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DEVELOPMENT OF AN INDOOR RESIDUAL SPARAY FOR MALARIA CONTROL

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Synopsis

The insecticide dichloro-diphenyl-trichloroethane (DDT) is widely used in indoor residual spraying (IRS) for malaria control owing to its longer residual efficacy compared to other World Health Organization (WHO) alternatives. It was envisaged that by investigating mechanisms of degradation of these alternative insecticides, a better understanding would be obtained on strategies of stabilising them and rendering their efficacy comparable with or better than DDT, hence providing less controversial and more acceptable and effective alternative insecticide formulations to DDT.

This study sought to investigate the reasons behind the reported longer lasting behaviour of DDT by exposing all the WHO approved insecticides to high temperature, high humidity and ultra-violet light. Interactions between the insecticides and some mineral powders in the presence of an aqueous medium were also tested. Simple insecticidal paints were made using slurries of these mineral powders whilst some insecticides were dispersed into a conventional acrylic paint binder. These formulations were then spray painted on neat and manure coated mud plaques, representative of the material typically used in rural mud houses, at twice the upper limit of the WHO recommended dosage range. DDT was applied directly onto mud plaques at four times the WHO recommended concentration and on manure plaques at twice WHO recommended concentration. All plaques were subjected to accelerated ageing conditions of 40 °C and a relative humidity of 90%.

In the accelerated ageing tests, Fourier transform infra-red (FTIR) interferograms showed that pyrethroids were the most stable insecticides compared to carbamates and DDT. High temperature oxidation, ultra violet light and humidity were ruled out as the cause of failure of the alternative insecticides. Gas chromatography (GC) interferograms showed that phosphogypsum stabilised the insecticides the most against alkaline degradation. Bioassay testing showed that the period of efficacy of these formulations was comparable to that of DDT sprayed at 4 times the upper limit of the WHO recommended dosage range. Bioassay testing also showed that these insecticidal "paint" formulations stabilised the insecticides on cattle manure coated surfaces as compared to DDT sprayed directly on similar surfaces.

Bioassay experiments indicated that incorporating insecticides into a conventional paint binder or adsorbing them onto phosphogypsum provided effective life spans, under accelerated ageing conditions, comparable to or exceeding that of DDT directly applied to typical soil substrates. Best results were obtained with propoxur in standard acrylic emulsion paint. Similarly, insecticides adsorbed on phosphogypsum and sprayed on cattle manure coated surfaces provided superior lifespans compared with DDT sprayed directly on a similar surface.

Key words: degradation; indoor residual spray; insecticidal paint; insecticides; malaria; mosquito

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To my girlfriend (Sicelokuhle), family and friends thank you very much for the moral support.

After climbing a great hill, one only finds that there are many more hills to climb…

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Nomenclature

Symbol	Property	Unit
ℜH	relative humidity	[-]
Φ	solids volume fraction	[-]
D_{AB}	diffusion coefficient	[$m^2.s^{-1}$]
k_c	mass transfer coefficient	[$m.s^{-1}$]
L	geometry characteristic length	[m]
P_A	vapour pressure of substance A	[$kg.m^{-1}.s^{-2}$]
Re	Reynolds number (VL/ν)	[-]
Sc	Schmidt number (ν/D_{AB})	[-]
Sh	Sherwood number (k_cL/D_{AB})	[-]
V	velocity	[$m.s^{-1}$]
Z	normal diffusion distance	[m]
P_{ow}	octanol water partition constant	[-]

List of acronyms

ACT	arteminism combination based therapy
ATR	attenuated total reflection
bw	body weight
ca	*circa* (approximately)
CAS	chemical abstract service
CbE	carboxylesterase
DBH	*p,p′*-dichlorobenzohydrol
DBP	*p,p′*-dichlorobenzophe-none
DDA	dichloro-phenyl-acetic acid
DDD	dichloro-diphenyl-dichloroethane
DDE	dichloro-diphenyldichloro-ethylene
DDM	dichloro-diphenyl-methane
DDNU	1, 1-ethenylidenebis (4-chlorobenzene)
DDOH	2, 2-bis (p-chlorophenyl) ethanol

DDT	dichloro-diphenyl-trichloroethane
DRIFT	diffuse reflectance infra red spectroscopy
DSC	dynamic scanning calorimetry
DT	decomposition temperature
DTA	differential thermal analyser
DT_{50}	the time it takes for 50% of the parent compound to degrade to by transformation [days]
et al.	*et alii* (and others)
EMS	electron ionisation mass spectroscopy
etc	*et cetera* (and so forth)
FTIR	Fourier transform infra-red
GMAP	global malaria action plan
h	hour
HPLC	high pressure liquid chromatography
ICP OES	inductively coupled plasma optical emission spectrometry
IRS	indoor residual spray
ITN	insecticide treated nets
IUPAC	international union of pure and applied chemistry
LD_{50}	lethal dose, 50%; the dose required to kill 50% of a test population after 14 days [$mg.kg^{-1}$ body weight]
LLITN	long lasting insecticide treated nets
min	minute
MP	melting point
NMR	nuclear magnetic resonance
PCPA	p-chlorophenylacetic acid
POP	persistent organic pollutant
R_1, R_2, R_3	hydrocarbon chain (aliphatic, aromatic or heterocyclic)
RBM	roll back malaria
ℜH, RH	relative humidity
SD	standard deviation
SEM	scanning electron microscope
TGA	thermo gravimetric analysis

TLC	thin layer chromatography
USA	United States of America
UV/ VIS	ultra-violet/ visible light
VP	vapour pressure
WHO	world health organisation
Wt.%	percentage of total weight
X	halogen substitute
XRF	X-ray fluorescence

1. Introduction

Malaria is an illness caused by a protozoan parasite of the plasmodium genus. There are over 200 species of the plasmodium parasites (Chavatte *et al.,* 2007; Perkins & Austin, 2008), of these four plasmodium species are responsible for human malaria i.e. *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi* (Cox-Singh *et al.,* 2007; Manguin *et al.,* 2010). *P. falciparum* is responsible for 80% of all reported cases and 90% of the fatalities.

The human malaria causing plasmodium parasites are exclusively transmitted by the female anopheles mosquito. There are about 484 species of anopheles mosquitoes (Harbach, 2004) of these about 70 have proven to be competent vectors involved in malaria transmission (Bruce-Chwatt, 1980:10).

During the progression of the malaria transmission cycle, sexual reproduction of the plasmodium parasite occurs between the male and female sexual gametes in the body of the anopheles mosquito. Anopheles female mosquitoes require protein in a blood meal from a human to produce their eggs. They feed on blood every 3 to 4 days, then resting in between feeding (Fradin, 1998). If this blood contains plasmodium gametocytes, a cycle of sexual reproduction will occur within the body of the mosquito to ultimately produce sporozoites. Some mature sporozoites will find their way to the mosquito's salivary glands. These sporozoites will infect the next host upon blood feeding by the anopheles mosquito. Human beings act as intermediate hosts of the parasite where only asexual reproduction of the gametes occurs. This sporogonic cycle lasts 10 – 14 days depending amongst other factors on the species of the plasmodium parasite. Such female mosquitoes remain infective for the rest of their lives (Manguin *et al.,* 2010).

Clinical symptoms of malaria vary depending on the type of parasite affecting the host. General symptoms of uncomplicated malaria include fever, chills, diarrhoea, vomiting, muscle pains, anaemia etc. In severe malaria, complications arise due to *P. falciparum* e.g. cerebral complications (coma, convulsions), kidney failure, fever, chills, low urine output, laboured breathing, fluid in the lungs, jaundice, shock, death etc (Castelli *et al.,* 2010). *P. vivax*, *P. ovale* and *P. malariae* have symptoms similar to *P. falciparum*. *P. vivax* can have longer periods of

latent activity and relapse after treatment. *P. ovale* has a more gradual onset of symptoms than *P. ovale*, with relapse less common. *P. malariae* has a more gradual onset, less severe symptoms than the former and death is very rare.

About half of the world population is at risk of contracting malaria. It infects about 240 million people a year and is responsible for about 863 000 deaths in 2009, and the majority of cases involved children under the age of 5 years old and pregnant women (WHO, 2009a). Malaria is endemic in 109 countries which mostly lie along the inter-tropical belt of Africa, Latin America and Asia (Figure 1). Sub Saharan Africa accounts for 86% of all global cases. In South Africa, malaria is prevalent in low altitude areas of Limpopo, Northern Province, Mpumalanga and north eastern parts of KwaZulu-Natal.

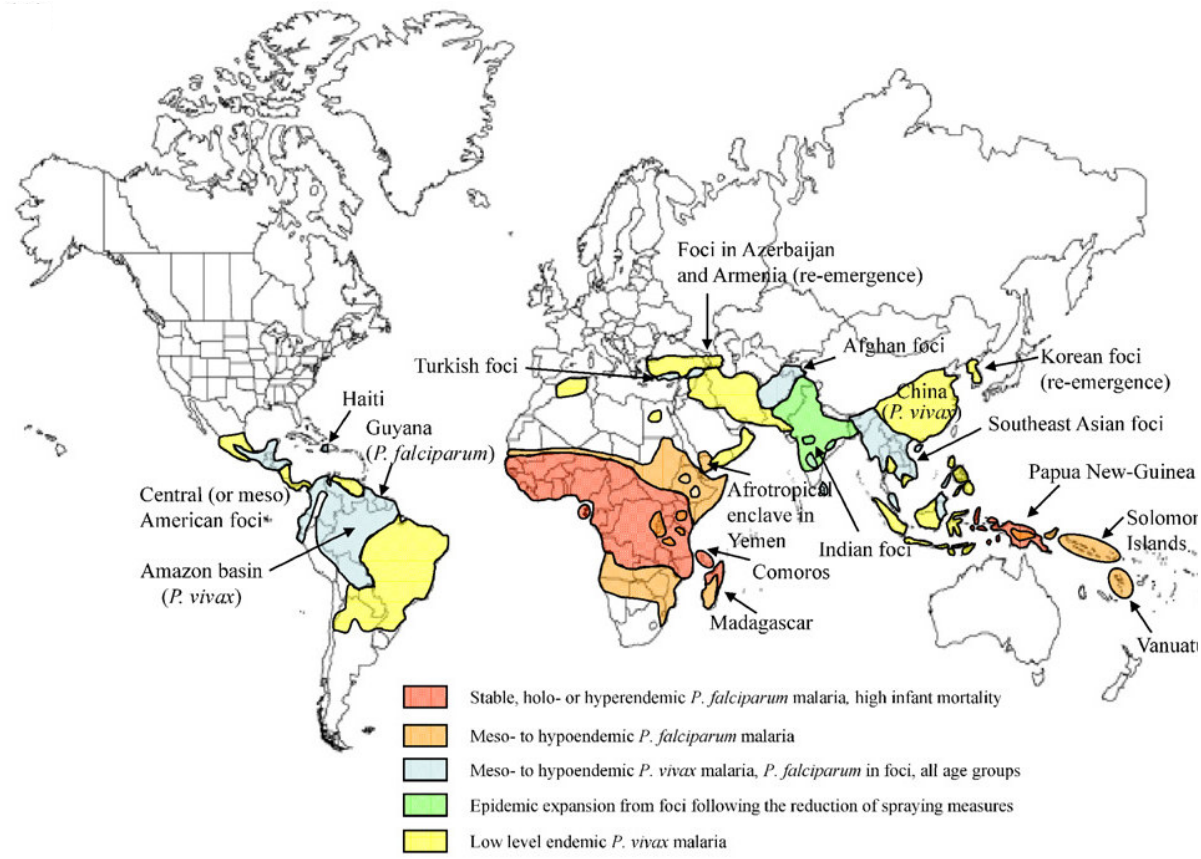


Figure 1: Malaria prevalence areas; adapted from Manguin *et al.* (2010)

There exists a significant statistical correlation between economic growth and malaria prevalence in malaria infested zones as compared to non malaria infested zones (Gallup &

Sachs, 2001). It has been shown that growth in income per capita from 1965-1990 for countries with severe malaria prevalence has averaged 0.4% per year, whereas growth in other countries has been 2.3%, more than 5 times higher. Western Europe and southern U.S.A experienced higher economic growth post malaria elimination period. The reasons for this strong statistical correlation may be due to the lack of foreign investment and limited tourism as well to do foreign investors and tourists will not risk infection by visiting holoendemic malaria areas. There may also be limited movement into and out of malaria areas and thus restricting trade and causing economic isolation, labour productivity may be low due to sickness or government investment is taken up by the health expenditures.

The roll back malaria (R.B.M) initiative reports that malaria costs Africa US$12 billion per annum in lost economic production (or a growth penalty of 1.3%), malaria control accounts for 40% of the health expenditure in some countries and half of the hospital admission and outpatient visits (GMAP, 2011). Malaria is clearly a heavy social and economic burden to Africa.

The WHO, African governments and donors at large have moved to mobilise resources to fight malaria because of the realisation that reduced economic disruptions will lead to increased productivity and household spending.The WHO has taken an integrated approach towards the control of malaria. The clinical control of the malaria parasite and the malaria vector control programmes has been combined to control the morbidity and mortality due to malaria.

WHO recommends the use of artemisinin based combination therapy (ACT) for the treatment of *P. falciparum* malaria, chloroquine and primaquine are recommended for the treatment of chloroquine sensitive *P. vivax*.

WHO has also approved twelve insecticides for use in IRS. Six of the insecticides are classified as pyrethroids (alphacypermethrin, betacyfluthrin, bifenthrin, deltamethrin, etofenprox, and lamdacyhalothrin), three as organophosphates (malathion, fenitrothion, pirimiphos-methyl), two as carbamates (propoxur, bendiocarb) and one as an organochlorine (DDT). The organochlorine, DDT, is the most preferred for IRS because it has an efficacy of

up to twelve months or more depending on the application surface whilst the rest of the insecticides have an efficacy of up to six months. The longer lasting efficacy of DDT provides for a low cost option as one spray cycle is required in a year as compared to two or more spray cycles for the alternative insecticides.

WHO has also recommended the use of insecticide treated nets (ITN). ITNs have proved to be a low cost option and simpler to implement compared to IRS programs. They require fewer resources and less sophisticated infrastructure to distribute. However ITN effectiveness is limited by the need to wash the nets from time to time, which gradually washes out the insecticide from the net fibres. The other disadvantage is that it only offers protection during sleeping time compared to the all day protection in IRS. However success has been reported in areas where ITNs have been implemented. Novel long lasting insecticide treated nets (LLITN) made of plastic blended with permethrin have been produced and exhibit insecticidal activity for about 3 – 5 years. Slow release mechanisms in the LLITNs eliminate the disadvantage of reduced efficacy after a wash cycle. Most African countries favour LLITN programs because they are easy and cheap to implement compared to IRS programs.

Vector control strategies such as larviciding and management of the environment are recommended if scientific research suggests that these may be very helpful.

1.1. Background to problem

The World Health Organization (WHO) Global Malaria Action Plan (GMAP) promotes IRS as a primary operational vector control intervention to reduce and ultimately eliminate malaria transmission. In some southern African countries DDT is regarded as the most effective insecticide for this purpose. Depending on the dosage and substrate nature, DDT can retain its efficacy against malaria vectors for up to 12 months. In South Africa DDT was temporarily replaced with the pyrethroid deltamethrin between 1996 and 1999. However, DDT was reintroduced in 2000 when malaria transmission reached epidemic proportions. The failure of the pyrethroid was attributed to a rise in mosquito resistance and the fact that it stays effective for much shorter time periods, i.e. up to a maximum of six months (Hargreaves *et al.,* 2000; WHO, 2011). Other WHO-approved pyrethroid, organophosphate and carbamate

alternatives also show similar limitations in effective IRS service life. Furthermore, repeated application of these alternative insecticides to provide for an all season round efficacy similar to that of DDT significantly increases the costs of IRS (Sadasivaiah, Tozan & Breman, 2007).

The Stockholm Convention on Persistent Organic Pollutants banned DDT for uses other than public health. In some developing countries DDT is currently widely used for indoor residual spraying. With DDT an annual application may suffice, whereas, the other WHO-approved alternatives may require up to 2-4 spray cycles. The problem with DDT is that it is a persistent organic pollutant (POP) accumulating in the environment. Consequently its use for indoor residual spraying is controversial and risky. Strict control measures must be implemented to prevent misuse and redirection of this active into other applications.

1.2. Aim

Develop a stable, non-controversial. environmentally friendly and affordable alternative formulation to DDT for indoor residual spraying.

1.3. Objectives

1. Investigate the causes of the *in situ* degradation of the alternative IRS insecticides.
2. From the insights developed on the *in situ* degradation mechanisms, develop an equivalent paint or “white wash” formulation and test for its effectiveness compared to DDT.

2. Literature review

It was of interest to review the physical and chemical properties of the approved insecticides and their mechanisms of degradation. This review was expected to highlight important pointers to consider during the planning of the laboratory investigative work.

2.1. Insecticides approved for IRS

2.1.1. Carbamates

Carbamates are esters derived from carbamic acid. Their general formula is shown in Figure 2.

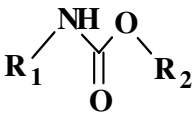


Figure 2: General molecular structure of a carbamate

R_1 represents an aliphatic moiety; R_2 represents an aromatic moiety for carbamate insecticides. Carbamates are neurotoxic and act on the target by reversibly inhibiting acetyl-cholinesterase enzyme required to breakdown acetyl choline, a nerve signal transmitter (Hassall, 1982:101). Failure to breakdown acetyl choline after it has transmitted a nerve signal leads to continuous signal transmission causing continuous involuntary muscle contraction and convulsions by the victim.

2.1.1.1. Propoxur

Technical propoxur is a white crystalline wettable powder. In solid form it exists in two crystalline modifications (modification 1 and 2). It contains more than 95% of the modification 1 crystal. Propoxur is characterized by using high pressure liquid chromatography (HPLC) retention time, infra red (IR) spectroscopy and mass spectroscopy (MS) (WHO, 2005). Figure 3 and Table 1 shows the molecular structure and physical and chemical properties of propoxur respectively.

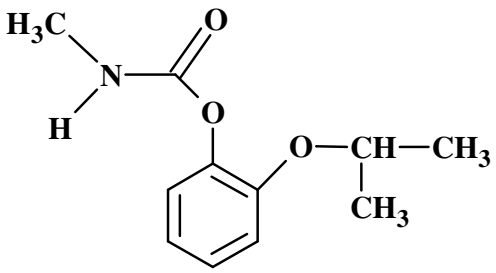


Figure 3: Molecular structure of propoxur

Table 1: Physical and chemical properties of propoxur (WHO, 2005)

IUPAC name	2-isopropoxyphenyl methylcarbamate
CAS registry number	114-26-1
Molecular formula	$C_{11}H_{15}NO_3$
Relative molecular mass	209.25
Thermal properties	MP is:
	Crystal modification I: 87.5 °C at 99.9% purity
	Crystal modification II: 90.0 °C at 99.9% purity
Density	1.17 $g.cm^{-3}$ at 20 °C
Vapour pressure	2.78×10^{-3} at 20 °C and 99.1% purity
Solubility in water	1.75 $g.l^{-1}$ at 20 °C and 99.8% purity
Octanol water partition coefficient	Log P_{ow} is 1.56 at 20 °C and 99.8% purity
Toxicity, LD_{50}	89.7 $mg.kg^{-1}$ bw (male rat)

2.1.1.2. Bendiocarb

Technical bendiocarb is a white wettable crystalline powder. Bendiocarb is analyzed by HPLC retention time and nuclear magnetic resonance (NMR) spectroscopy. Figure 4 and Table 2 shows the molecular structure and physical and chemical properties of bendiocarb respectively.

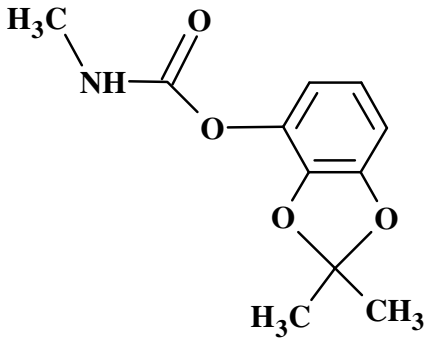


Figure 4: Molecular structure of bendiocarb

Table 2: Physical and chemical properties of bendiocarb (WHO, 2009b)

IUPAC name	2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate
CAS registry number	22781-23-3
Molecular formula	$C_{11}H_{13}NO_4$
Relative molecular mass	223.4
Thermal properties	MP is 129 °C at 98.5% purity
Density	1.203 ± 0.06 $g.cm^{-3}$ at 20 °C
Vapour pressure	4.6×10^{-3} Pa at 25 °C and 99.8% purity
Solubility in water	0.28 $g.l^{-1}$ at pH 7 and 99.3% purity
Octanol water partition coefficient	Log P_{ow} is 1.7 at 25 °C and 99% purity
Toxicity, LD_{50}	45-48 $mg.kg^{-1}$ bw (male rat)

2.1.2. Pyrethroids

The general structure of synthetic pyrethroids is shown in Figure 5. Pyrethroids make one of the largest groups of insecticides used in the world. They are very popular for use in agriculture and public health because they are very potent as insecticides. They can easily be metabolised via enzymatic processes in the body. Most house hold insect control chemicals contain pyrethroids as the active ingredient.

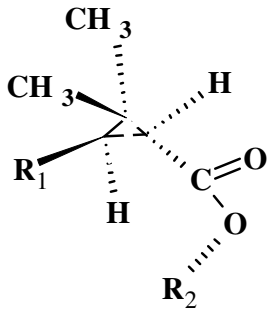


Figure 5: General molecular structure of a synthetic cyclopropane pyrethroid

Natural pyrethroids have very good insecticidal properties and combined with low mammalian toxicities they are ideal for application in agriculture and public health systems. However they are very unstable as they contain extensive photolabile centres within their molecular framework that makes them susceptible to oxidation and photo-degradation. Synthetic pyrethroids are engineered to reduce the number of these photolabile centers by introducing ethoxy benzene groups and halogen susbtitution to the alkene side chains to reduce oxidative and photo degradative mechanisms, whilst maintaining the stereochemistry associated with insecticidal activity (Elliot, 1976; Elliot, 1989). Pyrethroids are neurotoxins which work by modifying the sodium channel from very brief opening to a prolonged opening causing hyper muscular activity (Narahashi, 2001; Costa *et al.,* 2008). Pyrethroids are susceptible to oxidation by mono-oxygenase enzymes. The use of synergists such as piperonyl butoxide, sesamex etc that inhibit this enzyme can increase the toxicity of pyrethroids markedly.

2.1.2.1. Bifenthrin

Technical bifenthrin is a white wettable powder. Bifenthrin shows two chiral centres. The cis-stereo isomers at the cyclopropane moiety are the most effective and are predominant in the final product. The different stereoisomers show different insecticidal activity. Bifenthrin can be analysed by gas chromatography (GC), IR spectroscopy and electron ionization mass spectroscopy (EMS) (WHO, 2010a). Figure 6 and Table 3 shows the molecular structure and physical and chemical properties of bifenthrin respectively.

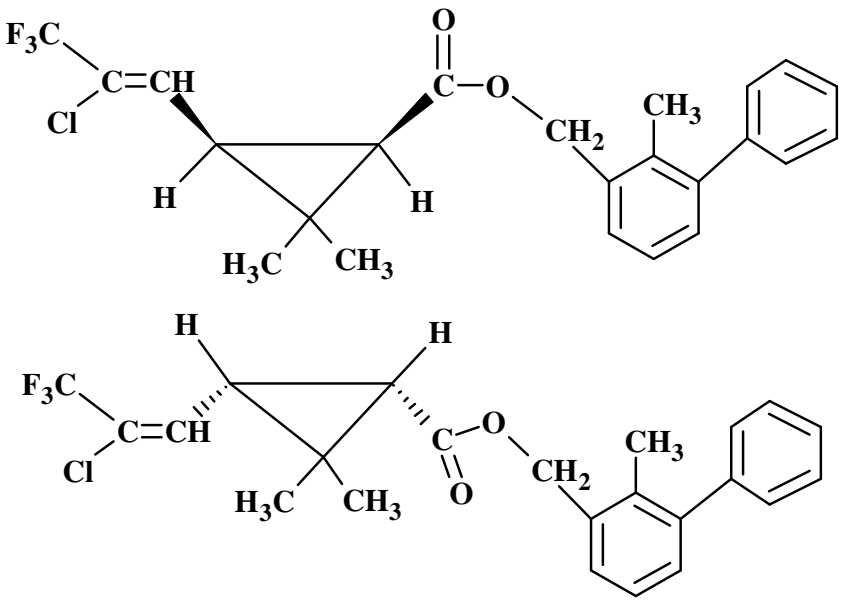


Figure 6: Molecular structures of bifenthrin cis/trans stereoisomers

Table 3: Physical and chemical properties of bifenthrin (WHO, 2010a)

IUPAC name	2-methylbiphenyl-3-ylmethyl(z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluroprop-1-enyl)-2-dimethylcyclopropanecarboxylate
CAS registry number	82657-04-3
Molecular formula	$C_{23}H_{22}ClF_3O_2$
Relative molecular mass	423.0
Density	1.262 $g.cm^{-3}$ at 20 °C
Vapour pressure	2.4×10^{-6} Pa at 25 °C 98.9% purity
Thermal properties	MP is 65-70 °C. Bifenthrin vapourises intact in the 215 – 225 °C temperature range
Solubility in water	Less than 0.1µg.l^{-1} at pH 2, 7 and 11 and 96.6% purity
Octanol water partition coefficient	Log P_{ow} > 6 at 25 °C and 96.5% purity
Toxicity, LD_{50}	55.5 $mg.kg^{-1}$ bw (male rat)

2.1.2.2. Lambdacyhalothrin

Technical lamdacyhalothrin is a white wettable powder with a low water solubility and non-volatile. Lambdacyhalothrin is a reaction mixture containing equal quantities of (S)-α-cyano-3-phenoxybenzyl (Z) - (1R, 3R) - 3 - (2-chloro-3,3,3-trifluoroprop-1-enyl) - 2,2 - dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl(Z)-(1S,3S)-3-(2-chlor-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclopropanecarboxylate. Lambdacyhalothrin is analysed by GC, NMR and IR spectroscopy (WHO, 2007). Figure 7 and Table 4 shows the molecular structure and physical and chemical properties of lamdacyhalothrin respectively.

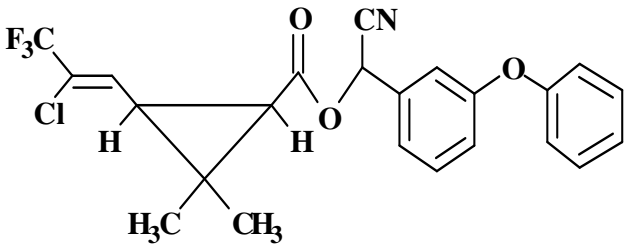


Figure 7: Molecular structures of lamdacyhalothrin

Table 4: Physical and chemical properties of lamdacyhalothrin (WHO, 2007)

CAS registry number	91465-08-6
Molecular formula	$C_{23}H_{19}ClF_3NO_3$
Relative molecular mass	449.9
Thermal properties	DT is 228-230 °C at 87.63% purity
Density	1.344 ± 0.06 $g.m^{-3}$ at 20 °C
Vapour pressure	2.8×10^{-7} Pa at 20 °C and 87.63% purity
Solubility in water	0.001 $mg.l^{-1}$ at pH 7 and 87.63% purity
Octanol water partition coefficient	Log P_{ow} is 6.28 at 25 °C and 87.63% purity
Toxicity, LD_{50}	91 $mg.kg^{-1}$ bw (male rat)

2.1.2.3. Etofenprox

Technical etofenprox is a white wettable powder. It is a non-ester pyrethroid, of which in most cases is the point of cleavage when it undergoes hydrolysis. It lacks centres of molecular assymmetry therefore does not show any stereo-isomerism. Etofenprox can be

characterized by GC and IR spectroscopy (WHO, 2010b). Figure 8 and Table 5 shows the molecular structure and physical and chemical properties of etofenprox respectively.

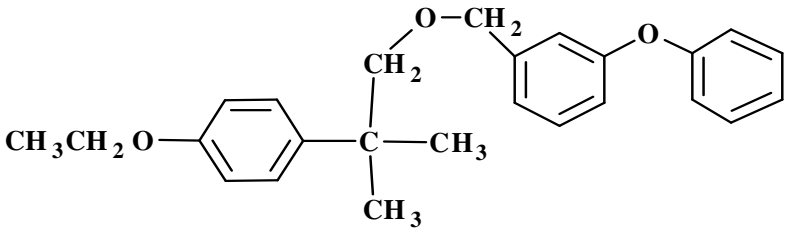


Figure 8: Molecular structure of etofenprox

Table 5: Physical and chemical properties of etofenprox (WHO, 2010b)

IUPAC name	2-(4-ethoxyphenyl)-2-methylpropyl -3-phenoxybenzylether
CAS number	80844-07-1
Molecular formula	$C_{25}H_{28}O_3$
Relative molecular mass	376.5
Thermal properties	37.4 °C (MP), 200 °C (DT) at 99% purity
Density	1.073 ± 0.06 $g.cm^{-3}$ at 20 °C
Vapour pressure	8.13×10^{-7} Pa at 25 °C and 99% purity
Solubility in water	22.5×10^{-6} $g.l^{-1}$ at pH 7 and 98% purity
Octanol water partition coefficient	Log P_{ow} is 6.9 at 25 °C and 99% purity
Toxicity, LD_{50}	>2000 $mg.kg^{-1}$ bw (male rat)

2.1.2.4. Betayfluthrin

Betacyfluthrin contains 3 chiral centers with 8 enantiomer forms, giving four pairs of diastereoisomers. Figure 9 and Table 6 shows the molecular structures and physical and chemical properties of betacyfluthrin respectively.

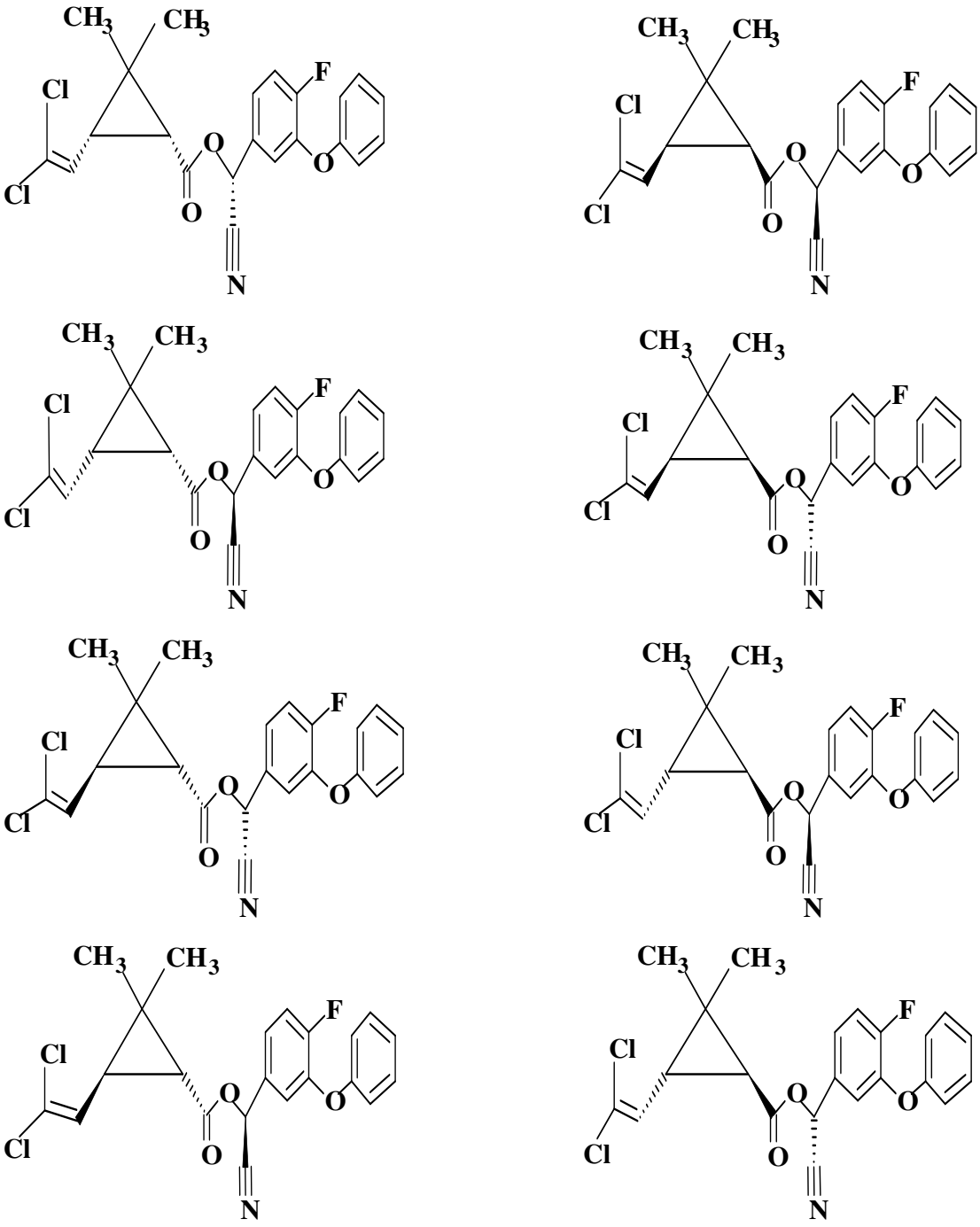


Figure 9: Molecular structures of betacyfluthrin enantiomers

It has therefore 4 CAS registry numbers i.e. 68359-37-5 (unstated stereochemistry), 86560-92-1 (diastereoisomer I), 86560-93-2 (diastereoisomer II), 86560-94-3 (diastereoisomer III), 86560-95-4 (diastereoisomer IV).

Table 6: Physical and chemical properties of betacyfluthrin (WHO, 2004a)

IUPAC name	(RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2, 2 dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate
CAS registry number	Diasteroisomer I: 86560-92-1 Diasteroisomer II: 86560-93-2 Diasteroisomer III: 86560-94-3 Diasteroisomer IV: 86560-93-5
Molecular formula	$C_{22}H_{18}Cl_2FNO_3$
Relative molecular mass	434.3
Thermal properties	MP is 77 °C (Weighted average for the four diastereoisomers)
Density	(g.cm^{-3} at 20 °C) Diastereoisomer I: 1.46, Diastereoisomer II: 1.373, Diastereoisomer III: 1.316, Diastereoisomer IV: 1.356
Vapour pressure	Diasteroisomer I: 2.1×10^{-6} at 25°C and 98.8%, Diasteroisomer II: 3.4×10^{-7} at 25°C and 97.4%, Diasteroisomer III: 4.7×10^{-7} at 25°C and 97.8%, Diasteroisomer IV: 2.0×10^{-7} at 25°C and 98.9% purity
Solubility in water	(solubility in µg.l^{-1} at pH 3/ pH 7) Diastereoisomer I: 2.5 2.2, Diastereoisomer II: 2.1/1.9, Diastereoisomer III: 3.2/2.2, Diastereoisomer IV: 4.3/2.9
Octanol water partition coefficient	(Log P_{ow} at 20 °C): Diastereoisomer I: 6.00, Diastereoisomer II: 5.94, Diastereoisomer III: 6.04, Diastereoisomer IV: 5.91
Toxicity, LD_{50}	20 mg.kg^{-1} bw (male rat)

2.1.2.5. Alphacypermethrin

Technical alphacypermethrin is a white wettable powder. Alphacypermethrin is described by IUPAC as a racemic mixture of (S) –α-cyano-3-phenoxybenzyl-(1R, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)-α-cyano-3-phenoxybenzyl-(1S,3S)-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate. Figure 10 and Table 7

shows the molecular structure and physical and chemical properties of alphacypermethrin respectively. Analysis of alphacypermethrin can be done by GC and IR spectroscopy.

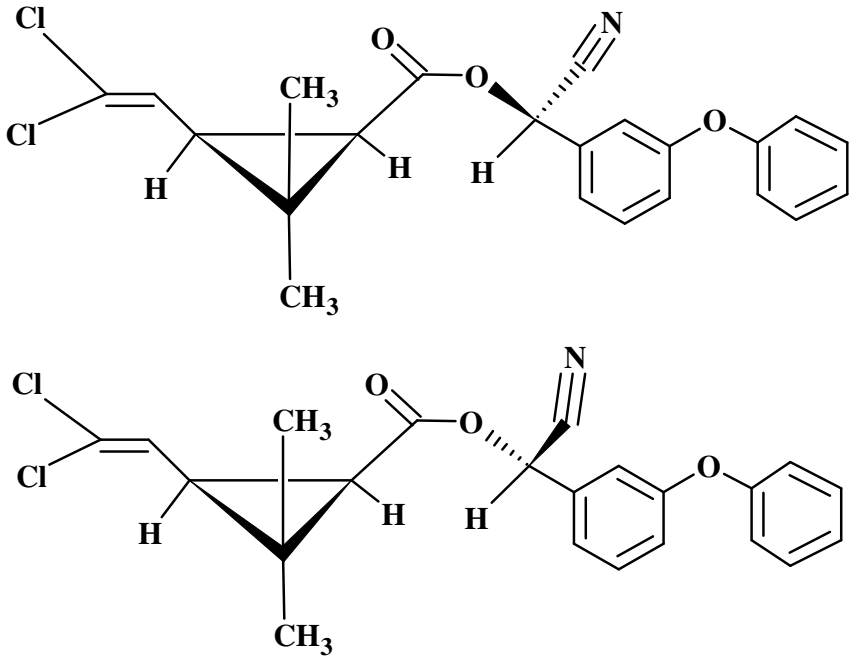


Figure 10: Molecular structure of alphacypermethrin

Table 7: Physical and chemical properties of alphacypermethrin (WHO, 2009c)

CAS registry number	67375-30-8
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Relative molecular mass	416.3
Thermal properties	MP is 81 – 83 °C at 95% purity
Density	1.329 ± 0.06 $g.cm^{-3}$ at 20°C
Vapour pressure	9.0×10^{-6} Pa at 25 °C and 95% purity
Solubility in water	6 $\mu g.l^{-1}$ at 20 ± 0.5 °C, pH ≈ 7 and 97.8% purity
Octanol water partition coefficient	Log P_{ow} is 6.64 at 25 °C and 95% purity
Toxicity, LD_{50}	360 $mg.kg^{-1}$ bw (male rate)

2.1.2.6. Deltamethrin

Technical deltamethrin is an off-white crystalline wettable powder. Deltamethrin has 8 stereoisomers and the isomers exhibit the structure in Figure 11. It is characterised by HPLC retention time, thin layer chromatography (TLC), IR, NMR and MS (WHO, 2010c). Table 8 shows the physical and chemical properties of deltamethrin respectively.

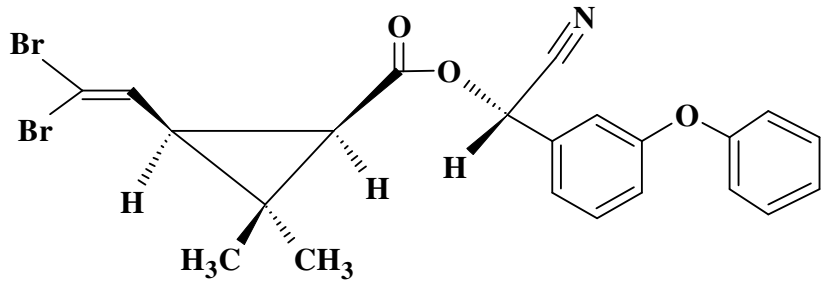


Figure 11: Molecular structure of deltamethrin

Table 8: Physical and chemical properties of deltamethrin (WHO, 2010c)

IUPAC name	(S)-α-cyano-3phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate
CAS registry number	52918-63-5
Molecular formula	$C_{22}H_{19}Br_2NO_3$
Relative molecular mass	505.2
Thermal properties	MP is 99 °C (decomposition temp >300 °C)
Density	1.595 ± 0.06 $g.cm^{-3}$ at 20 °C
Vapour pressure	1.24×10^{-7} Pa at 25 °C and 98% purity
Solubility in water	0.0907 $mg.l^{-1}$ at 25 °C and 98% purity
Octanol water partition coefficient	Log P_{ow} is 4.61 at 25 °C and 98% purity
Toxicity, LD_{50}	87.4 $mg.kg^{-1}w$ (male rat)

2.1.3. Organophosphates

This class of insecticide is characterised by the triphosphoric ester group (Figure 12).

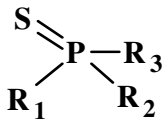


Figure 12: General molecular structure of an organophosphate

Where R_1, R_2 and R_3 are aryl, alkyl and heterocyclic groups respectively that are bonded to the phosphorus atom directly or through and oxy or sulphur atom. Their mode of activity is through the irreversible inhibition of enzyme acetyl-cholinesterase; an enzyme required to breakdown acetylcholine. The lack of reversibility in the deactivation of the enzyme makes organophosphates more neurotoxic than carbamates.

2.1.3.1. Malathion

Technical malathion is a colourless oily liquid with a characteristic pungent smell. Malathion is identified by GC retention time and IR spectroscopy (WHO, 2004b). Table 9 and Figure 13 shows the molecular structure and physical and chemical properties of malathion respectively.

Table 9: Physical and chemical properties of malathion (WHO, 2004b)

IUPAC name	*S*-1,2-bis(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate
CAS registry number	121-75-5
Molecular formula	$C_{10}H_{19}O_6PS_2$
Relative molecular mass	330.36
Thermal properties	DT > 174 °C at 99.1% purity
Density	1.272 ± 0.06 $g.m^{-3}$ at 20 °C
Vapour pressure	4.5×10^{-4} Pa at 25 °C and 98.9% purity
Solubility in water	148 $mg.l^{-1}$ at 25 ºC and 98.4% purity
Octanol water partition coefficient	log P_{ow} is 2.7 at 25 ºC and 98% purity
Toxicity, LD_{50}	1768 $mg.kg^{-1}$ bw (male rat)

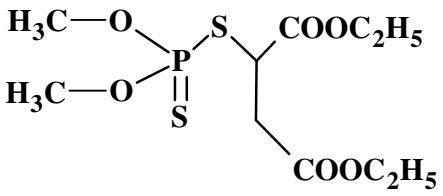


Figure 13: Molecular structure of malathion

2.1.3.2. Pirimiphos-methyl

Technical pirimiphos-methyl is an orange oily liquid with a characteristic pungent smell. Pirimiphos-methyl is identified using ultra-violet/visible (UV/VIS) spectroscopy, IR spectroscopy, NMR and MS (FAO, 2007). Table 10 and Figure 14 show molecular structure and physical and chemical properties of pirimiphos-methyl respectively.

Table 10: Physical and chemical properties of pirimiphos methyl (FAO, 2007)

IUPAC name	*O*-2-diethylamino-6-methylpyrimidin-4-yl-*O*,*O*-dimethyl phosphorothioate
CAS registry number	29232-93-7
Molecular formula	$C_{11}H_{20}N_3O_3PS_2$
Relative molecular mass	305.3
Thermal properties	MP is -20 °C at 99.1%
Density	1.229 ± 0.06 $g.m^{-3}$ at 20 °C
Vapour pressure	2.0×10^{-6} at 20 °C and 99% purity
Solubility in water	10 $mg.l^{-1}$ at pH 7, 20 °C and 99% purity
Octanol water partition coefficient	Log P_{ow} is 4.2 at pH 7, 25 °C and 99% purity
Toxicity, LD_{50}	1414 $mg.kg^{-1}$ bw (male rat)

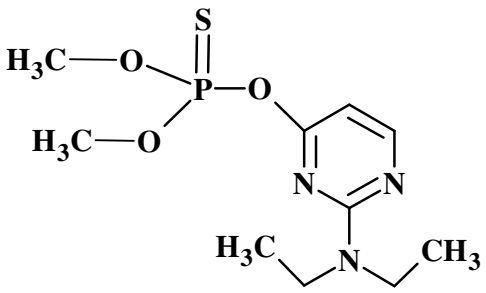


Figure 14: Molecular structure of pirimiphos-methyl

2.1.3.3. Fenitrothion

Technical fenitrothion is a reddish-brown oily liquid with a characteristic pungent smell. It can be identified by using HPLC retention time and IR spectroscopy. Table 11 and Figure 15 shows its molecular structure, physical and chemical properties.

Table 11: Physical and chemical properties of fenitrothion (WHO, 2010d)

IUPAC name	*o*,*o*-dimethyl *o*-4-nitro-*m*-tolyl phosphorothioate
CAS registry number	112-14-5
Molecular formula	$C_9H_{12}NO_5PS$
Relative molecular mass	277.25
Thermal properties	MP is -1 °C ± 1 °C
Density	1.229 ± 0.06 $g.cm^{-3}$ at 20 °C
Vapour pressure	1.57×10^{-3} Pa at 25 °C and 99.1% purity
Solubility in water	19.0 $mg.l^{-1}$ at 20 ± 0.5 °C and 99.1% purity
Octanol water partition coefficient	Log P_{ow} is 3.319 ± 0.080 at 25 °C and 99.3% purity
Toxicity, LD_{50}	1700 $mg.kg^{-1}$ bw (male rat)

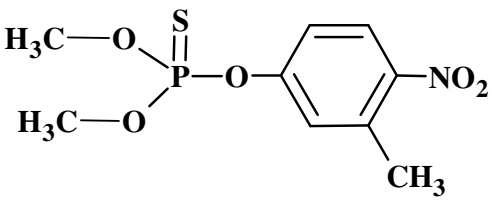


Figure 15: Molecular structure of fenitrothion

2.1.4. Organochrine (DDT)

Technical DDT consists of white crystalline wettable powder. DDT has two isomers *o,p'*-DDT and *p,p'* – DDT, the latter showing the most insecticidal activity isomer. It is identified using GC retention time and MS (WHO, 2009d). It is a neurotoxin that acts by modifying the membrane of nerve axons preventing the efflux of sodium ions out of the axons during the restoration of nerve polarities after a signal has been transmitted. This action causes the nerve to continuously transmit signals and thus causing hyper excitability of the muscles of the victim. Table 12 and Figure 16 show the molecular structure and physical and chemical properties of DDT respectively.

Table 12: Physical and chemical properties of DDT (WHO, 2009d)

IUPAC name	*p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane. *o,p'*-DDT: 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane Mixture: 1,1,1-trichloro-2,2-bis(chlorophenyl) ethane
CAS number	50-29-3
Molecular formula	$C_{14}H_9Cl_5$
Relative molecular mass	354.5
Melting point	109 °C
Density	0.98 $g.m^{-3}$ at 20 °C
Vapour pressure	2.5×10^{-5} Pa at 25 °C
Solubility in water	0.0055 $mg.l^{-1}$ at 25 °C
Octanol water partition coefficient	Log P_{ow} is 6.19 at 25 °C
Toxicity, LD_{50}	113 $mg.kg^{-1}$ bw (male rat)

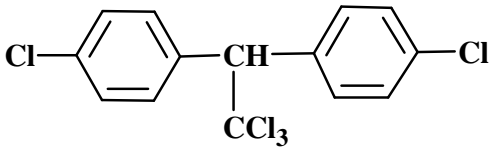


Figure 16: Molecular structure of DDT

2.1.5. Property comparisons of IRS insecticides

Figures 17, 18, 19 and 20 show plots of selected physical and chemical properties of IRS insecticides.

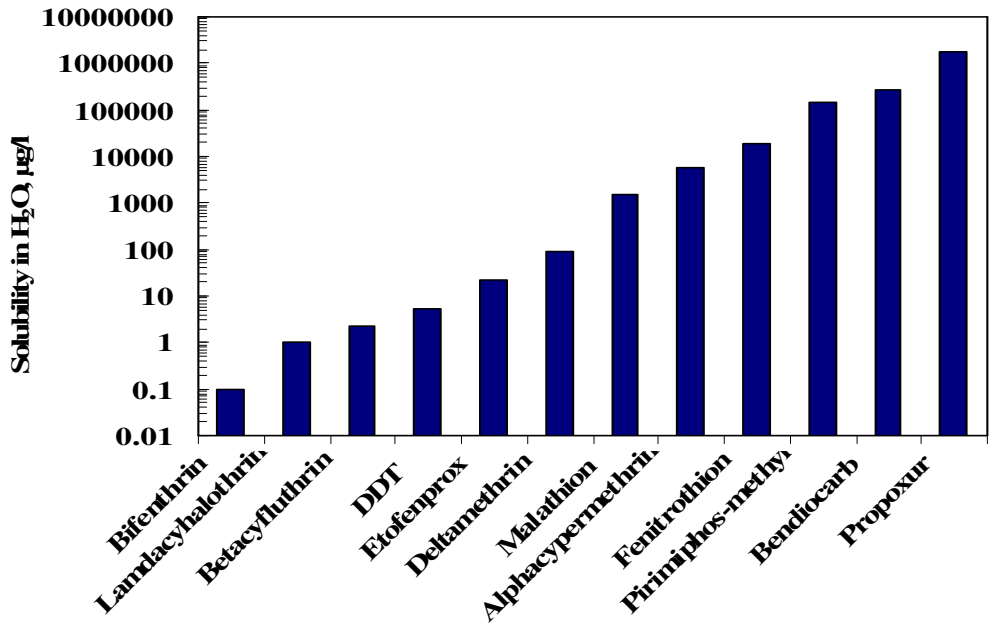


Figure 17: Plot of water solubility of IRS insecticides. Carbamates and organophosphates show markedly higher solubilities as compared to DDT and pyrethroids.

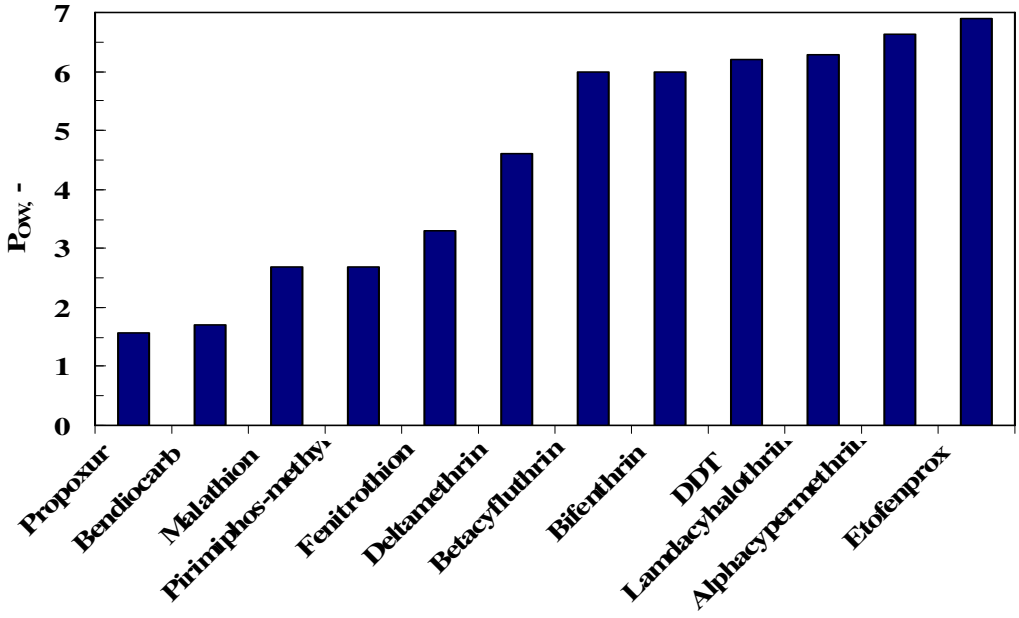


Figure 18: Plot of octanol/water partition constants (P_{OW}) of IRS insecticides. The higher octanol/water partition constants of pyrethroids and DDT show that they are more soluble in non polar solvents such as acetone.

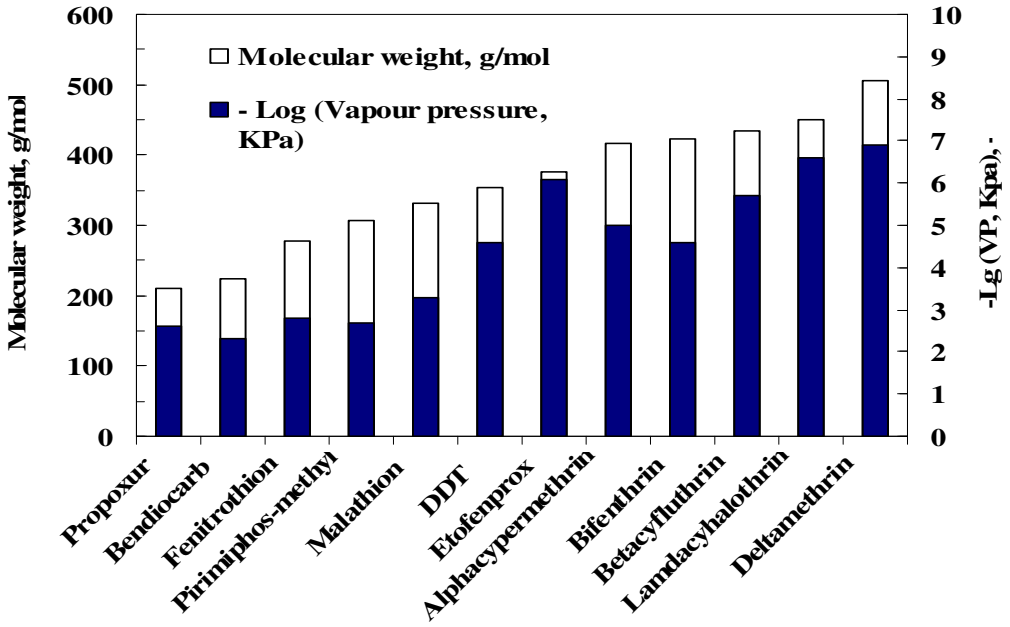


Figure 19: Plot of molecular weight and –Log (Vapour pressure, Kpa) plots of IRS insecticides. Pyrethroids have the lowest vapour pressure [highest –log (vapour pressure)] suggesting that they are the least fugitive class of insecticides. There appears to be no correlation between molecular weight and vapour pressure. Van Der Waals forces of attraction may not play significant role in insecticide molecule to molecule interactions.

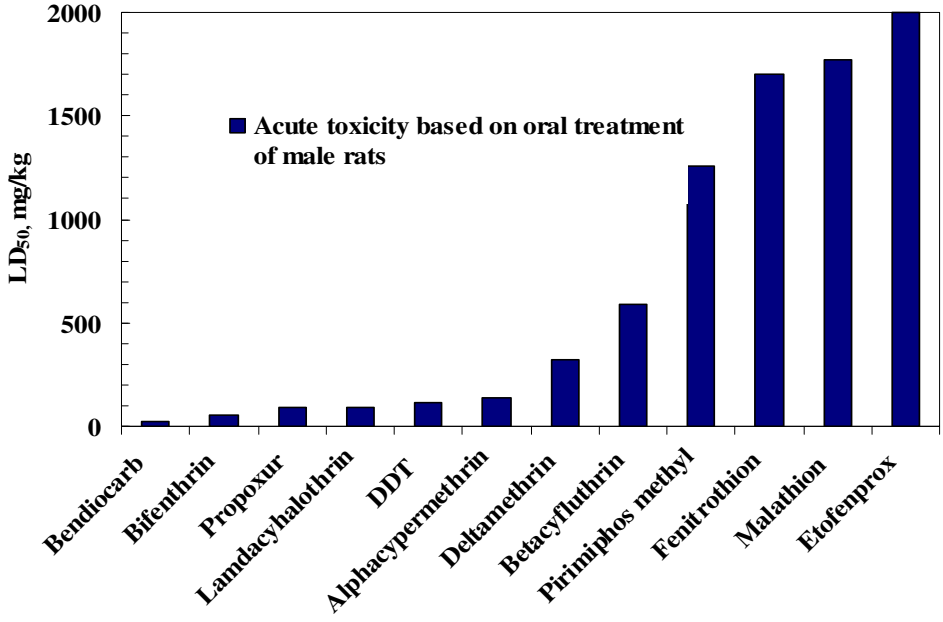


Figure 20: Plot of lethal dose to 50% deaths (LD_{50}) in male rats within 14 days of dosage. Carbamates, DDT, lamdacyhalothrin and alphacypermethrin show very high toxicities.

The IRS insecticides have been classified as moderately hazardous to humans. They are rapidly metabolised by carboxylesterase enzymes to non toxic by products, with the exception of DDT, and thus do not accumulate in the body. There is no evidence of mutagenicity, teratogenicity, carcinogenicity and genotoxicity found in the use of these insecticides in public health. Acute toxicity is higher via the oral and inhalation route than dermal route. Prolonged exposure leads to damage of the peripheral nervous system.

2.2. Insecticide degradation mechanisms

This investigation considered the reasons why DDT is reportedly more stable than its alternatives. Such understanding could aid development of strategies suitable for stabilising safer alternatives. Micro-encapsulated formulations of insecticides in materials such as polymeric capsules have been tested with great success (Mosqueira *et al.,* 2010a; Mosqueira *et al.,* 2010b). These results show that shielding the insecticides from the outside environment stabilises them from degradation. However these formulations are expensive.

Literature studies showed that in the outside environment, insecticides are susceptible to photo-degradation, thermal-degradation, hydrolytic/oxidative degradation and microbial-degradation.

2.2.1. Photodegradation

2.2.1.1. Carbamates

Carbamate photodegradation involves the cleavage of the carbamic acid ester to form the corresponding alkyl phenyl ether or alkyl phenol ether (Silk, Semeluk & Unger, 1976; Schwack & Wolfgang, 1992). Carbamates have been shown to isomerise on irradiation through the photo-Fries re-arrangement. The photodegradation of carbamates has also been shown to produce unidentified cholinesterase inhibitors as products of decomposition (Crosby, Leitis & Winterlin, 1965). The photodegradation pathway of propoxur proposed is shown in Figure 21.

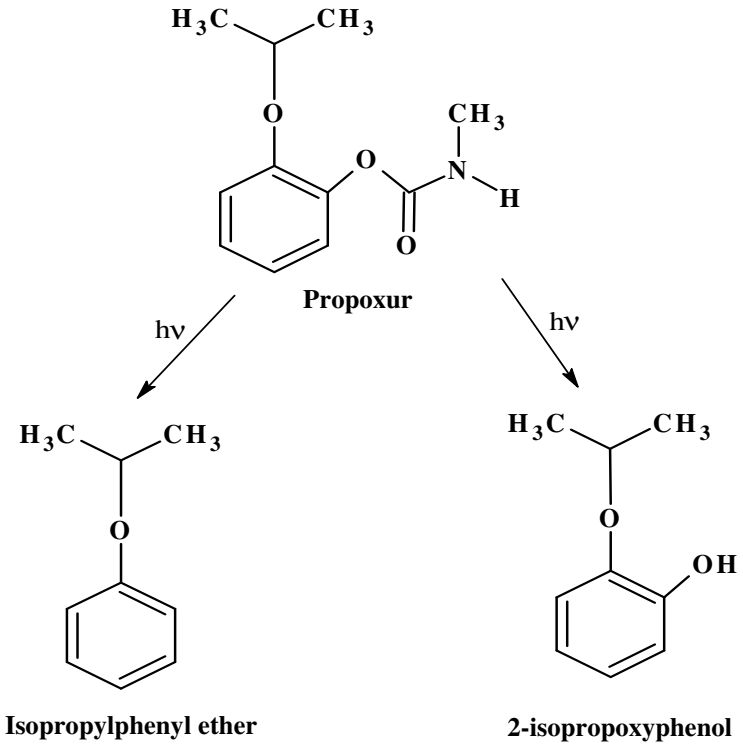


Figure 21: Photodegradation pathway of propoxur

2.2.1.2. Pyrethroids

All pyrethroids are structurally similar except for etofenprox. They all have the characteristic carboxylic ester moiety, the dihalogen substituted vinyl moiety and the phenoxy ether group. Etofenprox contains the phenoxy ether group only. The carboxylic ester moiety is very susceptible to photodegradation and hence decarboxylation of these insecticides is the main route of photodegradation (Ueda, Gaughan & Casida, 1974; Ruzo & Casida, 1982).

However photodecarboxylation of these pyrethroids is a minor transformation process accounting for not more 15% of the transformation products (Ruzo, 1983; Ruzo *et al.,* 1987). The amount of decarboxylation depends on the halogen substituents on the vinyl moiety i.e. the more electronegative the halogen the more the decarboxylation. The main process of transformation of these pyrethroids is photoisomerisation (cis/trans and E/Z) on the triplet diradicals formed on exposure to ultra-violet light (>300 nm) (Figure 22) e.g. deltamethrin (Ruzo, Holmstead & Casida, 1977), cypermethrin, cyhalothrin (Ruzo, 1983). Cyfluthrin is subject to photolysis rapidly forming of 4-fluorophenoxybenzaldehyde via ester hydrolysis and the release of cyanide ion from the corresponding cyanohydrin (Katagi, 2004).

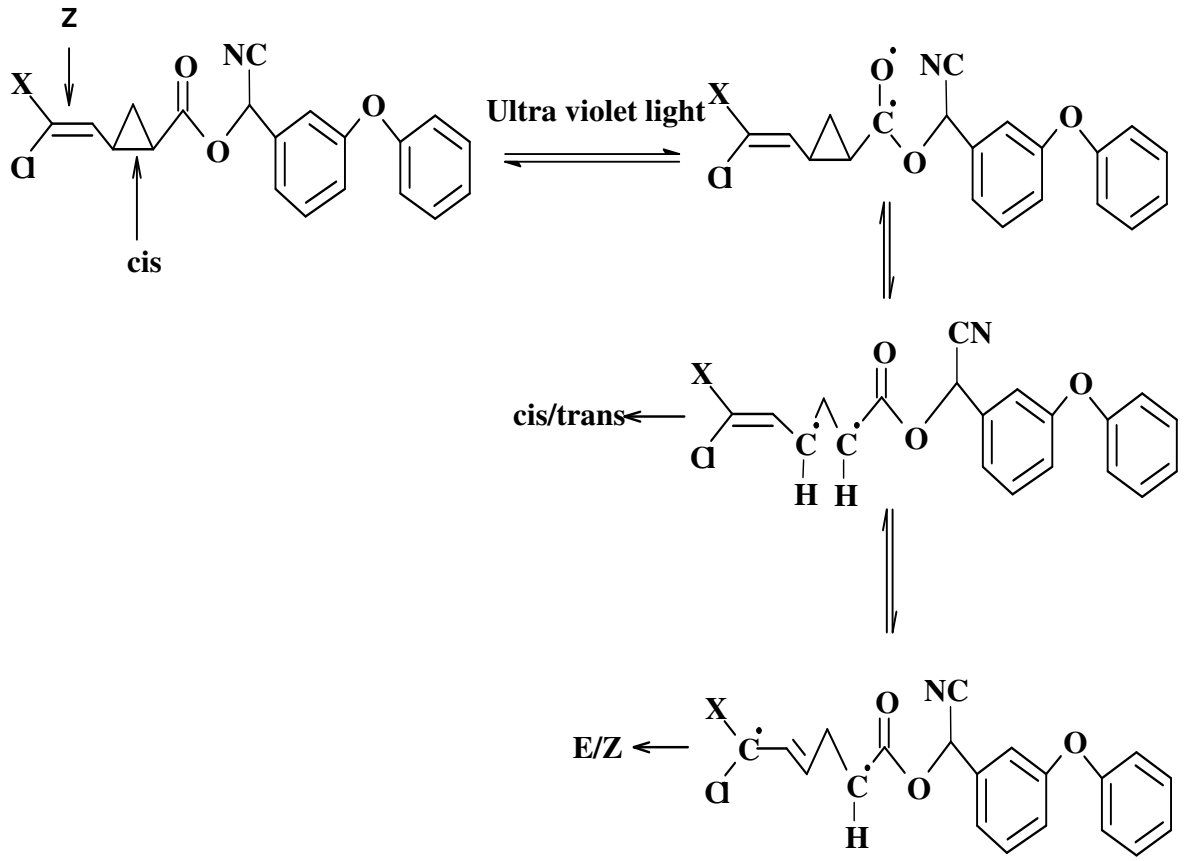


Figure 22: Photodegradation pathway of a general pyrethroid

Etofenprox exhibits an extended π-aromatic system and hence there is some absorption at wavelengths > 290nm. Products of etofenprox degradation depend on the irradiation conditions. In the presence of oxygen, etofenprox undergoes benzylic oxidation to form an ester. In de-aerated conditions it degrades to form an alkane linkage in place of the ether linkage (Class, Casida & Ruzo, 1989). The proposed degradation pathways are shown in Figure 23.

2.2.1.3. Organophosphates

On exposure to sunlight or UV radiation fenitrothion undergoes photo-oxidation on the benzene methyl group to form a carboxylic group. It may also undergo oxidation on the ─P═S moiety group to form the oxon and ester cleavage to form a corresponding phenol (Ohkawa, Mikami & Mayambo, 1974).

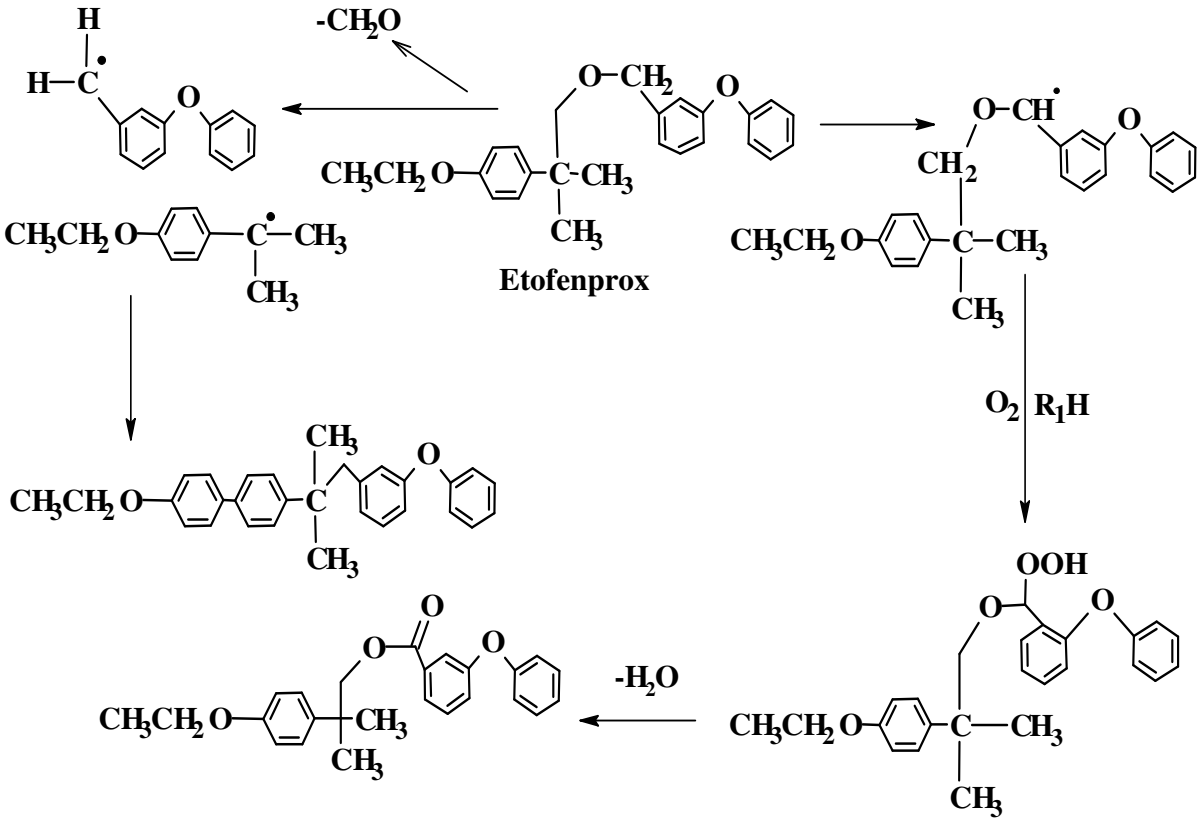


Figure 23: Photodegradation pathway of etofenprox

Malathion is generally stable to photolysis. This may be due to a lack of chromophores to absorb radiation in the UV/visible range (Katagi, 2004). Pirimiphos methyl generally photodegrades rapidly to form 2-diethylamino-6-methylpyrimidin-4-ol as the major degradate (FAO, 2007).

2.2.1.4. Organochlorine (DDT)

DDT undergoes homolytic cleavage of the C─Cl bond on the trichloromethyl group to form DDD as the product (Mosier, Guenzi & Miller, 1969; Lin & Chang, 2006). DDE may be produced due to exothermic effects of the initial photodechlorination stage. The overall photodechlorination (Figure 24) is proposed to be dominated by a sequential dechlorination pathway with each successive dehalogenation proceeding more slowly.

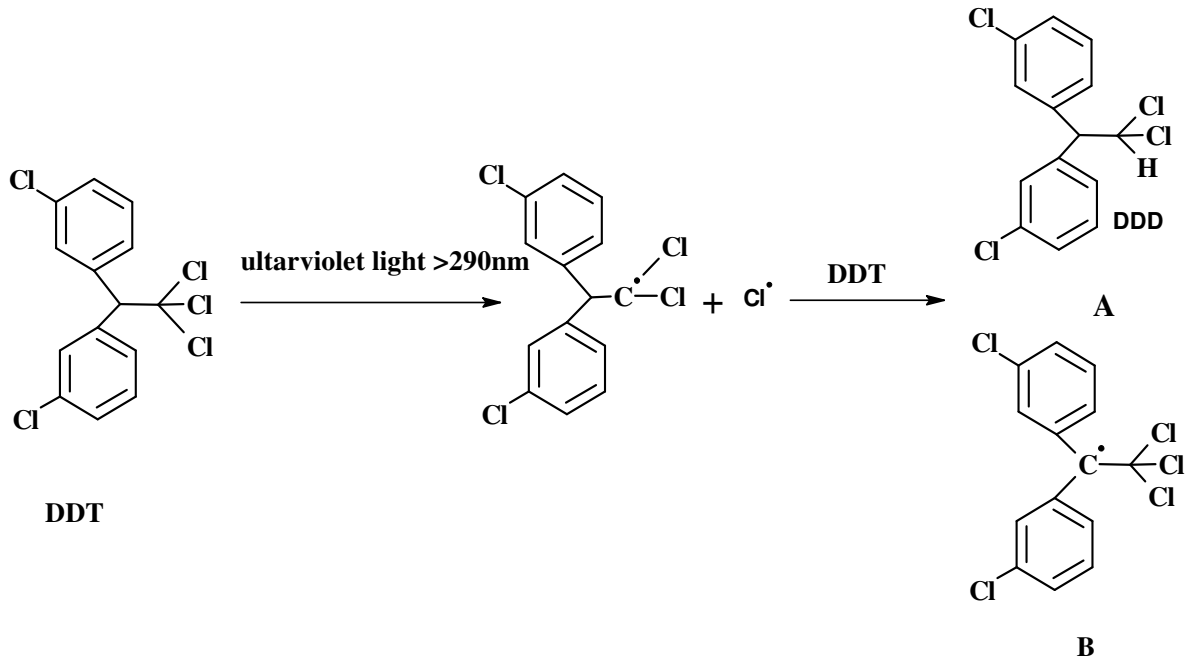


Figure 24: Photodegradation pathway of DDT

2.2.2. Thermal degradation

2.2.2.1. Carbamates

Phenyl carbamates (bendiocarb and propoxur) have been shown to degrade to methylisocyanate and the corresponding phenol (Figure 25) (Müller & Stan, 1990).

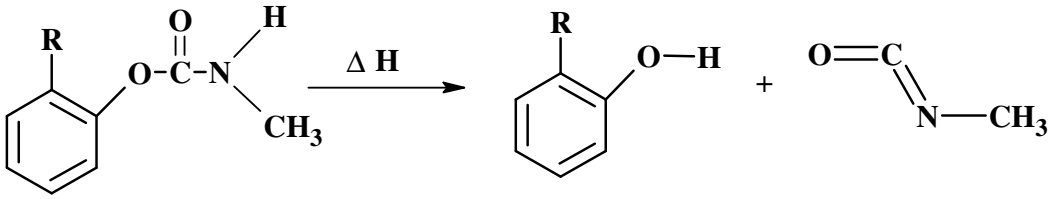


Figure 25: Thermal degradation pathway of phenyl carbamates

2.2.2.2. Pyrethroids

Under thermal exposure, pyrethroids transform by isomerisation, ester cleavage and finally primary oxidation of the final products (Audino, Licastro & Zerba, 2001). Secondary oxidation of pyrethroids (pyrolysis) has been reported (Senneca, Scherillo & Nuuziata, 2007) and is observed to be associated with production of CO, CO_2 and H_2O at 300-400 °C.

2.2.2.3. Organophosphates

Organophosphates have been shown to initially isomerise to S-alkyl organophosphates before they eventually decompose (Figure 26). This has been observed to occur for malathion at 150 °C (Mcpherson & Johnson, 1956).

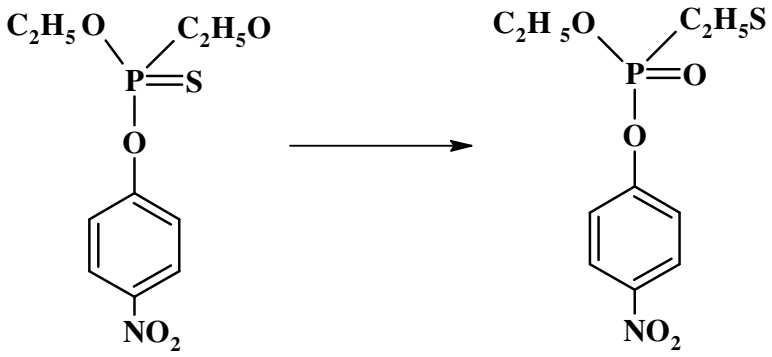


Figure 26: Schematic of parathion undergoing isomerisation to S-alkyl organophosphate

2.2.2.4. Organochlorine (DDT)

The primary step in thermal decomposition of *p,p'*-DDT is the elimination of HCl, resulting in the formation of *p,p'*-DDE at 152 °C (Lubkowski *et al.,* 1988). DDE starts to volatilize at the onset of the process. At higher temperatures side reactions take place causing partial carbonization of the sample. Last thermal event, which is exothermic, is accompanied by oxidation of the residue. The decomposition temperature is dependent on the type of DDT, for *o,p'*-DDT the decomposition starts at higher temperatures. This mechanism is similar to that of photodechlorination.

2.2.3. Microbial degradation

Research into microbial degradation of insecticides has been primarily on the fundamentals of biodegradation and bioremediation techniques to detoxify environments (Singh & Walker, 2006). The process of microbial degradation is affected by the availability of a co-metabolic substrate (Aislabie & Lloyd-Jones, 1995). This substrate can be a carbon source, nutrient source or both. The presence of alternative carbon substrates and nutrients generally increases the rate of biodegradation. This is supported by the observed increased DDT degradation in highly fertilised soils (Boul *et al.,* 1994). Microbiological degradation of insecticide relies on the availability of organisms that can secrete degrading enzymes.

Generally bacteria and fungi have the ability of producing these enzymes under aerobic and anaerobic conditions. Carbamates, pyrethroids and organophosphates have a common ester moiety that is a route to bacterial enzymatic biotransformation. The ester moieties are classified as carbamic linkage (for carbamate ester linkages), carboxylic linkage (pyrethroid ester linkages) and triphosphate linkage (organophosphate ester linkages). The enzyme groups that hydrolyse these ester moieties are carboxylesterases for carbamic and carboxylic esters, and phosphotriesterases and carboxylesterases for triphosphates esters (Sogorb & Villanova, 2002). DDT is a chlorinated organic molecule and does not possess any ester linkage. It is degraded by oxygenase and dehydrogenase enzymes (Singh *et al.,* 1999).

There is a period of adaptation that is required for degrading microbia to establish itself before any significant degradation of the insecticide is observed (Arbeli & Fuentes, 2007). This acclimation period entails the building of viable microbial colonies that have a capacity to induce enhanced degradation (Aislabie & Lloyd-Jones, 1995). This acclimation period is also affected by the availability of co-metabolic substrates for growth.

2.2.3.1. Carbamates

The products of carbamate enzymatic degradation (Figure 27) are carbamic acid and an alcohol (Sogorb & Villanova, 2002). The carbamic acid immediately decomposes to CO_2 and methyl amine. Tables 13 and 14 show examples of carbamate degrading bacteria and fungi.

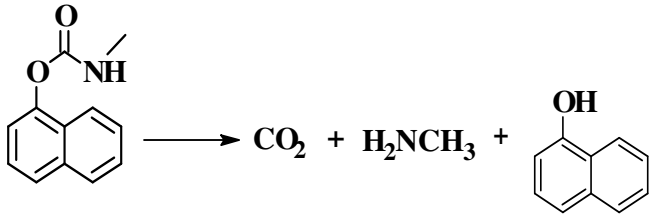


Figure 27: Degradation pathway of carbaryl by bacteria

Table 13: Examples of carbamate degrading bacteria

Pseudomonas cepacia (Venkateswarlu, Chendraya & Sethunathan, 1980)
Pseudomonas aeruginosa (Chapalmadugu & Chaudry, 1993)

Table 14: Examples of carbamate degrading fungi

Gliocladium roseum (Liu & Bollag, 1971)
Aspergillus flavus (Bollag & Lin, 1972)
Aspergillas terreus (Bollag & Lin, 1972)
Culcitalna sp. (Sikka, Miyazaki & Lynch, 1975)
Halosphaeria sp. (Sikka *et al.*, 1975)
Fusarium solani (Bollag & Lin, 1972)
Rhizopus sp. (Bollag & Lin, 1972)
Penicillium sp. (Bollag & Lin, 1972)

Bendiocarb is rapidly degraded in soil with a half life of 1-10 days by the hydrolytic cleavage of the methyl carbamate group to form the benzodioxol-4-ol (WHO, 2009b). Propoxur is moderately persistent in the soil under aerobic and anaerobic soil conditions (WHO, 2005).

2.2.3.2. Pyrethroids

The route of biodegradation is through the hydrolysis of the ester group by esterase enzymes. For the carboxylic ester groups, these enzymes are called carboxyesterases. The enzymatic hydrolysis of the carboxylic ester linkage is via the reversible acylation of the enzyme, which then causes the release of the alcohol moiety of the ester and acylated enzyme. A nucleophilic attack of the acylated enzyme by water then terminates the reaction cycle, releasing the corresponding carboxylic acid moiety and the free enzyme (Sorgorb & Villanova, 2002). The reaction mechanism is illustrated in Figure 28.

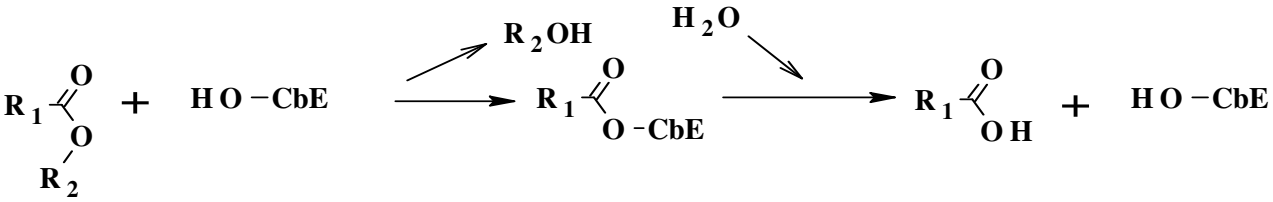


Figure 28: Degradation pathway of pyrethroids by bacteria

It has been reported that the pyrethroids of pyrethrin and allethrin are de-activated by a combination of hydrolysis, oxidation and reduction. The most efficient route of degradation is via hydrolysis. Table 15 shows examples of pyrethroid degrading bacteria.

Table 15: Examples of pyrethroid degrading bacteria

Achromobacter sp. (Maloney, Maule & Smith, 1988)
Bacillus cereus (Sakata, Mikami & Yamada, 1992)
Pseudomonas fluorescens (Grant, Danielle & Betts, 2002)
Serratia plymuthica (Grant *et al.,* 2002)
Pseudomonas sp. (Yu & Fan, 2003)
Stenotrophomonas acidaminiphila (Lee *et al.,* 2004)
Aeromonas sobria (Lee *et al.,* 2004)
Erwinia carotova (Lee *et al.,* 2004)
Yersinia frederiksenii (Lee *et al.,* 2004)
Trichoderma viride (Saikia & Gopal, 2004)

2.2.3.3. Organophosphates

Enzymes involved in the detoxification of organophosphates are phosphotriesterases and carboxylesterases. Phosphotriesterases are more efficient detoxifiers than carboxyesterases (Sogorb & Villanova, 2002). The degradation pathway is shown in Figure 29 and 30. Table 16 shows a list of organophosphate degrading bacteria.

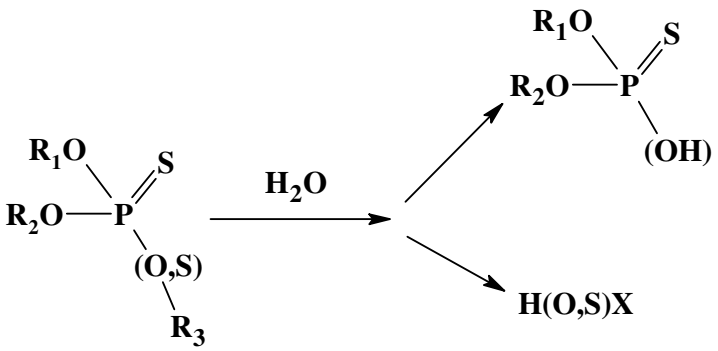


Figure 29: Degradation pathway of an organophosphate by bacteria

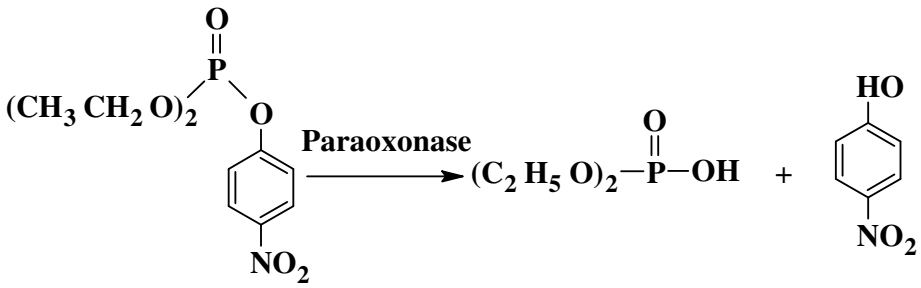


Figure 30: Degradation pathway of paraoxon by bacteria

The most studied enzyme is paraoxonous that hydrolyses paraxon and produced by *Pseudimonas diminuta*.

Table 16: Examples of organophosphate degrading bacteria

Pseudomonas diminuta (Sedar *et al.,* 1982)
Pseudomonas stuzeri (Doughton & Hsieh, 1967)

2.2.3.4. Organochlorine (DDT)

DDT has been found to degrade aerobically and anaerobically. Under aerobic conditions DDT can degrade to 4-chloro-benzoic acid. Anaerobically, it degrades ultimately to dichlorobenzophenone (DBP) (Aislabie, Richards & Boul, 1997). Several anaerobic bacterial species that can degrade DDT and its residues have been cited. Examples of such bacteria are shown in Table 17. DDT is de-chlorinated under reducing conditions to yield dichloro-diphenyl-dichloroethane (DDD) (Johnsen, 1976). Under anaerobic conditions, DDD can be further de-chlorinated to yield unsym-bis- (4 – chlorophenyl) ethylene (DDNU). DDNU is subsequently oxidised to 2, 2 – bis (p-chlorophenyl) ethanol (DDOH). DDOH is then further oxidised to yield dichlorophenylacetic acid (DDA).

DDA is de-carboxylated to dichloro-diphenyl-methane (DDM) which is further metabolised to DBP or alternatively undergo cleavage of one of the aromatic rings to form p – chlorophenylacetic acid (PCPA). A summary of the proposed pathway is shown in Figure 31. Under anaerobic conditions DBP was not further metabolised (Pfaender & Alexander, 1972).

Table 17: Examples of DDT degrading bacteria

Alcaligenes eutrophus (Nadeau *et al.,* 1993)
Agrobacterium tumefaciens (Johnson, Goodman & Goldberg, 1967)
Arthrobacter sp. (Patil, Matsumura & Boush, 1970)
Bacillus cereus (Johnson *et al.,* 1967)
Bacillus coagulans (Langlois, Collins & Sides, 1970)
Bacillus magaterium (Plimmer, Kearney & Von Endt, 1968)
Bacillus subtilis (Johnson *et al.,* 1967)
Clostridium pasteurianum (Johnson *et al.,* 1967)
Clostridium michiganense (Johnson *et al.,* 1967)
Enterobacter aerogenes (Langlois *et al.,* 1970)
Erwinia amylovora (Johnson *et al.,* 1967)
Escherichia coli (Langlois *et al.,* 1970)
Hydrogenomas sp. (Focht & Alexander, 1970)
Kurthia zapfii (Johnson *et al.,* 1967)
Micrococcus sp. (Plimmer *et al.,* 1968)
Serratia marsescens (Mendel & Walton, 1966)
Streptomyces annomoneus (Chacko, Lockwood & Zabik, 1966)
Streptomyces aureofaciens (Chacko *et al.,* 1966)
Streptomyces viridochromogens (Chacko *et al.,* 1966)
Xanthomonas sp. (Chacko *et al.,* 1966)

Aerobic degradation is through the oxidation of DDT by dioxygenase to produce a phenolic derivative (2, 3 – dihydrodiol – DDT) that undergoes meta cleavage to yield 4 – chlorobenzoic acid (Nadeau *et al.,* 1993). Figure 32 shows two proposed mechanisms for this biotransformation (Aislabie & Lloyd-Jones, 1995). The first mechanism leads to the production of DDE and the second mechanism leads to the production of 4 – chlorobenzoic acid.

DDE is a metabolite of aerobic systems in soils (Boul *et al.,* 1994; Aislabie, Richards & Boul, 1997). The concentration of DDE in irrigated soils is significantly lower than in less irrigated soils.

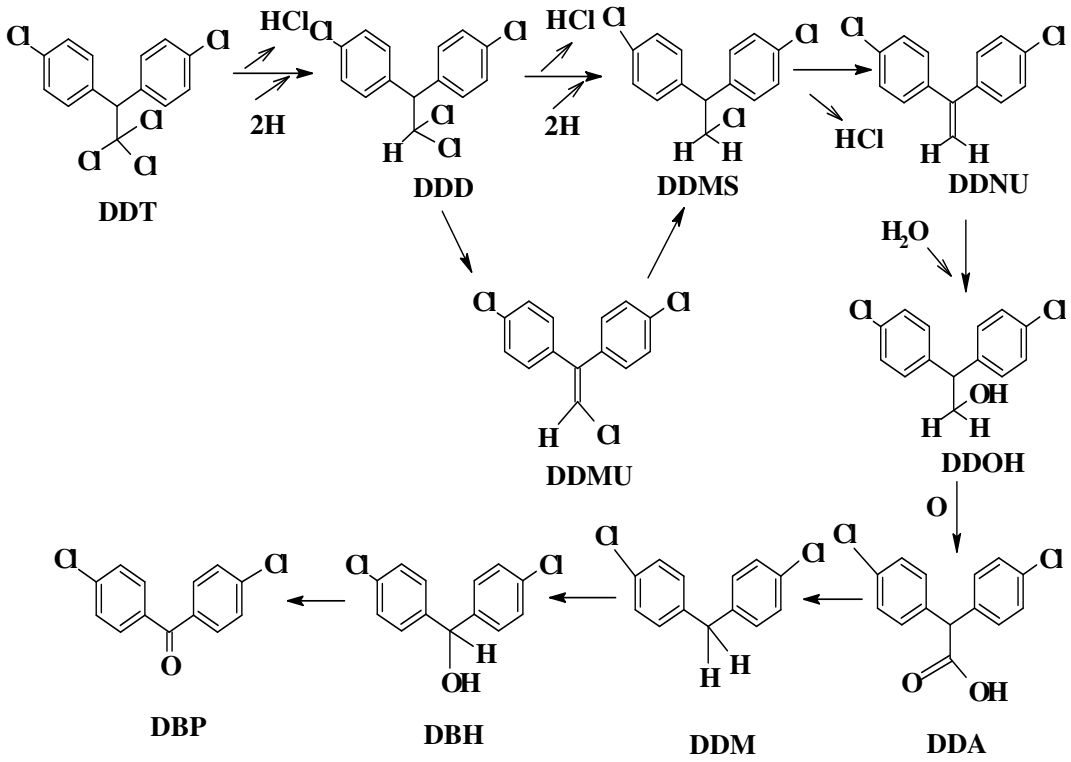


Figure 31: Anaerobic degradation pathway of DDT by bacteria

Fungi with the ability to degrade the chemical complex lignin usually found in plants have been found to be able to degrade DDT. This is due to the ability of the enzyme ligninase secreted by lignolytic fungi to degrade DDT (Bumpus & Aust, 1987; Fernando, Aust & Bumpus, 1989; Aust, 1995). The proposed degradation pathway is shown in Figure 33. Biodegradation of DDT by lignolytic fungi has been shown to depend on the availability of carbon (Fernando *et al.,* 1989). Examples of DDT degrading bacteria are shown in Table 18.

Table 18: Examples of DDT degrading fungi

Phanerochaete chryssosporium (Bumpus & Aust, 1987)
Trichoderma viride (Matsumara & Boush, 1968)

DDT persists in soils years after application (Boul *et al.,* 1994). However in highly irrigated soils DDT persistence was observed to be markedly less. This was attributed to the creation of anaerobic conditions in flooded soil and hence promoting biodegradation of DDT by anaerobic bacteria. There is lower DDT persistence in soils with high fertiliser applications.

This is attributed to the high biomass availability in such soils, promoting co-metabolism of DDT by the degrading microbes.

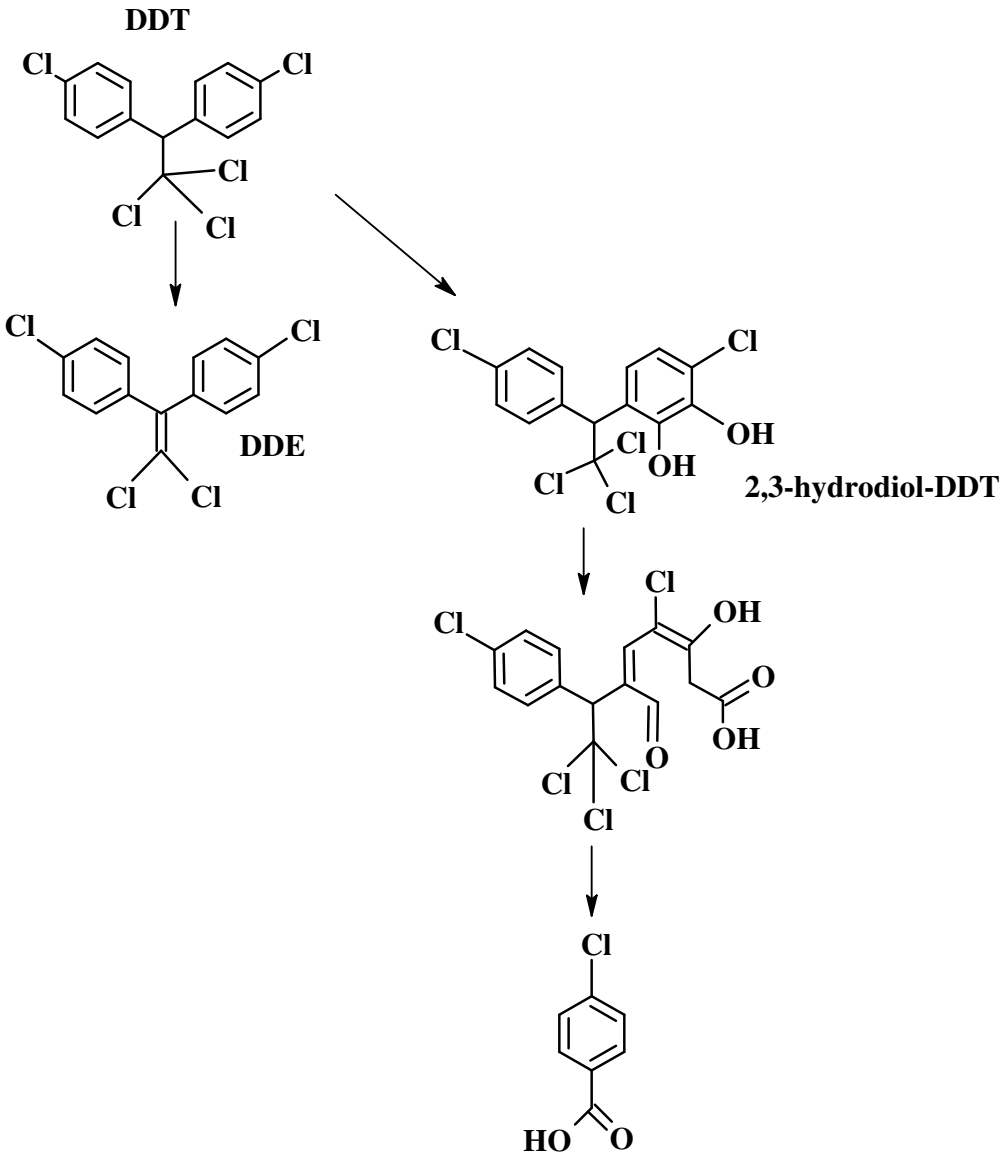


Figure 32: Aerobic degradation pathway of DDT by bacteria

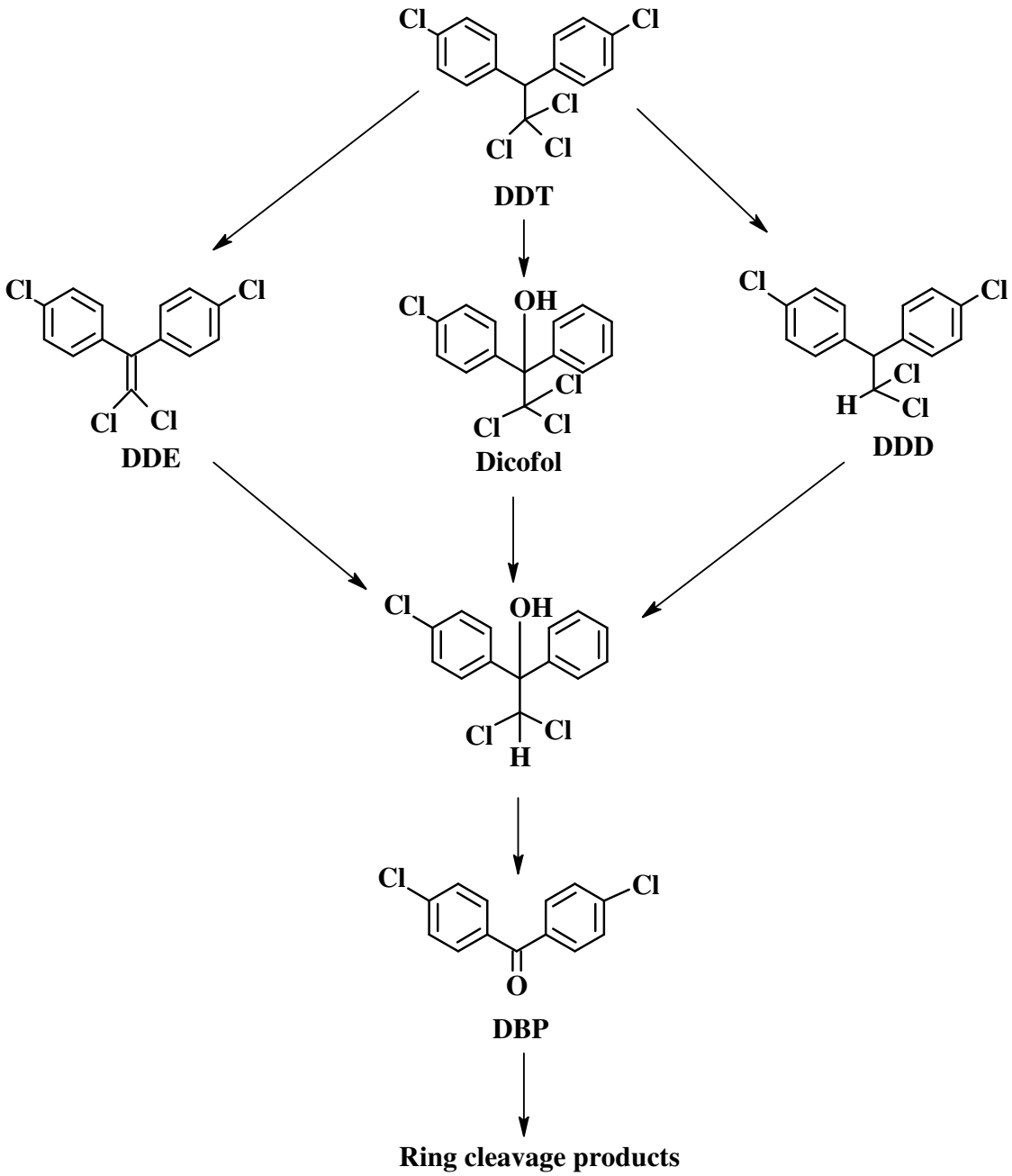


Figure 33: Fungi degradation pathway of DDT

2.2.4. Hydrolytic and oxidative degradation

Plots of the half life (DT_{50}) values against pH (Figure 34) shows that the WHO approved insecticides are more stable at lower pHs. However, bifenthrin, deltamethrin and etofenprox are reported to be stable in alkaline environments despite ester linkages (WHO 2010a; WHO 2010b and WHO 2010c).This is surprising particularly in the case of bifenthrin

and deltamethrin; they both contain ester linkages that theoretically should be susceptible to alkaline hydrolysis. Pyrethroids, organophosphates and carbamates degrade via hydrophilic attack of the carboxylic and carbamic ester linkages (Christenson, 1964; Camilleri, 1984; Caldwell & Raushel, 1991). DDT undergoes alkaline dechlorination to give DDE (Wolfe *et al.,* 1977).

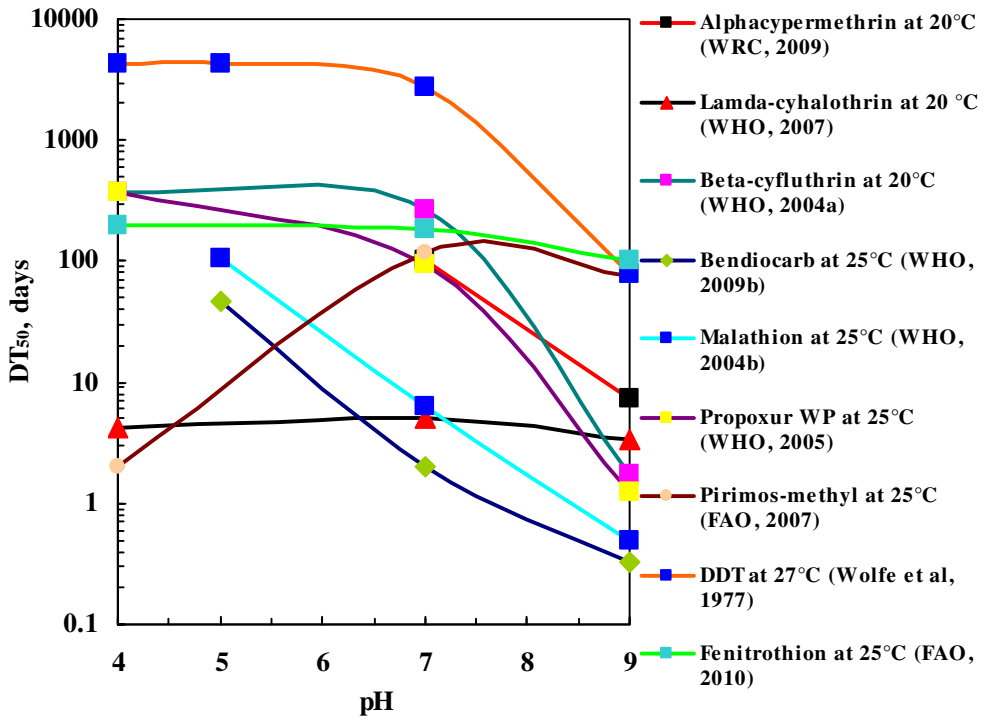
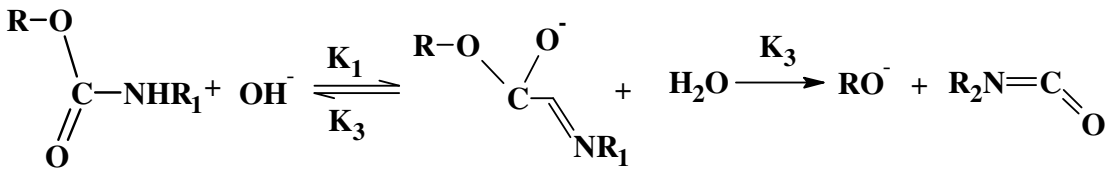


Figure 34: Plot of half life (DT_{50}) values of WHO approved insecticides

2.2.4.1. Carbamates

The alkaline hydrolysis of carbamates is via the cleavage of the ester bonds due to the hydroxyl attack on the carbonyl atom (Christenson, 1964). Primary carbamates hydrolyse much faster (order of days) than secondary carbamates (order of years). The proposed mechanism is shown in Figure 35 (representative of bendiocarb) and Figure 36 (representative of propoxur). Referring to Figure 34, bendiocarb hydrolyses faster than propoxur across the pH range considered.

1. 

2. $R_1N{=}C{=}O + H_2O \xrightarrow{K_4} R_1{-}NH{-}COOH$

3. $R_1{-}NH{-}COOH \xrightarrow{K_5} CO_2 + R_1{-}NH_2$

4. $CO_2 + 2OH^- \xrightarrow{fast} CO_3^{2-} + H_2O$

Figure 35: Alkaline hydrolysis pathway of primary carbamates

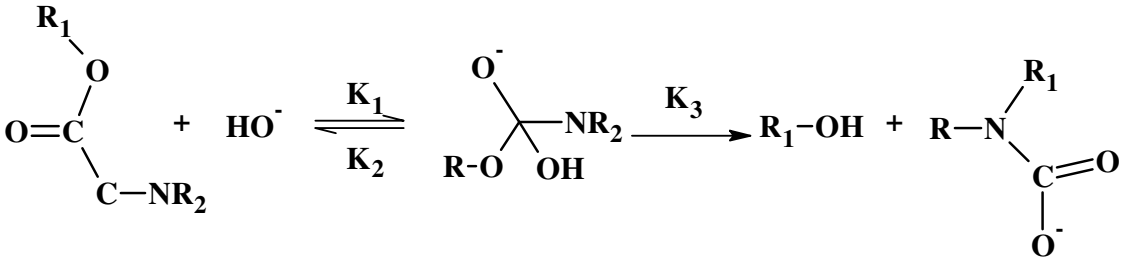


Figure 36: Alkaline hydrolysis pathway secondary carbamates

2.2.4.2. Pyrethroids

Pyrethroids are hydrolysed by the nucleophilic attack by the hydroxyl group on the ester group (Camilleri, 1984). This reaction is also the rate determining step and the mechanism is valid at low concentrations of the pyrethroid. The reaction is pseudo first order. The reaction pathway is illustrated in Figure 37.

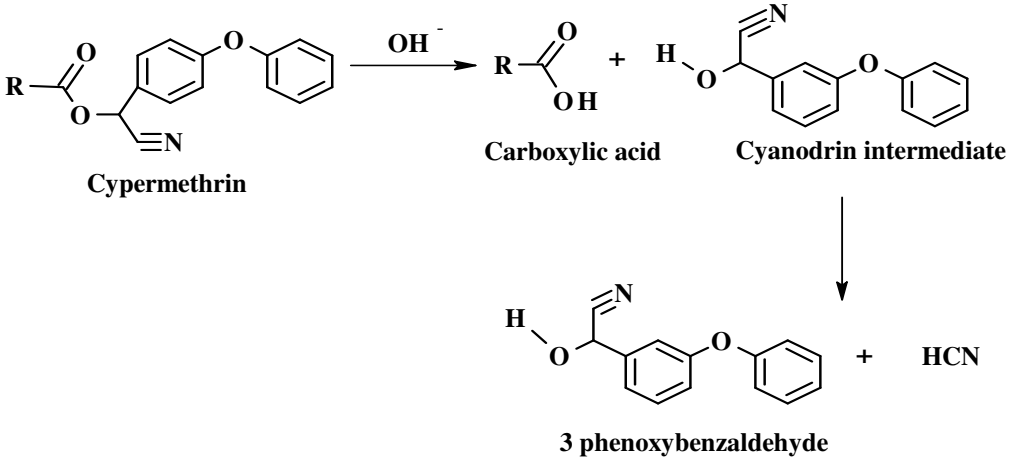


Figure 37: Alkaline hydrolysis pathway of alphacypermethrin

2.2.4.3. Organophosphates

The general reaction pathway proposed by Caldwell and Raushel (1991) is shown in Figure 38.

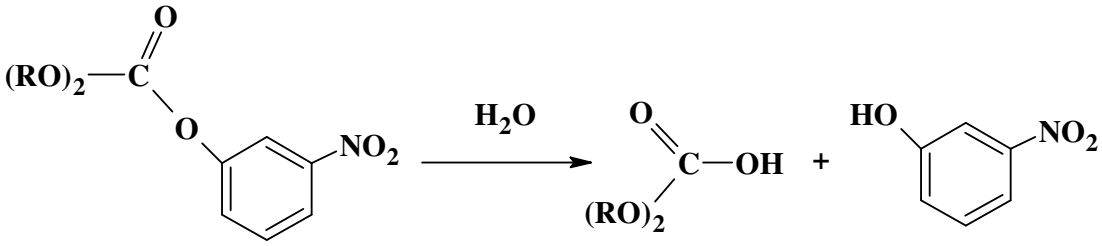


Figure 38: General alkaline hydrolysis pathway of organophosphates

Three possible mechanisms were proposed (Figure 39) as follows:

Mechanism 1: Dissociative is characterised by the elimination of the leaving group resulting in electropositive phosphorus susceptible to nucleophilic attack.

Mechanism 2: Addition elimination reaction whereby a nucleophilic attack leads to a pentasubstituted phosphorane intermediate followed by the collapse of the structure to products.

Mechanism 3: Direct displacement.

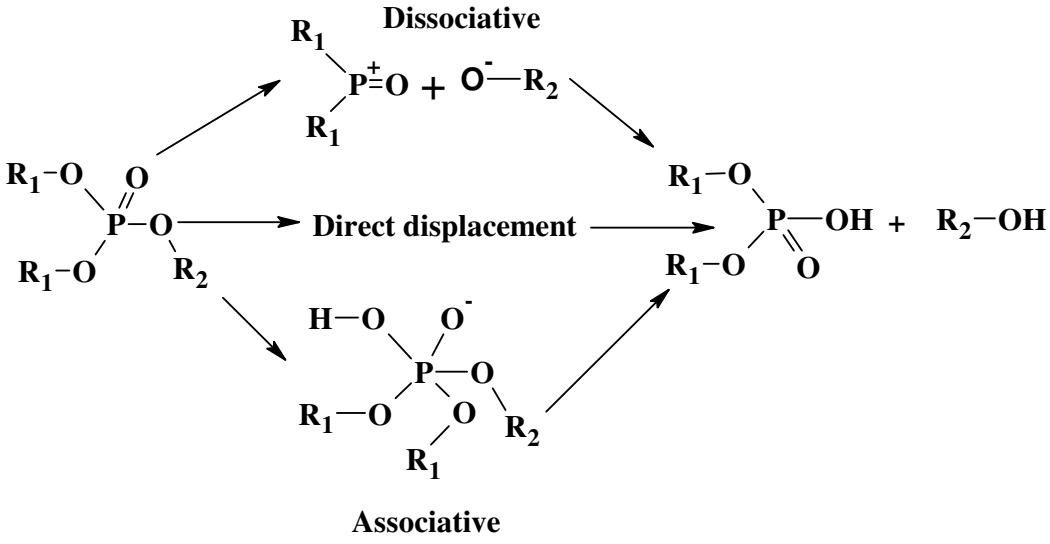
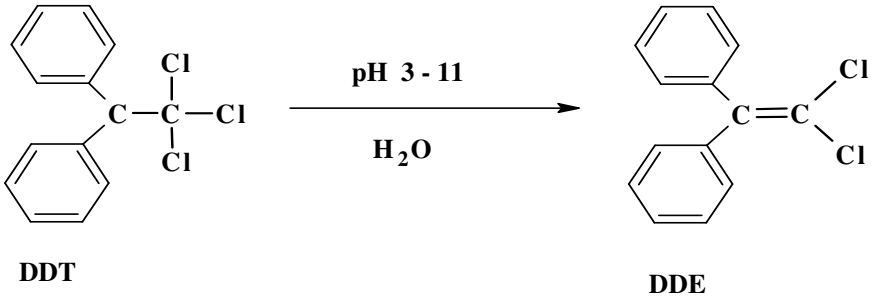


Figure 39: Proposed routes for alkaline hydrolysis pathways of organophosphates

The associative mechanism was experimentally found to be the most dominant

2.2.4.4. Organochlorine (DDT)

DDT undergoes hydrolysis to give DDE (Wolfe *et al.,* 1977). This reaction is first order and extremely slow at acidic pH and significantly fast at alkaline pH. DDE itself is stable to any further hydrolysis. Referring to Figure 34, DDT has a half life of ca. 10 000 days at pH 7 and a half life of ca. 100 days at pH 9. The mechanism of hydrolysis is illustrated in Figure 40.



DDT DDE

Figure 40: Alkaline hydrolysis pathway of DDT

2.3. Insecticide stabilisation using mineral powders

Mineral powders such as gypsum, montmorillonite, kaolin, attapulgite, diatomite etc have been used as insecticide carriers in granular insecticide formulations (Goss, Taylor & Kallay, 1994; Murray, 2000). These mineral powders have been shown to have the ability to slowly release the adsorbed insecticide into the environment (Peterson, Adams & Cutkomp, 1970; Atkinson, 1989; Bruna *et al.,* 2008).

Lagaly (2001) presents an excellent review on pesticide-clay interactions and formulations. Clay minerals are very suitable materials for formulating insecticide laden clay formulations. Absorptive forces play an important role in insecticide clay formulations. Generally good absorptive forces are required to make stable formulations. Surface morphology of the mineral particle also affects the availability of the insecticide on the surface. Mesopores on the particle have the ability of absorbing the insecticide and making it less available to the target environment. Flat surface morphology generally tends to avail more of the insecticide to the environment. These absorptive forces can be manipulated with surface modification of the clay mineral.

Insecticides adsorbed on mineral powders have also been shown to be stabilised by deactivating the surface activity of the mineral powders thereby reducing the catalytic

degradation of the insecticides (Kallay, Goss & Stein, 1993). Electron rich additives such as polyethylene glycol have been reported to be very effective deactivators. Another useful property of mineral powder carriers is that they have the ability to stabilise photolabile and thermolabile insecticides using UV and thermal absorbing additives (Margulies, Rozen & Cohen, 1988; Casal *et al.,* 2000; El-Nahhal *et al.,* 2001).

3. Experimental

The literature survey highlighted the following:

- Insecticides are generally insoluble in polar solvents and soluble in non-polar solvents.
- Pyrethroids are the least volatile of all the insecticides.
- All insecticides are relatively unstable to ultra-violet light except for pyrethroids.
- Under thermal stress, carbamates degrade to the corresponding phenol and methyl isocyanate. Pyrethroids isomerise and then degrade on the carboxylic moiety before ultimately undergoing pyrolysis. Organophosphates initially isomerise to S-alkyl organophosphates before they decompose. DDT undergoes an elimination reaction to form *p,p'*-DDE before volatilizing.
- All insecticides are biodegradable provided there is availability of degrading microbia and optimum conditions.
- Insecticides are generally unstable under alkaline conditions except for DDT.

3.1. Planning of investigation

The aim of the investigation was to determine the reasons why WHO – approved alternatives to DDT do not last as long as DDT in the field. Preliminary literature studies suggested that the loss of efficacy of WHO – approved insecticides may be caused by several degradation pathways including pH mediated hydrolysis owing to high humidity, photo degradation, thermal degradation and microbiological degradation.

It was envisaged that comparative appraisal of the WHO – approved insecticides for their time limited efficacy compared to DDT under accelerated conditions of temperature, ultra-violet light, humidity, alkaline and microbiologically active environments would provide indicators as to the *in situ* mechanisms of degradation that are dominant. With this understanding, it was expected that suitable strategies to stabilise and improve longevity and effectiveness of these insecticides could be recommended. The ultimate aim of this investigation was to develop a long-lasting, environmentally friendly and cost effective pesticide-based "whitewash" or equivalent "paint" to substitute DDT in indoor residual spraying.

The basic concept of the paint development was to use a solid carrier to adsorb the insecticide into its structure thereby protecting it from the outside environment. It was also expected that the solid carrier should provide a pH buffering effect to limit pH mediated hydrolysis. This could be achieved by using mineral powders that show acidic surface activities. The preferred solid carrier was expected to have micro pores into which the insecticide would be adsorbed. The micro pores would then provide a protective cover from ultra-violet light for the insecticide molecules whilst buffering the insecticide from alkaline mediated hydrolysis. It was then postulated that a dynamic process will develop whereby the insecticide on the surface of the solid particle will constantly be replenished by the insecticide adsorbed inside the solid surface by a process of mass transfer as the insecticide on the surface was depleted by normal wear and scuffing. The mineral powder was to be cheap and locally available in abundance.

Several mineral powders were studied for suitability as insecticide carriers. The two most promising candidates were phosphogypsum, a by product of the phosphoric acid manufacturing process, and attapulgite used to absorb excess moisture during oil well drilling.

The stability and effectiveness of the formulations were tested by using WHO recommended small scale bioassay tests and the results evaluated relative to DDT. Successful formulations would then allow DDT to be phased out permanently.

The investigation was to be divided essentially into two stages.

Stage 1 of the investigation was directed towards shedding light on the reasons why the WHO-approved DDT alternatives do not last. A proper understanding of the *in situ* degradation mechanisms of these insecticides will lead to rational strategies for improving both performance and longevity. Interaction of the insecticide with paint ingredients was also studied in order to evaluate the suitability of each inorganic mineral short-listed for use.
Activities earmarked for this stage were as follows:

1) Ageing of WHO-approved insecticides under accelerated conditions of
    - Temperature

- Ultra-violet light
- Humidity
- Microbiologically active environment

The insecticides were applied on neat surfaces. The time-dependant degradation was followed using a non-destructive characterisation technique i.e. infra red spectroscopy. Artificial weathering techniques employed the use of a QUV weathering tester, oven and a humidity incubator. Thermal analysis techniques such as TGA, DSC and DTA were employed on the insecticides. Neat substrate surfaces were used as controls.

2) Study the mineral powders short-listed for "white wash" or "paint equivalent" and their interaction with insecticide.

3) The soil samples collected from Mozambique used for plastering walls of dwellings were also characterised. Activities carried out for this investigation were as follows:

   - Characterise short-listed mineral powders for particle size, surface morphology, trace elements and pH of surface moisture.

   - Evaluate the interaction of the short-listed mineral powders with insecticides in the presence of moisture.

   - Collect representative samples of different soils used in construction of informal dwellings in rural areas. Determine their mineralogical composition. Prepare plaques based on these soils and characterize their surface properties, e.g. pH.

Stage 2 of the investigation was to develop a stable, cost effective and environmentally friendly "white wash" or "paint equivalent" based insecticide paint formulation to substitute DDT in indoor residual spraying. The development of inorganic paint slurry entailed the following:

- Rheology of the paint slurries at different solids volume fraction was studied. Effects of a suitable dispersant were considered since there was considerable agglomeration in the slurries.

- Prepare insecticide paint formulations based on the different insecticides under investigation. The interaction of the paint with a paint binder was tested also. The paint was supposed to adhere on the surface to prevent mass removal due to abrasion and scuffing.

- Perform spraying tests on prepared soil plaques. This activity was to allow the sprayer to familiarise with the spray gun to be used, suitable air pressure and nozzle settings and hand movement speeds during spraying.

- Conduct preliminary insecticide paint formulation test trials. Small scale bioassay tests, prescribed by WHO, were used to track the time dependant efficacy of the treated soil plaques. The treated test surfaces were subjected to accelerated ageing conditions of humidity and temperature. A statistical regression was performed in order to compare the time dependant efficacy of the formulations to the standard DDT formulation. Techniques that could detect the insecticide on the paint coated surface were established so as to evaluate the distribution of the insecticide on the paint.

- Use the insights and information from the above to formulate more persistent IRS techniques.

The main objective of this study was to prove the concept of using mineral powder slurries to stabilise the alternative insecticides. The experimental set ups served to indicate the comparative performance of each class of insecticides against DDT under simulated weather conditions and the effect of using a mineral powder on this performance.

3.2. Materials

Phosphogypsum is a waste product generated in the production of phosphoric acid. It was chosen because it is readily available in immense quantities and because the phosphoric acid impurity imparts an acidic nature to this filler. Phosphogypsum powder was supplied by Gypsum Industries. Attapulgite was supplied by G&W Base Minerals. The particles of this mineral have an elongated shape with parallel channels through the lattice that impart a high adsorption capacity and a high surface area (Murray, 2000). Calcium bentonite powder was supplied by G&W Base Minerals. Dellite 67G organoclay, a montmorillonite intercalated with ditallowdimethylammonium, was supplied by Laviosa.

Table 19 show the insecticides used and their suppliers.

Table 19: Suppliers of insecticides

Insecticide class	Insecticide	Supplier
Organochlorine	DDT	Avima
Organophosphate	Malathion	Agro China
	Fenitrothion	Agro China
	Pirimiphos-methyl	Avima
Carbamates	Bendiocarb	Bayer
	Propoxur	Avima
Pyrethroids	Alphacypermethrin	Bilag
	Bifenthrin	Arysta Life Science
	Cyfluthrin	Bayer
	Deltamethrin	Targos
	Lambdacyhalothrin	Avima
	Etofenprox	Agro China

A sulphonated mimosa extract (Mimosa WS supplied by Mimosa Extract Company) was used as dispersant. Makeean Polymers MCP 503 acrylic copolymer emulsion was used as binder. The emulsion had a solids content of 56 wt.%, a pH of 7.5 – 8.5.

Polytetraflouroethylene (teflon) sheet sample holders (2 cm in diameter) were supplied by Life & Analytical Sciences (Pty) Ltd.

Ethanol (96% Univar rectified), Methanol (Univar) and acetone Univar were supplied by Merck.

Soil samples used to prepare substrate surfaces were from the Thomo and Makoya locations in Mozambique.

3.3. Equipment

3.3.1. Spraying equipment

Spraying of insecticide paint on substrate was done by use of a spray gun from Aircraft Pneumatic Systems, model SG AS 1001A. The spray gun has a reservoir of 600 cm^3, a nozzle diameter of 1.4 mm and a working pressure of 4 bars. The feed to the spray gun is by gravity. Air pressure of 2 bars was used for spraying and the spray pattern adjustment was such that the widest spray width was achieved. For mud substrates sprayed with an acetone solution of DDT, a smaller spray gun from Aircraft Pneumatic Systems, model number SG A138 was used. This spray gun has a reservoir of 22 cm^3, a nozzle diameter of 0.8 mm and a working pressure of 3.45 bars. The feed to the spray gun is via suction. The compressed air used for spraying was from a common manifold with a maximum compressed air pressure of 6 bars.

3.3.2. Characterisation equipment

3.3.2.1. pH

A calibrated Russell RL060P portable pH meter was used to measure the pH imparted by the mineral powders on the distilled water.

3.3.2.2. Particle size

The particle size analyser used was a mastersizer 2000 from Malvern instruments. The instrument can measure a particle size range of 0.02 µm to 2000 µm.

3.3.2.3. Element analysis

Major element analysis was executed on a fused bead using a Panalytical Axios ARL9400XP+ X-ray fluorescence spectrometer. Analyses were performed on the Uniquant software platform.

3.3.2.4. Gas chromatography

The GC make was a Varian Star model 3400CX with an Agilent HP 5 GC column, 30 m in length and 0.25 mm internal diameter and a film thickness of 0.25 µm. The carrier gas was helium and the gas split ratio was 1:30. A flame ionization detector was used.

3.3.2.5. Infra red spectroscopy

Characterisation of the insecticides in humidity, temperature and ultra-violet light conditions was done by a Perkin Elmer Spectrum 100 Fourier transform infrared spectrometer with a transmission attachment; the resolution was 1 cm^{-1} and 20 scans were obtained per sample. The substrate surface for insecticide ageing tests used was polytetrafluoroethylene (teflon).

For the characterisation of insecticide distribution on paint coated surfaces; Fourier transform infrared (FTIR) reflection spectra were recorded with a Brüker Hyperion microscope, using a 15× objective, coupled to a Vertex 70v spectrometer also from Brüker. A liquid nitrogen cooled FPA (focal plane array) detector was used to detect the infrared signal and 32 interferograms were averaged over the range 600-4000 cm^{-1} at a resolution of 1 cm^{-1}.

3.3.2.6. Electron microscopy

Surface morphology of mineral powders was examined by low resolution scanning electron microscopy (SEM) using a JEOL (Tokyo) 500 SEM instrument. Powder sample coating used a SEM Polaron E5200 from Polaron equipment (Ltd).

3.3.2.7. Thermal analysis

Differential Thermal Analysis (DTA) was carried out using a Shimadzu DTA-50 instrument. Approximately 5 mg of the sample and standard (α-Al_2O_3) where measured out in alumina sample pans. 500 µm thick copper disks were placed at the bottom of the sample pans, these acted as heat sinks to protect the detector from high temperature rises that might occur.

3.3.2.8. Rheology

The viscosities of the paint slurries dispersions were determined at 25 °C on an Anton Paar Physica UDS 200 rheometer using a cone-and-plate geometry. The gap was set at 0.051 mm and the diameter of the disk was 50 mm. The operating software platform was RHEOPLUS/32 V3.40 21002828-33025.

3.3.2.9. Standard soil analysis

The analysis of cations in soil samples was done by a Spectro Genesis Fee inductively coupled plasma optical emission spectrometer (ICP-OES) for Ca^{2+}, Mg^{2+} and Na^{+}.

The pH of the soil in water was measured by a Consort C830 multi-parameter analyser.

3.3.3. Ageing equipment

3.3.3.1. Oven ageing

A Labcon economy oven, type EFDO was used for high temperature ageing. The temperature controller regulated the temperature to within ± 0.2 °C.

3.3.3.2. Ultra-violet light ageing

UV degradation of insecticides deposited on a polytetraflouroethylene (teflon) substrate was subjected to dry-cycle artificial weathering in a QUV tester fitted with A340 UV lamps, irradiance of 0.67 $W.m^{-2}$ at 63 °C.

3.3.3.3. Humidity ageing

A labcon forced circulation incubator model FSIM was used. The incubator controlled the temperature to within ± 0.2 °C and humidity was controlled to within ± 2% ℜH.

3.4. Methods

3.4.1. Mineral powder characterisation

3.4.1.1. Scanning electron microscopy

A small quantity of the each mineral powder was placed onto carbon tape on a metal sample holder. Excess powder was removed using light hand shaking of the coated carbon tape. The samples were then coated five times with gold under argon gas. The samples were coated for a total of 90 seconds, three times for 30 seconds at 60 second intervals.

3.4.1.2. Surface pH

A gram of each filler was added to 10 ml of distilled water in a 32 ml polytop® and shaken for 48 hours. The distilled water was bubbled with nitrogen for 20 minutes to remove dissolved carbon dioxide. After shaking, the pH of the supernatant liquid was then measured.

3.4.1.3. Element analysis

For major element analysis, a milled sample (<75 μm) of the neat or exchanged material was calcined at 1000 °C for at least 3 hours to oxidize Fe^{2+} and S and to determine the loss of ignition (L.O.I.). Glass disks were prepared by fusing 1 g roasted sample and 8 g 12–22 flux consisting of 35% $LiBO_2$ and 64.71% $Li_2B_4O_7$ at 1050 °C. The glass disks were then analysed. All elements in the periodic table between Na and U were analysed, but only elements found above the detection limits were reported.

3.4.1.4. Particle size distribution

A small amount of water was mixed with the dry mineral powder to make a watery paste. A drop of dish washing liquid was added to aid de-agglomeration of the sample. A portion of this sample was introduced into the particle size analyser using a small spatula.

3.4.2. Soil sample characterisation

3.4.2.1. pH

10 g of dried soil was added to a 60 cm^3 beaker. 25 ml of de-ionised water was then added. The mixture was stirred rapidly for 5 seconds with a glass rod. The mixture was allowed to stand for 60 minutes and then stirred again. The pH of the liquid mixture was then measured.

3.4.2.2. Cation composition (Ca^{2+}, Mg^{2+} and Na^{+})

5 g of dry soil sample was placed in a 100 cm^3 extraction bottle. 50 cm^3 of ammonium acetate (NH_4OAc) cooled to 20 ± 2 °C was added to the soil in the extraction bottle. The mixture was shaken horizontally on a reciprocating shaker at 180 oscillations per minute for 30 minutes. The sample was rapidly filtered through a buchner funnel with suction, discarding the first few drops. The resulting extract was analysed using ICP-OES.

3.4.2.3. Nitrate content

A KCl extract was prepared by weighing 50 g of soil into a 100 ml schott bottle. 100 ml of 1 M KCl was added and the mixture shaken for 60 minutes. A Whatman no. 2 filter paper was used for filtering the mixture. The filtrate was collected into a plastic container. 25 ml of boric acid indicator was added to a 250 ml beaker. 50 ml of the KCl extract was added in the flask. Dervardas alloy was then added to the flask and the flask was then placed in a buchi. The solution was distilled using the buchi stopping when the solution started to boil. A half beaker full distillate was collected and titrated with 0.01 M HCl.

3.4.2.4. Carbon content

1 g of "air dry" soil was weighed in a 500 ml Erlenmeyer flask. 10 ml of 0.167 M $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4 was added into the flask. The solution was allowed to cool for 30 minutes. 150 ml of water and 10 ml concentrated H_2SO_4 was then added to the flask. The solution was allowed to stand for 30 minutes. The mixture was then titrated with Fe-solution and a carbon indicator to evaluate the amount of carbon.

3.4.2.5. Particle size distribution (sand, silt and clay)

A 400ml beaker was weighed and the mass noted. 20 g of "air dry" soil was then added to the beaker. The soil was then transferred to a mixing flask. The beaker was washed to ensure that all soil was transferred into the flask. 10 ml of calgon (a solution of sodium hexa-metaphosphate and sodium carbonate) and enough water were added into a flask so that the blades of the mixer were covered. Mixing was done for 5 minutes and contents were transferred to a clear 2 litre measuring cylinder through a 54 µm sieve. The sieve was rinsed with water until sand only remained on the sieve. The sand was then transferred into a weighed beaker and then oven dried at 105 °C and re-weighed to obtain mass of sand. The underflow was then added to a 2 litre cylinder and made up to the mask with water. The contents were mixed and 3 hydrometer readings were taken at 50 seconds, 8 hours and 16 hours respectively to obtain percentage composition of silt and clay.

3.4.3. Thermal analysis

Approximately a 5 ± 0.5 mg insecticide was placed in an aluminium pan. The sample was heated from 25 to 350 °C at 10 °C.min^{-1} in air flowing 20 ml.min^{-1}.

3.4.4. Insecticide on mineral powder ageing

Since most insecticides are effectively insoluble in water, the following procedure was used to explore the effect of mineral powders on hydrolytic stability. Stock solutions containing ca. 1% insecticide were prepared by first dissolving 8 g bendiocarb or alphacypermethrin in 800 g acetone and then adding 32 g of distilled water. The six short listed mineral powders were added (1 g) to two sets of six schott glass bottles. To this was added 21 g

of the respective insecticide stock solutions. The top of the bottles were sealed with parafilm before capping and then wrapped in aluminium foil to prevent light exposure. The bottles were shaken at a shaker speed of 180 cycles per minute during the ageing period at ambient conditions. Two sets of solutions (without mineral powders) for each insecticide were used as reference and control standards respectively. The reference standard sample was stored in a fridge and the negative control was kept under the same conditions as the test samples. A small amount of sodium ethoxide, a highly alkaline solution, was sparingly added to another pair of schott glass bottles containing the bendiocarb and alphacypermethrin stock solutions. These samples were prepared in order to confirm the instability of both insecticides in highly alkaline environments and also as a reference of the possible hydrolysis products found in other test samples.

The degradation of the insecticide in the presence or absence of mineral powders was followed using a GC. For bendiocarb the initial oven temperature was 120 °C, a holding time of 1 minute, a ramp rate of 25 °C.min^{-1}, final temperature of 250 °C and a final holding time of 10 minutes. The injector temperature was 200 °C while the detector temperature was set at 265 °C. For alphacypermethrin the initial oven temperature was 245 °C, holding time of 0 minutes, a ramp rate of 25 °C.min^{-1}, final temperature of 275 °C and a final holding time of 10 minutes. The injector and detector temperatures were 250 °C and 250 °C respectively.

3.4.5. Temperature, ultra-violet light and humidity ageing

A small amount of ca. 1% insecticide solution for each of the insecticides was deposited onto a sample holder (polytetrafluoroethylene IR card). The treated IR cards including the control reference were put in a humidity incubator at a relative humidity of 90% and a temperature of 60 °C. Another set of samples was put in an oven at a temperature of 80 °C. The last set of ageing samples was put in a ultra-violet weathering tester. The ageing period in the humidity chamber was 168 hours in between FTIR characterisations. In the oven and UV weathering tester, the ageing period was 50 hours in between FTIR characterisation. Figures 41, 42 and 43 show the experimental set ups under the different ageing conditions.

Figure 41: Experimental set up for accelerated temperature ageing

Figure 42: Experimental set up for ultra-violet light ageing: note the infra red card is facing away from the UV lamps.

Figure 43: Experimental set up for accelerated humidity ageing

3.4.6. Paint formulation

To evaluate the most optimum phosphogypsum to water ratio for the slurry, 300 ml of water was added to different masses of phosphogypsum in six 500 cm^3 polyethylene bottles with screw caps as shown in Table 20.

Table 20: Mass of phosphogypsum added to 300 ml of water for different solids volume fraction

Sample	Phosphogypsum, kg	Solids volume fraction, Φ
1	0.0773	0.1
2	0.174	0.2
3	0.298	0.3
4	0.464	0.4
5	0.696	0.5
6	1.044	0.6

The densities used in evaluating volume fraction were 2.32 $kg.m^{-3}$ for phosphogypsum and 1 $kg.m^{-3}$ for water. The samples were adequately mixed using a high speed mixer. The shear rate dependant viscosity of the samples was then studied on the rheometer.

Once the most desirable slurry concentration was chosen further rheology studies were performed, this time studying the variation of rheological properties of the paint with the dispersant concentration. 298 g of phosphogypsum was added into another set of 6 polyethylene bottles. Mimosa WS dispersant was added as follows; 0 g (0 wt.%), 1.5 g (0.5 wt.%), 3 g (1.5 wt.%), 4.5 g (1.5 wt.%) and 6 g (4.5 wt.%). 300 ml of distilled water was added into each bottle, mixed well with a high speed mixer. The samples were then studied for the shear rate dependant rheology.

3.4.7. Spray surfaces

For preparation of a standard soil surface for spraying with the simple insecticide formulations; the soil samples were mixed with warm water to form a paste. This paste was then poured into a high density polyethylene (HDPE) substrate holder (diameter of 11.6 cm, height of 2 cm and diameter of 15.5 cm, height of 2.5 cm). The mud substrates were then oven dried at 90 °C for 24 hours and allowed to sufficiently cool to room temperature before spraying. A set of the dried soil plaques were coated with fresh cattle manure; this represented the microbiologically active surfaces typically found in rural mud huts.

3.4.8. Insecticide formulations for bioassaying and ageing

At least one insecticide from each class was chosen for bioassay testing. These were alphacypermethrin for pyrethroids, bendiocarb and propoxur (carbamates) and DDT (organochlorine). Organophosphates were excluded because they were considered too unstable. Insecticides were dissolved in just enough acetone to completely dissolve the insecticides. Since the insecticides are all water insoluble, the insecticides precipitated on the gypsum when the solution was dispersed in water slurry of gypsum. The dispersant concentration was optimized on the basis of the viscosity of the 50 wt.% phosphogypsum slurry (solids volume fraction of 0.3). Furthermore, carbamate insecticides dissolved in acetone were dispersed into a standard acrylic paint binder. Experiments conducted at a later stage established that more

stable dispersions were obtained by a slight modification of the procedure. In this method the insecticide was dissolved in just sufficient acetone to allow complete wetting of the dry phosphogypsum powder. This mixture was then slurried into water using vigorous stirring and then adding the same amount of dispersant. This second set of dispersion formulations was sprayed onto the cattle manure-coated mud plaques. The following three types of "paints" formulations were prepared and tested:

- **Gypsum "white wash"**. Insecticide-coated phosphogypsum slurry (50 wt.% solids with 1 wt.% dispersant) as is. The final insecticide content based on dry solids was 0.37 wt.% (alphacypermethrin) and 9 wt.% (bendiocarb). The dispersions were sprayed on soil surfaces and cattle manure coated surfaces.
- **Gypsum "white wash" with minimal acrylic binder**. Phospogypsum (45 wt.%), paint binder (4.5 wt.%), dispersant (0.9 wt.%), water (45 wt.%) plus bendiocarb (4.5 wt.%). The final insecticide content based on dry solids was 8%. This "paint" was sprayed on standard soil surfaces.
- **Acrylic paint**. Insecticide (10 wt.% solution in acetone) was dispersed directly into the acrylic paint binder (56 wt.% solids). The final insecticide content was 15.2 wt.% based on dry solids. Carbamate insecticides (bendiocarb or propoxur), dissolved in a minimal amount acetone, were mixed into the neat acrylic emulsion. This "paint" was sprayed on standard soil surfaces.

There were two sets of investigations to be made. The first investigation was to study the time dependant effectiveness of the above formulations sprayed on mud surfaces comparable to surfaces in rural dwellings. The second investigation was to study the time dependant effectiveness of the above formulations on fresh cattle manure coated surfaces.

Surface spraying. Each "paint" was sprayed on both mud and cattle manure plaques at concentrations equivalent to twice the mean WHO recommendations (Table 21) (Najera & Zaim, 2001). DDT was applied as a ca. 10% acetone solution at twice the WHO recommended on mud and cattle manure plaques. However the DDT performed poorly on mud plaques. This was a baffling observation, however DDT seems to bind strongly onto clay particles on application, then deactivated and released slowly from the adsorbing surfaces (Peterson,

Adams & Cutkomp, 1970). The "see-saw" activity behaviour of DDT sprayed on mud surfaces was also confirmed by the Limpopo health authorities. It was therefore decided to spray the DDT at four times the WHO concentration in order to obtain 100% mortality on the neat bioassay tests. For each formulation, triplicate surfaces were treated and the results were used to calculate the average and standard deviation. The treated surfaces were then subjected to accelerated ageing in a humidity cabinet set at 90% relative humidity and 40 °C. WHO laboratory-scale bioassays methods were used to track the residual efficacy against mosquitoes. Appendix A shows a summary of paint formulation compositions and amounts sprayed on the respective test surfaces.

Table 21: WHO recommended dosage range

Insecticide class	**Insecticide**	**IRS dosage, g.m^{-2}**	**Duration of effectiveness, months**
Organochlorine	DDT	1 - 2	>6
Organophosphate	Malathion	2	2-3
	Fenitrothion	2	3-6
	Pirimiphos-methyl	1 - 2	2-3
Carbamates	Bendiocarb	0.1 – 0.4	2-6
	Propoxur	1 - 2	3-6
Pyrethroids	Alphacypermethrin	0.02 – 0.03	4-6
	Bifenthrin	0.025 – 0.05	3-6
	Betacyfluthrin	0.02 – 0.05	3-6
	Deltamethrin	0.02 – 0.025	3-6
	Lambdacyhalothrin	0.1 – 0.3	3-6
	Etofenprox	0.02 – 0.03	3-6

Bioassay testing. In bioassay testing; 25 female *An. arabiensis* mosquitoes (KGB colony housed at the National Institute for Communicable Diseases, Johannesburg), 3-5 days old and non blood fed, were exposed to the samples of the treated and untreated substrates surfaces each respectively for 30 minutes (Figure 44). Percentage knockdown was recorded after 1 hour from the start of exposure and mortality rates were recorded after 24 hours from

the time of initial exposure (WHO, 2006). In between bio assay testing, substrate surfaces were kept in a humidity chamber at a temperature of 40 °C and a relative humidity of 90%.

Figure 44: Experimental setup for bioassay testing

3.4.9. Insecticide formulations for FTIR characterisation

The gypsum "white wash" was also sprayed on microscope slides. Another formulation containing 50% insecticide by weight were prepared and used to obtain a reference interferogram. It was of interest to determine the distribution of insecticide on the paint surface.

4. Results

4.1. Mineral powder characterisation

4.1.1. Element analysis

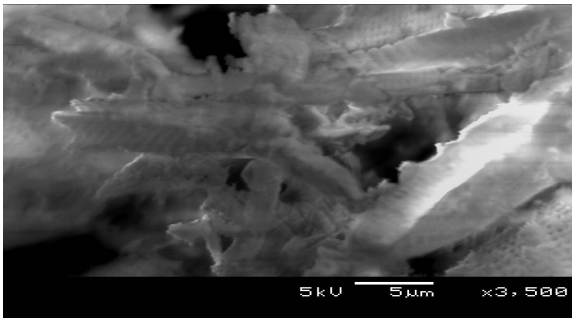
Results of X-ray fluorescence are shown in Table 22.

Table 22: XRF analysis results of major trace elements

	Attapulgite	Bentonite	Calcined diatomite	Organoclay	Raw diatomite	Phosphogypsum
SiO_2	68.77	62.98	89.42	67.65	86.45	0.70
TiO_2	0.46	0.37	0.31	0.11	0.35	0.03
Al_2O_3	11.55	20.82	7.86	21.47	7.11	0.37
Fe_2O_3	4.80	7.29	0.89	4.85	0.98	0.12
MnO	0.07	0.05	0.01	<0.01	0.01	<0.01
MgO	12.17	5.07	0.12	2.77	0.36	0.72
CaO	1.26	2.12	0.10	0.84	0.53	44.64
Na_2O	<0.01	0.12	0.15	0.81	0.47	0.39
K_2O	0.64	0.62	0.74	0.08	0.87	<0.01
P_2O_5	0.01	0.05	0.02	0.44	0.01	0.70
P_2O_5	0.04	<0.01	0.01	<0.01	0.01	<0.01
Cr_2O_3	0.04	<0.01	0.01	<0.01	0.01	<0.01
V_2O_5	0.02	0.03	0.02	0.02	0.02	<0.01
ZrO_2	0.06	0.33	0.28	0.25	2.25	49.82
SO_3	<0.01	<0.01	<0.01	0.63	0.50	<0.01
Cl	<0.01	<0.01	<0.01	<0.01	<0.01	0.53
SrO	<0.01	<0.01	<0.01	<0.01	<0.01	1.39
F	68.77	62.98	89.42	67.65	86.45	0.70
Total	95.40	93.3	101.00	85.40	97.10	96.10

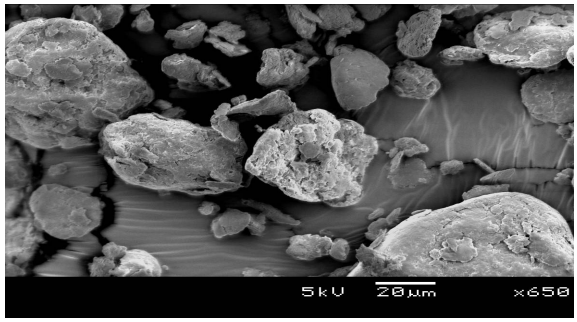
4.1.2. Scanning electron microscopy

The observed surface morphology for each mineral powder is summarised in Figure 45.



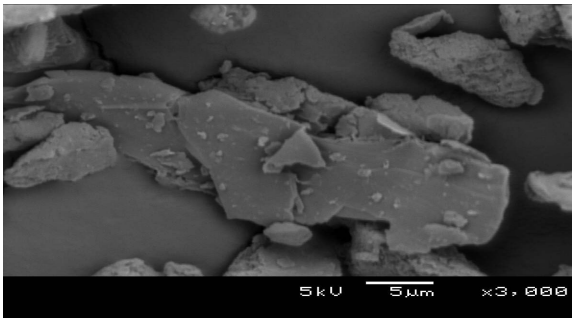
Attapulgite

Leafy platelets with perforated surface.



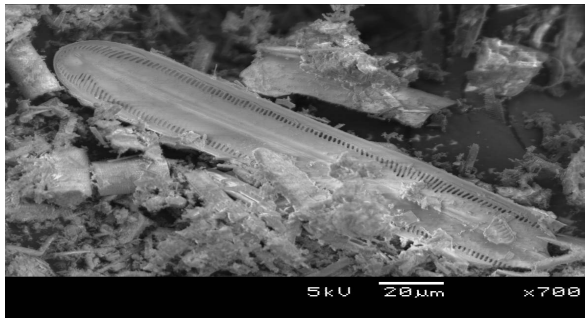
Organoclay

Agglomerated particles.



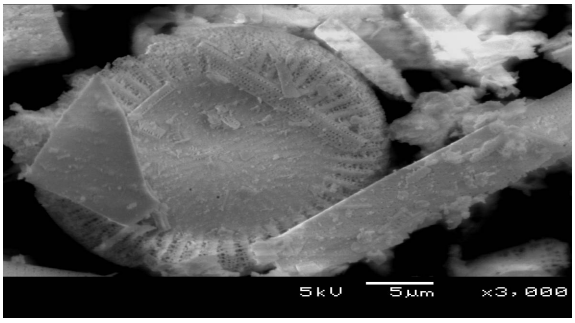
Bentonite calcium

Platy particles



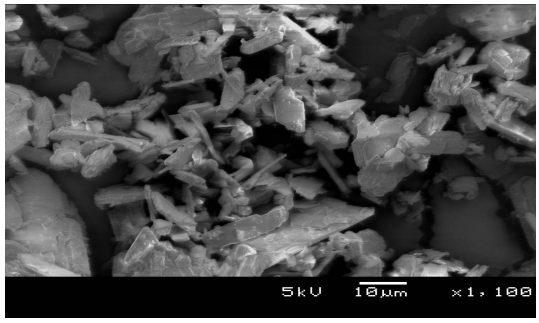
Raw diatomite

Particles vastly mixed in shapes and sizes. Larger leafy platelets with perforated surfaces are observed.



Calcined diatomite

Platy particles of mixed shapes and sizes.



Phosphogypsum

Leafy non-agglomerated platelets.

Figure 45: Scanning electron microscope images of the candidate mineral powders

Appendix B shows the SEM images of all the mineral powders considered in this investigation.

Attapulgite, calcined diatomite and raw diatomite contained micro pores that could be used as protective covers. The micro pores might however retard the slow release of the insecticide (Lagaly, 2001). Phosphogypsum and bentonite calcium can also provide sufficient surface to adsorb a high amount of insecticide.

4.1.3. Surface pH

The results (Figure 46) indicated that the pH imparted on distilled water by mineral powders range from pH 4.2 for phosphogypsum to pH 9.6 for organoclay. The most acidic mineral powder was phosphogypsum at pH 4.2. This was expected since phosphogypsum is a by-product of the phosphoric acid manufacturing process.

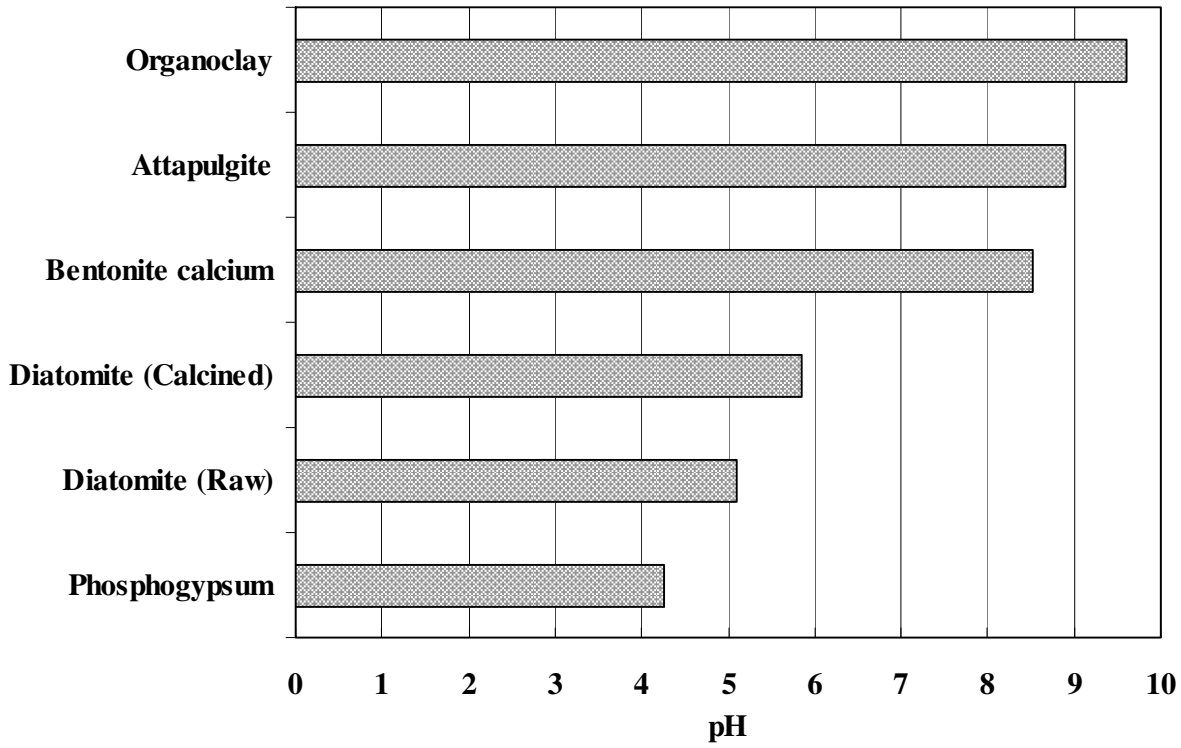


Figure 46: pH imparted on distilled water by shortlisted mineral powders

This pH is optimum as the pH mediated hydrolysis of insecticides is lowest at this pH. Calcined diatomite and raw diatomite also showed acidic pHs. The next promising mineral powder, attapulgite, showed a very basic pH, this may accelerate pH mediated hydrolysis. It was decided to shelve attapulgite for this part of the project.

4.1.4. Particle size analysis

Figures 47, 48, 49, 50, 51 and 52 show the particle size distribution of the mineral powders under consideration. The results indicate significant particle agglomeration. This is seen particularly for the phosphogypsum results; the highest peak indicates particle agglomeration.

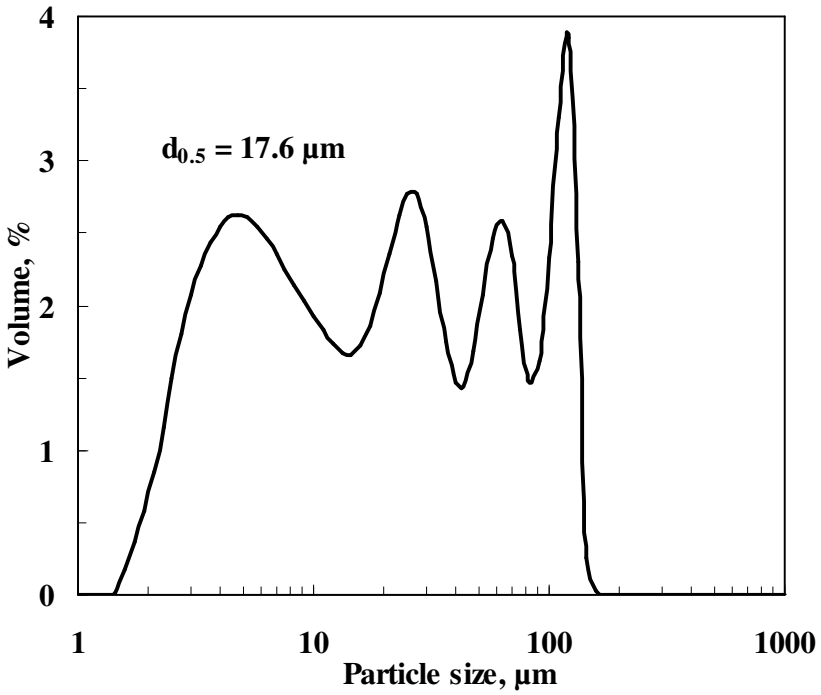


Figure 47: Particle size distribution of phosphogypsum

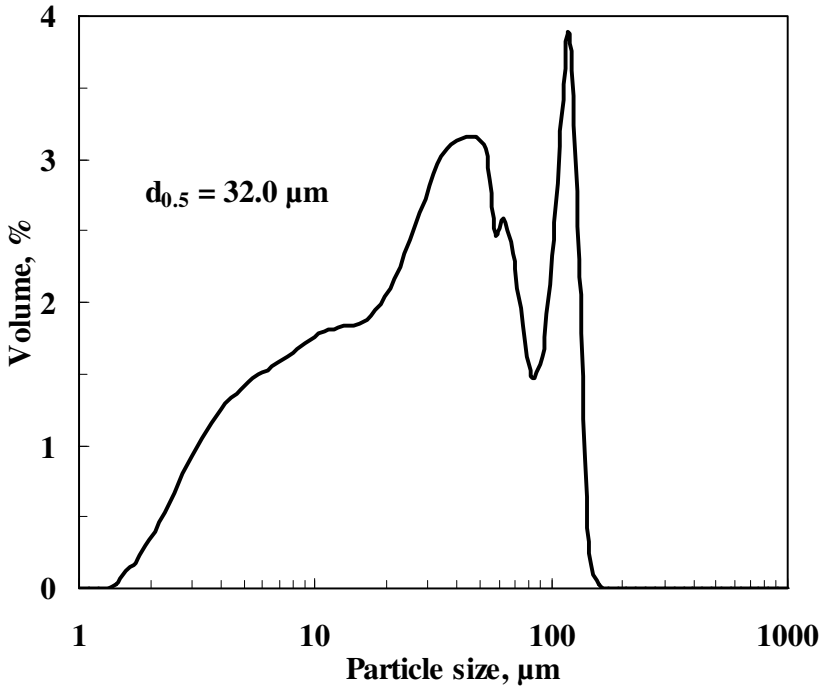


Figure 48: Particle size distribution of attapulgite

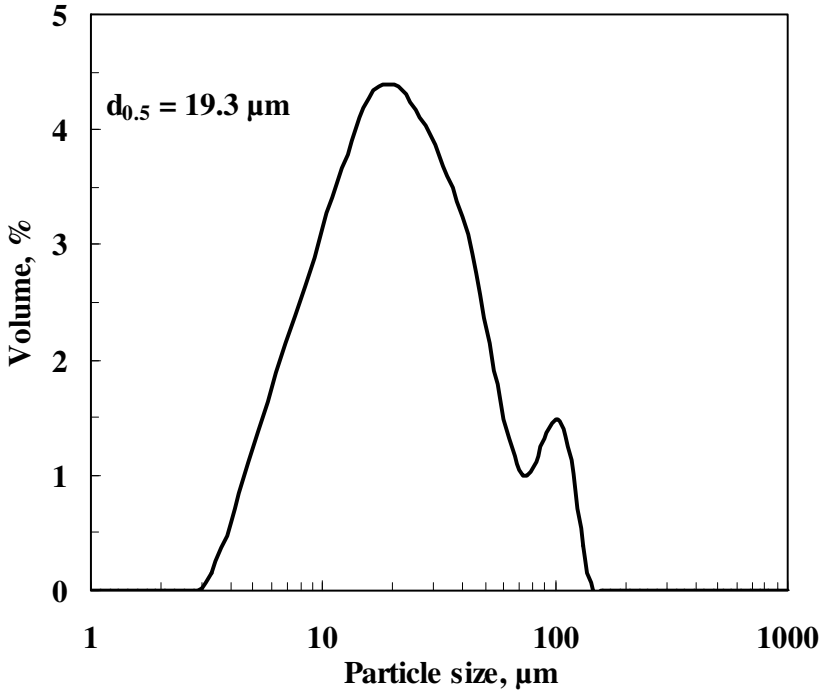


Figure 49: Particle size distribution of diatomite raw

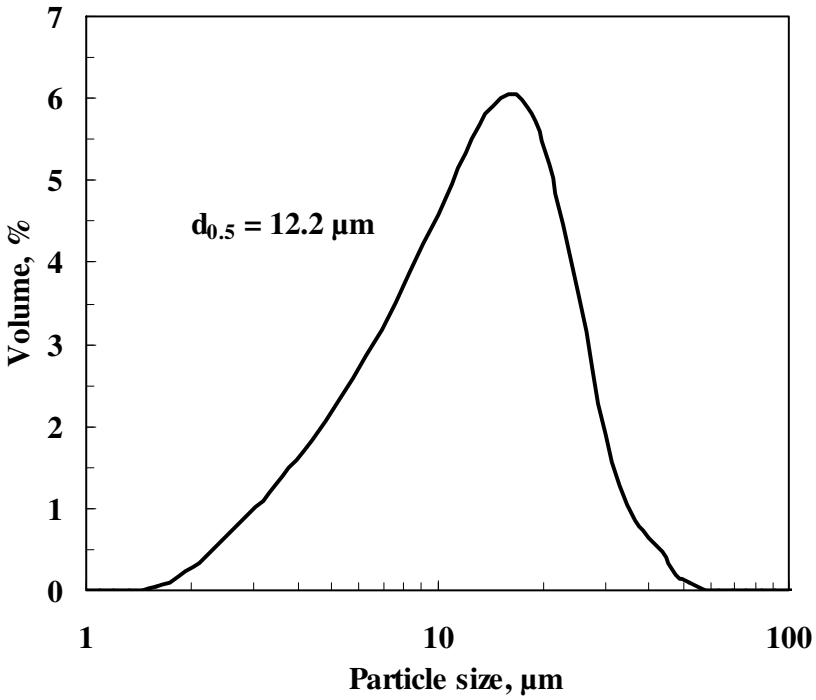


Figure 50: Particle size distribution of organoclay

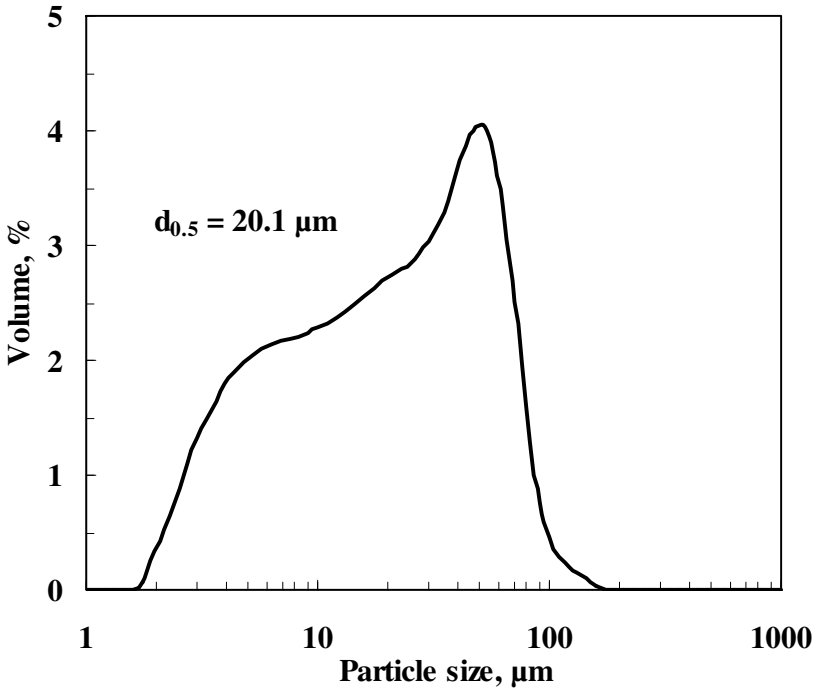


Figure 51: Particle size distribution of bentonite calcium

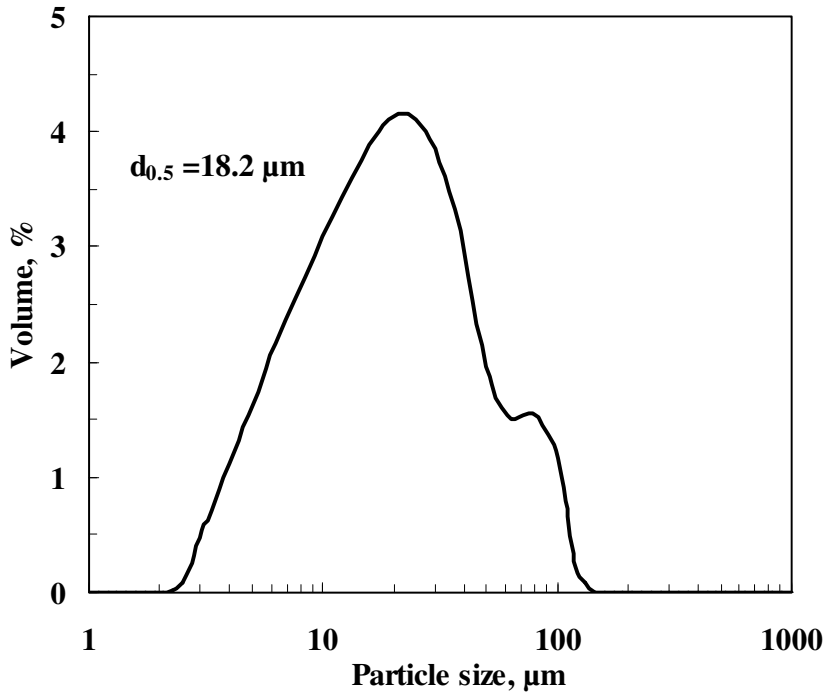


Figure 52: Particle size distribution of calcined diatomite

4.2. Soil sample characterisation

Tables 23, 24, 25 and 26 show results for the standard soil analysis and X-ray fluorescence analysis. Appendix C shows full X-ray fluorescence analysis results for the different soil types.

Table 23: Standard soil analysis results (A)

Sample	Coarse sand, %	Silt, %	Clay, %	Total, %
Thomo	59.8	25	10	95.1
Makoya	75.1	10	10	94
NAM 1	14	17.5	62.5	94

Table 24: Standard soil analysis results (B)

Sample	C,%	NH_4, $mg.kg^{-1}$	NO_3, $mg.kg^{-1}$
Thomo	1.15	1.51	1.01
Makoya	1.07	1.34	7.78
NAM 1	0.61	1.4	25.09

Table 25: Standard soil analysis results (C)

Sample	pH	P Bray I, mg.kg^{-1}	Ca, mg.kg^{-1}	K, mg.kg^{-1}	Mg, mg.kg^{-1}	Na, mg.kg^{-1}
Thomo	7.1	2.7	1687	454	342	103
Makoya	6.5	1.1	1585	122	359	126
NAM 1	5.1	0.3	269	172	349	56

Table 26: X-ray fluorescence analysis results

Sample	MAKOYA	NAM 01	THOMO
SiO_2	70.99	48.35	69.62
TiO_2	0.62	0.98	0.93
Al_2O_3	13.63	26.03	12.05
Fe_2O_3	2.89	12.87	5.75
MnO	0.05	0.09	0.13
MgO	0.69	<0.01	0.87
CaO	1.87	0.07	1.62
Na_2O	4.40	<0.01	2.94
K_2O	1.49	0.16	1.67
Total	96.74	88.84	95.78

4.3. Characterisation of insecticides

4.3.1. Thermal analysis of insecticides

Thermal analysis results are presented in Table 27. All the insecticides show evaporation temperatures above 150 °C. For the insecticides that exhibit oxidation induction, the temperature at which this occurs is at least above 180 °C. This implies that these insecticides are generally stable to heat exposure in the field of application.

Table 27: Dynamic temperature analyzer results

Pesticides			Melting		Evaporation		
Sample	Amount, mg	Theoretical mp, °C	*Tm onset, °C*	*ΔH, J.g^{-1}*	*Onset, °C*	*ΔH, J.g^{-1}*	*OIT onset, °C*
DDT	5.02	109	52	-10.28	225	-492.03	N/A
			79	-26.66			
Bifenthrin	5.02	68-70	66	84.73	249	-258.96	N/A
Betacyfluthrin	5.01	93	69	-14.94	N/A	N/A	310
			87	-39.62			
Bendiocarb	5.02	128	97	-3.24	179	533.86	N/A
			127	-143.67			
Alphacypermethrin	5.01	78-81	82	-113.45	N/A	N/A	312
Propoxur	5.03	85	85	-136.60	194	518.89	N/A
Etofenprox	5.03	35-38	36	-104.59	N/A	N/A	180
Deltamethrin	5.01	98-101	94	-109.06	N/A	N/A	280
Lamdacyhalothrin	5.02	49	48	-76.88	254	519.92	N/A

4.3.2. Temperature, ultra-violet light and humidity ageing

The results obtained for thermal ageing, UV degradation and resistance to high humidity are summarized in Table 28. As a group, the pyrethroids showed the greatest resistance to degradation whether mediated by elevated temperature, UV light exposure or high humidity.

Selected members of this class survived more than 250 h oven ageing at 80 °C, up to 150 h 0.67 W.m^{-2} QUV exposure at 63 °C and all were still present after 168 h high humidity ageing at 40 °C and 90% ℜH. In contrast, DDT and the other classes were either completely degraded or lost after only 50 h of exposure to high heat or UV light. The rest of FTIR interferograms not presented in this section are shown in Appendix D.

Table 28: Summary of insecticide degradation under high temperature, ultra-violet light and high humidity

Insecticide class	Insecticide	Heat ageing[1]	UV degradation[2]	Humidity[3]
Organochlorine	DDT	<50 h lost	<100 h lost	lost
Organophosphate	malathion	<50 h lost	<50 h lost	-
	fenitrothion	<50 h lost	<50 h lost	-
	pirimiphos-methyl	<50 h lost	<50 h lost	lost
Carbamates	bendiocarb	<50 h lost	<50 h lost	lost
	propoxur	<50 h lost	<50 h lost	lost
Pyrethroids	alphacypermethrin	>250 h present	>250 h present	present
	bifenthrin	<50 h lost	<150 h lost	present
	cyfluthrin	>250 h present	<150 h lost	present
	deltamethrin	>250 h present	<150 h lost	present
	lambdacyhalothrin	<150 h lost	<150 h lost	present
	etofenprox	<200 h lost	<150 h lost	present

[1] Oven ageing at 80 °C in 50 h intervals; [2]Dry cycle UV degradation in a QUV at 63 °C and 0.67 $W.m^{-2}$ in 50 h intervals; [3]168 h Humidity ageing at 40 °C and 90% ℜH

4.3.2.1. Temperature ageing

The FTIR interferograms (Figure 53, 54, 55, and 56) showed that the pyrethroid insecticides were still present after 250 h exposure to a temperature of 80 °C.

However, a reduction and broadening in the characteristic absorption peaks is apparent after about 150 h suggesting partial volatilisation and possibly isomerisation of the parent compound. No DDT, organophosphate or carbamate was detectable by FTIR on the sample substrates after 50 h at this temperature.

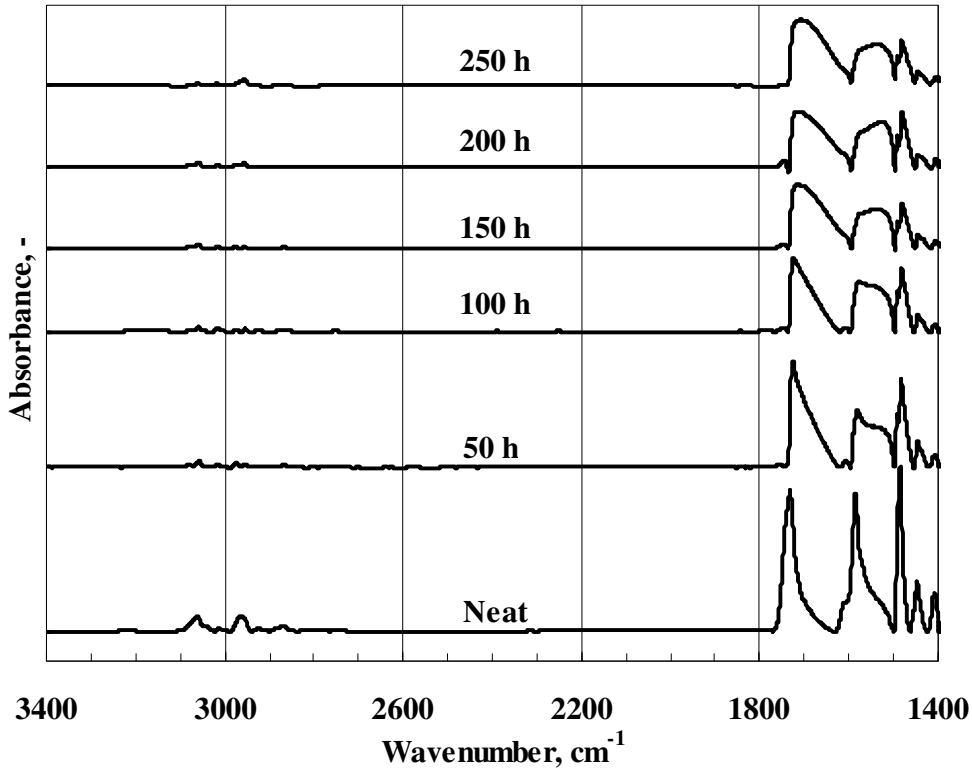


Figure 53: IR interferograms of alphacypermethrin in oven ageing at 80 °C

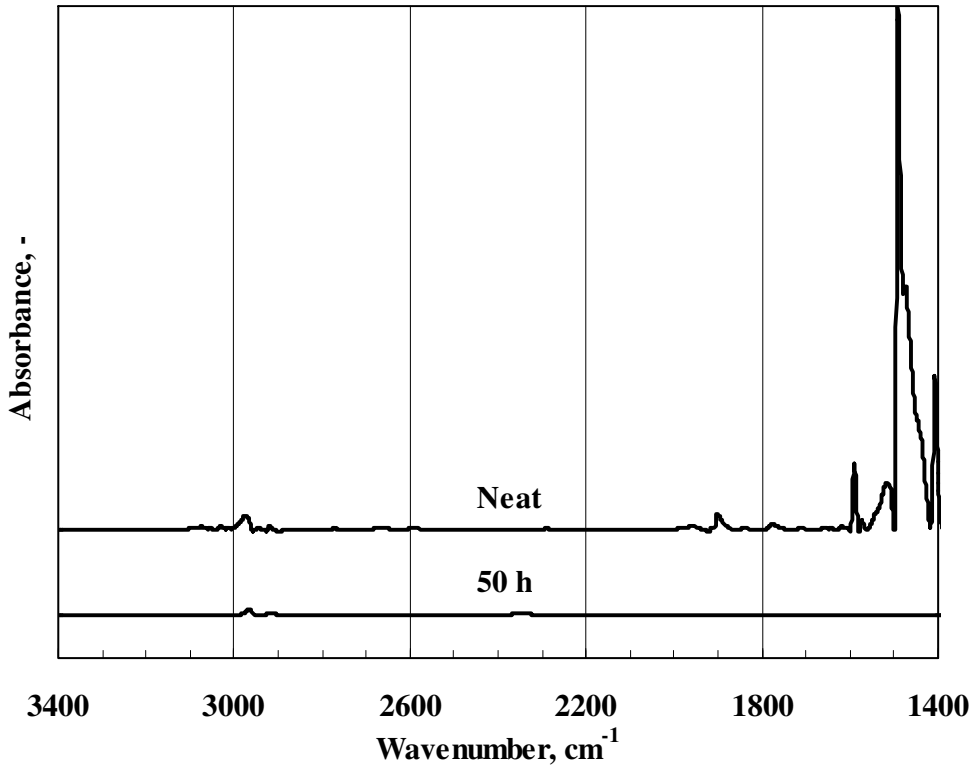


Figure 54: IR interferograms of DDT in oven ageing at 80 °C

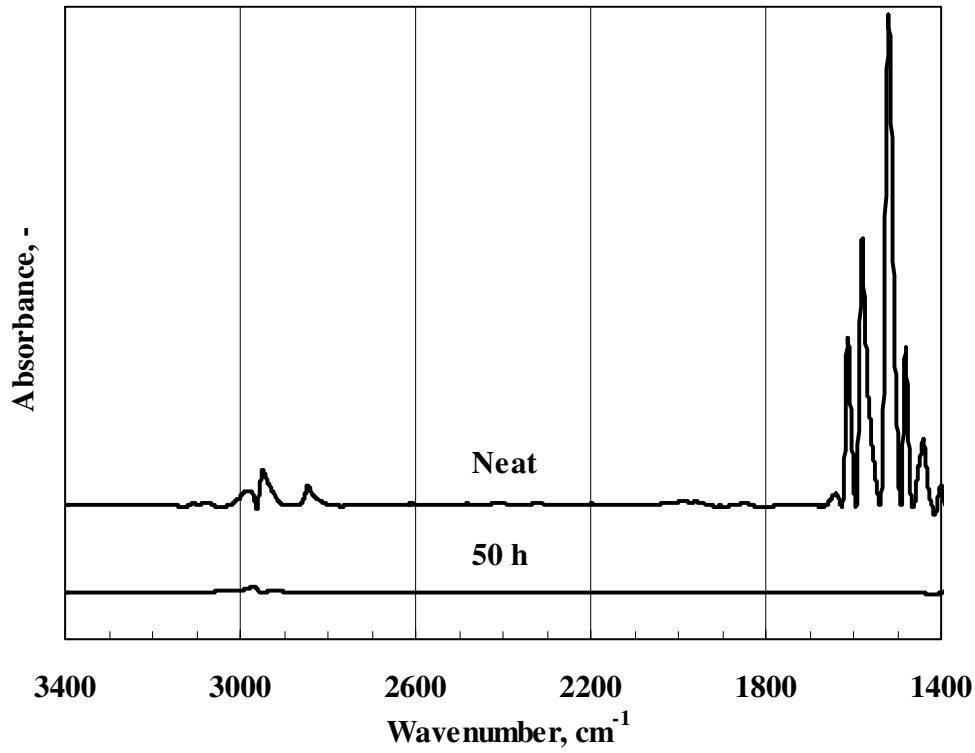


Figure 55: IR interferograms of fenitrothion in oven ageing at 80 °C

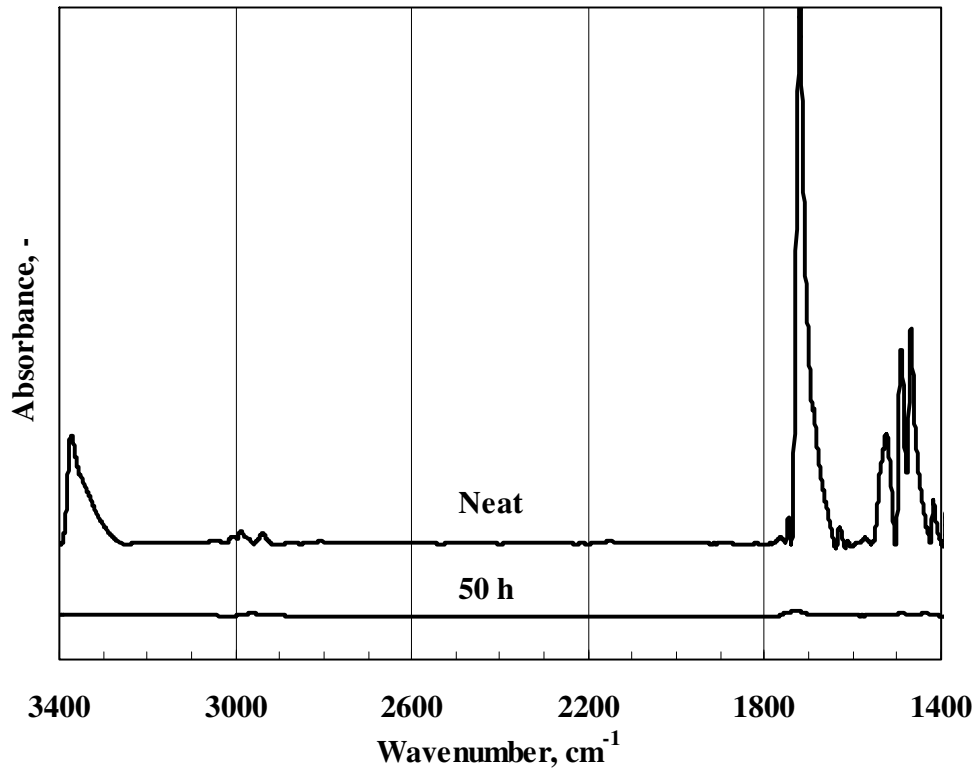


Figure 56: IR interferograms of bendiocarb in oven ageing

4.3.2.2. Ultra-violet light ageing

The FTIR interferograms showed that pyrethroids were relatively stable to ultra-violet light compared to other classes of insecticides. Pyrethroids (Figure 57) degraded gradually with no observable metabolite and were still detectable after 250 h. Another key observation is the apparent broadening of the absorption band particularly at wave number ca. 1724 cm^{-1} at 50 h and more pronounced to include the absorption band at ca. 1579 cm^{-1} at 100 h ageing. The observed broadening of the adjacent bands may have been due to formation of carboxylic and aromatic by-products with slightly shifted absorption bands that superposed with those of carbonyl and aromatic functional groups of the parent material (Segal-Rosenheimer & Dubowski, 2008). Deltamethrin UV (appendix D) ageing showed products of degradation after 50 h of ageing at the ca. 2969 cm^{-1} absorption band (assigned to aliphatic alkane functional groups) and ca. 2355 cm^{-1} absorption band (assigned ─CN functional group).

DDT (Figure 58) degraded gradually with the characteristic ─C=C─ absorption aromatic absorption bands diminishing in amplitude. An unidentified functional group was detected at ca. 2358 cm^{-1} gradually increasing with the decrease in the aromatic ─C=C─ stretching bands. The stepwise elimination of the ─CCl could not be confirmed as the absorption bands (>1400 cm^{-1}) were out of the spectral detection range. The increased intensity of the absorption band at ca. 2968 cm^{-1} suggests that the aromatic rings were oxidized to phenols or quinones.

Fenitrothion absorption peaks diminished significantly in the first 50 h of ageing (Figure 59). Phenols and possibly HO─P═O groups were detected as products of degradation at an overlapping absorption band (broad) of ca. 2970 cm^{-1}. The phosphoric groups as products of degradation were confirmed by the absorption band observed at ca. 2343 cm^{-1}. Another emergence of a broad peak at ca. 2970 cm^{-1} and disappearance of the aromatic absorption bands between 1500 and 1600 cm^{-1} strongly suggests that some of the by products are phenols.

Bendiocarb (Figure 60) undergoes degradation to produce ═CH functional groups at ca. 2960 cm^{-1} absorption band and possibly ─CN functional groups at ca. 2360 cm^{-1} absorption band.

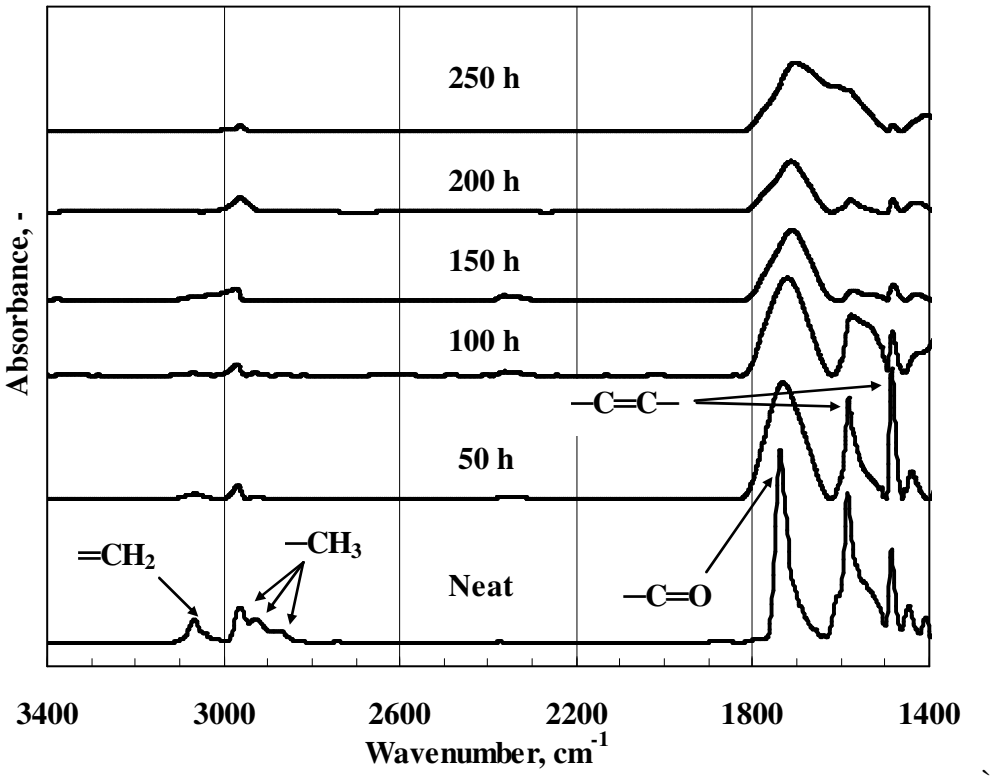


Figure 57: IR interferograms of alphacypermethrin in UV ageing

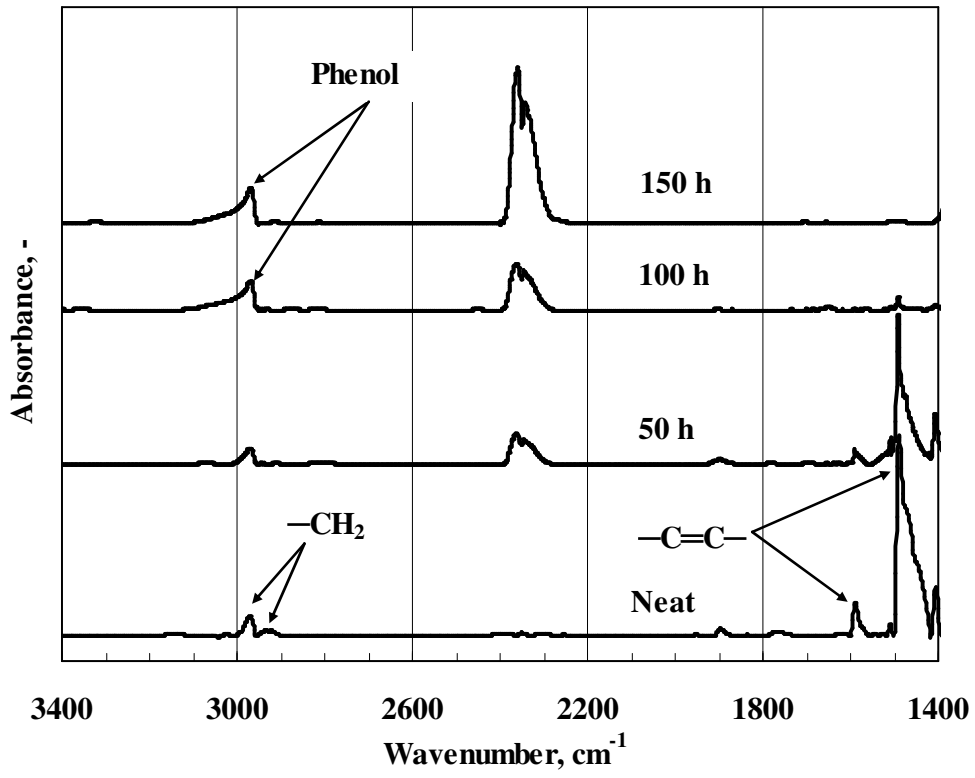


Figure 58: IR interferograms of DDT in UV ageing

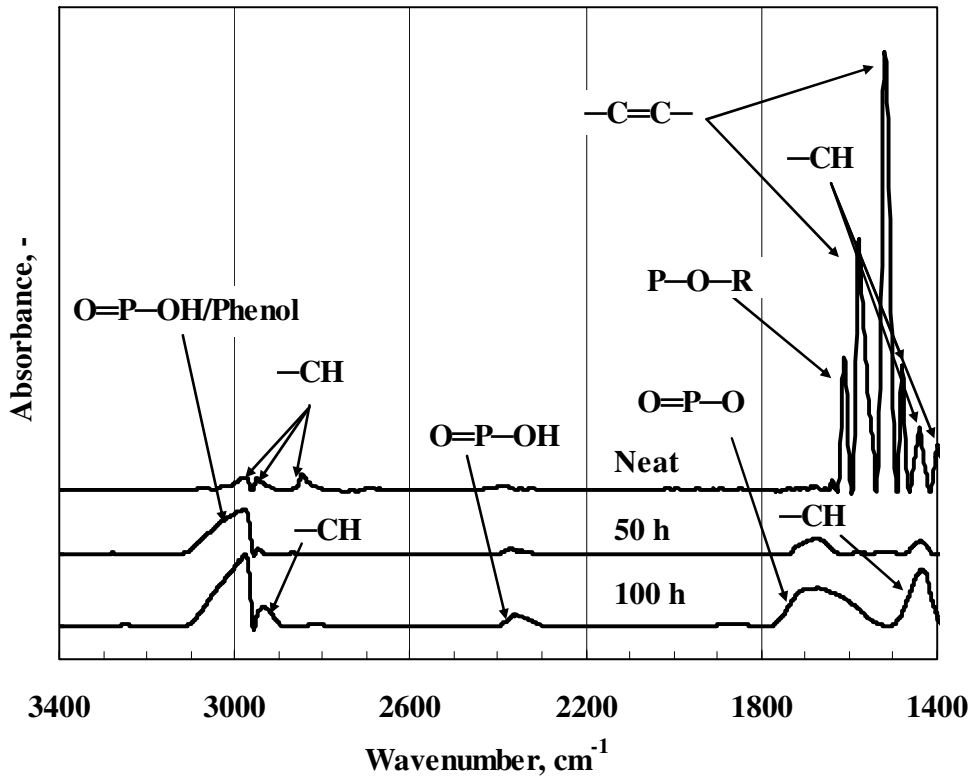


Figure 59: IR interferograms of fenitrothion in UV ageing

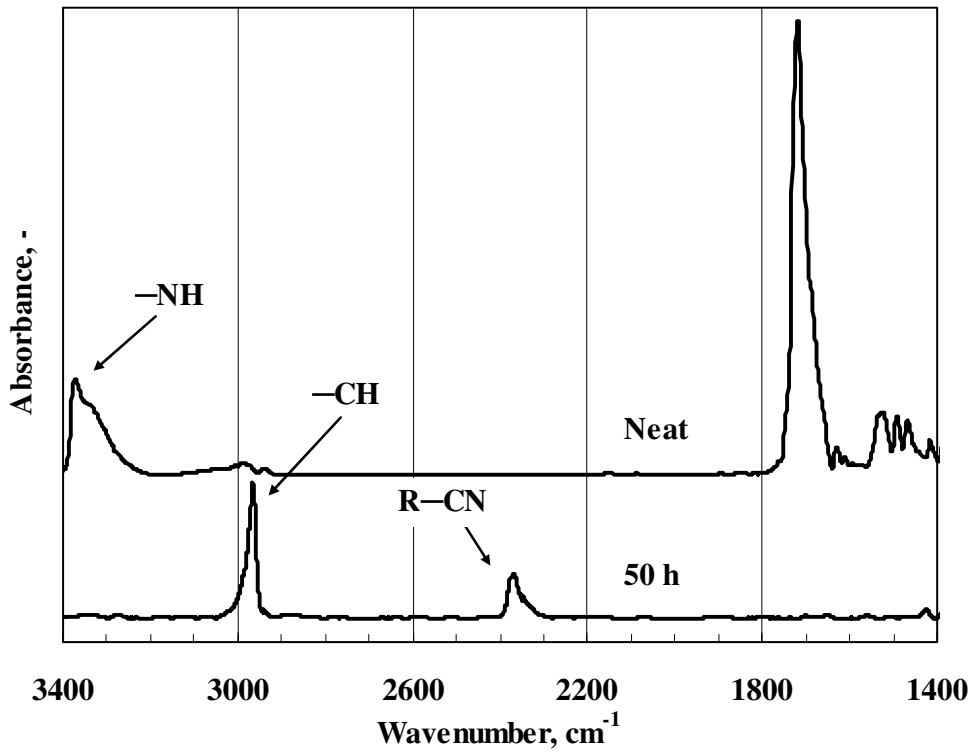


Figure 60: IR interferograms of bendiocarb in UV ageing

4.3.2.3. Humidity ageing

The samples were exposed to a relative humidity of 90% and 60 ^{o}C for 168 h. FTIR interferograms showed that pyrethroids (Figure 61) were stable to high humidity whilst the DDT (Figure 62), carbamates (Figure 63) and organophosphates (Figure 64) were undetectable after the ageing period.

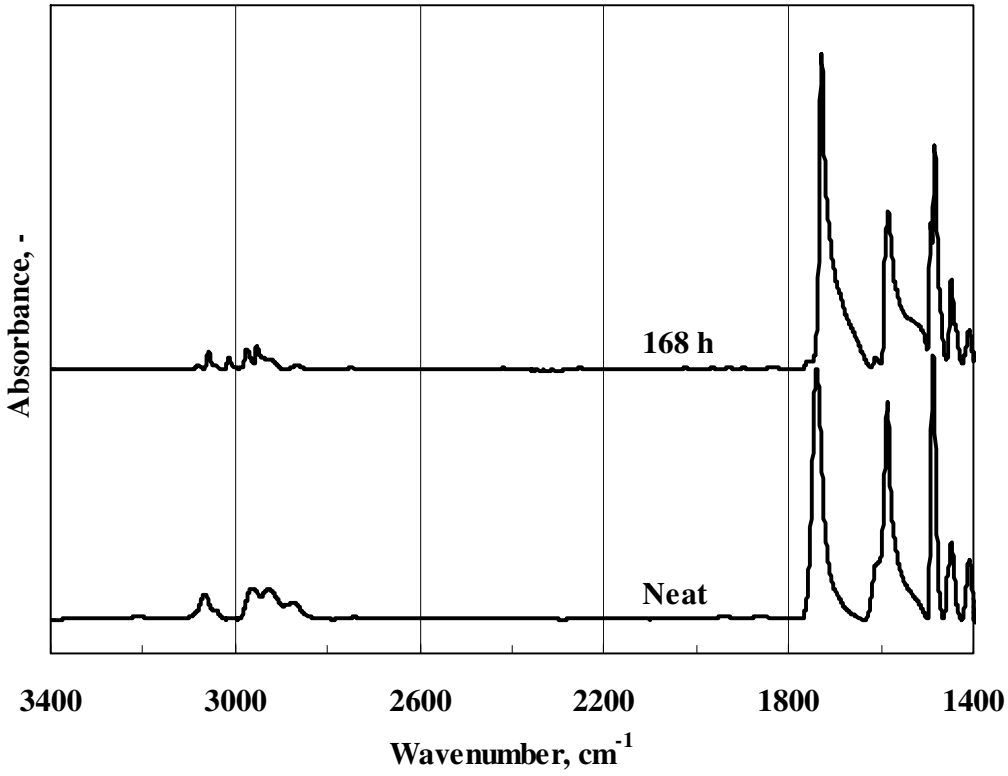


Figure 61: IR interferograms of alphacypermethrin in humidity chamber at an ℜH 90%, 60 °C

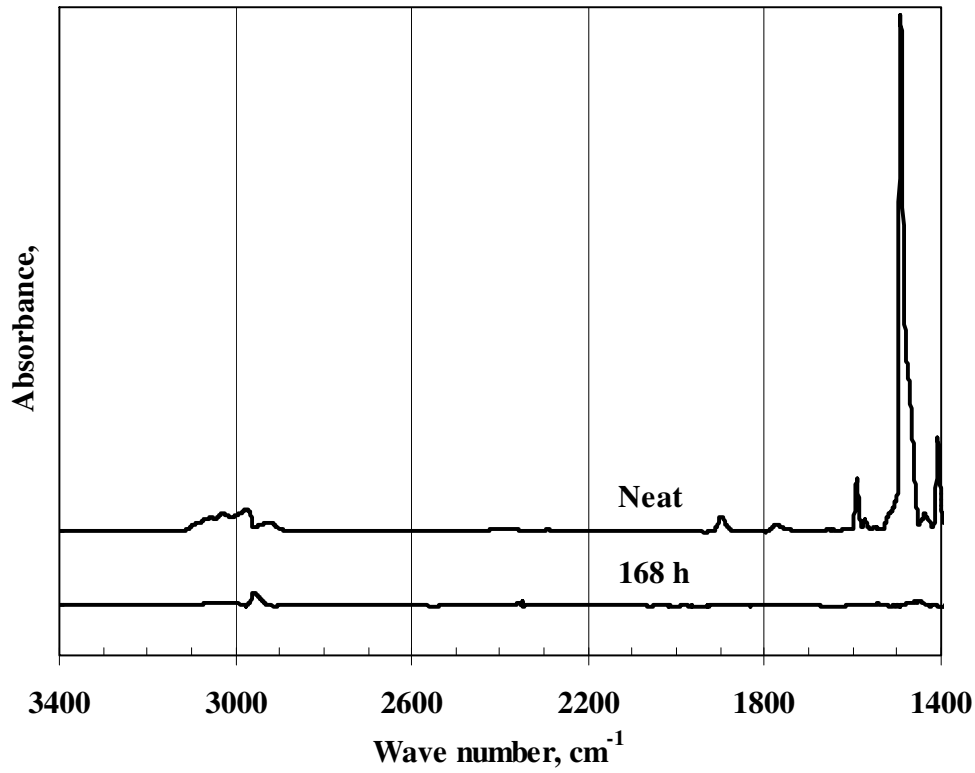


Figure 62: IR interferograms of DDT in humidity chamber at an ℜH 90%, 60 °C

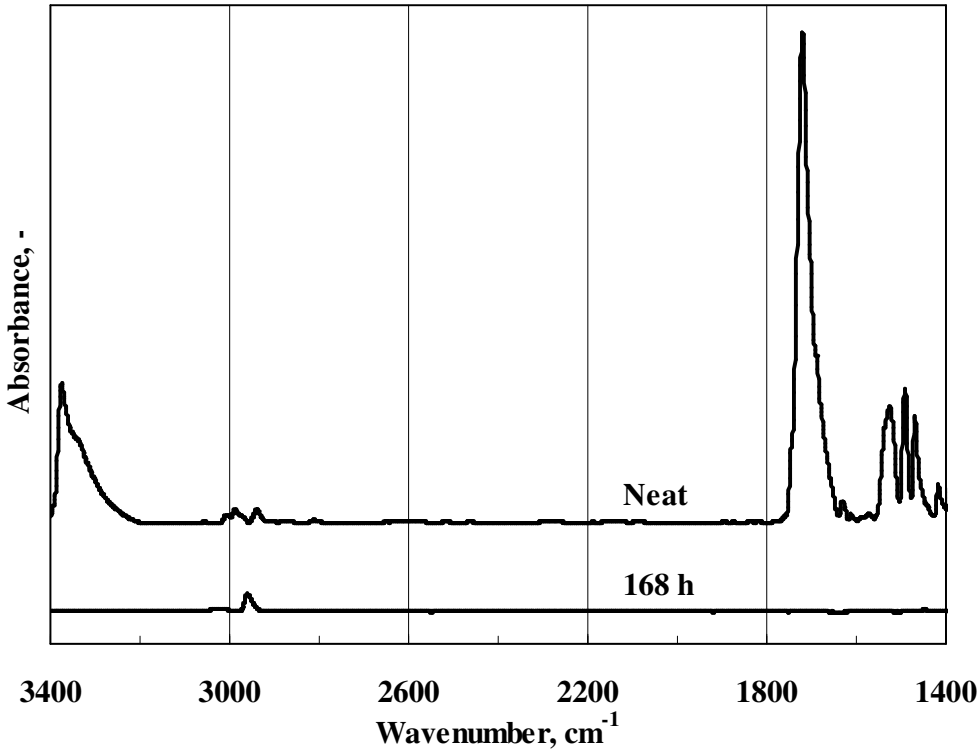


Figure 63: IR interferograms of bendiocarb in humidity chamber at an ℜH 90%, 60 °C

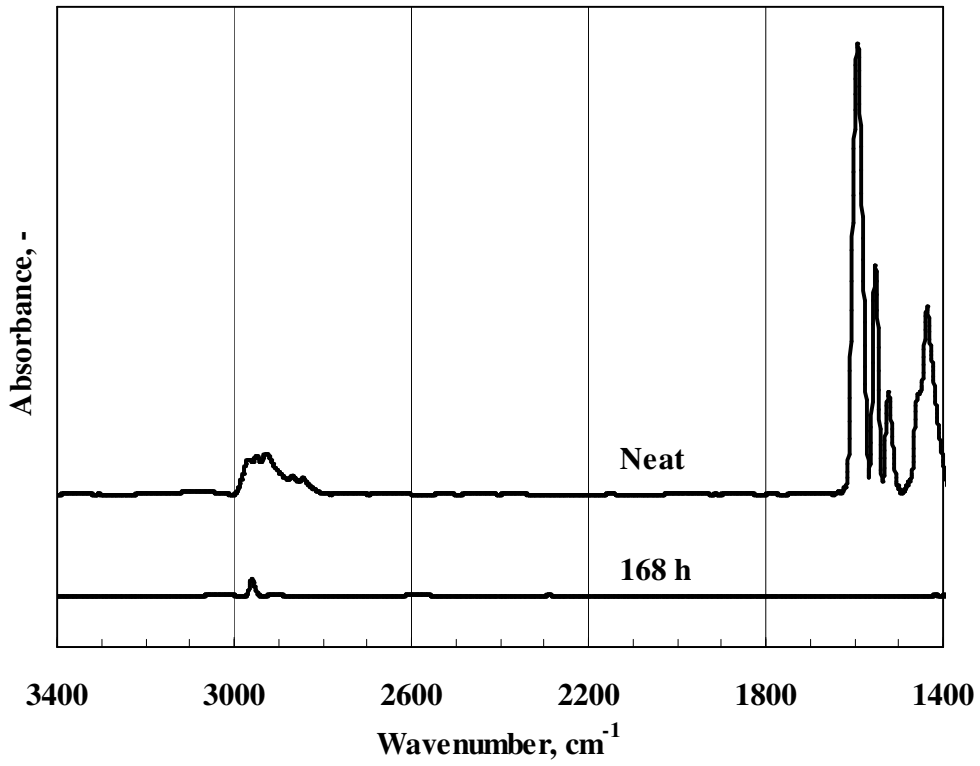


Figure 64: IR interferograms of pirimiphos-methyl in humidity chamber at an ℜH 90%, 60 °C

4.4. Insecticide on mineral powder ageing

Figure 4 presents the effect of the mineral fillers on the persistence of bendiocarb (Figure 65) and alphacypermethrin (Figure 66) solutions in acetone containing 1% water. Adding catalytic amounts of sodium ethoxide caused, as expected, rapid hydrolysis of both insecticides. Bendiocarb was quite stable in this medium. No change in concentration was detected in this medium when aged at room temperature (Control sample).

In contrast, the alphacypermethrin Control sample degraded over time. The concentration of both insecticides decreased rapidly in the presence of the organoclay mineral powder. This does not necessarily mean that it was degraded in the presence of this substance. Co-intercalation of the insecticides into the clay galleries can also remove them from the liquid phase. Adding phosphogypsum appears to have improved the hydrolytic stability of alphacypermethrin whereas the opposite appears to be true for bendiocarb.

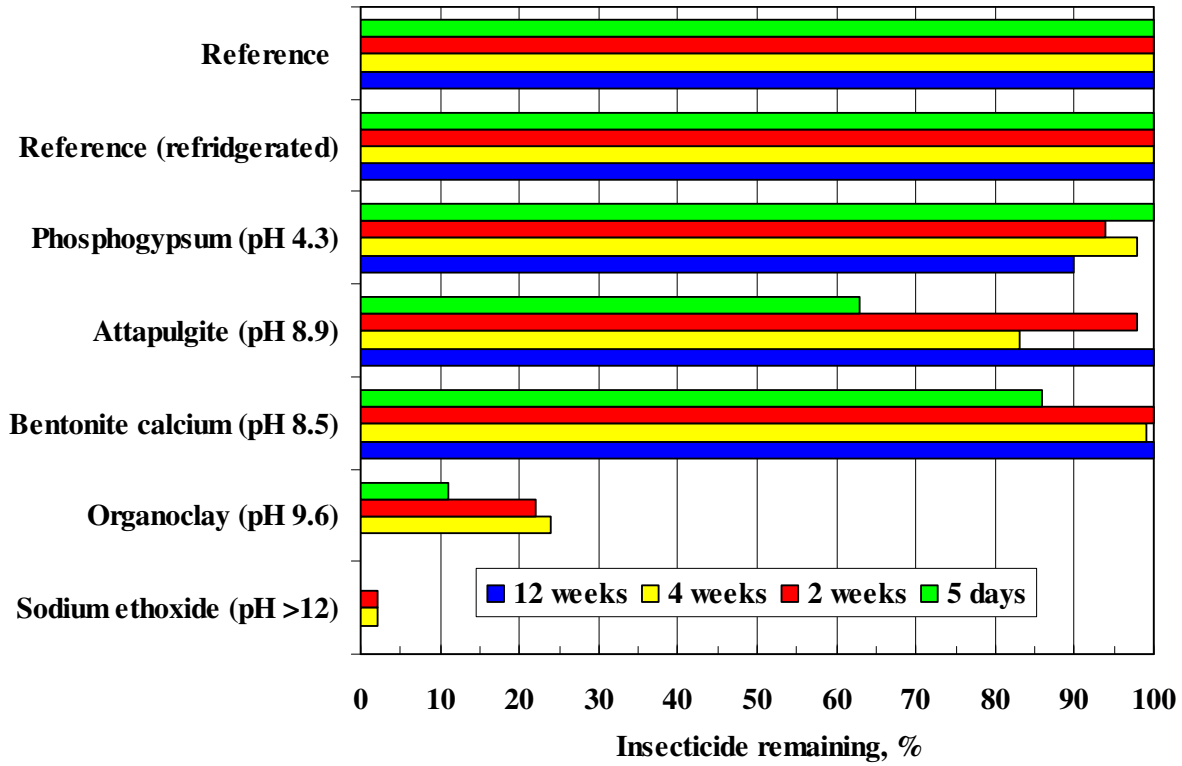


Figure 65: Ageing plots of bendiocarb on different mineral powders

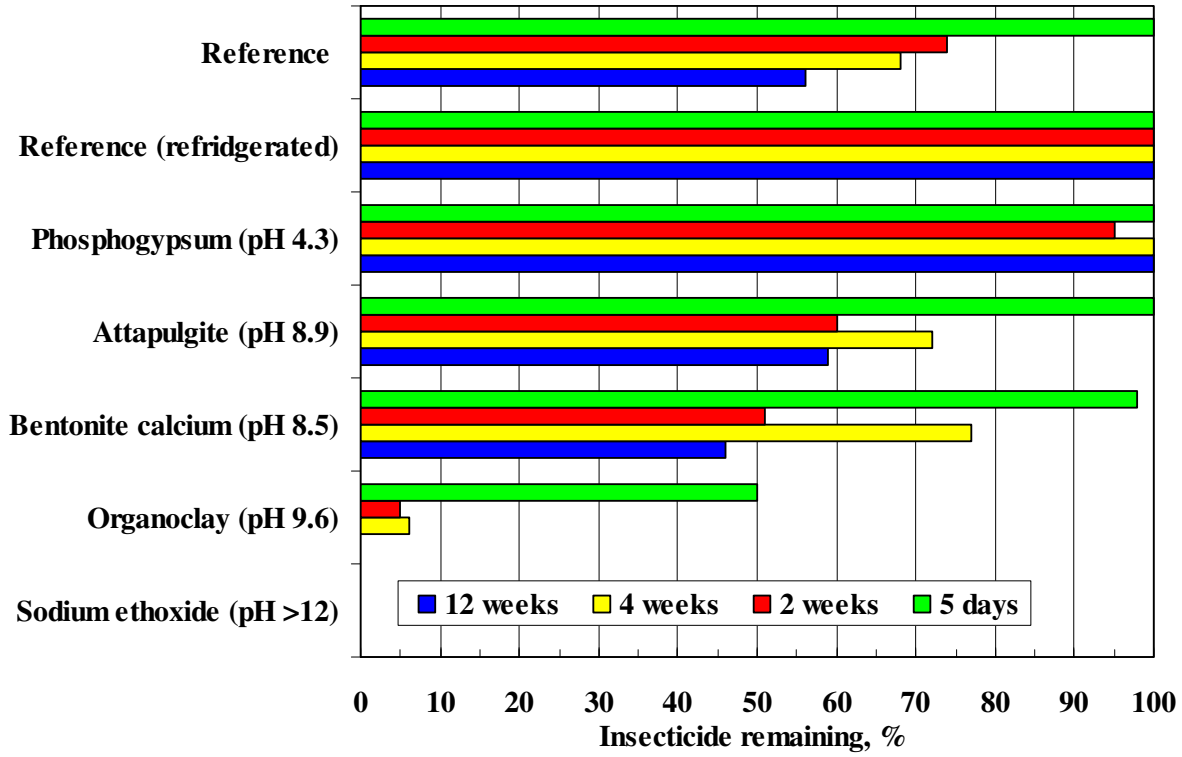


Figure 66: Ageing plots of alphacypermethrin on different mineral powders.

4.5. Paint rheology

The basic paint formulation was envisaged to be mineral powder slurry with insecticide precipitated on it. For the chosen mineral powder phosphogypsum, rheological behaviour at different solids volume concentration showed that the paint slurries were shear thinning in behaviour (Figure 67), suggesting that particles were agglomerated. Slurry with a solids volume fraction of 0.3 gave a uniform and sticking paint coating and also generally discharged well from the spray gun. This slurry was chosen for the paint formulation.

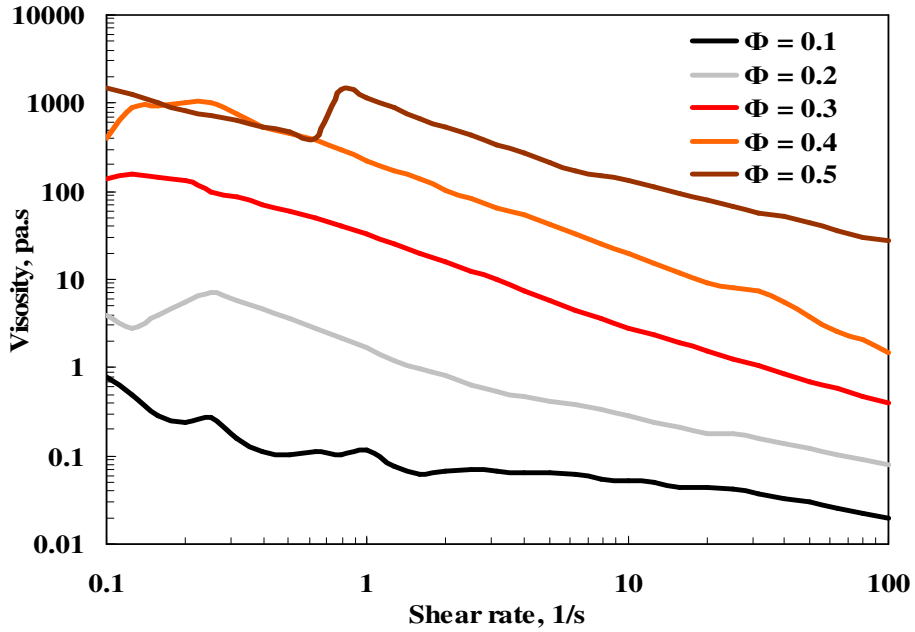


Figure 67: Viscosity plots of phosphogypsum slurries at different solids volume fraction (Φ)

It was recommended to add the dispersant, Mimosa WS powder (58% tannins and 39% non tannins) to assist in the de-agglomeration of the slurry particles. In the second phase of the project, emphasis should be placed on the rheology studies of the paint slurry in order to find an optimum dispersant concentration. For the paint formulations used in this project, 2% dispersant was used. However, results obtained (Figure 68) indicate that higher dispersant concentrations should have been used to de-agglomerate the paint slurry. The above results were not repeated and only used as a general guide for the formulation of the paint.

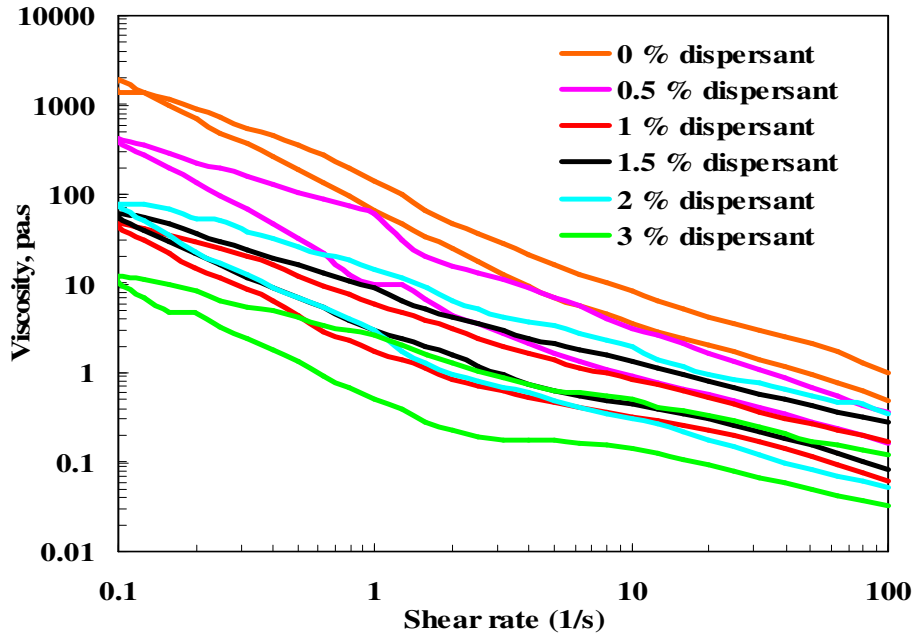


Figure 68: Viscosity profiles of phosphogypsum slurry (Φ = 0.3) at different dispersant concentrations

4.6. Insecticide distribution on paint

It was difficult to get a characterisation technique that could detect the insecticide on a treated (painted) surface. IR only managed to detect bendiocarb at 10 wt.% concentrations and failed to detect alphacypermethrin at 0.5 wt.%. Figure 69 shows 9 regularly spaced measuring spots used obtain IR interferograms on a treated microscope slide. Absorption due to bendiocarb was isolated at all the points (Figure 70). Bendiocarb appears to be distributed fairly evenly in the paint formulation.

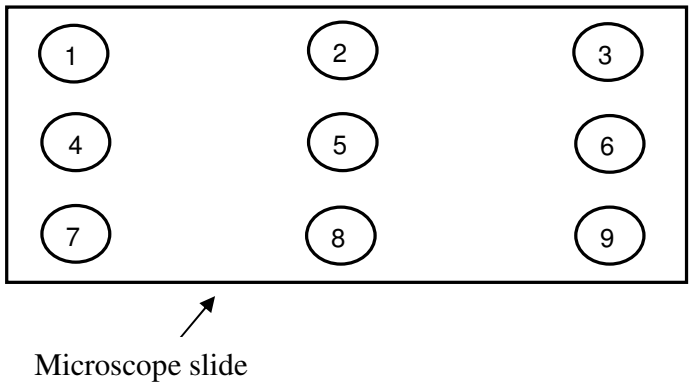


Figure 69: Schematic of a distribution of points measured by FTIR in reflectance mode

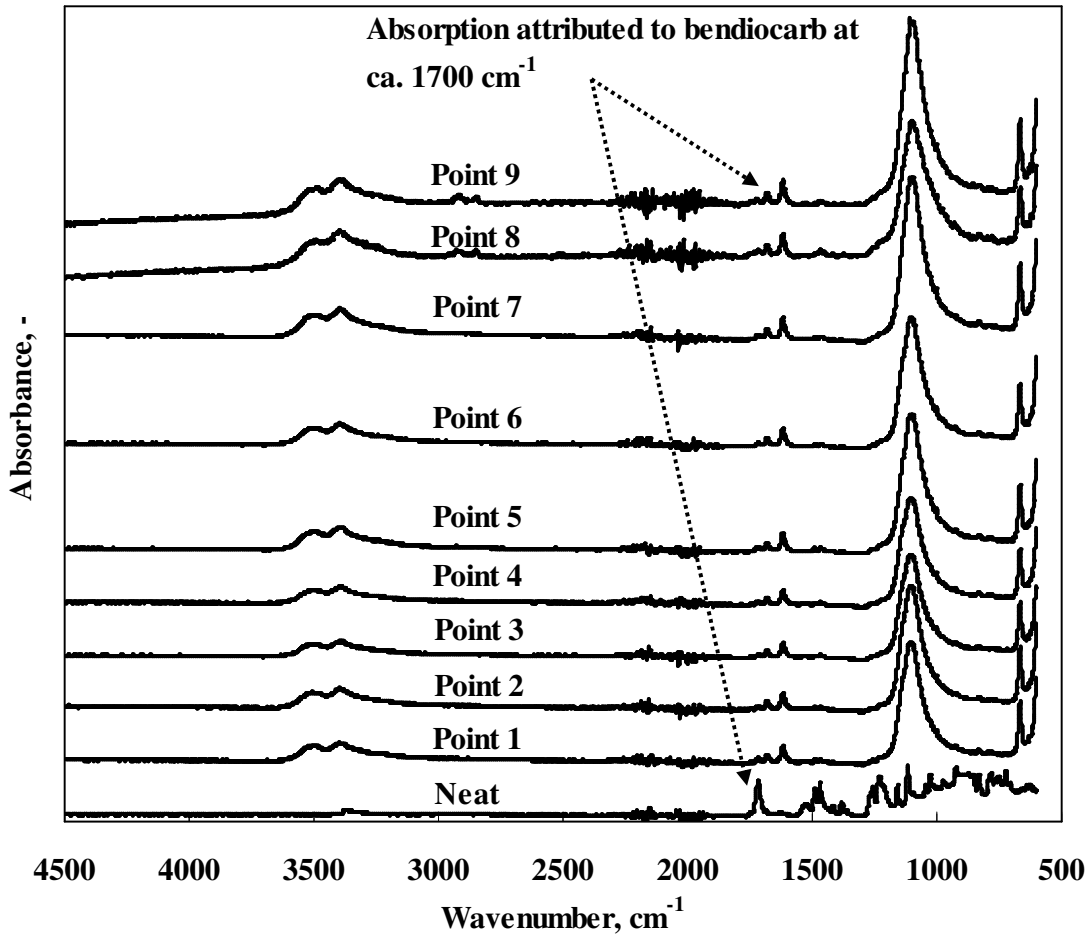


Figure 70: Distribution of bendiocarb in a paint formulation sprayed on a microscope slide

4.7. Bioassay

The bioassay results in Figures 71, 72, 73, and 74 show a considerable scatter. Raw results are presented in appendix E.

The WHO effectiveness criterion for IRS is a mortality exceeding 80% after 24 hours following a 30 minute exposure. The knock down after 60 minutes only provides a secondary indication of effectiveness. The mortality data were fitted using logistic regression.

The time to 80% mortality was estimated from the fitted equations (Figure 75).This resulted in the following ranking (estimated time to failure in days indicated in brackets):

DDT on manure (29.3) ≈ DDT on mud (31.3) < bendiocarb + binder on mud (38.3) ≈ alphacypermethrin + gypsum on mud (38.7) ≈ bendiocarb + gypsum on mud (39.4) ≈ bendiocarb + gypsum + paint binder on mud (39.6) < propoxur + paint binder on mud (50.3).

Raw results for the fitting of the statistical models on bioassay knockdown and mortality data are shown in Appendix F.

Alphacypermethrin + gypsum and bendiocarb + gypsum on manure test samples were not ranked as their knockdown and mortality rates were still at 100% on the 8 month bioassay test. This result is pertinent on the method of paint formulation. The initial formulations sprayed on mud plaques were made by dissolving the insecticide in just enough acetone and adding slurry of water and phosphogypsum to this mixture. The second set of formulations sprayed on cattle manure coated plaques was made by dissolving the insecticide in enough acetone to just wet the phosphogypsum powder to form slurry before adding water. The latter method uses excessive acetone but gave more stable formulations.

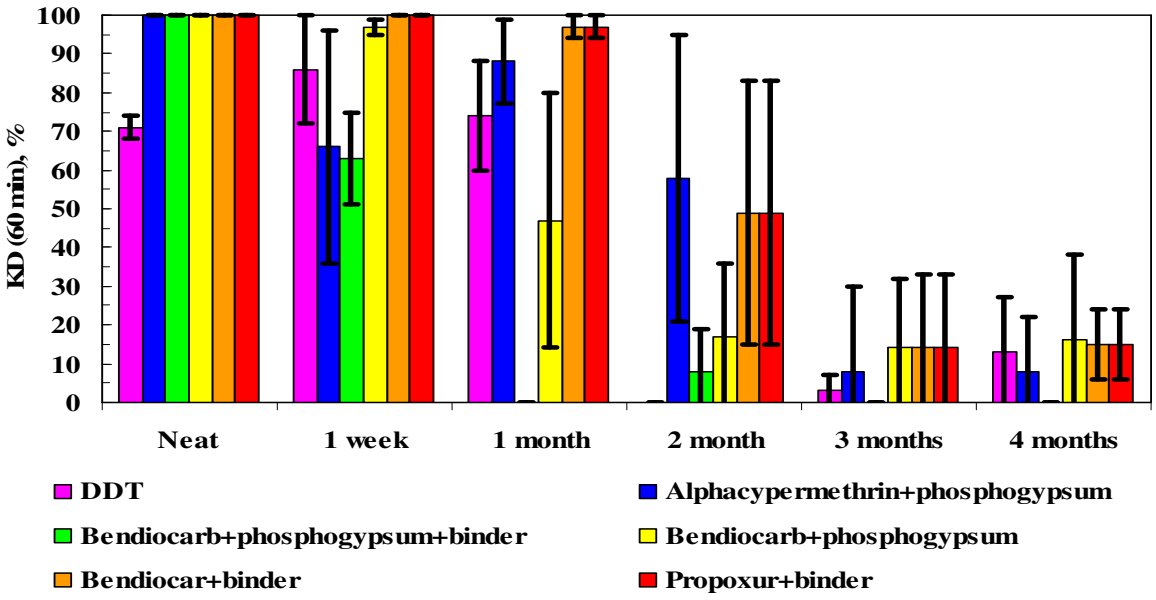


Figure 71: Bioassay results for knock-down after 60 minutes for mud surfaces treated with insecticides and aged at 40 °C and 90% ℜH for different time periods.

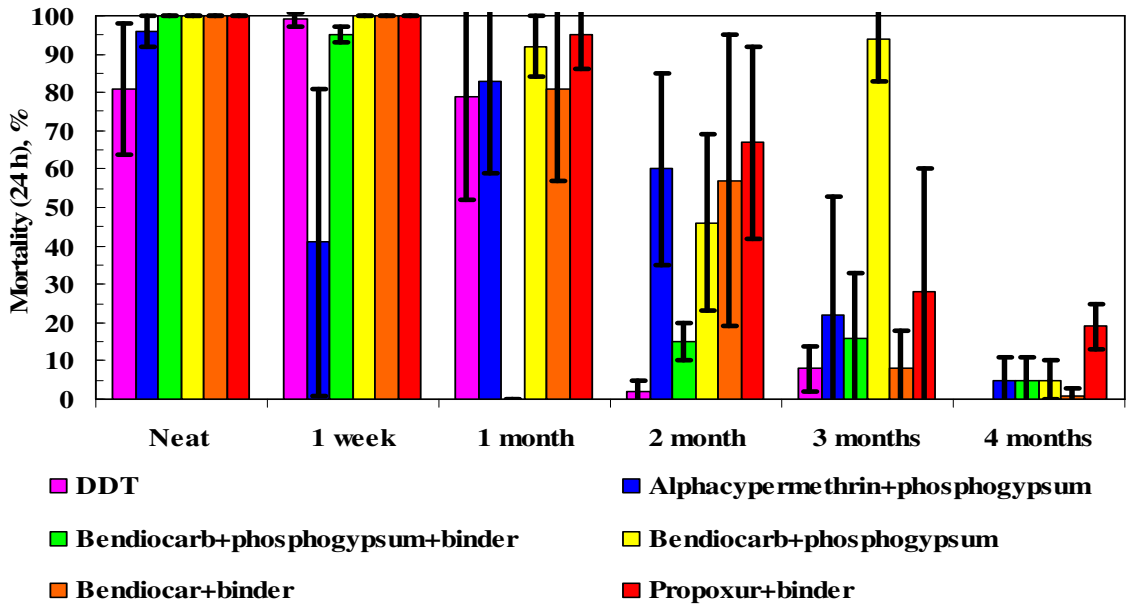


Figure 72: Bioassay results for mortality after 24 hours for mud surfaces treated with insecticides and aged at 40 °C and 90% ℜH for different time periods.

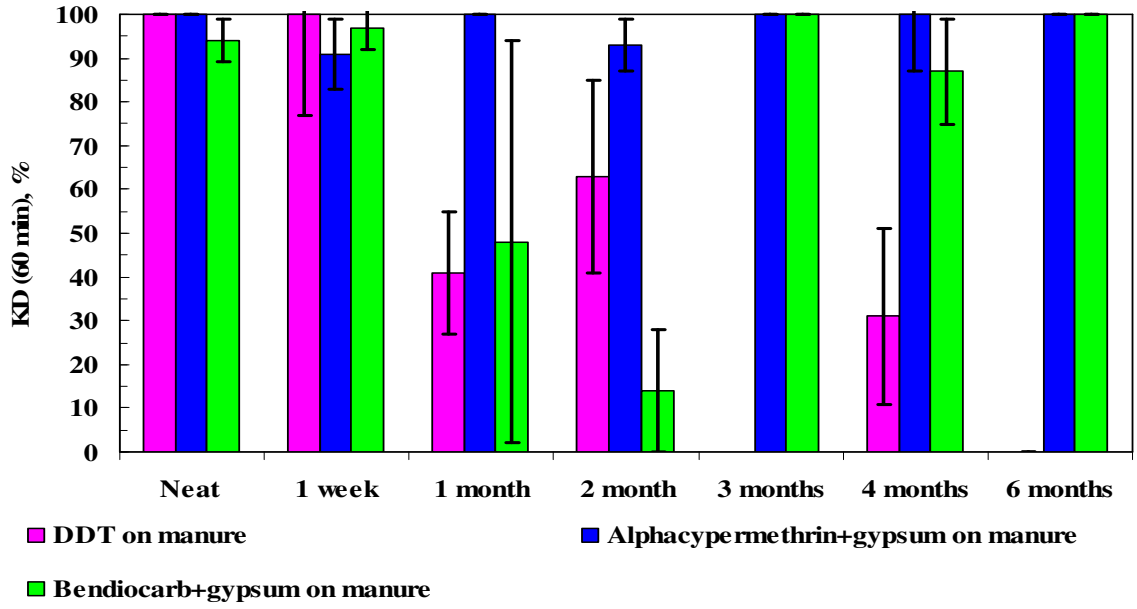


Figure 73: Bioassay results for knock-down after 60 minutes for manure samples treated with insecticides and aged at 40 °C and 90% ℜH for different time periods

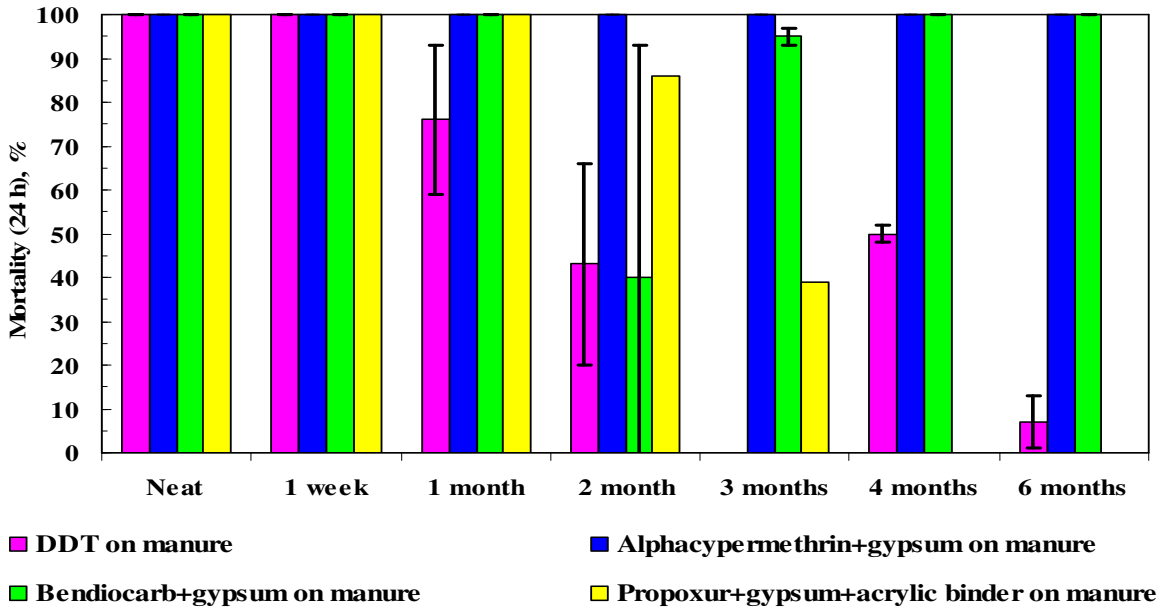


Figure 74: Bioassay results for mortality after 24 hours for manure samples treated with insecticides and aged at 40 °C and 90% ℜH for different time periods.

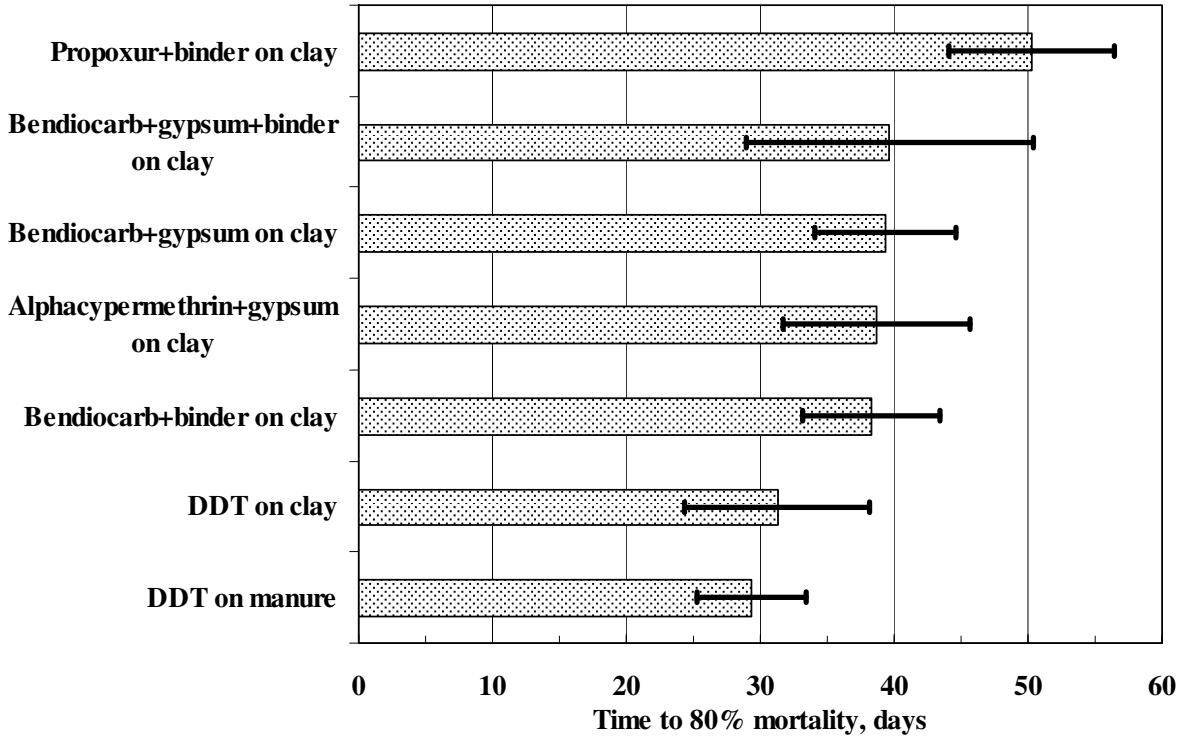


Figure 75: Mortality 80% cut-off determined from logistics fits with 95% confidence intervals

5. Discussion

5.1. Loss of insecticide

IRS relies on the fact that female mosquitoes tend to settle on the walls inside dwellings where they may rest for a few days after they have taken a blood meal. The insecticides used in IRS are contact poisons that are applied to the inside walls of dwellings to kill the female mosquitoes in this resting phase. The activity of the insecticides deposited on such walls decreases over time. This may be due to volatilisation or degradation processes, e.g. biodegradation, photodecomposition and alkaline hydrolysis. Volatilization occurs by evaporation for liquid insecticides and by sublimation for solid insecticides. The rate of vaporization increases with the ambient temperature and the degree of ventilation. In general, it can be estimated from equation (1) (Focke, 2003):

$$n_A = Sh\frac{D_{AB}P_A M_A}{zRT} \qquad [1]$$

where n_A is mass flux in kg.m^2.s^{-1}; Sh is the dimensionless Sherwood number; D_{AB} is the diffusion coefficient in m^2.s^{-1}; P_A is the vapour pressure of the insecticide in Pa; z is a characteristic length of the system measured in m the diffusion distance, taken here as half the width of the house; R is the gas constant 8.314 J.mol^{-1} K^{-1}; T is the absolute temperature in K; and M_A is the molar mass of the insecticide expressed in kg.mol^{-1}.

The rate of loss is least in the absence of any air movement. In this scenario the rate of mass loss is determined by rate of diffusion of the insecticide through the stagnant air layer and the Sherwood number assumes the value of unity while z is the diffusion distance in m normal to the wall surface. For preliminary estimation purposes it is taken as half the width of the house.

The diffusion coefficients in air of the present insecticides are not known but were estimated using Fuller's method (Reid, Prausnitz & Poling: 587). Vapour pressure data at 25 °C are summarised in Figure 19. Assuming a diffusion distance of 2 m, ambient temperature of

25 °C, using the WHO IRS dosage recommendations and applying equation (1) leads to the following estimates for the bioactive life in years:

Fenitrothion, 136; pirimiphos-methyl 110; bendiocarb, 11; propoxur 96; malathion 457; DDT, 7726; and alphacypermethrin 319.

Raising the ambient temperature has a dramatic effect. For example, at 40 °C the persistence of fenitrothion is reduced to 24 years. Ventilation further increases the rate of volatilization. In order to get a rough estimate of the impact of this factor, a flat plate approximation model for the wall is assumed with the air flow parallel to the wall at a velocity V in $m.s^{-1}$. For this geometry the characteristic length z corresponds to the length of the wall L and the Sherwood number is given by equation [2] (Bird, Steward & Lightfoot: 407):

$$Sh = 0.667\sqrt{\mathrm{Re}}Sc^{1/3} \qquad [2]$$

where Re is the Reynolds number (VL/ν); Sc is the Schmidt number (ν/D_{AB}); ν is the kinematic viscosity in $m^2.s^{-1}$.

Typical velocities that could be encountered inside a house range from ca. 0.001 $m.s^{-1}$ to about 1 $m.s^{-1}$. Using these values and typical property values for air at ambient conditions reveals that the active life times indicated above could be reduced by 4 to 100 times. This means that, with the possible exception of bendiocarb, the loss of activity of the WHO insecticides experienced in the field cannot be attributed to volatilization! The implication is that the loss of activity over time must be associated with degradation processes.

5.2. Ageing processes

In general organic insecticides degrade when exposed to elevated temperatures (thermal degradation); sunlight (photodegradation); moisture (e.g. hydrolysis), and microbial attack. The laboratory tests used in this study simulated relatively extreme conditions of temperature, ultra-violet light exposure, humidity, alkalinity and microbiologically activity. The temporal performance of neat and stabilized WHO-approved insecticides samples were compared to that

of neat DDT. It was found that DDT alternatives stabilized through incorporation in an acrylic binder or precipitated on acidic filler outlasted DDT when applied to cattle manure coated surface and performed at least at a similar level when applied to soil surfaces.

5.2.1. Temperature, ultra-violet light and humidity ageing

In general organic insecticides degrade when exposed to elevated temperatures (thermal degradation); sunlight (photodegradation); moisture (e.g. hydrolysis), and microbial attack. Thus laboratory tests simulating extreme conditions of temperature, ultra-violet light exposure, humidity, alkalinity and microbiologically activity were used in this study. The temporal performance of neat and stabilized WHO-approved insecticides samples were compared to that of neat DDT. Through these tests a low-cost way of stabilizing the insecticides was identified.

The loss of DDT and carbamate insecticide activity under the ageing condition of 80 °C is attributed to a combination of thermal degradation and volatilization. The latter speculation is based on the vapour pressure data for the insecticides (Figure 19). The pyrethroids class of insecticides have the lowest vapour pressure whilst the carbamates, organophosphates, and DDT have the highest, second highest and third highest vapour pressure respectively. The vapour pressure data explains why the pyrethroids are generally less fugitive than the rest of the insecticides. The low vapour pressure makes the pyrethroid group of insecticide the least volatile as compared to the rest of the insecticides and hence evaporation is slower than the rest. The present observations, however, suggest that pyrethroids are more stable towards thermal exposure compared with DDT and carbamates.

The progression of transformation of insecticides under UV light appears to be consistent with the literature. Differences may have been due to dissimilar ageing conditions insecticides were exposed to. On a comparative basis however, pyrethroids clearly exhibit significantly greater resilience to UV radiation compared to the rest of the insecticide classes.

Accelerated ageing tests indicate that pyrethroids are a far more stable class of insecticides when exposed to typical environmental elements of degradation compared with the other classes of insecticides. This strongly suggests that high temperatures, ultra-violet light

and humidity are not the dominant mechanisms of oxidative/hydrolytic degradation of insecticides in the field.

5.2.2. Insecticide ageing in the presence of a mineral powder

Plots of the half-life (DT_{50}) values against pH (Figure 34) show that the WHO-approved insecticides are more stable at lower pH values. It has been shown that, except for DDT, the other insecticides tested are very sensitive to pH-mediated hydrolysis. This is especially true at elevated pH values. Figure 66 shows that some potential paint fillers have a stabilizing effect on alphacypermethrin dissolved in acetone containing ca. 1% water. Similar results were obtained with bendiocarb although in this case greater stabilities were achieved. The stabilizing effect appeared to correlate with the surface pH of the mineral powders (indicated in brackets) suggesting they acted as acidic pH buffers. Phosphogypsum showed the best results. It is a cost-effective option and preliminary spray tests showed that it sticks well to a variety of vertical surfaces that are encountered in rural dwellings.

pH mediated hydrolysis is the only mechanism of degradation isolated that can degrade all the other classes of insecticides significantly rapidly relative to DDT. In recent work it was found that alphacypemethrin intercalated in bentonite formulations led to premature failure if the bentonite surface was not modified to an acidic pH of 4.5 using acetic acid (unpublished). Malaria control authorities in South Africa prefer to spray DDT on mud walls commonly found in rondavels (appendix G) whilst they spray pyrethroids on western style painted cement walls (Hargreaves *et al.,* 2000). This preference is due to the fact DDT has the ability to persist longer on mud walls compared to pyrethroids. It is highly likely that in the field of application, surface chemical properties of the walls being sprayed with the insecticides play a significant role in the breakdown of the insecticides through water mediated hydrolysis.

5.2. Bioassay

Dwellings in deep rural areas commonly feature mud and cattle manure coated walls. Bioassays were used to follow the time-dependent efficacy of the stabilized insecticides applied

onto such surfaces under accelerated laboratory ageing conditions of high humidity and elevated temperature. The considerable variability in the data makes statistically reliable comparisons difficult. However, the results suggest that the carbamates bendiocarb and propoxur perform well when incorporated into an ordinary paint binder, while bendiocarb and alphacypermethrin precipitated on phosphogypsum also performed well in the accelerated ageing test conducted at 40 °C and 90% relative humidity.

6. Conclusions and recommendations

Comparative tests under accelerated ageing conditions indicate that temperature, ultra-violet light and humidity do not play a significant role in the persistence of IRS insecticides in the environment. It is likely that water mediated hydrolysis is more important. Bioassay experiments indicated that insecticides incorporated in a conventional paint binder or adsorbed onto phosphogypsum provided effective life spans comparable to or exceeding that of DDT directly applied to typical soil substrates or cattle manure surfaces and subjected to accelerated ageing conditions. Adsorbing the insecticide on phosphogypsum mineral powder provided a suitable buffered pH environment which minimized pH mediated hydrolysis. This resulted in a significantly improved persistence of carbamates and pyrethroids compared to a standard DDT spray formulation. A conventional paint binder has also shown to be useful in providing a buffered environment that stabilizes carbamates and pyrethroids against water mediated hydrolysis. The binder gives a glossy and translucent coating that may be more acceptable to users who do not want to alter the color of their walls. These findings may allow replacement of DDT, a persistent organic pollutant, with WHO-approved insecticides.

- Recommendations for the next phase:

During phase 1 of this project, a number of IRS formulations were developed and tested. The efficacies and lengths of effectiveness of these formulations were found to be comparable to the standard DDT formulation under accelerated conditions of temperature, ultra-violet light and humidity. However further work needs to be done to optimise these formulations. A summary of the proposed work to be done is as follows:

1. Minimise the amount of solvent used in the formulations; there is a need to reduce the amount of acetone used to wet the insecticide.

2. Determine the most optimum insecticide loading on the solid carrier taking into account the amount of paint to be sprayed and the residual effectiveness of the formulations. This may entail evaluating the amount of insecticide required to achieve a monolayer coating on the solid surface and loading the formulation to values much higher than the

monolayer coating requirement. A higher insecticide loading reduces the paint formulation to be sprayed to amounts practicable for transportation.

3. Determine the most effective loading of acrylic paint binder on the solid carrier taking into account the length of the residual effectiveness. An acrylic binder has been found to minimise the volatility of the insecticides and thus lengthening its effectiveness. However a large amount of binder has shown to reduce effectiveness of the insecticide by masking it, hence the need to optimise.

4. Perform spray tests with varying air pressure, spray gun nozzle opening, slurry concentration and sprayer hand speed to achieve the most optimum spray delivery on the substrate. There is need for preciseness in terms of delivering a particular mass of spray per unit of area. This will also serve as a practice run for the actual spraying in the field trials.

7. References

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8. Appendix

8.1. Appendix A: Insecticidal paint formulations and spray results

8.1.1. Alphacypermethrin + phosphogypsum

	Mass, g	Wt.% of total formulation
Alphacypermethrin	0.076	0.2
Water	20	48.7
Gypsum	20	48.7
Acetone	0.6	1.5
Dispersant	0.4	1.0
Total	41.076	

The above formulation was sprayed on mud plaques of surface area 0.01887 m^2 (d = 15.5 cm). The required dosage was 0.06 $g.m^{-2}$. The target amount of paint to be sprayed was 0.61 g.

Alphacypermethrin + phosphogypsum	Mass of paint sprayed, g	Mass of insecticide sprayed, m^2
Sample 1	0.58	0.06
Sample 2	0.55	0.05
Sample 3	0.59	0.06

The above formulation was again sprayed on a fresh cattle dung coated mud substrate with a surface area of 0.0102 m^2 (Φ = 11.4 cm). The required dosage was 0.06 $g.m^{-2}$. The mass of insecticide to be sprayed was 0.33 g.

Alphacypermethrin + phosphogypsum on cattle manure	Mass of paint sprayed, g	Mass of insecticide sprayed $g.m^{-2}$
Sample 1	0.43	0.08
Sample 2	0.35	0.06
Sample 3	0.36	0.07

8.1.2. Bendiocarb + phosphogypsum

	Mass, g	Wt.% of total formulation
Bendiocarb	2	3.8
Water	10	19.1
Gypsum	20	38.2
Acetone	20	38.2
Dispersant	0.4	0.8
Total	52.4	

The above formulation was sprayed on mud plaques of surface area 0.01887 (d = 15.5 cm).the required dosage was 0.8 $g.m^{-2}$. The target amount of paint to be sprayed was 0.4 g.

Bendiocarb + phosphogypsum	Mass of paint sprayed, g	Mass of insecticide sprayed, $g.m^{-2}$
Sample 1	0.49	0.99
Sample 2	0.51	1.03
Sample 3	0.54	1.09

This formulation was sprayed on cattle manure coated mud plaques with a surface area of 0.0102 m^2. The required dosage was 0.8 $g.m^{-2}$. Amount of insecticide paint to be sprayed was 0.22 g.

Bendiocarb + phosphogypsum on manure	Mass of paint sprayed, g	Mass of insecticide sprayed, m^2
Sample 1	0.30	1.12
Sample 2	0.29	1.08
Sample 3	0.31	1.15

8.1.3. Bendiocarb + binder + phosphogypsum

	Mass, g	Wt.% of total formulation
Bendiocarb	2	3.68
Gypsum	20	36.8
Dispersant	0.4	0.7
Water	20	36.8
Binder	2	3.7
Acetone	10	18.4
Total	54.4	

This formulation was sprayed on mud plaques of surface area 0.01887 m^2 (d = 15.5 cm).the required dosage was 0.8 $g.m^{-2}$. The target amount of paint to be sprayed was 0.54 g.

Bendiocarb + binder + phosphogypsum	Mass of paint sprayed, g	Mass of insecticide sprayed, $g.m^{-2}$
Sample 1	0.58	0.85
Sample 2	0.58	0.85
Sample 3	0.67	0.98

8.1.4. Bendiocarb + binder

	Mass, g	Wt.% of formulation
Bendiocarb	2.8	6.5
Acetone	12	28.0
Binder	28	65.4
Total	42.8	

This formulation was sprayed on mud plaques of surface area 0.01887 m^2 (d = 15.5 cm).the required dosage was 0.8 $g.m^{-2}$. The target amount of paint to be sprayed was 0.23 g.

Bendiocarb + binder	Mass of paint sprayed, g	Mass of insecticide sprayed, $g.m^{-2}$
Sample 1	0.25	0.86
Sample 2	0.21	0.72
Sample 3	0.20	0.69

8.1.5. Propoxur + binder

	Mass, g	Wt.% of total formulation
Propoxur	2.8	6.9
Acetone	10	24.5
Binder	28	68.6
Total	40.8	

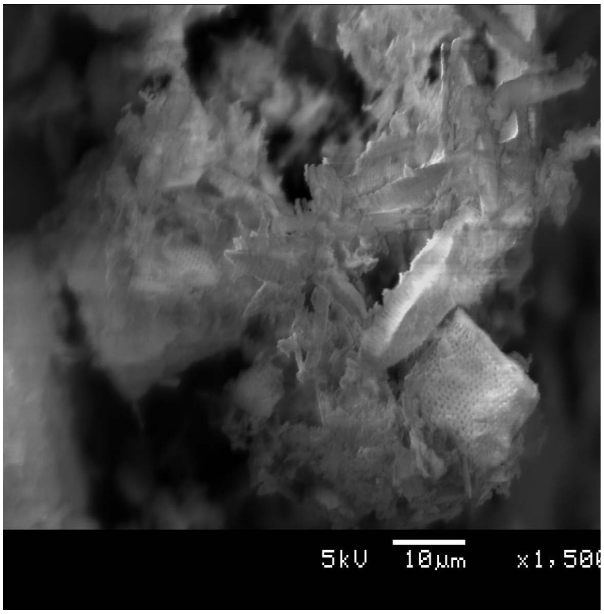
This formulation was sprayed on mud plaques of surface area 0.0104 m^2 (diameter = 11.5 cm).The required dosage was 4 $g.m^{-2}$. The target amount of paint to be sprayed was 0.604 g.

Propoxur + binder + formulation	Mass of paint sprayed, g	Mass of insecticide sprayed, $g.m^{-2}$
Sample 1	0.57	3.78
Sample 2	0.63	4.18
Sample 3	0.58	3.84

8.1.6. DDT

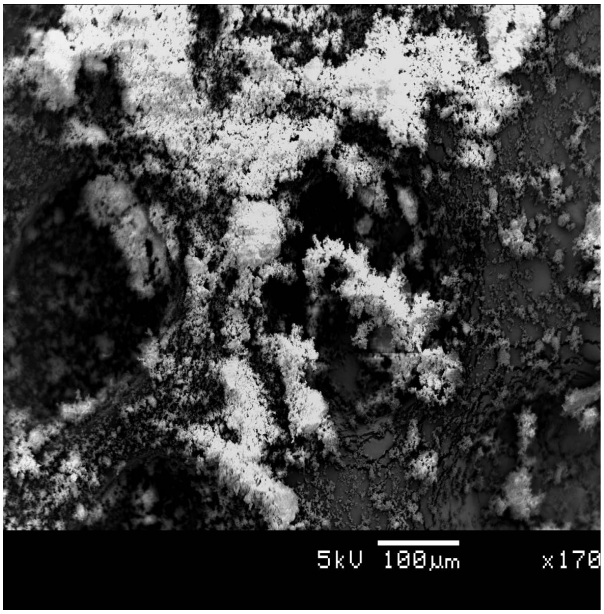
A 10% solution of DDT was made by adding 1 g of DDT in 9 g of acetone. This formulation was sprayed on substrate of surface area 0.010568 m^2 (d = 11.6 cm) required dosage was 8 $g.m^{-2}$. The target amount of solution to be sprayed was 0.8 g.

8.2. Appendix B: Scanning electron microscopy of all mineral powders



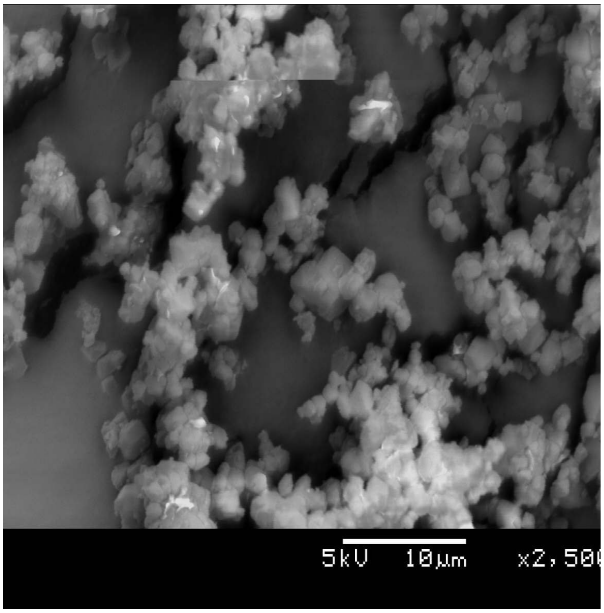
Attapulgite

Leafy platelets, appears to have a perforated surface



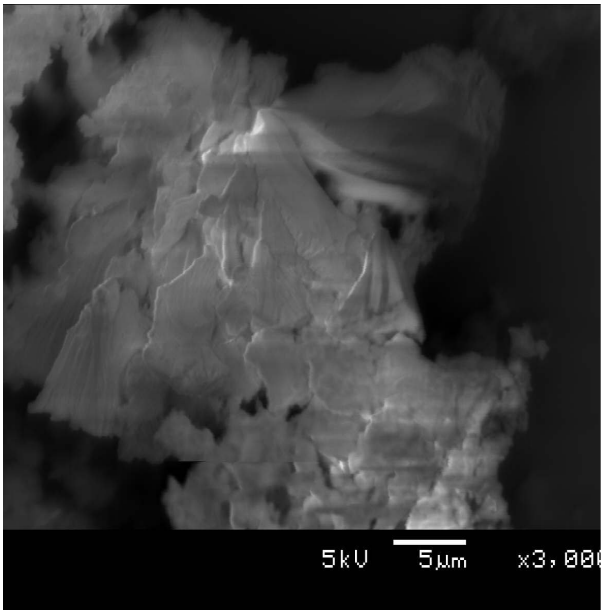
Calcium hydroxide

Fluffy and agglomerated particles



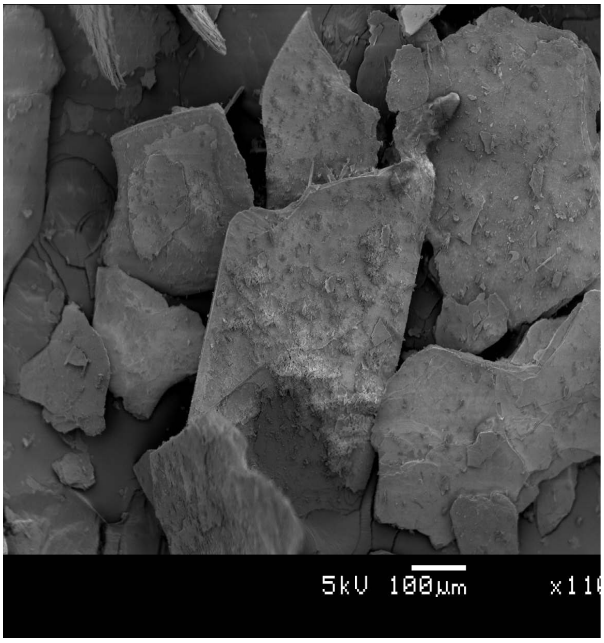
Calcium sulphate

Spherical agglomerated particles



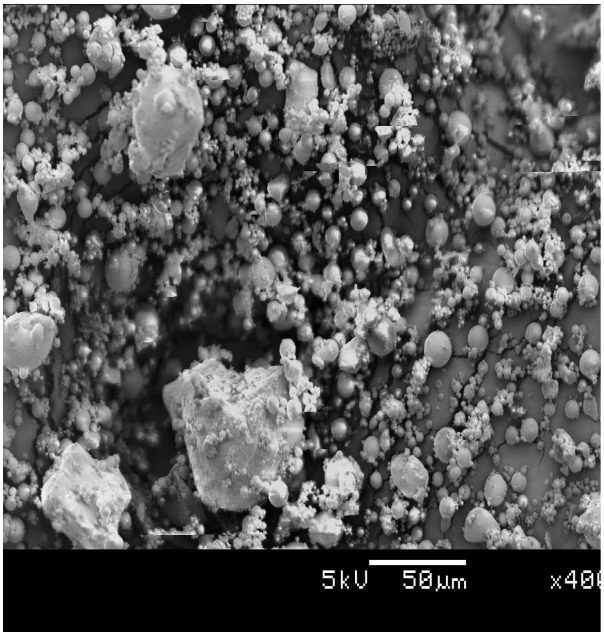
Hydrated lime

Platelets appear stack close to each other (agglomerated)



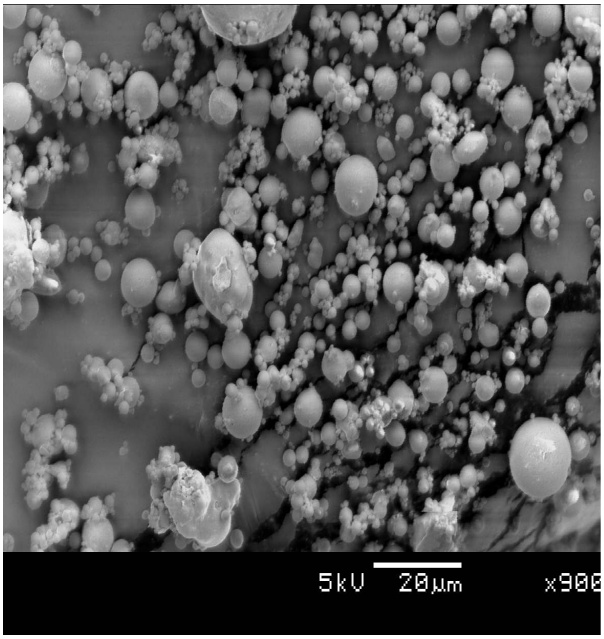
Leach flogopite

Large platelets



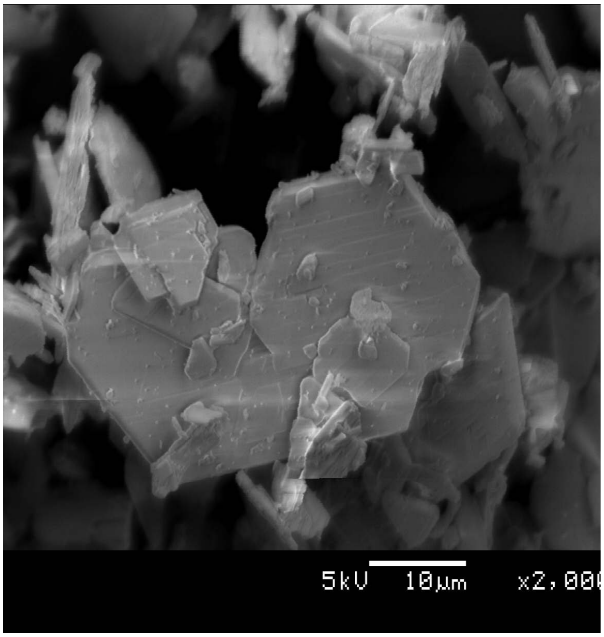
Plasfil 15

Smooth spherical particles



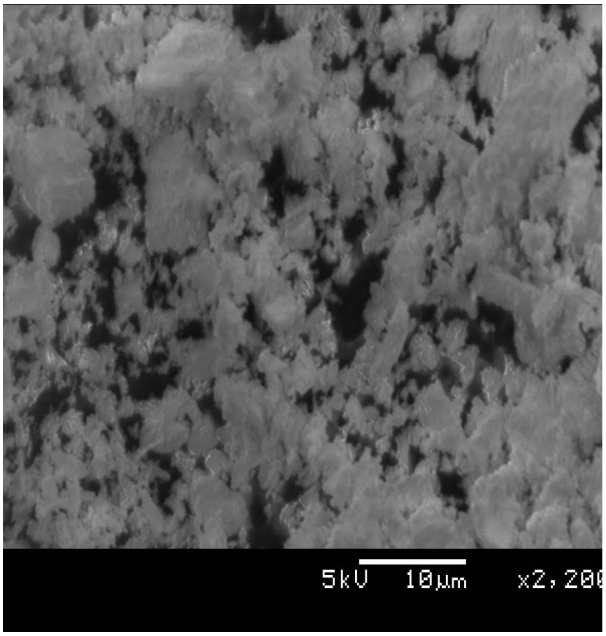
Plasfil 5

Smooth spherical particles



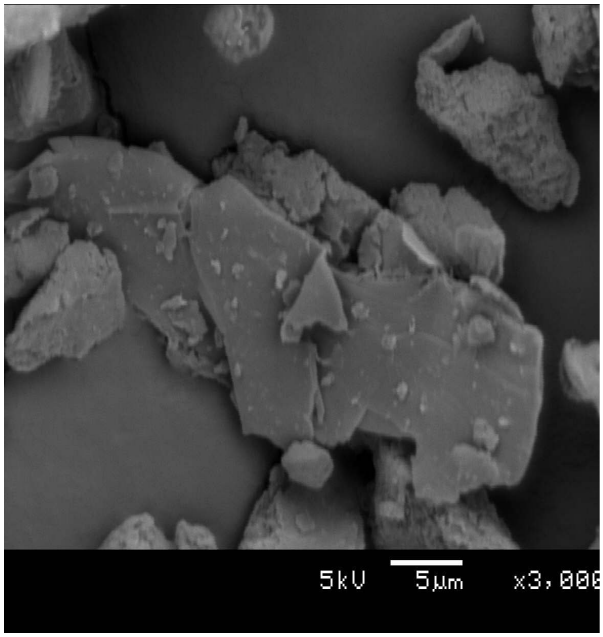
Super special

Smooth surfaced platelets



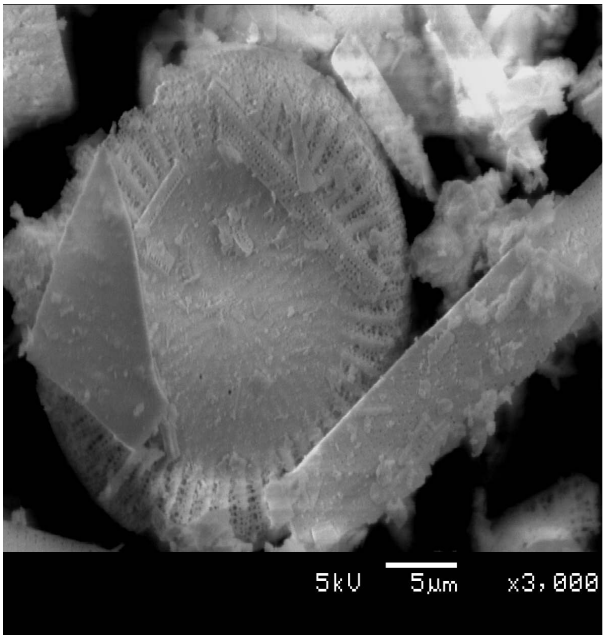
Wykan 12

Flaky particles, with sections of heavy agglomeration



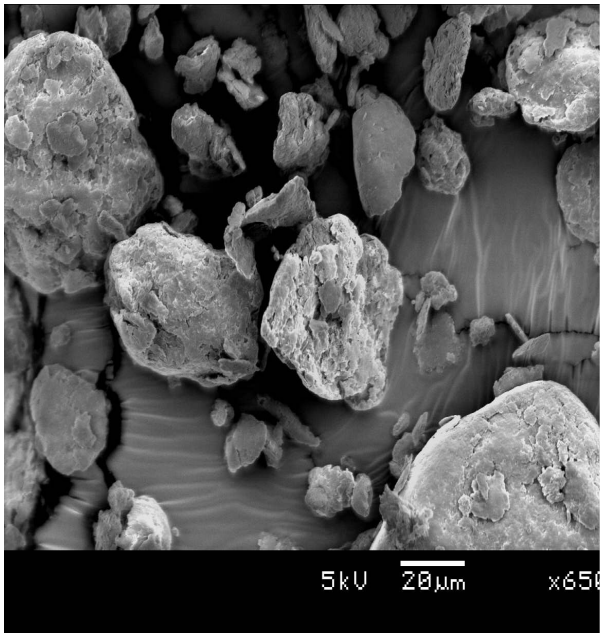
Bentonite calcium

Platy particles



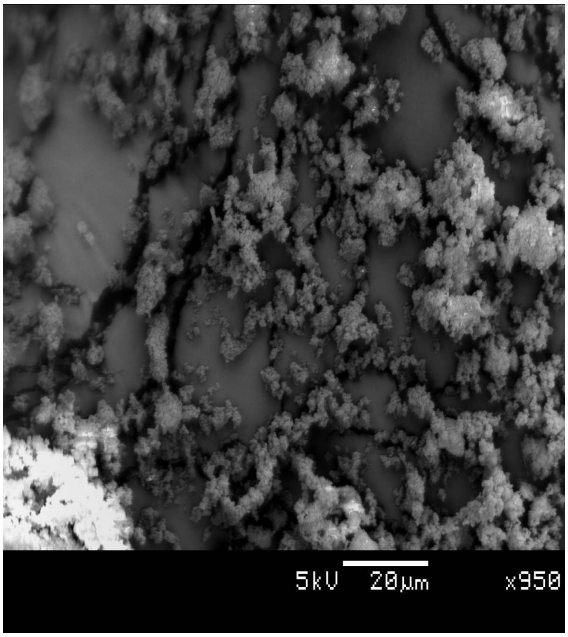
Calcined diatomite

Particles with different orientation and shapes



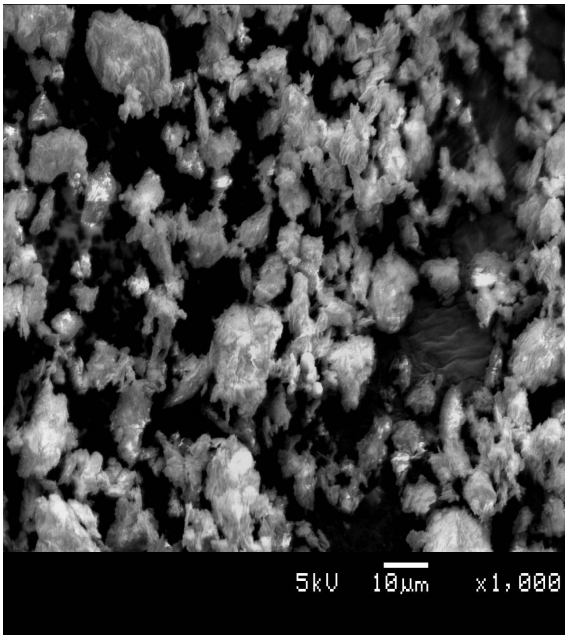
Organoclay

Clumped up particles that form spherical agglomerates



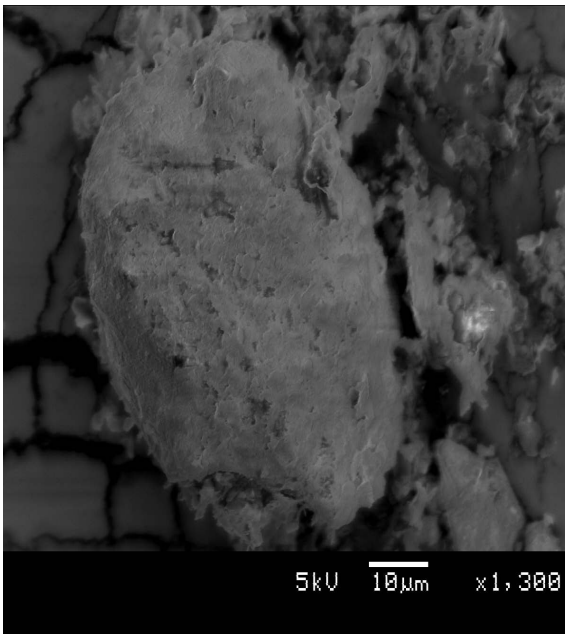
Hydrated dolomite

Fluffy agglomerated particles



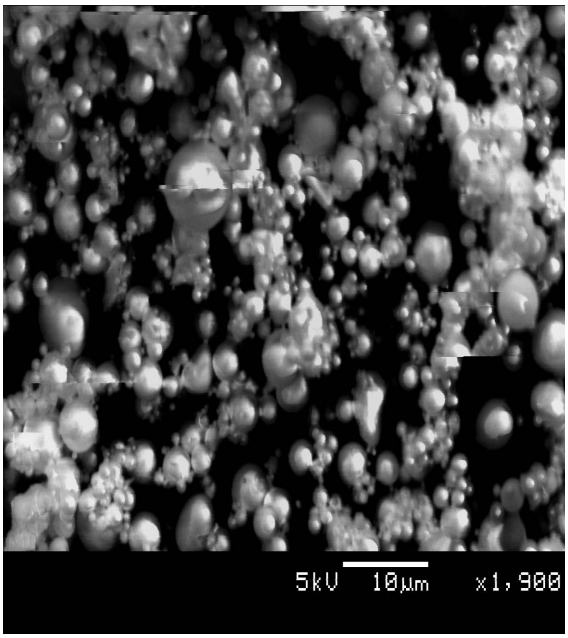
Kaolin

Agglomerated particles



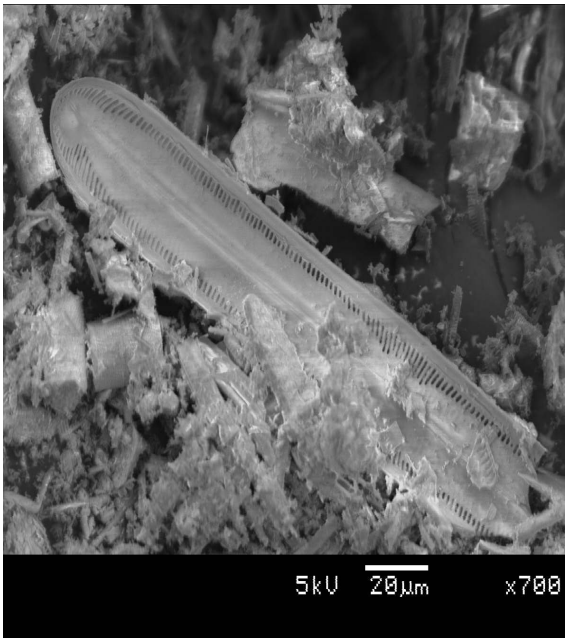
Mica 325

Agglomerated particles forming huge masses



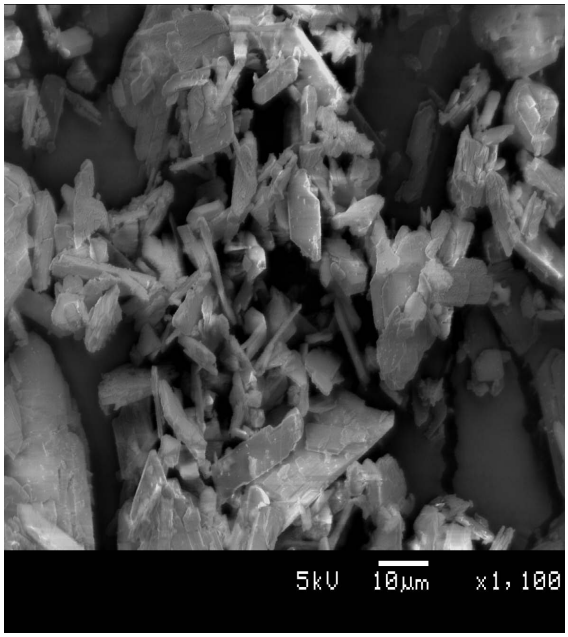
Plasfil 5 calcined at 500°C.

Spherical particles



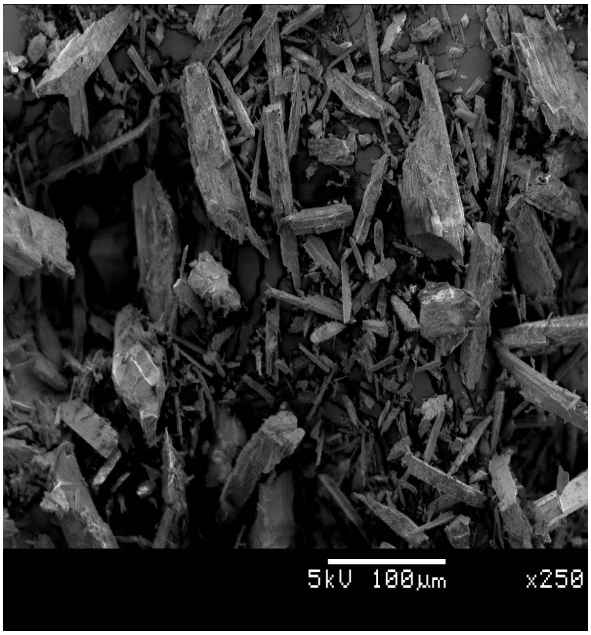
Raw diatomite

Several shapes and sizes were observed. The surface appeared to be perforated for most of the particles



Phosphogypsum

Non-agglomerated platelets



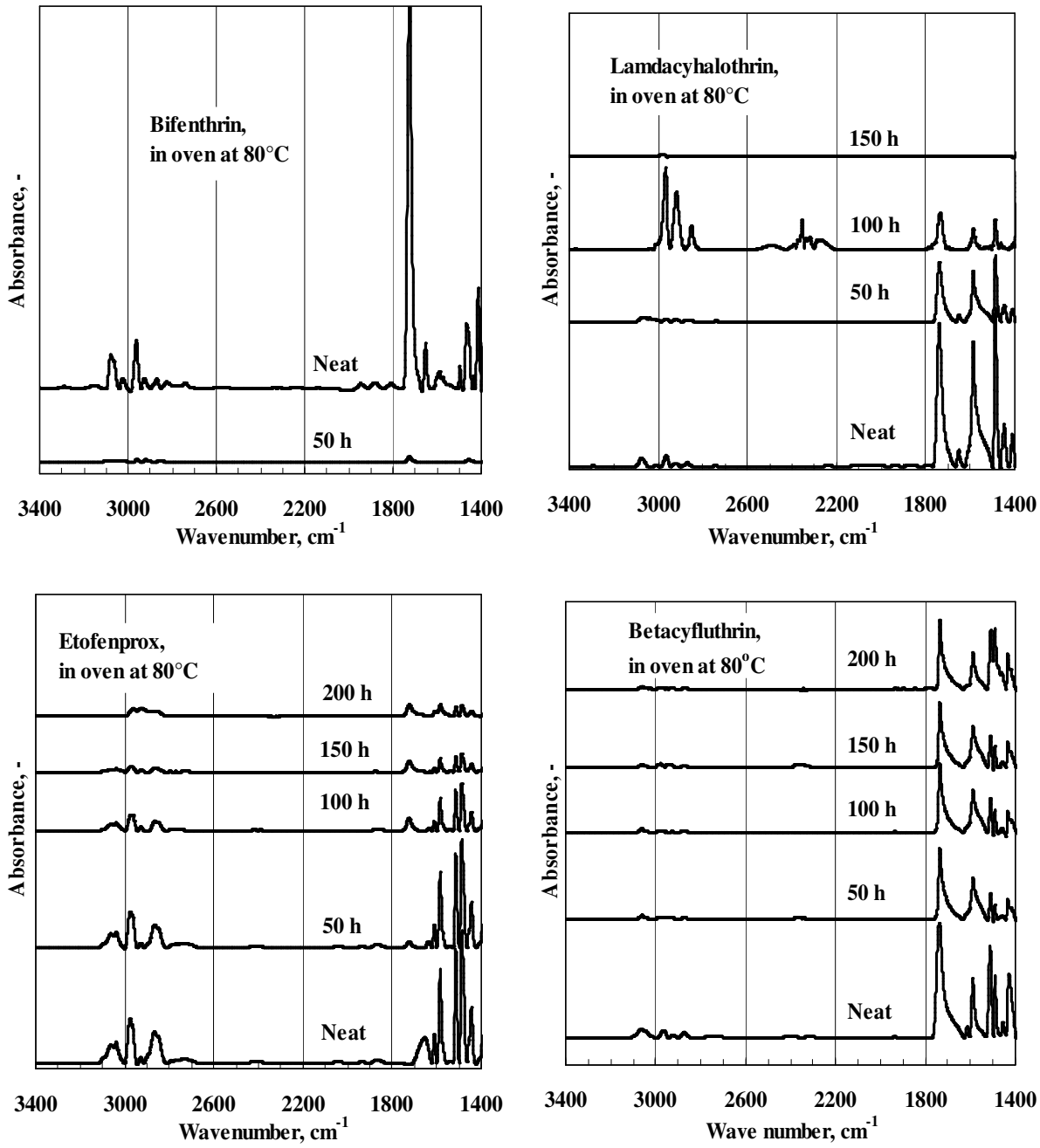
Wallastonite

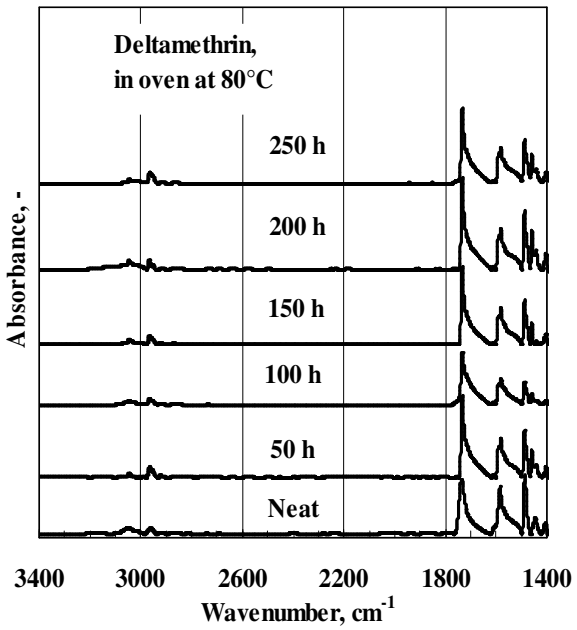
Needle like particles

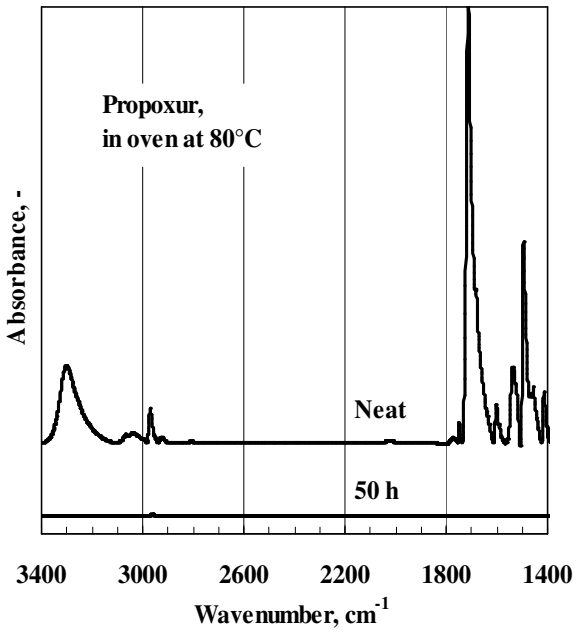
8.3. Appendix C: X-ray fluorescence analysis of soil samples

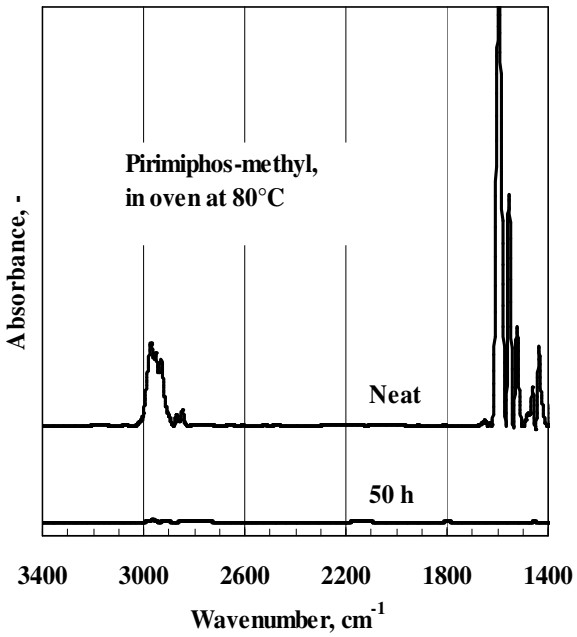
Sample	MAKOYA	NAM 01	THOMO
SiO_2	70.99	48.35	69.62
TiO_2	0.62	0.98	0.93
Al_2O_3	13.63	26.03	12.05
Fe_2O_3	2.89	12.87	5.75
MnO	0.05	0.09	0.13
MgO	0.69	<0.01	0.87
CaO	1.87	0.07	1.62
Na_2O	4.40	<0.01	2.94
K_2O	1.49	0.16	1.67
P_2O_5	<0.01	0.05	0.06
Cr_2O_3	0.02	0.01	0.04
NiO	0.01	<0.01	0.01
V_2O_5	0.01	0.01	0.01
ZrO_2	<0.01	0.18	0.01
SO_3	<0.01	<0.01	<0.01
WO_3	<0.01	<0.01	<0.01
BaO	0.04	<0.01	<0.01
Cl	<0.01	<0.01	<0.01
CuO	<0.01	<0.01	0.02
ZnO	<0.01	0.02	<0.01
Rb_2O	<0.01	<0.01	<0.01
Y_2O_3	<0.01	0.02	<0.01
SrO	0.06	<0.01	0.05
Total	96.74	88.84	95.78

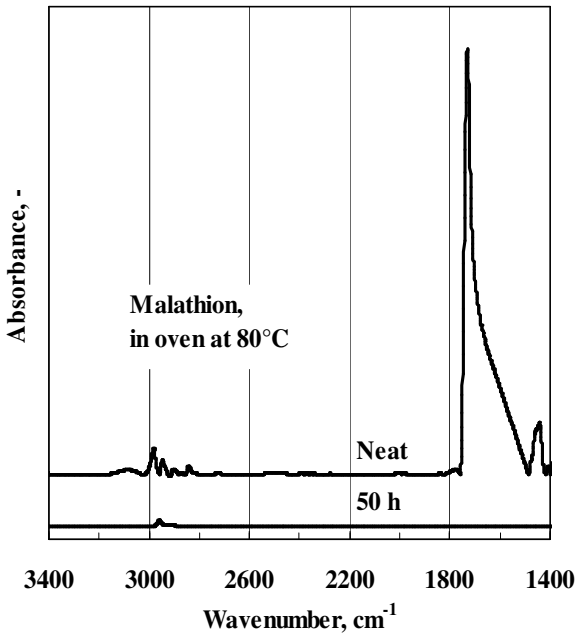
8.4. Appendix D: FTIR interferograms of WHO approved insecticides for temperature, ultra-violet light and humidity ageing tests

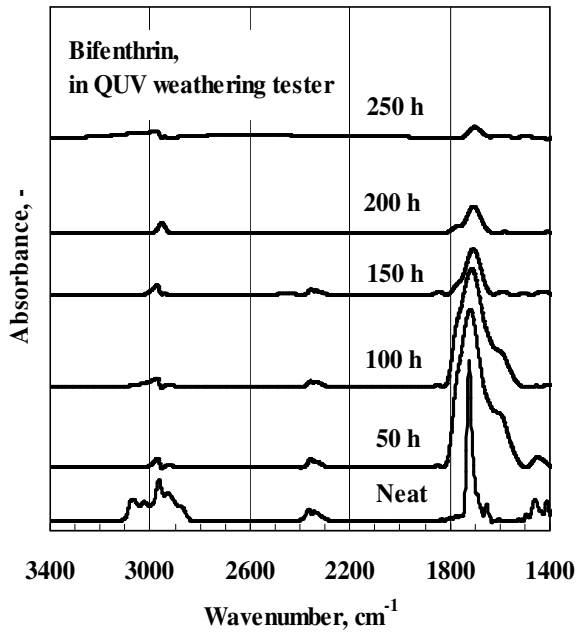


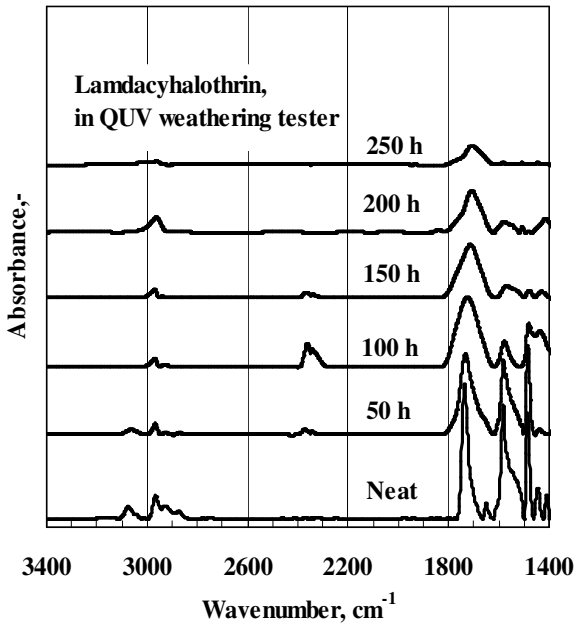

Deltamethrin,
in oven at 80°C
250 h
200 h
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

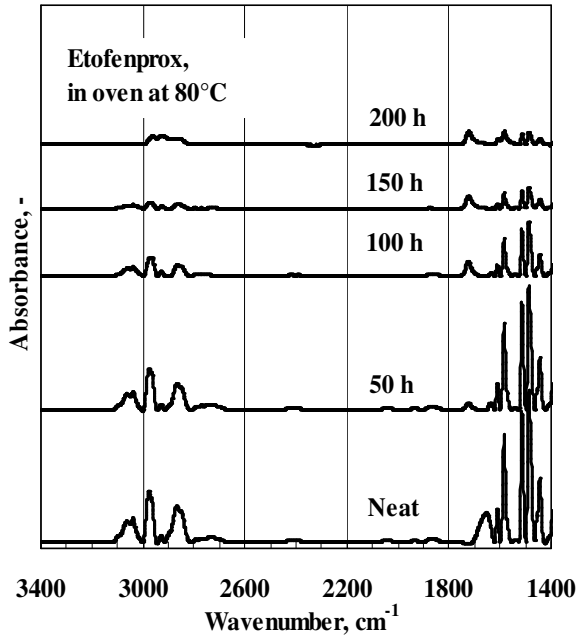

Propoxur,
in oven at 80°C
Neat
50 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

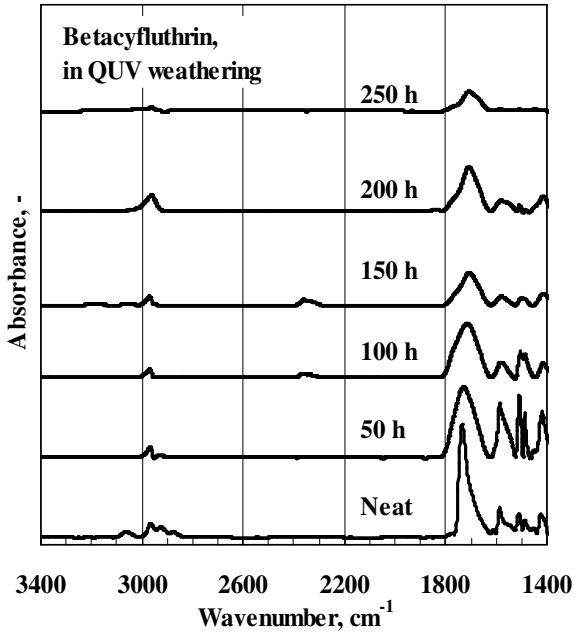

Pirimiphos-methyl,
in oven at 80°C
Neat
50 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

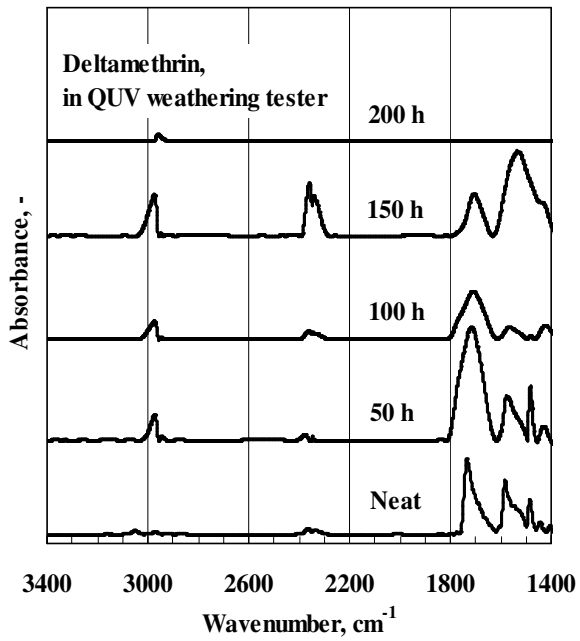

Malathion,
in oven at 80°C
Neat
50 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

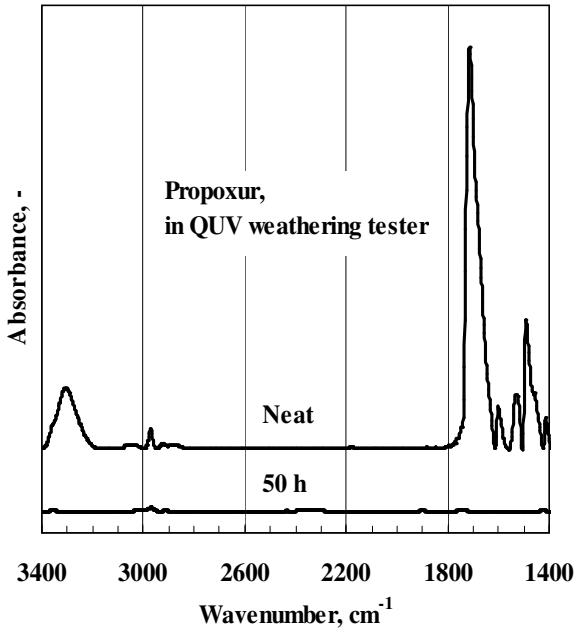

Bifenthrin,
in QUV weathering tester
250 h
200 h
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

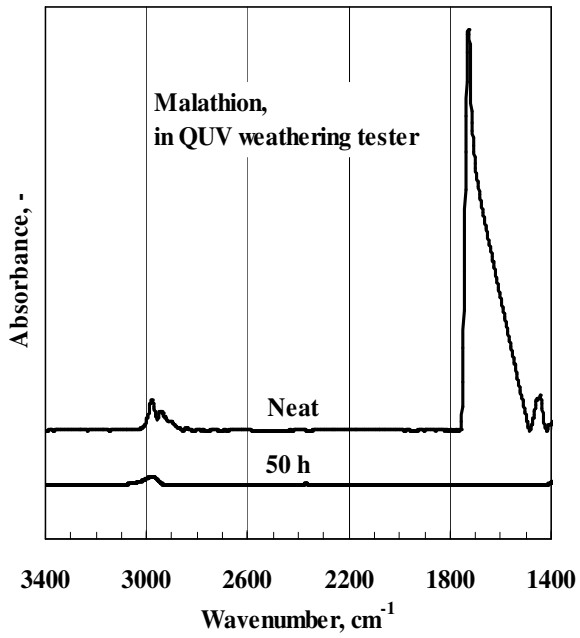

Lamdacyhalothrin,
in QUV weathering tester
250 h
200 h
150 h
100 h
50 h
Neat
Absorbance,-
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

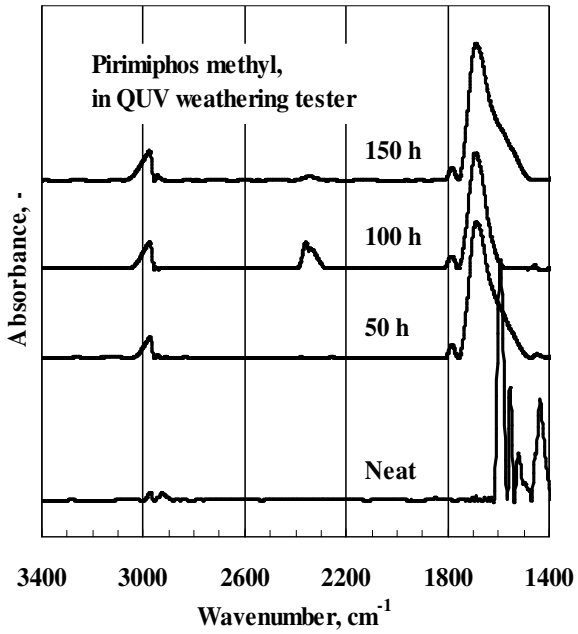

Etofenprox,
in oven at 80°C
200 h
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

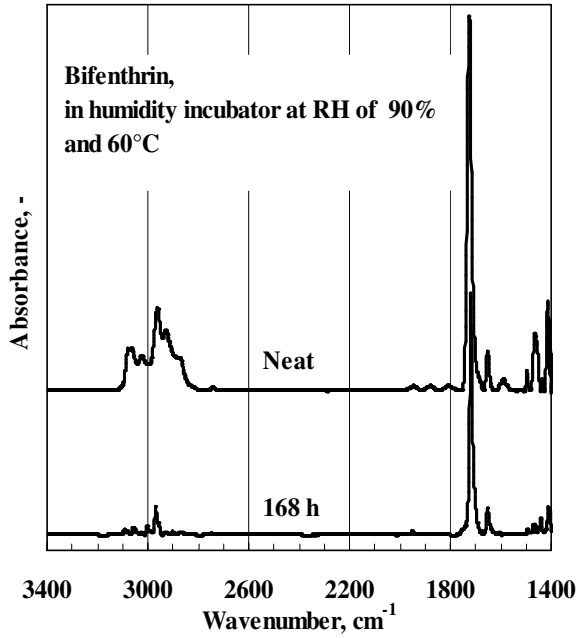

Betacyfluthrin,
in QUV weathering
250 h
200 h
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

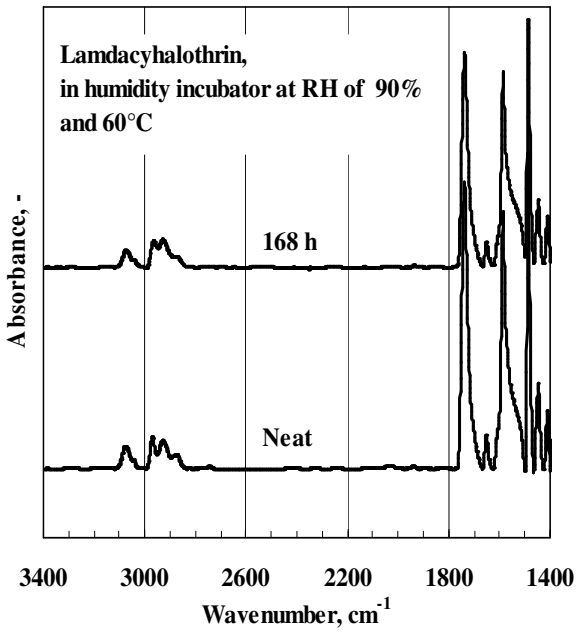

Deltamethrin,
in QUV weathering tester
200 h
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

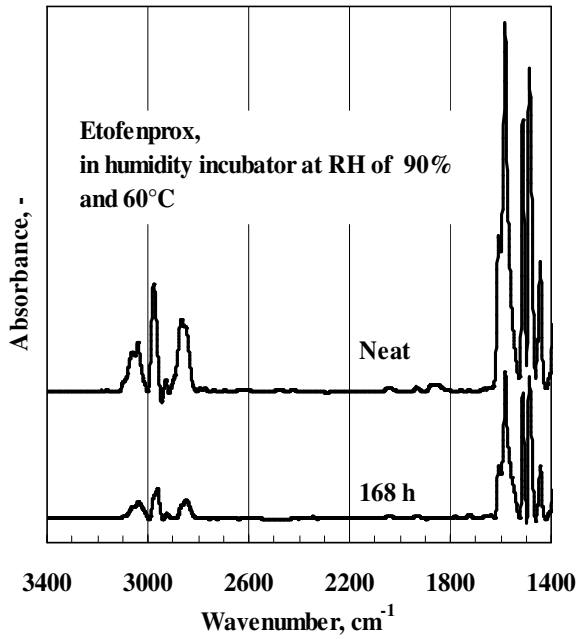

Propoxur,
in QUV weathering tester
Neat
50 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

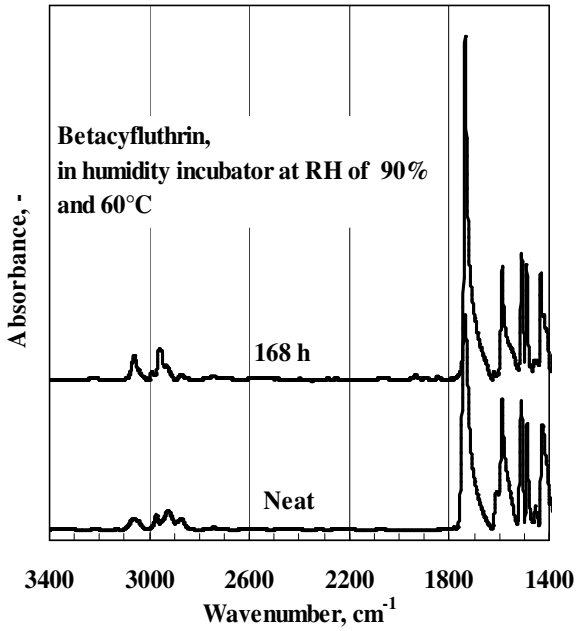

Malathion,
in QUV weathering tester
Neat
50 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

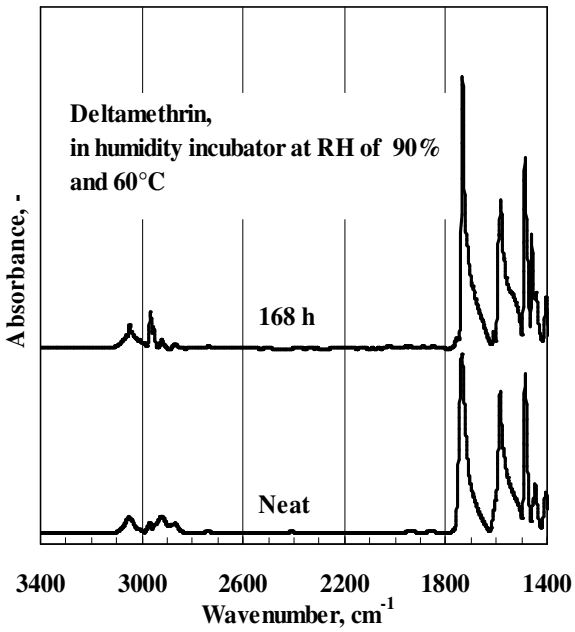

Pirimiphos methyl,
in QUV weathering tester
150 h
100 h
50 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

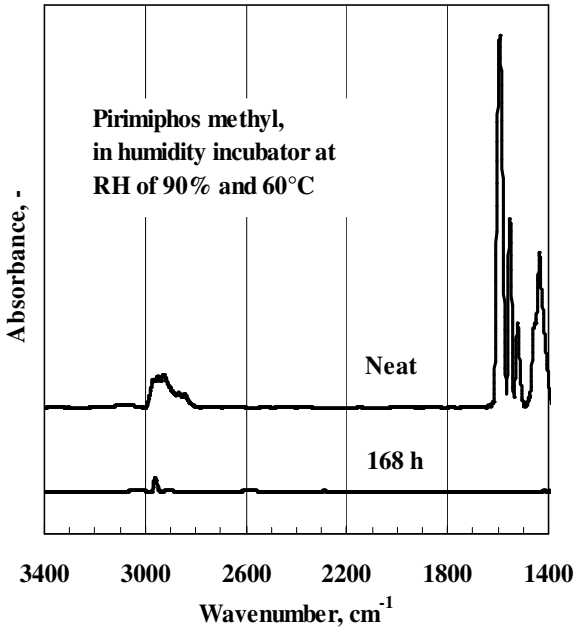

Bifenthrin,
in humidity incubator at RH of 90%
and 60°C
Absorbance, -
Neat
168 h
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1


Lamdacyhalothrin,
in humidity incubator at RH of 90%
and 60°C
Absorbance, -
168 h
Neat
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1


Etofenprox,
in humidity incubator at RH of 90%
and 60°C
Absorbance, -
Neat
168 h
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1


Betacyfluthrin,
in humidity incubator at RH of 90%
and 60°C
Absorbance, -
168 h
Neat
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1


Deltamethrin,
in humidity incubator at RH of 90%
and 60°C
168 h
Neat
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1


Pirimiphos methyl,
in humidity incubator at
RH of 90% and 60°C
Neat
168 h
Absorbance, -
3400
3000
2600
2200
1800
1400
Wavenumber, cm-1

8.5. Appendix E: Raw bioassay results

8.5.1. Control

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	0	0	0	0	0	0	0	0	0	0
1 week	0	0	0	0	0	0	0	0	0	0
1 month	0	0	0	0	0	0	0	0	0	0
2 months	0	15	8	8	8	4	27	12	14	12
3 months	0	0	0	0	0	0	0	0	0	0
4 months	0	0	0	0	0	4	16	8	9	6

8.5.2. DDT

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	71	74	68	71	3	75	100	68	81	17
1 week	70	92	96	86	14	96	100	100	99	3
1 month	88	61	73	74	14	100	48	88	79	30
2.5 months	0	0	0	0	0	5	0	0	2	3
3 months	8	0	0	3	4	15	4	4	8	7
4 months	0	12	28	13	14	0	4	4	3	2

8.5.3. Alphacypermethrin

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	96	92	100	96	0
1 week	83	83	32	66	30	42	80	0	41	40
1 month	100	85	78	88	11	97	97	56	83	24
2 months	100	42	32	58	37	85	58	36	60	24
3 months	23	0	0	8	13	58	4	4	22	31
4 months	25	0	0	8	14	0	4	12	5	6

8.5.4. Propoxur + binder

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	100	100	100	100	0	100	100	10	100	0
1 month	100	100	92	97	4	100	100	85	95	9
2 months	33	88	25	49	19	64	4	15	28	32
3 months	36	0	7	14	19	64	4	15	28	32
4 months	36	0	8	15	19	16	8	4	9	6

8.5.5. Bendiocarb + phosphogypsum + binder

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	76	62	52	63	12	96	96	92	95	2
1 month	0	0	0	0	0	0	0	0	0	0
2 months	4	21	0	8	11	12	13	21	15	5
3 months	0	0	0	0	0	36	4	8	16	18
4 months	0	0	0	0	0	0	4	12	5	6

8.5.6. Bendiocarb + phosphogypsum

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	100	96	96	97	2	100	100	100	100	0
1 month	82	42	17	47	33	100	92	83	92	8
2 months	8	39	4	17	19	40	71	26	46	23
3 months	34	7	0	14	18	100	100	81	94	11
4 months	42	8	0	16	22	8	0	8	5	5

8.5.7. Bendiocarb + binder

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	100	100	100	100	0	100	100	100	100	0
1 month	84	79	100	88	11	88	54	100	81	24
2 months	29	54	89	57	30	39	31	100	57	38
3 months	0	0	0	0	0	4	0	19	8	10
4 months	0	0	0	0	0	0	0	4	1	2

8.5.8. Alphacypermethrin + phosphogypsum on manure

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	**2**	**3**			**1**	**2**	**3**		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	100	87	86	91	8	100	100	100	100	0
1 month	100	100	100	100	0	100	100	100	100	0
2 months	100	88	92	93	6	100	100	100	100	0
3 months	100	100	100	100	0	100	100	100	100	0
4 months	100	100	100	100	0	100	100	100	100	0
6 months	100	100	100	100	0	100	100	100	100	0
7 months	100	100	100	100	0	100	100	100	100	0

8.5.9. Bendiocarb + phosphogypsum on manure

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	92	91	94	5	100	100	100	100	0
1 week	100	100	90	97	5	100	100	100	100	0
1 month	13	100	31	48	46	100	100	100	100	0
2 months	28	0	15	14	14	100	0	19	40	53
3 months	100	100	100	100	0	96	96	92	95	2
4 months	100	85	76	87	12	100	100	100	100	0
6 months	100	100	100	100	0	100	100	100	100	0
7 months	100	100	100	100	0	100	100	100	100	0

8.5.10. DDT on manure

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	100	100	100	100	0	100	100	100	100	0
1 week	100	100	100	100	0	100	100	100	100	0
1 month	57	29	38	41	14	91	79	58	76	17
2 months	88	47	54	63	22	53	60	17	43	23
4 months	9	40	45	31	20	52	48	50	50	2
6 months	0	0	0	0	0	12	0	8	7	6

8.5.11. Control (Untreated cattle manure surface)

Ageing period	Knock down after 60 min, %			Mean knockdown after 60 min, %	SD	Mortalities after 24 h, %			Mean mortality after 24 h, %	SD
	1	2	3			1	2	3		
Neat	0	0	0	0	0	0	0	0	0	0
1 week	0	4	0	1	2	4	11	0	5	6
1 month	0	0	0	0	0	0	4	0	1	2
2 months	0	0	0	0	0	0	0	0	0	0
3 months	0	0	0	0	0	0	0	0	0	0
4 months	0	0	0	0	0	0	0	0	0	0
6 months	0	0	0	0	0	0	0	0	0	0
7 months	0	0	0	0	0	0	0	0	0	0

8.6. Appendix F: Logistics regression of bioassay knockdown and mortality

8.6.1. DDT on clay (10 wt.% in acetone)

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 3.5771$ $\beta = -0.0701$

Indices: $\lambda_1 = e^{\alpha} = 35.7687$ $\lambda = e^{\beta} = 0.9323$

Odds	Time	Probability
21.902	7	0.956
5.028	28	0.834
0.086	86	0.079
0.020	107	0.019

80% cut-off point time and 95% confidence interval: 31.3 24.4 – 38.1

95% cut-off point time and 95% confidence interval: 9.0 -0.4 – 18.4

8.6.2. Alphacypermethrin + phosphogypsum

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 3.4276$ $\beta = -0.0610$

Indices: $\lambda_1 = e^{\alpha} = 85.9744$ $\lambda = e^{\beta} = 0.9409$

Odds	Time	Probability
85.974	0	0.989
56.107	7	0.982
15.594	28	0.940
2.828	56	0.739
0.335	91	0.251
0.088	113	0.080

80% cut-off point time and 95% confidence interval: 50.3 44.1 – 56.5.

95% cut-off point time and 95% confidence interval: 24.8 15.4 – 34.1.

8.6.3. Propoxur + binder

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 4.4540$ $\beta = -0.0610$

Indices: $\lambda_1 = e^{\alpha} = 85.9744$ $\lambda = e^{\beta} = 0.9409$

Odds	Time	Probability
85.974	0	0.989
56.107	7	0.982
15.594	28	0.940
2.828	56	0.739
0.335	91	0.251
0.088	113	0.080

80% cut-off point time and 95% confidence interval: 50.3 44.1 – 56.5

95% cut-off point time and 95% confidence interval: 24.8 15.4 – 34.1

8.6.4. Bendiocarb + binder

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 4.1667$ $\beta = -0.0727$

Indices: $\lambda_1 = e^{\alpha} = 64.5054$ $\lambda = e^{\beta} = 0.9299$

Odds	Time	Probability
64.505	0	0.985
38.791	7	0.975
8.436	28	0.894
1.103	56	0.525
0.087	91	0.080
0.018	113	0.017

80% cut-off point time and 95% confidence interval: 38.3 33.1 – 43.4

95% cut-off point time and 95% confidence interval: 16.8 9.1 – 24.6

8.6.5. Bendiocarb + phosphogypsum + binder

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 3.7406$ $\beta = -0.0594$

Indices: $\lambda_1 = e^{\alpha} = 42.1222$ $\lambda = e^{\beta} = 0.9423$

Odds	Time	Probability
42.122	0	0.977
27.797	7	0.965
0.190	91	0.159
0.051	113	0.049

80% cut-off point time and 95% confidence interval: 39.6 28.9 – 50.4

95% cut-off point time and 95% confidence interval: 13.4 -1.4 – 28.2

8.6.6. Bendiocarb + phosphogypsum

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 4.2930$ $\beta = -0.0738$

Indices: $\lambda_1 = e^{\alpha} = 73.1852$ $\lambda = e^{\beta} = 0.9288$

Odds	Time	Probability
73.185	0	0.987
43.648	7	0.978
9.259	28	0.903
1.171	56	0.539
0.017	113	0.017

80% cut-off point time and 95% confidence interval: 39.4 34.1 – 44.6

95% cut-off point time and 95% confidence interval: 18.3 10.0 – 26.5

8.6.7. DDT on manure (10 wt.% in acetone)

Model: Odds = $\lambda_1\lambda^t$

Logit parameters: $\alpha = 4.5319$ $\beta = -0.1072$

Indices: $\lambda_1 = e^{\alpha} = 92.9392$ $\lambda = e^{\beta} = 0.8983$

Odds	Time	Probability
92.939	0	0.989
48.837	6	0.980
4.614	28	0.822
0.540	48	0.351

80% cut-off point time and 95% confidence interval: 29.3 25.3 – 33.4

95% cut-off point time and 95% confidence interval: 18.3 8.1 – 21.5

8.7. Appendix G: DDT spraying in Venda area of Limpopo province, South Africa

Note the white flow marks are due to DDT