Comparison of cell based bioassay and GC/MS dioxin analysis in fly ash

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Introduction

Combustion, instead of deposit on landfills, is considered a solution to the problems with the huge amounts of waste produced by the households every year. However, in the incineration processes polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are formed, which are regarded as the most toxic compounds ever emitted to the environment by mankind. The PCDD/Fs are mostly bound to the fly ash, which in Sweden usually is deposited on controlled landfills. This is a short term solution since the PCDD/Fs are very persistent in the nature and there is a considerable risk of leaching from the landfills. A solution is to treat the fly ash thermally under low oxygen conditions, which has proven to reduce the PCDD/F content with about 95% [1]. However, there is a risk for formation of unwanted degradation products during the treatment that is harmful in the same manner as the dioxin molecules. A cheap and fast way to detect such degradation product would be to use a chemically activated fluorescent expression (CAFLUX) bioassay that emits an enhanced green fluorescent protein (EGFP) upon PCDD/Fs exposure. The amount of EGFP emitted is thereafter converted to TCDD toxic equivalents (TEQ) using a TCDD calibration curve.

The aim of this study was to examine if a CAFLUX assay could be used as a screening method to detect PCDD/Fs in fly ash along with potential harmful transformation products. The results from the CAFLUX bioassay were compared to Gas chromatography/ Mass spectrometry (GC/MS) data.

Materials and methods

The fly ash sample was from the municipal solid waste (MSW) incinerator at Dåvamyran, Umeå, Sweden. Two independent sub samples were prepared originating from the same homogenous sample. For each sub sample; two untreated and three treated fly ash extracts were prepared.

The fly ash was extracted according to *Liljelind et.al* [2].Each extract was split into a “GC/MS” fraction with tetradecane as final solvent and a “cell” fraction with DMSO as final solvent. To the “GC/MS” fraction were isotopically labeled standards added.

The mouse hepatoma cells (H1G1.1c3), modified by *Nagy et.al* [3] were grown in alpha-minimum essential media supplemented with 10% fetal bovin serum (FBS), 0.5% penicillin/streptomycin (PEST) and approximately 2% Geneticin (G418). The cells were maintained in cell flasks at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. When the cells were 80-90 % confluent, they were transferred into a clear-bottomed 96-well micro plate with approximately 70000 cells in 200 µl media to each well and were allowed to attach in 37 °C for 24 h.

On every plate, nine 2,3,7,8-TCDD standards of 0.02 through 125 pg in 2 µl dimethylsulfoxide (DMSO) were added along with a blank (only DMSO). To the remaining wells 2 µl of diluted sample extract was added, which gave a final concentration of 1% DMSO. The plates were incubated at 33 °C for 24 h. The induction of the EGFP was measured in a Floustar Galaxy Meter at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

The GC-MS analyses were performed using a Micromass Ultima GC high-resolution MS system operating in selected ion recording mode with electron ionisation. Two ions of the molecular ion isotope distribution cluster were monitored for each homologue. Quantification was performed according to the isotope-dilution technique.

Results and discussion

The GC/MS analyses showed that the total TEQ for the untreated fly ash were around 2700 pg/g for Sub sample 1 and 3000 pg/g for Sub sample 2. The total TEQ for treated fly ash were around 83 pg/g for Sub sample 1 and 100 pg/g for Sub sample 2 (table 1 a and b). The data from the GC/MS demonstrate a significant decrease of PCDD/Fs in treated fly ash of around 95%. This is in agreement with earlier experiments [1].

Table 1 a. TEQ values obtained by GC/MS analysis of the extracts from Sub sample 1

Sub sample 1 ***Untreated fly ash***	***TEQ (pg/g)***	***Sub sample 1*** ***Treated fly ash***	***TEQ (pg/g)***
A	2500	C	70
B	2800	D	90
		E	90
Mean	2700		83

Table 1 b. TEQ values obtained by GC/MS analysis of the extracts from Sub sample 2

Sub sample 2 ***Untreated fly ash***	***TEQ (pg/g)***	***Sub sample 2*** ***Treated fly ash***	***TEQ (pg/g)***
A	2900	C	160
B	3000	D	90
		E	60
Mean	3000		100

Figure 1 shows dilution curves for treated and untreated fly ash for the CAFLUX bioassay. The more contaminated untreated fly ash resulted in a higher EGFP induction as compared to the treated fly ash for the same dilution. To measure the PCDD/F content in a sample, wells with an induction of about 25000 was used, which corresponds a dilution of approximately 0.001 (1 to 1000) for the untreated fly ash and a dilution of 0.04 (1 to 25) for the treated fly ash. At the highest PCDD/F concentrations, the curves are descending due to cytotoxicity.

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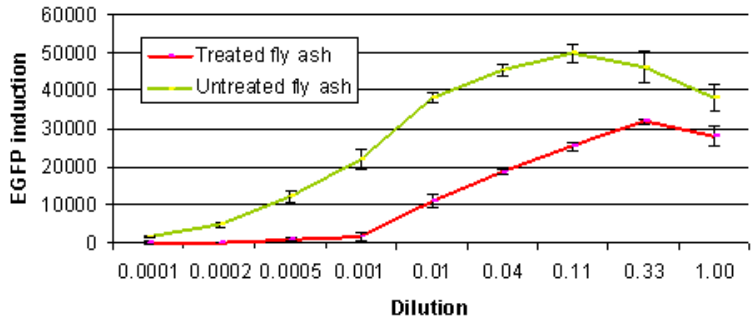

Figure 1. *Dilution curves for one treated and one untreated fly ash sample in a CAFLUX bioassay*

The PCDD/F content in the fly ash was significantly reduced by the thermal treatment according to the student's t-test ($p>0.05$). This was shown by both methods.

In Figure 2, the two analytical methods are compared against each other. In untreated fly ash, one CAFLUX test failed and no data is shown. The other was significant higher than the GC/MS-data. The TEQs obtained for CAFLUX in treated fly ash were generally slightly higher than GC/MS values, however not significant proven.

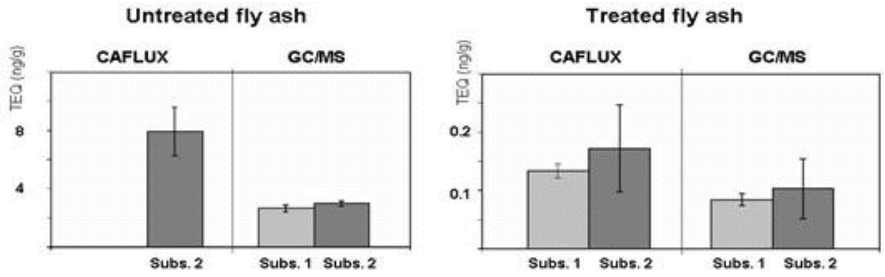

Figure 2. *Comparison of the two methods, CAFLUX and GC/MS.*

The slightly higher TEQ value generated from CAFLUX could indicate that some non-dioxin Ah-R agonists are formed during the thermal treatment of the fly ash.

Another possibility could be that relative efficient potency (REP) for each congener in CAFLUX is higher than WHO-TEF which might give a higher TEQ-value when calculating GC/MS data. The WHO-TEF is based on a number of different *in vivo* and *in vitro* studies and this value might therefore differ from the effect in a specific organism. A TEF obtained in a single *in vivo* or *in vitro* experiment is called the REP. There are no CAFLUX-REP data available but since the 2,3,7,8-TCDD pathway of CALUX is similar to CAFLUX, REP data for chemically activated luciferase expression (CALUX) has been used instead.

When the REP values were used instead of the WHO-TEF, the CAFLUX data seems to be more similar to the GC/MS data for the untreated fly ash (figure 3). The treated fly ash gave GC/MS data slightly higher than the CAFLUX data when REP was used. Thus, there is no indication of formation of any PCDD/F degradation products with "dioxin-like" toxicity.

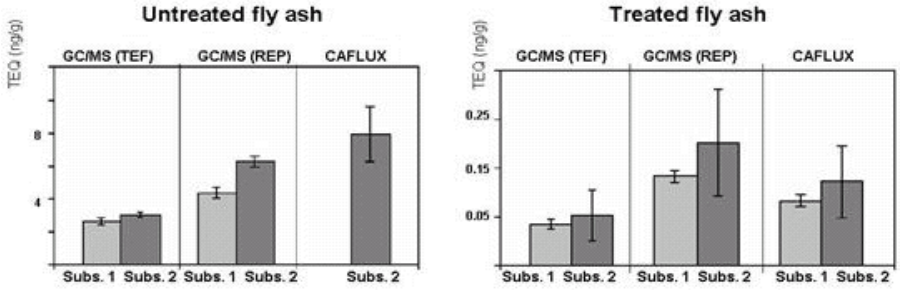

Figure 3. *TEQ from GC/MS calculated with TEFs and REPs compared to CAFLUX analysis*

Conclusions

Both the GC/MS analysis and the CAFLUX data suggest that the thermal treatment of the fly ash strongly reduce the toxic potential of the fly ash.

Therefore, we strongly encourage the use of both techniques. GC/MS provides an accurate measure of the PCDD/F degradation efficiency and the CAFLUX data can be used to check weather degradation products with dioxin-like effects are formed.

It seems possible that a CAFLUX bioassay can be used as a good screening method to detect PCDD/Fs in fly ash and furthermore that CAFLUX can meet the requirements on such a screening tool (30% variation within the samples analysis and tolerable deviation from the GC/MS data). However, there are some problems with the bioassay that have to be solved. The variation of 30% which is established by the European Union Commission [4] as the limit for screening dioxins in food and feed could not be fully proved. This should be done before the method could be accepted.

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