A COMPARISON AND DISCUSSION OF TWO DIFFERING METHODS OF MEASURING DIOXIN-LIKE COMPOUNDS: GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND THE CALUX BIOASSAY - IMPLICATIONS FOR HEALTH STUDIES

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Introduction

High-resolution Gas Chromatography/Mass Spectrometry (GC/MS) has become the "gold standard" for identifying dioxins and dioxin-like compounds in a sensitive and specific fashion. In addition to the analytical approach to dioxin exposure assessment, a recently developed *in vitro* chemically activated luciferase gene expression (CALUX) bioassay allows the quantification of dioxin-like ligand present in human serum (or in environmental samples) through measurement of chemically induced luminescence using cells transfected with halogenated aromatic hydrocarbons (HAH)-inducible luciferase vector.[1,2] This biological approach can, in theory, be a more cost-effective and faster method of quantifying dioxin-like compounds. Only 1ml of blood sample is required for CALUX bioassay rather than 25-200 ml required for GC/MS. In addition, the turn around time has been estimated to be weeks for CALUX and months for GC/MS analyses. Costs have been estimated to be five to ten times higher for GC/MS than the bioassay. It was hoped that CALUX results would be similar to GC/MS results and that CALUX could be used for screening with higher CALUX blood and environmental samples to be confirmed by congener specific GC/MS.

In this study, we analyzed human blood samples collected from exposed residents living in a heavily industrialized area using both methods. We hypothesize that CALUX bioassay as done here, without prior clean up such as column chromatography, will select total Ah receptor sensitive chemicals, thereby yielding a higher total dioxin-like compound measurement. Thus, biological activity along with contamination will yield higher dioxin induction equivalency (IEQ) in human blood or other samples than the dioxin toxic equivalency (TEQ) from PCDDs, PCDFs, and coplanar PCBs alone.

Materials and methods

In the course of a consultation in 1997 regarding potential human contamination with dioxins from a vinyl chloride facility located in the southern U.S.A., whole blood samples were collected from nine adults. A pooled blood sample used as a control was collected in the same year from surplus hospital blood of adults residing in the same area.

GC/MS Protocol: The blood samples were frozen and shipped on dry ice to ERGO laboratory. Analytical methodology involving capillary column cleanup and high resolution GC/MS has been previously described.[3] WHO 1998 TEFs were used in determining total TEQ of dioxin-like compounds.[4]

CALUX Protocol: The method using mouse hepatoma (Hepa1c1c7) (H1L1.1c2) cells containing a stably transfected HAH-inducible luciferase reporter plasmid pGudLuc1.1 was described previously.[2] Cells exposed to dioxin-like compounds (Ah receptor agonists) induce firefly luciferase activity in a time-, dose-, and AhR-dependent manner. [2] Blood samples were extracted in three volumes of hexane, dried under a stream of nitrogen and resuspended to 1/10th the original volume. Cells were incubated with 2. L of sample extract for 4 hours prior to analysis of luciferase activity. Induction equivalents (IEQs) were calculated for each sample relative to standard luciferase induction curve generated using 2,3,7,8-TCDD.

Results and Discussion

Results are shown in Figure 1 not lipid adjusted, but on a whole or wet weight basis. The two methods generated different results. GC/MS TEQ ranged from 0.04 – 0.52 pg/g or ppt, while CALUX IEQ ranged from 97 – 823 ppt. The CALUX-bioassay consistently showed higher readings than obtained by GC/MS. These variations suggest that each method provides different assessment of dioxin-like exposure.

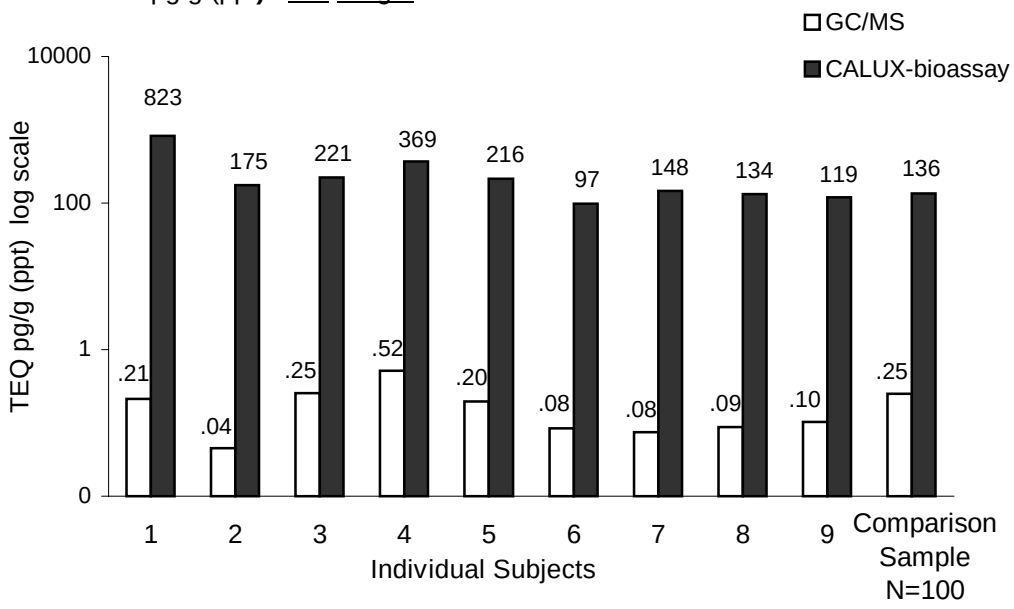
There are fundamental differences between the derivation of GC/MS analytical TEQ and the CALUX determination of IEQ. GC/MS TEQ is based on *in vivo* studies, whereby relative potencies are based on administered dose. Consequently, pharmacokinetic differences between the chemicals and binding affinity to the Ah receptor are maximized and absorption, elimination, and distribution are compared to 2,3,7,8-TCDD. In contrast, the pharmacokinetic differences between chemicals are minimized in the CALUX bioassay due to their limited metabolism in the H1L1.1C2 cells. Hence, the relative potency of HAHs in the *in vitro* CALUX bioassay is determined predominately by their binding affinity to the Ah receptor. (The relative potency of other chemicals are dependent upon their Ah receptor binding affinity and metabolic stability.) Thus, *in vivo* validity of a measure may not be the same as *in vitro* validity.

Moreover, each method provides unique interpretation. For instance, while GC/MS provides congener specific analysis, interaction of chemicals in the biologic system can not be assessed. On the other hand, while analysis using CALUX bioassay quantifies the overall dioxin-like IEQ level, it does not provide congener specific analysis, and measurement is limited to the metabolic capabilities of the cell line used.

The higher reading obtained in the CALUX bioassays additionally suggests that there are other biologically active compounds in human blood samples which can activate the Ah receptor pathway in the CALUX bioassay, thereby increasing the total dioxin-like measurement. Possible compounds include dietary compounds such as indole and tryptophan metabolites products[5,6], heme breakdown products[7], as well as environmental compounds such as PAHs, PBBs, PBNs, azo and azoxybenzenes, and HCB[7,8]. These and other chemicals are known to interact and activate the Ah receptor, but have not been assigned TEF or IEF values. In addition, PCBs have

been known to act as Ah receptor antagonists in a species-specific manner.[2] The presence of these antagonists will be observed and included in the CALUX bioassay, but not in the GC/MS analytical TEQ methodology. The lack of chemical cleanup of human blood samples prior to CALUX analysis, unlike that in the GC/MS approach, will clearly result in artificially higher CALUX relative activities (IEQ). Thus, the presence of other Ah receptor agonists or antagonists will contribute to the difference in dioxin-like compounds measured using these two approaches.

Figure 1. TCDD toxic equivalents (TEQ) of dioxins, dibenzofurans and coplanar PCBs in blood based on high resolution (GC/MS) vs. TCDD induction equivalents (IEQ) using the (CALUX) bioassay



CALUX test provided higher values than the TEQ sum from GC/MS in this case report for human blood. Although the CALUX bioassay could provide a fast and less expensive biological test for dioxin-like compounds, the presence of other compounds in human blood which can interact with and activate the Ah receptor can result in overestimation of the actual level of HAHs. It should also be emphasized that the chemical laboratory performed a careful chemical cleanup procedure prior to GC/MS analysis, whereas the blood did not go through a chemical clean up procedure prior to analysis using the CALUX bioassay. We conclude that although the CALUX bioassay is a useful approach which allows detection of total Ah receptor activation activity present in a given sample and may provide a better representation of the potential dioxin-like toxicity present than GC/MS for PCDDs, PCDFs, and PCBs alone, it cannot be used as a direct screening substitute for GC/MS analysis with the methodology used in this study.

Inclusion of an appropriate chemical clean-up procedure will likely increase the specificity of HAH detection by the CALUX method in human blood and other samples.

We conclude that CALUX bioassay may be useful in providing different information than we originally desired and at this time, however, without inclusion of appropriate sample clean up procedures, the CALUX bioassay is not a selective screening test for dioxins and dibenzofurans present in human blood samples.

This paper does not necessarily reflect official U.S.E.P.A. policy.

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