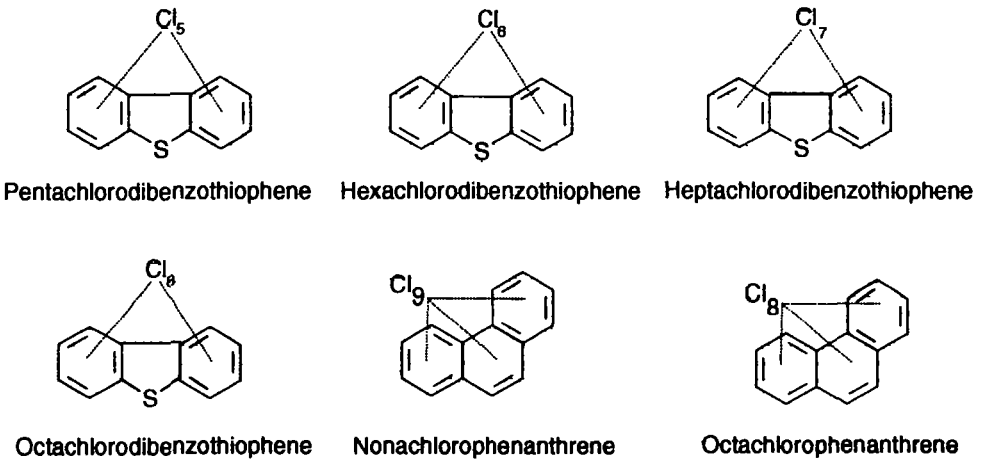

Figure 1: Compounds identified in soil sample 4 (Tab. 2)

Figure 1 shows some substances that were identified in soil sample 4 (Tab. 2), which exhibits a large ratio between bioassay and chemical analysis. These compounds, which probably explain partly the ratio between EROD-TEQ values and TEQ values obtained from chemical

analysis, have been identified with GC/MS scan runs by comparing the absolute molecular weight and the halogen-cluster with reference spectra [12]. However, a full proof for the presence of these compounds would require comparison with internal standards.
Nevertheless, it is not the aim of the bioassay to replace chemical analysis. In conclusion, the bioassay is a rapid screening method for large sample numbers. The bioassay results comprise the biological response to all persistent HAH. This argument reflects the greatest advantage of this bioassay with regard to human risk assessment. The quantification of single PCDD/F congeners or identification of hitherto unknown toxic substances remains the domain of GC/MS. Ideally, chemical analysis and bioassay could complement each other for an improved risk assessment.

Acknowledgement

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