Syllabus:

1) Pesticide classification on use, chemical nature, formulation, toxicity and action etc.
2) Various methods in Pesticide Formulation Analysis
3) Principles, operation and application of various chromatographic techniques
4) Pesticide Dissipation, Residue Dynamics, Different methods/ Steps in residue analysis
5) Confirmative analytical techniques in residue analysis
6) Different terminologies used in Pesticide Residue Analysis
7) Maximum Residue Levels in pesticide
8) Pesticide Management

1. Pesticide classification on Use, Chemical nature, Formulation, Toxicity and Mode of Action etc.

Pesticide: Pesticide is a substance which kills a pest.

Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other microorganisms on or in living man or other animals, which the administrator declares to be pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant.

As per the Insecticides Act 1968, any substance which is in the schedule, **or** Such other substances as the central government may, after consultation with the Board, by notification in the official gazette, include in the schedule from time to time **or** Any preparation containing any one or more of such substances is a pesticide.

The Pesticides can be classified in many ways on the basis of use, toxicity, mode of entry, mode of action, chemistry and formulations.

The classification based on the basis of use can be as follows:

Acaricides, Algicide, Antifeedants, Avicides, Bactericides, Bird repellents, Chemosterillant, Fungicides ,Herbicide softeners, Herbicides, Insect attractants, Insect repellents, Insecticides, Mammal repellents, Mating disrupters, Molluscicides, Nematicides, Plant activators, Plant growth regulators, Rodenticides, Synergists, Virucides and Miscellaneous

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Acaricides: are the substances that are used to kill mites and ticks, or to disrupt their growth or development. and some of the examples are DDT, dicofol, , carbofuran, methiocarb, Propoxur, abamectin, milbemectin, flufenoxuron, , chlorpyrifos, oxydemeton methyl, Phorate, Phosalone, fenpyroximate, Fipronil, bifenthrin, cyhalothrin, fluvalinate , permethrin, chlorfenapyr

Algicide: are the substances that are used to kill or inhibit algae. Some of the examples are copper sulfate, diuron, isoproturon, isoproturon, oxyfluorfen, simazine

Antifeedants: are the chemicals which prevent an insect or other pest from feeding. Some of the examples are chlordimeforn, fentin and azadirachtin.

Avicides: are the chemicals that are used to kill birds. The list include fenthion, strychnine.

Bactericides: are the compounds that are isolated from or produced by a microorganism (e.g. a bacterium or a fungus), or a related chemical that is produced artificially. which are used to kill or inhibit bacteria in plants or soil. Some of the examples are copper hydroxide, kasugamycin, streptomycin, tetracycline .

Bird repellents: are the chemicals which act as the bird repellants and some of the examples are copper oxychloride, diazinon, methiocarb, thiram, ziram

Chemosterillant: are the chemicals that renders an insect infertile and thus prevents it from reproducing. Some insects that mate only once can be controlled or eradicated by releasing huge numbers of sterilised insects, which act as sterilizing substances for the insects. All of these act in one of the three ways-

(a) they inhibit the production of egg or spam. If it fail then go to the second stages

(b) cause death of the spam or eggs

(c) If these steps are failed totally then these bring about lethal mutation on the spam or eggs material and severally damage the genetic material and chromatin material of eggs and spam. This produce zygote ,but the off springs will totally lost their reproduction ability.

Ex: diflubenzuron

Fungicides: are the chemicals which are used to prevent, cure eradicate the fungi . Some of the examples are cymoxanil , carpropamid, metalaxyl , metalaxyl-M , carboxin, aureofungin, kasugamycin , streptomycin , validamycin, kasugamycin, carbendazim , thiabendazole, thiophanate-methyl, cyproconazole, difenoconazole , flusilazole, tebuconazole , triadimefon, Bordeaux mixture, copper oxychloride, iprodione , captan, ferbam , thiram , ziram. mancozeb, maneb , metiram , propineb , zineb, isoprothiolane, tridemorph, edifenphos , fosetyl-Al, fenarimol, tricyclazole

Herbicide softeners: A chemical that protects crops from injury by herbicides, but does not prevent the herbicide from killing weeds. Examples are benoxacor , cloquintocet, cyometrinil, cyprosulfamide

Herbicides: are the substances that are used to kill plants, or to inhibit their growth or development. Some of the examples are alachlor, butachlor, metolachlor, pretilachlor, methabenzthiazuron, pendimethalin, oxyfluorfen, imazethapyr, anilofos, glyphosate, oxadiargyl, oxadiazon, 2,4-D , clodinafop , cyhalofop, quizalofop , Paraquat, atrazine, isoproturon, linuron, metoxuron, chlorimuron, sulfosulfuron.

Insect attractant: A chemical that lures pests to a trap, thereby removing them from crops, animals or stored products.
Ex Gossyplure, Gyplure, Muscalure (name ends with lure as they lure the pests)

Insect repellents: A chemical that deters an insect from landing on a human or an animal. Some of the examples are Citronella oil, Permethrin
Insect Growth regulator: A substance that works by disrupting the growth or development of an insect. Some of the examples are. Diflubenzuron, buprofezin

Insecticides: A pesticide that is used to kill insects, or to disrupt their growth or development. Some of the examples are azadirachtin, pyrethrins, carbofuran, carbosulfan, methomyl, buprofezin, diflubenzuron, fenoxycarb, abamectin, emamectin, milbemectin, spinosad, cartap, clothianidin, imidacloprid , thiamethoxam, Acetamiprid, Thiacloprid, DDT, Lindane, Endosulfan, dichlorvos, monocrotophos, phosphamidon, demeton-O-methyl , Ethion, Malathion, phorate, Dimethoate, Phosalone, azinphos-methyl, chlorpyrifos, pirimiphos-methyl, quinalphos, triazophos, cyfluthrin, cyhalothrin ,lambda-cyhalothrin, cypermethrin , alpha-cypermethrin , cyphenothrin , deltamethrin , fenpropathrin, esfenvalerate, fluvalinate , imiprothrin, tofenprox, chlorfenapyr, clothianidin thiamethoxam , Thiacloprid, isoprothiolane

Mammal repellents: A chemical that deters mammals from approaching or feeding on crops or stored products.

Mating disrupters: are the chemicals that interfere with the way that male and female insects locate each other using airborne chemicals (pheromones), thereby preventing them from reproducing.

Molluscicides:are the substances used to kill slugs and snails. Some of the examples are copper sulfate, metaldehyde, thiacloprid, thiodicarb,

Nematicides: are the chemicals which are used to control Nematicides. Some of the examples are abamectin, benomyl , carbofuran , carbosulfan, methyl bromide, fenamiphos, phosphamidon, chlorpyrifos , dimethoate, phorate, triazophos.

Plant growth regulators: are the substances that alters the expected growth, flowering or reproduction rate of plants. Fertilizers and other plant nutrients are excluded from this definition. 2,4-D,α-naphthaleneacetic acid, ethephon, metoxuron, gibberellic acid, chlormequat, paclobutrazol, triacontanol, are some of the examples.

Rodenticides: are the substances used to kill rats and related animals. Some of the examples are strychnine, bromadiolone, coumachlor, coumatetralyl , warfarin, zinc phosphide, Lindane, aluminium phosphide

Synergists: A chemical that enhances the toxicity of a pesticide to a pest, but that is not by itself toxic to the pest. Example: piperonyl butoxide

Virucide: an agent having the capacity to destroy or inactivate viruses. Example: Ribavirin (not available in India)

Miscellaneous: aluminium phosphide, sodium cyanide.

Biologicals: Viruses, bacteria, fungi, and plants Nematodes, insects and other parasites or predators.

Classification on the basis of the chemistry

A large number of group of chemicals are available in the list pesticides but we will confine to the pesticides registered in India.

a) Insecticides : The insecticides available can be classified as Organo halogen, Organophosphorous, Carbamates, Pyrethroids, Neonicotinoids, Miscellaneous pesticides, Spinosyns (spinosad) neriestoxin (cartap), Fiproles (or Phenylpyrazoles)(Fipronil), Pyrroles (chlorfenapyr) , Quinazolines (fenazaquin), Benzoylureas (diflubenzuron an IGR), Antibiotics (abamectin) etc.,

b) Fungicides: The fungicides available are aliphatic nitrogen fungicides(dodine), amide fungicides(carpropamid), acylamino acid fungicides (metalaxyl), anilide fungicides (carboxin), antibiotic fungicides (kasugamycin), methoxyacrylate strobilurin fungicides (azoxystrobin), aromatic fungicides (chlorothalonil), carbamate fungicides or benzimidazole fungicides (carbendazim), conazole fungicides (triazoles) (hexaconazole), copper fungicides (COC), dicarboximide fungicides (famoxadone), dichlorophenyl dicarboximide fungicides(iprodione), dinitrophenol fungicides (dinocap), dithiocarbamate fungicides(mancozeb), dithiolane fungicides (isoprothiolane), morpholine fungicides(tridemorph), Sulphur compounds etc.,

c) Herbicides: The herbicides are anilide herbicides(flufenacet), chloroacetanilide herbicides (butachlor), pyrimidinyloxybenzoic acid herbicides (bispyribac), benzothiazoleherbicides (methabenzthiazuron), dinitroanilineherbicides (pendimethalin), nitrophenyl ether herbicides (oxyfluorfen), halogenated aliphatic herbicides (dalapon), imidazolinone herbicides(imazethapyr), organophosphorus herbicides (anilofos), phenoxyacetic herbicides (2,4-D), aryloxyphenoxypropionic herbicides (clodinafop), quaternary ammonium herbicides (Paraquat), chlorotriazine herbicides (atrazine), triazolone herbicides (carfentrazone), Urea herbicides (methabenzthiazuron), phenylurea herbicides (isoproturon), sulfonylurea herbicides (chlorimuron).

d) Rodenticides: Inorganic Rodenticides: (Zinc Phosphide,Aluminium Phosphide,Magnesium Phosphide) coumarin Rodenticides (organic) (bromadiolone,coumachlor , coumatetralyl)

Organochlorine Pesticides

This group consists of, the polychlorinated derivatives of cyclohexane (Lindane), polychlorinated biphenyls (DDT, dicofol) and polychlorinated cyclodiene (Endosulfan).

Properties

Physical: Solids which possess low volatility
Low solubility in water, high solubility in oils, fats,lipids etc.,
Not prone to environmental degradation.

Chemical: Isomerism is a common phenomenon, Ex. Gamma HCH
Stable over a wide range of pH

Toxicity: Possess a high acute toxicity as well as chronic toxicity

Compound	LD50 (oral) mg/Kg
HCH	1000
dicofol	684-809
lindane	88-91
endosulfan	70-110

By and large these group of chemicals exhibit low selectivity

Biological stability: Not rapidly degraded by the enzyme, not rapidly exerted, but get stored in the fatty tissues.

Behaviour in the field: These chemicals are non systemic, act as contact and stomach poisons. Lindane exhibits slight fumigant action. Persist in the environment for long time, results in pesticide residue problem in the environment and bio magnification.

Effect of OC's in the environment: Insecticides can kill bees , pollination decline and the loss of bees that pollinate plants, and colony collapse disorder (CCD).

A number of the organochlorine pesticides have been banned from most uses worldwide, and globally they are controlled via the Stockholm convention on persistent organic pollutants. (POP's)

These include: aldrin, chlordane, DDT, dieldrin, endrin,heptachlor, mirex and toxaphene

Organo phosphorous pesticides

These are the esters of derivatised phosphoric acid, thiophosphoric acid and dithio phosphoric acids which are called phosphates, thiophosphates and dithiophosphates respectively. Some of the examples of each class of pesticides are as follows:

	Group	Example
i.	Phosphates	monocrotophos,phosphamidon, DDVP
ii.	Thiophosphates or	methyl parathion, fenitrothion, Phosphorothiates oxy demeton methyl
iii.	Dithiophosphates or phosporodithioates	dimethoate, phorate, Phosalone

Based on the organic moiety attached to the phosphoric acid these grouped can also be classified in to aliphatic, phenyl and heterocyclic derivatives.

Properties

Physical: These compounds are available as liquids or semi solids and posses significant vapour pressure and comparatively volatile. Some compounds have slight solubility in water (MCP and Phosphamidon are soluble in water). Sunlight brings about modification of the toxicity of these molecules either way.

Chemical: These compounds which are esters of phosphoric acid are not stable in alkaline pH, but stable over narrow range of pH. Thiophosphates and dithiophosphates undergo molecular rearrangements. Forms isomers with increased toxicity((may take place in storage as well as on application in the field) and under go oxidation to give oxo compounds with increased toxicity. Sulphur atoms in side chain may be oxidised to sulfoxide or sulfones which more toxic than parent molecule.

The organo phosphorous pesticides under go conversion of one pesticide in to another pesticides also takes place. The following are some of the examples.

Trichlorfon ⇒ dichlorvos

Formothion ⇒ dimethoate

Acephate ⇒ methamidophos

Toxicity:

Exhibits acute extreme toxicity to slight toxicity (Phorate1.5 to 3.7 mg/Kg, temephos 8600mg/Kg). LD50 values may change with the purity of the compound (as the impurities present some times are more toxic than the parent compd.) and they do exhibit low chronic toxicity. The undergo rapid conversion in to low fat soluble metabolites which are excreted.

Biological stability: The OP compounds undergo enzymatic degradation and the metabolites are fat insoluble and easily excreted. Bio magnification is almost absent and chronic toxicity is in significant

Behaviour in the field: Some of the OP compounds show systemic activity and chemicals like dichlorvos exhibits fumigant action too. Persists for shorter duration and do not pose environmental problem like organo halogen pesticides.

Carbamates

The Carbamates are esters of either carbamic acids or thiocarbamic acids. And the Carbamates may be further subdivided in to three sub-groups as given under,

	Group	Example
i.	Aryl N methyl carbamate	Carbaryl, Propoxur
ii.	Hetero cyclic mono or dimethyl Carbamates	carbofuran
iii.	carbamoylated oximes	methomyl
iv.	Thiocarbamates	cartap hydrochloride (neriestoxin group of insecticide)

Properties :

Physical : The organo carbamates are available as non volatile solids. carbaryl, carbofuran have very low water solubility (40-6000ppm) where as Cartaphydrochloride is hygroscopic. And these compounds under go degradation by the environmental factors.

Chemical : These compounds are unstable in alkaline medium.

Toxicity: The OC compounds exhibit moderate to extreme toxicity. And they do not display chronic toxicity.

carbaryl	moderately toxic
Propoxur and cartap	highly toxic
Methomyl & carbofuran	extremely toxic

Biological stability The OC compounds undergo enzymatic degradation and rapidly metabolised and excreted. Bio magnification is almost absent and Chronic toxicity is in significant.

Behaviour in the field:

Carbaryl& Propoxur	contact pesticides
Carbofuran, methomyl & Cartap	systemic pesticides

methomyl is well taken up by leaves
Carbofuran is well taken up by the roots (soil applied)
and Cartap is taken by both roots and leaves.

Pyrethroids

The living organisms do contain naturally a large number of chemicals some of which give them protection from foreign invasive and many such chemicals have been isolated, identified and evaluated of their biological activity. The flowers of chrysanthemum contain compounds called pyrethrins which are found to have possessed very good pesticidal activity but are found to be less stable in the environment. The pyrethrins are chemically the esters of chrysanthemic acid and pyrethric acid (contains dimethyl cyclopropane group) with alcohols, namely pyrethrolone, cinerolone and jasmolone. Thus there are a total of six esters as shown below.

Ester	Acid portion	Alcohol portion	% content in pyrethrum extract
Pyrethrin –I	chrysanthemic acid	pyrethrolone	35
Cinerin-I	chrysanthemic acid	cinerolone	10
Jasmolin-I	chrysanthemic acid	Jasmolone	5
Pyrethrin –II	Pyrethric acid	pyrethrolone	32
Cinerin-I	Pyrethric acid	cinerolone	14
Jasmolin-I	Pyrethric acid	Jasmolone	4

Synthetic Pyrethroids: Allethrin was the first synthetic pyrethroid developed in 1949, followed by resemethrin. However they have failed to contain the desired properties and proved to be highly photo labile. The first photo sable pyrethroid developed was permethrin. This was followed by cypermethrin, deltamethrin, and fenvalerate. Now world over plenty of synthetic Pyrethroids have been synthesized and put to use in plan- protection, The latest of the series in India is…The synthetic Pyrethroids contain halogenated derivative of dimethyl cyclopropane carboxylic acid and cyano phenoxy benzyl alcohol . Fenvalerate is an exception with the acid portion being p-chlorophenyl isopropyl acetic acid instead of cyclopropane carboxylic acid. In case of permethrin alcohol portion does not have cyano - group, but it is simply phenoxy benzyl alcohol.

Properties

Physical: The Pyrethroids are present as volatile and non volatile solids or semisolids. They are insoluble in water

Chemical: These compounds present in different isomeric forms Cis isomers are found to be more toxic. Individual isomers also are being marketed (e.g. alphamethrin, deltamethrin). The pyrethroid chemicals are unstable in alkaline medium.

Toxicity: The toxicity of these chemicals ranges from 80 to 4000mg/Kg body weight and toxicity varies with the ratio of isomers and test animals/species. Toxicity to the insects can be increased by synergists (e.g.. Piperonyl butoxide, sesamex) (9:1)

Neonicotinoids

Neonicotinoids are modified structures from nicotine that have come in to existence with improved bio efficacy.

- Acetamiprid
- Clothianidin
- Imidacloprid
- Thiacloprid
- Thiamethoxam

(2nd generation- thianicotinyl)

Properties:

Physical: These compounds are present as colour less to pale yellow crystals with almost negligible solubility in water with exception of thiamethoxam which has got slight solubility in water.

Chemical: These compounds are weak bases and are stable in acid conditions. These chemicals under go hydrolysis in alkaline solutions.

Toxicity: These compounds mostly belong to moderate to high toxic group. They do not have chronic toxicity and not mutagenic and teratogenic. However some of the compounds have been reported to be harmful to honeybees by direct contact but no problems expected when not sprayed into flowering crops.

Miscellaneous pesticides

The following are new arrivals in to the India from different class of chemical group and with high potential of biological activity . The chemicals like Spinosad and abamectin are biological origin . Many of these chemicals are required to use in very low dose of active ingredient to achieve the pest control and do not bio accumulate.

Spinosyns (spinosad- Contact and stomach poison)
neriestoxin (cartap)
Fiproles (or Phenylpyrazoles)(Fipronil- Broad spectrum insecticide with
contact action)
Pyrroles (chlorfenapyr-Used against insects and mites stomach and stomach contact action)
Quinazolines (fenazaquin-Contact acaricide)
Benzoylureas(diflubenzuron a Contact insect growth regulator IGR)
Antibiotics (abamectin - Contact and stomach poison-insecticide and acaricide)

Classification of Herbicides

Herbicides can be classified on the basis of application as Foliar &Soil applied. From the behaviour of these chemicals on application , they may be classified as contact and systemic herbicides. The foliar applied herbicides may be either contact or systemic but the soil applied herbicides are mostly systemic which is taken by the plant through the roots and translocate through out the plant. There is another type of classification by their action that is selective and non selective. The non selective herbicides will kill all the plants without any selection where as selective herbicides specific for specific species and in certain crops. Further these herbicides are classified on the basis of time of application and their activity as pre emergent and post emergent. The pre emergent herbicides are applied in to the soil before the weeds are germinated where as the post emergent applied only after the germination.

The herbicides can also be classified further on the basis of their mode of action as follows:

1. Growth regulators
2. Amino acid synthesis regulators
3. Lipid synthesis inhibitors
4. Seedling growth inhibitors
5. Photosynthetic inhibitors
6. Cell membrane disrupters
7. Pigment Inhibitors

Growth regulators:

These are the compounds which disrupts hormone balance and protein synthesis by which the plant ultimately dies. (phenoxy acetic herbicides, aryloxyphenoxypropionic herbicides). These are primarily broad leaf killers.

eg.2,4-D

Amino acid synthesis regulators: These are the compounds which inhibits specific enzyme which is responsible for synthesis of amino acids (Acetolactate synthase)

e.g. Imidazolinone herbicides (Imazethapyr)
pyrimidinylsulfonylurea herbicides (Bensulfuron Methyl)
Amino acid derivatives (inhibits EPSPS enzyme) (Glyphosate)

Lipid synthesis inhibitors: Lipid synthesis inhibitors prevents formation of fatty acids which are essential for production of lipids. Aryloxy phenoxypropionic herbicides inhibits ACCase which is responsible for fatty acid formation (e.g. Clodinafop-propargyl).

Seedling growth inhibitors: Seedling growth inhibitors are shoot growth inhibitors, root growth inhibitors. These chemicals inhibits cell division, and lipid or protein synthesis, in the seedling
e.g. Acetanilide herbicides (Butachlor)

Photosynthetic inhibitors: Photosynthetic inhibitors inhibits electron transfer in photosynthesis and hence conversion of sun light in the chemical energy e.g. Triazines (Atrazine).

Cell membrane disrupters: These group of chemicals disrupts cell membrane . e.g. Bypyridyliums or quaternary ammonium salts (Paraquat).

Pigment Inhibitors: These chemicals prevents formation of pigments necessary for photosynthesis. Bleaching or whitening of leaves occur. e.g. Isoxazolidones (Clomazone).

The herbicides which are being used now in India belongs to the following class of chemicals :

1. anilide herbicides (flufenacet)
2. chloroacetanilide herbicides (metolachlor)
3. chlorotriazine herbicides (atrazine)
4. pyrimidinylthiobenzoic acid herbicides (Pyrithiobac sodium)
5. phenylurea herbicides (Isoproturon)
6. pyrimidinylsulfonylurea herbicides (Bensulfuron Methyl)
7. dinitroaniline herbicides (Fluchloralin Pendimethalin)
8. imidazolinone herbicides (Imazethapyr)
9. organophosphorus herbicides(anilofos)
10. oxadiazolone herbicides (oxadiargyl)
11. benzothiazole herbicides (methabenzthiazuron)
12. phenoxyacetic herbicides (2,4-D)
13. aryloxyphenoxypropionic herbicides (Clodinafop-propargyl)
14. quaternary ammonium herbicides (Paraquat)
15. Unclassified herbicide
16. halogenated aliphatic herbicides
17. oxadiazolone herbicides
18. Nitrophenyl ether (Oxyflourfen)
19. Isoxazolidones (Clomazone)

Fungicides

Chemicals which act on fungal pathogens are fungicides and chemicals which act on bacterial pathogens are bactericides.

The fungicides can be classified basically as inorganic fungicides, organo metallic fungicides and organic fungicides. The examples for inorganic fungicides are Sulphur and copper compounds. Methoxy

ethyl mercuric chloride which is under restricted use pesticides list is an example for organometallic fungicide. And all other synthetic chemicals like alachlor, oxyfluourfen etc., are organic fungicides .

Classification based on field behaviour of these chemicals is contact and systemic fungicides. Among the contact fungicides there are two sub-classes of fungicides (i)Non therapeutic Or Non eradicant Or Protectant and (ii)Therapeutic Or Eradicant. And all the systemic fungicides belongs to Protective as well as curative fungicides.

Non therapeutic Or Non eradicant Or Protectant: These chemicals do have limited mobility and applied prior to onset of the infection. Thorough coverage is essential and acts to control further spread of the disease if applied after the infection. These are comparatively less persistent. Ex: Copper fungicides, Sulphur, Dithio Carbamates.

Therapeutic or Eradicant: Therapeutic property is exhibited only at optimum or higher concentration but the same chemical exhibits only protective action at lower concentrations. Ex: Lime Sulphur, Edifenphos, Captan, Dinocap, Chlorothalonil.

Systemic: These chemicals act as protective as well as curative fungicides. They are absorbed by the plant and translocated mostly upward direction, better control and persistence in the plant which gives protection for longer duration. Some of he chemicals are known to stimulate defense mechanism in the plants. Ex. TPM which do not show fungicidal activity in -vitro but is a very good fungicide**.**

Few of the examples of this category are Carbendazim, Fenarimol, TPM, Carboxin, Triazoles, Antibiotics)

Copper compounds: Copper sulphate (acidic) is water soluble and all other compounds are water insoluble.

Ex: Copper oxy chloride (alkaline) water in soluble (50%WP & 56% Oil based) Cuprous oxide
Bordeaux mixture (1% of 1:1 copper sulphate and lime in water) (alkaline) water insoluble, this should not have any free copper.

Sulphur compounds: Sulphur is a fungicide and acaricide and available as WP(80%) and DP(85%) (40% CS). Lime sulphur (22% solution) is prepared by boiling with lime which is a red coloured char solution. Highly alkaline and unstable, converted in to $CaSO_4$ $Ca(OH)_2$, Ca CO_3 H_2S and Sulphur. Essentially it is a calcium polysulphide ($CaS.S_x$ + CaS_2O_3) Water :Sulphur: lime is 17:2:1

Phthalimide fungicides: The captan fungicide belongs to the phthalimide group and its mode of action is multi-site contact activity. This is a protective and curative (contact) fungicide.

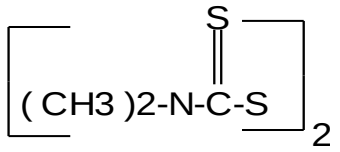
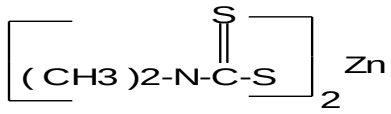
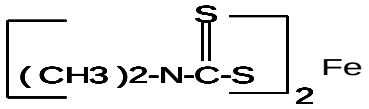
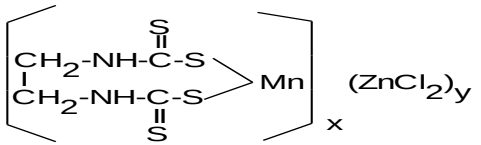
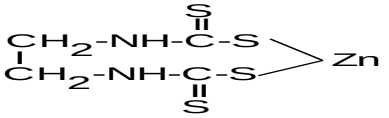
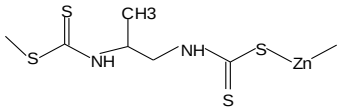
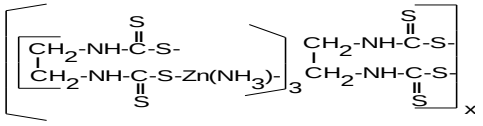
Dithio thio carbamates are used as seed dresser as well as foliar applied, as protective fungicide. Mancozeb is available in combination with metalaxyl. moderately toxic. moderately persistent.

Thiram is environmentally stable and displays persistence in plants up to 1 to 1.5 months and in soil for 6 to 24 months.

Benzimidazole fungicides: These group of chemicals inhibits mitosis by binding to beta-tubuline.These chemicals are systemic with protective and curative action, Slightly toxic. Unstable in alkaline medium and stable in acidic conditions. Benomyl and thiophanate methyl get converted in to carbendazim in the plant system in the presence of water, sunlight and enzymatic action. Benomyl is more persistent than carbendazim and TPM

Dinitrophenol fungicides: These compounds are contact fungicides with protective and curative action Their mode of action is through uncouples mitochondrial oxidative phosphorylation . Ex.Dinocap

dithio carbamate fungicides

Name	Molecular formula	Molecular weight	Structure
A. Alkyl dithio carbamates			
1. Thiram	$C_6H_{12}N_2S_4$	240.4	
2. Ziram	$C_6H_{12}N_2S_4Zn$	305.8	
3. Ferbam	$C_9H_{18}FeN_3S_6$	418.9	
polymeric dithio carbamate fungicides			
B. Alkylene dithiocarbamates			
1. Zineb	$C_4H_6N_2S_4Zn$	275.74	
2. Mancozeb	$C_4H_6N_2S_4Mn\ Zn$		
3. Propineb	$C_5H_8N_2S_4Zn$	290	
4. Metiram	$[C_{16}H_{33}N_{11}S_{16}Zn_3]_x$	$(1088.7)_x$	

Organophosphorous fungicides: Edifenphos, Fosetyl-Al and iprobenfos (IBP) (Kitazin) are the Organophosphorous fungicides . Among these edifenphos is a contact fungicide. Highly toxic chemical. It under go enzymatic hydrolysis in the plant and moderately persistent.

Fosetyl-Al (True systemic) blocks development of mycellium and sporulation and iprobenfos which is a phospho lipid biosynthesis inhibitor are systemic fungicides and reasonably persistent .

Conazole fungicides (triazoles): These compounds are basically Sterol synthesis inhibitors . These are available as non volatile solids with low solubility in water. These are systemic fungicides with protective and curative action. Triazole fungicides undergo hydrolysis in the plant and soil and converted to respective hydroxy compounds.

Imidazole, oxazole and strobillurin fungicides: Imidazole and oxazole fungicides inhibits mitochondrial respiration process. The example for imidazole is Fenamidone and oxazole femoxadone which are systemic fungicides and under go photo degradation in aqueous conditions. Azoxystrobin belongs to strobillurin class and its mode of action is the same as imidazoles and oxazoles

Pyrimidine fungicides: Pyrimidine fungicides inhibits the Ergo sterol bio synthesis and these chemicals are systemic fungicides. fenarimol is an example which is available as Fenarimol 12%EC.

Anilide fungicides: Anllide fungicides are systemic chemicals few of the examples belong to this group are as follows.

i. Carpropamid 27.8% SC : Mode of action :Nucleic acid synthesis (inhibition of melanin), Enhances crop resistance by increasing phyto alexin production following rice blast infection,

ii. Thifluzamide: Inhibits succinate de hydrogenase in the tricarboxylic acid cycle Thifluzamide 24% SC used in rice against sheath blight, Rhizoctoni a solani

Dithiolane fungicides: Isoprothiolane belongs to this group of chemical which is a fungicide and plant growth regulator . It's a Systemic fungicide which inhibits penetration and elongation of infection hyphae by inhibiting formation of infection peg or cellulase secretion. Used against blast in rice.

Strobilurin fungicides: This group of chemicals are systemic fungicides and they inhibit mitochondrial Respiration. Azoxystrobin , Pyraclostrobin are presently registered for use in India

Antibiotic fungicides: Streptomycin, kasugamycin and validamycin belong to the antibiotic fungicides which are used in agriculture. Their mode of action is mainly inhibition of protein synthesis .These chemicals are systemic and are reasonable persistent. Kasugamycin, Streptomycin sulphate (sesqui salt), Validamycin, (Streptomycin Sulphate 9%+ Tetracycline Hydrochloride 1%) SP are some of the examples belong to this group of chemicals.

The availability of pesticides in the market for application or use give raise to another type of classification, that is on the basis of type of formulation. Solid formulations for example, WP, SP, DP, Granules etc., and the liquid formulations for example, EC,SC, AF, ULV. The domestic pesticide formulations also are available in different formulations like LV, aerosol, Gels, mats, coils etc.

The Pesticide Formulations

Formulations : "Putting together of components in appropriate relationships or structures, according to a formula."

Formulations are commercially produced for drugs, cosmetics, coatings, dyes, alloys, cleaning agents, foods, lubricants, fuels, fertilizers, pesticides and many others.

Pesticide Formulations: "Pesticide formulations are mixtures of technical grade pesticide (s) with inert diluents and auxiliary chemicals".

Necessity: To understand the necessity of the pesticides to formulate, we better understand the Characteristics of technical grade Pesticides first.

Characteristics of technical grade Pesticides:

High purity: very low quantity of chemical need to be sprayed over large surface area which is very difficult and cannot be uniform

Solids or liquids or thick viscous pasty materials: spraying or broad casting is very difficult over the large area

Low water solubility : dilution and application /broadcasting is not possible

High bio efficacy: Many a times very less quantity of chemical is more than sufficient to achieve the desired pest control but spreading over the large area becomes difficult and can lead to inappropriate application leading to undesirable problems

Toxic to mammals in varying degree: Some of the chemicals are highly to extremely toxic which will be fatal for the operator , farm workers and the farming community besides environmental hazards if we use as such. This is more so particularly with those comounds which have their dermal toxicity is also very high/extreme- for eample phorate and carbofuran etc.,

Low selectivity: All the pesticides do not have inherent property to adhere and translocation in to the plant system/insect but requires to be modified to achieve the selectivity)

Low storage stability: Some of the compounds do not possess storage stability unless a suitable stabilisers are added

As has been seen above the characteristics of the technical pesticides do not allow them to be applied directly the technical grade pesticides need to be formulated to achieve the desired properties and use them advantageously.

Advantages of Pesticide Formulations:

- Facilitates easy handling
- Enables the farmer to dilute water insoluble pesticides in water
- Helps in achieving more uniform application
- Reduced toxic hazards
- Improved efficacy and selectivity
- Enhanced storage stability
- Cheaper

Basically there are Solid, liquid , gas and gel/paste formulations, each of which can be further divided in to various sub classes of formulations on the basis of their physico chemical properties. The solid formulations are available as powders (wettable powders, soluble powders, dusting powders and water soluble powders for seed treatment), granules (extruded granules, coated granules , encapsulated granules, water dispersible and micro granules), ready use cakes (Bromadiolone cake), coils (mosquito coils) tablets (soluble tablets and tablets for bait) Mats (mosquito control) and chalk (chlorpyrifos and cypermethrin chalks for domestic pest control).

The liquid formulations consists of Emulsifiable concentrates (e.g. chlorpyrifos EC, cypermethrin EC) , soluble liquids (e.g. phosphamidon and monocrotophos), suspension concentrates or flowable concentrate (SC), aqua flows (e.g. Carbaryl AF), Emulsified in water (e.g. butachlor EW), oil miscible liquid (e.g. fenitrothion OL) , flowable for seed treatment (FS) , and ready to use liquids namely Ultra Low Volume ULV, liquid vaporizers - LV (mosquito control refills)& Aerosols (mainly domestic pest control in India)

All the formulations will contains some inert material/filler material as diluent. The pesticide is mixed with the diluent to a desired level and then added some adjuvants to bring about the desired physico chemical properties and stabilisers to the product besides some colouring dye.

The following are some of the diluents used in different formulation:

Sl.No.	Type of formulation	Preferred diluent
1.	Emulsifiable concentrate	Aromax, xylene, solvent C IX , Cyclohexane, butanol
2.	Soluble liquids	Isopropyl alcohol, Cyclohexanone
3.	Water dispersible powders	China clay
4.	Granules	Bentonite, coarse sand
5.	Dusting powders	Soap stone, talc

The adjuvants are usually a class of chemicals known as surface active agents which are available as anionic, cationic, nonionic, Amphotylic and water insoluble. The concentration of these surface active agents usually present in the range of 5% to 20 % Depending on the function they perform the S.A.As are called as wetting agents, spreading agents, dispersing agents and emulsifiers.

In solid formulations, stabilisers are used to enhance the stability of the active ingredient or to enhance the shelf life of the product . Some of the chemicals which are used as stabilisers are , glycols, urea, hexa methyl tetra amine. In almost all the formulations, Epichlorohydrin is used as stabiliser.

Apart from the stabilisers, some miscellaneous substances also are used in certain formulations to enhance their shelf life. Some of the substances are listed below.

Sl. No.	Substance	Formulation	Purpose
1.	Rosin	Coated granules	To coat or cover the granules
2.	Carboxy methyl cellulose	WP	Viscosity modifier to improve stability of the suspension
3.	Calcium silicate	WP	Anti caking agent
4.	Zinc oxide, lamp black, kaolin	DP (Sulphur)	Anti balling agent
5.	Dye, colouring matter	Several formulations	Warning agent or indicator
6.	Gum resin	Granules	Binder

Compatibility of Pesticides

It has been a practice among our farming community to mix pesticide formulation , with another pesticide formulation or micronutrients, or fertilizers and spray such tank mixture in the field . This is done for various reasons and list them few.,

1. Simultaneous control of different pests that maybe present in the field.
2. To reduce the dose of one chemical on account of synergistic effects of other ingredients of mixture.
3. To save on labour cost, time, water.
4. To reduce the wear and tear of spray equipment.

But this type of unscientific practices can lead to catastrophic results as such tank mixtures **may not be compatible** and can lead to many problems like

- the very purpose of pest control is not achieved due to decrease in the bio efficacy (or toxicity).
- the spray fluid is non homogenous , breaks or settles down resulting in erratic/inadequate or no spray of pesticide.
- this leads to pest developing resistance to such chemicals.

- the toxicity may increase enormously which becomes hazardous to the farmer as well as the non target organisms.
- it ultimately leads to environmental contamination of the pesticide.
- may cause phyto toxicity
- the farmer will be at great loss of his investment as well as yield .

Therefore it is essential that the scientific principles, merits and demerits of the tank mixtures be made to known to every field/ extension workers.

Compatibility may be defined as ability of two or more chemicals to exist together in one medium in perfect harmony.

And the ideal requirements for calling a mixture to be compatible are as follows:

- Physical compatibility
- Chemical compatibility
- Physico-chemical compatibility
- Absence of phyto toxicity
- Absence of increase in toxicity to non-target organism.
- No reduction in bio efficacy

The factors that effect compatibility :

- Difference in composition of products mixed.
- Sequence of mixing.
- Degree of agitation.
- Quality of water used in mixing and preparing the spray fluid.
- Spraying equipment and its design.
- Temperature.
- Crop and its variety.
- Quality of different components of the mixture and their concentrations.

As has been seen above there are many variable factors that effect the compatibility of pesticide formulations, it is difficult to suggest which pesticides with what other chemical or substance or what concentration can be compatible as a guide , the mixing of pesticide formulations with any other as a tank mix the field level should discouraged and the farmers may be advised to use only those pre mixed / combinations pesticides which have been approved by the CIB& RC and are available in the market.

Toxicity of Pesticides

The toxicity of a pesticide is its capacity to cause injury to a living system, may be a human body, or parts of the body (such as the lungs or the respiratory system); a pond, a forest and those creatures that live there.

The toxicity of a pesticide is dependent on a number of factors.

1. Dose: It is the quantity of a pesticide that a surface, plant, or animal is exposed to.
2. Time: How often the exposure occurs.

Thus, the how much of the substance is involved and how often the exposure to the substance occurs gives rise to two different types of toxicity - acute and chronic toxicity.

***Acute* toxicity** refers to how poisonous a pesticide is to a human, animal, or plant after a single short-term exposure. A pesticide with a high acute toxicity is deadly even when a very small amount is absorbed. Acute toxicity may be measured as acute oral toxicity, acute dermal toxicity, and acute inhalation toxicity.

***Chronic* toxicity** is the delayed poisonous effect from exposure to a pesticide. Chronic toxicity of pesticides concerns the general public, as well as those working directly with pesticides because of potential exposure to pesticides on/in food products, water, and the air.

Routes of Entry: There are three specific ways in which pesticides may enter your body.

i. Dermal Route

Wet, dry, or gaseous forms of pesticides can be absorbed through the skin. Oil or paste forms allow greater absorption than water-based pesticides. The eyes, eardrums, scalp and groin areas absorb pesticides more quickly than other areas on the body. Once they are absorbed through skin, pesticides enter the blood stream and are carried throughout the body.

ii. Inhalation Route

Dusts, spray mist, or fumes can be drawn into lungs as one breathes. Inhalation can occur during the mixing, fumigating or spraying etc. The larger particles tend to stay on the surface of the throat and nasal passages but smaller particles can be inhaled directly into the lungs and enter the blood stream.

iii. Oral Route

Pesticides can enter the body through the mouth (ingestion). This can occur when hands are not properly washed before eating, swallowing by mistake or purposefully. Ingested materials can be absorbed anywhere along the gastrointestinal tract (mainly by the small intestine). Once absorbed, they the blood stream and circulate throughout the body.

The toxicity of a pesticide depends on the qualities of exposed individual as different individual characteristics affect the person's responds. Some examples of these individual qualities include:

- **Health conditions**: Individuals with poor health condition are more sensitive.
- **Age:** youngest and oldest individuals tend to be most sensitive.
- **Gender/sex**: Females are more sensitive.
- **Body size**: the effect of a dose is closely related to body weight.

Effects of Toxicity

a. Local or systemic (Both effects can occur with some pesticides.)

Local effects refer to those that take place at the site of contact with a material *e.g*. skin inflammation, irritation of the mucous membrane lining the lungs due to inhalation of toxic fumes etc. **Systemic effects** occur away from the original point of contact when pesticides are distributed throughout the body, or "system".

b. Immediate or delayed (Both effects can occur with some materials.)

Immediate toxic effects are those which are experienced upon or shortly after exposure. (*e.g.* a sneezing attack in response to inhaling pesticides during mixing). **Delayed effects** occur after some time has passed. *E.g*. Tumors may not be observed in chronically exposed people for 20 to 30 years after the original exposure to a cancer-causing or "carcinogenic" chemical.

c. Reversible or irreversible

Reversible effects are not permanent and can be changed or remedied. *E.g*. Skin rash, nausea, eye irritation, dizziness, etc. **Irreversible effects** are permanent and cannot be changed once they have occurred. *E.g*. Injury to the nervous system, birth defects, mutations, cancer etc.

d. Additive, antagonistic, or synergistic

An **additive** effect is one in which the combined effect of two pesticides is equal to the sum of the effects of each (i.e. 2 + 2 = 4). An **antagonistic** effect occurs when the toxic effect of the combination of pesticides is less than what would be predicted from the individual toxicities (i.e. 2 + 2 = 3). A

synergistic effect occurs when the combined toxic effect of two pesticides is much greater, or worse, than the sum of the effects of each by itself (i.e. 2 + 2 = 5).

e. Exposure to pesticides may also result in the following:

- **Reproductive effects:** effects on the reproductive system
- **Teratogenic effects:** effects on unborn offspring (birth defects)
- **Carcinogenic effects:** produces cancer in living animal tissues.
- **Mutagenic effects:** permanent effects on genetic material that can be inherited.
- **Neurotoxicity:** poisoning of the nervous system, including the brain.
- **Immunosuppression:** blocking of natural responses of the immune system responsible for protecting the body.

Measurement of Toxicity: Animals like rats, rabbits, mice, guinea pigs etc. are used to test pesticide toxicity. Due to some differences between the ways human and animals body work, toxicity studies are just guidelines for estimating and comparing toxic effects of pesticides.

a. Acute Toxicity Measures

Experimental doses are given orally, as well as put on the eyes, skin, and in the air that the test animals breathe. The animals are then observed carefully for changes.

Lethal Dose Fifty (LD_{50}): The amount of a pesticide that can kill half of the animals in a laboratory test. The smaller the LD_{50} value of a pesticide means it more poisonous.
Measured in units as **Milligrams per kilogram** (mg/kg)

Lethal Concentration Fifty (LC_{50}): It is a measure of "acute inhalation toxicity" of a pesticide. The concentration of a pesticide that causes half of the animals to die is called LC_{50}. Measured in milligrams per liter (mg/l) or ppm and sometimes in milligrams per cubic meter (mg/m3).

b. Chronic Toxicity Measures
There is no standard measure for chronic toxicity studies. Often the length of the experiment is in days, months, or years and the amount of each dose is stated.

Classification is based on toxicity levels: as per the CIB RC classification there are four types of classifications. They are:

TABLE

Classification of the Insecticides	Medium lethal dose by the oral route acute toxicity LD 50 mg/kg.. body weight of test animals	Medium lethal dose by the dermal route dermal toxicity LD 50 mg/kg. Body weight of test animals	Colour of identification band on the label
1	2	3	4
1. Extremely toxic	1-50	1-200	Bright red
2. Highly toxic	51-500	201-2000	Bright yellow
3. Moderately toxic	501-5000	2001-20000	Bright blue
4. Slightly toxic	More than 5000	More than 20000	Bright green

Mode of Action of Insecticides

Study of Mode of Action is helpful to understand how human systems function in order to see similarities and differences between humans and the pests we try to control. It also helps to prevent development of pesticide resistance in the target pest(s) - Development of pest resistance can be avoided or delayed by rotating pest control chemicals that work through different modes of action

Insecticides and miticides generally target the

1. Nervous system
2. Growth and development
3. Energy production of insects

1 Nervous system

This system has two components:

i.) The peripheral nervous system to receive and transmit incoming (*taste, smell, sight, sound, and touch*) and outgoing signals to the muscles and other organs, effectively telling them how to respond

ii.) The central nervous system (CNS) to interpret the signals and coordinate the body's responses and movements

A **neuron** is a single nerve cell, connects with other neurons and muscle fibers (the basic units of muscles) through gaps at the end of each neuron. The gap between neurons, or between a neuron and a muscle fiber, is called a **synapse**. Incoming signals (*the pain from a sharp object, the sight of a predator, or the odor of food, etc*.) are transformed by the neuron into an electrical charge (called **ions**) which then travels down the length of the neuron through –

1. Sodium channels,
2. Potassium channels,
3. Calcium channels, and
4. Chloride channels.

When an electrical charge reaches the end of the neuron, it stimulates a chemical messenger, called a **neurotransmitter,** to be released from the end of the neuron which crosses the synapse and binds to a receptor on the receiving end of the next neuron. Binding to the receptor causes the signal to be converted back into an electrical charge in the second neuron, and the signal is transmitted along the length of that neuron and the neurotransmitter is absorbed back into its originating neuron, and the nerve cell is then in a resting stage until the next signal is received. This process repeats over and over until the signal has reached the CNS (the brain and spinal cord in humans and a series of ganglia, or nerve bundles, in the insect) to be interpreted. Impulses from the CNS to the peripheral nervous system continue in the same way until the signal reaches the appropriate muscles or organs. Both humans and insects have many different neurotransmitters that work at different sites throughout the nervous system. Among them (both insects and humans have) acetylcholine (ACh) and gamma-aminobutyric acid (GABA) are important targets of some insecticides. ACh can either excite or inhibit its target neurons – depending on the particular neuron and the specific receptors at the site. In contrast, GABA is an inhibitory neurotransmitter – when GABA is the neurotransmitter activated at a synapse, the nerve impulse stops.

a. Cholinesterase Inhibition

Organophosphate and Carbamate Insecticides

Bind to the enzyme that is normally responsible for breaking down ACh after it has carried its message across the synapse. The **cholinesterase** is not available to help break down the ACh, and the neurotransmitter continues to cause the neuron to "fire," or send its electrical charge. This causes **over-stimulation** of the nervous system, and the insect dies.
Cholinesterase inhibition by carbamates is somewhat reversible.Organophosphate poisoning is not reversible - the insecticide does not release the bound cholinesterase.

b. Acetylcholine Receptor Stimulation

Neonicotinoid insecticides **mimic** the action of the neurotransmitter, acetylcholine (ACh). Cholinesterase is not affected by these insecticides, but the nerve is continually stimulated by the neonicotinoid and leads to **over-stimulation** of the nervous system leads to poisoning and death. A closer mimic for the insect's ACh than for human ACh - more specificity for insects and less ability to poison humans.

c. Chloride Channel Regulation

Avermectins bind to the chloride channel and activate it causing an inhibitory effect, which, when excessive, results in the insect's death. Cyclodiene group of Organochlorine insecticides affect the chloride channel by inhibiting the GABA receptor. The GABA receptor can no longer close the chloride channel for which it acts as a gate. The end result is **over-stimulation** of the nervous system.

d. Sodium Channel Modulators

Pyrethrins and **Pyrethroids** act on tiny channels through which sodium is pumped to cause excitation of neurons.They prevent the sodium channels from closing, resulting in continual nerve impulse transmission, tremors, and eventually, death. ***Lindane*** also act as a Sodium channel modulator

2. Growth and Development

Insects shed their skin (Molting) in order to grow and to develop into their next life stage. Insecticides target the insect's growth and development either through interfering with Hormones or Blocking the production of a structural component (Chitin) of the exoskeleton (Cuticle)

e. Chitin Synthesis Inhibitors (CSIs)

Chitin synthesis inhibitors (*e.g. novaluron, diflubenzuron*) , block the production of chitin. An insect poisoned with a CSI **cannot molt** & **reach the adult stage (cannot reproduce)** and the insect dies. CSIs are not considered toxic to humans.

b. Insect Growth Regulators (IGRs)

IGRs attack the insect's endocrine system, which produces the hormones (***juvenile hormone)*** needed for growth and development into an adult form. Insects poisoned with IGRs cannot molt or reproduce, and eventually they die. (e.g ***pyriproxyfen)***. **Azadirachtin** interferes with synthesis of ***Prothoracicotropic hormone*** *(PTTH)*
Humans do not make or use the hormones insects use in molting. Because of this, IGRs are considered to have little human toxicity.

4. Energy Production

Organisms generate energy from the food they take in and store the energy in molecules known as ***adenosine triphosphate* (*ATP)*.**
The energy stored in the ATP molecules can then be used to do the body's work such as:

- Thinking,
- Moving,
- Growing,
- Synthesizing chemicals and Structures that the body needs.

Some insecticides inhibit or disrupt energy production. The insect stops eating and even moving, and dies (e.g. ***Dicofol***). Some insecticides block feeding (***Azadirachtin*).**

On the basis of mode of action, they can also be classified as contact and stomach insecticides systemic or translocated and with fumigant action.

The contact pesticide acts on the pest when the pest comes in to contact are chew the plant material. Some examples are Endosulfan, Malathion, fenvalerate. Where as the systemic pesticide is moved within a plant from the point of application to the point where the insect will contact or ingest it . Some examples are monocrotophos, carbofuran. Some of the chemicals have fumigant action apart from either of the above properties, for example DDVP, Lindane, chlorpyrifos.

2. Various Methods in Pesticide Formulation Analysis

Titrimetry

Titration, also known as titrimetry, is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analyte. Because volume measurements play a key role in titration, it is also known as volumetric analysis. A reagent, called the titrant or titrator is prepared as a standard solution. A known concentration and volume of titrant reacts with a solution of analyte or titrand to determine concentration.

Small volumes of the titrant are then added to the titrand and indicator until the indicator changes, reflecting arrival at the endpoint of the titration. Depending on the endpoint desired, single drops or less than a single drop of the titrant can make the difference between a permanent and temporary change in the indicator. When the endpoint of the reaction is reached, the volume of reactant consumed is measured and used to calculate the concentration of analyte.

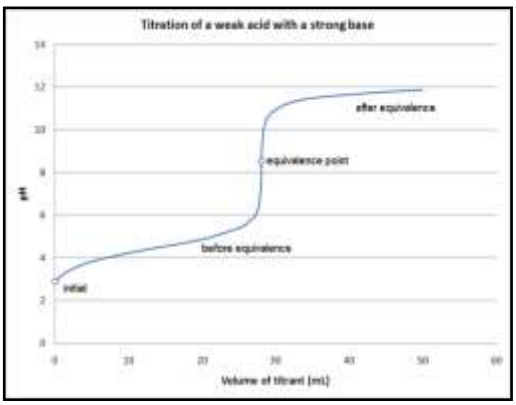
It is appropriate to know the following terminology used in the titrimetric analysis.

Titrant. The solution containing the active agent with which a titration is made.

Titrand: The solution containing the active agent which is titrated.

Equivalence-point. The point in a titration at which the amount of titrant added is chemically equivalent to the amount of substance titrated. (Stoichiometric (stoicheiometric)-point and Theoretical end-point are synonymous with Equivalence-point.)

End-point. The point in a titration at which some property of the solution (as, for example, the colour imparted by an indicator) shows a pronounced change, corresponding more or less closely to the equivalencepoint. The end-point may be represented by the intersection of two lines or curves in the graphical method of end-point determination.



TITRATION CURVE

Standard substance:

Primary standard. A substance of high purity which, by stoichiometric reaction, is used to establish the reacting strength of a titrant, or which itself can be used to prepare a titrant solution of accurately known concentration.

Secondary standard. A substance used for standardizations, whose content of the active agent has been found by comparison against a primary standard.

Standardisation: The process of finding the concentration or an active agent in a solution, or the reacting strength of a solution in terms of some substance, usually by titration of a known amount of the substance which is pure or has a known reaction value.

Indirect Titration: A titration (acid—base or other type) in which the entity being determined does not react directly with the titrant, but indirectly via the intermediacy of a stoichiometric reaction with another titratable entity.

Standard solution. A solution having an accurately known concentration of the active substance, or an accurately known titre.

Primary standard solution: A standard solution prepared from a primary standard substance whose concentration is known from the weight of that substance in a known volume (or weight) of the solution.

Secondary standard solution. A solution whose concentration or titre has been obtained by standardization, or which has been prepared from a known weight of a secondary standard substance.

Titration error. The difference in the amount of titrant, or the corresponding difference in the amount of substance being titrated, represented by the expression:

(End-point value — Equivalence-point value)

Types of titrations:

1. ACID-BASE. A titration involving the transfer of protons (Bronsted—Lowry) or electron-pairs (Lewis) from one of the reacting species to the other in solution.

2. NON-AQUEOUS. A titration (acid—base or other type) in which the solvent medium is one other than water and in which the concentration of the latter is minimal

2. COMPLEXIMETRIC (COMPLEXOMETRIC). A titration involving the formation of a soluble complex between a metal ion and a complexing agent.

3. IODIMETRIC. Titration with, or of, iodine Some authors restrict iodimetry to titration with a standard solution of iodine, and iodometry to titration of iodine; such restrictions are not recommended.

4. OXIDATION-REDUCTION (REDOX). A titration involving the transfer of one or more electrons from a donor ion or molecule (the reductant) to an acceptor (the oxidant).

5 PRECIPITATION. A titration in which the entity being titrated is precipitated from solution by reaction with the titrant.

Acid-Base titrations: Before starting the titration a suitable pH indicator must be chosen. The equivalence point of the reaction, the point at which equivalent amounts of the reactants have reacted, will have a pH dependent on the relative strengths of the acid and base used. The pH of the equivalence point can be estimated using the following rules:

- A strong acid will react with a strong base to form a neutral (pH=7) solution.
- A strong acid will react with a weak base to form an acidic (pH<7) solution.
- A weak acid will react with a strong base to form a basic (pH>7) solution.

When a weak acid reacts with a weak base, the equivalence point solution will be basic if the base is stronger and acidic if the acid is stronger. If both are of equal strength, then the equivalence pH will be

neutral. However, weak acids are not often titrated against weak bases because the colour change shown with the indicator is often quick, and therefore very difficult for the observer to see the change of colour.

Acid–base titration is performed with a phenolphthalein indicator, when it is a strong acid – strong base titration, a bromthymol blue indicator in weak acid – weak base reactions, and a methyl orange indicator for strong acid – weak base reactions.

Different methods to determine the equivalence point include:

pH indicator: This is a substance that changes color in response to a chemical change. An acid-base indicator (e.g., phenolphthalein) changes color depending on the pH. Redox indicators are also frequently used. A drop of indicator solution is added to the titration at the start; when the color changes the endpoint has been reached, this is an approximation of the equivalence point. Some of the pH indictors are listed below.

Indicator	Color on acidic side	Range of color change	Color on basic side
Bromophenol Blue	Yellow	3.0–4.6	Blue
Methyl Orange	Red	3.1–4.4	Yellow
Congo red	Blue	3.0 - 5.0	Red
Bromocresol Green	yellow	3.8 – 5.4	Blue
Methyl Red	Red	4.4–6.3	Yellow
Litmus	Red	5.0–8.0	Blue
Bromothymol Blue	Yellow	6.0–7.6	Blue
Phenolphthalein	Colorless	8.3–10.0	Pink
Alizarin Yellow	Yellow	10.1–12.0	Red

Potentiometer: A potentiometer can also be used. This is an instrument which measures the electrode potential of the solution. These are used for titrations based on a redox reaction; the potential of the working electrode will suddenly change as the equivalence point is reached.

pH meter: This is a potentiometer which uses an electrode whose potential depends on the concentration of H_3O^+ present in the solution. This is an example of an ion selective electrode. This method allows the pH of the solution to be measured throughout the titration. At the equivalence point there will be a sudden change in the measured pH. It can be more accurate than the indicator method, and is very easily automated.

Conductance: The conductivity of a solution depends on the ions that are present in it. During many titrations, the conductivity changes significantly. (For instance, during an acid-base titration, the H_3O^+ and OH^- ions react to form neutral H_2O. This changes the conductivity of the solution.) The total conductance of the solution depends also on the other ions present in the solution (such as counter ions). Not all ions contribute equally to the conductivity; this also depends on the mobility of each ion and on the total concentration of ions (ionic strength). Thus, predicting the change in conductivity is harder than measuring it.

Color change: In some reactions, the solution changes colour without any added indicator. This is often seen in redox titrations, for instance, when the different oxidation states of the product and reactant produce different colours.

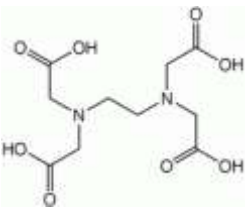
Complexometric titrations: A simple example in this type can be the titrations involving Silver nitrate in titrating the cyanide ion ;

$$Ag^{+} + 2\ CN^{-} \leftrightarrow [\ Ag\ (CN)_2]^{-}$$

using the indicator potassium iodide ion and ammonia solution

$[Ag(NH_3)_2]^{+} \leftrightarrow AgI + 2\ NH_3$ the formation silver iodide gives turbidity to the solution.

Another important complexometric titration is the titrations involving ethylenediaminetetra acetic acid (EDTA) in determining the metal ions



$M^{2+} + H_4Y \rightarrow MH_2Y + 2H^{+}$

Commonly used indicators in the EDTA titrations are organic dyes such as Fast Sulphon Black, Eriochrome Black T, Eriochrome Red B or Murexide.

- **Iodometric Titration**: Free iodine is titrated against standard reducing agent such as sodium thiosulfate solution. Usual reagents are sodium thiosulfate as titrant, starch as an indicator (it forms blue complex with free iodine molecules), and an iodine compound (iodide or iodate, depending on the desired reaction with the sample).The color change at the end point is usually blue to colorless. The principal reaction is the reduction of iodine to iodide by thiosulfate (One of the examples for redox titrations)

$$I_2 + 2\ S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2\ I^{-}$$

Redox titrations; As the name indicates both oxidation and reduction simultaneously takes place in the molecules to their respective oxidation/reduction states , The chemical reaction proceeds with transfer of electrons (simultaneous loss and gain of electrons) among the reacting ions in aqueous media.

Some Redox titrations are named after the reagent

Permanganate titrations

Potassium permanganate is used as an oxidizing agent. The medium is maintained acidic by the use of Dil. H_2SO_4. Potassium permanganate acts as self-indicator Used in the estimation of ferrous salts, oxalic acid, oxalates, H_2O_2 etc. Solution of the Potassium permanganate should be standardized first using standard oxalic acid or sodium oxalate .

$$2KMnO_4 + 5\ H_2C_2O_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 10\ CO_2 + 8H_2O$$

$$2MnO_4^{-} + 5\ C_2O_4^{2-} + 6H^{+} = 2Mn^{2+} + 10\ CO_2 + 8H_2O$$

Dichromate titrations

Potassium dichromate is used as an oxidizing agent in acedic medium and sulphuric acid is used to maintain the medium acidic. Potassium dichromate solution can be directly used for titrations. This type

of titration is mainly use in the estimation of Ferrous salts and Iodides. In these titrations of potassium dichromate versus ferrous salt, either an external indicator (Potassium ferricyanide) or an internal indicator (Diphenyl amine) can be used.

Iodimetric titrations also are redox titrations

Precipitation reactions: The argentometric titrations involving Silver nitrate in determining halogens is a classical example for precipitation reactions. The titrant react with the analyte forming an insoluble material and the titration continues till the very last amount of analyte is consumed.

$$Ag^+ + Cl^- = AgCl$$

The first drop of titrant in excess will react with an indicator resulting in a color change and announcing the termination of the titration.

$$2\ Ag^+ + CrO_4^{2-} = Ag_2CrO_4$$

This reaction should be carried out in faintly alkaline solution within the pH range of 6.5 to 9.0. In acid solution , the chromate under goes the following reaction, where in no colour change can be observed.

$$CrO_4^{2-} + 2H^+ \leftrightarrow HCrO_4^- \leftrightarrow Cr_2O_7^{2-} + H_2O$$

Nonaqueous titration is the titration of substances dissolved in non aqueous solvents. It is suitable for the titration of very week acids and very weak bases.

E.g. The titration of organic bases with perchloric acid in anhydrous acetic acid. If a very strong acid such as perchloric acid is dissolved in acetic acid, the latter can function as a base and combine with protons donated by the perchloric acid to form protonated acetic acid.

Since the $CH_3COOH_2^+$ ion readily donates its proton to a base, the titration of a base is accomplished . The reactions involved can be expressed as furnished.

I. $HClO_4 \rightleftharpoons H^+ + ClO4^-$

$CH_3COOH + H^+ \rightleftharpoons CH_3COOH2^+$ (onium ion)

$HClO_4 + CH_3COOH \rightleftharpoons CH_3COOH_2^+ + ClO_4^-$

II. $C_5H_5N + CH_3COOH \rightleftharpoons C_5H_5NH^+ + CH_3COO-$

$CH_3COOH_2^+ + CH3COO^- \rightleftharpoons 2CH_3COOH$

III. $HClO_4 + C_5H_5N \rightleftharpoons C_5H_5NH^+ + ClO_4^-$

Popularly used indicator used in this titrations is crystal violet , which shows different colours in different medium as given under;

violet blue-green yellowish-green

PREPARATION OF STANDARD SOLUTIONS

1. **Preparation of 0.1N silver nitrate:**

Weigh about 17.0 grams of A.R. Silver nitrate and dissolve in 1000 ml water. This is standardized against sodium chloride using potassium chromate indicator (The Mohr titration).

Standardisation:

Weigh out accurately 0.1000 to 0.1500 grams of A.R. Sodium Chloride and dissolve in sufficient amount of water (50-100 ml). Add 1 ml of the indicator. Add the silver nitrate solution slowl from the burette, swirling the liquid constantly, until the red colour formed by the addition of each drop begins to disappear more slowly. Continue the addition dropwise until a faint but distinct change in colour occurs. This faint reddish-brown colour should persist after brisk shaking.

Preparation of potassium Chromate indicator solution:

Dissolve 5 gms of A.R. Potassium Chromate in 100 ml water.

$$\text{Normality of Silver Nitrate} = \frac{\text{Weight of NaC1} \times 1000}{\text{Volume of } AgNO_3 \times 58.46}$$

2. Preparation of 0.1N Potassium Thiocyanate:

Weigh out about 10.5 gms. of A.R. Potassium Thiocyanate and dissolve it in 1 litre of water.

Standardisation:

Pipette 25 ml of the standard 0.1N silver nitrate into a 250 ml conical flask adds 5 ml of 6N-nitric acid and 1 ml of ferric indicator solution. Run in the potassium thiocyanate solution from the burette. Continue the addition until one drop of the thiocyanate solution produces a faint brown colour, which no longer disappears upon shaking.

Normality of potassium

$$\text{Thiocyanate solution} = \frac{\text{Normality of } AgNO_3 \times 25}{\text{Volume of thiocyanate added}}$$

The ferric indicator solution consists of a cold, saturated solution of A.R. ferric ammonium sulphate in water (about 40% to which a few drops of 6N – nitric acid has been added.

3. <u>Preparation of 0.1N Sodium Thiosulphate solution :</u>

Weigh about 25.0 grams of A.R. Sodium Thiosulphate $Na_2S_2O_3$. 5 H_2O, dissolve in 1 litre of water. If the solution is to be kept for more than a few days, add 0.1 gram of Sodium Carbonate or 3 drops of Chloroform.

Standardisation:

Potassium Iodate can be used for the standardization of Sodium Thiosulphate. Weigh out accurately 0.14-0.15 gm of pure dry potassium iodate, dissolve it in 25 ml water, add 2 g of iodate-free potassium iodide and 5 ml of 2 N sulphuric acid. Titrate the liberated iodine with the thiosulphate solution when the colour of the solution has become a pale yellow, dilute to 200 ml with water, add 2 ml of starch solution and continue the titration until the colour changes from blue to colourless.

$$\text{Normality of Sodium Thiosulphate} = \frac{\text{Wt. of Pot.iodate} \times 1000}{\text{Vol. of thiosulphate} \times 35.67}$$

4. <u>Preparation of 0.1N Iodine:</u>

Dissolve 20 g. of iodate free potassium iodide in 30-40ml water. Weigh out about 12.7 g. of iodine on a watch glass on a rough balance and transfer it into the concentrated Potassium iodide solution. Shake until all the iodine has dissolved. Dilute it by water so that the total volume becomes 1 litre.

Standardisation:

Transfer 25 ml of the iodine solution to a 250 ml conical flask, dilute to 100ml and add standard sodium thiosulphate solution from a burette until the soln. has a pale yellow colour. Add 2 ml of starch solution and continue the addition of the thiosulphate solution slowly until the solution is just colourless.

$$\text{Normality of Iodine} = \frac{\text{Normality of } Na_2S_2O_3 \times \text{vol. of } Na_2S_2O_3}{25}$$

Preparation of Starch solution:

Make a paste of 1.0 g. of soluble starch with a little water, and pour the paste with constant stirring, into 100ml of boiling water and boil for 1 minute. Allow the solution to cool and add 2-3 g. of potassium iodide.

5. Preparation of 0.1N Sodium Hydroxide:

Dissolve about 4.0 g. (20 pellets) of Sodium Hydroxide in 1 litre of water.

Standardisation:

This is standardized using Potassium Hydrogen Phthalate. Weight out accurately about 0.6-0.7 g. of A.R. Potassium Hydrogen Phthalate into a 250 ml conical flask, add 75 ml of water and shake gently until the solid has dissolved. Titrate this against Sodium hydroxide, using Phenolphthalein as indicator. The end point is the appearance of pink colour.

$$\text{Normality of NaOH} = \frac{\text{Wt. of potassium Hydrogen Phthalate} \times 1000}{\text{Volume of NaOH} \times 204.22}$$

6. Preparation of 0.1N Hydrochloric acid:

Measure out by means of a graduated cylinder 9 ml of pure concentrated hydrochloric acid into a 1000 ml measuring cylinder containing about 500ml water. Make up to the litre mark with water and thoroughly mix by shaking.

Standardisation:

Weigh out accurately 0.2 g. of pure sodium carbonate into a 250 ml conical flask, dissolve it in 50-75 ml water and add 2 drops of methyl orange indicator. Titrate against hydrochloric acid from the burette until the colour of the methyl orange becomes orange or a faint pink.

$$\text{Normality of H C1} = \frac{\text{Weight of } Na_2CO_3 \times 1000}{\text{Volume of HC1} \times 53}$$

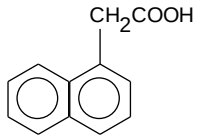
ALPHA NAPHTHYL ACETIC ACID

(ANA OR NAA)

Type of pesticide: Plant growth regulator

Molecular formula: $C_{12}\ H_{10}\ 0_2$ **Molecular weight:** 186.2

Structure:

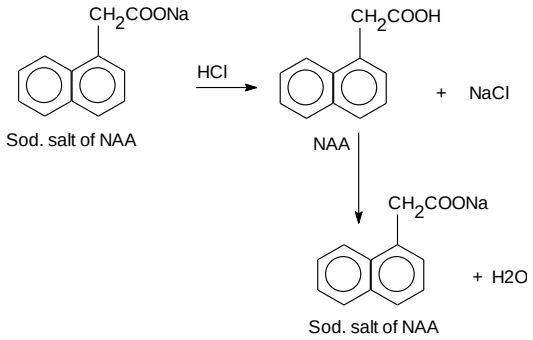


Registered products: Technical – 98%

S.L. – 4.5%

Principle: Alcohol present in the SL sample is evaporated off by heating on a hot water bath. The residue is redissolved in water and acidified to release NNA from sodium salt. NNA is extracted into solvent ether and washed with distilled water till free from mineral acid.After evaporation of ether, residue of NAA is redissolved in neutral alcohol and titrated against standard NaOH.

Reactions:



Calculations:

1 GE of NaOH = 1 gm of NAA

Therefore 1000 ml of 1(N) NaOH = 1 GE of NaOH

= 186.2 NAA

Therefore t ml of (n) NaOH $= \frac{186.2 \times t \times n}{1000}$

Therefore % AI $= \frac{18.62 \times t \times n}{W}$

Where W = weight in gms of the sample taken for analysis

T = titration reading

N = normality

Outline of the procedure:

(1) Technical grade sample: 3 gms of sample, weighed accurately is dissolved in 40 ml of neutralized methanol and titrated against 0.5 (N) standard NaOH. Solution using phenolphthalein as indicator.

(2) S.L. Sample: 10 gm of sample is weighed accurately in a beaker and heated on a boiling water bath till solvent is evaporated off and dry residue is obtained. This residue is dissolved in minimum quantity of water* and acidified using 50 ml of 5 pH buffer solution (prepared by dissolving 2.035 gms of citric acid and 2.924 gms of Na2 HPO4 in 200 ml water). The precipitate of ANA is extracted in a separating funnel, quantitatively into 50 ml of ether**. Separate the water layer and reextract it twice more using 50 ml ether each time. Combine all ether layers and heat the ether layer on boiling water bath till solvent is evaporated off leaving dry residue. Dissolve the residue in 50 ml of neutralized methanol and titrate with standard 0.1(N) Na OH using phenolphthalein as indicator.

Precautions:

*ANA has solubility in water to the extent of 420 mg/1. If large quantity of water is used, then there could be loss of AI.

**Ether is highly volatile, inflammable and explosive. Hence, extraction should be done in cooler room. The vapour of ether should be released from time to time during extraction to prevent development of pressure and bursting of separating funnel used for extractions. Further there should be no naked flame or spark while working with ether. Never heat ether layer on hot plate or gas burner.

(3) Identity test: UV spectroscopy:
(Described in subsequent sections)

Ref: IS : 13070 : 1991 - NAA – Technical

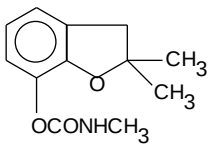
IS : 13138 : 1991 - NAA – SL.

CARBOFURAN

Type of pesticide: Systemic insecticide belonging to carbamate group

Molecular formula: $C_{12}H_{15}O_3N$ **Molecular weight**: 221.3

Structure:



Registered products: Tech i) 75%

ii) 90%

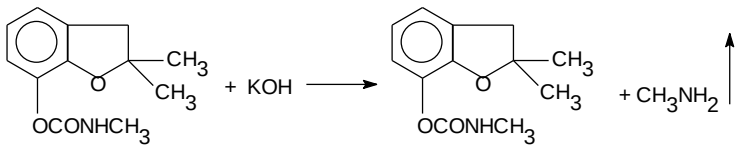
Formulations i) 3% CG

ii) 50% SP for Govt. use

Principle: One gm mole of carbofuran on alkaline hydrolysis in glycolic medium under reflux releases one gram mole of methl amine which is collected quantitatively; in 2% Boric acid solution containing of

standard HCl is equivalent to hydrogen ion absorbed by methyl amine, original green colour is restord bromo cresol green indicator. The boric acid methyl amine complex is then titrated with std. HCl. When the volme. Thus hydrogen ions absorbed by methyl amine is determined which is the basis for cabculation of AI.

Reactions:



$CH_3NH_2 + HCl \longrightarrow CH_3NH_3^+ Cl^-$

Additional product

Calculations:

1 gm. Eq. of HCl = 1 gm. Eq. of methyl amine

Or 1000 ml of 1 N HCl = 221.3 gm of carbofuran

$$\text{T ml of N HCl} = \frac{221.3 \times t \times N}{1000} \quad \text{gm of AI}$$

$$\text{Hence \% AI} = \frac{22.13 \times t \times N}{W}$$

Where t= titration reading in ml

N = normality of HCl

W = weight of sample taken

Outline of the procedure:

0.4 to 0.6 gm of AI weighed accurately; is hydrolysed for 60 minutes under relfux and stream of nitraogen with 50 ml of 2 N glycolic KOH. The amine liberated is absorbed in 150 ml of 2% Boric acid solution containing bromocresol green as indicator and estimated by titration against standard 0.1 N HCl.

Ref: IS: 11019 – 1984 for carbofuran technical

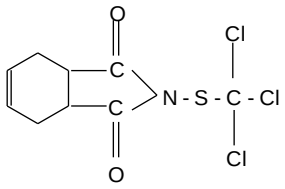
IS: 9360 – 1980 for granules.

CAPTAN

Type of pesticide: A contact / protective fungicide with eradicant properties, belonging to phthalimide group.

Molecular formula: $C_9H_8Cl_3NSO_2$ **Molecular weight:** 301

Structure:

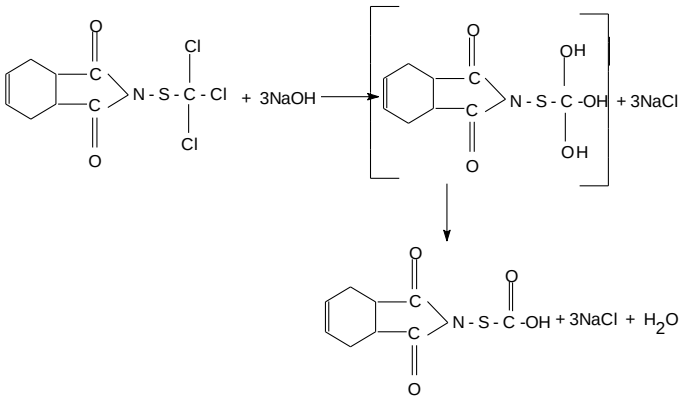


Registered products: Technical 90%
WP 50%
WS 75%
DS 75%

Principle:

Captan is hydrolysed with Sodium hydroxide in a medium of 1:1 mixture of methanol and acetone under reflux when 3 equivalence of ionic chloride are liberated per mole of a.i. The ionic chloride is estimated by Volhard's method. A.I content is calculated by multiplying per cent chloride (corrected free chloride), with a suitable convernsion factor.

Reactions:



$NaCl + AgNO_3$ ---$AgCl + NaNO_3$

Calculations:

1 gm equivalent of Silver nitrate = 1 gm equivalent of NaCl = 1/3 mole of a.i

1000ml of 1 N Silver nitrate = 100.3 gms of a.i

't' ml of N normal Silver nitrate = $\frac{100.3 \times t \times n}{1000}$

$$\% \text{ A.I} = \frac{10.03 \times t \times n}{W}$$

Multiply the above relationship by a suitable dilution factor, if necessary depending upon procedure.

Outline of the procedure:

1 gm a.i is taken in 250 ml volumetric flask and 125ml acetone is added. Shaken well to dissolve the a.i and make up to the mark with Methanol. To the 50ml aliquot, 50ml of 0.3N solution of aqueous Sodium hydroxide is added and refluxed for 1 hr.

After cooling add 5ml Hydrogen peroxide (30%) and boil gently for 10 min. If it's still coloured, repeat the above exercise. Destroy the excess alkalinity by using 1:1 Nitric acid and phenolphthalein. Add

10ml of Nitric acid in excess, 25ml standard solution of Silver nitrate and 5ml Nitro-benzene and 1 ml Ferric alum indicator and titrated against standard Potassium thiocyanate solution to a faint brick red colour (y ml).

Carry out a blank titration with 50ml aliquot of stock solution without hydrolysis (x ml) Calculate't' value by deducting y ml from x ml. and use dilution factor 5.

Precautions:

Captan is extremely unstable in solution. Hence blank titration for estimating free Chloride should be done in the beginning, within half an hour of solution preparation.

Ref:

IS: 11785-1986 for WP

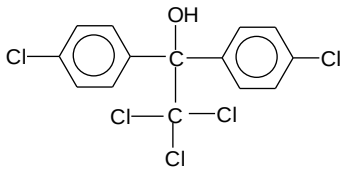
IS: 14251-1995 for Tech

DICOFOL

Type of pesticide: An acaricide with insecticidal action.

Molecular formula: $C_{14}H_9Cl_5O$ **Molecular weight:** 370.5

Structure:



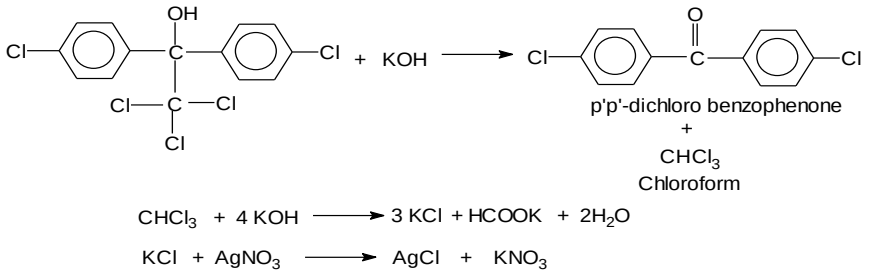
Registered products: Technical 82% min

EC 18.5%

Principle:

One mole of dicofol on hydrolysis with alkali under reflux yields 3 moles of ionic Chloride which is estimated by Volhard's method. Free Chloride is also estimated and the correction is applied.

Reactions:



$CHCl_3 + 4\,KOH \longrightarrow 3\,KCl + HCOOK + 2H_2O$

$KCl + AgNO_3 \longrightarrow AgCl + KNO_3$

Calculations:

3 gm equivalents of Silver nitrate = 3 gm equivalents of KCl = 1 gm mole of Dicofol

1 gm equivalent of Silve nitrate = 1/3 mole of a.i = 123.5 gms of a.i

1000ml of 1 N normal Silver nitrate = 123.5 gms of a.i

't' ml of normal solution $= \frac{123.5 \times t \times n}{1000}$

$$\% \text{ A.I} = \frac{123.5 \times t \times n \times 100}{1000 \times W} = \frac{12.35 \times t \times n}{W}$$

Outline of the procedure: 0.5 gms of a.i refluxed with 50 ml of 0.5 N ethanolic KOH for 90 minutes with 3 to 4 drops of ethanol as a seal. After cooling 50ml ethanol (95%) is added and swirled. The volume is made up to 300ml with distilled water, and ethanol is evoparated on water bath. Excess alkali is neutralized with 4N Nitric acid using Phenolphthalein as indicator and add 10ml Nitric acid in excess followed by 50ml of standard Silver nitrate (0.1N) is added. The excess Silver nitrate is back titrated with potassium thiocyanate.

Run a blank determination through all these steps of the procedure using all the reagents except sample.

Ref: IS: 5279-1978 for EC

IS: 5278-1969 for Technical

COPPER COMPOUNDS

Type of pesticide: Contact and protective fungicide

Molecular formula and Structural formula:

S.No.	Name	Structural formulae	Molecular formulae
1.	Copper oxy chloride	$3Cu(OH)_2CuCl_2$	$Cu_4H_6O_6Cl_2$
2.	Copper sulphate	$Cu\ SO_4\ 5\ H_2O$	$Cu\ H_{10}O_9S$
3.	Cuprous oxide	Cu_2O	Cu_2O

Registered products:

S.No.	Name	% A.I (Copper content)			
		Tech.	DP	WP	Oil based
1.	Copper oxy Chloride	57	4	50	40,50 & 56
2.	Copper Sulphate	25	--	--	--
3.	Cuprous Oxide	80	--	--	--
4.	Copper Hydroxide	--	--	50	--

Note: Since Copper is fungicidal component in the formulation, declared percentages are in terms of Copper content which is estimated.

Principle: The Copper oxy chloride which is insoluble in water is digested with 1:1 HCl (or conc. Nitric acid) to form water soluble cupric ions (i.e. Cu Cl_2) which when treated with Potassium iodide liberate one equivalent amount of Iodine. Thus liberated Iodine is estimated by standard Sodium thiosulphate solution using starch solution as indicator.

Reactions:

$$3Cu(OH)_2CuCl_2 + 2\,HCl \text{ ---- } 4CuCl_2$$

$$2CuCl_2 + 4\,KI \text{ ---- } 2CuI + 4\,KCl + I_2$$

$$I_2 + Na_2S_2O_3 \text{ ---- } 2\,NaI + Na_2S_4O_6$$

Calculations:

2 equivalents of Sodium thiosulphate = 2 equivalents of Iodine

= 2 equivalents of Copper

One equivalent of Sodium thiosulphate = 1 equivalent of Copper

= 63.5 gms of Copper

1000 ml of 1 N Sodium thiosulphate = 63.5 gms of Copper

't' ml of N normal Sodium thiosulphate $= \frac{63.5 \times t \times n \text{ gms.}}{1000}$

$$\% \text{ Copper content} = \frac{6.35 \times t \times n \times D}{W}$$

Where t = volume in ml of Sodium thiosulphate consumed

n = normality of Sodium thiosulphate

D = Dilution factor

W = Weight in gms of sample taken

Procedure: Weigh accurately a quantity of sample which contains 0.2 gm of a.i in a 250 ml iodine flask, add 2 to 3 ml conc. Nitric acid, 20 ml distilled water, allow the material to dissolve by shaking, then boil

for 3 to 5 minutes. Cool the flask, add 1 gm. of Urea and boil again for about 5 min. Then cool and add Sodium carbonate in small quantities with swirling until a faint precipitate or a blue colour appears. Add 10% acetic acid solution till the precipitate redissolves and solution becomes green. Add 5 ml of 30% KI solution and titrate the brown solution with 0.1N standard Sodium thiosulphate to a pale straw yellow colour. Add 1 ml of 1% starch solution, 1 to 2 gms of Potassium or Ammonium thiocyanate and continue the titration until the blue colour just discharged.

Precautions: The boiling or digestion of the sample with acid solution should be for sufficient duration to convert completely water insoluble Copper to water soluble Copper. Hence the boiling of the sample with acid may be done for longer duration if necessary.

References:

IS 1486-1978 for Copper Oxychloride Technical

IS 1506-1977 for Copper Oxychloride DP

IS 1507-1977 for Copper Oxychloride WP

IS 1665-1977 for Cuprous Oxide WP

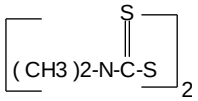
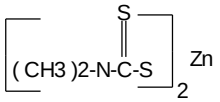
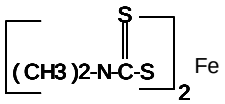
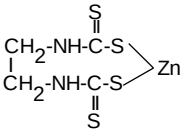
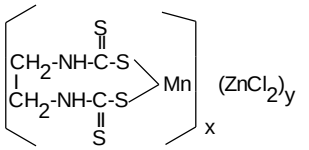
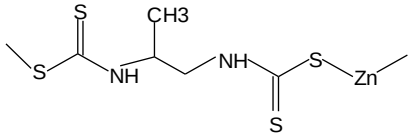
IS 1669-1960 for Cuprous Oxide DP

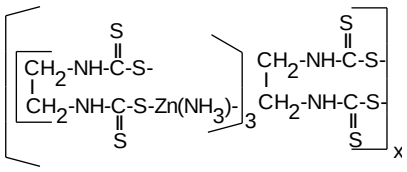
IS 1682-1973 for Cuprous Oxide Technical

IS 12873-1990 for Copper Oxychloride OP (oil based)

DITHIOCARBAMATES

Type of pesticide: They are protective and contact fungicides belonging to group of derivatives of dithiocarbamic acid

Name	Molecular formula	Molecular weight	Structure
A. Alkyl dithiocarbamates i. Thiram	$C_6H_{12}N_2S_4$	240.4	
ii. Ziram	$C_6H_{12}N_2S_4Zn$	305.8	
iii. Ferbam	$C_9H_{18}FeN_3S_6$	418.9	
B. Alkylene dithiocarbamates i. Zineb	$C_4H_6N_2S_4Zn$	275.74	
ii. Mancozeb	$C_4H_6N_2S_4Mn\ Zn$		
iii. Propineb	$C_5H_8N_2S_4Zn$	290	

iv. Metiram	$[C_{16}H_{33}N_{11}S_{16}Zn_3]_x$	$(1088.7)_x$	

Registered products:

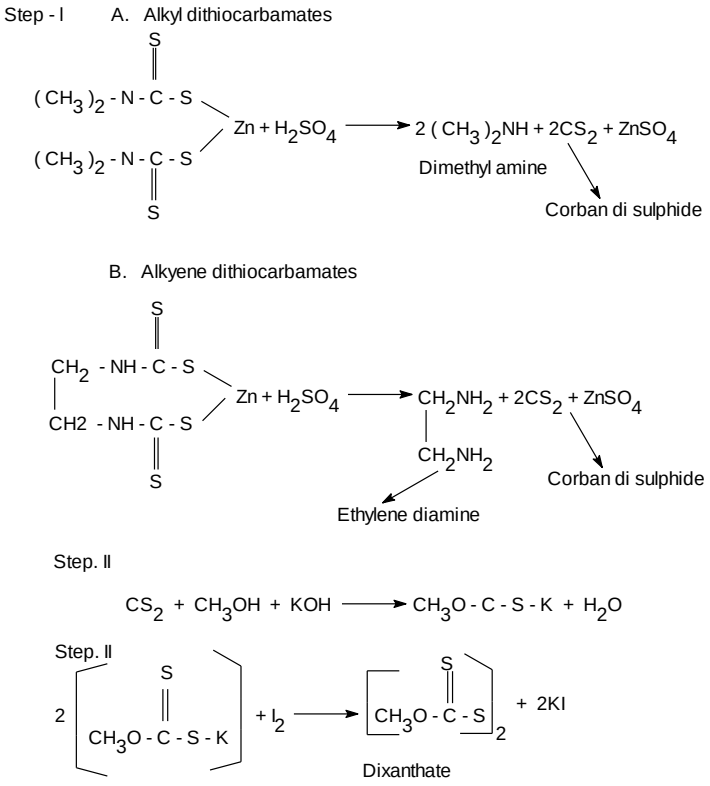
S.No.	Name	% A.I in different products				
		Tech	WP	CS	DS	WS
1.	Ferbam	81	75	-	-	-
2.	Mancozeb	85	75	-	-	-
3.	Thiram	95&98	-	-	75	75
4.	Zineb	80	75	-	-	-
5.	Ziram	95	80	27	-	-
6.	Propineb	80	70	-	-	-
7.	Metiram	--	75	--	--	--

*Combination of Mancozeb and Metalaxyl (containing 64% Mancozeb) as WP is also registered.

CS: Colloidal suspension DS: Dry seed dressers WS: Wet seed dressers

Principle: One mole of dithiocarbamate on decomposition with hot boiling dilute acid liberates two moles of carbondisulphide (3 moles in case of Ferbam, 8 moles in case of Metiram) which, after removal of Hydrogen sulphide (produced from impurities) by absorption in Cadmium sulphate/Lead acetate solution, is converted quantitatively in methanolic KOH to Potassium xanthate. This Potassium xanthate is quantitatively estimated in acetic acid medium by titration against standard Iodine solution using starch solution as indicator.

Reactions:



In the case of alkyl bisdithiocarbamates, Ziram, Thiram, Ferbam. A.I content can also be estimated on the basis of dimethylamine content.

Calculations:

a) Thiram, Ziram, Mancozeb, Propineb and Zineb
2 equivalents of Iodine = 2 equivalents of CS2 = 1 mole of a.i

1 equivalent of Iodine = ½ mole of a.i

1000ml of 1 N Iodine solution = ½ mole of a.i

't' ml of N normal Iodine solution = $\frac{\text{mol. Wt. x t x n}}{2 \times 1000}$ gms of a.i

% A.I = $\frac{\text{mol.wt. x t x n}}{20\ W}$

Where 't' = volume of standard Iodine solution consumed by the sample.

N = normality of Iodine

W = weight in gms of sample taken.

b) Ferbam:

3 moles of CS_2 = 1 mole of a.i

1 mole of CS_2 = 1/3 mole of a.i

1 equivalent of Iodine = 1 mole of $CS_{2=}$ 1/3 mole of a.i

% A.I = $\frac{\text{mol.wt. x t x n}}{30W}$

c) Metiram:

8 moles of CS_2 = 1 mole of a.i

1 mole of CS_2 = 1/8 mole of a.i

1 equivalent of Iodine = 1 mole of $CS_{2=}$ 1/8 mole of a.i

$$\% \text{ A.I} = \frac{\text{mol.wt. x t x n}}{80W}$$

Procedure:

Take 100 to 150 ml of 1 N Sulphuric acid in reaction kettle. Take 25ml Lead acetate (Zinc acetate in case of Propineb) in first absorber and keep it hot (approximately 70 deg.C). Take 25ml 2 N methanolic KOH in the second absorber keep it chilled below 10 deg.C.

Assemble the train of apparatus. Connect it to the vacuum line and maintain a regular flow of air through the assembly. Heat the acid in the kettle and keep it boiling. Transfer sufficient quantity of sample accurately by weighing 0.2 to 0.3 gms of a.i into the reaction flask. Digest for one hour and forty-five minutes. After the digestion is complete, quickly disconnect the KOH trap. Transfer quantitatively with distilled water the contents of the trap in to a 500ml iodine flask. Neutralise it with 30% acetic acid using phenolphthalein indicator, add 1ml excess acid and titrate immediately with 0.1N Iodine solution using starch solution as indicator. End point is colourless to blue.

In case of Thiram the acid used is acetic acid (1:1) and Zinc oxide mixture instead of 1.1N Sulphuric acid and 19.5% Cadmium sulphate solution is used instead of Lead acetate in the trap.

Precautions:

1. The final titration of Xanthate against standard Iodine solution should be done very rapidly within two to three minutes because xanthate is unstable in neutral or faintly acidic medium.
2. The sample should be brought in contact with only hot acid not with cold acid, because in cold acid only one and not two moles of carbon disulphide is released per mole of a.i.

References:

IS 3898-1981 for Zineb Technical

IS 3899-1981 for Zineb WP

IS 3900-1975 for Ziram Technical

IS 3901-1975 for Ziram WP

IS 4320-1982 for Thiram Tech

IS 4766-1982 for Thiram WP

IS 4783-1982 for Thiram seed dressing formulation

IS 8707-1978 for Mancozeb Technical

IS 8708-1978 for Mancozeb WP

IS 11010-1984 for Ziram colloidal suspension

IS 12501-1988 for Ferbam Tech

IS 12562-1988 for Ferbam WP

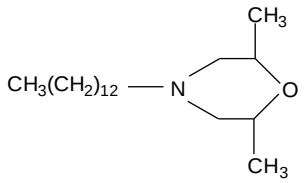
IS 13692-1993 for Mealaxyl-Mancozeb WP

TRIDEMORPH

Type of pesticide: Systemic fungicide belonging to morpholine group

Molecular formula: $C_{19}H_{39}NO$ Molecular weight: 297.5

Structure:



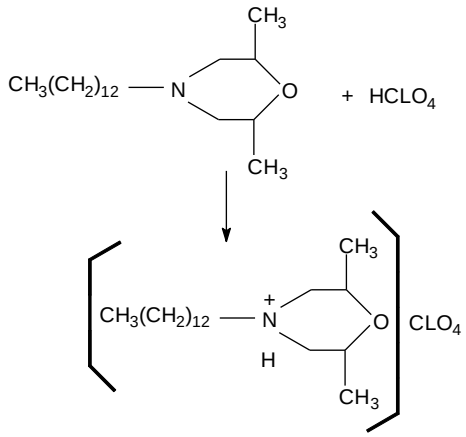
Registered products: Tech. 95%

EC 75%

Method of Analysis: Acid base titration in non-aqueous medium

Principle: AI is titrated against perchloric acid in aqueous medium like acetic acid, when one gram equivalent perchloric acid is consumed b one gram mole of a.i.

Reactions:



Calculations:

One-gram eqvt. Of perchloric acid = 1 gm mole of a.i. 1000ml of 1N perchlorc acid

= 297.5 gms. of a.i

$$\% \text{ a.i} = \frac{29.75 \times t \times n}{W}$$

Where t = Titration reading in ml. of std. perchloric acid

N = normality of perchloric acid

W = weight in gms. of sample

Outline of the procedure:

Weigh accurately the sample equivalent to 1gm a.i in a flask. Dissolve in 50 ml acetic acid. Add 10ml acetic anhydride. Keep it for five minutes then titrate it against std. perchloric acid.

Ref:

IS: 9667-1980 for Tridemorph Tech.

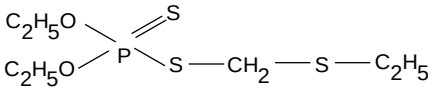
IS: 9656-1980 for Tridemorph EC.

PHORATE

Type of pesticide: Systemic insecticide belonging to organophosphorus group

Molecular formula: $C_7H_{17}O_2PS_3$ **Molecular weight:** 260.4

Structure:

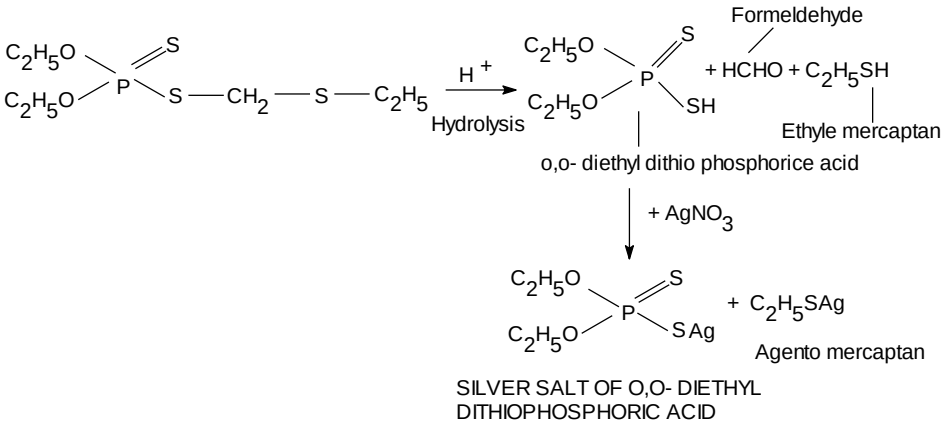


Registered products: Technical 90%

CG 10%

Principle: One mole of a.i on hydrolysis in acidic medium yields one mole each of diethyl dithio phosphoric acid and ethyl mercaptan, which consume two equivalents of Silver nitrate. From the quantity of Silver nitrate consumed by a.i alone, obtained after correcting for impurity interferences, a.i content is calculated.

Reactions:



Calculations:

Gm equivalents of Silver nitrate = 1 gm mole of a.i

1gm equivalent of Silver nitrate = ½ mole of a.i = 130.2 gms

1000ml of 1 N Silver nitrate = 130.2 gms of a.i

't' ml of N normal Silver nitrate = $\frac{130.2 \times t \times n}{1000}$

$$\% \text{ A.I} = \frac{13.02 \times t \times n}{W}$$

Where t = volume in ml of standard Silver nitrate solution consumed by a.i alone

N = normality of Silver nitrate

W = weight of sample taken

Outline of the procedure:

Step I Preparation of sample stock solution

Sufficient quantity of the sample equivalent to 2.5 gms of a.i is extracted with acetone quantitatively and made up to 250ml in a volumetric flask.

Step II Estimation of a.i and impurities

To a flask containing 150 ml water acidified with 5 drops of Nitric acid, add by pipette 50ml of standard Silver nitrate solution and maintain at 50^0C. After attaining constant temperature, add by a pipette 50ml of sample stock solution, and carry out hydrolysis at 50^0C. for 15 minutes. Titrate the excess silver nitrate against standard thiocyanate solution using 1 ml 10% Ferric nitrate solution as indicator (x ml).

Step III Estimation of impurities

Transfer by pipette 50ml of sample stock solution in to a 500 ml separating funnel containing 150ml enzene. Extract this solution thrice, each time by 50ml of 0.1N KOH in aqueous solution of 10% Potassium nitrate followed by one washing with 50ml distilled water. Collect the aqueous layer and acidify with concentrated Nitric acid till acidic to Congo red paper. Add 5 drops of acid in excess and keep it in waer bath maintained at50^0C till contents of the flask attained constant temperature. Then add by pipette 5 ml standard Silver nitrate and keep it in the water bath at 50^0C for 15 minutes. Then cool to room temperature and titrate the excess Silver nitrate against std. thiocyanate solution using 1 ml of 10% Ferric nitrate as indicator (y ml)

Calclate‘t’ value as shown below.

‘t’ = $(50F_1 – xF_2) – (5F_1 – yF_2)$

Where F1 = ratio of normality of Silver nitrate to 0.1

F2 = ratio of normality of thiocyanate to 0.1

Use a dilution factor of 5 while calculating final result.

Ref:

i) IS: 7976-1976 for Technical
ii) IS: 9359-1995 for Gr.

SULPHUR

Type of pesticide: Contact and protective fungicide, acaricide.

Molecular formula: S **Molecular weight:** 32.06

Registered products:	Technical	99.5%
	DP	85%
	WP	70% & 80%
	CS	40%
	Solution	22%

Principle: Sulphur is converted to thiosulphate by refluxing with Sodium sulfite. Excess Sodium sulfite is destroyed by Formaldehyde and then the thiosulphate is estimated by titration with standard Iodine solution using starch solution (1%) as indicator.

Reactions:

$$S + Na_2SO_3 \longrightarrow Na_2S_2O_3$$

$$2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6$$

Calculations:

2 gm equivalent of Iodine = 2 gm mole of Hypo

= 2 gm mole of Sulphur

1 gm equivalent of Iodine = 1 gm mole of sulphur = 32.06 gms of Sulphur

1000ml of 1 N Iodine solution= 32.06 gms of Sulphur

't' ml of N normal Iodine solution = $\frac{32.06 \times t \times n}{1000}$

If dilution is made the, Sulphur content % by mass = $\frac{3.2\ 06 \times t \times n \times d}{W}$

Outline of the procedure: Weigh accurately sufficient quantity of sample to contain 0.1 gm of Sulphur in a 250ml flat bottom flask add 30ml to 40ml distilled water, 2 gms of sodium sulfite and few drops of liquid paraffin. Reflux for 40 minutes, cool the flask and remove, add 10ml formaldehyde, 10ml acetic acid solution and titrate immediately with standard Iodine using freshly prepared Starch solution. ('t' ml).

Carry out a reagent blank without sample as per above procedure.

References:

IS 6444-1979 for Sulphur DP

IS 3383-1982 for Sulphur WP

ALUMINIUM PHOSPHIDE

Type of pesticide: Phosphine (fumigant) releasing product used in rodent control and stored grain protection.

Molecular formula: AIP **Molecular weight:** 57.96

Registered products: Tablets 56%

55%, 57%, 60% for export

Reactions: $2AIP + 3\ H_2SO_4$ Nitrogen atmosphere > $Al_2(SO_4)_3 + 2PH_3$

$2PH_3 + 8(O)$ ---------- > $2\ H_3PO_4$

Calculations:

16gm equivalents of Oxygen = 2 gm mole of Phosphine = 2 gms mole of a.i

1 gm equivalent of Oxygen = 1/8 gm mole o a.i = 7.24 gms of a.i

1000ml of 1 N solution = 7.24 gms of a.i

't' ml of N normal solution $= \frac{7.24 \times t \times n}{W}$

% A.I $= \frac{0.724 \times t \times n}{W}$

Where 't' = quantity in ml of oxidant solution

n = normality

W = weight in gms of sample

Outline of the procedure:

0.2 to 0.3 gms of material is decomposed with 10% Sulphuric acid in inert atmosphere. Phosphine liberated is swept by continuous stream of Nitrogen in to 200ml of standard 0.5N Potassium permanganate solution measured accurately and distributed in three different gas absorption bottles. Initially decomposition is carried out for 30 to 45 minutes at room temperature. Then the flask is heated on water bath maintaining at 60 deg.C for another 15 minutes. Transfer quantitatively permanganate solution into a 1 lit. beaker and add 200ml (sufficiently excess Oxalic acid) standard Oxalic acid (0.5N) solution. Titrate the excess Oxalic solution against standard Potassium permanganate solution.

% A.I $= \frac{0.724 \times (200 + A)\, N_1 - (200\, N_2)}{W}$

Where A = sample titration reading

N1 = normality of KMnO4

N2 = normality of Oxalic acid

W = Weight of sample

Precautions:

1. Phosphine is highly toxic and inflammable gas. Hence decomposition should be carried out only in inert atmosphere.
2. Introduce sample into reaction flask only after removing air by sweeping apparatus with Nitrogen gas for 15 minutes.

References: IS 6438-1980

ZINC PHOSPHIDE

Type of pesticide: Acute oral rodenticide

Molecular formula: Zn_3P_2

Molecular weight: 258

Registered products: Technical 80% min.

-do- 20% (for export)

1% & 2% (household products)

Principle:

One mole of a.i on acidic decomposition in an inert atmosphere yields two moles of phosphine which is oxidized by absorption in Potassium permanganate solution. From the quantity of permanganate solution consumed by phosphine, quantity of a.i is calculated.

Reactions: $Zn3P2 + 3\ H_2SO_4$ Nitrogen atmosphere > $3\ ZnSO_4 + 2PH_3$

$2PH_3 + 8(O)$ ---------- > $2\ H_3PO_4$

Calculations:

16 gms equivalent of (O) = 2 gm mole of Phosphine = 1 gm mole of a.i

1 gm equivalent of (O) = 1/16 gm mole of a.i = 16.13 gms of a.i

1000 ml of 1 N solution = 16.13 gms of a.i

't' ml of N normal solution = $\frac{16.13 \times t \times n}{1000}$

% A.I = $\frac{1.613 \times t \times n}{W}$

Where t = Quantity in ml of oxidant solution

N = Normality of oxidant solution

W = Weight in gms of sample

Outline of the procedure:

Weigh accurately 0.5 gms of sample and analyse as per method outlined under aluminium phosphide described earlier.

In order to remove adulteration by inorganic sulphides, phosphine carried by nitrogen is passed through 100ml of 1 N Sodium hydroxide solution before passing through Potassium permanganate solution.

Reference: IS 1251-1988 for Technical

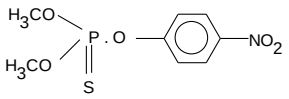
METHYL PARATHION

Type of pesticide: Contact insecticide belonging to organophosphorus group

Molecular formula: $C_8H_{10}O_5NPS$

Molecular weight: 263.21

Structure:



Registered products: Technical 92%

Technical concentrate 80%

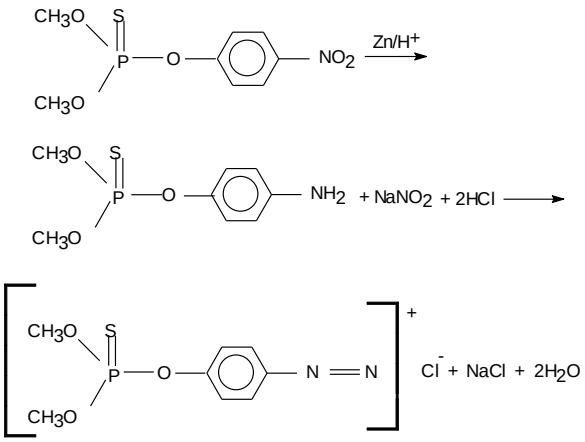
DP 2%

EC 50%

Principle:

The phenolic impurities mainly para nitrophenol are separated from a.i by washing the ethereal sample solution with dilute chilled sodium carbonate A.I is reduced to amino derivative which is estimated by diazotization titration.

Reactions:



Calculations:

1 gm equivalent of Sodium nitrite = 1 gm mole of a.i = 263.21 gms of a.i

1000 ml of 1N Sodium nitrite = 263.21 gms of a.i

't'ml of n normal Sod. Nitrite = $\frac{263.21 \times t \times n}{1000}$ gms of a.i

$$\% \text{ A.I} = \frac{26.321 \times t \times n}{W}$$

Where 't' = ml of standard solution of Sodium nitrite consumed by the sample

N = normality of sodium nitrite

W = weight of the sample

Outline of the procedure:

1 gm a.i * equivalent of sample is dissolved in 100ml ether contained in a separating funnel and wash repeatedly, very carefully using each time 10 to 20 ml of chilled 1% Sodium carbonate solution till the aqueous solution is free from yellow colour. Then ether layer is taken in to a beaker and mixed with acid mixture (9:1 Acetic acid and HCl), add about 2 gms of Zinc dust or granules and heat it on water bath for about 1 hr. and the solution in the beaker is colourless. Destroy the excess Zinc by adding conc. HCl and heating. Cool and chill it to about O^0C, add 5 gms of Sodium bromide or Potassium bromide and titrate it slowly and carefully aganst standard Sodium nitrite solution using starch iodide paper as external indicator.

* AI is quantitatively extracted from solid formulations with ether before washing.

References:

IS 2570-1980 for Technical

IS 9372-1980 for Technical concentrate

IS 8760-1978 for DP

IS 2865-1978 for EC

Physico-chemical Characteristics

Even though A.I content is the most important parameter in a formulation, there are certain other parameters which have profound influence on field bio efficacy of the formulation and hence are as much important as A.I content. These parameters are of physico-chemical nature. So testing of samples for A.I content alone is not sufficient measure of quality of the product. This does not reflect on field performance when used at any time within expiry date and hence is incomplete analysis. All samples have to be tested for physico-chemical parameters also after estimation of a.i content. Some of the important physico-chemical parameters are listed below.

Sl. No.	**Parameter**	**Application for**
1.	Acidity/Alkalinity	All samples
2.	Particle size	DP/WP/Gr
3.	Bulk density	DP
4.	Suspensibility	WP
5.	Emulsion stability	EC
6.	Water runoff test	Coated Gr
7.	Attrition test	Coated Gr
8.	Flash point	EC

BIS has compiled and published methodologies for testing of these characteristics (IS 6940-1982, Methods of test for pesticides and their formulations). For the purpose of training a beginner, simplified and condensed methodologies are given in this section.

Acidity and Alkalinity

a) Preliminary qualitative test:

Mix 0.5gms or ml of sample with 1ml water and find out whether the sample is acidic or alkaline by using litmus or pH paper.

b) Quantitative analysis:

i. For liquid formulations: 10gms sample, dispersed or dissolved in 100ml of water is titrated against 0.05 N Sodium hydroxide or HC1 using either bromo cresol purple (1% alcoholic solution) methyl red (1% aqueous solution).

Alternatively one may determine the end point using pH meter in which case end point is pH of mixture of 50ml of acetone and 5ml of standard buffer solution (100ml of 2N acetic acid 100ml of 1N Sodium hydroxide).

ii. For solid formulation: 10gms of sample is mixed with 25ml acetone and warmed gently on a hot water bath. Cool, dilute it with 75ml water and allow it to stand for 1 hour with intermittent agitation. Decant or filter the liquid and titrate.

iii.

c) Calculations:

$$\text{Acidity (as \% Sulphuric acid)} = \frac{4.9 \times t \times n}{W}$$

$$\text{Alkalinity (as Sodium hydroxide)} = \frac{4.0 \times t \times n}{W}$$

Particle Size

a) DP: 10gms of sample is weighed and transferred to a test sieve (75 micron or 200 mesh) and shaken in a rotap shaker for 20 min. Material passing through or retained on the sieve is weighed.

b) WP: 10gms of sample is mixed with 100ml of water, allowed to stand for half minute and agitated gently with glass rod for half minute. Transfer the slurry on to sieve (75 or 45 micron as required) rinsing with tap water. Pass running water till all solids pass through. Weigh the material retained on the sieve after drying in a tarred gooch crucible.

c) Granules: 100gms of sample is sieved for 15 min. through set of sieves of upper and lower particle size declared on the label.

d) Calculations:

$$\text{Material passing through the sieve} = \frac{W1-W2 \times 100}{W1}$$

Where W1 = Weight in gm of sample

W2 = Weight in gm of sample retained on the sieve.

Bulk Density

a) Find the weight of 100ml of sample filled in a measuring cylinder and calculate the density D^1
b) Stopper and gently tap or drop the cylinder 20 times on a felt pad or any other soft surface through a height of 15 cm. Measure the volume and calculate the density D^2
c) Requirement: D^2 should not be more than $(D^1 + 0.6D^1)$

Suspensibility:

Definition: Percentage of A.I held in the suspension of known concentration prepared in standard hard water, after keeping for 30 minutes to stand for sedimentation if any.

Step - I Preparation of suspension

Weigh in a beaker enough quantity of sample to give (on mixing with water) 250ml of suspension containing 0.5% or 2.5% a.i as per relevant specification requirement. Add about 50ml of standard hard water of 342 ppm. Of Calcium carbonate (Dissolve 304 mgs of Calcium chloride and 139mgs of magnesium chloride in 1 lit. of water) and stir gently with glass rod. Transfer the slurry quantitatively in to a 250ml Stoppard measuring cylinder using standard hard water and diluting it up to the mark, Stopper the cylinder and turn it quickly (30 times in a minute end over end. Allow the suspension to settle for 30 minutes.

Step - II Sucking the suspension

Gently remove by mild suction 225ml of suspension without disturbing bottom 25ml (within 15 seconds) and discard it.

Step - III Estimation

Transfer the bottom 25ml of suspension/sediment and estimate A.I content by relevant method given in the specification.

Step. IV Calculation

$$\% \text{ Suspensibility} = \frac{1000\,(W^1 - W^2)}{9\,W^1}$$

Where W^1 = Weight in gm of a.i taken for test

W^2 = Weight of a.i estimated in the bottom 25ml sediment

Emulsion Stability

2 or 5ml of sample is emulsified with standard hard water and diluted to 100ml in a Stoppard measuring cylinder. Allow it to stand for one hour on a vibration free bench. Observe the emulsion for creaming at the top and sediment at the bottom and measure the same, which should not exceed 2ml.

Water Runoff Test

Soak 10gms of sample for 15 min. in 50ml of water contained in a burette or glass tube fitted with a stopcock. After the specified time drain off the water and estimate the a.i contained in the water.

Attrition Test

Determine the a.i in the residue on 75 micron sieve after shaking 100gms of sample for 2 hrs. on rotap sieve shaker.

Flash point

Fill the cup (sample holder) of Abel's closed cup flash point apparatus with the sample up to the recommended level. Close the cup. Set up the test flame as very small dot or button. Measure the temp. of the sample. Now introduce the test flame in to empty space above the surface of sample in the closed cup and check whether the vapour contained in the empty space flashes or not. By repeating the above process at different temperatures (by either heating or cooling the sample). The flash point of the sample should be above 24.5 deg.C.

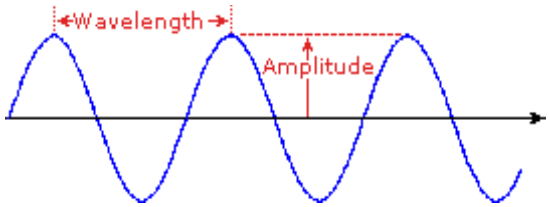
Spectroscopy

Spectroscopy is the science related to the study of interaction of radio magnetic energy with the atoms, ions or molecules. When the electromagnetic radiation falls on the matter part of the radiant energy is absorbed and remaining is transmitted are scattered or reflected. If the scattered or reflected radiation is kept at least and constant, the measurement of transmitted radiation to that of incident radiation is possible and the amount of absorbed radiation can be deduced out of it. The relationship can be plotted to obtain spectrum called absorption/transmission spectrum. This type of study is known as molecular absorption Spectroscopy. The instruments used for this study are Spectrophotometers .

The excited atoms ,ions or molecules on irradiation with a beam of electromagnetic radiation, relax to lower energy levels by giving up their excess energy as photons (fluorescence or phosphorescence) to attain stability and such emitted radiant energy can be measured and plotted as a

spectrum which is called emission spectrum and the study the emission spectroscopy. The instruments used for this study are Fluorometers and Spectrofluorometers.

We will confine our discussion to the molecular absorption spectroscopy in this chapter.



Important terminology used :

1.Wave length : maybe defined as distance between two troughs or crests of a wave (denoted as lambda) and expressed in micron( ), millimicron/ nanometer(nm)

2.Micron: 10^{-4} Cm

3.Millimicron/Nanometer:10^{-7}Cm
4.Wave number ( ): is defined as number of waves per centimeter. 1/ wavelength.
5.frequency ( ) may be defined as number of waves that passes through a unit in unit time.

$$V = C/\lambda$$

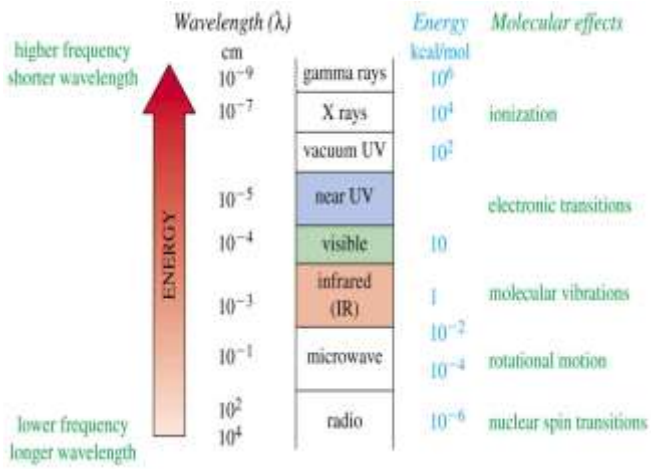
6.velocity of light: $C = 3 \times 10^{10}$ Cm/sec

7.E = Energy
8.Planks constant = h = 6.623×10^{-27} erg sec/mol
9.Monochromatic light: The light which consists of polychromatic/multi wavelength radiation is resolved using a prism or diffraction grating to give single wavelength radiation which is called monochromatic light.

The electromagnetic radiation consists of a broad spectrum of radiations which are named on the basis of their wave length or frequency as Gamma , X rays, Ultraviolet, Visible, Infrared rays and Hertzian, Radio waves and Audible frequencies.

The radiation which are important for absorption spectroscopy are tabulated here under.

Sl.No	Type of radiation	Wave length	Energy
1.	Ultra violet (UV)	200 to 375 nm	More
2.	Visible (Vis.)	400 to 760 nm	Medium
3.	Infrared (IR)	1 micron to 50 micron	Less



Every radiation has got a defined amount of energy which is given by the relationship E = hC/ wavelength, where C is the speed of light and h planks constant whose product is constant and hence the energy is associated with each wave length is unique and characteristic of it. The type of change that is brought about in the energy level of the molecule by each monochromatic light is specific as that of their energy. They may be listed as follows,

Sl.No	Type of radiation	Type of energy level change
	Infra red (IR)	Change in vibrational and rotational energy level
	Visible (Vis)	Change in electronic level leading to excitation.
	Ultra violet (UV)	Change in electronic level leading to excitation.

Application : The molecular absorption spectroscopy can be applied for the purpose of qualitative and quantitative analysis.

Qualitative Analysis:

As each molecular orbital in the molecule require a specific energy to excite to the higher energy level, it absorbs a specific electromagnetic radiation or monochromatic light and becomes the qualitative parameter for identification. Usually it is the lambda maximum and the shape of the spectrum of the unknown compared with a known pure reference standard spectrum or compared with the spectra available in the literature for its identification.

Quantitative analysis:

The quantitative analysis is based on two laws namely (i) Lamberts law. and (ii) Beer's law and the absorption is proportional to the concentration of the solute present in the solution within the limits of the above laws.

Lamberts law : states that when monochromatic light passes through a transparent medium, the in intensity of the emitted light decreases exponentially as the thickness of the absorbing medium increases arithmetically, or that any layer of given thickness of the medium absorbs the same fraction of the light incident upon it.

Beer's law: state that the intensity of a beam of monochromatic light decreases exponentially as concentration of absorbing substance increases arithmetically.

Combining both the laws we have Beer-Lamberts law , $A = ecl = \log I_0/I_t = \log 1/T = - \log T$

Where A= absorbance

e=molar absorption coefficient (when concentration is expressed in mole dm^{-3})

c= concentration

l= path length

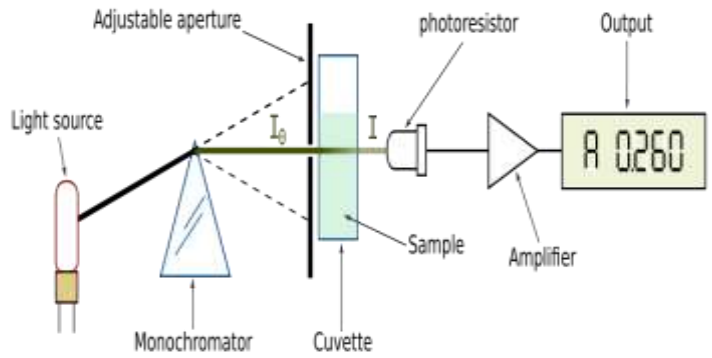
For any given molecule the molar absorption coefficient is constant and if a constant path length cell is used then A is proportional to the concentration. And this principle is used for quantitative estimation of the substances of known by comparing with the response/absorbance of a pure reference standard in a single point calibration method or can draw a calibration curve of the reference standard and read out the unknown concentration from its absorbance.

Deviations from the Beer's law:

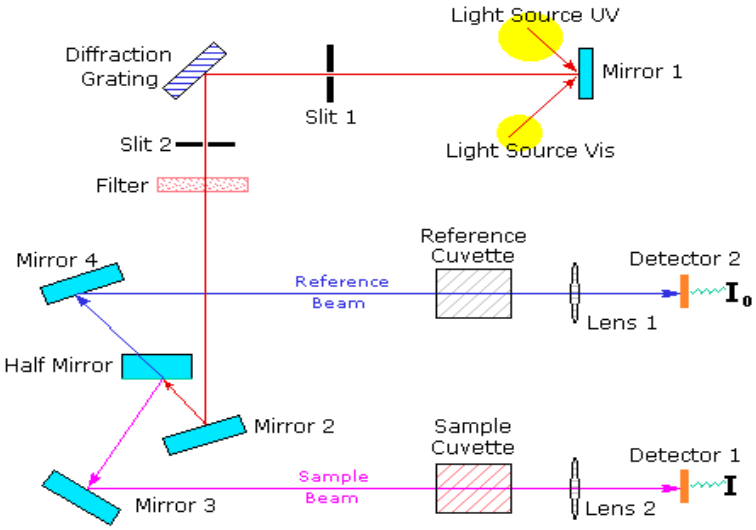
1. It is most reliable when radiation is monochromatic.
2. It is valid or linear relationship is observed only up to a particular concentration. Hence quantitative analysis should be done within this range of linearity validity.
3. Solutions should be absolutely clear, even trace quantity of fine particles floating in solution will give erratic results.
4. The cells should have absolutely transparent surfaces because Beer-Lamberts law assumes minimum reflection of light.

UV-Visible Spectrophotometer

The schematic diagram of the UV-Visible Spectrophotometer is as follows.



Single beam spectrophotometer



Double beam spectrophotometer

The UV-Visible Spectrophotometer consists of mainly a source, monochromator, Beam splitter, sample path and reference path, cell holders and cells and the detector. The signal from the detector is fed to an internal microprocessor or external personal computer, which will analyse the data, present in the desired spectral form and generate the reports.

Source of light : The UV-Visible Spectrophotometer is equipped with two types of sources to give the radiant energy to cover both the ranges of UV and Visible as no single source will be able to cover both the ranges. The deuterium lamp is provided to supply the UV range of light, where as the tungsten lamp provides the visible range**.**Both the lamps are aligned in such a way to give continuous spectrum of UV and Visible range and the change over takes place automatically without any hindrance in scanning and recording the complete range from 200 nm to 1100nm.

Monochromator: Earlier days the colorimeters were manufactured using filters as monochromators and need to be changed depending on the requirement of the sample under investigation and the data arrived are not so accurate.

Wavelength of absorbance maximum (nm)	**Color Absorbed**	**Color Remaining**
380-420	Violet	Green-yellow
420-440	Violet-blue	Yellow
440-470	Blue	Orange
470-500	Blue-green	Red
500-520	Green	Purple
520-550	Yellow-green	Violet
550-580	Yellow	Violet-blue
580-620	Orange	Blue
620-680	Red	Blue-green
680-780	Purple	Green

The modern spectrophotometers are manufactured using either a prism or dispersive grating as the monochromator, which will resolve spectrum to give an accuracy of 0.1nm. The monochromator is

made to move on rotating base with the help of a motor to project the required wave length through the slit.

Beam splitter:

The spectrophotometers are manufactured as single beam and double beam instruments. The single beam instruments are used in routine testing facilities where much accuracy is not involved and they are less expensive than the double beam spectrophotometers. The double beam spectrophotometers employ a design where the monochromatic light beam is split in to two and allowed to pass through two paths one for reference solution and another for the sample solution. The two beams are made to reach the detector simultaneously by employing a chopper, resulting in accurate measurement of the transmission or absorption of light that takes place in the sample solution.

Detector:

The most popular detector used in the Spectrophotometers is photomultiplier tube, which converts the energy of the photons which falls on it in to an electrical energy and fed to the amplifier. The amplifier transmits the data though the microprocessor connected to it which displays the transmission or absorption values or it can plot the spectrum in case of scanning.

Photo Diode Array detector:

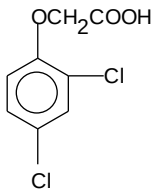
The latest of the detector is the Photo Diode Array detector which simultaneously collects the data points through out the scanning wave lengths and process electronically to give the spectrum within no time. This enables to identify the unknowns easily with much faster rate and can simultaneously obtain the spectrum and absorption data for different wavelengths in a single run. This particular detector is employed successfully in HPLC to monitor the unknowns where the quantity of sample available is much less and in dynamic state.

DICHLOROPHENOXY ACETIC ACID (2,4-D)

Type of pesticide: Selective translocated herbicide belonging to phenoxy group

Molecular formula: $C_8 H_6 O_3 Cl_2$ Molecular weight: 221

Structure:



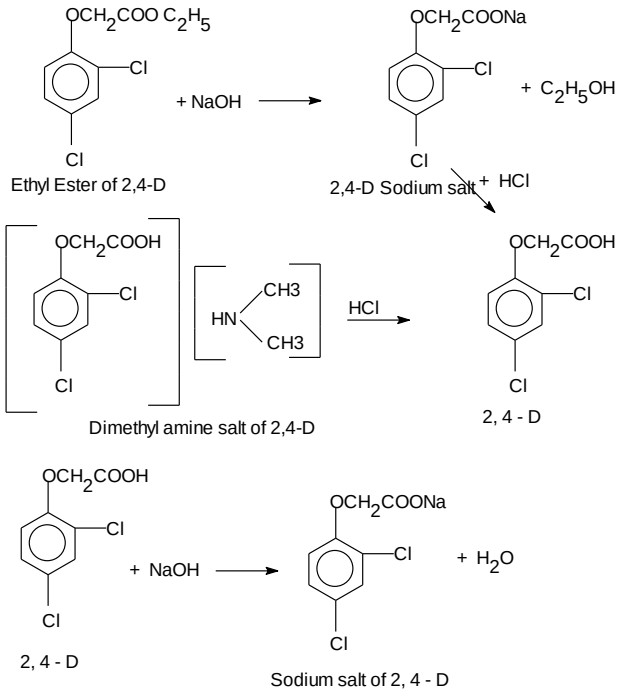
Registered products:

S.No.	Product	Acid Equivalent	Active Ingredient
1.	2,4-D Technical	97%	97% (As acid)
2.	Ethyl Ester of 2,4-D – Technical	90%	80% (As acid) 6% Free Acid (Max)
3.	Granules	4%	4.5% (As ester)
4.	W.P	18.5%	20.9% --do--
5.	E.C	3%	38.3% --do--
6.	SL	58%	69.8% (As salt)

7.	SP	80%	88% --do--

Principle of analysis: Ester is converted to sodium salt. Salts (Sodium and amine salt) are acidified to release 2,4-D acid which is extracted, washed and is titrated against standard sodium hydroxide solution.

Reactions:



Calculations: 1 GE of NaOH = 1 gm of 2, 4-D

1000 ml of 1 (N) Na OH = 221 gm of 2, 4-D

$$\% \text{ AI as} = \frac{2.21 \times t \times n}{W}$$

Where t = titration reading in ml

N = normality of Na OH

W = weight of sample in gms.

$$\text{Ester content \%} = \text{2, 4-D} \times \frac{249}{221}$$

$$\text{Dimethyl amine salt content \%} = \text{2, 4-D} \times \frac{65.9}{221}$$

$$\text{Sodium salt content \%} = \text{2, 4-D} \times \frac{243}{221}$$

Introduce suitable dilution factor, if necessary, depending upon procedure:

Outline of the procedure:

(1) Technical 2,4-D

(a) A.I content: 5 gms of the sample, weighed accurately is dissolved in 50 ml of neutralized alcohol. Add 50 ml water and titrate against standard 1(N) NaOH using bromothymol blue (0.04% in alcohol) as indicator.

(b) Determination of free 2,4-D phenols:

i) Preparation of standard solution:

Dissolve 100 mg of pure 2,4-Dichlorophenol in 10 ml. acetone and dilute to 500 ml with water. Pipette 10 ml and dilute to 100 ml with water.

ii) Preparation of sample solution:

Weigh 200 mg of sample and dissolve in 60 ml of standard 0.1(N) NH_4 OH and dilute to 500 ml with distilled water.

iii) Colour development:

Pipette 50 ml of sample solution; add 0.5 ml of standard 4-amino-phenazone solution (2% in water) and 0.25 ml of potassium ferricyanide (7% in water) shake well.

Pipette 10 ml of standard solution, add 5 ml of standard ammonium hydroxide solution, (0.1N) dilute to 50 ml with water. Add 0.5 ml of standard 4-amino-phenazone and 0.25 ml of potassium ferricyanide.

Measure absorbance in spectrophotometer.

2)Amine salt of 2,4-D

1 gm of 2,4-D equivalent sample is weighed accurately, dissolved in 10 ml wate, acidified with dilute Hcl (1:1), precipitate of 2,4-D extracted thrice with ether (25 ml each time), ether layer washed with minimum quantity of water till free from chloride ions, ether distilled off, residue dissolved in 50 ml neutral alcohol and 50 ml water and titrated against standard 0.1(N) NaOH using bromothymol blue as indicator.

(2) Sodium Salt: a stock solution of the sample is prepared by dissolving 10 gm in 250 ml water. Pipette out 25 ml into separating funnel, acidify with 1.5 ml of dilute HCl (1:1), extract the acid with ether, distill off the ether and titrate the residue of 2,4-D dissolved in 60 ml netutral alcohol against 0.1(N) Na OH using broothymol blue as indicator.

(3) Ester: About 1 gm of 2,4-D equivalent sample is refluxed for 1 hour with 1.5 gm KOH, 80 ml isopropanol and 20 ml water.

If the sample is EC: then extract the refluxed solution twice with petroleum ether. Then aqueous solution is first neutralized with few drops of HCl (1:1) using phenolphthale-in and then made faintly alkaline with NH4 OH (1:1). Then add 3 ml of 0.1% ba cl2. Shake continuously; dilute it to 250 ml mark in a volumetric flask. Filter and pipette 100 ml into a separating funnel for further analysis.

If the sampe is technical: then transfer the entire solution after reflux into a separating funnel. Acidify the solution with 3 ml HCl (1:1) extract 2,4-D with ether and proceed as mentioned under Sl. No. 3 above.

Identity test:

(1) For 2,4-D (Acid By TLC:

Spot on a silica gel-G (250 micron) plate, 10 microlitres of 5% solution, develop with chloroform+acetic acid (95:5) Dry the plate at 1000c for 20 minute. Spray with bromocresol green solution (0.04% in alcool).

(2) For 2,4-D (Ester) : GLC method (described in subsequent section)

(3) For amine salt: 1 ml of sample is mixed with 5 ml of 1% sodium nitro prusside 2.5 ml of 10% Acealdehyde and 5 ml of 2% sodium carbonate shake well. A dark blackish to brownish colour is produced afgter 5 minutes.

References:

IS: 4321-1989 for 2,4-D (Technical)

IS: 1488-1989 for 2,4-D sodium (Technical)

IS: 7233-1991 for 2,4-D Ester (Technical)

IS: 10243-1993 for EC containing 2,4-D Ester

IS: 10244-1992 for WP containing 2,4-D Ester

IS: 13513-1992 for Gr containing 2,4-D Ester

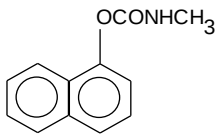
IS: 1827-1989 for SL containing amine salt of 2,4-D

CARBARYL

Description: Contact insecticide belonging to carbamate group.

Molecular Formula: $C_{12}H_{11}O_2N$, Molecular Weight: 201

Structural Formula :



Registered Products:

Technical		- 97%
DP		- 5% / 10%
WP		- 50% / 85%
Flowable		- 42%
Granule	- 4%	
LV		- 40%
Combination		- 4% each
Production		
(Carbaryl + Lindane)		

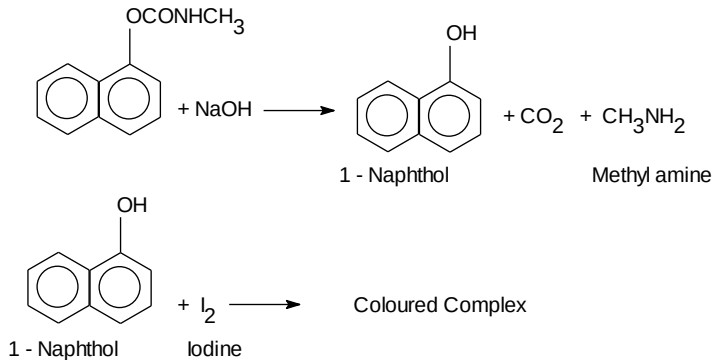
Method of Analysis: a. Visible spectroscopy
b. HPLC* for estimation of Beta carbaryl.

a. Visible spectroscopy:

(i) **Principle:** Carbaryl, on alkaline hydrolysis, yields one mole each of 1-Naphthol, methylamine**, and carbondioxide per mole of A.I. The reaction product, 1-Naphthol, is reacted with iodine solution to yield

purple red colour which is measured at 540 nm. The colour intensity (Absorbance) of test sample is compared with that of standard reference sample and % AI content in test sample is calculated.

\Reactions:



iii) **METHOD OUTLINE**

a) Weigh, in two different flasks, enough quantity of reference standard and test sample equivalent to 60 mgm A.I.
b) Add 10 to 15ml methanol followed by 1 ml of 0.5 (N) potassium hydroxide solution in methanol and boil it for about 2 to 3 minutes. Add few more ml of methanol, if required, to prevent drying up of flask.
c) Cool it to room temperature and dilute the solutions to 100 ml in a volumetric flask, filtering if necessary, with methanol.
d) Pipette out 5 ml of these two solutions into two different 500 ml volumetric flasks, add about 300 ml water, followed by 20 ml of 0.01 (N) aqueous iodine solution. Shake well and dilute upto the mark. Prepare a reagent blank.
e) Measure absorbances of these two solutions at 540 nm against reagent blank.

f) **Calculations:**

$$\text{AI content \% (m/m)} = \frac{W1}{A1} \times \frac{A2}{W2} \times P$$

Where W1 = weight in gms of reference standard

A1 = absorbance of reference standard

A2 = absorbance of test sample

W2 = weight in gms of test sample

P = purity of reference standard

iii) **I.S. Specifications:**

IS : 7539 – 1975 for technical

IS : 7121 – 1973 for WDP

IS : 7122 – 1984 for DP

IS : 9368 – 1980 for granules

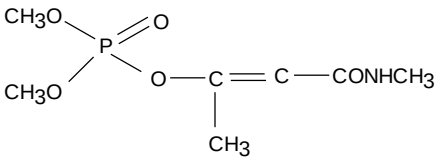
IS : 11784 – 1986 for carbaryl & GAMMA BHC granules

MONOCROTOPHOS

Description: Systemic insecticide belonging to Organophosphorus group

Molecular Formula: $C_7H_{14}O_5NP$ Molecular weight: 223

Structural Formula:



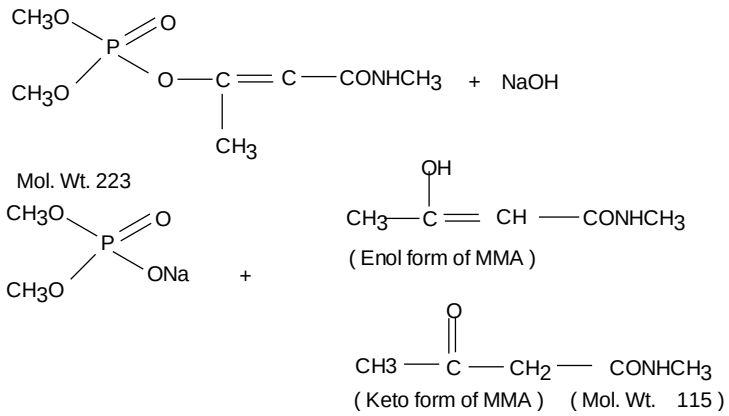
Registered Products: Technical concentrate – 68% / 74%, SL – 36%

Method of Analysis: Visible spectroscopy.

Principle:

Monocrotophos, on alkaline hydrolysis, yields one molecule each of 0,0-Dimethyl phosphoric acid, aceto acetic acid monomethyl amide (MMA) forms. After acidifying, MMA is reacted with Ferric ions to yield purple red colour which is measured at 544 nm. The colour intensity (Absorbance) of test sample is compared with that of standard reference sample of Monomethyl Amide of Aceto Acetic Acid (MMA), and % AI content in test sample is calculated. Thus, obtained value is corrected for interfering impurity in the test sample, namely free MMA, in test sample, estimated separately without hydrolysis of the sample.

Reactions:



OUTLINE OF METHOD

(I) **Estimation of total MCP content.**

(A) Processing of sample.

(i) Weigh enough quantity of sample equivalent to 2.9 to 3.0 gms of AI into a 100 ml volumetric flask, dissolve it in methanol and dilute it upto mark with methanol.

(ii) Transfer 10 ml of this solution to 250 ml volumetric flask and add 10 ml of 5 (N) aqueous sodium hydroxide solutions. Keep it for 30 minutes at room temperature.

(iii) Neutralise the free alkalinity with 1(N) HNO_3 and dilute upto the mark.

(B) Preparation of MMA standard solution.

Weigh 0.15 gms of MMA and dilute it with methanol in a 250 ml volumetric flask.

(C) Colour development.

Transfer 10 ml of solution A (iii) and B above into two 100 ml volumetric flasks. Add to both flasks 10 ml of 5% methanolic acetic acid, 50 ml methanol and 10 ml of 4% aqueous ferric chloride solution. Then add only to the flask containing MMA standard solution; 10 ml of sodium nitrate solution (prepared by neutralising 10 ml of 5(N) NaOH with 1(N) HNO_3 and diluting to 250 ml in a volumetric flask).

Dilute the contents of both flasks with methanol upto the mark and measure absorbance at 544 nm against reagent blank.

(D) Calculation of total MCP content

% MCP (TOTAL)

$$= \frac{W_1}{A_1} \times \frac{A_2}{W_2} \times P \times 19.41 = (X)$$

(II) **Estimation of free MMA**

(a) Transfer adequate volume (usually 2 to 4 ml) from stock solution of test sample (prepared as under A (i) above) into a 100 ml volumetric flask. Develop colour as before and measure absorbance (A3) at 544 nm against reagent blank.
(b) Calculate MCP equivalent to free MMA impurity in the sample as shown below:

MCP equivalent to free MMA.

$$= \frac{W1}{A1} \times \frac{A3}{W2} \times P \times 0.776 = (Y)$$

III. **Final calculation**

(i) % A.I (w/w) in test sample = (X) - (Y)

(ii) % Free MMA (w/w) in test sample = $\frac{Y}{1.94}$

IV. **IS Specifications:**

IS : 8025 – 1990 for technical.

IS : 8074 – 1990 for S.L

Additional points:

(1) Reference standard to be used is MMA and not technical monocrotophos because with the latter, it is not possible to estimate free MMA.
(2) Technical MMA is highly hygroscopic. Hence it should be weighed quickly to minimize absorption of moisture. Alternatively, aqueous solution of MMA of known concentration may be used as reference standard.
(3) It is the Enol form of MMA which produces colour with ferric ion. Keto form of MMA does not produce colour with ferric ions. Hence about 10 minutes time should be given after addition of

ferric ion before measuring absorbance. This will enable complete conversion of Keto to Enol form failing which erratic results will be obtained.

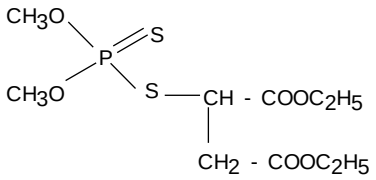
(4) While determining free MMA in the sample, absorbance values of standard and sample should be as close as possible. If not, volume of stock solution of the sample, to be taken may be proportionately reduced.

MALATHION

Description: Contact insecticide belonging to Organophosphorus group.

Molecular Formulation: $C_{10}H_{19}O_6PS_2$ **Molecular Weight:** 330

Structure Formula :



Registered Products:

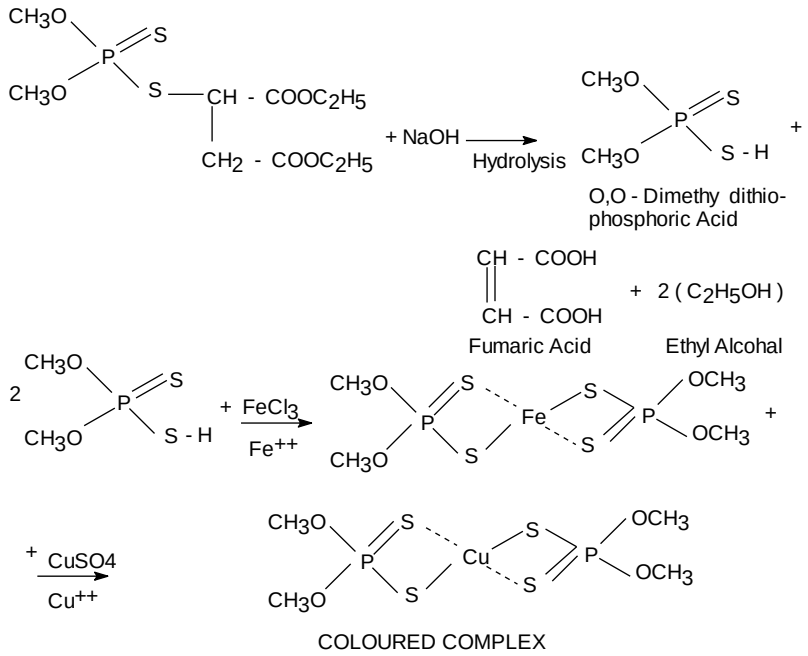
Technical	- 95%
DP	- 5%
WP	- 25%
EC	- 50%
ULV	- 96%

Method of Analysis: Visible Spectroscopy.

(i) Principle:

Malathion, on alkaline hydrolysis, yields one mole each of 0, 0-Dimethyl Dithiophosphoric acid, fumaric acid and two moles of ethanol per mole of AI. After acidifying, 0,0-Dimethyl Dithiophosphoric acid (DMTA) is complexed with cupric ions to yield a yellow coloured complex which is extracted with carbon tetrachloride and colour intensity (Absorbance) is measured* at 420 nm. The absorbance value of test sample is compared with that of standard reference sample and % AI content in test sample is calculated.

Reactions:



Precautions:

Coloured complex is unstable and hence absorbance of sample and standard should be measured at the same time lapse after addition of cupric ions.

METHOD OUTLINE

i) Weigh enough quantity of reference standard and test sample to contain 100 mgm AI. Dilute to 100 ml with ethanol/methanol in 100 ml volumetric flasks.

ii) Transfer 10 ml of these two solutions into two 100 ml volumetric flasks, add 1 ml acetonitrile and dilute upto the mark with methanol.

iii) Transfer 15 ml of these two solutions into two different 250 ml separating funnels. Add 2 ml of aqueous 0.5 (N) sodium hydroxide solutions. Allow the reaction (Hydrolysis) to proceed at room temperature for 2 minutes.

iv) Add 75ml of ferric reagent* and keep it for 5 minutes.

v) Add, either by pipette or by volumetric flask, exactly 50 ml of carbon tetrachloride.

vi) ** Add, by fast delivery pipette, 2 ml of 1.5% aqueous copper sulfate solution. Shake vigorously for 1 minute. Measure within 5 minutes absorbance of yellow coloured carbon tetrachloride layer at 420 nm against solvent.

vii) Calculations:

$$\text{AI content \% (W/W)} = \frac{W1}{A1} \times \frac{A2}{W2} \times P$$

Where W1 = Weight in gms of reference standard

A1 = Absorbance of reference standard

A2 = Absorbance of test sample

W2 = Weight in gms of test sample

P = Purity of reference standard

* Dissolve 4 gms of ferric chloride and 160 ml of conc. HCl in 500 ml water. Dilute 25 ml of this solution to 1 litre with water.

** Since colour is unstable, first process reference standard followed by test sample solution.

IS Specifications:

IS : 1832 – 1978 for technical

IS : 2567 – 1978 for EC

IS : 2568 – 1978 for DP

IS : 2569 – 1978 for WDP

3. Principles, operation and application of various chromatographic techniques

Chromatography can be defined as a process in which separation of chemicals in a mixture is accomplished by the differential adsorption /partition in two immiscible phases.

Or

Chromatography is a technique to separate the components of a mixture based on differential migration caused by a mobile phase over a stationary phase.

Chromatography word is derived from the Greek Word, Chromos meaning Colour, Graph of Colours meaning Chromatography

Types of Chromatography

1. Column Chromatography – separates liquid sample in a liquid solvent (M.P.) and a column composed of solid beads (S.P.)

2. **Paper Chromatography** – separates dried liquid samples with a liquid solvent (M.P.) and a paper strip (S.P.)

3. **Thin Layer Chromatography** – separates dried liquid samples with a liquid solvent (M.P.) and a glass plate covered a thin layer of adsorbent (S.P.)

4. **Gas Chromatography** – separates vaporized samples with a carrier gas (M.P.) and a column composed of a liquid or of solid beads (S.P.)

5. **High Performance Liquid Chromatography** – separates liquid samples with a liquid solvent (M.P.) and a column composed of solid beads (S.P.)

Chromatographic Methods Classification

Based on

I. Geometry of system

   a. In **column chromatography** - the stationary phase is contained in tube called the Column.
   E.g. Column Chromatography, GLC and HPLC

   b. **Planar Chromatography** - in this geometry the stationary phase is configured as a thin two dimensional sheet.

   In paper chromatography - a narrow strip of paper serves as a stationary phase.

In thin Layer Chromatography – a thin film of a stationary phase of solid particles bound together for mechanical strength with a binder such as calcium sulfate, is coated on a glass plate or plastic or metal sheet.

II. Mode of Operation

- **Development Chromatography** – The Flow of mobile phase is stopped before solute reach the end of the bed of stationary phase. Eg. Paper chromatography & Thin Layer Chromatography.

- **Elution Chromatography** – This method employed with columns involves solute migration through the entire system. Detector continuously monitors the amount of solute emerging out of the column Eg. Column Chromatography, GLC & HPLC.

III Based on the Phases involved

Mobile Phase	**Stationary Phase**	**Type of Chromatography**
Gas	Liquid	Gas Liquid Chromatography
Gas	Solid	Gas Solid Chromatography
Liquid	Solid	Liquid Solid Chromatography
Liquid	Liquid	Liquid Liquid Chromatography

IV Principles of Separation

Adsorption Chromatography – Chromatography in which separation is based on difference between the adsorption affinities of the sample components for the surface of an active solid.

Partition Chromatography – Chromatography in which separation is based on differences between the solubility of sample components in the Stationary phase and Mobile Phase.

The Affinity Chromatography – In this type of chromatography an affinity ligand specific for a binding site on the target molecule, is coupled to an inert chromatography matrix.
Under suitable binding conditions this affinity matrix will bind molecule according to its specificity only. All other sample components will pass through the medium unabsorbed.
After the wash step, adsorbed molecules are released and eluted by changing the conditions towards dissociation or by adding an excess of a substance that displaces the target molecule from a affinity ligand.

ION Exchange Chromatography - Chromatography in which separation is Based on difference in the ion – exchange affinities of the sample component anions or cations are covalently attached to stationary phase usually a resin.

Exclusion Chromatography - Stationary phase used are porous material. The mechanism used for separating solute molecules according to their size is known as exclusion or size exclusion chromatography.

Filtration performed in aqueous solvents on water soluble species are described as Gel Filtration Chromatography (**GFC**).

Filtration performed on non – aqueous solvents on species soluble in organic solvents are described as Gel permeation Chromatography (**GPC**).

Features of Chromatography

- Two mutually immiscible phases
- One phase is stationary and the other one is mobile
- The Mixture is partitioned between the stationary and mobile phase
- Dependent upon the relative attraction of each component for the two phases
- Sample components are gradually separated into bands in the mobile phase
- At the end of the process separated components emerge in order of increasing interaction with the stationary phase.

Application of Chromatography

I. Qualitative Analysis : is based on the retention time of the solute in the column (R_t)
II. Quantitative Analysis: is based on the response of the solute measured in terms of height or area (based on Peak Height or based on Peak Area) .

Uses of Chromatography

a. Used by Scientist to

i. Analyze – Examine a mixture, its components and their relations to one another
ii. Identify – Determine the identity of a mixture of components based on known components
iii. Purify – Separate components in order to isolate one of interest for further study
iv. Quantify – Determine the amount of the components present in the sample

b. <u>In Real Life</u>

i. Pharmaceutical company – Determine amount of each chemical found in new product
ii. Hospital – Detect blood or alcohol levels in a patients' blood stream
iii. Law Enforcement – To compare a sample found at a crime scene, to sample from suspect.
iv. Environmental Agency – Determine the level of pollutants in the environmental samples.
v. Manufacturing plants – To purify a chemical needed to make a product.

<u>Principles of Gas Chromatography</u>

Gas chromatography (GC) or gas liquid chromatography (GLC) involves a sample being vaporized & injected into the chromatographic column containing liquid stationary phase. The sample is transported through the column by a flow of inert gaseous mobile phase (carrier gas). Separation takes place due to differential migration caused by a mobile phase over a stationary phase.

GLC is operated at high temperature, hence it is must that, samples to be analyzed by this technique should be thermally stable at operating temperature and volatile in nature. All the basic components of the GLC are heated in different manner with some purpose.

Temperature of Injector port is usually maintained 50^0C higher than the boiling point of the least volatile component of the sample, Column is heated slightly above the average boiling point of sample & Detector temperature is kept highest to prevent condensation of compounds at detector which may lead to loss of sensitivity.

Gas chromatograph mainly consists of injector, column, detector & recorder. Diagram -Typical gas chromatograph

Chemical introduced in vapors phase and pass through the column of liquid stationary phase. Separation takes place due to their differential partition in two phases in to different degree.

Partition Co-efficient (K)

The ratio of the Concentration Of a chemical in liquid phase to the concentration In gaseous phase.

$$K = \frac{C_l}{C_g}$$

Where, K=PartitionCoefficient

C_l = Conc. of chemical in liquid phase

C_g = Conc. of chemical in gaseous phase

GLC is a dynamic system where there is a continuous flow of carrier gas, Time taken by the chemical to come out from the column is called as 'Retention Time' which is constant under the set of parameters, hence Retention Time (RT) Forms the basis for identification. Length of column plays important role in separations of mixture. Column should be sufficiently long to get good separation.

If it is too much long, there will be complete separation but,

- RT will increase (Longer Run Time)
- Gives broad peak (Peak Broadening)

Effects of Various Parameters on Retention Time (RT)

1. Column Length
   - Increase in length - RT will increase
   - Decrease in length - RT will decrease

4. Temperature of Column
   - Increase in temperature - RT will decrease
   - Decrease in temperature - RT will increase

3. Carrier Gas Flow rate
   - Increase in flow rate - RT will decrease
   - Decrease in flow rare - RT will increase

4. Concentration of Stationary Phase
   - Increase in concentration - RT will increase
   - Decrease in concentration - RT will decrease

5. Particle size of Solid Support
   - Smaller the size - RT will increase
   - Larger the size - RT will decrease

Column Efficiency

Theoretical Plate (n)

Chemical passes through mobile phase to stationary phase and vice-versa. The junction is called as Theoretical Plate.

$$N = 16 (Rt/W_b)^2$$

Where,

N = No. of Theoretical plates
R_t = Retention time
W_b = Width of the peak at base

More the no. of n, column will be more efficient.

Height Equivalent to Theoretical Plates (HETP)

$$HETP = \frac{l}{n}$$

Where, l = Length of the column
n = No. of Theoretical plates

- Value of HETP is less, Efficiency will be more.
- Ideal length of Theoretical plate is 1 mm.

Resolution

Separation of two chemicals in a mixture is called Resolution.

$$R = \frac{Rt_2 - Rt_1}{0.5 (Wb1 + Wb2)}$$

Carrier gas

The carrier gas must be chemically inert. The carrier gas is either nitrogen, helium, hydrogen or argon. The choice depends on factors such as availability, cost, consumption, purity & type of detector employed.

When detectors like FID & NPD are employed, in addition to carrier gas two more gases are required as fuel gases i.e. hydrogen & zero air. As these gases are used from high pressure cylinders, its supply is controlled through regulators and flow meters. Though, these gases are highly purified, it is passed through the adsorbents like silica, charcoal & molecular sieve to protect gas chromatograph from moisture & pollutants.

Sample injection port

The injector can be used in one of two modes – split or split less. The injector contains a heated chamber containing a glass liner into which the sample is injected using a micro syringe with a hypodermic needle. The needle is inserted through a self-sealing silicone rubber septum. The carrier gas enters the chamber & can leave by three routes in split mode. The sample vaporizes to form a mixture of carrier gas, vaporized solvent & vaporized solutes.

Columns

Column is a heart of chromatograph, as actual separation is effected in the column. There are two types of columns – Packed & Capillary (also known as open tubular column).

Packed columns contain a finely divided, inert, solid support material coated with liquid stationary phase. Most packed columns are 0.5 to 2 meter in length & have a inner diameter of 2 to 3 mm.

Capillary column have an inner diameter of a few tenth of mm. They can be of two types,

i. Wall coated open tubular (**WCOT**) – columns have the stationary phase coated directly on to the inner wall of the tubing.

ii. Support coated open tubular (**SCOT**) – columns have a finely divided layer of solid support material deposited on the inner wall, on to which the stationary phase is then coated.

SCOT columns are less efficient than WCOT columns & both types of capillary columns are more efficient than packed columns.

In 1979 a new type of WCOT column was devised – The fused silica open tubular (**FSOT**) column. These have much thinner wall than glass capillary column. These columns are flexible & can be wound into coils. They have the advantage of physical strength, flexibility & low reactivity

Detectors

Detector is a transducer that transforms chemical or physical properties of an analytes into an electrical signal. The function of detector is to sense and measure the small amounts of the separated components present in the carrier gas stream leaving the column. The output from the detector is fed to a device which produces a trace called **chromatogram**.

The choice of detector depends on the concentration level to be measured and the nature of separated components. Detectors are of two types,

i. **Destructive** – Sample which comes in contact of detector are destroyed & can not be used for other detector.

ii. **Non destructive** – After sensing it remains undestroyed & can be used for other detectors. FID & TCD are used for pesticide formulation analysis, whereas, ECD, NPD,FPD & PID are used for pesticide residue analysis.

Properties of detectors

Each detector has different sensitivity, linearity, stability & selectivity.

Sensitivity

This is usually defined as the detector response (mV) per unit concentration of analyte (mg / mL). It is an amount of analytes that can produce signal equal to twice of noise (baseline).

Linearity:

The linearity refers to the concentration range over which the signal is directly proportional to the amount (or concentration) of analyte. A large linear range is a great advantage, but detectors with a small linear range may still be used because of their other qualities.

Stability

An important characteristics of a detector is the extent to which the signal output remains constant with time, assuming there is a constant input. Lack of stability can be exhibited in two ways: by baseline noise or by drift, both of which will limit the sensitivity of the detector.

Universal or selective response

A universal detector will respond to all the components present in a mixture. In contrast, a selective detector senses only certain components in a sample. This can be advantageous if the detector responds only to components of interest. The quantitative determination of component in GLC using different type of detectors are based upon the measurement of the recorded peak area or peak height.

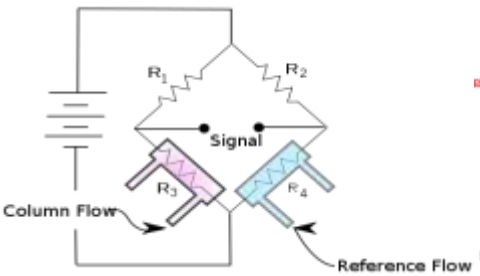
Hot Wire Detector

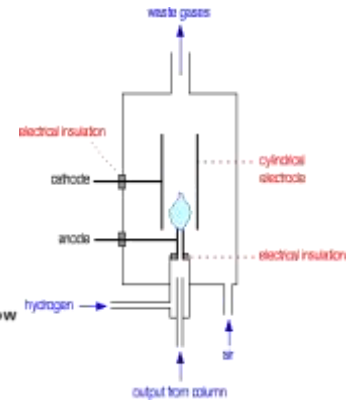
The Hot wire detector is also known as Thermal conductivity detector. In the detector two pairs of matched filaments are arranged in a Wheatstone bridge circuit; two filaments in opposite arms of the bridge are surrounded by the effluent from the chromatographic column. When pure carrier gas passes over both the reference and sample filaments, the bridge is balanced, but when a vapour emerges from the column, the rate of cooling of the sample filament changes and the bridge becomes unbalanced. The extent of this imbalance is a measure of the concentration of vapour in the carrier gas which is fed to the recorder producing the chromatogram.

In case of TCD, helium and hydrogen are the best carrier gases, as their thermal conductivities are much higher than any other gases. On safety grounds helium is most preferred because of its inertness.

TCD is universal, non destructive, simple and rugged detector.

Flame Ionization Detector

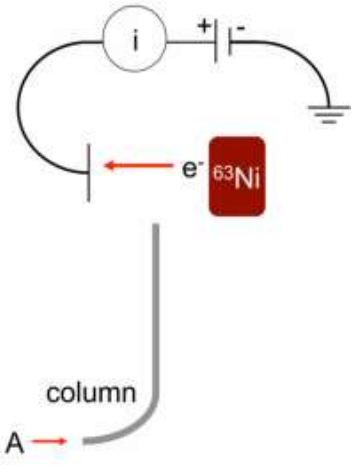




The effluent from the column is mixed with hydrogen & burned in the air to produce a flame over a tip of the jet. As separated compounds emerges out of the column, burns in hydrogen rich flame and produces ions which are collected at electrodes resulting in increased conductivity & increase in current, which is proportional to the quantity of chemical. FID is nearly a universal detector for gas chromatography of organic compounds, coupled with high sensitivity, stability, fast response and wide linear response range. This has made it the most popular detector.

Electron Capture Detector

The ECD operates by ionization of the carrier gas from a radioactive source (H^3 or Ni^{63}) splitting off slow moving electrons. This ionization gives a background current commonly referred as standing current. When halogenated compound passes through the detector, a certain amount of the free electrons are captured, resulting decrease in standing current which is proportional to the quantity of the chemical, is displayed as a peak on the recorder.



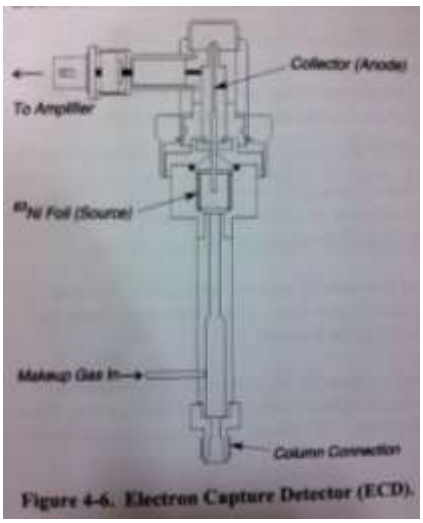


Figure 4-6. Electron Capture Detector (ECD).

Nitrogen Phosphorus Detector

This contain an electrically heated a silica or ceramic bead fused to platinum wire is impregnated with rubidium salt and placed between the jet and the collector electrode. The temperature of the bead electrically heated by passing the current through platinum wire maintain at 600-800 ^{0}C temperature. While plasma (flame) is sustained in the region of bead, when the flow of H_2 gas is reduced to 1-2 ml / min.

NPD works on same principle as in case of FID. Presence of alkali bead makes it selective and more sensitive to nitrogen and phosphorus containing compounds.

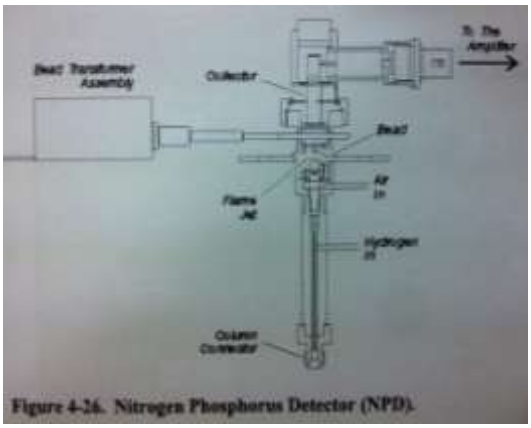


Figure 4-26. Nitrogen Phosphorus Detector (NPD).

Flame Photometric Detector

It is based upon the atomic emission of light at 526 nm for ‘P’ and 394 nm for ‘S’. when compounds containing these species are burnt in a hydrogen rich flame, the light collected with a mirror, filtered to a specific wave length and measured by a photo multiplier tube.

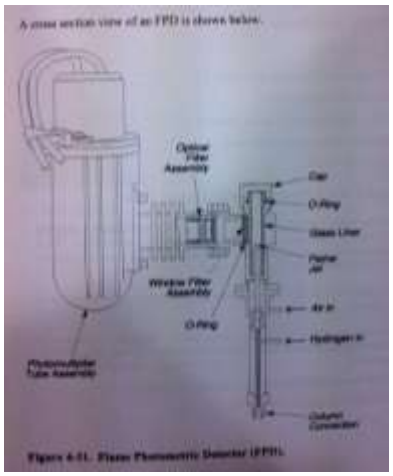
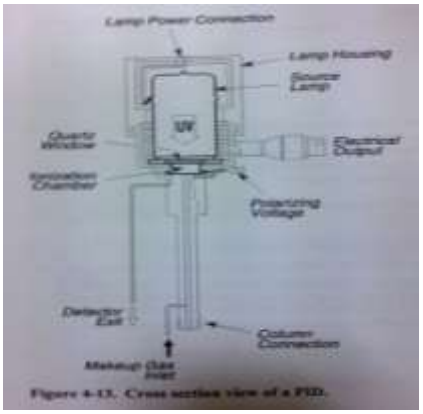


Photo Ionization Detector

It works as an ionization detector similar to FID or ECD and the response results from the collection and amplification of ions at a positively charged collector electrode using a conventional high-impedance amplifier.

As organic solute molecules elute from the column, ions are produced by irradiating the elute with light from a high-intensity ultraviolet lamp.



Important Detector – A Comparison

Detector	Principle of operation	Selectivity (mic. gm)	Sensitivity (mic.gm)	Linearity	Minimum detectability (g/sec)	Carrier gas	Remarks
TCD	Measure of thermal Conductivity	Universal	1	10^4	10^{-5}	H_2, N_2 He_2, CH_4	Non destructive, simple, inexpensive & rugged
FID	H_2, O_2 Flame	Universal	10^{-1}	10^7	$2X\ 10^{-11}$	He, N_2	Destructive
ECD H^3,Ni^{63}	N_2 + ß ---- e^{-1} e + sample	Selective	10^{-7}	$5X10^2$	10^{-13}	N_2	Non-destructive
AFID (NPD)	Alkali modified H_2, O_2 flame	Selective	P 10^{-7} N – 10^{-3}	10^3	$1.5X10^{-14}$ $1X10^{-12}$	He, N_2	Destructive
FPD	H_2, O_2 flame	Selective	P – 10^{-5} S	10^5	$1X10^{-12}$ $8X10^{-11}$	He, N_2	Destructive

GAS CHROMOGRAPHIC COLUMNS

The chromatographic column is the heart of the instrument.. The packed column has an internal diameter of 2-4mm and has tubular construction of either glass or stainless steel . The column is packed with particles, which act either as retarding stationary phase or may be coated with a thin film of organic material, which may act as the stationary phase. There are two major types of capillary columns. One is termed porous layer open tubular [PLOT] column, in which inner surface of the column has embedded layer that contains stationary phase. The other type is a wall – coated open tubular [WCOT] column in which a thin film organic stationary phase is bonded directly onto the inner surface of the column. WCOT capillary columns are used for gas chromatographic analysis of trace organic compounds. He inner diameter of capillary columns ranges from about 0,18 mm 0.53 mm. columns with an id of 0.35mm are known as mega bore and are some what unique in that they can be used in GC systems configured for either capillary or packed columns. The i.d.of packed columns ranges from 2 to 4 mm. The separation power of respective columns is given by total chromatograhic plate counts. These values are calculated for typical lengths of 20m for a capillary and 2m for a packed column. The capillary column has 10-30 times more separating power than packed columns . If column efficiencies are compared, calculated by normalizing chromatographic plates to length of columns [i.e plates/ m],. capillary columns are slightly more efficient than packed columns. The high separating power of capillary columns is achieved primarily by column lengths that can be achieved in column technology.

The column capacity i.e mass of individual solutes that can be injected into columns is measured in nanograms[ng] , for very narrow bore 0.18 mm capillary column is limited to about 100 ng per component and increases to 1,000-2,000 ng for megabore [0.53mm].columns. By comparison, capacity for packed columns is considerably higher, of the order of 10,000 ng . The packed column is usually employed when sample injection volume is large. Generally packed columns are unfavorable for trace analysis due to baseline instabilities as consequence of pressure changes during dosing procedure as the result of excessive column bleed at high sensitivities

Capillary columns made from fused silica and synthetic quartz, coated on the out side with polyamide, which make them flexible and easy to handle. Stationary phases are cross-lined polymers bonded to interior column wall, effectively eliminating column bleed. Length of the capillary columns ranges from 10-60 m, however, 15 or 30m columns are commonly used for residue work.

Heating a column without carrier gas flow or exposing it to any oxygen at temperature 100oC even for shorter periods can damage phases rapidly and irreversibly. Efficiency increases as capillary columns bore size decreases. However, sample capacity decreases with decreasing bore size. For narrow bore, gas flow requirement is at or less than 0.9ml/min, for traditional capillary columns, it is at or less than 3ml/min. and for wide bore capillary columns, it is at or less than 6ml/min..However, wide bore columns can accommodate 20-30 ml/min. gas flow without generating excessive column head pressure. It is important the column should not be stressed in the oven and that couplings should not be over tightened. It is good practice to carry out a leak test on the system frequently; but avoid using soap solution for leak test of column since this may contaminate column. Ethyl acetate is known to be a suitable solvent because of its volatility. Most problems of GC comes from leak and contamination.

GAS CHROMATOGRAPHY STATIONAY PHASES

The stationary phase of the column system is chosen after considering polar characteristics of the analytes, their volatility range and column temperature programme. A diverse range of stationary phase is available for WCOT capillary columns, 100% dimethylpolysiloxane polymer that is chemically bonded on to the interior wall of the column, this is a non-polar stationary phase. This basic polymeric matrix can be modified to create slightly more polar phase by incorporating phenyl group into the structure. The 5% phenyl polymer composition is one of the most widely used separation phase. A much more polar phase can be created by incorporating a cyanopropylphenyl group into the basic dimethylpolysiloxane polymer. Nonpolar phases are more stable and chromatographically robust than polar phases, which tend to have a lower temperature tolerance and are more susceptible to oxidative damage if air is introduced into the column.

DB-5 is non-polar column with 5% -phenyl , methylpolysiloxane, DB-1 has most nonpolar siloxane stationary phas, surface bonded with 100% dimethylpolysiloxane. Db-5 is similar to DB-1 excepting that 5% of the methyl group is substituted with phenyl group, which is more polar. DB-17 has a medium polar stationary phase(5% [phenyl-methyl polysiloxane). Generally more polar the stationary phase is, better is the separation.

Film thickness ranges 0.1 – 5.0 um, and normally columns with 1.0 or 1.5um film thickness are used in pesticide determination.

Polar stationary phases (cyanopropyl phenyl) are difficult to coat on to column walls, and hence are usually available only 1.0um film thickness. They tend to bleed more than their non-polar counterparts. Heating a column without carrier gas flow or exposing it to oxygen at temperature at or above 100C, even for shorter period, can damage phases rapidly and irrseversibly. The stationary phases fall into two categories i.e Liquids and solids, giving rise to GLC and GSC. Liquid stationary phases are by far most frequently used reliable among the two types.

Liquid Stationary phases: Liquid stationary phases are used in both packed and capillary columns . In either case, the properties which they need and the way in which they work are the same and also the same type of compounds are used in both types of columns.. The liquid stationary phase is a liquid which is coated on a solid support and packed in a column. When the column is heated, the liquid layer on the solid support is activated and it comes in contact with the mixture of compounds in gaseous form and due to affinity the components in the mixture tend to remain in liquid or in gaseous phase.

Properties for stationary phase:

It must be non-volatile, and it should be thermally stable. and chemically inert i.e it should not react with the components of the mixture to be identified. It should have high molecular mass such molecules with high molecular mass have low volatilities. Saturated hydrocarbons,silicones ethers,esters and amides are choosen. It should have polar molecules with strong inter molecular attraction..

When a mixture of chemical is introduced in a vapour phase and pushed through a column, the two immiscible phases act on it at different degrees and partition takes place.

The partition coefficient (K) defined as the ratio of concentration of chemical in the stationary phase to that of mobile phase.

$$K = \frac{CL}{CG}$$

Chemically the stationary phases are organo-silicates with four different groups of configuration. R1,R2,R3,R4 occupied by phenyl, methyl (CH3), cyanopropyl (C3H6CN) and tri-flluoro-propyl (C_2H_4CN)etc.

Physically stationary phases are thick viscous gummy substances having high boiling point, non-volatile, inert and thermally stable.

Solid stationary phases -

Solid stationary phases are less commonly used,but are attractive because they offer much greater selectivity and in fact are
Often essential for analysis of gases and metals.

Solids stationary phases can be divided into two groups,i). those which operate by adsorption and ii) those which operate on the molecular sieving principle.

Adsorption onto a solid surface is not a uniform principle process. Some areas of the surface adsorb molecules more rapidly and more strongly than others and in such areas the adsorbed molecules cluster more thickly. Such areas are known as active sites and they tend to be found by cracks and crevices. This non-uniformity leads to non-linear adsorption and tailing. b) Forces responsible for adsorption - a) hydrogen bonding b) dipole – dipole attraction , c) dipole induced dipole attraction ,d) dispersion forces.

ii. Molecular sieves.

Examples of solid stationary phases:-

1. Alumina
2. Carbon black
3. Zeolite
4. Silica gel
5. Porous polymers

THE SOLID SUPPORT:

The support is a solid material on which the liquid stationary phase is coated in a packed column. The support should have following properties. 1. Chemical inertness, thermal stability, non-adsorbancy.2. Mechanical strength, uniform particle size, large surface area per unit volume.

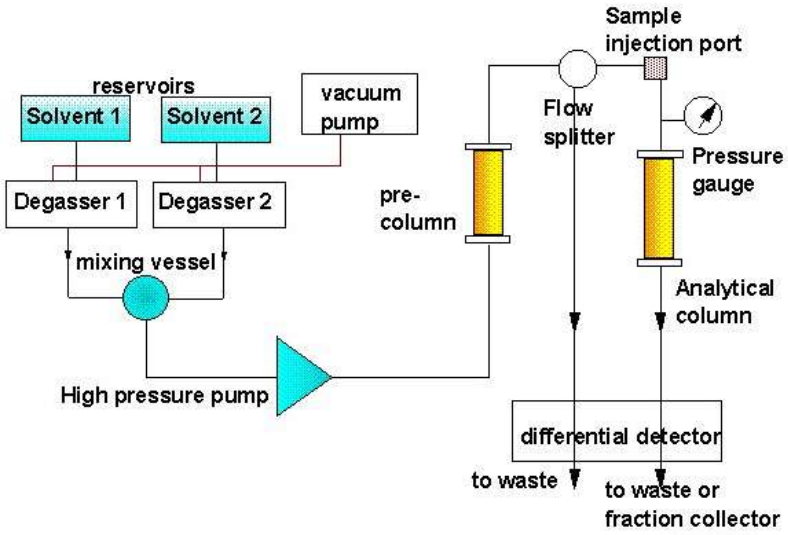
The most important group of support are the diatomaceous earth based materials eg. Kieselguhr, celite, chromosorb,crushed firebrick.

Diatomaceous earth is very porous and therefore has a high surface area, enabling it to absorb a large amount of liquid stationary phase (up to 20- 30%) whilst still remaining a free flowing powder. Chemically diatomaceous earth consists of about 95% SiO_2, the remainder being Al_2O_3 and other metal oxides. It is chemically inert and weak adsorbent , and can interfere with GLC. It does not have great mechanical strength naturally .

High Performance Liquid Chromatography

Early liquid chromatography (LC) was carried out in glass column and with a diameter of 10 to 150mm . The columns were packed with 50cm to 500cm lengths of solid particles coated with an adsorbed liquid that formed stationary phase. To ensure reasonable flow through the column, the particle size were kept at 150 to 200 micro meter, even then the flow rates were at best few tenths of milliliter per minute. Thus separation times were too long –often several hours. It is only after sixties that technology came in to existence to make suitable pumps which can pump efficiently through 3 to 10 micron columns. With the change in particle change and the efficient pumps the terminology of High Pressure Liquid Chromatography has changed in to High Performance Liquid Chromatography. Now we are in the era of Fast Liquid Chromatography where the reduced length of the column , reduced particle size of 1.7 microns stationary phase, and pumps capable of pumping with high pressures made it possible to resolve very complicate matrix with ease and less time.

HPLC schematic diagram:



Solvent reservoir: The solvent reservoirs are filled with the respective HPLC grade solvents which needs to be filtered (if not already filtered) and degassed. Each of the solvent needs to be connected to the pumps through the inert tubing and needs priming and purging of air and earlier solvent from the tubing and the pump head. Utmost care should be taken while preparing mobile solvents and purging as in many instances it is the mobile solvent(s) that effects system operation in a number of ways such as high system back pressure, flow-related baseline noise, shifting retention times, abnormal peak shapes and incorrect qualitative /quantitative results.

Solvent preparation:

Filtration: The filtration of the solvent is done using clean glass flasks and appropriate choice of filter membranes with pore size of 0.45 microns or less and preserved in sealed containers. If premixing of solvents is required it must be done using a clean and properly rinsed measuring cylinders/pipettes in dust free atmosphere.

***Solvent degassing*:** Proper degassing is essential to obtain reproducible retention times, stable pump operation, stability in the baseline, stable operating pressures and enhanced sensitivity. The following are some of the degassing methods.

1. Vacuum filtration: Vacuum filtration through 0.45 micron or less pore size membrane is one method to degas the solvents but takes a longer time and may not be efficient alone.

2. Sonication: Sonication technique is used high energy sound waves to drive energy in to solvent and cause submicron sized gas to aggregate. As the gas bubbles aggregate, they become large enough to float out of the solvent. However as in the case of vacuum filtration the Sonication alone is not enough to bring out the desired degassing as it takes longer times. There fore a combination of vacuum filtration and Sonication `be used to achieve the degassing of solvents within a minute.

3. Sparging: Sparging with helium though the solvents will saturate the solvents with helium and leaves a blanket of helium has over the solvent which will prevent the degassed solvents from absorbing the atmospheric gases.

4. In line degassing: The solvents are made to flow through a gas permeating chamber kept under vacuum and such degassed solvents are pumped through the system. This problem do not suffer the re saturation of gasses and can maintain a steady base line.

HPLC Solvents:

The solvents used in HPLC are analytical pure, free from suspended particles and also degassed to give steady base line and without any interference in the analysis. Its physico chemical properties should hinder neither the process of chromatography nor the identification of the solute under investigation. For example if the detector used is spectrophotometer the solvents should be transparent enough at the wave length of interest for the solute under investigation. The following are some of the important solvents used in HPLC with important properties.

Sl.No.	Solvent	Miscibility	UV cut off point	RI	Polarity index
1.	Hexane	29			0.0
2.	Cyclohexane	28	200	1.427	0.0
3.	n-Decane	29		1.412	0.3
4.	Octane	29	215	1.404	0.4
5.	Carbon tetra chloride		265	1.466	1.7
6.	Tri ethanolamine	26			1.8
7.	Iso – propyl ether	26		1.368	2.2
8.	Toluene	23	285	1.496	2.3
9.	Benzene	21	280	1.501	3.0
10.	Methylene chloride	20	232	1.424	3.4
11.	Chloroform	19	245	1.443	3.4
12.	Tetra hydro furan	17	225	1.408	4.2
13.	Ethyl acetate	19	260	1.37	4.3
14.	1-Propanol	15	210	1.380	4.3
15.	2-Propanol	15	205	1.380	4.3
16.	Ethanol	14	210	1.361	5.2
17.	Pyridine	16		1.510	5.3
18.	Acetone	15, 17	330	1.359	5.4
19.	Acetonitrile	11.17	190	1.344	6.2
20.	Acetic acid	14	230 (1% of MP)	1.372	6.2
21.	Dimethyl formamide	12		1.428	6.4
22.	Methanol	12	205	1.329	6.6
23.	Formamide	3			7.3
24.	Water	--	180	1.330	9.0

It is common practice to prepare the water for HPLC in their own laboratory. It is prepared by distillation of distilled water or de ionised water twice preferably in quartz distillation unit, filtered and stored in closed in sealed borosilicate bottles. The water is degassed before use just like any other HLC solvent.

Mobile phase modifiers: Apart from the usual mixtures of mobile phases for achieving separation in the process of chromatography, to avoid tailing of the peaks , mobile phase modifiers like water, isopropyl alcohol, to non polar mobile phase and tetra hydrofuran /acetic acid to polar mobile phases are added.

Pump: The pumps are operated preferably wet and without air bubbles. The flow settings are done as per the requirement and the programming of the gradient flow is done carefully. The system leaks if any be checked and rectified by identifying the problem. The pressure monitoring and safety pressure limits also are maintained while running the pump.

Injector port: The injector port is used in HPLC is a two stage injector port, where sample is loaded in one stage and injected in the second stage. Moreover the injector consists of an automatically adjusted pre calibrated sample loop which will allow only fixed amount of sample solution on to the column every time which enables the analyst to introduce the sample without any injection error as in the case of GLC, where much practice is required to obtain accuracy in the injections. However, certain precautions need to be taken while injecting the sample like, using appropriate syringe, proper cleaning , sufficient amount of sample (always excess than sample loop size), clean syringe and without air bubbles.

Column: The choice of column is dependent on the type of molecules under investigation and is maintained always in wet conditions. The assembling and detaching the column from the system requires caution. The appropriate column and the compatible solvents may be identified while changing the column and operating the system. The column requires to be equilibrated before actually use for analysis to obtain reproducible retention times and areas. After use the solvent is allowed to run through column to see the contaminants from the previous injection (s) flushed out and if necessary the proportion of the polar solvent in the system be increased and run for some time so as to clean the column. The highly contaminated columns requires regeneration using the series of solvents in sequence as recommended. The columns are stored in a cool and vibration free shelves/cupboards.

Column maintenance:

The columns are shipped in a solvent suitable for the column by the manufacturer when supplied. The column requires to be equilibrated before use in a sequential order and finally set the required mobile phase. The following is one of the examples to follow to use .,

Silica column

Shipping solvent → Dichloromethane → isopropyl alcohol → Methanol →
Mobile phase → Dichloromethane

The equilibrium may be done by using a minimum of 50 volumes of column of each solvent given and whenever the column needs to be regenerated the same cycle be repeated. When column is not in use the column may be washed with appropriate intermediate solvents and finally preserved in hexane or cyclohexane by end capping.

C_{18} or C_8 columns:

Shipping solvent → 50% aq solvent → Methanol → Mobile solvent

The equilibrium may be done by using a minimum of 50 volumes of column of each solvent given and whenever the column needs to be regenerated the same cycle be repeated. When column is not in use the column may be washed with appropriate intermediate solvents and finally preserved in methanol by end capping.

The columns may be tested for their efficiency using appropriate chemicals and the operating conditions based on use or the manufacturers certificate.

Detectors:

The detectors used in HPLC depends on the solute under investigation and their physico chemical properties with which the identity and quantification is most suitable. Commonly used detectors are , UV-Vis. Spectrophotometer/ Photo Diode Array (PDA) detector, Fluorescence detector, Refractive Index(RI) detector, Mass Spectrometer and triple quad MS/MS detector.

The following table shows the commonly used detectors for different applications.

Sl.No.	Detector	Types of molecules	Application
1.	UV-Vis. Spectrophotometer, PDA	Organic molecules, bio- molecules, except carbohydrates	Any molecule that absorbs light between 170 and 700 nm, PDAs are typically used either when the molecules in the mixtures absorb at different wavelengths or when the e max is unknown. PDA can be used for determining the e max.
2.	Fluorescence	Aromatic compounds (PAHs)	Generally used for applications that require extremely high sensitivity (e.g. Residue analysis)
3.	Refractive Index	Carbohydrates, Polymers	Molecules that do not have a UV chromophore and can not be analysed by UV-Vis.
4.	Mass spectrometer	Organic molecules, Bio molecules	Used to detect and determine the mass on any molecule that can be ionized and is within the mass range of the specific MS
5.	Triple quad MS/MS	Organic molecules, Bio molecules	As above but allow more detailed structural studies to be performed.

The UV-Vis Spectrophotometer detector is used widely used for quality control work in organic analysis including Pesticide Quality control. For investigational and monitoring of Pesticide Residues work the PDA is preferred. For conformational analysis the MS and MS/MS are the ultimate choice.

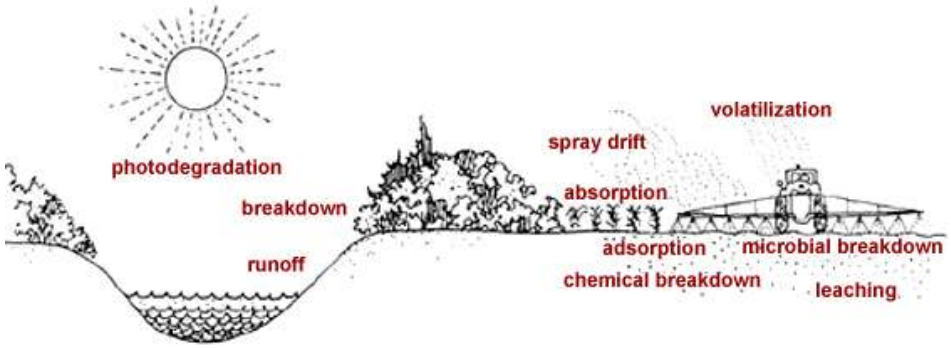
4. Pesticide Dissipation, Residue Dynamics, Different methods/ Steps in residue analysis

Pesticide Dissipation

Releasing of pesticides into the environment can be beneficial (leaching of some herbicides into the root zone can give you better weed control) and sometimes harmful as not all of the applied chemical reaches the target site (runoff can move a herbicide away from target weed). The chemical is wasted, weed control is reduced, and there is more chance of damaging other plants and polluting soil and water. Or some of the pesticide may drift downwind and outside of the intended application site.

Dissipation of pesticides is defined as loss of pesticide residues from an environmental compartment due to *degradation* and transfer to another environmental compartment. The dissipation includes various processes like adsorption, transfer, breakdown and degradation.

Picture: Various Dissipation Processes



A. Transfer Processes

Adsorption is the binding of pesticides to soil particles. The amount a pesticide is adsorbed to the soil varies with the type of pesticide, soil, moisture, soil pH, and soil texture. Pesticides are strongly adsorbed to soils that are high in clay or organic matter. They are not as strongly adsorbed to sandy soils.

Most soil-bound pesticides are less likely to give off vapours or leach through the soil. They are also less easily taken up by plants. For this reason you may require the higher rate listed on the pesticide label for soils high in clay or organic matter.

Volatilization is the process of solids or liquids converting into a gas, which can move away from the initial application site. This movement is called vapour drift. Vapour drift from some herbicides can damage nearby crops.

Pesticides volatize most readily from sandy and wet soils. Hot, dry, or windy weather and small spray drops increase volatilization.

Where recommended, incorporating the pesticide into the soil can help reduce volatilization.

Spray Drift is the airborne movement of spray droplets away from a treatment site during application.

Spray drift is affected by:

- spray droplet size - the smaller the droplets, the more likely they will drift
- wind speed - the stronger the wind, the more pesticide spray will drift
- distance between nozzle and target plant or ground - the greater the distance, the more the wind can affect the spray

Drift can damage nearby sensitive crops or can contaminate crops ready to harvest. Drift may also be a hazard to people, domestic animals, or pollinating insects. Drift can contaminate water in ponds, streams, and ditches and harm fish or other aquatic plants and animals. Excessive drift also reduces the pesticide applied to the target and can reduce the effectiveness of a treatment.

Runoff is the movement of pesticides in water over a sloping surface. The pesticides are either mixed in the water or bound to eroding soil. Runoff can also occur when water is added to a field faster than it can be absorbed into the soil. Pesticides may move with runoff as compounds dissolved in the water or attached to soil particles.

The amount of pesticide runoff depends on:

- the slope
- the texture of the soil
- the soil moisture content
- the amount and timing of a rain-event (irrigation or rainfall)
- the type of pesticide used

Runoff from areas treated with pesticides can pollute streams, ponds, lakes, and wells. Pesticide residues in surface water can harm plants and animals and contaminate groundwater. Water contamination can affect livestock and crops downstream.

Pesticide runoff can be reduced by:

- using minimum tillage techniques to reduce soil erosion
- grading surface to reduce slopes

- diking to contain runoff
- leaving border vegetation and plant cover to contain runoff

Pesticide losses from runoff are greatest when it rains heavily right after you spray. Reduce the chances of runoff by watching the weather forecast. If heavy rain is expected, delay spraying to avoid runoff. Irrigate according to label instructions.

Leaching is the movement of pesticides in water through the soil. Leaching occurs downward, upward, or sideways. The factors influencing whether pesticides will be leached into groundwater include characteristics of the soil and pesticide, and their interaction with water from a rain-event such as irrigation or rainfall. These factors are summarized in the table below.

Leaching can be increased when:

- the pesticide is water soluble
- the soil is sandy
- a rain-event occurs shortly after spraying
- the pesticide is not strongly adsorbed to the soil.

Absorption is the uptake of pesticides and other chemicals into plants or microorganisms. Most pesticides break down once they are absorbed. Pesticide residues may be broken down or remain inside the plant or animal and be released back into the environment when the animal dies or as the plant decays.

Some pesticides stay in the soil long enough to be absorbed by plants grown in a field years later. They may damage or leave residues in future crops.

Crop Removal through harvest or grazing may remove pesticide residues.

B. Degradation or Breakdown Processes

Degradation is the process of pesticide breakdown after application. Pesticides are broken down by microbes, chemical reactions, and light or photodegradation. This process may take anywhere from hours or days to years, depending on environmental conditions and the chemical characteristics of the pesticide. Pesticides that break down quickly generally do not persist in the environment or on the crop. However pesticides that break down too rapidly may only provide short-term control.

Microbial breakdown is the breakdown of chemicals by microorganisms such as fungi and bacteria.

Microbial breakdown tends to increase when:

- temperatures are warm
- soil pH is favourable
- soil moisture and oxygen are adequate
- soil fertility is good

Chemical breakdown is the breakdown of pesticides by chemical reactions in the soil. The rate and type of chemical reactions that occur are influenced by:

- the binding of pesticides to the soil
- soil temperatures
- pH levels - Many pesticides, especially the organophosphate insecticides, break down more rapidly in alkaline soils or in spray tank water with a high pH level.

- Soil moisture

Photodegradation is the breakdown of pesticides by sunlight. All pesticides are susceptible to photodegradation to some extent. The rate of breakdown is influenced by the intensity and spectrum of sunlight, length of exposure, and the properties of the pesticide. Pesticides applied to foliage are more exposed to sunlight than pesticides that are incorporated into the soil. Pesticides may break down faster inside plastic-covered greenhouses than inside glass greenhouses, since glass filters out much of the ultraviolet light that degrades pesticides.

Hydrolysis: Degradation of pesticides due to water

Sampling

Sampling is the act, process, or technique of selecting a suitable sample, or a representative part of a population for the purpose of determining parameters or characteristics of the whole population It is the process of taking portion(s) of a food or other substance for laboratory analysis so that the resulting analytical data and conclusions can be applied to the original food or substance sampled. The samples should always be a representative and be sufficiently large. Selecting a representative sample and correctly submitting it are critical to obtaining acurate information from the analysis. For **produce**, it is important several portions are collected from different areas of the field, bin, etc, for compositing in the laboratory, in order to determine the average pesticide level in the commodity.

1.1 Sampling of Fruit, Vegetables and Grains

The following procedures should be followed while sampling of Fruit, Vegetables and Grains (recommended by the Codex Alimentarius Commission and the Food and Agriculture Organization)

- Separate samples should be collected for distinct lots of produce - different fruits, vegetables or grains; different cultivars or varieties; areas of crop which have had different chemical treatments, or which have been sprayed on different days; produce sourced from different growers for repacking or processing.
- Do not freeze fresh produce. As a general rule all samples, especially samples of perishable fresh produce, should be kept cool BUT NOT FROZEN. However, samples of already frozen foods should be kept frozen until they reach the laboratory.
- Individual fruits and vegetables should not be cut or divided.

1.1.1 Taking a Sample of Produce in the Field

Superimpose an imaginary grid on the field dividing it into approximately 100 areas, and randomly select 10 of these to form a representative sample. Collect o.5 to 1 kg from each of these 10 areas and combine them to form the sample of desired size (Table 1-4)

Each plant or fruit should have an equal chance of being chosen. Even when using the best possible sampling techniques there will be variability between different samples. For a better estimate of the residue status of a crop, take duplicate samples.

- Sample the parts of the crop that normally constitute the marketable produce.
- Avoid taking diseased or under-sized crop parts or produce at a stage when it would not normally be harvested.
- Take samples in such a way as to be reasonably representative of typical harvesting practice.
- Take care not to remove surface residues during handling, packing or preparation.
- Sample and bag the required weight of samples in the field and do not sub-sample.

- Keep records of the samples taken and the method used.

1.1.1.1 Fruits and tree nuts

- Circle each tree or bush and select fruit from all segments of the tree or plant, high and low, exposed and protected by foliage. For small fruits grown in a row, select fruit from both sides, but not within 1 metre of the end of the row.
- Select the quantity of the fruit according to its density on the tree or plant, i.e. take more from the heavily laden parts.
- Take both large and small fruits where appropriate, but not so small or damaged that they could not be sold.

Table 1: Sampling of fruits

Commodity	**Quantity, method of collection**
Citrus fruits e.g. orange, lemon, mandarin, grapefruit	A total of 12 fruits from several places on 4 individual trees. (If this produces a sample weight of less than 2 kg, more fruit should be taken to yield a 2 kg sample)
Pome fruits e.g. apples, pears	
Large stone fruit e.g. apricots, peaches, plums	
Miscellaneous fruit e.g. guavas, mangoes, papayas, pomegranates, kiwifruit, litchi	
Small stone fruit e.g. cherries	A total of 1 kg from several places on 4 trees
Grapes	A total of 12 bunches, or parts of 12 bunches, from separate vines to give at least 1 kg
Raspberries and other small berries	A total of 0.5 kg from 12 separate areas or bushes
Strawberries, Gooseberries	A total of 1 kg from 12 separate areas or bushes
Miscellaneous small fruits e.g. olives, dates, figs	A total of 1 kg from several places on 4 trees
Pineapples	A total of 12 fruits
Bananas	A total of 24 fruits. Take two fingers each from top, middle and lowest hand.
Tree nuts e.g. walnuts, chestnuts, almonds	A total of 1 kg
Coconut	A total of 12 nuts

1.1.1.2 Bulb, root and tuber vegetables

- Take samples from all over the plot, excluding 1 metre at the edges of the plot and the ends of the rows.
- Adhering soil is to be removed by brushing and, if necessary, gentle rinsing with cold running water.
- Trim off tops according to local agricultural practice. Details of any trimming should be recorded. Where the tops are not used as animal feed (carrots, potatoes) they should be discarded; otherwise (e.g. turnips, beets) they should be bagged separately.

Table 2: Sampling of bulb, root and tuber vegetables

Commodity	**Quantity, method of collection**
Sugar beets	12 plants
Potatoes	12 tubers or more (sample weight: at least 2 kg)

Other root crops e.g. carrots, red beet, sweet potato, turnip, radish	12 roots or more (sample weight: at least 2 kg)
Bulb onions	12 plants
Spring onions	24 plants or more (sample weight: at least 2 kg)
Garlic	12 bulbs from 12 plants (sample weight: at least 2 kg)

Brassica and leafy vegetables, stalk and stem vegetables, legume vegetables and fruiting vegetables

- Take the sample from all parts of the plot, leaving 1 metre at the edges and ends of rows.
- Adhering soil need to be removed by brushing and/or gentle rinsing with cold water.
- Do not trim except for the removal of obviously decomposed or withered leaves. Details of any trimming should be recorded.

Table 3: Sampling of other vegetables

Commodity	**Quantity, method of collection**
Large Brassica crops e.g. cabbage, cauliflower	12 plants
Cucumbers	12 fruits from 12 separate plants
Melons, gourds, pumpkins, watermelons	12 fruits from 12 separate plants. In case of large crops, the sample size may be reduced to 5 units.
Egg plants (aubergines)	12 fruits from 12 separate plants
Sweet corn	12 ears (the sample should weigh at least 2 kg – where necessary take a larger number of items to produce a 2 kg sample)
Mushrooms	12 items (the sample should weigh at least 0.5 kg)
Tomatoes, Peppers	24 fruits from small-fruiting varieties, 12 from large fruiting varieties. (the sample should weigh a minimum of 2 kg)
Spinach	1 kg from 12 plants

1.1.1.3. Cereals

- Cut stalks 15 cm above the ground and remove the grain from the straw.
- Care should be taken to avoid contamination when mechanical methods are used to separate the parts of the crop. The operation is best carried out in the laboratory.
- If the plots are harvested mechanically, take not less than twelve grab samples of grain and straw from the harvester at uniform intervals over the plot.
- Do not sample within 1 metre of the edges of the plot.

Table 4: Sampling of cereals

Commodity	**Quantity, method of collection**
Cereal grains e.g. wheat, rice, barley, oats, and other small grain cereals; maize (off the cob)	1 kg
Straw of the above crops	0.5 kg
Maize cobs	12 or more ears (the sample should weigh at least 2 kg)

1.1.1.5 Grasses, forage and animal feed

- Cut with shears at normal harvest height (usually 5 cm above the ground) the vegetation from not less than twelve areas uniformly spaced over the entire plot, leaving 1 metre at the edges of the plot.
- Crops which are harvested mechanically can be sampled by taking not less than twelve grab samples from the harvester at uniform intervals over the plot.
- Take 1 kg of sample for green forage and 0.5 kg for dry hay.

1.1.1.6 Spices, Herbs and tea leaves

The freshly harvested produce is not normally required for tea although herbs should be sampled fresh (sample size 0.5 kg). For tea and dry leaves, the sample size can be reduced to 0.2 kg.

1.1.2 Taking a Sample of Harvested Produce

Each laboratory sample should be made up of several individual sub-samples. As far as possible, sub-samples should be taken randomly throughout the lot and should be of a similar size. For example: sub-samples would consist of a single fruit or vegetable for larger items; a single bunch or bundle for grapes, a small scoop of produce for small items (peas, berries etc). If produce has been packaged, sub-samples should be taken from more than one box in the lot.

For cereals and other materials shipped in bulk, take a number of sub-samples (at least 10) from different places. Residues on grains are typically due to post harvest use of fumigants. Sampling of bulk lot can be done using the large sector trier to probe the surface of the bulk lot in several places. A sample size of 5 kg should be taken from 5 locations.

In some cases, it may be possible to do this by taking small grab samples while it is being unloaded, or else use a grain sampling probe which allows for sampling at different depths.

The minimum number of sub-samples to be taken is given in Table 7. The minimum quantity that should be sent to the laboratory is given in Table 8.

Table 7: Subsampling

Weight of lot (kg)	**Minimum number of sub-samples**
Less than 50	3
50 – 500	5
More than 500	10
For product in bulk which can be assumed to be well mixed or homogenous e.g. grains, the laboratory sample should consist of at least 10 sub-samples taken as described above.	
For processed products in cans, bottles, packages or other small containers, especially when the weight of the lot is unknown, the following sampling plan may be followed	
Number of cans, packages or containers in the lot	**Minimum number of sub-samples**
1-25	1
26-100	5
More than 100	10

Table 8: Sample quantity required

Commodity	Examples	Minimum quantity required
Small or light products, unit weight up to about 25 g	Berries, Peas etc.	1 kg
Medium sized products, unit weight usually between 25 and 250 g	Apples, Pears, Citrus, Potatoes	1 kg (at least 10 units)
Large sized products, unit weight over 250g	Cabbage, Melons, Cucumbers, grapes (bunches)	2 kg (at least 5 units)
Grains and oilseeds		1 kg

1.2 Soil Sample

General rules for sampling soils

- Individual blocks or paddocks or areas of paddocks likely to have been subjected to different chemical treatments should be sampled separately.
- The area sampled should be of the one soil type.
- Do not mix different soil types.
- Preferably, soil should be collected using a core-type sampling tool.
- Targeted sampling from small areas, e.g. around power poles, fence posts, old tree stumps or old dip sites, may be required to identify residue 'hot spots'.

There are two aspects of soil sampling:

i. As an environmental sample for analysis of pesticide residues in routine monitoring

ii. To know the dissipation rate of pesticides in case of supervised field trial.

Soil samples should be taken from growing fields in the grid pattern uniformly distributed, so that each area of the field is sampled. A 3x3 grid with 9 total sample portions is suggested for smaller fields, with 4x4 (16 sample portions) for the medium sized fields, and 5x5 and even larger grids are used for very large fields. Each sample site represents 1 portion of the total sample, and at each site, 2 soil plugs about 15 cm deep and 3-5cm in diameter are to be taken. The 2 plugs, when combined, become sample portion of that sample site. Combine different sample portions from the whole field in a clean container such as a bucket and mix thoroughly. Remove about 1/2 - 1 kg of soil and package up for transport to the laboratory.

Another common soil sampling method for a field or other area is to take 5 portions in a Z pattern.

1.2.1 Collection of Surface Soil Samples

Collection of samples from near-surface soil can be accomplished with tools such as spades, shovels, trowels, and scoops. Surface material is removed to the required depth and a stainless steel or plastic scoop is then used to collect the sample. This method can be used in most soil types but is limited to sampling at or near the ground surface.

- Carefully remove the top layer of soil or debris to the desired sample depth with a pre-cleaned spade.
- Using a pre-cleaned, stainless steel scoop, plastic spoon, or trowel, remove and discard a thin layer of soil from the area which came in contact with the spade.
- Place the sample into a stainless steel, plastic, or other appropriate homogenization container, and mix thoroughly to obtain a homogenous sample representative of the entire sampling interval. Then, either place the sample into appropriate, labeled containers and secure the caps tightly.

1.2.2 Sampling at Depth with Augers and Thin Wall Tube Samplers

This system consists of an auger, or a thin-wall tube sampler, a series of extensions, and a "T" handle.

- Attach the auger bit to a drill rod extension, and attach the "T" handle to the drill rod.
- Clear the area to be sampled of any surface debris (e.g., twigs, rocks, litter). It may be advisable to remove the first three to six inches of surface soil for an area approximately six inches in radius around the drilling location.
- Begin augering, periodically removing and depositing accumulated soils onto a plastic sheet spread near the hole. This prevents accidental brushing of loose material back down the borehole when removing the auger or adding drill rods.
- After reaching the desired depth, slowly and carefully remove the auger from the hole. When sampling directly from the auger, collect the sample after the auger is removed from the hole.
- Remove auger tip from the extension rods and replace with a pre-cleaned thin wall tube sampler. Install the proper cutting tip.
- Carefully lower the tube sampler down the borehole. Gradually force the tube sampler into the soil. Do not scrape the borehole sides. Avoid hammering the rods as the vibrations may cause the boring walls to collapse.
- Remove the tube sampler, and unscrew the drill rods.
- Remove the cutting tip and the core from the device.
- Discard the top of the core (approximately 1 inch), as this possibly represents material collected before penetration of the layer of concern. Place the remaining core into the appropriate labeled sample container. Sample homogenization is not required.
- If volatile organic analysis is to be performed, transfer the sample into an appropriate, labeled sample container with a stainless steel lab spoon, or equivalent and secure the cap tightly. Place the remainder of the sample into a stainless steel, plastic, or other appropriate homogenization container, and mix thoroughly to obtain a homogenous sample representative of the entire sampling interval. Then, place the sample into appropriate, labeled containers and secure the caps tightly.

It is recommended to chill soil samples to 4°C for transport to the laboratory.

1.3 Water Sample

Collect 3 litres of water to represent 1 sample from 1 water source. From a water source like pond or small lake, collection of water from a single point is sufficient. But if it is a stream, collect samples from different locations.

1.4 Milk

A total sample size of 2 litres is sufficient. Most recommended containers are glass jar. Where pouched milk is to be sampled, a minimum of three 500 ml pouches of similar nature need to be taken.

1.5 Processed food

Codex recommendation for sampling of processed packaged food-

Number of retail units in the lot	Minimum no. of retail units to be sampled
1-25	1
26-100	5
100-250	10
Over 250	15

1.6 Eggs and Egg Products

Shell-eggs can be sampled at the primary producer or at the packer prior to shipment. The sample size for the unpackaged shell-eggs at the producer is 6-dozen eggs. They are quite fragile and if packaging is not available for transport to the laboratory, then the eggs can be broken, and the magma is placed in glass jars and the shells are discarded. For sampling at packer, take 1 dozen eggs for 15 cases or fewer. For 16 cases or more, take a total of 10 dozen eggs, each from 10 cases selected at random.

Transport of samples

The laboratory sample must be placed in a clean, inert container which provides secure protection from contamination, damage and leakage. The container should be sealed, securely labeled and the sampling record must be attached. The use of marker pens containing organic solvents should be avoided for labeling bags containing samples to be analyzed for fumigant residues, especially if an electron capture detector is to be used.

Rapid transportation to the laboratory, preferably within one day, is essential for samples of most fresh products. Very fragile or perishable products (e.g. ripe raspberries) may have to be frozen to avoid spoilage and then transported in "dry ice" or similar, to avoid thawing in transit. Samples that are frozen at the time of collection must be transported without thawing. Samples that may be damaged by chilling (e.g. bananas) must be protected from both high and low temperatures. The condition of samples delivered to the laboratory should approximate to that acceptable to a discerning purchaser, otherwise samples should normally be considered unfit for analysis.

Water and Soil samples should be chilled at 4°C immediately after collection for transport to the lab. The glass jars used for sample collection should be rinsed thoroughly with methanol and dried.

Precautions to be taken during sampling, transportation, storage and sample preparation:

Samples must be separated from each other, and from other sources of potential contamination, during transit to, and storage at, the laboratory.

- Take care not to remove surface residues during handling, packing or preparation. To provide a representative sample of the raw commodity, adhering soil may have to be removed from some crops, such as root crops. This may be done by brushing and if necessary gentle rinsing with cold running water
- Make sure that sampling tools and bags are clean. Use new bags and containers of suitable size and adequate strength. The bags or containers should be made of materials which will not interfere with the analysis.
- Avoid contamination of the sample by hands and clothes which may have been in contact with pesticides.
- Do not allow the samples to come into contact with containers or equipment (including vehicles) that have been used for transporting or storing pesticides.

- In case of residue trial, avoid sampling at the plot borders because of the possibility of spray drift or overlap, especially where the plot is small and particularly when various pesticides and dosages are applied to adjacent areas.
- Avoid cross-contamination of crop and soil samples.
- Incase of residue trial, sampling should proceed from the control to the lowest treatment and so on to the highest treatment
- Pest control in, or near, the laboratory must be restricted to pesticides that will not be sought as residues.

C. Storage of Sample

Usually the samples are stored in deep freezer maintained at -10°C or below. Alternatively, instead of storing the sample, the extract of the sample may be stored in a refrigerator. Though later method is better, a control sample extracts fortified with pesticides need to be stored along with samples to know the total degradation of pesticides during storage.

Sample Preparation

Lot

A quantity of a materials, delivered at one time and known, or presumed, by the sampling officer to have uniform characteristics such as origin, producer, variety, packer, type of packing, markings, consignor, etc. In case of food material, a suspect lot is one which, for any reason, is suspected to contain an excessive residue. A non-suspect lot is one for which there is no reason to suspect that it may contain an excessive residue.

Primary sample

One or more units taken from one position of a lot.

(a) The position from which a primary sample is taken in the lot should preferably be chosen randomly but, where this is physically impractical, it should be from a random position in the accessible parts of the lot.

(b) The number of units required for a primary sample should be determined by the minimum size and number of laboratory samples required.

Bulk sample

The combined and well mixed aggregate of the primary samples taken from a lot is called bulk sample. The primary samples must contribute sufficient material to enable all laboratory samples to be withdrawn from the bulk sample.

Laboratory sample

The sample sent to, or received by, the laboratory. It is a representative quantity of material removed from the bulk sample. The laboratory sample may be the whole or a part of the bulk sample. Units should not be cut or broken to produce the laboratory sample(s).

Analytical sample

The material prepared for analysis from the laboratory sample, by separation of the portion of the product to be analysed and then by mixing, grinding, fine chopping, etc., for the removal of analytical portions with minimal sampling error.

Analytical portion

A representative quantity of material removed from the analytical sample, of proper size for measurement of the residue concentration.

On receipt, each laboratory sample must be allocated a unique reference code by the laboratory. Sample preparation, sample processing and sub-sampling to obtain analytial portions (an analyzable sample portion that represents the whole sample) must take place before visible deterioration occurs. Canned, dried or similarly processed samples should be analysed within the stated shelf life. If a single analytical portion is unlikely to be representative of the ana-lytical sample, replicate portions must be analysed, to provide a better estimate of the present residues.

The first step is to reduce the total sample (when necessary) to a manageable portion i.e. sub sampling. Determine what portion of the sample can be used for analysis, i.e. for raw and partially processed foods (this is called as edible portion) and for processed foods (this is the required portion). Both represent part of food that is normally consumed. A reserve portion of the original unprepared sample is necessary or desirable, and hence be stored for future use, such as a check analysis or for additional analysis required for confirmation test. The final step is to physically homogenize sub sample aggregate by chopping, grinding, blending etc.

Soil samples are always non-uniform in both particle size and composition. Hence to ensure proper extraction, the soil sample must be ground to a uniformly small particle size.

Where appropriate, the analytical sample should be processed under special conditions, e.g. at sub-zero temperature, to minimize adverse effects. Where processing could affect residues and where practical alternative procedures are not available, the analytical portion may consist of whole units, or segments removed from whole units. If analytical portions are to be stored before analysis, the method and length of time of storage should be such that they do not affect the level of residues present. Additional portions must be withdrawn for replicate and confirmatory analyses, as required.

Portion of raw agricultural commodities to be analysed for pesticide residues:

Sl. No.	Substrate	Portion to be analysed
1.	Root and Tuber vegetables	Where separate tolerances are established for root and tuber, analyze top and root separately. Where a tolerance is established including tops and/or with tops, analyse whole commodity after removing adhering soil by lightly rinsing in running water.
2.	Leafy Vegetables	Whole commodity after removing and discarding obviously decomposed or withered leaves. Remove adhering soil from leaves if any.
3.	Brassica	Whole commodity after removing and discarding obviously decomposed or withered leaves; excepting removal and discarding all leaves from cauliflower and broccoli.

4.	Fruiting Vegetables	Whole commodity after removing and discarding stems and husks.
5.	Cucurbit Vegetables	Whole commodity after removing and discarding stems
6.	Cereal Grain	Whole commodity (grain), excepting for fresh corn: Include kernels plus cob after removing and discarding husk.
7.	Eggs	Whole commodity after removing and discarding shells
8.	Fish	Edible portion of the commodity after removing and discarding scales, fins, viscera; bones and skin (if inedible)

In case of processed foods, analayse the whole processed commodity including any liquid or other medium in which the commodity is packed. If any inedible medium is present, discard that medium.

Sample Extraction

The analysis of pesticide residues in food samples is increasingly becoming an important technique. Extraction is a fundamental process in pesticide residue analysis. Extraction may be defined as the procedure or step adopted to isolate the pesticides from the samples. This is accomplished by emplying suitable solvents which remain in contact with the sample for a specific duration. Extraction and clean-up methods vary greatly with the matrix used. It largely depends on the moisture, fat and sugar content of the commodity. The quantity of sample to be taken for extraction will depend upon the expected levels of residues as well as the sensitivity of technique or tool employed, in the subsequent step of identification and quantification. In general, 50 to 250g of solids are usually recommended whereas for liquids it may range between 2ml (in case of blood) to 5 litre (in case of water).

Ideal extraction procedure should be capable of extracting 100% of pesticides only, leaving behind other materials. However this is not the actual practice. An extraction method should satisfy the following requirements:

- Rapid
- Small solvent usage
- Good recovery: 40 to 120%.
- Robust: RSD of the recoveries less than 20% at LOQ.

Depending upon the type of samples, different extraction methods can be followed. The following table lists the extraction methods, the matrix types, the techniques, and the analyte categories.

Sl. No.	Matrix Type	Extraction Technique	Analytes
1	Aqueous	Separatory Funnel Liquid-Liquid Extraction, Continuous Liquid-Liquid Extraction, Solid-phase Extraction (SPE)	Semivolatile and Nonvolatile Organics
2	Solids	Soxhlet Extraction, Automated Soxhlet Extraction,	Semivolatile and

		Pressurized Fluid Extraction (PFE), Microwave Extraction, Ultrasonic Extraction,	Nonvolatile Organics
3	Solids	Supercritical Fluid Extraction	Semivolatile and Organochlorine Pesticides
4	Air Sampling Train	Separatory Funnel & Soxhlet Extraction	Semivolatile Organics
5	Fruits and Vegetables	Blending and Separatory Funnel Liquid- Liquid Extraction	Semivolatile and Nonvolatile Organics

Test portions should be disintegrated thoroughly during extraction to maximize extraction efficiency. Temperature, pH, etc., must be controlled if these parameters affect extraction efficiency, analyte stability or solvent volume.

1. **Liquid-liquid extraction:** also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent such as hexane, ether, dichloromethane, chloroform, ethyl acetate etc. It is performed using a separatory funnel, also known as separation funnel, separating funnel, or colloquially sep funnel. Most organic solvents float on top of an aqueous phase, though important exceptions are most halogenated solvents.

   In this method, the two phases i.e. a liquid sample and the extracting solvent are added through the top of a separatory funnel with the stopcock at the bottom closed. The funnel is then closed and shaken gently by inverting the funnel multiple times; if the two solutions are mixed together too vigorously emulsions will form. The funnel is then inverted and the tap carefully opened to release excess vapor pressure. The separating funnel is set aside to allow for the complete separation of the phases. The top and the bottom tap are then opened and the two phases are released by gravitation.

Separatory Funnel Extraction Procedure

1. *Inspect the separatory funnel*: The Teflon stopcocks work better than the ground glass stopcock. Make sure that the stopper fits snugly in the top of the flask

2. *Support the separatory funnel in a ring on a ringstand*: The rings are located on the back shelves and they come in many sizes. Choose a proper size ring before setting the funnel in it.

3. *Add the liquid to the separatory funnel.:*Place a stemmed funnel in the neck of the separatory funnel. Add the liquid to be extracted, then add the extraction solvent. The total volume in the separatory funnel should not be greater than three-quarters of the funnel volume. Insert the stopper in the neck of the separatory funnel.

4. *Shake the separatory funnel*: Pick up the separatory funnel with the stopper in place and the stopcock closed, and rock it once gently. Then, point the stem up and slowly open the stopcock to release excess pressure. Close the stopcock. Repeat this procedure until only a small amount of pressure is released when it is vented.

Now, shake the funnel vigorously for a few seconds. Release the pressure, then again shake vigorously. About 30 sec total vigorous shaking is usually sufficient to allow solutes to come to equilibrium between the two solvents.

5. *Separating the layers:* Let the funnel rest undisturbed until the layers are clearly separated. While waiting, remove the stopper and place a beaker or flask under the sep funnel.

6. Carefully open the stopcock and allow the lower layer to drain into the flask. Drain just to the point that the upper liquid barely reaches the stopcock. If the upper layer is to be removed from the funnel, remove it by pouring it out of the top of the funnel.

7. Store your separatory funnel with the cap (stopper) separate from the funnel. Otherwise it can become irreversibly attached to the separatory funnel.

The organic product will be soluble in an organic solvent (organic layer) while the inorganic substances will be soluble in water (aqueous layer). The organic solvent used for extraction must meet a few criteria:

1. Should readily dissolve substance to be extracted.
2. Should not react with the substance to be extracted.
3. Should not react with or be miscible with water (the usual second solvent).
4. Should have a low boiling point so it can be easily removed from the product.

Distribution ratio: The distribution ratio (*D*), sometimes referred to as the **partition coefficient (P**) is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium. It is equal to the concentration of a solute in the organic phase divided by its concentration in the aqueous phase. Depending on the system, the distribution ratio can be a function of temperature, the concentration of chemical species in the system, and a large number of other parameters. The phrase "Partition Coefficient" is now considered obsolete by IUPAC, and "partition constant," "partition ratio," or "distribution ratio," are all more appropriate terms that should be used.

Removal of water: Although the criteria state that the organic solvent chosen should not be miscible with water, some solvents dissolve a small amount. A drying agent such as sodium sulfate or magnesium sulfate must be used, through which the organic solvent containing the dissolved product is passed through before collecting.

2. Soxhlet Extraction: A Soxhlet extractor was invented in 1879 by Franz von Soxhlet for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

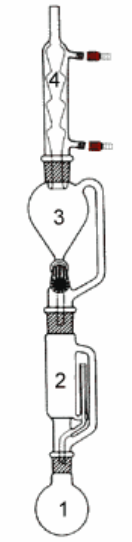


Fig.1. Soxhlet Extractor

The sample is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent and then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, condenser which ensures that the solvent vapour cools, and drips back down into the chamber housing the sample and the chamber slowly fills with warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. During each cycle, a portion of the non-volatile compound dissolves in the solvent. This cycle may be allowed to repeat many times, over hours or days. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. In some device (Fig.1) a funnel (3) allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask (1) is then evaporated and the sample concentrate is taken for analysis.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

3. Automated Soxhlet extraction: Several solvent extraction systems, automated or semi-automated, based on the Soxhlet device are on the market to allow fast and effective determination of organic compounds in food, soil etc. As example **"Soxtec Systems**" by FOSS which performs boiling, rinsing and solvent recovery. Similarly, **Soxtherm extractors** from **Gerhardt GmbH** was developed to reduce extraction times. The sample to be analyzed is weighed into cellulose thimbles and inserted in the extraction device. Except diethyl ether, all solvents may be used (about 15 ml per sample), with a 75% recovery of the solvent after the extraction which is completed in 30 to 60 min,

depending on the application. The **Büchi** Extraction System B-811 is an automated system which can be used to perform an extraction according to the original Soxhlet principle. Four different extraction methods are possible without making any changes to the unit: Soxhlet standard, Soxhlet warm, hot extraction and continuous extraction. The system has an inert gas supply to avoid oxidation during extraction and to accelerate the evaporation and drying process even with high boiling point solvents (up to 150°C). In microwave-assisted Soxhlet extraction, uses two sources of energy, namely microwaves, applied on the extraction chamber of a modified Soxhlet, and electrical heating applied on the distillation flask.

4. Super-critical fluid extraction (SFE):

SFE makes use of a supercritical fluid as the extraction solvent. Carbon dioxide is the most common solvent in SFE because it is safe, unreactive, readily available, relatively inexpenpensive, has a low critical pressure and temperature point (74 atm and 31°C). A key property of supercritical fluid is that, fluid density, which is related to solvating power, in SFE can be controlled by changing extraction pressure and temperature. Also, supercritical fluids have low viscosity even at high fluid density, which allows good penetration into the matrix. Concentration of analytes after extraction is fast and convenient because supercritical CO_2 becomes a gas after depressurization.

Disadvantages include large capital cost and method development can be time consuming.

4. Solid Phase Extraction

Solid phase extraction is a chromatographic technique used to prepare samples for subsequent analysis by removing interfering substances that may be present. This is done either by retaining the substance of interest and washing off everything else or by retaining the interfering substances and eluting the product of interest. Though the method is commonly used to clean up a sample before injection, today it is one of the most widely used sample preparation methods. The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube.

The SPE sample preparation technique is frequently carried out in a small, disposable, plastic column (cartridge) that looks like a 10-mL medical syringe. The column is packed with a small amount (< 1.0 g) of sorbent (e.g. C_{18}-silica) which is held in place by frits.

It can be attached to a vacuum manifold, which increases the solvent flow rate through the cartridge. A collection tube is placed beneath the SPE cartridge to collect the liquid that passes through the column.

6. Solid Phase Micro Extraction: The SPE method can also be implemented by using a micro-pipette tip, a 96-well plate, a disk, or even coated fibers, the later has its own name - "solid-phase microextraction" or SPME.

7. Accelerated Solvent extraction (ASE): ASE also known as **Pressurized Liquid extraction (PLE),** is similar in principle to Soxhlet extraction, except that elevated temperatures and pressures are used in enclosed vessels, which allows extraction by a small amount of solvent (<50 mL) to be completed in a very short time (<20 min). Application of ASE has been reported for the extraction of various organic compounds from different environmental samples including a few pesticides in soil, including chlorinated and organophosphorus insecticides, the herbicide and the fungicide hexaconazole.

8. Microwave Assisted extraction (MAE): MAE has been applied to soil, food, vegetables, oil, eggs, dairy products, sediments and other samples for extraction of polycyclic aromatic hydrocarbons (PAHs), pesticide residues, trace elements etc. MAE of pesticide residues is applied generally when the pesticide residue level in samples is very low (ppm or ppt). It uses less extraction solvent for a same amount of sample (about 1/10). However, it needs a different solvent from the conventional method to extract the same pesticide. Studies have shown that, using iso-octane, n-hexane / acetone, benzene / acetone (2; 1), methanol / acetic acid, methanol / n-hexane, iso-octane / acetonitrile etc. as solvent, with a certain moisture condition of the soil or sediment, the extracted organochlorine pesticide residues are the same whether using MAE method for 3 minutes or the Soxhlet extraction method for 6 hours. Microwave

extraction method has been applied to other samples of extracting pesticide residues such as: meat, eggs and dairy products; soil and sand; dust, water and sediments; vegetables etc.

NEW TECHNIQUES

Quick, easy, cheap, effective, rugged, and safe (QuEChERS)

Many food samples contain a range of pesticides. These pesticides can be acidic, basic and neutral, and the sample matrix may contain components such as lipids, sterols, etc. Due to increasing throughput requirements and cost pressures, a multi-residue method should meet the following criteria:

- Fast to perform — Use as few process steps as possible
- Easy to execute — Avoid laborious process steps such as evaporation/reconstitution
- Use a minimum amount of solvents and sorbents — Reduce costs and environmental impact

By minimizing the number of steps in multi-residue method, greater accuracy in results and a greater throughput of samples can be achieved.

The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003. The method was modified to address some problematic pesticides by including a buffered extraction system. The EN method 15662:2007 is a European variation to the QuEChERS method.

The QuEChERS technique is:

- Quick – Sample throughput is high and it can detect a range of pesticides in a single extraction
- Easy – No need for automation during the process and laborious process steps are eliminated
- Cheap – It uses less sorbent and solvent than other multi-residue methods
- Effective – It gives high recoveries and accurate results for a range of pesticide types
- Rugged – can detect a range of pesticides, such as polar and pH-dependent compounds
- Safe – Extraction is carried out using acetonitrile instead of chlorinated solvent.

This method is a sample preparation technique using solvent extraction of high-moisture samples with acetonitrile, ethyl acetate or acetone and partitioning with magnesium sulfate alone or in combination with other salts ($MgSO_4$, NaCl and buffering citrate salts to induce liquid- liquid partitioning), followed by sample clean-up using a dispersive solid-phase extraction (SPE) technique.

Although the EN and AOAC are similar methods, they do have several differences. First, the extraction buffered system in the EN method uses sodium chloride, sodium citrate and disodium citrate sesquihidrate instead of sodium acetate in the AOAC extraction step. Second, in the dispersive SPE step, the EN method uses 25 mg PSA per mL of extract rather than 50 mg PSA per mL of extract as stated in the AOAC method.

The first step (sample extraction) involves adding a homogenized food sample to a 50-ml centrifuge tube containing magnesium sulfate and a salt such as sodium chloride or sodium acetate. Upon addition of acetonitrile and extensive shaking and centrifugation of the tube, the magnesium sulfate induces a phase separation between the acetonitrile and water layers. The compounds of interest transfer into the acetonitrile layer. A portion of this acetonitrile layer is next transferred to a clean-up tube. This tube is typically 2 or 15 ml in volume and contains a combination of sorbent materials in different amounts. Magnesium sulfate is present to remove any excess water from the sample. Primary/secondary amine (PSA) is used for the removal of organic acids and polar pigments, while endcapped C18 is used for lipid and sterol removal. Graphitized carbon block is used if substances such as chlorophyll are present and may break down during analysis. Following sample extraction and clean-up, a portion of the sample can then be injected directly onto an LC or GC system without any need for evaporation, reconstitution or derivatization. This technique was initially developed for analysis of fruit and vegetable products. However, research has shown that it can be successfully utilized with other matrices such as milk, honey and rice.

Clean-up

The steps or procedure adopted to isolate the pesticides from other impurities or co-extractives present in the sample extract are called clean-up. The degree or level of clean-up should be very high so that highly sensitive, expensive instruments used in subsequent steps are not adversely affected. During estimation, the co-extractive may cause:

- Extra peaks
- Poor peak resolution
- Reduce efficiency of the column

In certain samples like water or soil, it may be possible to use uncleaned extract directly for the next step because of absence of co-extractives. With the development of new extraction techniques and analytical methods that are less affected by co-extractives, clean-up becomes less important.

Different clean-up methods are:

i) Liquid-liquid partitioning
ii) Column Chromatography
iii) Solid Phase Extraction
iv) Chemical clean-up
v) Gel-permeation/Size Exclusion Chromatography
vi) Sweep co-distillation

Liquid-liquid and liquid-solid partitioning, in which separation is based on polarity, are the primary means of clean-up in residue analysis.

Liquid-liquid partitioning

Liquid-liquid partition clean-up using acetonitrile and **dimethylformamide-hexane** were amongst the earliest methods developed to remove lipid materials.

Column chromatography

The widely used material in column-liquid chromatographic sample preparation for OC analysis are florisil, alumina and silica. Alumina has also been mixed/impregnated with other compounds to aid clean-up, e.g. alumina/silver nitrate column to remove carotenoids, elemental sulphur, and some interfering organosulphur compounds, impregnated alumina with sulphuric acid to improve lipid removal etc. Besides the type of adsorbent, their mesh size and column dimensions also play important role in separation efficiencies.

Solid-phase Extraction

The solid-phase cartridge was developed soon after HPLC when high quality sorbent materials become available. The cartridges are used for extraction of water samples, but a large part of their use has been for clean-up of extracts. The mechanism of separation in SPE is the same as in HPLC. The type of solvent and solid phase material can be varied to achieve the desired separation. Some common SPE stationary phases are florisil, alumina, carbon, C-8, C-18 etc. The recent developments include polymer based

phases, and SPE disk format. Although the disk format can become clogged more easily than cartridges, the disks allow faster throughput for large sample volumes.

The drawback with the use of certain sorbents in solid-phase techniques is the cost. Because, the sorbents are often most expensive materials and most cartridges are designed to be discarded after a single use.

Chemical clean-up

Most chemical clean-up techniques reported have used fairly harsh acidic conditions, based on concentrated sulphuric acid or chromic acid to remove lipids and elemental sulphur. Sulphuric acid can be used successfully to clean-up extracts for the determination of the more robust organochlorines. Compounds in the Drin group (Aldrin, Endrin, Dieldrin) are destroyed by this treatment, as are most other oxidisable organics. Alternatively, the co-extracted lipid material may be modified by saponification with ethanolic potassium hydroxide prior to separation on alumina or silica gel. This has the advantage of removing the lipid hydrolysis products by back extraction into an aqueous phase and reducing to lipid loading to any secondary clean-up column. Saponification of tissue prior to extraction may also improve the recovery for some organochlorines.

Gel-permeation Chromatography

GPC was first developed in the late 1960's and early 1970's, and began to be used in pesticide regulatory methods in the late 1970's and early 1980's. GPC also known as size-exclusion chromatography (SEC), where the separation is based on molecular weight (size), separates compounds with large molecular weight, such as lipids and proteins, from smaller analytes, such as pesticides using a porous stationary phase material packed in a column

The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. Commercial gels like Sephadex, Bio-Gel (cross-linked polyacrylamide), agarose gel and Styragel are often used based on different separation requirements. Carbon-foam has been used very successfully in the separation of very closely related OCs groups

Samples are dissolved in an appropriate organic solvent (cyclohexane, toluene, ethyl acetate etc.) and after filtering, it is injected onto a column. The separation of multi-component mixture takes place in the column. The constant supply of fresh eluent to the column is accomplished by the use of a pump.

GPC can be automated in series with SFE, and studies have been performed that utilize supercritical CO_2 mixed with liquids as the mobile phase in GPC.

Sweep co-distillation

The potential of sweep co-distillation in the routine clean-up of fatty tissue has only recently been practically realized. The recent designs allow up to 10 fat samples **(**1 g of fat) to be injected into a heated (235^{O}C) fractionation tube containing silanised glass beads. The OCs are distilled in a stream of pure nitrogen into a cold florisil (deactivated with 1% water) trap on the outside of the oven, which is subsequently detached and eluted with hexane or hexane-diethyl either to remove the groups of OCs. Until recently is technique was unreliable because of sample loss by degradation and adsorption. However improved silanisation techniques on the glass packing now allow excellent recoveries from 1 g of fat at 0.02-0.1 mg/kg range for OCs and 0.10 mg/kg for total PCBs. Good yields were also obtained for

the Organophosphorus (OPs) such as Fenchlorophos and Chlorophyriphos, and for pentachlorophenol, which clearly indicate the future possibilities for the current systems.

AUTOMATION

The recent concerns for labour reduction and ease of use have led to a push for automation in clean-up techniques. The automated clean-up instrument can be combined in series with automated SPE/PLE and/or analytical instrument.

Clearly, there must be some justification for the financial outlay and technical expertise required for automation. However in an area of trace analysis which is growing so rapidly and which at present is so labour intensive, that justification is generally not too hard to find.

A. Identification and Quantification

Identification and Quantification are the most important steps of whole pesticide residue analysis process. Since the job of Residue Analysis is to detect and estimate trace levels of toxic pesticide residues in large quantity of samples, which may be equated to job of searching for needle in hay stack, the analytical technique adopted should be extremely sensitive. In addition, it would be more desirable if the technique is specific with repeatability, reproducibility, cheap and quick.

A.1 Techniques used for identification and quantification of pesticide residues are:

- Gas-liquid chromatography (GLC) with various detectors (FPD, NPD, ECD etc.)
- High-performance (pressure) liquid chromatography (HPLC) with various detectors like DAD, Fluorescent etc.
- GLC coupled with Mass-Detector (GC-MS or GC-MS^n)
- HPLC coupled with Mass-Detector (LC-MS or LC-MS^n)

A.2 Steps involved in identification and quantification of pesticide residues are:

- Preparation of pesticide standards, calibration solutions
- Selection of GC/HPLC columns*
- Selection of GC/HPLC detectors*
- Method Validation
- Concentration/dilution of sample aliquot for injection
- Calculation of residue concentration

A.2.1 Preparation of Pesticide standards, calibration solutions, etc.

a) Identity, purity, and storage of standards

"Pure" standards of analytes and internal standards should be of known purity and each must be uniquely identified and the date of receipt recorded. They should be stored at low temperature, preferably in a freezer, with light and moisture excluded, i.e. under conditions that minimize the rate of degradation. The pure standard may be retained if its purity is shown to remain acceptable. The identity of freshly acquired "pure" standards should be checked if the analytes are new to the laboratory.

b)Preparation and storage of stock standards

When preparing stock standards solutions, the identity and mass (or volume) of the standard and the identity and amount of the solvent (or other diluents) must be recorded. The solvent(s) must be appropriate to the analyte (solubility, no reaction) and method of analysis. The concentrations must be corrected for the purity of the "pure" standard.

Not less than 10 mg of the standard should be weighed using a 5 decimal place balance. The ambient temperature should be that at which the glassware is calibrated, otherwise preparation of the standard should be based on mass measurement. Volatile liquid analytes should be dispensed by weight or volume (if the density is known) directly into solvent. Gaseous (fumigant) analytes may be dispensed by bubbling into solvent and weighing the mass transferred, or by preparing gaseous dilutions (e.g. with a gas-tight syringe, avoiding contact with reactive metals).

Stock standards must be labeled properly, allocated an expiry date and stored at low temperature in the dark in containers that prevent any loss of solvent and entry of water. Currently available data show that stock standards of the large majority of pesticides in toluene and acetone are stable for at least 5 years in the freezer when stored in tightly closed glass containers.

When a stock standard is prepared for the first time, and for suspensions (e.g. dithiocarbamates) and solutions (or gaseous dilutions) of highly volatile fumigants that must be prepared freshly, the accuracy of the solution should be compared with a second solution made independently at the same time.

c) Preparation, use and storage of working standards

When preparing working standards, a record must be kept of the identity and amount of all solutions and solvents employed. The standards must be labeled indelibly, allocated an expiry date and stored at low temperature in the dark in containers that prevent any loss of solvent and entry of water. Septum closures are particularly prone to evaporation losses (in addition to being a source of contamination) and should be replaced as soon as practicable after piercing, if solutions are to be retained.

d) Testing and replacement of standards

Whenever any standard reaches its expiry date or is replaced, its purity should be checked. Existing stock and working solutions may be tested against newly prepared solutions by comparing the detector responses obtained from appropriate dilutions of individual standards or mixtures of standards. The purity of an old "pure" standard may be checked by preparing a new stock standard and comparing the detector responses obtained from freshly prepared dilutions of old and new stock standards. The means from at least five replicate measurements for each of two solutions should not normally differ by more than ±5%. The mean from the old (existing) solution is taken to be 100%. However, if the number of replicate determinations required to distinguish a difference of ±5% is unacceptably large for problematic analytes, the acceptable range may be increased to ±10%. The use of an internal standard may reduce the number of replicate injections required to achieve a ±5% difference. If a response of the old standard differs by more than ±5% (or ±10% in the case of problematic analytes) from the new, storage time or conditions must be adjusted as necessary on the basis of the results.

A.2.2 Method Validation

Within laboratory method validation should be performed to provide evidence that a method is fit for the purpose for which it is to be used. All procedures (steps) that are undertaken in a method should be validated, if practicable.

For multi-residue methods validation, representative matrices may be used. However, representative matrices must be chosen carefully on the basis of their biological or "analytical" similarity. This may be with regard to their water, lipid or sugar contents, pH, etc. So, for example, oranges may be chosen as being representative of citrus fruits, and lettuce as representative of green leafy vegetables, etc.

The method must be tested to assess different criteria such as linearity, sensitivity, mean recovery (as a measure of trueness or bias), precision etc.

a) Calibration

i. Calibration with standard (Linearity Check)

The most straightforward method for quantitative chromatographic analysis involves preparation of a series of standard solutions that approximate composition of unknown. It is done to detect differences in response due to different concentration. Chromatograms for the standards are then obtained, and peak heights or areas are plotted as the function of concentration. This plot is called **Calibration Curve.** It is a straight line and can be represented with a regression equation.

Y=mX + C

Where, Y = Peak Area

X = Concentration

m = Slope of the curve

C = Constant

Responses used to quantify residues must be within the dynamic range of the detector. Extracts containing high-level residues may be diluted to bring them within the calibrated range but, where calibration solutions must be matrix-matched the concentration of matrix extract may have to be adjusted.

Residues below the lowest calibrated level (LCL) should be considered uncalibrated, and therefore reported as <LCL, whether or not a response is evident. The calibration curve should not be forced through the origin.

Single level calibration may provide more accurate results than multilevel calibration if the detector response is variable with time. When single-level calibration is employed, the sample response should be within ±10% of the calibration standard response if the MRL is exceeded.

b) Reagent Blank

Analysis of reagent blanks should identify sources of interference in the equipment or materials used.

Ideally, there should be no signal values when control tests are run with the samples. Practically and specially with agricultural commodities, this however never happens. Very often signals of "apparent residues" or blank signals appear due to unremoved co-extractives in the extraction process, or impurities in the solvents or reagents or instrument noise.

Volumetric equipment, such as flasks, pipettes and syringes, must be cleaned scrupulously; separate glassware, syringe etc., should be allocated to standards and sample extracts, in order to avoid cross-contamination.

Where an internal standard is used, unintended contamination of extracts or analyte solutions with the internal standard, or *vice versa*, must be avoided.

Equipment, containers, solvents (including water), reagents, filter aids, etc., should be checked as sources of possible interference. Rubber and plastic items (e.g. seals, protective gloves, wash bottles), polishes and lubricants are frequent sources. Vial seals should be PTFE-lined. Extracts should be kept out of contact with seals, especially after piercing, by keeping vials upright. Vial seals must be replaced quickly after piercing, if re-analysis of the extracts is necessary.

Interference from natural constituents of samples is frequent. The interference may be peculiar to the determination system used, variable in occurrence and intensity, and may be subtle in nature. If the interference takes the form of a response overlapping that of the analyte, a different clean-up or determination system may be required. If it is not practicable to eliminate interference, or to compensate for it by **matrix-matched calibration**, these analytes must be considered in the interpretation of results.

c) Sensitivity

i) Limit of Detection (LOD)

LOD is defined as the lowest concentration of a pesticide residue that can be identified positively using a particular method in a particular matrix. This concentration is recommended to be three standard deviations above the measured average difference between the sample and blank signals which corresponds to the 99% confidence level.

Samples that do not bear residues at or above the LOD are referred to as "non-detects" (NDs).

Sometimes other related (more specific) concepts like **Method Detection Limit** (**MDL**) and **Instrument Detection Limit** (**IDL**) are used instead of LOD. The **Method Detection Limit** (**MDL**) refers to the lowest concentration which can be reliably detected in either a sample or a blank and the **Instrument Detection Limit** (**IDL**) refers to the smallest signal above background noise than an instrument can reliably detect.

ii) Limit of Quantitation (LOQ)

LOQ is defined as the level above which quantitative results may be obtained. It is the lowest tested concentration at which an acceptable mean recovery (normally 70-110%) and acceptable RSD (normally <20%) are obtained. Samples that do not bear residues at or above the LOQ are often referred to as "nonquantifiable."

To determine LOD and LOQ, fortification experiments should be conducted using a control sample and at least 5 evenly spaced fortification levels, with at least 2 replications. Each replicated sample should be individually fortified. All the analysis should be carried out consecutively. Where drift is exhibited, the values should not be taken into consideration.

Lower Limit of Method Validation (LLMV). There are cases in which a laboratory does not stringently determine the LOD and LOQ of a particular substrate/method/ equipment combination but, rather, a "Lower Limit of Method Validation" (LLMV) is reported that could be higher than the true LOQ within the capability of the method. The LLMV is simply the lowest concentration at which the method was validated.

d) Recovery:

The portion of the compound, recovered in the final determinative step, after processing of the sample (Extraction and Clean-up) is called recovery. In pesticide residue analysis studies it is of utmost importance that the method used for analysis ensures recovery of the pesticides to the maximum possible extent, which in turn reflects acceptance of the method employed for analysis.

In order to validate the residue analytical methods for crops, food, feed and environmental samples, recovery studies are carried out at specified fortification levels. The analytical material is fortified with the pesticide in a suitable solvent, and the solution is allowed to absorb into the sample. The fortified

control sample is then extracted and analysed in accordance with the specified method. Then recovery is calculated as:

Recovery (%) = (Recovered Concentration/Added Concentration) x100

Recovery may be subjected to considerable variation induced by dissimilar handling during processing/clean-up and by remnants of interfering substances in the final determination step even when same methodology was followed. So, several recovery experiments should be run to know the **range of variation of the recovery** from which the recoveries mean and its standard deviation are calculated. The recovery and its range of variation are also greatly dependent upon the amount of pesticides in the analyzed material. A minimum of 5 replicates is required (to check the precision) at both the reporting limit (to check the sensitivity of the method), and at least at another higher level, perhaps an action level, for example the MRL. With the aid of recovery mean, the analytical results can be converted to a non-loss *i.e* Theoritical Recovery (TR) by applying recovery Factor (RF).

If the analytical method does not permit determination of recovery (for example, direct analysis of liquid samples, SPME, or headspace analysis), the precision is determined from repeat analyses of calibration standards. The bias is usually assumed to be zero, although this is not necessarily so. In SPME and headspace analysis, the trueness and precision of calibration may depend on the extent to which the analyte has equilibrated, particularly with respect to the sample matrix.

Where practicable, recovery of all analytes determined should be measured with each batch of analyses. If this requires a disproportionately large number of recovery determinations, the minimum acceptable frequency of recovery may be as given in Table 1.

In cases where blank material is not available or where the only available blank material contains an interfering compound at an acceptably low level, the spiking level for recovery should be ≥3 times the level present in the blank material. The analyte (or apparent analyte) concentration in such a blank matrix should be determined from multiple test portions. If necessary, recoveries should be corrected by blank values.

The determination system must be calibrated with the representative analytes for every batch of analyses. The minimum frequency for calibration of representative and all other analytes is given in Table 1.

Table 1. Minimum frequencies for calibration and recovery Representative analytes

Minimum frequency of calibration	Calibration in each batch of analyses. At least at the level corresponding to the reporting limit.
Minimum frequency of recovery	Determination in each batch of analyses. At least at the level corresponding to the reporting limit.

Acceptable limits for single recovery should normally be in the range of 70-110% (in certain cases 60–140%) and may be adjusted using repeatability (validation) and intra laboratory reproducibility (routine on-going recovery) data. Recoveries outside this range usually require re-analysis of the batch but may be acceptable in certain justified cases.

The recovery study also helps in achieving various validation parameters of analysis. These parameters also required for the registration of plant protection products and their a.i. The parameters are:

1. **Trueness:** There are various approaches to determine trueness of methods. The most common is the performance of recovery experiments. According to the EC guidance document, the mean recovery should be in the range of 70-110%. In justified cases, recoveries outside this range are

also acceptable. In such cases, the recovery factor is generally applied to the detected residue concentration.

2. **Repeatability:** Repeatability is defined as the **precision** under conditions where independent test results are obtained with the same method on the identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. Normally, it is estimated by the relative standard deviation (RSD) of the recoveries, which should be lower than 20% per commodity and fortification levels.

3. **Reproducibility:** It is defined as the validation of the repeatability of recovery, from representative levels, by at least one laboratory, which is independent of the laboratory which initially validated the study.

4. **Specificity:** Specificity is defined as the ability of the method to distinguish between the analyte being measured and other substrates. For this, blank value must be reported using representative matrices. They should not be higher than 30% of the LOQ.

The highest precision for quantitative chromatography is obtained by using **internal standard** (I.S.)**,** because uncertainties introduced by the sample injection can be avoided. In this procedure, a carefully measured quantity of an internal standard substance is introduced into each standard and sample, and the ratio of the analyte peak area to the I.S. peak area are recorded. The I.S. peak should well be separated from the peaks of all other components in the sample. With a suitable I.S., precisions of 0.5 to 1% are reported.

A.2.5 Dilution/Concentration of sample Extract

Great care must be exercised when extracts are evaporated to dryness, as trace quantities of many analytes can be lost in this way. A small volume of high boiling point solvent may be used as a "keeper" and the evaporation temperature should be as low as practicable. Frothing and vigorous boiling of extracts, or dispersion of droplets, must be avoided. A stream of dry nitrogen or vacuum centrifugal evaporation is generally preferable to the use of an air stream for small-scale evaporation, as air is more likely to lead to oxidation or to introduce water and other contaminants.

Where extracts are diluted to a fixed volume, accurately calibrated vessels of not less than 1 ml capacity should be used and further evaporation avoided. Alternatively, an internal standard may be used, particularly for small volumes. Storage of extracts in a refrigerator or freezer will minimize degradation of compounds.

A.2.6 **Calculation of results**

a) Chromatographic integration

Chromatograms must be examined by the analyst and the baseline fitting checked and adjusted, as required. Where interfering or tailing peaks are present, a consistent approach must be adopted for the positioning of the baseline for standards as well as for all sample peaks. Peak height or peak area data may be used; whichever yields the more accurate and repeatable results.

b) Calculation of concentration Using Calibration Curve

The concentration of a pesticide residue can be calculated based upon the calibration curve. The pre-requisite for this method is that the peak area should fall within the linear range of the curve. Then

the concentration can be calculated on basis of the slope of the calibration curve using the regression equation:

Y=mX + C

Where, Y = Peak Area

X = Concentration

m = Slope of the curve

C = Constant

Limitation of this method is:

- frequent re-standardization is necessary
- Uncertainty in the volume of sample

c) Calculation of concentration Using Peak Area of Standard

The amount of pesticide residues present in a sample can be calculated using the formula:

$$\mathbf{R = (P_1/P_2)} \times \mathbf{C} \times \mathbf{(D/W)}$$

Where,

R = Pesticide Residue in ppm or μg/g or μg/ml

P_1 = Peak area of sample aliquot

P_2 = Peak area of the standard solution

C = Concentration of standard solution (μg/ml)

D = Volume of the sample extract (ml)

W = Weight of the sample portion used for analysis (g or ml)

In this case, the sample response should be within ±10% of the response of standard concentration used for calculation.

In general, residues data are not to be adjusted for recovery. If they are adjusted for recovery (in case of low recovery, generally <60%), then this must be stated. In this case they should be adjusted using the mean value from 3 recoveries performed in the same matrix, and analysed in the same batch of samples.

d) Qualifying results with uncertainty data

It is a requirement under ISO/IEC 17025 that laboratories determine and make available the uncertainty associated with analytical results. Measurement uncertainty is a quantitative indicator of the confidence in the analytical data and describes the range around a reported or experimental result within which the true value can be expected to lie within a defined probability (confidence level). Uncertainty ranges must take into consideration all sources of error. Laboratories should have available sufficient data derived from method validation/verification, inter-laboratory studies (e.g. proficiency tests) and in-house quality control tests, which are applied to estimate the uncertainties.

Estimates of typical uncertainty are based on previous data and may not reflect the uncertainty associated with analysis of a current sample. Uncertainty data relate primarily to the analyte and matrix used to generate them and should be extrapolated to other analytes and matrices with caution. Uncertainty tends to be greater at lower levels, especially as the LOQ is approached. It may therefore be necessary to generate uncertainty data for a range of concentrations if typical uncertainty is to be provided for a wide range of residues data.

Replicate analyses of a specific sample combined with concurrent recovery determinations, can improve the accuracy of the single-laboratory result and justify the use of a refined figure for the measurement uncertainty.

5. Confirmative analytical techniques in residue analysis

Principles of confirmation

Negative results (residues below the reporting limit) can be considered confirmed if the recovery and LCL measurement for the batch are acceptable. Positive results (residues at or above the reporting limit) usually require additional confirmation. Unusually high residues must be identified by combination of techniques, available and must be quantitatively confirmed by analysis of at least one additional test portion. Different combinations of clean-up, derivatization, separation, and detection techniques may also be used to support confirmation.

For confirmation of residues generally used methods are

- Use of selective detectors with GC or LC such as ECD, FPD, NPD, DAD and fluorescence, offer only limited specificity.
- The use of dual column, i.e. a second column of different polarity in chromatography.
- The use of a highly specific detection system, such as mass spectrometry.

The use of combination of different polarity columns, can only provide limited confirmatory evidence. These limitations may be acceptable for frequently found residues, especially if some results are also confirmed using a more specific detection technique.

Confirmation by mass spectrometry (MS)

Mass spectrometric determination is considered as the most specific method of confirmation. The term "confirmation by mass spectrometry" normally refers to over-whelming evidence that a sample actually contains the analyte, i.e. proof of identity. MS of residues is usually carried out in conjunction with a chromatographic separation technique to simultaneously provide:

i) retention time;

ii) ion mass/charge ratio; and

iii) abundance data

For GC-MS procedures, the chromatographic separation should be carried out using capillary columns. For LC-MS procedures, the chromatographic separation can be performed using any suitable LC column. Diagnostic ion chromatograms should have peaks (with minimum 3 data points exceeding, S/N 3:1) of similar retention time, peak shape and response ratio to those obtained from a calibration standard analysed in the same batch. Where chromatograms of unrelated ions show peaks with a similar retention time and shape, or where unrelated ion chromatograms are not available (e.g. with SIM), additional confirmation may be required. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon to quantify or identify residues.

Careful subtraction of background spectra may be required to ensure that the resultant spectrum of the chromatographic peak is representative. Whenever background correction is applied, this must be applied uniformly throughout the batch and should be clearly indicated. Where ions unrelated to the analyte in a peak-averaged "full-scan" spectrum (i.e. from *m/z* 50 to 50 mass units greater than the "molecular ion") do not exceed a quarter of base peak intensity in EI spectra, or one-tenth for all

other ionization methods, the spectrum may be accepted as sufficient evidence of identity. Where unrelated ions exceed these limits, and they derive from chromatographically overlapping species, additional evidence should be sought. Intensity ratios for principal ions should be within the tolerance limits shown in Table 3. The ion that shows the best signal-to-noise ratio and no evidence of significant chromatographic interference, should normally be used for quantification. In such cases, MS/MS or MS-MS^n can provide good evidence of identity.

Where the increased sensitivity obtained by scanning a limited mass range or by SIM, the general minimum requirement is for data from two ions of *m/z* >200; or three ions of *m/z* >100, preferably including the molecular ion. For a few analytes, where these minimum requirements may not be achievable, ions with *m/z* <100 may also provide supporting evidence. However, ions arising from common moieties may be of little use, as are cationised molecules or adducts, such as $[M+NH_4]^+$, formed in LC-MS. Intensity ratios obtained from the more characteristic isotopic ions, e.g. those containing Cl or Br, may be of particular utility. The selected diagnostic ions should not exclusively originate from the same part of the parent molecule.

For full scan and SIM the relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion or transition, should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. Table 3 below indicates the maximum tolerances.

Table 3. Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques

Relative intensity (% of base peak)	**EI-GC-MS (relative)**	**CI-GC-MS, GC-MS^n, LC-MS, LC-MS^n (relative)**
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10%	± 50 %	± 50 %

Larger tolerances are more likely to lead to a larger percentage of false positive results. Likewise, if the tolerances are decreased, then the likelihood of false negatives increases.

When full scan spectra are recorded in single mass spectrometry, a minimum of four ions should lie within the maximum permitted tolerances for the relative ion intensities (Table 3). **Computer-aided library searching** may be used. In this case, the comparison of mass spectral data in the test samples with that of the calibration solution has to exceed a critical match factor. This factor should be determined during method validation for every analyte. Variability in the spectra caused by the sample matrix and the detector performance must be checked.

Confirmation by an independent laboratory Where practicable, confirmation of results in an independent expert laboratory provides strong supporting evidence of quantity. If different determination techniques are used, the evidence will also support identification.

6. Different terminologies used in Pesticide Residue Analysis

Pesticide Residue:

Any substance or mixture of substances in food resulting from the use of a pesticide and includes any specified derivatives such as degradation and conversion products, metabolites, reaction products, and impurities that are considered to be of toxicological significance. It is express in mg/Kg.

Metabolism:

Sum total of all physical and chemical process that take place within an organism; chemical changes that occur for a pesticide within an organism. It includes uptake and distribution within the body, changes (biodegradation) and elimination of pesticides and their metabolites.

Metabolite:

After the pesticide enter the plant or animal body, it is normally converted into parts

Dissipation and persistence

The first step is the initial phase in which the disappearance of the residue is fast. This phase is called "Dissipation". The second phase, in which there is a slow decrease in the amount of residue, is known as "persistence".

No observable adverse effect level (NOAEL)

It is the highest dose of substance that does not cause any detectable toxic effects in experimental animal studies. It is expressed in mg/kg of body weight per day. To determine NOAEL values the sub chronic and chronic studies are carried out in different species as given under.

Sub chronic

90-Day Feeding Study

- Rodent (Rat, Mouse)
- Non-rodent (Dog)
- Dermal (Depending on Use Pattern)
- Inhalation
- Neurotoxicity

Chronic

One- or Two-year Oral Study

- Rodent (Usually Rat)
- Non-rodent (Dog)
- Life-time Oncogenicity Study
- Reproductive
- Multi-generation (Rat, Mouse), Fertility, Reproduction
- Teratogenicity (Rat, Mouse, Rabbit)
- In-Vitro Mutagenicity and Mechanistic Studies

Acceptable daily intake (ADI):

It is an estimate of the amount of a pesticide, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.

$$\text{ADI for human beings (mg/kg body weight)} = \frac{\text{NOAEL animal studies (mg/kg body weight)}}{\text{100 (safety factor)}}$$

PHI (Pre Harvest Interval):

The pre harvest interval is the time interval from the application of pesticide to harvest under good agricultural practice so as to see that the pesticide residue falls below the MRL value.

Maximum Residue Level (MRL)

Is the maximum concentration of a pesticide residue in or on a food, agricultural commodity or animal feed, resulting from the use of a pesticide according to Good Agricultural Practice (GAP). The concentration is expressed in milligrams of pesticide residue per kilogram of the commodity. Under the PFA Act, MRL or Tolerance Limits (TLs) are fixed considering MRLs based on supervised trials conducted in India as well as the dietary habits of our population.

Toxicity studies of pesticides in animal are carried out to determine No observed Adverse Effect Level (NOAEL). Data are evaluated and NOAEL is calculated from chronic study. It is usually expressed in terms of milligrams of that particular pesticide per kilogram of body weight. From this NOAEL, the **Acceptable Daily Intake (ADI)** is calculated by dividing with a safety factor of 100. Therefore ADI, which is expressed in terms of mg/kg body weight, is an indication that if a human being consumes that amount of pesticide every day, throughout his lifetime, it will not cause appreciable health risk on the basis of well known facts at the time of the evaluation of that particular pesticide. ADIs are derived from the results of long term feeding studies with laboratory

MRL is therefore a dynamic concept dependant on extant knowledge and is therefore required to be renewed from time to time.

Terminal residues of a particular pesticide on a treated crop are estimated from supervised trials, to assess the maximum residue limit which the pesticide leaves when used as per the **Good Agricultural Practice (GAP).**

Thus, the above three parameters i.e. ADI, terminal residues as per Good Agricultural Practice on the crop and the diet pattern of the population are the critical inputs needed to derive the maximum residue limits (MRLs) of pesticides in food commodities.

7. Maximum Residue Level of Pesticides

The **Codex Alimentarius Commission (CAC),** established by FAO and WHO in 1963 to develop harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair trade practices in the food trade.

- The Codex Alimentarius officially covers all foods, whether processed, semi-processed or raw, but far more attention has been given to foods that are marketed directly to consumers.
- Contains general standards covering matters such as food labelling, food hygiene, food additives and pesticide residues (MRL value for different commodity) .
- The Codex Alimentarius is recognized by the World Trade Organization as an international reference point for the resolution of disputes concerning food safety and consumer protection

JMPR - Joint FAO/WHO Meetings on Pesticide Residues provide independent scientific expert advice to the Commission and its specialist Committee on Pesticide Residues.

Codex India

The **Food Safety and Standards Authority of India** (FSSAI), Ministry of Health and Family Welfare has been designated as the nodal point for liaison with the Codex Alimentarius Commission. The FSSAI has been established under the Food Safety and Standards Act, 2006 as a statutory body for laying down science based standards for articles of food and regulating manufacturing, processing, distribution, sale and import of food so as to ensure safe and wholesome food for human consumption.The **National Codex Contact Point (NCCP)** has been constituted by the **FSSAI** for keeping liaison with the CAC and to coordinate Codex activities in India. The NCCP is located at Food Safety and Standards Authority of India (Ministry of Health and Family Welfare), FDA Bhawan, Kotla Road, New Delhi -110002, India. It coordinates and promotes Codex activities in India in association with the **National Codex Committee** and facilitates India's input to the work of Codex through an established consultation process

Core Functions of NCCP-INDIA:

- Coordinate all relevant Codex activities within India
- Receive all Codex final texts (standards, codes of practice, guidelines and other advisory texts) and working documents of Codex Sessions and ensure that these are circulated to those concerned
- Promote Codex Activities throughout India
- Build capacity in country to effectively take up Codex work
- Keep track of international food standards work and give comments and data to ensure that international food standards elaborated are practicable for local manufactures and do not hinder exports of food
- Undertake study and research work
- Encourage food manufacturers to improve quality and hygiene management to meet requirements of international food standards; and
- Disseminate information of food standards and food laws to relevant government agencies, primary producers, manufacturers, exporters, consumers and concerned organizations.
- Advise government on the implications of various food standardization, food quality and safety issues
- Provide important inputs to the government so as to assist in ensuring quality and safety of food to the consumers
- Cooperate with other local/regional or foreign organizations dealing with activities relating to food standardization.

The Food Safety and Standards Act, 2006

The FSSA aims to establish a **single reference point** for all matters relating to food safety and standards, by moving from multi- level, multi- departmental control to a single line of command.

FSSAI and the State Food Safety Authorities shall enforce various provisions of the Act.

APEDA

The Agricultural and Processed Food Products Export Development Authority (APEDA) was established by the Government of India under the Agricultural and Processed Food Products Export Development Authority Act & came into effect from 13th February, 1986

Functions of APEDA

- Fixing of standards and specifications for the scheduled products for the purpose of exports;
- Carrying out inspection of fruits and fruit products, Milk and Poultry products, meat and meat products in slaughter houses, processing plants, storage premises, conveyances or other places where such products are kept or handled for the purpose of ensuring the quality of such products;
- Improving of packaging of the Scheduled products;
- Improving of marketing of the Scheduled products outside India;

8. Principles of Pesticide Management

Pesticide Management is the regulation of the import, manufacture, export, sale, transport, distribution, quality and use of pesticides with a view to

(i) control pests;

(ii) ensure availability of quality pesticides;

(iii) allow its use only after assessing its efficacy and safety;

(iv) minimize the contamination of agricultural commodities by pesticide residues;

(v) create awareness among users regarding safe and judicious use of pesticides, and

(vi) to take necessary measures to continue, restrict or prohibit the use of pesticides on the basis of reassessment with a view to prevent its risk on human beings, animals or environment, and for matters connected there with or incidental.

Pesticide Management is an activity carried out within the overall framework of the Plant Production and Protection Division of FAO. It is designed to work together with member countries and other International Organizations as a partner to introduce sustainable and environmentally sound agricultural practices that reduce health and environmental risks associated with the use of pesticides.

In March 2007 FAO and WHO signed a Memorandum of Understanding on cooperation in a *Joint Programme For The Sound Management Of Pesticides* to provide unified, coordinated and consistent advice and support to their Member States and to other stakeholders on sound management of pesticides. The "FAO/**WHO Joint Meeting on Pesticide Management**" (JMPM) is an expert *ad hoc* body administered jointly by FAO and WHO: the JMPM advises on matters pertaining to pesticide regulation, management and use, and alerts to new developments, problems or issues that otherwise merit attention from one or both Organizations. The JMPM consists of members drawn from the **FAO Panel of Experts on Pesticide Management** and the WHO Panel of Experts on Vector Biology and Control, which are statutory advisory bodies of the respective Organizations

The International Code of Conduct on the Distribution and Use of Pesticides

The **International Code of Conduct on the Distribution and Use of Pesticides** is the worldwide guidance document on pesticide management for all public and private entities engaged in, or associated with, the distribution and use of pesticides. It was adopted for the first time in 1985 by the Twenty-fifth Session of the FAO Conference. It focuses on

i. Risk reduction,

ii. Protection of human health and the environmental, and

iii. Support for sustainable agricultural development by using pesticides in an effective manner and applying IPM strategies.

Particular concerns are given for countries where living and working conditions make pesticide use more risky.

The Code is designed to provide standards of conduct and to serve as a point of reference in relation to sound pesticide management practices, in particular for government authorities and the pesticide industry. Following the adoption of the Rotterdam Convention in 1998 and in view of the changing international policy framework, as well as the persistence of certain pesticide management problems, particularly in developing countries, in 1999 FAO initiated the update and revision process of the Code.

The 12 Articles of the Code, plus supporting technical guidelines and a new Annex consisting of references to international policy instruments related to the Code, represent an up-to-date standard for pesticide management. This embodies a modern approach, leading to sound management of pesticides which focuses on risk reduction, protection of human and environmental health, and support for sustainable agricultural development by using pesticides in an effective manner and applying IPM strategies.

In addition, the revised Code includes the life-cycle concept of pesticide management.

1. Objectives of the Code:

The objectives of this Code are to establish voluntary standards of conduct for all public and private entities engaged in or associated with the distribution and use of pesticides, particularly where there is inadequate or no national legislation to regulate pesticides.

The Code describes the shared responsibility of many sectors of society to work together so that the benefits to be derived from the necessary and acceptable use of pesticides are achieved without significant adverse effects on human health or the environment.

The Code addresses the need for a cooperative effort between governments of pesticide exporting and importing countries to promote practices that minimize potential health and environmental risks associated with pesticides, while ensuring their effective use

The entities which are addressed by this Code include international organizations, governments of exporting and importing countries, pesticide industry, application equipment industry, traders, food industry, users, and public-sector organizations such as environmental groups, consumer groups and trade unions

The Code recognizes that training at all appropriate levels is an essential requirement in implementing and observing its provisions. Therefore, governments, pesticide industry, users of pesticides, international organizations, non-governmental organizations (NGOs) and other

parties concerned should give high priority to training activities related to each Article of the Code.

The standards of conduct set forth in this Code:

i. **E**ncourage responsible and generally accepted trade practices;

ii. **A**ssist countries which have not yet established regulatory controls on the quality and suitability of pesticide products needed in that country to promote the judicious and efficient use of such products and address the potential risks associated with their use;

iii. **P**romote practices which reduce risks in the handling of pesticides, including minimizing adverse effects on humans and the environment and preventing accidental poisoning resulting from improper handling

iv. **E**nsure that pesticides are used effectively and efficiently for the improvement of agricultural production and of human, animal and plant health

v. **A**dopt the "life-cycle" concept to address all major aspects related to the development, regulation, production, management, packaging, labelling, distribution, handling, application, use and control, including post registration activities and disposal of all types of pesticides, including used pesticide containers

vi. Promote Integrated Pest Management (IPM) (including integrated vector management for public health pests)

vii. Include reference to participation in information exchange and international agreements identified in Annex 1, in particular the *Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade.*

2. Pesticide management

i. Governments have the overall responsibility to regulate the availability, distribution and use of pesticides in their countries and should ensure the allocation of adequate resources for this mandate.

ii. Pesticide industry should adhere to the provisions of this Code as a standard for the manufacture, distribution and advertising of pesticides, particularly in countries lacking appropriate legislation and advisory services.

iii. Governments of pesticide exporting countries should, to the extent possible:

   a) Provide technical assistance to other countries, especially those lacking technical expertise in the assessment of the relevant data on pesticides;

   b) Ensure that good trading practices are followed in the export of pesticides, especially to those countries with limited or no regulatory schemes.

iv. Pesticide industry and traders should observe the following practices in pesticide management, especially in countries without legislation or means of implementing regulations:

   a) Supply only pesticides of adequate quality, packaged and labelled as appropriate for each specific market

   b) **I**n close cooperation with procurers of pesticides, adhere closely to provisions of FAO guidelines on tender procedures

   c) Pay special attention to the choice of pesticide formulations and to presentation, packaging and labelling in order to reduce risks to users and minimize adverse effects on the environment

d) Provide, with each package of pesticide, information and instructions in a form and language adequate to ensure effective use and reduce risks during handling

e) Be capable of providing effective technical support, backed up by full product stewardship to field level, including advice on disposal of pesticides and used pesticide containers, if necessary;

Retain an active interest in following their products to the end-user, keeping track of major uses and the occurrence of any problems arising from the use of their products, as a basis for determining the need for changes in labelling, directions for use, packaging, formulation or product availability.

Pesticides whose handling and application require the use of personal protective equipment that is uncomfortable, expensive or not readily available should be avoided, especially in the case of small-scale users in tropical climates. Preference should be given to pesticides that require inexpensive personal protective and application equipment and to procedures appropriate to the conditions under which the pesticides are to be handled and used.

v. National and international organizations, governments and pesticide industry should take coordinated action to disseminate educational materials of all types to pesticide users, farmers, farmer organizations, agricultural workers, unions and other interested parties. Similarly, users should seek and understand educational materials before applying pesticides and should follow proper procedures.

vi. Concerted efforts should be made by governments to develop and promote the use of IPM. Furthermore, lending institutions, donor agencies and governments should support the development of national IPM policies and improved IPM concepts and practices. These should be based on scientific and other strategies that promote increased participation of farmers (including women's groups), extension agents and on-farm researchers.

vii. All stakeholders, including farmers and farmer associations, IPM researchers, extension agents, crop consultants, food industry, manufacturers of biological and chemical pesticides and application equipment, environmentalists and representatives of consumer groups should play a proactive role in the development and promotion of IPM.

viii. Governments, with the support of relevant international and regional organizations, should encourage and promote research on, and the development of, alternatives posing fewer risks: biological control agents and techniques, non-chemical pesticides and pesticides that are, as far as possible or desirable, target-specific, that degrade into innocuous constituent parts or metabolites after use and are of low risk to humans and the environment.

ix. Governments and the application equipment industry should develop and promote the use of pesticide application methods and equipment that pose low risks to human health and the environment and that are more efficient and cost-effective, and should conduct ongoing practical training in such activities.

x. Governments, pesticide industry and national and international organizations should collaborate in developing and promoting resistance management strategies to prolong the useful life of valuable pesticides and reduce the adverse effects resulting from the development of resistance of pests to pesticides.

3. Testing of pesticides

Pesticide industry should:

i. **E**nsure that each pesticide and pesticide product is adequately and effectively tested by recognized procedures and test methods so as to fully evaluate its efficacy, behaviour, fate, hazard and risk with regard to the various anticipated conditions in regions or countries of use;

ii. **E**nsure that such tests are conducted in accordance with sound scientific procedures and the principles of good laboratory practice (15);

iii. **E**nsure that the proposed use pattern, label claims and directions, packages, technical literature and advertising truly reflect the outcome of these scientific tests and assessments;

iv. **P**rovide at the request of a country, methods for the analysis of any active ingredient or formulation that they manufacture, and provide the necessary analytical standards;

v. **P**rovide advice and assistance in the training of technical staff involved in the relevant analytical work. Formulators should actively support this effort;

vi. **C**onduct residue trials prior to marketing, at least in accordance with Codex

4. Reducing health and environmental risks

Governments should:

i. **I**mplement a pesticide registration and control system.

ii. **P**eriodically review the pesticides marketed in their country, their acceptable uses and their availability to each sector of the public, and conduct special reviews when indicated by scientific evidence.

iii. **C**arry out health surveillance programmes of those who are occupationally exposed to pesticides and investigate, as well as document, poisoning cases.

iv. **P**rovide guidance and instructions to health workers, physicians and hospital staff on the treatment of suspected pesticide poisoning.

v. **E**stablish national or regional poisoning information and control centres at strategic locations to provide immediate guidance on first aid and medical treatment, accessible at all times.

vi. **P**rovide extension and advisory services and farmers' organizations with adequate information about practical IPM strategies and methods, as well as the range of pesticide products available for use;

vii. **I**mplement a programme to monitor pesticide residues in food and the environment

Pesticide industry should:

i. **C**ooperate & provide poison-control centres and medical practitioners with information about pesticide hazards and on suitable treatment of pesticide poisoning;

ii. **M**ake every reasonable effort to reduce risks posed by pesticides by:

- **M**aking less toxic formulations available;
- **I**ntroducing products in ready-to-use packages;
- **D**eveloping application methods and equipment that minimize exposure to pesticides;

- **U**sing returnable and refillable containers where effective container collection systems are in place;
- **U**sing containers that are not attractive for subsequent reuse and promoting programmes to discourage their reuse, where effective container collection systems are not in place;
- **U**sing containers that are not attractive to or easily opened by children, particularly for domestic use products;
- **U**sing clear and concise labelling.

iii. **H**alt sale and recall products when handling or use pose an unacceptable risk under any use directions or restrictions.

Government and industry should cooperate in further reducing risks by:

i. Promoting the use of proper and affordable personal protective equipment.

ii. **M**aking provisions for safe storage of pesticides at both warehouse and farm level.

iii. **E**stablishing services to collect and safely dispose of used containers and small quantities of left-over pesticides.

iv. **P**rotecting biodiversity and minimizing adverse effects of pesticides on the environment (water, soil and air) and on non-target organisms.

To avoid unjustified confusion and alarm among the public, concerned parties should consider all available facts and should promote responsible information dissemination on pesticides and their uses.

In establishing production facilities of a suitable standard in developing countries

5.Regulatory and technical requirements

Governments should:

i. **I**ntroduce the necessary legislation for the regulation of pesticides.

ii. Conduct risk evaluations and make risk management decisions based on all available data or information, as part of the registration process;

iii. Improve regulations in relation to collecting and recording data on import, export, manufacture, formulation, quality and quantity of pesticides;

iv. Permit pesticide application and personal protective equipment to be marketed only if they comply with established standards;

v. Detect and control illegal trade in pesticides;

vi. When importing food and agricultural commodities, recognize good agricultural practices in countries with which they trade.

6. Availability and Use

Governments should use (where appropriate) the WHO classification of pesticides by hazard as the basis for their regulatory measures and associate the hazard class with well-recognized hazard symbols. When determining the risk and degree of restriction, the type of formulation and method of application should be taken into account.

Two methods of restricting availability can be exercised by the responsible authority: not registering a product or, as a condition of registration, restricting the availability to certain

groups of users in accordance with a national assessment of the hazards involved in the use of the product.

Prohibition of the importation, sale and purchase of highly toxic and hazardous products, such as those included in WHO classes Ia and Ib, may be desirable if other control measures or good marketing practices are insufficient to ensure that the product can be handled with acceptable risk to the user.

7. Distribution and trade

Governments should:

i. **D**evelop regulations and implement licensing procedures to ensure that, those involved in the sale of pesticides are capable of providing buyers with sound advice on risk reduction and efficient use.

ii. **T**ake the necessary regulatory measures to prohibit the repackaging or decanting of any pesticide into food or beverage containers and rigidly enforce punitive measures that effectively deter such practices;

iii. **E**ncourage a market-driven supply process to reduce the potential for accumulation of excessive stocks.

Pesticide industry should:

i. **T**ake all necessary steps to ensure that pesticides entering international trade conform at least to relevant FAO, WHO or equivalent specifications.

ii. Ensure that pesticides manufactured for export are subject to the same quality requirements and standards as those applied to comparable domestic products;

iii. Encourage importing agencies, national or regional formulators and their respective trade organizations to cooperate in order to achieve fair practices as well as marketing and distribution practices that reduce the risks posed by pesticides.

iv. Recognize that a pesticide may need to be recalled by a manufacturer and distributor when its use, as recommended, represents an unacceptable risk to human and animal health or the environment, and act accordingly;

v. Endeavour to ensure that pesticides are traded by and purchased from reputable traders, who should preferably be members of a recognized trade organization;

vi. Ensure that persons involved in the sale of pesticides are trained adequately, hold appropriate government licences (where such licences exist) and have access to sufficient information, such as material safety data sheets, so that they are capable of providing buyers with advice on risk reduction and efficient use;

vii. Provide, consistent with national requirements, a range of pack sizes and types that are appropriate for the needs of small-scale farmers and other local users, in order to reduce risks and to discourage sellers from repackaging products in unlabelled or inappropriate containers.

The procurer (government authority, growers' association, or individual farmer) should establish purchasing procedures to prevent the oversupply of pesticides and consider including requirements relating to extended pesticide storage, distribution and disposal services in a purchasing contract.

8. Information Exchange

Governments should:

i. **P**romote the establishment or strengthening of networks for information exchange on pesticides through national institutions, international, regional and sub-regional organizations and public sector groups;

ii. **F**acilitate the exchange of information between regulatory authorities to strengthen cooperative efforts. The information to be exchanged should include:

- **A**ctions to ban or severely restrict a pesticide in order to protect human health or the environment, and additional information upon request;
- **S**cientific, technical, economic, regulatory and legal information concerning pesticides including toxicological, environmental and safety data;
- **T**he availability of resources and expertise associated with pesticide regulatory activities.

In addition, governments are encouraged to develop:

i. **L**egislation and regulations that permit the provision of information to the public about pesticide risks and the regulatory process;

ii. **a**dministrative procedures to provide transparency and facilitate the participation of the public in the regulatory process.

International organizations should provide information on specific pesticides (including guidance on methods of analysis) through the provision of criteria documents, fact sheets, training and other appropriate means.

9. **Labelling, packaging, storage and disposal**

i. All pesticide containers should be clearly labelled in accordance with applicable guidelines, at least in line with the FAO guidelines on good labelling practice.

ii. Industry should use labels that:

- **C**omply with registration requirements and include recommendations.
- **I**nclude appropriate symbols and pictograms whenever possible, in addition to written instructions, warnings and precautions in the appropriate language or languages.
- Include, in the appropriate language or languages, a warning against the reuse of containers and instructions for the safe disposal or decontamination of used containers.
- Clearly show the release date (month and year) of the lot or batch and contain relevant information on the storage stability of the product.

iii. Pesticide industry, in cooperation with government, should ensure that:

- **P**ackaging, storage and disposal of pesticides conform to the relevant FAO, UNEP9, WHO guidelines or regulations or to other international guidelines, where applicable.
- **P**ackaging or repackaging is carried out only on licensed premises where the responsible authority is satisfied that staff are adequately protected against toxic hazards, that the resulting product will be properly packaged and labelled, and that the content will conform to the relevant quality standards.

iv. Governments should take the necessary regulatory measures to prohibit the repackaging or decanting of any pesticide into food or beverage containers and rigidly enforce punitive measures that effectively deter such practices.

v. Governments, with the help of pesticide industry should inventory obsolete or unusable stocks of pesticides and used containers, establish and implement an action plan for their disposal, or remediation in the case of contaminated sites, and record these activities.

vi. Pesticide industry should be encouraged, with multilateral cooperation, to assist in disposing of any banned or obsolete pesticides and of used containers, in an environmentally sound manner, including reuse with minimal risk where approved and appropriate.

vii. Governments, pesticide industry, international organizations and the agricultural community should implement policies and practices to prevent the accumulation of obsolete pesticides and used containers.

10. **Advertising**

i. Governments should control, by means of legislation, the advertising of pesticides in all media to ensure that it is not in conflict with label directions and precautions.

ii. Pesticide industry should ensure that:

- **A**ll statements used in advertising are technically justified;
- **A**dvertisements do not contain any statement or visual presentation which is likely to mislead the buyer, in particular with regard to the "safety" of the product, its nature, composition or suitability for use, official recognition or approval;
- **P**esticides which are legally restricted to use by trained or registered operators are not publicly advertised through journals other than those catering for such operators, unless the restricted availability is clearly and prominently shown.
- **N**o company or individual in any one country simultaneously markets different pesticide active ingredients or combinations of ingredients under a single brand name;
- Advertisements do not misuse research results, quotations from technical and scientific literature or scientific jargon to make claims appear to have a scientific basis they do not possess;
- **C**laims as to safety, including statements such as "safe", "non-poisonous", "harmless", "non-toxic" or "compatible with IPM," are not made, with or without a qualifying phrase such as "when used as directed".
- **S**tatements comparing the risk, hazard or "safety" of different pesticides or other substances are not made;
- **M**isleading statements are not made concerning the effectiveness of the product;
- Advertisements do not contain any visual representation of potentially dangerous practices, such as mixing or application without sufficient protective clothing, use near food or use by or in the vicinity of children;
- **A**dvertising or promotional material draws attention to the appropriate warning phrases and symbols as laid down in the FAO labelling guidelines.
- **T**echnical literature provides adequate information on correct practices, including the observance of recommended application rates, frequency of applications and pre-harvest intervals;
- All staff involved in sales promotion are adequately trained and possess sufficient technical knowledge to present complete, accurate and valid information on the products sold;

- Advertisements and promotional activities should not include inappropriate incentives or gifts to encourage the purchase of pesticides.

11. **Monitoring and observance of the Code**

- The Code should be published and should be observed through collaborative action on the part of governments, individually or in regional groupings, appropriate organizations and bodies of the United Nations system, international, governmental and non-governmental organizations and the pesticide industry.
- The Code should be brought to the attention of all concerned in the regulation, manufacture, distribution and use of pesticides, so that governments, individually or in regional groupings, pesticide industry, international institutions**,** pesticide user organizations, agricultural commodity industries and food industry groups (such as supermarkets) that are in a position to influence good agricultural practices, understand their shared responsibilities in working together to ensure that the objectives of the Code are achieved.
- All parties should observe this Code and should promote the principles and ethics expressed by the Code, irrespective of other parties' ability to observe the Code. Pesticide industry should cooperate fully in the observance of the Code and promote the principles and ethics expressed by the Code, irrespective of a government's ability to observe the Code.
- Independently of any measures taken with respect to the observance of this Code, all relevant legal rules, whether legislative, administrative, judicial or customary, dealing with liability, consumer protection, conservation, pollution control and other related subjects, should be strictly applied.
- FAO and other competent international organizations should give full support to the observance of the Code.
- Governments, in collaboration with FAO, should monitor the observance of the Code and report on progress made to the Director-General of FAO.
- NGOs and other interested parties are invited to monitor activities related to the implementation of the Code and report these to the Director-General of FAO.
- Governing Bodies of FAO should periodically review the relevance and effectiveness of the Code. The Code should be considered a dynamic text which must be brought up to date as required, taking into account technical, economic and social progress.

Obsolete Pesticides

Half a million tonnes of obsolete pesticides are scattered throughout the developing world. These toxic chemicals, often stored outdoors in leaking containers, are seeping into the soil and water.

Eliminating these dangerous stocks is a development priority. Rural communities can't hope to develop if the soil and water are contaminated with pesticides. Their people can't hope to prosper if they are suffering from severe illnesses caused by pesticide poisoning. FAO's Programme on the Prevention and Disposal of Obsolete Pesticides is working to inform the world about the dangers of obsolete pesticide stocks. It collaborates with developing countries to prevent more obsolete pesticides from accumulating and assists them dispose of their existing stockpiles.

Pesticides that can no longer be used for any purpose are dangerous toxic waste. Moreover, there are discarded pesticide containers. These old containers can be as dangerous as the pesticides themselves. In developing countries, they are often used to store food or water. FAO's Programme on the Prevention and Disposal of Obsolete Pesticides assists developing countries deal with these toxic containers.

- ***FAO Pesticide Disposal Series 14: The Preparation of Inventories of Pesticides and Contaminated Materials***
- ***FAO Pesticide Disposal Series 15: Environmental Managment Tool Kit for obsolete pesticides(EMTK)***
- ***FAO Pesticide Disposal Series 16: Environmental Managment Tool Kit for obsolete pesticides(EMTK)***

Often stockpiles of old pesticides are poorly stored and toxic chemicals leak into the environment, turning potentially fertile soil into hazardous waste. The Programme also provides strategies for handling contaminated soil.

Pesticides: Around a thousand active ingredients are used to manufacture the wide array of pesticides in countries all over the world. What's more, these ingredients come in many thousands of different formulations. All these formulations degrade over time. The chemical by-products that form as the pesticide deteriorates can be even more toxic than the original product.

Obsolete pesticide stockpiles are often poorly stored and the containers corrode and leak. In a single storage facility, chemicals from many different products may blend together to create a toxic quagmire.

Because of this tremendous chemical complexity, there is no single solution that can be applied to clean up obsolete pesticide stocks.

Containers: Wherever pesticides are used, empty containers are generated. Obviously, no country can eliminate the problem of used pesticide containers in a single, or even a series, of disposal operations. It's an ongoing problem; one that poses a serious threat to the environment and public health. In many developing countries, empty pesticide containers are highly valued property. Even though it is usually impossible to remove all traces of toxic chemicals from pesticide containers, people often use them for storing fuel or even food and water. This is clearly an unsafe practice that must be discouraged.

Dangers of improper disposal: When measures are taken to dispose of containers, often they are not appropriate. For example, many pesticide suppliers and national authorities recommend the burying or burning of waste pesticides and empty containers. But buried chemical waste can contaminate soil and groundwater, while burning pesticides and containers releases highly toxic fumes. Often pesticides, empty containers and contaminated materials are dumped in landfills or other general waste collection sites. Most of these sites aren't designed to prevent toxic materials from leaking into the ground or being washed out by rain into water bodies. In developing countries such sites are also usually scavenged and useful items such as pesticide containers are reclaimed.

A responsibility of industry and the government

Most pesticide users can't dispose of pesticides and related waste materials safely. Under the **International Code of Conduct on the Distribution and Use of Pesticides,** manufacturers and distributors of pesticides are expected to provide facilities that allow pesticide users to

dispose of empty containers and pesticide-related waste materials safely. National and local authorities must also help with the disposal of farmers' and householders' pesticide-related waste. They can do this by establishing schemes for collecting small quantities of pesticides, used containers and contaminated materials. The FAO Programme provides technical advice and guidance to assist developing countries take the necessary to reduce the risks posed by used pesticide containers.

Contaminated soil: Obsolete pesticides are often improperly stored. Liquid pesticides can leak out of corroded drums into the soil and groundwater and end up polluting local lakes and rivers. The wind can spread pesticide powders over a wide area. Once pesticides enter soil they spread at rates that depend on the type of soil and pesticides, moisture and organic matter content of the soil and other factors. A relatively small amount of spilled pesticides can therefore create a much larger volume of contaminated soil. For example, approximately 30 tonnes of pesticides buried on a site in Yemen in the 1980s contaminated over 1500 tonnes of soil. Obviously, this can pose a serious health and environmental threat to nearby communities.

Cleaning up contaminated water and soil is a desirable part of any obsolete pesticide disposal operation. But dealing with contaminated soil is a costly, technically complex and difficult task. Every site is different. First the extent of the contamination and the impact on the local environment must be determined. This requires an understanding of the chemical properties of the pesticides. Often the pesticides are unknown and samples must be analysed. Depending on the results of the chemical analysis and risk assessment, there are three basic ways of dealing with contaminated soil and water:

- removing the contamination by excavating the soil and pumping-up of groundwater;
- containing the contamination by covering contaminated soil with buildings, asphalt or another impermeable layer, and preventing contaminated groundwater from flowing downstream;
- preventing human contact with the contamination by covering the contamination with clean soil, fencing-off contaminated areas and closing contaminated wells.

Removing contamination is more expensive than containing it, which in turn is more expensive than taking protective measures. Containment and protective measures are effective only for as long as they are maintained and their proper maintenance may be difficult to ensure over a long period of time. The FAO Programme has published a reference manual for assessing soil contamination to assist developing countries to make sound decisions about how to deal with the problem in the most cost-effective manner. FAO is also working to develop cost effective methods for dealing with pesticide contaminated soil in developing countries

Why do we have this problem?

The only way we can permanently eliminate the dangers posed by old and unwanted pesticide stocks is to make sure that no more stocks accumulate. That's why it is essential to understand the reasons behind the build-up of existing obsolete pesticide stockpiles. As the philosopher George Santayana said, *"Those who cannot learn from history are doomed to repeat it."*

The major factors that have resulted in the creation of large quantities of old and unused pesticides in developing countries are:

i. Pesticide Bans

In many countries, when a range of products has been banned or withdrawn for health or environmental reasons stocks remain where they are stored and eventually deteriorate. Good practice in such cases requires pesticide regulatory authorities to allow a phase out period when products are banned or restricted so that existing stocks can be used up before the restriction is fully applied.

ii. The ban on POPs pesticides

As the world became more aware of the dangers of POPs chemicals, these pesticides were banned from donor-funded locust campaigns in the late 1970s. Many POPs pesticides, dieldrin in particular, were widely used in campaigns to eradicate locusts in Africa. When the POPs pesticides were banned, little thought was given to the fate of the remaining stock. Existing data indicate that more than 20 percent of obsolete pesticides stockpiles consist of POP pesticides which are nearly 30 years old. They are poorly stored and are leaking into the environment and contaminating soil and water. Also, because they are very persistent, POPs pesticides can be effective for a long time. As a result, the pesticides are sometimes stolen and sold illegally.

There are many cases where highly hazardous pesticides, which are not permitted for use in industrialized countries, are exported to developing countries. For a pesticide to be banned, it has to be registered first. Some pesticide companies have not registered or re-registered products which they knew would have not have been authorized in their own country but continue to produce and export the same products to developing countries. There are also cases of pesticide manufacturers increasing exports of products that have been banned or restricted in their own countries, possibly in order to use up existing stocks or to compensate for depleted local markets.

Authorities within the country must identify the root cause for the accumulation of the stocks and adopt measures to ensure that no more stocks will accumulate. By following the International Code of Conduct on the Distribution and Use of Pesticides, governments can ensure that useless or unwanted pesticides don't enter the country and that the pesticides that are allowed in are stored and managed safely. The FAO Programme on Prevention and Disposal of Obsolete Pesticides has published several guidelines specifically related to the prevention of obsolete stocks.

Problem in Clearing of Obsolete Pesticides

Disposal of obsolete pesticides currently costs between 3 and 5 USD per kilogram or litre of pesticide or contaminated material. This covers the costs of repackaging, site clean-up, overland transportation, shipment to Europe and incineration in dedicated high-temperature hazardous waste incinerators. To date about 3 000 tonnes of obsolete pesticides have been disposed of from 14 countries at a cost of almost 14 million. On the basis of global estimate totalling 250 000 tonnes of obsolete pesticides, about 1.25 billion would be needed to destroy all the stock.

Pesticide quality specifications

The "**Joint Meeting on Pesticide Specifications**" (JMPS) is an expert ad hoc body administered jointly by FAO and WHO, composed of scientists collectively possessing expert knowledge of

the development of specifications. Their opinions and recommendations to FAO/WHO are provided in their individual expert capacities, not as representatives of their countries or organizations.

The primary function of the JMPS is to produce recommendations to FAO and/or WHO on the adoption, extension, modification or withdrawal of specifications. FAO is publishing specifications for pesticides and their related formulations, as well the accompanying manual on the development and use of these specifications.

The purpose of these publications is to provide

- a normal standard of quality for the buying and selling of pesticides
- assistance in the official approval and acceptance of pesticides
- protection for responsible vendors against inferior products
- a link between biological efficacy and specification requirements
- an international point of reference;

Insecticide Act-1968

The Insecticide Act-1968 has come in to existence in India as act of 46 of 1968, Dt. 2-9-1968. The enactment of act took place on the basis of recommendation of Inter-ministerial Committee, headed by DR. M.S. THACKER on "the Kerala & Madras Food-poisoning Cases Enquiry Commission's report" under Justice J.C. Shah, then a sitting judge of Bombay High court. Dr M.S. THACKER committee recommended Short-term and Long-term measures on the basis of the enquiry commission's report which were accepted and the enactment of the Insecticide Act took place.

The constitution of Kerala & Madras Food-poisoning Cases Enquiry Commission under Justice J.C. Shah took place due to the accidents occurred in April & May, 1958 , in Kerala(by Folidol contamination) & Tamil Nadu, causing Food Poison due to which many people died and suffered due to the their toxic effects.

The Government has enacted the Insecticide Act 1968 on the basis of long term measures to regulate

- Import
- Manufacture
- Sale
- Transport
- Distribution
- Use

of pesticides with a view to prevent risk to human beings, animals and for matters connected therewith.

The Salient features of the Bill of the are Establishment of CIB and RC Licensing of persons, Establishment of CIL, Prohibition of import, manufacture, sale, etc ,Regulation of transport and storage of insecticides so as to prevent cases of accidental contamination of food..Provision of taking immediate action by way of prohibition of sale, distribution and use of any insecticide if it involved risk to human beings and vertebrate animals.

The IA-1968 act comprises of **38** Sections in it dealing various aspects of the pesticides in its life cycle and the act is executed following the **46** rules provided in the Insecticide Rules 1971 and the Insecticides (Price, Stock Display and Submission of Reports) Order - 1986 . The rules are being amended from time to time to suit and improve so as to serve the nation and farmers in particular and the following are the amendements existing to the Insecticide Rules 1971.

a) The Insecticides(1st Amendment) Rules – 1993
b) The Insecticides (2nd Amendment) Rules – 2006

As per the Act The Central Insecticides Board (CIB) is the Apex Advisory Body, which is headed by **Director General, Health services, and a**dvises the Central & State Governments on technical matters relating to Risk to human beings or animals involved in the use of insecticides and measures necessary to prevent such risk; and manufacture, sale, storage, transport and distribution of insecticides with a view to ensure safety to human beings and animals.

The Technical Executive Body under the act is Registration Committee which is Headed by Agricultural Commissioner, Govt. of India . The Registration Committee Registers insecticides after scrutinizing their formulae and verifying claims, made by applicants with regard to their efficacy and safety to human beings and animals; and other functions as assigned by or under the Act.

The Registration of the insecticides is done under the Act in different categories depending on the purpose and type operations intended by the applicant. They are three Types of registration as furnished below.

- Provisional [9(3B)]
- Regular [9(3)]
- Repeat [9(4)

The registration committee ensures the efficacy and safety of the products registered on scrutiny of the data submitted by the registrants through its panel of experts on the following before issuing the registration.

Ensuring Efficacy and Safety

- Chemistry
- Bio-efficacy / Residues
- Toxicity
- Packing & Packaging

Ensuring infrastructural facilities for

- Manufacture
- Stock
- Distribution
- Sale
- Exhibit for Sale
- Commercial Pest Control Operations

The CIB & RC periodically reviews the pesticide usage and its effects and considers the feed back from the general public, scientific community and research bodies and through the media and satisfy through its own technical expert committees and take necessary steps to impose prohibition on certain pesticides pertaining to Import, Manufacture, Sale, Stock or exhibit for sale, Distribution, Transport and Use or Caused to be used as it deemed fit for the reasons of Public Safety.

Regulation of Pesticide-usage restricted and banned pesticide

The Ministry of Agriculture regulates the manufacture, sale, transport and distribution, export, import and use of pesticides through the 'Insecticides Act 1968' and the rules framed there under. Through this Act, a Central Insecticides Board (CIB), has been set up to advise the Central and state governments on technical matters and for including insecticides into the Schedule of the Act. The approval of the use of pesticides and new formulations to tackle the pest problem in various crops is given by the Registration Committee (RC) while the Union Ministry of Health and Family Welfare monitors and regulates pesticides residue levels in food through Prevention of Food Adulteration Act. The existing MRLs listed in the Prevention of Food Adulteration Act and Rules, are incorporated in the Food Safety and Standards Regulations, 2010.

In India, the pesticides regulations are governed under the following Acts/Rules:

- The Insecticides Act 1968 and Rules 1971
- Prevention of Food Adulteration Act 1954
- The Environment (Protection) Act 1986
- The Factories Act 1948
- Bureau of Indian Standards Act
- Air (Prevention & Control of Pollution) Act 1981
- Water (Prevention & Control of Pollution) Act 1974
- Hazardous Waste (Management & Handling) Rules 1989

The Pesticides Management Bill, 2008 was introduced in the Rajya Sabha on October 21, 2008 but this bill shall come into force after notification in the official gazette.

Restricted and Banned pesticide

The Central Insecticide Board (CIB) & Registration Committee (RC) scrutinizes and periodically reviews all pesticides and their usage - some are banned from registration itself when it causes serious environmental and public health concerns. Currently 230 pesticide have been registered by the Registration committee India has banned 28 pesticides for manufacture, import and use (**Table no. 2**). The "main reason for banning" pesticide was because of health hazard to human beings, animals and damage to the environment".

India has currently banned for use of two pesticides and formulations - the neurotoxicant nicotin sulfate and the Bangalore based manufactured broad-spectrum protective contact fungicide captafol 80 per cent powder - but their manufacture is allowed for export (**Table no. 3**). Four pesticide formulations has banned for import, manufacture and use (**Table no. 4**). Seven pesticides have been placed in the "Withdrawn List" as these pesticides are likely to cause risk to human beings and animals as their safety cannot be fully established for one of complete data asked for from the pesticide industry (**Table no. 5**). Another 18 pesticides have been "refused registration" as the pesticide industry has failed to submit "complete data" about their products (**Table no. 6**).

Some pesticides are meant for "Restricted Use" which means that they can be used only for prescribed purposes and by authorised personnel by obtaining the appropriate Government license. Therefore 13 pesticides have been listed for restricted use in India.

Endosulfan : Endosulfan has been banned by the supreme Court of india w.e.f. 13-05-2011 for production, use & sale all over India till further orders vide ad-Interim order in the Writ Petition (Civil) No. 213 of 2011.

Lindane : Banned vide Gazette Notification No S.O. 637(E) Dated 25/03/2011) Lindane has been Banned for Manufecture, Import or Formulate w.e.f. 25th March, 2011 and banned for use w.e.f. 25th March, 2013).