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Biomarkers of Environmental Pollutants of PCDD/Fs, dl-PCBs, PBDD/Fs, PBB, PAH

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Introduction

In the Netherlands people concerned about adverse health effects related to emissions of dioxins and other POPs by industrial activities enable NGO ToxicoWatch for research. Starting research ToxicoWatch use the biotechnological technique of Chemical Activated LUciferase gene eXpression (CALUX), a bioassay for detection of specific chemicals. CALUX bioassay is beside a cost-efficient analyse technique, less complicated in sample preparation, so a broad set of samples could be analysed.

Three bioassays of CALUX were used in this cases: DR CALUX, PAH CALUX and the ER CALUX. DR CALUX for determining dioxins and dioxin like PXB's (PCDD/F, dl-PCBs, PBDD/Fs, PBBs), PAH CALUX for the toxic response of Polycyclic Aromatic Hydrocarbons (PAH) and the ER CALUX to measure Estradiol EQuivalents (EEQs)/ L. In this abstract we highlight some examples of CALUX application and the results.

Materials and methods

Eggs of backyard chicken have been analysed for dioxins (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) by DR CALUX® (BioDetection Systems, Amsterdam and RIKILT, Wageningen (both in the Netherlands) and by chemical GC-HRMS analysis (MAS, Münster, Germany, Nofalab, Schiedam (the Netherlands) and RIKILT, Wageningen (the Netherlands)).

Liver hare was taken out of dead animals and cooled send to the BDS laboratory. Livers from the location in the Hague were already dissected. Water was sampled in one-litre brown glass containers. Caps were from polyethylene (PE-LD 4) to avoid any contamination of additives.

Sampling of dust deposits from window frames, gutters, roof coverings verandas and filter/air diffusers. Samples are pooled back to 5 and send to laboratory. Pooling of gutter-samples on basis of equally weight. Samples of wool (10 gram) are extracted with internal 13C standards. Clean-up is performed with a multi-layer silica and an aluminumoxide column. Samples of wool have been analysed for all PCDD/F congeners by HRGC-HRMS, State Key Laboratory for Clean Energy Utilization, Institute for Thermal Power Engineering, Zhejiang University in China. Bioassays DR CALUX; 100-gram wool, 2-times cold shake extraction 180 ml hexane and clean-up with 2 big acid silica gel clean-up columns. Bioassay PAH-CALUX: 100-gram wool, 2-times cold shake extraction 180 ml hexane and clean-up with a basic alumina column (8%) water and 210 ml pentane solvent.

DR CALUX® bioanalysis: The procedure for the BDS DR CALUX® bioassay has been described in detail previously1. Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in α-MEM culture medium, supplemented with 10 % (v/v) FCS under standard conditions (37^0 C, 5 % CO_2, 100 % humidity). Cells were exposed in triplicate on 96-well microtiterplates containing the standard 2,3,7,8-TCDD calibration range, a DMSO blank. Following a 24 hours incubation period cells were lysed. A luciferine containing solution (Glow Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

CASES/RESULTS

Cases of CALUX application, results and comments

1. Emissions of an incinerator

The first case was how to determine local dioxin deposition in the environment of an incinerator. Eggs of backyard chicken (n=23) were sampled in a radius of 700 – 13.000 m and analysed with DR CALUX.
The results show strong elevated levels of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofuranes (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in eggs within a radiation of 2 kilometres of the location of the waste incinerator. In order to find the source of this pollution, a study of continuous sampling of flue gas (AMESA®) follow. Following studies of eggs of backyard chicken in other parts of the Netherlands (Rikilt, n=63) verified the results of CALUX with GC-MS, no false positive or negative.
The results of the longterm sampling of flue gases make it very likely the dioxins originated from the waste incinerator, because of many shutdowns and start-ups (> 5 times a year), structural use of bypasses during failures (regular) and measured heterogeneous levels of temperatures and oxygen in the post combustion zone. Also comes clear that dioxin emissions are strongly correlated with particulate dust emissions, taking place in just a few minutes, most of the time bypassing one or two Air Pollution Control Devices.

2. Hare from Kings Garden

A point of attention is the choice of a reference location in a study of possible local pollution. In the environment of the incinerator dead animals (birds, sheep) were found and so also dead hare. The hypothesis was tested if there could be found a relation lethality and PCDD/F content, so the liver was analysed with DR CALUX. On finding some data, one needs also data from a reference location. ToxicoWatch was able to get hare (n=6) living in the royal garden from the King of the Netherlands, ZKH Willem Alexander, Wassenaar/the Hague, the Netherlands.

The hare livers have been analysed by DR CALUX for PCDD/Fs- and dl-PCB-BEQ. The results of the hare liver (PCDD/F: 1.2 pg BEQ/g wet weight; PCDD/F/dl-PCB-BEQ: 1.7 pg BEQ/g wet weight) show 3-times higher levels than the EC guidelines for livers of terrestrial animals (0.5 pg PCDD/F/dl-PCB-TEQ/g wet weight). CALUX results of the 'reference' hare from 'Kings garden' were verified with GC-MS (see table 1). Remarkable is 98% contribution of PCB 126 to the 1,75 pg TEQ dl-PCB. Explanation for the PCB 126 source could not be given. Evidence for relation with the incinerator could not be verified.

3. Endocrine disrupting activity in water

As residue of incineration of 228.000 ton of waste, 37.500 ton of bottom ash is produced. Bottom ash was transported in open trucks to boats for transport to Belgium. When bottom ash was loaded into the boats, no protection of wind-blown dust was taken and dust contaminates water of the harbour. Samples of water were taken several hours after loading of bottom ash has taken place. Reference samples were taken along the shore 15 km outside the harbour. The results show high endocrine disrupting activity of 5,3 ng 17ß-estradiol eq/l near the loading place of bottom ash in the harbour of Harlingen. The reference location measured 0,45 ng 17ß-estradiol eq/l, a factor 3 above the mean of drinking water. A counter study of the government (Rijkswaterstaat) two weeks after loading bottom ash in ships show no EDC activity. Measures were taken to reduce dust deposition in the water, covering during transport and wetting the ash.

4. Dust deposition
Studies had been undertaken with PAH CALUX to determine local contamination of PAH. The results show high doses of PAH in deposited dust on window panes and roof gutters, exceeding many times background levels. Problems arise in source determination when chemical analyses be taken, without bioassays analyses and comparison isn't possible anymore. Chemical analyses have however shortcomings in its limitations of congeners, most of the time only 16. The toxicity of PAH is determined by summing the concentrations, although the relative potencies can differ a factor 10,000. The toxicity of PAH is determined by summing the concentrations, although the relative potencies can differ a factor 10,000. The chemical analysis approach of 16 PAH needs to be updated to total toxic profile, comparable with the TEQ-model of the dioxins. One cannot sum naphthalene and benzo(a)pyrene equally, while there's with a difference of relative potency of more than 10.000.

PAH refer to such a broad class of compounds that vary widely in toxicity, that the concept of REP has to be applied to facilitate risk assessment and regulatory control. Thereby also needed a standardisation towards GC-MS instead of HPLC fluorescent, because of a higher sensitivity.

5. Wool
The object of this study are sheep grazing around the environment of a waste incinerator near the UNESCO protected Waddenzee. The hypothesis to be tested, if wool can be used as a bio-indicator of POPs pollution in the environment of a waste incinerator. If so, emissions can possibly be measured near waste incinerators, where only sheep (wool) can used as bio-indicators. The research of the waste incinerator in Harlingen offers an unique opportunity to test this hypothesis, because of the availability of many research data from emission and immision of POPs. Samples of wool are taken from several locations; near this incinerator, heavy traffic highway, urban agriculture and samples from a region without any industrial activity in the surroundings. Analyses are performed by GC-HRMS of all dioxin and furan congeners and bioassays are performed by DR CALUX®.

Conclusions:
The bioassay of CALUX can be used for numerous purposes, easy, cost efficient and can be used in many applications. It's an excellent tool in screening pilots and show results comparable with chemical analyses like GC-MS, without notable false positive or negative results. PAH CALUX should be promoted more in PAH research to reduce the risks of these harmful substances to the environment.

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