A Proposed General Mechanism for Rodent Liver Carcinogenesis by Persistent Organohalogen Compounds at the Maximum Tolerated Dose

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Introduction

The formerly commercial organohalogen compounds (OHC) and their contaminants include a number of chemical species, notably, the PCBs, PCDDs, PCDFs, HCB, DDT, chlordane, lindane, dieldrin, etc., that have been found to be persistent in living organisms and in the general environment. Such persistent OHCs have attracted concern because of their widespread distribution, potential for bioaccumulation in environmental food chains, and ability to produce cancer in laboratory rodents when tested at the maximum tolerated dose (MTD) (1).

The implications of MTD carcinogenicity in rodents as regards cancer risk to lightly exposed humans have remained controversial, primarily, because the mechanisms by which MTD "carcinogens" produce their effects have remained elusive. Although the persistent OHCs are generally not mutagenic, they are all carcinogenic in rodents at the MTD (1). In conventional two-stage hepatotumorigenicity tests, they are not tumor initiators, but instead tumor promoters (2). For the persistent OHCs, as for other tumor promoters, there is extensive evidence that such promotion is somehow mediated by reactive oxygen species (ROS), such as superoxide ($\cdot O_2^-$) or hydrogen peroxide (3-5). However, the precise mechanisms by which such agents transmit their signals, or how their formation is increased by OHC exposure, or whether they continue to be produced under the conditions of a lifetime MTD bioassay, have remained undefined.

To address such issues, we commissioned parallel, multidose MTD bioassays of the four most widely used PCB compositions, namely, Aroclors 1016, 1242, 1254, and 1260, for chronic toxicity and tumorigenicity in male and female Sprague-Dawley (S-D) rats (6), and then undertook to characterize PCB levels and compositions (7) and a variety of biochemical responses (8) in the preserved tissues. The bioassayed Aroclors varied widely in both bioaccumulability (9) and toxic equivalency (TEQ), so that distinctions between the contributions of these common OHC attributes to the individual biochemical and histological changes observed could be made via inter-Aroclor comparisons.

Results

It was found that the liver tumor counts in the twenty-two 50-animal 24-month dose groups could be well described ($r^2 = 0.97$) by a mathematical model that used hepatic accumulation of low-*ortho* PCBs, adipose total PCB, and rat sex as the independent variables. An alternative

model using hepatic TEQ instead of low-*ortho* PCBs gave a slightly poorer correlation ($r^2 = 0.91$). The modelling indicated that the observed hepatotumorigenicity must be proceeding by two distinguishable processes. The first of these occurred in proportion to hepatic accumulation of low-*ortho* (coplanar or "near-coplanar") PCB congeners, which are known to be Ah-receptor agonists, and, like the hepatotumorigenesis or tumor promotion induced by other coplanar OHCs, occurred only in the S-D females. The other processes, which occurred in both sexes in proportion to total lipid PCB loading, independently of PCB coplanarity or TEQ, is presumably similar to that induced by other types of persistent non-coplanar OHCs as well.

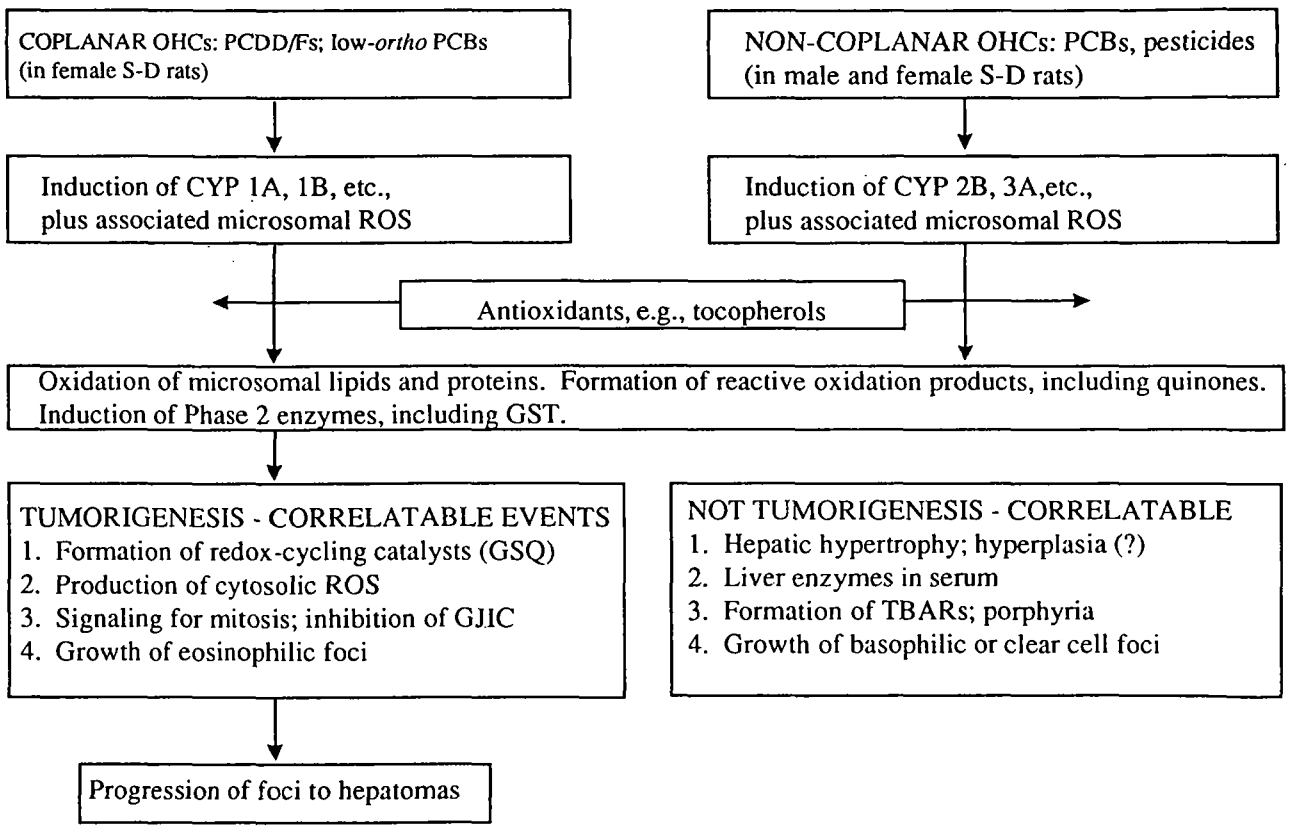
Both sexes exhibited strong and usually dose-saturated inductions of CYP1A1 and 2B1/2 proteins and the associated MROD, EROD, PROD and BROD activities. These inductions all declined somewhat over the 24-month dosing period, suggesting receptor down-regulation. Both sexes exhibited progressive (time-dependent) increases in microsomal ROS production and porphyria suggestive of alterations in enzyme activities. There were also dose-dependent increases in glutathione S-transferase (GST) activity, cytosolic ROS production, and incidence of eosinophilic foci, and decreases in glutathione peroxidase, GPx. The cytosolic ROS production, which correlated closely with tumor count (8), resulted from redox cycling by soluble, low molecular weight species that are being hypothesized as quinone-glutathione conjugates (GSQ) on the bases of chemical activity and dependence on GST/QR (for quinone reductase) ratios. Work aimed at more specific GSQ identification is in progress. A proposed general mechanism for explaining these and other observations is presented as Figure 1.

Discussion

According to the proposed mechanism, the key step in rodent liver tumorigenesis by the persistent OHCs (or other tumor promoters) is production of cytosolic ROS. This is mostly produced by redox cycling GSQs, which result from the chronic oxidative stress consequent to prolonged maximal induction of the Phase 1 (oxidative) enzymes by the persistent OHCs. Such induction is not unique to the OHCs; instead, it is a general property of all persistent lipophilic compounds in the 250 ± 200 MW range (10). The generality of this property explains why about half of all chemical compounds, whether genotoxic or non-genotoxic, natural or synthetic, can produce or promote tumors in rodents when tested at the MTD (2, 11).

The proposed process would not be expected to respond to low doses of the OHCs, even those producing modest increases in P450 cytochrome (CYP) levels and microsomal ROS production. Both CYP induction and microsomal ROS production are normal physiological processes. At moderate levels, such oxidant production, and hence also any mitogenic signaling processes that are mediated by ROS or ROS-derived products, remain controllable by antioxidants, such as tocopherols, as has been recently reported for dieldrin-mediated tumor production (5). It is only when the dose levels become so high as to overwhelm the cells' various defenses against oxidative stress - as must be often the case in MTD bioassaying, judging from the remarkable incidence of positive "carcinogenicity" findings - that tumor promotion occurs. For the persistent OHCs, at least, there is no evidence of other mutagenic or regulatory activities that would confer cancer risk at exposure levels substantially below the MTD.

Fig. 1. Proposed Interrelationships among Rat Liver Responses to Persistent OHCs



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