APPLIED ENTOMOLOGY
TOXICOLOGY OF INSECTICIDES

Dileep K. Singh
Department of Zoology
University of Delhi
Delhi-110007
INDIA
Email: dileepksingh@gmail.com
dksingh@zoology.du.ac.in

Content:
INTRODUCTION
TOXICOLOGY OF INSECTICIDES
GROUP CHARACTERISTICS AND FUNCTION OF INSECTICIDES
INSECTICIDES
MODE OF ACTION
METABOLISM OR DEGRADATION OF INSECTICIDES
TOXICOLOGICAL SYMPTOMS
THERAPY AND ANTIDOTES
SUMMARY
TERMINOLOGIES
1. INTRODUCTION:

Indian economy is mainly an agriculture based. Approximately 18 percent of the food grains go waste because of the plant pathogens, pests, weeds and rodents. To minimize these losses different pesticides are used. Pesticides are agrochemicals, used for preventing, repelling, mitigating or destroying any pests. It includes insecticides, fungicides, rodenticides and herbicides etc. Among these insecticides are of chemical or biological origin that controls the insect. Control may results in the form of killing the insect or otherwise preventing it from destructive behaviors. Insecticides are either natural or synthesized and are applied to target pests in a myriad of formulations (EC, WP, SP, FP, G etc.) and delivery systems (sprays, baits, slow-release diffusion, dust, etc.). In recent years, the bacterial genes coding for insecticidal proteins have been incorporated into various crops that dealt with the mortality of the pests feeding on them. Many other eco-friendly methods for insect pest control viz. IPM (Integrated Pest Management), use of bio-pesticides etc., are becoming popular. Bio-pesticides and IPM have a good growth in the future, as there is growing concern for the eco-friendly agriculture and could be achieved through Good Agriculture Practice.

History of insecticides may go back as far as 2500 BC, when the Sumerians used sulphur as an acaricide as well as an insecticide. In 1500 BC, first descriptions of cultural controls especially manipulation of planting dates was observed. In China, in about 1200 BC, chalk and wood ash were used to control the insects in enclosed spaces, and plant extracts were used for the treatment of stored grain. In addition, arsenic sulphide was used to control human lice. It is widely accepted that the ancient Greeks and Romans used sulphur, fumigants, oil sprays, oil and bitumen sticky bands, oil and ash, and other preparation for insect control. Pliny and Elder advocated the insecticidal use of arsenic and referred to the use of soda and olive oil for the treatment of legumes. Pliny reported the use of sulphur in 77 AD. In 300 A.D. first records of the use of biological control by predatory ants (*Oecophylla smaragdina*) was seen in citrus groves with bamboo bridges to move between trees to control caterpillar and beetle pests.

Progress with insecticides came with the introduction of botanicals such as pyrethrum, derris, quassia, and tobacco leaf infusion around the 16th century. Perhaps the first organic insecticide was nicotine that was applied in its natural form as crushed tobacco leaves for the control of aphids as early as 1763. The most successful botanical has been pyrethrum, a mixture of natural esters extracted from *Chrysanthemum* flowers grown principally in Kenya.

The first stomach poison was probably arsenic, which was mixed with honey in the mid 1600 on ant bait. In the late 1810, Paris green which is copper salt of arsenic was used in 1867 to control Potato Colorado Beetle, codling moth and other leaf-eating insects when dusted on plant foliage. In 1892 lead arsenate was introduced as one of the most effective inorganic insecticides for control of pests such as gypsy moth, apple maggot, and various soil insects. One serious drawback in the application of most of these compounds was their high toxicity to mammals. Other inorganic compounds containing mercury, tin, or copper were also used as stomach poisons during this period. In 1888, the first significant success in biological control was achieved on the suggestion of C.V.Riley of California. The Vedalia beetle, *Rodolia cardinalis* was introduced into California from Australia for the control of cottony-cushion scale, *Icerya purchasi* on citrus orchards. An inventory of insecticides used in the 19th century would include sulphur, arsenicals, fluorides, soaps, kerosene and various botanicals, of which nicotine, rotenone, pyrethrum, sabadilla and quassia appear to have been most widely used. The use of biological control agents had also begun.

The first four decades of the 20th century saw a significant progress in the synthesis of insecticides. Chemical insect control changed dramatically following the discovery of insecticidal properties of dichlorophenyl trichloro ethane that later on popularly known as DDT. This was done by Paul Muller of J.R. Geigy Company in 1939. Its first important use was to control malaria and typhus by the Western allies during World War II. This marked the era of the chlorinated hydrocarbon insecticides, with the subsequent synthesis of hexachlorocyclohexane (HCH) and the cyclodiene compounds. These chlorinated
hydrocarbons were welcomed at the beginning, but their stability and their hydrophobicity resulted in the contamination of the environment and bioconcentration in the body of many animals, thus they were restricted in use or banned later on. The first generation insecticides, i.e the chlorinated hydrocarbons are still in use, though in developed countries, their use has been restricted or banned. India has recently banned the use of DDT and HCH in agriculture.

Synthesis of organophosphorous insecticides was subsequently done on a world wide scale (starting from Germany). Three of these compounds HETP, Parathion and Schradan attributed to Gerhard Schrader, were extensively used. They were not persistent, and, in attempts to lower the mammalian toxicity and increase the efficacy, hundreds of other OP insecticides have been synthesised. Thus the most important group of insecticides is still the organophosphorous compounds.

The existence of another class of insecticides, the carbamates, was foreshadowed by Swiss workers in 1940, but the first major success was with the introduction of the American insecticide carbaryl in 1950. It was followed by synthesis of many other carbamates. They are all nerve poisons and specifically acetylcholinesterase inhibitors, as are the organophosphates.

Pyrethrum use levies high cost on the farmer, thus numerous analogues of the compound were prepared with the hope of finding an inexpensive replacement. The period between 1949 and the early 1970s saw the development of a number of synthetic pyrethrin analogs. The first synthetic pyrethroid was introduced in 1976, and since then they have become the second largest class of insecticide used today. These compounds are effective at very low rates. They are among the least toxic to mammals.

Insecticide development till this time was guided mostly by chemorational design. Gradually biorational design based on basic understanding of physiology and ecology of insects and crops, become popular. These include the insect growth regulators (IGR), such as chitin synthesis inhibitors, juvenile hormone mimics, ecdysone agonists, pymetrozine, and other novel agents such as pheromones, Bacillus thuringiensis, avermectins, formamidines.

In spite of severe environmental pressures, the world market for pesticides has grown phenomenally. There is a boom in the global pesticide market and new insecticides, herbicides and fungicides and their formulations are being introduced with greater level of activity, however, with conscious efforts for minimizing the hazards to the human beings and the environment.

India produces 46000 metric tones of Technical Grade pesticides in a year and pesticide industry is recent and the largest in Asia and is the twelfth largest in the world. It uses about 3 % of the total pesticides used in the world in terms of values. However, per hectare consumption of pesticides in India is very low. Insecticides are used predominantly and most important crops having larger share of pesticides are cotton and rice. Within the crop segments cotton is king for the pesticides industry with over 50% consumption leaving the gamut of so many other crops from the benefits of chemical crop protection. In insecticides organophosphates (50 %) dominates the Indian market followed by pyrethroids (19 %), organochlorine (18 %), carbamates (4 %) and bio-pesticides (1 %).

Table 1. Different groups of pesticides, their examples and current status for use.

<table>
<thead>
<tr>
<th>Pesticides Class and Types</th>
<th>First Used</th>
<th>Examples</th>
<th>Current Status</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organochlorines</strong> (insecticide, acaricide, HCB &amp; PCP are fungicides)</td>
<td>1942</td>
<td>Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor, Lindane, Methoxychlor, Toxaphene,</td>
<td>Lindane, methoxychlor and pentachlorophenol are registered in Canada. The other products have been discontinued in Canada, but they</td>
<td>Persistent, bioaccumulative, affect the ability to reproduce, develop, and to withstand environmental stress by depressing the nervous, endocrine and immune systems</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th><strong>Organophosphates</strong> (insecticide, acaricide)</th>
<th>Very early 1940s</th>
<th>Schradan, Parathion, Malathion</th>
<th>Schradan was discontinued in 1964 and resulted in a move toward less toxic groups (e.g. malathion, parathion)</th>
<th>Non-persistent, systemic (cholinesterase-inhibiting), not very selective, toxic to human</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carbamates</em> (Fungicide, insecticide, acaricide)</td>
<td>First appeared in 1930 but large-scale use in mid-1950s</td>
<td>Carbaryl, Methomyl, Propoxur, Aldicarb</td>
<td>Aldicarb was discontinued in 1964, the others are registered in Canada. Although carbamates share a mode of action with the organophosphates, their effects are reversible and they are biotransformed in-vivo</td>
<td>Non-persistent, cholinesterase-inhibiting, not very selective, toxic to birds and fish</td>
</tr>
<tr>
<td><strong>Phenoxy</strong> (Herbicide)</td>
<td>Large-scale marketing and distribution began in 1946</td>
<td>2,4-D, 2,4,5-T</td>
<td>2,4-D is widely used. 2,4,5-T banned in Canada. Selective effects on humans and mammals are not well known. 2,4-D: potential to cause cancer in laboratory animals. 2,4,5-T: is the source of a toxic contaminant dioxin</td>
<td></td>
</tr>
<tr>
<td><em>Pyrethroids</em> (insecticide)</td>
<td>1980</td>
<td>Fenpropanthrin, Deltamethrin, Cypermethrin</td>
<td>Fenpropanthrin is not registered in Canada, unlike the two other pesticides. Target-specific: more selective than the organophosphates or carbamates, generally not acutely toxic to birds or mammals but particularly toxic to aquatic species.</td>
<td></td>
</tr>
</tbody>
</table>

There are 190 pesticide molecules registered for the use in India under section 9(3) of Insecticides Act, 1968 (as on 7th March, 2005) and out of it, 8 pesticides come in the category of Restricted Pesticides. There are 12 biopesticides registered under Insecticides Act, 1968. There are 25 pesticides banned from manufacture, import and use.

Certain Indian Companies have made substantial investments and have made a name and reputation for themselves in the world markets. India is now recognized as an important source for supply of generic products. A very large number of units in the small-scale sector are involved in formulations and sell their...
products essentially on regional basis. Some of the leading pesticide industries of India are Bayer India, Excel Industries, Monsanto India, Rallis India, Syngenta India, United Phosphorus, etc. The industry manufactures two main types of products:

(a) Technical grade pesticides (the basic concentrated chemical compound). Technical grade pesticides are both manufactured locally as well as imported.

(b) Formulations from these technical grade pesticides (the usable form of pesticides).

Table 2. Pesticides registered for the use in India under section 9(3) of Insecticides Act, 1968 (as on 3rd August, 2006).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Pesticide</th>
<th>Sl. No.</th>
<th>Name of the Pesticide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,4-Dichlorophenoxy Acetic Acid</td>
<td>96</td>
<td>Glyphosate</td>
</tr>
<tr>
<td>2</td>
<td>Acephate</td>
<td>97</td>
<td>Hexaconazole</td>
</tr>
<tr>
<td>3</td>
<td>Acetamiprid</td>
<td>98</td>
<td>Hydrogen Cyanamid</td>
</tr>
<tr>
<td>4</td>
<td>Alachlor</td>
<td>99</td>
<td>Imiprothrin</td>
</tr>
<tr>
<td>5</td>
<td>Alphacypermethrin</td>
<td>100</td>
<td>Imazethapyr</td>
</tr>
<tr>
<td>6</td>
<td>Alphanaphthyl Acetic Acid</td>
<td>101</td>
<td>Imidacloprid</td>
</tr>
<tr>
<td>7</td>
<td>Aluminium Phosphate</td>
<td>102</td>
<td>Iprobenfos (Kitazin)</td>
</tr>
<tr>
<td>8</td>
<td>Anilophos</td>
<td>103</td>
<td>Indoxacarb</td>
</tr>
<tr>
<td>9</td>
<td>Atrazine</td>
<td>104</td>
<td>Iprodione</td>
</tr>
<tr>
<td>10</td>
<td>Aureofungin</td>
<td>105</td>
<td>Isoprothiolane</td>
</tr>
<tr>
<td>11</td>
<td>Azadirachtin (Neem Products)</td>
<td>106</td>
<td>Isoproturon</td>
</tr>
<tr>
<td>12</td>
<td>Bacillus thuringiensis (B.t. &amp; Bs.)</td>
<td>107</td>
<td>Kasugamycin</td>
</tr>
<tr>
<td>13</td>
<td>Barium Carbonate</td>
<td>108</td>
<td>Lambdacyhalothrin</td>
</tr>
<tr>
<td>14</td>
<td>Beauveria bassiana</td>
<td>109</td>
<td>Lime Sulphur</td>
</tr>
<tr>
<td>15</td>
<td>Bendiocarb</td>
<td>110</td>
<td>Lindane</td>
</tr>
<tr>
<td>16</td>
<td>Benomyl</td>
<td>111</td>
<td>Linuron</td>
</tr>
<tr>
<td>17</td>
<td>Beta Cyfluthrin</td>
<td>112</td>
<td>Lufenuron</td>
</tr>
<tr>
<td>18</td>
<td>Bifenthrin</td>
<td>113</td>
<td>Magnesium Phosphide Plates</td>
</tr>
<tr>
<td>19</td>
<td>Butachlor</td>
<td>114</td>
<td>Mancozeb</td>
</tr>
<tr>
<td>20</td>
<td>Carbaryl</td>
<td>115</td>
<td>Malathion</td>
</tr>
<tr>
<td>21</td>
<td>Carbendazim</td>
<td>116</td>
<td>Mepiquate Chloride</td>
</tr>
<tr>
<td>22</td>
<td>Buprofezin</td>
<td>117</td>
<td>Metalaxyl</td>
</tr>
<tr>
<td>23</td>
<td>Benfuracarb</td>
<td>118</td>
<td>Metaldehyde</td>
</tr>
<tr>
<td>24</td>
<td>Captan</td>
<td>119</td>
<td>Metasulfuron Methyl</td>
</tr>
<tr>
<td>25</td>
<td>Carbanil</td>
<td>120</td>
<td>Methabenzthiazuron</td>
</tr>
<tr>
<td>26</td>
<td>Carbofuran</td>
<td>121</td>
<td>Methomyl</td>
</tr>
<tr>
<td>27</td>
<td>Carbofuran</td>
<td>122</td>
<td>Methoxy Ethyl Mercury Chloride (MEMC)</td>
</tr>
<tr>
<td>28</td>
<td>Carbosulfan</td>
<td>123</td>
<td>Methyl Bromide</td>
</tr>
<tr>
<td>29</td>
<td>Carboxin</td>
<td>124</td>
<td>Methyl Chlorophenoxy Acetic Acid (MCPA)</td>
</tr>
<tr>
<td>30</td>
<td>Carpropamid</td>
<td>125</td>
<td>Methyl Parathion</td>
</tr>
<tr>
<td>31</td>
<td>Cartap Hydrochloride</td>
<td>126</td>
<td>Metolachlor</td>
</tr>
<tr>
<td></td>
<td>Chemical Name</td>
<td></td>
<td>Active Ingredient Name</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------</td>
<td>---</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>33</td>
<td>Chlorofenvinphos</td>
<td>127</td>
<td>Metoxuron</td>
</tr>
<tr>
<td>34</td>
<td>Chlorfenapyr</td>
<td>128</td>
<td>Metribuzin</td>
</tr>
<tr>
<td>35</td>
<td>Chlorimuron ethyl</td>
<td>129</td>
<td>Metiram</td>
</tr>
<tr>
<td>36</td>
<td>Chloromequat Chloride (CCC)</td>
<td>130</td>
<td>Milbemectin</td>
</tr>
<tr>
<td>37</td>
<td>Chlortalonil</td>
<td>131</td>
<td>Monocrotophos</td>
</tr>
<tr>
<td>38</td>
<td>Chlorpyrifos</td>
<td>132</td>
<td>Myclobutanil</td>
</tr>
<tr>
<td>39</td>
<td>Chlorpyriphos Methyl</td>
<td>133</td>
<td>Novaluron</td>
</tr>
<tr>
<td>40</td>
<td>Cinnmethyline</td>
<td>134</td>
<td>Nuclear polyhydrrosis virus of Helicoverpa armigera</td>
</tr>
<tr>
<td>41</td>
<td>Clodinafop-propargyl(Pyroxofop-propargyl)</td>
<td>135</td>
<td>Oxadiargyl</td>
</tr>
<tr>
<td>42</td>
<td>Clomazone</td>
<td>136</td>
<td>Oxadiazon</td>
</tr>
<tr>
<td>43</td>
<td>Copper Hydroxide</td>
<td>137</td>
<td>Oxycarboxin</td>
</tr>
<tr>
<td>44</td>
<td>Copper Oxychloride</td>
<td>138</td>
<td>Oxydemeton-Methyl</td>
</tr>
<tr>
<td>45</td>
<td>Copper Sulphate</td>
<td>139</td>
<td>Oxyflucoxin</td>
</tr>
<tr>
<td>46</td>
<td>Coumaranil</td>
<td>140</td>
<td>Paclorbutrazole</td>
</tr>
<tr>
<td>47</td>
<td>Coumatetralyl</td>
<td>141</td>
<td>Paraquat dichloride</td>
</tr>
<tr>
<td>48</td>
<td>Cuprous Oxide</td>
<td>142</td>
<td>Penconazole</td>
</tr>
<tr>
<td>49</td>
<td>Cyfluthrin</td>
<td>143</td>
<td>Pendimethalin</td>
</tr>
<tr>
<td>50</td>
<td>Cyhalofop-butyl</td>
<td>144</td>
<td>Permethrin</td>
</tr>
<tr>
<td>51</td>
<td>Cymoxanil</td>
<td>145</td>
<td>Phenthoate</td>
</tr>
<tr>
<td>51</td>
<td>Cymoxanil</td>
<td>146</td>
<td>Phorate</td>
</tr>
<tr>
<td>52</td>
<td>Cypermethrin</td>
<td>147</td>
<td>Phosalone</td>
</tr>
<tr>
<td>53</td>
<td>Cyphenothrin</td>
<td>148</td>
<td>Phosphamidon</td>
</tr>
<tr>
<td>54</td>
<td>Dazomet</td>
<td>149</td>
<td>Primiphos-methyl</td>
</tr>
<tr>
<td>55</td>
<td>Deltamethrin (Decamethrin)</td>
<td>150</td>
<td>Prallethrin</td>
</tr>
<tr>
<td>56</td>
<td>Diazinon</td>
<td>151</td>
<td>Pretilachlor</td>
</tr>
<tr>
<td>57</td>
<td>Dichloro Diphenyl Trichloroethane (DDT)</td>
<td>152</td>
<td>Profenophos</td>
</tr>
<tr>
<td>58</td>
<td>Dichloropropene and Dichloropropene mixture (DD mixture)</td>
<td>153</td>
<td>Propanil</td>
</tr>
<tr>
<td>59</td>
<td>Dichlorvos (DDVP)</td>
<td>154</td>
<td>Propergite</td>
</tr>
<tr>
<td>60</td>
<td>Diclofop-Methyl</td>
<td>155</td>
<td>Propetamphos</td>
</tr>
<tr>
<td>61</td>
<td>Dicofol</td>
<td>156</td>
<td>Propiconazole</td>
</tr>
<tr>
<td>62</td>
<td>Difenocenazole</td>
<td>157</td>
<td>Propineb</td>
</tr>
<tr>
<td>63</td>
<td>Difenhiuron</td>
<td>158</td>
<td>Propoxur</td>
</tr>
<tr>
<td>64</td>
<td>Diflubenzuron</td>
<td>159</td>
<td>Pyrethrins (pyrethrum )</td>
</tr>
<tr>
<td>65</td>
<td>Dimethoate</td>
<td>160</td>
<td>Quinalphos</td>
</tr>
<tr>
<td>66</td>
<td>Dimethomorph</td>
<td>161</td>
<td>Quizalofop ethyl</td>
</tr>
<tr>
<td>67</td>
<td>Dinocap</td>
<td>162</td>
<td>S-bioallethrin</td>
</tr>
<tr>
<td>68</td>
<td>Dithianon</td>
<td>163</td>
<td>Sirmate</td>
</tr>
<tr>
<td>69</td>
<td>Diuron</td>
<td>164</td>
<td>Sodium Cyanide</td>
</tr>
<tr>
<td>70</td>
<td>Dodine</td>
<td>165</td>
<td>Spinosad</td>
</tr>
<tr>
<td>71</td>
<td>D-trans Allethrin</td>
<td>166</td>
<td>Streptomycin + Tetracycline</td>
</tr>
<tr>
<td>72</td>
<td>Edifenphos</td>
<td>167</td>
<td>sulfosulfuron</td>
</tr>
<tr>
<td>73</td>
<td>Endosulfan</td>
<td>168</td>
<td>sulphur</td>
</tr>
<tr>
<td>74</td>
<td>Ethephon</td>
<td>169</td>
<td>Tebuconazole</td>
</tr>
<tr>
<td>75</td>
<td>Ethion</td>
<td>170</td>
<td>Temephs</td>
</tr>
<tr>
<td>76</td>
<td>Ethofenprox (Etofenprox)</td>
<td>171</td>
<td>Thiobencarb (Benthiocarb)</td>
</tr>
<tr>
<td>S. No.</td>
<td>Name of the Biopesticide</td>
<td>S. No.</td>
<td>Name of the Biopesticide</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------</td>
<td>-------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td><em>Bacillus thuringiensis</em> var. <em>israelensis</em></td>
<td>4.</td>
<td><em>Bacillus sphaericus</em></td>
</tr>
</tbody>
</table>

Table 3. Bio-pesticides registered under Insecticides Act, 1968

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the pesticide</th>
<th>S. No.</th>
<th>Name of the pesticide</th>
<th>S. No.</th>
<th>Name of the pesticide</th>
</tr>
</thead>
</table>

Table 4. Restricted pesticides for use in agriculture.
Table 5. Pesticides banned for manufacture, import and use.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aldrin</td>
<td>10. Heptachlor</td>
<td>19. Toxafen</td>
</tr>
<tr>
<td>3</td>
<td>Calcium Cyanide</td>
<td>12. Nitrofen</td>
<td>21. Chlorobenzilate</td>
</tr>
<tr>
<td>4</td>
<td>Chlordane</td>
<td>13. Paraquat Dimethyl Sulphate</td>
<td>22. Dieldrine</td>
</tr>
<tr>
<td>5</td>
<td>Copper Acetoarsenite</td>
<td>14. Pentachloro Nitrobenzene</td>
<td>23. Maleic Hydrazide</td>
</tr>
<tr>
<td>6</td>
<td>Clbromochloropropane</td>
<td>15. Pentachlorophenol</td>
<td>24. Ethylene Dibromide</td>
</tr>
<tr>
<td>7</td>
<td>Endrin</td>
<td>16. Phenyl Mercury Acetate</td>
<td>25. TCA (Trichloro acetic acid)</td>
</tr>
<tr>
<td>8</td>
<td>Ethyl Mercury Chloride</td>
<td>17. Sodium Methane Arsonate</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ethyl Parathion</td>
<td>18. Tetradike</td>
<td></td>
</tr>
</tbody>
</table>

2. TOXICOLOGY OF INSECTICIDES

Toxicology is defined as the study of the adverse effects of chemicals on living systems. Here insecticide is a toxic chemical. Toxicity of chemicals depends on the nature of toxicant, routes of exposure (oral, dermal and inhalation), dose and organism. Toxicity of insecticides usually expressed in terms of LD$_{50}$ or LC$_{50}$. Its values are expressed in term of milligram per kilogram body weight or ppm respectively.

**2.1. LD$_{50}$ (Lethal Dose 50 percent):**

The term LD$_{50}$ is expressed, as the single exposure dose of the poison per unit weight of the organism required to kill 50% of the test population, where the population is genetically homogeneous. It is applied in a particular way under stated experimental conditions. It is usually expressed in terms of mg poison per kilogram body weight of the experimental animals. Under certain conditions, the term micrograms per insect (µg/insect) may be used when chemical is applied topically to the insect.

The LD$_{50}$ can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

It is a frequently used to measure of acute toxicity of an insecticide on an organism.

It is also important to know that the actual LD$_{50}$ value may be different for a given chemical depending on the route of exposure e.g., oral, dermal, inhalation. For example, some LD$_{50}$ in rat for dichlorvos, an insecticide commonly used in household pesticide strips, are listed below:

1. Oral LD$_{50}$(rat): 56 mg/kg
2. Dermal LD$_{50}$(rat): 75 mg/kg
3. Intraperitoneal LD$_{50}$(rat): 15 mg/kg
4. Inhalation LC$_{50}$(rat): 1.7 ppm (15 mg/m$^3$), 4-hour exposure
5. In general, the smaller the LD$_{50}$ value, the more toxic is the chemical. The opposite is also true, larger the LD$_{50}$ value, the lower the toxicity.

**Table 5.** Toxicity scale for pesticides.

<table>
<thead>
<tr>
<th>Category</th>
<th>LD$_{50}$ oral mg/kg(ppm)</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely toxic</td>
<td>1 mg/kg(ppm) or less</td>
<td>Parathion, aldicarb</td>
</tr>
<tr>
<td>Highly toxic</td>
<td>1-50 mg/kg(ppm)</td>
<td>Endrin</td>
</tr>
<tr>
<td>Toxicity Level</td>
<td>Concentration</td>
<td>Chemical</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Moderately toxic</td>
<td>50-500 mg/kg (ppm)</td>
<td>Carbofuran</td>
</tr>
<tr>
<td>Slightly toxic</td>
<td>500-1000 mg/kg (ppm)</td>
<td>Malathion</td>
</tr>
<tr>
<td>Non-toxic (practically)</td>
<td>1-5 gm/kg</td>
<td></td>
</tr>
</tbody>
</table>

2.2. **LC\(_{50}\) (Lethal Concentration 50 percent):**

LC\(_{50}\) is the concentration of the chemical in the external medium (usually air or water surrounding experimental animals), which causes 50% mortality of the test population, where the population is genetically homogeneous. This value is used when the exact dose given to the individual is difficult to be determined. LC\(_{50}\) is expressed as the percent of active ingredient of the chemical in the medium or as parts per million (ppm).

2.3. **Dose response relationships:**

Toxicity of chemical is determined by quantifying the response on test animal to a series of increasing dose. The relationship between animal and administered dose can be graphically presented as dose-response relationship curve. Dose response relationships means the relationship between the dose of a chemical substances administered or received and the incidence of an adverse health effect in exposed population.

In toxicology, the dose is very important which determine its impact on organism. It is the dose which makes substances a poison. The right dose differentiates a poison and a remedy. At high doses, all the chemicals are toxic, at judicious doses they are useful and at very low doses they do not have a detectable toxic effect.

A dose-response relationship is based on the following important assumptions:

- There is always a threshold dose below which no effect occurs.
- Once effect occurs, response increases as dose increases.
- Once a maximum response is reached, any further increases in the dose will not result in any increased effect.

![Diagram](image.png)

Figure 1. Typical sigmoid cumulative dosage-response curve for a toxic effect which is symmetrical about the average (50 percent response) point.

When a genetically homogeneous population of animals of the same species and strain is exposed to a toxicant, the proportion exhibiting a particular toxic effect will increase as the dosage increases. This is
shown schematically (Figure 1.) as a cumulative distribution curve, where the number of animals responding is plotted as a function of the dosage given (as a \( \log_{10} \) function). A few individuals respond to relatively low doses (constituting a hyperreactive group), most respond to medium dose, and a small number required a relatively high dose (constituting a hyporeactive group) before they are affected. The lowest dose at which an effect is discernable is referred to as the no observable effect level (NOEL).

2.4. Carcinogenic, Mutagenic and Teratogenic effects:

To understand the effects of pesticides on organisms, studies on long term exposure of pesticides at the doses that do not immediately kill the organism are required. These are referred as long-term, or chronic, studies. The consequences of chronic exposure to toxicants can results in different responses in the exposed organisms. The following responses are typically evaluated for pesticides.

Carcinogenic effect:
Carcinogenesis is the production or increase in cancer frequency in the test organisms relative to exposure to a toxicant.

A number of pesticides have been reported to induce tumors in mice and rats in laboratory tests. DDT and other chlorinated hydrocarbon insecticides have been shown to cause marked changes in the liver of various rodents, and these changes may progressed to tumor formation in some species, notably in the mouse. However, DDT failed to produce detectable tumors in pesticide industry workers who absorbed DDT for 19 years or more at rates hundreds of time higher than those found in the general population.

Mutagenic effects:
Mutagenicity refers to the induction of permanent changes in the amount or structure of genetic material of cells or organisms, which can be transmitted to the next generations. Changes induced in cells by a mutagen can cause cancer, while damage to the egg and sperm can cause adverse reproductive and developmental outcomes.

There are a large number of assays for mutagenesis such as point mutations in bacteria, yeast and mold, mammalian cells in vitro, tests for chromosomal aberrations, etc. The Ames Test with *Salmonella typhimurium* is a popular assay procedure and is used extensively by many investigators and chemical industries to test the safety of new products before their introduction in the market.

Teratogenic effects:
Teratogenesis is a medical term from the Greek, literally meaning *monster-making*, which derives from teratology, the study of the frequency, causation, and development of congenital malformations misleadingly called *birth defects*.

Teratogenesis has gained a more specific usage for the development of abnormal cell masses during fetal growth causing physical defects in the fetus. Such deformities can also be observed in rodent fetuses by direct inspection or with the aid of a microscope.

Teratogenesis does not include toxic injury to organs after they are fully formed.

Methods of testing chemicals on insect and evaluation of toxicity:
There are several methods of administering a chemical to an insect. A commonly employed methods are,

2.5.1. Topical application
Where the insecticide is dissolved in a relatively nontoxic and volatile solvent such as acetone, and is then allowed to come in contact with a particular location on the body surface. The results are expressed as micrograms of active ingredient per insect (\( \mu\)g AI/ insect) or (\( \mu\)g AI/g insect). The advantages of this method are,

1. The high degree of precision and reproducibility that can be attained.
2. The large number of tests that can be performed in a relatively short time.
3. The small number of insects (10-20) required per replication.
4. The simple and inexpensive equipment needed.
5. The very small amount of chemicals and solvents used.
6. The fact that the LD$_{50}$ values obtained for any species are reasonably constant and reproducible from laboratory to laboratory, provided that identical conditions of testing are maintained.

2.5.2. Injection Method:
Injection method is used when knowledge of the exact amount of insecticide inside the body of the insect is required.
Very fine stainless steel needles of 27 or 30 gauge (0.41 or 0.30 mm in diameter) are used. Small glass needles of 0.1-0.16 mm in diameter may be used for injection to small insects.
The insecticide is commonly dissolved in propylene glycol or peanut oil and injection is made intraperitoneally (into the body cavity).
Care must be taken to avoid bleeding by the insects.

2.5.3. Dipping Method:
This method is employed when topical application or injections are impractical, e.g., with small plant feeding insects, housefly larvae, insect eggs, etc.
The insects are dipped in aqueous solutions, emulsions, or suspensions of the chemical for short periods of time.
In this case, the LC$_{50}$ is used to express the results.

2.5.4. Contact or Residual Method:
The insecticide in a volatile solvent is applied to a glass container such as a vial or a jar. The solvent is allowed to evaporate by rotating the container so that the insecticide is spread evenly over the entire surface leaving a residual film. Alternatively, the insecticide is applied evenly on a glass, filter paper, wood panel or other types of building materials and allowed to dry before exposing the insects to the residual deposits. The deposits are expressed as mg or g of active ingredient per square meter (mg or g AI/m$^2$).

Feeding and Drinking Method:
These methods are used to evaluate the toxicity of ingested chemicals. They may be classified as unlimited availability of food or drink or as limited dose feeding.
Unlimited feeding includes: textiles tests for moth-proofing, treated flour or grain, media for fly larvae, sprayed or dusted foliage, poison baits.
Limited dose feeding include: coated leaves disks, sandwiches, squares, strips and pellets.
Drinking methods include: sugar syrups and drinking through membranes such as plant juices or blood. In addition, there are methods for screening chemical attractants and repellents, for screening animal sprays, dusts, dips and dressings, and techniques for evaluating systemic insecticides against livestock insects.

2.6. Evaluation of Toxicity in insects and animals:
The toxicity evaluation process or toxic interactions of any chemical and any given biological system are dose related. At extremely high concentrations, most chemicals have toxic effects on biological systems. The toxicology of poisonous chemicals can be termed as the science of doses.
In order to assess the susceptibility of any population to a certain poison, probit units of percent mortalities are customarily plotted against a logarithmic scale of dosages. This method of computation yields a straight line which facilitates the determination of the LD$_{50}$ and other values on the plot (figure 2).
Figure 2. Plot of log$_{10}$ dose versus probit value of percent mortality showing effective dose (ED), toxic dose (TD), and lethal dose (LD).

If there is natural mortality in the controls, adjusted mortality is used according to Abbott’s formula (Abbott 1925) as follows:

Corrected Mortality Percent = \frac{(P - P_0) \times 100}{(100-P_0)}

Where P is the percent mortality of treated insects and P$_0$ is the percent mortality of insects in the untreated control. This adjusted value is permissible when mortality in the controls does not exceed 20 percent or when mortality is based on a large number of replications.

Evaluation of toxicity in higher animals is different from that of insects because the number of available animals for testing usually is limited. While the process for determining LD$_{50}$ is identical, greater emphasis is placed on qualitative rather than quantitative aspects of poisoning. Another characteristic of toxicological tests in higher animals is that, in most cases, the overriding concern is the evaluation of safety for man.

Selection of the test animal is usually based on convenience and cost. For ordinary testing of LD$_{50}$ values rats or mice are the animals of choice. The animals should be healthy and of acceptable genetic homogeneity. Factors influencing toxicity includes, duration of exposure, route of administration, species, individual variation, age, sex, population density temperature, and nutrition.

3. GROUP CHARACTERISTICS AND FUNCTION OF INSECTICIDES

Insecticides can be defined as any substance or mixture of substances used for killing, repelling or controlling insect pests. It can be classified into following groups depending on their chemical structure.

3.1 Group characteristics and functions of Organochlorine (OC) insecticides:

Organochlorine Insecticides (also known as chlorinated hydrocarbons) are mainly characterized by,

- Presence of carbon (organo-), chlorine and hydrogen and sometimes oxygen atoms including a number of C-Cl bonds.
- Presence of cyclic carbon chains including benzene ring.
- Lack of any particular active intramolecular sites.
- They are polar and liposolistic (soluble in fat).
- Chemically unreactive, it is that they are highly persistent in the environmental, resistant to microbial degradation and have a tendency to concentrate in the lipid rich tissues, thereby causing its bioconcentration, and biomagnification in the food chain.

Organochlorines group were first used in the 1940s. From about 1945 to 1965, organochlorines were used extensively in all aspects of agriculture and forestry, in protecting wooden buildings and protecting humans from a wide variety of insect pests. After realisation of the fact that they are highly persistant, legal action to phase out this class of insecticide was undertaken. Common examples include DDT, Lindane, Endosulfan.
Group characteristics and functions of organophosphorous insecticides:
Organophosphorous insecticides (often refer to as organo phosphates) are neutral ester or amide derivatives of phosphorous acids carrying a phosphoryl (P-O) or thiophosphoryl (P-S) group. Certain fluorides and chlorides are also used commercially to develop organophosphorus insecticides, however only one phosphoric acid is known for its insecticidal properties. It was Gerhard Schrader (and his co-workers) of Germany who discovered the insecticidal properties of first OP pesticides in 1937, insecticide was named after its discoverer, Schrandn with general formula $R_1, R_2, and R_3$ are alkyl groups, and Ary is inorganic or organic radical (Cl, F, SCN, CH$_3$COO).

OP insecticides identified by single characteristic i.e., they act by inhibiting cholinesterase enzyme. OP compounds are manufactured at very high temperatures (150-200°C), thus they commonly contain isomers or bioproducts which are responsible for their unpleasant odour, and for their anticholinesterase activity. Ops are readily activated and degraded in mammals and by micro-organisms and therefore do not accumulate thus OP insecticides are non-persistant and are quite biodegradable. It is because of this quality that OP insecticides have largely replaced the persistant Organochlorine insecticides becoming one of the largest groups of insecticides in use today. Early products such as parathion had very high mammalian toxicities (LD$_{50}$ rat <5 mg/kg body weight) which made them very hazardous to use. However more recent Ops are much less toxic, e.g. pirimiphos methyl (LD$_{50}$ rat 2000 mg/kg), and are widely used in agriculture. OP pesticides have different names in different countries. Thus rules were adopted by agreement of British Chemical Society and American Chemical Society in 1952. OP compounds are named as derivative of their corresponding parent compound (acids or hydrides).

HYDRIDES
- Phosphine $\text{H}_3\text{P}$
- Phosphine Oxide $\text{H}_3\text{PO}$
- Phosphine Sulphide $\text{H}_3\text{PS}$
- Phosphorane $\text{H}_5\text{P}$

TRIVALENT ACIDS
3.3. Group characteristics and functions of carbamates:

Carbamate are anticholinesterase insecticide and are synthetic derivative of physostigmine, also known as eserine, which is a principle alkaloid of plant, *physostigma benenosum*, calabar bean.

Chemically, they are esters of carbamic acid, HOOC.NH₂.

Carbamates with insecticidal (and related) properties possess the general structure:

\[
\begin{align*}
\text{R} & \quad \text{O} \quad \text{R}_2 \\
\text{R}_3 & \quad \text{O} \quad \text{CN} \quad \text{R}_1 \\
\end{align*}
\]
R₁, and R₂ are hydrogen, methyl, ethyl, propyl or other short chain alkyls and R₃ is phenol, napthyl ring or other cyclic hydrocarbons or oxime derivative.

There are 3 major sub-groups of carbamates:
Sub-group 1, comprises N-methyl carbamate esters of phenols that is the compounds with a hydroxyl group attached directly to a phenyl or napthyl ring e.g. Carbaryl (1-napthyl N-methyl carbamate)
Sub-group 2, similarly comprises N-methyl and N-dimethyl esters of heterocyclic phenols e.g. Carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl N-methyl carbamate).
Sub-group 3, contains oxime (the OH group of which has been carbamylated) e.g. Aldicarb.

Their distinctive feature is their low toxicity to mammals (exception is aldicarb) and broad spectrum to insect control (used widely for lawn and garden insects).

Carbamate insecticides possess both contact and stomach toxicity. They are nerve poisons that inhibit acetylcholinesterase at nerve synapses, cause rapid twitching of muscles, incoordination (ataxia), convulsions, paralysis and death.

Carbamates are degraded by many enzyme catalysed reactions, primarily through hydrolysis, oxidation and conjugation.

Carbamate insecticides came into use later than both the organochlorines and the organophosphates and are less widely used than the others. Their use is diverse, some are used extensively for forest protection, while others are widely used against insect pests of potatoes and grains. Examples are carbaryl (Sevin) and aldicarb (Temik), etc.

---

3.4. Group characteristics and functions of pyrethroids:
Pyrethroids are natural esters formed by combination of 2 carboxylic acid and 3 keto acid. It is extracted from plant chrysanthemum cinerariaefolium, in which flower contains on an average 1.3 percent pyrethrins that was first used as powder in around 1851.
Pyrethrum concentrate was prepared from flower by extracting with petroleum ether or methanol or acetone or dichloromethane or ethylene dichloride. Technical pyrethrum contains 20-30 percent toxic ingredient. LD$_{50}$ is around 1500 mg/kg body weight for rat, but very toxic for insects. There are four principal active ingredients in pyrethrum flowers, known as pyrethrins I and II and cinerins I and II. All 4 are esters comprising an acid containing a 3C ring joined to an alcohol containing a 5C ring. In addition, small quantities of jasmolins I and II are present, these differ from the pyrethrins only in that one double bond in the side chain of the alcohol moiety of pyrethrins is saturated. The acid present in compounds designated by I is called chrysanthemic acid while in those designated by II is called pyrethric acid.

Although natural source pyrethrum has been used for hundreds of years, Synthetic Pyrethroid (SP) group of insecticides was introduced more recently, in the early 1970s. SPs are structural analogues of the natural pyrethrums. They are more stable to light and possess a higher insecticidal activity, almost ten times that of most organophosphates and carbamate insecticides. They are nerve poisons and affect the nerve axon, causing repetitive discharge of nerves which results in eventual paralysis.

The stability and activity of the synthetic pyrethroids are reflected in their increased use during the last two decades on fruits, vegetables and corn. The high insecticidal activities of these chemicals allow relatively small amounts to be applied. They also have good biodegradability due to the ester linkages. Thus because of these two reasons their environmental residues are uncommon. Their principle disadvantage is the very broad insecticidal activity which tends to eliminate many beneficial. Also all pyrethroids are lipophillic in nature, and practically insoluble in water. Examples are allethrin and permethrin.

3.5. Group characteristics and functions of other plant origin

Plants are known to produce a diverse range of secondary metabolites such as alkaloids, flavonoids, polyacetylenes, terpenoids, etc. Many of these chemicals protect the plant from pests and pathogens. More than 2400 plant species belonging to 235 families have been reported to possess pest control properties. Botanical insecticides are these secondary metabolites derived from plants. Flowers, leaves and roots are finely ground and used, or toxic ingredients of plants are extracted and used alone or in mixture. This method of insect control is being used for centuries. Advantages of using botanicals is that they are safe to
natural enemies, and being biodegradable do not leave toxic residues. For a chemical to be termed as an ideal botanical pesticide it must possess the following additional characteristics in addition to high insecticidal activity:

Should be safe to environment i.e. biodegradable
Should have low toxicity to mammals
Plant availability should be ensured
Isolation of active compounds should be economical

(i) Nicotine: The main source of nicotine are the two species *Nicotiana tabacum*, and *N. rustica*, the latter being more abundant in India. Free nicotine is a colourless or pale-yellow oily liquid. It has an odour of pyridine, because of its high volatility free nicotine is mainly used as a fumigant. In agriculture nicotine is used as nicotine sulphate which acts as a stomach poison. Addition of alkaline compounds such as soap and calcium caseinate at the time of spraying, liberates the nicotine more quickly, making it a more effective contact insecticide or fumigant. LD$_{50}$ of nicotine sulphate to rat is oral 83 mg/ kg body weight and dermal 285 mg/ kg body weight. Nicotine is a nerve poison and mimics acetylcholine at the nerve synapse.

(ii) Rotenone: It is mainly obtained from the roots of two species of *Derris* which grow in far east and some species of *lonchocarpus* which grow in Amazon valley in South America. Natives throughout the tropics have used rotenone-containing plants as fish poisons. Mode of action of rotenone poisoning derives from the ability of rotenone to inhibit the respiratory metabolism or the electron transport system between the NADH dehydrogenase and the coenzyme Q at complex I. Rotenone is both a contact as well as a stomach poison. It has very low toxicity to mammals, and thus is particularly useful in killing external parasites of livestock such as lice, fleas and ticks in dust form. It can be used as a lotion for chiggers, as an emulsion for scabies, and as a spray against cattle grubs and mange for dogs. The only drawback associated with the use of rotenone as an effective botanical is that it deteriorates in storage and has slow action against some insects.
(iii) Others: Neem (oil extracts of neem seed kernel), Chinaberry (a close relative of Neem tree), Pongram, Custard apple (powdered seeds of custard apple), Rynia (roots of shrub), Limonene (citrus peels), Sabadilla (seeds of lily).

3.6. **Group characteristics and functions of bioinsecticides**

Bioinsecticides involve the use of natural biological control agents either by introducing new species into the environment of a pest or by increasing the effectiveness of those already present. Traditionally, this method was employed to control insect pests by parasitoids, predators and pathogens.

A parasite is an organism which at one time or other lives in the body of the host and may or may not kill the host, after it has completed its development. A parasitoid is an organism which completes its life cycle on the host and then kills it. A predator on the other hand is a free living animal and kills its prey immediately.

In 1949, E.A. Steinhaus coined another term 'microbial control'. It employs those microorganisms or their products that are capable of attacking or killing pest insects.

The advantage of the use of microorganisms for insect pest control are: they have minimum effect on non-target organism as they are highly host specific. In addition to this microorganisms have a natural capability of causing diseases at epizootic levels due to their persistance in soil and their efficient transmission. Drawbacks associated with its use are: microbial pesticides are not economically viable. Also they are relatively slow acting.

Bioinsecticide use involves three major techniques, viz. introduction, conservation and augmentation.

- **Introduction** involves the release of bioinsecticides during the season when the pest is to be controlled, and during the availability of the stage of development against which it is most effective.
- **Conservation** means the avoidance of measures that destroy natural enemies and the use of measures that increase their longevity and reproduction or the attractiveness of an area to natural enemies.
- **Augmentation** includes all activities designed to increase numbers or effect of existing natural enemies. These objectives may be achieved by releasing additional number of a natural enemy into a system or modifying the system in such a way as to promote greater numbers or effectiveness. These releases have to be done periodically. This may be done in two ways, 
  - **Inoculative releases**: releases are made as infrequently as once a year. The purpose of it is to re-establish a specie of natural enemy which is otherwise less in number due to unfavourable conditions. In his case the control is expected from the progeny and subsequent generations, and not by the release itself. 
  - **Inundative releases**: Involves mass culture and release of natural enemies to suppress the pest population directly. These are most economical against pests with one or atmost few discrete generations every year.

3.7. **Group characteristics and functions of neonicotinoids and nitrogenous insecticides**

The neonicotinoids, are the newest major class of insecticides, derived synthetically from nicotinoids. It includes the following chemicals, acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam etc.

These biotransformations involve some activation reactions but largely detoxification mechanisms.

In contrast to nicotine, epibatidine, and other ammonium or iminium nicotinoids, which are mostly protonated at physiological pH, the neonicotinoids are not protonated and have an electronegative nitro or cyano pharmacophore. These substitutions increase the hydrophobicity. Thus the neonicotinoids are systemic poisons as well.

Mode of action of neonicotinoids is they act as agonists at the insect nicotinic acetylcholine receptor (nAChR) whereas the botanical insecticide nicotine acts at the same target without the neonicotinoid level of effectiveness or safety.
Fundamental differences between the nAChRs of insects and mammals confer remarkable selectivity for the neonicotinoids. Nicotinoids, with no ionisation have poor binding affinity with the insect nAChR. The presence of electron donating atom, increases the binding affinity with the insect nAChR. Thus the neonicotinoids have higher toxicity to insects in contrast to nicotinoids. On the contrary, vertebrate nAChR prefers the presence of one unit of positive charge for stronger binding. The ionized nicotine binds at an anionic subsite in the mammalian nAChR. Thus resulting in high toxicity for mammals of nicotinoids.

![Chemical structures of Imidacloprid and Acitamiprid](Imidacloprid.png)

3.8. **Group characteristics and functions of fumigants:**
Fumigants are insecticides in the form of gases that are slightly heavier than air and have ability to spread to all areas of a sealed structure. The ideal fumigant would have the following characteristics,

- Easily and cheaply generated
- Harmless to foods and commodities
- Inexpensive
- Non-explosive, Non-flammable
- Non-persistent
- Insoluble in water
- Easily diffuses and rapidly penetrates commodity
- Stable in the gaseous state (will not condense to a liquid)
- Easily detected by human senses

Unfortunately, no one fumigant has all the above properties, thus we have to look for the best possible option. The toxicity of a fumigant depends on the respiration rate of the target organism. Generally, lower the temperature, lower is the respiration rate of the organism which tends to make the pest less susceptible. Fumigation at lower temperatures thus requires a higher dosage rate for a longer exposure period than fumigation at higher temperatures. Fumigants vary greatly in their mode of action. Some kill rapidly while others kill slowly. In sub lethal dosages, some fumigants may have a paralyzing effect on the pest while others will not allow the pest to recover. Some fumigants have no effect on commodities while others are detrimental even at low concentrations. Commodities vary in their sorption of fumigants and in the effort required to aerate the commodities after fumigation.

![Ethylene dibromide](Ethylene_dibromide.png)
4. INSECTICIDES

**ORGANOCHLORINES**

Organochlorine insecticides can be classified into the following groups based on their chemical structures,

- **Cyclodiene series**: They are very stable in the environment and form epoxides responsible for their high persistence e.g.: chlordane, aldrin, heptachlor, endosulfan, dieldrin, isodrin, endrin.
- **Halogenated aromatic compounds**: DDT, DDE, dicofol, methoxychlor
- **Cycloparaffins**: BHC/HCH, lindane
- **Chlorinated terpenes**: Polychloropinenes

### 4.1.1. DDT: 2,2-bis-(p-chlorodipheny)-1,1,1-trichloroethane

An organochlorine insecticide, prepared by reacting chloral (or its alcholate or hydrate) with chlorobenzene in presence of sulfuric acid, oleum or chlorosulfonic acid. Its chemical structure is,

![Chemical Structure of DDT](image)

Trade or other names: DDT, Anofex, Gyron Cesarex, Chlorophenothane, Guesapon, Guesarol, Gexarex, etc.

Appearance: Technical product p,p’-DDT is white tasteless, almost odorless crystalline solid.

Empirical formula: \( C_{14}H_9Cl_5 \)

Molecular Weight: 354.49

Solubility: acetone(58 gm/100ml), Cyclohexane(116 gm/100ml), benzene(106 gm/100ml), carbontetrachloride (45 gm/100ml), ethylether(28 gm/100ml), petroluemether(4-10 gm/100ml), ethanol(2 gm/100ml) and water(0.0012 ppm).

Melting Point: 108.5-109°C

Vapor pressure: \( 1.5 \times 10^{-7} \) mm Hg at 20°C

ADI: 0.005 mg/kg/b.w/d (man)

Technical grade: DDT is actually a mixture of three isomers of DDT, principally the p,p’-DDT isomer (85%), with the o,p’-DDT and o,o’-DDT isomers typically present in much lesser amounts.

Toxicity, single dose: Rat (male): Oral LD50 = 250 mg/kg, Dermal 250-500 mg/kg in oil, 3000 mg/kg as powder. Rat (female): Dermal 2510 mg/kg powder.

Common formulations: Wettalbe powders, dusts, aerosols, smoke candles, E.C., etc. In house hold formulations of DDT are combined with synergized pyrethrins. Concentrations of solid and liquid formulations are mostly 20%-25%.

Mode of action: Central nervous system stimulant producing hyperactivity and tremor, convulsions may occur but are less common than any other organochlorine pesticides.

Breakdown in Soil and Groundwater: DDT is very highly persistent in the environment, with a reported half life of between 2-15 years and is immobile in most soils. Routes of loss and degradation include runoff, volatilization, photolysis and biodegradation aerobic and anaerobic.

The insecticidal properties of DDT were discovered by Paul Muller of J.R.Geigy, A.G. in Switzerland in 1939. DDT and its metabolites accumulate in body fat and other tissues, either as DDT, DDD or DDE. Under normal circumstances a plateau level is reached where intake and storage are in equilibrium with excretion, therefore the amount stored in fat will remain constant. It is cumulative in natural environment.

BHC/HCH: 1,2,3,4,5,6-hexachlorocyclohexane
BHC, benzene hexachloride was first prepared in 1825 by Michael Faraday, who did not recognize its insecticidal properties. It is produced by the chlorination of benzene under u.v. light. Insecticidal properties lie in α isomer. Its chemical structure is,

\[
\begin{align*}
\text{BHC} & : \quad \text{C}_6\text{H}_6\text{Cl}_6 \\
\end{align*}
\]

Trade or other names: Benhexachlor, benzene hexachloride, BHC, HCH, hexachloran, hexachlor.
Appearance: Technical HCH is off-white to brown, amorphous powder with a characteristic musty odor.
Empirical formula: \( \text{C}_6\text{H}_6\text{Cl}_6 \)
Molecular Weight: 290.80
Water Solubility: 7.3 mg/L at 25°C.
Melting Point: Crude BHC begins to melt at 65°C
Vapor pressure: 0.06 mm Hg at 40°C α isomer, 0.17 mm Hg at 40°C β isomer),
Technical grade: Technical HCH consists of various stereoisomers (molecules with a unique structural arrangement, but identical chemical formulas) viz., α isomer constitutes 65-70% (158°C M.P), β isomer constitutes 5-6% (200°C M.P), γ isomer constitutes 13% (108-111°C M.P), δ isomer constitutes 6% (129-130°C M.P), and ε isomer constitutes 3-4% etc.
Toxicity, single dose: Rat: Oral LD50 = 500 mg α isomer/kg, 6000 mg β isomer/kg, 1000 mg δ isomer/kg. The β isomer constitutes 5-6% (200°C M.P), γ isomer constitutes 13% (108-111°C M.P), δ isomer constitutes 6% (129-130°C M.P), and ε isomer constitutes 3-4% etc.
Common formulations: Emulsifiable concentrate, wettable and dustable powder. The concentration of γ isomer should be stated.
Mode of action: The α and γ isomers are stimulants of central nervous system, with the principal symptom being convulsions. The β and δ isomers are depressants of the central nervous system.
Breakdown in Soil and Groundwater: BHC is highly persistent in most soils, with a field half-life of approximately 289 days. When sprayed on the surface, the half-life was typically much shorter than when incorporated into the soil. It is very stable in water environment, and is resistant to photodegradation. It will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin, and food.

4.1.3. LINDANE: 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
In 1912, Van der Linden discovered four isomers. Insecticidal properties lie in γ isomer, so pure form of γHCH is named as Lindane in honor of Van der Linden. It is produced by the selective crystallization of crude HCH.

\[
\begin{align*}
\text{Lindane} & : \quad \text{C}_6\text{H}_6\text{Cl}_6 \\
\end{align*}
\]

Trade or other names: lindane, Agrocide, Ambrocide, gamma-hexachlor, gamma benzene hexachloride, Gamaphex, gamma-BHC, Gamma-Col, gamma-HCH, Gammexane
Appearance: Lindane is a colorless crystal compound.
Emperical formula: C₆H₆Cl₆  
Molecular Weight: 290.80  
Solubility: 10 mg/L at 25 °C in water, slightly soluble in petroleum oils, soluble in acetone, aromatic and chlorinated hydrocarbons.  
Melting Point: 112.9°C  
Vapor pressure: 9.4 x 10⁻⁶ mm Hg at 20°C  
Technical grade: Technical lindane is comprised of the gamma-isomer of hexachlorocyclohexane.  
Toxicity, single dose: Rat: Oral LD₅₀ = 88-91 mg/kg, dermal 900-1000 mg /kg  
ADI: 0.008 mg/kg/day  
Common formulations: Suspension, emulsifiable concentrate, fumigant, seed treatment, wettable and dustable powder, and ultra-low volume (ULV) liquid.  
Mode of action: Lindane are stimulants of central nervous system, with the principal symptom being convulsions.  
Breakdown in soil and groundwater: Lindane is highly persistent in most soils, with a field half-life of approximately 15 months. When sprayed on the surface, the half-life was typically much shorter than when incorporated into the soil.  

4.1.3. ENDOSULFAN:  
6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzadioxathiepin 3-oxide.  
Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. It is formed by the reaction of thionylchloride with thiodan.  

\[
\begin{align*}
\text{Cl} & \quad \text{C} & \quad \text{Cl} \\
\text{Cl} & \quad \text{C} & \quad \text{Cl} \\
\text{SO} & \quad \text{CH}_2 & \quad \text{CH}_2 \\
\text{Cl} & \quad \text{Cl} & \quad \text{Cl}
\end{align*}
\]

Endosulfan  
Trade or other names: Thiodan, Afidan, Beosit, Cyclodan, Devisulfan, Endocel, Endocide, Endosol, Hexasulfan.  
Appearance: Pure endosulfan is a colorless crystal. Technical grade is a yellow-brown color.  
Emperical formula: C₉H₆Cl₆O₃S  
Molecular Weight: : 406.96  
Solubility: It is practically insoluble in water, moderately soluble in most organic solvents  
Melting Point: Technical material, 70-100°C  
Vapor pressure: 1 x 10⁻⁵ mm Hg at 20°C  
ADI: 0.006 mg/kg/b.w/d  
Technical grade: Technical endosulfan is a mixture of two isomers viz., α isomer constitutes 70% (106°C M.P), and β isomer constitutes 30% (212°C M.P).  
Toxicity, single dose: Rat: Dermal LD₅₀ = 30-79 mg/kg.  
Common formulations: Emulsifiable concentrate, granules, wettable and dustable powder, and ultra-low volume (ULV) liquid. Combinations are available with other pesticides, e.g. Dimethoate and parathion-methyl at various concentrations.  
Breakdown in soil and groundwater: Endosulfan is moderately persistent in the soil with an average field half-life of 50 days. The two isomers have different degradation times in soil. The half-life for the alpha-isomer is 35 days, and is 150 days for the beta-isomer under neutral conditions. These two isomers will persist longer under more acidic conditions. The compound is broken down in soil by fungi and bacteria. Endosulfan does not easily dissolve in water, and has a very low solubility.  

ORGANOPHOSPHORUS PESTICIDES  
Organophosphorous pesticides includes following groups:
Pyrophosphates: schradan, TEPP, Dialkylarylphosphate, phosphorothioate, phosphorothionate, phosphorothiolate, phosphorothiolothioate: Maximum organophosphorous pesticides belong to this group. They are very toxic and their LD$_{50}$ value varies from 5-55/kg body wt. Metabolites are more toxic than parent compounds. P=O analogs are less stable than P=S analogs, eg, Parathion

Phosphorohalides and cyanides: this group includes mostly nerve gases such as mipafox

4.2.1. MALATHION: Diethyl (dimethoxy thiophosphorylthio) succinate

Malathion is a nonsystemic, wide-spectrum organophosphate insecticide. It was one of the earliest organophosphate insecticides developed and introduced in 1950. Malathion is suited for the control of sucking and chewing insects on fruits and vegetables, and is also used to control mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice.

\[
\text{CH}_3\text{O} \hspace{1cm} \text{P} \hspace{1cm} \text{O} \hspace{1cm} \text{CH}_3\text{O}
\]

Malathion

Trade or other names: Malathion is also known as carbophos, maldison and mercaptothion.

Appearance: Technical malathion is a clear, amber liquid at room temperature

Empirical formula: C$_{10}$H$_{19}$O$_6$PS$_2$

Molecular Weight: 330.3

Solubility: 145 mg/L at 25°C in water, miscible with most organic solvents.

Melting Point: 2.85°C

Vapor pressure: 4 x 10$^{-5}$ mm Hg at 30°C

ADI: 0.02 mg/kg/b.w/d

Technical grade: The Technical grade is 95% pure.

Toxicity, single dose: Rat: Oral LD$_{50}$ = 2800 mg/kg

Common formulations: Suspension, emulsifiable concentrate, wettable and dustable powder, and ultra-low volume (ULV) liquid. Malathion may also be found in formulations with many other pesticides.

Breakdown in soil and groundwater: Malathion is of low persistence in soil with reported field half-lives of 1 to 25 days. Degradation in soil is rapid and related to the degree of soil binding.

4.2.2. PARATHION: O,O-diethyl O-4-nitrophenyl phosphorothioate

Parathion is a broad spectrum, organophosphate pesticide used to control many insects and mites. It has non-systemic, contact, stomach and fumigant actions. It is produced by the condensation of O,O-diethyl phosphorochloridothioate with sodium 4-nitrophenoxide.

\[
\text{CH}_2\text{H}_5\text{O} \hspace{1cm} \text{P} \hspace{1cm} \text{O} \hspace{1cm} \text{C}_2\text{H}_5\text{O}
\]

Parathion

Trade or other names: Trade names include, Alkron, Alleron, Aphamite, Corothion, E-605, Ethyl parathion,

Appearance: Pure parathion is a pale yellow liquid with a faint odor of garlic at temperatures above 6°C.

Technical parathion is a deep brown to yellow liquid

Empirical formula: C$_{10}$H$_{14}$NO$_5$PS

Molecular Weight: 291.3

Solubility: 24 mg/L at 25°C in water, slightly soluble in petroleum oils, miscible with most organic solvents.

Boiling Point: 157-162°C /0.6 mm Hg

Vapor pressure: 3.78 x 10$^{-5}$ mm Hg at 20°C
ADI: 0.006 mg/kg/b.w/d
Technical grade: Technical grade is 96-98 % pure.
Toxicity, single dose: Rat (male): Oral LD50 = 13 mg/kg, Dermal 21 mg/kg, Rat (female): Oral LD50= 3.6 mg/kg, Dermal 6.8 mg/kg.
Common formulations: Emulsifiable concentrate, granules, wettable and dustable powder, smokes and aerosol concentrates.
Breakdown of Chemical in Soil and Groundwater: Parathion has little or no potential for groundwater contamination. It binds tightly to soil particles and is degraded by biological and chemical processes within several weeks. Degradation is faster in flooded soils. Photodegradation may occur on soil surfaces. Sunlight can convert parathion into the active metabolite paraoxon, which is more toxic than parathion.

4.3. CARBAMATES

Carbamates are a group of organic compounds sharing a common functional group with the general structure -NH(CO)O-. The parent compound of all carbamates is called carbamic acid or NH₂COOH and examples are Carbaryl (SEVIN), Oxamyl (VYDATE) Carbofuran (FURADAN) Thiodicarb (LARVIN) Methomyl (LANNATE)

4.3.1. CARBARYL: 1-napthyl methylcarbamate

Carbaryl is a contact insecticide of carbamate group with slight systemic properties, produced by the reaction of 1-napthol with methyl isocyanate or with carbonyl chloride and methylamine.

\[
\text{H}_2\text{N} \quad \text{C} \quad \text{O} \\
\text{N} \quad \text{C} \quad \text{O} \\
\text{H} \quad \text{C}_12\text{H}_11\text{NO}_2
\]

Carbaryl

Trade or other names: Carbaryl, Adios, Bugmaster, Carbamec, Carbamine, Crunch, Denapon, Dicarban
Appearance: Carbaryl is a solid that varies from colorless to white or gray, depending on the purity of the compound. But pure carbaryl is colorless crystalline solid.
Empirical formula: C₁₂H₁₁NO₂
Molecular Weight: : 201.2
Solubility: 120 mg/L at 30°C in water, soluble in most organic solvents such as dimethylformamide and dimethyl sulfoxide.
Melting Point: 142°C
Vapor pressure: < 4 x 10⁻⁵ mm Hg at 25°C
ADI: 0.01 mg/kg/b.w/d
Technical grade: Technical product is almost 99% or slightly less. It is compatible with most other pesticides except those strongly alkaline, such as Bordeaux mixture or lime sulphur, which hydrolyse it to 1-napthol.
Toxicity, single dose: Rat (male): Oral LD50 = 850 mg/kg, Dermal > 4000 mg/kg.
Common formulations: Emulsifiable concentrate, granules, wettable and dustable powder, bait pellets, micronised suspensions in molasses, in non-phytotoxic oil or in aqueous media and as true solutions in organic solvents.
Breakdown in soil and groundwater: Carbaryl has a low persistence in soil. Degradation of carbaryl in the soil is mostly due to sunlight and bacterial action.

PYRETHROIDS

Depending on their mode of action pyrethroids are of the types, Type I and Type II pyrethroids.
Type I pyrethroids include natural pyrethrins, allethrin, resmethrin.
Type II pyrethroids include cypermethrin, deltamethrin, fenvalerate

4.4.1. CYPERMETHRIN:
(RS)-a-cyano-3-phenoxybenzyl(IRS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-carboxylate
Cypermethrin is a synthetic pyrethroid of stomach and contact action, produced by the esterification of a-hydroxy-3-phenoxy-phenylacetonitrile with 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid.

Trade or other names: Ammo, Arrivo, Barricade, Basathrin and Super.
Appearance: Pure isomers of cypermethrin form colorless crystals. When mixed isomers are present, cypermethrin is a viscous semi-solid or a viscous, yellow liquid
Empirical formula: C\textsubscript{22}H\textsubscript{19}Cl\textsubscript{2}NO\textsubscript{3}
Molecular Weight: 416.3
Solubility: 0.01-0.2 mg/L at 21°C in water, 103g/l in hexane, >450 g/l in acetone at 20°C, soluble in Cyclohexane, ethanol, xylene, chloroform.
Melting Point: 60-80 °C (pure isomers)
Vapor pressure: 3.8 x 10\textsuperscript{-8} mm Hg at 70°C (for pure compound).
ADI: 0.05 mg/kg/b.w/d
Technical grade: Technical cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties.
Toxicity, single dose: Rat: Oral LD\textsubscript{50} = 303-4123 mg/kg (depending on the carrier and condition used).
Common formulations: It is available as an emulsifiable concentrate or wettable powder
Breakdown in soil and groundwater: Cypermethrin is moderately persistent in soils. In aerobic conditions, its soil half-life is 4 days to 8 weeks. When applied to a sandy soil under laboratory conditions, its half-life was 2.5 weeks

4.5. Insect Growth Regulators
They are synthetic compounds that control insects by disrupting normal growth and development of their larvae nymphs, pupae or adults rather than by toxic action and finally lead to their death.
Growth and moulting of immature insects is regulated by three main groups of brain hormones ecdysone alpha, beta, juvenile hormones. Antagonists and analogs of insect growth regulators IGR such as juvenile hormones (JH), ecdysones, chitin synthesis inhibitors and other related compounds are used as means for insect growth regulation.
Insect growth regulators are eco-friendly in nature. They have low mammalian toxicity and high selective toxicity for insects.

![Diflubenzuron structure](image1)

**4.5.1. Inhibitors of Chitin Synthesis**

![Diflubenzuron structure](image2)  
Diflubenzuron (>4640 mg/kg)

![Teflubenzuron structure](image3)  
Teflubenzuron (>5000 mg/kg)

These compounds are classified as benzoylphenylureas and possess a number of halogen substituents. Diflubenzuron is the prototypical compound although in this series, second generation compounds also exist. Water solubility of these compounds is extremely low (< 1ppm). Insects exposed to these compounds are unable to form normal cuticle because the ability to synthesize chitin (polysaccharide of N-acetylglucosamine) is lost.

**4.5.2. Juvenile Hormone Mimics**

![Juvenile hormone I structure](image4)  
Juvenile hormone I
Juvenile hormones are lipophilic sesquiterpenoids containing an epoxide and methyl ester groups. The juvenile hormone mimics are compounds bearing a structural resemblance to the juvenile hormones of insects. Both compounds are soluble in organic solvents and have extremely low toxicity to mammals. Methoprene (>30,000 mg/kg) which possesses a phenoxybenzyl group instead of a carbon chain which bears a close structural resemblance to juvenile hormones, and fenoxycarb, which possesses a phenoxybenzyl group instead of a carbon chain with an epoxide.
Exposure to these compounds at molting results in the production of insects containing mixed larval/pupal or larval/adult morphologies. The efficacy of these compounds is greatest when normal juvenile hormone titers are low, namely, in the last larval or early pupal stages. Timing of application is important for successful control. Another useful property of these compounds is that, in adults, they disrupt normal reproductive physiology and act as a method of birth control.

4.6. Toxins from Bacillus thuringiensis

*Bacillus thuringiensis* (Bt) forms a crystalline inclusion body during sporulation that contains a number of insecticidal protein toxins. When consumed by the insect, the inclusion is dissolved in the midgut and releases δ-endotoxins. Mixtures of different δ-endotoxins are usually present in the inclusion and individual toxin proteins are designated with the prefix *cry.*

The toxin proteins contain a few hundred to over 1000 amino acids. After they are ingested, the δ-endotoxins are cleaved to an active form by proteases within the midgut. The active toxins bind specifically to the membranes of the midgut epithelia and alter their ion permeability properties by forming a cation channel or pore. Ion movements through this pore disrupt potassium and pH gradients and lead to lysis of the epithelium, gut paralysis, and death.

4.7. ANTIFEEDANTS

Antifeedants are substances (aversive chemicals) which make the crop plant distasteful to the insect attempting to feed on it. Presence of antifeedant compound renders the host plant unpalatable to insect, inhibiting feeding. Some antifeedants inhibit feeding because they are toxic, others because they have a bad taste. They might also prevent insects to lay eggs. Bordeaux mixture is a feeding deterrent to fleas, beetles, leaf hoppers. Pymetrozin a pyridine azomethrine, is an effective on aphids, leaf hoppers and whitefly. It is systemic has long residual activity and is non-injurious to natural enemies and the environment.

4.8. REPELLENTS

Repellents are chemicals which cause insects to make oriented movement away from source of chemical or its vapors thus preventing insect from reaching target. Applying of these chemicals to skin, clothing, or other surfaces discourages insects (and arthropods in general) from landing or climbing on that surface. Common insect repellents include: oil of lemon, eucalyptus, picaridin or icaridin (a piperidine derivative), dimethyl phthalate, butopyronoxyl, citronella, DEET.
Some insect repellents, particularly permethrin, are insecticides. Natural permethrin is known for its repellent properties. It is highly effective when applied to livestock to repel tsetse flies, ticks. DEET is the most commonly used and most effective repellent against biting flies, chiggers, and certain species of mosquitoes.

Insect repellents help prevent and control the outbreak of insect-borne diseases such as malaria, Lyme disease, bubonic plague, and West Nile fever. Insects commonly serving as vectors for disease include fleas, flies, mosquitoes, and ticks.

### 4.9. ATTRACTANTS

Attractants are chemicals which cause insects to make oriented movement towards its source or chemicals acting in vapor phase causing an insect to move towards its source or zone of preferred concentration.

Attractant can be of the type: poison baits, traps, ovipositional-type attractant.

- **Poison baits** – Attractants can be used in bait alone or in conjunction with a poison.
- **Trap** – is a device for immediate killing of insects or retaining them for later destruction. Synthetic attractant medlure and trimedlure used as attractant in traps for fruit flies.

- **Oviposition lure** are substances that attract gravid females and induce them to lay eggs. Houseflies are attracted to ammonia and green bottle flies are attracted to ammonium carbonate for ovipositional purposes.

### 4.10. SYNERGISTS

Synergists are compounds, when mixed with pesticides increases toxicity to several folds. The combined effect of multiple exposure is considerably greater than the sum of the effects from the individual components. This phenomenon is known as synergism or potentiation.

- **Synergism** means when both chemicals (have an effect individually) have more additive effect when together.
- **Potentiation** means when one chemical has an effect but the second chemical does not but enhances the effect of the former chemical on combined exposure, eg. sesame oil enhanced insecticidal activity of pyrethrum and the active compounds were identified as sesamin, sesamolin.

Effectiveness of insecticide synergist is expressed by ratio of LD$_{50}$ of insecticide alone to that of insecticide with synergist.

### 4.11. BOTANICAL INSECTICIDES

Botanical insecticides are naturally occurring chemicals extracted from plants. Natural pesticidal products are available as an alternative to synthetic chemical formulations but they are not necessarily less toxic to humans. Some of the most deadly, fast acting toxins and potent carcinogens occur naturally. Botanical insecticides break down readily in soil and are not stored in plant or animal tissue.

Citrus oil (limonene, linalool) are extracts from citrus peels primarily used as flea dips, but have been combined with soaps as contact poisons against aphids and mites. They evaporate quickly after application and provide no residual control.
Nicotine concentrate is very poisonous if inhaled. It is derived from tobacco and is commonly sold as a 40 percent nicotine sulfate concentrate. Nicotine is a fast acting contact killer for soft bodied insects, but does not kill most chewing insects.

Pyrethrin is a fast acting contact poison derived from the pyrethrum daisy. It is very toxic to cold blooded animals. Pyrethrin is effective on most insects, but does not control mites. It rapidly breaks down in sunlight, air and water.
Rotenone is derived from the roots of over 68 plant species. It has a short residual time. Rotenone is a broad spectrum poison mainly used to control leaf-eating caterpillars and beetles.

![Rotenone](image)

Ryania is a slow acting stomach poison. It has a longer residual than most botanicals. Toxicity to mammals is moderate.

Sabadilla is derived from the seeds of South American lilies. It is a broad spectrum contact poison, but has some activity as a stomach poison. It is most effective against true bugs such as harlequin bugs and squash bugs.

Neem is a relatively new product on the market. It is derived from the neem tree that grows in arid tropical regions. Extracts from the neem tree have been reported to control over 200 types of insects, mites, and nematodes.

![Azadirachtin](image)

### 4.12. BIOPESTICIDES

Biopesticides are certain types of pesticides derived from such natural materials such as animals, plants, bacteria, and certain minerals. For example, canola oil and baking soda have pesticidal applications and are considered biopesticides. Biopesticides fall into three major classes:

4.12.1. Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient.
Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi that control certain weeds, and other fungi that kill specific insects, e.g., *Beauveria bassiana*.

The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or Bt. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae.

4.12.2. Plant Incorporated Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest.

4.12.3. Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones that interfere with mating as well as various scented plant extracts that attract insect pests to traps.

**4.13. NEONICOTINOIDs**

The neonicotinoids are the only major new class of insecticides developed in the past three decades. Neonicotinoids commonly include *acetamiprid, clothianidin, dinotefuran, imidanclorprid, nitenpyram, thiacloprid, and thiamethoxam*.

![Thiamethoxam, Clothianidin, Dinotefuran](image)

They have outstanding potency and systemic action for crop protection against piercing-sucking pests, and they are highly effective for flea control on cats and dogs. They are readily absorbed by plants and act quickly, at low doses, on piercing-sucking insect pests (aphids, leafhoppers, and whiteflies) of major crops. The neonicotinoids are poorly effective as contact insecticides and for control of lepidopterous larvae.

They are used primarily as plant systemics, when applied to seeds, soil, or foliage they move to the growing tip and afford long-term protection from piercing-sucking insects, e.g., for 40 days in rice. IMI and nitenpyram are highly effective flea control agents on cats and dogs, and are administered as oral tablets or topical spot treatments while the nicotinoids are structurally similar to the neonicotinoids, they primarily differ by containing an ionizable basic amine or imine substituent.

**5. MODE OF ACTION OF PESTICIDES**

Majority of the insecticides attack the nervous system. Which shows irreversible damage, more so than any other tissue in the body. Other poisons whose primary target is elsewhere may also produce their ultimate effect on the nervous system. For example the heart poisons like atropine and poison which block the oxygen-carrying capacity of blood like carbon monooxide are lethal because of the brain damage that follows deprivation of the brain’s great oxygen requirement. Understanding the mechanism of action of pesticides is a major and fundamental task for pesticide toxicologists, as the knowledge of the mode of action is of vital importance for pharmacologists.

In order to understand how insecticides exert their toxic effects, it is essential to have some fundamental understanding of the physiology and biochemistry of the mammalian and insect nervous system.
5.1. Conduction of nerve impulse: There are two quite different modes of transmission in the nervous system, axonal transmission and synaptic transmission.

5.1.1. Axonal transmission: Axon is the part of the neuron, which is specialised for carrying nerve impulses or action potentials rapidly without changing the size or pattern of the impulse as it moves along.

Under normal conditions, extracellular fluid surrounding the axonal membrane has a high concentration of Na$^+$ and a low concentration of K$^+$ ion. Hence neurons possess a transmembrane voltage of about -60mV on the inner side of the cell. This is known as the Resting Membrane Potential (RMP).

A stimulation causes axonal membrane to become permeable to Na$^+$, this causes sodium influx resulting inside membrane transiently positive. This constitutes the rising phase of the action potential. However, the sodium channels start closing quickly, usually within 1m sec. The membrane now becomes permeable to K$^+$ ion, and because of its higher concentration on the inside, rushes out (potassium efflux), constituting the falling phase of the action potential.

The ability to perform all these events depends upon the maintenance of gradient across the membrane. This is done by Na$^+$/K$^+$ pump which maintains the gradient in the first place, and secondly compensates for the leakage that occurs during impulse transmission.

5.1.2. Synaptic transmission: When an impulse has passed along an axon, it must cross a synapse to stimulate another neuron. Transmission across a synapse involves a chemical transmitter which is stored in vesicles in the end of the axon (unlike transmission across an axon, which is electrical transmission). The transmitter becomes attached at receptor sites to the post-synaptic membrane. This causes a change in the ion permeability, which leads to membrane depolarisation generating an action potential.

The two most common types of neurotransmitters are, Acetylcholine and Norepinephrine. Universally, the synapse which utilizes acetylcholine is called cholinergic, while the one utilising norepinephrine are called adrenergic synapse.

In order to restore the sensitivity of the synapse, the transmitter must be eliminated, so that the receptor can return to its resting state.

At cholinergic junctions this is done by cholinesterase which hydrolyses acetylcholine into inactive components, choline and acetate.

At adrenergic junction, the corresponding degrading enzyme is monoamino-oxidase.

Central nervous system (CNS) refers in mammals to the brain and the spinal cord and in insects to the chain of ventral ganglia. The brain is the integrating center of all body activities. The peripheral nervous system consists of the somatic and the autonomic systems.

The somatic system handles those movements characterised by reaction to environmental stimuli and the corresponding muscle response. Transmission across the synapse and at the neuromuscular junction is cholinergic.

The autonomic division of the nervous system innervates all the effectors of the body except the skeletal muscles. There are two divisions in the autonomic nervous system, the sympathetic and the parasympathetic. Sympathetic system has adrenergic system while parasympathetic system uses cholinergic system. Beside these there are many other chemicals which act as transmitters in the central nervous system. Gamma-aminobutryic acid (GABA) is one of them.

Insect nervous system is analogous to that of mammals. There are however some differences. Firstly, there are histochemical, enzymological and physiological evidences that neuromuscular junction of insects are not cholinergic. Instead Glutamic acid stimulates and GABA supresses muscle contraction as transmitters at the neuromuscular junction. Also there is no distinct autonomic system. Insect nerve also show no distinct myelination.

Acetylcholine was established as the transmitter at the insect nervous system synapse, although GABA, glutamic acid, glycine, biogenic amines such as dopamine, norepinephrine, serotonin and tryptamine do occur in insect nervous system. Octopamine is unique to the insect system.
5.2. **GABA receptor complex:**

GABA receptor complex or GABA receptor chloride ionophore is a single complex protein having at least three distinct interacting components both in mammals and in insects. GABA is released from the presynaptic endings of stimulated inhibitory neurons and diffuse across the synaptic cleft. Now it binds to the GABA receptor complex at the postsynaptic site. This increases the chloride permeability of the axon thus causing hyperpolarization of the nerve fibre. All these events lead to the deactivation of the postsynaptic cell.

Thus activation of GABA receptors produces inhibition at a variety of sites, while inhibition of GABA-induced chloride ion permeability causes excessive release of acetylcholine at presynaptic sites or stimulates glutamine action, which may account for stimulant and convulsant effects of the inhibitors.

5.3. **Mode of action of Organochlorine insecticides:**

5.3.1. DDT group: DDT is a slow acting neurotoxicant. It shows a negative correlation with temperature i.e. its insecticidal potency increases with the decrease in temperature. Its exact biochemical mechanism has not been elucidated yet although it is suggested that the ATP-dependent portion of the Na⁺/Ca⁺ exchange may be involved. It is now established that DDT acts primarily on neurons and interferes with the axonal transmission. DDT prolongs the closure of sodium gated channels, thereby increasing the depolarisation after-potential. When this has increased to a certain level, a sudden burst of repetitive discharge or a trail of impulse is provoked by a single stimulus. This leads to hyperexcitability of the nervous system resulting in tremors, paralysis and even death. DDT has been shown to cause the release of neurohormones which might be involved in its toxicity.

5.3.2. Hexachlorocyclohexane (HCH): Among its isomers, only gamma HCH or lindane has high toxicity towards insects and other organisms. It is a more acute nerve poison than DDT. There is negative correlation of its toxicity with temperature, but not as pronounced as in DDT poisoning. Practically nothing is known about the biochemical basis of insecticidal action of lindane. It is a better inhibitor of Na⁺, K⁺ and Mg⁺ ATPase than DDT. Lindane causes accumulation of acetylcholine in nerves of insects but it does not inhibit the enzyme cholinesterase. The mechanism of action was established as blocking of the GABA-gated chloride channels.

5.3.3. Cyclodiene group: Like most organochlorine insecticides, cyclodiene also are neurotoxicants. But unlike other organochlorine insecticides they show a positive correlation with temperature i.e. their toxicity is enhanced with increase in temperature. There is a characteristic ‘lag period’ between the administration of the poison and the appearance of poisoning symptoms in case of cyclodiene insecticides. Cyclodiene compounds cause an excessive release of acetylcholine, but do not block the enzyme cholinesterase [AChE]. There is evidence that they interact with ATPases from nerve cord and muscle. Cyclodiene compounds are particularly more dangerous because of their high oral and dermal toxicity.

5.4. Mode of action of Organophosphorous insecticides:

(a)
Organophosphorous [OP] insecticides have structural complementarity with AChE enzyme, thus they mimic the gross molecular shape of acetylcholine. OPs react with a serine hydroxyl group within the enzyme active site, phosphorylating this hydroxyl group and yielding a hydroxylated leaving group (fig.). This process inactivates the enzyme and blocks the degradation of the neurotransmitter acetylcholine. The synaptic concentration of acetylcholine then builds up and hyperexcitation of the CNS occurs. The signs of intoxication include restless, hyperexcitability, tremors, convulsions, and paralysis. In insects, the effect of OPs are confined to the CNS, where virtually all of the cholinergic synapses are located. The phosphorylation of acetylcholinesterase by OPs is persistent, reactivation of the enzyme can take many hours or even days.

5.5. Mode of action of Carbamate insecticides:

Carbamates react with acetylcholinesterase in the same manner as that of OP compounds. They also bind to the enzyme cholinesterase forming a reversible complex. In this case, the reaction yields a carbamylation of the serine hydroxyl group (figure 4). Complex decomposes into stable carbamylated enzyme (enzyme rendered inhibited) and an hydroxylated leaving group. Finally carbamylated enzyme is hydrolysed to regenerate the free enzyme and methylcarbanic acid. Only difference between the two group of insecticides is that phosphorylated enzyme (in the case of OP compounds), hydrolyses at a much slower rate as compared to the carbamylated enzyme (in the case of carbamates). Thus animals showing carbamate poisoning recover within hours after exposure to carbamates, unlike the OP compounds.
5.6. Synthetic Pyrethroids:

Pyrethroids are typically esters chrysanthemic acid having a high degree of lipophilicity (fat solubility). Pyrethroid control and action are classified as Type1 or Type2, depending on the poisoning symptoms.

5.6.1. Type1 pyrethroids: This group includes a non-alpha-cynopyrethroids, including natural pyrethrins, allethrin, tetramethrin, etc. They are characterised by whole body tremors similar to that in DDT. These insecticides prolong the sodium current during excitation, cause depolarisation after potential to increase. When the after potential exceeds the membrane threshold, repetitive action potentials are generated, leading to hyperexcitation. This is followed by tremors, paralysis and even death.

Type2 pyrethroids: This group includes alpha-cynopyrethroids including cypermethrin, deltamethrin, fenvalerate. They produce a distinctly different syndrome, characterised by sinus writhing, convulsions, accompanied by profuse salivation. They also act on the sodium channels, prolonging the sodium current to a greater extent than type1. Thus they depolarize the nerve membrane more strongly than type1, because of membrane depolarisation, nerve fibres do not initiate repetitive discharges, but sensory neurons discharge bursts of impulses and synaptic transmission is disturbed. The nerve conduction is eventually blocked due to membrane depolarisation. At higher concentrations type2 pyrethroids bind to the chloride ionophore component of the GABA receptor complex and inhibit the GABA dependent chloride flux.

These classifications of type1 and type 2 pyrethroids are not absolute because there is continuous transition from type1 to type2 structures. Some pyrethroids such as fenfluthrin, cyphenothrin have an intermediate position in their effects on the axon.

5.7. OTHER MODES OF ACTION:

5.7.1. Metabolic inhibitors: Certain chemicals affect the electron transport chain thus disrupting the normal metabolic pathway. Examples are rotenone (slows heartbeat, depresses respiration and oxygen consumption, and causes paralysis and death) and arsenicals (inhibit respiratory enzymes).

5.7.2. Muscle poisons: Certain chemicals have a direct action on the muscle tissue. Examples are rynia and sabadilla which increases oxygen consumption, followed by paralysis and death.

5.7.3. Alkylating agents: Certain chemicals react directly with chromosomes and enzymes in the cells. Examples are fumigants such as methyl bromide and ethylene dibromide.

5.7.4. Physical toxicants: Certain chemicals mechanically block the physiological processes. Examples are oil (blocks respiratory openings in insects) and boric acid and silica gel (effects insect cuticle causing dehydration and death).

5.7.5. Cytolytic (cellular) toxins: Certain chemicals cause cells to rupture and disintegrate. Example is *Bacillus thuringiensis*, which is ingested by insect larvae and disrupts cells in the gut (causing paralysis of gut and cessation of feeding)
6. METABOLISM OR DEGRADATION OF INSECTICIDES: PHASE-1 AND PHASE-2 REACTIONS

All living organisms possess defence mechanisms intended to protect them from the deleterious effects of foreign compounds, including pesticides. When the toxic substances (pesticides) enter an organism, the immune systems elicits against it and convert many compounds into less toxic or harmless products, this phenomenon is termed as detoxification. Consequently, metabolism is sometimes referred to as detoxification process. However, it has been found that the body converts some compounds into more toxic substances, and so the term ‘activation’ is often used to describe this phenomenon. The type of change that occurs depends on the chemical structure of the compound, but other factors such as species of animal, method of administration, and diet may also be involved. Some compounds that are very polar or are insoluble in both water and lipids are not metabolized by the body and are excreted unchanged. Metabolism is typically a two stage process. These are,

- Phase 1 reactions – Phase 1 reactions normally add a functional (polar reactive) group to the foreign molecule which enables the phase 2 reaction to take place.
- Phase 2 reactions – Phase 2 reactions are conjugation reactions and involve the covalent linkage of the toxin or phase 1 product to a polar compound.

Conjugation processes usually required cooperation between membrane bound enzymes in the microsomes and other enzymes and cofactors present in the cytosol. The phase 2 products are generally more water soluble than the original compound and so are more readily excreted, usually in the bile or in urine.

6.1. PHASE 1 REACTIONS OF PESTICIDE METABOLISM

Phase 1 reactions are catalyzed by the cytochrome P450 group of enzymes and other enzymes which are associated with endoplasmic reticulum. Phase 1 reactions include,

- Microsomal oxidation
- Extramicrosomal oxidation

6.1.1. Microsomal oxidation

The NADPH-requiring general oxidation system, commonly referred to as the “Microsomal oxidase system” (also known as monooxygenase or mixed function oxidase system, MFO), is located in the microsomal portions of various tissues, particularly in the liver.

The monooxygenase system gets its name from the way the atoms from the oxygen molecule are separated from one another and end up in different substances. These enzymes are capable of inserting one of the two oxygen atoms from an oxygen molecule into an appropriate substrate, R-H. The other oxygen atom eventually reduced into a water molecule:

\[ R-H + O_2 + [2H] \rightarrow R-OH + H_2O \]

6.1.1.1. Microsomes

Microsomes are accurate morphological and biochemical replica of the endoplasmic reticulum of the intact cell. Chemically they are composed predominantly of lipoprotein, the lipid of which accounts for approximately 40% of the pellet’s weight, phospholipids, lipositol, plasmagen, and fatty acids. They also contain 12% of the total cellular protein and approximately 50% of the ribonucleic acid (RNA). It is the high content of RNA associated with ribosomes which imparts basophilic staining properties to the fraction.

Although the smooth and rough endoplasmic reticulum are part of the same anastomosing system, they are morphologically and biochemically distinct. Both types perform microsomal oxidations, although smooth endoplasmic reticulum has higher activity.
In both cases, activity is associated with the enzymatic components on the membrane itself since various solubilizers and organic solvents disrupt activity whereas ribonuclease has no effect.

6.1.1.2. Components of microsomal oxidases
Mixed function oxidase system, MFO is characterized by:
requiring NADPH as a cofactor,
involving an electron transport system with cytochrome P450, and
being capable of oxidizing many different kinds of substrates (i.e., substrate nonspecificity).
The major components of the system which play the central role in oxidation are:
a flavoprotein, *NADPH-cytochrome c reductase*, and
a unique cytochrome, *cytochrome P450*

6.1.1.3. NADPH-cytochrome c reductase
NADPH-cytochrome c reductase is recognized as a mediator of electron flow from NADPH to the oxygen-activating enzyme.
It is stable flavoprotein, having molecular weight of 70,000 dalton and containing 2 moles of FAD per mole, and is commonly assayed by the reduction of artificially added electron acceptors such as cytochrome c or neotetrazolium in the presence of NADPH.
The level of NADPH-cytochrome c reductase can be increased by the induction of microsomal oxidases and that antibody to NADPH-cytochrome c reductase inhibits oxidative metabolism.
NADPH-cytochrome c reductase has been found in the microsomal fraction of mammalian tissues such as liver, adrenal cortex, spleen, kidney, heart, and lung as well as in the tissues of insects.

6.1.1.4. Cytochrome P450
Cytochrome P450 is carbon monoxide binding pigment of microsomes, are actually hemoprotein of b-cytochrome type.
Cytochrome P450 consists of a single polypeptide having a molecular weight of 45,000 daltons. It contains 1 mole of ferrirprotoporphyrin IX, the iron of which is bound to four pyrrole nitrogens and two amino acid ligands, possibly cysteine and histidine.
The localization of this hemoprotein, however, is not totally restricted to the microsomal fraction, nor, for that matter, to any specific tissue or group of animals. Microbes and insects, in addition to mammals, are known to contain cytochrome P450.
In mammals, it has been located in the microsomal fraction of extrahepatic organs such as the kidney, lung, and placenta and has also been reported in the mitrochondria of the adrenal cortex and corpus luteum.
Cytochrome P450 is also found in the particulate fraction of yeast. In almost all vertebrates species which have liver, cytochrome P450 is most important for oxidation of xenobiotic compounds.
It is also found in skin, nasal mucosa and gastrointestinal tract presumably reflecting defence mechanism at portal of entry.
Cytochrome P450 is the common oxygen-activating enzyme for the entire family of microsomal mixed-function oxidases.

6.1.1.5. Catalytic events of microsomal oxidase system
The process of microsomal oxidation integrates the transfer of electrons from NADPH with the binding of substrate and oxygen at cytochrome P450. Two separate one-electron reductions are involved i.e.
the first occurs after the initial complexing of the substrate with oxidized cytochrome P450 and
the second on formation of the reduced cytochrome P450/substrate/oxygen complex.
Subsequently to catalysis, oxidized cytochrome P450 is regenerated by dissociation of the hydroxylated product and water. The mechanism of microsomal oxidation involves three basic events i.e.
substrate binding,
reduction, and
oxygen binding and activation.

6.1.1.5.1. Substrate binding
Substrate binding is the initial reaction in the microsomal oxidase system in which electrons from NADPH integrates the binding of substrate and oxygen at cytochrome P450.

6.1.1.5.2. Reduction
The overall process of microsomal oxidation requires the transfer of two electrons from NADPH through a series of redox components to cytochrome P450. The expected 1:1 ratio for substrate oxidation and NADPH oxidation can be known from the general equation:

\[ S + O_2 + XH_2 \rightarrow SO + H_2O + X \]

Reduction occurs in two separate one-electron steps. Involved in the reduction of the cytochrome P450-substrate complex via NADPH-cytochrome c reductase is monitored in the presence of carbon monoxide by the appearance of absorbance at 450 nm. The second electron is introduced at the level of the oxygenated cytochrome P450-substrate complex (oxycytochrome P450).

The route of the second electron from a reduced pyridine nucleotide to oxy cytochrome P450 reputedly involves cytochrome b₅, a component of the microsomal electron transport system usually associated with fatty acid desaturation.

6.1.1.5.3. Oxygen binding and Activation
Binding of carbon monoxide to cytochrome P450 in competition with oxygen is an indication of the role of this cytochrome in oxygen activation. In fact, along with the requirements for NADPH and oxygen, inhibition by carbon monoxide is an important criterion for cytochrome P450-mediated microsomal oxidation. Subsequent to its binding to cytochrome P450, the oxygen molecule is activated and split, one atom being inserted into the substrate and the other reduced to water. The mechanism by which cytochrome P450 effects the introduction of oxygen into the substrate could conceivably involve the generation of a free radical or the direct insertion of atomic or molecular oxygen into the substrate.
6.2. Microsomal oxidation of insecticides
Most organic insecticides and synergists are subject to microsomal oxidase system. Many of them possess multiple sites at which oxidation can occur, and consequently a combination of several transformations can take place with any particular compound (Figure 7).
The reactions catalyzed by this system include,

- O-, S-, and N- Alkyl Hydroxylation
- Desulfuration
- Epoxidation
- Thio ester oxidation
- Aromatic hydroxylation

6.2.1. O-, N-, and S- Alkyl Hydroxylation

O-, N-, and S- Alkyl Hydroxylation is an important pathway of organophosphates metabolism in which an alkyl group adjacent to a hetero atom such as oxygen, sulfur, and or nitrogen is a potential target for microsomal hydroxylation, but because of the electronegativity of the hetero atom, the reaction often leads to dealkylation.

These reactions usually takes place in microsomes, however, these may also possibly occur in cytoplasm in minor quantity when specific enzymes are present.

Enzymes responsible for these reactions are dealkylase, desulfurase, and hydrolase.

6.2.2. O- dealkylation

Dealkylation of O-alkyl groups of the ester or ether structures of insecticides occurs readily, but does not take place by simple replacement of an alkoxy group with a hydroxy group. Instead, an unstable α-hydroxyl intermediate is produced which spontaneously releases an aldehyde in the case of a primary alkyl group and a ketone in the case of a secondary alkyl group (Figure 8).
6.2.3. S-dealkylation
Microsomal S-demethylation of several methylthio compounds has been reported, and its involvement in aldicarb metabolism has been inferred from the in vivo conversion of the methylthio carbon to carbon dioxide in the housefly. However, no S-demethylation of aldicarb has been detected in the rat liver in vitro system.

6.2.4. N-dealkylation
N-dealkylation occurs in the metabolism of many organophosphates and carbamates. Unlike O-dealkylation, this reaction often yields a fairly stable N-a-hydroxy alkyl derivative, probably because nitrogen is less electronegative than oxygen. The metabolite may then undergo nonoxidative cleavage to a dealkylated product and an aldehyde (Figure 10).

In the case of N-methyl hydroxylation, further oxidation to an N-formyl derivative has sometimes been noted, although the nature of the oxidase for this step has not been defined.

The activation of the phosphoramidate insecticide, schradan to N-hydroxymethyl schradan is a classical example of N-dealkylation. It is likely that the N-hydroxymethyl derivative is an intermediate in stepwise N-demethylation reactions of this compound.
6.2.5. Desulfuration

Desulfuration is one of the most commonest metabolic pathway of organophosphorus pesticides which contains phosphorothioate and phosphorodithioate esters.

- $P \rightarrow S$ structure of organophosphorus pesticides are desulfurated to their corresponding $P \rightarrow O$ analogues by microsomal oxidases of mammals and insects.

3. The detached sulfur is apparently bound covalently to microsomal macromolecules and is eventually excreted as inorganic sulfate.

Desulfuration, however, represents only part of the microsomal oxidation of these compounds. These esters are concurrently hydrolysed to $P \rightarrow S$ acids and the corresponding leaving groups by the oxidase system.

Oxidative desulfuration of $P=S \rightarrow P=O$ is always lead to more toxic products. These changes are responsible for increase in their toxicity towards the cholinesterase enzyme where phosphorothioate activation to phosphate, an important and direct cholinesterase inhibitor.

This type of oxons are reported in parathion, dimethioate, abate etc (fig.5). The formation of oxons require NADPH and molecular oxygen with microsomes. The eliminated S is absorbed in microsomes and excreted in urine.

$P = S \rightarrow P = O$
6.2.6. Thioether or Sulfur Oxidation
Sulfoxidation of phorate, aldicarb takes place in microsomes where one atom of oxygen is attached with S, forming sulfuroxide and when two atoms of oxygen are attached with S, forming sulfone (Figure 12). Enzyme responsible in this reaction is called sulfoxidase, they are named after the pesticide name, such as phorate sulphoxidase etc. These are soluble in organic solvent and responsible for mixed function oxidase reaction.
Usually the alkyl sulfur in pesticide is rapidly oxidized to sulfoxide and more slowly to sulfones. This type of reaction is very limited in insecticide metabolism. Recently, it was observed that these sulfoxides enter into phase 2 reaction, where they conjugate with glutathione and finally become highly polar and excrete from body.

6.2.7. Aromatic hydroxylation or NIF shift
The NIF shift (named after the National Institute of Health, where it was discovered) is a characteristic of aromatic hydroxylation by all mixed function oxidases.
During such hydroxylation reactions, the hydrogen atom replaced by the hydroxyl group is not always expelled from the molecule, but may migrate to an adjacent position in the ring (Figure 13). The degree of hydrogen retention varies with different substrates. Substitution other than hydrogen (e.g., halogen) may behave in a similar manner. Such retention is not observed in the well-known electrophilic substitution reactions.

![Figure 13. Aromatic hydroxylation](image)

6.2.8. Epoxidation

Epoxidation is an important microsomal reaction in which stable and environmentally persistant epoxides of dihydrodiols are formed. It is one of the important pesticide degradation reaction in case of cyclodiene compounds e.g., heptachlor, aldrin, isodrin, when this reaction occurs, the oxygen gets detached to the place where chlorine is absent, but double bond is present (figure 14).

These epoxides may further go for hydroxylation reaction and form trans-diols. Enzymes responsible for this reaction is epoxidase which is present in microsomes. However, it is also reported from cytoplasm. These epoxides are quite stable metabolites and responsible for pollution in environment. Epoxides are subjected to hydration to form dihydrodiols, by epoxide hydrases. Trans hydrodiol are also catalysed by other enzyme epoxide isomerase. From here, they move into phase 2 reaction, where they conjugate with glucoronic acid and become highly polar and excreted from body.
6.3. EXTRAMICROSOMAL METABOLISM OF INSECTICIDES
Extramicrosomal metabolism contributes minor pathways for the pesticides metabolism. Here the reaction is decided according to the enzyme involve.
Extramicrosomal metabolism includes the following reactions:

- Phosphotriesterase hydrolysis
- Carboxylesterase hydrolysis
- Pyretheroids hydrolysis
- Carboxylamide hydrolysis
- Nitroreductase
- Carbamate hydrolysis
- Epoxide hydrases
- Dechrolination and Dehydrochrolination

Phosphotriesterase hydrolysis

Degradation of organophosphorus pesticides by Phosphotriesterase is an important mechanism for pesticide detoxification outside of the microsome, where enzyme attack in the phosphorus ester, an anhydride bond hence enzyme term as Phosphotriesterase.

There are 3 possible reactions in cytoplasm for detoxification- First, second and third reactions.
1\textsuperscript{st} reaction lead to the formation of dialkyl phosphorothioic acid
2\textsuperscript{nd} reaction lead to the formation of dialkyl phosphoric acid

Figure 14. Epoxidation
3rd reaction lead to the formation to the metabolite desalkyl derivative and an alcohol. This type of reaction takes place in mammalian kidney and liver.

\[
\begin{align*}
(1) \quad (RO)_2P - X + H_2O & \rightarrow (RO)_2POH + HX \\
(2) \quad (RO)_2P - X + H_2O & \rightarrow (RO)_2POH + HX \\
(3) \quad (RO)_2P - X + H_2O & \rightarrow (RO)(HO)P - X + ROH \\
\end{align*}
\]

dialkyl phosphorothioic acid

dialkyl phosphoric acid

desalkyl derivative

\[ \text{Nitroreductase} \]

The reduction of a number of nitro-containing organophosphorus compounds such as parathion, sumithion, and EPN is one of the minor detoxification reactions. The reduction of the nitro group occurs through the enzyme nitroreductases.

Through this reaction, parathion is hydrolyzed in vivo to p-aminophenol, conjugated with glucoronic acid to an appreciable extent and become highly polar which excrete finally in the urine as p-aminophenyl glucoronide.

\[ \text{Phosphotriesterase} \]

\[ \text{Nitroreductase} \]

\[ \text{Phase 2} \]

This type of enzymatic activity is reported in mammalian kidney, spleen, lungs, and erythrocytes, and also in avian kidneys.

NADPH and a high concentration of FAD (1.23 moles) are necessary as cofactors but this reaction is not affected by the presence or absence of oxygen.

\[ \text{Carboxyl ester hydrolysis (Organophosphorus Compounds)} \]

\[ \text{malathion} \]

\[ \text{malathion} \alpha-\text{monoacid} \]
The hydrolysis of both aromatic and aliphatic esters is catalyzed by carboxyl esterase, -esterase, or aliesterase. But these enzymes are not responsible for the hydrolysis of choline esters. Carboxylesterases are very important in the metabolism of a number of types of insecticides, however, most importantly concern with the organophosphorus insecticides, malathion, acethion, etc. The hydrolysis of malathion and acethion by this said enzyme involves cleavage of the carboxyester group to form a water-soluble nontoxic product. In this reaction malathion and acethion forms the metabolite malathion monoacid and acethion monoacid respectively. Only one carbethoxy group of malathion was found to be hydrolysed by carboxylesterase and formed monoacid. This enzyme is found to be widely distributed in mammalian tissues e.g. kidney, lung, spleen, etc. The said enzyme is also reported in microorganisms.

Carboxyl ester hydrolysis (Pyrethroids)

Carboxyl ester hydrolysis (Pyrethroids) is very much similar to that of organophosphorus Compounds where ester linkage of compound $\left[^{14}C\right]$allethrin, $\left[^{14}C\right]$pyrethrum is the major target site of hydrolysis. Enzymes responsible are carboxyl esterases, β-esterases, and aliesterases, very much similar with that of organophosphorus compounds. These enzymes are reported in kidney, lung, and spleen etc.

Carboxylamide Hydrolysis

The number of amide containing organophosphorus pesticide have been reported to metabolise by carboxylamide hydrolysis to their corresponding carboxylic acid derivative e.g. dimethoate, in this case, the product form is monocarboxylic acid which is the direct result of amidase action. The dimethoate monoacid was initially isolated from the urine however it was also reported in various vertebrate tissue.

Amidase activity is reported from various mammalian tissues, lung, muscle and pancreas but not at all from brain, spleen, and blood. It is reported that hydrolyzing activity is highest in rabbit and sheep, followed by dog, rat and steer, and low activity is found in pig, mouse, and guinea pig. Most of the hydrolytic activity was localized in both rat and sheep liver microsomes. Amidase activity was also reported from human liver and also play important role in detoxification of this compound. Enzyme activity is maximum in pH-9.0 and molecular weight is 2.3-2.5 lakh Dalton. Carboxylamidases, like the carboxylesterases, can hydrolyze only phosphorothionate insecticides and are inhibited by the corresponding phosphate analogues.

Carbamate hydrolysis

Carbamate hydrolysis for ester group is reported by the enzyme hydrolases. It is a potential pathway for carbamate detoxification.
Carbaryl has been shown to hydrolyze by plasma albumin fraction from several mammalian and avian sources. The same enzyme is shown to hydrolyze the ethyl, propyl, i-propyl carbamates. It appears that in both microsomal and extramicrosomal reactions, the hydrolysis of carbamate takes place in the similar manner and it is of minor importance in carbamate metabolism.

**Epoxide Hydrases**

Epoxidation is a common phenomenon of pesticide metabolism in cyclodiene insecticide where the insecticide oxidized to form epoxide rings in presence of enzyme epoxidase and they further go for hydrolysis by enzyme epoxide hydrases and form corresponding trans hydrodiols. Reactions involve here is similar as microsomal metabolism, except that enzyme is changed. This reaction is responsible for the deactivation of certain labile epoxides, which may be responsible for carcinogenesis, and also for the detoxification of certain aromatic and olefinic xenobiotics. The enzymes which mediate this reaction is highest in pig and rat and mainly associated by liver followed by kidney, intestine, and lung. Cleavage of epoxide ring of certain cyclodiene insecticide and their analogs has been well demonstrated in housefly.

**Reductive dechlorination and Dehydrochlorination.**

Reductive dechlorination and dehydrochlorination are the two most common metabolic pathways by which chlorinated hydrocarbon insecticide (e.g. DDT) is degraded.

Reductive dechlorination reaction is characterized by removal of chlorine atom and its replacement with a hydrogen atom e.g. dechlorination of DDT to DDD. Dehydrochlorination reaction is also characterized by the removal of chlorine atom but in this reaction, a hydrogen atom from the adjacent carbon is also removed along with the chlorine atom e.g. dehydrochlorination of DDT to DDE. The enzymes responsible are dechlorinase and dehydrochlorinase which are glutathione dependent.
6.4. PHASE 2 REACTIONS

Phase 2 reaction involves conjugation of natural or foreign compounds or their metabolites with readily available, endogeneous conjugating agents (e.g., glucuronic acid, sulfate, acetyl, methyl, glycine) to form conjugates.

Conjugation process may be viewed as a normal biochemical reaction serving as a dual role in intermediary metabolism which is responsible for detoxification of pesticides. Being a biosynthetic process, conjugation is generally energy dependent, so directly or indirectly linked with high energy compounds.

Phase 2 reactions are of the following types:

Type I: Pesticide / metabolite + Activated conjugating agent → Conjugated product

Here, pesticide conjugates into endogenous substance which is already activated by high energy compound and finally forms a conjugated product.

Type I reactions include such conjugations as methylation, acetylation, and the formation of glucuronides, glucosides, and sulfates.

The sites at which these enzymatic reactions occur are distributed throughout the body, although the liver constitutes the principal site.

Type II: Activated Pesticide /metabolite + conjugating agent → Conjugated product

In this case, pesticide is first activated with the high energy compound and then conjugates with the conjugating agent forming the product.

Type II reactions consist of amino acid conjugations, which occur only in the liver and or the kidney.

Type III: Reactive Pesticide / metabolite + reduced glutathione → Conjugated product

Here, the reactive pesticide or their metabolites conjugates with the conjugating agent forming conjugated product. No activation with the energy compound is required.

In this type of conjugation, the pesticides or their metabolites possess certain chemical groups such as halogens, alkenes, NO₂, epoxides, aliphatic and aromatic compounds.

In general, conjugated products are ionic, polar, less lipid soluble, less toxic and easily excretable from body. Among the above three types of conjugating reactions, Type I is very common, and occurs in almost all pesticides.

Types of reaction include are:
- Glucoside conjugation
- Glutathion-s-transferase conjugation
- Sulphate conjugation
- Phosphate conjugation
- Methyl transferase conjugation
- Glycine conjugation
- Cysteine conjugation

**Glucoside conjugation**

Glucosidation is very important reaction in the pesticide metabolism. This type of conjugation is found both in plants and animals where reactive intermediate is derived from the universal energy fuel, glucose. The supply of this molecule is less likely to be depleted than of amino acids and other proteins.

Glucosidation is a major pathway of conjugation reaction because it has a great capacity to react with the wide range of molecules.

In order to conjugate the pesticide molecule with glucose, a high energy endogenous molecules are required, which first activated the glucose and then this glucose are available for conjugation with the pesticide.

This reaction is of two types:
- Glucoronic acid conjugation
- Glucose conjugation
**Glucoronic acid conjugation**

\[
\text{D-glucose-1-Pi + UTP} \xrightarrow{\text{UDPG pyrophosphorylase}} \text{UDP-\(\alpha\)-D-glucose + PPi ~ (a)}
\]

\[
\text{UDP-\(\alpha\)-D-glucose + 2NAD + H}_2\text{O} \xrightarrow{\text{UPDG dehydrogenase}} \text{UDP-\(\alpha\)-D-glucuronic acid + 2NADH + H}_2\text{O} ~ \text{(b)}
\]

In glucoronic acid conjugation, the reaction intermediate is UDPG (Uridine diphosphate Glucose), which further changes into UDPGA (Uridine diphosphate Glucuronic acid), and this conjugate with the pesticide in presence of the enzyme glucoronyltransferase.

\[
\text{UDP-\(\alpha\)-D-glucuronic acid + ROH} \xrightarrow{\text{glucoronyltransferase}} \text{RO-\(\beta\)-D-glucuronic acid + UDP + H}_2\text{O} ~ \text{(c)}
\]

Reactions (a) and (b) are catalyzed by enzymes present in the nuclear and soluble fraction of the liver, respectively.

The enzyme responsible for reaction (c), UDP glucoronyltransferase is located in the microsomal fraction. Glucuronide formation occurs mainly in the liver, although other organs and tissues such as kidney, intestines, and skin also possess enzyme activity.

A wide variety of chemicals can be conjugated with glucuronic acid, the most common functional groups involved being the hydroxyl, carboxyl, and amino moieties.

**Glucose conjugation**

\[
\text{D-glucose-1-Pi + UTP} \xrightarrow{\text{UDPG pyrophosphorylase}} \text{UDP-\(\alpha\)-D-glucose + PPi}
\]

\[
\text{UDP-\(\alpha\)-D-glucose + ROH} \xrightarrow{\text{glucoronyltransferase}} \text{RO-\(\beta\)-D-glucose + UDP + H}_2\text{O}
\]

Glucose conjugation was regarded as most important reaction in pesticide detoxification, both in animals and plants.

In this reaction, glucose is first activated in presence of enzyme pyrophosphorylase and then conjugated with pesticide in presence of glyoxyl transferase and form the product which is highly polar and thus excreted from body.

In mammals, glyoxyl transferase is located primarily in liver microsomal fraction while in insect, it is distributed to the subcellular level.

**Glutathion Conjugation (Mercapteric Acid Formation)**

This is type III reaction, here neither pesticide nor conjugating agent is get activated, but both are reactive. The main enzyme involved in this reaction is Glutathion-s-transferase which are group of enzymes that catalyze conjugation of electrophilic xenobiotic compounds with endogenous reduced glutathione. In this reaction, formation of mercapturic acid involves 4 important steps:

\[
\text{RX + GSH} \xrightarrow{\text{GSH-s-transferase}} \text{RSG + HX}
\]

\[
\text{RSG} \xrightarrow{\text{\(\alpha\)-glutamylytransferase}} \text{R-Cys-Gly + glutamate}
\]
In this type of reaction, the substrate is first conjugate with reduced glutathione (GSH) in presence of enzyme Glutathion-s-transferase which further conjugate with cysteine and glycine to form cys-gly conjugate, and then in presence of peptidase form premercapturic acid.

Subsequently cys-gly conjugate is acetylated to form mercapturic acid which becomes highly polar and eliminated in urine.

This Glutathion-s-transferase is present in soluble fraction of mammalian liver and/or kidney, the glutamyl transferase is more active in kidney than liver.

Glutathion-s-transferase are involved in wide variety of electrophillic insectides conjugation which can be metabolized by glutathione dependent reaction e.g. lindane, DDT and also many organophosphorus pesticide that are dealkylated or dearylated.

Glutathion-s-transferase work for binding protein and serve as a storage place for toxic compound that have lipophillic nature.

In certain strains of insects Glutathion-s-transferase plays an important role in development to resistance to pesticide e.g Housefly.

This reaction is also reported in mammals, reptiles, birds, fishes and invertebrates.

**Phosphate Conjugation**

Although the biosynthesis of phosphate esters is a common occurrence in intermediary metabolism, the conjugation of foreign compounds with phosphate is rarely encountered in nature.

Insects appear to be the major group of animals in which phosphate conjugation has been studied to any extent.

In insects phosphate conjugation is reported in several members of coleopterans, Lepidoptera and hymenoptera.

It is reported that an active phosphotransferase in insects catalyzed the phosphorylation of 4-nitrophenol in the presence of ATP and Mg$^{2+}$. This enzyme is present in the high speed supernatant (100,000g) of gut tissue homogenates of the Madagascar cockroach and tobacco hornworm and of whole body homogenates of the housefly.

It is possible that ATP may serve as the activated conjugating agent in the enzymatic phosphorylation of foreign compounds by analogy with other type 1 conjugations.

$$\text{ROH} + \text{ATP} \xrightarrow{\text{phosphotransferase}} \text{ROPO}_3^{2+} + \text{ADP}$$

Among mammals, phosphate conjugation is reported in human and dog.

There are also reports concerning the formation of phosphate conjugates in fungi and the primitive arthropod peripatus.

**Sulphate conjugation**

Sulphate ester formation readily occurs in phenolic hydroxyl, alcoholic hydroxyl and aromatic amino group.

Sulphate ester in biological conjugation is in reality half ester which is completely ionized and highly soluble in H$_2$O.
Conjugation by sulphate formation requires two stable activations. The sulphate ion is activated by ATP-sulfurylase, in the following reactions:

\[
\text{ATP-sulphate} \\ \text{ATP + SO}_4^{2-} \rightarrow \text{Adenosine-5'-phosphosulphate (APS) + PPI} \]

\[\text{ATP-adenylyl sulphate} \]

\[
\text{APS + ATP} \rightarrow 3’\text{-phosphoadenosine-5’-phosphosulphate (PAP)} + \text{ADP} \\
\text{3’-phosphotransferase} \\
\text{ROH + PAPS} \rightarrow 3’\text{-phosphoadenosine-5,6-phosphosulphate (PAPS)} + \text{ROSO}_3\text{H} \\
\text{sulphotransferase}
\]

This is the second type reaction, in which the reaction requires the biosynthesis of an active intermediate, \(3’\text{-phosphoadenosine-5,6-phosphosulphate (PAPS)}\).

In the second activation reaction, APS kinase catalyzed the formation of activated sulphate PAPS through the reaction (b).

Sulphate conjugates are formed by transfer of sulphate moiety from PAPS in presence of enzyme sulphotransferase in form of aryl or alkyl sulphates.

Enzymes responsible for reaction (a) and (b) are located in the soluble fraction of the cell.

Reaction (c) occurs with a very broad spectrum of natural and foreign substrates which include phenols, steroids, arylamines, chondroitin, choline, tyrosine methyl ester, luciferin, galactocerebroside, and heparin.

It is reported that there is a family of at least 12 sulfotransferases which catalyze the PAPS-dependent sulphate conjugation in various organisms.

In general, this enzyme system is located in the soluble fraction of the cell and the liver, while the presence of sulphotransferases in the gut tissues of the southern armyworm (Prodenia eridania) has also been reported.

This enzyme system is active toward 4-nitrophenol as well as toward several naturally occurring mammalian, insect, and plant steroids, including cholesterol, \(\alpha\)-ecdysone, and \(\beta\)-sitosterol.

\[\text{Methylation} \]

\[
\begin{align*}
\text{nicotine} & \rightarrow \text{methylnicotine} \\
\text{CH}_3 & \text{CH}_3
\end{align*}
\]

Methylation is also known as Methyl transferase reaction.

Bio-methylation is an important reaction for metabolism of endogenous compounds or exogenous compounds containing \(\text{O}_2\), \(\text{S}\), \(\text{N}\), as a functional group.

The co-enzyme that are possible source of methyl group are as adenosyl methionine, 5-methyl etra hydropholic acid, vitamin \(\text{B}_12\).

The basic reaction involves the transfer of activated methyl group from S-adenosyl methionine to substrate to form methylated product and S-adenosyl homocysteine.

The substrate for this reaction are primary, secondary and tertiary amines and azo hetero cycles, phenolic and thiol compounds to form nitrogen and oxygen methyl conjugates.
In general, methylated products are less water soluble than their parent compound. The enzymes responsible for methylation is reported from both plants and animals. This very enzyme can further be classified as 5-adenosyl 6-methionine dependent methyl transferase or N-O adenosyl 6-methionine dependent transferase.

**Glycine conjugation**

Aromatic and some aliphatic carbocyclic acids are often conjugated with amino acid in various organisms, the most widely occurring reaction involving amino acid glycine. Glycine conjugation occurs in two stages:

Activation of substrate (RCOOH) through an enzyme system which requires ATP and coenzyme A.

Condensation of activated substrate with glycine.

\[
\text{acyl synthetase} \\
\text{RCOOH} + \text{ATP} \xrightarrow{\text{acyl synthetase}} \text{RCO-AMP} + \text{PPI} \\
\text{acyl thiokinase} \\
\text{RCO-AMP} + \text{CoA-SH} \xrightarrow{\text{acyl thiokinase}} \text{RCO-S-CoA} + \text{AMP} \\
\text{acyl-CoA:} \\
\text{RCO-S-CoA} + \text{Glycine} \xrightarrow{\text{Glycine N-acyl transferase}} \text{RCO-Gly} + \text{CoASH}
\]

Normally these enzymatic reactions take place in the mitochondrial fraction of liver and kidney cells. Rat intestinal preparations have also been shown to be active in glycine conjugation. Glycine conjugation occurs in mammals, insects, amphibians, some birds, and reptiles. In certain species where glycine conjugation is absent, it appears to be replaced by conjugations involving other amino acid such as ornithine, arginine, and glutamine.

**Cysteine conjugation or Amino acid conjugation**

This is also a very important phase II reaction where the metabolic product of pesticide conjugate with cysteine before the formation of mercapturic acid and it become a highly polar compound and finally excreted from the body.

Cysteine conjugation is mainly reported in cyclodiene insecticides where they degrade by epoxidation and finally they form trans di-hydrodiol and this conjugate with amino acid cysteine at 4\textsuperscript{th} position e.g. aldrin. This type of conjugation is reported in mammals, fish, bird, reptiles, plants etc.

7. TOXICOLOGICAL SYMPTOMS

Pesticides are a diverse group of substances with a potential for varied toxic effects. They can enter the human body in three ways: by absorption, through the skin or eyes (dermally), through the mouth (orally), and by breathing into the lungs (inhalation).

7.1. Dermal exposure results in absorption immediately after a pesticide comes in contact with skin or eyes. Absorption continues as long as the pesticide remains in contact with the skin. The rate at which dermal absorption occurs is different for each part of the body. The relative absorption rates are determined by comparing each respective absorption rate with the forearm absorption rate.

7.2. Oral exposure may result in serious illness, severe injury, or even death, if a pesticide is swallowed. Pesticides can be ingested by accident, through carelessness, or intentionally.

7.3. Respiratory exposure is particularly hazardous because pesticide molecules can be rapidly absorbed by the lungs into the bloodstream. Pesticides can cause serious damage to nose, throat, and lung tissue if inhaled in sufficient amounts. Vapors and very small amount of pesticide pose the most serious risks. Lungs can be exposed to pesticides by inhalation of powders, airborne droplets or vapors. Handling concentrated wettable powders can pose a hazard if inhaled during mixing. The hazard from inhaling
pesticide spray droplets is fairly low when dilute sprays are applied with low pressure application equipment. This is because most droplets are too large to remain airborne long enough to be inhaled. However, when high pressure, ultra low volume (ULV), or fogging equipment is used, the potential for respiratory exposure is increased. The droplets produced during these operations are in the mist- or fog-size range and can be carried along with the air currents for a considerable distance.

Toxicity of insecticides: In an organism toxicity can be classified as,

Acute toxicity of a pesticide refers to the effects from a single exposure or repeated exposure over a short time, such as an accident during mixing or applying pesticides. Various signs and symptoms are associated with acute poisonings. A pesticide with a high acute toxicity can be deadly even if a small amount is absorbed. It can be measured as acute oral toxicity, acute dermal toxicity or acute inhalation toxicity.

Chronic toxicity refers to the effects of long-term or repeated lower level exposures to a toxic substance. The effects of chronic exposure do not appear immediately after first exposure and may take years to produce signs and symptoms. Examples of chronic poisoning effects may include:

- Carcinogenicity- produce cancer.
- Mutagenicity- cause genetic changes.
- Teratogenicity- cause birth defects.
- Oncogenicity-ability to induce tumor growth (not necessarily cancers).
- Liver damage-death of liver cells, jaundice (yellowing of the skin), fibrosis and cirrhosis.
- Reproductive disorders-reduced sperm count, sterility, and miscarriage.
- Nerve damage-accumulative effects on cholinesterase depression associated with organophosphate insecticides.

Allergic sensitization-development of allergies to pesticides or chemicals used in formulation of pesticides.

The effects of chronic toxicity, as with acute toxicity, are dose-related. In other words, low-level exposure to chemicals that have potential to cause long-term effects may not cause immediate injury, but repeated exposures through careless handling or misuse can greatly increase the risk of chronic adverse effects. Poisoning signs can be seen by others, for example, vomiting, sweating, or pin-point pupils. Symptoms are any functional changes in normal condition which can be described by the victim of poisoning, and may include nausea, headache, weakness, dizziness, and others.

7.4. Poisoning of Organochlorine pesticides

Organochlorine pesticides are not readily biodegradable and persist in the environment. These materials affect the nervous system as stimulants or convulsants. Nausea and vomiting commonly occur soon after ingesting organochlorines.

Early signs and symptoms includes, apprehension, excitability, dizziness, headache, disorientation, weakness, a tingling or pricking sensation on the skin, and muscle twitching. This is followed by loss of coordination, convulsions similar to epileptic seizures, and unconsciousness. When chemicals are absorbed through the skin, apprehension, twitching, tremors, confusion, and convulsions may be the first symptoms.

No specific antidotes are available for organochlorine poisoning. Remove contaminated clothing immediately, and then bathe and shampoo the person vigorously with soap and water to remove pesticide from the skin and hair. Persons assisting a victim should wear chemical resistant gloves and be careful to avoid becoming contaminated by the pesticide. If the pesticide has been ingested, empty the stomach as soon as possible by giving the conscious patient ipecac and water or by inserting a finger into the throat.

7.5. Poisoning of Organophosphorus pesticides

These chemical groups affect humans by inhibiting acetyl cholinesterase, an enzyme is required for proper functioning of the nervous system. The effects of OP pesticides are rapid. Symptoms begin shortly after exposure. Exposure to this class of insecticide may pose serious risks for persons with reduced lung
function, convulsive disorders, etc. In some cases, alcoholic beverage consumption may exacerbate the pesticide effects.

Signs and symptoms associated with mild exposures to organophosphate insecticides include:
- headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps and diarrhea,
- blurred vision associated with excessive tearing,
- contracted pupils of the eye,
- excessive sweating and salivation,
- slowed heartbeat, often fewer than 50 per minute,
- rippling of surface muscles just under the skin.

These symptoms may be mistaken for those of flu, heat stroke or heat exhaustion, or upset stomach. Moderately exposure of organophosphate and carbamate insecticide poisoning cases exhibit all the signs and symptoms found in mild poisonings, but in addition, the victim:
- is unable to walk,
- often complains of chest discomfort and tightness,
- exhibits marked constriction of the pupils (pinpoint pupils),
- exhibits muscle twitching,
- has involuntary urination and bowel movement.

Severe poisonings are indicated by incontinence, unconsciousness and seizures.

The order in which these symptoms appear may vary, depending on how contact is made with the pesticide. If the product is swallowed, stomach and other abdominal manifestations commonly appear first, if it is absorbed through the skin, gastric and respiratory symptoms tend to appear at the same time. Fortunately, good antidotes are available for victims of organophosphate or carbamate poisoning at emergency treatment centers, hospitals, and many physicians' offices. As with all pesticide poisonings, time management is extremely critical. If a pesticide is swallowed, obtain prompt medical treatment. If a dermal exposure has occurred, remove contaminated clothing, wash exposed skin and seek medical care.

7.6. Poisoning of Carbamate pesticides

These pesticides also are cholinesterase inhibitors (nerve poisons) and range in toxicity from low to mild toxicity. Symptoms may include:
- **Mild exposure** - constricted pupils, salivation (slobbering), profuse sweating
- **Moderate exposure** - fatigue, uncoordinated muscles, nausea, vomiting
- **Severe exposure** - diarrhea, stomach pain, tightness in the chest.

7.7. Poisoning of Plant derived and Synthetic pesticides

**Synthetic Pyrethroids**

Pyrethroids are synthetically produced compounds that mimic the structure of naturally occurring pyrethrins. Some may be toxic by the oral route, but usually ingestion of pyrethroid insecticide presents relatively little risk. Very large doses may rarely cause incoordination, tremors, salivation, vomiting, diarrhea, and irritability to sound and touch. Most pyrethroid metabolites are promptly excreted by the kidney. Pyrethroids are not cholinesterase inhibitors. Crude pyrethrum is a dermal and respiratory allergen. Skin irritation and asthma have occurred following exposures. The refined pyrethrins are less allergenic, but appear to retain some irritant and/or sensitizing properties.

In cases of human exposure to commercial products, the possible role of other toxicants in the products should be considered. The synergists, such as piperonyl butoxide, have low toxic potential in humans, but organophosphates or carbamates included in the product may have significant toxicity. Pyrethrins themselves do not inhibit the cholinesterase enzyme.

Systemic toxicity by inhalation and dermal absorption is low. There have been very few systemic poisonings of humans by pyrethroids. Dermal contact may result in skin irritation such as stinging, burning, itching, and tingling progressing to numbness.

**Rotenone**
This naturally occurring substance is present in many plants. It is formulated as dusts, powders, and sprays for use in gardens and on food crops. Although rotenone is toxic to the nervous systems of insects, fish, and birds, commercial rotenone products have presented little hazard to humans.

7.8. Poisoning of Bioinsecticides pesticides

*Bacillus thuringiensis* (Bt). From studies involving deliberate ingestion by human subjects, it appears possible that the organism can cause inflammation of the digestive tract. No irritation or sensitization effects have been reported in workers preparing and applying commercial products.

7.9. Treatment and Therapy to Pesticide Toxicity

Persons who are frequently involved with pesticides should become familiar with these important steps:
- Recognize the signs and symptoms of pesticide poisoning.
- Get immediate help from a local hospital, physician, or the nearest poison control center.
- Identify the pesticide to which the victim was exposed. Provide this information to medical authorities.
- Have a copy of the pesticide label present when medical attention is begun. The label provides information that will be useful in assisting a pesticide poisoning victim.

Treatment for poisoning with OP and carbamate insecticides involves the use of atropine which counteracts the muscarinic effects, keeping the individual alive. For OP poisoning events, pralidoxime is given to prevent “aging” of the enzyme/pesticide complex. If aging is prevented, normal enzyme activity will slowly be restored as the insecticide molecule disassociates from the enzyme.

8. THERAPY AND ANTIDOTES

Antidotes are remedy or other agent used to neutralize or counteract the effects of a poison. Medical antidotes are available to neutralize the poisoning effects of the pesticides. Taken improperly, however, these antidotes can be more dangerous than the effects of the pesticide itself. Medical antidotes should be prescribed or given only by a physician. There are no known antidotes for some pesticides. Once a lethal dose has been ingested, the effects are irreversible and terminal.

The enzyme cholinesterase regulates the chemical transmission of nerve impulses, and the poison victim will die without it. Both carbamate and organophosphate pesticides attack this enzyme in the blood and make it useless. After a physician has determined the patient’s base level of cholinesterase, a simple blood test will show if this level has decreased. If the cholinesterase level has decreased, the patient has been overexposed to either organophosphate or carbamate pesticide. One should avoid further contact with these pesticides until his cholinesterase level has returned to normal. In severe cases, medical antidotes must be given.

8.1. Types of Antidotes

8.1.1. Cholinesterase reactivators: These medications are used as antidotes to reverse the inhibition of acetylcholinesterase. The effectiveness of oxime compounds is attributed to their 2-formyl-1-methylpyridinium ions. Pralidoxime (Protopam, 2-PAM) - Nucleophilic agent that reactivates phosphorylated AChE by binding to the OP molecule. These antidotes are used to treat muscle weakness and respiratory muscle weakness in known or suspected OP exposure and must be administered into the patient within 24 h. The earlier this medication is administered, the better the result. Because it does not significantly relieve respiratory center depression or decrease muscarinic effects of AChE poisoning, concomitantly administer atropine to block effects of OP poison on these areas. Signs of atropinization might occur earlier with addition of 2-PAM to treatment regimen. Action of barbiturates potentiated by AChE inhibitors, antagonism with neostigmine, pyridostigmine, and edrophonium, morphine, theophylline, aminophylline, succinylcholine, reserpine, and phenothiazines can worsen condition of patients poisoned by OP insecticides or nerve agents. Rapid injection can cause tachycardia, laryngospasm, muscle rigidity, pain at injection site, blurred vision, diplopia, impaired accommodation, dizziness, drowsiness, nausea, tachycardia, hypertension, and hyperventilation, can precipitate myasthenia crisis in patients with myasthenia gravis and muscle rigidity in healthy volunteers, decrease in renal
function increases serum drug levels because 2-PAM is excreted in urine, can transiently increase creatinine phosphokinase level, SGOT and/or SGPT levels increase in 1 of 6 patients.

8.1.2. Anticholinergics such as atropine, cause pharmacologic antagonism of excess anticholinesterase activity at muscarinic receptors. Oximes reverse the inhibition of AChE and nicotinic effects, including muscle paralysis.

8.1.3. Atropine (Atropair) - Used for GI or pulmonary distress in known or suspected OP or carbamate poisonings. Continue until bronchoconstriction and bronchorrhea is controlled. High doses may be required in first 24 h of treatment. Treatment may be required for 48 h in severe cases.

8.1.4. Pralidoxime (2-PAM, Protopam) - Indications include muscle weakness (especially respiratory) in known or suspected OP poisoning. Must be used early in poisoning, before OP-AChE bond has aged, to be effective. May help prevent intermediate and delayed neuromuscular and neuropsychiatric OP syndromes.

8.1.5. Diazepam (Valium) - Depresses all levels of CNS (eg, limbic and reticular formation), possibly by increasing GABA activity. Effects potentiated by phenothiazines, narcotics, barbiturates, MAOIs, and other antidepressants. Caution with other CNS depressants, low albumin levels, or hepatic disease (may increase toxicity), monitor for respiratory depression with high or repeated doses.

8.1.6. Lorazepam (Ativan) - DOC for treatment of status epilepticus because persists in CNS longer than diazepam. Rate of injection not to exceed 2 mg/min. May be administered IM if IV access not available. Monitor for respiratory depression with high or repeated doses, contains benzyl alcohol, which may be toxic to infants in high doses, caution in renal or hepatic impairment, myasthenia gravis, organic brain syndrome, Parkinson disease, or inhibited benzodiazepine metabolism and clearance (eg, in use of nicotine or cimetidine).

8.2. Methods of administration of the Antidotes for different groups of pesticides

8.2.1. Organophosphates

Inject atropine sulphate intravenously in a dose of 2-4 mg./kg body weight for an adult (0.04 to 0.08 mg/kg body weight for children) every 5-10 minutes until signs of atropinisation occur e.g. Dry mouth and usually dilated pupils. Maintain atropinisation for atleast 24-48 hours and carefully observe the patient as further atropinisation is stopped. It may be necessary to recommence treatment if signs of poisoning return.

Convulsions and anxiety can be treated with 5-10 mg. of diazepam injected intramuscularly. While keeping the patient fully atropinised, administer also an oxime, if available, cholinesterase reactivator e.g. 2-PAM 1000-2000 mg/kg body weight IM (IntraMuscular) or IV (IntraVenous) for adults (25 mg/kg body weight for children), or Toxogonin (Merck) 250 mg for adults (4-8 mg/kg body weight for children). Repeat if necessary after 1-2 hours.

Morphine, phenothiazines, Succinylchloride, xanthenederivative, epinephrine and barbiturates are contraindicated.

9.2.2. Carbamates

Atropine therapy as indicated for organophosphorous compounds.

Oximes such as 2-PAM, P2S, Toxogonin should not be administered.

Convulsions can be treated with diazepam (valium, Roche)

8.2.3. Organochlorines

Obtain and secure an unobstructed air way by suction, if necessary, from pharynx and trachea. If necessary, give artificial respiration.

Control convulsions by administering anticonvulsants like diazepam or paraldehyde, soluble barbiturates (Phenobarbital upto 0.7 gm per day or pentobarbital 0.25 to 0.5 gm per day)

10% Calcium gluconate to be given IntraVenously.
In severe cases, it is necessary to protect vital organs like liver by injecting corticosteroids and kidney by dialysis.
Give fat free diet with high proteins, carbohydrates and calcium.
Adrenalin derivatives are contraindicated, since they may induce ventricular fibrillation.
Avoid giving morphine, theophyline or aminophylline.
Patients who have had one or more convulsions should be kept under close observation for at least 24 to 48 hours.

8.2.4. Synthetic Pyrethroids
In case of severe skin exposure in handling or application, typical sensations of exposed skin, especially of face may appear which can be described as tingling, burning or numbness. These sensations will wear off in the course of a few hours.
The treatment is symptomatic.
In case larger amounts have been ingested, perform gastric lavage.
Administration of activated charcoal followed by saline Cathartic with Sodium sulphate solution.
Control seizures with injectable diazepam or barbiturates.

9. SUMMARY

9.1. Toxicology of insecticides
The toxicity of insecticides to an organism is usually expressed in terms of the LD$_{50}$ (lethal dose 50 percent) and LC$_{50}$ (50 percent lethal concentration). And the interaction of toxic chemical with a given biological system is dose-related. So, it is the dose which makes substances poison. The right dose differentiates a poison and a remedy. At high doses, all the chemicals are toxic, at appropriate intermediates doses they are useful and at low enough doses they do not have a detectable toxic effect. There are certain pesticides (e.g. DDT) known today, because of the long term exposure to them at doses that do not immediately kill the organism showed severe effects like Carcinogenic, Mutagenic and Teratogenic effects.

Insects administered chemicals by several methods including topical application, Injection Method, Dipping Method, Contact or Residual Method, Feeding and Drinking Method. The susceptibility of insect population to a certain poison is assessed by constructing dosage-mortality curve in which the logarithmic scale of dosages is plotted against the probit units of percent mortalities at a given period of time.

Insecticides classification, group characteristics with example and mode of action

<table>
<thead>
<tr>
<th>GROUP CHARACTERISTICS AND MODE OF ACTION</th>
<th>EXAMPLE WITH CHARACTERISTICS OF INDIVIDUAL PESTICIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organocholine (OC) insecticides:</strong> They are characterized by</td>
<td></td>
</tr>
<tr>
<td>Presence of carbon (organo-), chlorine and hydrogen, and sometimes oxygen atoms including a number of C-Cl bonds.</td>
<td></td>
</tr>
<tr>
<td>Presence of cyclic carbon chains including benzene ring.</td>
<td></td>
</tr>
<tr>
<td>Lack of any particular active intramolecular sites.</td>
<td></td>
</tr>
<tr>
<td>Polar and liposolic (soluble in fat).</td>
<td></td>
</tr>
<tr>
<td>Chemically unreactive</td>
<td></td>
</tr>
<tr>
<td>Mode of action: organochlorine insecticides are neurotoxicants, inhibitor of Na$^+$, K$^+$ and Mg$^+$ ATPase. Most of them causes accumulation of</td>
<td></td>
</tr>
<tr>
<td><strong>DDT:</strong> 2,2-bis-(p-chlorodi pheny)-1,1,1-trichloroethane</td>
<td></td>
</tr>
<tr>
<td>Trade or other names: DDT, Anofex, Gyon Cesarex, Chlorophenothane, Guesapon, Guesarol, Gexarex, etc.</td>
<td></td>
</tr>
<tr>
<td>Appearance: Technical product p,p’-DDT is white tasteless, almost odorless crystalline solid.</td>
<td></td>
</tr>
<tr>
<td>Empirical formula: C$_{14}$H$_9$Cl$_5$</td>
<td></td>
</tr>
<tr>
<td>Molecular Weight: 354.49</td>
<td></td>
</tr>
<tr>
<td>Melting Point: 108.5-109°C</td>
<td></td>
</tr>
<tr>
<td>Vapor pressure: 1.5 x 10$^{-7}$ mm Hg at 20°C</td>
<td></td>
</tr>
<tr>
<td>ADI: 0.005 mg/kg/b.w/d (man)</td>
<td></td>
</tr>
<tr>
<td>Toxicity, single dose: Rat (male): Oral LD50 = 250 mg/kg, Dermal 250-500 mg/kg in oil, 3000 mg/kg as</td>
<td></td>
</tr>
</tbody>
</table>
acetylcholine in nerves of insects but it does not inhibit the enzyme cholinesterase.

Organophosphorous insecticides (OP) They are neutral ester or amide derivatives of phosphorous acids carrying a phosphoryl (P-O) or thiophosphoryl (P-S) group. Certain fluorides and chlorides are also used commercially to develop OP, however only one phosphoric acid is known for its insecticidal properties. OPs are manufactured at very high temperatures (150-200°C), thus they commonly contain isomers or bioproducts which are responsible for their unpleasant odour, and for their anticholinesterase activity. Mode of action: OPs react with a serine hydroxyl group within the enzyme active site, phosphorylating this hydroxyl group and yielding a hydroxylated leaving group. This process inactivates the enzyme and blocks the degradation of the neurotransmitter acetylcholine.

**PARATHION**: O,O-diethyl O-4-nitrophenyl p
Trade or other names: Trade names include, Alkron, Alleron, Aphanite, Corothion, E-605, Ethyl parathion,
Appearance: Pure parathion is a pale yellow liquid with a faint odor of garlic at temperatures above 6°C. Technical parathion is a deep brown to yellow liquid
Empirical formula: C_{10}H_{14}NO_{5}PS
Molecular Weight: : 291.3
Solubility: 24 mg/L at 25°C in water, slightly soluble in petroleum oils, miscible with most organic solvents.
Boiling Point: 157-162°C /0.6 mm Hg
Vapor pressure: 3.78 x 10^{-5} mm Hg at 20°C
ADI: 0.006 mg/kg/b.w/d
Toxicity, single dose: Rat (male): Oral LD50 = 13 mg/kg, Dermal 21 mg/kg, Rat (female): Oral LD50= 3.6 mg/kg, Dermal 6.8 mg/kg.

**CARBARYL**: 1-napthyl methylcarbamate
Trade or other names: Carbaryl, Adios, Bugmaster, Carbamec, Carbamine, Crunch, Denapon, Dicarban
Appearance: Carbaryl is a solid that varies from colorless to white or gray, depending on the purity of the compound. But pure carbaryl is colorless crystalline solid.
Empirical formula: C_{12}H_{11}NO_{2}
Molecular Weight: : 201.2
Solubility: 120 mg/L at 30°C in water, soluble in most organic solvents such as dimethylformamide and dimethyl sulphoxide.
Melting Point: 142°C
Vapor pressure: < 4 x 10^{-5} mm Hg at 25°C
ADI: 0.01 mg/kg/b.w/d
Toxicity, single dose: Rat (male): Oral LD50 = 850 mg/kg, Dermal > 4000 mg/kg.

**PYRETHRIDS**: natural esters formed by combination of 2 carboxylic acid and 3 keto acid. It is extracted from plant chrysanthemum cineriaefolium.
Pyrethrum concentrate was prepared from flower by extracting with petroleum ether or methanol

**CYPERMETHRIN**: (RS)-a-cyano-3-phenoxybenzyl (I RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-carboxylate
Trade or other names: Ammo, Arrivo, Barricade, Basathrin and Super.
Appearance: Pure isomers of cypermethrin form
or acetone or dichloromethane or ethylene dichloride. Technical pyrethrum contains 20-30 percent toxic ingredient. LD$_{50}$ is around 1500 mg/kg body weight for rat, but very toxic for insects. There are four principal active ingredients in pyrethrum flowers, known as *pyrethrins I and II* and *cinerins I and II*. In addition, small quantities of *jasmolins I and II* are present, these differ from the pyrethrins only in that one double bond in the side chain of the alcohol moiety of pyrethrins is saturated. Mode of action: They are nerve poisons and affect the nerve axon, causing repetitive discharge of nerves which results in eventual paralysis.

<table>
<thead>
<tr>
<th>Colorless crystals. When mixed isomers are present, cypermethrin is a viscous semi-solid or a viscous, yellow liquid</th>
<th>Empirical formula: C$<em>{22}$H$</em>{19}$Cl$_2$NO$<em>3$ Molecular Weight: 416.3 Solubility: 0.01-0.2 mg/L at 21°C in water, 103g/l in hexane, &gt;450 g/l in acetone at 20°C, soluble in Cyclohexane, ethanol, xylene, chloroform. Melting Point: 60-80°C (pure isomers) Vapor pressure: 3.8 x 10$^{-8}$ mm Hg at 70°C (for pure compound). ADI: 0.05 mg/kg/b.w/d Toxicity, single dose: Rat: Oral LD$</em>{50}$ = 303-4123 mg/kg (depending on the carrier and condition used).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botanical insecticides: Naturally occurring chemicals extracted from plants. They are known to produce a diverse range of secondary metabolites such as alkaloids, flavonoids, polyacetylenes, terpenoids, etc. Should be safe to environment i.e. biodegradable Should have low toxicity to mammals Plant availability should be ensured Isolation of active compounds should be economical Mode of action: Certain chemicals affect the electron transport chain thus disrupting the normal metabolic pathway. Examples are rotenone (slows heartbeat, depresses respiration and oxygen consumption, and causes paralysis and death) and arsenicals (inhibit respiratory enzymes).</td>
<td><em>Nicotine</em>: The main source of nicotine are the two species <em>Nicotiana tabacum</em>, and <em>N. rustica</em>, the latter being more abundant in India. Free nicotine is a colourless or pale-yellow oily liquid. It has an odour of pyridine, because of its high volatility free nicotine is mainly used as a fumigant. In agriculture nicotine is used as nicotine sulphate which acts as a stomach poison. Addition of alkaline compounds such as soap and calcium caseinate at the time of spraying, liberates the nicotine more quickly, making it a more effective contact insecticide or fumigant. LD$_{50}$ of nicotine sulphate to rat is oral 83 mg/ kg body weight and dermal 285 mg/ kg body weight. Nicotine is a nerve poison and mimics acetylcholine at the nerve synapse.</td>
</tr>
<tr>
<td>Bioinsecticides: Natural biological control agents either by introducing new species into the environment of a pest or by increasing the effectiveness of those already present. Traditionally, this method was employed to control insect pests by parasitoids, predators and pathogens. Bioinsecticide use involves three major techniques, viz. introduction, conservation and augmentation.</td>
<td><em>Bacillus thuringiensis</em> (Bt) forms a crystalline inclusion body during sporulation that contains a number of insecticidal protein toxins. When consumed by the insect, the inclusion is dissolved in the midgut and releases d-endotoxins. Mixtures of different d-endotoxins are usually present in the inclusion and individual toxin proteins are designated with the prefix cry. The toxin proteins contain a few hundred to over 1000 amino acids. After they are ingested, the d-endotoxins are cleaved to an active form by proteases within the midgut.</td>
</tr>
<tr>
<td>Neonicotinoids and nitrogenous insecticides:</td>
<td>Nitenpyram:</td>
</tr>
</tbody>
</table>
derived synthetically from nicotinoids. It includes the following chemicals, acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam etc. Mode of action: they act as agonists at the insect nicotinic acetylcholine receptor (nAChR) whereas the botanical insecticide nicotine acts at the same target without the neonicotinoid level of effectiveness or safety.

They are highly effective flea control agents on cats and dogs. administered as oral tablets or topical spot treatments.

<table>
<thead>
<tr>
<th>Fumigants: Fumigants are insecticides in the form of gases that are slightly heavier than air. The ideal fumigants: Easily and cheaply generated Harmless to foods and commodities Inexpensive Non-explosive, Non-flammable Non-persistent Insoluble in water Easily diffuses and rapidly penetrates commodity Stable in the gaseous state (will not condense to a liquid)</th>
<th>Chloropicrin -CCl3No2 Packaged in a liquid form and turns into a gas as it is exposed to air. The residues left behind after fumigation with chloropicrin tended to stay on the grain. Chloropicrin is an intense lacrimator or “tear gas.” Breathing chloropicrin fumes, even for a very short period of time, can lead to severe lung injury and death. Because of toxicity and residues, chloropicrin is not recommended for fumigating empty grain storage facilities.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insect Growth Regulators: Synthetic compounds which interfere with growth of insect’s larvae, nymphs, pupae or adults and finally lead to their death. Growth and molting of immature insects is regulated by three main groups of brain hormones ecdysone alpha, beta, juvenile hormones. Antagonists and analogs of insect growth regulators</td>
<td>The juvenile hormone mimics: Compounds bearing a structural resemblance to the juvenile hormones of insects. Juvenile hormones are lipophilic sesquiterpenoids containing an epoxide and methyl ester groups. Two insecticidal mimics of juvenile hormone are methoprene, and fenoxycarb.</td>
</tr>
<tr>
<td>Antifeedants: Substances (aversive chemicals) which make the crop plant distasteful to the insect attempting to feed on it.</td>
<td>Pymetrozin: a pyridine azomethrine, is an effective on aphids, leaf hoppers and whitefly it is systemic has long residual activity and is non- injurious to natural enemies and the environment.</td>
</tr>
<tr>
<td>Repellents: Chemicals which cause insects to make oriented movement away from source of chemical or its vapors preventing insect from reaching target it would attach.</td>
<td>DEET: The most commonly used and most effective repellent against biting flies, chiggers and certain species of mosquitoes.</td>
</tr>
<tr>
<td>Attractants: Chemicals which cause insects to make oriented movement towards its source or chemicals acting in vapor phase causing an insect to move towards its source or zone of preferred concentration.</td>
<td>Oviposition lure: Substances that attract gravid females and induce them to lay eggs. Houseflies are attracted to ammonia and green bottle flies are attracted to ammonium carbonate for ovipositional purposes.</td>
</tr>
</tbody>
</table>
9.2. Pesticide Metabolism: Metabolism is typically a two stage process. These are: Phase 1 reactions and Phase 2 reactions

9.2.1. Phase 1 reactions – Phase 1 reactions normally add a functional (polar reactive) group to the foreign molecule which enables the phase 2 reaction to take place. These reactions are catalyzed by the cytochrome P450 group of enzymes and other enzymes which are associated with endoplasmic reticulum. Phase 1 reactions include:

1. Microsomal oxidation: The reactions catalyzed by this system include:
   - O-, S-, and N- Alkyl Hydroxylation
   - Desulfuration
   - Epoxidation
   - Thio ester oxidation
   - Aromatic hydroxylation

2. Extramicrosomal oxidation: Extramicrosomal metabolism contributes minor pathways for the pesticides metabolism. Here the reaction is decided according to the enzyme involved. Extramicrosomal metabolism includes the following reactions:
   - Phosphotriesterase hydrolysis
   - Carboxylesterase hydrolysis
   - Pyrethroids hydrolysis
   - Carboxylamide hydrolysis
   - Nitroreductase
   - Carbamate hydrolysis
   - Epoxide hydrases
   - Dechrolination and Dehydrochrolination

9.2.2. Phase 2 reactions – Phase 2 reactions are conjugation reactions and involve the covalent linkage of the toxin or phase 1 product to a polar compound. They are of the following types:

   Type I: Pesticide / metabolite + Activated conjugating agent → Conjugated product
   Type II: Activated Pesticide / metabolite + conjugating agent → Conjugated product
   Type III: Reactive Pesticide / metabolite + reduced glutathione → Conjugated product

In general, conjugated products are ionic, polar, less lipid soluble, less toxic and easily excreted from the body. Among the above three types of conjugating reactions, Type I is very common, and occurs in almost all pesticides. Types of reaction include are:

- Glucoside conjugation
- Glutathion-s-transferase conjugation
- Sulphate conjugation
- Phosphate conjugation
- Methyl transferase conjugation
- Glycine conjugation
- Cysteine conjugation
10. TERMINOLOGIES

Abiotic: The nonliving components of a system, such as temperature, mineral nutrients, water, soil type, sunlight, and air pollutants.

Absorption: The assimilation of molecules into cells, e.g., the process by which plants take up nutrients through their roots.

Acaricide: A pesticide toxic to mites and ticks (pest arthropods that belong to the class Acarina).

Acceptable daily intake (ADI): Estimate of the amount of a substance in food or drinking water, expressed on a body mass basis (usually mg/kg body weight), which can be ingested daily over a lifetime by humans without appreciable health risk.

Accumulation: Successive additions of a substance to a target organism, or organ, or to part of the environment, resulting in an increasing amount or concentration of the substance in the organism, organ, or environment.

Acetylcholine: A chemical that functions as a synaptic neurotransmitter in the nervous system of animals.

Active ingredient (a.i): Chemicals in a pesticide formulation that is biologically active as toxins. A given pesticide formulation may have more than one active ingredient.

Acute effect: Effect of short duration and occurring rapidly (usually in the first 24 h or up to 14 d) following a single dose or short exposure to a substance or radiation.

Additive effect: The efficacy of a pesticide mixture that is equal to the sum of the toxicities of the individual pesticides (cf., antagonism and synergism).

Adjuvant: Any nonpesticidal substance in a pesticide formulation that improves the physical, chemical, or biological properties of the active ingredient, or improves application efficacy.

Administration: Application of a known amount of a substance to an organism in a reproducible manner and by a defined route.

Adverse effect: Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Aerobic: A process or an organism that requires oxygen.

Aerosol: Very fine droplets (0.1 to 5 m in diameter) suspended in air, generated by a container pressurized with a gas propellant, or aerosol generators such as fogging machines or ultra-low-volume (UVL) equipment.

Agitate: Stirring or shaking a pesticide mixture so that the components will not separate or settle in the application tank.

Antibiotic: Chemicals produced by microbes that inhibit, or are toxic, to other microbes.

Antibody: Protein molecule produced by the immune system (an immunoglobulin molecule) which can bind specifically to the molecule (antigen or hapten) which induced its synthesis.

Anticoagulant: A substance that inhibits blood clotting resulting in internal hemorrhaging, this is the mode of action of a major class of rodenticides.

Antidote: Substance capable of specifically counteracting or reducing the effect of a potentially toxic substance in an organism by a relatively specific chemical or pharmacological action.

Antigen: Substance or a structural part of a substance which causes the immune system to produce specific antibody or specific cells and which combines with specific binding sites (epitopes) on the antibody or cells.

Antifeedant: Materials that inhibit or stop pest feeding. A control method often used for clothes moth larvae and termites.

Asphyxia: Condition resulting from insufficient intake of oxygen: symptoms include breathing difficulty, impairment of senses, and, in extreme, convulsions, unconsciousness and death.

Asphyxiant: Substance that blocks the transport or use of oxygen by living organisms.
Ataxia: Unsteady or irregular manner of walking or movement caused by loss or failure of muscular coordination.

Attractant: Chemical substances or devices used to lure insects or other mobile pests to areas where they can be trapped or killed. Attractants are based on feeding, oviposition, or mating behavior of insects.

*Bacillus thuringiensis* (*Bt*): Soil-inhaling bacterium that produces an insecticide effective against larval stages of many species of Lepidoptera, although some strains are effective against various beetle, mosquito, and black fly larvae.

Bacterial insecticide: Bacteria pathogenic to insects (e.g., *Bt* or *B. popilliae*). Applied using application techniques also used for chemical pesticides.

Bait: A pesticide formulated with an attractive food substance and containing a small amount of toxic active ingredient (usually about 5%). Baits are primarily used for control of mollusks, cutworms, and rodents.

Bioaccumulation: Progressive increase in the amount of a substance in an organism or part of an organism which occurs because the rate of intake exceeds the organism's ability to remove the substance from the body.

Bioactivation: Any metabolic conversion of a xenobiotic to a more toxic derivative.

Bioassay: an experiment in which test organisms are exposed to different

Bioconcentration: Process leading to a higher concentration of a substance in an organism than in environmental media to which it is exposed.

Biodegradation: Decomposition or breakdown of a substance through the action of microorganisms (such as bacteria or fungi) or other natural physical processes (such as sunlight).

Biological control or biocontrol: Regulating pest populations by using natural enemies such as herbivores, predators, parasitoids, and parasites.

Biomagnification: Sequence of processes in an ecosystem by which higher concentrations are attained in organisms at higher trophic levels (at higher levels in the food web), at its simplest, a process leading to a higher concentration of a substance in an organism than in its food.

Biomineralization: Complete conversion of organic substances to inorganic derivatives by living organisms, especially micro-organisms. Volume, in a medium such as water.

Biotic insecticide: natural or introduced enemies of a pest including predators and parasites that are applied using standard pesticide application techniques.

Biotransformation: Any chemical conversion of substances that is mediated by living organisms or enzyme preparations derived there from.

Botanicals: Pesticides derived from plants, such as pyrethrum, rotenone (derris) ryania, and nicotine.

Brand name: The name that manufacturers use for commercial purposes.

Carcinogen: A chemical or biological agent which produces, accelerates or increases frequency of cancers.

Carcinogenicity: Process of induction of malignant neoplasms by chemical, physical or biological agents

Central nervous system: The part of the nervous system that consists of the brain and the spinal cord.

Chemical name: The name that specifies the chemical structure of a compound.

Cholinesterase inhibitor: Substance which inhibits the action of acetylcholinesterase and related enzymes which catalyse the hydrolysis of choline esters: such a substance causes hyperactivity in parasympathetic nerves.

Chronic effect: Consequence which develops slowly and has a long-lasting course (often but not always irreversible).

Combined effect of poisons: Simultaneous or successive effect of two or more poisons on the organism by the same route of exposure.

Conjugate: Derivative of a substance formed by its combination with compounds such as acetic acid, glucuronic acid, glutathione, glycine, sulfuric acid etc.

Contaminant: A substance that is either present in an environment where it does not belong or is present at levels that might cause harmful (adverse) health effects.
Cytochrome P-450: Haemoproteins which form the major part of the enzymes concerned with the mono-oxygenation of many endogenous and exogenous substrates. The term includes a large number of iso-enzymes which are coded for by a superfamily of genes. Endogenous substrates of these enzymes include cholesterol, steroid hormones and the eicosanoids, the exogenous substrates are xenobiotics. Strictly, the cytochrome P450 family are not cytochromes but are haem-thiolate proteins.

Cytoplas: Fundamental substance or matrix of the cell (within the plasma membrane) which surrounds the nucleus, endoplasmic reticulum, mitochondria and other organelles.

Cytotoxic: Causing damage to cell structure or function.

Cytochrome: Haemoprotein whose characteristic mode of action involves transfer of reducing equivalents associated with a reversible change in oxidation state of the haem prosthetic group: strictly, the cytochrome P450 family are not cytochromes but haem-thiolate proteins.

Dehydrogenase: Enzyme which catalyses oxidation of compounds by removing hydrogen.

Dermal: Pertaining to the skin

Dermatitis: Inflammation of the skin: contact dermatitis is due to local exposure and may be caused by irritation, allergy or infection

Detoxification: Process, or processes, of chemical modification which make a toxic molecule less toxic.

Dissipation: Reduction in the amount of a pesticide or other compound which has been applied to plants, soil etc. (used when it is not clear whether this is by mineralization degradation, binding, or leaching)

Dosage: Dosed expressed as a function of the organism being dosed and time, for example mg/(kg body weight)/day

Dose: The total amount of the compound given to or taken by an organism

Dose-response relationship: Association between dose and the incidence of a defined biological effect in an exposed population

Drug: Any substance which when absorbed into a living organism may modify one or more of its functions. The term is generally accepted for a substance taken for a therapeutic purpose, but is also commonly used for abused substances.

Ecotoxicology: Study of the toxic effects of chemical and physical agents on all living organisms, especially on populations and communities within defined ecosystems, it includes transfer pathways of these agents and their interactions with the environment

Effective concentration (EC): Concentration of a substance that causes a defined magnitude of response in a given system: EC50 is the median concentration that causes 50 % of maximal response

Effective dose (ED): Dose of a substance that causes a defined magnitude of response in a given system: ED50 is the median dose that causes 50 % of maximal response

Effluent: Fluid, solid or gas discharged from a given source into the external environment.

Elimination: Expulsion of a substance or other material from an organism (or a defined part thereof), usually by a process of extrusion or exclusion, sometimes after metabolic transformation

Endoplasmic reticulum: Intracellular complex of membranes in which proteins and lipids, as well as molecules for export, are synthesized and in which the biotransformation reactions of the mono-oxygenase enzyme systems occur: may be isolated as microsomes following cell fractionation procedures.

Environment: Aggregate, at a given moment, of all external conditions and influences to which a system under study is subjected.

Excretion: Discharge or elimination of an absorbed or endogenous substance, or of a waste product, and/or their metabolites, through some tissue of the body and its appearance in urine, faeces, or other products normally leaving the body.

Exposure: Contact with a substance by swallowing, breathing, or touching the skin or eyes.

Fertility: Ability to conceive and to produce offspring: for litter-bearing species the number of offspring per litter is used as a measure of fertility. Reduced fertility is sometimes referred to as subfertility.
Fetus (often incorrectly foetus): Young mammal within the uterus of the mother from the visible completion of characteristic organogenesis until birth: in humans, this period is usually defined as from the third month after fertilisation until birth (prior to this, the young mammal is referred to as an embryo).

Fumigant: Substance that is vaporized in order to kill or repel pests.

Gene: Structurally a basic unit of hereditary material, an ordered sequence of nucleotide bases that encodes one polypeptide chain (following transcription to mRNA).

Half-life (half-time) ($t_{1/2}$): Time in which the concentration of a substance will be reduced by half, assuming a first order elimination process or radioactive decay.

Harmful substance: Substance that, following contact with an organism can cause ill health or adverse effects either at the time of exposure or later in the life of the present and future generations.

Herbicide: Substance intended to kill plants.

Hormone: Substance formed in one organ or part of the body and carried in the blood to another organ or part where it selectively alters functional activity.

Hydrophilic: Describing the character of a molecule or atomic group which has an affinity for water.

Hydrophobic: Describing the character of a molecule or atomic group which is insoluble in water, or resistant to wetting or hydration.

Hyper-reactivity: Term used to describe the responses of (effects on) an individual to (of) an agent when they are qualitatively those expected, but quantitatively increased.

Hypersensitivity: State in which an individual reacts with allergic effects following exposure to a certain substance (allergen) after having been exposed previously to the same substance.

Immune complex: Product of an antigen-antibody reaction that may also contain components of the complement system.

Immune response: Selective reaction of the body to substances that are foreign to it, or that the immune system identifies as foreign, shown by the production of antibodies and antibody-bearing cells or by a cell-mediated hypersensitivity reaction.

Inhalation: The act of breathing.

Inherently biodegradable: Class of compounds for which there is unequivocal evidence of biodegradation (primary or ultimate) in any test of biodegradability.

Insecticide: Substance intended to kill insects

Intake: Amount of a substance that is taken into the body, regardless of whether or not it is absorbed: the total daily intake is the sum of the daily intake by an individual from food, drinking-water, and inhaled air.

Larvicide: Substance intended to kill larvae

Latent period/lag period: Delay between exposure to a disease-causing agent and the appearance of manifestations of the disease: also defined as the period from disease initiation to disease detection.

Lethal dose: Amount of a substance or physical agent (radiation) that causes death when taken into the body by a single absorption (denoted by LD).

Lethal: Deadly, fatal, causing death.

Lethal concentration: Concentration of a potentially toxic substance in an environmental medium that causes death following a certain period of exposure (denoted by LC).

Lipophilic: Having an affinity for fat and high lipid solubility; a physicochemical property which describes a partitioning equilibrium of solute molecules between water and an immiscible organic solvent, favouring the latter, and which correlates with bioaccumulation.

Long-term exposure: Continuous or repeated exposure to a substance over a long period of time, usually of several years in man, and of the greater part of the total life-span in animals or plants.

Malignant: Tending to become progressively worse and to result in death if not treated

Metabolic activation: Biotransformation of a substance of relatively low toxicity to a more toxic derivative

Metabolism: The conversion or breakdown of a substance from one form to another by a living organism.

Metabolite: Any product of metabolism.
Microcosm: Artificial test system that simulates major characteristics of the natural environment for the purposes of ecotoxicological assessment: such a system would commonly have a terrestrial phase, with substrate, plants and herbivores, and an aquatic phase, with vertebrates, invertebrates and plankton.

Microsome: Artefactual spherical particle, not present in the living cell, derived from pieces of the endoplasmic reticulum present in homogenates of tissues or cells: microsomes sediment from such homogenates when centrifuged at 100,000 g and higher: the microsomal fraction obtained in this way is often used as a source of mono-oxygenase enzymes.

Mono-oxygenase: Enzyme that catalyses reactions between an organic compound and molecular oxygen in which one atom of the oxygen molecule is incorporated into the organic compound and one atom is reduced to water.

Mutagen: Any substance that can induce heritable changes (mutations) of the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof)

Mutagenicity: Ability of a physical, chemical, or biological agent to induce heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof)

Mutation: Any relatively stable heritable change in genetic material that may be a chemical transformation of an individual gene (gene or point mutation), altering its function, or a rearrangement, gain or loss of part of a chromosome, that may be microscopically visible (chromosomal mutation), mutation can be either germinal and inherited by subsequent generations, or somatic and passed through cell lineage by cell division.

Neuron(e): Nerve cell, the morphological and functional unit of the central and peripheral nervous systems

Neurotoxicity: Able to produce chemically an adverse effect on the nervous system: such effects may be subdivided into two types

Non-target organism: Organism affected by a pesticide although not the intended object of its use

No-observed-adverse-effect-level (NOAEL): Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure

No-observed-effect-level (NOEL): Greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure

Paralysis: Loss or impairment of motor function.

Peroxisome: Organelle, similar to a lysosome, characterized by its content of catalase, peroxidase and other oxidative enzymes.

Persistence: Attribute of a substance that describes the length of time that the substance remains in a particular environment before it is physically removed or chemically or biologically transformed.

Pest: Organism that may harm public health, which attacks food and other materials essential to mankind or otherwise, affects human beings adversely.

Pesticide: Strictly a substance intended to kill pests: in common usage, any substance used for controlling, preventing, or destroying animal, microbiological or plant pests.

Pesticide residue: Pesticide residue is any substance or mixture of substances in food for man or animals resulting from the use of a pesticide and includes any specified derivatives, such as degradation and conversion products, metabolites, reaction products and impurities considered to be of toxicological significance.

Pharmaceuticals: Drugs, medical products, medicines, or medicaments.

Pheromone: Substance used in olfactory communication between organisms of the same species eliciting a change in sexual or social behaviour.

Poison: Substance that, taken into or formed within the organism, impairs the health of the organism and may kill it.
Potency: Expression of chemical or medicinal activity of a substance as compared to a given or implied standard or reference.

ppb: Parts per billion.

ppm: Parts per million.

Probit: Probability unit obtained by adding 5 to the normal deviates of a standardized normal distribution of results from a dose response study: addition of 5 removes the complication of handling negative values.

Random sample: Subset of a population that is arrived at by selecting units such that each possible unit has a fixed and determinate probability of selection.

Receptor: High affinity binding site for a particular toxicant.

Repellent: Substance used mainly to repel blood sucking insects in order to protect man and animals: also used to repel mammals, birds, rodents, mites, plant pests, etc.

Resistance (in toxicology): Ability to withstand the effect of various factors including potentially toxic substances.

Rodenticide: Substance intended to kill rodents.

Route of exposure: Means by which a toxic agent gains access to an organism by administration through the gastrointestinal tract (ingestion), lungs (inhalation), skin (topical), or by other routes such as intravenous, subcutaneous, intramuscular or intraperitoneal routes.

Secondary metabolite: Product of biochemical processes other than the normal metabolic pathways, mostly produced in micro-organisms or plants after the phase of active growth and under conditions of nutrient deficiency.

Side-effect: Action of a drug other than that desired for beneficial pharmacological effect.

Symptom: Any subjective evidence of a disease or an effect induced by a substance as perceived by the affected subject.

Synapse: Functional junction between two neurones, where a nerve impulse is transmitted from one neurone to another.

Syndrome: Set of signs and symptoms occurring together and often characterizing a particular disease-like state.

Synergism: Pharmacological or toxicological interaction in which the combined biological effect of two or more substances is greater than expected on the basis of the simple summation of the toxicity of each of the individual substances.

Synergistic: effect a biologic response to multiple substances where one substance worsens the effect of another substance. The combined effect of the substances acting together is greater than the sum of the effects of the substances acting by themselves.

Systemic effect: Consequence that is of either a generalized nature or that occurs at a site distant from the point of entry of a substance: a systemic effect requires absorption and distribution of the substance in the body.

Target organ(s): Organ(s) in which the toxic injury manifests itself in terms of dysfunction or overt disease.

Teratogen: Agent that, when administered prenatally (to the mother), induces permanent structural malformations or defects in the offspring.

Teratogenicity: Potential to cause or the production of structural malformations or defects in offspring.

Threshold: Dose or exposure concentration below which an effect is not expected.

Topical: Pertaining to a particular area, as in a topical effect that involves only the area to which the causative substance has been applied.

Toxic substance: Material causing injury to living organisms as a result of physicochemical interactions.

Toxic: Able to cause injury to living organisms as a result of physicochemical interaction.

Toxicology: Scientific discipline involving the study of the actual or potential danger presented by the harmful effects of substances (poisons) on living organisms and ecosystems, of the relationship of such
harmful effects to exposure, and of the mechanisms of action, diagnosis, prevention and treatment of intoxications
Toxin: Poisonous substance produced by a biological organism such as a microbe, animal or plant
Transgenic: Adjective used to describe animals carrying a gene introduced by micro-injecting DNA into the nucleus of the fertilized egg
Uptake: Entry of a substance into the body, into an organ, into a tissue, into a cell, or into the body fluids by passage through a membrane or by other means.
Xenobiotic: Any substance interacting with an organism that is not a natural component of that organism.