The role of metabolism in the toxicokinetics of persistent organic pollutants

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Introduction

Our society is today using an almost endless number of halogenated organic compounds. Numerous of these will end up as environmental pollutants, although with varying degrees of persistency (resistence to metabolism or degradation). Some are persistent only in soil or at lower trophic levels, and some in the whole food chain, including in top carnivores. However, in international fora (e.g., CLRTAP), only 14 compounds are agreed on as being POPs (persistent organic pollutants). Logically, if this definition is accepted, all other compounds should then be regarded as non-POPs.

For non-persistent chemicals, it is well-known and accepted that metabolism is the key modulator of distribution, disposition, excretion, and toxicity. However, also the toxicokinetics of POPs are dependent on metabolism, and not only metabolism related to the excretion of the compound, but on metabolism resulting in formation of toxic reactive intermediates (bioactivation) or formation of persistent metabolites. Both reactive and persistent metabolites can contribute considerably to the toxicity profile. Using DDT and PCB as examples, this presentation will illustrate how metabolism and formation of metabolites may alter an already complex toxicokinetic profile. Several of the biological effects exerted by these compounds are of course caused by the parent compounds, e.g., the neurotoxicity of DDT and the Ah-receptor-dependent effects of the coplanar PCBs, but this presentation will focus on some effects dependent on metabolism or metabolites.

Discussion

Formation of reactive intermediates in the metabolism of DDT Because POPs are rather resistent to metabolism, the metabolism often proceeds via formation of short-lived reactive intermediates. There are often species differences in, e.g., tissues mediating the bioactivation. For DDT (2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane), the metabolism has been proposed to involve some 10 reactive intermediates (1). The first transformations of DDT into DDE or DDD involves dehydrochlorination and reductive dechlorination, respectively (2). Of these, the latter proceeds via a radical mechanism (3).

The further metabolism of (p,p'-and o,p')-DDD has in some tissues been shown to proceed via oxidation at the ethane side-chain carbon 1, yielding a reactive acyl halide (DDA-Cl) (4,5). The target tissues for this bioactivation are the lungs (6) and the adrenal cortex (7). The adrenocortical bioactivation of DDD results in cytotoxicity, and DDD has consequently been used as a cytostatic drug in the treatment of adrenal tumours, both in human and veterinary medicine. A possible involvement of DDD in the generation of the adrenocortical hyperplasias observed in Baltic grey seals (8) has also been discussed (9). Both DDT and DDD cause lung tumours in bioassays on mice (2). Since DDD is not mutagenic, the mechanism for the carcinogenicity may involve the increased cellular proliferation caused by the pulmonary bioactivation of DDD (10).

DDE is the major DDT-compound found in biota, and this metabolite is the cause of the egg-shell thinning observed in, e.g., predatory birds in areas of high DDT exposure. The mechanism seems to be inhibition of prostaglandin synthetase, a key enzyme in the transport of calcium from the eggshell gland to the egg (11).

The metabolism of POPs often involves epoxidation of an aryl ring, as is the case for DDE. Hydroxylated DDE, presumably formed from the epoxide, has been found in biota (12). More importantly, the epoxide is conjugated with glutathione. The conjugate is still lipophilic, and is transformed via several steps, involving enterohepatic circulation and the gut microflora, into methylsulfonyl-DDE (13). 3-Methylsulfonyl-DDE is probably the most potent adrenocorticolytic compound known, although there are great species differences in sensitivity (14). The mechanism underlying the toxicity is a mitochondrial bioactivation mediated by CYP11B1, a steroid (11β-)hydroxylase previously not known to be involved in metabolism of xenobiotics (15). The reactive intermediate has been shown to bind both to protein and DNA in adrenocortical cell cultures, and to be deactivated by conjugation with glutathione.

Tissue-specific accumulation of PCB-metabolites and the relation to toxicity Tissue-specific accumulation of metabolites may affect the biological half-life and toxicological effects of the parent chemical considerably. When it comes to metabolites of PCBs, there are numerous examples of tissue-specific retention, sometimes also clearly related to toxicity.

Hydroxylated PCBs (OH-PCBs) are frequently formed in the metabolism of PCBs, and some of them (e.g., 4-OH-2,3,3',4'-tetraCB and 4-OH-2,3,5,3',4'-pentaCB) have been shown to bind to transthyrotin (TTR), a transport protein for thyroxin present in plasma (16). Although not conclusively shown yet, it is suspected that the thyroxin homeostasis will be affected by the elimination of thyroxin by OH-PCBs from their common binding sites on TTR. OH-PCBs (e.g., 4-OH-2',4',6'-triCB) also bind to the estrogen receptor (17), and OH-PCBs (e.g., 4-OH-2',4',6'-triCB) have consequently *in vivo* been shown to affect the sexual differentiation of turtle-eggs (18). Another point of potential interference with

the reproduction concerns the accumulation of one hydroxylated metabolite of 3,4,3',4'-tetraCB in the uterine fluid of pregnant mice (19).

The other group of common PCB-metabolites is the methylsulfonyl-PCBs. When it comes to distribution and accumulation, they have generally properties not shared by the parent PCBs. For one subgroup of 4-methylsulfonyl-PCBs, characterized by chlorines in 2,5,4'-positions (20), the accumulation in pulmonary (non-ciliated Clara) cells have been shown to increase their lifetime substantially. The mechanism for this accumulation involves binding to a uteroglobin-like, 'PCB-binding' protein being synthesized in these cells (21). The existence of these methylsulfonyl-PCBs in brochiolar lavage-fluid from Japanese 'Yusho'-patients suffering from pulmonary disorders has led to speculations regarding their involvement in the etiology of this disorder (22).

It has recently been shown that the induction of cytochrome P450 that has been believed to be caused by PCBs, in some cases actually are caused by their methylsulfonyl-metabolites. For one of the most common PCBs, CB101, its 3-methylsulfonyl-metabolite is some 700 times more potent than CB101 itself in inducing CYP 2B1, 2B2, 3A2 and 2C6 (23). It also appears that methylsulfonyl-PCBs may affect the glucocorticoid homeostasis by acting as antagonists at the glucocorticoid receptor (GR). *In vitro*, especially 4-substituted methylsulfonyl-PCBs with at least 3 chlorines in ortho-position, and one chlorine in 4'-position, have an affinity to the GR in the low micromolar range (24).

Metylsulfonyl-PCBs are metabolites, and as such they are presumed to have lower bioconcentration factors (BCF) than their parent PCBs. However, a mixture of the methylsulfonyl-PCBs generally present in biota has a similar BCF as technical PCB (Clophen A50) in chronically exposed mink (25), showing a high persistency of these metabolites.

In conclusion, the metabolism of POPs often proceeds via reactive intermediates, which in some cases may be cytotoxic in the tissue where they are formed. The stable metabolites of POPs are often nearly as persistent as the parent compound, and the metabolites may change the distribution, toxicity and elimination of the parent compound significantly, e.g., by binding to tissue macromolecules. Thus, even for the most persistent POPs, metabolism needs to be kept in mind in toxicity testing and risk assessments.

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