APPLYING TEFS IN RISK ASSESSMENT (AN UPDATE ON SYSTEQ, II)

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Introduction

The European Union Seventh Framework-sponsored research project SYSTEQ is aimed at improving the database for the generation of 'systemic TEFs', i.e., Toxicity Equivalency Factors for 'dioxins' and 'dioxinlike' compounds which not only take into consideration the biochemical potency a compound as 'dioxinlike' but also the biological (or toxicological) potency of the compound in a relevant organism, ultimately in humans.
This approach is not completely new since former expert panels already took into consideration 'kinetic' features of certain 'dioxins' without applying a certain 'factor'. In fact the derivation of TEFs is an interactive procedure which over the years turned from a 'common sense' approach among a group of experts into a more scientifically-based procedure (van den Berg *et al.*, 1998; 2006). This became possible by the expansion and quantitative use of the database on biological and toxicological effects of dioxins more extensively used in later TEF derivations. In turned out that the 'relative potency' approach (REP approach) is the most suitable since it attributes any compound tested in a certain biological model with a potency relative to TCDD which has to be tested in the same model, of course. Thus, a single REP for a certain congener is usually derived from an individual study (publication). A few additional prerequisites and pitfalls are usually discussed during this procedure:

a. These may include a certain minimum number of data points used for the calculation of a dose (concentration)-response-curve and parameters describing these curves. Such parameters include the height of the maximum effect level and the slope of the curve at any given data point. Although many dose (concentration)-response-curves have a sigmoidal shape and do not differ considerably in their slope, e.g., at the EC_{50} value, the maximum responses can be very different. Here, qualifying limits which define if and when a dataset is suitable for risk assessment need to be determined in the future. This problem is particularly evident, if EC_{50} values cannot be estimated since the maximum effect of a certain congener does not reach 50% of the maximum TCDD effect.

b. Another issue is the fact that REPs for a certain congener can vary over more than one order of magnitude when all available REPs from different models are considered. Such variation may even occur among related models such as CYP1A induction assays in primary cells in culture and/or permanent cell lines. In a few cases even the 'identical' model used in different laboratories led to pronounced differences in REP estimates. Finally, the same model such as human hepatocytes in primary culture may elicit considerable differences between preparations probably reflecting inter-individual variability (Schrenk *et al.*, 1995).
All consultancies dealing with this problem decided to assess each individual study using certain quality criteria. In case of consideration of a study, REPs from 'identical' models should be weighted equally, whereas different models seem to require a selective weighting procedure given different weight to the datasets. A priority list for such an approach could look as follows:
A: human *in vivo* data
B: animal data
C: human primary cells
D: animal primary cells
E: human cell lines
F: animal cell lines
G: subcellular models (receptor binding, microsomal metabolism etc.)
H: in silico calculations based on theoretical considerations, not on experimental data

c. in principle, REPs derived from *in vitro* data do not (or to a very low extend) take into account toxicokinetic properties of a congener. This issue is particularly tackled by SYSTEQ including *in vitro* REPs of relevant parameters in conjunction with *in vivo* data in animals (and humans) with simultaneous determination (or estimate) of target levels (tissue concentrations etc.) of the congener.

Materials and methods

PCDDs (TCDD, 1-PnCDD, 1,6-HxCDD, 1,4,6-HpCDD), PCDFs (TCDF, 4-PnCDF, 1,4-HxCDF, 1,4,6-HpCDF) and PCBs (77, 105, 118, 126, 153, 156) were from Dow, Chemical Company, USA. All compounds were analyzed and if necessary purified by the Department of Chemistry at Umeå University, Sweden. Consumables were obtained from Greiner (Frickenhausen, Deutschland), chemicals were from Sigma Aldrich (Steinheim, Germany) unless noted otherwise.
Primary rat hepatocytes were obtained from male Sprague Dawley rats (Charles River; Sulzfeld, Germany) and incubated with the aforementioned congeners as described elsewhere (Neser *et al.*, 2011). TCDD (1 nM) served as positive control for EROD induction. EROD activity was measured after 24 h of treatment.

Results and discussion:

The *in vitro* analysis of EROD induction is one of the most widely used methods aimed at a quantitative measurement of Ah receptor (AhR)-driven activation of gene expression. A set of typical concentration-response curves is shown in Figure 1.

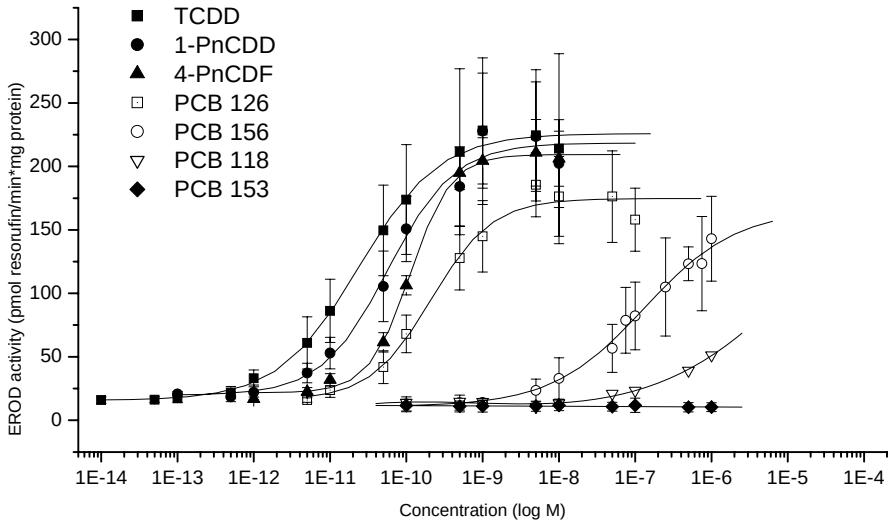

Figure 1: EROD activity (pmol resorufin/min*mg protein) in primary rat hepatocytes after 24 h of treatment with TCDD, 1-PnCDD, 4-PnCDF, PCB 118, PCB 126, PCB 153, and PCB 156. EROD activity displayed including background levels.

Figure 1 shows the original EROD activity data which exhibit a certain background activity without test compound added. This activity, which might be due to the presence of endogenous inducers and/or inducer-independent expression, is usually subtracted trying to identify the pure compound-dependent effect. Furthermore, it is obvious that certain inducers, e.g. PCB 118, can lead to very flat induction curves. This circumstance was dealt with by applying the EC_{20} concept (Neser er al., 2011) which determines the

concentration leading to a 20% induction compared to the full-scale effect of TCDD. Since the slopes (shapes) of the induction curves obviously were not identical in all cases (Fig. 1), EC_{50} and EC_{20}-derived REPs may differ which is in fact shown in Neser *et al.* (2011). It is a matter of further discussion if the EC_{20} approach is more suitable for risk assessment since relevant (human) exposure-related levels usually are much lower than those employed in the induction assays making a lower concentration appearing more relevant than a higher one.

In summary, the choice of EROD induction (or the related induction of CYP1A mRNAs) as a major *in vitro* parameter for the derivation of TEFs appears plausible because CYP1A induction is

- the most robust biochemical response to AhR activation
- the most sensitive response to AhR in many experimental systems
- tightly related to AhR activation in a well-understood manner
- relatively easy to measure and standardize between laboratories

The choice of hepatoma cells and hepatocytes appears plausible because

- liver is a major target organ of ‘dioxin’ toxicity
- liver cells strongly respond to 'dioxins’
- rat (and human) hepatocytes are easily available and relatively ‘uniform’ although zonal and inter-individual (humans) differences exist
- hepatic levels of ‘dioxins’ have been determined in a number of animal studies
- blood levels and liver levels in humans may follow a certain ratio (needs to be investigated)
- liver cancer in rodents is an endpoint which has been considered in risk assessment

However, the following questions need to be tackled when extending the database for future risk assessment

- is the variability among human hepatocyte preparations form various donors reflecting the *in vivo* variability in humans or does it depend on other factors?
- is ‘the’ human hepatocyte the ‘golden standard’ for *in vitro* AhR activation?
- are the effects on gene expression identical between *in vivo* and *in vitro* (e.g. CYP1A1 vs. CYP1A2 induction)?
- does the lower sensitivity of CYP1A induction in human liver cells reflect a lower sensitivity towards ‘dioxins’ in humans in general when compared to rodents?
- is the liver cell model suitable for risk assessment if other endpoints (in animals) are considered being more sensitive (development effects etc.)
- which is the relevance of background gene expression of AhR-dependent genes?
- which is the quantitative link between AhR-dependent CYP1A induction and liver cancer?
- which is the relationship between hepatic ‘dioxin’ levels and AhR-related effects in humans?

Acknowledgements:

We gratefully acknowledge the European Union Seventh Framework Project SYSTEQ under grant agreement number FP-ENV-226694. The Dow Chemical Company financially afforded the purchase of compounds.

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