A. **Organophosphate Pesticide-Malathion**:

Organic compounds of phosphorous are the essential constituents of protoplasm and play important roles for maintenance of life, for example nucleic acids, nucleotides, coenzymes, metabolic intermediates and phosphatides. On the other hand, many organophosphorous compounds (opc) are artificially produced for the practical uses as lubricants, oil additives, plasticizers and pesticides. Op pesticides include not only insecticides but also fungicides, herbicides and others. It is surprising to know that such great variety in chemical, physical and biological properties are governed by the selection of groups attached to the phosphorous atom.

The research in the field of organic chemistry of phosphorous was first undertaken by Lassaigne in 1820 to prepare phosphate esters. The chemistry of opc was developed extensively by Michaelis in Germany during the later part of 19th century and the beginning of this century. Schrader discovered the first insecticide organophosphorous ester Schradan (OMPA) in 1941. The opc are neutral ester or amide derivatives of phosphorous acids carrying a phosphoryl (P-O) or thiophosphoryl (P-S) group.

![General formula of an organophosphorous compound.](image)

In due course of great advancement of agricultural practice and scientific knowledge, various op pesticides such as TEPP (Bladan), parathion, chlorthion, fenthion, fenitrothion, malathion were discovered.
Organophosphorous compounds (opc) are poisons with a neuroparalytic and enzymatic action. The basis of their toxicity lies in the capacity of their selective effect on enzymes of nerve tissues - cholinesterase, which leads to excessive accumulation of acetylcholine in the organisms, giving rise to complex poisoning symptoms. Opcs also inhibit other enzyme-esterase, protease, peroxidase and slightly increase the activity of catalase.

Op insecticides vary widely in chemical and biological properties. Many are fast acting, quickly degraded insecticides. Others are stable enough for use as systemic toxicants in plants and animals. Their toxicity to mammals ranges from very high for parathion and disulfoton (rat, oral LD$_{50}<$10 ppm) to very low (>1000 ppm) for ronnel and malathion.

Compared to the organochlorines, op pesticides are much less environmentally persistent, much more biodegradable, less subject to biomagnification and usually unstable in the presence of sunlight. The range of uses of ops is very broad, extending from food and fibre crops to forest insects, medical entomology, the control of internal and external parasites of mammals and a wide range of household insects.

Op insecticides are toxic because they inhibit acetylcholinesterase, which breaks down acetylcholine, a major transmitter of nerve to nerve and nerve to muscle nerve impulses in most animals. If acetylcholinesterase is not present, impulses continue to be transmitted, a situation which rapidly destroys the normal ability of animals to respond to external stimuli.
In mammals poisoned by OP pesticides, death apparently occurs from asphyxiation, a consequence of inhibition of acetylcholinesterase at neuromuscular junctions. The precise cause of death in insects is unknown but almost certainly different. This is because unlike mammals, breathing in insects depends mainly on diffusion processes.

**Malathion:**

Chemical name -

[0,0 dimethyl S (1,2 dicarbethoxy ethyl phosphorodithioate)]

Chemical structure -

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{S} \quad \text{O} \\
\text{P.SCH-C-OCH}_2\text{CH}_3 & \\
\text{CH}_3\text{O} & \quad \text{CH}_2\text{C}-\text{OCH}_2\text{CH}_3 \\
& \quad \text{O}
\end{align*}
\]

*Figure II. 2: Chemical structure of malathion.*

- Chemical formula - $\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$
- Molecular weight : 330.4
- Melting point : 2.85°C
- Boiling point : 156-157°C/0.7 mm Hg
- Vapour pressure : $4 \times 10^{-5}$ mm Hg/30°C
- Specific gravity : 1.23
- Refractive index : 1.4985
**Toxicological Property of Malathion**

- Toxicity assignment of registered pesticides: normal drugs
- LD₅₀ of acute oral toxicity/wt. (kg): rat 2800 mg
- Percutaneous LD₅₀: Rabbit 4100 mg
- Fish toxicity LC₅₀: Carp 9.0 ppm/48h.
- Fish toxicity rank of registered pesticide: B
- Intake quantity permitted/wt ([kg/day](by Pesticides residues in Foods, FAO and WHO)): 0.02 mg
- Quantity for pesticide residue regulated by - Ministry of Welfare:
  - Unpolished rice: 0.1 ppm
  - Fresh vegetables: 0.5 ppm
  - Pesticide residue in stored food (by EPA): 0.5 ppm

Malathion is also known as carbophos, maldison and mercapothion. It has many other trade names such as cythion, karbofos, celthion, dielathion, maltox, el-4049, emmaton and exathion.

**Use of Malathion:**

Malathion is a non-systemic op insecticide widely used for a general purpose of controlling the sucking and chewing insects of fruits and vegetables and for controlling mosquitoes and flies. It is also used to eliminate animal ectoparasites.

**Persistence of Malathion in the Environment:**

Malathion is released into the environment through its production, formulation and widespread use as insecticide for agricultural and household applications. It is applied in ground and aerial
sprays, as aerosols, foggers, baits, paints, pet colours, animal dips, animal dust bags and cattle feed blocks. Malathion does not enter the ground water level.

Hydrolysis of malathion is a major route of degradation in aquatic system and increases with increasing alkalinity and temperature. Microbial activity and interaction with sediments may increase degradation. Malathion is readily converted to malaxon in the environment under the influence of oxygen and sun light. Malaxon is 40 times more acutely toxic than malathion but malaxon also breaks down to non toxic compounds. In soil break down is quicker than in water and is fastest in highly alkaline soil with high moisture content. Half life of malathion in soil is 24 hrs to 6 days, in water - 1.5 days to 21 weeks, in Air 1.5 days.

**Metabolism of Malathion: Degradation through Hydrolytic Cleavage of Side-Chains:**

Malathion appears to degrade mainly through hydrolytic pathways (Seume and O' Brien, 1960). The importance of the hydrolytic degradation at its carboxyesterase in animals has been determined by Cook *et al.* (1958) and they found that malathion mono and dicarboxylic acids are formed as a result of malathion incubation with animal systems. O' Brien (1960) proposed that strong carboxyesterase action in some animal systems can explain malathion's selective toxicity and this view is supported by experimental evidence on malathion resistant insects which invariably show high carboxyesterase activities compared to their susceptible counterparts (Matsumura and Brown 1961; Matsumura and Hogendijk, 1964). Malathion resistance is usually
specific, extending only to other pesticides (Dauterman and Matsumura, 1962). Malathion is hydrolysed by carboxyesterase mainly in mammals by addition of water to yield alcohols and acids and thereby detoxified.

Figure II 3: Metabolic fate of malathion.
Plate II 1: Mechanism of cholinergic neurotransmission showing the toxicity of organophosphates which inhibit acetylcholinesterase (AChE) (Drawn after Jason Weeks).
During hydrolysis of P-O and P-S bonds, various phosphoric, thiophosphoric and dithiophosphoric acid derivatives are formed in absence of GSH or NADPH in vitro. By the action of mixed function oxidase (MFO) malathion is converted to its oxon analogue malaxon which then gets converted into nontoxic substances.

Matsumura and Boush (1966) demonstrated that malathion can be hydrolysed by an enzyme preparation from Trichoderma viride.

**Malathion Poisoning:**

Malathion, introduced by American Cyanamid Company in 1950, is one of the safest of the organophosphates, however human poisonings still occur. Namba et al. (1970) have summarised the general trend of malathion poisoning.

In Japan, there were 63 poisonings, resulting in ten deaths, from occupational handling or accident and 480 poisoning cases during 1957-1961 and 1965-1966 respectively (Anonymous, 1967). In Guyana, there were reports of 46 deaths by accidents during 1959-1964 (Mootoo and Singh, 1966). Man may be more susceptible to malathion poisoning than experimental animals (Matsumura, 1973). There are various reports of death in adults after ingestion of about 5, 25, 35 and 70 gm of malathion (Faraga, 1967).

Poisoning occurs due to dermal absorption. It is not clear whether the estimated oral dose of malathion for man is lower than for experimental animals because of greater human susceptibility or because of the presence of organic solvents that accelerate absorption (particularly xylene) in commercial preparations.
Synthesis of Malathion:

Malathion, has been acknowledged as the first OP insecticide with high selective toxicity. It is synthesized by the addition of dimethyl hydrogen phosphorodithioate to diethyl maleate. This reaction is catalysed by small amount of base. A catalytic amount of hydroquinone is usually added to the mixture in order to prevent polymerization of maleate.

\[
\begin{align*}
\text{Dimethyl} & \quad \text{Diethyl} \\
\text{hydrogen} & \quad \text{maleate} \\
\text{phosphorodithioate} & \quad \text{Malathion}
\end{align*}
\]

Figure II. 4: Synthesis of malathion

Synergistic Effect of OP Pesticide:

Many other insecticides act synergistically to increase the toxicity of malathion when used in a mixture. Frawley et al. (1957) found that EPN (an OP pesticide) potentiated markedly the mammalian toxicity of malathion. DuBois (1961) reported that 4 out of 50 tested combinations of OP insecticides were synergistic towards female rats. The synergistic pairs were malathion - EPN, malathion - coumaphos, malathion-trichlorfon and azinphos-methyl trichlorfon. The first pair strongly inhibited the enzymatic detoxification of malathion by carboxyester hydrolysis in vivo. EPN may be converted in vivo into its oxon which is a good inhibitor of carboxyesterase rather than...
cholinesterase and the inhibition of this enzyme causes the increase of the malathion toxicity. Infact, any compound which selectively inhibits carboxyesterase, might potentiate malathion. Murphy et al. (1959) tested tri-orthotolyl phosphate (TOTP) which was not used as an insecticide but as a plasticizer, found synergistic activity of TOTP in potentiating malathion toxicity.

Diazinon and other op pesticides as well as carbamate pesticides can increase malathion's toxicity.

Various op pesticides have been observed to exert various histopathological and biochemical changes in fish.

Rashatwar and Ilyas (1984) observed histopathological and biochemical changes in Nemacheilus denisonii under phosphamidon exposure.

Biochemical alterations following exposure to nuan on hepatic tissues in Labeo bata was studied by Medda (1994) and recorded the amount of protein, DNA, RNA and glutamin in the liver. Similar type of hypoproteinemic response in Labeo rohita and Cirrhina mrigala was also observed by Medda et al. (1992) under the exposure of phosphamidon and nuan. Effect of op pesticides on glutaminase and synthetase activity in the brain of rat was studied by Nag (1992).

Kumar and Ansari (1986) observed degeneration of hepatic cells in zebra fish exposed to 0.9 mg/l of malathion for a period of four months and noted skeletal deformities (Kumar and Ansari, 1984). Ramalingam (1988) reported congestion in the venous sinusoids in the liver of Sarotherodon mossambicus on malathion exposure. Hepatotoxicity of malathion was observed as necrosis formation in the liver of bluegill sunfish (Richmonds and Dutta, 1988). Barlas (1992)
noted increase of kupffer cells in the liver of mice receiving 4.16 mg/kg dose of oral administration of commercial malathion for 15 weeks. In fish, malathion was seen to cause histopathological changes in the brain, liver, gill and kidney (Cook et al. 1976). Detectable liver injury in the fish *H. fossilis* exposed to 30 ppm of rogor have been observed by Gadhia (1989).

Early biochemical changes were observed by Rosenbaum et al.(1988) in toad embryos on exposure to op pesticide malathion.

Dhanapakiam and Premlatha (1994) investigated the histopathological changes in the kidney of *Cyprinus carpio* under malathion and sevin exposure and noted that malathion exposure had caused severe intensity of histopathological changes than sevin treatment and observed necrosis in the fish kidney exposed to LC₅₀ dose of malathion for 15 days. Dubale and Shah (1984) reported necrosis in the kidney of malathion exposed *Channa gachua*. Singh and Sahai (1988) also reported similar effects on kidney, liver and gills of malathion exposed *Puntius ticto*. Shah and Dubale (1983) studied the necrotic changes induced by malathion in fish. Disturbed metabolic activity was reported by Sahai and Thakur (1989) in the saccus vasculosus of *Notopterus notopterus* and *Mystus vitattus* under the stress of malathion and noted depletion of glycogen content in the cerebro-spinal fluid and adverse effect of malathion toxicity on the pituitary gland structure.

Exposure of catfish *H. fossilis* to different concentrations of op pesticide dimethoate resulted in cellular destruction and necrosis of the alimentary canal (Awasthi and Gadhia, 1983).
Largescale degeneration of kidney tissues have been reported by Ozelmas and Akay (1995) at 1, 2 and 3 ppm of malathion exposure which showed dose dependent increase. They also reported adverse effects of malathion toxicity on liver and alimentary canal.

Singh (1985) also reported such changes in optic tactum of *Rasbora daniconius* and *Puntius ticto* under treatment of oc and op insecticides.

Bora and Yadav (1996) studied protein content and activity of alkaline phosphatase in the muscle and gills of both male and female *H. fossilis* fishes exposed to rogor. Kabeer Ahmmad Sahib *et al.* (1982) investigated the basic proteins in *T. mossambica* under sublethal malathion impact and reported higher levels of basic protein. Kabeer Ahmmad Sahib (1979) recorded higher rate of incorporation of 14C-glutamic acid in the tissues of fishes exposed to malathion for 48 hours. The sodium, potassium and calcium ion content in all the tissues of malathion exposed *Tilapia mossambica* was studied by Kabeer Ahmmad Sahib *et al.* (1981).

Singh and Singh (1992) and Singh (1992) studied the impact of malathion and γ BHC on steroidogenesis and lipid metabolism of catfish *H. fossilis*. Mukhopadhyay and Dehadrai (1978) studied the effect of malathion toxicity on catfish *Clarias batrachus* and reported impairment of drug metabolism in liver and gill of the fish by inducing mixed function oxidase activity. Mukhopadhyay and Dehadrai (1980 a, 1980 b) studied certain biochemical parameters in *C. batrachus* and assessed various physiological indices at cellular and sub cellular levels under subacute exposure of malathion.
Hasan and Ahmed Khan (1985) investigated the methyl parathion induced dose related alteration in lipid levels and lipid peroxidation in various regions of rat brain and spinal cord. Kurisaka (1985) studied lipid peroxidation by paraquat in human being.

Comparatively higher level of polyunsaturated fatty acids (PUFA) in fish lipids than in other animal lipids have been reported in different fish species (Ackman et al., 1974, Sen et al., 1976). Mukhopadhyay et al. (1984) evaluated the phospholipids and fatty acid content in the liver of C. batrachus under sublethal malathion stress and reported high level of PUFA in liver lipid of the fish and no marked alteration of fatty acids over the control. Singh and Singh (1980) evaluated short term effect of two op pesticides on lipid and cholesterol content of liver, ovary and blood serum of H. fossilis during prespawning phase.

The toxic effect of malathion and phosphamidon on the air breathing fish Channa striatus have been studied by means of graphical method and probit analysis by Chaudhury et al. (1984). Haider and Inbaraj (1986) evaluated the relative toxicity of technical and commercial formulation of malathion and endosulfan by determining their LC$_{50}$ value in C. punctatus and found that commercial formulation of malathion and endosulfan were 1.8 and 1.88 times more toxic than their technical materials. Ravikumar and Gupta (1988) observed the effect of malathion and chlordane in silver carp and common carp and noted progressive mortality of silver carp fingerlings exposed to malathion stress at 1.75 mg/l and reported greater resistance of silver carp and common carp fingerlings than gangetic carps to malathion.
Konar and Ghosh (1981) studied the effect of various organophosphorus insecticides on fish and fish food organisms. Weiss (1959) recorded various responses of fish in response to sublethal exposure of organophosphorus pesticides. Pickering et al. (1962) reported the effects of toxicity study on fathead minnows (*Pimephales promelas*) to an organophosphorus delnav for a period of 30 days and observed that toxicity increased with length of exposure up to 15 days but detected no further increase during an additional 15 days.

Holland and Lowe (1966) studied the effect of prolonged sublethal exposure of malathion on an estuarine common scianid, *Leiostomus xanthurus* and reported development of resistance in these fishes. Ghosh and Chatterjee (1989) studied the influence of nuvan on the organic reserve of murrel *Channa punctatus*.

Mohanachari et al. (1980) elucidated the inhibitory effect of malathion on the activity of sheep liver arginase. Kumar and Gupta (1986) recorded loss of equilibrium, convulsion, coma and death in *Catla catla* exposed to very high dose of chlordane and malathion. Kabeer Ahmad Sahib and Ramana Rao (1980) studied the toxicity potential of malathion on *Tilapia mossambica* and reported malathion's relative toxicity to *T. mossambica* (LC$_{50}$ = 5.6 ppm) fathead minnows (LC$_{50}$ = 23 ppm), blue gills (LC$_{50}$ = 0.12 ppm) and guppies (LC$_{50}$ = 0.88 ppm) and suggested that this difference of toxicity may be due to differences in the capacity of the fish to tolerate brain cholinesterase inhibition.

Excessive mucus secretion from gills and skin of blue gill fish on exposure to malathion has been reported by (Richmonds and Dutta, 1989; Dutta and Marcelino, 1990) and from the skin of *Puntius sarana* (Moitra and Lai, 1989).
Dauterman and Matsumura (1962) reported the effect of malathion analogues upon resistance and susceptibility of *Culex tarsalis*. Mechanism of resistance to malathion in green rice leafhopper was studied by Kojima *et al.* (1963). Biochemistry of malathion resistance in *culex* was worked out by Matsumura and Brown (1961), while Matsumura and Hogendijk (1964 a,b) studied the process of enzymatic degradation of malathion and parathion in resistant and susceptible strains of *Musca domestica*. Cook *et al.* (1958) investigated the activity and inhibition of enzyme malathionase. Seume and O’Brien (1960) studied the hydrolytic pathway of degradation of malathion by rat tissue preparation and its subsequent potentiation by EPN in body organs of *Channa punctatus*. Kabeer Ahmmad Sahib *et al.* (1983) evaluated the effects of sublethal malathion on dehydrogenase enzyme system of *T. mossambica*.

**Op pesticides exert effects on the antioxidant system.** Datta *et al.* (1992) evaluated the effects of op pesticide phosphamidon on antioxidant defence system of human erythrocytes. Thomas *et al.* (1992) studied the effect of monocrotophos on superoxide dismutase activity in liver and kidney of *H. fossilis*. Vaid and Mishra (1997) investigated the effect of vitamins macraberin forte against malathion poisoning in fish brain homogenates by employing reversed phase high performance liquid chromatography (HPLC) and noted macraberin forte vitamin's antioxidant function against malathion poisoning.

Combined toxicity of chlordane, malathion and furadan was studied by Gupta *et al.*(1994) in *Notopterus notopterus* and reported nine out of twelve combinations of these pesticides to be synergistic, two antagonistic and one additive in nature. Several workers (Durham,
1967; Stevens et al., 1972; Springfield, 1972) reported induction phenomenon in mammals caused by synergistic effect of malathion, parathion, OMPA, EPN with SKF - 525 A, sesamex, piperonyl butoxide, paraxon, disulfon and carbaryl. Similar works on potentiation of malathion and malaxon by different opcs such as triorthotolyl phosphate (TOTP), EPN, dipterex etc were carried out by Murphy and Dubois (1957,58); Murphy et al. (1959), Murphy (1967), Cohen and Murphy (1971).

Haematological picture of the fish are also adversely affected by op pesticides. Such changes in the blood parameters have been observed in fishes (Verma et al., 1981; Goel et al., 1982; Misra and Srivastava, 1983; Dabral and Chaturvedi, 1983; Nath and Banerjee, 1995; Bhatia et al., 1996).

Pesticide residues in food stuff are often a matter of major concern to the public. Several workers have detected the presence of residues of op pesticides in food stuff (Samanta et al., 1994; Garcia et al., 1995).

According to a report the daily intake of total chlorinated organics dropped by 22% (Corneliussen, 1970), while that of op residues rose by 14% (Duggan and Corneliussen, 1972).

The possible teratogenic potential of op pesticides have been reported by Khera, 1966; Khera et al., 1965, 1966; Khera and Bedok, 1967; Greenberg and LaHam, 1969; Miniel et al., 1970 in various species of birds. Marliac et al. (1965) confirmed that several insecticides are teratogenic, although diazinon was about 300 fold more toxic to the chick embryo than to the rat. Eggs from hens fed for 3 weeks on basal diets containing malathion and carbaryl (both singly and in
combination) at doses of up to 600 ppm showed a concentration dependent decrease in hatchability (Ghadiri et al., 1967).

Malathion, one of the least acutely toxic opcs to mammals, is teratogenic to the chick embryo in ovo (McLaughlin et al., 1963; Walker, 1967; Greenberg and LaHam, 1969). McLaughlin et al. (1963) reported that malathion lowered the percentage of hatching and produced abnormalities including short legs and bleaching effect on the chick embryo. Injections of 3.99 mg malathion into the yolk sac of 4 to 5 days old incubated eggs consistently produced deformed chicks with a combination of sparse plumage, micromelia, overall growth retardation and beak defects (Greenberg and LaHam, 1969).

Walker (1967, 1971) observed that malaxon was more teratogenic than malathion at a higher level. Walker (1968) reported abnormalities such as shortened tibiofibulae and toes, sparse or clubbed down and hooked beaks in embryos from malathion treated eggs. Wilson and Walker (1966) observed that concentrations of malathion above 1 μg/ml (3.0 x 10⁶ M) inhibited the growth rate and peak cell production in cultured 14 days old chick fibroblasts and cell populations rapidly decreased in cultures containing 50 μg/ml.

Wilson et al. (1973) demonstrated that malathion, malaxon, parathion and paraxon inhibit the growth of cultured chick embryo pectoral muscle cells. The inhibition of cell growth by malathion can be attributed to its ability to inhibit protein synthesis (Gabliks and Friedman, 1965 a, b), a mechanism already proposed to account for its teratogenic action (Greenberg and LaHam, 1969).
Studies with malathion and diazinon in the wistar rat (Dobbins, 1967) revealed that malathion (205.4 mg/kg) administered on day 10 of gestation period and diazinon (63.8 mg/kg) on day 9, both produced morphological malformations manifested primarily as hydronephrosis and hydrourereter. The intraperitoneal administration of toxic doses of malathion (600-900 mg/kg) to female sherman rats neither affected the weight of the foetus nor produced malformation (Kimbrough and Gaines, 1968).

Wistar rats exposed continuously to 240 ppm malathion showed a decrease in litter size and weight and an increase in the incidence of ring-tails (Kalow and Martin, 1961).

Czeizel et al. (1973) studied human chromosome anomalies in 31 acute op intoxication cases from exposure to malathion, dimethoate, mevinphos, dichlorovos, methyl parathion, trichlorfon and diazinon. The frequency of chromosomal breaks temporarily increased significantly in acutely intoxicated patients. It was suggested that these temporary somatic chromosome mutations might have significant long term consequence for the actually intoxicated individual. Of the insecticides shown to cause human chromosome aberrations, malathion was assessed as being the most dangerous (Czeizel et al., 1973).

Malathion has been found to affect the reproductive system and reproductive success of fish.

Ghosh and Dutta (1985) studied the effect of malathion toxicity on the female reproductive system of H. fossilis and reported that malathion exposure effectively inhibits fertilization of eggs at 2 ppm and inhibition enhanced at 10 ppm and prolonged exposure to 2 ppm for 6 months produced extensive damage in ovarian follicles. Such
ovarian recrudence was observed by Saxena and Garg (1978) in *Channa punctatus* on treatment with LC$_{50}$ dose of fenitrothion and carbaryl. Sadhu and Mukhopadhyay (1985) studied the comparative effect of malathion and carbofuran on testis of *Clarius batrachus* and noted that malathion accumulation in the tissues of the testis exerts no apparent structural change.

Zutshi *et al.* (1990) investigated the effect of sublethal malathion at 0.05, 0.25 and 0.5 ppm concentration on the testis of freshwater gobiid and reported blockage of all the spermatogenic activity at spermatid level by malathion.

Zutshi *et al.* (1990) also reported significant alterations in cholesterol contents in testicular tissue in *G. giuris* by malathion. Similar result also have been obtained by Singh and Singh (1980) and Ghosh and Chatterjee (1989) with other op pesticides. Malathion is capable of exerting toxic effects on the testicular hormones in terms of impairment of synthesis and secretion which affected the reproductive potential of *G. giuris* (Zutshi *et al.*, 1990).

Sakaguchi (1972) reported liver damage and hepatic necrosis in fish under malathion stress. Pascual *et al.* (1991) studied the breeding success of blue tit, living in the forest treated with cypermethrin and malathion. Sarma and Nath (1996) investigated the subcellular responses of malathion on oocytes of *H. fossilis* and noted deleterious changes.

Bhattacharya (1997) observed acute toxicity of BHC, malathion, parathion, phosphamidon and DDVP in the form of significantly delayed breeding, reduced rate of feeding, growth, respiration and observed internal abnormalities in *Catla catla, Labeo*
rohita, Anabas testudineus and Hypopthalmichthys molitrix. Anomalous changes in oogenesis of freshwater leech Poecilobdella viridis under sublethal stress of heptachlor, monocrotophos and fenvalerate was observed by Naik et al. (1996). Effect of sublethal malathion on ovarian histopathology in Sarotherodon mossambicus was studied by Shukla et al. (1984).

Durham and Wayland (1962) analysed the nature and physiological function of cholinesterase and symptoms of organophosphorous poisoning, mechanism of inhibition of cholinesterase, measurement of cholinesterase activity. Durham et al. (1965) revealed that the inhibition of blood cholinesterase activity serves as a sensitive measure of incipient toxic effect. Coppage et al. (1975) determined the brain AChE inhibition in marine teleost fish Lagodon rhomboides by op pesticide naled and correlated it with diagnosis of anticholinesterase poisoning.

Santolucito and Whitcomb (1971) while studying the effects of anticholinesterase compounds, considered blood primarily as the index of exposure. Pope et al. (1991) observed that developing mammals are more sensitive than adults to a variety of organophosphates, which act in vivo by inhibition of cholinesterase (ChE). Swamy et al. (1992) studied the development of behavioural tolerance to the opc monocrotophos and change in AChE and suggested the de novo synthesis of cholinesterase resulting in the development of behavioural tolerance, as was evident by the recovery trend.

Barron and Woodburn (1995) investigated the ecotoxicology of chlorpyrifos and found that species sensitivity varies considerably across the kingdom and phyla.
Kurtz (1976) studied the behavioural changes in rats treated with 25, 50, 100 and 150 mg/kg malathion intraperitonially.

Mendoza and Shields (1977) studied the effect of intragastric incubation of malathion on esterases of liver, brain and kidney of suckling rats and noted marked inhibition in brain esterase. In addition, \( I_{50} \) value of malathion based on the inhibition of brain AChE was determined and used this value to confirm the LC\(_{50} \) value obtained by an acute toxicity test. Kabeer Ahmad Sahib and Ramana Rao (1980) reported significant decrease in AChE activity in tissue of \( T. \ mossambica \) by sublethal (2ppm) malathion exposure. Coppage et al. (1975) observed similar inhibition of AChE in fish brain exposed to sublethal concentration of malathion for 72 hours.

Several workers have investigated the effects of different op pesticides on AChE enzyme activity of fish, rat, daphnia, starling, crayfish, man and rabbit (Gaal et al., 1980; Rao et al., 1983; Kozlovskaya et al., 1984, Ghosh, 1989; Galli et al., 1994; Fryday et al., 1995; Chang and Lin, 1995; Deng a et al., 1995; Ramesh et al., 1996; Boone and Chambers, 1977)

Op induced Delayed Neuropathy (OPIDN) is a syndrome which is characterised by a delay in manifestation of poisoning symptoms from one to three weeks after intoxication and this response can be produced by a single dose of op compound.

Subchronic dietary exposure to technical grade of op ester EPN induced delayed neurotoxicity in mallard (\( Anas \ platyrhynchos \)) was observed by Hoffman et al. (1984).
B. Superoxide Dismutase:

Superoxide dismutase acts as a defence against endogenous superoxide radical. The enzyme acts to scavenge the molecules of superoxide anion that are formed during biological reductions but escape from a specific enzyme's active site, thus generating an adventitious potential oxidizing agent of cellular constituents.

\[
\begin{align*}
O_2 + 2H^+ & \rightarrow O_2^- + H_2O_2 \\
H_2O_2 & \rightarrow O_2^- + H^+ (\text{the form at physiological pH})
\end{align*}
\]

The dismutase removes the free superoxide anion radical by accelerating its biomolecular recombination. In this dismutation reaction, one molecule of superoxide undergoes 1-e\(^-\) reduction to \(H_2O_2\), the other undergoing 1-e\(^-\) oxidation back to molecular oxygen.

\[
O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2
\]

The enzyme has a very high turnover number, which was estimated at \(3 \times 10^6\) moles (mole enzyme\(^{-1}\)) with a Km for superoxide at \(5 \times 10^{-4}\) M (Fridovich, 1972, 1975). Expressing the catalytic coefficient as K cal/Km, (Walsh, 1979) reported a biomolecular rate of \(10^8\) M\(^{-1}\) sec\(^{-1}\), a value that is slightly less than the diffusion limit. As a catalyst, superoxide dismutase simply accelerates the non-enzymatic
rate of chemical dismutation. These non-enzymatic disproportionation of two molecules of anion is relatively slow, probably due to electrostatic repulsions. The enzyme presumably overcomes these by binding anion and protonating it at the active site before dismutation.

Superoxide dismutases have been purified from both prokaryotic and eukaryotic sources. The prokaryotes had been found to contain superoxide dismutases based on manganese or iron but not on copper. The copper-zinc enzyme was considered to be characteristic of eukaryotes.

The bovine erythrocyte enzyme has two Cu$^{2+}$ and two Zn$^{2+}$ ions and has 32,000 mol. wt. The Zn and Cu atoms are in close proximity, sharing a common imidazole group of a histidine residue as ligand. The metals were thought to be involved in both binding and electron transfer between oxygen species. Fridovich (1972, 1975) reported a reasonable scheme for redox function of the copper, the zinc is not essential for activity.

\[
\text{Enz} - \text{Cu}^{2+} + O_2 \rightleftharpoons \text{Enz Cu} + O_2 \\
\text{Enz} - \text{Cu}^{2+} + O_2 \rightleftharpoons \text{Enz Cu}^{2+} + H_2O_2
\]

The copper-zinc SOD was first reported in *Photobacter leiognathi* by Puget and Michelson in 1974. A wide range of bacteria was recently surveyed for their contents of superoxide dismutases, with cyanide sensitivity as an indicator of the copper-zinc SOD and $H_2O_2$ sensitivity to detect iron-SOD (Britten et al., 1978). Some bacterial species contain Fe-SOD apparently located in the periplasmic space.
between the inner and outer cell walls (Fridovich 1972, 1975). Some other bacteria contain Mn SOD and still others has both but none had a Cu-zn SOD. Since *P. leiognathi* is a symbiont having been isolated from a special gland of the pony fish, it was supposed that it obtained the genetic information coding for the cu-zn-SOD from its host fish.

The Mn-SOD is a dimer of 40,000 mol.wt. with one manganese ion apparently as Mn (Fee and Valentine, 1977) per subunit. It has the same spectrum of activity as the enzyme from eukaryotic cytoplasm. Subsequent studies established that eukaryotic mitochondria contain a manganoenzyme analogous to the major *E.coli* superoxide dismutase.

Superoxide dismutase enzyme supresses reactions caused specifically by $O_2^{-}$ and is effective in the ng ml$^{-1}$concentration range. It is an extremely sensitive and specific assay for detecting whether $O_2^{-}$ is formed during some biological process. Among the systems thus reported to produce superoxide are-

1. aldehyde oxidase, sulfite oxidase.
2. flavoenzyme dehydrogenase during their slow reoxidation by $O_2$.
3. reduced iron - sulfur chromophores being autoxidized in ferredoxins.
4. a dioxygenase (tryptophan 2,3-dioxygenase)is inhibited by superoxide dismutase.
5. In fruit, ethylene acts as a ripening hormone, its production is attended by formation of $O_2^{-}$ (Fridovich, 1972, 1975).
Fridovich (1972, 1975) proposed the general superoxide theory of oxygen toxicity. This enzyme protects oxygen metabolising enzymes against the deleterious effects of free O$_2^-$ such as sulfhydryl oxidation or unsaturated lipid oxidations. It has long been known that the obligate anaerobes are killed on exposure to oxygen. Fridovich suggested that this oxygen toxicity might arise from the absence of superoxide dismutase in the anaerobes. In their normal metabolism they do not generate O$_2^-$ and thus they have no need for a scavenging device. Indeed, in a species survey all aerobic bacteria examined had a detectable dismutase, but the anaerobes had no superoxide dismutase activity. Hewitt and Morris (1975) found some SOD in 14 out of 16 obligate anaerobes. Some of these, i.e. Chlorobium thiosulfatophilum and Clostridium perfringens, had about one third of the activity found in anaerobically grown E.coli, others i.e. Clostridium acetobutylicum and C. pasteurianum, had only trace amounts of activity.

Tally et al. (1977) addressed the question of the oxygen tolerance of obligate anaerobes and classified 22 strains on the basis of this tolerance. Strains which were very sensitive to oxygen lethality had little or no SOD, whereas oxygen tolerant anaerobes did contain
SOD. Hatchikian et al. (1977) reported SOD in several strains of Desulfovibrio but not in others. They considered the possibility that SOD in anaerobes might be a recent acquisition, perhaps via plasmid transfer, rather than an ancient retained characteristics. Fridovich has suggested that aerobic organisms evolved this enzyme in response to the challenge of O₂ production generated when green plants began to photosynthesize and to convert H₂O to O₂.

**Control of Biosynthesis of SOD:**

Greater resistance towards oxygen toxicity is conferred by elevated intracellular levels of SOD as a consequence of increased exposure to oxygen. The phenomenon has been observed in *Streptococcus faecalis* (Gregory and Fridovich, 1973a), *E.coli* B (Gregory and Fridovich, 1973b), *E. coli* K 12 (Hassan and Fridovich, 1977a), yeast (Gregory et al., 1974) and rat liver (Crapo and Tierney, 1974). In this mechanism of O₂ induced intracellular SOD elevation there exist some doubt, whether the actual inducer is O₂ or O₂⁻ or some other compound uniquely derived from O₂. Circumstances leading to induction of SOD at fixed pO₂ exclude the possibility of O₂ itself being the inducer. Under three very different sets of conditions, induction of the manganese containing SOD (Mn SOD) of *E. coli* is observed with increase in the rate of production of O₂⁻ at fixed pO₂.

Under different sets of conditions as (Hassan and Fridovich, 1977b) change of respiration rate in glucose limited chemostate culture, shifts between fermentative and oxidative metabolism by substrate exhaustion switch over technique (Hassan and Fricovich 1977c) and methyl viologen electron shunting technique between normal electron
transport pathway and cyanide insensitive respiration and O₂ in E. coli cultures under fixed pO₂ results in profound induction of SOD (Hassan and Fridovich 1977d), which is dramatically absent in anaerobic conditions and leads to the conclusion that O₂⁻ itself or some unique product of O₂⁻ is the inducer rather than O₂.

C. Glutathione Peroxidase (GPx):

The evolution of antioxidant defence mechanisms are probably critical to the survival of aerobic life forms. Glutathione peroxidase (GSH-Px) is one of the antioxidant defence enzymes of the body which protects the cells from oxidative damage.

The molecular weight of GSH-Px is about 85,000 and it consists of four apparently identical subunits and contains four gram atoms of selenium/mol. The enzyme bound selenium can undergo a substrate induced redox change and is obviously essential for activity. The enzyme is highly specific for GSH (glutathione) but reacts with many hydroperoxides.

Glutathione peroxidase (glutathione : H₂O₂ oxido-reductase) discovered in 1957 by Mills, was reviewed by Ganther et al., (1976), Flohe' et al., (1976) and Flohe' (1976). Flohe' (1979) summarised the essential characteristics of the enzyme which are still under investigation. Various possible roles of GSH-Px in biological systems were reported to reveal that the function of GSH-Px (GPx) might be relevant to both acute and chronic alterations of mammalian tissues.

Addition of catalytic amounts of various xenobiotics results in extensive oxygen uptake and the rapid oxidation of GSH or NAD(P)H in a peroxidase H₂O₂ reaction system (Subrahamanyam and O'Brien, 1976).
1985, 1987). However, no oxygen uptake occurred in absence of xenobiotics, although at much higher concentrations of GSH, \(H_2O_2\) and peroxidase, GSH oxidation can occur at \(pH\) 8 when the thiol group exists as a thiolate ion in absence of xenobiotics (Harmon et al., 1986).

GSH also increases hydrocarbon evolution during the microsomal catalysed metabolism of the hydrozines imipramiazid, phenelzene, phenylhydrazine, methyl hydrazine and procarbazine. Covalent bonding by these hydrazines to proteins following metabolic activation however, is decreased by GSH. This suggests that carbon and phenyl radicals oxidise GSH to thiyl radicals (Kahyanaraman and Sinha, 1985) and the respective hydrocarbons are formed in the process. Moldeus et al. (1983) first reported the trapping of a thiyl radical with 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) in a peroxidase, \(H_2O_2\), GSH reaction system with \(p\)-phenetidine or acetaminophen as redox mediators (Ross et al., 1984, 1985). No thiyl radicals could be trapped during the oxidation of GSH to GSSG by \(H_2O_2^-\) catalysed by the selenoenzyme glutathione peroxidase (GPx). Furthermore, glutathione peroxidase prevented the formation of thiyl radicals as observed with horse radish peroxidase at high \(pH\), GSH and peroxidase concentration (Harmon et al., 1986). This indicates the occurrence of following reaction-

\[
GS^- + Se^{2+} \rightleftharpoons GP \rightleftharpoons GS^- + Se^{3+} \rightleftharpoons GP
\]

The enzyme is highly specific for glutathione but catalyses the reduction of various hydroperoxides to alcohols including \(H_2O_2\), ethyl hydroperoxides, \(t\)-butyl hydroxide, cumene hydroperoxide,
thymine hydroperoxide, hydroperoxides of unsaturated fatty acids and the corresponding esters, hydroperoxides of steroids and nucleic acids and prostaglandin biosynthesis (Flohe/ and Gunzler 1974, Flohe/ et al., 1976). Studies with various SH compounds indicate that both carboxylic groups of the GSH molecule contribute to substrate binding, since the activity decreases considerably if the γ-glutamyl residue of GSH is substituted by a β-aspartyl or N-acetyl residue or if the glycine residue is replaced by a methoxy or amide group.

Table II 1 : Kinetic constants of GSH peroxidase of bovine erythrocytes. [(Data are taken from Flohe/ et al. (1972) and Gunzler et al. (1972)].

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Buffer System</th>
<th>pH</th>
<th>$\Phi_1 \times 10^{-8}$ mol sl$^{-1}$</th>
<th>$\Phi_2 \times 10^{-6}$ mol sl$^{-1}$</th>
<th>$K_{+1} \times 10^{-7}$/ mol$^{-1}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>0.05 M Potassium phosphate</td>
<td>7.0</td>
<td>0.56</td>
<td>1.27</td>
<td>17.86</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>0.25 M-MOPS</td>
<td>7.7</td>
<td>0.94</td>
<td>0.83</td>
<td>10.6</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>0.25 M-MOPS</td>
<td>6.7</td>
<td>1.70</td>
<td>2.19</td>
<td>5.88</td>
</tr>
<tr>
<td>Ethyl hydroperoxide</td>
<td>0.25 M-MOPS</td>
<td>6.7</td>
<td>3.3</td>
<td>2.24</td>
<td>3.09</td>
</tr>
<tr>
<td>Cumene hydroperoxide</td>
<td>0.25 M-MOPS</td>
<td>6.7</td>
<td>7.8</td>
<td>2.24</td>
<td>1.28</td>
</tr>
<tr>
<td>t-butyl hydroperoxide</td>
<td>0.25 M-MOPS</td>
<td>6.7</td>
<td>13.5</td>
<td>2.24</td>
<td>0.75</td>
</tr>
</tbody>
</table>

According to the criteria of SDS electrophoresis (Flohe/ et al., 1971a), number of tryptic pcptides, number of active sites (Flohe/ et al., 1976) and preliminary x-ray crystallography (Ladenstein and Epp, 1977), the enzyme was reported to be consisting of four identical subunits which are not co-valently linked.
Several investigators have reported on the amino acid composition of GSH peroxidase. The content of cysteine residues is uncertain however the data do not show any characteristic deviations from the amino acid pattern of other proteins (Gunzler, 1974; Nakamura et al., 1974). According to Flohe et al. (1971c), the enzyme does not contain co-enzyme as haems or flavins typical for other peroxidases, instead 4g atom of selenium/mol were found to be isolated from bovine (Flohe et al., 1973), ovine (Oh et al., 1974) and human blood (Awasthi et al., 1975) and rat liver (Nakamura et al., 1974). Wendel et al. (1975) by means of ESCA spectroscopy demonstrated that enzyme bound selenium can change its redox state when natural substrates are added. The ESCA spectra supports the assumption that the selenium of the GSH-reduced enzyme is present in oxidation state 0 or -2 and shifts to a higher state of oxidation on addition of H$_2$O$_2$. This finding is in accordance with the report by Forstrom et al. (1978) suggesting seleno-cysteine as the active site of GSH peroxidase. The ratio of cysteine to selenocysteine is uncertain.

**Kinetics of GSH Peroxidase**:

The kinetics of GSH peroxidase is described by an initial rate equation (1) analogous to that of other peroxidases -

\[
\frac{d[A]}{dt} = V = \left[ E_0 \left( \frac{1}{k_{+1}[A]} + \frac{1}{K_{+2}[B]} + \frac{1}{K_{+3}[B]} \right) \right]^{-1} \quad \text{(1)}
\]

Where \([A]\) is the concentration of hydroperoxide; \([B]\) is the concentration of reductant (GSH), $K_{+i}$ is the constant for the reaction
of reduced enzyme with the hydroperoxide; $K_{+2}$ and $K_{+3}$ are the rate of constants of the oxidized enzyme species with GSH; and $[E_0]$ is the total enzyme concentration. The Dalziel equation (1957) (4) is possible by transformation of equation (2) and (3) which is satisfied, by the experimental data for GSH peroxidase from both bovine blood (Flohe et al., 1972; Gunzler et al., 1972) and rat liver (Chiu et al., 1975).

$$
\frac{[E_0]}{V} = \frac{\varnothing_1}{[A]} + \frac{\varnothing_2}{[B]} \quad (4)
$$

Equation (4) describes a ping-pong mechanism without kinetically-relevant central complexes. The enzyme goes through consecutive steps of oxidation and reduction during the catalytic cycle.

**Mechanism of Action:**

It is deduced from the kinetic analysis of GSH peroxidase that in physiological condition removal of hydroperoxide is largely independent of fluctuations in the cellular concentration of GSH. However, the system abruptly collapses if the rate of hydroperoxide formation exceeds that of regeneration of GSH. By these considerations, the pathophysiological manifestation of disorders in GSH metabolism and pentose phosphate shunt may be explained (Flohe', 1976).

The present view of the mechanism of GSH peroxidase is based on the following facts.
(a) Lack of specificity with respect to hydroperoxidase (Flohe et al. 1976).

(b) High specificity for GSH (Flohe et al. 1971b).

(c) Selective inhibition by iodoacetate of the substrate reduced enzyme only (Flohe and Gunzler, 1974).

(d) Increased binding of p-chloromercuri-benzoate by the enzyme on reduction by GSH (Flohe et al., 1971C).

(e) The ping-pong kinetics (Flohe et al., 1972; Gunzler et al., 1972; Chiu et al., 1975).

(f) The identification of a selenol as functional group (Forstrom et al., 1978).

(g) The reactivity of the enzyme bound selenium with the physiological substrate (Wendel et al., 1975).

The catalytic cycle can be formulated as follows.
Table II 2 - Some characteristic examples of RSH oxidation by H$_2$O$_2$ catalysed by GSH peroxidase. The results are obtained at H$_2$O$_2$ concentrations (1 m mol/l) (Data are taken from Flohe' et al.)(1971b).

<table>
<thead>
<tr>
<th>Glutathione analogue</th>
<th>catalytic activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Variations of the $\gamma$ glutamyl residue</td>
<td></td>
</tr>
<tr>
<td>$\gamma$ - Glu - Cys - Gly (GSH)</td>
<td>100.0</td>
</tr>
<tr>
<td>$\beta$ - Asp - Cys - Gly</td>
<td>7.6</td>
</tr>
<tr>
<td>Cys - Gly</td>
<td>6.8</td>
</tr>
<tr>
<td>$N$ - AC - Cys - Gly</td>
<td>2.7</td>
</tr>
<tr>
<td>(b) Variations of the glycine residue</td>
<td></td>
</tr>
<tr>
<td>$\gamma$ - Glu - Cys - Ghy</td>
<td>100.0</td>
</tr>
<tr>
<td>$\gamma$ - Glu - Cys - OMe</td>
<td>26.0</td>
</tr>
<tr>
<td>$\gamma$ - Glu - Cys - NH$_4$</td>
<td>1.4</td>
</tr>
</tbody>
</table>

A reduced form of the enzyme E reacts with a hydroperoxide in an uncomplicated bimolecular reaction. Neither the kinetics nor substrate specificity studies indicate that an enzyme-substrate complex is involved in the catalytic step. The oxidized enzyme (F) then forms a complex with GSH. This complex however, is rapidly transformed into a new intermediate (G) in an intramolecular reaction. Complex formation in the second step is supported by the high specificity of the enzyme for GSII, but not by the kinetic analysis. The intriguing observation that limiting Michaelis constants or limiting maximum velocities for GSH cannot be achieved despite high specificity, however is compatible with the assumption that the formation of the complex (F.GSH) is much
slower than the intramolecular transformation into the intermediate G and the second molecule of G regenerates E from intermediate, whereby a process analogous to step 2 has to be assumed.

As to the chemical nature of the three enzymes, E, F and G, the following ideas were proposed (Flohe/ 1979) E most likely represents the enzyme containing a largely dissociated selenol function of the selenocysteine residue (E-Sc\(^{-}\)). The selective inhibition of E by iodoacetate which does not show the p\(^{H}\) dependence typical for the reaction with SH groups (Gunzler, 1974) strongly supports this assumption. The results of the ESCA studies (Wendel et al., 1975) and the binding of an additional 4 mol of p-chloromercuri-benzoate to the GSH-reduced enzyme (Flohe/ et al., 1971) also point out to a selenol function. Finally preliminary x-ray crystallography has revealed that the enzyme bound selenium is exposed at the surface of the molecule and is thus in an excellent position to react with various hydroperoxides.

In F the selenium may be oxidised to a selenic acid derivative (E-SeOH) (Ganther et al., 1976). However, a mixed selenosulphide (R Se-SR\(^{-}\)) cannot be excluded at the moment as an alternative. Both forms would readily react with thiols to generate finally disulphides and selenols. The most likely intermediate in both types of reactions would be a mixed selenosulphide consisting of the enzyme bound selenium and GSH (E-Se.SG). Such a compound may represent the kinetic entity G. Despite all these, the reaction mechanism of glutathione peroxidase can not be precisely described and it needs more extensive study.
The definitely established functions of GSH peroxidase in living systems are scanty. From experiments of nature such as genetic disorders it was reported that human red blood cells deficient in GSH peroxidase are highly susceptible to peroxidative drug metabolites or xenobiotics. GSH peroxidase deficiency results in a clinical condition very similar to favism ie G6PDH deficiency (Necheles, 1974). Similarly rat erythrocytes made deficient in GSH peroxidase by means of low selenium diet are prone to peroxide induced haemolysis (Rotruck et al., 1972). Several observations proved the essential contribution of GSH peroxidase to the integrity of the red cell membrane.

Perfusion studies have demonstrated that exogenous hydroperoxides including \( \text{H}_2\text{O}_2 \) are metabolized by rat liver via GSH peroxidase (Sies et al., 1972, 1974). The hydroperoxides infused into the liver mimic to some extent the hydroperoxide originating endogenously outside the peroxisomal compartment. \( \text{H}_2\text{O}_2 \) generated within the peroxisomes by urate infusion usually results in compound I formation of catalase without the decrease of NADPH dependent fluorescence and without the marked GSSH release typical for GSH peroxidase function. These experiments were interpreted as showing that GSH peroxidase is responsible for removing \( \text{H}_2\text{O}_2 \) (as well as other hydroperoxides) in cell compartment low or free of catalase. In rat liver the compartments primarily protected by GSH peroxidase are cytosol and mitochondrial matrix space and the enzyme is hardly detectable in microsomes, nuclei and the peroxisome which probably contain the entire catalase of the cell (Flohe' and Schlegel, 1971).
Prevention of Lipid Peroxidation by Glutathione Peroxidase:

*In vitro,* GSH peroxidase prevents the oxidative break down of unsaturated lipids of biomembranes. Flohe *et al.* (1976) reported that this enzyme plays a role in the defence against oxidative damage of organisms living in aerobic conditions. This view is predominantly based on the following observation of various investigators in different laboratories.

(a) GSH peroxidase can reduce esters of hydroperoxy-fatty acids (Little and O'Brien, 1968).

(b) Endogenous mitochondrial GSH peroxidase prevents lipid peroxidation and irreversible high amplitude swelling of rat liver mitochondria (Flohe and Zimmermann, 1970).

(c) In isolated inner membranes of rat liver mitochondria, purified GSH peroxidase prevents the oxidative degeneration of phospholipids and the concomitant formation of malonaldehyde (Flohe and Zimmermann, 1974).

(d) Bovine blood GSH peroxidase added to illuminated chloroplasts inhibits swelling and malonaldehyde formation (Flohe and Menzel, 1971).

(e) *In vivo* inhibition of GSH peroxidase by repeated administration of cadmium salts results in an accumulation of degradation products of unsaturated lipid in rat testes (Omaye *et al.*, 1975).

(f) Conditions requiring a high rate of lipid peroxide removal, such as the ingestion of lipid peroxides (Reddy and Tappel, 1974) or exposure to ozone (Chow and Tappel, 1972) lead to increased GSH peroxidase activity.
In rats deficient in selenium and consequently in GSH peroxidase, lipid peroxidation can be detected in vivo by monitoring the evolution of ethane. The effect can be inhibited partially by selenium alone and more consistently by a combined treatment with selenium and tocopherol (Hafeman and Hoekstra, 1977).

Low selenium and GSH peroxidase levels were detected in Finnish children suffering from neuronal ceroid lipofuscinosis (Westermarck, 1977).

The theoretical ability of GSH peroxidase to scavenge mutagens is reinforced by a number of observations relating selenium supply to the incidence of cancer. Though any well defined role of selenium in mammals is obscure apart from its being an integral part of GSH peroxidase, the beneficial effects of selenium are suggestive of an optimised removal of hydroperoxides (Schwarz 1976). Supplementation with subtoxic doses of selenium consistently decreases the incidence of tumours in several models of chemical carcinogenesis as well as in mice developing spontaneous mammary tumours (Schrauzer et al., 1977; Jacobs et al., 1977).
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