DEVELOPMENT OF A NEUROTOXIC EQUIVALENCE SCHEME OF RELATIVE POTENCY FOR ASSESSING THE RISK OF PCB MIXTURES

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Abstract
PCBs produce adverse effects in humans and animals by several modes of action – the best known mode is binding of dioxin-like PCBs to the aryl hydrocarbon receptor. But other PCB congeners have different modes of action. Di-ortho-substituted non-coplanar PCBs interfere with intracellular signaling pathways dependent on Ca^{2+} homeostasis and produce changes in protein kinase C translocation, modulation of neurotransmitter release and uptake, formation of reactive oxygen species, and thyroid-related effects. Here, we propose a scheme of relative potency estimates (REP) for the PCB congeners not considered in the TEF scheme for dioxin-like PCBs. Because a number of the modes of action presented here for the ortho-substituted non-coplanar PCB congeners have been implicated in neurotoxic effects, this relative potency scheme is referred to as the Neurotoxic Equivalent (NEQ) scheme. The NEQ values were developed in a similar way to the well-known TEF values for dioxin-like PCB congeners. The NEQ scheme has three main benefits: (1) encouragement of discussion regarding the different modes of action and related PCB congener potency differences; (2) prediction of the toxicity of PCB mixtures and identification of other specific modes of action of the non-coplanar PCBs; and (3) improvement of future risk assessments of PCB mixtures.

Introduction
PCBs produce adverse effects in humans and animals by several modes of action. The best known mode of action involves the binding of a set of twelve coplanar and mono-ortho-PCB congeners to the aryl hydrocarbon (Ah) receptor. Another mode of action that has become linked to certain diortho- PCB congeners is interference with intracellular signaling pathways that are dependent on Ca^{2+} homeostasis and the consequent cellular, organ-level and organismal effects. These non-coplanar congeners produce adverse effects including changes in protein kinase C translocation, changes in cellular dopamine (DA) uptake, and formation of reactive oxygen species. These endpoints may be related by similar cellular or biochemical mechanisms, or the endpoints may be separate but occur in parallel fashion and appear to be related on an organismal level. These cellular endpoints likely underlie the well known neurotoxic effects of PCBs.[1,2,3,4,5]

The development of a relative potency scheme based on the endpoints mentioned above and the determination of whether this scheme could predict the NDL toxicity of different PCB congeners or mixtures could serve to clarify the relationship of these various cellular and biochemical effects in producing the toxic effects observed at the organismal level. Recently, a similar approach indicated the carcinogenic potency of Aroclor mixtures was driven by at least two different modes of action.[5] Hence, the goal of this current work is to propose and develop an initial relative potency scheme for NDL congeners [referred to hereafter as the Neurotoxic Equivalent (NEQ) scheme] that can be tested and modified as new data become available and should ultimately lead to a better understanding of the toxicity of individual PCB congeners and PCB mixtures. If successful, this scheme would, in turn, pave the way for the development of other relative potency schemes to predict PCB congener toxicity occurring by other modes of action.[6]

Although the TEF approach considers the impact of the presence of the twelve coplanar and mono-ortho-dioxin-like-PCBs, this approach does not account for the remaining 197 congeners. Filling this data gap for risk assessment of PCBs has become even more pressing since the release of the NAS review of EPA's dioxin reassessment.[7] The lack of cancer slope factor or reference dose for dioxin that is both scientifically credible and acceptable to US regulatory agencies renders moot any risk assessment for PCBs based solely on dioxin-like effects. In fact, recent work suggests that dioxin-like effects of PCB mixtures may contribute a lesser proportion of the risk associated with these mixtures and, thus, the risk associated with NDL effects becomes more important.[4,6,8,9] These findings also emphasize the need for an assessment method for the NDL PCB congeners.

The mode of action that has been of greatest interest for NDL PCB congeners involves changes in Ca^{2+} homeostasis.[4] In sum, a relative potency scheme for the non-coplanar ortho-substituted PCB congeners would enable risk assessors to adjust the toxicity estimate for a PCB mixture based on the amounts of ortho-substituted non-coplanar congeners present in the mixture. This adjustment method would predict the toxicity of a PCB mixture based on the amounts of the relevant PCB congeners in the mixture in the same way that the dioxin TEF scheme predicts dioxin-like toxicity of a mixture.

Materials and Methods

Toxicity estimates of various Aroclor mixtures relative to that of Aroclor 1016 and Aroclor 1254 are developed by combining congener-specific Neurotoxic Equivalence (NEQ) potency values obtained by consideration of relative potency estimates (REPs) from several sources as a measure of the potential for neurotoxic effects for each PCB congener.[4,10,11.12,13] These congener-specific NEQ values can be used along with measurements of the composition of various Aroclor mixtures to estimate the relative neurotoxic potency of each mixture. Adjusted reference doses for various Aroclor mixtures are developed that include consideration of both DL and NDL effects. PCB homologue analysis is one of more economical (and hence more common) methods of PCB analysis used in environmental investigations. Hence, NEQ values representative of homologues would be much more useful for risk assessment than congener-specific values. Therefore, the congener-specific NEQ values are also used to estimate the neurotoxicity for PCB homologues for use in the risk assessment of weathered environmental PCB mixtures that possess different homologue compositions than the original Aroclor mixtures.

The effects considered were [^{3}H]phorbol ester binding as a measure of PKC translocation in rat cerebellar granule cells, inhibition of microsomal and mitochondrial Ca^{2+} sequestration in rat cerebellum, reduction in dopamine content in PC-12 cells *in vitro*, and binding to the ryanodine receptor (RyR1) in rabbit skeletal muscle.[10,11,12,13] The measures of potency were EC_{50} or EC_{2x} values measured *in vitro*. These studies provided five measures of potency for a relatively large range of PCB congeners. For each of the individual effects, a neurotoxic REP value was calculated for each congener as the ratio between the lowest concentration producing a given effect level (e.g. EC_{50} value) in the particular study and the concentration producing that same effect level; hence, the congener with the lowest effective concentration would have a neurotoxic REP value of 1 and those for the less potent congeners would range between 1 and 0. The PCB congeners with the three highest NEQ values are 2,2',5,6'-tetraCB (PCB-53, NEQ=1.0), 2,2',3,4',6-pentaCB (PCB-95, NEQ=0.991) and 2,3,3',4',6-pentaCB (PCB-110, NEQ=0.971). Hence, the NEQ scheme represents the neurotoxicity of a given PCB congener relative to PCB-53 in the same fashion that the dioxin TEQ scheme represents Ah-receptor-related toxicity relative to 2,3,7,8-TCDD.

To obtain PCB homologue-specific NEQ values, the congener composition of each homologue was used along with congener-specific NEQ values. These homologue-specific NEQs were developed to take advantage of the lower cost homologue analysis for PCB mixtures instead of full congener analysis and thus enable flexibility in environmental decision making. To obtain Aroclor-specific NEQ values and adjusted reference doses, the congener composition of each mixture was used with the congener-specific NEQ values and the published reference doses for Aroclor 1016 and Aroclor 1254 were adjusted.[6]

Results and Discussion

Because of space considerations, the congener-specific NEQ values are not presented here; rather, the reader is referred to the original work.[6]

Comparison of the Effects of Single Congeners and Mixtures to the NEQ Predictions

The NEQ scheme can predict the effects of single PCB congeners on membrane integrity and consequent viability in rat cerebellar granule cell as well as dose-dependent changes in dopamine content of PC-12 cells and primate brain.[14,15,16] Measures of relative potency for the Aroclors 1016, 1254 and 1260 based on PKC translocation are remarkably consistent with the relative potency estimates predicted for the three mixtures using the NEQ scheme.[6,10] In a study of hyperactivity and impulsiveness in rats fed either Aroclor 1248 or contaminated fish from the Saint Lawrence River in Quebec, behavioral deficits in both groups were similar relative to those of a control group.[17] The total NEQ for fish obtained in Quebec was determined to be 0.309 mg NEQ/mg mixture and for

Aroclor 1248 was 0.363 mg NEQ/mg Aroclor, consistent with these behavioral results.[18]

NEQ Values specific for PCB Homologues

Because homologue analysis is one of more economical (and hence more common) methods of chemical analysis for PCBs used in environmental investigations, NEQ values representative of homologues rather than single congeners would be more useful for risk assessment. Percent compositions of individual congeners within each homologue groups was obtained from Aroclor composition data.[19,20,21] Within each homologue group, the average percentage of each detected congener in the mixtures Aroclor 1016, Aroclor 1221, Aroclor 1232, Aroclor 1242, Aroclor 1248, Aroclor 1254, Aroclor 1260, Aroclor 1262, and Aroclor 1268 was multiplied by its NEQ value. The total NEQ content within each homologue group is a measure of that group's neurotoxic potential and these homologue-specific NEQ values are shown below in **Table 1**.

Table 1. Homologue-Specific NEQ Values

Homologue	*NEQ (mg/mg homologue)*
DiCB	0.184
TriCB	0.284
TetraCB	0.383
PentaCB	0.541
HexaCB	0.310
HeptaCB	0.205

NEQ specific values for Aroclor Mixtures

To obtain estimates of the relative potency of the various Aroclor mixtures, the composition values for each congener were multiplied by the congener-specific NEQ values. The sum of these products for each Aroclor mixture represents a measure of neurotoxicity relative to PCB-53, the most potent neurotoxic congener in the present scheme.

Table 2 below shows the neurotoxic potencies, TEQ and adjusted RfDs relative to both Aroclor 1016 and Aroclor 1254.[22,23] Because Aroclor 1254 contains over 4 ppm dioxin-like TEQ, TEQ related effects were also considered as a possible contributor to the neurotoxicity of this mixture and the geometric mean of the relative TEQ and NEQ concentrations was used to adjust the RfD value, acknowledging the fact that TEQ present in the mixture may contribute to some neurotoxic effects observed. Because Aroclor 1016 contains only tiny amounts of TEQ, adjusting RfDs for other Aroclor mixtures that contain much greater amounts of TEQ would bias the adjusted RfD specific for neurotoxicity toward the TEQ contribution. Hence, mixture specific RfDs derived on the basis of Aroclor 1016 use their respective NEQ values only.

Table 2. Neurotoxic Potency of Aroclor Mixtures and Adjusted RfDs relative to Aroclor 1254 and Aroclor 1016

Mixture	*Aroclor 1254 Adjusted RfD (mg PCB/kg-day)*	*Aroclor 1016 Adjusted RfD (mg PCB/kg-day)*
Aroclor 1016	2E-04	7E-05
Aroclor 1221	5E-04	3E-04
Aroclor 1232	4E-05	1E-04
Aroclor 1242	2E-05	6E-05
Aroclor 1248	1E-05	5E-05
Aroclor 1254	2E-05	6E-05
Aroclor 1260	7E-05	1E-04
Aroclor 1262	9E-05	1E-04
Aroclor 1268	4E-04	1E-03

One cannot help but note the striking consistency between the two sets of adjusted reference doses. This fact suggests that the NEQ scheme can make reasonable predictions of the relative toxicity of various PCB mixtures.

We believe this proposed NEQ scheme will likely produce three beneficial outcomes. First, we expect the proposal of this NEQ scheme to foster open discussion as to how different modes of action can be utilized to predict congener potency differences for the various effects they produce. Second, we expect that evaluation and scrutiny of the ability of proposed NEQ scheme to predict the toxicity of PCB mixtures will assist in the identification of other specific modes of action underlying the effects produced by PCBs. Third, we anticipate that a providing a quantitative scheme to begin assessing the toxicity of the non-coplanar PCB congeners present in a mixture will significantly improve future risk assessments of PCB mixtures and provide a better understanding of the associated uncertainties.

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