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POLYHALOGENATED COMPOUNDS OF APPROPRIATE CONFIGURATION INTERACT WITH MAMMALIAN OR BACTERIAL CYP ENZYMES TO INCREASE BILIRUBIN AND UROPORPHYRINOGEN OXIDATION *IN VITRO*

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Previous work has shown that polyhalogenated compounds are associated in man and experimental animals with a toxic variety of *Sporadic Uroporphyria*, a disorder of liver heme metabolism characterized by the accumulation of uroporphyrin. This is thought to arise through an oxidative stress mechanism, whereby an intermediate of the heme biosynthetic pathway, uroporphyrinogen is oxidised and the corresponding porphyrin, uroporphyrin, which cannot be metabolized, accumulates in large amounts (reviewed by De Matteis, 1998).
In agreement with such an oxidative mechanism, we have previously shown that liver microsomes isolated from animals treated with either 3-methylcholanthrene or phenobarbital respond to the *in vitro* addition of PCBs with increased oxidation of uroporphyrinogen or bilirubin, another easily oxidizable molecule (Zaccaro *et al*., 2001). Planar isomers, such as 3,4,3',4'-tetrachlorobiphenyl (TCB), are active with CYP1A whereas di-*ortho*-substituted, non-planar isomers, as 2,4,2',4'-TCB, are active with CYP2B (De Matteis *et al*., 2002).
We have now confirmed, with pure genetically overexpressed, rat CYP1A1 (Supersomes, Gentest Corporation), that the increased bilirubin degradation is caused by the addition of the planar 3,4,3',4'-TCB; in contrast, 2,4,2',4'-TCB, the non-planar isomer, was almost inactive (see Table).
To gain more information on the mechanisms, in the absence of lipid peroxidation and other membrane-related effects, we tested a pure soluble bacterial enzyme, CYP 102 - BM3*. Dodecanoic acid, the normal substrate, and its polyhalogenated analogue (perfluorododecanoic acid) will both stimulate NADPH oxidation, but only the perfluoro analogue will also increase uroporphyrinogen and bilirubin oxidation (see Table). This suggests that with the normal substrate NADPH may be used mostly for monooxygenation, whereas with the halogenated analogue, a bilirubin oxidizing species, is produced instead, probably by an uncoupling mechanism.

CYP tested	Further addition	Rate of bilirubin degradation
Rat CYP1A1 (supersomes)	none (control)	34.9 ± 8 (4)
	3,4,3',4'-TCB (990 nM)	106.8 ± 11.5 (4)***
	2,4,2',4'-TCB (990 nM)	52.8 ± 5.6 (3)*
Bacterial CYP 102 (BM3)	none (control)	48.6 ± 20.2 (3)
	perfluorododecanoic acid (950 nM)	536.3 ± 55.6 (3)**
	dodecanoic acid (950 nM)	55 ± 14.1 (3)

Table. The CYP indicated (32.5 pmol/ml) was incubated with bilirubin in presence of NADPH .
(*P< 0.05; **P< 0.01; ***P< 0.001, all compared with corresponding controls.)

We conclude that halogenated substrate analogues can interact with different CYPs to increase production of oxidative species. These findings may have implications for CYP-mediated toxicity of polyhalogenated compounds, including hepatic uroporphyria. They may also help develop a treatment for severe jaundice caused by unconjugated bilirubin, which is very neurotoxic.
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References

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