Introduction
INTRODUCTION

Organophosphorus (OP) compounds are widely used in agriculture, medicine and industry. OP pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors. The widespread use of pesticides in public health and agricultural programs has caused severe environmental pollution and potential health hazards including severe acute and chronic cases of human poisoning (Ellenhorn et al., 1997; Abdollahi et al., 1999; Jalali et al., 2000; Moghadamnia and Abdollahi, 2002). The World Health Organization estimates that the incidence of pesticide poisoning in developing countries has doubled between 1987 and 1997 (WHO, 1997).

In general, it has been observed that OP pesticides are responsible for death in more than 70% cases of intentional poisonings (mainly attempted or successful suicides), which make up a large proportion of the poisonings by pesticides of high toxicity in certain developing countries (Anonymous, 1990). Exposure to OP is also a potential cause of longer-term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration (Nigg and Knaak, 2000). A considerable number of these agrochemicals are reported to be carcinogenic in laboratory animals (Greim and Wolff, 1984). Epidemiological evidences of carcinogenic effects of some of the pesticides in animals are also known (Newell et al., 1984).
MALATHION

Malathion is a pesticide that is used to kill insects on agricultural crops, on stored products, on golf courses, in home gardens and in outdoor sites where trees and shrubs are grown at home; it is also used to kill mosquitos and Mediterranean fruit flies (medflies) in large outdoor areas. Additionally, malathion is used to kill fleas on pets and to treat head lice on humans. Usually, it is sprayed on crops or sprayed from an airplane over wide land areas, especially in the states of California and Florida. The Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) allow a maximum amount of 8 parts per million (ppm) of malathion to be present as a residue on specific crops used as foods. The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. Malathion has been found in at least 21 of the 1,623 current or former NPL sites. However, the total number of NPL sites evaluated for this substance is not known. As more sites are evaluated, the sites at which malathion is found may increase. This information is important because exposure to this substance may harm us because these sites may be sources of exposure. Malathion is one of the most frequently used organophosphorothioate (OPT) insecticides in the world, both in agriculture and in residential settings; in addition, it has been used in malaria eradication programs in Africa and Central America or in wide-scale pest control, including the Mediterranean fruit fly in the United States, through aerial applications. The reason for such widespread use lies in its
relatively low toxicity to mammals and high selectivity toward insects, paralleled by a moderate persistence in the environment, when compared with other OPTs (Wauchope et al., 1992).

CHEMICAL IDENTITY OF MALATHION

Information regarding the chemical identity of malathion is depicted in Table 1. and physical and chemical properties is depicted in Table 2. Malathion comes in two forms: a pure form of a colorless liquid and a technical-grade solution (brownish-yellow liquid), which contains malathion (>90%) and impurities in a solvent. The technical grade malathion smells like garlic. Malathion is a manufactured chemical, and so it is only found in the environment as a result of its manufacture or use. As many as 14 impurities have been identified in technical-grade malathion. The identities of the impurities and their percent (w/w) in technical grade malathion were found to be as follows: S-1,2-ethyl-0,S-dimethyl phosphorodithioate (isomalathion; 0.2%), S-1,2-bis(ethoxycarbonyl)- ethyl-0,O-dimethyl phosphorothioate (malaxon; 0.1%), diethylfumarate (DEF; 0.9%), O,S,S-trimethyl phosphorodithioate (0.003–1.2%), O,O,S-trimethyl phosphorothioate (0.04%), O,O,S-trimethyl phosphorodithioate (1.2%), O,O,O-trimethyl phosphorothioate (0.45%), diethylhydroxysuccinate (0.05%), ethyl nitrite (0.03%), diethyl mercaptosuccinate (0.15%), diethyl methylthiosuccinate (1.0%), O,O-dimethylphosphorothioate (0.05%), diethyl ethylthiosuccinate (0.1%), and sulfuric acid (0.05%). Malathion is formulated as an emulsifiable concentrate (EC), a dust (D), a wettable powder (WP), a ready-to-use (RTU)
Table 1 Information about chemical identity of malathion

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS Nomenclature</td>
<td>Diethyl[(dimethoxyphosphino-thiol)thio]butanedioate</td>
<td>CAS 2001</td>
</tr>
<tr>
<td>Common name</td>
<td>Malathion</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td>Synonym(s)</td>
<td>1,2-Di(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td>Registered trade name(s)</td>
<td>Cekumal</td>
<td>Farm Chemicals Handbook 2000</td>
</tr>
<tr>
<td></td>
<td>Fyfanon&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td></td>
<td>Mallikol&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Farm Chemicals Handbook 2000</td>
</tr>
<tr>
<td></td>
<td>Maltox&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;PS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td>Chemical structure</td>
<td>![Butanedioic acid, [(dimethoxyphosphinothiol)thio]- diethyl ester (malathion)]</td>
<td>Howard and Neal 1992</td>
</tr>
</tbody>
</table>

Identification numbers:

- CAS registry: 000121-75-5 Howard and Neal 1992
- NIOSH RTECS: WM8400000 HSDB 2001
- EPA hazardous waste
- OHIM/TADS
- DOT/UN/NA/IMCO: NA 2783; Malathion HSDB 2001
- shipping: 665 HSDB 2001
- NCI

*CAS = Chemical Abstracts Service, RTECS = Registry of Toxic Effects of Chemical Substances.*

America/International Maritime Dangerous Goods Code, EPA = Environmental Protection Agency,
### Table 2 Physical and chemical properties of malathion

<table>
<thead>
<tr>
<th>Property</th>
<th>Information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular weight</strong></td>
<td>330.36</td>
<td>Howard and Neal 1992</td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td>Colorless liquid (pure form)</td>
<td>Matsumura 1985</td>
</tr>
<tr>
<td></td>
<td>Deep brown to yellow</td>
<td>Budavari 1998; NIOSH 1997</td>
</tr>
<tr>
<td><strong>Physical state</strong></td>
<td>Liquid</td>
<td>Matsumura 1985</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>2.9 °C</td>
<td>Budavari 1998</td>
</tr>
<tr>
<td><strong>Boiling point</strong></td>
<td>156–157 °C</td>
<td>Budavari 1998</td>
</tr>
<tr>
<td><strong>Boiling point pressure</strong></td>
<td>0.7 torr</td>
<td></td>
</tr>
<tr>
<td><strong>Density:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 25 °C</td>
<td>1.23 g/cm³</td>
<td>Budavari 1998</td>
</tr>
<tr>
<td><strong>Odor</strong></td>
<td>Garlic-like</td>
<td>NIOSH 1997</td>
</tr>
<tr>
<td></td>
<td>Mercaptan</td>
<td>Farm Chemicals Handbook 1999</td>
</tr>
<tr>
<td><strong>Odor threshold:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water at 60 °C</td>
<td>1.0 mg/L</td>
<td>Fazzalari 1978</td>
</tr>
<tr>
<td>Air</td>
<td>13.5 mg/m³ (low)</td>
<td>Ruth 1986</td>
</tr>
<tr>
<td></td>
<td>13.5 mg/m³ (high)</td>
<td></td>
</tr>
<tr>
<td><strong>Solubility:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water at 20 °C</td>
<td>145 mg/L</td>
<td>Tomlin 1997</td>
</tr>
<tr>
<td>Organic solvent(s)</td>
<td>Miscible with alcohols, esters, ketones, ethers,</td>
<td>Budavari 1996</td>
</tr>
<tr>
<td></td>
<td>aromatics, and vegetable oil; limited solubility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in paraffin hydrocarbons</td>
<td></td>
</tr>
<tr>
<td><strong>Partition coefficients:</strong></td>
<td></td>
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</tr>
<tr>
<td>Log $K_{ow}$</td>
<td>2.30</td>
<td>Hansch et al. 1995</td>
</tr>
<tr>
<td>Log $K_{w}$</td>
<td>2.89</td>
<td>Chou et al. 1977; Freed et al. 1978</td>
</tr>
<tr>
<td>Log $K_{oc}$</td>
<td>3.25</td>
<td>Buyukozmen 1999</td>
</tr>
<tr>
<td><strong>Vapor pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 25 °C</td>
<td>5.03x10⁻⁶ torr</td>
<td>Watanabe 1993</td>
</tr>
<tr>
<td>at 30 °C</td>
<td>3.36x10⁻⁶ torr</td>
<td>SRC 2000</td>
</tr>
<tr>
<td>at 25 °C</td>
<td>7.9x10⁻⁶ torr</td>
<td>Kim et al. 1984</td>
</tr>
<tr>
<td><strong>Henry's law constant (25 °C)</strong></td>
<td>4.8x10⁻³ atm m²/mol</td>
<td>Fandinger et al. 1990</td>
</tr>
<tr>
<td><strong>Autoignition temperature</strong></td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td><strong>Flashpoint</strong></td>
<td>163 °C</td>
<td>Farm Chemicals Handbook 1989</td>
</tr>
<tr>
<td><strong>Flammability limits</strong></td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td><strong>Conversion factors</strong></td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td><strong>Explosive limits</strong></td>
<td>Containers of malathion may explode in a fire</td>
<td>U.S. Coast Guard 1984–1985</td>
</tr>
</tbody>
</table>

*Penfot-Marsens closed cup test

*The conversion factor for ppm to mg/m³ is: ppm = (mg/m³) (24.45 L/mole)/(g/mole)
liquid, and a pressurized liquid. The quantity of active ingredient (ai) in EC and RTU formulations is variable and can contain up to 82 and 95%, respectively (Brown et al., 1993b; EPA 2001a).

**ROUTES OF ENTRY OF MALATHION TO THE HUMAN SYSTEM**

Most people are not exposed to malathion in the air that they breathe or on things that they touch, unless they live near areas being sprayed. The people who are at the greatest risk of being exposed to malathion are those who work with this chemical. These include farm workers, chemical sprayers, and people who work in factories that make malathion or other products that contain the chemical. They are exposed to malathion on things they touch where it can pass through their skin, or by breathing it after it has been sprayed. Overexposure to malathion may cause severe poisoning or death. Persons may be exposed to dangerous amounts if they go into fields too soon after spraying.

For the general population, the most likely way that malathion can enter the body is by eating or drinking contaminated food or water or through dermal contact with contaminated plants, soils, or surfaces such as playground equipment or pavements. It can also enter our body if we breathe air containing malathion during or after it has been sprayed for public health uses. Individuals can also be exposed to malathion if they live near landfills where malathion has been dumped or near water containing malathion that washes off nearby land or that is accidentally spilled. The greatest amounts of
malathion are expected to be present near or on the farms where malathion is used. After spraying, some malathion can be transported by the wind or fog to areas away from where it is used, but the amounts present at these locations are not expected to be at dangerous levels.

MAJOR HEALTH PROBLEMS OF MALATHION

Malathion interferes with the normal function of the nervous system. Exposure to high amounts of malathion in the air, water, or food may cause difficulty in breathing, chest tightness, vomiting, cramps, diarrhea, watery eyes, blurred vision, salivation, sweating, headaches, dizziness, loss of consciousness, and death.

If persons who are exposed accidentally or intentionally to high amounts of malathion are rapidly given appropriate treatment, there may be no long-term harmful effects. If people are exposed to low levels of malathion below those that affect the function of the nervous system, few or no health problems seem to occur.

Malathion toxicity, in a manner similar to all OPTs, depends on its bioactivation to the toxic metabolite malaoxon (Thompson et al., 1989; Sultatos, 1994), which inhibits acetylcholinesterase (AChE) Forsyth and Chambers, 1989), causing the accumulation of acetylcholine within synapses and the consequent over stimulation of postsynaptic receptors.
BIOACTIVATION OF MALATHION

The effect has been attributed to the potent inhibition exerted by isomalathion on hepatic and serum carboxylesterase activity (Aldridge et al., 1979; Talcott et al., 1979a,b); in these conditions, the major pathway of malathion metabolism is the cytochrome P$_{450}$-catalyzed bio-activation, resulting in a dramatic increase in the production of malaoxon. In recent years a number of papers have been published on the activity of human P$_{450}$s involved in the bio-transformation of some members of the OPT class (Butler and Murray, 1997; Mutch et al., 1999; Kappers et al., 2001; Tang et al., 2001; Vittozzi et al., 2001; Buratti et al., 2002, 2003). The information on the bio-activation of malathion, which has linear groups as substituents at the thioether sulfur, could indicate whether other OPTs with different chemical structure are bioactivated by the same isoform(s). This information represents the basis for the selection of metabolic biomarkers to be used to identify individuals potentially characterized by different production of toxic metabolites and consequent different susceptibilities to the toxic effects induced by the entire class of OPT insecticides.

Indeed, mammalian carboxylesterases catalyze rapid degradation of malathion to monoacid and diacid derivatives (Kutz et al., 1992; USEPA, 2000); the reaction efficiently competes with the cytochrome P$_{450}$-catalyzed formation of malathion phosphate triester malaoxon, which in turn can be degraded by carboxylesterase, and dimethylthiophosphate, a detoxication product (Fig. 1).
Fig. 1 The pathways of malathion bioactivation. DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; MMA, malathion monocarboxylic acid; MDA, malathion dicarboxylic acid.
METABOLISM OF MALATHION

Absorption of ingested malathion is rapid, followed by efficient biotransformation and elimination, mostly in urine. Malathion requires for its acute toxicity the bioactivation to the ultimate neurotoxic metabolite, malaoxon. It is the level of this metabolite at the target site that determines acute toxicity. Although the liver is the richest source of the bioactivation enzyme among various mammalian organs, the source organ of malaoxon responsible for acute toxicity has not been determined. The overriding factor that makes the mammalian toxicokinetics of malathion unique is the rapid hydrolytic cleavage of carboxylic ester linkages that counters the buildup of the neurotoxic metabolite malaoxon.

Malathion also undergoes various other forms of biotransformation. Both malathion and malaoxon are subject to phosphate linkage hydrolysis as well as glutathione-linked cleavage, both of which are detoxicative. Carboxylesterase is quite active in rat blood, but not in human blood (Main and Braid, 1962).

Malathion concurrently encounters three types of metabolic modifications in animals, a) oxidative, b) hydrolytic, and c) the elimination of a methyl group catalyzed by glutathione S-transferase (Fig. 2). The most important metabolite of the former biotransformation is malaoxon, the ultimate neurotoxic molecule responsible for the acute toxicity. Among the latter reactions, hydrolysis of one of the two carboxylic ester linkages
Fig. 2 Metabolic Pathways of Malathion
abolishes the potential of acute toxicity and is mainly responsible for the well-known low acute toxicity of malathion to mammals.

**Oxidative Metabolism**

While lacking in detailed analysis with malathion, the oxidative reaction catalyzed by mixed function oxidase is considered as general one for phosphorothioate esters and involves cytochrome P-450. In the case of the well-studied phosphorothioate parathion, the reaction involves CYP2B (Wolf *et al.*, 1990). Although malaoxon is toxicologically the most important product of this enzyme reaction, it is one of the products arising from the putative sulfur oxide intermediate. In a few dialkyl aryl phosphorothioates studied in detail, such as parathion and diazinon, the sulfur oxide intermediate undergoes a rearrangement to yield "oxon" on the one hand, and hydrolysis to yield dialkyl phosphorothioic and dialkyl phosphoric acid on the other (Nakatsugawa, 1992). This scheme is likely to apply to malathion as well, and it would be expected that dimethyl phosphorothioic acid and dimethyl phosphoric acid arise as products from the rearrangement of the sulfur oxide intermediate. Probably reflecting the technical difficulty in the presence of carboxylesterase activity, the process of oxidative malathion metabolism has not been studied specifically. Oxidative metabolism of parathion demonstrated in the pig skin (Chang *et al.*, 1994) suggests similar reactions for malathion.
Carboxylester Hydrolysis

A group of urinary metabolites in malathion-exposed animals is produced by the hydrolysis of the succinate ester moiety. Included in this group are α- and β-malathion monocarboxylic acid (O,O-dimethyl-S-(1-carboxy-2-carbethoxy)ethyl phosphorodithioate and O,O-dimethyl-S-(1-carbethoxy-2-carboxy)ethyl phosphorodithioate, respectively), α- and β-malaoxon monocarboxylic acid (corresponding α- and β-analogs of malathion monocarboxylic acids), and malathion dicarboxylic acid. Enzymes involved in producing these metabolites are called carboxylesterases after the type of ester linkages they target. Multiple forms of carboxylesterases are widely distributed in mammalian tissues. Even the brain tissue has a detectable level of carboxylesterase activity as observed in female mice (Sakai and Matsumura, 1968). In the rat, the liver contains the highest level of carboxylesterase among various organs. Total enzyme activities (in terms of nmol/minute) among the various organs were 370 for lung, 4,720 for kidney, 24,000 for liver, and 7,490 for serum. Intestinal villi and brain homogenates revealed little activity. Three-fourths of the hepatic carboxylesterase were found in the microsomal fraction. In rats, however, carboxylesterase in the serum play an important role as the hepatic carboxylesterase (Talcott, 1979).

Malaoxon is hydrolyzed by a carboxylesterase, and its acute toxicity increases when this enzyme is inhibited (Dauterman and Main, 1966). The kinetics of carboxylesterase is complicated since the substrate malaoxon inhibits carboxylesterase (Main and Dauterman, 1967). Malathion dicarboxylic acid is a major urinary metabolite of malathion, but the enzyme
that yields this metabolite by hydrolyzing the second carboxylester linkage has not been studied. In rats, the dicarboxylic acid was produced more than monocarboxylic acid, with the ratio of mono/dicarboxylic acids decreasing with the decreasing dosage. Malathion monocarboxylic acids were more abundant than the dicarboxylic acid, and dimethyl phosphorothioic acid was the main alkylphosphate metabolite.

**Phosphoric ester Hydrolysis**

Urine of malathion-treated animals often contains significant amounts of dimethyl phosphorus esters such as dimethyl phosphoric acid, dimethyl phosphorothioic acid, and dimethyl phosphorodithioic acid. Pathways leading to these metabolites, however, have not been clarified.

Dimethyl phosphoric acid is the anticipated metabolite in the hydrolysis of malaoxon. Such an enzyme resistant to the inhibitory action of organophosphates, or A-esterase, was detected in the serum of 100 human subjects. The assay determined the free thiol-containing leaving group of malaoxon. Contribution of A-esterase to the detoxication of malaoxon appears less than for other neurotoxic organophosphates assayed (Sams and Mason 1999). Dimethyl phosphoric acid is also a potential product of the oxidative metabolism as previously mentioned.

Dimethyl phosphorothioic acid would be assigned to the oxidative metabolism as the sole source in the case of related dimethyl phosphorothioate like methyl parathion since no hydrolytic enzymes have been found to yield this metabolite. Hydrolytic characteristics of malathion,
however, makes it difficult to interpret the data. According to Mattson and Sedlak (1960), "malathion is readily hydrolyzed by acid chiefly to \( O,O \)-dimethylphosphorothionic acid and by alkali chiefly to \( O,O \)-dimethylphosphorodithioate". The same authors also noted that "phosphorodithioate is rather unstable and is converted at least partially to the phosphorothonate". In the absence of further information, the possibility may exist that dimethyl phosphorothioic acid is partially derived from malaoxon hydrolysis.

Dimethyl phosphorodithioic acid can only arise from malathion, and not from malaoxon. The enzymes responsible for this metabolism have not been studied. In a gavage study using doses of 0.069–69 mg/day in 10-fold increments for 3 days to 400–450 g male Sprague-Dawley rats, dimethyl phosphorothioic acid was the dominant phosphate metabolite, followed by dimethyl phosphorodithioic acid and dimethyl phosphoric acid (Bradway and Shafik 1977). The proportion of the latter two metabolites seems to shift with the dose, with dimethyl phosphoric acid dominating at lower doses and dimethyl phosphorodithioic acid being the dominant metabolite at the highest dose.

**Glutathione-linked Metabolism**

Mouse liver homogenate contains a glutathione (GSH) S-transferase which demethylated malathion to yield demethyl malathion (Nomeir and Dauterman, 1978). This may account for an earlier observation of a 10-fold enhancement of malathion metabolism by GSH with mouse liver homogenate
in which esterases were suppressed by DFP (di-isopropyl fluorophosphate) (Bhagwat and Ramachandran, 1975). A substantial yield of demethyl malathion in rat liver homogenate has also been reported though involvement of GSH is unknown (Matsumura and Ward, 1966).

Hepatocytes isolated from male Wistar rats were used to study the depletion of GSH by malathion, isomalathion, and trimethyl phosphorus esters (Malik and Summer, 1982). Isomalathion was the most effective. GSH depletion by malathion was greatly increased when carboxylesterase was pre-inhibited by isomalathion, indicating the greater involvement of GSH-linked metabolism of malathion under those conditions.

MECHANISM OF ACTION

Most organophosphates are highly lipid-soluble agents and are well absorbed from the skin, oral mucous membranes, conjunctiva and gastrointestinal and respiratory routes. The onset, severity and duration of poisoning are determined by the dose, route of exposure, physicochemical properties of the organophosphate, (e.g. lipid solubility) rate of metabolism, (whether transformation in the liver is required before the compound becomes toxic) and whether the organophosphorylated cholinesterase ages rapidly (Schnaite, et al., 2003). The inactivation of the cholinesterases occurs in fast blood and in a wide range of nerve, neuromuscular skeletal, smooth and cardiac) and glandular tissues where these enzymes have a role in cell-to-cell communication and the hydrolysis of xenobiotics. These enzymes have possibly as yet unidentified roles such as cell development and growth.
The inhibition of AChE leads to the accumulation of acetylcholine, the neurotransmitter at all ganglia in the autonomic nervous system and at many synapses in the brain, skeletal neuromuscular junctions, at some postganglionic nerve endings of the sympathetic nervous system and adrenal medulla. The role of butyryl cholinesterase in the body is yet to be fully identified, but it is known to be involved in the hydrolysis of many therapeutic agents (e.g. suxamethonium, esmolol, procaine and cocaine). There are many other roles speculated for butyryl cholinesterase and these include cellular differentiation and growth, as a scavenger in xenobiotics exposure and as a modulator in lipid metabolism.

The key reactions taking place between organophosphates and AChE are indicated in Fig. 3. The consequences of inhibition of other enzyme systems by organophosphorus compounds are as yet uncertain. A variety of tissue carboxylesterases exist in the serum, liver, intestine and other tissues. Although inhibition of one specific carboxylesterase (neuropathy target esterase) has toxic sequelae (Moretto and Lotti, 1998), no direct deleterious effects of inhibition of other carboxylesterases have been demonstrated. However, carboxylesterases may contribute markedly to the metabolic degradation of organophosphorus insecticides and inhibition of these enzymes may potentiate the toxicity of organophosphorus compounds such as the nerve agents. The search for effects of inactivation or changes in other physiological systems continues. The following effects of organophosphorus agents have been demonstrated in animals and are theoretically possible effects in man (Karalliedde, 1999).
Fig. 3 Mechanism of Action
1. Inactivation by phosphorylation of other β esterases.

2. Altering the release of neurotransmitters, e.g. α-aminobutyric acid (GABA) and glutamate.

3. Increasing the number of GABA and dopamine receptors.

4. Acting as agonists at M2/M4 muscarinic receptors.

5. Inhibition of mitochondrial enzymes, respiration and ATP generation.

6. Induction of mast cell degranulation, probably causing the release of histamine or histamine-like compounds.

7. Inhibition of nitric oxide.

8. Interference with surfactant in the lung.

9. Inhibition of phospholipase A₂.

10. Interference with humoral and cellular immunity, e.g. the function of T lymphocytes.

**Alcohol**

Alcohol is used as a solvent in industry but it is heavily consumed by a large number of people as a component of potentially intoxicating beverages. Alcohol acts as both a general anaesthetic and as a nutrient. (Morgan, 1982) A population based case control study in India reported that alcoholism was significantly associated with suicide and as much as 34% of the study subjects could be diagnosed as alcoholics (Vijayakumar, 1999). Pesticides are consumed along with alcohol by depressed farmers in many self poisoning cases as evidenced in forensic cases. The effect of alcohol with other
hepatotoxic agents is a recognized factor (Zimmerman, 1978) but with malathion it is a novel approach.

**Pharmacology of Ethanol**

Ethanol is a weakly charged molecule that moves easily through the cell membranes, rapidly equilibrating between blood and tissues. Congeners found in alcohol beverages may contribute to body damage with heavy drinking; these include low molecular weight alcohols (eg. Methanol and Butanol), aldehydes, esters, histamine, phenols, tannins, iron, lead and cobalt. The daily amount of alcohol established in cirrhotic patients is normally in the range of 160g to 220g/day (Wilson, 1991).

**ETHANOL METABOLISM**

Ethanol is absorbed from the gastrointestinal tract by simple diffusion because of its small size and low solubility in lipids. Diffusion is rather slow from the stomach and consequently most (70% to 80%) ingested ethanol is absorbed from the duodenum and upper jejunum (Salaspuro, 1992).

The diffusion of alcohol through the cell boundaries is a rather slow process, but the distribution of alcohol by the blood flow is very fast. In organs with dense vascularization and rich blood supply, such as brain, lungs and liver, alcohol rapidly equilibrates with the blood (Salaspuro, 1992).

Only two to ten percent of that absorbed is eliminated through the kidneys and lungs; the rest is oxidized in the body, principally in the liver. The rate of removal of ethanol from the blood is, indeed, remarkably
decreased or halted by heptectomy or procedures damaging the liver (Thompson, 1956). Extrahepatic metabolism of ethanol is small (Forsander and Raiha Niels, 1960).

**Pathways of ethanol oxidation**

The hepatocyte contains three main pathways for ethanol metabolism, each located in different subcellular compartments; the alcohol dehydrogenase (ADH) pathways of cytosol, the microsomal ethanol oxidizing system (MEOS) located in endoplasmic reticulum and catalase located in the peroxisomes.

**Alcohol dehydrogenase pathway**

Alcohol dehydrogenase (ADH) catalyses the oxidation of alcohols, including ethanol, to their corresponding aldehydes according to the following scheme:

\[
\text{ADH} \quad \text{CH}_2\text{CH}_2\text{OH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+
\]

ADH has a broad substrate specificity, which includes dehydrogenation of steroids (Okuda and Takigawa, 1970) and omega oxidation of fatty acids; (Bjorkhem, 1972) and these compounds may represent the “physiologic” substrate for ADH.

In ADH mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor form (NADH) and acetaldehyde is produced. The association of the NADH enzyme complex had been shown to be a rate –
limiting step in this reaction (Theorell and Chance, 1951). As a net result, the first step in the oxidation of ethanol generates an excess of reducing equivalents in the cytosol, primarily as NADH. Thus, in normal rats given ethanol there is a marked shift in the redox potential of the cytosol as measured by the change in the lactate: pyruvate ratio (Domschke et al., 1974). The altered redox state, in turn is responsible for a variety of metabolic abnormalities.

The agent known to enhance ethanol elimination both *invivo* and *invitro* is fructose, which increases the capacity of the respiratory chain to oxidize NADH derived from the oxidation of ethanol and acetaldehyde. Corticosteroids enhance the rate of ethanol elimination, by increasing the conversion of NADH to NAD$^+$ as a result of enhanced gluconeogenesis (Salaspuro, 1992).

**Microsomal ethanol oxidizing system (MEOS)**

The existence of an independent MEOS pathway is now generally accepted although its quantitative contribution to total ethanol metabolism still remains in dispute (Lieber, 1988). MEOS is located in the endoplasmic reticulum, requires NADPH and oxygen and is relatively insensitive to catalase inhibition. The scheme of the reaction is as follows:

\[
\text{MEOS} \\
\text{CH}_3\text{CH}_2\text{OH} + \text{NADPH}^+ + \text{H}^+ + \text{O}_2 \rightarrow \text{CH}_3\text{CHO} + \text{NADP}^+ + 2\text{H}_2\text{O}
\]
A microsomal system capable of methanol oxidation had been described (Orme-Johnson and Ziegler, 1965) but its capacity for ethanol oxidation was extremely low. Ziegler (1972) concluded that this system is clearly different from the cytochrome P450 dependent system and involves the H\textsubscript{2}O\textsubscript{2} - mediated ethanol peroxidation by catalase. However, a MEOS with a rate of ethanol oxidation ten times higher than reported by Orme-Johnson and Ziegler (1965) was subsequently described by Lieber and Decarli (1970).

Studies of MEOS with different alcohols as substrates are of particular interest. The NADPH dependent MEOS was found capable of metabolizing methanol, ethanol, n-propanol and n-butanol to their respective aldehydes in hepatic microsomal preparations as well as in column fractions, that contained the microsomal components cytochrome P450, NADPH-cytochrome-c-reductase and phospholipids which exhibited no ADH or catalase activity (Teschke et al., 1975), but unlike ethanol is not a substrate for catalase – H\textsubscript{2}O\textsubscript{2}. The latter finding is in excellent agreement with previous reports regarding the substrate specificity of catalase (Chance and Oshino, 1971).

**Catalase Mediated Ethanol Oxidation**

Catalase is a hemoprotein located in the peroxisomes of most tissues. It is generally accepted that the H\textsubscript{2}O\textsubscript{2}-mediated ethanol peroxidation by catalase is limited by the rate of H\textsubscript{2}O\textsubscript{2} generated rather than the amount of catalase itself. It may play a minor role in alcohol metabolism according to the following scheme.
CH₃CH₂OH +H₂O₂ → CH₃CHO+2H₂O

Hypoxanthine + H₂O+O₂ → Xanthine + H₂O₂

The physiological rate of H₂O₂ production had been estimated to be 3.0 to 3.6μmole/hr/g of liver, which represents 2% of the in vivo rate of ethanol oxidation (Lieber and DeCarli, 1973).

*Terminalia chebula* (Tc)

*Terminalia Chebula* is called the "king of medicines" and is always listed first in the Ayurvedic meteria medica because of its extraordinary powers of healing. In Ayurveda it is considered to destroy all diseases and eliminate all waste from the body. At the same time, it is known to promote tissue growth and health. The medical properities of *Terminalia chebula* and other several herbal plants have been documented in the ancient Indian literature (Japtap and Karkera, 1999; Kaur *et al*., 1998; Trease and Evans, 1983; Inamdar and Rajarama, 1954; Saleen *et al*., 2002). *Terminalia chebula* has been used extensively as a drug against a number of diseases (Awasthi and Nath 1968; Ram Chandra Reddy *et al*., 1990).

**Habitat**

The tree is found all over India chiefly in deciduous forests and areas of light rainfall, up to about 1500 m mean sea level (MSL).
Macroscopic identification

A large tree, young branchlets, leaf buds, and leaves with long, soft, shining, rust colored, sometimes silvery hair. Flowers are dull white or yellowish in color with a strong offensive smell. Fruits are ovoid, wrinkled and ribbed longitudinally.

Parts used in Medicine: Fruits

Terminalia chebula tree
Terminalia chebula fresh fruits
Terminalia chebula dry fruit

VERNACULAR NAMES

Tamil : Kadukkai
English : Black Myrobalan, Chebulic Myrobalan
Hindi : Harad
Sanskrit : Haritaki
Telugu : Karkchettu
Marathi : Harade
Malayalam : Divya, Katukka, Kayastha putanam
Chemical composition

It contains anthraquinone glycoside, chebulic acid, tannic acid, terchebin, vitamin C (Fruits), arachidic acid, behenic acid, linoleic acid, oleic acid, palmitic acid and stearic acids (Fruit kernels), chebulin occurs in the flowers.

Taxonomy

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
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<tbody>
<tr>
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<td><em>Terminalia</em> L.</td>
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<tr>
<td>Species</td>
<td>Terminalia chebula (Gaertner) Retz</td>
</tr>
</tbody>
</table>

Anti-oxidant activity of *Terminalia chebula*

The ethanol extract from the fruit of Terminalia chebula (Combretaceae) exhibited significant inhibitory activity on oxidative stress (Na et al., 2004). *T.chebula* has antioxidant properties (Sharma et al., 1999). The extract of *T.chebula* shows excellent anti-oxidant activity *in vitro* system and anti-oxidant activity of this extract is tested by studying the inhibition of
radiation induced lipid peroxidation in rat liver microsomes. The extract of *T. chebula* is found to restore anti-oxidant enzyme superoxide dismutase (SOD) from the radiation induced damage (Priyadarsini *et al.*, 2003). *T. chebula* has the potential to play a role in the hepatic prevention of oxidative damage in living systems (Lee *et al.*, 2005). The aqueous extract of the fruit of *T. chebula* is evaluated for their in vitro antioxidant activity and results showed *T. chebula* has greater radical scavenging activity (Naik *et al.*, 2005).

To identify promising sources of antioxidants *T. chebula* is studied for total phenolic contents and antioxidant activity. The leaves bark and fruits of *T. chebula* are found to have high total phenolic contents (72.0-167.2 mg/g) and high antioxidant activity (69.6-90.6%) (Bajpai *et al.*, 2005). *T. chebula* is investigated for anti-lipid peroxidation, anti-superoxide radical formation and free radical scavenging activities. The results showed pure compounds of *T. chebula* exhibited antioxidant activity at different magnitudes of potency (Cheng *et al.*, 2003).

**Nutritive value of the *Terminalia chebula***

The edible fruit tissue of the *T. chebula* was analyzed for certain organic and mineral nutrients. Compared with commercial apples, the tissue contained 10.3 and 14.5 times more vitamin C and protein, respectively. Of the 14 macro and micro nutrients studied, the minimum Recommended Dietary Allowance (RDA) for Se, K, Mn, Fe and Cu can be met (100, 63.5, 32, 30 and 28.5%, respectively) if 100 g of the raw fruit is eaten. Aspartic
*T. chebula* is reported to significantly reduce serum cholesterol, aortic sudanophilia and the cholesterol contents of the liver and aorta in cholesterol-fed rabbits (Thakur et al., 1988).

**Anti-mutagenic activity of *Terminalia chebula***

The extract of *T. chebula* and its fraction were highly significant against S-9 dependent mutagen (Grover et al., 1998). The extract of plant *T. chebula* has been reported to inhibit the mutagenicity of sodium azide and 4-nitro-o-phenylene-diamine in salmonella assay (Grover and Bala, 1992). Extract of *T. chebula* inhibited the mutagenicity induced by both direct and indirect acting mutagens, but the inhibition was greater for S-9 dependent mutagens (Arora et al., 2002).

**Inhibitory action of Water soluble Fraction of *Terminalia chebula* (WFTC) on systemic and local anaphylaxis.**

WFTC may contain compounds with actions that inhibit systemic and local anaphylaxis and inhibits the anaphylactic degranulations of mast cells. The WFTC administrated rats are protected from IGE mediated local anaphylaxis. This finding suggests that WFTC may be applicable to the treatment of allergic skin reaction (Lee et al., 2001).

**Anti-caries activity of *Terminalia chebula***

In India the role of *T. chebula* in decreasing the prevalence of caries has been common knowledge for many years (Chopra and Handa, 1958). On examining the list of plants recommended for a particular dental therapeutic
purpose, it is evident that the ripe fruit of *T.chebula* is valuable in the prevention and treatment of several diseases of the mouth such as dental caries, spongy and bleeding gums, gingivitis, and stomatitis (Date and Kulkarni, 1995). The extract of *T.chebula* may be an effective agent in the treatment of carious teeth, owing to its ability to inhibit the growth and accumulation of Streptococcus on the surface of the tooth. This would prevent the accumulation of acids on the surface of the teeth and thus preventing the further demineralization and the break down of the tooth enamel (Jagtap *et al.*, 1999).

**Anti-diabetic activity of *Terminalia chebula***

Oral administration of the extract of *T.chebula* reduced the blood sugar level in normal and alloxan diabetic rats significantly within four hours. Continued, daily administration of the *T.chebula* Produced a sustained effect (Ramadasan Kuttan *et al.*, 2002). Mechanism of action of this drug in reducing the diabetic is not known. It may be possible that this extract may reduce the effect of inflammatory cytokine release during diabetes, which may be one of the causative agents for the tissue distraction and insulin resistance (Saghizadeh *et al.*, 1996).

**SCOPE OF THE STUDY**

The organophosphorus insecticides are widely studied in agriculture. Malathion is said to be widely used as a suicidal chemical in villages in India. Here is a belief in local population that pesticides mixed with alcohol will have additive effect in suicidal attempts. However, studies on Malathion with
ethanol have not received much attention as yet. It is therefore, worthwhile to investigate the toxic reactions in mice with reference to malathion poisoning either combined with ethanol or alone.

*T. chebula* has been reported to exhibit a variety of biological activity, including anticancer (Saleem *et al.*, 2002), antidiabetic (Sabu and Kuttan, 2002), antimutagenic (Arora *et al.*, 2002) and antioxidant (Hua-Yew Cheng, *et al.*, 2003) activities etc., Village physicians use *Terminalia chebula* fruit extract as an antidote for malathion and other pesticide poisoning.

Hence, the present study was undertaken to evaluate the antidotal activity of *T. chebula* against malathion induced toxicity in Swiss albino mice. This is the first of its kind on the antidotal activity of aqueous extract of *T. chebula* against malathion poisoning.