Practice No. 4

Pesticides – insecticides

Insecticides:

- Preparations used against insect pests in all developmental forms and stages
- Used in agriculture, medicine (as antiparasitics), industry and the household
- Division: according many criteria
  - route of application: fumigation; spray – effective after contact, feed; with local or systemic effect
  - type of effect: immediately effective, after chronic/repetitive intake or with residual activity
  - chemical structure: chlorinated insecticides
    organophosphates and carbamates
    pyrethrins and pyrethroids
    phenylpyrazoles
    neonicotinoids
    others

Chlorinated insecticides

- To this group belong DDT, HCH, aldrin, endrin, heptachlor, toxafen etc.
- They are very stable and accumulate in the environment
- Transport on long distances and contamination of remote areas
- Nowadays, beside a few exceptions, not used. Most of them banned. But there are still old resources, residues in water, soil, food chain

1) Polycyclic chlorinated insecticides:

- all are banned/abandoned (aldrin, endrin, heptachlor, toxafen etc.), usually from 1950-1970´s

- 3 types of action in animals:
  - acute poisoning with high dose: neurotoxicity – not seen nowadays
  - chronic poisoning with higher doses: similar signs to acute poisoning, but longer and gradual coming; again not seen nowadays
  - ingestion of residues from food: deposition in fatty tissues and elimination to milk – alteration of next food chain links mainly in carnivores and omnivores. This type of effect can be seen, health changes are non-specific, mainly genotoxicity, immunotoxicity etc. are expected

- Mechanism of action is usually inhibition of GABA receptors and thus hyperexcitation
- Treatment is symptomatic

HCH (hexachlorocyclohexane):

- Several forms, γ isomer called lindan is the most toxic one
- Used as an ectoparasitic agent e.g. against scabies

- Banned recently, so acute poisonings from older resources are still possible
- Inhibits probably GABA system and Na/K ATPase, which leads to excitation
- Clinical signs: diarrhoea, vomiting, hyperactivity of muscles, convulsions
- Treatment is symptomatic

DDT:

- In the Czech Republic banned since 1974, in USA since 1968, but still manufactured in a few developing countries (main producer is India)
- The most used historical pesticide (millions of tons), and it still persists in soil, sediment and food chain
- Accumulation in fat
- Slow conversion (decades): DDT ⟶ DDE ⟶ DDD
- Acts both after contact and eating
- DDT and DDD affect nervous system – destroy Na^+ channels, which leads to their prolonged open stage and longer/repetitive depolarisation of neurons (hyperexcitation). After chronic intake alteration of calcium metabolism and malacia (softening) of eggshells in birds and reptiles
- Pathological examination doesn't show any specific damage, only typical mousy smell from GIT
- DDE metabolite in not neurotoxic, but has xenoestrogennic effect (endocrine disruptor). Right now the most detected metabolite (> 50%)

Organophosphates (OP) and carbamates (CARB)

- They have systemic effect – applied on the surface of plant and absorbed to its tissues, and usually have residual activity - act for days to weeks, it is necessary to keep the protection period
- They enter in the animal organism very easily and in every route – inhalation, ingestion, skin contact
- Division:

  organophosphates – fluoro+cyano phosphates: war gases synthesized in 1930´s: sarin, soman, tabun etc.; all strictly banned by different peace agreements
  - derivates of pyrophosphoric acid: pesticides, insecticides - trichlorfon, dichlorvos; less used nowadays
  - derivates of thiophosphoric acid: a little bit less toxic to mammals, but still of high toxicity: parathion, metathion, diazinon. In the body undergo metabolic activation to oxons (e.g. paraoxon) with higher toxicity (example of bioactivation)

  carbamates – very toxic: oxamyl, carbofuran – agriculture, some banned
  medium toxicity: propoxur, pirimicarb – antiparasitics, agriculture
  low toxicity for mammals: carbaryl - antiparasitics, agriculture

- both OP and CARB inhibit several enzymes in the organism, the most important is Acetylcholinesterase (ACHE) !!!

This enzyme is found in nervous system and erythrocytes. In blood we find similar enzyme butyrylcholinesterase – we can measure it as a biomarker of poisoning, but it may be decreased also during pregnancy, acute infection or kidney diseases. In the poisoning the decrease of enzyme activity is more than 50%, which is not seen in other situations.

- Organophosphates blocks enzymes irreversibly, carbamates reversibly !!!

Mechanism of action:

- due to blockage of the enzyme, which decomposes acetylcholine, there cummulates a great amount of active acetylcholine on synapses – amplified cholinergic effect on smooth muscles (muscarinic receptors) and neuromuscular platelet (nicotinic receptor)

The course of intoxication:

1. stage: PRODROMAL
 - non-specific signs: fear, headache, anxiety, agression, vision impairment
2. stage: MUSCARINIC
 - fluently follows and blends together with the first stage
 - activation of parasympaticus, increased activity on muscarinic receptors in GIT and glands: hypersalivation, lacrimation, sweating, nausea, vomiting, colic, spontanneous defecation and emiction, miosis !!! – usually syndrome called SLUD (salivation, lacrimation, urination and defecation)
3. stage: NICOTINIC
 - again follows and blends with the two previous stages. Prognosis unfortunately bad.
 - hyperactivity of muscles, begins on head and neck, pure clonic seizures, exitus due to exhaustions and respiration centre paralysis

Warning: often delayed-onset neurological consequences even if the animal survives !!!

Pathological examination:

- No significant signs except for typical body position in birds
- Characteristic chemical smell of cadaver in case of carbamates

Treatment:

- Usually in human medicine we use so called reactivators of acetylcholinesterase – oxims – eg. pralidoxim. These, when administered in first or beginning of second stage, bind covalently with organophosphates and protect ACHE . Later, when there is a firm covalent bond between enzyme and OP already made – ineffective. We don't administer them in carbamate intoxication – pointless, no covalent/irreversible bond!
- **Atropine**: the main antidote !!! Administration as soon as possible, after so called atropine test. In poisoning proved, then 25 % of dose i.v. (till strong mydriasis), and then we administer atropine i.m. or s.c., repetitively. Next dose is given when miosis starts to occur again.
- **Treat convulsions** with diazepam or methocarbamol (barbiturates can be used in severe cases, but have a risk of further inhibition of respiratory centre), as convulsions are what exhausts an animal.
- We recommend oxygen inhalation, fluid therapy, glucose etc.

Pyrethrins and pyrethroids

- Pyrethrins – insecticides originally isolated from a "daisy like" looking plant *Pyrethrum cinerariaefolium*. Not very stable, so usually replaced by new substances – pyrethroids
- Pyrethroids are synthetic derivates of natural pyrethrins
- Used alone or in combination, most often with organophosphates or carbamates, or potentiated by piperonylbutoxide
- They are relatively less toxic to mammals **with exceptions** - young animals, cats and ferrets. Higher sensitivity of these animals is caused by their decreased activity of conjugation enzyme glucuronyl-S-transferase
- Highly toxic also for fish and bees !!!
- They act both as contact and feed poisons, in insects act quickly - many of them cause so called knock down effect
- Pyrethroids have two types – T (tremor) and CS (choreoatethosis+salivation)

Mechanism of action:
- influence mainly Na^+ channels, which cannot close after the impulse, and that causes hyperexcitability of neurons and muscles
- also Ca^{2+} channels can stay opened and GABA receptors are inhibited in CS type of pyrethroids

Clinical signs:
- within 1-3 hours; hypersalivation, nausea, vomiting (all in CS type), muscle trembling, ataxia, dyspnoe. If the poisoning is via inhalation route, the course of poisoning is much faster with possible lung oedema and it has bad prognosis

Pathological examination:
- nothing specific, death usually occurs only in sensitive species (cats, ferrets, fish)

Treatment:
- emetics, absorbents – activated charcoal, diazepam or other anticonvulsants, atropine to decrease GIT signs in present, fluid therapy

Phenylpyrazoles

- the mainly used substance from this group is **fipronil**
- it is already banned in agriculture due to residual activity and toxicity for bees and fish, and also banned in food producing animals
- fipronil is used as an ectoparasitic agent in companion animals (Frontline)
- phenylpyrazoles block GABA receptors – in overdose increased irritability, problems with coordination, tremor, convulsions
- **Fatal** for rabbits, higher risk of poisoning is described in guinea pigs

Neonicotinoids

- many substances used in agriculture
- problems mainly with substance called **imidacloprid**
- imidacloprid is highly toxic to bees
- poisonings in mammals are rare with non-specific signs
- mechanism of action is either activation or inhibition of nicotinic receptors on muscles which leads to hyperexcitation or paralysis respectively

Other insecticides

- Bioracional insecticides – synthetic analogues of insect hormones – juvenile or feromones
- not toxic, but some signs of usually GIT irritation or skin damage occur due to solvents and additives in the products

Stockholm convention on persistent organic polutants (POPs) 2001

- International convention on termination or restriction of use of these chemicals (POPs), which accumulate in the environment and are badly degradable
- all POPs are lipophilic and spread on long distances in form of vapours or solid particles
- originally 12 substances or their mixtures (called Dirty dozen), divided into three so called Annexes
- right now 28 substances classified in this convention

- Annex A: aldrin, dieldrin, chlordan, endrin, heptachlor, HCH, mirex, toxafen (all pesticides), but also PCBs, pentachlorbenzene etc. (industrial chemicals) – requirement of absolute termination and prohibition of production and use of them
- Annex B: DDT – requirement of maximum restriction of production and use. It is impossible to eliminate its use at the moment in some countries of Africa and Asia – agent effective on Anopheles mosquito, a vector of malaria. Very cheap, rotation system with other insecticides. These countries must ask for exception.
- Annex C: polychlorinated dibenzodioxins and dibenzofurans (PCDDs and PCDFs) and some other chemicals – these are not substances manufactured intentionally, but produced as non-purpose by-products in industry or during burning of waste. Requirement of new technologies, which will restrict their production and release.

Practical work: Determination of the activity of butyrylcholinesterase in blood plasma
Principle of detection of DDT in samples – gas chromatography