بسم الله الرحمن الرحيم

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Pesticides Toxicity

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Time table of Toxicology Course (First-term, 2023-2024)

Item No	Subject	Hours	Staff member	Weeks
1	Introduction of Toxicology	4	Prof. Eman Ezz Eldawela	1,2
2	Pesticides	4	Prof. Doha Y. Ahmed	3,4
3	Mycotoxins	4	Prof. Z. M. Zaky	5,6
4	Irritant poisons	4	Prof. A.A.Sharkawy	7,8
5	Corrosive toxicants	2	Dr. Heba Fawzy	9
6	Poisonous plants	4	Prof. /Th. A. Ibrahim	10,11
7	Animal poisons	2	Dr. Mohammed Abdelhadi	12
8	Eco-Toxicology	2	Dr. Mohammed Abdelhadi	13
9	Feed additives & drug toxicity	2	Prof. Manal Abd Ellatif	14
Total hours		28	Total weeks	14

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What is the purpose of studying pesticides?????

- To know different types of pesticides?
- How they kill pests?
- Their toxic effects to the pest?
- The toxic effect in human and animals?
- ✓How to diagnose and treat the toxicity??

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➢ Objectives:

1. Definition and history of pesticides.
2. Different types of pesticides.
3. The ideal pesticide.
4. Mode of action.
5. Diagnosis and treatment of pesticide toxicity.

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✓ DEFINITIONS

Pest: is any harmful, destructive, or troublesome animal, plant or microorganism.

Pesticides:- Chemicals used for eradication of pests.

Pesticide: is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.

US Environmental Protection Agency (EPA)

Pesticide: any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insects, rodents, nematodes, fungi, or weeds or any other form of life declared to be pests. … and any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA – 1947)

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- **Historical Events**
  - **1930's – "ginger jake paralysis" alcoholic Jamaican ginger.**

+ A neurotoxic organophosphate compound (TOCP).

- **1962 – "Silent Spring" by Rachel Carson exposed the hazards of DDT.**
- **1975 – Workers made ill from Kepone (insecticide) manufacture in Hopewell, Virginia. (chlorinated insecticide).**

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Classification of pesticides:

- **According to the source:**

1- **Natural: sulfur, nicotine, arsenic, pyrethrin.**

2- **Synthetic:** (chemical groups) :

- **organophosphates,**
- **organochlorines,**
- **Carbamates,,,,**

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- According to the target pest:

- **Insecticides (kill insects):**
  - Organochlorines.
  - Organophosphates.
  - Carbamates.
  - Pyrethroids.
- **Herbicides (kill plants).**
- **Rodenticides (kill rodents).**
- **Fungicides (kill fungus).**

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- **Pesticides**
- **are designed to kill – and for insecticides this usually means toxicity to the nervous system.**
- **A small amount of pesticide is fatal to an insect primarily because of the insect's small size and high rate of metabolism.**
- **For an insect a small exposure can represent a very large dose on a body weight basis. This same small amount is relatively less harmful to an animal of much larger size, because it is a small dose based on body weight.**
- **Is there an ideal pesticide??????**

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➢ The ideal pesticide:

- **The ideal pesticide would be:**
- **Highly specific (It should not kill any organism other than the target pest) and should be non toxic to the host animal.**
- **Quick acting,**
- **Degrade rapidly to non-toxic materials in the environment.**
- **It should prevent the development of resistance in the organism it is designed to kill.**
- **Provide no or a minimum residue in the host animal and animal tissues.**
- **Destroy all target pests at every stage of their life cycle.**
- **Cost-effective.**

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Application of pesticides

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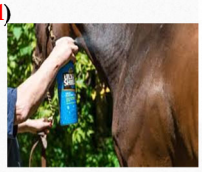
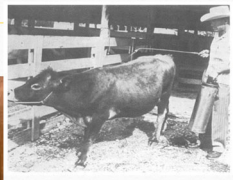
➢ **Routes of exposure: (human and animal)**

- Ingestion(oral): feed stuff or drinking water.
- Inhalation (lung)
- Skin (dermal)





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Insecticides

➢ **Insecticides (kill insects):**

➢ **Definition:** chemicals used for eradication or repelling of insects.

1. Organochlorines (Ocs) = Chlorinated hydrocarbons.
2. Organophosphates (OPC)
3. Carbamates.
4. Pyrethroids.
5. Neonicotinoids (newer class of insecticides).

- Most modern chemical insecticides act by poisoning the nervous system.
- The central and peripheral nervous system of insects is fundamentally similar to that of mammals.???

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1. Organochlorine insecticides Chlorinated hydrocarbons

Definition: they are hydrocarbon compounds with variable degrees of chlorination.

- The chemical structure of organochlorines is diverse but they all contain chlorine, which places them in a larger class of compounds called chlorinated hydrocarbons.

Examples

DDT, methoxychlor, aldrin, dieldrin, endrin, heptachlor, kepone, lindane, chlordane, toxaphene, mirex, kalthane and methoxychlor.

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➢ Organochlorines (OC) are a group of chlorinated compounds widely used as synthetic insecticides.

➢ These chemicals belong to the class of persistent organic pollutants (POPs) with high persistence in the environment.

➢ These compounds are known for their high toxicity, slow degradation and bioaccumulation.

➢ Even though many of the compounds which belong to OC were banned in developed countries, the use of these agents has been rising.

➢ Though pesticides have been developed with the concept of target organism toxicity, often non-target species are affected badly by their application.

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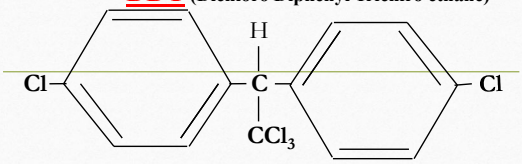
✓ **DDT** (Dichloro Diphenyl Trichlro ethane)



- DDT was used World War II (1945) to limit the spread of the insect (mosquito) born diseases malaria and typhus .
- Effective but very persistent.
- Highly soluble in fat.

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➢ General properties of DDT & Ocs:

1. Highly persistent in the environment.
2. Lipophilic (highly soluble in fat) and tend to be stored (sequestered) in fat and adipose tissue.
3. They have low solubility in water.
4. Very long half life. In soil 2–15 years, in an aquatic environment is about 150 years.
5. DDT in soil usually breaks down to form DDE or DDD.

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- **Absorption of Ocs:**
  - Absorbed from the skin, mucous membrane and gastrointestinal tract.
  - Accumulated or sequestrated in adipose tissues.
  - The depletion of body fat can release them into the bloodstream.
- **Metabolism**
  - Takes place in the liver by MFOs.
  - Their metabolites are more toxic than the parent compounds,
  - Ex.: DDT breaks down to form DDE or DDD

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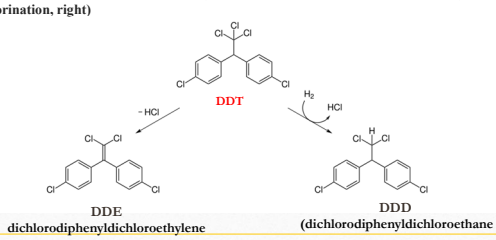
Metabolism of DDT

- Degradation of DDT to form DDE (by elimination of HCl, left) and DDD (by reductive dechlorination, right)



DDE
dichlorodiphenyldichloroethylene

DDD
(dichlorodiphenyldichloroethane

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Excretion:

- Excreted in Feces
- A small Part excreted in Urine & bile
- ✓ The main route of excretion is Milk & Eggs.
- *Storage : Adipose tissues

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Mechanism of Action

1- Stimulation of nervous system:

by interfering with the normal nerve impulses.

- Interfere with sodium channel kinetics in the nerve membrane by increasing the flow of sodium ions through the cell membrane of neurons.
- causing voltage-gated sodium channels to remain open, leading to persistent depolarization and hyperactivity in the nervous system.

2- Organochlorines disrupt the movement of ions such as Ca++, Cl– , Na+ and K+ into and out of the nerve cells, which leads to hypersensitivity and death.

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- The DDT like compounds work on the peripheral nervous system. At the axon's sodium channel, they prevent gate closure after activation and membrane depolarization. Sodium ions leak through the nerve membrane and create a destabilizing negative "after potential" with hyper excitability of the nerve. This leakage causes repeated discharges in the neuron either spontaneously or after a single stimulus.
- They are neurotoxins leading to tremors, convulsions and death.

3- Inhibition of neurotransmitter Gamma amino butyric acid (GABA).

4- Reproductive dysfunction especially in birds.

Some of them are estrogenic in nature, causing reproductive dysfunction in both birds and animals.

5- Carcinogenic and teratogenic effects in long term exposure.

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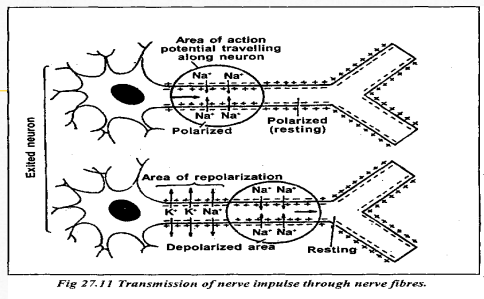

Fig 27.11 Transmission of nerve impulse through nerve fibres.

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Clinical signs:

1- Neurological signs: Poisoned animals become hyperesthetic and exhibit motor tremors and convulsions. Animals seems to be hypersensitive.

2- Fasciculation of the face and cervical muscles.

3- Spasms of eyelids, muscles of fore quarters and finally the hind quarters (continually or intermittently).

4-Clonic-tonic convulsions or seizures then death.

5-Some animals: behavioral signs; coordinate and stumble while walking, jump imaginary objects, walk aimless or move in circles.

6-Continuous chewing movements with increase salivation.

7-Poisoned animal may be comatose then death or may regain consciousness and fully recover.

8-Simple stomach animals show vomiting ,salivation and froth accumulation at the mouth.

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Diagnosis and differential diagnosis:

Diagnosis

1- Circumstantial evidence (case history).

2- Clinical signs.

3- Chemical analysis of fat (deposit fat or milk fat, liver, kidney) and stomach contents using chromatographic techniques.

Differential diagnosis:

- It should be differentiated from viral encephalomalacia, rabies, brain abscesses and BSE in cattle (Caw Mad Syndrome).
- lead, strychnine, fluoroacetate and food poisoning in cases of cattle, dogs and cats.

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Treatment:

- There is no specific antidotes for organochlorine poisoning.
- Light anesthesia to control convulsive seizures and other hyperactivity signs.
- Sodium Phenobarbital.
- Diazepam is also used to control convulsions or muscle spasms as its sedative effects.
- Activated charcoal in cases of oral toxicity is highly recommended.

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Prophylaxis:

1 - Prevent further exposure.

2 – Increase the excretion of Ocs:

Simultaneous feeding of Phenobarbital and activated charcoal.

-Activated charcoal tends to trap the insecticides from the gut.

-Phenobarbital stimulates liver microsomal enzymes which increase the rate of detoxification of chlorinated insecticides.

- Phenobarbital (10 mg/kg B.W.)
- Activated charcoal (2g/ kg B.W.)
- For one month decreases the body burden of organochlorine compounds by 50%.
- 3- Loss of body fat.

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✓ Phenomena related to organochlorine insecticides: (Special effects)

1. • Bioaccumulation and Biomagnification
2. • Egg shell thinning.
3. • Enzyme induction.

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1. Bioaccumulation and Biomagnification

- Bioaccumulation takes place in a single organism over the span of its life, resulting in a higher concentration in older individuals.
- Bioaccumulation means the uptake and retention of pesticide from the environment by an organism results in an elevation of concentration and causes deleterious effects.
- Or, it is the gradual accumulation of substances, such as pesticides, in a single organism regardless of its position in the food chain.
- Ex. :
- When DDT enters aquatic bodies, it gets build up in the body of fish and this is known as bioaccumulation.

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✓ Biomagnification: (Biological magnification)

- What is biomagnification in simple words?
- The process by which a compound (such as a pollutant or pesticide) increases its concentration in the tissues of organisms as it travels up the food chain.
- Biomagnification is defined as the process by which a compound becomes concentrated in the tissues of organisms as it makes its way up the food chain.
- Examples:……

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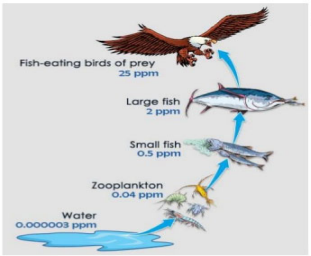
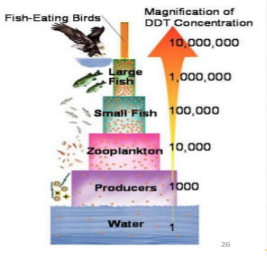
Biomagnification





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Biological magnification

Top of food chain (birds)
200,000 ppm
↑
Large fish 100,000 ppm
↑
Plant eating fish 50,000 ppm
↑
Planktons 500 ppm
↑
Lake sediment 1ppm

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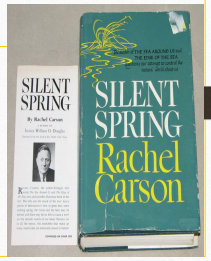
2. Eggshell Thinning Phenomenon

- It was noticed that a decline in the population of birds in some regions.
- The regions of population decline coincide with areas where DDT and dieldrin are widely applied.
- DDT and its metabolites DDD and DDE in a level of 10 ppm reduce eggshell thickness by 20%, although there is sufficient calcium in the diet.
- There are many theories for the explanation of eggshell thinning phenomenon.



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Eggshell Thinning Phenomenon:

➢ This phenomenon occurs due to:

- 1- Inhibition of Carbonic anhydrase enzyme which is very important for calcium precipitation from blood to oviduct (shell formation).
- 2- Inhibition of Ca metabolism either from the diet or even from the circulating calcium.
- 3- Inhibition of Ca ATPase enzyme.
- 4- Induction of microsomal enzymes from the liver leading to breakdown of sex hormones (estrogen).
- 5- Feed back mechanism to the hypothalamus which interferes with hormone production.

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➢ Results of Shell Thinning Phenomenon

1- Loss of eggs either due to failure to lay eggs or breakage of the eggs that are laid.

2- High mortality of the embryos (reduced hatching %) .

3- High mortality rate of hatched chicks

4- Change in nesting behavior of the exposed birds leading to eating of broken eggs or destruction of birds nest by the mother itself.

5- Disturbances in the ratio of large to small individuals in the population.

DDT makes birds' shells thin

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3. Enzyme induction

- A number of drugs or chemicals can cause increase in liver microsomal enzymes activity, this in turn can increase the metabolic rate of the same or other chemicals.
- OC induces increased activity of hepatic enzyme systems, for example, mixed function oxidase (MFO) induction.
- Induction of liver microsomal enzymes is an important aspect of detoxification of fat-soluble toxicants. So low doses can be beneficial.

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This phenomenon lead to:

1- Synergistic or antagonistic effects through stimulation of enzyme systems responsible for metabolizing other pesticides.

2- Antagonistic storage of lipophilic insecticides in animal tissue due to accelerated metabolic detoxification which may results in lowered chronic toxicity for certain insecticides.

3- Increased hormone turnover by induced enzymes, causing disturbances to endocrine relationships which may lead to physiological alterations in certain bird species, for example, thin-eggshell phenomenon.

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Enzyme Induction

Exposure to Environmental chemicals

(DDT)

↓

Enhance the enzymatic activity

Increase P450, Glutathione S-transferase

Epoxide hydrolase & others

↓

Increase rate of detoxification

↓

Increase or decrease the biological effects of chemicals.

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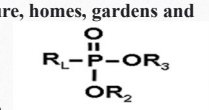
2. Organophosphorous compounds (organophosphates)

- Organophosphorus compounds are derivatives of phosphoric, phosphonic or phosphinic acids whose oxygen atoms bound directly to the phosphorus atom.
- Organophosphates are used in agriculture, homes, gardens and veterinary practices.

Examples:

Malathion, parathion, Mipafox, dimefox, Nuvacron, guthion, diazinon, TPN, TOCP, nerve gases – tabun, sarin, soman,…….



Organophosphate "insecticide"

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- Environmental characteristics:

✓Rapidly degrade in outdoor environment (last longer in doors),

✓do not bioaccumulate.

- Organophosphates, while environmentally more suitable than the organochlorines, present their own challenges.? Foremost was their toxicity to mammals, including humans.
- Absorption:-
- Skin GIT. Eyes,,, Lungs.
- Excretion:-

Urine, Bile , Feces (Rapidly).

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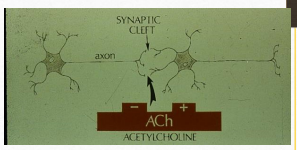
Mechanism of toxic action:

✓- This class of insecticides is commonly referred to as antiacetylcholinesterase.

✓-Irreversible Inhibition of acetylcholinesterase enzyme (AchE) in the nerve endings.

✓ - Accumulation of Ach (neurotransmitter) in the nerve synapse.



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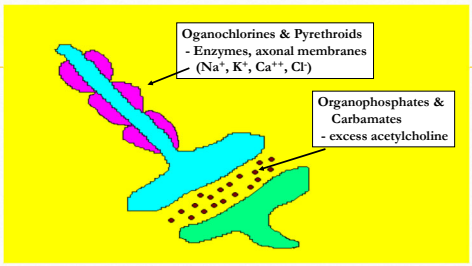
Mechanism of Action



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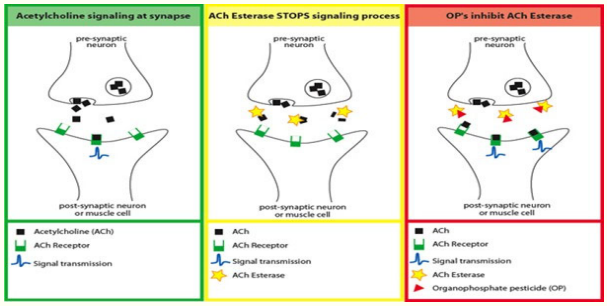
Mechanism of action



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AChE Phosphorylation

- **Acetylcholinesterase is inhibited by Phosphorylation of the active site (Esteratic site) and blocking of the enzyme.**
- **Acetylcholine (ACh), therefore remains in the synapse between pulses, leading to repeated firing of the postsynaptic nerve.**
- **Accumulated ACh gives the clinical signs of muscarinic, nicotinic and CNS affection of Organophosphorous compounds toxicity.**
- **The kinetics of the enzyme will reach zero within 10 hours i.e. all enzyme will be in the form of OP-AChE complex (phosphorylated enzyme), this situation is called aging phenomenon.**

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Clinical signs

- **1- Acute toxicity: due to accumulation of Ach (cholinergic crisis).**
- Signs of organophosphate poisoning can be divided into three broad categories:
  - **A- Muscarinic effects**
  - **B- Nicotinic effects.**
  - **C- CNS effects.**

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➢ Muscarinic effects:

Excessive salivation (Drooling).

lacrimation,

Sweating,

Diarrhea,

Urination (Micturition).

Bradycardia, hypotension,

Miosis (pin point pupil),

Bronchoconstriction.

These muscarinic effects are blocked by atropine.

Figure 1 Calf intoxicated with carbofuran, note the sialorrhea and protrusion of the tongue.

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Acute OP poisoning

MUSCARININC FEATURES	NICOTINIC FEATURES	CNS FEATURES
D iarrhoea	Muscle weakness	Fatigue
U rination	Muscle fasiculations	Confusion
M iosis	Muscle paralysis	Unconsciousness
B ronchorrhea B ronchospasm		Seizues
E mesis	Hypertension	Ataxia
L acrimation	Tachycardia	Resp. depression
S alivation S weating		

.Bronchorrhea:

.Excess fluid secretions in airway .

.Obstructions of upper and lower airways.

.Pulmonary edema--hypoxia—death.

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Nicotinic effects: (at neuromuscular junction).
-Muscular twitching and tremors.
-Fasciculation of muscles especially diaphragm and respiratory muscles.
-Muscular weakness.
-Muscle paralysis.

➢ For nicotinic signs of acetylcholinesterase inhibitor toxicity, think of the days of the week:
Monday = Mydriasis
Tuesday = Tachycardia
Wednesday = Weakness
Thursday = Hypertension
Friday = Fasciculations

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CNS effect (depression):

- Restlessness followed by depression
- Depression of respiratory muscles, then asphyxia and coma.
- Convulsions
- Respiratory failure.

Chronic Toxicity:-
*Fasciculation
*Weakness
*Loss of weight

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Diagnosis of OP Poisoning

- 1. Case history or evidence of ingestion of the poison.
- 2. Clinical signs.
- 3. PM lesions.
- 4. Clinical improvement after atropine injection.
- 5. Laboratory diagnosis:
- Inhibition of ACHE in vitro.
- Presence of PNP (para nitophenol) in urine (yellow) of poisoned animal with methyl parathion.
- Urinary dialkyl phosphate (DAP) and dimethylthiophosphate (DMTP) metabolites.

✓ Feed stuffs: Isolation and identification of OP using chromatography (TLC or GC).

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➢ Cholinesterase Blood Tests

- Two cholinesterase enzymes
  - RBC
    - "true"/ acetylcholinesterase
  - Plasma
    - "pseudo"/ butyrylcholinesterase
- Percentage of possible inhibition:
  - It should be not less than 30-40% inhibition to be specific for Organophosphate toxicity.

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✓ Treatment

- 1. Atropine sulfate injection: to stop the muscarinic effect (salivation, lacrimation, bronchial secretion , diarrhea….
- 2. Enzyme reactivators (Oximes): 2-PAM
- 3. Activated charcoal (500 – 1000 g).

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Atropine Sulphate:

0.2 mg/Kg body weight
IV, IM and SC
Maximum dose is:
65 mg for average horse
80 mg for average cattle
2 mg for average dog
Repeat the dose after 6 hours if needed

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Oximes: Enzyme reactivators

- Pharmacological antidote to reverse the inhibited enzyme.
- Pralidoxime (2-PAM) : Pyridine Aldoxime Methiodide.
- Oximes are used for reactivation of the inhibited AchE enzyme.
- Treatment is effective within 48 hours.
- Enzyme aging occurs if not treated.
- 2-PAM reactivates cholinesterase.
- Cholinesterase levels rise.

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Delayed Neurotoxicity (DNT)

- It means paralysis of the legs in man and birds or of the hind limbs in quadrupeds.
- This paralysis is two to three weeks after poisoning by OP and treatment.
- DNT takes place due to the damage of myelin sheath surrounding axons of the nerves or nerve demyelination.
- Hens are highly susceptible.
- Examples:
- TOCP: Tri Ortho Cresyl Phosphate
- TEPP: Tetraethyl pyrophosphate

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3- Carbamates

Def:- Derivative s of carbamic or thiocarbamic acids.

- Carbamates are a class of insecticides structurally and mechanistically
- similar to organophosphate (OP) insecticides.

- Carbamates are used as sprays or baits to kill insects by affecting their brains and nervous systems.

➢ This class of insecticides is commonly referred to as antiacetylcholinesterase.

➢ Not persistent in environment

Examples:

Sevin (carbaryl), Baygon (propoxur), Temik (aldicarb)

Toxicity

Aldicarb is very toxic, others less toxic

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Carbamates

❖ Used also as nerve and war gases.
❖ Similar mode of action to OP's as AChE inhibitors.
❖ Reversible inhibition of AChE by carbamylation of acetylcholinesterase at neuronal synapses and neuromuscular junctions.
❖ Short residual effects.
❖ Variable toxicity in different species.
❖ Do not accumulate internally or in the environment.
❖ Atropine sulfate alone is the specific antidote.

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Clinical signs: are similar to OP toxicity.

Treatment:

1. Atropine sulphate.

✓ 2. Contraindication to use oximes:?????

1) Due to formation of Oxime–Carbamate complex (more Toxic) because it increases the inhibition of Ach E.
2) No need for oximes (reversible inhibition of AchE).

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4- Pyrethroids

- The name pyrethroid means "pyrethrum-like" and refers to the origin of this class of pesticides.
- Pyrethrum is one of the oldest known insecticides and comes from the dried and crushed flowerheads of *Chrysanthemum*.

Chrysanthemum flowers

- 1- Natural insecticides : pyrethrins.
- 2- Synthetic: pyrethroids.

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- **Synthetic Pyrethroids** : are divided into two groups according to their chemical structures:
- A- **Type I pyrethroids**: are devoid of α- cyano moiety at the alpha-position (Permethrin). causes reversible block of sodium channels.
- B- **Type II pyrethroids:** have an alpha-cyano moiety (cypermethrin).
- cause reversible block of sodium channels and inhibition of GABA . This group is more neurotoxic.

✓ **Mechanism of action:**

- Interacting with voltage-gated sodium channels in neurons.
- They have action like DDT as they block ion channels in the nervous system but they have high selectivity towards the insects rather than host animals.

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Characteristics of pyrethroids

- Low in toxicity to mammals and birds;
- High arthropod toxicity (require very low doses to kill insects).
- High in toxicity to fish if applied directly to water;
- Fast-acting;
- Dissolve very poorly in water.
- Pyrethroid common names almost always end in *-thrin*
- Ex.: Permethrin, deltamethrin, Cyhalothrin, cypermethrin, ……

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Advantages:

1- Selective toxicity: are extremely toxic to insects and they are of low toxicity or even harmless to mammals.

Insect / mammal toxicity ratio is very high.

2- Rapid detoxification in mammals.

3- Lack of cumulative toxicity.

4- It is not stable so it is not harmful to environment with exception in case of improper disposal to aquatic because fish are very sensitive to pyrethroids.

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➢ Clinical signs:-

Acute:-

- Skin Itching, burning sensation.
- GIT Salivation.
- CNS Tremors and weakness.
- Lung Dyspnea , may lead to death.

➢ **TREATMENT**

➢ - Symptomatic treatment:

- CNS depressant (Diazepam)
- Activated charcoal &
- Atropine sulfate.

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5. Neonicotinoids
(newer class of insecticides)

- The name literally means "new nicotine-like insecticides".
- Neuro-active insecticides chemically similar to nicotine.
- Ex. Acetamiprid, Imidacloprid, Clothianidin, Dinotefuran, Thiocloprid, thiamethoxam.
- The mode of action of neonicotinoids is similar to the natural insecticide nicotine. They selectively bind and interact (interfere) with the insect nicotinic acetylcholine receptor site.
- Because the neonicotinoids block a specific neural pathway that is more abundant in insects than in warm-blooded animals, these insecticides are selectively more toxic to insects than mammals. This target site selectivity is a major factor in the favorable toxicological properties of neonicotinoids.
- Treatment: no specific antidote, symptomatic treatment.

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