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## A Heuristic Model for the Molecular Mechanism of Action for Dioxin-Like Compounds: A View Inside the "Black Box"

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### Abstract

Investigations regarding the mechanism of action of dioxin-like compounds have focused heavily on the binding of the parent compound to the cytosolic, arylhydrocarbon (Ah) receptor. According to the currently embraced model, toxicity of dioxin-like compounds is attributed to increased transcription and protein synthesis in response to the binding of the receptor-ligand complex to a specific enhancer sequence in DNA. The specific events following the change in transcription which lead to toxicity are not explicitly known. While there are many strengths to this model which are supported by the literature, Ah receptor binding in and of itself, does not appear to be capable of explaining all effects observed. For example, *in vitro* studies have reported rapid changes in response to dioxin that cannot possibly be attributed to Ah receptor binding. Additionally, there are naturally occurring ligands (*i.e.*, polycyclic aromatic hydrocarbons, indole(b)carbazole) which are able to bind the receptor but do not produce the same spectrum of dioxin-like symptoms. Lastly, the current model cannot account for why humans appear to be less susceptible than laboratory animals to the effects of dioxin-like compounds, despite the fact that these compounds are more persistent in human tissues. In this paper, a mechanism of action is offered as a possible explanation to resolve these apparent inconsistencies. The literature was reviewed for specific information to support the existence of an alternative mechanism of action. Information is presented which suggests that the mechanism of action involves a direct molecular interaction between very potent metabolites of dioxin-like compounds and components of the mitochondrial respiratory chain. This interaction equates to a persistent uncoupling of the mitochondrial respiratory chain, resulting in the generation of oxygen free radicals from the cells reducing equivalents (NADH) at the expense of adenosine triphosphate (ATP) production. Under this alternative model, the role of Ah receptor binding in toxicity is not a causative one, but is instead redefined as greatly enhancing toxicity via increasing metabolic activation. The implications of this proposed mechanism on the risk assessment of dioxin-like compounds are discussed.

### Introduction

In general, halogenated aromatic compounds, such as hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins/dibenzofurans (PCDDs/PCDFs), produce similar toxic effects in animals as well as in man, although there are notable differences in species and tissue sensitivity, and relative potency. While initial interest focussed on the potential

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carcinogenicity of these compounds; increasing attention is now being paid to more subtle effects of these compounds on immune function, reproduction and development, neurological function, and endocrine system. Numerous hypotheses have been advanced to explain the mechanism(s) of action of these compounds, including binding to and activation of a soluble intracellular protein, the arylhydrocarbon (Ah) receptor by these compounds; however, questions still remain as to how this interaction relates to toxicity. While no single theory is likely to suffice for all of the observed effects of these compounds, it is conceivable that many of the effects may be explained through a common mechanism. In order to satisfy requirements, such a hypothesis must explain certain general observations that distinguish many members of this class of compounds: (1) the remarkably potent nature of various halogenated aromatic compounds in inducing effects at very low doses; (2) the variety of tissues affected by these compounds; and (3) the differences in species response.

### Experimental Methods

Two hypotheses are offered regarding an alternative mechanism of action for dioxin-like compounds. These are as follows:

Hypothesis #1: *The mitochondria is the primary target for dioxin-like compounds.*

Hypothesis #2: *The toxicity of these compounds is attributable to specific dihydroxylated or quinone metabolites.*

Regarding Hypothesis #1, the scientific literature was reviewed for HCB, PCBs, and PCDD/PCDFs specifically to (1) illuminate any patterns in the spectrum of toxic effects observed in animals and man which may be linked to a mitochondrial origin, (2) direct evidence of change in mitochondrial function or structure as a result of exposure. Regarding Hypothesis #2, the scientific literature was reviewed to (1) determine whether or not haloquinone compounds are produced in biological systems, and (2) review the physical-chemical properties of these metabolites which may be important determinants of their toxic potential.

### Results and Discussion

*Evidence Supporting Mitochondrial Involvement* - The mitochondria is an organelle present in virtually all plant and animal cells. The primary function of the mitochondria is cellular respiration resulting in the generation of a high-energy phosphate bond in ATP. However, mitochondria perform a number of additional functions in the cell, including roles in heme synthesis, steroid hormone synthesis, heat production, and calcium sequestration. It is perhaps more than coincidence that exposures to HCB, PCBs, and PCDDs/PCDFs are associated with alterations in each of these processes in producing hepatic porphyria (<sup>1</sup>Cam and Nigogosyan, 1963; <sup>2</sup>Kuiper-Goodman et al. 1977; <sup>3</sup>Goldstein et al. 1982; and <sup>4</sup>Cantoni et al. 1981), endocrine system modulation (<sup>5</sup>Dibartolomeis et al. 1987; <sup>6</sup>Foster et al. 1992; <sup>7</sup>Barsotti et al. 1979; <sup>8</sup>Kleeman et al. 1990; <sup>9</sup>Moore et al. 1985), and a mysterious "wasting syndrome" (<sup>10</sup>Smith et al. 1987; <sup>11</sup>Hanberg et al. 1989; <sup>12</sup>Moore et al. 1979). Thus, the mitochondria, as a common element in each of these endpoints, conceptually serves as an ideal target for halogenated aromatics in producing some of the adverse effects associated with these chemicals. Taking cell architecture into consideration, the mitochondria also appears to be an especially likely target for lipophilic compounds since

mitochondria are typically and Kish, 1995). These lip respiration, may also serve mitochondrial involvement functional changes of the n (<sup>14</sup>Mollenhauer et al., 1975 Braselton, 1987), and PCE on the mitochondria have t investigations (<sup>20</sup>Bagchi an also been reported in work et al., 1985).

*Evidence Supporting the 1* PCBs, and PCDDs/PCDFs to form dihydroxy metabol 1989; <sup>25</sup>To-Figueras et al. <sup>29</sup>Poiger et al. 1989). Whil been considered to be less that these metabolites are t HCB, tetrachloro-1,4-quin porphyrinogenic effects of Similarly, several quinone 1 nitrogen and sulfur nucleop property in their ability to 1 reactions. It is this propert Ubiquinone, for example, membrane. The controlled difference across the mitoc 1, the haloquinone metabol structure to ubiquinone.

Table 1. Comparison of Aromatic Compounds





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Chemical Structure	10
Molecular Weight	5
Redox Potential	

Although similar in structure, haloquinone compounds are

mitochondria are typically found in close proximity to lipid droplets within the cell (<sup>13</sup>Kleinman and Kish, 1995). These lipid droplets, which typically serve as a fuel source for mitochondrial respiration, may also serve as cellular reservoirs for lipophilic compounds. In support of a mitochondrial involvement in the mechanism of action for dioxin-like, ultrastructural and functional changes of the mitochondria have been observed in animals exposed to HCB (<sup>14</sup>Mollenhauer *et al.*, 1975), PCBs (<sup>15</sup>Williams *et al.* 1993; <sup>16</sup>Narasimhan *et al.* 1991; <sup>17</sup>Ebner and Braselton, 1987), and PCDDs/PCDFs (<sup>18</sup>Johnson *et al.* 1994; <sup>19</sup>Weber *et al.* 1987). Similar effects on the mitochondria have been reported for PCBs and PCDDs/PCDFs following *in vitro* investigations (<sup>20</sup>Bagchi and Stohs, 1993; <sup>21</sup>Thome *et al.* 1995). Mitochondrial derangement has also been reported in workers accidentally exposed to a mixture of PCBs and PCDDs (<sup>22</sup>Schechter *et al.*, 1985).

*Evidence Supporting the Formation and Involvement of Dihydroxylated Metabolites - HCB, PCBs, and PCDDs/PCDFs* indicates that they are metabolized by cytochrome P450, albeit slowly, to form dihydroxy metabolites or haloquinones (<sup>23</sup>Mehendale *et al.* 1975; <sup>24</sup>van Ommen *et al.* 1989; <sup>25</sup>To-Figueras *et al.* 1991; <sup>26</sup>Koga *et al.* 1989; <sup>27</sup>Sawata *et al.* 1982; <sup>28</sup>Pluess *et al.* 1987; <sup>29</sup>Poiger *et al.* 1989). While the metabolites of halogenated aromatic compounds have generally been considered to be less toxic than their parent compounds, a review of the literature suggests that these metabolites are biologically significant. For example, the dihydroxylated metabolite of HCB, tetrachloro-1,4-quinone, has been identified as the toxic species responsible for the porphyrinogenic effects of the parent chemical (<sup>30</sup>Billi *et al.*, 1986; <sup>24</sup>van Ommen *et al.*, 1989). Similarly, several quinone metabolites of PCBs were have been reported to react with cellular nitrogen and sulfur nucleophiles (<sup>31</sup>Amaro *et al.* 1996). Quinone compounds, possess a unique property in their ability to participate in one and two electron oxidation-reduction (redox) reactions. It is this property that makes them essential for electron transport in biological systems. Ubiquinone, for example, is present in relatively large amounts in the inner mitochondrial membrane. The controlled redox cycling of ubiquinone is important in maintaining the potential difference across the mitochondrial membrane which is used to generate ATP. As shown in Table 1, the haloquinone metabolites of HCB, PCBs, and PCDDs/PCDFs are similar in chemical structure to ubiquinone.

**Table 1. Comparison Between Ubiquinone and Quinone Metabolites Halogenated Aromatic Compounds**

	Ubiquinone	HCB	PCB	PCDD
Chemical Structure				
Molecular Weight	592 - 864	248	~324	~354
Redox Potential	+0.10	+0.71	+0.7 - 0.8	TBD

Although similar in structure, haloquinones differ from ubiquinone in three important ways: (1) haloquinone compounds are smaller, and therefore should be more mobile than ubiquinone in a

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bilipid membrane, (2) haloquinones are persistent and are likely to have longer biological half-lives than ubiquinone, and (3) haloquinones have a greater redox potentials (+0.7 to + 0.8) (<sup>32</sup>van Ommen *et al.*, 1986;<sup>31</sup>Amaro *et al.* 1996) than that of ubiquinone (approximately +0.04 volts) (<sup>13</sup>Kleinsmith and Kish, 1995). The larger redox potentials of the haloquinones indicates that they are much better electron acceptors than ubiquinone. Thus, based solely on an evaluation of their physical-chemical properties, haloquinones are capable of producing a persistent uncoupling or "short circuit" of the respiratory chain, giving rise to oxygen free radicals at the expense of ATP production. The potential impacts of this uncoupling are two-fold:

- (1) *Oxidative Stress* - The production of reactive oxygen species results in the oxidation of key cellular molecules and lipid peroxidation. The generation of reactive oxygen species has been implicated in the toxicity of HCB (<sup>33</sup>Smith and de Matteis, 1990), PCBs (<sup>34</sup>Smith *et al.* 1995; <sup>21</sup>Thome *et al.* 1995), and PCDDs (<sup>35</sup>Wahba *et al.* 1989; <sup>36</sup>Alsharif *et al.* 1994; <sup>20</sup>Bagchi and Stohs, 1993). As such, oxidative stress may play an important role in porphyria (oxidation of heme precursors), tumor initiation (single strand DNA breaks), and tumor promotion.
- (2) *Loss of Reducing Equivalents from Catabolic Reactions* - When reducing equivalents from NADH are diverted, the electric potential difference across the mitochondrial membrane is not maintained. In this state, mitochondrial ATP generation is decreased, and all ATP-dependent reactions of the cell (*i.e.*, maintenance of cytosolic calcium levels, synthesis of cyclic AMP) are adversely affected. The mitochondria becomes much less efficient in generating ATP from food molecules (carbohydrates, lipids) and body stores. As such, this mechanism is consistent with the mysterious "wasting" of skeletal mass observed in animals exposed to halogenated aromatic compounds. The impacts of the loss of ATP on the levels of cAMP and calcium alone are likely to have significant biological consequences in terms of altered kinase activities, phosphorylation capacities, and cellular signalling cascades.

It is proposed here that the role of Ah receptor binding is one of enhancing metabolic activation. In this way, the toxicity of poorly metabolized compounds (such as TCDD) is more highly dependent upon interaction with the receptor, than is the toxicity of compounds which are more readily metabolized without cytochrome P-450 induction (such as HCB). A comparison between the proposed model the mechanism of action for dioxin-like compounds, and the existing model is provided in Figure 1. The proposed model is intended to serve as a tool to direct additional research, in the hopes of developing better methods for evaluating potential health risks. Several important issues regarding the risk assessment of these chemicals which require further discussion, specifically regarding (1) tissue- and individual susceptibility, (2) interspecies extrapolation, and (3) dose-response relationships.

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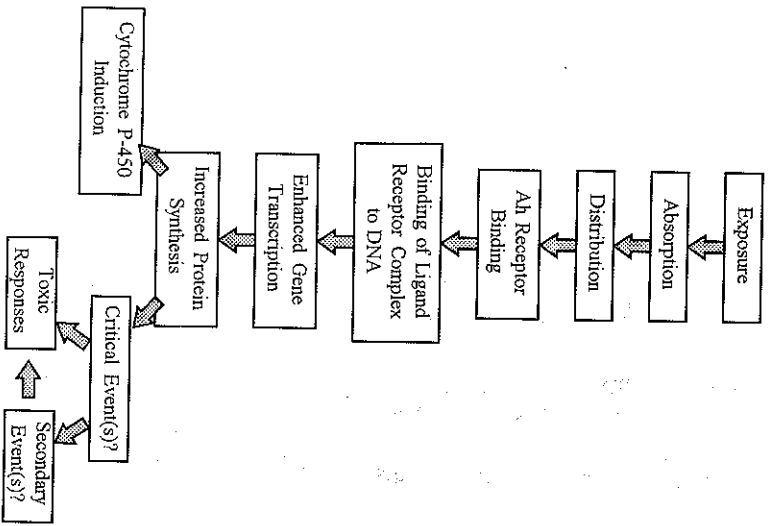
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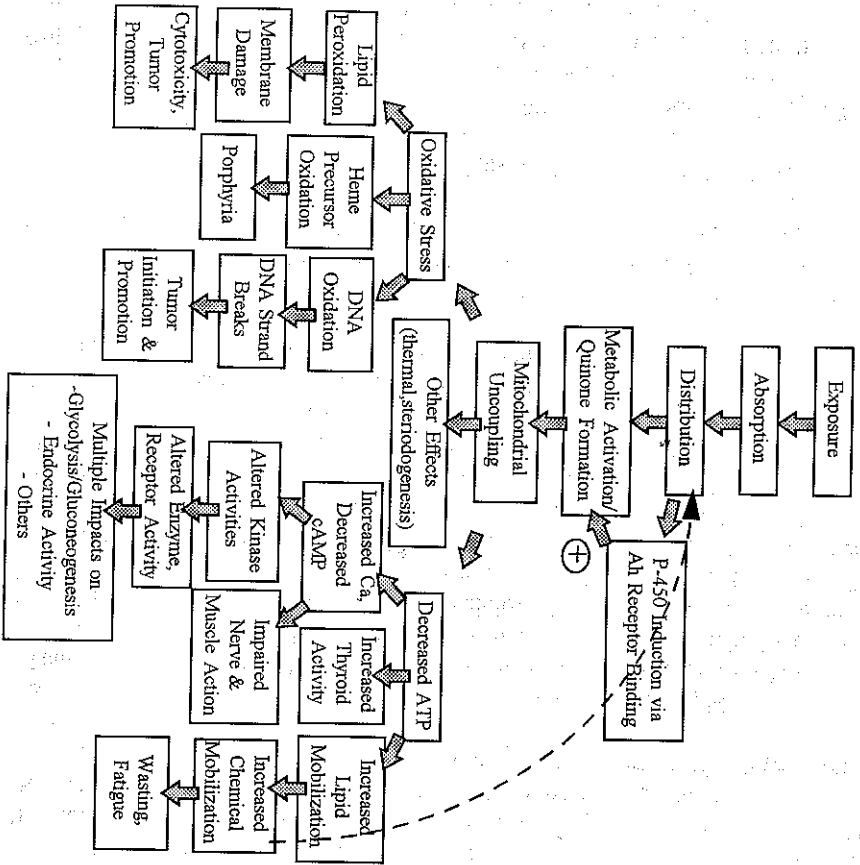
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**Figure 1. Comparison of Current and Proposed Models for the Mechanism of Action for Dioxin-Like Compounds**

Current Model



Proposed Model



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Endometrial cancer is the fifth leading cancer in the United States its number since 1980. Nevertheless, endometrial cancer is partly and various factors comparable to those of estrogens in the development of an increased incidence suggests that cigarette menopause, and other estrogens, and other factors present in cigarette smoking (and antitumorogenic) studies have confirmed the rodent/tortoise based antiestrogens cancer. This report is estrogen-responsive future studies.

Ishikawa, HEC-1-ary hydrocarbon (A) from cells treated with Ah comparable with Ah-dependent CYP1A1 lines after treatment with ECC-1 cells transiently indicate that endometrial cancer is the fifth leading cancer in the United States its number since 1980. Nevertheless, endometrial cancer is partly and various factors comparable to those of estrogens in the development of an increased incidence suggests that cigarette menopause, and other estrogens, and other factors present in cigarette smoking (and antitumorogenic) studies have confirmed the rodent/tortoise based antiestrogens cancer. This report is estrogen-responsive future studies.

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