Synergistic effects of mixtures of dioxin-like and non-dioxin-like compounds in rodents

Angélique P.J.M. van Birgelen[1] and Linda S. Birnbaum[2].

[1]National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA; [2]US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC 27711, USA.

Introduction

Toxic equivalency factors (TEFs) are used for estimating the dioxin activity of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) in order to facilitate risk assessment of these compounds. The common mechanism of action of these compounds is based on binding to the Ah-receptor and their common toxic and biochemical responses as observed for 2,3,7,8-TCDD. The compounds included in this TEF scheme are A) structurally related to PCDDs and PCDFs, B) binding to the Ah-receptor, C) resulting in dioxin-specific biochemical and toxic responses, and D) persistent and accumulate in the food chain[1)].

Additivity of the TEF concept

The TEF concept is based on additivity of the individual dioxin-like congeners in mixtures. Additivity has been reported for mixtures of dioxin-like compounds for tumor promotion, lethality, body weight loss, teratogenicity, fish early life stage mortality, immunotoxicity, and cytochrome P450 induction[2-9)]. Various biochemical and toxic effects were accurately predicted after exposure to food-like mixtures of dioxin-like compounds in female B6C3F1 mice and female Sprague Dawley rats using relative potency values based on cytochrome P450 induction in female B6C3F1 mice. Additivity was found for cytochrome P450 induction in liver, lung, and skin[6)]. Furthermore, the immunotoxic response as measured by the primary antibody plaque-forming cell response to the T cell-dependent antigen sheep red blood cells was well predicted in mixtures of dioxin-like compounds[8)]. In addition, the decrease in hepatic retinoid levels was shown to be predicted based on relative potencies based on cytochrome P450 induction[10)].

However, non-dioxin-like compounds have other patterns of toxic and biochemical effects which can interfere with the effect of dioxin-like compounds when co-administered. Both synergistic and antagonistic effects have been reported after co-administration of dioxin-like and non-dioxin-like compounds. For example, synergistic interactions have been reported for cytochrome P450 induction, tumor promotion, lethality, thyroid hormone metabolism, hepatic porphyrin accumulation, and *in vitro* mutagenicity[2,3,10,11)]. These synergistic effects have been attributed to multiple mechanisms and/or are associated with alterations in distribution of the administered compounds. On the other hand, antagonistic effects have been reported for cytochrome P450 induction, immunotoxicity, and teratogenicity[2,3)]. For immunotoxicity it has been shown that two competing mechanisms are involved, leading to functional antagonism[12)]. In addition, several studies suggest that some antagonistic effects are related to alterations in the tissue distribution of the administered compounds.

Synergistic effects of mixtures on hepatic porphyrin accumulation

Exposure of a binary mixture containing 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) to female Sprague Dawley rats for 13 weeks resulted in an 800-fold hepatic porphyrin accumulation compared to controls[9]. The individual compounds did not result in a dose-dependent increase in hepatic porphyrin accumulation. The proposed mechanism for this synergistic effect is based on a TCDD-dependent induction of hepatic cytochrome P4501A2, which is involved in the oxidation of uroporphyrinogen III to uroporphyrin III[14,15] and a PCB 153-dependent induction of δ-aminolevulinic acid synthetase. This synergistic effect has also been found after co-administration of 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexabromobiphenyl, both *in vivo* (rats) as well as *in vitro* (chicken hepatocytes)[16,17].

This same mechanism could explain why mono-ortho substituted PCBs, which are mixed type inducers, have a greater efficacy in hepatic porphyrin accumulation than PCDDs, PCDFs, or planar PCBs[18]. Furthermore, a mixture of dioxin-like compounds including mono-ortho substituted PCBs resulted in hepatic porphyrin accumulation up to 4000 times control levels. The same dose of TCDD resulted in hepatic porphyrin accumulation of about 15 times control levels. Moreover, addition of the non-porphyrinogenic PCB 153 to the mixture of the dioxin-like compounds (including the mono-ortho substituted PCBs) resulted in a hepatic porphyrin accumulation up to 6800 times control levels[13]. These synergistic effects clearly indicate that multiple mechanisms must play a role in hepatic porphyrin accumulation.

Synergistic effects of mixtures on thyroid hormone metabolism

Exposure to a binary mixture containing 2,3,7,8-TCDD and PCB 153 to female Sprague Dawley rats for 13 weeks resulted in a synergistic decrease in total and free thyroxin plasma levels[18]. This decrease was about 2-fold more than what would have been expected from the single congeners. Although this synergism was not as drastic as found after co-administration of these compounds for porphyrin accumulation, it can be suggested that multiple mechanisms play a role in this decrease. In rodents, a decrease in plasma thyroid hormone levels is correlated with induction in hepatic thyroxin glucuronidation (T4-UGT)[20,21]. Various classes of UDPGT inducers - including TCDD and phenobarbital inducible classes - have been shown to increase hepatic T4-UGT activity and decrease serum thyroid hormone levels[22]. Both enzyme inducers represent dioxin-like compounds and di-ortho substituted PCBs, respectively. It can be suggested that multiple UDPGT isozymes were induced after co-administration of PCB 153 and TCDD, resulting in a more than additive induction of T4-UGT and subsequently to a more than additive decrease in plasma thyroxin levels.

The same mechanism might be involved in the strong synergistic decrease in plasma thyroxin levels after exposure to a mixture of dioxin-like compounds including mono-ortho substituted PCBs in comparison to TCDD alone[23]. Dose-dependent hepatic T4-UGT induction was found in this experiment with both TCDD and the mixture of dioxin-like compounds. The dose of TCDD which induced hepatic T4-UGT activity was about 5-fold higher than for the mixture containing the dioxin-like compounds. This slight synergistic effect in hepatic T4-UGT induction may not fully explain the synergistic decrease in plasma thyroxin levels after exposure to the mixture of dioxin-like compounds. The dramatic decrease in thyroxin levels suggests that an additional mechanism plays a bigger role than the induction of T4-UGT in the decrease in plasma thyroxin levels. As suggested by Brouwer and co-workers, metabolites of PCBs play a role in the reduction in plasma thyroid hormone levels by binding to the transport protein of thyroxin, transthyretin (TTR)[24,25]. One of these metabolites, 2,3,3',4',5-pentachloro-4-biphenylol, has been found in high concentrations in plasma from dams, fetuses, weanling rats, and young adult rats after pre- and postnatal exposure to Aroclor 1254[26]. Since this metabolite can be formed by metabolism of 2,3,3',4,4'-pentachlorobiphenyl (PCB

105) or 2,3',4,4',5-pentachlorobiphenyl (PCB 118), it can be suggested that hydroxylated metabolites may have contributed in the decrease in plasma TT4 levels in the mixture of dioxin-like compounds with high concentrations of PCB 105 and PCB 118.

The synergistic effect observed for the decrease in plasma thyroxin levels after mixture exposure suggests that multiple mechanisms play a role in comparison to exposure to TCDD. These mechanisms probably include the induction of multiple isozymes of UDPGT and the involvement of PCB-metabolites.

Further research

The synergistic effect on hepatic porphyrin accumulation occurred at high dose levels. However, the contribution of other di-ortho substituted PCBs apart from PCB 153 needs to be addressed, since di-ortho substituted PCBs are the most abundant congeners in environmental and human samples. In addition, other environmental contaminants, such as hexachlorobenzene (HCB) are highly porphyrinogenic. What would happen with the porphyrinogenicity of an environmental dioxin-like mixture including di-ortho PCBs and HCB ? Since HCB binds to the Ah receptor, bioaccumulates, and results in dioxin-like effect, such as induction of hepatic cytochrome P4501A1, P4501A2, porphyrin accumulation, decrease in plasma thyroxin levels, and induction of liver tumors, HCB should be included in the TEF scheme[27-33]. Using a relative potency value based only on Ah-receptor binding, HCB could contribute for over 10% of the total TEQ in human milk samples[32,34].

The same questions can be asked for the effect on plasma thyroid hormone levels. What will other di-ortho substituted PCBs do ? How much is the contribution of other compounds, such as HCB ? Do these effect occur at exposure levels which are close to human exposure levels, especially when alterations in plasma thyroid hormone levels have been found in infants[35,36]?

Hepatic porphyrin accumulation and alterations in thyroid hormone status are two examples of interactive effects which are suggested to occur through multiple mechanisms. However, multiple mechanisms are involved in more endpoints, such as an increase in liver weight and tumor promotion. Great care has to be considered when evaluating these effects. Since the current risk assessment of dioxin-like compounds is still driven by the 2-year bioassay of Kociba and co-workers[37] and the 3-generation study of Murray and colleagues[38], more attention should be given to possible interactive effects in these paradigms. The National Toxicology Program has planned various 2-year bioassays to address this question for carcinogenicity[39].

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