THE INFLUENCE OF CHEMICAL IMPURITY ON ESTIMATING RELATIVE POTENCY FACTORS FOR PCBs.

Michael DeVito[1], Nigel Walker[2], and Linda S. Birnbaum[1]

1 National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina, USA, 27711.
2 National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA, 27709.

Introduction

In 1998, the WHO published the proceedings of a workshop that provided recommended TEF values for dioxin-like chemicals[1]. The dioxin-like chemicals examined at the workshop consisted of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls. TEF values were assigned to particular chemicals based on an examination of available data comparing the relative potency of a chemical to either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or 3,3',4,4',5-pentachlorobiphenyl (PCB 126). The assignments of TEF values were based on expert judgement and a scheme to rank the relative impact of a particular data set on the TEF value.

One of the criticisms of this approach is that examination of the WHO database indicates that for some chemicals there is a large range in the published relative potency factors (RPFs) from individual studies. For example, the range of RPFs for PCB 77 compared to TCDD varies from 0.14 for immunotoxicity in mice to 0.000003 for enzyme induction in mice. However, for other chemicals the range of RPFs is less than two orders of magnitude. There are several possibilities that could explain the variability in the RPF values. Study designs could play a significant role in this variability due to pharmacokinetic differences between the test chemical and TCDD. Rapidly metabolized chemicals may be more potent in an acute exposure compared to subchronic exposures. Another possibility could be that different endpoints may have different RPF values and comparisons of the RPFs across endpoints could possibly increase variability. Species differences could also play a role. Methods of RFP calculation could also play a role since in many cases only a single dose level of a test agent or of the positive controls (TCDD or PCB 126) were used rather than a comparison of dose response curves. While these are all possibilities, the relative importance of these different variables is difficult to define quantitatively because the data is often from different laboratories and that may also be a reason for the variability in the RPFs.

Further examination of the WHO database also demonstrates that the variability in RPF values is not consistent between classes of chemicals. For example, the range of variability for PCDDs and PCDFs is much less than that of the mono-ortho-PCBs and PCB 77 in particular. One contributing factor in the variability may be chemical purity of the test compound. Typically, purity of these chemicals from commercially available sources is at least 98% and above. However, because the mono-ortho PCBs and PCB 77 are weak dioxin-like chemicals, relatively high doses are required in order to observe biological effects. This may result in minor chemical contaminants with higher potency than the test article playing an important role in the biological effects observed. The present study describes two instances where test chemicals with purity greater than 98% were used to determine RPF values, only to have minor contaminants confound the study results.

Methods and Materials

Study 1:

60 day old female B6C3F1 mice were exposed to PCB 126, PCB 77 or PCB 118, 5 days/week for 13 weeks and terminated 3 days after the last dose. Hepatic EROD activity and PCB tissue concentrations were determined. PCB chemical purity was greater than 98% as determined according to the supplier.

Study 2:

60 day old female Sprague-Dawley rats were treated with either PCB 126 or PCB 118 5 days/week for 31 weeks. Hepatic EROD and hepatic tissue concentrations of PCBs were determined at the end of the study. PCB chemical purity was greater than 98% as determined according to the supplier.

Results and Discussion

In study 1 exposure to all three PCBs resulted in dose dependent induction of hepatic EROD activity. In animals exposed to PCB 77 at the highest exposures (100 mg/kg/d), 4 out of 5 animals died before the study was terminated. Chemical analysis of the tissues by GS-MS indicated that animals receiving PCBs 126 and 118 did not have significant concentrations of other PCBs, PCDDs or PCDFs. In contrast, in the mice receiving PCB 77, liver concentrations of PCB 126 were greater than those of PCB 77. Comparisons of the PCB 126 liver concentrations in the animals receiving PCB 126 alone to those receiving PCB 77 indicate that the animals exposed to PCB 77 actually received over 30 times the dose of PCB 126 compared to the animals receiving PCB 126 alone. Chemical analysis of the PCB 77 dosing solution demonstrated that the concentration of PCB 77 was within 5% of the target concentration of 10 mg/ml. In addition, PCB126 was found at a contaminate level of 0.035% in the PCB 77 dosing solution. It should be noted that the PCB 126 and PCB 77 studies were done approximately 6 months apart and it is unlikely that cross-contamination of dosing solutions or inadvertent dosing of the PCB 77 animals with PCB 126 occurred.

In study 2, similar findings of PCB 126 contamination occurred in the animals treated with PCB 118. Initial chemical purity checks indicated that both the PCB 126 and PCB 118 purities were greater than 98%. Following, 31 weeks of exposure, dose dependent changes in EROD were observed. However, the relative potency of PCB118 was almost an order of magnitude higher than predicted from its WHO TEF of 0.0001. Subsequent analysis of the PCB 118 dosing solution demonstrated a PCB 126 contamination at 0.8% (w/w). Consequently the TEQ of the "PCB118" was almost an order of magnitude higher than expected.

Because of the high level of PCB 126 contamination in the dosing solutions, RPF values cannot be accurately estimated for either PCB 77 in mice or PCB 118 in rats based on these studies. In both of these studies the PCB 126 contamination is high enough to be a significant contributor to the observed dioxin-like effects. If chemical analysis of the tissues and dosing solutions were not performed in order to specifically examine for low level contamination of other dioxin-like chemicals, this contamination problem would not have been detected and estimates of the RPFs for either 77 or 118 from these studies would be over-estimated. It should be noted in contrast to the rat study, in the mouse study, there was no significant contamination of the PCB 118 dosing solution by PCB 126 or other high potency PCDDs or PCDFs.

One of the implications of this study is that it may explain the large variability in the RPF values for the some of the mono-ortho PCBs and PCB 77. Minor contamination of less than 0.1% resulted in over estimations of the RPFs for PCBs 77 and 118 in the present study. The fact that this contamination was observed in the rat PCB 118 study and not the mouse PCB 118 study, suggests that these contaminants may not be consistently present in chemicals obtained from different suppliers or in different lots from the same supplier. It is possible that in the published studies where the mono-ortho PCBs and PCB 77 demonstrate relatively high potency that these effects may, in part, be due to minor contaminants that have high dioxin like potencies, such as PCB 126.

While the potential for minor contaminants to confound studies examining the RPFs of dioxin-like chemicals has been demonstrated in the present study, it may be difficult to determine how often this problem has occurred in the published literature. Because the contamination problem does not appear to be occurring consistently, it is difficult to go back to the published literature and determine if a particular study is compromised because of minor contaminants. Most of the published studies estimating RPFs for these chemicals do not provide data on the concentration of minor contaminants in the dosing solution or tissue disposition data for compounds with an assigned TEF. One recommendation would be to incorporate information on purity or "dioxin" tissue concentrations as part of the scaling when weighing the particular studies in TEF assignments. Another recommendation would be that any new study examining RPFs of dioxin-like chemicals should provide sufficient information on purity of the test compound and/or tissue analysis of WHO TEF assigned compounds to ensure that the data is not confounded by the presence of minor contaminants.

Because expert judgement was used in assigning TEF values, it is difficult to determine how this information would alter the present TEF values for the mono-ortho PCBs and PCB 77. The large variance in the RPFs for the PCBs, other than PCB 126, may be due to a variety of reasons, such as differences in experimental design, or species and endpoint examined. However, these differences are also present in the databases for the PCDDs and PCDFs. Minor chemical impurities would be one of the variables that would impact the mono-ortho PCBs and PCB 77 to a greater degree than the PCDDs and PCDFs. Because these chemicals are approximately ten thousand times less potent than TCDD, minor contaminants may influence the potency estimates. In contrast, most of the PCDDs and PCDFs are ten to 100 times less potent than TCDD and minor contaminants may not significantly influence their potency estimates. A better understanding of the reasons for this variability in the RPF database would provide for a clearer understanding of the uncertainty in risk estimates using the TEF methodology.

References

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