Risk assessment of dietary dioxins – Species differences in the aryl hydrocarbon receptor (AhR)

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Background

Dioxins and dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that are often detected as contaminants in food. There are many different types of dioxins and PCBs which have different levels of toxic potential, with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) recognised as being the most potent. There are concerns that dietary exposure to dioxins and dioxin-like compounds can lead to reproductive effects, immune deficiency and an increased risk of cancer. The initial aim of the project was to identify knowledge gaps to further refine and reduce uncertainty in the risk assessment of dioxins. Particular emphasis was placed on understanding the underlying toxicokinetic and toxicodynamic factors used in the risk assessment for dioxins.

In the course of the project methodology was developed that enabled the measurement of the dissociation constant (the ability of a contaminant to bind to a cellular receptor) for the aryl hydrocarbon receptor (AhR). The methodology is suitable for use in both rat and human recombinant In Vitro cell lines.

The project was extended in 2008 to further investigate how dioxins and dioxin-like compounds interact with the AhR, specifically focusing on how AhR activation may be affected if there is more than one dioxin or dioxin-like compound present and to determine what the effect might be on toxicological potency.

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Research Approach

In order to measure the activation of the AhR, induction of the enzyme Cytochrome P450 1A1 (CYP1A1) was measured in RNA from rat Liver cells (H4-IIE-C3) and human carcinoma cells (MCF-7) using quantitative Real-Time PCR. Measurement of the induction of CYP1A1 RNA allowed the construction of a concentration-response curve. The concentration-response curve can be used to determine the agonistic potency of the compounds, with antagonism measured by treating the cell lines with TCDD together with differing concentrations of the antagonist.

The project extension aimed to investigate partial agonism of the AhR. The potency of three dioxin-like compounds that are often detected as contaminants in food will be assessed. These dioxin-like compounds have been identified as having antagonistic properties and may reduce the agonistic function of TCDD on the AhR.

CYP1A1 RNA from rat liver cells (H4-IIE-C3) and human carcinoma cells (MCF-7) will be used to assess the agonistic and antagonistic properties of each contaminant alongside TCDD (utilising the method identified earlier in the project). The potency values, also known as the toxic equivalency factor (TEF), will be calculated using a mathematical model known as a quantitative structure activity relationship (QSAR) approach.

Comparison of the AhR between species (rat and human) will allow for an improved understanding of AhR activation and CYP1A1 induction. The results will enable the Agency to validate and improve the risk assessment process and will provide additional valuable information on the toxicity of the selected contaminants. The results will also provide valuable information on the activation of the AhR and the resulting toxicological responses from exposure to more than one dioxin or dioxin-like compound.

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Results

The structure-activity relationships of several families of compounds were investigated to improve our understanding of the requirements for binding and of an AhR ligand to be an agonist or antagonist. Many of the novel 2-amino-isoflavones described in this study were not only active ligands (agonists/antagonists) of the AhR, but they also produce unusual species differences in response. These analyses have shown that even the slightest substitutions in chemical structure can significantly alter not only the potency of the compound but also its antagonistic potential.

The potency data, together with occurrence data, should help inform regulators which compounds should be measured in environmental and food samples for purposes of risk assessment which should result in a better estimate of our overall exposure to AhR agonists. With regards to the current WHO TEFs, (ranking system for toxicity of species), this study has shown that PCB 105 and PCB 118 are essentially antagonists at all but the highest of concentrations and therefore suggests these TEFs should be decreased. Consideration should be taken regarding the antagonistic properties of the PCBs and PXBs in relation to their ability to reduce the potency of other more potent AhR agonists.

In terms of species differences between rat and human, an estimated 15-fold reduction in the potency of these compounds to activate human AhR was observed in all of the compounds tested in this study. The study also identified a more promising alternative method which should eliminate the background mouse AhR response allowing a more accurate comparison of the infected AhRs. During this study, 5F 203, was once again shown to be significantly more potent in human (equal to TCDD) than in rat highlighting its usefulness in species comparison.

In conclusion, the data derived in this study will help to improve our overall understanding of the mechanism of AhR activation by environmental pollutants and allow more focused risk assessment on these compounds.

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Published Papers

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