Conventional insecticides for management

Introduction

- Pesticides play a vital role in the control of a huge variety of organisms that are pests of human.
- The word ‘pesticide’ refers to a substance used to kill undesirable animals or plants.
- As such, pesticides are toxic materials that effect death by interfering with the normal life processes of pest organisms.
- ‘Pesticide’ is derived from Latin ***pestis*** (a plague, pestilence or contagious disease) and ***cidere*** (to kill).
- Pesticide is a general term that embraces many specific and particular types.

Types of pesticides and their target organisms

Insecticides	Insects
Rodenticides	Rodents (rats and mice)
Miticides (acaricides)	Mites
Herbicides (weedicides)	Weeds
Bactericides	Bacteria
Algicides	Algae
Fungicides	Fungi
Molluscicides	Molluscs (snails and slugs)
Avicides	Birds (from Latin ***avis***)
Ovicides	Eggs (from Latin ***ovum***)
Piscicides	Fish (from Latin ***piscis***)
Termiticides	Termites

Other chemicals that have similar roles like pesticides

• Attractants	Attract insects
• Chemisterilants	Sterilize insects.
• Defoliants	Remove leaves.
• Dessicants	Drying of plants, or pests.
• Disinfectant	Destroy or inactivate harmful microorganisms.
• Feeding stimulants	Cause insects to feed more vigorously.
• IGRs	Retard the normal growth processes of insects.
• Pheromones	Attract insects or vertebrates.
• Repellents	Repel insects, mites, ticks, etc.

Insecticide Nomenclature

- Formal process of naming insecticides.
- Designated by 3 names: the approved common name, trade name and the chemical name.
- Common name: fipronil.
- Trade name: Regent(R), Termidor(R)
- Chemical name: *(+)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile.*

Common chemical name versus trade name

Chemical name	Trade name
propoxur	Baygon®
chlorpyrifos	Dursban®, Lentrek®
lamda-cyhalothrin	Icon®
alpha-cypermethrin	Fendona®
fipronil	Termidor®, Goliath®
bifenthrin	Biflex®
chlorfenapyr	Phantom®, Terminator®
lufenuron	Instar®
hexaflumuron	Sentricon®
deltamethrin	Crackdown®

Rules and regulations on use of pesticides

There are several laws, rules and regulations governing the control of pesticides in Malaysia:

- ❑ Pesticides Act 1974 (amended 2004).
- ❑ Food Act 1983 and the Food Regulations 1985 (amended 1995).
- ❑ Environmental Quality Act 1974 (amended 1985 and 1996).
- ❑ Occupational Safety and Health Act 1994.
- ❑ Hydrogen cyanide Fumigation Act 1953 (revised 1981).

Pesticide Act 1974 (amended 2004)

- Main act for the control of pesticides in Malaysia, and is implemented by Pesticides Board, which has its secretariat in the Department of Agriculture.
- To ensure that pesticides imported, manufactured and sold in the country are of good quality, and that will not cause adverse effects on man, food crops and the environment.
- All pesticides imported or manufactured in this country must be registered with Pesticides Board. A pesticide will be evaluated on aspects of product chemistry/quality, toxicology including ecotoxicology, efficacy and its uses, residue chemistry/effects, environmental fate, packaging labelling, and risk-benefit before it may be registered.
- Pesticides are registered for a period of 5 years after which they have to be re-registered before they can continue to sell, import or manufacture.

Pesticide Act 1974 (amended 2004)

Various rules and regulations have been created and implemented to ensure the objective of the Act is achieved. The following 7 rules and regulations have been implemented:

- ❑ Pesticides (registration) rules 1976.
- ❑ Pesticides (importation for educational and research purposes) rules 1981 (amendment 1987).
- ❑ Pesticides (Labelling) Regulations 1984.
- ❑ Pesticides (Licensing for sale and storage for sale) Rules 1988.
- ❑ Pesticides (Highly toxic Pesticides) Regulations 1996 (amendment 2004).
- ❑ Pesticides (Pest Control Operator) Rules 2004.

Sales of Pesticides

Under the Pesticides Rules 1988, all premises, which sell and store for sale of pesticides have to be licensed, to ensure:

- ❑ Only registered pesticides are stored, displayed and sold.
- ❑ Pesticides are stored and handled properly and safely in order to minimize contamination of food and the environment and harm to humans and livestock.
- ❑ Misuse or abuse of pesticides is minimized by creating greater public awareness on the need to use and store pesticides correctly.
- ❑ Under Pesticides (Exemption Order) 2004, the following pesticides (classes II, III and IV on label) can be sold or stored without the need for a license:

  Bti formulations, liquid for control of ticks and fleas, fly, cockroach and ant baits, insect repellent, insecticidal aerosols, liquid vaporiser, coils, mats, temephos granules, etc.

Labelling

- Pesticides (Labelling) Regulations 1984 have provisions for the stipulation on the type of information that should be stated on the label. These includes:

- ❑ Trade name of product.
- ❑ Active ingredient(s), concentrations and formulation.
- ❑ Direction for users and recommended concentrations.
- ❑ Toxicity class.
- ❑ Pre-harvest interval.
- ❑ Symptom of poisoning.
- ❑ First aid and medical treatment.
- ❑ Pre-cautionary statements.
- ❑ Guide to disposal of empty container(s).
- ❑ Date of manufacture.

Advertisement

- Pesticides (Advertisement) Regulation 1996 stipulates that advertisement on pesticides must have prior approval from the Pesticide Board before they can be published.
- The objective is to ensure all pesticides advertisement does not mislead, and provide only useful information to prospective purchasers, to minimize pesticide risk and promote healthy competition among pesticide companies.
- Statement which imply the indiscriminate, unnecessary and excessive use of pesticides would not be permitted. Advertisement should not show pesticide within reach of children, nor should children be shown using or endorsing pesticides in any way.

The action of insecticides

- Insecticides kill insects by disrupting important life processes. This toxic action relies on:

❑ Route of entry of insecticides into insect body.

❑ Mode of action of the insecticide inside the insect body.

Routes of entry

- Insecticides may enter insect body via cuticle (dermal entry), via mouth (oral entry) or via breathing system (respiratory entry).
- **Dermal entry** - penetration of insect cuticle by an insecticide is often referred as "contact action"; hence insecticides that rely heavily on this method are referred as contact poisons.
- The contact of insecticide with the insect may arise from: (1) direct and immediate contact that occurs when insects are wetted by aerosol (mist or fog) droplets, or (2) contact that occurs when crawling insects walk on surfaces that have been treated.

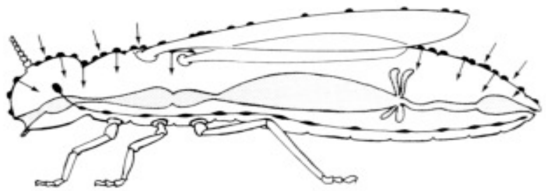


Figure 8.1 1. the direct and immediate contact that occurs when insects are 'wetted' by aerosol (mist or fog) droplets; or

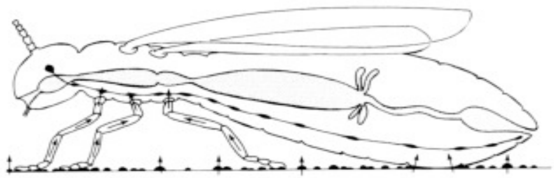


Figure 8.2 2. the contact that occurs when crawling insects walk on surfaces that have been treated so as to leave an insecticidal deposit.

Oral entry

- Oral entry of insecticides relies on ingestion of the poison into the digestive system.
- Insecticides that rely heavily on this mode of entry are called 'stomach poisons'.
- Entry is effected via (1) being incorporated in a poisonous bait that is ingested by pest, or (2) being ingested during the process of grooming, when the insect's body may be contaminated by the insecticides.
- Most contact poisons can also act as stomach poisons when ingested. Some other stomach poisons have little or no contact action on insects (eg. boric acid, arsenic).
- Systematic insecticides are designed, so that when administered to the host plant or animal, they are translocated throughout the host. When parasitic pests feed on the host, the poison will be ingested.

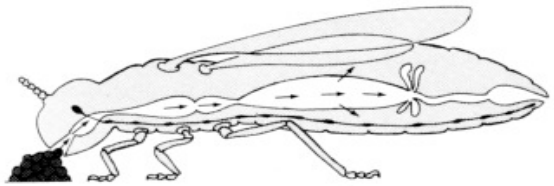


Figure 8.3 1. being incorporated in a poisonous bait that is ingested by the pest; or

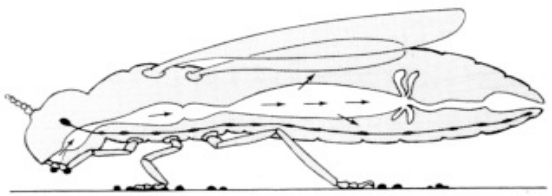


Figure 8.4 2. being ingested during the process of grooming, when the insect's body may be contaminated by the insecticide (eg when the places where cockroaches hide are dusted).

Respiratory entry

- Insecticides that rely largely on being 'breathed in' by the insect to cause death are referred as respiratory or inhalation poisons.
- All fumigants are inhalation poisons.
- They act by being present as toxic molecules in the atmosphere.
- Fumigants are described as 'volatile' (capable of easy and rapid conversion to gaseous state).
- Fumigation techniques rely on containing the toxic vapours for a period of time, as relatively airtight conditions are required.
- All insecticides have some degree of volatility, but most of them at very low level.

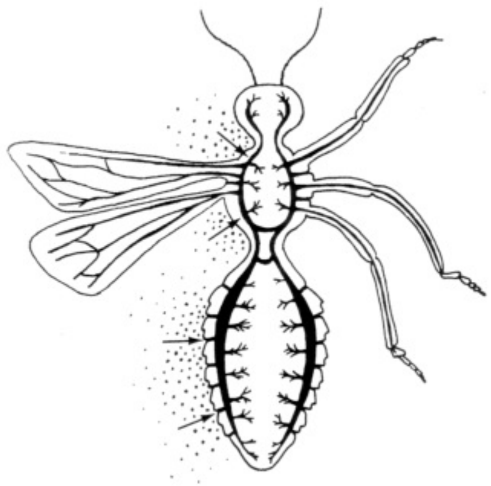


Figure 8.5 Respiratory entry of insecticides

Toxicity of insecticides (1)

- Interaction between insecticide and living organisms is dose-related.
- Toxicity is usually expressed in LD_{50} (= amount of toxicant that will killed 50% of the test organisms).
- Usually expressed in mg /kg body weight of test animals.
- When exact dose cannot be determined, LC_{50} value (= lethal concentration that killed 50% of test insects) is used. Example: when we test mosquito larvae in the water.

Toxicity of insecticides (2)

- Besides dose, toxicity can also be expressed in time.
- LT_{50} or KT_{50} = time taken for a certain concentration of insecticide to cause 50% mortality of test insects.
- When expressing using time, the dose/concentration is usually standardized.

Toxicity of insecticides (3)

- LD_{50} ↑, insecticide toxicity ↓
- Example:

  LD_{50} for cypermethrin = 500 mg/kg

  LD_{50} for temephos = 8600 mg/kg

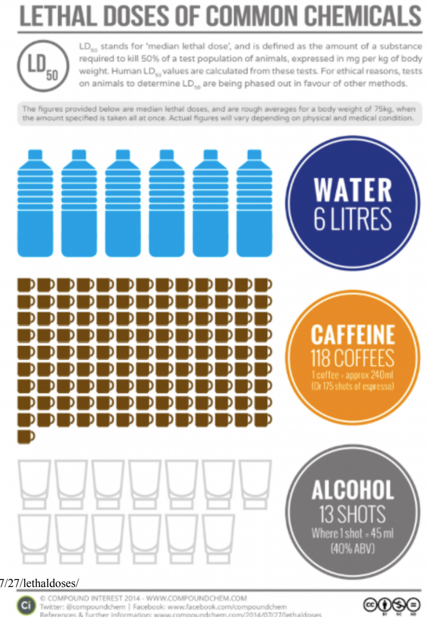
  Hence, cypermethrin is more toxic than temephos.

How to measure insecticide toxicity

- **Acute toxicity** – rapid or short expression of toxic effects – derived from single dose of insecticide within a short period of time (eg. one day)
- **Sub-chronic toxicity** — several doses.
- **Chronic toxicity** – long-term expression of toxic effects – usually results of several doses of insecticides applied over a long period of exposure (eg. 6 months, 1 year).
- Application can be done either through skin, intravenous, intramuscular, subcutaneous or ingestion.

Table 3. The LD50 (mg/kg of a body weight) of some substances found around the home. (The Merck Index, 11th edition, 1989.)

Substance	LD50	Use
absolute alcohol	10,600	beverage, preservative
acetone	10.7	fingernail polish remover
aspirin	1,000	drug, pain
caffeine	355	constituent in coffee, colas
ethylene glycol	8,540	antifreeze
propylene glycol	24,000 - 30,000	antifreeze
ibuprofen	626	drug, pain
nicotine	0.3	constituent in tobacco
table salt	3,750	food additive
vitamin A	7,910	vitamin
warfarin	323	rodenticide, anticoagulant



http://www.compoundchem.com/2014/07/27/lethaldoses/

TOXICITY OF SOME MATERIALS

MORE TOXIC

Material	Acute Oral LD_{50} (rat) in mg/kg (see above)
Harvester ant venom	0.66 EXTREMELY TOXIC (but "organic")
Paper wasp venom	2.4
Honey bee venom	2.8
Yellow jacket venom	3.5
Nicotine	10
Toxaphene	29
Gasoline	50
Fipronil	95 (see Termidor below)
Diazinon®	100
Caffeine	200
Pyrethrums	200 to 2,600 (more below)
Sevin®	650
Aspirin	1,200
Malathion®	1,375
Household bleach	2,000
TimBor®	2,500 and at 5% is 50,000
Borax	2,500
Table Salt-------------	**3,320** ----------------------------------
Boric Acid	3,500
Baking Soda	4,200
DeltaDust	5,050
d-Limonene (the active ingredient in orange oil)	5,300
Grain Alcohol	14,000
Sugar	30,000
Niban®	60,000+
Termidor® (as 0.06% Fipronil spray)	3,252,936 = about 60 Gal/Kg for an adult

LESS TOXIC

Substance	Oral LD50	Class	Toxicity Category
d-limonene	nontoxic	botanical (citrus)	5
diatomaceous earth	nontoxic	desiccant	5
methoprene	34,000	insect growth regulator	4
fenoxycarb	10,000	insect growth regulator	4
hydroprene	> 5,100	insect growth regulator	4
d-phenothrin	10,000	pyrethroid	4
piperonyl butoxide	7,500	synergist	4
abamectin, avermectin b1	4,200	natural toxin	4
hydramethylnon	> 5,000	aminohyrazone	4
tetramethrin	4,640	pyrethroid	3
borax	3,000	mineral	3
resmethrin	2,000	pyrethroid	3
pyrethrins	1,500	botanical	3
malathion	885	organophosphate	3
acephate	886	organophosphate	3
allethrin	425	pyrethrin	3
diazinon	300	organophosphate	3
trichlorfon	144	organophosphate	3
permethrin	4,000	pyrethroid	2
cyfluthrin	500	pyrethroid	2
fenvalerate	451	pyrethroid	2
cypermethrin	247	pyrethroid	2
chlorpyrifos	135	organophosphate	2
propoxur	95	carbamate	2

Classification of toxicity (based on LD_{50} values)

- 1 mg/kg or less – extremely hazardous.
- 1 – 50 mg/kg – highly hazardous.
- 50 – 500 mg/kg – moderately hazardous.
- 500 – 5000 mg/kg – slightly hazardous.
- 5000 – 15000 mg/kg – relatively non-hazardous.
- >15000 mg/kg – relatively harmless.

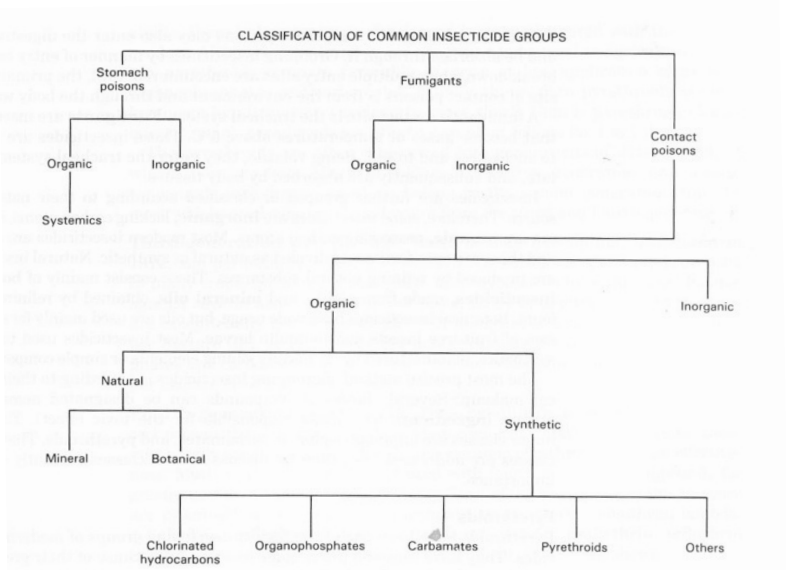


Figure 11.2 Classification of common insecticide groups. Initial divisions are based on site of encounter.

Inorganic insecticides

- Inorganic insecticides are of mineral origin and contain no carbon in their make-up.
- Being mostly crystalline solids, they have been used largely as baits or dusts, and mostly rely on a stomach poison action.
- The use of inorganics as insecticides has declined over the last few decades because:
  - ❑ high mammalian toxicity.
  - ❑ Great stability which can lead to accumulation of toxic compounds.
  - ❑ Recent development of safer synthetic insecticides that can replace them.
- Few inorganics are used at present. Arsenic trioxide is used as dust against termites; boric acid is used as active in cockroach bait product; silica aerogels (very finely ground silica formulation) are used in cockroach control as physical abrasives that cause dehydration.

Botanical insecticides (1)

- Sometimes referred to as natural, or plant-derived insecticides, are extracted from specific parts of certain plants.
- Nicotine extracts from tobacco were used as insecticides as early as the late 1600.
- The most widely used botanical insecticide is pyrethrum (containing pyrethrin I and II, and cinerin I and II) from the flowers of a daisy-like plant *Chrysanthemum cinerariaefolium*. The main areas of use include control of various household and industrial insect pests. Pyrethrin has the following characteristics:
- ❖ Broad spectrum of activity.
- ❖ Low mammalian toxicity.
- ❖ Rapid breakdown after application.
- ❖ Fast knockdown, flushing and repellent properties.
- Pyrethrin is a rather expensive insecticide and often formulated with piperonyl butoxide.
- Rotenone is a botanical insecticide extracted from roots of certain South American plants. It is used mainly for control of plant pests and animal ectoparasites. It is usually formulated as a dust (derris dust).

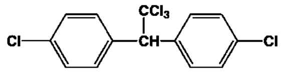
Botanical insecticides (2)

- **Azadiractin** -- also known as neem insecticide, and extracted from the seeds of neem tree, Azadirachta indica (Meliaceae).
- Deter insect feeding and oviposition, and interfere with growth, development and reproduction.
- Excellent against target organisms, and safe towards nontarget arthropods, fish and livestock.
- D-limonene -- extracted from citrus peel, constituting of 98% orange peel oil.
- Useful against external parasites of pets such as fleas, lice, mites and ticks.

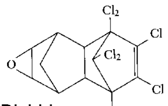
Classification of synthetic insecticides

- Chlorinated hydrocarbons (eg. DDT) and cyclodienes
- Organophosphates (eg. chlorpyrifos)
- Carbamates (eg. propoxur)
- Pyrethroids (eg. deltamethrin)
- Organosulphurs (eg. tetradifon)
- Formamidines (eg. chlordimeform, amitraz)
- Organotins (eg. cyhexatin)
- Neonicotinoids (eg. imidacloprid)
- Phenylpyrazoles (eg. fipronil)
- Pyrroles (eg. chlorfenapyr)
- Spinosyns (eg. Spinosad)
- Benzoylureas (usually Chitin Synthesis Inhibitors) (eg. dimilin, hexaflumuron)
- Juvenile hormone analogues (eg. pyriproxyfen)

Chlorinated hydrocarbons (CHs)



DDT

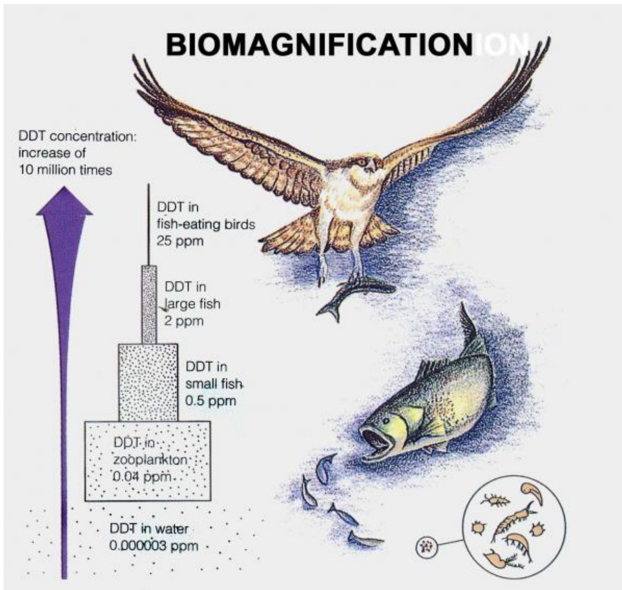


Dieldrin

- Started with discovery of DDT in 1939.
- Usually chemically unreactive and are not water-soluble.
- Very persistent in the environment.
- Most candidates had been banned for usage.
- Eg. DDT, chlordane, dieldrin, endrin, etc.

DDT and relatives

- DDT had been cancelled for usage in many countries in the world today because of its stability and fat solubility.
- Sublethal doses ingested by animals will not be metabolized, but instead **stored in the body fat.**
- Because of its persistency, it can move from the bottom of the trophic level to the top trophic level, a phenomenon known as **biomagnification**.

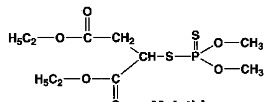


Source: http://bwbearthenviro2011.wikispaces.com/file/view/48e0c2e877e27Biomagnification1copy_org.jpg/341273764/48e0c2e877e27Biomagnification1copy_org.jpg

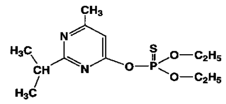
Cyclodienes

- Cyclodiene -- developed after DDT and HCH. eg. chlordane, aldrin, dieldrin, heptachlor, endrin, mirex and endosulfan.
- Very persistent, stable in the soil, and under sunlight as well.
- Higher mammalian toxicity than DDT.

Organophosphates (OPs)



Malathion



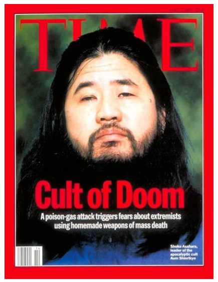
Diazinon

Remember the movie Schindler List in 1993?

- First discovered by the Germans during the 2nd WW as toxic agents (nerve gases) for chemical warfare (eg. tabun, sarin, soman).
- Characteristics: (1) more toxic to higher animals than CHs, (2) chemically unstable and non-persistent in the environment.
- Candidates that have/had been used widely: chlorpyrifos, malathion, diazinon, fenitrothion, etc.
- Majority of OPs are contact and oral poisons.

Tokyo Subway Sarin Gas Attack

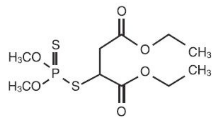
20 March 1995



MASSACRE BY POISON GAS

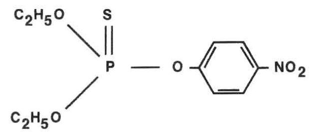
Many die, 1,200 collapse in Tokyo Tube attack

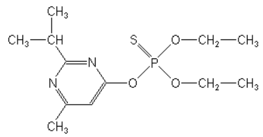
Organophosphates



malathion

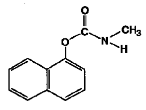
PARATHION INSECTICIDE
diethyl-p-nitrophenyl monothiophosphate





diazinon

Carbamates (CARBs)

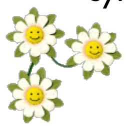


Carbaryl

- The first CARB was developed in 1951, but was not effective and high cost.
- Candidates that are used widely today: carbaryl, carbofuran, propoxur and bendiocarb.
- Characteristics: (1) high mammalian toxicity, (2) broad spectrum for insect control.

Pyrethroids (1)

- Pyrethrum (a botanical insecticide) – extracted from chrysanthemum flower showed good knockdown in the early 1940s.
- However, it is too costly to extract and unstable under sunlight.
- Based on the chemical structure of pyrethrum, the chemical analogues of pyrethrum were synthesised.

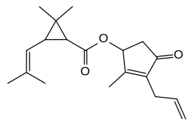


Andr'oid'

"Man-like" or Robot

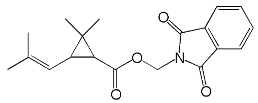
Various generations of pyrethroids (1)

- **1st generation**



  - allethrin (available commercially in 1949)
  - effective against flies and mosquitoes, but not against cockroaches.
  - photo-unstable.

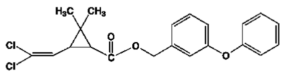
- **2nd generation**



  - tetramethrin (1965).
  - better knockdown on flying insects and synergizable with other compounds.
  - other candidates: resmethrin, bioresmethrin, d-trans allethrin, phenothrin.
  - photo-unstable.

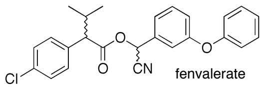
Various generations of pyrethroids (2)

- **3rd generation**



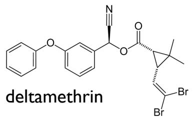
Permethrin

  - permethrin (1972) and fenvalerate.
  - photostable and with residual properties.
  - exceptional insecticidal activity.



fenvalerate

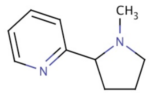
- **4th generation**



deltamethrin

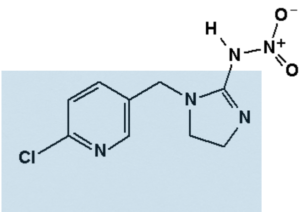
  - 10x more active than 3rd generation.
  - photostable, residual and superb insecticidal activity.
  - candidates: cypermethrin, cyfluthrin, bifenthrin, deltamethrin, esfenvalerate.

Neonicotinoids

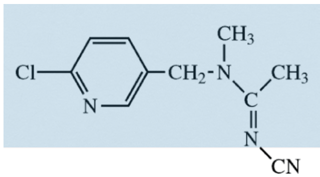


- Resemble natural product **nicotine**.
- eg. imidacloprid, acetamiprid, thiamethoxam, thiacloprid, clothianidin, dinotefuran, etc.
- Imidacloprid is a systemic and contact insecticide.
- Low mammalian toxicity and good environmental characteristics.

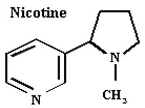
Neonicotinoids



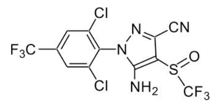
imidacloprid



acetamiprid

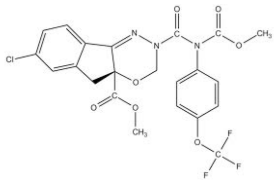


Phenylpyrazoles



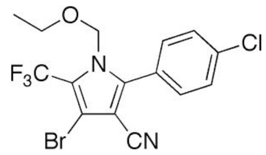
- Relative new class of insecticide with only a single material, fipronil.
- Effective against a wide variety of insects.
- Good for treating soil, and foliar insects, as well as for seed treatment. It is also widely used by urban area against cockroaches, ants (in baits) and termites.

Oxidiazines



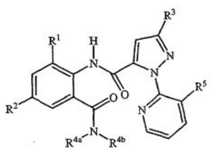
- New class with only a single candidate -- indoxacarb.
- low mammalian toxicity and little effect on birds and aquatic organisms.

Pyrroles

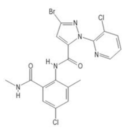


- New group of insecticide, having both contact and stomach modes of action.
- Slow acting.
- Only candidate: chlorfenapyr.

Diamide/ Ryanoids



Basic structure of ryania

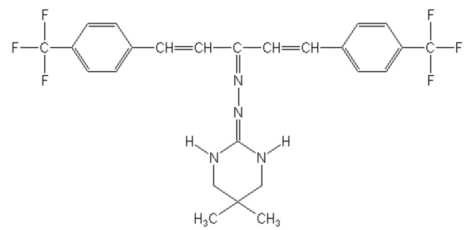


chlorantraniliprole

- Resemble ryania, a botanical insecticide extracted from *Ryania speciosa*.
- Only one candidate: chlorantraniliprole under trade name of Rynaxpyr, and Altriset.
- Low toxicity to human.

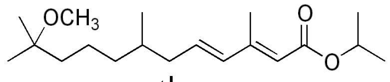
Amidinohydrazone

- Contain an amidino and a hydrazone group.
- The only candidate in this group: hydramethylnon.
- Controls pest ants and cockroaches.

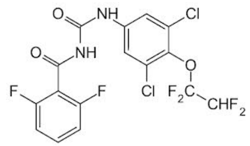


Insect growth regulators (IGRs)

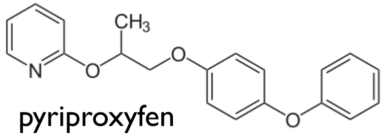
- Chemical substances that **disrupt insect growth and development**, resulting in the death of insects.
- 2 main types of IGRs: juvenoids, benzoylphenylureas.
- The most common ones are juvenoids and benzoylphenylureas.
- **Juvenoids** (Juvenile Hormone Analog) -- structurally similar to the insect juvenile hormones. eg. methoprene, hydroprene, pyriproxyfen etc.
- **Benzoylphenylureas** -- mainly chitin synthesis inhibitors that inhibit formation of chitin, an important component of insect exoskeleton. eg. hexaflumuron, bistrifluron, chlorfluazuron, lufenuron, etc.



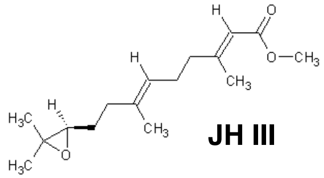
methoprene



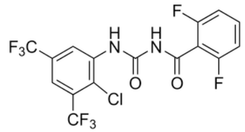
hexaflumuron



pyriproxyfen



JH III

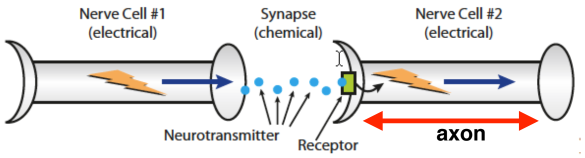


bistrifluron

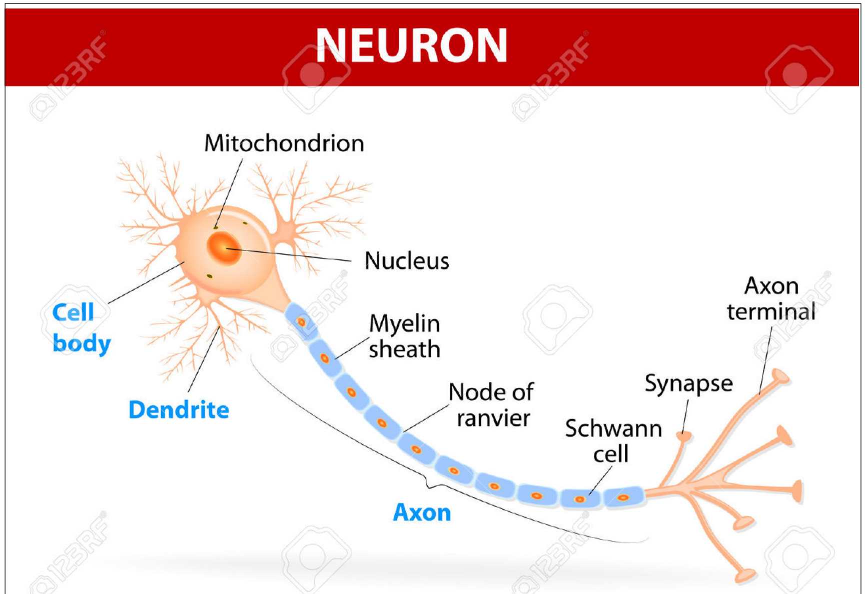
Fumigants

- Fumigants are pesticides that are volatile and exert their toxic action as poisonous gases in an enclosed space.
- They are usually stored as liquids under pressure in industrial cylinders (although phosphine is also available in pellet, tablet and some other forms).
- The materials that may be fumigated are usually those where some penetration of insecticidal action is required, as in treating timber or grain to kill the pests within.
- Insects are killed when breathing in the toxic vapours.
- In Malaysia, only a license fumigators can conduct a fumigation operation.
- Examples of common fumigants: methyl bromide, hydrogen cyanide, chloropicrin, carbon disulphide, phosphine, sulfuryl flouride.

Insect neurobiology (1)

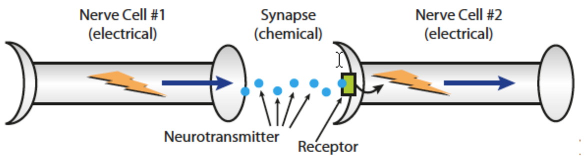


- The nervous system in insects is composed of a series of interconnected cells, called **neurons**, along which travel electrical charges called **impulses**.
- Impulses are carried from one end of a nerve cell across the synapse by a chemical messenger called a **neurotransmitter (NT).**

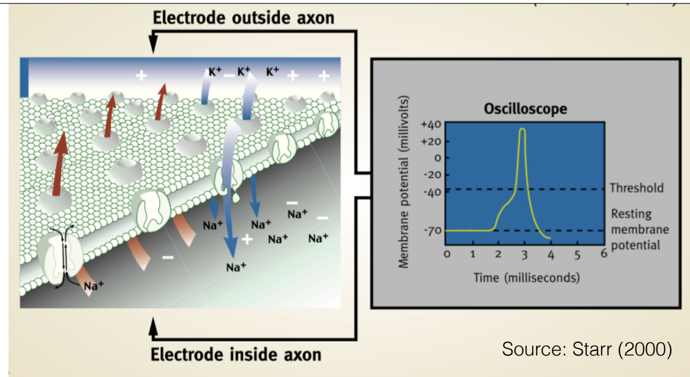


http://previews.123rf.com/images/designua/designua1311/designua131100012/23684899-Anatomy-of-a-typical-human-neuron-axon-synapse-dendrite-mitochondrion-myelin-sheath-node-Ranvier-and-Stock-Vector.jpg

Insect neurobiology (2)



- NTs include acetylcholine (ACh), gamma amino butyric acid (GABA) and glutamate.
- NTs are released from the pre-synapse region, move across the synapse and are received by the post-synapse region at the receptor sites.
- When the NTs successfully bind to its receptor site, this triggers an impulse in the next nerve cell.

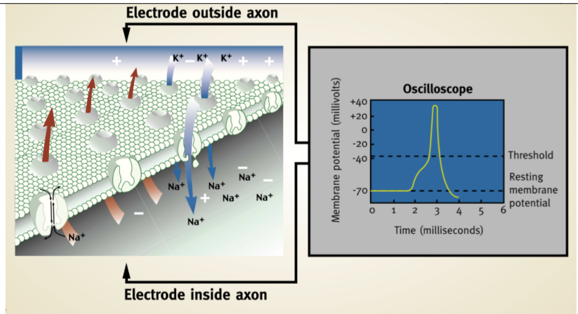


Source: Starr (2000)

Messages are passed along axon via electrical impulses, this is achieved by altering the normal charge of the axon.

Under resting conditions there is a large number of Na^+ ions on the outside of the axon and very few inside.

Also, there are a large number of K^+ ions on the inside and very few outside. This results in a positive charge on the outside of the axon and a negative charge on the inside of the axon.



- As a message is sent along the axon, a sodium channel is opened allowing increased amounts of sodium to enter inside the axon.
- At the same time a potassium channel is opened, allowing increased amounts of potassium on the outside of the axon.
- This will alter the charge of the axon on the outside to negative, and on the inside to positive.
- Once the message has passed by, the sodium and potassium channels shut, restoring the outside of the axon to a positive charge and the inside to a negative charge.

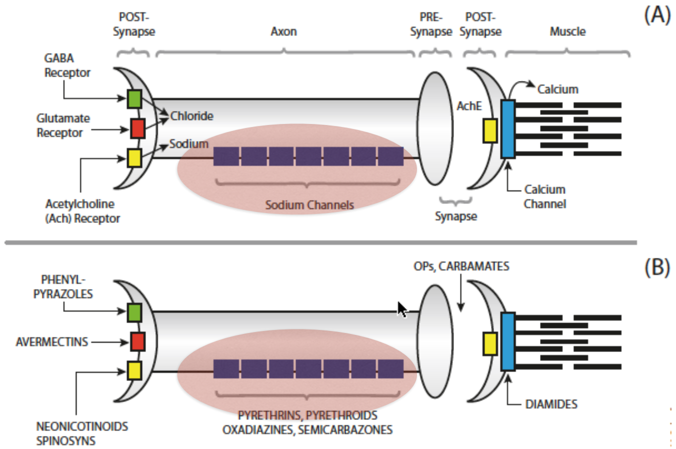
Insecticide mode of actions

- **Insecticides that target insect nervous system:**(1) Target site: Sodium Channels. (2) Target site: Acetylcholine system, (3) Target site: Chloride channel.
- **Insecticides that DO NOT target insect nervous system:** (1) Muscular calcium channel toxins. (2) Insect growth regulators, (3) Inhibitors of energy production, (4) Desiccants, (5) Alimentary toxin.

Insecticides that <u>target</u> the insect nervous system

Target site: (1) Sodium channels

(Chemical class: Pyrethrins and Pyrethroids)



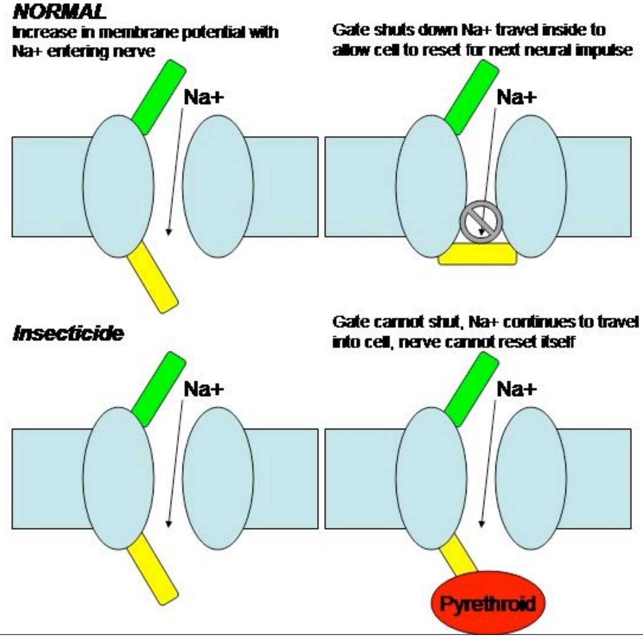
Target site: (1) Sodium channels

(Chemical class: Pyrethrins and Pyrethroids)

- Both pyrethrins and pyrethroids disrupt normal nerve function in **axon**.
- They affect the **sodium channels** by delaying the rate at which they close — thus **increasing the flow of sodium ions into the cell.**
- This mode of action results in uncontrolled, uninterrupted nerve firing resulting in tremors and shaking in insects.

A German cockroach intoxicated by deltamethrin

Warning: Several cockroaches were harmed in the making of this video



Target site: (1) Sodium channels

(Chemical class: Oxidiazines)

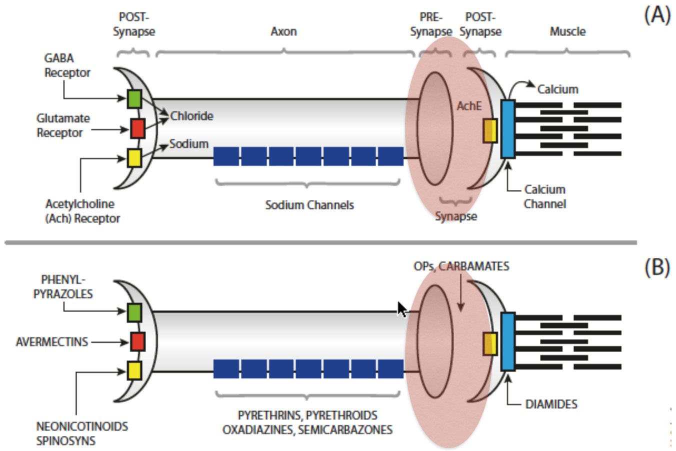
- When indoxacarb enters the insects, it is bioactivated into a new molecule.
- After activation, the newly formed molecule (metabolite) targets the sodium channels along the nerve axon.
- The metabolites **tightly binds to the sodium channel and completely blocks sodium ions flow into the nerve cells** (completely the opposite of pyrethrins and pyrethroids).
- Poisoned insects appear paralysed and limp and incapable of movement.

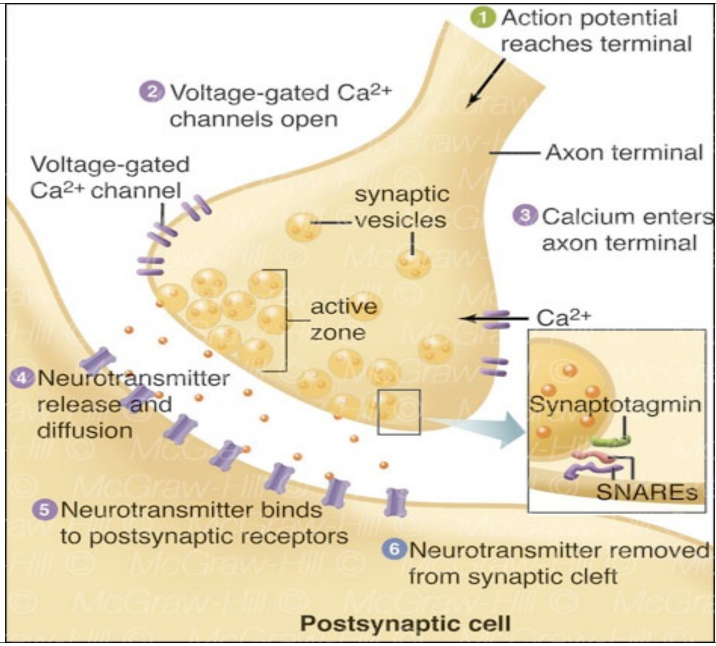
A German cockroach intoxicated by indoxacarb

Warning: Several cockroaches were harmed in the making of this video

Target site: (2) Acetylcholine System

(Chemical class: Organophosphates and Carbamates)





Target site: (2) Acetylcholine System

(Chemical class: Organophosphates and Carbamates)

- OP and CARB act by inhibiting the acetylcholinesterase (**AChE**) enzyme in the nervous system.
- AChE removes the ACh from its receptor at post-synapse, and converts it into acetate and choline. This **prevents overstimulation** of the nervous system.
- OP and CARB tie-up AChE, preventing it from removing ACh from the receptor site.
- Without AChE, the stimulated nerve will continuously produce impulses.

A German cockroach intoxicated by chlorpyrifos

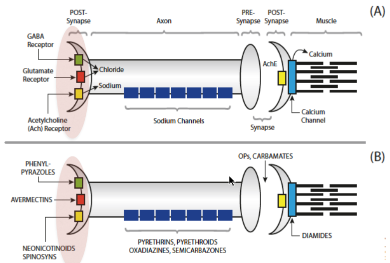
Warning: Several cockroaches were harmed in the making of this video

A German cockroach intoxicated by propoxur

Target site: (2) Acetylcholine System

(Chemical class: Neonicotinoids)

- Active ingredients: imidacloprid, dinotefuran, thiamethoxam, chlothianidin and acetamiprid.
- Mimicking ACh.
- Target the insect nervous system by **binding to ACh receptor** on the post-synapse nerve cell for a long time resulting nerve hyperstimulation.
- This causes symptoms of tremors and hyperactivity.



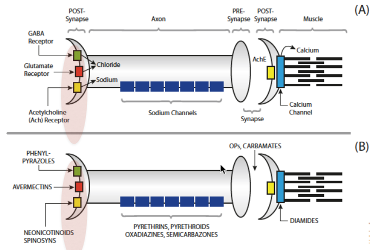
Warning: Several cockroaches were harmed in the making of this video

A German cockroach intoxicated by imidacloprid

Target site: (3) Chloride Channels

(Chemical class: Phenylpyrazole)

- Active ingredient: fipronil.
- Work by **mimicking GABA** (gamma- aminobutyric acid), an inhibitory signal sent from the pre-synaptic nerve terminal.
- They bind to the post-synaptic receptor for the GABA signal. When this receptor is "stuck" open, it allows an influx of chloride ions into the post-synaptic neuron. This will dampen the effect on nerve impulses firing.

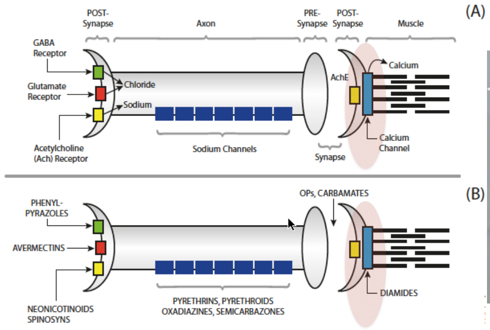


Warning: Several cockroaches were harmed in the making of this video

A German cockroach intoxicated by fipronil

Insecticides that <u>DO NOT</u> target the insect nervous system

(I) Muscular Calcium Channel Toxins
(Chemical Class: Diamide)



A German cockroach intoxicated by chlorantraniliprole

Warning: Several cockroaches were harmed in the making of this video

- Active ingredient: Chlorantraniliprole.
- **It binds and stimulate muscular calcium channels**, causing uncontrolled calcium release and resulting **muscle contractions**.

(II) Insect Growth Regulators

- 2 groups - **Juvenoid** or juvenile hormone analogs (eg. pyriproxyfen, methoprene, hydroprene) and **Benzoylphenylureas or** chitin synthesis inhibitors (eg. diflubenzuron, triflumuron, hexaflumuron, bistrifluron).
- <u>JHA</u> mimics the natural insect juvenile hormone which presence in immature insects keeps them from becoming adults.
- <u>CSI</u> prevents the formation of chitin by <u>blocking chitin synthase</u> which is responsible for conversion of some chemicals into chitin (an important component of insect exoskeleton)

A German cockroach nymph affected by juvenoid

(III) Inhibitors of Energy Production
Chemical Class: Amidinohydrazone

- Active ingredient: hydramethylnon.
- **<u>Prevents mitochondria from producing energy for the cell</u>**, and organism is unable to conduct its normal activities.
- Insects affected by hydramethylnon die slowly as energy becomes depleted.

(III) Inhibitors of Energy Production
Chemical Class: Pyrrole

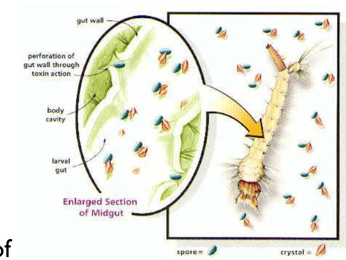
- Active ingredient: chlorfenapyr.
- Chlorfenapyr must be **converted** (through activation process) by enzymes in insect to an active form.
- The metabolite has the mode of action like hydramethylnon, and it **destroys the mitochondria’s ability to provide energy** to the insects.

(IV) Insecticides that Act via Desiccation
Chemical Class: Dehydrating dust

- Example: Silica gels and diatomaceous earth.
- Absorb the thin wax layer on insect exoskeleton that normally prevent insects from losing water through desiccation.
- By absorbing the wax layer, they increase the permeability of the exoskeleton, resulting the insect death by dehydration.

(V) Insecticides Acting as Alimentary Toxin
Chemical Class: *Bacillus thuringiensis*

- Bti H-14 spores and parasporal crystals must be ingested by the mosquito larvae to cause mortality.
- Following ingestion, the parasporal crystals are solubilised in the alkaline larval midgut (pH 9.0 - 10.5) and endotoxins are released.
- These toxins then tightly bind to the insects gut cells resulting the formation of large pores that increase water permeability of the cell membrane causing large uptakes of water, swelling and eventual rupture of gut cells.
- This destroys the insect’s gut lining preventing feeding.



Do you know ?
Human gut pH is acidic (pH < 6) and thus will not be affected by Bti.

- ; this will follow by proteolytic activation

INSECTICIDE RESISTANCE (1)

- **Defined as an ability of an insect population to withstand an insecticide concentration that would normally kill a normal population.**
- **Inherited, not acquired after-born.**
- **Can occur when insect population is subjected to insecticide selection pressure for a long time, or at frequent basis.**

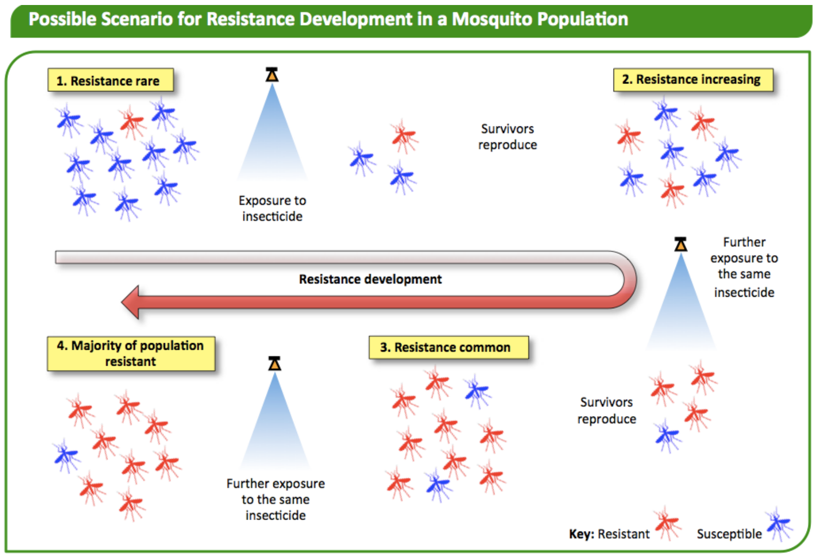
Immunity

VS

Resistance

- Acquired after born.
- Limited to that individual.
- No involvement of gene(s)

- Inherited
- Could be passed on.
- Involves resistance gene(s)



Source: http://www.irac-online.org/documents/irm-mini-vector-booklet/?ext=pdf

INSECTICIDE RESISTANCE (2)

- Insecticide candidates within the same class can become useless, eventhough they have never been used (CROSS RESISTANCE).
- Can also cross-resistant between classes of insecticides with similar mode of action.
- Example:

  A population of flies is resistant to propoxur. They will also become resistant to bendiocarb, and may also resistant to chlorpyrifos.

Mechanisms of insecticide resistance in urban insect pests

Metabolic resistance
- Elevated esterases
- Enhanced P450 monoxygenases
- Glutathione S-transferase

Reduced penetration

Target site resistance
- Altered acetylcholinesterase
- *kdr*-type resistance
- *rdl*-mutation

Behavioural resistance
- Bait aversion
- Glucose aversion

An insect population may possess more than one resistance mechanism -- multiple resistance

The mechanism may confer resistance to more than one class of insecticide -- cross resistance

Insecticide classes and resistance mechanisms in the German cockroach

Classes	Metabolic (Esterase)	Metabolic (P450 Monooxy	Metabolic (GST)	Reduced penetration	Target site (altered AChE)	Target site (*kdr*-type)	Target site (*rdl*-mutation)	Behavioral resistance
Pyrethroids	x	x		x		x		
DDT			x	x		x		
Organophos-phates	x	x	x	x	x			
Carbamates	x	x		x	x			
Cyclodienes/ fipronil		x (fipronil only)		x			x	x
Neonicoti-noids		x						x
Oxidiazines		x						x

Glucose aversion and bait aversion

- Early 90s: Some strains of German cockroaches in the USA avoiding cockroach baits due to glucose aversion.
- In another words, cockroaches avoid to feed on bait because they are aversed to glucose, which is an important attractant in bait.
- The problem was solved by changing the sugar attractant in bait to other sugar compounds.
- However, more recently (2004/05), some strains of German cockroaches are avoiding many major sugar attractants such as glucose, fructose, sucrose and maltose.
- So far, the incidences of bait aversion are only limited to the USA, and South Korea.

Glucose aversion in the German cockroach

Source: Wada-Katsumata et al. (North Carolina State University)

Resistance management strategies

- Rotation
- Mixture
- Non-chemical IPM

Rotation

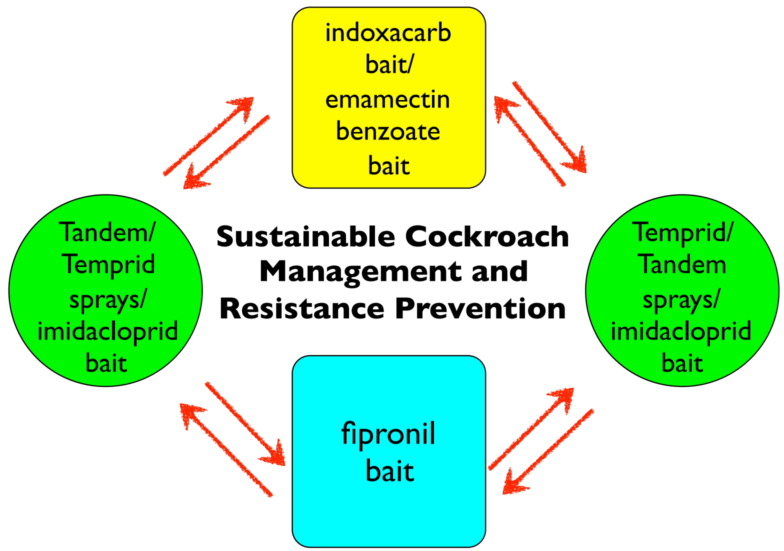
- Strategy based on the **rotation over time of two or more insecticide classes** with different mode of actions.
- This approach assumes that if resistance to each insecticide is rare, then multiple resistance will be even rarer.
- Also, once a population has become resistant to a particular insecticide class, the insects will be overcome by another insecticide class of different mode of action.

Mixture

- A **single formulation containing 2 or more insecticides of different mode of actions**, or different insecticide formulations being applied in the same spray tank.
- This approach assumes that if the resistant population survives one insecticidal mode of action, it will be killed by the other.
- If resistance to each insecticide is rare, then multiple resistance will be even rarer.
- **Tandem** (lambda-cyhalothrin + thiamethoxam) and **Temprid** (betacyfluthrin and imidacloprid) are examples of mixture formulations.
- Mixture could also be a integration between an insecticide and an IGR, or a **synergist** (enzyme inhibitor, eg. PBO).

Sustainable Cockroach Management and Resistance Prevention

- Should incorporate **both rotation and mixture** strategies.
- The same product should not be used for more than 3 months to prevent insects becoming resistant to the active ingredients.
- Could involve (1) rotation between bait formulations of different active ingredients, or (2) rotation between mixture formulation and bait.



Resistance management for *Aedes* mosquitoes

- **Pyrethroid resistance in *Aedes*** — use of pyrethroids should be temporary halted.
- Switch to **<u>organophosphorus insecticide</u>** for fogging (eg. pirimiphos-methyl, temephos).
- Larviciding with either <u>IGR (eg. pyriproxyfen) or *Bti.*</u>
- Use non-chemical IPM approaches including source reduction and MMF (monomolecular surface film).

Properties of insecticides (1)

- Before examining the main types of insecticides in common used, it is important to know the various properties of insecticides that can influence the end use, and the effectiveness of the product.
- ***Toxicity*** - its capacity to poison. For control of certain pests, extensive tests are carried out on new insecticides to determine their toxicity to those pests.
- ***Hazard*** - This is the risk of harm that may result from handling and use of pesticides. A highly toxic pesticide may be used in a relatively low hazard manner (eg. soil injection), whereas a less toxic pesticide may be used in a high hazard manner (eg. thermal fogging).
- ***Spectrum of activity*** - refers to range of pests against which the product is active. Insecticide that kill many types of insects are said to be broad spectrum, while those that are more specific in nature are narrow spectrum.

Properties of insecticides (2)

- ***Mammalian toxicity*** - determined by testing with test animals (usually rats). The relative measures of mammalian toxicity of an insecticide are given as: oral LD50 (mg/kg that is in mg of poison per kg of body weight of the rat). Dermal LD50 (mg/kg), and inhalation LC50 (usually in mg/m^3 for 4 hr).
- ***Persistence*** - biological activity of most insecticides begins to decline after application. When the lost of activity is slow, the insecticide is said to be persistent, stable or residual. By chemical nature, insecticides may have stabilities ranging from hours to tens of years. Environmental factors can shorten the residual life of an insecticide, as well as presence of sunlight, moisture and heat.
- ***Volatility*** - refers to the ease with which it converts into a gaseous state. Volatile insecticides (eg. dichlorvos) can act as inhalation poisons.
- ***Repellency*** - the ability to keep insects away from a treated area.

Properties of insecticides (3)

- ***Flushing action*** - the tendency to excite the insects from a treated area, causing them to leave their harbourages. Flushing agents are sometimes mixed with surface spray chemicals to encourage contact with treated surfaces.
- ***Knockdown action*** - refers to the rapid incapacitation of insects by a quick-acting insecticide. Pyrethrins and certain synthetic pyrethroids are regarded as knockdown agents.
- ***Compatibility*** - refers to whether or not two chemicals can be mixed without undesirable outcome. Pesticides should only be mixed when label directions indicate the compatibility of one product with another.
- ***Phytotoxicity*** - refers to the degree to which it is poisonous to plant life.
- ***Withholding period*** - period of time which must elapse between the treatment of a crop with a pesticide, and the harvesting of the crop.

Insecticide Formulations and Applications

- Insecticides are manufactured as relatively pure materials (90 - 99%) and this reasonable pure form is also known as 'technical material'.
- It may be waxy solid, in crystalline powder, or a viscous oily liquid.
- Because of high toxicity, cost, problems with dilutions and other factors, technical material is seldom useful to end user in its pure form.
- Usually, the pure insecticide is processed into a form that will facilitate its safe and efficient application.
- Different formulations have different characteristics, which are important in their end use.
- The type of formulation chosen for a given pest problem can play an important role in the effectiveness of the insecticide.

Components in formulations

- ***Active ingredient*** - the biologically active component. Usually, the insecticide labels indicate it by giving the common chemical name and its proportion of the net contents.
- ***Diluent*** - any component that is added to reduce the concentration of the insecticide. Eg. water, oil, talc, clay, etc.
- ***Solvent*** - a liquid in which a chemical may be dissolved. Some chemicals dissolve readily in water, while others require special solvents, such as aromatic hydrocarbons (eg. toulene xylene). Solvents in liquid formulation usually evaporate after application, leaving an insecticide residue.

Components in formulations (2)

- ***Surfactants*** - also known as surface active agents. These are chemicals introduced into a liquid to affect its wetting, spreading and dispersibility or emulsifiability properties. In general, they are detergent-like substance. Two types of surfactant:
  - ❑ ***Emulsifiers*** - facilitate the mixing of oil-based liquids in water. Normally oil and water cannot mix. With addition of emulsifiers, oil (plus insecticide) will form tiny globules that are evenly dispersed throughout the water (often giving the mix a milky appearance).
  - ❑ ***Wetting agents*** - facilitate the mixing of solid insecticide particles in water. Normally, a dry powder will not mix evenly in water. When a wetting agent is added, its effect will allow powder to disperse evenly in the water.
- ***Synergist*** - a substance that do not have insecticidal property, but when formulated together with an insecticide, it will increase the potency of the insecticide. Example: piperonyl butoxide.

Types of formulations

- **Oil concentrates.**
- **Emulsifiable concentrates (EC).**
- **Wettable powders (WP).**
- **Suspension concentrates (SC).**
- **Micro-encapsulated concentrate (ME).**
- **Dusts.**
- **Aerosols.**
- **Baits.**
- **Granules.**
- **ULV**
- **Coil.**
- **Liquid vaporiser.**
- **Mat**

Oil concentrates

- This is the simplest liquid formulation.
- It is not as widely used now as they were 20 years ago.
- Oil concentrates comprise the insecticide itself, dissolved in a strong solvent.
- It can be further diluted by the end user with an oil-based solvent/diluent (eg. kerosene, diesel oil). Water cannot be used.
- One good example of oil concentrate is the premium grade malathion used in dengue operation.

Emulsifiable concentrates (EC)

- Very commonly used in urban pest control practices.
- Most insecticides dissolve in strong oil-based solvents (eg. toulene), but this mixture is not mixable with water. To facilitate the mixing, an emulsifier is added.
- Insecticide+solvent+emulsifier = EC.
- When the product is diluted with water, this results in an 'oil in water' emulsion, in which globules of insecticide and solvents are evenly dispersed in the water.
- Advantages of EC:
  - ❑ Cheap and non-flammable.
  - ❑ Spray is often less visible on surfaces than other formulations.
- Disadvantages of EC:
  - ❑ Insecticide emulsion when sprayed onto porous surfaces will be absorbed.
  - ❑ Maybe phytotoxic.

Wettable powder (WP)

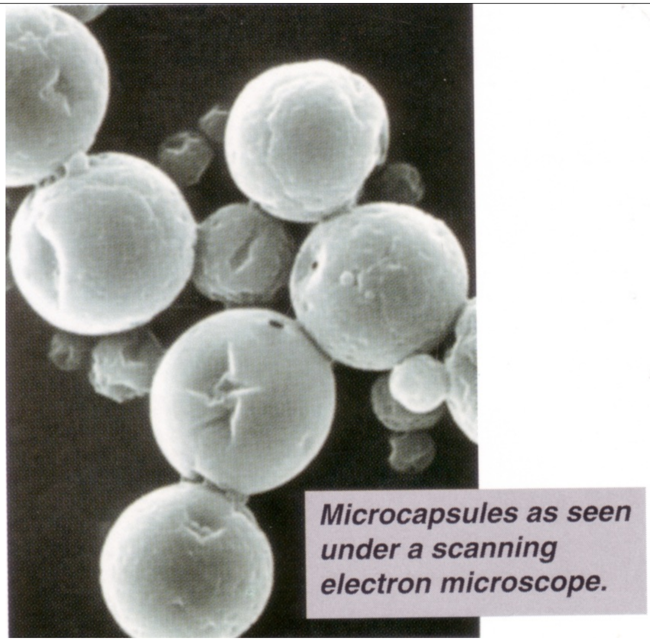
- Also known as water-dispersable powder.
- Suited for insecticide whose technical form is insoluble in water and other solvents.
- They contain finely divided insecticide mixed with a suitable solid diluent (eg. talc, diatomaceous earth) and wetting agents (detergent-like substance that enable the powder to mix with water to form a suspension).
- Insecticide+diluent (eg. talc)+wetting agent = WP.
- WP mixtures are relatively unstable, the solid particles after time will settle to the bottom of the tank. Frequent agitation is necessary.
- Advantages:
  - ❑ Can be diluted in water, cheap, non-flammable.
  - ❑ Effective on porous surface.
  - ❑ Unlikely to be phytotoxic.
- Disadvantages:
  - ❑ Leaves a visible deposit on surfaces.

Suspension concentrates (SC)

- Insecticide+diluent+wetting agent = SC.
- It is almost similar to WP, but WP is a dry formulation, while SCs are supplied as a liquid concentrate.
- SC tends to have a finer diluent, contains deionised water, may contain a bactericide and may also have anti-freeze agent.
- It is important to agitate the liquid concentrate before use, and agitate the spray mixture frequently duirng use to ensure the material does not settle down.

Microencapsulated concentrate (ME)

- This product incorporates an insecticide in a permeable covering.
- The insecticide is released from the capsules over an extended period of time, or when the covering is damaged (during grooming, or pick-up by insects).
- Advantages:
  - ❑ Long residual activity.
  - ❑ Good pick-up by insects and may be ingested during grooming.
  - ❑ No smell.
  - ❑ Not phytotoxic.
- Disadvantages:
  - ❑ Require regular agitation during use.
  - ❑ Expensive.
  - ❑ May leave a stain on some surfaces.



Microcapsules as seen under a scanning electron microscope.

Dusts

- Dry mixture of finely ground insecticide with a diluent (eg. fine talc or clay particles).
- Mainly used in the control of household pests and usually applied to relatively inaccessible areas (eg. cracks, crevices, wall voids, etc).
- Advantages:
  - ❑ Useful in areas where wet sprays are not permissible (eg. electric switchboxes, motors, ceiling voids).
  - ❑ Not absorbed by porous surfaces and remain on surfaces to be picked up by crawling insects.
- Disadvantages
  - ❑ Hazardous, conspicuous, and unsightly deposits if not applied properly.
  - ❑ Deposits are not fixed and can easily be blown away or removed physically.
  - ❑ Should not be used above ground level, if there is a chance of them falling to contaminate food, utensils, etc.

Aerosols

- Self-contained system that, with the operation of the nozzle or gun, emit a space-spray present as droplets in the air, which contain the active insecticide.
- The formulation contains active ingredient (which may contain a synergist), an oil solvent and the propellant, which is usually a hydrocarbon type.
- Aerosol formulations were initially used only for the control of flying insect, but they can now be used against crawling insects.
- This is achieved by producing heavier droplet size for the purpose of residual surface treatment.

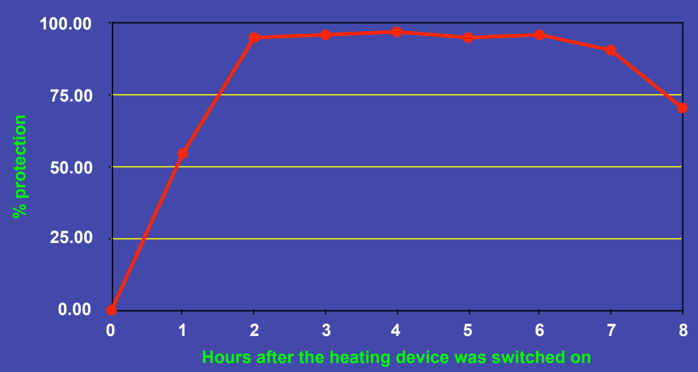
Mosquito Coil

- Easy to use.
- Insecticide+wood powder +malachite green+ other additives.
- Continuous protection for 8 hours with consistent performance.

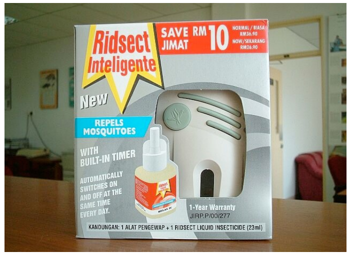
Mosquito Mat

- **Clean (smoke-less).**
- **One piece of mat lasts about 8 hours.**
- **The heater should be switched on 2 hours earlier to ensure optimal temperature is achieved at the time of sleep (140 – 160^{o}C).**
- **Inconsistent performance.**

Protection against Culex mosquitoes by mosquito mat



Electric Liquid



- Clean.
- A bottle of liquid solution lasts 30, 60 or even up to 90 days.
- The heater should be switched on 2 hours before time of sleep.
- More expensive.

Repellent

- For protection against blood-sucking or biting insects.
- Used outdoor.
- Insecticide/repellent+cream + moisturizer.
- Example of active ingredient: DEET.
- Can also be found in the form of soap bar.

Insecticidal lacquer

- Insecticide (1 - 4%) is incorporated in a resin formulation so that when it is painted onto surfaces, crystals of insecticide continue to remain on the surface for months to even years.
- The material is painted or sprayed onto surfaces and may be active for several months.

Smoke generators

- The insecticide is formulated with a pyrotechnic materials (eg. sodium chlorate) and a burnable fuel (eg. sugar).
- When the mixture is ignited, the insecticide is vapourised.
- Smoke generators are suitable only for enclosed spaces.

Baits

- Insecticide is mixed with an attractive food in acceptable proportions.
- They rely the insects in finding the bait, or there will be competing alternative food sources and they can be time consuming.
- However, they are extremely useful in sensitive environments.
- In addition to cockroach, ant, fly baits, termite bait is also available.

Gel bait application.

Granules

- Insecticide is sprayed onto clay granules and then distributed equally.
- Granules are used mostly in crop pest control and are useful in the control of soil-dwelling insect pests.

ULV (ultra low volume) formulations

- Insecticides that are oily liquids in their pure form may be supplied in an oil solvent for ULV application.
- ULV-dispersing equipment breaks up the insecticides onto such tiny particles that only small amounts of concentrated insecticide are needed.

Impregnated resin

- Volatile insecticides, such as dichlorvos (OP) may be impregnated into resin strips that when kept in an enclosed environment will emit vapours that will kill insects by inhalation poisoning.

Choice of formulations

- The choice of formulation is very much influenced by a number of factors:
  - ❑Pest to be controlled.
  - ❑Convenience of use.
  - ❑Application equipment available.
  - ❑Nature of surfaces to be treated.
  - ❑Particular conditions surrounding the treatment.
  - ❑Price

Pest to be controlled

- Many different products and formulations are registered for various uses, and the operator must ensure that the product selected is appropriate for the purpose.

Convenience of use

- Some operator prefer to use the premeasuured sachet that some wettable powders are packed in, or even water soluble bags, instead of measuring out amounts of liquid emulsifiable concentrate from a drum or bottle.

Application equipment available

- An operator who does not have a dust dispenser may mist a roof cavity space, while an operator who has a powderful dust applicator may avoid the inherent risk of misting in the roof space, and instead apply a uniform, light film of dust throughout.

Nature of surfaces to be treated

- When surface sprays are to be applied for residual control of crawling insects, the type of surface is an important consideration.
- EC application may allow the insecticide to be absorbed by porous surface.
- WP, SC and ME, on the other hand, deposit the insecticide on the surface, but will leave a stain residue.

Particular conditions surrounding the treatment (1)

- Where insect harbourages are in close association with electrical switches, wiring, applicance, wet sprays must be excluded for reasons of safety.
- Where animals are bred or kept (eg. pet shops, aquarium), sprays and dust may need to be excluded. Baits and traps may be acceptable.
- Where areas to be treated are constantly occupied by humans, space-spray systems are excluded. The most viable alternative may be a low-volatility, low mammalian toxicity, wettable powder that is considered safe.

Particular conditions surrounding the treatment (2)

- Where the pests are contained and protected (as is the case of timber borers in an article of furniture), the best solution would be to fumigate the article. The fumigant gas penetrates the timber to ensure killing of the pests.
- Where insect pests spend much of their time hidden in cracks and crevices (eg. cockroaches), there may be a choice of treatments:
  - ❑ A non-residual flushing chemical.
  - ❑ A residual WP insecticide.
  - ❑ A residual EC insecticide.
  - ❑ A residual dust insecticide.

Price

- The cost of formulations of insecticide varies, depending on relative costs of manufacture.
- Operators choosing between products on the basis of cost should ensure that they base calculations on final “out of the nozzle” costs, rather than, for example, cost per sachet or liter of concentrate. Labour costs for application also warrant consideration.

Principle of Insecticide Testing, and Probit Analysis

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Introduction

- Test insects may exhibit either a GRADED OR A QUANTAL response.
- Graded response – when test insects show a range of measurable reactions (eg. weight loss, irritability, etc).
- Quantal response is when organism shows an all-or-none type of reaction (eg. dead or alive).
- Insect – insecticide interaction is dose-related.
- The higher the concentration, more insects will be killed.

What are the other examples of quantal response?

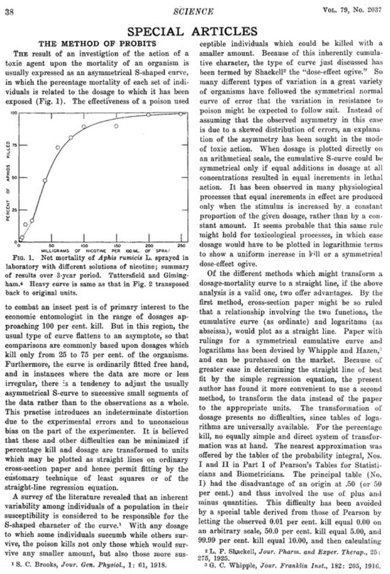
Flipping a coin — head or tail
Testing a beauty product — rash or no rash
Success or failure
Heart attack or no heart attack

Introduction (2)

- A quantal response can usually be employed because the percentage insect responding to a given insecticide will increase with increasing dose/ concentration.

Principle of probit analysis

- A statistical procedure commonly used in analysis of bioassays (eg. testing of an insecticide with a dose range which results in a sequential mortality increase of insect pests).
- Insects respond to insecticide action according to normal distribution.



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SPECIAL ARTICLES

THE METHOD OF PROBITS

The result of an investigtion of the action of a toxic agent upon the mortality of an organism is usually expressed as an asymmetrical S-shaped curve, in which the percentage mortality of each set of individuals is related to the dosage to which it has been exposed (Fig. 1). The effectiveness of a poison used

Fig. 1. Net mortality of *Aphis rumicis* L. sprayed in laboratory with different solutions of nicotine; summary of results over 3-year period. Tattersfield and Gimingham.[4] Heavy curve is same as that in Fig. 2 transposed back to original units.

to combat an insect pest is of primary interest to the economic entomologist in the range of dosages approaching 100 per cent. kill. But in this region, the usual type of curve flattens to an asymptote, so that comparisons are commonly based upon dosages which kill only from 25 to 75 per cent. of the organisms. Furthermore, the curve is ordinarily fitted free hand, and in instances where the data are more or less irregular, there is a tendency to adjust the usually asymmetrical S-curve to successive small segments of the data rather than to the observations as a whole. This practise introduces an indeterminate distortion due to the experimental errors and to unconscious bias on the part of the experimenter. It is believed that these and other difficulties can be minimized if percentage kill and dosage are transformed to units which may be plotted as straight lines on ordinary cross-section paper and hence permit fitting by the customary technique of least squares or of the straight-line regression equation.

A survey of the literature revealed that an inherent variability among individuals of a population in their susceptibility is considered to be responsible for the S-shaped character of the curve.[1] With any dosage to which some individuals succumb while others survive, the poison kills not only those which would survive any smaller amount, but also those more susceptible individuals which could be killed with a smaller amount. Because of this inherently cumulative character, the type of curve just discussed has been termed by Shackell[2] the "dose-effect ogive." So many different types of variation in a great variety of organisms have followed the symmetrical normal curve of error that the variation in resistance to poison might be expected to follow suit. Instead of assuming that the observed asymmetry in this case is due to a skewed distribution of errors, an explanation of the asymmetry has been sought in the mode of toxic action. When dosage is plotted directly on an arithmetical scale, the cumulative S-curve could be symmetrical only if equal additions in dosage at all concentrations resulted in equal increments in lethal action. It has been observed in many physiological processes that equal increments in effect are produced only when the stimulus is increased by a constant proportion of the given dosage, rather than by a constant amount. It seems probable that this same rule might hold for toxicological processes, in which case dosage would have to be plotted in logarithmic terms to show a uniform increase in kill or a symmetrical dose-effect ogive.

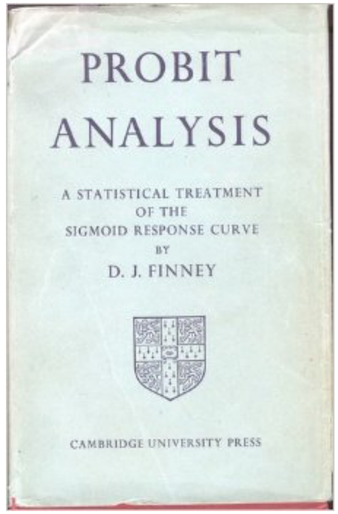
Of the different methods which might transform a dosage-mortality curve to a straight line, if the above analysis is a valid one, two offer advantages. By the first method, cross-section paper might be so ruled that a relationship involving the two functions, the cumulative curve (as ordinate) and logarithms (as abscissa), would plot as a straight line. Paper with rulings for a symmetrical cumulative curve and logarithms has been devised by Whipple and Hazen,[3] and can be purchased on the market. Because of greater ease in determining the straight line of best fit by the simple regression equation, the present author has found it more convenient to use a second method, to transform the data instead of the paper to the appropriate units. The transformation of dosage presents no difficulties, since tables of logarithms are universally available. For the percentage kill, no equally simple and direct system of transformation was at hand. The nearest approximation was offered by the tables of the probability integral, Nos. I and II in Part I of Pearson's Tables for Statisticians and Biometricians. The principal table (No. I) had the disadvantage of an origin at .50 (or 50 per cent.) and thus involved the use of plus and minus quantities. This difficulty has been avoided by a special table derived from those of Pearson by letting the observed 0.01 per cent. kill equal 0.00 on an arbitrary scale, 50.0 per cent. kill equal 5.00, and 99.99 per cent. kill equal 10.00, and then calculating

[1] S. C. Brooks, *Jour. Gen. Physiol.*, 1: 61, 1918.

[2] L. F. Shackell, *Jour. Pharm. and Exper. Therap.*, 25: 275, 1925.

[3] G. C. Whipple, *Jour. Franklin Inst.*, 182: 205, 1916.

- The idea of probit analysis was originally published in Science by Chester Bliss, an entomologist with Connecticut Agricultural Experiment Station in 1934, to find an effective pesticide to control insects that fed on grape leaves.
- By plotting the response of the insects to various concentrations of pesticides, he could visually see that each pesticide affected the insects at different concentrations, i.e. one was more effective than the other.
- However, there was no statistically sound method to compare this difference. The most logical approach would be to fit a regression of the response versus the concentration, or dose and compare between the different pesticides.
- Yet, the relationship of response to dose was sigmoid and at the time, regression was only used on linear data.
- Therefore, Bliss developed the idea of transforming the sigmoid dose-response curve to a straight line.
- In 1952, David Finney, a professor of statistics at the University of Edinburgh took Bliss' idea and wrote a book called Probit Analysis.



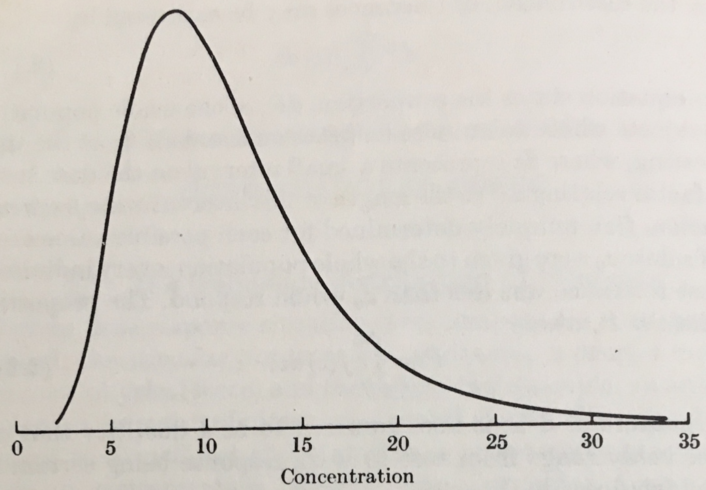


Fig. 2.1. Typical frequency distribution for the tolerance concentrations of a population. (The area between any two ordinates represents the proportion of subjects having tolerances between these two limits.)

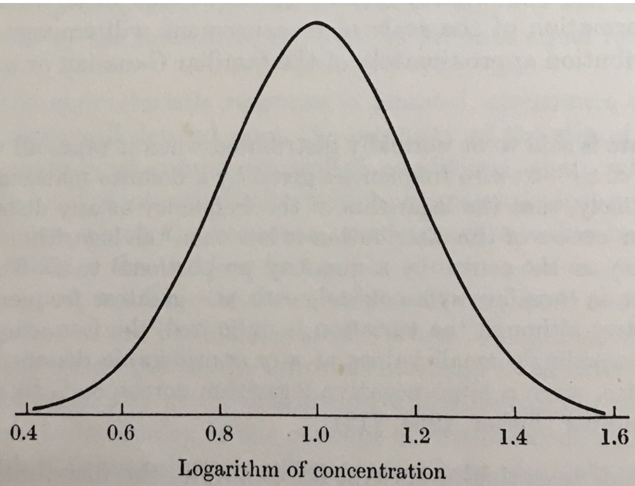


Fig. 2.2. Normal frequency distribution for the logarithms of the tolerance concentrations in Fig. 1.

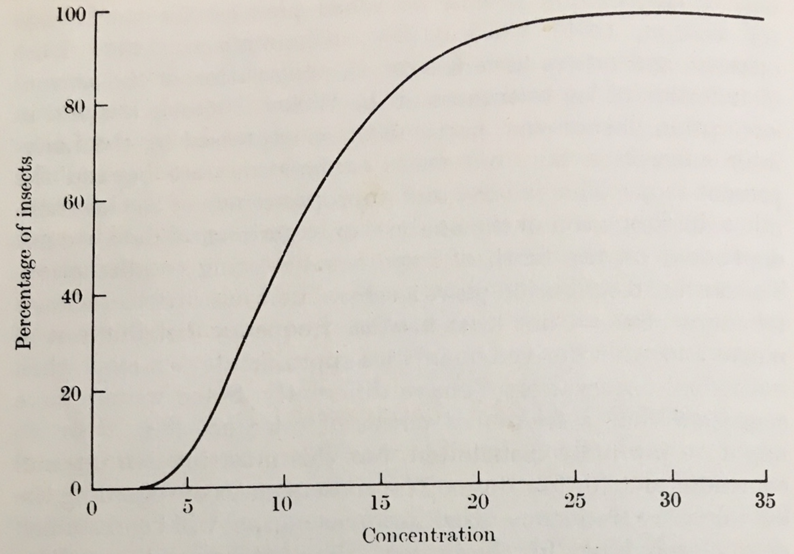


Fig. 2.3. Sigmoid curve derived from Fig. 1, showing percentage of subjects with tolerances less than a specified value.

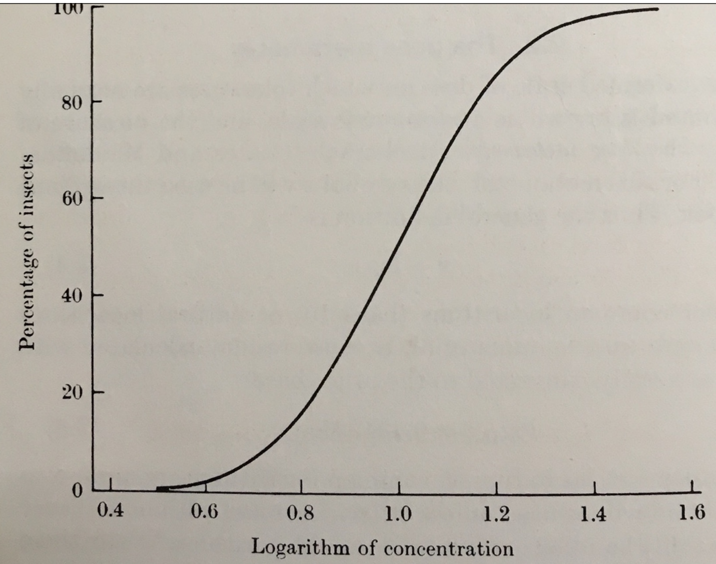


Fig. 2.4. Normal sigmoid curve derived from Fig. 2, showing percentage of subjects with log tolerances less than a specified value.

Transformation from percentage to probit

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

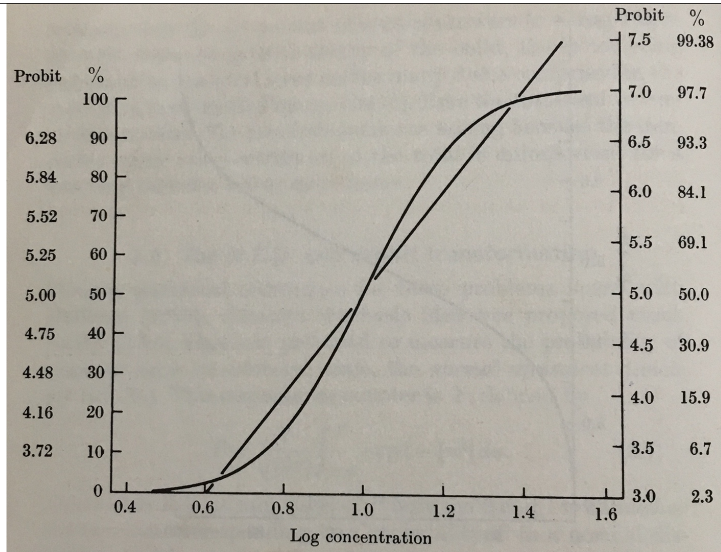
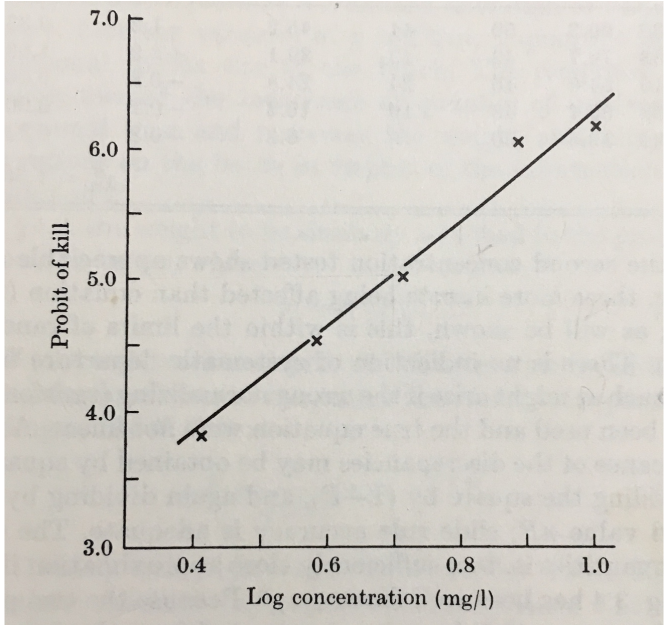


Fig. 3.2. Effect of the probit transformation. The normal sigmoid curve in Fig. 2.4 is transformed to a straight line when the ordinates are measured on a scale linear in probits instead of in percentages.



Transformation to probit (probability unit)

- Transformation could be done using a mathematical model.
- For those who are interested in the complicated equation, please contact me.

Probit Analysis Program

- There are many programs available to everyone. Some can be downloaded from the internet.
- A few common ones:

  Polo-Plus

  Quantal

  SPSS

  SAS

From the computer-generated results, you can get:

- Test for heterogeneity – an indicator to the scatterness and sufficiency of data points.
- Test for paralellism – comparison of the slopes of multiple regression lines.
- Slope of graph – in absence of parallelism test, values of slope can be used to confirm whether relative efficacy can be determined.
- Dose response – WHAT YOU WANT ! ($LT_{50,}$ 95% fiducial limit, etc).

Interpreting toxicity results

Bait	n	LT_{50} (95% FL) (days)	LT_{95} (95% FL) (days)	Slope ± SE	χ^2	df
A	100	1.9 (1.7 – 2.1)	6.5 (6.0 – 6.9)	3.4 ± 0.1	3.45	5
B	100	2.3 (2.0 – 2.6)	5.4 (4.7 – 6.1)	2.2 ± 0.5	4.32	5
C	120	4.5 (4.2 – 4.8)	8.9 (8.5 – 9.3)	3.1 ± 0.2	4.67	5

n = number of test insects used in the evaluation.
LT = lethal time (time needed to achieve 50 or 95% mortality).
95% FL = 95% fiducial limit = the interval where the lethal time most likely fall within.
Slope = slope of the probit line.
Comparison is usually at 50% response level because this will reflect the concentration where the majority of the insects respond to the insecticide.

Relative performance

Bait	n	LT_{50} (95% FL) (days)	LT_{95} (95% FL) (days)	Slope ± SE	χ^2	df
A	100	1.9 (1.7 – 2.1)	6.5 (6.0 – 6.9)	3.4 ± 0.1	3.45	5
B	100	2.3 (2.0 – 2.6)	5.4 (4.7 – 6.1)	2.2 ± 0.5	4.32	5
C	120	4.5 (4.2 – 4.8)	8.9 (8.5 – 9.3)	3.1 ± 0.2	4.67	5

Bait A = B > C
A = B: Their 95% FLs overlapped.
A, B > C: Their 95% FLs do not overlap.

Abbot's Formula

- When untreated group die from natural or some unknown causes, it is important to correct this discrepancy.
- If the mortality rate is more than 10%, experiment will have to be repeated.
- If mortality rate is less than 20%, it is important to perform a correction to realign the results. We use Abbot's Formula:

$$P_c = 100\ [(P_t - P_u)/(100 - P_u)]$$

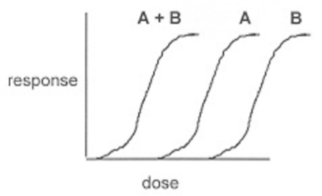
where, P_c = corrected % mortality; P_t= observed % mortality in treatment group; P_u = untreated % mortality in control group.

Relative efficacy

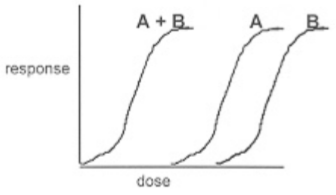
- If comparison between two products (A and B) were to be made, it can be done by dividing the LT_{50} of the product B with that of product A.
- However, it has to fulfill the following:
- both probit lines should be parellel.
- both population tested are homogenous population.
- Why ?

Interaction of chemicals

- Additive effects
- Synergistic effects
- Antagonistic effects

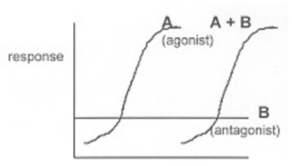


The combined effect of 2 chemicals is predictable based the known toxicity of both compounds. No specific interactions occur….
1 + 1 = 2



The combined effect of 2 chemicals may be greater than the sum of the 2 effects — this is known as synergism. The synergist piperonyl butoxide is added to some insecticides to greatly enhance their toxicity to insects.
1 + 1 = 10

c. **Antagonism....**



The toxic effect of a chemical, A can be reduced when given another chemical B.

Discussion in class

- chi-square goodness of fit test.

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

where:

O_i = an observed frequency (i.e. count) for bin i

E_i = an expected (theoretical) frequency for bin i, asserted by the null hypothesis.

- Insecticide resistance - homogeneity and heterogeneity (based on normal curve to linear probit line).
- Discriminating dose/concentration.
- Hardy-Weinburg and gene frequency ($S + R = 1$, $S^2 + 2RS + R^2 = 1$).

Percentage Points of the Chi-Square Distribution

Degrees of Freedom	Probability of a larger value of x^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72
12	3.571	5.226	6.304	8.438	11.340	14.85	18.55	21.03	26.22
13	4.107	5.892	7.042	9.299	12.340	15.98	19.81	22.36	27.69
14	4.660	6.571	7.790	10.165	13.339	17.12	21.06	23.68	29.14
15	5.229	7.261	8.547	11.037	14.339	18.25	22.31	25.00	30.58
16	5.812	7.962	9.312	11.912	15.338	19.37	23.54	26.30	32.00
17	6.408	8.672	10.085	12.792	16.338	20.49	24.77	27.59	33.41
18	7.015	9.390	10.865	13.675	17.338	21.60	25.99	28.87	34.80
19	7.633	10.117	11.651	14.562	18.338	22.72	27.20	30.14	36.19
20	8.260	10.851	12.443	15.452	19.337	23.83	28.41	31.41	37.57
22	9.542	12.338	14.041	17.240	21.337	26.04	30.81	33.92	40.29
24	10.856	13.848	15.659	19.037	23.337	28.24	33.20	36.42	42.98
26	12.198	15.379	17.292	20.843	25.336	30.43	35.56	38.89	45.64
28	13.565	16.928	18.939	22.657	27.336	32.62	37.92	41.34	48.28
30	14.953	18.493	20.599	24.478	29.336	34.80	40.26	43.77	50.89
40	22.164	26.509	29.051	33.660	39.335	45.62	51.80	55.76	63.69
50	27.707	34.764	37.689	42.942	49.335	56.33	63.17	67.50	76.15
60	37.485	43.188	46.459	52.294	59.335	66.98	74.40	79.08	88.38

Common pitfalls in bioefficacy test

Assuming experimental procedures are followed correctly, discrepancy of test results can still happen if the following factors are not considered:

- Homogeneity of test insects
  - age (1 – 2 weeks old: cockroaches).
  - virgin or non-virgin females?
  - strain (susceptible).
  - food (standardized medium or food).
  - rearing density.
- Accuracy in recording
  - standardized criteria for knock-down of insects.
  - discrepancy in recording.

LT_{50} or LT_{95}

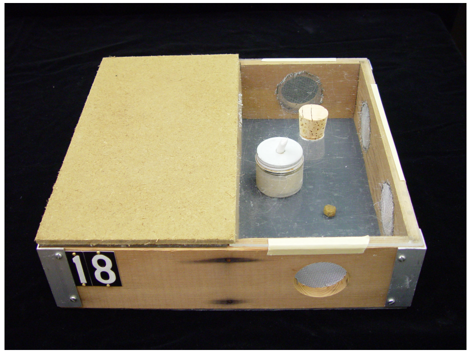
- LT_{50}
  – time when 50% test insects were knocked down.
  - used for comparison between products.
  - generally more acceptable because it is the point where majority of test insects would respond to product treatment.
- LT_{95}
  - time when 95% test insects were knocked down.
  - rarely used.

Lab or field test?

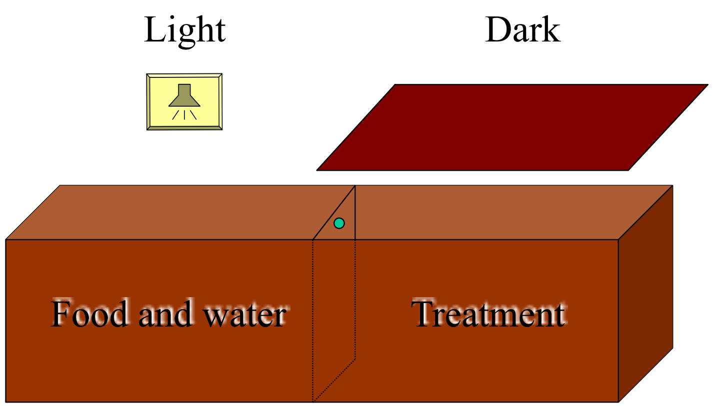
Lab
- Only tells basic (& relative) product performance (eg. LT/KT values, 24-h mortality, repellency).
- Cheaper and quick.
- Test insect conditions are standardized.
- Susceptible (or resistant) insects.

Field
- ‘Real world’ (provide results in % reduction of insects)
- Expensive and time consuming to do.
- Conditions are not standardized.
- Mixed of susceptible and resistant test insects.

Ebeling choice box - Predicting field efficacy in the laboratory for cockroach products



Ebeling choice box - Predicting field efficacy in the laboratory for cockroach products



$$PI = 1 - \frac{\underline{\text{no. alive} + \text{no. alive in light side}}}{\text{no. dead} + \text{total no. insects}} \times 100$$

When PI is:
100 = complete mortality, no repellency.
- 100 = no mortality, complete repellency.
0 = control set, no mortality and no repellency.

The PIs should be taken on daily basis and plot over time

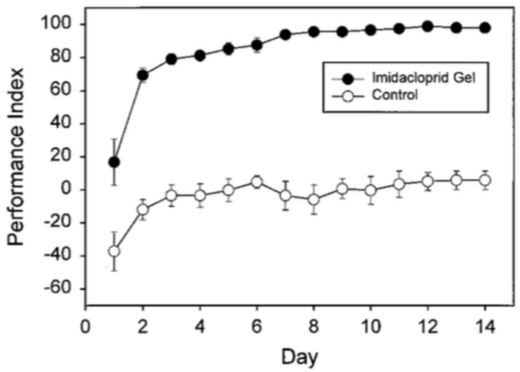


Fig. 3. Performance index relationships for 2.15% imidacloprid bait determined in Ebeling choice boxes and for untreated control boxes. Points represent means of 6 replicate boxes, each containing 20 adult male German cockroaches.

Source: Appel & Tanley (2000)