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Epigenetic Mechanisms of Action of Carcinogenic Organochlorine Pesticides

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Many of the most widely used chlorinated cyclic hydrocarbon compounds have been found to be carcinogenic in experimental laboratory rodents (Table I).

Table I. Carcinogenicity of Chlorinated Cyclic Hydrocarbon Pesticides

Compound	Principal Target Organ		References
	Mouse	Rat	
Aldrin	liver		1
Chlordane	liver, uterus		2
Chlorobenzilate	liver	NS[a]	3
DDT	liver, lung	liver	3,4,5
Dieldrin	liver	NS	6, 7
Heptachlor	liver	thyroid	8
Hexachlorobenzene	liver		9
Hexachlorocyclohexane (BHC), lindane	liver	liver	10,11
Kepone	liver	liver, thyroid	12
Mirex	liver	liver	3,13
PCB	liver	liver	14,15

[a] no significant increase in neoplasms

Cyclic hydrocarbons with chlorine substituents that block ring oxidation are resistant to biodegradation and thus accumulate in the environment and persist for long periods in animals once they are absorbed. The persistence of organochlorine pesticides

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together with their animal carcinogenicity has given rise to concern that exposed humans would be at risk of cancer development from these chemicals (16,17, 18). Indeed, extrapolation of dose-response effects from rodents to humans predicts substantial cancer causation (16). However, epidemiologic studies of highly exposed groups have failed to reveal any significant increase in cancer occurrence (19,20) and no increase in cancer incidence has been associated with pesticide usage (21). Such a discrepancy suggests that the mechanism of action of chlorinated cyclic hydrocarbons may be different from that of other carcinogens which produce cancer in both experimental animals and humans (22,23). This possibility is further supported by the unusual situation that all carcinogens of this structural type have the liver as their principal target organ. For carcinogens that are activated to reactive metabolites, members of a structural type almost always affect more than one organ and often the principal organ affected varies with the specific compound. For these and other reasons, we have suggested that chlorinated cyclic hydrocarbons may be carcinogenic to rodents by indirect mechanisms (22,23,24).

Mechanisms of Carcinogenesis

Chemical carcinogens are defined operationally by their ability to induce tumors in exposed animals. A highly diverse collection of chemicals is capable of producing this effect, including organic and inorganic chemicals, solid state materials, hormones and immunosuppressants. The heterogeneity of structures represented makes it improbable that all chemicals would act through a single mechanism. Therefore, Weisburger and Williams (23) have proposed a classification that separates chemical carcinogens into two major categories, genotoxic and epigenetic (Table II).

Table II Classes of Carcinogenic chemicals

Type	Example
A. Genotoxic	
1. Direct-acting or primary carcinogen	Ethylene imine, bis-(chloromethyl)ether

2.	Procarcinogen or secondary carcinogen	Vinyl chloride, benzo(a)pyrene,2-naphtylamine, dimethylnitrosamine
3.	Inorganic carcinogen	Nickel, chromium
B. Epigenetic		
4.	Solid-state carcinogen	Polymer or metal foils, asbestos
5.	Hormone	Estradiol, diethylstilbestrol
6.	Immunosuppressor	Azathioprine,
7.	Cocarcinogen	Phorbol esters, pyrene, catechol, ethanol, n-dodecane,
8.	Promoter	Phorbol esters, bile acids, saccharin

Carcinogens that interact with and alter DNA are classified as genotoxic. Thus, the genotoxic category contains the chemicals that function as electrophilic reactants as originally postulated by the Millers (25). Also, because some inorganic chemicals have displayed such effects they have tentatively been placed in this category. The second broad category designated as epigenetic carcinogens comprises those chemicals for which no evidence of direct interaction with genetic material exists. This category contains solid state carcinogens, hormones, immunosuppressants, cocarcinogens and promoters.

This classification and the underlying concepts, if ultimately validated, have major implications for risk extrapolation to humans of data on experimental carcinogenesis. Genotoxic carcinogens, as a consequence of their effects on genetic material, pose a clear qualitative hazard. These carcinogens are occasionally effective after a single exposure, are often carcinogenic at low doses, act in a cumulative manner, usually produce irreversible effects, and produce combined effects with other genotoxic carcinogens having the same target organ. In contrast, with some types of epigenetic carcinogens, it is known that the carcinogenic effects occur only with high and sustained levels of exposure that lead to prolonged physiologic abnormalities, hormonal imbalances, or tissue injury. In such cases, the effects are often entirely reversible upon cessation of exposure. Because of these features, the risk from expo-

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sure to epigenetic carcinogens seems to be of a quantitative nature.

Thus, a major element in assessing the potential hazard of a chemical is to evaluate its potential genotoxicity.

Lack of Genotoxicity of Organochlorine Pesticides

The genetic effects of organochlorine pesticides have been examined in a number of *in vitro* short-term tests (Table III).

Table III. Activity in Short-term Tests Measuring DNA Interaction of Carcinogenic Organochlorine Compounds

Compound	DNA Damage	DNA Repair	Mutagenesis	
			Bacterial	Mammalian
DDT	-a	-b,d	-f	-b
DDE	ND	-b	-g	ND
Dieldrin	-a	-b,+c	-e,f	+c
Chlordane	ND	+c,-d	ND	-b,+c
Heptachlor	ND	ND	-e,-h	-b
Kepone	ND	-b	-h	-b

a) Swenberg (26), b) Williams (24), c) Hart (27,28), d) Flamm (29), e) Marshall (30), f) Shirasu (31), g) Ames (32), h) Schoeny (33).

Although the results have been predominantly negative, their significance has been minimized by the frequent suggestion that lack of activity is simply a consequence of the absence of appropriate metabolism in the *in vitro* tests.

In our laboratory we have developed several tests for genotoxicity utilizing liver-derived cells (34,35). Since the organochlorine pesticides have the liver as their principal target organ, these tests represent the ideal system in which to evaluate the genotoxicity, as well as other effects, of these compounds.

The hepatocyte primary culture (HPC)/DNA repair test assesses the capability of chemicals to undergo

covalent interaction with DNA by measurement of autoradiographic DNA repair elicited as a result of the DNA damage (36,37). The freshly isolated hepatocytes used in this test retain a high level of activity for biotransforming xenobiotics and thus the test responds to a wide spectrum of structural types of carcinogens requiring metabolic activation (34,35). Our previous reports of lack of genotoxicity of organochlorine pesticides in the rat liver HPC/DNA repair test (24,38) have been extensively confirmed (Table IV).

Table IV. HPC/DNA Repair Results

Compound	grains/nucleus[a]		
	Rat	Mouse	Hamster
2',3-Dimethyl-4-aminobiphenyl	60	25	>100
Biphenyl	-	-	-
Chlordane	-	-	-
DDT	ND	-	-
Mirex	ND	-	-
Kepone	-	ND	ND

[a] - = zero; ND = not done

In addition, since the organochlorine pesticides are sometimes more active on mouse liver, these results were extended (38,39) to the mouse liver derived HPC/DNA repair test, as well as the hamster liver derived test (Table IV).

Another liver-derived test for genotoxicity is the adult rat liver epithelial cell (ARL)/hypoxanthine-guanine phosphoribosyl transferase (HGPRT) mutagenesis assay (40,41). This test assesses mutagenicity at the HGPRT locus through measurement of conversion of liver epithelial cells to HGPRT-deficient mutants that are resistant to 6-thioguanine. As with the HPC/DNA repair test, the cells in this assay possess intrinsic metabolic capability for the biotransformation of activation-dependent carcinogens (34). In spite of a mutagenic response to three genotoxic carcinogens, the organochlorine pesticides were all non-mutagenic in this assay (24) (Table V).

Table V. ARL[a]/HGPRT Mutagenesis Assay Results

Compound	Concentration molar[b]	Induction of HGPRT deficient mutants
Aflatoxin B_1	10^{-6}	+
3-Methyl-4- dimethyl-aminoazobenzene	10^{-5}	+
2-Aminofluorene	10^{-4}	+
Chlordane	2.5×10^{-5}	-
Kepone	10^{-5}	-
Heptachlor	10^{-5}	-
Hexachloro-cyclopentadiene	10^{-6}	-
Endrin	3×10^{-3}	-
DDT	10^{-4}	-

[a] line ARL 6
[b]-highest nontoxic dose that was negative or lowest dose that was positive.

The consistent lack of genotoxicity of organochlorine pesticides in liver derived tests strongly supports the negative data obtained in other tests. Thus, it appears that these chemicals are not genotoxic carcinogens.

Epigenetic Mechanism of Action of Organochlorine Pesticides.

At least one organochlorine pesticide, DDT, has been shown to be a liver tumor promoter (42), enhancing the carcinogenic effect of 2-acetylaminofluorene when given after the carcinogen. Thus, we have postulated that the organochlorine pesticides may be carcinogenic through a mechanism of tumor promotion (22,24,38). All of the inbred strains of rats and mice used for carcinogen bioassay have a spontaneous incidence of liver tumors which in the case of some mouse strains is quite high (22). As part of this

condition, these animals also have a higher incidence of lesions regarded as preneoplastic or potentially neoplastic. Thus, we postulated that the promoting effect of organochlorine pesticides would enable the pre-existing abnormal liver cells to progress to a higher frequency of tumor development than would occur under control conditions.

The mechanism of the promoting effect of chemicals when administered after a primary carcinogen is not yet known. A compelling concept is that tumor promoters may act on the cell membrane. Under normal conditions, the cells composing a tissue are in homeostasis in which the requirements for cell growth to balance cell loss are regulated throughout the tissue. The regulation probably occurs through cell to cell communications. Interruption of such communications could permit cells with an abnormal genotype to proliferate beyond the normal growth requirements, that is to form a neoplasm. Recently, several groups (43,44) have reported in vitro studies which show that tumor promoters are capable of blocking intercellular communication. We have extended these studies to the use of liver-derived cells to study liver tumor promoters (38).

The test system involves the measurement of inhibition of metabolic cooperation in mixed liver cell cultures. Metabolic cooperation in cell culture involves the cell-to-cell transfer through gap junctions of a metabolic product from enzyme-competent to enzyme-deficient cells, as with the transfer of phosphoribosylated 6-thioguanine (TG) from HGPRT-competent cells to HGPRT-deficient cells. In this case, HGPRT-deficient cells, such as those comprising an ARL-TG resistant strain, are not affected by the addition of TG to the medium because they lack the purine salvage pathway enzyme to convert TG to the mononucleotide, but are killed when cocultivated with HGPRT-competent cells as a result of transfer of the toxic metabolite. As shown in Table VI, the colony forming efficiency of HGPRT-deficient ARL-TG^r is comparable in control medium to that in TG-containing medium.

Table VI. Inhibition of Metabolic Cooperation between Hepatocytes and an ARL TG Resistant Strain by the Liver Tumor Promoter DDT

Condition	TG resistant colonies per flask[a]	
	- hepatocytes	+ hepatocytes
ARL 14-TG resistant cells	126[b]	-
+ TG	110	63
+ TG + DDT 10^{-7}	103	86
+ TG + DDT 10^{-6}	101	112
+ TG + DDT 10^{-5}	105	117
+ TG + DDT 10^{-4}	61	24

[a] 500 TG resistant cells were cocultured with 0.75×10^6 hepatocytes.
[b] Average of three flasks.

When HGPRT-competent cells, such as freshly isolated hepatocytes, are co-cultivated with TG resistant cells at ratios high enough to achieve significant cell to cell contacts, the HGPRT-competent cells metabolize the TG and transfer the mononucleotide to the TG resistant cells, thereby killing the TG resistant cells as well as themselves. Consequently, as shown in Table VI, the co-cultivation of hepatocytes with TG resistant cells in the presence of TG reduces the recovery of the colonies from TG resistant cells. The approach developed by Trosko and associates (44) and applied by us to liver (38) involves measurement of the ability of tumor promoters to inhibit this process and produce an increase in the recovery of TG resistant cells in the co-cultivation system. As shown in Table VI, the addition of DDT to co-cultivated hepatocytes and TG resistant cells exposed to TG restores the recovery of the mutant cells beginning at 10^{-7}M and reaching 100% at 10^{-6} and 10^{-5}M.

Conclusions

The studies described provide evidence for the

lack of genotoxicity of carcinogenic organochlorine pesticides and demonstrate an effect on the intercellular lipid layer of the cell membrane. This process may differ from that of other liver tumor promoters such as phenobarbital. We have reported (45) that phenobarbital alters the activity of certain membrane associated enzymes such as gamma glutamyltranspeptidase and have suggested that phenobarbital modifies gene expression to produce a biochemical change in the composition of the cell membrane. Thus, both types of tumor promoters may achieve the same inhibition of intercellular communication by different processes.

The concept that the carcinogenicity of organochlorine pesticides is due to their promoting action as a result of effects on the cell membrane has important implications. Inhibition of intercellular communication presumably would not occur without substantial accumulation of the compounds in the cell membrane. Thus, the carcinogenicity of these compounds only at high dose levels would be explained. Furthermore, cessation of exposure would lead to elimination of the compounds and restoration of intercellular communication. This would suggest that the carcinogenic effects, unlike those of genotoxic carcinogens, would be entirely reversible up to a point.

The absence of observable human carcinogenic effects following exposure to organochlorine pesticides is interpretable in light of the proposed epigenetic mechanisms of action. It could be that human exposures have been insufficient to achieve the cellular levels required to effectively inhibit intercellular communication. Certainly, this would seem to be the case for exposures of the general population. It could even be that human cells are more efficient in intercellular communication and thus more resistant to the effects of inhibitors. A third possibility is that the exposed human populations lack the background of genetic alterations in the liver needed to give rise to neoplasms in response to a promoting agent.

These concepts and interpretations require rigorous documentation. Nevertheless, sufficient evidence is now available to suggest that projections of the carcinogenic risks from organochlorine pesticide exposure require re-evaluation in light of newer developments.

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