The role of toxicokinetics in the occurrence of interactive effects between PCDDs, PCDFs and PCBs.

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Introduction

The development of the TEF concept has made it possible to assess the biological activity of complex mixtures of 2,3,7,8-substituted PCDDs or PCDFs and non- or mono-ortho PCBs[1]. One of the principles of the TEF concept is the assumption that the Ah-mediated toxicity mechanism is additive for the contribution of every component that is present in a mixture. However, the occurrence of mixture interactions between some PCDDs, PCDFs and PCBs has been reported in laboratory studies, both *in vivo* and *in vitro*[2-12]. The most extensively studied effect is CYP1A dependent enzyme induction. In the *in vivo* studies, teratogenicity and immunological responses are also frequently studied parameters. The results of some studies appear to be conflicting since additivity, synergism, potentiation and antagonism have all been reported. In addition, most of the *in vivo* studies are not backed by toxicokinetic data.

Thus, some questions regarding the interactive effects between these compounds arise:

1) What kind of interactive effects have been observed until now ?
2) Which congeners are involved ?
3) What is the role of toxicokinetics in the occurrence of these effects ?
4) Are interactive effects on toxicokinetics relevant for risk assessment ?

Interactive effects, a brief overview.

The effects of mixtures of PCDDs, PCDFs and PCBs on the induction of CYP1A dependent enzyme activities, like AHH or EROD, have been reported *in vitro* using isolated hepatocytes or cell lines. Both additivity and antagonism have been reported[9,11]. These results seem to be in accordance with the Ah-mediated model for induction of CYP1A dependent enzyme activities[1].

In contrast, the results from *in vivo* studies are not easy to catagorize, since effect parameters, species, mixtures and dose regimens differ throughout the studies.

In mice, several studies have reported on the effects of mixed dosage on teratogenicity parameters such as the appearance of hydronephrosis or cleft palate after exposure during gestation. For mixtures of PCCDs and/or PCDFs additivity is usually observed[4,12]. For binary mixtures of TCDD and 2,2',4,4',5,5'-HxCB (PCB 153), a di-ortho, 'non-dioxin' PCB, antagonism has been observed[3,10]. In one study, this was paralled by a decrease of TCDD concentrations in the foetal tissue[3].
Studies on interactive effects on immunologic parameters in both mice and rats, have reported additive or antagonistic effects after dosage of mixtures[5,6]. Mixtures usually consisted of TCDD and either a single PCB or an Aroclor mixture. No toxicokinetic data are reported in any of these studies.
Studies on interactive effects between these compounds on induction of CYP1A dependent enzyme activities, like AHH or EROD, show varying results. Additivity, antagonism and synergism have all been observed[2,3,7]. Many of these studies reported on the effects of PCB 153 cotreatment on EROD induction by PCDDs, PCDFs or non- and mono-ortho PCBs. Toxicokinetic measurements in some of these studies have showed, that synergistic effects on the hepatic EROD induction are always accompanied by an increased hepatic concentration of the inducing congener[6,8]. One study has also shown that PCB 153 cotreatment could cause an antagonistic effect on TCDD induced EROD activity. But in this case the effect was not based on a change of hepatic TCDD levels[3]. In addition, one study has demonstrated that dosage of a PCDD/PCDF mixture may increase the hepatic concentration of some components when compared to dosage of the single compounds[8]. This phenomenon is likely a reflection of the dose dependent tissue distribution of PCDDs and related compounds observed in some other studies[13]. Binding to the hepatic CYP1A2 protein is probably responsible for this effect[14]. However, in the light of the previous explanation, the similar effects of PCB 153 cotreatment remains to be explained since PCB 153 is considered to be unable of CYP1A2 induction. It has been postulated that induction of Ah receptor levels by di-ortho PCBs may play a role here[2].

Discussion

Although the mechanisms behind the interactions between PCDDs, PCDFs and PCBs cannot be fully elucidated yet, combining the data from the studies discussed above allows for a preliminary hypothesis on the occurrence of interactive effects *in vivo*. The dose dependent body distribution plays a key role here (figure 1).
At low PCDD/PCDF/PCB doses, induction of CYP1A2 will occur in the liver and the number of hepatic binding sites increases with dose (dose range I). As a result, an overproportional fraction of the absorbed dose will be deposited into the liver. This has been shown in a study with TCDD in rats[13]. The observed increase of hepatic deposition after mixed dosage is another aspect of this phenomenon. A greater fraction of each component is deposited into the liver due to an increased CYP1A2 induction by the total

PCDD/PCDF/PCB dose. In this dose range, Ah-mediated responses are directly proportional to the hepatic concentration but not to the administered dose. Thus, an apparent synergism may occur when effects are related to the dose. In addition, this overproportional deposition to the liver may lower extrahepatic concentrations and related biologic responses. This will likely be the cause for the apparent antagonism that was observed in teratogenic and immunologic studies with mixtures.
After saturation of CYP1A2 induction, these effects will disappear (dose range II).
When the dose is further increased, a competition for the available hepatic binding sites may lead to apparent antagonistic responses (dose range III). In addition, in this high dose range, a competition for the Ah-receptor may lead to antagonistic effects of Ah-mediated responses. Some *in vitro* studies using high concentrations seem to confirm this. However, in the *in vivo* situation, this situation may never be observed due to mortality of the animals in this dose range.
In summary, we conclude that toxicokinetic mixture interactions may be a cause for non-additive effects on some biologic responses of PCDDs, PCDFs and PCBs. This is dependent on the observed dose range. Their relevance for risk assesment seems not easy to predict. However, it appears that synergistic responses are more likely to occur within the lower hepatic CYP1A2 protein induction range. Antagonism may be found at dose levels lying above environmental background exposure to these compounds.

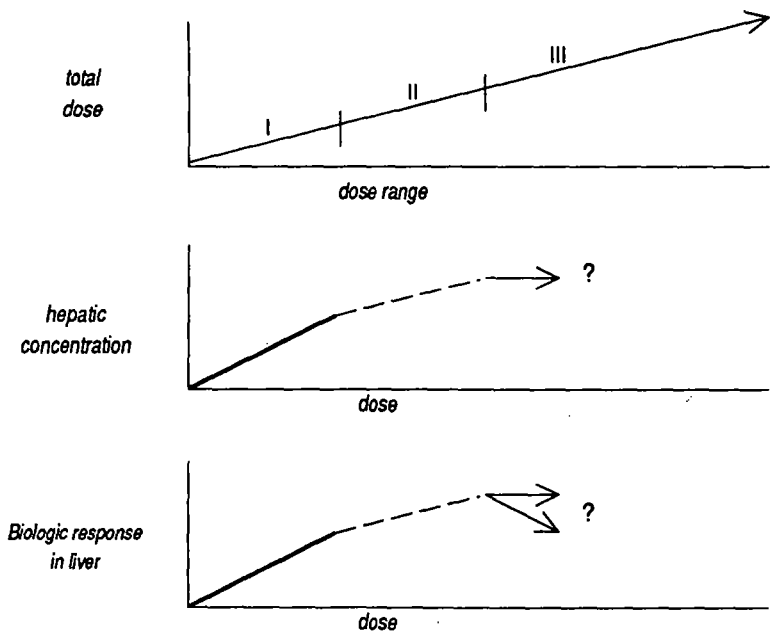

Figure 1: Relation between dose, hepatic concentration and Ah-mediated response.

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