Bioassay for TCDD equivalents (TEQs) in environmental samples: requirements for cleanup and comparison to chemical analysis.

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Introduction

Polyhalogenated aromatic hydrocarbons (HAH) are of great concern because of their ubiquitous distribution in the environment, their persistence, and high toxic potential. The biologically most active group of HAH largely consists of dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs). At present, the toxic potential of HAH mixtures is determined by identifying and quantitating individual isomers and congeners and multiplying their actual amounts with their toxic potencies. By convention, the latter are expressed relative to the toxic potency of 2,3,7,8-TCDD. The total toxic potencies of HAH mixtures are given as TCDD equivalents (TEQs).

The chemical-analytical techniques available for quantitating HAH are too elaborate and costly to screen large numbers of environmental samples for the presence of these compounds. Thus routine practice restricts the analysis to chlorinated PCDD/Fs disregarding equally toxic fluorinated, brominated and mixed halogenated PCDD/Fs, biphenyls or other halogenated derivatives such as azo- and azoxy compounds, biphenylethers and naphthalines.

HAH have in common that they bind to a cytosolic receptor protein and induce the synthesis of several gene products including cytochrome P4501A1 (cyp1A1). The toxic potencies of HAH have been shown to strongly correlate with their capacity for inducing cyp1A1 in cultured cells[1)]. On this basis, a bioassay has been established measuring the induction of cyp1A1 as surrogate for TEQs predominantly using rat hepatoma cells H4IIE[1),2),3)]. Our first attempts to apply this bioassay to complex environmental matrices

were greatly hampered by the fact that the test materials contained i) large amounts of cyp1A1 inducers other than HAH and ii) agents which are toxic to the cells.

The present studies were aimed at establishing a simple and rapid procedure for eliminating the compounds interfering with the HAH bioassay. To this end we developed a single step cleanup procedure using a sandwich column. The procedure was tested on samples of compost, soil and sludge and validated against TEQs derived from parallel chemical analysis.

Methods

Test material: The environmental samples were derived from the following locations: compost: bio-waste compost from a compost plant, Institute of Agriculture, Technical University Munich; soil: standard soil from Speyer, FRG; sludge from sewage sludge plant Selblitztal, FRG.

Sample preparation and extraction: The compost samples were pulverized in a Retsch mill during cooling with liquid nitrogen. The soil and sludge samples were freeze-dried. About 20 g aliquots were quantitatively extracted in a soxhlet apparatus using toluene for 24 hours. For chemical analysis, the samples were spiked prior to extraction (isotope dilution method) with seventeen $^{13}C_{12}$-labelled PCDD/Fs.

Determination of PCDD/F TEQ by chemical analysis: Cleanup and quantification were done as described elsewhere[4] using capillary HRGC/HRMS. The MS-measurement was conducted with high resolution at a Finnigan MAT 95 (R=10000) instrument for isomer specific measurement.

Cleanup procedure for the bioassay: Concentrated crude extract was applied to a column filled with n-hexane from bottom to top with 10 g silica, 20 g silica (44 % conc. sulfuric acid w/w), 40 g silica (4 % water w/w). The column was topped with Na_2SO_4. The silica type was active and 63 - 200 µm. Samples were eluted by 870 ml n-hexane and the eluate was reduced by evaporation (550 mbar, 343 K) to 2 - 3 ml. The extract was stepwise transferred into a vial and evaporated to dryness under a stream of nitrogen. Then the sample was redissolved with 500 µl of DMSO:isopropanol (4:1 v/v).

Bioanalytical procedures: H4IIE/T cells were routinely grown in Dulbecco's Minimum Eagles Medium with 5 % calf serum[3]. They were seeded at a density of $1x10^5$/50 mm culture dish. When the cells reached about 70 % confluency, they were exposed to the environmental samples (5 µl per 1 ml medium) or standards of 2,3,7,8-TCDD. Cells were harvested 72 h after exposure and cyp1A1 activity was determined by the method of Pohl and Fouts[5]. Biological TEQs were calculated as described elsewhere[6].

Table 1: TEQs of compost determined by bioassay or chemical analysis

	compost #1	compost #2	compost #3
biol. TEQ no cleanup	176,5 ± 28,8[a]	n.d.[b]	n.d.
biol. TEQ prior cleanup	18,3 ± 2,0	22,6 ± 0,5	12,0 ± 0,9
TEQ[c] (PCDD/F) GC/MS	4,2	5,1	2,63

[a] mean ± standard deviation of triplicate cultures, [b] not determined, [c] TEQ according to NATO/CCMS

Results and Discussion

Three samples of compost were analyzed for TEQs by the bioassay and by chemical techniques. As shown in Table 1, both types of analyses yielded low but significant TEQ values in these samples.

The biological TEQs measured after prior cleanup were 4-5 times higher than those obtained by chemical analysis. This is most likely due to the fact that the bioassay encompasses the measurement of HAH which are not included in the chemical analysis. The crude sample of compost (#1) seemed to have a very high TEQ value, indicating the presence of large amounts of cyp1A1 inducers sensitive to sulfuric acid. These might be unsubstituted aromatic polycyclic hydrocarbons or plant constituents such as indole-3-carbinol known to possess a marked potential for inducing cyp1A1.

Similarly to the samples of compost, samples of soil and sludge showed a good relation of biologically and chemically derived TEQs provided the material was passed first through the sandwich column (Table 2). In this case, biological and chemical TEQs

Table 2: TEQs of various environmental samples determined by bioassay or chemical analysis

	soil	sludge #1	sludge #2
biol. TEQ no cleanup	11,9 ± 2,2	155,9 ± 39,6	220,1 ± 56,3
biol. TEQ prior cleanup	3,8 ± 1,2	43,4 ± 5,2	50,1 ± 7,8
I-TEQ (PCDD/F) GC/MS	2,1	26,4	25,8

differed only by a factor of 2 suggesting a smaller contribution of HAH other than dioxins to the TEQs as compared to compost. The TEQs of crude samples of soil and sludge were 6 - 9 times higher than the samples processed by column chromatography.

The results demonstrate that environmental samples may contain large amounts of compounds which can interfere with the bioassay for TEQs and have to be removed prior to the biological analysis. This pertains in particular to samples of compost which are notoriously difficult to analyze for AOX[7]. The cleanup can be achieved by a single step procedure using conventional column chromatography. Under these conditions, the bioassay offers a useful tool for assessing the presence of toxic, dioxin-like substances in complex environmental matrices complementing the chemical analysis or alerting to the need of such an analysis.

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