A Short-term Bioassay in Environmental Biomonitoring: Cytochrome P450IA1 Induction in a Mouse Hepatoma Cell Line

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Introduction

Polycyclic aromatic compounds (PACs) are ubiquitous and persistent in the environment, being primary released from incomplete combustion processes, solid wastes and waste water. In the atmosphere, PACs can occur in a gaseous form or bound to particles formed in combustion processes (fly ash). Fly ash contains several PACs including polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs).

PCDDs/PCDFs are almost planar compounds inducing xenobiotic enzymes, e.g. cytochromes P450. The inducible cytochrome P450IA1 activity has been suggested to be associated with an increased risk for environmental pollutant-induced mutagenesis and carcinogenesis[1].

Long-term studies on laboratory animals demostrate the carcinogenicity most reliably. However, they are very expensive and time-consuming and their sensitivity may be limited. For these reasons, there is an interest in quick and reliable short-term bioassays. These bioassays could be used in the estimation of the hazards of complex environmental pollutants.

In this paper we summarize the results from our studies using Hepa-1 cell culture as a short-term bioassay in environmental biomonitoring. Cytochrome P450IA1 is the only form of P450 known to be expressed in these cells, and its inducibility by several model chemicals has been reported[2,3]. The increased activity of this isozyme was used as an indicator of inducing xenobiotics present in fly ashes from different combustion processes.

Materials and Methods

The fly ash samples from combustion of coal[4], heavy fuel oil, peat, hazardous waste, biosludge[5] and polyvinylchloride (PVC) plastic material[6] were prepared for the bioassay with an efficient and quick method, which is practical also in routine work.

The fly ashes were extracted in Soxhlet with toluene. Toluene was removed by a rotary evaporator and the residues were dissolved in n-hexane and shaken with concentrated sulphuric acid to remove other organic compounds than hydrocarbons. The extract was further fractionated by a procedure described elsewhere[5]. In this fractionation method, basic aluminium binds neutral, planar aromatic compounds which can be eluted from the column with specific solvent mixtures. Two fractions are collected. Fraction I mainly contains non-planar, fraction II planar aromatic compounds. The fractions were dried under a nitrogen gas stream and the residues were dissolved in acetone.

The cell line exposed to the fractions was a subclone Hepa-1c1c7 of a mouse hepatoma cell line Hepa-1. The induction of cytochrome P450IA1 was detected as aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin O-deethylase (EROD) activities. The details are described elsewhere[5,3].

PCDD/PCDF analysis of the fractions was performed with a high resolution mass spectrometer (HP 5) (HRGC/HRMS). The quantitation was made with a selective ion recording method using the VG 70 SE mass spectrometer (resolution 10.000)[5,6].

Results and Conclusions

The inducing power of the fly ash extracts was located in fraction II containing planar PACs. Comparison of different combustion processes is shown in Table 1.

Table 1. Comparison of different combustion processes. The biological effects of fly ash extracts are calculated as half maximal induction values (ED50, expressed as original fly ash per ml of growth medium during the exposure). The PCDD/PCDF concentrations are expressed as Nordic TCDD equivalents[7](NE, pg/g).

Fly ash/ Fraction II	ED50/AHH mg/ml	ED50/EROD mg/ml	PCDD/PCDF pg/g (NE)
Coal	n.i.	n.i.	n.d.
Heavy fuel oil	n.i.	n.i.	n.d.
Peat	n.i.	n.d.	0.6
Hazardous waste	2.0	3.1	72
Biosludge	2.5	2.5	2114
PVC plastic material	7.1	6.4	415

n.i. not inducing
n.d. not determined

The emissions of planar aromatic compounds from combustion of coal, heavy fuel oil or peat were small, as judged from the lack of the enzyme-inducing capacity of the fly ash extracts. This indicates that the combustion conditions of these power plants are effective enough to destroy the planar compounds possibly present in the original fuel or formed during the combustion processes.

The ED50 value of the fly ash extract from hazardous waste combustion was of the same magnitude as for the fly ash extract from combustion of biosludge, but smaller than that of the fly ash extract from combustion of PVC plastic material. Comparison of the ED50 values of these different fly ash extracts with their Nordic TCDD equivalents shows no apparent correlation. More TCDD equivalents were present in the fly ash from biosludge and PVC plastic material than in that from hazardous waste combustion. The explanation could be that probably other planar inducing compounds than PCDD/Fs are present in the fly ash from hazardous waste combustion.

However, the biological effect and chemical data of the fly ashes from biosludge and PVC plastic material are in good accordance: the more TCDD equivalents, the stronger inducing effect. This implicates that the sole inducers in these fly ashes are PCDDs/PCDFs.

It can be concluded that TCDD equivalents (cytochrome P450IA1 inducing compounds) can be detected in this bioassay, but all the inducers are not detected in chemical analysis of PCDDs/PCDFs.

References

1 Nebert D W. The Ah locus: Genetic differences in toxicity, cancer, mutation, and birth defects. Crit Rev Toxicol 1989;20:153-174.

2 Kärenlampi S O, Tuomi K, Korkalainen M, Raunio H. Induction of cytochrome P450IA1 in mouse hepatoma cells by several chemicals. Phenobarbital and TCDD induce the same form of cytochrome P450. Biochem Pharmacol 1989;38:1517-1525.

3 Kopponen P, Mannila E, Kärenlampi S. Induction of aryl hydrocarbon hydroxylase AHH by two previously uncharacterized pentachlorinated biphenyls in a mouse and a rat hepatoma cell line. Chemosphere 1992;24:201-10.

4 Kopponen et al., unpublished.

5 Kopponen P, Törrönen R, Tarhanen J, Ruuskanen J, Kärenlampi S. Cytochrome P450IA1 induction in a mouse hepatoma cell culture as an indicator of polycyclic organic compounds in fly ash. Chemosphere 1991;22:895-904.

6 Kopponen P, Törrönen R, Ruuskanen J, Tarhanen J, Vartiainen T, Kärenlampi S. Comparison of cytochrome P450IA1 induction with the chemical composition of fly ash from combustion of chlorine containing material. Chemosphere 1992;in press.

7 Ahlborg U G. Nordic risk assessment of PCDDs and PCDFs. Chemosphere 1989;19:603-8.