DIOXIN-LIKE COMPOUNDS ANALYSED BY DR CALUX® IN WIPE SAMPLES OF A REMEDIATION CASE IN A FORMER ALUMINIUM RECYCLING PLANT IN THE NETHERLANDS

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Introduction
Many contaminated sites are globally monitored only for PCDD/Fs, in case metal recycling processes are involved.

We report here on a dioxin case in the Netherlands, which has been already investigated earlier in 2003 by the Dutch RIVM Institute[1]. They analyzed only the PCDD/Fs in emission gas, soil and wipe samples by chemical analysis. The chemical PCDD/F analyses of the wipe dust samples showed that the deposition was elevated up to 500 meters in the surroundings of the aluminum recycling plant, as a result of the emissions from the stored filtering material.

Motivation for this study is the remediation and demolition of the former aluminum recycling plant. An important precondition of this project is a high level of reuse of recycling materials such as wood, iron and concrete. The project was divided in phases. Phase 1 is the cleanup of the contaminated site. Loose materials with no value were transported to authorized waste processors. Wood and iron materials were on-site cleaned to be recycled. To separate clean materials from polluted materials, and to determine the cleaning level of the buildings, surface was sampled with cotton wipes and analyzed.

In this study, we used the DR CALUX® bio-analysis for measuring Total-TEQ levels in wipe samples from several storage rooms, recycling materials (iron and wood) from demolition of the building structures, as well as from water and soil samples from this former aluminum recycling plant.

Several thousands of mixed halogenated PXDD/Fs, PXBs, PXNs and X-PAHs (X= Cl, Br, J and F) exist and can be formed in such thermal processes. Many of them may be classified as persistent organic pollutant (POP), while only 29 PCDD/PCDF/dl-PCB congeners are routinely measured and monitored[2,3]. Some studies using environmental samples (soils, sediments etc.) involving effect-based analysis tools for dioxin-like compounds (such as DR CALUX®) showed that they give a good prediction for environmental safety and human health impacts[4]. The use of cell and nuclear receptor based screening methods for monitoring dioxins/dl-PCBs in environmental samples allows the (pre)-selection of samples suspected of being contaminated above limit values, as well as the discovery and evaluation of so far not regulated emerging dioxin-like pollutants.

Cell based bioassays such as the CALUX technology have been evaluated, accepted and used in National regulations for environmental samples for the presence of dioxins and related compounds in e.g. the Netherlands (50 ng CALUX-TEQ/kg sediment d.w.)[5], Norway (25 ng CALUX-TEQ/kg sediment d.w.)[6], Japan (JIS K 0463; 150 ng CALUX-TEQ/kg sediment d.w) or the USA (EPA 4435).

Methods and materials
Sample shipment: All wipe, water and soil samples were received from Envita Almelo in February to May 2014 and analyzed in the laboratory of BioDetection Systems bv (BDS).

Wipe samples: Characteristic and representative aliquots of wipe samples are described in table 1. The typical

sampling surface of the recycling materials (wood and iron) was ca. 0.01 or 0.09 m^2 depending on surface.

In the outdoor area, as well for the cleaned rooms of the buildings, per area of max. 500 m^2 one wipe sample was taken. The surface area was 30 x 30 cm with a surface of 0.09 m^2. The samples have than be stored at 4ºC and send to BDS.

Sample extraction and clean-up for DR CALUX® bioassay The procedure for the DR CALUX® bioassay by BDS was described in detail previously[7]. Briefly, the wipe samples were extracted with hexane. Approximately 10-20 gram of wipe samples was used for the clean-up. The clean-up was conducted firstly by a multilayer silica gel/sulfuric acid chromatography. The fractions were carefully evaporated by a stream of nitrogen, and replaced with 50 µL of dimethylsulfoxide (DMSO).
DR CALUX® bioanalysis: The procedure for the BDS DR CALUX® bioassay was described in detail previously3,4. 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˚C, 5% CO2, 100% humidity). Cells were exposed in triplicate on 96-well micro-titer plates containing the standard 2,3,7,8-TCDD calibration range and a DMSO blank. Following a 24 hour incubation period, cells were lysed. A luciferine containing solution (Glow Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3). All results have been corrected for the procedure blank.

Discussion about suitable trigger value?
It was reported in 2003, that the current investigated aluminium recycling plant in the Netherlands did emit dioxins through a stack[1]. Also, dioxin containing dust particles are emitted from filtering material removed from the flue gas, stored on the site of the plant. The concentrations and deposition of dioxins around the plant were calculated using emission data and a dispersion model.

Furthermore, soil and wipe dust samples were taken in the surroundings. Except for the location nearest to the plant (at about 200 m) the measured concentrations in soil (1.6 to 7.6 ng WHO-TEQ/kg d.m.) did not significantly differ form the background value[1]. The analyses of the wipe dust samples (results ranged between 99 to 521 pg WHO-PCDD/F-TEQ per m^2) showed that the deposition was elevated up to 500 m in the surroundings, as a result of the emissions from the stored filtering material[1]. Not many results of wipe samples of background locations are published and we would refer to a typical background level of between 5 to 25 pg WHO-TEQ/m^2. It is recommended to use a dioxin norm of 25 pg TEQ/m^2 as trigger value.

In Germany, in the Regulation for Fire case remediation a value of 10 ng I-TEQ/m^2 for such wipe samples in indoor contaminations is used. Lindert and Fiedler reviewed in 1999 several fire cases with a wide range of PCDD/F results (80 to 15.000 ng PCDD/F-TEQ/m^2) in wipe sample[9].

Results and Discussion
In our study, we therefore applied the trigger value of 25 pg TEQ/m^2 suggested from RIVM[1].
Using this trigger value for our study with DR CALUX® analysis shows that many wipe samples in the here investigated locations were above this norm (see Table 1).

In this study the wipe samples from different locations (e.g. wood storage, iron storage, concrete floor) showed Total-TEQ levels by DR CALUX® ranging from 14 to 400.000 pg TEQ/m^2 (Table 1).

One waste water sample did also show elevated levels of 17 ng TEQ/l water (Table 1).

All tested soil samples have been below the here applied norm of 55 ng TEQ/kg d.w. (Table 1).

Table 1: DR CALUX® analysis of several different wipe, water and soil samples from an aluminium recycling plant in the Netherlands. Individual analysis of wipe samples with their samples surface are listed.

Samples	**Sampled surface (m^2)**	**DR CALUX-TEQ (pg TEQ/m^2)**
Wipe samples		
Wipe sample M3, wood	0.002	570
Wipe sample M4, concrete floor	0.09	540
Wipe Sample M5, Iron from hall	0.09	2100
Wipe Sample M6, Iron from hall	0.09	580
Wipe Sample M7, cleaned area	0.09	100
Wipe Sample M8, floor cleaned area	0.09	47
Wipe Sample M9, cleaned concrete floor hall 1	0.09	15
Wipe Sample M10, Iron from hall 1	0.09	2000
Wipe Sample G6, cleaned with high pressure	0.09	100
Wipe Sample M11, concrete floor, cleaned with high pressure	0.09	230
Wipe Sample H2, Wood, cleaned with water	0.01	22000
Wipe Sample H3, Wood, cleaned with high pressure	0.0025	400.000
Wipe Sample M12, floor cleaned with soap	0.09	110
Wipe Sample Y7 to Y23, Steel storage	0.09	1 to 50
Sample H4, Wood from roof loc. C	0.01	20.000
Soil samples		
Soil Samples G1 to G2	Soil samples	All below norm of 55 ng TEQ/kg
Water samples		
Waste Water Sample WW1		17 ng TEQ/l water

Conclusions

The demand for monitoring of remediation sites will continually rise due to more National and International standards with the ultimate goal to reduce these dioxin-like compounds and POPs (such as e.g. polyhalogenated PXDD/Fs and PXBs) significantly. More efforts will be promoted by international organisations to receive more data about the dioxins, dioxin-like PCBs and new emerging dioxin-like compounds (such as mixed halogenated PXDD/DFs and PXBs) for such remediation cases to proper ensure the safety for the environment, wildlife and human health.

Our results show that the DR CALUX® bioassay for screening of dioxins/dl-PCBs and not yet regulated new dioxin-like compounds (such as PXDD/Fs and PXBs) in wipe, water and soil samples is an important tool to separate the

bulk of unpolluted samples from the few percentage of polluted samples exceeding limit values. Such cell based screening technologies also offer an alternative tool for monitoring/screening of all kinds of environmental samples (wipe, soils, sediments, water and bio-wastes) for regulated and not yet regulated dioxin-like compounds (such as dioxin-like and polyhalogenated PCBs/PBBs or mixed PCl-B-DD/DFs).

References

1. Mennen MG, Fortezza F, Dusseldorp A, Hoogerbrugge R, den Boer AC and Groenemeijer GS (2003). RIVM Report 609023004/2003
2. Behnisch PA, Hosoe K, Sakai S (2003); *Environ. Int.* 29: 861-877
3. Janosek J, Hilscherova K, Blaha L, Holoubek I (2006); *Toxicology in Vitro* 20: 18-37
4. Yu X, Zennegg M, Engwall M, Rotander A, Larsson M, Wong MH, Weber R (2008); *Organohalogen Compounds* 70, 813-815
5. Rectificatie Chemie-Toxiciteit-Toets verspreiding zoute baggerspecie (2004); Staatscourant 5 juli, nr. 125
6. Veileder for risikovurdering av forurenset sediment (2005). TA-2085. Rev 1, juni
7. Besselink HT, Schipper C, Klamer H, Leonards P, Vethaak H, Felzel E., Murk AJ, Thain J, Hosoe K, Schoeters G, Legler J, Brouwer B (2004); *Environm. Toxicol. Chem*., 23: 2781-2789
8. Richtlinien für den Umweltschutz VDS 2357: 2007-04 (05). Publisher Gesamtverband der Deutschen Versicherungswirtschaft GDV.
9. Lindert M and Fiedler H (1999). UWSF - Z. Umwe|tchem, Okotox. 11 (1) 39 – 47.