An Article Review

The Interaction of Pesticides with Bioenergetic Processes and Protein Kinases in Cell Biology

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Abstract:

Since the chemiosmotic theory was proposed by Peter Mitchell in the 1960s, a major objective has been created to elucidate the mechanism of coupling of the transmembrane proton motive force. Recently, significant progress has been made towards establishing the complete structure of ATP synthase which is the master enzyme in the bioenergetics in cell biology. ATP synthase contains a rotary motor involved in biological energy conversion. Respiratory complexes in mitochondria and eubacteria, and photosynthetic complexes in chloroplasts and photosynthetic eubacteria use energy derived from the oxidation of nutrients and from light, respectively, to generate a transmembrane proton motive force (pmf). Numerous pesticides were found to interact with the processing of bioenergetics in cells to inhibit ATP formation.

Kinases catalyze transferring the Pi from the ATP to specific protein to turn it phosphorylated protein. This step of protein phosphorylation by kinasaes play a central role in signal transduction pathway in cell biology such as protein synthesis, metabolism and hormone-receptor interactions. Exogenous compounds such as organochlorine, organophosphorous and pyrethroid insecticides thought to mimic the action of hormones on their receptor and influence endocrine and kinases activity.

Introduction:

The sun is the ultimate source of energy for all life on the planet earth. That energy, sunlight, is trapped by photosynthetic organisms and used to convert CO_2 into the organism's cellular material which is composed of mainly proteins, carbohydrates, and lipids, but also smaller amounts of nucleic acids, vitamins, coenzymes, and other different cellular compounds (Bergantino et al 2003). Some of these products of photosynthesis (carbohydrates) are, in turn, utilized by no photosynthetic organisms, mainly animals, as a source of energy for growth, development, and reproduction. Other essential compounds that cannot be synthesized by animals (certain amino acids, fatty acids, and vitamins) are also provided by the

photosynthetic organisms (mainly higher plants) when they are consumed by animals as food (Rizhsky et al 2003). Cells generate their energy through the absorption of light quanta in plants and oxidation of these foods by respired oxygen in animals, in both cases the redox energy is coupled to the synthesis of ATP. ATP synthase uses the pmf to make ATP from ADP and inorganic phosphate (Pi). As summarized in Figure 1, the enzyme has two major structural domains, known as F1 (factor 1) and Fo (factor oligomycin).The F1Fo-ATP synthase complex (also called F1Fo-ATPase) plays a central role in energy transformation in most organisms (Muller 2003, Kawasaki-Nishi et al 2003, Stalz et al 2003, Helfenbein et al 2003, Palmgren et al 2003 Buch-Pedersen et al 2003). It is composed of two major domains, a globular F1 catalytic domain and a membrane-bound Fo proton-translocating linked together by central stalk domain. The synthesis of ATP requires an electrochemical proton gradient across the inner mitochondrial membrane, which is driven by the transport of protons back into the matrix through the Fo domain. When a cell is deprived of oxygen, the electrochemical gradient across the inner membrane collapses, and the enzyme switches its catalytic activity from ATP synthesis to ATP hydrolysis (Walker 1994, Fillingame 1999, Cabezon *et al.* 2000 Nishi et al 2003, Guerra et al 2003, Shao et al 2003, Angevine and Fillingame 2003).

Animal mitochondria and plant chloroplasts are seats of such essential biological processes. Many of the known commercial pesticides interfere with the generation of energy through these processes.

It would be worth to emphasize the difference between ATPases and protein Kinases. Both of which use ATP, in the case of ATPases, the ATP is hydrolyzed into ADP, Pi and free energy. On the contrary, protein kinases are catalyze the transfer of the terminal phosphate group of ATP to the hydroxyl group of serine, therionine, or tyrosine residues of substrate proteins (Nestler and Greengard 1984). It plays a fundamental role in protein phosphorylation in almost all types of cells. These phosphorylation and dephosphorylation reactions have been recognized as regulatory mechanisms in many of metabolic reactions (Yanagita *et al.* 1987). Moreover, Kinases play a crucial role in regulation of most, if not all, biological processes in cells, i.e., glycolysis, protein synthesis, photosynthesis and so on. Both classes of enzymes, ATPases and kinases are intimately related to the bioenergetics aspect of cells. In the following we would be focused on the interaction of pesticides with both systems.

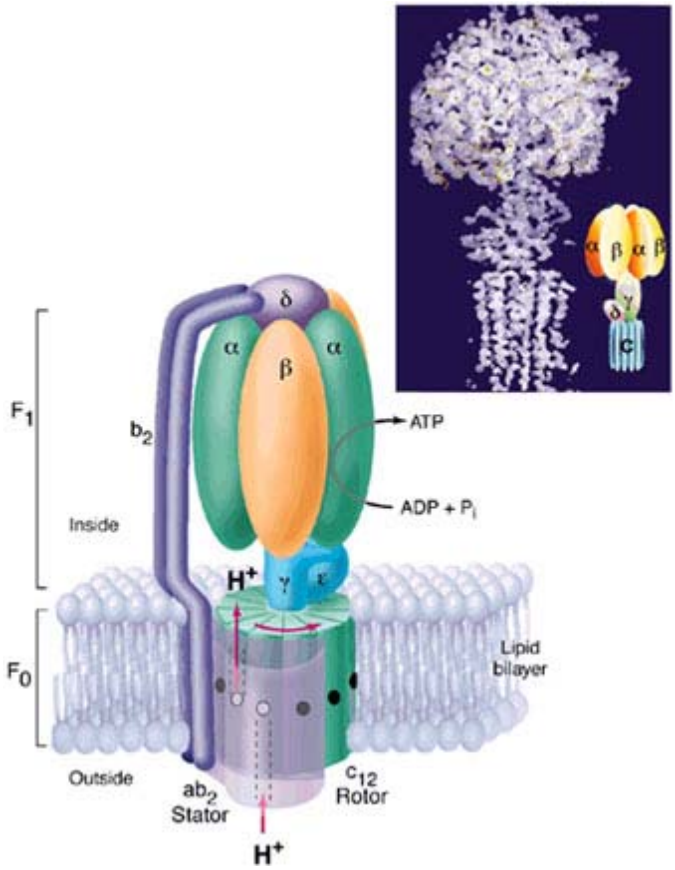

Figure (1) : ATP synthase complex. This structure adapted from Fillingame 1999

Interference of herbicides with photosynthesis:

Plant can make high-energy compounds and carbohydrates by capturing the energy of light through a process so-called photosynthesis which is defined as the process whereby light energy is converted to chemical energy by green plants. Unlike animals and many microorganisms, green plants are able to use photosynthesis to make all their basic components. In photosynthesis, plants combine carbon dioxide, water, and the energy in sunlight to build carbohydrate, which becomes the chief internal source of building materials and the energy to drive metabolic processes.

Herbicides and non-herbicidal inhibitors that affect the photochemically induced reactions of isolated chloroplasts can be divided into the following classes depending on the effects imposed (Moreland 1976).

1. Electron transport inhibitors: Electron transport inhibition results from the removal or inactivation of one or more of the intermediate electron transport carriers by certain herbicides such as the chlorinated phenylureas, phenmedipham, and triazines (Ohki *et al.* 1999, Dayan *et al.* 2000).
2. Uncouplers: Uncouplers are compounds that dissociate electron transport from ATP formation; hence, they inhibit phosphorylation but not electron transport such as perfluidone, chlorpromazine, atebrin, derivatives of carbonyl cyanide phenyl hydrazones, and some phenols.
3. Inhibitory Uncouplers: The action of the inhibitory uncouplers can be explained by considering two sites of action, one associated with photosystem II, where they act much like diuron, and 4,6-dinitro-*o*-cresol (DNOC), bromo-nitrophenols (Trebst and Draber 1995) and a second site of action associated with the phosphorylation pathways, such as dinoseb (Younis and Mohanty 1980), N-phenyl-carbamates, 3,5-dihalogenated 4-hydrobenzo-nitriles, and substituted 2,6-dinitroanilines.
4. Electron Acceptors: Compounds classified as electron acceptors are able to compete with some component of the electron transport pathway and subsequently undergo reduction such as diquat and paraquat which compete with FRS (ferredoxin-reducing substance) to accept electrons as shown in Fig. 2 (Franqueira *et al.* 1999).

The decrease of number of ring nitrogen atoms of 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines on herbicidal activity and inhibition of photosynthetic electron transport (PET) was assayed using thylakoids from *Spinacia oleracea*. Three 2-benzylamino-4-methyl-6-trifluoromethyl-1,3,5-triazines, nine pyrimidines with a benzylamino-, methyl- and trifluoromethyl-group, 2-benzylamino-6-methyl-4-trifluoro methyl pyridine and N-benzyl-3-methyl-5-trifluoromethylaniline were synthesized and assayed. 2-(4-Bromobenzylamino)-4-methyl-6-trifluoromethylpyrimidine exhibited the highest PET inhibitory activity against *Spinacia oleracea* thylakoids of all compounds tested. The 2-benzyl aminopyrimidines and 2-methyl pyrimidines having a 4-halobenzyl amino group exhibited higher

PET inhibition than atrazine and 2-trifluoromethyl pyrimidines against *Spinacia oleracea* thylakoids. These PET inhibitory active compounds also exhibited a strong and similar inhibition both against atrazine-resistant. The herbicidal activity of 4-(4-bromobenzylamino)-2-methyl-6-trifluoromethyl pyrimidine was equivalent to that of known herbicides like simetryne, simazine or atrazine (Ohki *et al.* 2001).

Amino- and urea-substituted thiazoles exhibited *in vivo* herbicidal activity on duckweed (*Lemna paucicostata* Hegelm. strain 6746) cultures and appeared to act via inhibition of photosynthetic electron transport system. A small number of the thiazole derivatives tested were active but only at relatively high concentrations. The most active structures were the amino-substituted thiazoles with isopropyl and n-butyl side chains and the urea-substituted thiazole with p-chlorophenyl side chain. Decreasing the length of the side chain had a negative effect on the PSII inhibitory activity. The urea-substituted series was as a group less active than the amino series, and the free acid series had no biological activity. The most active compounds competed for the same binding site as atrazine on PSII. Computer modeling highlighted the structural similarities between some of the thiazoles and the commercial herbicides diuron and atrazine (Dayan *et al.* 2000).

The effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis by a freshwater green alga, *Scenedesmus quadricauda* Berb 614, were determined. Within the concentration range 0.02-200 mg/l, paraquat was more toxic than glyphosate and 2,4-D to the growth, photosynthesis and chlorophyll-a synthesis. The presence of 0.02, 0.2 or 2 mg/l of 2,4-D was not toxic to the alga. Algal growth, photosynthesis and chlorophyll-a synthesis were stimulated by the presence of low concentrations (0.02 or 0.2 and 0.02 mg/l, respectively) of 2,4-D and glyphosate. The presence of 0.02 or 0.2 mg/l of paraquat, 2 mg/l of glyphosate or 20 mg/l of 2,4-D was significantly inhibitory to the three test parameters, whereas the presence of 2 or more mg/l of paraquat, 20 or more mg/l of glyphosate or 200 mg/l of 2,4-D completely inhibited algal growth, photosynthesis and chlorophyll-a synthesis (Wong 2000). The herbicide paraquat is a redox active compound known to generate superoxide anions in mitochondria and the cytosol of yeast and mammalian cells leading to the formation of several reactive oxygen species (Tien and Knoops 2003).

Novel 2-(benzylamino)-4-methyl-6-(trifluoromethyl) – 1 , 3, 5 - triazines have the same 1,3,5-triazine skeleton as atrazine, although some of them, for example, 2-(3-chlorobenzylamino)-4-methyl-6-(trifluoromethyl)-1,3,5-triazine-[pI(50)(spinach) = 7.21], show a >3 times stronger photosynthetic electron transport inhibitory activity than atrazine [pI(50)(spinach) = 6.72]. The new triazines have only one amino group at the triazine ring, and their molecular shapes are different from atrazine. The replacement of the bound [(14)C]atrazine by 1,3,5-triazines was tested to determine whether the novel 1,3,5-triazine analogues exhibit the same binding pattern at the D1-protein as atrazine. It was found that [(14)C]atrazine bound to the D1-protein was replaced by the triazine tested by a clearly competitive interaction. Obviously, the novel 1,3,5-triazines are attached to the same binding niche as atrazine (Ohki *et al.* 1999). In a reaction of central importance to the energetics of photosynthetic bacteria, light-induced electron transfer in the reaction centre (RC) is coupled with the uptake of protons from the cytoplasm at the binding site of the secondary quinone (QB). It has been established by X-ray crystallography that the triazine herbicide terbutryn binds to the QB site (Lancaster and Michel 1999).

The effect of paraquat herbicide on freshwater microalga *Chlamydomonas eugametos* was studied in function of different parameters such as growth, elemental composition, total lipids, and photosynthetic pigments content and others assayed by flow cytometry (cell viability, cell volume, and granularity). The study reveals that paraquat concentrations above 0.15 μM are toxic for the microalga *C. eugametos*, inducing an inhibition of all the physiological parameters analyzed and strong structural changes (Franqueira *et al.* 1999). However, lower concentrations cause alterations in certain cellular components that are especially sensitive to the toxic action of the herbicide; so total lipids and photosynthetic pigments content are affected by concentrations such low as 0.037 μM . Taking into account these results, these parameters are better indicators of the cellular state than data on biomass or growth rate (Ye et al 2003).

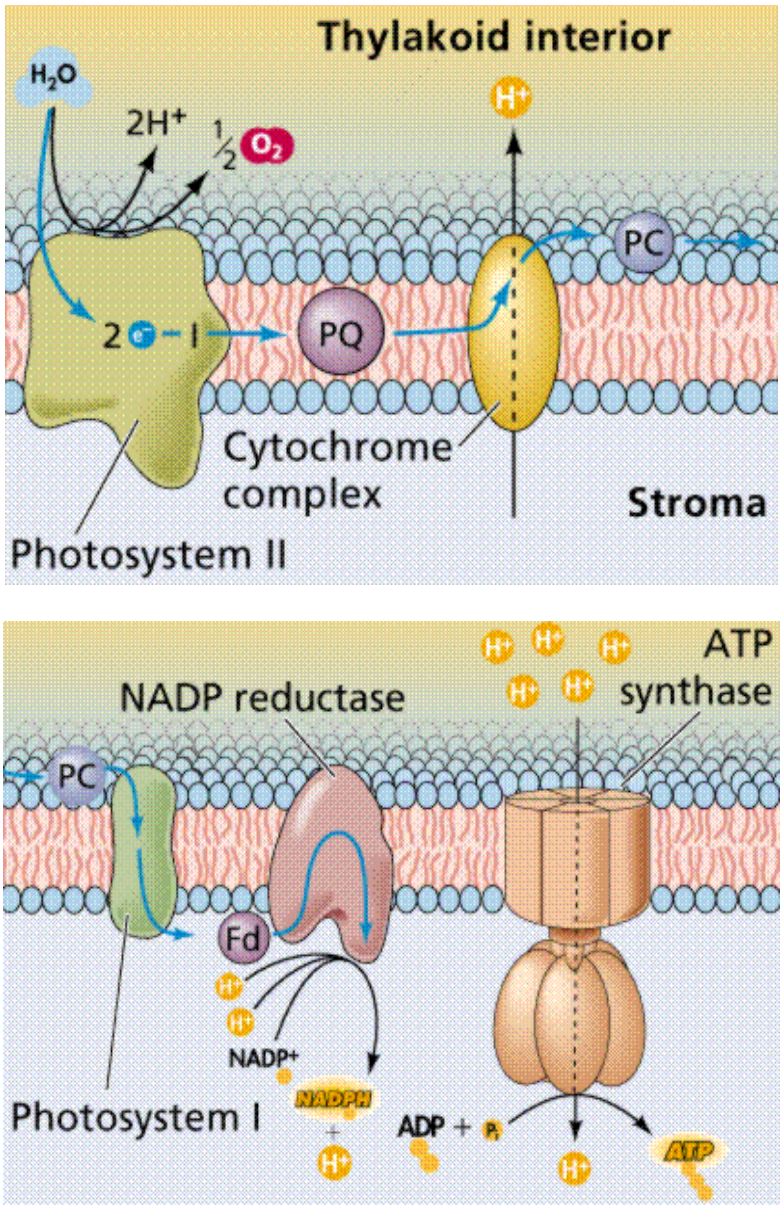

Fig (2): Interaction of Electron acceptor herbicide compounds to compete with some component of the electron transport pathway and subsequently undergo reduction such as diquat and paraquat which compete with ferredoxin-reducing substance (Fd) to accept electrons as shown in Fig. 2 (Franqueira *et al.* 1999).

Respiratory chain and pesticide interactions:

Respiration is composed of a number of different steps, many of which are affected by insecticides. Organic foodstuffs contain mixtures of carbohydrates, fats and proteins and these are broken down to sugars, acetate (as acetyl COA) and aminoacids, respectively. Sugars are then converted by glycolysis to pyruvic acid, an intermediate which is also accessible from fats (via glycerol) and aminoacids. The reactions of glycolysis take place in the cytoplasm of cells and pyruvate is subsequently transported into the mitochondrion where it is converted by the pyruvate dehydrogenase multi-enzyme complex to acetyl COA. The acetyl COA formed is then broken down further in the tricarboxylic acid (TCA) cycle . The pyruvate dehydrogenase complex and the analogous α-oxoglutarate dehydrogenase complex which occurs in the TCA cycle and use a number of cofactors. One of these is lipoic acid which is reduced to the corresponding dithiol during the reaction sequence, and it is likely that arsenic-containing compounds produce an arsenic species which inhibits this enzyme activity by combining with this dithiol (and probably other cellular dithiols as well) (Webb 1966). It has been proposed that the actual toxicant is arsenous acid, $HAs(OH)_2$, and that this is formed by direct reduction of arsenites and by two step reduction of arsenates (Knowles and Benson 1983).

An alternative possibility for the arsenate ion is that it mimics a phosphate ion and becomes incorporated into key high-energy containing intermediates which rapidly broken down again (Slater 1963). The net result is the futile recycling of intermediates instead of a supply of the required phosphorylated products.

During the flow of electrons down this chain, energy is trapped and coupled to the synthesis of ATP from ADP and inorganic phosphate, in a process called oxidative phosphorylation. The mechanism of the coupling of ATP synthesis to electron transport is a subject of continuing research and debate but most biochemists favour the “chemiosmotic”theory (Mitchell 1961) which states that, during the passage of electrons down the chain of respiratory carriers, protons are transported across the inner mitochondrial membrane from inside to outside. The resulting proton electrochemical gradient drives ATP synthesis via an ATPase enzyme which spans the membrane. (The enzyme is called an ATPase because, in the reverse direction, it can hydrolyse ATP.).

The respiratory electron-transport chain in the mitochondrial membrane has been resolved into five complexes. The insecticide rotenone interferes with the activity of complex I, it inhibits electron-transport catalyzed by NADH-CoQ Reductase, but it also has effects elsewhere on the carrier chain and on cell division. Furthermore, Nicotine bound to complex I of the respiratory chain and inhibited the NADH-Ubiquinone reductase activity (Cormier *et al.* 2001). Furthermore, The death of dopaminergic neurons induced by systemic administration of mitochondrial respiratory chain complex I inhibitors such as 1--methyl-4-phenylpyridinium (MPP(+); given as the prodrug 1--methyl-1,2,3,6-tetrahydropyridine) or the pesticide rotenone have raised the question as to whether this family of compounds are the cause of some forms of Parkinsonism. The neurotoxic potential of another complex I inhibitor, annonacin, the major acetogenin of *Annona muricata* (soursop), atropical plant suspected to be the cause of an atypical form of Parkinson disease in the French West Indies (Guadeloupe). When added to mesencephalic cultures for 24 h, annonacin was much more potent than MPP(+) and as effective as rotenone) in killing dopaminergic neurons (Lannuze et al 2003). *in vitro* study identified endosulfan as a chemical inducing a loss of secretory responses in teleost adrenocortical steroidogenic cells and alterations in the activity of enzymes known to be involved in oxidative stress pathways. Moreover, the significant increase in lipid hydroperoxides levels provided further evidence for endosulfan-induced oxidative stress (Dorval et al 2003).

Cyanide, which is used as a fumigant, carbon monoxide and sodium azide, also inhibit electron-transport, but at latterly step (complex IV), that is catalyzed by cytochrome oxidase. Paraquat depresses respiratory activity through partial inhibition of mitochondrial complexes III and IV (Palmeira *et al.* 2001). Dinoseb and 2,4-D strongly inhinited succinate dehydrogenase and cytochrome c reductase, complex III (Palmeira *et al.* 1994). Compounds of the first class, including for example dinitro-*o*-cresol (DNOC) and other dinitrophenol derivatives, increase the cellular respiration by decreasing mitochondrial membrane potential, then uncouple ATP synthesis from electron transport i.e. electron flow occurs, but ATP is not produced (Sibille *et al.* 1998). Uncouplers are typically lipid soluble acids and, on the basis of the "chemiosmotic" theory , it is supposed that they act by destroying the proton gradient referred to above (Mitchell and Moyle 1967). Thus, the protonated acid enters the mitochondrion and dissociates. Since the resulting

anion can effectively delocalize its charge, it is deemed able to recross the membrane, pick up another proton, carry it in, and so on. One interpretation of this scenario is that the uncoupler needs no specific binding site, but this has been called into question lately and a specific site has been proposed (Hatefi 1980). The second class of compounds that affect oxidative phosphorylation includes acaricides such as trialkyltin compounds (Bragadin *et al.* 2000, Desaiah *et al.* 1973), and diaryl sulphides and related compounds (Desaiah *et al.* 1972). It is thought that they inhibit the ATPase directly although the molecular details of how this occurs are not known, however, most likely this effect is to inhibit both electron transport and ATP synthesis. Uncoupling--reductions in ATP synthesis accompanied by increased respiration--was found to be induced by 1 mM of the classic uncoupler 2,4-dinitrophenol (2,4-DNP) at pH 7.0 and 8.0. At pH 4,5 and 6.0, the ATP synthesis and respiration were strongly inhibited by both 2,4--DNP and the chlorophenoxy herbicides tested (Loffhagen et al 2003). Strobilurin fungicides have a broad spectrum activity against all major foliar pathogens of wheat. In addition to this extraordinary fungicidal activity side-effects have been reported which result in higher yields of cereals, e.g. the reduction of respiration, delayed leaf senescence, activation of nitrogen metabolism as well as increased tolerance against abiotic stress factors (Beck et al 2002).

Interaction of chlorinated hydrocarbon pesticides with the energy dependent ion pumps:

Matsumura and Patil (1969) found that DDT selectively inhibited the activity of a Na^+,K^+, Mg^{2+}-ATPase found in the nerve ending fraction of the rat brain. Moreover, All DDT-related compounds were found to produce greatest inhibition on ATPases of both honey bee nerve and muscle tissues. (Koch *et al.* 1969).

In 1978, Younis *et al.* accomplished solubilization and purification of mitochandrial F1-ATPase from cockroach coxal muscles. They purified an active soluble Mg^{2+}- dependent ATPase from a particulate mitochondrial ATPase . Their work revealed that although the particulate enzyme was highly sensitive to DDT, 98% inhibition by 2μM concentration, the soluble enzyme activity was not affected by this concentration of DDT. This result initiated a very interesting point regarding the mode of action of DDT. It is obvious , that the factor(s) responsible for DDT action is in the membrane

sector of the ATPase and its action is not on the soluble enzyme. Recently, they isolated a new target protein for DDT from insect mitochondria (Younis *et al.* 2002).

The organochloride insecticide DDT (2,2-bis(p-chlorophenyl)-1,1-trichloroethane) depresses the phosphorylation efficiency of mitochondria.The inhibitory action of DDT on phosphorylation efficiency may result from: (1) a direct effect on the ubiquinol-cytochrome c segment of the redox chain; (2) direct action on the ATP-synthetase complex; (3) partial inhibition of the phosphate transporter. DDT preferentially interacts with phosphorylation process in relation to respiration. High concentrations of DDT induce destruction of the structural integrity of mitochondria (Moreno and Madeira 1991).

The effects of organochlorine pesticides: o,p'-DDT, p,p"-DDT, methoxychlor, and lindane on ATPase activities of microsomal fractions of bovine oviductal and endometrial cells were investigated (Tiemann and Kuchenmeister 1999). After 10 min preincubation with the four organochlorines, a significant inhibition was found only with o,p"-DDT at 32 μM (27.9%) and 64 μM (35.6%) in the oviductal microsomal fraction and at 64 μM (32.2%) in that of the endometrium. Increasing the preincubation time to 30 min, the Mg2+ ATPase in the endometrial fraction was significantly inhibited by all four pesticides at 64 μM , but in the oviductal fraction only at 64 μM o,p"-DDT. It is suggested that organochlorine pesticides can have an influence on cells responsible for reproduction. The effects of DDE (2,2-bis(p-chlorophenyl)-1,1-dichloroethylene), the major metabolite of DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane), on rat liver mitochondrial bioenergetic activities were examined. DDE up to 40 nmol/mg protein affected the proton motive force generating system. In fact, DDE interacted with succinate dehydrogenase (complex II), decreasing respiration and membrane potential. In this concentration range, the permeability of the inner membrane to protons remained intact. Only higher concentrations (≥ 80 nmol/mg) increased permeability to protons, uncoupling oxidation from phosphorylation. The phosphorylative system was not affected because the rate of ATP synthesis was unchanged. In addition, data from carbonyl cyanide m-chlorophenylhydrazone-uncoupled rotenone-inhibited preparations or submitochondrial particles indicated that FoF1 ATPase activity is not affected by DDE. Therefore, DDE inhibition of complex II and putative

inhibition of succinate translocation explain the depression of mitochondrial respiration. The use of appropriate substrates and assay conditions indicates that complexes I, III and IV were not affected by DDE. The uncoupling of oxidative phosphorylation at high concentrations (> 80 nmol DDE/mg protein) was probably related to deleterious effects on the integrity of the mitochondrial membrane. (Ferreira *et al.* 1997).

The organochlorine pesticide DDT is a liver tumor promoter and a potent inhibitor of intercellular communication (Hansen and Matsumura,2001a and Shinomiya and Shinomiya, 2003). Present knowledge of the mechanism by which DDT inhibits intercellular communication is limited but it has been suggested that increased intracellular free calcium induced by DDT could be of importance (Hansen and Matsumura,2001b). As the effects of calcium are closely associated with the multifunctional protein calmodulin (CaM) in most cells the potential binding of DDT to CaM and subsequent effects on CaM-stimulated Ca2+/Mg2+-ATPase activity were studied (Warngard *et al.* 1988). DDT inhibited CaM-stimulated Ca2+/Mg2+-ATPase activity and bound to CaM in a manner similar to established CaM-inhibitors. Subsequently an in vitro assay for measuring inhibition of metabolic cooperation between 6-thioguanine (TG)-sensitive and TG-resistant Chinese hamster (V79) cells was used to investigate the possible involvement of CaM in the regulation of intercellular communication. Calmidazolium (CzM), a potent CaM inhibitor, was tested alone or in combination with the tumor promoters 12-O-tetradecanoyl phorbol-13-acetate (TPA) or DDT known inhibitors of intercellular communication. The results showed that CzM alone was without effect with regard to inhibition of metabolic cooperation but potentiated the response induced by TPA, an effect not noticed with DDT. These results suggest different mechanisms of action of TPA and DDT on metabolic cooperation and support the hypothesis that with calcium CaM may be of importance for drug-induced inhibition of intercellular communication and tumor promotion (Warngard *et al.* 1988).

Investigations to determine the inhibitory activity on the Ca(2+)-transport-ATPase of human erythrocyte membranes were performed with various organochlorin compounds. Some of the compounds investigated display an inhibitory effect on the Ca(2+)-transport-ATPase at very low concentrations. The *in vitro* results obtained in this enzyme assay can be correlated directly with the results of other *in vitro* assays and with the

results of *in vivo* investigations in different species in which an inhibitory effect on various biological functions is observed. Therefore, an inhibitory effect on the Ca(2+)-transport-ATPase indicates a toxic effect of these compounds to cell functions. (Janik and Wolf 1992).

The effects of a short-term *in vivo* administration of two liver tumor promoters (phenobarbital and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane on rat liver endoplasmic reticulum Ca(2+)-ATPase were investigated. The specific activity values of this membrane-bound enzyme significantly decreased by 51% for phenobarbital-treated rats and by 48% for 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane-treated rats compared with control animals. The depression of liver endoplasmic reticulum Ca(2+)-ATPase appears to be a manifestation of the toxicological effect of tumor promoters (Adenuga *et al.* 1992).

In vitro inhibition of Ca2+-ATPase by DDT homologs was studied using maternal brush-border membranes from human term placentas as an enzyme source. At 10 μM concentration many of the compounds tested inhibited this enzyme. The order of effectiveness of inhibition was as follows: p,p'-DDE greater than p,p'-DDD greater than p,p'-DDT greater than methoxychlor. Both p,p'-DDOH and p,p'-DDA did not inhibit the placental Ca2+-ATPase. Assays using varying concentrations (0.3 μM to 0.1 mM) of p,p'-DDT were also performed. The inhibition of human placental Ca2+-ATPase ranged from 12% for 0.3 μM p,p'-DDT to 69% for 30 μM p,p'-DDT. Higher concentrations of this pesticide failed to cause further enzyme inhibition (Treinen and Kulkarni 1986).

Inhibitors interfering with protein kinases:

Protein kinases are phosphoryl transferases. They transfer the terminal phosphate group of ATP to hydroxyl group of serine, threonine, and tyrosine residue of acceptor substrate protein . The study of protein kinases has resulted in their classification into two general groups based upon amino acid which is phosphorylated from their activity, serine/ threonine kinases and tyrosine kinases (Nestler and Greengard 1984) . The tyrosine kinases are generally composed of kinases which act as receptors for growth factors. The serine/threonine protein kinases composed of the Ca^{+2}-/ phospholipid-dependent protein kinase, Ca^{+2}-calmodulin -dependent protein kinase, cAMP-dependent protein kinase, polypeptide-dependent protein kinase and protein kinase II. Protein kinases have been described in many tissue types including mammalian, yeast, insects and plants. Protein phosphorylation and

dephosphorylation have been widely recognized as regulatory mechanisms of metabolism , membrane function , and structural as well as contractile protein (Abdel-Ghany *et al.* 1989). Some protein kinases are known to play a role in the regulation of normal cell metabolism such glycogen synthesis, while a number of protein kinase activities were shown to be associated with growth factor receptors (Abdel-Ghany et al 1993 and Abo-El-Saad 1991) . It has been found that certain pesticides interact with several known protein kinases as anew target. In this regard, the effects of several derivatives of phenolic compounds have been studied on different protein kinases (Abo-El-Saad 1991). Some of these compounds were among the early known generation of insecticides, over 100 years ago, such as dinitro-*o*-cresol (DNOC) and herbicides such as dinoseb. Non-aromatic insecticides commonly used to combat insect pests on stored products i.e. aluminum phosphide (phostoxin) and sodium flualuminate (cryolite) which generates Al^{3+} as metabolite. Recently, it has been found that Al^{3+} inhibits human brain tau (micotubule associated protein) protein phosphorylation at low concentrations, whereas, at concentrations higher than 100 μM caused aggregation of tau protein preventing its entry in SDS- gel electrophoresis (El-Sebae *et al.* 1993, Abdel-Ghany *et al.* 1993). Moreover, Pyrethroids such as deltamethrin was found to inhibit protein phosphorylation from rat and housefly brain and increased the level of depolarization-induced protein phosphorylation (Enan and Matsumura 1993a, b, Leng and Xiao 1995).

It has been investigated the effects of a type II pyrethroid insecticide, deltamethrin, on changes in the protein phosphorylation pattern associated with neurotransmitter release in rat brain synaptosomal preparations. Deltamethrin was found to stimulate directly the activity of the protein kinase C/phosphoinositide pathway at very low concentrations. This action resulted in an increase in the intracellular concentration of inositol 1,4,5-triphosphate (IP3) and free calcium, as well as an increase in overall and specific protein phosphorylation within the synapse. Particularly noticeable was the deltamethrin-induced increase in phosphorylation on two very acidic proteins (87 and 48 kDa proteins) and one basic 38 kDa protein (Enan and Matsumura 1993a). These results are consistent with those of a previously reported study in which deltamethrin caused an increase in neurotransmitter release which was accompanied by increased intrasynaptosomal free Ca2+ levels and protein phosphorylation activities. Together all these observations support the view that calcium-sensitive

proteins involving synaptic transmission are the major action targets of type II pyrethroids (Enan and Matsumura 1993b) .Deltamethrin causes a significant change in protein phosphorylation activities which follow depolarization. The most significant change caused by deltamethrin was the prolonged elevation of the level of phosphorylation on a number of key synaptic proteins beyond the normal time of their recovery to the dephosphorylated state. The best marker proteins reacting to deltamethrin in this manner were calcium-calmodulin dependent protein kinase (Matsumura *et al.* 1989).

It has been proposed that both DDT and pyrethroids inhibit the affinity of calcium to phosphorylated proteins (Matsumura 1986). Next, he found that the DDT-sensitive Ca^{2+}-ATPase is not actually an ATPase, but is a protein kinase-phosphatase system in phosphorylating and dephosphorylating the Na^{+}/Ca^{2+} exchange protein. It was shown subsequently that Ca^{2+}-stimulated ATP hydrolysis via a membrane phosphorylation-dephosphorylation process have been found to be highly sensitive to the action of by pyrethroid insecticides (Clark 1986).

A number of microbial inhibitors for various protein kinases have been identified, including sturosporine, UCN-01, calphostin, and genistein. Sturosporine is the most potent inhibitor of protein kinases *in vitro* with an IC_{50} value of 2.7 nM for protein kinase C, 8.2 nM for PKA, 6.4 nM for tyrosine kinase of v-src and 630 nM for tyrosine kinase of epidermal growth factor receptor (Tamaoki 1993).

Quercetin, a bioflavonoid which occurs in free or conjugated form in fruits and vegetables and found to have an effect on insect behavior (Abo-El-Saad and Abo-Seda 1993) and inhibits the activity of tumor promoters in carcinogenicity (Fujiki and Sugimura 1983). It was found to have an inhibitory effect on the G-type casein kinase and other protein kinases (Cochet *et al.* 1982, Abo-El-Saad 1991).

The biochemical and physiological interactions of gossypol, a terpenoid compound found in cotton plants, on lepidopterous insects have been studied by several investigators (Sherby 1979, El-Sebae *et al.* 1980, 1981). In addition, Abo-El-Saad (1998) was found that gossypol dramatically inhibits tyrosine kinase activity. In a subsequent work, calcium-dependent protein kinase from rice seeds has found to be induced by gibberellin (Abo-El-Saad and Wu 1995) which is known as terpenoid compound and

commonly utilized as plant growth regulator in some fruit yards such as vineyards.

It has been found that dichlorvos enhanced the phosphorylation of 55- and 280-kDa proteins (Choudhary *et al.* 2001). These two proteins were identified as tubulin and microtubule associated protein-2 (MAP-2) by immunoblotting. This study showed that dichlorvos induced hyperphosphorylation of tubulin and MAP-2 which in turn destabilizes microtubule assembly, and may ultimately result in axonal degeneration leading to dichlorvos induced delayed neurotoxicity

In the human prostate cancer cell lines LNCaP and PC-3, erbB-2 kinase was activated by pesticides of different chemical classes: (1) the organochlorine insecticides beta-hexa-chlorocyclohexane (beta-HCH), o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT), and heptachlor epoxide; (2) the pyrethroid insecticide trans-permethrin, and (3) the fungicide chlorothalonil. o,p'-DDT also causes phosphorylation of mitogen-activated protein kinase (MAPK) (Tessier *et al.* 2001).

The effects of *in vivo* administration of the cyclodiene tumor promoter heptachlor epoxide on mouse liver protein kinase C were studied in male B6C3F1 mice by protein kinase C activity assays and Western blotting under conditions known to increase the incidence of hepatocellular carcinoma because protein kinase C is thought to be critical in phorbol ester-induced tumor promotion (Zou and Matsumura 2003). Under these test conditions, 20 ppm dietary heptachlor epoxide for 1-20 days increased cytosolic and decreased particulate total protein kinase C activities, while 10 ppm had no effect. Further, total cytosolic and particulate protein kinase C activities were decreased within 1 hour by 10 mg/kg intraperitoneal (i.p.) heptachlor epoxide. Western blotting showed that conventional protein kinase C alpha and beta isoforms were unaffected by heptachlor epoxide. Particulate novel protein kinase C epsilon, however, was selectively down-regulated by 1, 10, and 20 ppm dietary heptachlor epoxide, whereas the cytosolic isoform was decreased by 1 and 10 ppm heptachlor epoxide for 10 days. The high-dose treatment for 24 hours also decreased particulate novel protein kinase C epsilon but increased the cytosolic titer (Hansen and Matsumura 2001a). Furthermore, the tyrosine kinase growth factor receptor was found to be probable critical pathway for heptachlor epoxide-induce tumor promotion (Hansen and Matsumura 2001b). Tyrosine kinase assays

showing that carbaryl, 1-naphthol and 2-naphthol were equally efficient at inhibiting tyrosine kinase activity as tyrphostin (specific tyrosine kinase inhibitor) (Renglin *et al.* 1999).

The organochlorine pesticide heptachlor constitutes a potential health hazard because of its persistence in nature, its reported contamination in food and milk, and its possible carcinogenic effects. As a tumor promoter, heptachlor induces human myeloblastic leukemia cells to differentiate, and also down-regulates the tumor suppressor gene p53 in human immune cells. In this study, the heptachlor signaling pathway in human lymphocytes was studied. Addition of heptachlor to human CEM x174 lymphocytic cells reduced the cellular levels of MAP kinase (MAPK, mitogen-activated protein kinase) cascade proteins, including ERK1 (a 44-kDa MAPK), ERK2 (a 42-kDa MAPK), a 85-kDa and a 54-kDa MAP kinase, MEK1 (a 45-kDa ERK kinase) and MEKK (a 78-kDa MEK kinase). However, heptachlor treatment caused a marked increase in the expression of the activated (Thr- and Tyr-dually phosphorylated) ERK1 and ERK2 in the cells. These studies indicate that mitogen-activated protein kinases are important intermediates in the signal transduction pathway of immune cells upon heptachlor exposure, and the observation of stimulation of activated MAP kinases without a simultaneous accumulation of basal enzymes may suggest the involvement of a negative feedback control mechanism in the pathway (Chuang and Chuang 1998).

It has been suggested that there is a positive correlation between increased incidence of breast cancer and the presence of organochlorine residues such as DDT and HCH in breast tissues in the United States. To study possible biochemical links between these two parameters, it has been examined the effect of o,p'-DDT, the most estrogenic congener of the DDT family of chemicals and beta-HCH on protein phosphorylation activities in MCF-7, a line derived from human breast cancer cells. Both of these organochlorine chemicals were found to be potent activators of protein kinases. Among kinases activated, protein tyrosine kinases (PTK) appear to be most affected as judged by the antagonistic action of genistein, a class-specific PTK inhibitor. Moreover, these organochlorines were found to activate PTK even under cell-free conditions, indicating that they are likely to interact directly with the target protein tyrosine kinase (Enan and Matsumura 1998).

The prenylated isoflavone warangalone from the insecticidal plant *Derris scandens* is a selective and potent inhibitor of rat liver cyclic AMP-dependent protein kinase catalytic subunit (cAK) (IC50 3.5 microM). The inhibition of rat liver cAK by warangalone is non-competitive with respect to both ATP and the synthetic peptide substrate (LRRASLG) employed in this study. Warangalone is a poor inhibitor of avian calmodulin-dependent myosin light chain kinase (MLCK), rat brain Ca(2+)- and phospholipid-dependent protein kinase C (PKC) and wheat embryo Ca(2+)-dependent protein kinase (CDPK) (Wang *et al.* 1997).

Epidermal growth factor (EGF) and its receptor (EGF-R) have been implicated as mediators for estrogen induced cellular growth. The action of the estrogenic pesticide methoxychlor (MXC) parallels the action of 17 beta-estradiol (E2) on uterine EGF-R has been examined. The results demonstrate that both E2 and MXC can stimulate the number of EGF-R binding sites without significantly altering the receptor binding affinity (Kd). Further, this stimulation is time dependent and is affected by dose (Metcalf et al 1996).

Polychlorinated hydrocarbons known to be nongenotoxic carcinogens were screened as activators of protein kinase C (PKC)-beta 1 either at high concentrations of Ca2+ or in the absence of Ca2+ (i.e., with 1 mM ethylene glycol-bis(beta-aminoethyl ether) N,N,N‹'N',-tetraacetic acid). Of those compounds tested, kepone and dicofol significantly stimulated PKC activity in the absence, but not the presence, of Ca2+. PKC activation was most pronounced in the presence of phosphatidylserine. Kepone and dicofol stimulated PKC activity 26% and 13%, respectively, as compared with the PKC activity (100%) stimulated by the tumor-promoting phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). (Rotenberg and Weinstein 1991).

The effects of pyrethroids and DDT on the alpha-subunit protein of the rat brain sodium channel were studied by using both native and exogenously added cAMP-dependent protein kinases. It was concluded that the alpha-subunit of the voltage-sensitive sodium channel protein is the only phosphorylatable protein present at the 260 kD molecular weight range on the sodium dodecyl sulfate-polyacrylamide gel electrophoretogram. Phosphorylation of the alpha-subunit was induced by depolarization, and this process was inhibited by 10(-6) to 10(-10) M 1R-deltamethrin, but not by 1S-deltamethrin, the latter being an inactive enantiomer of the former.

DDT produced a similar effect, but only at a higher concentration ranges (Ishikawa *et al.* 1989).

Various chlorinated hydrocarbons, many of which are known hepatic tumor promoters, have been evaluated for their ability to stimulate protein kinase C (PKC) activity *in vitro*. Chlordane, kepone, toxaphene, heptachlor, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane, the polychlorinated biphenyl Aroclor 1254, aldrin, 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT) and gamma-hexachlorocyclohexane (lindane) were the most potent stimulators of PKC activity. Of these compounds, chlordane was the most potent organochlorine pesticide (Moser and Smart 1989). Moreover, Administration of rats by DDT and endosulfan signifigantly increased the activity of choline kinase (Narayan *et al.* 1989). The results clearly indicate that pesticides as well as cadmium chloride and sodium dichromate can modulate a vital component of the cell signaling pathway, namely PKC activity. PKC may be a target of free radicals and oxidative stress, leading to altered cell proliferation and differentiation (Bagchi *et al.* 1997).

Conclusion:

The goal of the present review is to collect information concerning ATPases and protein kinases which play a centeral role in many biological processes in cell. Interaction of pesticides with these enzyme resulted in many problems and health defect. Thus, certain compounds such as organochlorine known to frequently accumulate in human adipose and breast tissues. An epidemiological study has indicated that exposure to beta-HCH could be one of the significant environmental risk factors for the development of human breast cancers. Additionally, beta-HCH has recently been identified as an environmental estrogen capable of activating estrogen receptor (ER) through a ligand-independent pathway. The similarity between lindane and inositol (1, 4, 5) phosphate (IP3) suggested that lindane releases Ca2+ from IP3-sensitive intracellular stores in macrophages and myometrial cells. On the other hand, in cellular bioenergetic processes, some pesticides such as lindane altered energetic metabolism of hepatic mitochondria and the inositol-phosphate synthesis in neuronal cells. These alterations may represent a potential risk for human health.

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تداخل المبيدات مع نظام الطاقة الحيوية والأنزيمات الناقلة في الخلية

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الأحساء – المملكة العربية السعودية

الملخص:

إن مصدر الطاقة المطلق عل كوكب الأرض لكل أنواع الحياة المعروفة لدينا هو الشمس ، فمن ضوء الشمس جعل الله سبحانه وتعالى سر الحياة حيث تُحَول هذه الأشعة الضوئية بواسطة الكائنات الذاتية التغذية مثل النبات في وجود الماء و ثاني أكسيد الكربون و العناصر الغذائية اللازمة للنبات لكي يتكون البروتينات والكربوهيدرات و الدهون و الأحماض النووية والفيتامينات و مركبات أخرى وهذه كلها نواتج تفاعل في غاية الأهمية والذي لم يقلد حتى الآن على سطح الكرة الأرضية و هو تفاعل التمثيل الضوئي ولو استطاعت البشرية بكل التكنولوجيا الحديثة أن تقلد هذا التفاعل لأذابت كل مشاكل البشرية. ناتج هذا التفاعل كما هو معروف يستخدم كمصدر طاقة للكائنات الغير ذاتية التغذية مثل الإنسان والحيوان للحصول على الطاقة اللازمة للنمو و التطور و الإنتاج والحصول على بعض المواد التي لا يستطيع الإنسان أن يخلقها مثل الفيتامينات.و على مستوى الخلية فان مصدر الطاقة لها يكمن في أكسدة الهيدروجين الناتج من غذاء الحيوان أو الإنسان بواسطة أكسيجين التنفس و يتكون الماء ، وأن أفضل مصدر لهذا الهيدروجين هو الناتج عن دورة كربس والتي سميت باسم مكتشفها و هو هانس أدولف كربس أو ما يطلق عليها دورة حامض الستريك في الخلية الحيوانية نجد أن هذه الدورة تحدث كاملة في إحدى مكونات الخلية الهامة جدا و التي يُطلق عليها محطة توليد الطاقة و هى الميتوكوندريا و لقد تم التعرف على نظم الأغشية البلازمية الإنزيمية للميتوكوندريا و تداخل عديد من المركبات الكيماوية سواء كانت مبيدات أو مركبات أخرى تعمل كمثبطات لأهم هذه النظم و هو أنزيم الأدينوسين ثلاثي الفوسفات والذي يعتبر من أهم النظم الحيوية على الإطلاق لما له من دور جوهرى في تكوين جزيئات الـATP الغنية بالطاقة اللازمة للعمليات الحيوية الأخرى.والمعقد الأنزيمي الخاص بتكوين جزيئات الـATP

يسمى F_1F_o-ATP synthase وهو الذي يلعب دور جوهري في تكوين الطاقة داخل الخلايا الحية ويتكون من جزئين الأول وهو كروي ويسمى F1 والثاني مرتبط بالغشاء البلازمي للخلية ويسمى بالـ Fo والاثنين معاً يرتبطان بوحدة مركزية تسمى الـ Stalk. وتخليق جزيئات الـATP تتطلب تدرج الكتروكيماوي للبروتونات خلال الغشاء البلازمي الداخلي للميتوكوندريا داخل المكون Fo وعندما ينقص الأوكسجين في الخلية فإنه يحدث وقف لتدفق البروتونات وعليه يحدث تغيير وظيفة الأنزيم من مكون لـATP الى محلل لها.

لعل من المنطق في هذه المقالة أن ننوه الى الفرق بين أنزيم الادينوسين ثلاثي الفوسفاتATPase والإنزيمات الناقلة Kinases . أن اهم أوجه الشبه بينهما أن كلاهما يستخدم مركب الادينوسين ثلاثي فوسفات ATP و لكن فى حالة الأول فانه يحلل هذه المادة و يتكون مركب ثنائي الفوسفات و الفوسفات الغير عضوى وتنطلق الطاقة الحرة أما الثاني فانه يحلل هذه المادة و ينقل مجموعة الفوسفات الحرة لمادة تفاعل متخصصة و لهذا فإن المادة تتحول إلي الصورة المفسفرة لكي تتم العمليات الحيوية مثل الهدم و البناء و التخليق الحيوي و غيرها من العمليات الفسيولوجية الأخرى مثل الشعور بالحب و الكراهية و الجوع و العطش و غيرها و لذا يعتبر هذا الإنزيم بمثابة مفتاح بيولوجي للعمليات الحيوية المختلفة و من هذا يتضح أهمية ارتباط كلا النظامين حيويا و سوف يتضح من هذه المقالة تداخل المبيدات مع نظم الطاقة الحيوية مثل التخليق الضوئي والمضخات الأيونية المعتمدة على الطاقة و الأكسدة الفوسفورية وكذلك مع الأنزيمات الناقلة.