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RANKING THE BIOASSAY TEFs AND REPs

Raymond C. Vaughan
Department of Geology, University at Buffalo, Buffalo, NY 14260, USA

Introduction

In vitro bioassays based on the Ah receptor are being used increasingly in environmental testing and screening.[1,2] CALUX, developed by Xenobiotic Detection Systems, Inc., is one of the best-known bioassay methods. Another, the Human Reporter Gene System (HRGS), has recently been designated EPA Method 4425.[3] In general, these bioassays have a broad response spectrum. They respond not only to dioxin-like compounds but also to polycyclic aromatic hydrocarbons (PAHs).[3,4] Cleanup can remove PAHs from environmental samples reasonably well, leaving "only" the dioxin-like compounds.[3,5] However, for soil and sediment samples, what remains after PAH cleanup tends to be a complex chemical mixture of dioxin-like compounds and other materials.

If such a complex mixture produces a high response in a bioassay, the chemicals that contribute to the response are not immediately identifiable. The response, typically expressed as a light signal, effectively integrates all components in the sample and provides no chemical-specific information. If 2,3,7,8-TCDD were the only contributor, the dose-response relationship would be straightforward (see Fig. 1), but the source of the bioassay response is less simple when the sample contains many chemicals that bind to the Ah receptor. The response in this case is approximately the sum of the contributions of the dioxin-like compounds in the sample. The generally accepted procedure is to multiply the concentration of each dioxin-like chemical by its toxic equivalency factor (TEF) or relative potency (REP), where either TEF or REP represents the potency or toxicity of the chemical relative to 2,3,7,8-TCDD. ("TEF" is often used generically but "REP" is preferred in a bioassay context.) The sum of these TEF-weighted or REP-weighted concentrations should then equal the bioassay response in toxic equivalents (TEQ). This is a useful general rule, although in some cases there may be synergism or antagonism that makes the bioassay response either greater or less than the sum of the TEF-weighted or REP-weighted concentrations.[2]

Methods and Materials

Various bioassay-based TEF or REP values have been published. Some of these values have been reviewed and compiled for this study. Where necessary, values have been calculated from relative concentration (EC25 or EC50) data.[2] Values reported in the literature for a given compound may vary considerably, reflecting different bioassay techniques and test conditions, but a general ranking of these TEF or REP values provides useful guidance. Fig. 2 shows a compilation of values for a various dioxin-like compounds, including some that fall outside the usual realm of dioxins, furans, and coplanar PCBs. Published sources are identified by number after the name of each compound in Fig. 2, and the range of reported values is represented by the solid bar on the graph. For comparison, Fig. 2 also includes a few candidate compounds that elicit no response and thus are not dioxin-like.

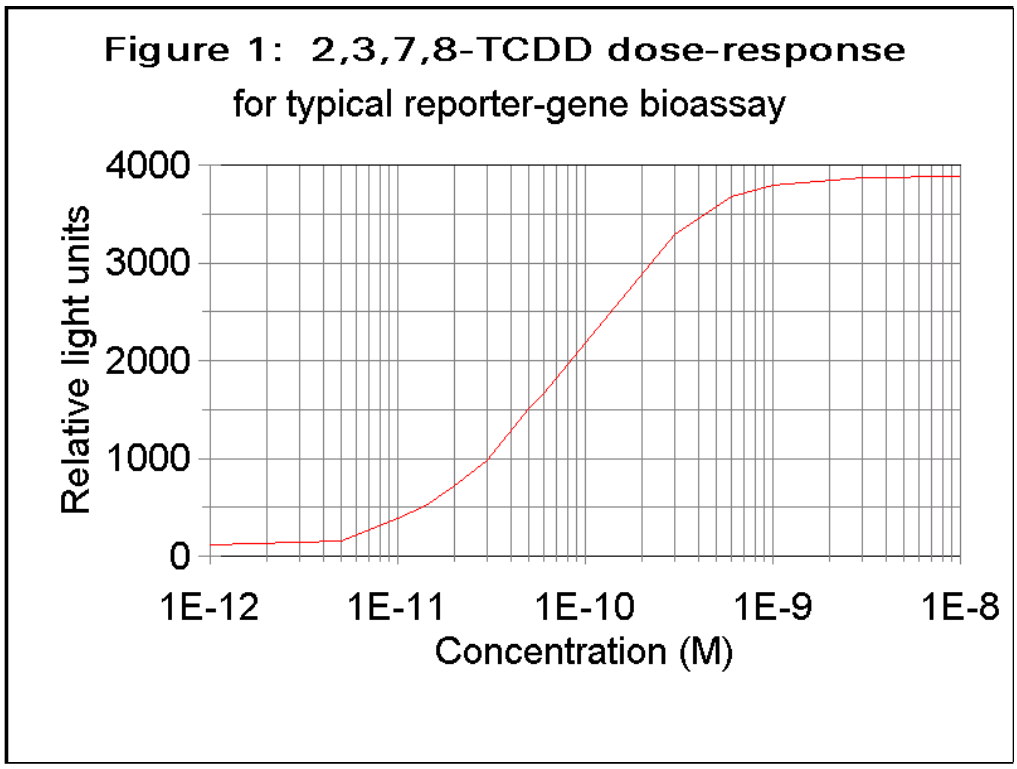


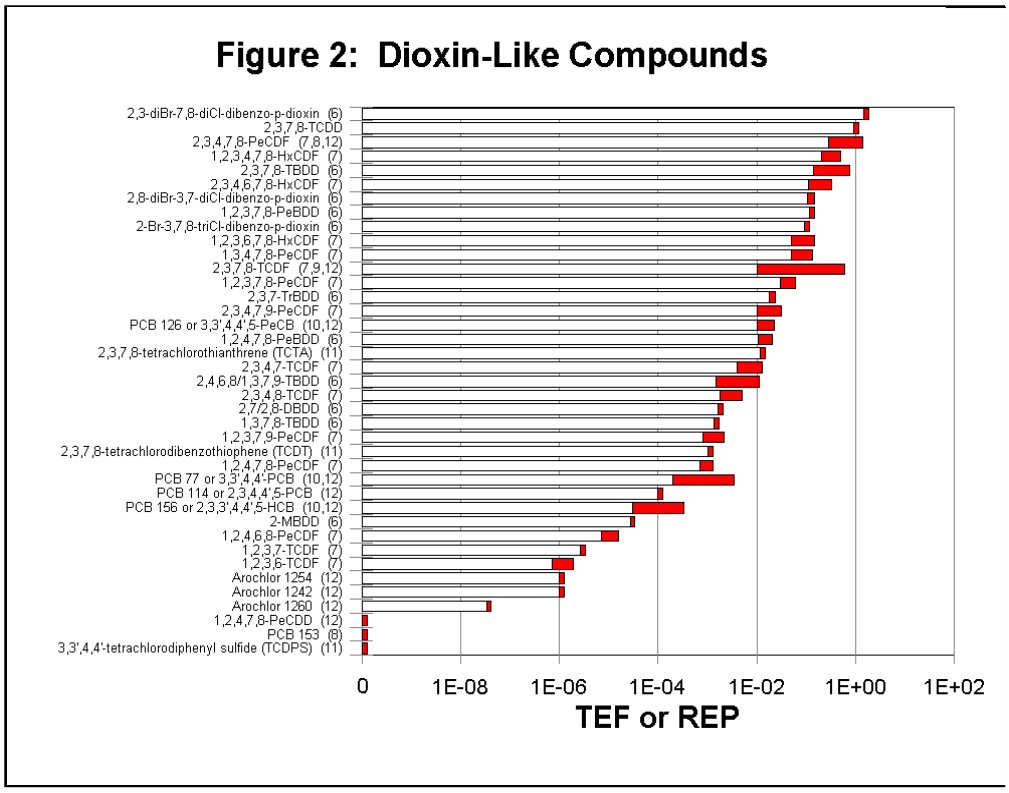
Fig. 3 shows a similar compilation of bioassay-based TEF or REP values for polycyclic aromatic hydrocarbons (PAHs). As above, references are indicated in parentheses after the name of each compound. The compounds included in Fig. 3 are representative; they do not necessarily include all compounds reported in that source.

Results and Discussion

The dioxin-like compounds in Fig. 2 show relatively narrow ranges of reported TEF/REP values. The broader ranges seen in Fig. 3 for the PAHs are due mainly to variations in either the bioassay incubation period or the response level at which PAH concentrations were compared to TCDD.[4] PAHs are especially sensitive to the incubation period. In the HRGS bioassay, for example, a maximal response for PAHs is seen after a 6-hr incubation period, after which the response tends to decrease by about a factor of 5 as the PAHs are degraded by enzymes.[3] For a given bioassay procedure, the reported range of responses would be much narrower. Despite the artificially broad ranges, the approximate overall TEF/REP ranking of these PAHs can be seen in Fig. 3.

In either Fig. 2 or Fig. 3, the TEFs or REPs represent not only the bioassay response relative to TCDD but also the toxicity that is assumed to be proportional to the bioassay response. The relationship between bioassay response and toxicity has not been fully established for all compounds that elicit a response, but an approximately proportional relationship (*within* each of the two categories, dioxin-like compounds and PAHs, but *not* between the two categories) is a reasonable assumption in the absence of definitive studies in vivo.

Several of the high-ranked dioxin-like compounds are bromo- or bromochlorodioxins, with 2,3-dibromo-7,8-dichloro-p-dioxin at the top of the list. These compounds have not been studied as extensively as 2,3,7,8-TCDD and the other chlorinated congeners but are beginning to receive more attention. One advantage of bioassay techniques is their sensitivity to low levels of these and similar chemicals for which highly sensitive and reliable GC-MS techniques may not yet be available. For this reason, bioassays may potentially provide an early warning of little-known or unknown compounds that have high dioxin-like activity, but this is speculative and must remain so until more work is done on complex chemical mixtures from soil and sediment samples that produce high bioassay responses.



Conclusions

Rankings of the TEFs or REPs for dioxin-like compounds and PAHs provide a useful guide not only to bioassay response but to known or presumed toxicity. Graphical rankings show at a glance the primary candidates responsible for high bioassay responses and, correspondingly, the primary candidates that may contribute to high toxicity in an environmental sample.

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Figure 3: Polycyclic Aromatic Hydrocarbons (PAHs)