Chapter - 2

Material & Method
2. Materials and Methods

2.1 General Information of Malathion

Malathion is a wide-spectrum insecticide that was introduced in 1950. It is used to control sucking and chewing insects on fruits and vegetables and also to control mosquitoes, flies, household insects, and animal parasites.

The chemical and physical properties of are given below.

Availability and Recommended Use of Malathion During ODS/DS. Malathion was primarily used as an outdoor spray during ODS/DS to control mosquitoes and flies.

2.1.1 Environmental Characteristics of Malathion.

Malathion displays little persistence in soil, with rapid degradation (Howard, 1991) and reported half-lives in the field ranging from one to 25 days (Wauchope et al., 1992). It does bind moderately to some soils and could contaminate groundwater or surface water in some cases. In air, malathion is rapidly broken down by sunlight, with a reported half-life of approximately one and one-half days (Howard, 1991).
### 2.1.2 Chemical Identity of Malathion

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical class</td>
<td>Organophosphate</td>
</tr>
<tr>
<td>Chemical name</td>
<td>Diethyl (dimethoxyphosphinothioyl) thiobutanedioate</td>
</tr>
<tr>
<td>Trade names</td>
<td>Carbophos; Celthion; Cythion; Dilathion; El 4049;</td>
</tr>
<tr>
<td></td>
<td>Emmaton; Exathios; Fyfanon; Hithion; Karbofos;</td>
</tr>
<tr>
<td></td>
<td>Malathion; Matrox</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{10}H_{19}O_{6}P_{2}</td>
</tr>
<tr>
<td>CAS Registry number</td>
<td>121-75-5</td>
</tr>
</tbody>
</table>

### 2.1.3 Physical and Chemical Properties of Malathion

<table>
<thead>
<tr>
<th>Property</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>330.36</td>
</tr>
<tr>
<td>Color/form</td>
<td>Clear, brown to colorless liquid</td>
</tr>
<tr>
<td>Odor</td>
<td>May be garlic-like</td>
</tr>
</tbody>
</table>

**actions lost substantial quantities**

- Water solubility at 25°C: 145 mg/L
- Partition coefficient ($K_{ow}$): 560
- Soil sorption coefficient ($K_{oc}$): 1,800
- Vapor pressure at 30°C: $4 \times 10^{-5}$ mm Hg
- EPA toxicity classification: Class III
- ACGIH TLV-TWA: 10 mg/m³ (skin)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIOSH REL-TWA</td>
<td>10 mg/m³ (skin)</td>
</tr>
<tr>
<td>NIOSH REL-STEI</td>
<td>NA</td>
</tr>
<tr>
<td>NIOSH IDLH value</td>
<td>250 mg/m³</td>
</tr>
<tr>
<td>OSHA PEL-TWA</td>
<td>15 mg/m³ (total dust) (skin)</td>
</tr>
<tr>
<td>EPA IRIS RfD</td>
<td>2 x 10² mg/kg/day</td>
</tr>
<tr>
<td>EPA IRIS RfCNA</td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity classification</td>
<td></td>
</tr>
<tr>
<td>ACGIH</td>
<td>A4</td>
</tr>
<tr>
<td>EPA</td>
<td>NA</td>
</tr>
<tr>
<td>IARC</td>
<td>3</td>
</tr>
</tbody>
</table>

NA = not available.

2.1.4 Malathion Residues.

Most studies of malathion residues have been conducted on edible products; however a few studies have focused on malathion residues on skin and fabrics. Patches of fabrics exposed to pesticide spray formulchemicals within four to six hours. Fabrics were cotton or 1:1 cotton-polyester blends, knitted or woven, un unfinished or finished. Deposition and retention of pesticide-bearing particulates appeared to depend on mechanical restrictions related to fabric weave and on the electrokinetic potential of fabric surfaces (Serat, 1982).
2.2 Experimental design:

Albino rats of either sex weighing between 152 to 200 gm. Were divided into different groups. Animas in each group maintained on specific diet. Rats were divided into following groups.

2.2.1A- Rat (male)

Group-I- **Intact (control):** The rats of this group received standard pallet diet food.

Group-II- The rats of this group received a food having a malathion concentration (16 ml./5000 ml. distilled water).

Group-III- The rats of this group received a cholesterol diet (400 mg./Kg. A body weight of cholesterol powder mixed with malathion of same concentration i.e.(16 ml./5000 ml.).

2.2.2 B - Rat (Female).

Group-I- **Intact (control):** The rats of this group received standard pallet diet food.

Group-II- The rats of this group received a food having a malathion concentration (16 ml./5000 ml. distilled water).

Group-III- The rats of this group received a cholesterol diet (400 mg./Kg. A
body weight of cholesterol powder mixed with malathion of same concentration i.e. (16 ml./5000 ml.).

Following parameters were considered:-

2.3 Organ and body weight relationship:-

The initial and final body weight of the animal was recorded. The reproductive tract of both and adrenal glands were taken out trimmed free of fat and each organ was weighed separately on electronic balance. The reproductive organ taken into account for study in male included testes, epididymes, ventral prostate, seminal vesicle, vas deferens, in female ovary, uterus and vagina.

Adrenal and body weight relationship:-

On the day of experiment, animal was sacrificed and adrenal glands were taken out and weighed before and after giving food having malathion concentration as well as malathion + cholesterol concentration.

Testes and body weight relationship:-

The weight of testes was also recorded before and after giving food with malathion concentration as well as malathion + cholesterol concentration and the relation between weight testes and body weight was studied.
Adrenal and gonad weight relationship:

The relationship between the weight of adrenal and the weight of testes or ovaries was studied by comparing weights before and after giving malathion concentrated food as well as malathion + cholesterol concentrated food.

2.4 Fertility Test:

Successful mating was achieved with all the animals prior to autopsy (male : female ratio 1:2). All female rats used for meeting studies were laparotomized on the 16th day of pregnancy. The number of implantations if any, were recorded. The female were then allowed to complete the pregnancy and the number of delivered pups and their morphology was observed (WHO, 1983).

2.5 Histological studies:

The Bouin’s fixed reproductive organs (testes, epididymides, seminal, vesicle, ventral prostate, vas deferens, ovary and uterus) were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 m) and staining (Harris haematoxyline and eosin). The adrenal tissue was used for electron microscopy.
2.6 Biochemical studies:

2.6.1 Tissue biochemistry:

1. Cholesterol was estimated in testes and adrenal glands in male rats and in female cholesterol was estimated in ovary in place of testes (Cf. Oser, 1965).

2. Glycogen was estimated in testes in male rats and in females glycogen was estimated in ovary in place of testes by the method of (Montgomery 1957).

3. Protein was estimated in the testes, cauda, epididymides, caput, epididymides, seminal vesicle, ventral prostate and vas deferens in male rats and in female protein was estimated in ovary and uterus (Lowry, et. al., 1951).

4. Sialic acid was estimated in testes, cauda epididymides, caput epididymides, seminal vesicle, ventral prostate and vas deferens in male rats and in female sialic acid was estimated in ovary and uterus (Warren, 1959).

5. Fructose was estimated in seminal vesicle (Mann, 1964).

6. Ascorbic acid was estimated in adrenal by the method of (Roe and Kuether 1943).
2.7 Haematological parameters:

1. RBC & WBC counting (Lynch et.al., 1969).

2. Haemoglobin (Drabkin’s Cynmethoglobin method by Fisher’s Haemophotometer).

3. Haematocrit (Wintrobe, 1930).


2.8 Serum biochemistry:

1. Total cholesterol (Zlatkis et.al., 1953).

2. High density lipoprotein (HDL) cholesterol (Burmstein et.al., 1970).


4. Triglycerides (Gottfried and Rosenberg, 1953).

5. VLDL-Cholesterol (Dedonder-Decoopman et.al., 1980).

6. LDL- Cholesterol (Shephred et.al., 1980).

7. Total protein (Lowry et.al., 1951).


2.9 Hormonal estimations-

Hormones like cortisol, FSH, LH, Prolactin, Testosterone was estimated in blood serum at different time periods like after (1, 6, 12, 18, 24, 30 days).

Statistical calculation:

All the values of body/organ weight biochemical estimation and histometry were expressed in terms of mean value ± standard error. The different treatment groups were compared with control group using student “t” test (Ispstein and Polya, 1970).

2.10 Histometric investigations-

For this, male and female rats were grouped in three groups i.e. group-I served as group of control male or female, group-II of animal treated by malathion for 15 days and lastly the group-III of consists of animals treated by malathion for 30 days. For the same time period animals was also treated by cholesterol (400mg/kg) diet + malathion concentration. On the day of experiment, animals were sacrificed and adrenal, ovary and testes were taken outside, washed thoroughly and cut into small pieces and fixed into glutaraldehyde and send to Jaslok hospital and research center at Mumbai.
Further processing of tissues were done in the laboratory of Electron Microscopy division of research center.

2.10.1 Essentials for electron microscopic study:-

One limitation of EM is the low penetration power of electrons. In current instruments, if specimen is more than 500nm (0.5 mm) thick, it appears almost totally opaque.

The specimen is generally deposited on an extremely fine film (7.5 to 15nm thick) of collodion, carbon, or other substance to support the specimen, and this film must be held up by a fine metal grid. For observation, under the EM the specimen is usually dehydrated and then placed in a vacuum.

2.10.2 Preparation of thin sections-EpoxyResins and ultramicrotomes:-

For thinner sections, hard embedding media are used. Those most often used are epoxyresins that impregnate the tissue and are tcatalyst.

To prepare the extremely thin sections, ultramichromomes are used. Several micrometromes have been designed that have a thermal or a mechanical advancing device. With both types, the thinnest section that can be made are of the order of 20nm. But for this, proper embedding and the sharpness of the cutting edge of the micrometome is required. Dimond knives are now in general use. Thin sectioning can be performed at low temperature with simple embedding in gelatin.