CHAPTER II

LITERATURE REVIEW

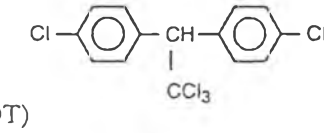
The literature review is divided into two parts. The first part concerns the chlorinated hydrocarbon insecticides with special emphasis on the DDT group; the principles of carcinogenesis are briefly discussed in the second part.

The Chlorinated Hydrocarbon Insecticides

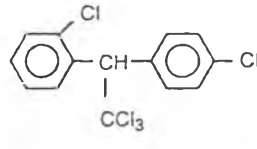
1. DDT and associated compounds (23-26)

DDT is a common name for 1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane or p,p'-DDT. Although DDT was first synthesized by Zeidler in 1874 it remained for Paul Muller to rediscover DDT in 1939 while searching for a contact poison against clothes moths and carpet beetles. The effectiveness of DDT against a variety of household and crop insect pests was quickly demonstrated, earning Muller a Nobel Price in 1948 for his research. DDT was first used to control malaria, typhus and other vector borne diseases; and in 1945 it was released for commercial use in agriculture. Its productions continued to increase until 1963 and then gradually decreased. The ban on the use of DDT is based on a number of ecological assesment and its

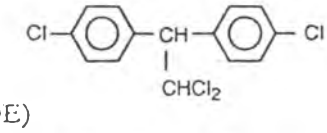
toxic effects. However,DDT is still used in agriculture and in vector control in some tropical countries. Technical-grade DDT,in which p,p'-DDT is the predominant component,is made by condensing chloral hydrate with chlorobenzene in the presence of sulfuric acid. A typical sample of technical DDT has the following constituents: p,p'-DDT, 77.1%; o,p'-DDT, 14.9%; p,p'-TDE (DDD), 0.3%; o,p'- TDE (DDD),0.1%; p,p'- DDE, 4%; o,p'- DDE, 0.1%; and unidentified product, 3.5%. The chemical structures of DDT and related compounds are shown below.



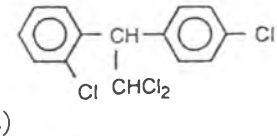
$C_{14}H_9Cl_5$ (*para,para'*-DDT) Mol. wt: 354.5



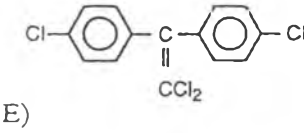
$C_{14}H_9Cl_5$ (*ortho,para'*-DDT) Mol. wt: 354.5



$C_{14}H_{10}Cl_4$ (*para,para'*-TDE) Mol. wt: 320.0



$C_{14}H_{10}Cl_4$ (*ortho,para'*-TDE) Mol. wt: 320.0



$C_{14}H_8Cl_4$ (*para,para'*-DDE) Mol. wt: 318.0

Figure 1. The chemical structures of DDT and related compounds

1.1 Chemical and physical properties

p,p'-DDT is a colorless crystalline solid, odorless or with weak aromatic odor and melts at 108-109 °C. It is soluble in acetone, benzene, cyclohexanone, diethyl ether, chloroform and other organic solvents, but practically insoluble in water. This chemical is stable to oxidation; corrosive to iron; and dehydrochlorinated at temperatures above its melting point to the non-insecticidal DDE, a reaction catalysed by iron (III) or aluminium chlorides, by ultraviolet light and, in solution, by alkali.

o,p'-DDT is a white crystalline solid and melts at 74-75 °C.It is slightly soluble in water, soluble in lipids and most organic solvents; and is stable to concentrated sulfuric acid.

p,p'- TDE is a colorless, odorless crystalline solid and melts at 109-110 °C. It is slightly soluble in water. Its stability is similar to that of p,p'-DDT but more slowly hydrolysed by alkalis.

o,p'- TDE is colorless crystals which melts at 76-78 °C.

p,p'- DDE is a white crystalline solid which melts at 88.4-90 °C. It is slightly soluble in water but soluble in lipids and most organic solvents. This chemical is stable to concentrated sulfuric acid, andmay be oxidized to p,p'- dichlorobenzophenone, a reaction catalysed by ultraviolet radiation.

1.2 Occurrence and expossure

The physicochemical properties of DDT and its metabolites (low volatility, chemical stability, lipid solubility, slow rate of biotransformation and degradation) enable the organisms to take them up rapidly. As these compounds are resistant to breakdown, they are readily absorbed by sediments and soils, which can act as both sinks and long-term sources of exposure. Organisms can accumulate these chemicals from the surrounding medium and from food. Uptake from water is generally more important for aquatic organisms, whereas food provides the major source in terrestrial fauna. DDT is stored in tissues, particularly in adipose tissue, and in liver,brain and muscle of mammals, fish, reptiles and birds; it is also stored in insects, algae, plankton and in the eggs of fish and birds. DDT has also been found in dairy products and human milk.

Several studies have been carried out of the exposure of workers manufacturing and formulating DDT and of those applying it. Indications of exposure have been determined either by direct or indirect measurements of DDT levels in blood and body fat, and by determination of DDA levels in urine. Using direct methods of measurement, estimations of potential dermal exposure ranged from 84 mg/hr for outdoor spraying to 1,755 mg/hr for indoor spraying. Value of 212 mg/hr were found during forest spraying and of 524.5 mg/hr for formulating plant workers.

Estimation of potential respiratory exposure ranged from 0.11 mg/hr for outdoor spraying to 7.1 mg/hr for indoor spraying, with values of 4.92 mg/hr for forest spraying and of 14.1 mg/hr for formulating plant workers.

1.3 Absorption,distribution, metabolism and excretion

DDT is absorbed by all routes; its fate and its metabolism in man was studied in volunteers receiving known quantities of technical-grade DDT (77% p,p', 23% o,p'), p,p'-2,2- bis (p- chlorophenyl) acetic acid (DDA), p,p'- TDE or p,p'- DDE. DDT, TDE or DDA ingested at 5, 10 or 20 mg per day for 21-183 days was partly excreted as DDA in urine most rapidly following DDA ingestion and least following DDT. Urinary excretion of DDA began within 24 hr of ingestion of DDT, TDE or DDA. Urinary DDA returned to its predose level two of three days after its administration but continued to be excreted slightly above the predose level for more than four mouths following termination of ingestion of TDE or DDT. DDE failed to produce any increase in DDA excretion. Dechlorination of DDT (administered to volunteers at 5,10 or 20 mg per day for 183 days) led to conversion to TDE (measured in serum and adipose tissue) and further metabolism to the readily excreted DDA. Dehydrochlorination of DDT yielded DDE, a stable metabolite. In two subjects who ingested technical-grade DDT, the conversion of p,p'-DDT to p,p'-DDE was limited, as assessed by measuring DDE concentrations in serum and adipose tissue.

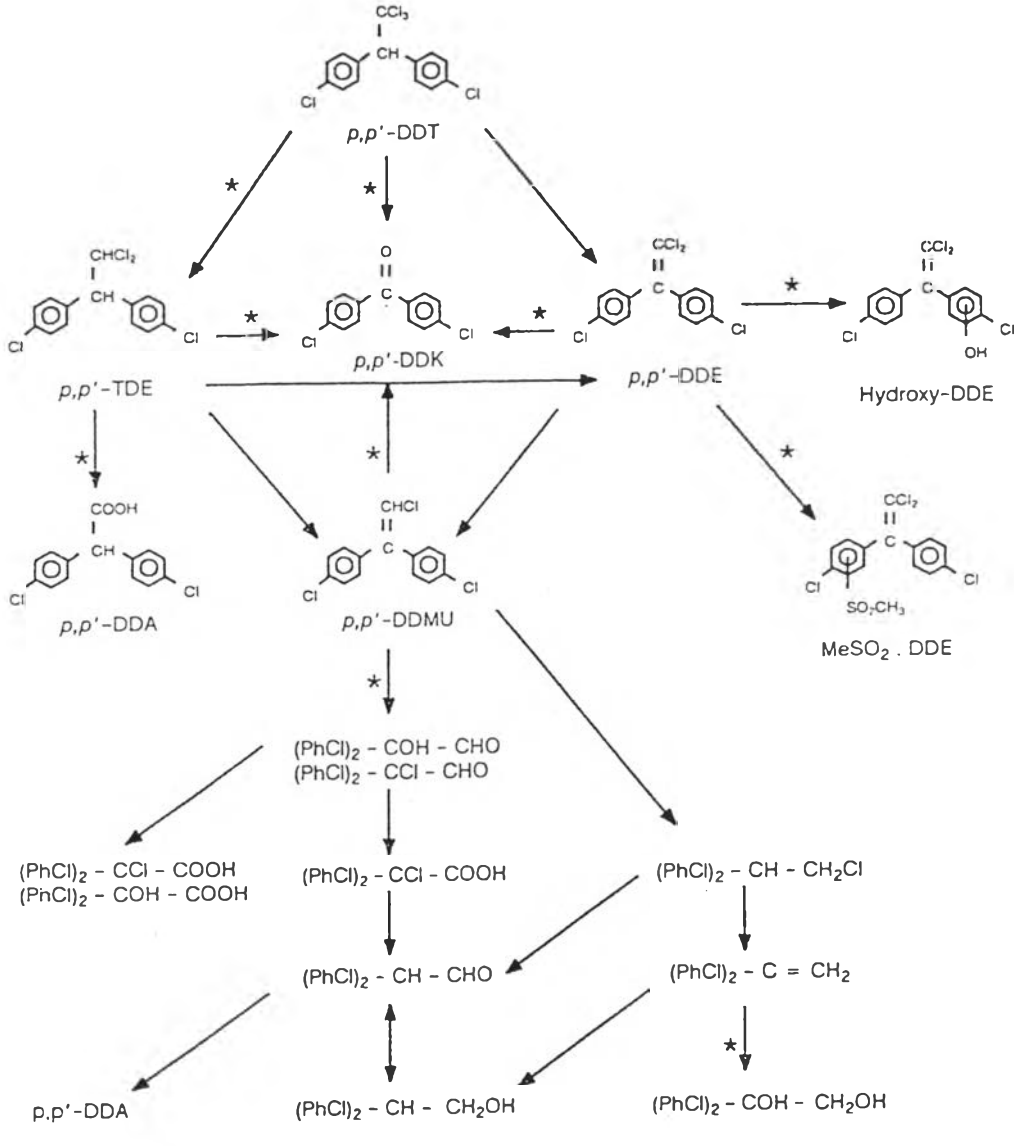
After oral administration of technical-grade DDT at 10 or 20 mg per day for six months, the level of o,p'- DDT was reported to decline more rapidly than of p,p'-DDT. After the treatment period, excretion of DDA declined sharply, despite a very slow decrease in serum and adipose tissue levels of DDT.

A positive dose-related correlation between exposure to DDT and urinary excretion of DDA has been observed, indicating that the urinary level of DDA could be used as a monitoring test of the recent exposure to DDT.

Several metabolic pathways leading from DDT to DDA have been proposed, and those suggested for the degradation of DDT, including areas at which reactive metabolites may be involved, are given in Figure 2. The biological half-time for DDT is about one month in dogs, two months in hens, three months in monkeys and approximately five weeks in rats. In the latter species, the half-time was reduced to five days under conditions of starvation for three days followed by a restricted diet. Most species, including humans but with the exception of rhesus monkeys, store DDE more tenaciously than they do DDT. DDA is the major and final water-soluble metabolite in the urine of rat. DDA is the major and final water-soluble metabolite in the urine of rat. DDA is the major and final water-soluble matabolite in the urine of rats, mice and rabbits.

In the main pathway from p,p'- DDT via p,p'- TDE to p,p'- DDA, the formation of two reactive intermediates is postulated, i.e., a free radical and an acid chloride. Both

intermediates are probably capable of binding covalently to cellular macromolecules. Other reactive intermediates in



p,p'-DDK, bis(4-chlorophenyl)ketone; DDA, *para,para'*-2,2-bis(*para*-chloro-phenyl)acetic acid; DDMU, 1-chloro-2,2-bis(*para*-chlorophenyl)ethene; PhCl, 4-chlorophenyl

Figure 2. Compilation of metabolic pathways proposed for DDT in rodents ; asterisks indicate where reactive intermediates are suggested to be formed

the metabolism of DDT include side - chain epoxides of DDE, 1-chloro-2,2-bis (p-chlorophenyl) ethane and 1,1-bis(p-chlorophenyl)ethane. Ring epoxides (arenoxides) may lead to the formation of the methylsulfone of DDE.

In a study in mice pretreated for five months with DDE and subsequently given radiolabelled DDE , however, most of the radiolabel in urine, feces and liver was bound to unchanged DDE and one phenolic metabolite. It was concluded that there was no indication for the metabolism of DDE to a reactive electrophillic species.

Several studies have indicated that the metabolic pathways are similar in various species including humans. Hamsters differ from mice, however, in that after dietary treatment with p,p'- DDT, they do not excrete DDE in the urine; furthermore, the relative DDE tissue levels in hamsters are much lower than those in mice. The metabolism of DDT is promoted by DDT itself in hamsters but not in mice. Monkeys fed diet containing up to 5000 ppm (mg/kg) of p,p'- DDT stored little or no DDE in bodyfat, but when DDE itself was fed (at 200 ppm in the diet), DDE was readily accumulated in body fat.

1.4 Carcinogenicity in experimental animals

DDT has been tested adequately for carcinogenicity by oral administration to mice, it caused liver-cell tumors, including carcinomas, in animals of each sex and hepatoblastomas in males. In one study, the incidence of lung carcinomas was increased, and in three studies the

incidence of malignant lymphomas was increased; the incidence of lymphoma was decreased in two studies. the incidence of liver tumors was increased in mice following subcutaneous injection of DDT. Oral administration of DDT to rats increased the incidence of liver tumors in female rats in one study and in male rats in two studies. In two studies in which DDT was administered orally to hamsters at concentrations similar to or higher than those found to cause liver tumors in mice and rats, some increase in the incidence of adrenocortical adenomas was observed.

A metabolite of DDT, p,p'-DDE, has been tested for carcinogenicity by oral administration in mice and hamsters. A second metabolite, TDE, was tested by oral administration in mice and rats. TDE increased the incidence of liver tumors in male mice and of lung tumors in animals of each sex in one of the two studies in mice. An increase in the number of thyroid tumors was observed in one study in male rats. DDE produced a high incidence of liver tumors in male and female mice in two studies. An increased incidence of neoplastic liver nodules was observed in one study in male and female hamsters.

1.5 Carcinogenicity in humans

Slight excess risks for lung cancer were observed among workers at two DDT producing factories in the U.S.A. A nested case- control study in one of these investigations found a slight deficit of respiratory cancer. No other cancer occured in sufficient numbers for analysis. In a

prospective cohort study in which exposures were estimated on the basis of serum levels of DDT, the risk for lung cancer rose with increasing concentration but was based on small numbers.

Several investigators have compared serum or tissue levels of DDT and/ or DDE among individuals with and without cancer, with inconsistent results.

Results from case-control studies of soft-tissue sarcoma do not point to an association.

An elevated risk for non-Hodgkin's lymphoma in relation to potential exposure to DDT was found in a study from Washington State in the U.S.A., but not for other agricultural exposures. An elevated risk for malignant lypmhomas was also found in a case-control study in northern Sweden, with adjustment for exposure to herbicides. The only study available found no association between exposure to DDT and primary liver cancer. In the U.S.A., a slight increase in the risk of leukemia occured among farmers who reported use of DDT and many other agricultural exposures. The relative leukemia risks in animals rose with frequency of DDT exposure.

Epidemiological data on cancer risks associated with exposure to DDT are suggestive but limitations in the assessments of exposure in the studies and the finding of small and inconsistent excesses complicate an evaluation. The slight excesses of respiratory cancer seen among cohorts exposed to DDT are based on differences of five or fewer cases between exposed and unexposed groups. In case-

control studies of lymphatic and haematopoietic cancers, exposure to agricultural pesticides other than DDT resulted in excesses as large as or longer than those associated with exposure to DDT. In most of the case-control studies, adjustment was not made for the potential influence of other exposures.

2. Chlorinated hydrocarbon insecticides other than the DDT group.

In this section, the carcinogenic risk of some organochlorine insecticides besides the DDT group are briefly considered.

2.1 Chlordane, heptachlor and heptachlor epoxide (27)

Chlordane, heptachlor are discussed together because of their close structural similarity and because technical-grade products each contain approximately 20 % of the other compound. Heptachlor epoxide is not normally present in commercial heptachlor but is apparently formed by biological and chemical transformation of heptachlor in the environment.

Chlordane and heptachlor have been used since the 1950s as insecticides; their uses are now largely restricted to underground control of termites. Chlordane, heptachlor and heptachlor epoxide have been tested for carcinogenicity by oral administration in several strains of mice and rats. These studies uniformly demonstrate increases in the incidence of hepatocellular neoplasms in

mice of each sex. Increases in the incidence of thyroid follicular-cell neoplasms were observed in rats treated with chlordane and technical-grade heptachlor. There is inadequate evidence in humans for the carcinogenicity of chlordane and of heptachlor.

2.2 Dieldrin (28)

Dieldrin was first synthesized in 1948, and commercial production in the United States was first reported in 1950. The only known use for dieldrin is as an insecticide, and one significant outlet is in the treatment of soil around structures for termite control. Dieldrin has been tested for carcinogenicity by the oral route in mice and rats. The hepatocarcinogenicity of dieldrin in mice has been demonstrated and confirmed in several experiments, and some of the liver-cell tumors were found to metastasize. The epidemiological study carried out on occupationally exposed workers dose not allow any conclusions to be made concerning the existence of an excess risk of developing cancer.

2.3 Toxaphene (29)

Toxaphene is polychlorinated camphenes. The exact composition of toxaphene is unknown. Technical toxaphene consists predominantly of polychlorinated camphenes with 4-12 chlorine atoms per molecule. Commercial production of toxaphene in the United States was first reported in 1947. Toxaphene is used as an insecticide. There is sufficient

evidence that toxaphene is carcinogenic in mice and rats. After oral administration, a dose-related increase in the incidence of hepatocellular carcinomas has been observed in male and female mice, and an increased incidence of thyroid tumors has been observed in male and female rats. There is inadequate evidence in humuns for the carcinogenicity of toxaphene. However, an increased frequency of chromosomal aberrations in the lymphocytes of workers exposed to toxaphene has been reported.

2.4 Methoxychlor (30)

Methoxychlor was first synthesized in 1893, and commercial production in the United States was first reported in 1946. The only known use for methoxychlor is as an insecticide. Domestic use of methoxychlor as a substitute for DDT is increasing in the United States. Methoxychlor was tested in one experiment in mice and in several experiments in rats by oral administration. The study in mice gave negative results. In at least four experiments in rats, dietary concentrations of 1000 mg/kg or more were used. An earlier suggestion that it was hepatocarcinogenic in rats was not confirmed in these more recent experiments. Thus, the available data do not provide evidence that methoxychlor is carcinogenic in experimental animals. No case reports or epidemiological studies are available to the IARC working Group for evaluating the carcinogenic risk of this insecticide in humans.

2.5 Chlordecone (31)

Synthesis of chlordecone was first reported in 1952, and commercial production in the United States was first documented in 1966. The only known use of chlordecone is as an insecticide. There is sufficient evidence that this chemical is carcinogenic in mice and rats. Oral administration of chlordecone produces hepatocellular carcinomas in male and female of both species. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard chlordecone as if presented a carcinogenic risk to humans.

Principles of Carcinogenesis (32-33)

1. Theories of carcinogenesis

To explain the causation of cancer, many theories have been propounded, all of which have sought in various ways to account for the phenotypic changes that typify the cancer cell. These changes include the following :

- tendency for relatively uncontrolled and unlimited proliferation, ultimately at the expense of the host
- transmissibility of the proliferative abnormality from one neoplastic cell to successive generations of daughter cells, as a relatively "stable" and "heritable" phenotype
- tendency for the proliferative abnormality to

progress with time toward increasing malignancy, associated with increasingly marked alterations in cell morphology,karyotype, antigen specificity, metabolism and other properties.

Although many theories of carcinogenesis have failed to stand the test of time, and none by itself has successfully accounted for all the observed aspects of neoplasia, several theories form the basis for contemporary concepts. These theories can be grouped under the four major mechanisms they invoke :

1. Somatic mutation
2. Aberrent differentiation
3. Virus activation
4. Cell selection

The somatic mutation theory attributes neoplasia to abnormality in one or more of the genes regulating growth and differentiation. According to this theory such genetic abnormalities can occur at any time life. The carcinogenic action of agents such as ionizing radiation and alkylating chemicals is ascribed to their mutagenic effects on exposed cells.

In contrast to the somatic mutation theory, which attributes carcinogenesis to abnormalities of genes or chromosomes, the aberrant differentiation theory supposed that such changes need not occur. Instead, it postulates that disturbances in gene regulation, through faulty repression or derepression, may cause a derangement of

growth and differentiation expressed in the form of cancer. Since the defect merely involves changes in the regulation of genes and not changes in their structure, it can be considered epigenetic rather than genetics.

Oncogenic viruses contain either DNA or RNA as their genome. Prevailing evidence favors the view that the virus exerts its oncogenic effect through integration of genetic information encoded in its nucleic acid into the genome of the infected host cell. In the case of DNA virus, the integration and subsequent transcription of viral nucleic acid may be analogous to processes that have been best characterized in lysogenic bacteriophages. For RNA virus, the process of integration is thought to involve a DNA intermediate, synthesized from viral RNA through the action of a virus-specified, RNA-directed, DNA polymerase, or "reverse transcriptase".

In cell selection theory, stimuli that increase the propability of cancer are thought to do so by favoring the proliferation of transformed cells which might not otherwise express their neoplastic proclivities. The theory is partially based on the evidence that under some conditions, carcinogenesis can be demonstrated to be a multistage process. The progression of the tumor toward malignancy is viewed as the sequential appearance and selective outgrowth of progressively more autonomous subpopulations of cells evolving through stepwise mutation-like changes, and proliferating under the influence of sustained selection pressure.

2. Initiation and promotion

In certain organs such as skin and liver, it has been shown that carcinogenesis can be divides into at least two stages. The classic example is skin. Typically, identical areas of the skin of a group of mice are painted once with benzo[a]pyrene. If no other subsequent treatment is used, no skin tumors develop. However, if the application of benzo[a]pyrene is followed by several applications of croton oil, many tumors subsequently develop. Applications of croton oil alone, i.e., no pretreatment with benzo[a]pyrene, do not result in skin tumors. Many other variants of this basic protocol have been carried out, permitting the following conclusions :

1. The stage carcinogenesis caused by application of benzo[a]pyrene is called initiation; this stage appears to he rapid and irreversible.It is presumed to involve an irreversible modification of DNA, perhaps resulting in one or more mutations. Benzo[a]pyrene is thus called an initiating agent.

2. The second, much slower (i.e., months or years) stage of carcinogenesis resulting from application of croton oil, is called promotion. Croton oil is thus a promoting agent, or promoter.

3. Most carcinogens are capable of acting as both initiating and promoting agents.

A large number of compounds, including phenobarbital and saccharin, can act as promotors in

various organs. The active agent of croton oil is a mixture of phorbol esters. The most active phorbol ester is 12-O-tetradecanoylphorbol-13-acetate(TPA) which has numerous effects. The most interesting finding has been that protein kinase C can act as a receptor for TPA. Stimulation of the activity of this enzyme by interaction with TPA may result in the phosphorylation of a number of membrane proteins, leading to effects on transport and other functions. Many tumor promoters appear to act by causing alterations of gene expression, but the precise mechanisms by which promoters influence the initiated cell to become a tumor cell remain to be determined.

3. Chemical carcinogens

Chemical carcinogens are defined operationally by their ability to induce neoplasms. They may be seperated into classes based on their chemical or biologic properties. These in turn can be placed in the two general categories : DNA-reactive (genotoxic) and epigenetic (table 1), where available information is sufficient. Otherwise, specific carcinogens or classes are left unassigned.

The DNA-reactive (genotoxic) category comprises carcinogens that chemically interact with DNA. The defining characteristic is a chemical property and, therefore, chemical assays of adduct formation are most definitive for identifying such agents. This category contains most of the "classic" organic carcinogens, and consists mainly of

carcinogens that function as electroplilic reactants. DNA-reactive carcinogens can be subdivided according to whether they are active in their parent form or require bioactivation.

Table 1. Classification of carcinogenic chemicals

CATEGORY AND CLASS	EXAMPLE
A. DNA-reactive (genotoxic) carcinogens	
1. Activation-independent organic	Alkylating agents
2. Activation-independent inorganic	Nickel, cadmium
3. Activation-dependent	Polycyclic aromatic hydrocarbon, arylamine, nitrosamine
B. Epigenetic carcinogens	
1. Promoter	Organochlorine pesticides, saccharin
2. Hormone-modifying	Estrogen, amitrole
3. Peroxisome proliferators	Clofibrate, diethylhexylphthalate
4. Cytotoxic	Nitrilotriacetic acid
5. Immunosuppressor	Cyclosporin A, azathioprine
6. Solid state	Plastics, asbestos
C. Unclassified	
1. Miscellaneous	Ethanol, dioxane

The second broad category, designated as epigenetic carcinogens, comprises those carcinogens for which there is evidence of an inability of the chemical to interact with genetic material and for which another biologic effect has been delineated that could be the basis for carcinogenicity. The category contains carcinogens characterized by their promoting activity, hormone-modifying activity, cytotoxicity, immunosuppressive action, or ability to induce peroxisome proliferation. This scheme dose not preclude

genotoxic carcinogens as such, or in the form of their metabolites, from also having epigenetic effects. Indeed, the potency of some carcinogens may reside in their promoting as well as genotoxic actions.