WEIGHT OF EVIDENCE EVALUATION OF THE MODE OF ACTION FOR PCB-PROMOTED RAT LIVER TUMORS USING THE HUMAN RELEVANCE FRAMEWORK

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Introduction

It is well established that sufficient exposure of rodents to mixtures of polychlorinated biphenyls (PCBs) (i.e., Aroclors) can promote liver tumors[1,2,3], although the mode of action (MOA) remained elusive. However, a recent publication[4] described the likely MOA by which PCBs promote hepatic tumorigenesis in Aroclor-dosed Sprague-Dawley rats. This study involved numerous biochemical measurements taken over the course of a chronic bioassay[1] with Aroclors 1016, 1242, 1254 and 1260 and showed that liver tumors were closely, consistently, and predictably correlated with the net hepatic cytosolic activity of redox-cycling quinones (RCQ) acting as catalysts for the production of reactive oxygen species (ROS; initially, $O_2^{\bullet -}$, then H_2O_2). This indicated a tumorigenic MOA whereby (1) tissue PCB/TEQ accumulations induce expression of mixed function oxidases (MFOs) and (2) MFOs convert endogenous substrates to RCQs, eventually resulting in ROS-mediated promotion of spontaneously initiated liver cells. Findings from this study are corroborated by the results of other bioassays with TCDD, individual PCB congeners and binary mixtures of PCB congeners[5]. Because a well-defined MOA can now be described for PCB promoted rat liver tumors, the totality of the data can be used in a systematic evaluation of the relevance of these tumors to human health and for risk assessment. This evaluation follows EPA's Guidelines[6] in conjunction with the methods of ILSI-RSI[7,8] as well as IPCS[9] which are essentially identical. This methodology provides a decision-logic based approach for determining the relevance of the PCB-induced cancer in animal studies to humans.

Methods

The evaluation process used in the human relevance framework (HRF) asks a series of questions concerning the significance of the animal MOA data to human health. The EPA[6] guidelines provide the issues that must be considered and addressed in order to conclude that a MOA is sufficiently established based on the weight of evidence (WOE) while the HR framework provides a logical and systematic basis for complying with the EPA guidelines. The first question addresses whether the WOE is sufficient to establish the MOA in animals and includes (a) description of the postulated MOA and (b) the identification of key events (i.e., empirically observable precursor step that is a necessary element of the MOA). Importantly the MOA data must fulfill the well-established Hill Criteria including demonstration of strength, consistency and specificity of association, temporal association, dose-response and biological plausibility. The second question asks whether the key events in the animal MOA are plausible in humans and includes a qualitative and quantitative concordance analysis of animal and human responses. Ultimately, if the animal MOA does not include a mutagenic component and it can be demonstrated that the key events do not exhibit linear dose-response relationships, a non-linear approach can then be justified for cancer risk assessment purposes.

Results and Discussion

While the key MOA events briefly described below were first described by Brown *et al.*[4], all are corroborated by other independent studies which facilitate fulfillment of the requirements of the HRF, particularly those pertaining to satisfying the Hill Criteria as described above. Space limitations preclude a comprehensive description of all relevant data until a full-length paper (presently in preparation) has been peer reviewed and published.

Key MOA Elements

Accumulation of ΣPCB/TEQ Accumulation of sufficient tissue levels of difficult to metabolize PCBs or PCB/TEQ, rather than formation of PCB metabolites, is the first key event in rat liver tumorigenesis. This is best demonstrated in the NTP bioassays[5], which unlike the high dose Mayes et al.[1] study, included doses down to 1% of the maximum dose administered. Sufficient PCB/TEQ accumulation is clearly correlated with the eventual promotion of spontaneously initiated hepatocytes.

Induction of mixed function oxidases (MFOs) It has been well known that induction of MFOs (e.g., CYP1A1) was an obligatory first step in the overall MOA. Numerous *in vivo* studies have demonstrated that PCBs can readily penetrate cell membranes, activate nuclear receptors (e.g., AHR, CAR, and PXR), and induce expression of both Phase 1 [(oxidative) enzymes; i.e., Cytochrome P450 (CYP) families 1-3)] and Phase 2 (detoxifying/conjugating) enzymes[4]. MFO induction as measured by EROD is clearly correlated with the eventual development of hepatic tumors[5] as shown in Figure 1 with an obvious threshold for this response.

Generation of redox cycling quinones (RCQ) from endogenous substrates As described in Brown *et al.*[4], low molecular weight molecules identified as glutathionylated estrogen catechols were found in pooled hepatic cytosols from Aroclor-treated female S-D rats. Formation of quinones having redox-cycling activity has been demonstrated to result from the MFO-mediated metabolism of estrogen[10,11]. While it was not possible to quantify RCQ levels in individual Aroclor/dose groups, abundant data demonstrate that estrogen predicts the sex differences in tumors since estrogen metabolites are known carcinogens. Other corroborative data include a lack of TCDD-induced hepatic foci in ovarectomized rats as well as TCDD-induced foci in males following estrogen supplementation[12,13,14]. The identity of the small molecule cytosolic substrate in males remains uncertain.

Formation of reactive oxygen species (ROS) [O_2•−] It is well established that RCQ (particularly as derived from estrogen metabolites) is a source for the generation of ROS potential[10,11]. As reported in Brown *et al.*[4] cytosolic RCQ-derived ROS (O_2•−) production was highly correlated with liver tumors in both sexes and for all time points examined (i.e., increased ROS was an early predictor of eventual tumors as well as a late life correlate). Similar findings were also observed in the NTP studies[15,16]. Figure 2 illustrates ROS potential vs. ΣPCB which essentially parallels liver tumors vs. ΣPCB as shown in Fig. 3. An association between tumor promotion (which depends upon mitotic stimulation) and ROS is well recognized and extensively reviewed[17,18,19]. More recently, mitotic stimulation has been shown to be mediated by H_2O_2 which acts as an intracellular ‘‘second messenger’’ by selectively oxidizing certain signaling proteins, e.g., the protein tyrosine phosphatases and kinases that mediate mitosis[20,21,22].

Dose-Response relationships among key events involved in tumor development The MOA, as delineated by the key events described above, is further strengthened by additional data demonstrating temporal and sequential dose-response relationships among these key events. These additional relationships include: (1) female liver EROD vs. PCB 126; (2) cytosolic ROS vs. ΣPCB; (3) ROS vs. EROD and cell proliferation; and, (4) cell proliferation and liver glutathione peroxidase (GPx) vs. ROS. The overall MOA is illustrated in Figure 4 which shows the sequence of key events (data not shown).

Possible Alternative Modes of Action

The HR framework calls for consideration of possible alternative MOAs for a particular chemical. The most likely alternative MOA with respect to PCBs would be the possibility that mutagenicity might play a role in the development of PCB-induced tumors. There is a consistent absence of positive findings in *in vitro* mutagenicity tests on PCBs[23,24,25] as well as no evidence of PCB–DNA interaction products in Aroclor-dosed animals[26,27,28]. Two other plausible modes of action, i.e., (1) cytotoxicity and regenerative proliferation and (2) microsomal futile cycling by PCB-induced P450s, can be ruled out since the former was not correlated with tumors in a chronic bioassay while the latter made only a minor contribution to ROS and tumors[4].

Can Human Relevance of the MOA be Excluded on the Basis of Fundamental Qualitative or Quantitative Differences in Key Events Between Experimental Animals and Humans?

Is the animal MOA qualitatively relevant to humans? This question must be answered in the affirmative since both animals and humans (1) have the same xenobiotic receptors (e.g., AHR, CAR, PXR), (2) have many of the same MFOs, (3) produce ROS as part of normal metabolic processes, and (4) have the same ROS-controlling enzymes (e.g., GPx, SOD, CAT).

Is the animal MOA quantitatively relevant to humans? This question must also be answered in the affirmative with the important caveat that the following quantitative differences (i.e., kinetic and dynamic factors) must also be accounted for in a human risk assessment context: (1) substantial species differences in AHR and PXR, (2) no CYP1A1 or other PCB metabolizing MFO induction in occupationally exposed humans, (3) minimal CYP sexual dimorphism in humans, (4) humans are more efficient in controlling/reducing ROS, (5) PCB levels to trigger MOA events not achieved in humans, (6) dramatic species differences in responsiveness to PCBs exist[29] and (7) the WOE from occupational epidemiological studies shows that PCB exposure does not increase the risk of cancer[30].

Biological Plausibility and Confidence - Are key events and their sequence consistent with current biological thinking?

All of the individual key events in the MOA (i.e., MFO-RCQ-ROS-mediated promotion of spontaneously initiated cells to hepatic tumors) are well known biological processes and all identified key events correlate with subsequent tumorigenesis. In addition, the sequence of events is consistent with the known biochemical characteristics of the key events (i.e., ROS → promotion via O_2•−/H_2O_2–mediated mitotic signaling) and all key events are corroborated by other bioassay data with Aroclors, PCB 126, 153, 126 + 153, and *in vitro* data.

Conclusions

Sufficient data can now describe the MOA for PCB-promoted rat liver tumors. The WOE supporting the postulated MOA fulfills the Hill Criteria and the comprehensive requirements of strength, consistency, specificity, temporality, and dose-response as well as the overarching criterion of being a biologically plausible MOA based on the known characteristics of the individual elements in the MOA. Importantly, since the MOA clearly fulfills the rigorous ILSI/IPCS HR Framework and none of the elements show linearity at low doses the EPA guidance[6] concerning alternative risk assessment approaches can be used, i.e., *"A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses."*

Acknowledgments

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Figure 1. Female liver tumor (i.e., adenoma and carcinoma) incidence at 24 months versus 14wk hepatic EROD activity from the NTP PCB congener/mixture studies (NTP, 2006)

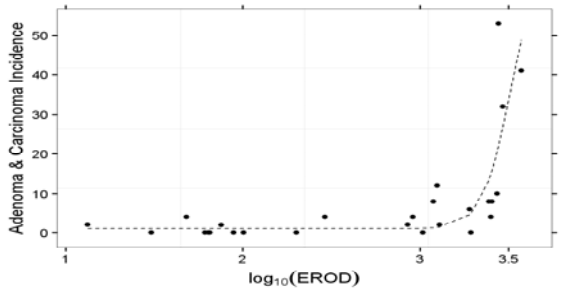

Figure 2. Liver cytosolic ROS potential versus hepatic PCB concentration at 6 months from the Brown et al. (2007) study

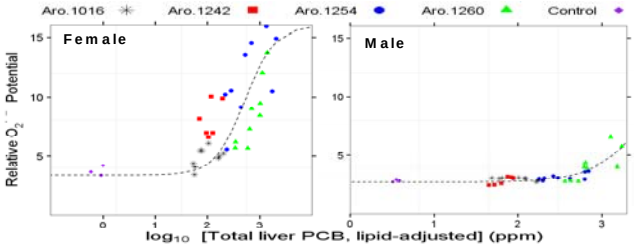

Figure 3. Liver tumor incidence at 24 months versus liver PCB concentration at 6 months in the Maves *et al.* (1998) study

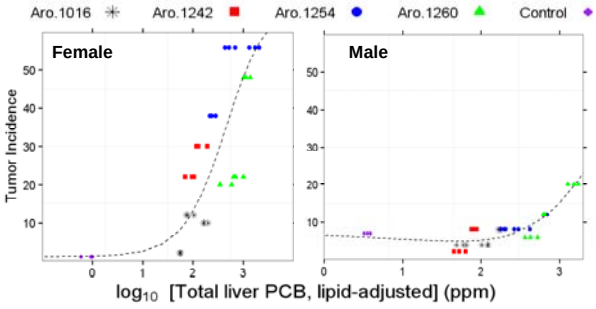

Figure 4. Demonstrated MOA for PCB-promoted rat liver tumors