Metabolism-dependent tissue-binding and toxicity of persistent environmental pollutants

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Persistent organic environmental pollutants are generally hydrophobic compounds characterized by low vapour pressure and high resistance towards degradation in the environment. Consequently, such compounds may bioaccumulate in organisms and biomagnify in food chains. The high residue levels attained in the fat tissues of humans, in mammals and birds of prey are to a major extent due to a low rate of metabolism of the pollutants in these species as well as in organisms at lower trophic levels of the food chain. Hence, biological effects induced by persistent environmental pollutants would generally be expected to be caused by the unchanged parent compound rather than by metabolites.

DDE and DDD are lipophilic DDT-metabolites present in the environment. More than 15 years ago Jensen and Jansson [1] identified methyl sulphone metabolites of PCB ($MeSO_2$-PCB) and DDE ($MeSO_2$-DDE) in fat tissue from Baltic grey seal and it is now well established that methyl sulphone metabolites are ubiquitously present in the environment [2, and references cited therein]. During early studies on the disposition of chlorinated hydrocarbons in experimental animals [3] it was observed that certain PCB congeners were accumulated in the respiratory tract of mice as neutral metabolites [4], later identified as PCB methyl sulphones [5]. In subsequent studies, the biosynthesis of PCB methyl sulphones was examined. The methyl sulphone metabolites are formed by glutathione (GSH) conjugation followed by subsequent metabolism during biliary excretion and entero-hepatic circulation of GSH-derived intermediates [6-10]. Stimulated by these early results, research on metabolism-dependent tissue-binding and toxicity of chlorinated hydrocarbons has been carried out. This work led to the identification of compounds that show long-term retention following reversible association to specific binding proteins in the tissues, and compounds that become irreversibly (covalently) bound to cellular macromolecules in target cells. Whereas several very potent toxicants have been found in the latter group, no compound in the former group has so far been associated with overt toxicity in short term bioassays. In this presentation metabolism-dependent tissue-binding and toxicity of some chlorinated environmental pollutants will be discussed.

Reversible tissue-binding of PCB methyl sulphones in airway epithelial cells

Studies on the tissue-localization of ^{14}C-labelled chlorinated biphenyls (CBs) showed that certain compounds were enriched in the tracheo-bronchial mucosa in mice [3,4,11]. The accumulated compounds

were reversibly bound in the airways but remained there at time-points when very low levels of radioactivity remained in other tissues including the body fat. The uptake in the airways was dose-dependent and seemed to be favoured by the presence of chlorine atoms in 2,4',5-position in the molecule [3,4]. Also the presence of chlorines in 2,3,6-position in one of the phenyl rings gave rise to accumulation in airways and lung tissue [12]. The methyl sulphone-metabolites present in extracts of whole lungs were substituted with the sulphone group in the 4-position (major compounds) and the 3-position (minor compounds) [5]. Two compounds, 2,4,5,2',4'-pentaCB and 2,4,5,2',4',5'-hexaCB were accumulated unchanged in the airways. Some of the brochial-seeking PCBs also showed affinity for the kidney proximal tubules. One of these, 2,4,2',4'-tetraCB , was metabolized to the 4-substituted methyl sulphone which became accumulated predominantly in the airways and 4,4'-bis-methylsulphonyl-tetraCB which showed a strong binding both in the airways and in the kidney proximal tubules [11,13]. Using this latter compound as a labelled ligand for high resolution microautoradiography, the nonciliated bronchiolar (Clara) cells were identifieed as the main target cell in the airways [14]. A selective labelling of goblet cells at higher levels of the airways (the tracheo-bronchial mucosa) was also observed. The PCB methyl sulphone-metabolite was associated with a 13 kDa binding protein in rat lung cytosol [15]; the protein has been cloned and shows homology to uteroglobin, a progesterone-binding protein present in rabbit uterus and lung [16]. The protein PCB-methyl sulphone complex was secreted into the airway lumen where at least 30 % of the lung burden was shown to be localized [14]. A similar $MeSO_2$-PCB-binding protein is present in human lung [17] and a large number of $MeSO_2$-PCBs have been found in human lung tissue [18]. The biological significance of this accumulation mechanism for PCB methyl sulphones in the airways of rodents and humans is not understood and the biological function of the binding protein remains unclear. The evaluation of a possible causal relationship to respiratory distress observed in PCB-exposed human subjects has to await demonstration of the biological function of the binding protein.

Covalent tissue-binding and toxicity of DDT-metabolites

The DDT-metabolite o,p'-DDD is a classical toxicant following metabolic activation and covalent binding in the adrenal cortex in humans and dogs and the compound is currently used for the pharmacological treatment of adrenocortical carcinoma and Cushings disease [19-21]. Studying the disposition of chlorinated hydrocarbons in lung tissue we observed that administration of o,p'-DDD gave rise to a high and tissue-specific covalent binding in the lung alveolar region in mice [22]. A reactive DDD metabolite, formed locally in a cytochrome P450-dependent reaction, seemed to result in an increased proliferation of type 2 pneumocytes [23]. No covalent binding of o,p'-DDD occurred in the adrenal cortex in mice. We observed, however, that administration of another persistent DDT metabolite, 3-methylsulphonyl-DDE ($MeSO_2$-DDE), resulted in a high and tissue-specific binding of a non-extractable residue in the adrenal *zona fasciculata* cells in mice [24]. No binding appeared in other endocrine organs such as ovaries or testicles. When mouse adrenal 300 g supernatants were incubated with $MeSO_2$-DDE, a concentration- and time-dependent covalent binding to protein was observed. Addition of cytochrome P-450 inhibitors to the incubation medium decreased the covalent binding, indicating that P450 was involved in an *in situ*

metabolic activation of $MeSO_2$-DDE to a reactive metabolite. Addition of reduced glutathione (GSH) to the incubations decreased the covalent protein binding, indicating that a reactive $MeSO_2$-DDE-metabolite was conjugated with GSH.

$MeSO_2$-DDE was found to be a highly potent adrenal toxicant that induces ultrastructural changes in the *zona fasciculata* following administration of low (3 mg/kg) single doses to mice [24,25]. Administration of low doses of $MeSO_2$-DDE to lactating mice resulted in decreased plasma corticosterone levels in the suckling offspring and the dam [27]. Since mitochondrial lesions were observed as soon as 6 hr after injection, the mitochondria were considered as primary targets for the $MeSO_2$-DDE-induced toxicity. The initial degenerative changes were present in the cristae with a subsequent reduction in the number of mitochondria [25]. The observed changes suggest that $MeSO_2$-DDE induces a disturbance in the energy metabolism which eventually results in massive cellular necrosis in the adrenal *zona fasciculata* . The tissue-specific toxicity of $MeSO_2$-DDE seems to be due to a cytochrome P-450 form that is specifically expressed in the adrenal *zona fasciculata* cells. Several observations favour the conclusion that the mitochondrial P450 11ß was responsible for the covalent binding of $MeSO_2$-DDE in the mouse adrenal cortex [25,28].

Comparative studies on adrenocorticolytic DDT-metabolites in mammals, birds and fish

The methylsulphonyl moiety of $MeSO_2$-DDE is required for irreversible binding *in vitro* or *in vivo* [24] and for adrenal toxicity *in vivo* [unpublished]. Interestingly, also a methylsulphonyl metabolite of hexachlorobenzene, methylsulphonylpentachlorobenzene, is selectively accumulated in the adrenal cortex of mice [29]. The finding that $MeSO_2$-DDE is a highly potent adrenocorticolytic agent in mice suggested that this and other structurally related adrenal toxicants including DDD could be etiologically important for adrenocortical hyperplasia [30] observed in Baltic seals [28]. However, since o,p'-DDD and p,p'-DDD were not activated and covalently bound in the adrenal cortex of mice and $MeSO_2$-DDE was not activated in the adrenal cortex of rats, we compared covalent binding and toxicity of these adrenocorticolytic DDT-metabolites in mammals, birds and fish. A compilation of data from these experiments and from the literature is shown in Table 1. As can be seen, both DDD isomers were covalently bound and toxic to the adrenal cortex in mink, while no binding of $MeSO_2$-DDE was found in this species [31]. Both o,p'-DDD and $MeSO_2$-DDE were covalently bound and toxic in the interrenal cells in birds (corresponding to mammalian adrenal cortex) [32]. Both compounds were covalently bound also to human adrenal cortical tissue in vitro [33]. Considering that o,p'-DDD is a classical toxicant in the adrenal cortex in humans, it seems likely that $MeSO_2$-DDE is an adrenocorticolytic agent in humans. o,p'-DDD and, less pronounced, $MeSO_2$-DDE were covalently bound to adrenal cortical tissue from grey seal [34].

METAB

2,6-Dichlorophenyl methyl sulphone; metabolism-dependent toxicity in the olfactory mucosa

A number of chlorinated benzenes including hexachlorobenzene are transformed to methyl sulphides, sulphoxides and sulphones following metabolism in the mercapturic acid pathway in rodents. Studying the disposition of $MeSO_2$-derivatives of chlorinated benzenes, some compounds were found to be accumulated in target tissues similarly to $MeSO_2$-PCBs. E.g., the distribution of 2,5-dichloro methyl sulphone resembles that of 4,4'-bis-($MeSO_2$)-2,2',5,5'-tetrachlorobiphenyl with high binding both in the trecheo-bronchial mucosa and the kidney proximal tubules in mice unpublished). Interestingly, $\alpha_{2\mu}$-globulin binds methyl sulphones such as 2,3-dichlorophenyl and 3,4-dichlorophenyl methyl sulphones [35]; this protein is considered to mediate nongenotoxic carcinogenicity in the kidney cortex in male rats. 2,6-Dichlorophenyl methyl sulphone is selectively enriched in the olfactory nasal mucosa in mice, but unlike most other $MeSO_2$-derivatives this compound seems to be irreversibly bound in the target cells unpublished). Studying this methyl sulphone further, we have recently observed that it is a potent toxicant that induces necrosis in the Bowmans glands in the nasal olfactory mucosa in mice. The pattern of nasal toxicity seems to be very similar to those observed after treatment with dichlobenil (2,6-dichloro-benzonitrile) or chlorthiamid (2,6-dichlorothiobenzamide), herbicides that become potent toxicants in the olfactory mucosa following metabolic activation *in situ* in mice and rats [36-38].

Table 1.
DDT-metabolites in the adrenal cortex of different species

	$MeSO_2$-DDE		o,p'-DDD/p,p'-DDD		
	Covalent binding in vitro	Toxicity in vivo	Covalent binding in vitro	Toxicity in vivo	Reference
Mouse	+	+	-	-	24-26)
Rat	-	-	-	-	unpubl)
Mink	-	?	+	+	31)
Otter	-	?	+	?	31)
Seal	(+)	?	+	?	34)
Dog	-	?	+	+	19,21,unpubl)
Chicken	+	+	+	+	32)
Duck	+	?	+	?	32)
Eider	+	?	+	?	32)
Cod	+	?	+	+	unpubl)
Man	+	?	+	+	33)

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