Bioanalytical detection methods (BDMs) and monitoring

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Introduction

A battery of *in vivo, in vitro* bioassays and ligand binding assays have been developed to detect the toxicity of the chemical family of halogenated aromatic hydrocarbons (HAHs), such as several PCDDs/PCDFs, PCBs, PCNs and brominated analogues of the above mentioned chemical groups always in comparison to the toxicity of 2,3,7,8-TCDD. Bioanalytical detection methods (BDMs) includes the AHH/EROD bioassay, recently new developed sensitive and specific enzyme immunoassays (EIAs), reporter gene assays (CALUX), the GRAB assay, the DELFIA Dioxin TEQ assay, the filtration assay with radiolabelled dioxins and the Ah-immunoassay (AhIA). Most of BDMs for the determination of TEQs are based on the assumption that dioxin-related compounds all act through the Ah receptor (AhR) signal transduction pathway (EROD, CALUX, CAFLUX) and/or based on antibodies (EIAs).

Several reviews and reports have been already published about BDMs for DLCs[1-16]. The aim of this review will be to describe principles and advantages/limitations of these BDMs to detect DLCs and will give some examples of their applications in field studies in comparison to the chemical analysis.

Biomonitoring design

This review focus on relative potencies (REPs) (or also named TEFs; NATO/CCMS, 1988) for single compounds and dioxin induction equivalents (IEQ)[7] or bio-TEQs[12] for complex mixtures of DLCs. The ratio $R_{b/c}$[10] describes the comparison between the bioanalytical (bio-TEQ or IEQ) and the chemoanalytical (TCDD- or I-TEQs) response.

The biomonitoring design depends on the choice of the BDMs, the applied clean-up method and the expected ratio between bioanalytical and chemical detection. If the ratio $R_{b/c}$ would be between 1 and 10 the results would be fully accepted by additional reanalysis of the AhR dependence with an AhR antagonist (like 4-amino-3-methoxyflavone). 10% of the negative samples should be further confirmed by chemical analysis. If the $R_{b/c}$ would be under 0.1 or higher than 10 the complex environmental mixture should be fractionated, the potential active compound characterisized by mass spectrometer libary and additional bioassay battery screening.

Advantages and Limitations of several biochemical detection methods

This literature report reviewed (Table 1 and 2) the state of the art knowledge about advantages/ limitations of several biochemical detection methods (EROD, CALUX, CAFLUX, EIAs, Ah-IAs, Ah receptor assays, yeast assay, PAP assay, DNA binding assay, DELFIA Dioxin TEQ assay, GRAB assay). The detection limits of several BDMs have been reported similar to the chemicals analysis: Micro-EROD (0.06)[14], chicken embryo hepatocytes (0.16)[15], CALUX (rat: 0.06; mouse: 0.64)[16] or enzyme immunoassays [DF1: 3-4; Sugawara et al. (1999)[17]: 0.5; all data in pg/well][6].

Application of the different bioanalytical technologies

Dioxin-like compounds are PCBs (which can assume a planar orientation), PXDD/DFs (X= chlor, bromine or fluorine), alkyl-substituted R-PCDD/PCDFs, PCNs, polychlorinated

dibenzothiophenes (PCDTs) and polychlorinated thianthrens (PCTAs). Additionally, some PAHs, terphenyls and polychlorinated terphenyls (PCTs), HCBs, and PBDEs are reported to influence the dioxinlike potency in environmental samples.

This review will focus on REP values of these DLCs analysed by different BDMs. This presentation gives a summary of the applications of these biochemical detection methods in field studies in industrial processes/products (incinerator processes, technical PCB-mixtures, sludge, Commercial/Consumer samples, Human and animal food), in the environment (water, air, soil, sediments) and in the uptake of the biota, wildlife and humans.

Conclusion

This literature study reviewed the principles of current biochemical technologies for DLCs and discussed there advantages/limitations. Most of the biodetection methods demonstrated comparable REP values to the I-TEF for DLCs (WHO, 1998). Also several studies from complex environmental samples like biosludges, PCB-mixtures or various samples from incinerators and the final uptake in wildlife/humans showed comparable data to the chemical analysis[13].

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Table 1: Some advantages and drawbacks of biodetection methods (BDMs) for dioxin-like compounds (DLCs)

BDMs	Advantages	Drawbacks	Application
***In vivo* biomarker**	Most defensible screening tool for wildlife, because of uncertainties of the *in vitro* bioassays in bioavailability and toxicokinetics. Necessary to confirm the *in vitro* results	Costly, time consuming, methods required cuthanasia or invasive surgical techniques for animals; ethical critical; at high doses competitive inhibition occurred.	
AHH/ EROD H4IIE cells	Most published data, most experienced, "golden standard of bioassays", no patent or "expensive cells"; Analysis the sum of biological relevant TEQ-detection of the persistent class of AhR active compounds is possible; Analysis of the catalytic activity of CYP1A1 reflects more the real effects on human/wildlife than immunoassays or luciferase induction (incorporated the effects of metabolism, serum binding, pharmacokinetics); Good linear correlation with *in vivo* assays; Metabolic capacity, because a long incubation time is possible (e.g. TEF for 2,3,7-TriCDD <0.001), with a resulting better linearity for samples with labile AhR binding compounds; More physiological relevant than CALUX; Bioassay quality: CV 29-38%;	Many chemicals are substrates for P4501A1 and can inhibit EROD activity (PCBs) leading to a lower induction. More narrow linear working range than the CALUX; More time consuming, HTPS would require faster and less expensive alternatives; Significant species; Sensitive to oxidative stress; *In vivo* season-dependent fluctuations in inducibility, low enzyme and mRNA stability.	REPs for PXDD/ Fs (X= Br, Cl, F); PCBs; PAHs Application: CU (R); Fl (B); S (R); F (R); C (B); CP (R); Sd (R); W (R);
CALUX	Induction of reporter genes through AhR binding, analysing luciferase and assayed on the basis of light production, for which extremely sensitive detectors exist; Analysis of the biological relevant sum of TEQ; Bioassay quality: CV 29%; Short chemical exposure time possible (about 5 h); Distinguishes between agonist/antagonist; Tissue- and species-specific (rat H4IIE-IF 25, mouse H1L1.1c7-IF 75; rat higher metabolic capacity than mouse; mouse greatest concentration of AhR); HTPS possible; Provides choice of reporter gene; cope with important biological effects (e.g. membrane passage; proteinbinding)	Specialised instrumentation; more expensive and limited usage. Short time assay has the risk of analysing labile AhR agonists; Stability of luciferase; Missing of possible tissue factors due to the transformation into a recombinant cell; Leaves out outer signal pathways; Induction for any compound capable for binding to the AhR- without clean up higher TEQs/false positive results are reported (e.g. in blood)	REPs:PCDD/F ; PCBs; PCNs; PBDE; Application: CU (B); Fl (B); S (R); F (B); C (B); Sd (R); PP (R)
CALUX-ERODcompariosn	Similar REPs for DLCs analysed by EROD and CALUX; CALUX 100 times faster, more stable response with better reproducibility; CALUX insensitive to substrate inhibition; CALUX higher selectivity; Maximum IF 3-times higher for CALUX; Luciferase more stable than EROD protein	CALUX not affected by posttranscriptional and –translational events (luciferase is foreign for the cells) and not be dependent on a functional CYP1A1 gene, although the AhR path is present	
CAFLUX	Enhanced green fluorescent protein as reporter gene allows longer kinetic than CALUX; Less complicated and cheaper than CALUX, since no expensive substrate or luminometer necessary; Non-destructive methods allows to follow the expression on a real-time basis.	Cumulative signal: very sensitive for low concentrations of Ah non-persistent agonists, but difficult to analyse only the persistent class of DLCs	

[MDL minimal detection limit; CR cross-reactivity; Fl fly ash; F food; S sludge; CP compost; C combustion gas; CU clinical use; W water; A air; S soil; Sd sediment; P PCB-mixtures; R in research; D in development; B business; PP paper; CV coefficient of variation; IF induction factor; CC correlation coefficient]

Table 2: Some advantages and drawbacks of biodetection methods (BDMs) for dioxin-like compounds (DLCs)

BDMs	Advantages	Drawbacks	Application
EIAs	Speed, rapid turnaround time, simplicity, low cost (about 1/10 of chemical analysis), parallel processing of many samples, easily HTPS and possible portable field use	Costly development, cross reacting compounds, non specific interferences and not standardised enough; Distinguishs not between metabolic stable/unstable DLCs.	
A) DF1 (CAPE) (pabs)	MDL: 4 pg/assay; Most frequently used EIA with most and detailed validation data; Most experienced with clean-up; in fly ash, wood and sediments lower $R_{b/e}$ than the Micro-EROD	Low CR value of 2,3,4,7,8-PCDF 0.17; High CR for 2,3,7-TriCDD (0.24); No CR with PCBs. H7/O8-CDD/DFs; Overestimation of the I-TEQ in fly ashes; Acceptable low false negative rates for soil	CR: PCDD/Fs PCBs, Appl.: Fl (B); S (B); F (B); Sd (R) ; CU (B)
B) DD3 (SDI) (mabs)	CR value of 2,3,4,7,8-PCDF (0.55) similar to the WHO-TEF (0.5); Lowest CR value for 2,3,7-TriCDD (0.14)	Not anymore frequently used; MDL: 80 pg/assay; Broadly specific; No validation data on real samples yet; low CR	-
C) Sugawara et al, 1998; Pabs	Minimal detection limit: 0.5 pg/assay	for 2,3,7-TriCDD (0.28) and 2,3,4,7,8-PCDF (0.03)	In process
D) RISc kit (SDI)	Highly specific for 2,3,7,8-TCDD; Mabs	MDL: 70 pg/assay; Application on real samples necessary;	-
Ah-IA (Paracelsian)	Hybrid assay containing a cloned AhR bound by an antibody; Minimal detection limit: 1 pg/assay; simple, cost effective (100$/sample).	Limited validation and application data Uses cell extract which requires careful handling, including frozen storage; Run time longer than DF1	Testing period for Fl, C, F, CU, S, Sd
Bioassays using AhR containing extracts (e.g. AhR ligand binding or GRAB assay)	Rapid, inexpensive, sensitive, simple; identification of all AhR agonists/antagonists; Species/tissue specific for all kind of AhR agonists; DNA binding activity correlates extremely well with its biological activity; Utilises species/tissue of choice; cell free system; Several studies with the AhR binding assay in comparison to *in vitro* and *in vivo*	Detects only AhR ligands (no information about biological activity), does not distinguish between agonist and antagonist; several false positives; Requires radiolabelled ligands (^{125}I-DBDD or ^{3}H-PCDDs/PCDFs) or gel separation and quantification of protein-bound from protein-free DNA GRAB requires ^{32}P-DNA preparation, handling, disposal;	Several AhR agonists; Paper and household products
DELFIA® Dioxin TEQ Assay™	Based on time-resolved fluorometry of lanthanide compounds such as europium which exhibits a unique fluorescence; Ultrasensitive, QA/QC routines, retained standard curves, LIMS compatible data-reduction, Based on a molecular cloning system and clinical instrumentation	Application on real samples necessary Clean up problem solved, but no details published yet Currently in the validation and application of several field studies; Use time-resolved fluorescence which requires an expensive instrument	PCDD/PCDF, co-planar PCBs, PAHs
Yeast bioassay	Human AhR and ARNT coexpressed in yeast reflects well the actual biology of the AhR complex; Study of ER and AhR signalling way possible	New development under validation and application Lack of background endogenous hormones/receptors; Transport differences of DLCs across cell membranes and different receptor populations than mammalian cells	S (R)
Cell proliferation (keratinocytes)	Analysis the hallmark of dioxinlike response: chloracne; sensitive, distinguish between agonist/antagonist	Variations within cell types with respect to coactivators, accessory proteins and growth factors will make a interlaboratoty study maybe difficult	

[MDL minimal detection limit; CR cross-reactivity; Fl fly ash; F food; S sludge; CP compost; C combustion gas; CU clinical use; W water; A air; S soil; Sd sediment; P PCB-mixtures; R in research; D in development; B business; PP paper; CV coefficient of variation; IF induction factor; CC correlation coefficient; pabs polyclonal antibody; mabs monoclonal antibody]