RELEASE KINETICS OF A SYNTHETIC TSETSE ALLOMONE BASED ON WATERBUCK ODOUR FROM A TYGON SILICON DISPENSER

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DECLARATION

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DEDICATION

This work is dedicated to my parents, Shem and Sarah Ndumbú.

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ABSTRACT

This study was conducted in order to develop an appropriate dispenser for the waterbuck-derived repellent for tsetse flies. The waterbuck-derived repellent consisted of pentanoic, hexanoic and heptanoic acids, guaiacol, geranylacetone, 2-undecanone and δ-octalactone. The reservoir tube of the dispenser was made from aluminium (or polypropylene) of diameter 10 mm and length 10 cm. The diffusion area was made from tygon silicon tubing of internal diameter 6.4 mm, outer diameter 9.6 mm, thickness 3.2 mm and length 2 cm (diffusion area 6.028 cm^2) or 4 cm (diffusion area 12.056 cm^2).

Preliminary trials were conducted under semifield conditions with a synthetic repellent (2-methoxy-4-methyl phenol) to determine the effect of surface area on the release rates. The rates were found to be directly related to the surface area of the tygon tubing. Increasing the surface area increased the weight loss of 2-methoxy-4-methyl phenol. These trials enabled the selection of appropriate lengths of the tubing to be used in both the laboratory and semifield trials with the waterbuck-derived repellent blend.

Laboratory tests were conducted in a two choice wind tunnel in which the windspeed was maintained constant at 20cm/sec while the room was maintained at 24 ± 1°C and 65 ± 5 % relative humidity. The compounds were dispensed either singly or as a blend from the dispensers with 6.028 cm^2 diffusion area. The weight loss of the individual compounds was assessed gravimetrically and the release of the individual compounds in the blend quantitatively determined by gas chromatography (GC). Zero-, first- and second-order rate models were tested to determine the release kinetics of the individual compounds and the blend. Comparison of the models using correlation

coefficients (r^2) indicated that the release of the individual compounds followed first-order kinetics while the release of the blend followed zero-order kinetics.

In the semifield trials, dispensers were placed either under direct sunlight or under the shade. Weight loss was assessed gravimetrically and the release of the blend compounds quantitatively determined by GC. The individual compounds dispensed singly followed first-order release kinetics while the blend of the compounds followed zero-order release kinetics. It's however interesting to note that the release of the individual components of the blend-mixture follows zero-order kinetics under semi-field conditions contrary to the behaviour exhibited by the individual components dispensed singly. The repellents placed in dispensers exposed to direct sunlight exhibited higher rate constants than those in the dispensers placed in the shade. The rate of release was found to be slightly higher during the first 24 hours and then became steady, obeying Fick's law of diffusion. The release rates were observed to depend on the surface area of the tygon tubing and generally increased with temperature. Semifield data was more variable than laboratory data due to the changing temperature conditions in the field.

Rate constants established under laboratory conditions were slightly lower than those obtained under semifield conditions. The results indicate that temperature could be the major environmental determinant of release rates with other variables like relative humidity having little or no effect. However, the magnitude of the effect of temperature on the release rates was not easily demonstrated with the field data. It is thus evident that the release of the compounds was not a simple function of temperature; with the release rates at higher temperatures being lower than would be expected. The zero-order rate

equation best described the release of the blend, which was found to be diffusion-controlled. Controlled release of the blend was therefore achieved using the dispenser.

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DCM: Dichloromethane

EAG chromatography: Electroantennographic chromatography

ECD: Electron Capture Detector

FAO: Food and Agriculture Organisation

FDA: Food and Drug Administration

FID: Flame Ionisation Detector

GC: Gas Chromatography

GMP: Good Manufacturing Practises

IAEA: International Atomic Energy Agency

ICIPE: International Centre of Insect Physiology and Ecology

ILRI: International Livestock Research Institute

k_o = zero-order rate constant (g day^{-1})

k_1 = first-order rate constant (day^{-1})

k_2 = second-order rate constant (g^{-1} day^{-1})

ppb: parts per billion

PVC: polyvinylchloride

R: gas constant (8.314J/Kmol)

r^2: Pearson Correlation Coefficient

SD: Standard Deviation

TCD: Thermal Conductivity Detector

UV: ultra violet

VTS: Volatile trapping system

WHO: World Health Organisation

ΔH = Enthalpy of vaporization (kJ)

CHAPTER ONE

1.0 INTRODUCTION

Tsetse flies belong to the genus *Glossina,* family *Glossinidae* in the order *Diptera.* The genus is subdivided into three subgroups i.e. *fusca, palpalis* and *morsitans* and the genus is currently considered to include 22 species and 14 subspecies (FAO, 1982). The *fusca* which is the largest subgroup consisting of 12 species and 4 subspecies is mainly associated with forest habitat and can be heavily infected with trypanosomes and maintain the sylvatic cycle among the wild host reservoirs (Oloo, 2000). The *palpalis* group which consists of 5 species and 7 subspecies is associated with the rain forests and extends into the savanna, where they are efficient transmitters of human sleeping sickness parasites. The *morsitans* group which comprises of 5 species and 3 subspecies is closely associated with transmission of the animal trypanosomiasis to both livestock and wildlife.

Tsetse flies have a fascinating reproductive biology because the entire egg and larval development takes place in the female (adenotrophic viviparity). During her lifespan, the female can give birth to a maximum of 8 to10 offsprings (Jordan, 1993) and the entire life cycle from egg to adult takes about 30 days.

Tsetse flies have shown varying degrees of specialization in their feeding habits (Moloo, 1993). In Kenya's Lambwe Valley, a study showed that the dominant *Glossina pallidipes* derived 80% of their feed from bushbuck, buffalo and bushpig, but hardly fed on the common game species such as oribi, impala, waterbuck and reedbuck. The most preferred host for the *fusca* group based on bloodmeal analysis is the bushpig, *Potamochoerus porcus,* hippopotamus, *Hippopotamus amphibious,* bushbuck, *Tragelaphus scriptus,* buffalo, *Syncerus caffer* and cattle, *Bos spp* (Moloo, 1993). For the

palpalis group, the most preferred hosts are man and bushbuck, followed by domestic pig, *Sus scrofa,* and monitor lizard, *Varanus niloticus* (Moloo, 1993; Gikonyo, 1999). The *morsitans* group prefers the warthog, *Pharcochoerus aethiopicus*, cattle, buffalo, followed by bushbuck, bushpig and man (Moloo, 1993). Host body size and mass are known to affect close range attraction and landing behaviour of tsetse but these cannot account for the feeding preference (Vale, 1974). The unpreferred hosts of tsetse have been shown to emit chemicals that repel flies from a distance and others that deter flies from feeding (Gikonyo *et al.,* 2002)

According to the World Health Organisation (1996), about 60 million people and 46 million cattle are at risk of sleeping sickness. Due to budget cuts by governments and donor agencies, only 3 to 4 million of those at risk are screened for the disease. According to the WHO (1996), conflict zones and remote areas, especially the zone between Southern Sudan and Angola, including the Democratic Republic of Congo, have been registering sleeping sickness as the leading cause of death ahead of HIV/AIDS.

Because of the tsetse fly, many African farmers are not able to use animals for ploughing (Okhoya, 2003). Apart from the limited use of draft power, soil fertility has also declined from lack of manure. In Asia, an estimated 50% of crop production benefits from the power of draught animals compared to Africa where only 5 to 10% of crop production benefits from animal draught power. This has adversely affected crop production in 10 million km^2 of fertile land in 32 countries in Africa.

1.1 Geographical Distribution of the Tsetse Fly

The distribution of the tsetse fly is determined mainly by climate, altitude, vegetation and presence of suitable hosts (Leak, 1998). Their geographical distribution lies between 15^0N and 20^0S and covers an area of about 10 million km^2 (Nash, 1969). The tsetse fly thus occupies vast areas of Africa that have a great potential for agriculture. A quarter of Kenya is infested by the tsetse fly (ILRAD, 1990). In Kenya, there are 8 species of the tsetse fly (Owaga *et al*, 1995). The species include *G.palpalis* Fuscipes, *G.pallidipes* Austeni, *G.sywnertoni* Austeni, *G.morsitans* Westwood causing human and animal trypanosomiasis, and *G.longipennis* Newstead, *G.austeni* Newstead, *G.brevipalpis* Newstead and *G.fuscipleuris* Austeni causing animal trypanosomiasis. The geographical distribution of the tsetse species in Kenya is shown in Figure 1.

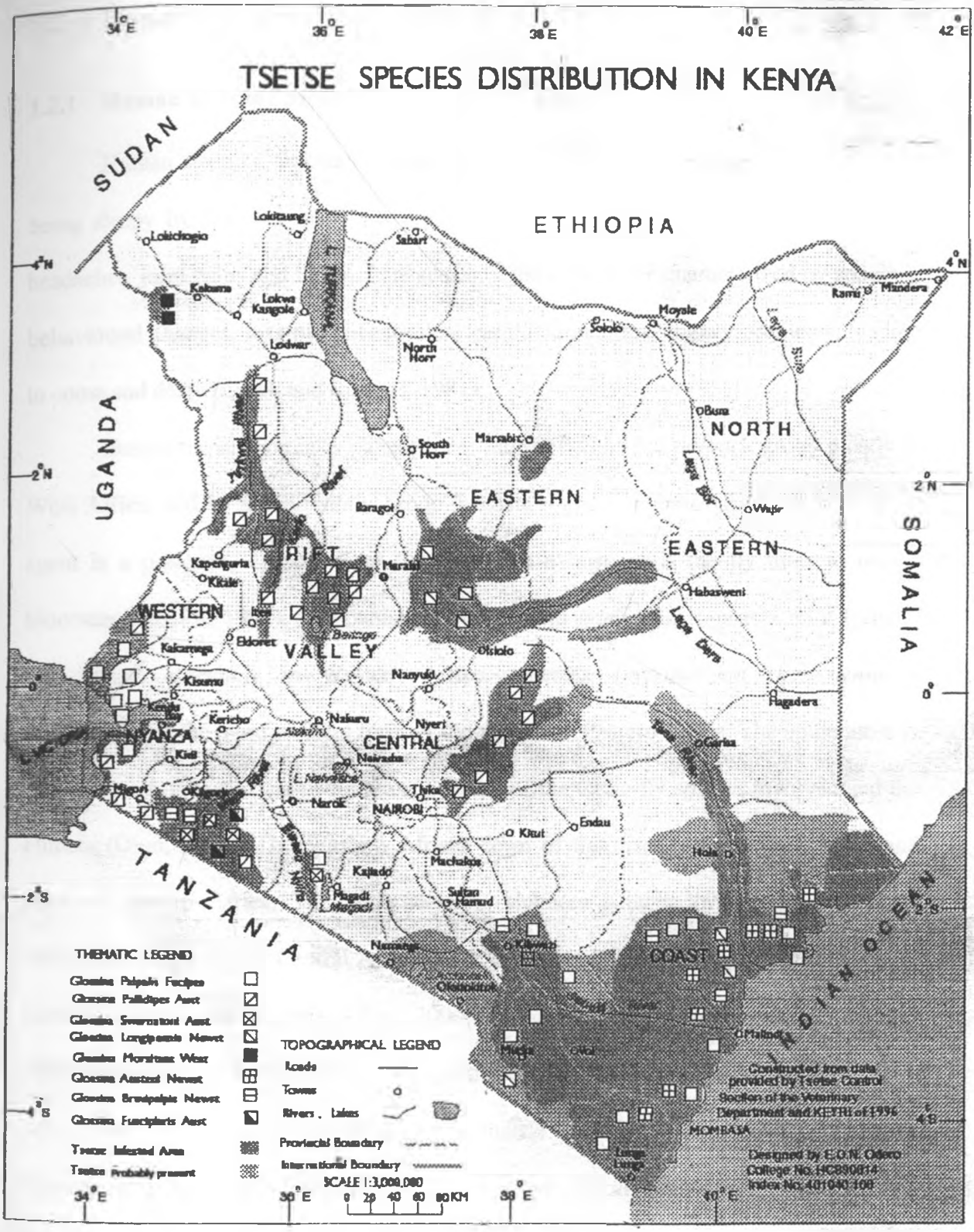

Figure 1: Geographical distribution of the tsetse fly in Kenya (Odero, E.O.N, 1999)

1.2 Impact of the Tsetse Fly in Africa

1.2.1 Human Sleeping Sickness

Human sleeping sickness is a fatal disease which is characterised by the patients being sleepy by day and restless at night. Other symptoms are high fever, weakness, headaches, joint pains and itches. Late stages of the disease are characterized by anaemia, behavioural changes, loss of concentration, cardiovascular and kidney problems, leading to coma and death (Leach and Roberts, 1981).

Human trypanosomiasis parasites are transmitted by the *palpalis* group mostly in West Africa, and by the *morsitans* group in Eastern and Southern Africa. The causative agent is a protozoa, *Trypanosoma*, which enters a host when the fly bites to take a bloodmeal (ICIPE, 1992). The parasite *Trypanosoma gambiense* causes a mild form of the disease especially in Western Africa, whereas *Trypanosoma rhodesiense* is responsible for the more virulent form of the disease in Eastern Africa. The epidemiology of the disease varies in the two regions because of the tsetse fly species involved and the climate (Oloo, 2000). The Western African form of the disease spread to Central and parts of Eastern Africa. The main sleeping sickness parasite in Eastern Africa, *T. rhodesiense* spread from Central Africa and the Zambezi Valley northwards to Tanzania, Uganda, Kenya and Ethiopia (Oloo, 2000). In Eastern Africa, both the *palpalis* and *morsitans* subgroups are involved in the transmission of the disease parasites.

The first records of human trypanosomiasis in Kenya date to early 20th century when an epidemic, which began in 1901 on the shores of Lake Victoria in Uganda spread into Western Kenya along the Nyanza coastline (Otieno and Darji, 1985). It spread up the tsetse infested rivers and *T. brucei gambiense* sleeping sickness became endemic in South

Nyanza. Insecticidal control of the vector, *Glossina fuscipes* between 1952 and in the period 1955-1957 put a stop to *T.b gambiense* sleeping sickness along the rivers (Willet *et al.*, 1965). It was not until 1953-54 that *T.b rhodesiense* was discovered in Western Kenya. In the period 1959-60, a similar disease appeared in the Lambwe Valley of South Nyanza which is inhabited by *G. pallidipes*. Lambwe Valley remains an active focus of sleeping sickness inspite of repeated insecticide spraying (Otieno and Darji, 1985). By 1996, the prevalence of the disease had returned to the 1930's levels in Africa (WHO, 1996).

1.2.2 Animal Trypanosomiasis

Animal trypanosomiasis is also known as "nagana". It is the most widespread livestock disease in Africa and the range of infection extends beyond the tsetse fly belt. The area infested with tsetse flies in Africa is estimated to be 10.8 million km^2 extending through 32 countries with 30% of the approximately 46 million cattle at risk (Oloo, 2000). The disease is caused by *T.brucei, T.vivax, T.theleiri, T.congolense* and *T.evansi* (Owen, 1991). The symptoms of the disease include pathological conditions like anaemia, lymphoid cell proliferation, immune suppression and circulatory disturbance, high temperature, progressive weakness and gradual wasting, leading to death (Leak, 1998). The disease interferes with animal reproduction causing abortion and infertility.

Death of about 3 million cattle annually has been reported with direct production losses estimated at between US $0.6-1.2 billion annually (ICIPE, 1992). Livestock production has been reduced by 20-40%. Urquhart (1984) suggested that 120 million extra cattle, 150 million extra sheep and 250 million extra goats could be reared if tsetse was eliminated and problems like water availability and disease control tackled.

1.3 Tsetse Control and Eradication

1.3.1 Tsetse Eradication

Tsetse eradication involves the removal of the fly over its entire distribution. This is possible when the area infested by the flies is not too large and is naturally isolated from other infested areas (Allsopp, 1984). Flies from outside an eradication area are prevented from entering the area by maintaining a deliberate man-made barrier of traps, targets, insecticide or clearing vegetation (Allsopp, 1984; FAO, 1986). Eradication of tsetse has been reported only in specific circumstances for example in the island of Principe, an isolated fly belt in Zululand, parts of northern Nigeria and the island of Unguja in Zanzibar (Saleh *et al.,* 1997). Tsetse control rather than eradication is the only practical way of reducing the level of the trypanosomiasis challenge which will in turn allow for effective landuse practises involving livestock.

1.3.2 Tsetse Control

Tsetse control aims at reducing disease challenge by maintaining the flies at low levels. This is achieved by use of several methods including bush clearing, game exclusion and control, bait technology, screens, traps and targets, ground and aerial spraying, use of pour-ons, use of repellents, use of predators and parasites, sterile insect technique, chemotherapy and rearing of trypanotolerant cattle. However, the high mobility of the savanna species means that re-invasion into control zones is a constant problem (Dransfield *et al.*, 1990; Brightwell *et al.*, 1992).

1.3.2.1 Bush Clearing

Cutting down the bush removes the cool, humid shades which are the resting sites of the tsetse. Bush clearing aims at making a barrier against spread of tsetse, and also cutting off infested areas to protect villages having a bad record of sleeping sickness (FAO, 1986). This exercise is not only too costly in terms of labour but it also promotes soil erosion and is no longer being used.

1.3.2.2 Game Exclusion and Control

The killing of game animals removes all or much of the food source of tsetse, and hence the main reservoir of trypanosomiasis. A major game destruction exercise in Zimbabwe successfully stopped an advance of *G.morsitans* (FAO, 1992). This method has proved to be unsuitable since tsetse flies can shift their feeding preferences once their regular food source is removed (FAO, 1992).

1.3.2.3 Bait Technology

Control of tsetse flies has been facilitated greatly by the development of efficient devices, baited with attractive odours (Odulaja et al., 1998). Vale (1977a), demonstrated in Zimbabwe that ox breath is an effective odour bait for *Glossina morsitans* and *Glossina pallidipes*. Other baits include 1-octen-3-ol (Hall *et al.,* 1984), acetone, butanone and phenols (Owaga, 1985; Torr *et al.,* 1997).

1.3.2.4 Screens, Traps and Targets

Screens are made from pieces of black or blue cloth that are treated with insecticide. They are placed in a tsetse habitat and kill the flies that come into contact

with them (Oloo, 2000). Impregnating the screens with 0.1 % deltamethrin gives it a knockdown effect that persists for upto 4 months and 0.6-0.8 % can last upto 12 months on a stable fabric (Torr et al., 1992).

Insecticide impregnated blue and black cloth targets are now in widespread use for the control of savanna tsetse and a variety of traps and targets are also being used for the control of the riverine species (Leak *et al.*, 1995). Odour baited traps have a great potential for tsetse control and as monitoring aids (Owaga, 1985). Targets differ from traps in having no holding cage where the flies are retained until they die and are only used for control when impregnated with insecticides.

1.3.2.5 Ground and Aerial Spraying

The aim of spraying is to place a persistent insecticide onto the natural resting places of the tsetse so that they are killed if they settle on the deposit (FAO, 1986). The insecticides used are dichloro-diphenyl-trichloroethane (DDT) and dieldrin. In Nigeria, ground spraying with DDT led to the reclamation of 32,000 sq.km of land from *G.morsitans submorsitans, G.palpalis palpalis* and *G.tachynoides* (Ford and Okiwelu, 1977). Application of insecticides from fixed wing aircraft or helicopters has been done on a large scale for tsetse control in Africa. Aerial spraying has been used successfully in Zululand (Ford and Okiwelu, 1977). Insecticides used are endosulfan, DDT and dieldrin. Application of these insecticides has however led to environmental concerns.

1.3.2.6 Use of Pour-Ons

Pour-ons are various formulations like deltamethrin (Decatix), alfacypermethrin (Renegade) and cyfluthrin (Cylence) which when applied to cattle give a tsetse knockdown above 50% for 5-24 days and 24-55 days in hot and cool months, respectively (Vale *et al.*, 1999). The average knockdown was 77-86% (deltamethrin), 74% (alfacypermethrin) and 59% (cyflumethrin) in a study by Vale *et al.*, (1999) in Zimbabwe. Work done in Burkina Faso and Zimbabwe shows that deltamethrin Spot On formulation persists for about 100 days (Bauer *et al.*, 1992).

1.3.2.7 Use of Predators and Parasites

Natural parasites and pathogens are used to reduce the insect population although no large-scale field application of this method to tsetse flies has been done (Oloo, 2000). Attempts have been made to contaminate the flies with fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Oloo, 2000), and to expose tsetse pupa and adults to bacteria, protozoa, viruses and nematode worms (FAO, 1982). Insect predators like *Syntomosphyrum* and *Mutilla* (both Hymenoptera) and *Thyridanthrax* (Diptera) (FAO, 1982; 1986) have also been tested.

1.3.2.8 Sterile Insect Technique

Wild tsetse flies are sterilised using hormones and insect growth regulators like diflubenzuron and juvenile hormone mimics like pyriproxyfen and triflumuron (Hargrove and Langley, 1990). These agents are placed on targets and traps where the flies pick them up and pass the chemicals to others in the field by physical contact (Oloo, 2000).

These chitin synthesis inhibitors disrupt the reproductive cycle by inhibiting chitin synthesis and causing abortion.

In sterile insect technique (SIT), sterilised males are released in a ratio that exceeds that of a natural population thereby increasing the chances of wild females mating with the sterilised males to produce non-viable offsprings. Temporary eradication has been achieved for *G.palpalis* in Burkina Faso (Politzar and Cuisance, 1982), *G.morsitans* in Mkwaja Ranch, Tanzania (Williamson et al., 1983) and in a 1500 sq.km agropastoral land in Nigeria (Oladunmade, 1990). *Glossina austeni* was declared eradicated from Unguja Island in Zanzibar at the end of 1997 (IAEA, 1997). In all cases, the wild population was first suppressed by over 90% using other control methods.

1.3.2.9 Chemotherapy and Rearing Trypanotolerant Breeds

Several drugs are used for treatment of *nagana* including Ethidium, Isomethamidium and Berenil (Leach and Roberts, 1981), which intercalate into the DNA and are suspected to be mutagenic. Human trypanosomiasis is treated in the early stages with either Pentamidine or Suramin and with other organic arsenicals like Arsobal, Melarsoprol and Mel B for the late stages of the disease (Okhoya, 2003). The later treatment is dangerous and may lead to 5 -10 % fatalities. Resistance to some drugs has been reported (Okhoya, 2003). Melarsoprol, which was developed 50 years ago, induces serious and sometimes fatal side effects. A new drug, Eflornithine, originally developed as an anticancer agent, has shown promising results against the *gambiense* form of the disease (Okhoya, 2003).

Trypanotolerant breeds of cattle like the *B.taurus* subtypes, N'Dama and Baoulè, and the *B.indicus* zebu breeds like the Orma Boran and the Maasai Zebu has been adopted to counter the tsetse challenge (Njogu *et al.*, 1985; Mwangi *et al.*, 1993).

1.3.2.10 Use of Repellents

The use of repellents is meant to prevent host-fly contact or the initiation of feeding by the fly. The search for repellents started in the 1940s when several compounds including plant extracts were tested, but they showed lack of persistence of the repellent activity (Hornby and French, 1973; Holden and Findlay, 1944; Findlay *et al.*, 1946). Later, other repellents such as N, N-dimethyl-m-toluamide (DEET), indalone, citronyl and those associated with hosts such as 2-methoxyphenol (from cattle urine), acetophenone, pentanoic and hexanoic acids (from cattle sebum) have shown variable repellence to tsetse flies (Schimdt, 1977; Wirtz *et al*, 1985; Torr *et al.*, 1996). However, none of these compounds is being used commercially due to lack of appropriate dispensing techniques on livestock.

1.4 Allomones from Waterbuck

Gikonyo *et al.*, (2000) studied the behaviour of caged individual teneral *G.m. morsitans* on waterbuck (a non host) and ox (a preferred host) and on feeding membranes with and without smears of different doses of waterbuck sebum. Flies that contacted the body of the waterbuck or areas of the membrane treated with different doses of sebum showed significant reluctance to feed, manifested by high proportions of flies departing, changing probing sites and general delays in the initiation of feeding compared to the ox and untreated zone of the membrane. This suggested presence of volatile and non-volatile allomones on waterbuck.

The odour composition of the preferred and non-preferred hosts revealed that waterbuck odour consisted of fewer aldehydes, but more phenolic components, δ-octalactone, moderate amounts of C_5-C_9 straight chain fatty acids and a series of methylketones (C_8-C_{13}) which were either not detected or present in trace amounts in the two preferred hosts (buffalo and ox). The preferred host odour comprised medium-chain, saturated or unsaturated aldehydes and phenols (Gikonyo *et al.*, 2002). It was proposed that the blend of waterbuck-specific odour compounds might function as a long- or medium-range allomone against tsetse flies. Subsequent wind tunnel experiments on *G.m. morsitans* corroborated the existence of a tsetse repellent blend in waterbuck body odour (Gikonyo *et al.*, 2003). Testing of the repellent in the field indicates that it reduces the number of tsetse flies attracted to the cattle and those that engorge (Bett, personal communication). There is therefore a need to develop an efficient method of dispensing the blend of repellent compounds from the waterbuck in the field.

1.5 JUSTIFICATION OF THE STUDY

Several methods have been used for the control of tsetse flies. Use of insecticides has undesirable effects on the environment and on non-target organisms (Manahan, 1994). Clearing bushland and elimination of wild hosts are unsustainable. Use of trypanocidal drugs is expensive and drug resistance has been reported, while vaccine development is being hampered by antigenic variation of trypanosomes. Other control methods are hampered by lack of resources as evidenced by the decreasing investment by governments and donors in tsetse control programmes.

An allomonal blend for the savanna group of tsetse flies was identified from waterbuck sebum and volatiles (Gikonyo *et al.*, 2000; 2002; 2003). Work currently going on in the field indicates that the waterbuck-based repellent blend reduces the proportion of flies attracted to the host (cattle) and those that feed by more than 80% (Bett, personal communication). The use of this natural repellent which is environmentally safe in conjunction with other control methods can lead to lower incidences of trypanosomiasis in cattle.

The aim of this project is to develop a suitable technology to dispense the waterbuck-based repellent for use by rural communities. Existing dispensers previously developed for pheromones are not suitable for use on cattle and they require modification to enable them to be used for tsetse control. In this project, a dispenser affordable to the rural poor communities who derive their livelihood on livestock will be developed. It should be easy to use, requiring minimum maintenance and should be consistent with the production practices of the rural pastoral communities who migrate from one place to another in search of pasture and water. For this reason, on-host dispensers would be more appropriate as opposed to off-host dispensers.

1.6 OBJECTIVES OF THE STUDY

1.6.1 Main Objective

To establish the release rates of the individual components and the blend of the waterbuck-based repellent (pentanoic, hexanoic and heptanoic acids, geranylacetone, 2-undecanone, δ-octalactone and guaiacol) under laboratory and field conditions with a view to developing a robust dispenser for the repellents.

1.6.2 Specific Objectives

1. Establish and develop an appropriate dispenser for the repellent.
2. Determine the release rates of the individual compounds of the repellent and in blend under laboratory conditions
3. Determine the release rates of the individual compounds of the repellent and in blend under field and semifield conditions
4. Establish the optimum release rates for the repellent.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 SEMIOCHEMISTRY

Semiochemicals (Gk. *semeon*, a signal) are chemicals that mediate information interactions between organisms. Semiochemicals are divided into allelochemicals and pheromones depending on whether interactions are interspecific or intraspecific, respectively. Pheromones are semiochemicals produced by individuals in a species that affect the behaviour of other individuals of the same species (Campion, 1984; Mori, 2001). Allelochemicals are significant to individuals of a species different from the source species, and they are subdivided into several groups depending on the behaviour involved in the response.

Pheromones are chemicals emitted by living organisms to send messages to individuals of the same species. Pheromone categories include sex pheromones which are produced by either male or female, to attract a mate for the purposes of mating. Aggregation pheromones attract conspecific insects but unlike sex pheromones, they attract individuals of both sexes for the purposes of aggregation. Anti-aggregation pheromones are produced by mass attacking insects like bark beetles as a means of avoiding overcrowding in the host tree. Oviposition and larviposition pheromones are chemicals that mediate information about laying eggs and depositing larvae respectively. Alarm pheromones are produced by many gregarious insects when attacked by a predator or parasite to warn conspecifics of the danger. Epidietic pheromones are used by insects to mark their oviposition site to help deter over exploitation of the resource, especially among the egg parasitic wasps that are solitary parasites. Pheromones were the first

semiochemicals to be studied and the class most widely explored is the sex pheromones, especially those produced by female moths (Lepidoptera), which are used to attract males for mating (Nordlund et al., 1981). Bombykol, the sex pheromone of the silkmoth was first synthesized in 1959 (Mori, 2001). Most pheromones consist of blends of two or more chemicals that need to be emitted at exactly the right proportions to be biologically active. The female effluvia or sex gland can contain additional compounds which are related to the pheromone components and whose biological function is often unclear. On the other hand, many attractants of male moths have been discovered simply by field screening of randomly selected chemical compounds. In several cases, it could later be shown that the attractants found using this technique were identical to the natural pheromone produced by the female. Attractants that mimic pheromones are referred to as parapheromones.

Allomones are allelochemicals that are produced by individuals of a species and affect the behaviour of individuals of another species to the benefit of the emitter. Kairomones are allelochemicals produced by individuals of a species that beneficially affect the behaviour of individuals of another species, to the detriment of the emitting species. Synomones are produced by individuals of a species and they affect the behaviour of individuals of another species to the benefit of both species. Apneumones are chemicals emitted by a non-living material on which one species is found to the detriment of the resident species. However, the chemicals emitted benefit the receiver (Mwangi, 2002; Francke and Sohulz, 1999). A summary of the categories and impact of the semiochemicals is shown in Table 1.

Semiochemicals are produced in trace amounts either as single compounds or in blend. With advances in the field of chemical ecology, it has become clear that semiochemicals possess great potential as components of pest management strategies (Golub and Weatherstorm, 1984). It has therefore become necessary to define precisely the blend emitted, the rates of production, release rates and to develop controlled release systems for use in control programmes (Baker and Linn, 1984). There have been many successful applications of semiochemicals to manage pests. They have been used to control bark beetles (Holsten *et al.*, 2002) and pine beetles in USA, sandfly in South and Central America, among other pests (Byers, 1988).

Table 1: Categories and impact of semiochemicals

CATEGORY	IMPACT OF THE SEMIOCHEMICAL	
	Emitter	Receiver
Intraspecific: Pheromones	+ / 0	+
Interspecific: Allomones	+	0 / -
Kairomones	-	+
Synomones	+	+
Apneumones	0	+

Key: + beneficial; - not beneficial; 0 no impact

2.2 HOST LOCATION BY TSETSE FLIES

Host selection by insects involves a series of steps: host-habitat finding, host finding, host recognition, host acceptance and host suitability (Strom *et al.*, 1999). These

steps lead to acceptance or rejection of a resource, in this case the host (Mozuraitis *et al.*, 2002). Tsetse flies locate their hosts by visual and olfactory cues (Gikonyo, 1999). The fly is attracted by the host's odour even when beyond it's visual range. In the vicinity of the host, visual cues like size, shape, colour and close range olfactory stimuli are used. On the host, the fly uses other stimuli sensed by touch, taste and thermoreception to determine suitability of the host and to locate a feeding site (Van der Goes van Naters *et al.*, 1998; Saini *et al.*, 1993). Thus tsetse flies locate stationary hosts beyond their visual range upwind (60-120 m) through odour-mediated anemotaxis (Vale, 1977a), with visual cues becoming important at close range (~10 m). Some of the long-range kairomones used by the tsetse fly to locate their hosts include carbon dioxide, acetone, 1-octen-3-ol (Hall *et al.*, 1984) and phenolic microbial breakdown products particularly, 4-methylphenol and 3-*n*-propylphenol from host skin and urine (Hassanali *et al.*, 1986; Gikonyo *et al.*, 2002).

2.2.1 Kairomones

In the 1970's and 1980's, research to identify tsetse fly attractants was conducted. The urine of several mammals was found to be attractive to various species of tsetse. Skin secretions were also found to contain olfactory attractants for tsetse flies. Components of ox breath attractive to tsetse flies such as carbon dioxide, acetone and 1-octen-3-ol were identified (Hall *et al.*, 1984). Carbon dioxide and acetone have been used to enhance trap catches. Catches were reported to increase further when the two attractants are used together (Vale, 1980). Hall *et al.*, (1984) showed that 1-octen-3-ol was the most potent olfactory stimulant in cattle breath.

Odours from cattle and buffalo urine increases by several times the numbers of *G. pallidipes* Austen and *G.m. morsitans* Westwood caught in traps or attracted to targets (Vale *et al.,* 1988). Much of the efficacy of the urine is due to phenols. Hassanali et al., (1986) identified seven phenols (phenol, 3-methoxyphenol, 4-methylphenol, 3-ethylphenol, 4-ethylphenol, 3-*n*-propylphenol and 4-*n*-propylphenol) from the urine of buffalo. 4-Methylphenol and 3-n-propylphenol were found to act synergistically and to be the most important for the attractancy of the urine (Vale *et al.,* 1988; Owaga *et al.*, 1988). According to Torr *et al.*, (1997), the most important attractants for practical purposes are acetone, butanone, 1-octen-3-ol and various phenols. Combinations of these have been used for tsetse control in Zimbabwe, Zambia and Kenya (Dransfield *et al.*, 1990)

2.2.2 Allomones

Defence by use of chemical substances or repellents is a well-known phenomenon to protect an organism from attacks by enemies (Mori, 2001). These substances are produced in trace amounts. The term 'repellent' is more widely used for chemicals, which elicit a combination of behavioural responses resulting in prevention of biting by an insect (Torr *et al.*, 1996). Davies (1985) suggested five sensory mechanisms by which these attraction inhibitors (repellents) might act:

(a) They interact with and inhibit the response of a sensory neuron to a normally attractive signal,

(b) They interact with their own specific receptors and can be attractants at low intensities. However, they become repellent at higher levels,

(c) They activate a receptor system that mediates a competing or inappropriate behaviour pattern,

(d) They activate specific 'noxious odour' repellent receptor, and

(e) They simultaneously activate several different receptor types that mediate various behaviour patterns so that any sensory signal specific to host finding is lost in the resulting barrage of sensory input.

Studies by Torr *et al.*, (1996), suggested that the repellents he studied might act by mechanism (e), causing a barrage of sensory input that jams any signal specific to host finding, whether olfactory, visual or other type.

In a study by Gikonyo et al. (2002) on the odour composition of waterbuck, certain waterbuck specific electroantennographic (EAG) active components, particularly 2-ketones and lactone were found to constitute a candidate allomonal blend in waterbuck odour. Some components, which were absent or present in trace amounts in odours of two preferred hosts, were present in the waterbuck odour, namely, δ-octalactone, 2-methoxyphenol (guaiacol), 3-isopropyl-6-methylphenol and a series of C_8-C_{13} methylketones. Guaiacol is moderately repellent to tsetse flies (Torr *et al.*, 1996; Vale *et al.*, 1988). In field studies, several C_6 and C_7 methylketones reduced tsetse fly catches although some of the lower homologues (C_3-C_4) were attractive (Vale, 1980). Thus Gikonyo *et al.*, (2002) concluded that the 2-methylketones in waterbuck odour together with guaiacol and the other two EAG-active constituents, acting additively or synergistically, could potentially constitute a long range allomonal barrier. Gikonyo *et al.*, (2003) demonstrated this synergistic activity in a wind tunnel.

Moderate amounts of C_5 –C_9 straight chain fatty acids were also present in the waterbuck odour (Gikonyo, 1999). Some of these acids reduced tsetse fly trap catches in the field (Vale, 1980), as did pentanoic and hexanoic acids (Torr *et al.*, 1996). Studies by

Torr *et al.* (1996) showed that low doses of 2-methoxyphenol, acetophenone, pentanoic and hexanoic acid reduce the catch of baited traps by 45-85 %. Lactic acid was also repellent when dispensed at 100mg/h. Results showed that 2-methoxyphenol was the most potent repellent of those tested, reducing trap catches by 85%. However, its repellent effect was not enhanced by adding either pentanoic acid or acetophenone. Adding 2-methoxyphenol and pentanoic acid to a trap resulted in a big decrease in trap efficiency. However, even though all the repellents studied halved the number of tsetse attracted to a host, none had an effect on landing and only pentanoic acid had a slight and significant effect on feeding.

Man produces a mixture of attractants and repellents (Vale, 1977). Vale showed that placing man adjacent to an ox halved the numbers of tsetse attracted and reduced the proportion that fed by 75%, giving an overall reduction in biting rate of 90% which was due to L-lactic acid.

2.3 Dispensers

A major consideration in constructing and developing dispensers is to ensure that the surface emits the compound or compounds of interest at a fairly constant rate over the intended period. This may be difficult for very volatile chemicals of low molecular weight, and so the dispenser as well as the length of time it is used before reloading and replacing it must be chosen properly (Baker and Cadre, 1984). A good dispenser ensures a steady and constant release rate of the odour for as long as the chemical is present in the dispenser. It should be sufficiently robust to operate under field conditions for long periods (FAO, 1992). The release rate should be independent of environmental conditions

such as temperature and wind strength. However in the field, release rates have been found to vary since temperature and humidity variations are large (Holsten *et al.*, 2002).

2.3.1 Types of Dispensers

The first dispensers that released pheromones used filter paper (Wood *et al.*, 1967) or metal tubes containing gas-liquid chromatographic packing to hold the compounds until they evaporate (Wood *et al.*, 1968). Several types of dispensers have since been developed for different semiochemicals.

One type uses various materials, such as rubber septa (Byers, 1988) and is widely used with moth pheromones. The release rates change dramatically and it is difficult to specify and vary the release rates without much measurement. A second method uses wicks dipped in a chemical (Tilden *et al*, 1979), but the inexact surface area and physical properties of wicks make it difficult to either specify or vary the release rates accurately. Another method uses semipermeable plastic vials to enclose the chemical (Byers, 1988). The release rate is constant and proportional to the thickness of the plastic and it's surface area. However, the release rates over several orders of magnitude are impractical due to the limitation of dispenser sizes.

Test tube dispensers have also been used. Their advantage is that the glass does not affect the chemical and the rate can be specified depending on the area of the aperture and the level of the liquid. However, a large array of tube sizes is needed to encompass several orders of magnitudes in release (Byers, 1988). Other types of dispensers include glass jars and bottles (FAO, 1992), that are most frequently used for ketones and urine of host animals in trapping of tsetse (Owaga, 1985). The volume of the bottle does not affect

the release rate as this is controlled by the size of the aperture. Dispensing through an aperture gives a high release rate for phenols and octenol.

Low-density polythene plastic tubing has been tested for dispensing mixtures of phenols and octenol (FAO, 1992). The chemicals diffuse through the walls of the tubing. One disadvantage is that the plastic tends to harden with age and exposure, hence reducing the release rate. Polythene satchets which are cheap and easy to make and whose release rate remains constant with age have also been used (FAO, 1986, 1992). The satchet can be filled first and then sealed, or the chemical can be introduced into a completed satchet using a syringe and the resulting small hole sealed (FAO, 1992).

Zeolite dispensers whereby zeolite powder is mixed with the repellents dissolved in solvent have been reported (Jaku et al., 2003). Synthetic zeolites with different pore diameters can be used. Polymer dispensers have also been used, including plasticised polyvinylchloride dispensers (Shailaja et al., 1997) and interpenetrating polymer network (IPN) beads of polyacrylamide-*g*-guar gum crosslinked with glutaraldehyde and loaded with the chemicals of interest (Kumbar et al., 2003). Polymeric hydrogels (xerogels) containing polyacrylamide loaded with the chemicals of interest have also been used (Bajpai and Rajpoot, 2001).

2.3.2 Factors Affecting Efficiency of Dispenser

An efficient dispenser releases the chemical of interest at a steady rate over the intended period. The rate and method of dispensing affects the efficiency of the dispenser. It is important to use the optimal release rates of different chemicals. The rates however, vary geographically and seasonally due to weather factors. Environmental factors affecting the efficiency of dispensers include temperature, wind speed, turbulence

and humidity. The optimal position for odour may vary in relation to wind direction (FAO, 1992).

Efficiency of a dispenser may be viewed as the degree to which the odours are able to attract or repel the flies. The repellence or attractiveness of the odour may change through the day due to activity of flies of different physiological states such as hunger (FAO, 1992).

2.4 Kinetics of Release of Semiochemicals

Knowledge of qualitative and quantitative release of chemicals from dispensers is of major importance to the understanding of chemical ecology of an organism. Generally, with laboratory and field tests, the experimenter wants to know:

(a) Whether the semiochemical remains stable during the test,

(b) The semiochemical's rate of release,

(c) Whether the release rate remains constant.

Several factors have been determined that affect the kinetics of the release of chemicals from dispensers including temperature, surface area and thickness of dispensers, blend ratio and age of dispenser material (Torr *et al.,* 1997; Holsten *et al.*, 2002; Byers, 1988).

Holsten *et al.* (2002) used bubble cap and bead dispensers to release verbenone and methylcyclohexanone under field conditions. They found that the release rates were strongly controlled by temperature inside the cap. The most likely mechanism suggested is that the chemical placed in the dispenser initially as a liquid vaporises into the atmosphere within the dispenser and then it is released through the membrane.

Vaporisation may possibly occur on the exterior membrane surface.) The vapour pressure inside the dispenser is close to the saturation vapour pressure, whereas the vapour pressure outside the membrane is negligible because the chemical is quickly removed by atmospheric turbulence. The saturation vapour pressure and the conductance of the membrane therefore control release rates (Holsten *et al.*, 2002). Membrane conductance remained constant unless the membrane integrity was disrupted, but the saturation vapour pressure increased exponentially with time.

Torr *et al.* (1997) found that in the absence of other factors, the release rate of a substance from the dispensers is determined by the rate of diffusion of the substance across the membrane and is governed by Fick's law. Most controlled release devices have been found to release substances by either first-order or zero-order kinetics (Chigwanda, 2003).

2.5 Fick's law of diffusion

The release of substances from membranes is governed by Fick's law (Torr *et al.*, 1997). Consider a substance whose concentration (c) is not uniform and varies in at least one direction (x). Since the concentration must be uniform at equilibrium, there will be a flow (flux) of the substance from regions of high concentration to regions of low concentration.

Consider a plane (figure 2) with an area A and width dx; assume that the concentration at x is c and at x+dx is c-dc. The flow of the chemical through the plane will be proportional to the area of the plane and the concentration difference per unit length, -dc/dx.

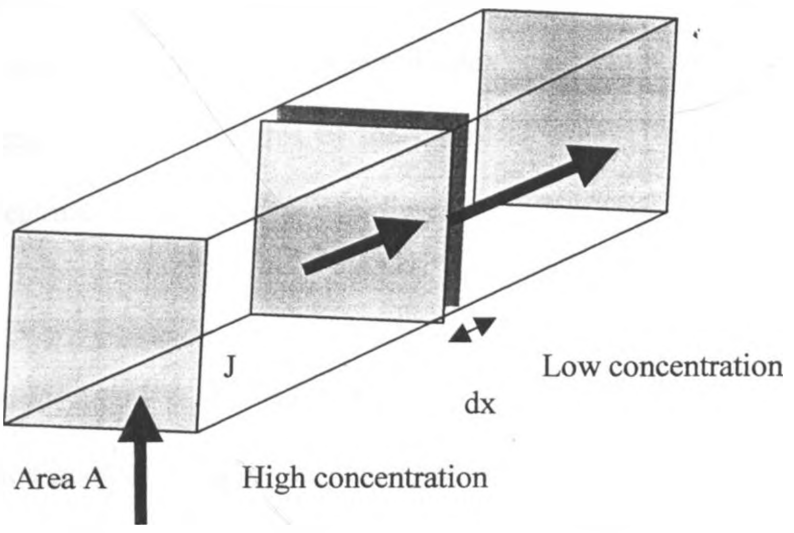

Figure 2: Diagram illustrating Fick's law of diffusion (Source: Noggle, J.H, 1996).

The diffusional flux (J) is defined as (Noggle, 1996);

$$J = \frac{1}{A}\frac{dn}{dt} = -D\frac{dc}{dt} \quad \text{..... 1}$$

Where n is the number of moles and the proportionality factor, D, is the diffusion coefficient or constant. If c is in moles per cm^3 and t in seconds, the units of D are cm^2/s or m^2/s. Equation 1 is called Fick's first law of diffusion.

2.6 METHODS OF DETERMINING RELEASE RATES

2.6.1 Gravimetric Method

This is the most commonly used method for determining release rates (Holsten *et al.*, 2002). It involves weighing the dispensers between periods of laboratory or field

exposure or measuring the load remaining in the dispensers (Brunner, 2002). After a specified duration, the dispensers are weighed on a sensitive scale. A limitation of this method is that contaminants such as dirt, water or pitch could cause weight changes. This is corrected by regressing the weights of the chemical against time. This method is widely used for determining release rates over time.

2.6.2 Volatile trapping system (VTS) method

This method involves trapping sufficient amounts of the chemicals released from the dispenser on an adsorbent material. The adsorbed chemicals are extracted with an appropriate solvent and then quantified by gas chromatography (Brunner, 2002). A VTS developed by Phero Tech Company has been shown to capture 90% of the target chemical based on use of known internal standards during the analysis (Brunner, 2002). The system employs columns packed with PoraPak® (ethylvinylbenzene-divinylbenzene copolymer that is cross-linked).

2.6.3 Residual analysis method

This method involves extracting all the chemical from the dispenser to determine the amount remaining after a specified period. It can be assumed that the difference in the amount remaining at each time period is a measure of the amount of chemical released (Brunner, 2002). The ingredients remaining in the dispenser are extracted and their quantity determined by gas chromatography. This procedure is repeated in 2-4 weeks intervals (Koch *et al.*, 2002). This method however suffers from several systematic errors

like weather effects whereby the release rate is dependent on wind and temperature, differences in weight resulting from changes in water content caused by changes in relative humidity and rainfall. However, according to Brunner (2002), this method has been shown to give 97% recovery of target chemicals based on internal standards used during analysis

Arn *et al.* (1997) describes a method for measuring release rates of individual components in a short time without affecting the dispenser. This method measures the release rate independent of weather effects and can be used to compare dispenser performance over different periods of use and climate conditions. In a wind tunnel, the dispenser is mounted in a tube inside a temperature-controlled air space. A stream of air with constant velocity is drawn through the tube by means of a suction pump. After passing over the tube containing the dispenser, the whole air stream is drawn through a filter cartridge containing an adsorbent material. After 1-4 hours of sampling, the cartridge is washed with a solvent, which in turn is analysed for its content by GC analysis.

2.6.4 Diffusion- dilution method

A method for releasing chemicals which combines the principles of volatile gas diffusion through a tube (Fick's first law) with Raoult's law of vapour pressures for mixtures of volatile liquids has been described by Byers (1988). Fick's differential equations describing diffusion can serve to determine the instantaneous rate of release of a chemical from a capillary tube.

$$\text{Release rate} = \frac{-\pi r^2 D(C_2 - C_1)}{x} \quad \text{.....2}$$

Where: r = radius of tube (cm), D = diffusion coefficient (cm^2/s), C_2 = chemical concentration (moles per litre), $C_1 = 0$ (assuming convection carries vapour away) and x = distance between tube opening and meniscus level of liquid (cm). More complicated equations are needed to describe the release over time as the level of liquid decreases in the tube (Brooks, 1980). In practise, however, it is usually more accurate to measure the release rate over the expected experimental period because one does not know precisely the diffusion coefficient (D) and other contributing factors like meniscus curvature, surface tension and temperature effects (Tilden and Bedard, 1985).

The concentration C_2 is related to the vapour pressure of the chemical and it can be varied according to Raoult's law, which states that the partial pressure of a volatile substance (chemical) is proportional to it's mole fraction in a solvent. The following equation can then be derived for purposes of diluting chemicals with solvent in order to obtain a specific chemical release rate (Byers, 1988):

$$x = fws\left(\frac{\frac{gsem - (fsem)(gsem)}{fwsem}}{fsem/gs}\right) \qquad \text{.....3}$$

where:

x mls = millilitres of solvent

fws = formula weight of chemical /molecular weight of solvent (grams)

gs =grams solvent per millilitre (density)

gsem = grams of chemical

fwsem = formula weight of chemical (grams)

fsem = mole fraction of chemical (0 <fsem< 1)

Byers (1988) stated that the mole percentages of chemical and solvent determine the corresponding release rates of each compared to respective neat solutions as controlled by the diffusion rate through the tube. A dispenser with a large reservoir is preferable because it remains constant in concentration and level, as does the release rate, during prolonged periods of release.

2.7 Gas Chromatography

Gas chromatography (GC) is one of the fastest and most useful separation techniques available in a laboratory. GC analysis is basically limited to organic compounds that are volatile and not thermally labile (Shugar and Ballinger, 1996). Gas chromatography requires that a sample be converted into or exist in the vapour state and be transported by an inert carrier gas through a column packed with either a liquid phase coated on a solid support (gas liquid chromatography) or simply a solid adsorbent with no liquid phase coating (gas solid chromatography). The temperature of the column and the flow rate of the carrier gas (mobile phase) affect the degree of separation (Skoog and Leary, 1992; Rubinson and Rubinson, 2000).

2.7.1 Basic Principles of Operation of a Gas Chromatograph

A typical gas chromatograph consists of a carrier gas supply, sample injection port, column, column oven, detector and a recorder/integrator system (Figure 3). In gas liquid chromatography, a sample is injected through the injection port onto the head of the chromatographic column and vapourised to a degree and rate dependent on its boiling point and the concentrated vapours are swept into the column. The column itself contains

a liquid stationary phase, which is adsorbed onto the surface of an inert solid, and it is of a given overall polarity. Separation occurs as the various compound vapours are selectively adsorbed by the stationary phase and then desorbed by the carrier gas. This sorption-desorption process occurs repeatedly as the compounds are transported through the column by the flow of an inert, gaseous mobile phase through the column to the detector. The compounds will be eluted from the column with those having a high affinity for the column packing being slower than those with a low affinity. The detector signals a chart recorder, which records the response, ideally in the shape of a Gaussian peak (Skoog and Leary, 1992).

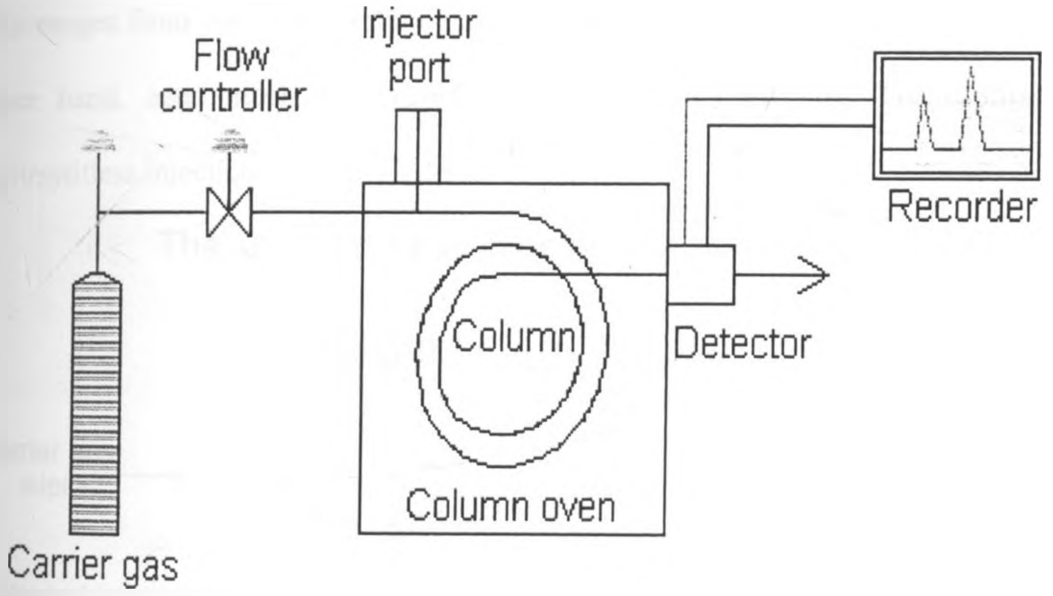

Figure 3: Schematic diagram of a gas chromatograph

2.7.2 Instrumental components of a gas chromatograph

a) Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent on its

compatibility with the detector in use. The carrier gas is passed through a molecular sieve trap to remove water and other impurities.

b) Sample injection port

The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. For optimum column efficiency, small quantities of sample are rapidly introduced onto the column. Slow injection of large samples causes band broadening and loss of resolution. The temperature of the injection port is usually about 50°C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} μL. For capillary GC, split/splitless injection is used (Figure 4).

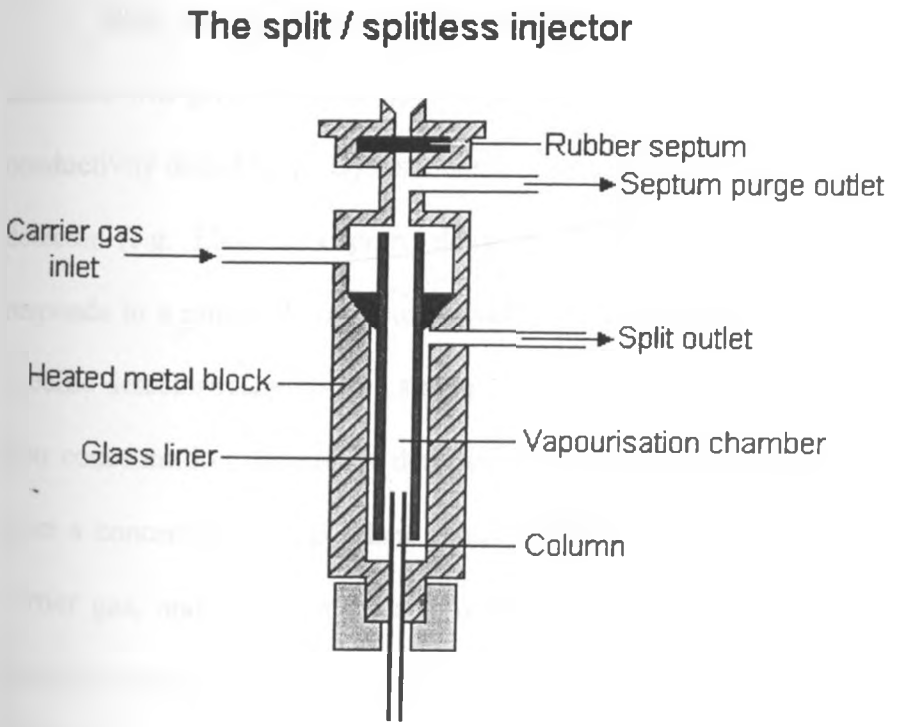

Figure 4: The split/ splitless injector

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes into the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column. In the splitless mode, the split valve is initially closed. The vapourised sample is slowly carried by carrier gas into the column and after a few seconds, the split valve is opened and residual vapours swept out of the system via the purge vent.

c) Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector (e.g. Thermal conductivity detector, TCD) responds to all compounds except the carrier gas, a selective detector (e.g. Electron capture detector, ECD and Flame ionization Detector, FID) responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound. Detectors can also be grouped into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the carrier gas, and does not usually destroy the sample. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector.

The flame ionization detector (Figure 5) is sensitive to all organic compounds, but it is not a universal detector. The effluent from the column is mixed with hydrogen and air, and ignited to produce a very hot (~2100°C) flame that can ionize carbon-containing compounds. A collector electrode with a DC potential is placed above the flame to measure its conductivity. As the effluent passes through the burner jet, the organic compounds are ionized and converted into positively charged ions, which are then attracted to the negatively charged collector ring above the flame jet. This creates a current flow of about ~ 10^{-12} amperes (Churacek, 1993) which then requires an electrometer for amplification of the signal. The ion current is roughly proportional to the number of carbon atoms present; therefore each compound requires a response factor.

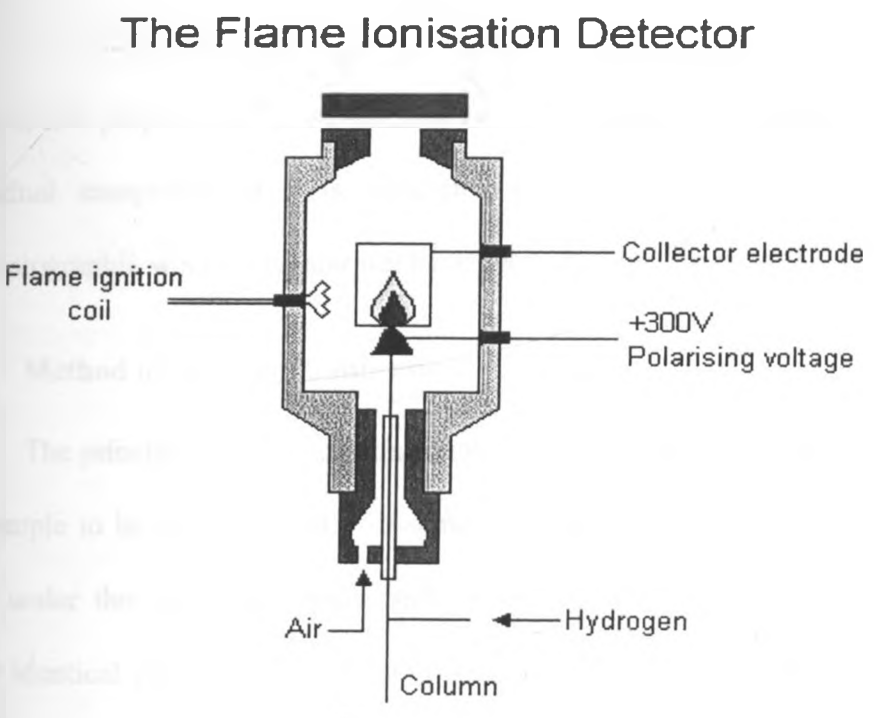

Figure 5: The flame ionization detector (FID)

FIDs are mass sensitive rather than concentration sensitive and hence changes in mobile phase flow rate do not affect peak area. The FID is a highly sensitive general

detector for the analysis of organic compounds and can detect concentrations in the range of 100 ppb. It has an excellent linear response range and can handle signal magnitude changes of 6 orders or more (low noise). It is also robust and easy to use, but unfortunately, it destroys the sample.

2.8 Survey of Gas Chromatographic Working Techniques

Quantitative determination is achieved by analyzing the peak area. The area under a chromatographic peak (A_i) is proportional to the amount of substance present (C_i) in the carrier gas (Ettre and Zlatkis, 1967).

$$A_i \propto C_i$$

$$A_i = f_i C_i \quad \text{.....4}$$

where f_i is a proportionality factor which depends mainly on the chemical nature of the individual component and is different for each type of detector. Several gas chromatographic working techniques have been employed in quantitative analysis.

2.8.1 Method of Absolute Calibration

The principle of this technique rests with separately injecting defined quantities of the sample to be analyzed and of a standard substance and subsequently comparing the areas under the chromatographic peaks (Novak, 1974). The injections must be made under identical conditions. If the sample contains a component that is not amenable to chromatography, or an unidentifiable portion, this technique is the only possible means for quantitative analysis of the chromatogram. This technique is very useful in cases

where it is necessary to eliminate errors caused by incomplete course of a reaction or by losses in sample adjustment prior to injection into the instrument.

2.8.2 Internal Standardization Technique

The principle of this technique is that a defined amount of the sample to be analyzed is mixed with a known amount of the standard and the resulting mixture is then injected into the chromatograph (Novak, 1974). The principal advantages of this method are that the exact knowledge or the reproducibility of sample volumes is unimportant, and that by fixing the concentration of the internal standard, calibration curves can easily be established because the ratio of the area of a peak to the area of the standard peak is directly proportional to the concentration of the respective component in the sample (Ettre and Zlatkis, 1967; Novak, 1974). Spiking samples with the internal standard thus helps to compensate for the imprecision inherent to GC methods like difficulty in the exact replication of conditions such as gas flow rate and injection volumes between trials (Rubinson and Rubinson, 2000).

The standard peak should be located in close proximity to but without overlapping the analyte peaks. The amount of standard to be added ought to be comparable to the content of the sample component to be determined, so that chromatography takes place under conditions corresponding to linear portions of the respective sorption isotherms and the maximum concentration of either compound in the column effluent will not exceed the linear range of detector response (Novotny, 1982). In this way, the reproducibility of measurements will be quite satisfactory even if operating conditions vary somewhat from run to run.

2.8.3 Standard Addition Technique

This technique is related to internal standardization. With regard to the procedure adopted for sample preparation for chromatography, the only difference is that no new component but the component to be determined is added as the standard to the sample. The substance present in the original sample and the substance added are viewed as different components. The concentration of the original component under analysis in the enriched sample will be lower. Two injections are required, one for the original sample to be analyzed and the other for a measured amount of the enriched sample. These injections must be run under identical conditions and with absolutely defined amounts of sample.

2.8.4 Internal Normalization

This technique may be taken as a special case of internal standardization where the role of the internal standard is performed by an arbitrary component of the original mixture under analysis. The ratio of the amount of standard and of the sample under analysis is determined. Thus neither is the absolute amount of sample injected defined nor is any standard added. This gives rise to limitations in the application of this technique. An example is when a part of the analyte is decomposed or is not eluted, or when some components of the mixture are not resolved.

2.8.5 Controlled Internal Normalization Technique

This method can be used to reduce the uncertainty of results associated with the internal normalization technique. To a sample to be analyzed, a measured amount of some control substance is added. The substance is chosen such that the respective

correction factor is known and the chromatographic peak of the substance does not coincide with any other peak in the chromatogram. This method has certain limitations also. The major limitation is that the determination of the component will be possible in cases where the component is a major constituent of the sample to be analyzed, or in other words, when the error of determination of the directly determinable portion is considerably smaller than the amount of the component. The lesser the content of the component, the greater will be the error involved in its determination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Dispenser Construction

A dispenser was designed that consisted of a repellent reservoir and a diffusion area which made up the dispensing unit whose top could be unscrewed for refilling (Figure 6). The upper part was a reservoir tube made of aluminium (or polypropylene), diameter 10 mm and length 10 cm, through which no significant diffusion of the repellent constituents could take place. The diffusion area was made from tygon silicon tubing (Cole-Palmer Co., Illinois) of internal diameter 6.4 mm, outer diameter 9.6mm, thickness 3.2 mm and variable length. When the length was 2 cm, total diffusion area was determined by calculation to be 6.028 cm^2. The tygon silicon tubing was taken as cylinder and its surface area calculated thus:

Surface area of a cylinder = area of top + area of bottom + area of side

$$= \pi r^2 + \pi r^2 + (h)\, 2\pi r$$

$$= 2\pi r^2 + 2\pi rh$$

Since the top and bottom of the tubing will be closed using an impermeable polypropylene screw caps, the total active surface area of the tubing will be equal to the surface area of the side.

Surface area of the tubing = area of side

$$= 2\pi rh$$

Where 2r is the outer diameter (OD) of the silicon tubing and h is the length of the tubing.

The dispensing unit was closed with screw caps made of polypropylene on both ends. The specifications of the tygon silicon tubing are as shown in Table 2.

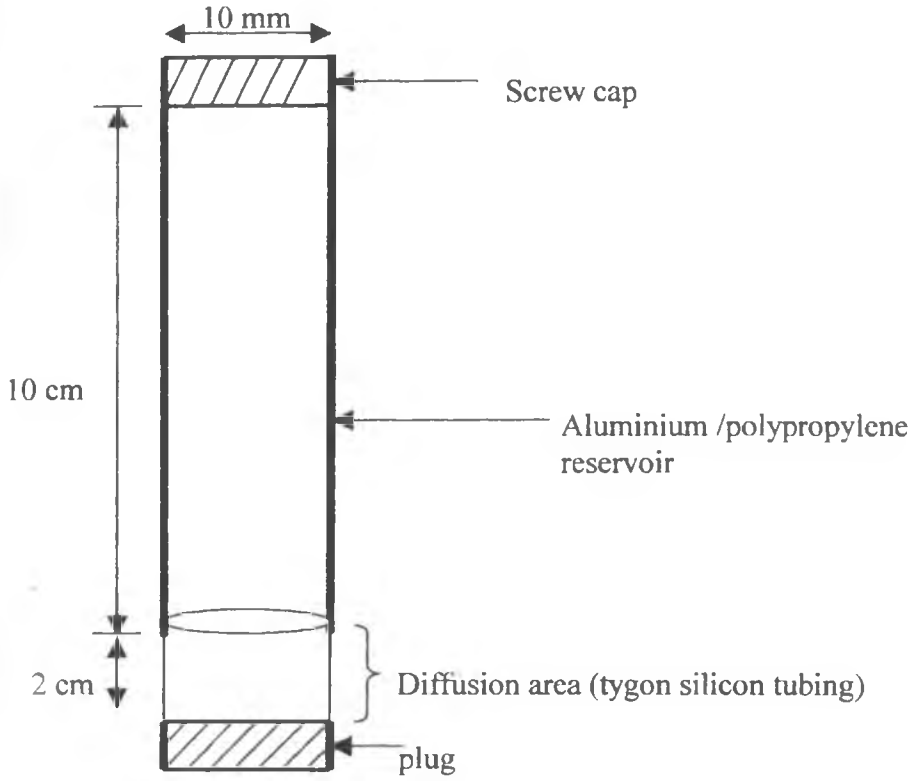

Figure 6: The repellent dispenser

Table 2: Specifications of the tygon silicon tubing

Formulation	colour	Operating temperature	Resin certification	Durometer (mm)	Dimensions		Chemical resistance						Permeability (approximate) Units: (cm^3-mm/sec-cm^2-cm Hg)			
					ID	OD	Weak Acids	Strong Acids	Weak Bases	Strong Bases	Ozone	UV	CO_2	H_2	O_2	N_2
Tygon ®	Transparent	-50 to 74 ° C	FDA, GMP	55 (A)	6.4	9.6	A	D	A	D	B	B	360	-	40	80

Key:

A= No damage after 30 days of constant exposure

B =Little or no damage after 30 days of constant exposure

D = Immediate damage

FDA = Food and Drug Administration (USA)

GMP = Good manufacturing Practices (USA)

ID = internal diameter

OD =Outer diameter

3.2 Reagents

Repellents and standards

Release kinetics of the following repellent compounds which fall into four groups were determined individually and in blend;

i. carboxylic acids (pentanoic acid, hexanoic acid and heptanoic acids),

ii. ketones (geranylacetone and 2-undecanone),

iii. phenol (guaiacol)

iv. δ-octalactone.

These chemicals were purchased from Sigma-Aldrich.

The physical properties of the repellent compounds are listed in Table 3. The standards used were: butanoic acid, octanoic acid, 2-dodecanone (assay 99.8 %, Sigma Aldrich, UK) and 2-methoxy-4-methyl phenol (assay 99.5%, EMEL T s.r.l, Italy).

Table 3: Physical properties of the repellent compounds

	Boiling point (^{o} C)	Molecular weight (g)	Relative density (water = 1)	Assay (%)	Vapour pressure (mm Hg) at 25 °C	Henry's Law Constant (atm L mol $^{-1}$) at 25 °C
Pentanoic acid reagent	186-188	102.13	0.94	99.8	0.196	$4.72x10^{-4}$
Hexanoic acid reagent	202-203	116.16	0.927	99.8	0.0435	$7.58x10^{-4}$
Heptanoic acid	223	130.19	0.918	99.7	0.0107	$6.5x10^{-4}$
2-undecanone	231.5	170.3	0.825	99.8	0.0414	$6.36x10^{-2}$
Guaiacol	205	124.14	1.129	99.9	0.103	$1.2x10^{-3}$
Geranylacetone	124	194.2	0.873	98.5	-	-
δ-octalactone	115	142.1	0.995	98.8	-	-

(Source: Weast, R. C., 1974)

NOTE: Henry's Law constant can take two forms, one describing volatilization i.e. water to air partitioning (atm L mol $^{-1}$) and the other dissolution i.e. air to water partitioning (mol L^{-1} atm^{-1}).

Dispensers

The dispensers used were the prototype dispensers constructed as described in Figure 6 with a tygon silicon tubing (Cole-Palmer, USA) of length, 4, 3 or 2 cm with diffusion area of 12.0586 cm ^{2}, 9.0439 cm ^{2} and 6.0288 cm ^{2}, respectively. The polypropylene tubes were obtained from Cole-Palmer while the aluminium tubes were obtained locally.

3.3 Apparatus Used for Laboratory and Semifield Trials

Environmental conditions

Temperature and relative humidity were monitored over 24 hours using a thermohygrometer (Wil.Lambrecht GmbH, Gottingen) for seven days. The data was collected hourly and the corresponding averages calculated.

Wind tunnel

Laboratory trials were conducted in a two choice cylindrical plexiglass wind tunnel 180 cm long and 24 cm internal diameter as illustrated in Figure 7. A duct 20 cm diameter in the middle of the tunnel was connected via a polyvinylchloride (PVC) pipe through which an air-extracting fan was mounted. Air flowed into the tunnel from either arm on switching the fan on, thereby making the middle of the tunnel downwind. The duct in the middle divided the tunnel into two equal arms with a 20 cm wide middle zone where air from either arm mixed. The upwind of the tunnel was closed with white PVC gauze, while the downwind end was closed with a metallic mesh cover.

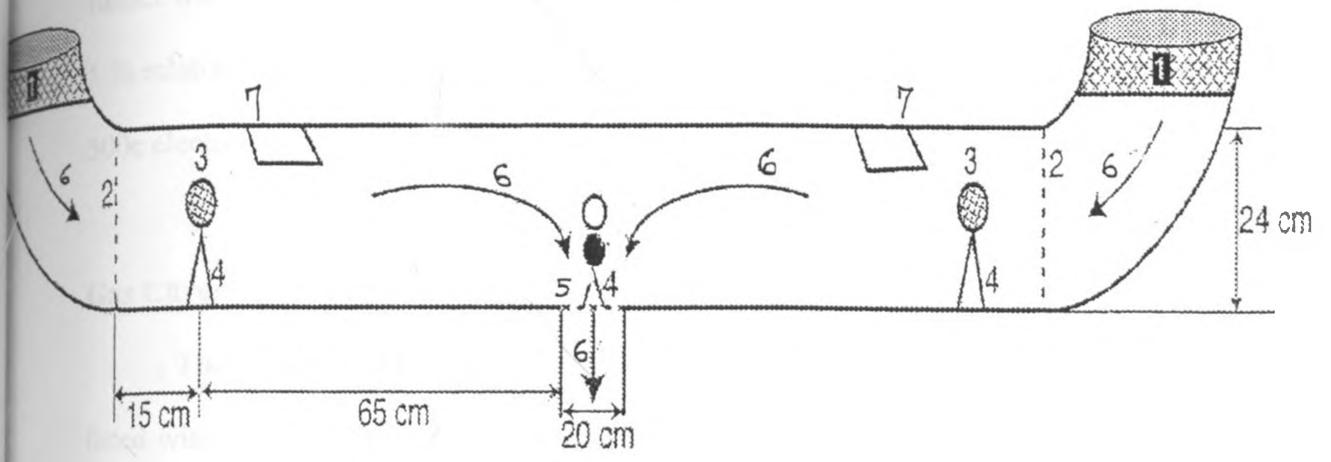

FIGURE 7: THE WIND TUNNEL

1. activated charcoal
2. PVC gauze
3. dispenser
4. metallic rack
5. metallic wire mesh
6. air flow direction
7. window for introducing dispenser

The two upwind ends of the tunnel were connected to activated charcoal (4-14 mesh, Sigma) air filters made of PVC. Two windows, one on either arm (15 cm x 10 cm) of the wind tunnel were used for introducing sample dispensers. The wind speed in the tunnel was maintained at 20 cm/sec while the room was maintained at 24 ± 1°C and 65 ± 5 % relative humidity. The laboratory conditions were determined using a Solomat MPM 500e electronic anemometer (Devon, UK).

Gas Chromatography

The samples were analysed on a Hewlett-Packard model 5890 gas chromatograph fitted with a carbowax 20M column (HP 30 m x 0.2 mm x 0.2 μm film thickness) and a flame ionization detector (FID).

3.4 Determination of Release Rates

3.4.1 Optimisation of the Dispenser

A study was conducted to determine the appropriate length of the tygon silicon tubing using a synthetic tsetse repellent (2-methoxy-4-mnethylphenol). The diffusion area was varied by using 4, 3 and 2 cm lengths of the tubing which gave different diffusion areas. The dispensers of different diffusion areas were filled with 2-methoxy-4-mnethylphenol leaving 2 cm vapour space above the repellent in the reservoir. The dispensers were then tightly closed with propylene plugs. They were then wrapped in aluminium foil, placed in a closed container and transported to the field. They were placed 2 m above the ground in the sun and under tree shade and their weights taken every 24 hours.

3.4.2 Determination of the Release Rates under Laboratory Conditions

The dispensers used were the prototype dispensers constructed as described in section 3.2.1, with a tygon silicon tubing of length, 2 cm and a diffusion area of 6.028 cm^2. Dispensers were filled in triplicate with the individual repellent compounds or a freshly prepared uniform blend of the repellent compounds listed in section 3.2. A 2 cm vapour space was left above the repellent in the reservoir. The dispensers were then tightly closed with propylene plugs. They were then wrapped in aluminium foil, placed in a closed container and transported to the wind tunnel room. The dispensers were each placed in the wind tunnel through the window on the upper side of either arm of the tunnel onto a metallic rack positioned 15 cm from the upwind ends such that they were mid-height of the wind tunnel, facing downwind. The dispensers were carefully removed after every 24 hours and their weights taken. For the dispensers containing the blend, 2 µl of the sample was removed and the dispenser weight recorded before they were returned to the wind tunnel. The removed sample was placed in a glass vial with teflon lined caps containing 1ml solvent (dichloromethane). The vials were stored in a freezer at –20°C.

3.4.3 Determination of the Release Rates under Semifield Conditions

Dispensers were filled in triplicate with the individual repellent compounds as described in section 3.4.2 and their weights taken before being placed in the open (semifield) at ICIPE, Kasarani. Dispensers were placed 2m above the ground in the sun and under tree shade and their weights taken after every 24 hours. Using the gravimetric method, release rates were determined for the individual compounds.

Two sets of dispensers with a tygon silicon tubing of length 4 cm and 2 cm were used. Equal amounts of freshly prepared blend of the seven repellent compounds was

introduced into the dispensers. Three replicate dispensers for each set were placed 2m above the ground in the sun and under tree shade. Every day, 2 µl of the sample was removed from each dispenser and placed in glass vials containing 1 ml of dichloromethane (DCM). The samples were stored in a freezer at –20°C to await analysis. The weight of the dispensers was determined before and after removal of the sample.

3.5 Gas Chromatographic Analysis of the Samples

3.5.1 Preparation of the Internal Standards

A mixture of butanoic and octanoic acid was prepared by dissolving 100 mg of each acid in DCM and diluting to 100 ml with the same solvent to make a stock solution containing 1000 µg/ml of each acid. Butanoic acid was used to quantify pentanoic and hexanoic acids while octanoic acid was used to quantify heptanoic acid.

A mixture of 2-dodecanone and 2-methoxy-4-methyl phenol was prepared by dissolving 100 mg of each compound in dichloromethane and diluting to 100 ml with the same solvent. 2-Dodecanone was used to quantify 2-undecanone, geranylacetone and δ-octalactone, while 2-methoxy-4-methyl phenol was used to quantify guaiacol

3.5.2 Preparation of the Standards

A standard stock solution containing pentanoic, hexanoic and heptanoic acids was prepared by dissolving 100 mg of each acid in DCM and diluting to 100 ml with the same solvent to make a stock solution containing 1000 µg/ml of each acid. Working solutions with 300 µg /ml internal standard compounds and 500, 400, 300, 200 and 100 µg /ml standard compounds were prepared from the stock solutions.

A standard stock solution containing 2-undecanone, geranylacetone, δ-octalactone and guaiacol was prepared by dissolving 100 mg of each compound in DCM and diluting to 100 ml with the same solvent. Working solutions with 200 µg/ml internal standard compounds and 400, 300, 200 100 and 50 µg/ml standard compounds were prepared from the stock solutions.

The working solutions were prepared using the formula;

$C_1V_1 = C_2V_2$ 5

Where:

V_1 is the initial volume (ml) of the stock solution corresponding to concentration C_1 (µg/ml)

C_1 is the concentration of the stock solution (µg/ml)

V_2 is the final volume (ml) of the working solution

C_2 is the final concentration of the working solution (µg/ml)

3.5.3 Sample Preparation

Samples obtained from laboratory and semifield dispensers containing the blend were prepared for analysis by spiking each one of them with 300 µg/ml of the acid internal standards and 200 µg/ml of the 2-dodecanone and 2-methoxy-4-methylphenol internal standards and topping up the mixture to 2ml with dichloromethane. The vials containing the mixtures were then shaken vigorously and sealed tightly.

3.5.4 Gas Chromatographic Analysis of the Samples

The samples were analysed on a Hewlett-Packard model 5890 gas chromatograph equipped with a split/splitless injection system, a carbowax 20M column (HP 30 m x 0.2

mm x 0.2 μm film thickness) and a flame ionization detector (FID). The column was operated under the following conditions: the oven temperature was initially at 90°C where it was held for 2 minutes, raised to 220°C at 8 °C/min and held there for 10 minutes. Nitrogen was used as the carrier gas at 0.8 ml/min.

Standard solutions (1.0 μl) were injected manually into the gas chromatograph in the splitless mode and the peaks in the chromatograms identified. Three injections were made for each sample. A calibration curve was prepared for each compound by plotting the concentration of the analyte against the average of the normalized peak area of the solute at each concentration. A regression of the calibration curve was fitted with the straight line equation, $y = mx + b$.

Samples (1.0μl) were injected into the gas chromatograph. This was repeated thrice. The concentration of the sample was obtained using the straight-line equation, $y = mx + b$.

3.6 Analysis of Release Data

The data were analysed for significance in difference in the release rates of repellent compounds from different lengths of the dispensers and the relationship between environmental variables and release rates by ANOVA and ANCOVA respectively using Statistical Analysis Software (SAS, 2003). The release data were fitted to zero-, first- and second-order rate equations in order to determine the corresponding release rates and hence the mechanism of repellent release from the dispensers. The Pearson correlation coefficient (r^2) which describes the proportion of variance in common between two variables was used to determine the release kinetics of the repellents, with the highest r^2 value being taken as the one that best describes the release kinetics of the repellents.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Optimisation of the dispenser and determination of the release kinetics of 2-methoxy-4-methyl phenol under semifield conditions

The study to optimise the dispenser was conducted using the synthetic repellent , 2-methoxy-4-methyl phenol in order to determine the appropriate length of the tygon silicon tubing to be used in subsequent kinetics studies involving the waterbuck odour based repellent blend. The diffusion area was varied using 4, 3 and 2 cm of the tygon silicon tubing and the corresponding release rates compared under semifield conditions. Three kinetic models were used to evaluate the release kinetics of 2-methoxy-4-methyl phenol:

The zero order equation expressed as,

$$A_t - A_o = -k_0 t \quad \text{.....6}$$

The first order equation expressed as,

$$\ln A_t - \ln A_o = -k_1 t \quad \text{.....7}$$

The second order equation expressed as,

$$\frac{1}{A_t} - \frac{1}{A_o} = k_2 t \quad \text{.....8}$$

Where:

A_t is the amount of repellent present (grams) in the dispenser at any time t,

A_o is the initial amount of repellent present (grams) in the dispenser at time t = 0,

k_0, k_1 and k_2 are the zero-, first- and second-order rate constants, respectively.

The experimental data obtained (Appendix 1) was fitted to the various kinetic models described above to establish the most appropriate model for the data. The best model was determined through the coefficient of correlation (r^2) values) (See Table 4).

Table 4: Correlation coefficient (r^2) values and the rate constants (with units as g day^{-1} for zero-order , day^{-1} for first-order and g^{-1} day^{-1} for second-order) for the various kinetic models tested to describe the release of 2-methoxy-4-methyl phenol (n=3).

LENGTH OF TYGON SILICON TUBING	ZERO-ORDER MODEL				FIRST-ORDER MODEL				SECOND-ORDER MODEL			
	SUN		SHADE		SUN		SHADE		SUN		SHADE	
	k_0	r^2	k_0	r^2	k_1	r^2	k_1	r^2	k_2	r^2	k_2	r^2
4 cm	0.2760	0.9187	0.2150	0.9884	0.0600	0.9084	0.0400	0.9880	0.0110	0.8951	0.0080	0.9856
3 cm	0.1480	0.9991	0.1560	0.9995	0.0520	0.9980	0.0530	0.9978	0.0187	0.9911	0.0180	0.9890
2 cm	0.0960	0.9979	0.0106	0.9990	0.0197	0.9972	0.0200	0.9980	0.0040	0.9967	0.0039	0.9963

The overall best fit (highest r^2) was obtained with the zero-order rate equation for the dispensers fitted with 4cm, 3 cm and 2 cm tygon silicon tubing (Figures 8 -10).

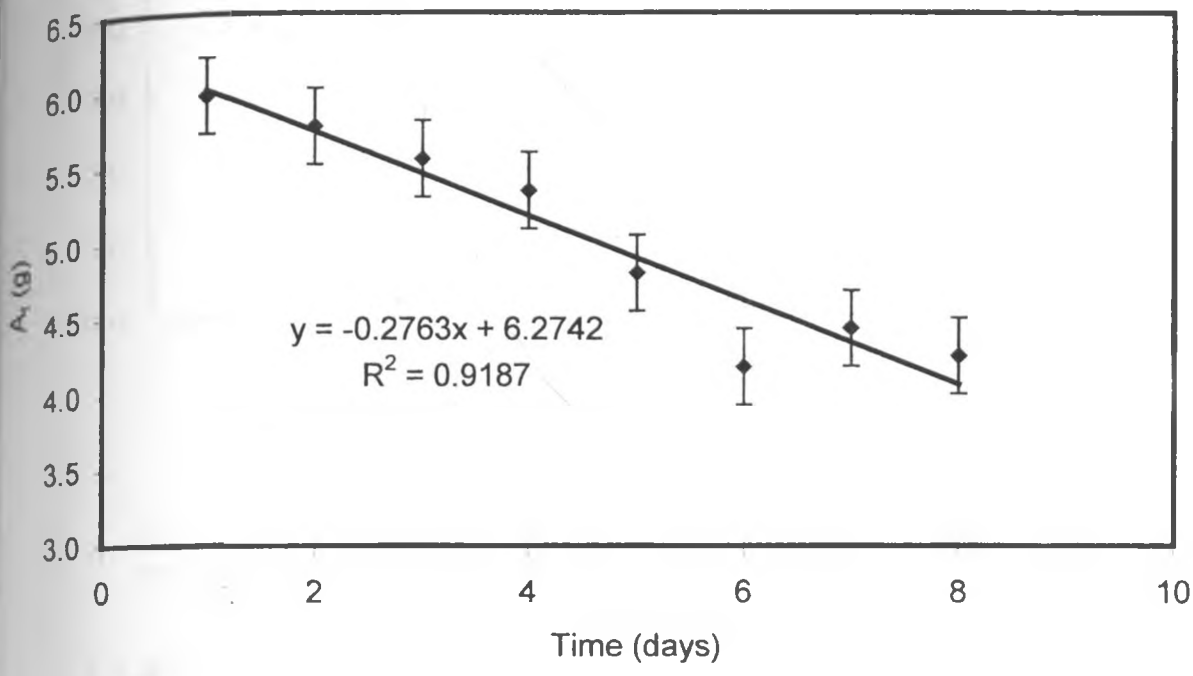

Figure 8a: Release kinetics of 2-methoxy-4-methyl phenol in the sun using 4 cm tygon tubing as described by the zero-order rate equation

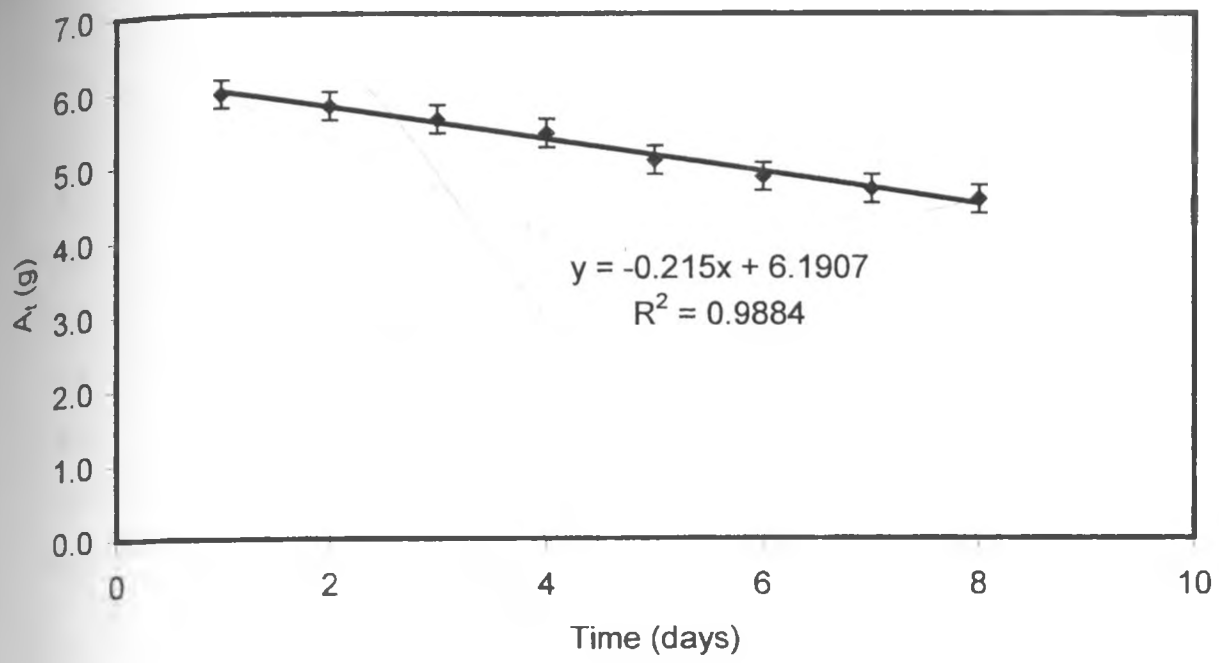

Figure 8b: Release kinetics of 2-methoxy-4-methyl phenol in the shade using 4 cm tygon tubing as described by the zero-order rate equation

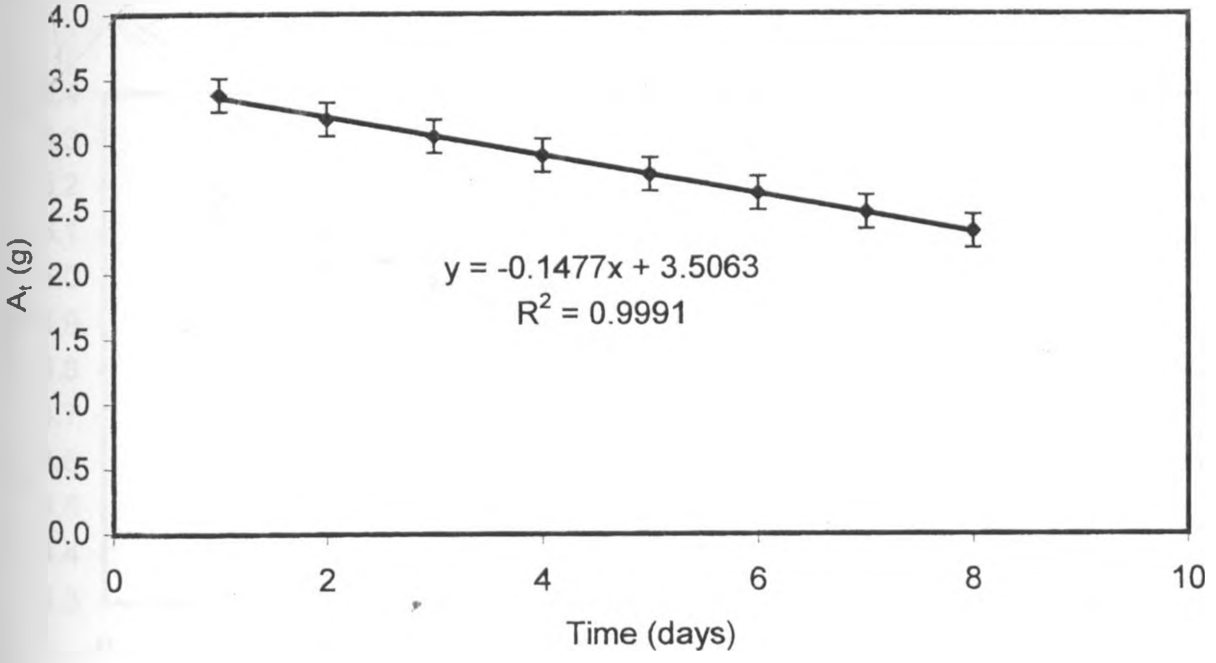

Figure 9a: Release kinetics of 2-methoxy-4-methyl phenol in the sun using 3 cm tygon tubing as described by the zero-order rate equation

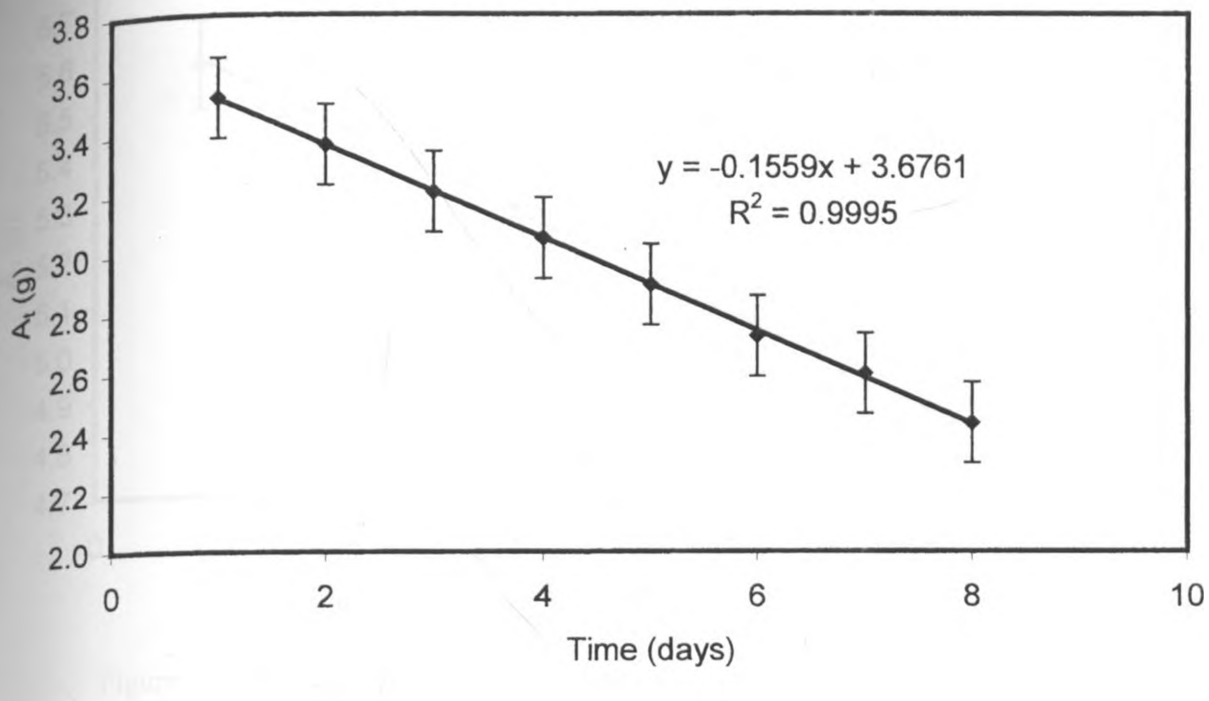

Figure 9b: Release kinetics of 2-methoxy-4-methyl phenol in the shade using 3 cm tygon tubing as described by the zero-order rate equation

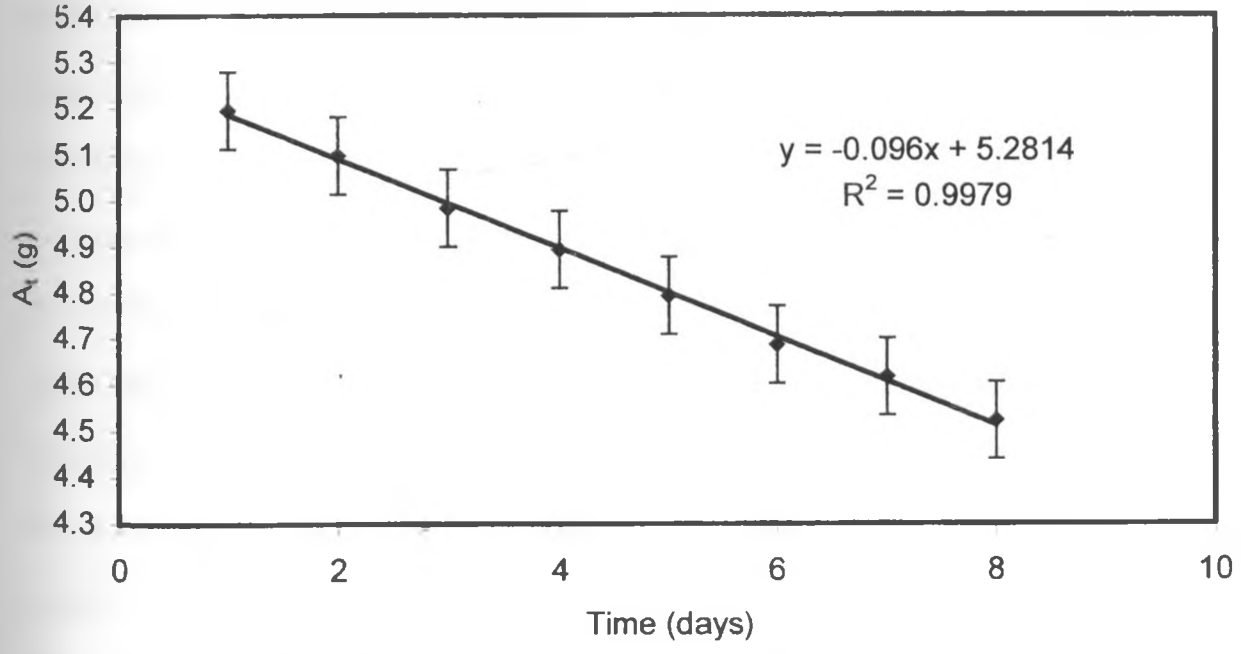

Figure 10a: Release kinetics of 2-methoxy-4-methyl phenol in the sun using 2 cm tygon tubing as described by the zero-order rate equation

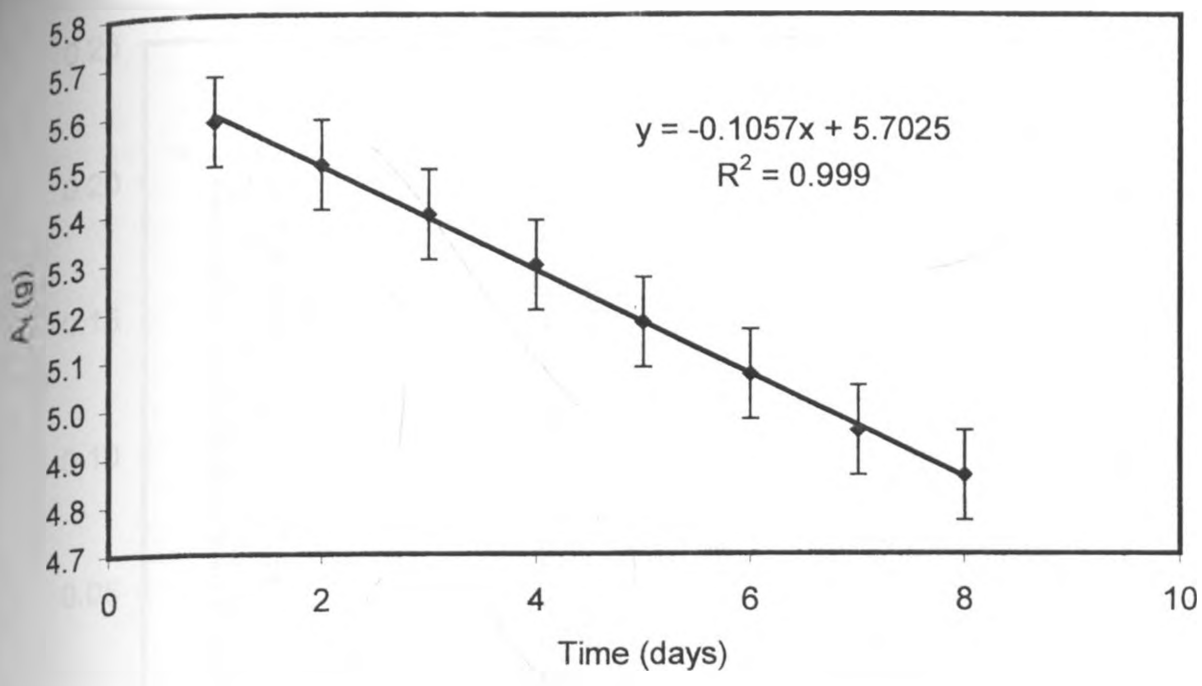

Figure 10b: Release kinetics of 2-methoxy-4-methyl phenol in the shade using 2 cm tygon tubing as described by the zero-order rate equation

The first-order and second-order models did not adequately describe the release of the repellent from the dispensers as evidenced by the relatively low correlation coefficient (r^2) values compared to those obtained using the zero-order model. The weight loss (assessed gravimetrically) was observed to be higher for the dispensers in the sun compared to those placed under the shade. This is evident when one examines the corresponding zero-order rate constants, where the rate constants were generally higher in the sun than under shade (see Table 4). The release rates were also found to be dependent on the surface area of the tygon silicon tubing (assumed to be proportional to length of tubing) as is evident from Figure 11a. It is clear from Figure 11a that the longer the silicon tubing, the greater the loss in weight. It is for this reason that a silicon tubing of length 2cm was selected for use in subsequent laboratory and semifield trials. The corresponding average weight losses over the entire period of the trials and zero-order rate constants for the different lengths of the tygon tubing are summarized in Table 5

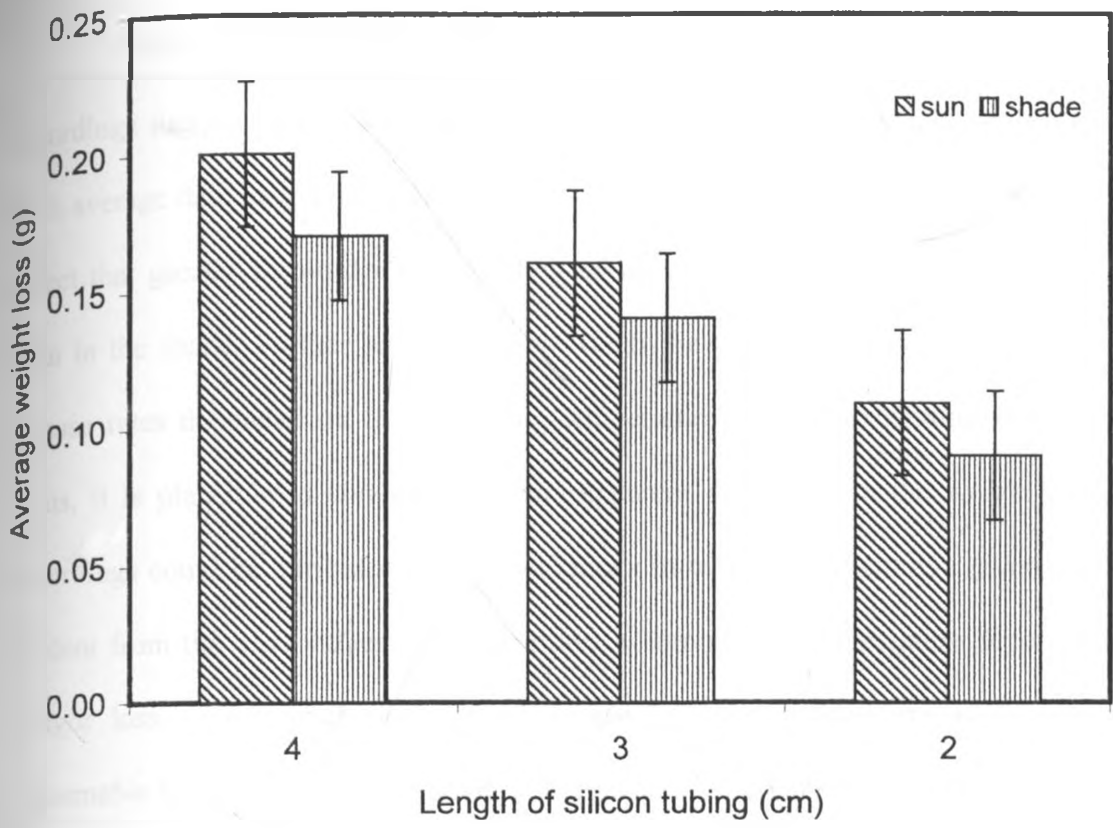

Figure 11a: Average weight losses of 2-methoxy-4-methyl phenol using different lengths of the tygon silicon tubing

Table 5: Average weight losses (± SD) and the corresponding zero-order rate constants of 2-methoxy-4-methyl phenol using different lengths of tygon silicon tubing (n=3)

Length of tygon tubing (cm)	Diffusion area (cm^2)	SUN		SHADE	
		AVERAGE WEIGHT LOSS (g)	RATE CONSTANT (g day^{-1})	AVERAGE WEIGHT LOSS (g)	RATE CONSTANT (g day^{-1})
4	12.056	0.2033 ± 0.0081	0.276	0.1714 ± 0.0099	0.215
3	9.042	0.1642 ± 0.0078	0.148	0.1463 ± 0.0066	0.156
2	6.028	0.1098 ± 0.0042	0.096	0.0991 ± 0.0048	0.011

Further examination of the weight loss data alongside daily temperature recordings suggest that there is a general tendency for the average weight loss to increase with average daily temperature (Figure 11b). This corroborates earlier observations to the effect that greater zero-order rate constant or weight losses are recorded under the sun than in the shade. Further scrutiny of the weight loss data revealed that the zero-order release rates depended on the prevailing temperature of the surrounding environment. Thus, it is plausible to conclude that any apparent deviations in the weight loss data recordings could be attributed to wide variations in temperature within a given day as is evident from the ±SD values of temperature. A summary of temperature dependence of weight loss of the synthetic repellent is given in Table 6. There is however, no discernable correlation between average weight loss and relative humidity (% RH) as is evident from Table 6.

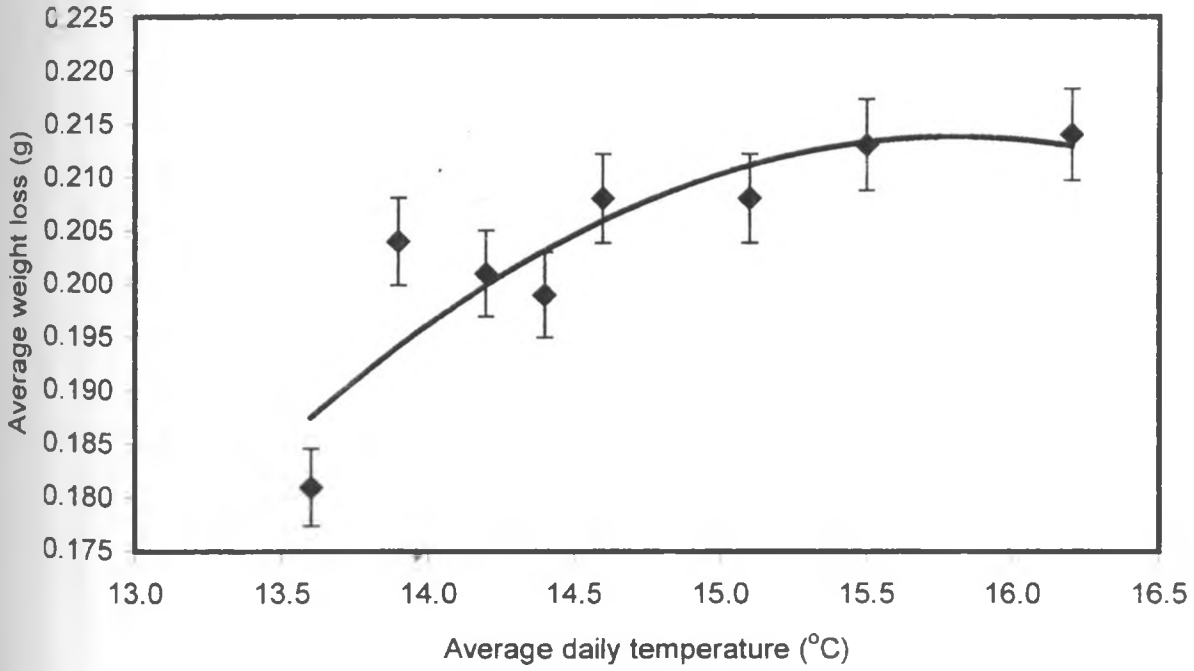

Figure 11b: Average weight loss of 2-methoxy-4-methyl phenol as a function of average daily temperature obtained with a 4 cm tygon silicon tubing

Table 6: Average weight losses (± SD) of 2-methoxy-4-methyl phenol from different silicon tubing lengths per day (n=3) against the prevailing average daily temperature (± SD) and the average % relative humidity (RH)

Length of tubing	DAY	Average weight loss in the sun (g)	Temperature (°C)	%RH	Average weight loss in the shade (g)	Temperature (°C)	%RH
4 cm	1	0.213 ± 0.003	15.5 ± 4.7	43.2	0.175 ± 0.004	13.4 ± 3.6	46.4
	2	0.208 ± 0.010	15.1 ± 3.6	42.7	0.173 ± 0.008	13.2 ±2.7	43.7
	3	0.214 ± 0.011	16.2 ± 5.3	43.1	0.182 ± 0.001	13.5 ±3.2	43.4
	4	0.208 ± 0.005	14.6 ± 3.7	46.9	0.181 ± 0.007	13.1 ±2.6	44.5
	5	0.204 ± 0.011	13.9 ± 3.3	44.8	0.170 ± 0.001	12.2 ±2.1	56.2
	6	0.199 ± 0.011	14.4 ± 3.6	44.9	0.170 ± 0.002	12.6 ± 2.5	56.8
	7	0.201 ± 0.014	14.2 ± 3.8	45.1	0.162 ± 0.014	12.3 ±2.2	55.7
	8	0.181 ± 0.002	13.6 ± 3.5	44.9	0.137 ± 0.001	12.1 ±2.3	57.3
3 cm	1	0.188 ± 0.018	15.5 ± 4.7	43.2	0.159 ± 0.002	13.4 ± 3.6	46.4
	2	0.163 ± 0.019	15.1 ± 3.6	42.7	0.154 ± 0.009	13.2 ±2.7	43.7
	3	0.165 ± 0.007	16.2 ± 5.3	43.1	0.146 ± 0.008	13.5 ±3.2	43.4
	4	0.163 ± 0.001	14.6 ± 3.7	46.9	0.136 ± 0.004	13.1 ±2.6	44.5
	5	0.166 ± 0.008	13.9 ± 3.3	44.8	0.135 ± 0.004	12.2 ±2.1	56.2
	6	0.162 ± 0.007	14.4 ± 3.6	44.9	0.152 ± 0.006	12.6 ± 2.5	56.8
	7	0.156 ± 0.002	14.2 ± 3.8	45.1	0.148 ± 0.011	12.3 ±2.2	55.7
	8	0.154 ± 0.006	13.6 ± 3.5	44.9	0.141 ± 0.008	12.1 ±2.3	57.3
2 cm	1	0.112 ± 0.009	15.5 ± 4.7	43.2	0.093 ± 0.002	13.4 ± 3.6	46.4
	2	0.098 ± 0.004	15.1 ± 3.6	42.7	0.089 ± 0.004	13.2 ±2.7	43.7
	3	0.113 ± 0.011	16.2 ± 5.3	43.1	0.102 ± 0.007	13.5 ±3.2	43.4
	4	0.116 ± 0.004	14.6 ± 3.7	46.9	0.110 ± 0.001	13.1 ±2.6	44.5
	5	0.110 ± 0.004	13.9 ± 3.3	44.8	0.103 ± 0.003	12.2 ±2.1	56.2
	6	0.107 ± 0.009	14.4 ± 3.6	44.9	0.096 ± 0.001	12.6 ± 2.5	56.8
	7	0.112 ± 0.003	14.2 ± 3.8	45.1	0.101 ± 0.007	12.3 ±2.2	55.7
	8	0.111 ± 0.005	13.6 ± 3.5	44.9	0.098 ± 0.005	12.1 ±2.3	57.3

4.2 Determination of Release Kinetics of Waterbuck-Derived Repellents under Laboratory Conditions

4.2.1 Release Kinetics of the Individual Repellent Compounds

The kinetics of release of the individual compounds were investigated under controlled conditions in a wind tunnel in the laboratory. The data collected (Appendix 2) was tested against zero-, first- and second-order kinetic models. The release of the individual compounds was found to follow first-order kinetics as evidenced by the higher correlation coefficients of the plots of the experimental data fitted to the various models (Table 7). The first-order release rate constants for the individual compounds were determined graphically from the corresponding straight line plots of the first-order model. The corresponding first-order plots for the compounds are shown in Figures 12 to 18.

Pentanoic acid, guaiacol, 2-undecanone and hexanoic acid had the highest first-order release rate constants of 0.081, 0.034, 0.03 and 0.02 day $^{-1}$, respectively, while δ-octalactone, heptanoic acid and geranylacetone had lower rate constants of 0.012, 0.008 and 0.007 day $^{-1}$, respectively.

The weight loss data as presented in Appendix 2 shows that higher weight losses and hence higher release rates were registered during the first two days of the trials, with the overall release rates (taken as average weight losses) showing a gradual decrease with time as would be expected (due to reduced residual repellents in the silicon tubing). This observation is also clearly evident from the first-order model plots of Figures 12 to 18.

Table 7: Correlation coefficient values (r^2) and rate constants for the various kinetic models used to describe the release of the individual repellent compounds under laboratory conditions (*)

COMPOUND	ZERO ORDER		FIRST ORDER		SECOND ORDER	
	k_o g day $^{-1}$	r^2	k_1 day $^{-1}$	r^2	k_2 g $^{-1}$ day $^{-1}$	r^2
Pentanoic acid	0.2829	0.9569	0.0808	0.9864	0.0225	0.9835
Hexanoic acid	0.0877	0.9669	0.0197	0.9788	0.0043	0.9750
Heptanoic acid	0.0462	0.9467	0.0082	0.9502	0.0017	0.9233
δ - Octalactone	0.0341	0.9063	0.0121	0.9217	0.0043	0.9190
2-Undecanone	0.1321	0.9147	0.0283	0.9620	0.006	0.9437
Geranylacetone	0.0366	0.9622	0.0069	0.9719	0.0013	0.9666
Guaiacol	0.1580	0.9914	0.0339	0.9978	0.0069	0.9965

*Laboratory conditions: Temperature 24 ± 1° C

Relative humidity 65 ± 5 %

Wind speed 20 cm / sec

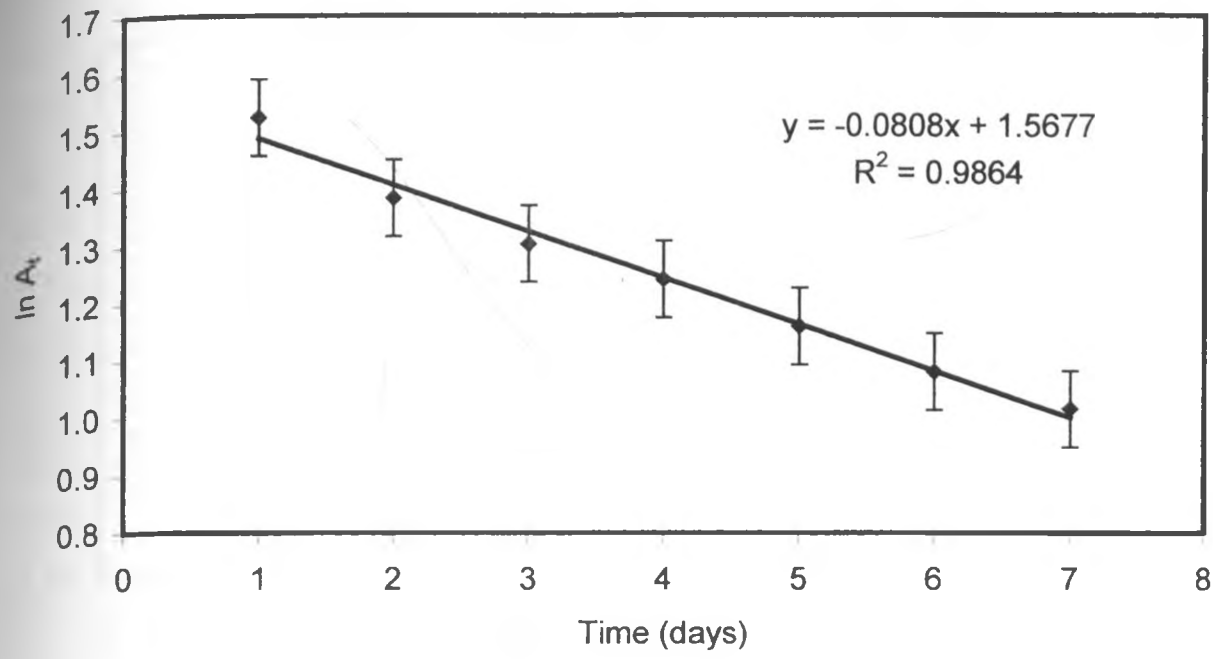

Figure 12: Release kinetics of pentanoic acid under laboratory conditions as described by the first-order rate equation.

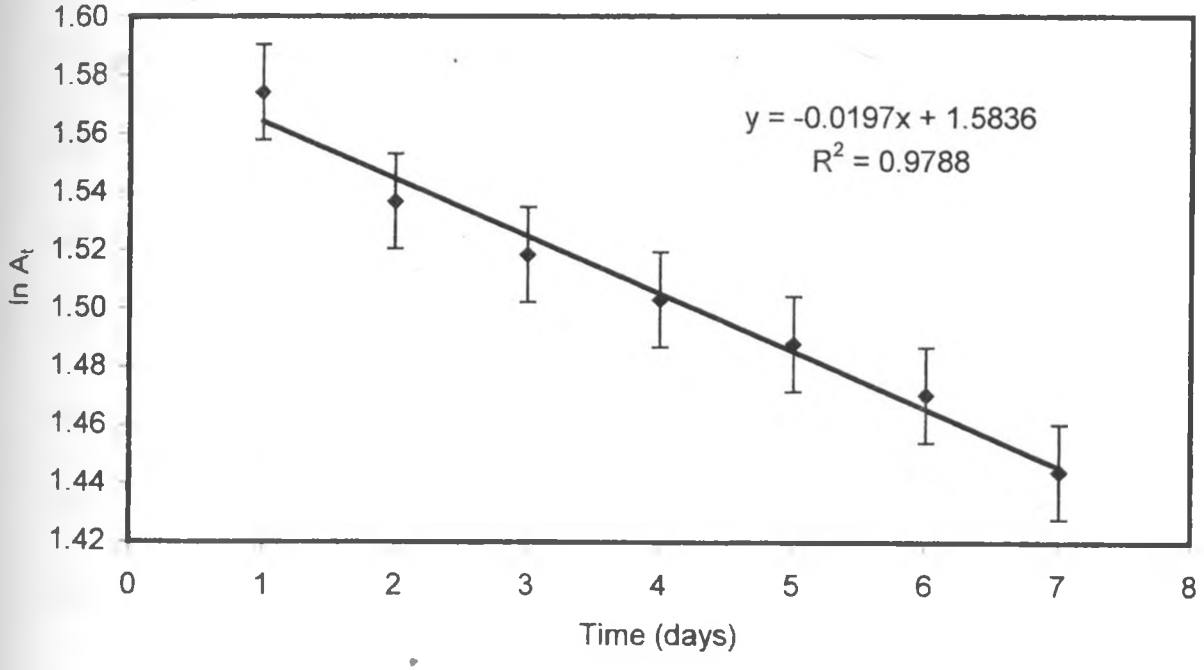

Figure 13: Release kinetics of hexanoic acid under laboratory conditions as described by first-order rate equation.

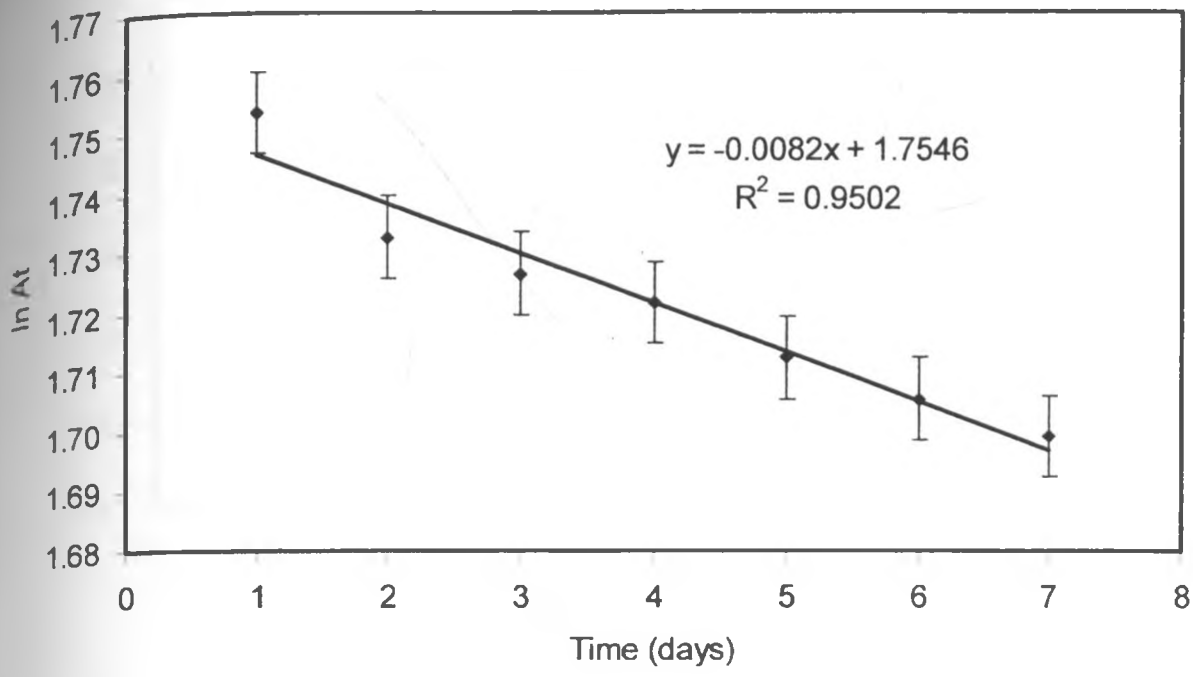

Figure 14: Release kinetics of heptanoic acid under laboratory conditions as described by the first-order rate equation

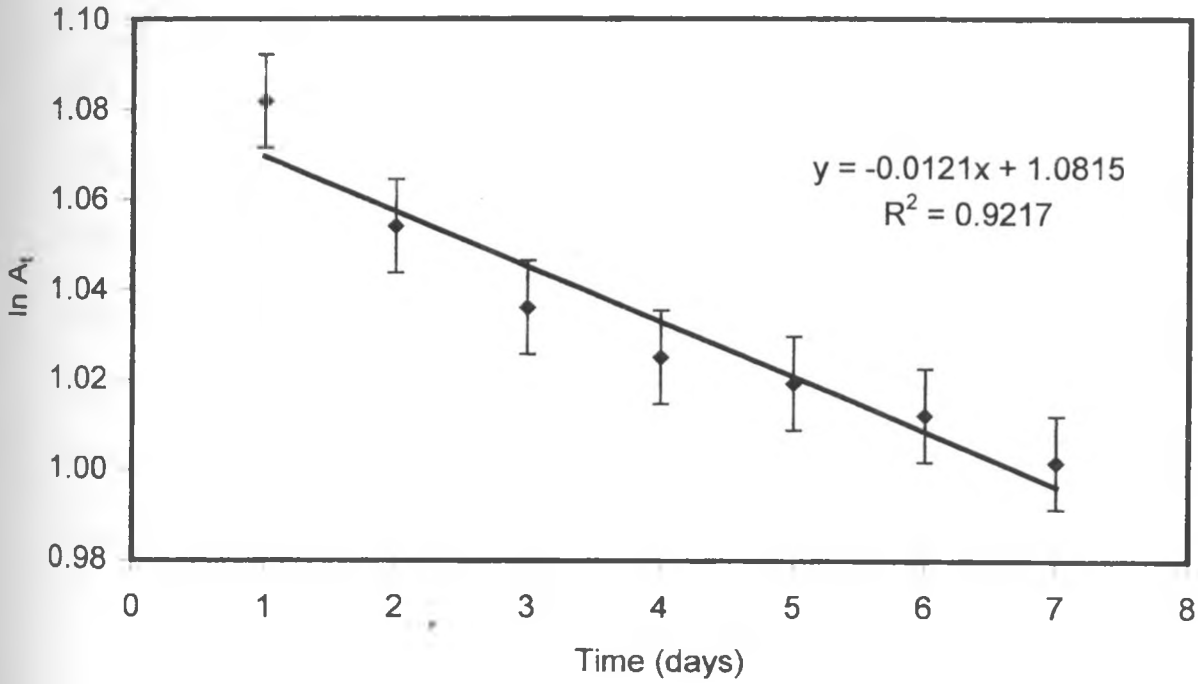

Figure 15: Release kinetics of δ-octalactone under laboratory conditions as described by the first-order rate equation.

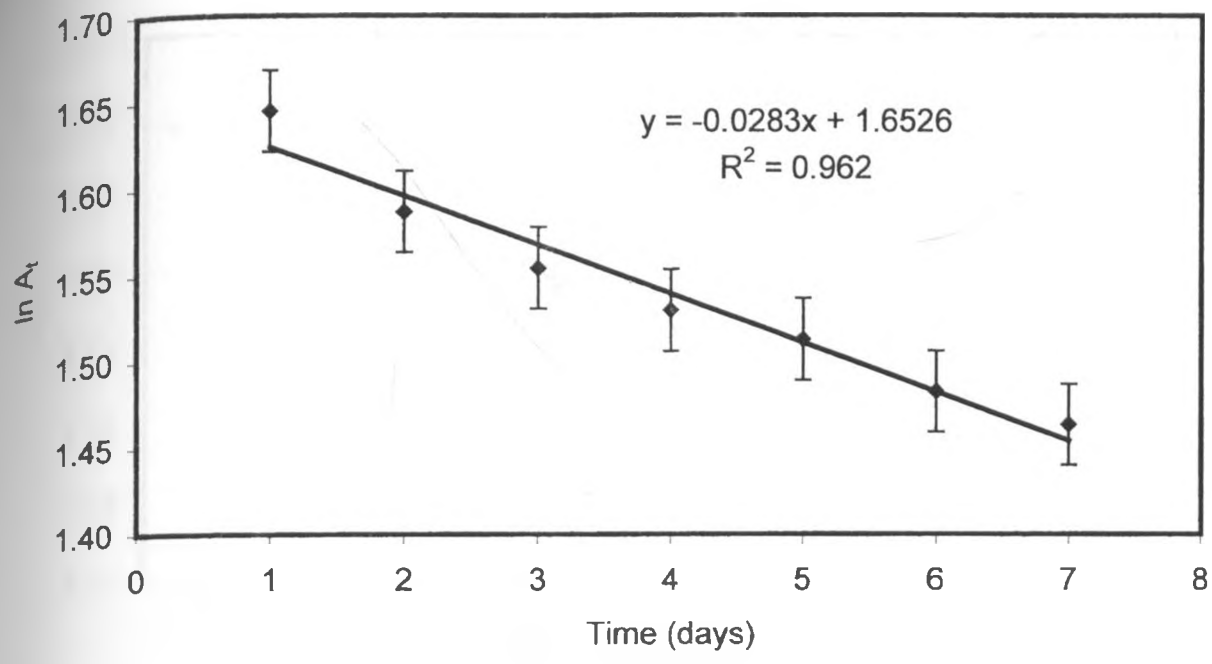

Figure 16: Release kinetics of 2-undecanone under laboratory conditions as described by the first-order rate equation

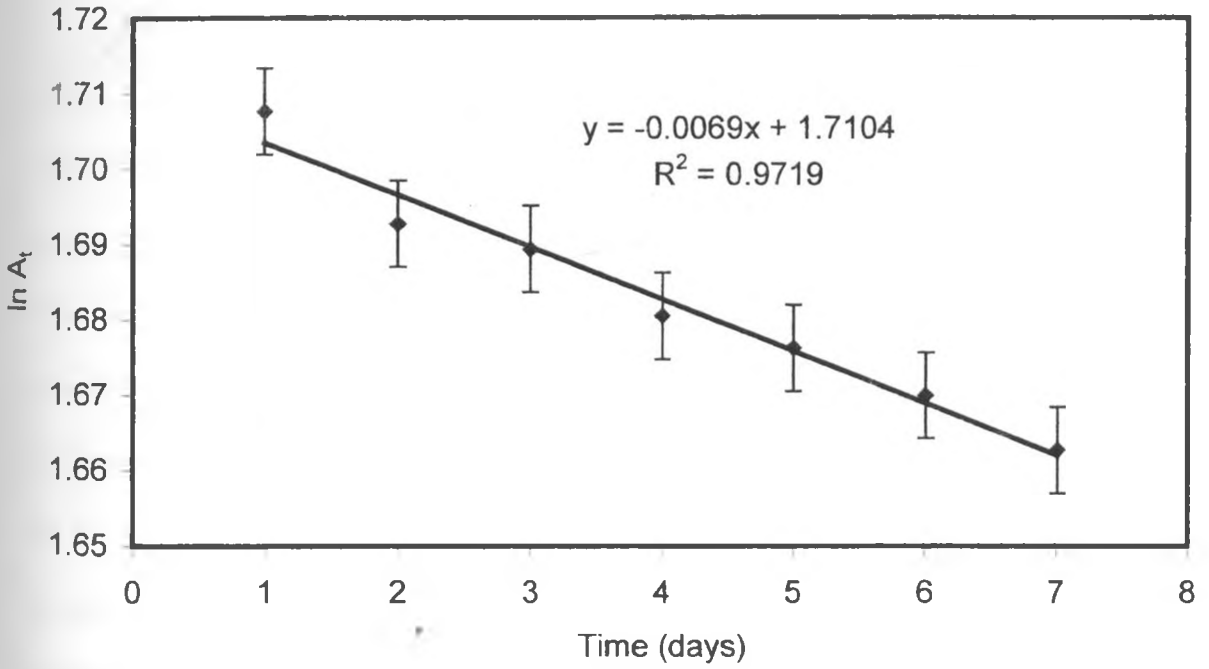

Figure 17: Release kinetics of geranylacetone under laboratory conditions as described by the first-order rate equation

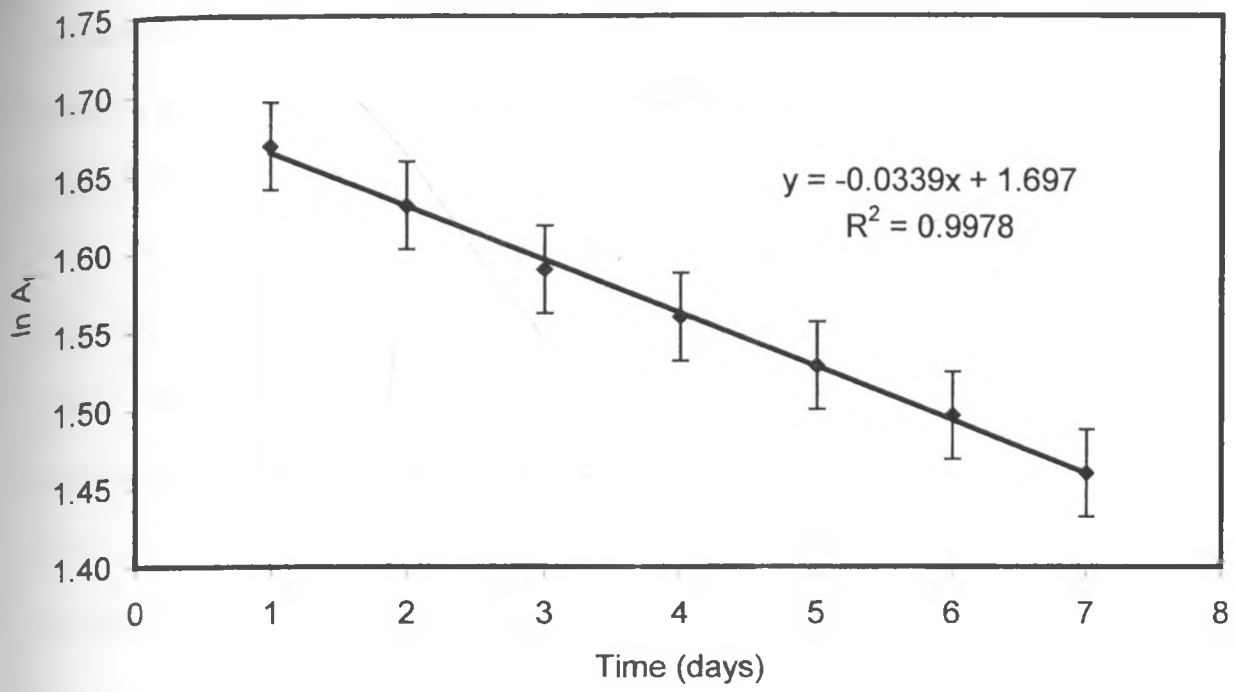

Figure 18: Release kinetics of guaiacol under laboratory conditions as described by the first-order rate equation

Close examination of Figures 12 to 18 indicates that the data point corresponding to day 1 in each figure appear to be consistently higher (slight in Figure 18) or above the regression lines drawn, thereby creating an illusion of an upward curvature around this point. This apparent initial curvature has also previously been observed and reported by other workers (Koch *et al.*, 2002) who attributed it to non-equilibrium status of the system during day 1 of the measurements. However, even with this data point included in the plots, the experimental data points are still consistent with first-order kinetics for all the compounds: The r^2 values are consistently higher with the first-order model compared to the other models (see Table 7). In fact, this anomalous data point has generally contributed to the relatively lower r^2 values. It is significant to note that with the day 1 data point omitted, the r^2 values improved but the orders of release became inconsistent. Similar observations have been previously made and reported. In a study to

evaluate dispenser performance, Koch *et al.* (2002) observed that the bulk release may yield the desired results but the individual component analysis may show differences in release kinetics.

From these findings, the compounds are released from the tubing following first-order release kinetics which is consistent with a Fickian diffusion model (Wells *et al.*, 2004). This is because the concentration within the diffusion volume (the dispenser) changes with respect to time, thus obeying Fick's law of diffusion (Eichie and Okor, 2002). Since the release follows first-order kinetics, the implication is that the concentration of the individual compounds in the mixture decreases exponentially with time (i.e. $A_t = A_o\, e^{-k_1 t}$) where A_t, A_o, k_1 and t are as defined in section 4.1 (Atkins, 1990).

4.2.2 Release kinetics of the Blend under Laboratory Conditions

The experimental data (Appendix 3) was fitted to zero-, first- and second-order kinetic models in an attempt to describe the cumulative release of the blend from the dispensers. The model that best fitted the experimental release data was determined based on the correlation coefficient value (r^2). The model that yielded the highest r^2 values was chosen as the model that best describes the blend release rate. Figure 19 shows the experimental data fitted to the zero-order kinetic model. A summary of the r^2 and rate constant values corresponding to data fitted to the zero-, first-, and second-order kinetic models is given in Table 8.

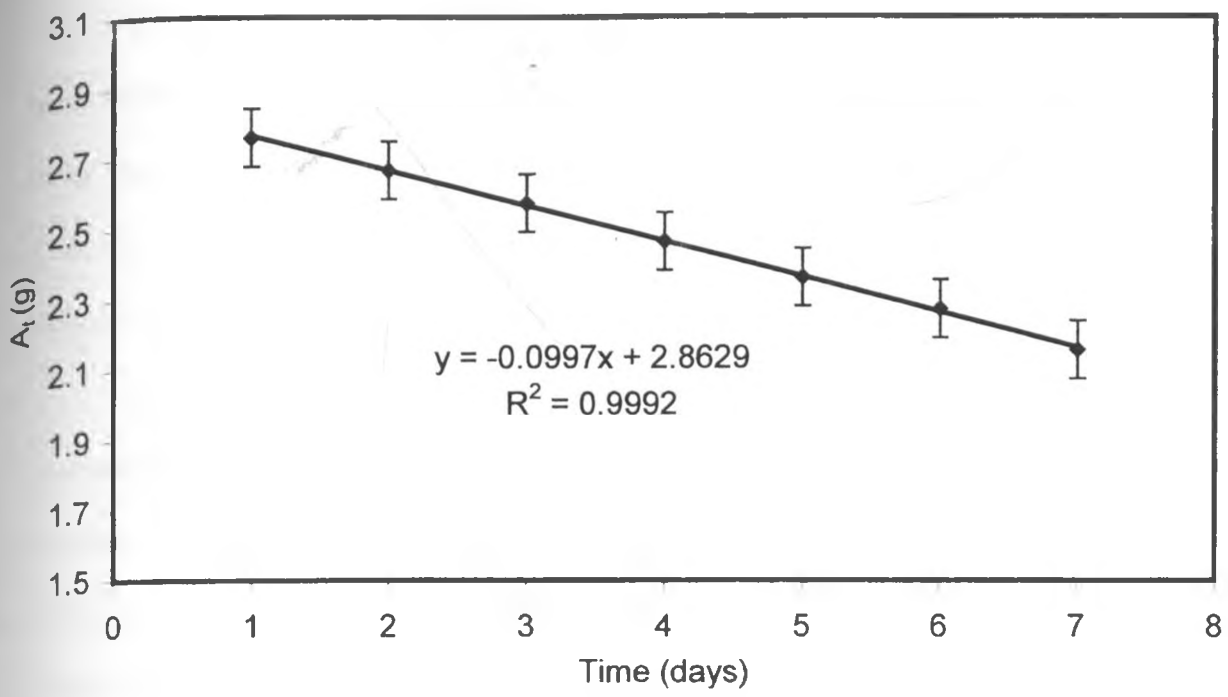

Figure 19: Release kinetics of the blend under laboratory conditions as described by the zero-order rate equation

Table 8: Correlation coefficient values (r^2) and rate constants for the various kinetic models used to describe the release of the blend under laboratory conditions

ZERO-ORDER MODEL		FIRST-ORDER MODEL		SECOND-ORDER MODEL	
k_o (g day^{-1})	r^2	k_1 (day^{-1})	r^2	k_2 (g^{-1} day^{-1})	r^2
0.0997	0.9992	0.0407	0.9969	0.0166	0.9921

The highest r^2 value (0.9992) obtained after fitting the data to the zero-order model indicated that the release process of the blend from the dispenser follows zero-order kinetics. The overall rate constant (0.0997 g day $^{-1}$) was determined graphically from the straight line plot of the zero-order equation (i.e. $A_t = A_o - k_0 t$ where $-k_0$ is the slope of the graph and k_o is the zero-order rate constant).

It is significant to note that under controlled laboratory conditions, the individual compounds follow first-order release kinetics whereas a uniform blend of the same compounds follow zero-order release kinetics. The explanation for this could lie in the differences in the chemical dynamics of the two systems since the environmental conditions in the wind tunnel were held constant. Such dynamics are best described by Raoult's law. In the paragraphs that follow, an attempt is made to postulate the happenings in this system under investigation.

According to Raoult's law, the partial pressure of a solvent over a solution, P_1, is given by vapour pressure of the pure solvent, P_1^o and the mole fraction of the solvent in the solution, X_1 (Chang, 1991).

$$P_1 = X_1 P_1^o, \quad \text{.....9}$$

In a solution containing only one solute, $X_1 = 1-X_2$, where X_2 is the mole fraction of the solute. Equation 9 can be rewritten as,

$$P_1 = (1 - X_2)P_1^O$$

$$P_1^O - P_1 = \Delta P = X_2 P_1^O \quad \text{.....10}$$

The decrease in vapour pressure, ΔP, is directly proportional to the concentration (measured in mole fraction) of the solute present. If both components of a solution are

volatile, the vapour pressure of the solution is the sum of the individual partial pressures. Raoult's law holds equally well in this case.

$$P_A = X_A P_A^o \quad \text{and,}$$

$$P_B = X_B P_B^o \quad \text{.....11}$$

where P_A and P_B are the partial pressures over the solution for components A and B, P_A^o and P_B^o are the vapour pressures of the pure substances and X_A and X_B are their mole fractions. The total vapour pressure is given by Dalton's law of partial pressure,

$$P_T = P_A + P_B \quad \text{.....12}$$

It follows that the vapour pressure of the mixed liquids will be dependent on the vapour pressures of the individual compounds and the molar fraction of each compound present (Byers, 1988).

This law is strictly valid under the assumption that the bonding between the components is equal to the bonding within the components. Therefore, comparing the actual measured vapour pressures to predicted values from Raoult's law allows information about the relative strength of bonding between components to be obtained. If the measured vapour pressure is less than the predicted value, then fewer molecules have left the solution than expected. This is due to the strength of bonding between the components being greater than the bonding within the individual component molecules, so that fewer molecules have enough energy to leave the solution. Conversely, if the vapour pressure is greater than the predicted value, then more molecules have left the solution than expected due to bonding between the component molecules being less strong than the bonding within each. The law assumes an ideal behaviour and gives a simple picture of the situation just as the ideal gas law does, with an assumption that the

physical properties of the components are identical. The more similar the components, the more their behaviour approaches that described by Raoult's law.

Non-ideality of the mixture will be manifest in the deviation of the activity coefficient of the mixture from unity and hence from Raoult's law. For a positive deviation, the vapour pressure for a given mixture is greater than would be expected and therefore the boiling point is lower. Since the vapour pressure is higher, the liquid evaporates more easily implying that some of the intermolecular bonds in the liquid must have been broken when the liquids were mixed. This would be the case, for example when hydrogen bonding is reduced. Negative deviations are observed when the vapour pressure is lower than would be expected from Raoult's law, meaning that the intermolecular forces increase on mixing the liquids. The attractive forces between molecules of different species are greater than between molecules of the individual species, thus the escaping tendency as measured by vapour pressure is reduced.

However, in practise, introducing other factors like meniscus curvature, surface tension and diffusion coefficient, which are not precisely known at any one particular instance, introduces complications as some researchers have reported (Browne, 1978; Byers and Wood, 1980; Byers, 1982; Tilden *et al.* 1983; Tilden and Bedard, 1985). Thus the observed decline in the release rates of the individual compounds with time can be attributed to the vapour pressure in the dispensers becoming diminished as the amount of the chemical compound decreases. This causes a reduction in the vapour pressure between that inside of the dispenser and the external atmosphere (wind tunnel environment), thus affecting the diffusion of the individual compounds (Holsten *et al.* 2002).

From these results, the kinetics of release of the blend are adequately described by the zero-order model while that of the individual compounds are best described by the first-order model. However, neither model clearly indicates the mechanism through which the release occurs: whether it is evaporation, diffusion or degradation (Mayer and Mitchell, 1998). For the blend under investigation in this study, chromatographic analysis shows that the seven compounds in the blend are still present at the end of the release period but in smaller quantities (see section 4.4.2).

4.3 Determination of the Release Kinetics under Semifield Conditions

4.3.1 Release Kinetics of the Individual Compounds

The dispensers containing the individual compounds were exposed to direct sunlight and others were placed under the shade. The experimental weight loss data (Appendix 4) were fitted to the zero-, first- and second-order kinetic models and the release kinetics of the compounds determined. The results are summarised in Table 9.

Table 9: Correlation coefficient values (r^2) and rate constants for various kinetic models used to describe the release of the individual repellent compounds under semifield conditions using a 4 cm length tygon silicon tubing

COMPOUNDS	ZERO ORDER				FIRST ORDER				SECOND ORDER			
	SUN		SHADE		SUN		SHADE		SUN		SHADE	
	k_0 g day $^{-1}$	r^2	k_0 g day $^{-1}$	r^2	k_1 day $^{-1}$	r^2	k_1 day $^{-1}$	r^2	k_2 g $^{-1}$ day $^{-1}$	r^2	k_2 g $^{-1}$day^{-1}	r^2
Pentanoic acid	0.8441	0.9925	0.6554	0.9982	0.3905	0.9782	0.2043	0.9907	0.2150	0.8767	0.0688	0.9572
Hexanoic acid	0.3958	0.9983	0.2690	0.9973	0.1003	0.9936	0.6110	0.9956	0.0257	0.982	0.0139	0.9914
Heptanoic acid	0.1726	0.9990	0.1625	0.9986	0.0492	0.9986	0.0454	0.9965	0.0140	0.9948	0.0132	0.9982
δ - octalactone	0.0795	0.9945	0.0528	0.9960	0.0259	0.9936	0.0168	0.9957	0.0080	0.9921	0.0050	0.9952
Geranylacetone	0.1560	0.9982	0.0775	0.9981	0.0314	0.9978	0.0145	0.9976	0.0060	0.9967	0.0027	0.9970
2-undecanone	0.6970	0.9963	0.4172	0.9945	0.3117	0.9656	0.1518	0.9807	0.2651	0.8254	0.0567	0.9530
Guaiacol	0.2539	0.9952	0.2535	0.9956	0.0422	0.9995	0.0401	0.9981	0.0061	0.9992	0.0064	0.9944

Pentanoic, hexanoic and heptanoic acids, δ-octalactone, 2-undecanone and geranylacetone were found to follow zero-order kinetics as indicated by the higher correlation coefficient values in both the sun and the shade. The carboxylic acids (i.e., pentanoic, hexanoic and heptanoic acid had higher rate constants than δ-octalactone and geranylacetone. Pentatonic acid registered the highest rate constants of 0.8841 and 0.6554 g day $^{-1}$ in the sun and shade, respectively (Figure 20). It was followed by hexanoic acid with 0.3985 and 0.269 g day $^{-1}$ in the sun and shade, respectively (Figure 21); and heptanoic acid with 0.1726 and 0.1625g day $^{-1}$ in the sun and shade (Figure 22) respectively. Geranylacetone yielded rate constants of 0.156 and 0.0775 g day $^{-1}$ in the sun and shade, respectively (Figure 23) while δ-octalactone had 0.0795 and 0.0528 g day $^{-1}$ in the sun and shade, respectively (figure 24). 2-Undecanone registered higher zero-order rate constants of 0.697 and 0.4172 g day $^{-1}$ in the sun and shade, respectively (Figure 25) than hexanoic and heptanoic acids, δ-octalactone, and geranylacetone. Guaiacol (Figure 26) however, follows first-order release kinetics in the semifield conditions, unlike the rest of the studied repellents.

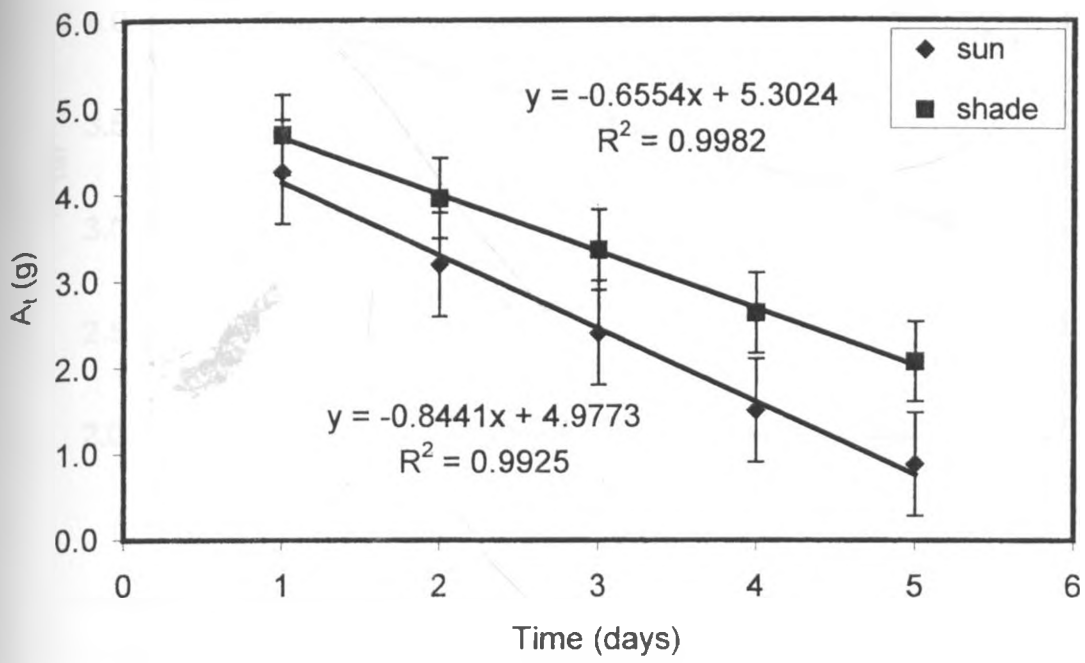

Figure 20: Release kinetics of pentanoic acid as described by the zero-order rate equation under semifield conditions

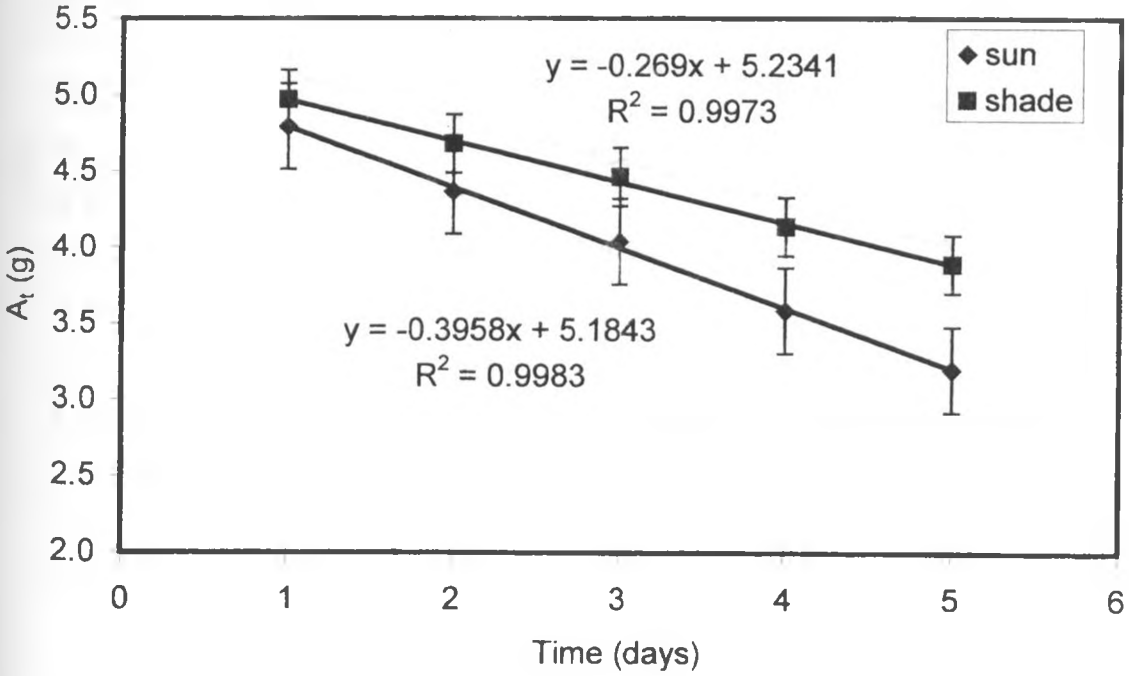

Figure 21: Release kinetics of hexanoic acid as described by the zero-order rate equation under semifield conditions

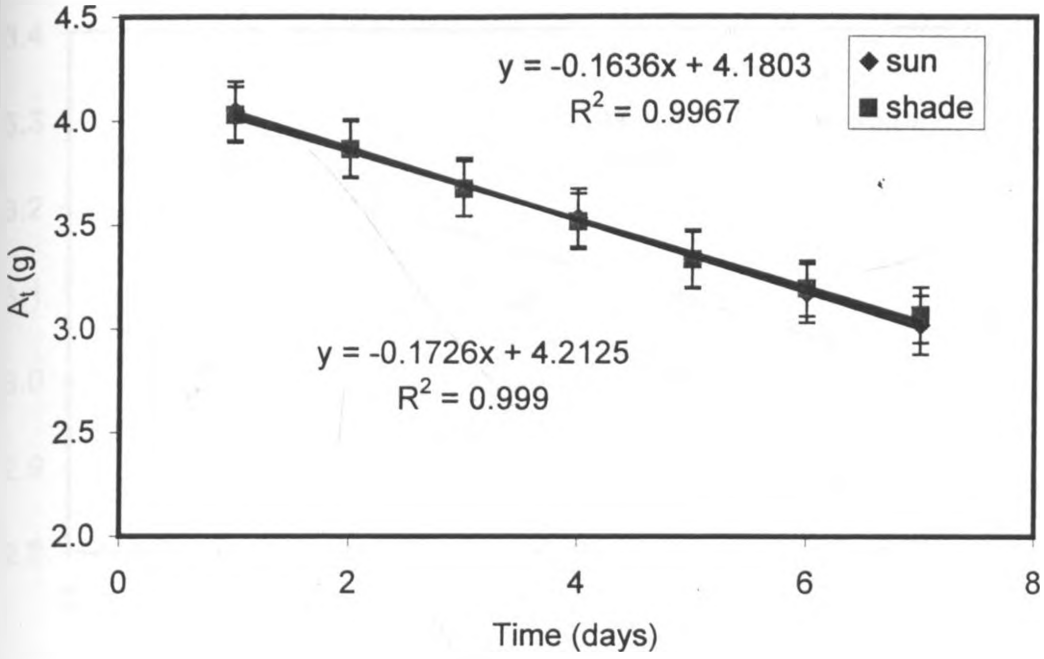

Figure 22: Release kinetics of heptanoic acid as described by the zero-order rate equation under semifield conditions

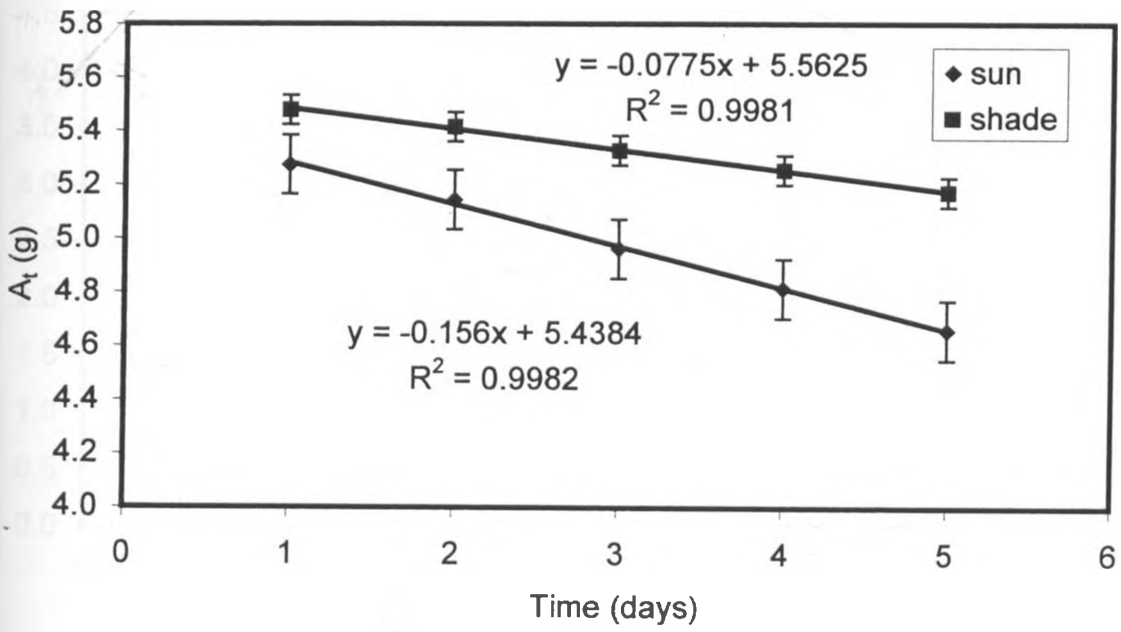

Figure 23: Release kinetics of geranylacetone as described by the zero-order rate equation under semifield conditions

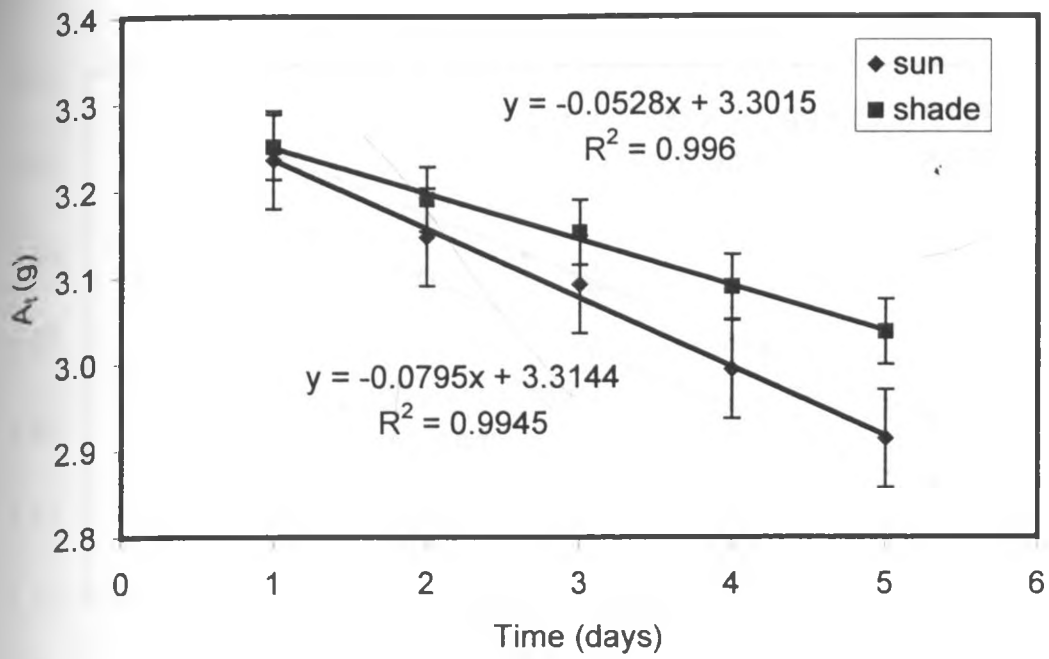

Figure 24: Release kinetics of δ-octalactone as described by the zero-order rate equation under semifield conditions

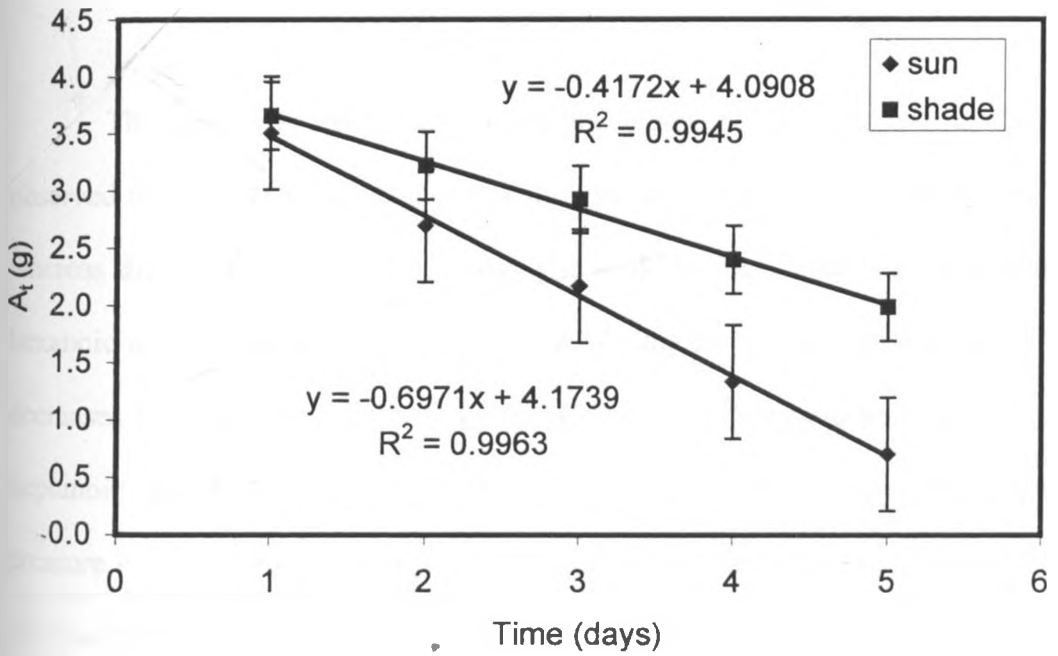

Figure 25: Release kinetics of 2-undecanone as described by the zero-order rate equation under semifield conditions

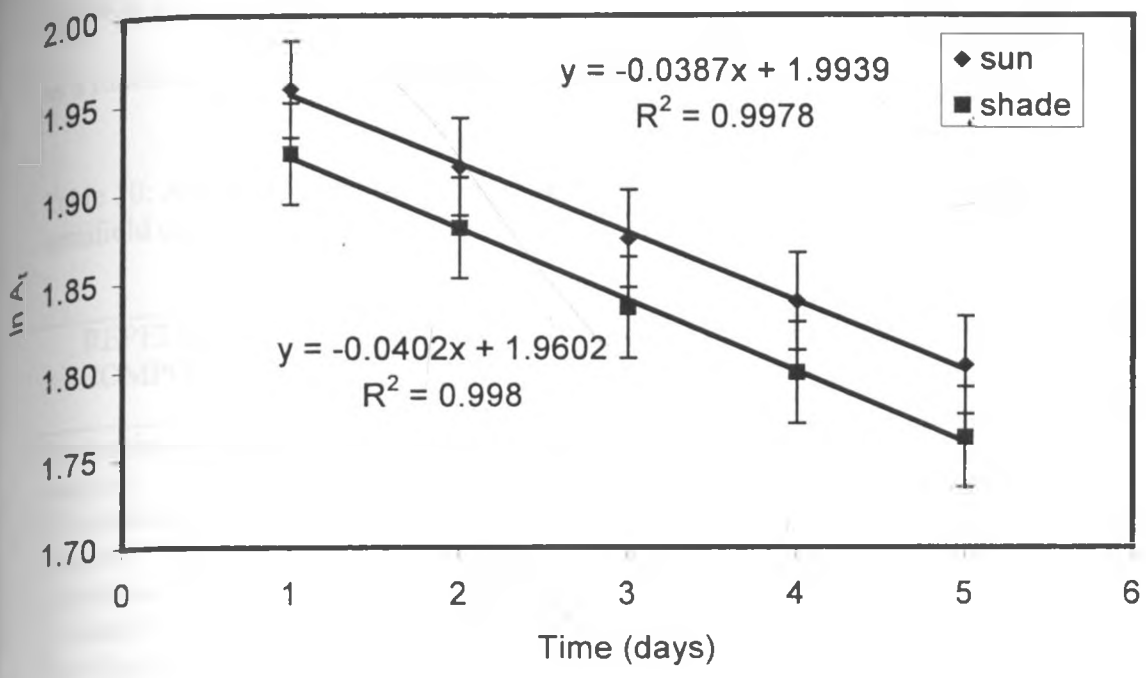

Figure 26: Release kinetics of guaiacol as described by the first-order rate equation in the semifield.

The zero-order rate constants for the release of the carboxylic acids were also observed to decrease with increasing boiling points of the respective acids. For instance, whereas the boiling points of the carboxylic acids increase from pentanoic acid through hexanoic acid to heptanoic acid (see Table 10), the zero-order rate constants in the sun decreases from pentanoic acid (0.8441 g day^{-1}) through hexanoic acid (0.3958 g day^{-1}) to heptanoic acid (0.1726 g day^{-1}). This trend is however not surprising since vapour pressure is inversely proportional to boiling point. The same argument, however does not seem to apply among non-homologous compounds. For instance, whereas 2-undecanone and guaiacol have boiling points of 232°C and 205°C, respectively, their corresponding zero-order release rate constants are 0.697 and 0.254 g day^{-1} respectively; a trend

opposite to that observed with the carboxylic acids. The corresponding weight loss data as a function of boiling points are given in Table 10.

Table 10: Average weight loss (± SD) of the individual repellent compounds under semifield conditions (n= 3)

REPELLENT COMPOUND	Boiling point (°C)	WEIGHT LOSS IN SUN (g)	WEIGHT LOSS IN SHADE (g)
Pentanoic acid reagent	186-188	0.89 ± 0.21	0.68 ± 0.09
Hexanoic acid reagent	202-203	0.39 ± 0.06	0.28 ± 0.03
Heptanoic acid	223	0.17 ± 0.01	0.15 ± 0.03
Guaiacol	205	0.25 ± 0.03	0.23 ± 0.03
δ-octalactone	115	0.08 ± 0.02	0.05 ± 0.01
Geranylacetone	124	0.14 ± 0.03	0.07 ± 0.02
2-undecanone	232	0.68 ± 0.01	0.39 ± 0.09

4.3.1.1 Effect of Ambient Temperature

In the laboratory experiments, experimental data were collected under controlled conditions of temperature, relative humidity and wind speed. These parameters are however expected to vary significantly under semifield or field conditions and thereby influence the release kinetics of the repellent chemicals. In fact, evidence of potential dependence of release rates on temperature can be seen from variations in weight loss data obtained from both the sun and under shade (Table 10). It is clearly evident from Table 10 that for all compounds studied, weight losses recorded in the shade are lower than corresponding weight losses in the sun. Thus it was necessary to take the daily temperature recordings over the entire duration of the experiments conducted under semifield or field conditions.

Temperatures and relative humidity were recorded continuously over the duration of the experiments and the trends of temperature recordings are shown in Figures 27 and 28. The figures show high variations: with the temperatures in the sun registering a high of 26.5°C on day 3 and a low of 7.0°C on day 6. In the shade, a high of 21.5°C was registered on day 1 and a low of 6.5°C registered on day 3. The mean relative humidity was 44.9% in the sun and 48.3% in the shade. The data obtained indicate that variations in relative humidity were very small.

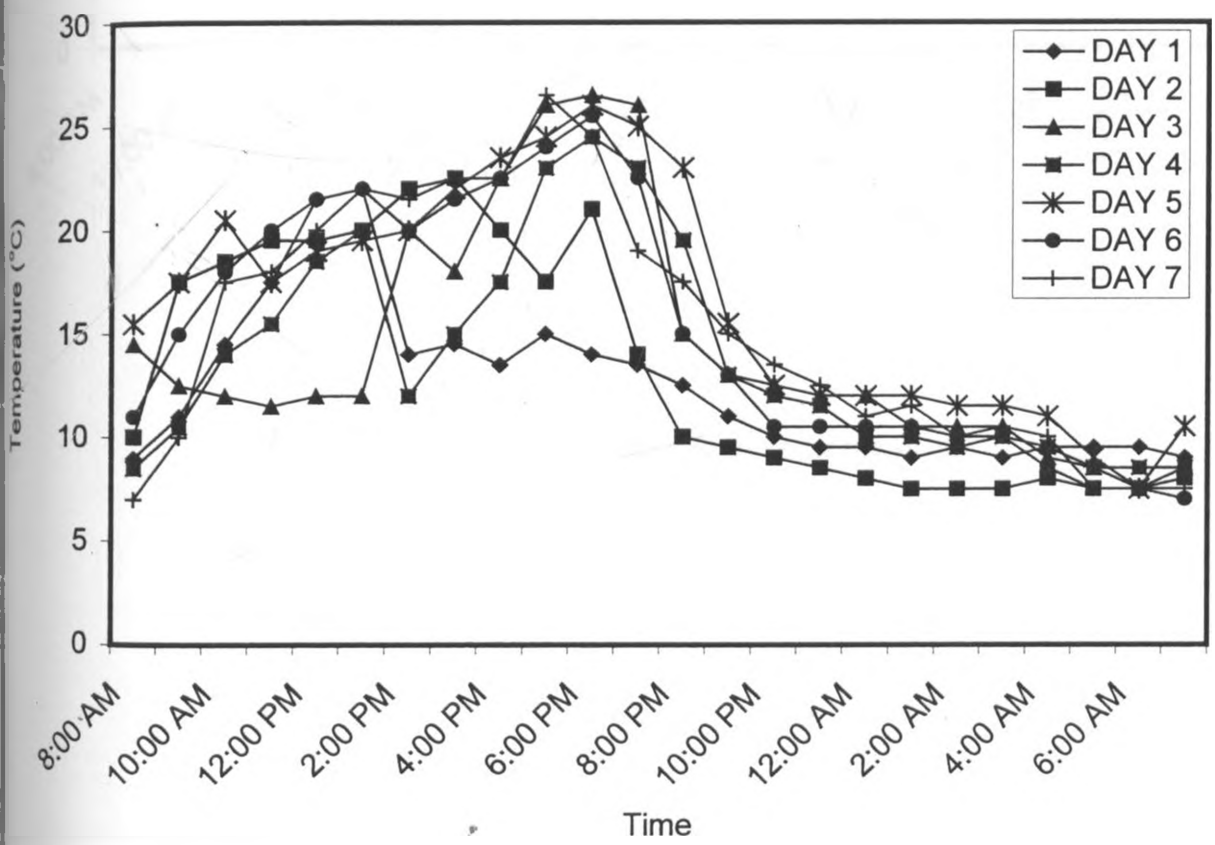

Figure 27a: Mean daily temperatures in the sun

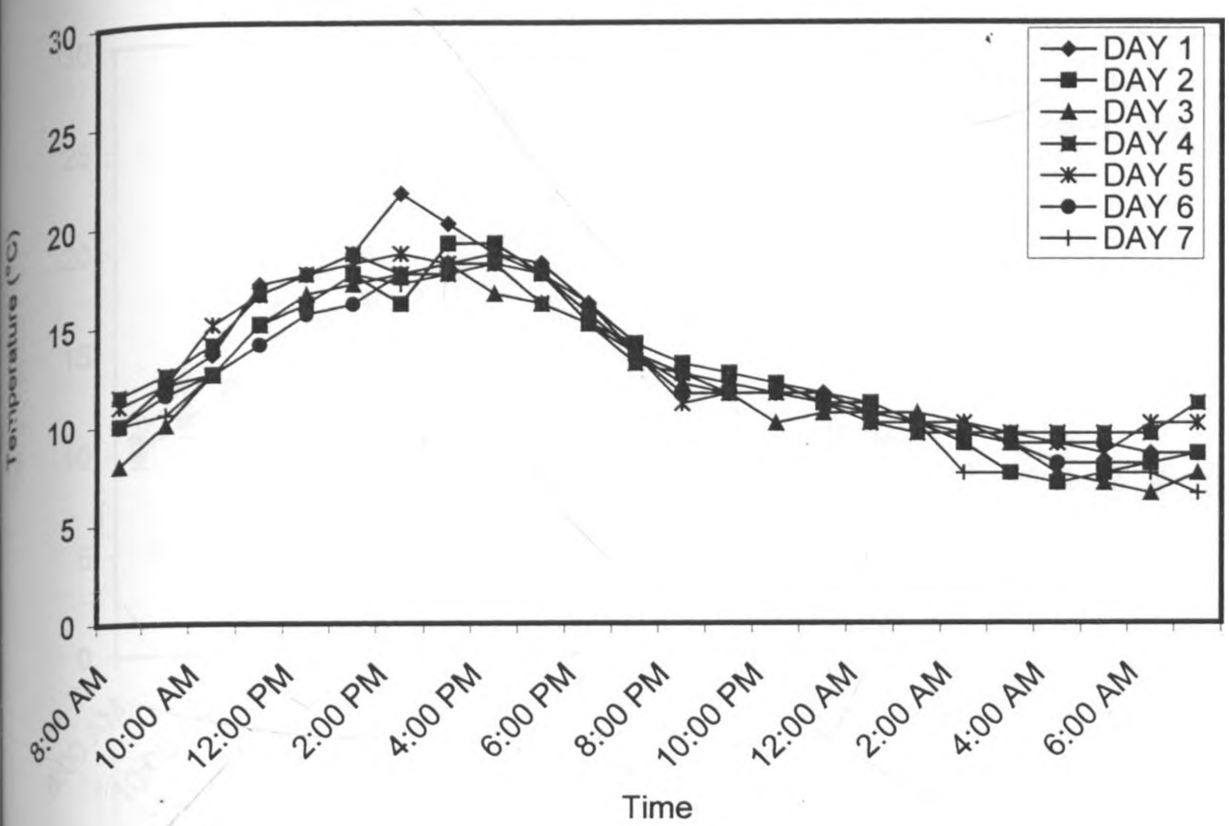

Figure 27b: Mean daily temperatures under the shade

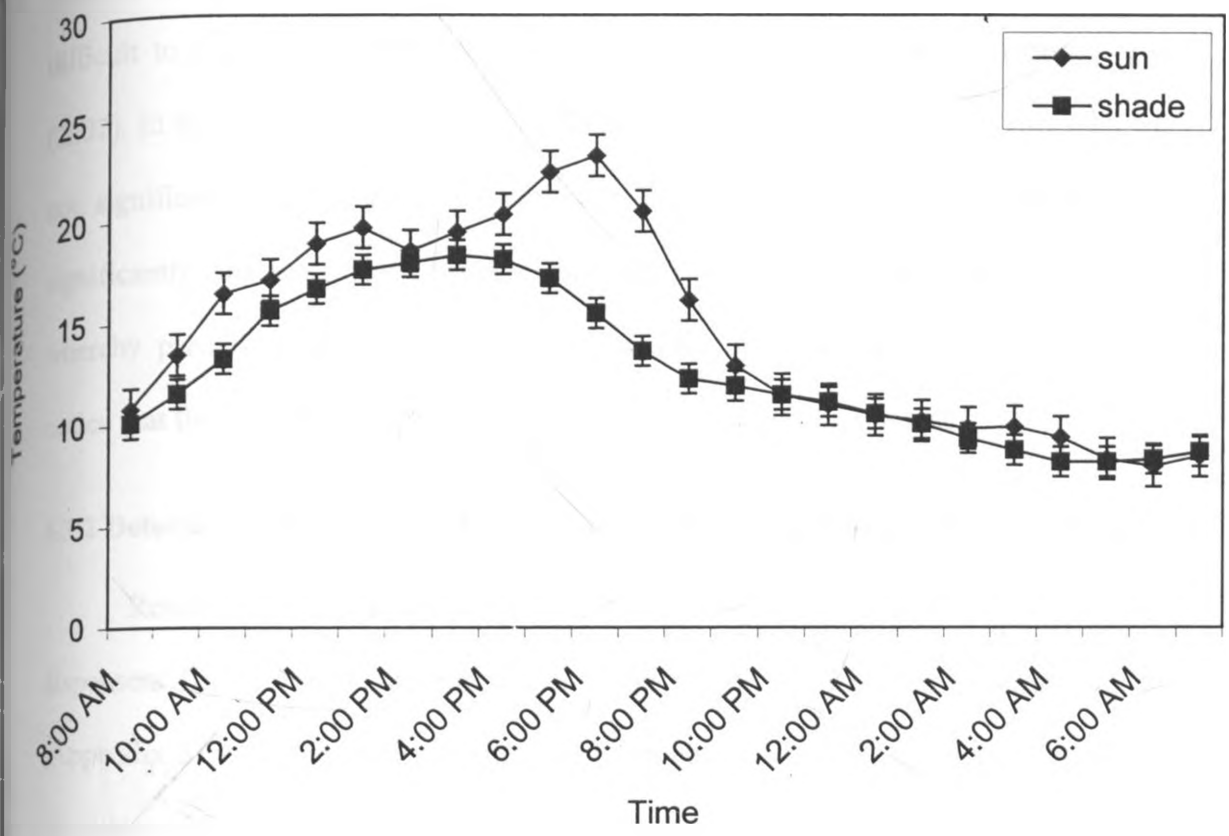

Figure 28: Average hourly temperatures in the sun and under shade over the 7 day period.

In view of the large variations in daily temperatures, it is plausible to assume that ambient air temperature has a profound effect on weight losses in the dispensers. In chemical kinetics for example, as the temperature is increased, the rate of most reactions increases. As a rough approximation, for most reactions occurring at around room temperature, the rate of reaction could increase by a factor of two to three for every 10°C rise in temperature (Atkins, 1990). Thus the temperature variations registered above would be expected to cause significant variations in the measured values of weight losses

and therefore the corresponding rate constants of release. Even though the rates are affected by the surrounding temperature, the magnitude of change is however more difficult to demonstrate with field data as was previously reported by Holsten *et al.*, (2002). In that study, Holsten *et al.* (2002) noticed that release rates from two sites were not significantly different from each other, although the average temperatures were significantly different. These workers attributed this to a compensatory phenomenon whereby partially compensating effects occur throughout the day (Byers, 1988). Also notice that the spread in temperatures reported in this study is quite wide.

4.3.2 Determination of Release Kinetics of the Blend under Semifield Conditions

Release kinetics of the blend were determined under semifield conditions using dispensers fitted with 4 cm and 2 cm tygon silicon tubing. The experimental data (Appendix 5) was fitted to zero-, first- and second-order kinetic models and the results are summarised in Table 11. The zero-order model best described the release kinetics of the blend as evidenced by the higher r^2 values compared to those obtained with first- and second-order models.

Table 11: Correlation coefficient values (r^2) and rate constants for various kinetic models used to describe the release of waterbuck-derived blend under semifield conditions

TUBING LENGTH (cm)	ZERO ORDER				FIRST ORDER				SECOND ORDER			
	SUN		SHADE		SUN		SHADE		SUN		SHADE	
	k_0 g day $^{-1}$	r^2	k_0 g day $^{-1}$	r^2	k_1 day $^{-1}$	r^2	k_1 day $^{-1}$	r^2	k_2 g $^{-1}$ day $^{-1}$	r^2	k_2 g $^{-1}$day^{-1}	r^2
4	0.1490	0.9997	0.2019	0.9996	0.0466	0.9976	0.0696	0.9951	0.0147	0.9921	0.0240	0.9840
2	0.1885	0.9846	0.1661	0.9578	0.0885	0.9628	0.078	0.9296	0.0420	0.9299	0.0376	0.8945

The zero-order plot corresponding to the dispenser with 4 cm length of tygon tubing yielded rate constants of 0.149 g day $^{-1}$ (r^2 = 0.9997) and 0.2019 g day $^{-1}$ (r^2 = 0.9996) in the sun and shade, respectively (Figure 29). For the dispenser fitted with 2 cm tygon tubing, the corresponding zero-order rate constants were determined as 0.1885 g day $^{-1}$ (r^2 = 0.9846) and 0.1661 g day $^{-1}$ (r^2 = 0.9578) in the sun and shade, respectively (Figure 30). It is significant to note that the r^2 values corresponding to the 2 cm tygon tubing were consistently lower than their 4 cm tygon tubing counterparts, even though the r^2 values for the zero-order model were still higher than those for first- and second-order models.

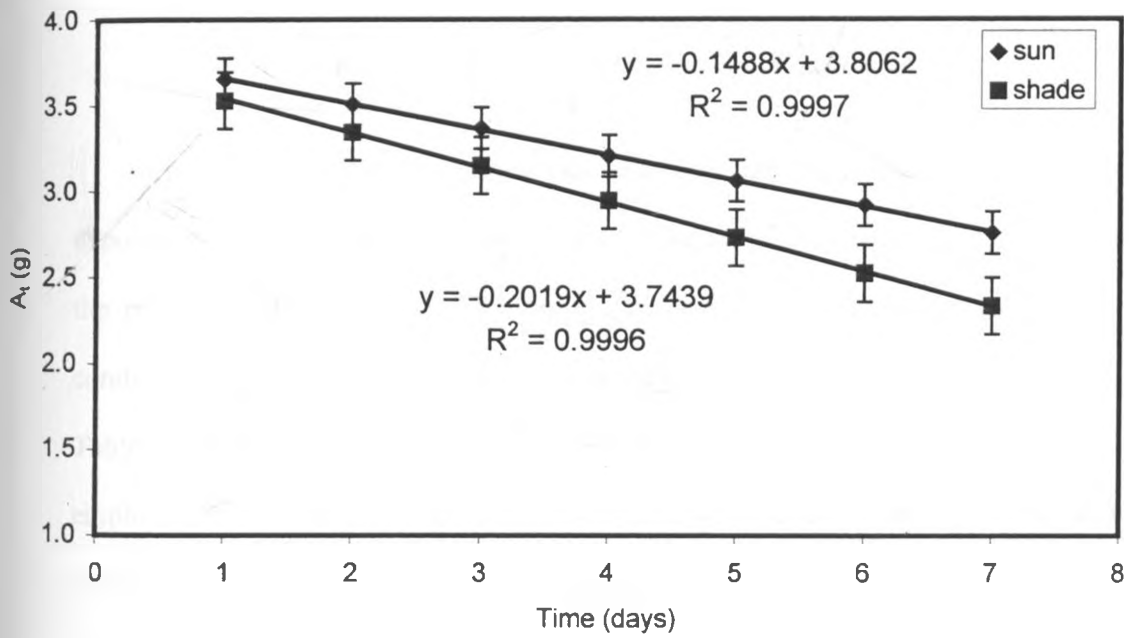

Figure 29: Release kinetics of the blend using 4cm tygon silicon tubing under semifield conditions as described by the zero-order rate equation.

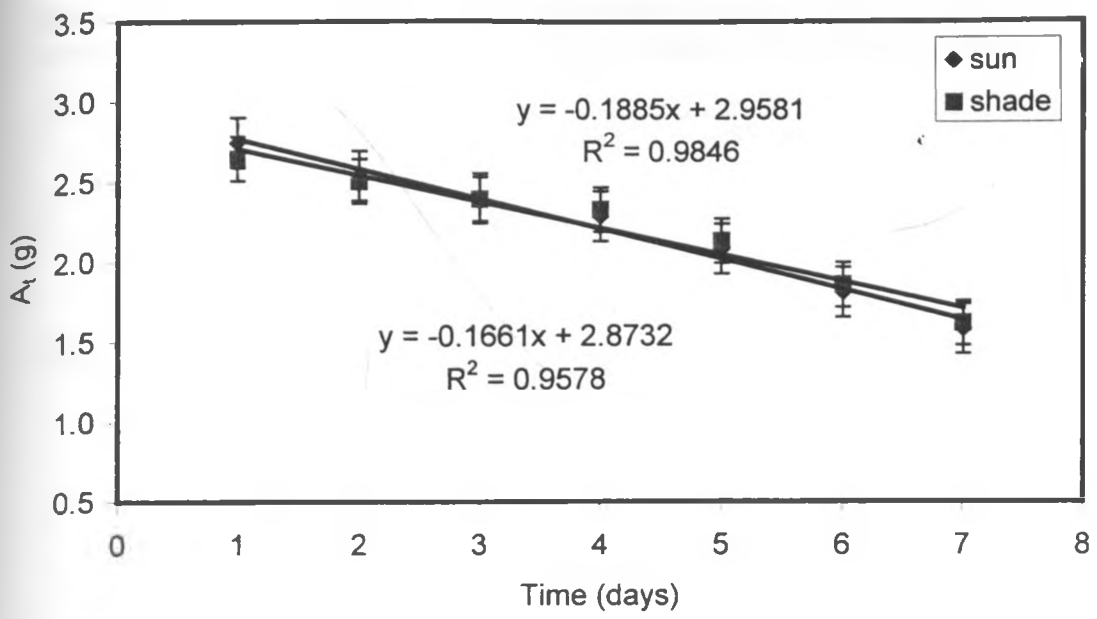

Figure 30: Release kinetics of the blend using 2cm tygon silicon tubing under semifield conditions as described by the zero-order rate equation

In all cases, the determined rate constants are higher for the dispensers exposed to direct sunlight suggesting that temperature is an important determinant in the release kinetics of the blend from the dispensers. The prevailing temperature conditions during these trials are as previously indicated in Figures 27a and 27b. Table 12 provides a summary of the weight loss data for the two types of dispensers employed. It is evident from this table that the weight loss data associated with the 4 cm tygon silicon tubing are nearly twice the values obtained with the 2 cm tygon silicon tubing. This illustrates the importance of surface area of the dispenser unit on release rates.

Table 12: Average weight loss (± Standard Deviation, SD) of the blend in sun and shade under semi-field conditions using different lengths of tygon silicon tubing (n=3)

DAY	WEIGHT LOSS (g)			
	4 cm TYGON SILICON TUBING		2 cm TYGON SILICON TUBING	
	SUN	SHADE	SUN	SHADE
1	0.107 ± 0.001	0.095 ± 0.009	0.073 ± 0.006	0.058 ± 0.006
2	0.136 ± 0.009	0.101 ± 0.009	0.065 ± 0.009	0.052 ± 0.009
3	0.129 ± 0.006	0.097 ± 0.012	0.061 ± 0.006	0.051 ± 0.003
4	0.139 ± 0.009	0.104 ± 0.006	0.061 ± 0.009	0.050 ± 0.003
5	0.131 ± 0.009	0.105 ± 0.006	0.056 ± 0.009	0.045 ± 0.003
6	0.118 ± 0.003	0.111 ± 0.036	0.051 ± 0.003	0.046 ± 0.009
7	0.142 ± 0.003	0.090 ± 0.006	0.049 ± 0.006	0.0456 ± 0.002

For the 4 cm tygon tubing, the weight losses on days 4 and 7 in the sun are relatively larger compared to the losses recorded in the other days, even though the average daily temperature for the two days were not very different at 13.8 ± 1.1°C and 15.2 ± 1.2°C, respectively: both temperatures lower than the highest average temperature which was recorded on day 5 at 16.6 ± 1.1°C (with a lower weight loss). As much as this may seem anomalous, similar findings have been reported previously. Holsten *et al.* (2002) noted that with field data, the magnitude of the effect of temperature on the release rates was more difficult to demonstrate; and they attributed this in part to non-uniform chemical release behaviour under actual environmental conditions due to deterioration of the release device. Another possible reason could be due to systematic errors associated with the standard method of determining the

chemical load remaining in the dispensers by weighing the dispensers between periods of field exposures. The errors may occur when dust particles stick to the dispenser surface or due to weight differences caused by changes in relative humidity and rainfall. Still, since the release rate depends on factors like temperature and wind, this procedure inseparably measures the effects of these and other weather effects.

Nevertheless, it is expected that the vapour pressure and thus the mole percentage of the compounds in the vapour state would increase during daytime and more so under the sun than in the shade due to the effects of temperature. Thus, the observed differences in the release rates between the sun and under the shade are therefore to be expected. Moreover, a compensatory effect has previously been reported (Byers, 1988) where a reverse effect that reduces the rate of release (e.g. lower temperature) occurs to counter an effect which increases the release rate due to higher temperatures. Byers (1988) noted that variation in temperature could probably have effects of up to 100 % on the vapour pressures over the daily temperature range. Thus the difference in the release rates in the semifield or the field can be explained by temperature differences (Holsten *et al.* 2002).

Olsson *et al.* (1983) has also suggested that the longer the chain length of the compound, the greater the dependence of the release of a compound (from a release device) on temperature. However, results of the weight analysis and by extension the release rates indicates that the weight losses between the days are not significant, but the weight differences between the dispensers are significant. Weight losses with the dispensers fitted with 4 cm of the tubing are nearly twice those fitted with 2 cm tubing.

The dispensers in this trial were made to be similar in all aspects as was practically possible in order to reduce the difference in the airflow along the surface

of the dispenser, which may interfere with the effective air velocity around the dispenser. If this interference in airflow occurs, small, although significant deviations in the release rates can occur (Van der Kraan and Ebbers, 1990). Air movement is assumed not to influence the properties of the dispenser itself, but to influence only the transport of vapour molecules from the boundary layer above the releasing surface. The main properties of the volatile compound that may be relevant to that process and might therefore affect the release rate are diffusivity and vapour pressure (Van der Kraan and Ebbers, 1990).

4.4 Quantitation of Repellent Release Kinetics by GC Analysis of the Samples

4.4.1 Calibration Curves

Multiple injections of a sample will usually result in different peak areas for a given component because it is difficult to exactly reproduce the injection volumes (Novotny, 1982; Rubinson and Rubinson, 2000). These variations are compensated for by adjusting (normalising) the component peak area by dividing it by the peak area of an internal standard since the internal standard has a known concentration in both sample and standard. Internal standards were chosen based on their structural similarity and chromatographic resolution with the compounds of interest (Qian and Reinecccius, 2003). In this way, the concentration of each component in the unknown can be determined by comparison of the normalised peak areas. The linear least squares equations obtained from the calibration curves were then used to quantify the individual compounds studied.

A calibration curve was constructed for each individual compound using standards of concentration 500, 400, 300, 200 and 100 μg/ml for the acids and 400, 300, 200 and 100 μg/ml for 2-undecanone, geranylacetone, δ-octalactone and

guaiacol. The chromatogram of the acid mixture and the mixture of 2-undecanone, geranylacetone, δ-octalactone and guaiacol are shown in Figures 31 and 32, respectively.

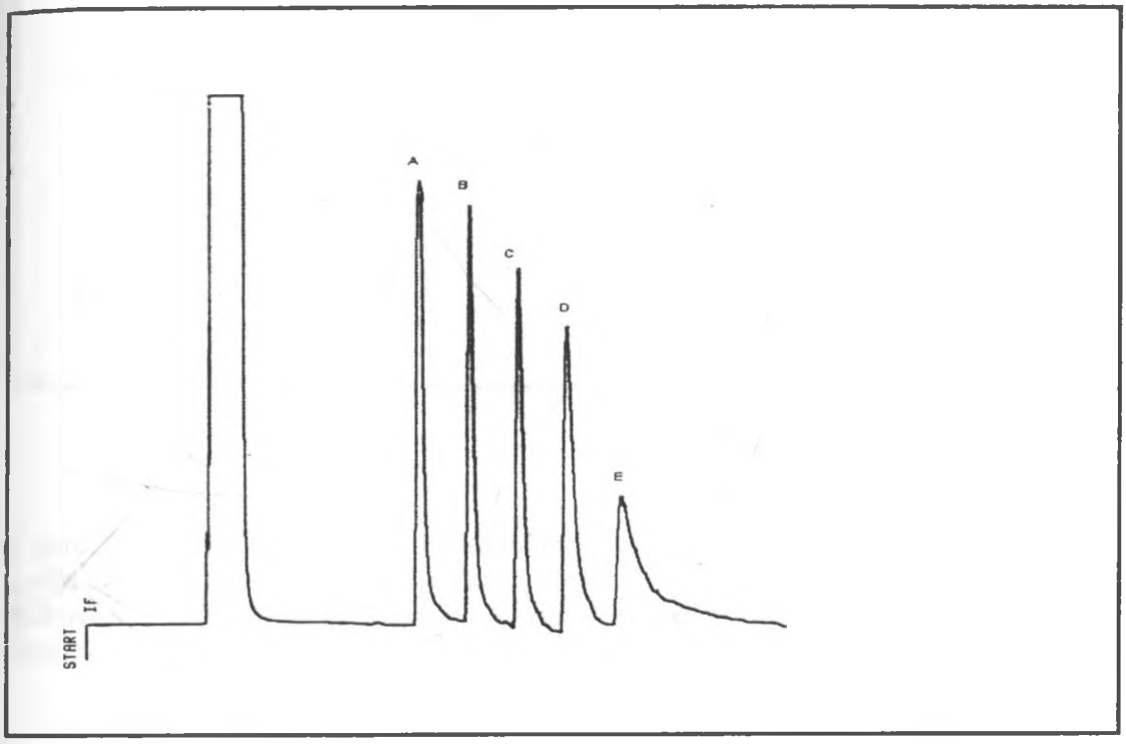

Figure 31: Gas chromatogram of the 500µg/ml standard mixture of butanoic (A), pentanoic (B), hexanoic (C), heptanoic (D) and octanoic (E) acids on a 30 m carbowax 20M column

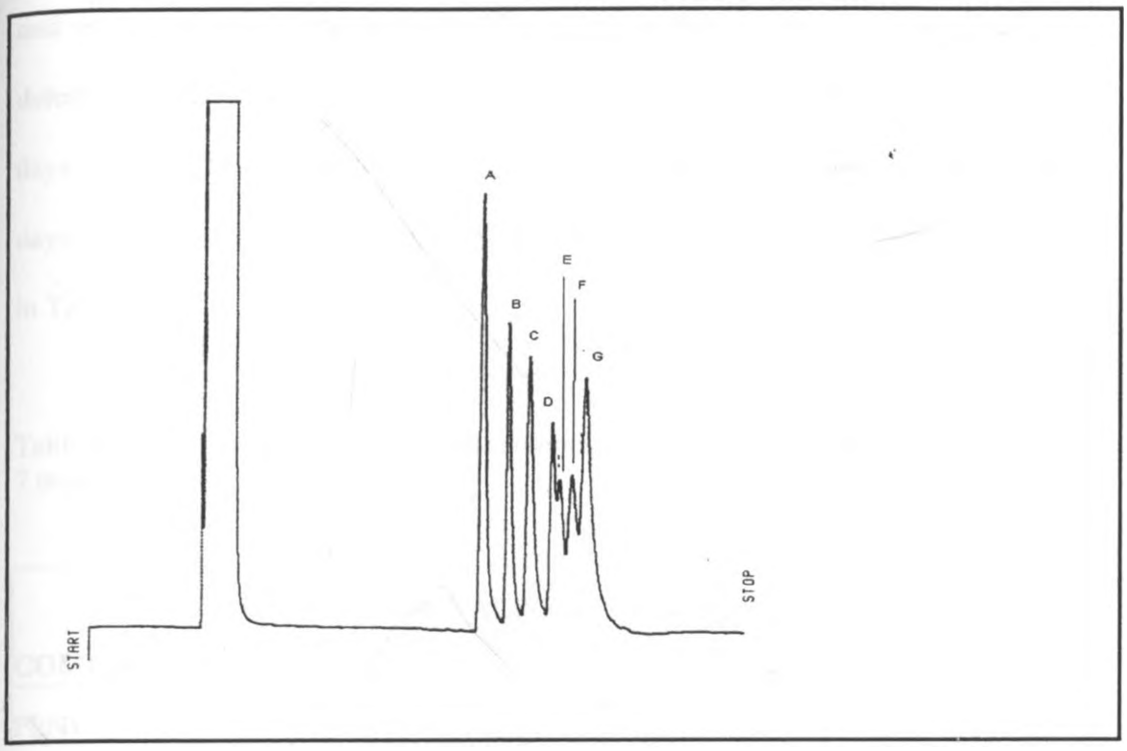

Figure 32: Gas chromatogram of the 400µg/ml standard mixture of 2-undecanone (A), guaiacol (B), 2-dodecanone (C), 2-methoxy-4-methylphenol (D), nerylacetone (E), geranylacetone (F) and δ-octalactone (G), on a 30 m carbowax 20M column. (E) is an impurity.

The quantitative data obtained for the various compounds together with their corresponding calibration plots are shown in Appendix 6 to Appendix 12. The standard calibration curves for all the analytes were reasonably good with linear correlation coefficients (r^2) greater than 0.970 for most of the compounds. The calibration curves were used to determine the amount of the individual compounds released over the sampling period in both laboratory and semifield trials.

4.4.2 Laboratory Trial Results

Samples were collected on days 1, 4 and 7 from the dispensers placed in the wind tunnel. The samples were then prepared as described in the experimental section

and run on the gas chromatograph. The corresponding peak areas were used to determine the concentration of the various compounds present in the samples on those days. Appendices 13, 14 and 15 show the chromatograms of the samples collected on days 1, 4 and 7, respectively. The normalized peak areas of the compounds are shown in Table 13.

Table 13: Normalised peak areas of the compounds in the dispensers on days 1, 4 and 7 under laboratory conditions

COMPOUND	NORMALISED PEAK AREAS		
	DAY 1	DAY 4	DAY 7
PENTANOIC ACID	0.4792	0.5092	0.4682
HEXANOIC ACID	0.4591	0.4596	0.3996
HEPTANOIC ACID	0.7069	0.6429	0.5983
2-UNDECANONE	0.6734	0.6644	0.6182
GERANYLACETONE	1.0963	1.1030	1.1058
δ-OCTALACTONE	0.7714	0.7565	0.7294
GUAIACOL	0.6532	0.5253	0.3579

The chromatograms show that the compounds under study are present on all the days of the sampling. Peak areas data show that the amounts of each compound present gradually decreases with time due to their release through the tygon silicon tubing of the dispenser. Using the linear least squares equation from the calibration curves of each compound, the residual amounts (g) of each compound present on days 1, 4 and 7 were determined and are given in Table 14. The results show differences in the residual amounts present as determined by gas chromatography versus the weights method as obtained by gravimetry (weighing the dispensers on a sensitive scale). The

gravimetric method consistently showed higher values. The differences can be attributed to loss of the volatiles during handling and sample preparation. Qian and Reineccius (2003) reported the same difficulty in the quantification of volatiles, especially carboxylic acids.

Table 14: Amount of individual compounds present in the dispensers on days 1, 4 and 7 under laboratory conditions

COMPOUND	AMOUNT PRESENT (g)		
	DAY		
	1	4	7
PENTANOIC ACID	0.3742	0.3557	0.2859
HEXANOIC ACID	0.3811	0.3408	0.2618
HEPTANOIC ACID	0.3603	0.2823	0.2233
2-UNDECANONE	0.3372	0.2956	0.2341
GERANYLACETONE	0.3525	0.3342	0.2998
OCTALACTONE	0.3877	0.3376	0.2823
GUAIACOL	0.4555	0.3497	0.2410
TOTAL (by chromatography)	**2.6485**	**2.2959**	**1.8282**
TOTAL AMOUNT PRESENT (by gravimetry)	2.7577	2.4629	2.1582
DIFFERENCE BETWEEN THE TWO METHODS	**0.1092**	**0.1670**	**0.3300**

The GC results were used to determine the amounts (g) of the individual compounds lost each day (i.e. the difference between the amounts present on each sampling day (Table 14) and the initial amounts present). The results are summarised in Table 15.

Table 15: Amount of the individual compounds lost under laboratory conditions by days 1, 4 and 7

COMPOUND	WEIGHT LOST (g) by indicated period		
	DAY 1	DAY 4	DAY 7
PENTANOIC ACID	0.0313	0.0498	0.1196
HEXANOIC ACID	0.0201	0.0604	0.1394
HEPTANOIC ACID	0.0359	0.1139	0.1729
2-UNDECANONE	0.0188	0.0604	0.1219
GERANYLACETONE	0.0266	0.0449	0.0793
OCTALACTONE	0.0416	0.0917	0.1470
GUAIACOL	0.0314	0.1372	0.2459

The data shown in Table 14 (3 for each compound) were tested against zero- and first-order kinetic models and used to determine the corresponding rate constants and hence the kinetics of release of each compound. The release of the compounds was found to follow zero-order kinetics (as evidenced by the smaller standard deviation among the calculated zero-order rate constants compared to those of first-order (see Table 16)). This is contrary to the results reported earlier when the individual compounds are studied singly as opposed to as a blend. Singly, the release of the individual compounds was found to follow first-order kinetics. This difference can be explained by the interactions taking place within the mixture of the compounds as discussed previously in sections 4.3.2 and 4.5. It is significant to note that data on total amount present obtained from gravimetric analysis was found to be fairly comparable to that obtained (i.e., sum total of individual components) from gas chromatographic estimation.

Table 16: Average (based on days 1, 4 and 7 calculated rate constants) zero- and first-order rate constants with their corresponding standard deviations for the individual compounds under laboratory conditions.

ANALYTE	Calculated zero-order rate constant	Calculated first-order rate constant
	k_0 (g day $^{-1}$)	k_1 (day $^{-1}$)
PENTANOIC ACID	0.0203 ± 0.0098	0.0543 ± 0.0241
HEXANOIC ACID	0.1840 ± 0.0028	0.0510 ± 0.0101
HEPTANOIC ACID	0.0297 ± 0.0057	0.0872 ± 0.0069
2-UNDECANONE	0.1710 ± 0.0019	0.0536 ± 0.0067
GERANYLACETONE	0.0164 ± 0.0088	0.0459 ± 0.0232
OCTALACTONE	0.0285 ± 0.0114	0.0739 ± 0.0242
GUAIACOL	0.0336 ± 0.0019	0.0833 ± 0.0169

4.4.3 Semifield Trial Results

Samples were collected on days 1, 4 and 7 from the dispensers exposed to the sun and those placed under the shade. Appendices 16, 17 and 18 show the gas chromatographic profiles of the samples collected on days 1, 4 and 7, respectively in the sun, while appendices 19, 20 and 21 show the GC profiles of the samples collected on days 1, 4 and 7, respectively in the shade. The normalized peak areas of the compounds are shown in Table 17.

Table 17: Normalised peak areas of the compounds in the dispensers on days 1, 4 and 7 under semifield conditions

COMPOUND	NORMALISED PEAK AREAS					
	DAY 1		DAY 4		DAY 7	
	SUN	SHADE	SUN	SHADE	SUN	SHADE
PENTANOIC ACID	0.3475	0.3417	0.3404	0.3757	0.3733	0.3956
HEXANOIC ACID	0.3201	0.3195	0.3289	0.3423	0.3922	0.3800
HEPTANOIC ACID	0.6069	0.6324	0.6271	0.6117	0.6625	0.5785
2-UNDECANONE	0.5036	0.4950	0.5053	0.5036	0.5182	0.5394
GERANYLACETONE	1.0739	1.0801	1.0745	1.0638	1.0620	1.0728
δ-OCTALACTONE	0.6632	0.6853	0.6818	0.6682	0.6596	0.6636
GUAIACOL	0.4423	0.3946	0.2923	0.4225	0.4152	0.3140

Using data obtained from the chromatograms, the quantities of the compounds present in the dispensers on days 1, 4 and 7 were determined. The results are summarised in Table 18.

Table 18: Amount (g) of individual compounds present in the dispensers on days 1, 4 and 7 under semifield conditions.

COMPOUND	Amount present (g)					
	DAY 1		DAY 4		DAY 7	
	SUN	SHADE	SUN	SHADE	SUN	SHADE
PENTANOIC ACID	0.4494	0.4886	0.3874	0.4050	0.3321	0.3408
HEXANOIC ACID	0.4564	0.4867	0.3998	0.4609	0.3533	0.3545
HEPTANOIC ACID	0.4882	0.4950	0.4544	0.4621	0.3863	0.3018
2-UNDECANONE	0.3688	0.3696	0.3149	0.3226	0.2799	0.2718
GERANYLACETONE	0.4702	0.4166	0.4418	0.3394	0.3575	0.3077
OCTALACTONE	0.5330	0.5397	0.4887	0.4426	0.4176	0.3535
GUAIACOL	0.5867	0.5715	0.4798	0.4718	0.3482	0.3176
TOTAL(by chromatography)	**3.3527**	**3.3677**	**2.9667**	**2.9043**	**2.4749**	**2.2476**
TOTAL AMOUNT PRESENT (by gravimetry)	3.6541	3.5298	3.2064	2.9455	2.7590	2.3333
DIFFERENCE BETWEEN THE TWO METHODS	**0.3015**	**0.1621**	**0.2397**	**0.0411**	**0.2841**	**0.0857**

The data obtained from gravimetric analysis were found to be fairly comparable to those obtained from gas chromatographic analysis, with the gravimetric data still consistently higher. The amounts lost on each of those days were computed and summarised in Table 19. Table 20 summarises the rate constants obtained, when both zero- and first-order kinetic models are assumed and tested.

Table 19: Amount of compounds lost by days 1, 4 and 7 under semifield conditions

	WEIGHT LOST (g) BY THE DAYS INDICATED					
	DAY 1		DAY 4		DAY 7	
COMPOUND	SUN	SHADE	SUN	SHADE	SUN	SHADE
PENTANOIC ACID	0.0848	0.0262	0.1468	0.1098	0.2021	0.3408
HEXANOIC ACID	0.0722	0.0227	0.1288	0.0485	0.1753	0.3545
HEPTANOIC ACID	0.0338	0.0081	0.0677	0.0410	0.1357	0.3018
2-UNDECANONE	0.1003	0.0824	0.1542	0.1295	0.1891	0.2718
GERANYLACETONE	0.0293	0.0648	0.0577	0.1420	0.1420	0.3077
OCTALACTONE	0.0327	0.0054	0.0770	0.1025	0.1481	0.3535
GUAIACOL	0.0549	0.0469	0.1618	0.1466	0.2934	0.3176

Table 20: Average (based on days 1, 4 and 7 calculated rate constants) zero- and first-order rate constants with their corresponding standard deviations for the individual compounds under semifield conditions

ANALYTE	k_0 (g day^{-1})		k_1 (day^{-1})	
	SUN	**SHADE**	**SUN**	**SHADE**
PENTANOIC ACID	0.0501 ± 0.0303	0.0262 ± 0.0013	0.1070 ± 0.0574	0.0570 ± 0.0042
HEXANOIC ACID	0.0432 ± 0.0254	0.0189 ± 0.0059	0.0914 ± 0.0484	0.0408 ± 0.0140
HEPTANOIC ACID	0.0234 ± 0.0091	0.0157 ± 0.0113	0.0482 ± 0.0167	0.0369 ± 0.0314
2-UNDECANONE	0.0553 ± 0.0394	0.0468 ± 0.0309	0.1379 ± 0.0898	0.1194 ± 0.0711
GERANYLACETONE	0.0213 ± 0.0075	0.0417 ± 0.0207	0.0463 ± 0.0149	0.0986 ± 0.0414
OCTALACTONE	0.0244 ± 0.0073	0.0195 ± 0.0122	0.0465 ± 0.0117	0.0413 ± 0.0276
GUAIACOL	0.0457 ± 0.0079	0.0422 ± 0.0052	0.0831 ± 0.0091	0.0806 ± 0.0139

From the standard deviation values corresponding to each calculated rate constant for both sun and under shade conditions, it is clear that the release of all the compounds investigated from the blend, follow zero-order kinetics. The variability in the calculated rate constants (i.e., from the standard deviation values) are however much higher for semifield conditions than was the case under laboratory conditions. This is attributable to the varying temperatures under the semifield conditions as is evident in Figures 33 and 34. Temperature was maintained constant under laboratory conditions.

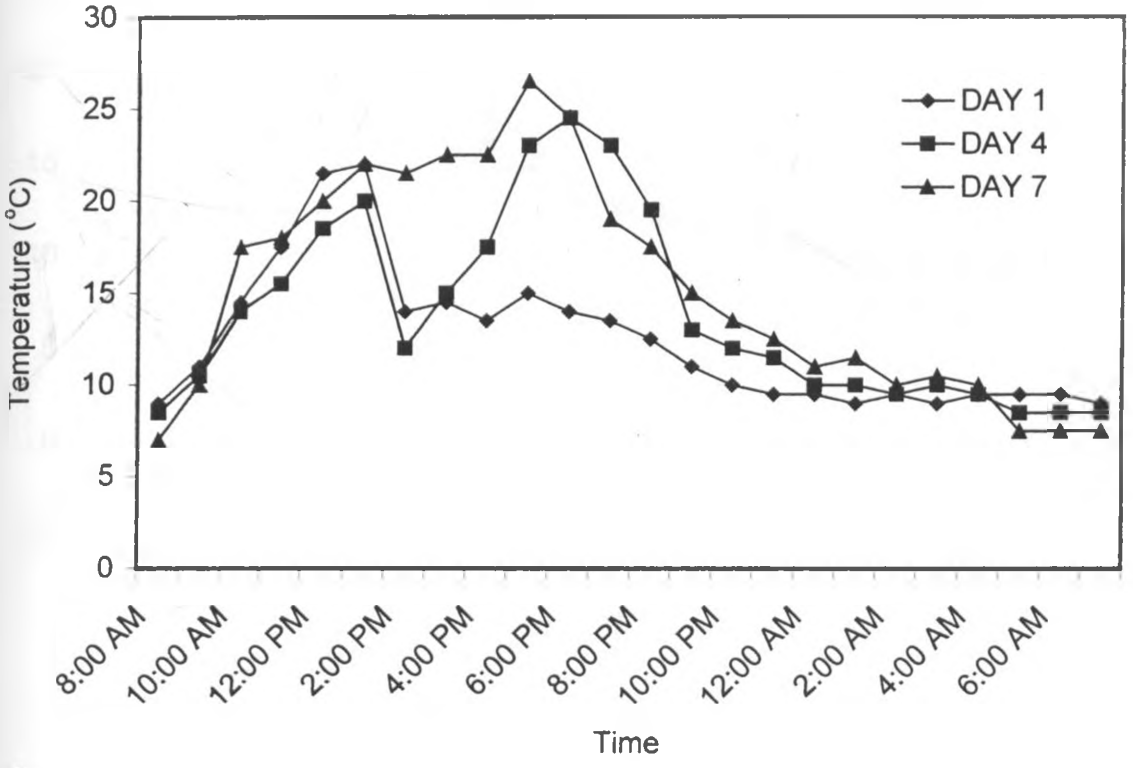

Figure 33: Temperature profiles for days 1, 4 and 7 in the sun.

It is also significant to note that the corresponding standard deviation values of the rate constants are generally much smaller under the shade than for those obtained in the sun. This is consistent with the earlier assertion that temperature could be playing a significant role in influencing the rate of release among other possible factors (Note that temperature variations in the shade are much less erratic than in the sun- compare Figures 33 and 34).

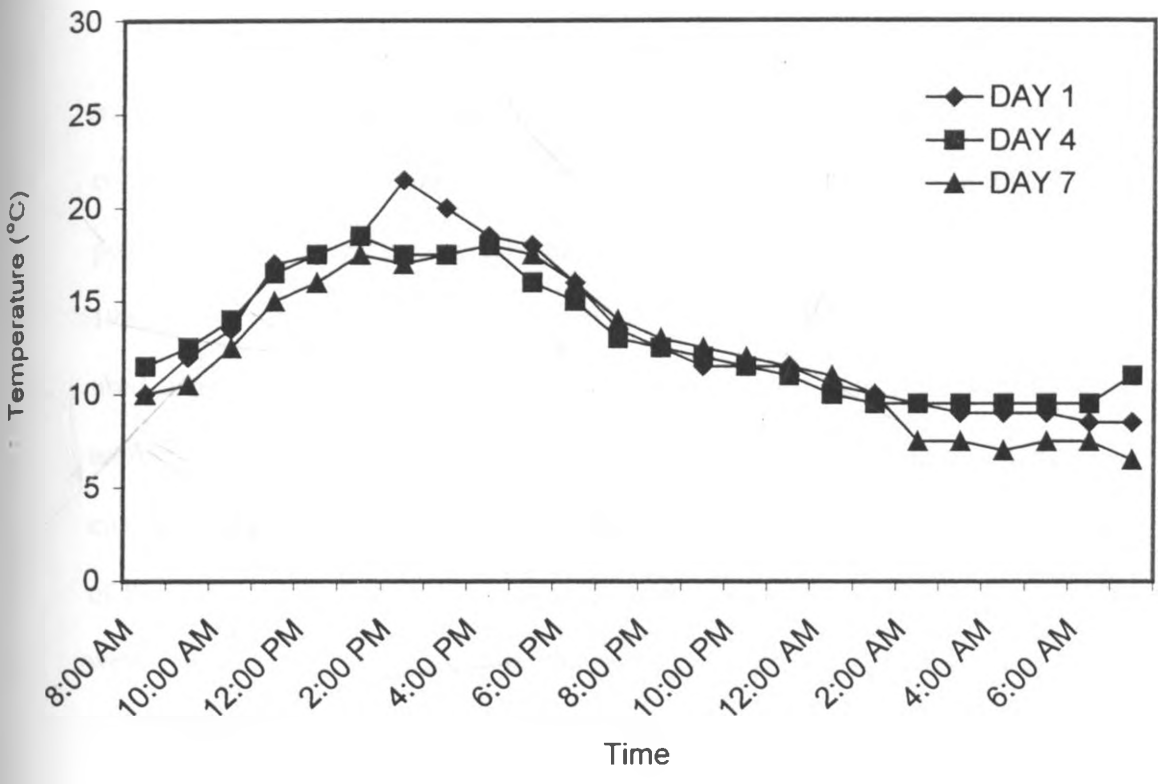

Figure 34: Temperature profiles for days 1, 4 and 7 in the shade

Recalling from earlier results that the individual compounds follow first-order kinetics when dispensed singly under semifield conditions, it is possible that the compounds are interacting with each other to affect the rate at which they volatilize and diffuse from the tygon tubing. Hence, the change in the release kinetics. This is discussed in detail in section 4.5 below.

4.5 Discussion of the Release Mechanism

The rate of release of the repellents is controlled by several factors including the active length of the dispenser and the conductance of the dispenser tubing. The conductance through the tubing will depend on temperature, since the logarithm of polymer permeability is inversely proportional to absolute temperature (O'Neill, 1980). The rate of release is proportional to the length of tubing of the dispenser unit used as has been shown in this study and the transfer conductance of the walls of the tubing. Bradley *et al.* (1995) reported that the complex relationship between conductance and temperature could be expressed as a Taylor series with the expansion of the series being about a mean operating temperature. The flux through the tubing wall is proportional to the concentration difference of the repellent within the inner and outer surfaces of the tubing (Suckling *et al.*, 1997). The coefficient of proportionality is called the conductance.

Temperature also affects volatilization, mainly through its effect on vapour pressure and the Henry's Law constant. The primary effect of temperature is on vapour density because an increase in temperature increases equilibrium vapour density, which in turn increases the rate of volatilization (Tinsley, 1979). Heath *et al.* (1986) studied the release of multicomponent pheromones and reported that the

release rates were based on the relative vapour pressures of the components. The flux of the volatile components across the tubing will also be determined by Fick's law of diffusion (Torr *et al.,* 1997), and thus the basic scheme of release will depend on the processes that control in one way or another the diffusion of the active agent through a polymer barrier (Zeoli *et al.,* 1982). Diffusion can occur in both the liquid and vapour phases, with liquid phase diffusion being much slower than vapour phase diffusion. Several steps are thought to occur during the release of the repellents from the dispenser to the atmosphere. McDonough (1997) explains that the steps are:

1. chemical passes from the liquid well into the outer dispenser wall,
2. chemical leaves the dispenser wall, and
3. chemical is carried away by air movement.

Bradley *et al.*, (1995) reported that the evaporation rate is controlled by the rate of diffusion of the repellent through the dispenser wall rather than by air speed, and therefore the rate limiting step is transmission through the wall rather than the transport across the aerodynamic boundary layer surrounding the dispenser. It follows that the rate of removal R, of the repellents from the tubing is the sum of the three processes as described by McDonough (1997); i .e.,

$$R = -\frac{dP_r}{dt} = k_1 P_{rs} - k_2 P_v + k_3 P_v \quad \text{.....13}$$

Where:

P_r is the repellent concentration in the tygon silicon tubing, P_{rs} is the repellent concentration in the outer surface of the tubing, P_v is the repellent concentration in the vapour over the tubing, k_1 is the rate constant for the movement of the repellents from the tubing into the vapour phase, k_2 is the rate constant for the condensation of the

repellent vapours back into the tubing, k_3 is the rate constant for the movement of the repellent vapours to positions in space where it cannot condense into the tubing.

It therefore follows that there are two limiting cases depending on the magnitude of k_2 and k_3 The processes involving k_2 and k_3 compete for P_v and the two limiting cases are:

a) k_3 is much greater than k_2.

Thus, condensation back into the tygon tubing is essentially suppressed, i.e., $k_2P_v = 0$. Then.

$$-dP_r / dt = k_1P_{rs} + k_3P_v \quad \text{.....14}$$

The rate-determining step would be diffusion across the tubing of the dispenser. The concentration of the repellents would be highest at the greatest distance from the surface and would be lowest in the surface as described by Fick's law of diffusion. Then the rate of evaporation would be proportional to $t^{1/2}$ which is the half life of the chemical (Zeoli *et al.*, 1982).

b) k_3 is much less than k_2 .

Thus, P_v and P_{rs} are in equilibrium with each other and $k_1P_{rs} = k_2P_v$. Therefore;

$$-\frac{dP_r}{dt} = k_3P_v$$

Because the repellents would be evenly distributed throughout the tubing,

$$k_1P_r = k_1P_{rs}$$

Therefore;

$$k_1P_r = k_2P_v \text{ and,}$$

$$P_v = k_1P_r / k_2$$

$$P_v = K\,P_r, \text{ where } K = k_1/k_2$$

Therefore;

$$-\frac{dP_r}{dt} = k_3 K P_r \quad \text{.....15}$$

Equation 15 is the equation of first-order evaporative loss.

The mechanism of release is independent of the dispenser and it is determined by the relative values of k_2 (which depends on the inherent tendency of the repellent compound molecules to condense) and k_3 (which depends on the rate of diffusion of the repellents and on transport across the boundary layer surrounding the dispenser tubing). Since k_2 and k_3 are independent of the dispenser, changing the dispenser would change k_1 and hence K but not the mechanism. Due to the influence of vapour pressure, it is apparent that the Classius-Clapeyron equation (Equation 16) applies (McDonough, 1997):

$$\log\frac{P_2}{P_1} = \frac{\Delta H}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad \text{.....16}$$

where; P_1 is the vapour pressure (mmHg) at temperature T_1; P_2 is vapour pressure (mmHg) at temperature T_2 ; T is temperature in Kelvin (K); ΔH = enthalpy of vaporisation in kJ/mol , and R = 8.314 J/K.mol. Thermodynamically, the temperature dependence of vapour pressure is related to the enthalpy of vaporization through the Classius-Clapeyron equation (logarithm of vapour pressure is inversely proportional to absolute temperature).

Thus, this information in combination with the results presented in this study can be used to develop a predictive model to give dependable estimates of the release rates of the dispensers in the field for both the individual compounds and the blend. It can also assist in determining the optimum replacement time of the dispenser contents. This is particularly useful during dose-response studies to determine the

exact ratio of the compounds in the blend required to achieve maximum repellency. At the start of the experiment, the ratio of the compounds in the blend was 1:1 and it changed with time as the compounds were depleted. The change in ratios was generally consistent, with 2-undecanone being depleted fastest followed by the carboxylic acids (pentanoic, hexanoic and heptanoic acids in that order) and geranylacetone. The phenol (guaiacol) was depleted the slowest followed by δ-octalactone by days 1 and 4. However by day 7, the ratios of the compounds present were such that δ-octalactone was being depleted the slowest followed by guaiacol with 2-undecanone, carboxylic acids and geranylacetone still being depleted the fastest in that order. Assuming a uniform release, it is estimated that the compound being depleted fastest (i.e., 2-undecanone) can last for 23 days. Calculations indicate that the dispenser fitted with 4 cm tygon silicon tubing is therefore expected to last for an average of 21 days under the semifield conditions. Using the waterbuck-blend ratio of the compounds pentatonic, hexanoic, heptanoic acids, 2-undecanone, geranylacetone, δ-octalactone and guaiacol as reported in Gikonyo *et al.*, (2002; 2003), the ratios of the compounds will change and thus the interactions of the compounds in the dispenser reservoir. There is thus a need for appropriate dose response studies.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

Laboratory and semifield studies showed that the release rates increased with temperature. The release rates measured under semifield conditions were slightly higher than those obtained under laboratory conditions. They also showed more variability than those obtained under laboratory conditions. This is due in part to the temperature variations in the field whereas in the laboratory, the temperature was held constant. The results indicate that temperature is a major determinant in the release rates since it affects the rate at which the compounds volatilize, thereby affecting their rates of diffusion. However, it is evident that the release rates, more so in the field, were not a simple function of temperature since the release rates at higher temperatures were consistently less than was expected.

The data obtained from gravimetric analysis were found to be fairly comparable to those obtained from gas chromatographic analysis. Under laboratory conditions, the release of the individual compounds followed first-order kinetics while that of the blend followed zero-order kinetics. In the semifield, the individual compounds dispensed singly also followed first-order release kinetics while the blend of the compounds followed zero-order release kinetics. However, it was noted that the release of the individual components of the blend-mixture followed zero-order kinetics under semi-field conditions contrary to the behaviour exhibited by the individual components dispensed singly. The zero-order rate constants follow a trend (either increasing or decreasing) among homologous compounds (i.e. carboxylic acids and ketones), an observation that was not made among non-homologous compounds. It is plausible to conclude that the compounds are interacting with each other to affect the rate at which they volatilize and diffuse from the tygon tubing. Based on the

results obtained in this study, the aluminium/polypropylene tygon silicon tubing dispenser can be used to dispense the waterbuck-derived repellent blend at zero-order kinetics.

RECOMMENDATIONS

Having determined the rate of release of the individual compounds in the blend, dose response studies need to be undertaken in the field to establish the exact blend ratio that gives both maximum repellency to the tsetse flies and optimum protection of cattle in the field. In addition, further experiments should be carried out using the GC analysis method on a larger number of days than just 7 days.

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Appendix 1: Experimental data for the synthetic repellent using different lengths of tygon silicon tubing under semifield conditions

Length of tygon silicon tubing	DAY	SUN			SHADE		
4 cm		A_t	ln A_t	$1/A_t$	A_t	ln A_t	$1/A_t$
	1	5.9612	1.7853	0.1678	5.9435	1.7823	0.0048
	2	5.7545	1.7500	0.1738	5.7710	1.7528	0.0049
	3	5.5408	1.7121	0.1805	5.5892	1.7208	0.0051
	4	5.3335	1.6740	0.1875	5.4090	1.6881	0.0053
	5	4.7952	1.5676	0.2085	5.0478	1.6190	0.0069
	6	4.1780	1.4298	0.2393	4.8287	1.5746	0.0072
	7	4.4319	1.4888	0.2256	4.6670	1.5405	0.0073
	8	4.2509	1.4471	0.2352	4.5298	1.5107	0.0071
3 cm	1	3.3795	1.2177	0.8212	3.5245	1.2597	0.2851
	2	3.1928	1.1609	0.8614	3.3656	1.2136	0.2987
	3	3.0598	1.1183	0.8942	3.2071	1.1654	0.3137
	4	2.9112	1.0686	0.9358	3.0512	1.1155	0.3299
	5	2.7650	1.0170	0.9832	2.8956	1.0632	0.3479
	6	2.6212	0.9636	1.0378	2.7229	1.0017	0.3676
	7	2.4740	0.9058	1.1040	2.5971	0.9544	0.3885
	8	2.3290	0.8454	1.1828	2.4323	0.8888	0.4156
2 cm	1	5.1948	1.6477	0.1925	5.5841	1.7199	0.1791
	2	5.0967	1.6286	0.1962	5.4948	1.7038	0.1820
	3	4.9833	1.6061	0.2007	5.3933	1.6852	0.1854
	4	4.8938	1.5880	0.2043	5.2904	1.6659	0.1890
	5	4.8243	1.5737	0.2073	5.1731	1.6435	0.1933
	6	4.6881	1.5450	0.2133	5.0671	1.6228	0.1974
	7	4.6194	1.5303	0.2165	4.9520	1.5998	0.2019
	8	4.5245	1.5095	0.2210	4.8599	1.5810	0.2058

Appendix 2: Experimental data for the individual repellent compounds using 2 cm of tygon silicon tubing under laboratory conditions

DAY	PENTANOIC ACID		
	A_t	ln A_t	$1/A_t$
1	4.5843	1.5226	0.2181
2	3.9482	1.3733	0.2533
3	3.6822	1.3035	0.2716
4	3.4623	1.2419	0.2888
5	3.1904	1.1601	0.3134
6	2.9203	1.0717	0.3424
7	2.7927	1.0270	0.3581
	HEXANOIC ACID		
	A_t	ln A_t	$1/A_t$
1	4.8251	1.5738	0.2072
2	4.6391	1.5345	0.2156
3	4.5510	1.5153	0.2197
4	4.4950	1.5030	0.2225
5	4.4276	1.4879	0.2259
6	4.3515	1.4705	0.2298
7	4.2394	1.4444	0.2359
	HEPTANOIC ACID		
	A_t	ln A_t	$1/A_t$
1	5.7745	1.7534	0.1732
2	5.6550	1.7325	0.1768
3	5.6208	1.7265	0.1779
4	5.5936	1.7216	0.1788
5	5.5423	1.7124	0.1804
6	5.5042	1.7055	0.1817
7	5.4699	1.6993	0.1828
	δ-OCTALACTONE		
	A_t	ln A_t	$1/A_t$
1	2.9500	1.0818	0.3390
2	2.8692	1.0540	0.3485
3	2.8127	1.0342	0.3555
4	2.7877	1.0252	0.3587
5	2.7715	1.0194	0.3608
6	2.7521	1.0124	0.3634
7	2.7236	1.0020	0.3672

Appendix 2: contd

DAY	2-UNDECANONE		
	A_t	ln A_t	$1/A_t$
1	5.2125	1.6511	0.1918
2	4.8393	1.5768	0.2066
3	4.7025	1.5481	0.2127
4	4.6122	1.5287	0.2168
5	4.5400	1.5129	0.2203
6	4.4060	1.4830	0.2270
7	4.3223	1.4638	0.2314
DAY	GUAIACOL		
	A_t	ln A_t	$1/A_t$
1	5.2980	1.6673	0.1888
2	5.0514	1.6197	0.1980
3	4.8900	1.5872	0.2045
4	4.7547	1.5591	0.2103
5	4.6095	1.5281	0.2169
6	4.4668	1.4967	0.2239
7	4.3068	1.4602	0.2322
DAY	GERANYLACETONE		
	A_t	ln A_t	$1/A_t$
1	5.5158	1.7076	0.1813
2	5.4344	1.6927	0.1840
3	5.4000	1.6864	0.1852
4	5.3686	1.6806	0.1863
5	5.3456	1.6763	0.1871
6	5.3123	1.6700	0.1882
7	5.2740	1.6628	0.1896

Appendix 3: Experimental data for the waterbuck-derived blend using 2 cm of tygon silicon tubing under laboratory conditions

DAY	A_t	ln A_t	$1/A_t$
1	2.7578	1.0144	0.3626
2	2.6642	0.9799	0.3753
3	2.5714	0.9445	0.3889
4	2.4629	0.9013	0.4060
5	2.3612	0.8592	0.4235
6	2.2731	0.8211	0.4399
7	2.1582	0.7693	0.4633

Appendix 4: Experimental data for the compounds under semifield conditions

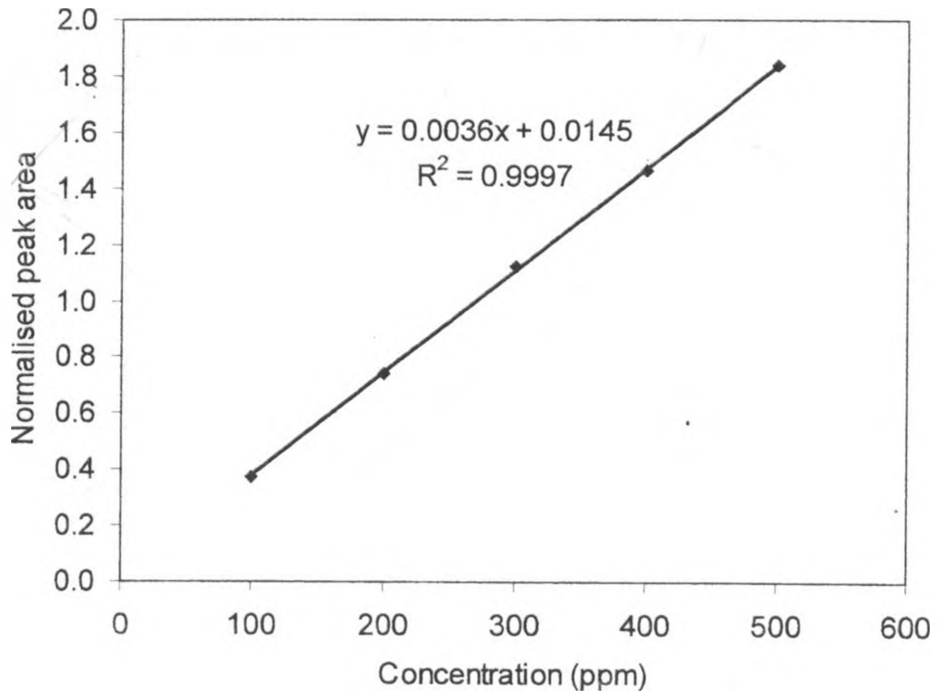
COMPOUND	DAY	SUN			SHADE		
		A_t	ln A_t	$1/A_t$	A_t	ln A_t	$1/A_t$
PENTANOIC ACID	1	4.2581	1.4488	0.2348	4.6819	1.5437	0.2136
	2	3.1870	1.1591	0.3138	3.9510	1.3740	0.2531
	3	2.3979	0.8746	0.4170	3.3549	1.2104	0.2981
	4	1.5010	0.4061	0.6662	2.6268	0.9657	0.3807
	5	0.8804	*0.1274*	1.1358	2.0672	0.7262	0.4837
HEXANOIC ACID							
	1	4.7896	1.5664	0.2088	4.9666	1.6027	0.2013
	2	4.3680	1.4743	0.2289	4.6782	1.5429	0.2138
	3	4.0395	1.3961	0.2476	4.4615	1.4955	0.2241
	4	3.5867	1.2772	0.2788	4.1369	1.4199	0.2417
	5	3.2015	1.1636	0.3124	3.8921	1.3589	0.2569
HEPTANOIC ACID							
	1	4.0477	1.3981	0.2471	4.0303	1.3938	0.2481
	2	3.8676	1.3526	0.2586	3.8664	1.3523	0.2586
	3	3.6821	1.3035	0.2716	3.6752	1.3016	0.2721
	4	3.5345	1.2626	0.2829	3.5171	1.2576	0.2843
	5	3.3347	1.2044	0.2999	3.3331	1.2039	0.3000
OCTALACTONE							
	1	3.2351	1.1740	0.3091	3.2502	1.1787	0.3077
	2	3.1459	1.1461	0.3179	3.1895	1.1598	0.3135
	3	3.0914	1.1286	0.3235	3.1512	1.1478	0.3173
	4	2.9936	1.0965	0.3340	3.0886	1.1277	0.3238
	5	2.9138	1.0694	0.3432	3.0367	1.1108	0.3293
2-UNDECANONE							
	1	3.5079	1.2550	0.2851	3.6601	1.2975	0.2732
	2	2.6973	0.9923	0.3707	3.2237	1.1705	0.3102
	3	2.1691	0.7743	0.4610	2.9261	1.0737	0.3418
	4	1.3347	0.2887	0.7492	2.4003	0.8756	0.4166
	5	0.7035	*0.3517*	1.4215	1.9858	0.6860	0.5036
GUAIACOL							
	1	7.0880	1.9584	0.1411	6.8358	1.9222	0.1463
	2	6.7852	1.9147	0.1474	6.5554	1.8803	0.1525
	3	6.5156	1.8742	0.1535	6.2677	1.8354	0.1595
	4	6.2881	1.8387	0.1590	6.0412	1.7986	0.1655
	5	6.0668	1.8028	0.1648	5.8252	1.7622	0.1717
GERANYLACETONE							
	1	5.2744	1.6629	0.1896	5.4793	1.7010	0.1825
	2	5.1436	1.6378	0.1944	5.4157	1.6893	0.1846
	3	4.9614	1.6017	0.2016	5.3285	1.6731	0.1877
	4	4.8139	1.5715	0.2077	5.2548	1.6591	0.1903
	5	4.6595	1.5389	0.2146	5.1724	1.6433	0.1933

Appendix 5: Experimental data for the waterbuck-derived blend under semifield conditions

BLEND	DAY	SUN			SHADE		
		A_t	ln A_t	$1/A_t$	A_t	ln A_t	$1/A_t$
4 cm	1	3.6541	1.2959	0.2737	3.5298	1.2612	0.2833
	2	3.5079	1.2550	0.2851	3.3443	1.2073	0.2990
	3	3.3677	1.2142	0.2969	3.1499	1.1474	0.3175
	4	3.2064	1.1651	0.3119	2.9455	1.0803	0.3395
	5	3.0606	1.1186	0.3267	2.7294	1.0041	0.3664
	6	2.9203	1.0717	0.3424	2.5232	0.9255	0.3963
	7	2.7590	1.0149	0.3625	2.3334	0.8473	0.4286
2 cm	1	2.7472	1.0106	0.3640	2.6468	0.9733	0.3778
	2	2.5392	0.9318	0.3938	2.5037	0.9178	0.3994
	3	2.3950	0.8734	0.4175	2.3916	0.8720	0.4181
	4	2.2813	0.8247	0.4383	2.3213	0.8421	0.4308
	5	2.0770	0.7309	0.4815	2.1268	0.7546	0.4702
	6	1.8076	0.5920	0.5532	1.8535	0.6171	0.5395
	7	1.5819	0.4586	0.6321	1.6184	0.4814	0.6179

Appendix 6: Pentanoic acid sample standards and butanoic acid internal standard

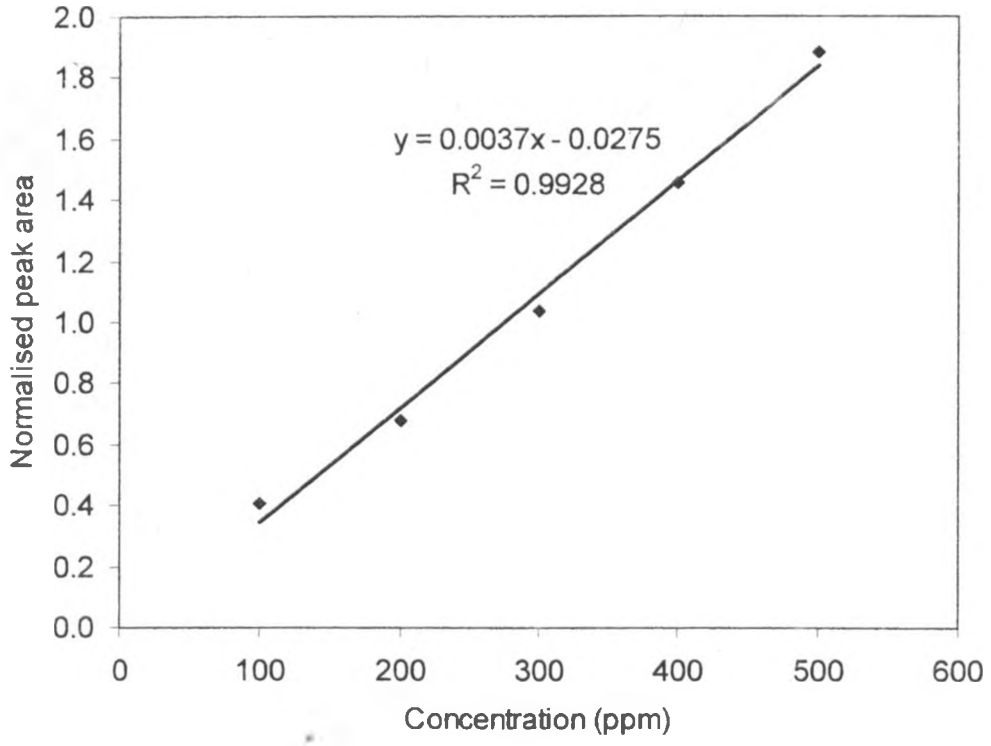
Concentration (μg/ml)	Pentanoic acid peak area	Butanoic acid (300μg/ml) peak area	Pentanoic acid peak area/Butanoic acid peak area
500	439927	239424	1.8374
400	309039	211252	1.4629
300	245267	217866	1.1258
200	201221	271308	0.7417
100	115324	307808	0.3747



Pentanoic acid calibration plot

Appendix 7: Hexanoic acid sample standards and butanoic acid internal standard

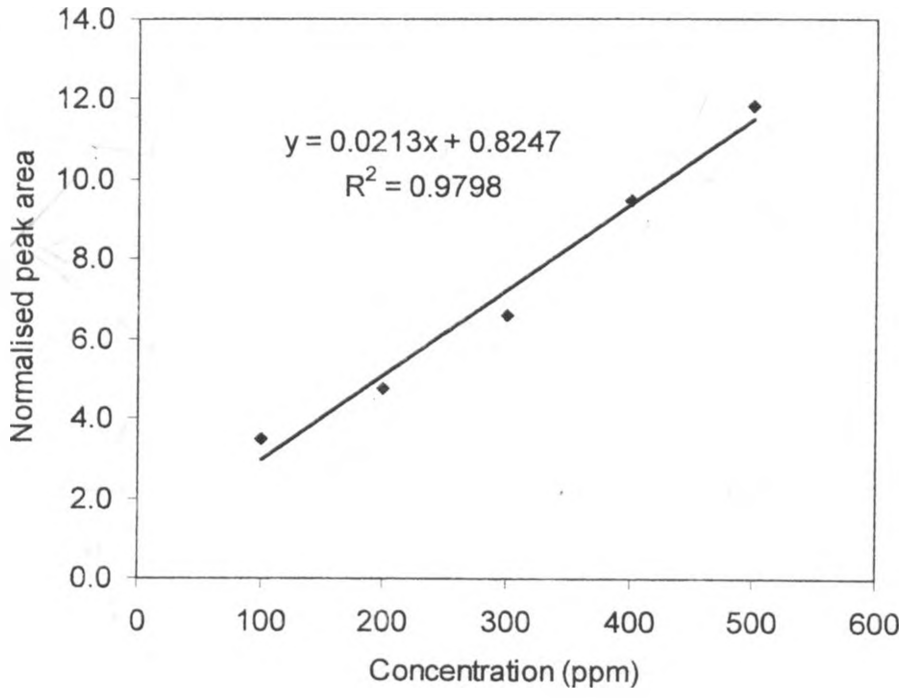
Concentration (μg/ml)	Hexanoic acid peak area	Butanoic acid (300μg/ml) peak area	hexanoic acid peak area/Butanoic acid peak area
500	450891	239424	1.8832
400	308327	211252	1.4595
300	226256	217866	1.0385
200	184114	271308	0.6786
100	124948	307808	0.4059



Hexanoic acid calibration plot

Appendix 8: Heptanoic acid sample standards and octamoic acid internal standard

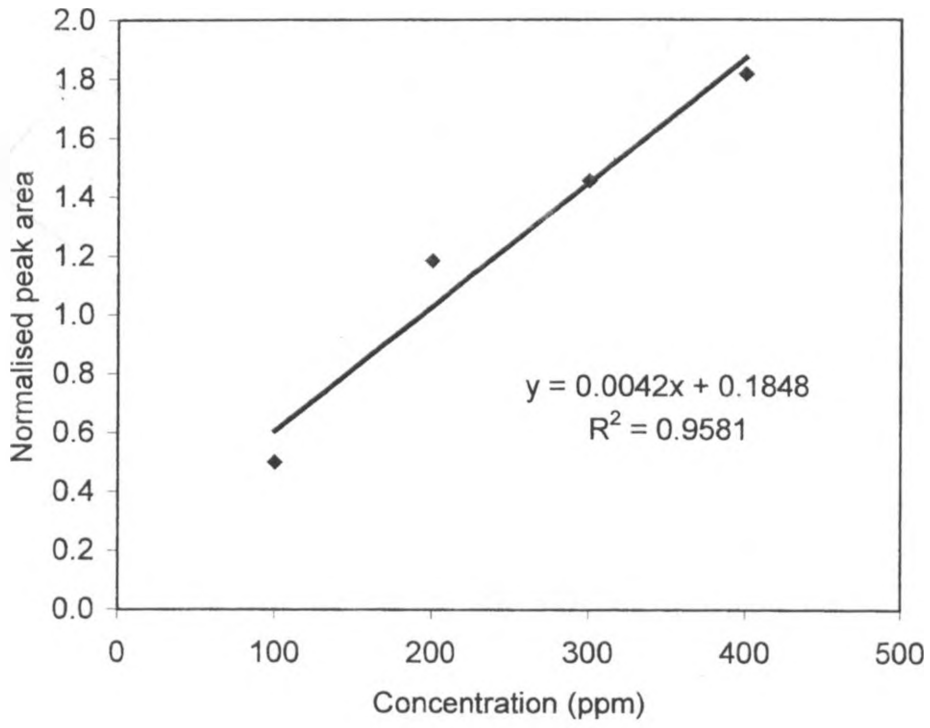
Concentration (μg/ml)	Heptanoic acid peak area	Octanoic acid (300μg/ml) peak area	heptanoic acid peak area/octanoic acid peak area
500	543153	45976	11.8138
400	461072	48659	9.4756
300	324813	49324	6.5853
200	227184	47792	4.7536
100	151663	43271	3.5050



Heptanoic acid calibration plot

Appendix 9: 2-undecanone sample standards and 2-dodecanone internal standard

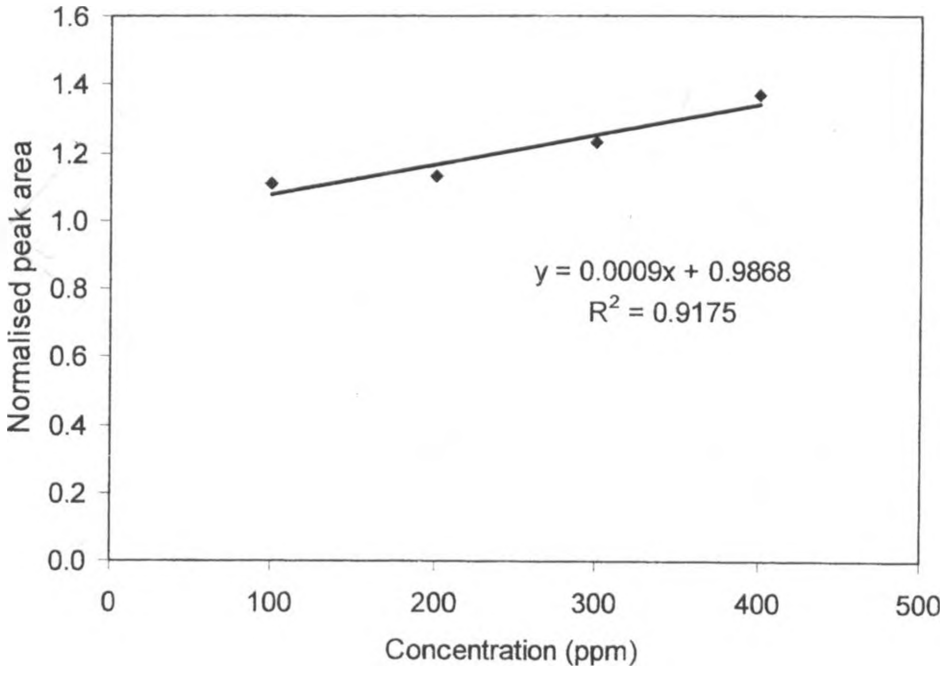
Concentration (μg/ml)	2-undecanone peak area	2-dodecanone (200 μg/ml) peak area	Normalised peak area
400	566391	311812	1.8165
300	512166	351855	1.4556
200	311658	263015	1.1849
100	132464	264649	0.5005



2-undecanone calibration plot

Appendix 10: Geranylacetone sample standards and 2-dodecanone internal standard

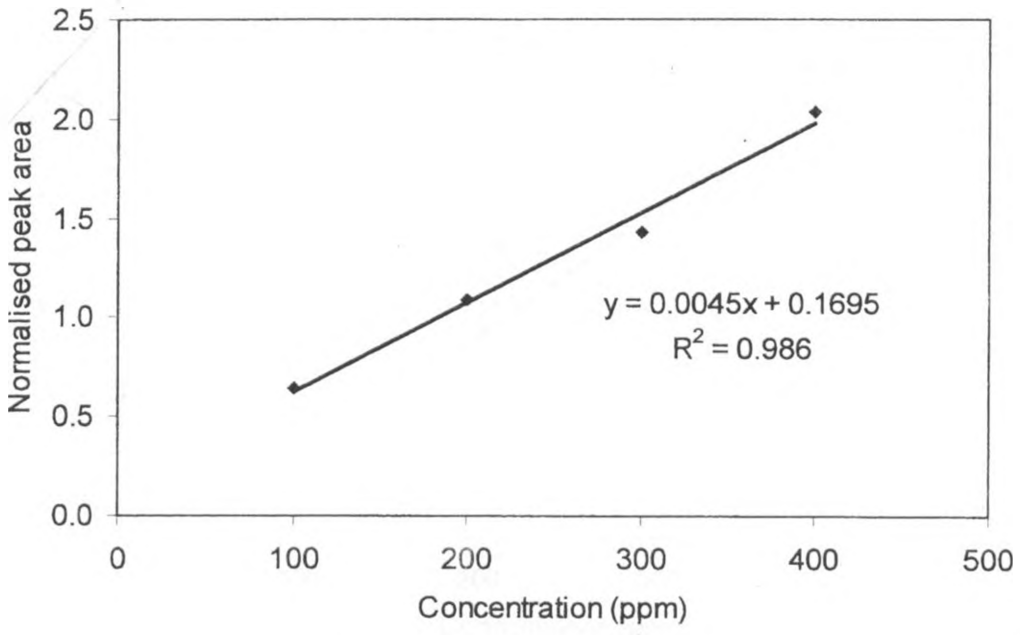
Concentration (μg/ml)	geranylacetone peak area	2-dodecanone (200 μg/ml) peak area	Normalised peak area
400	427208	311812	1.3701
300	445714	363016	1.2278
200	376468	333016	1.1305
100	303928	274649	1.1066



geranylacetone calibration plot

Appendix 11: δ-octalactone sample standards and 2-dodecanone internal standard

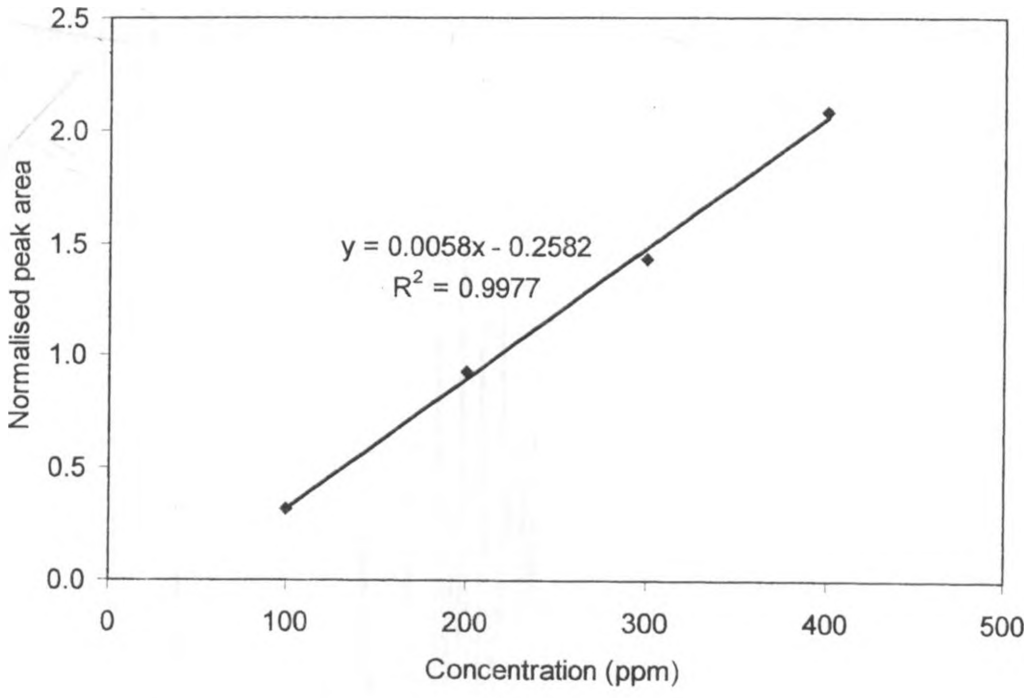
Concentration (μg/ml)	δ-octalactone peak area	2-dodecanone (200 μg/ml) peak area	Normalised peak area
400	636102	311812	2.0400
300	501951	351855	1.4266
200	369274	338141	1.0921
100	170289	264649	0.6435



δ-octalactone calibration plot

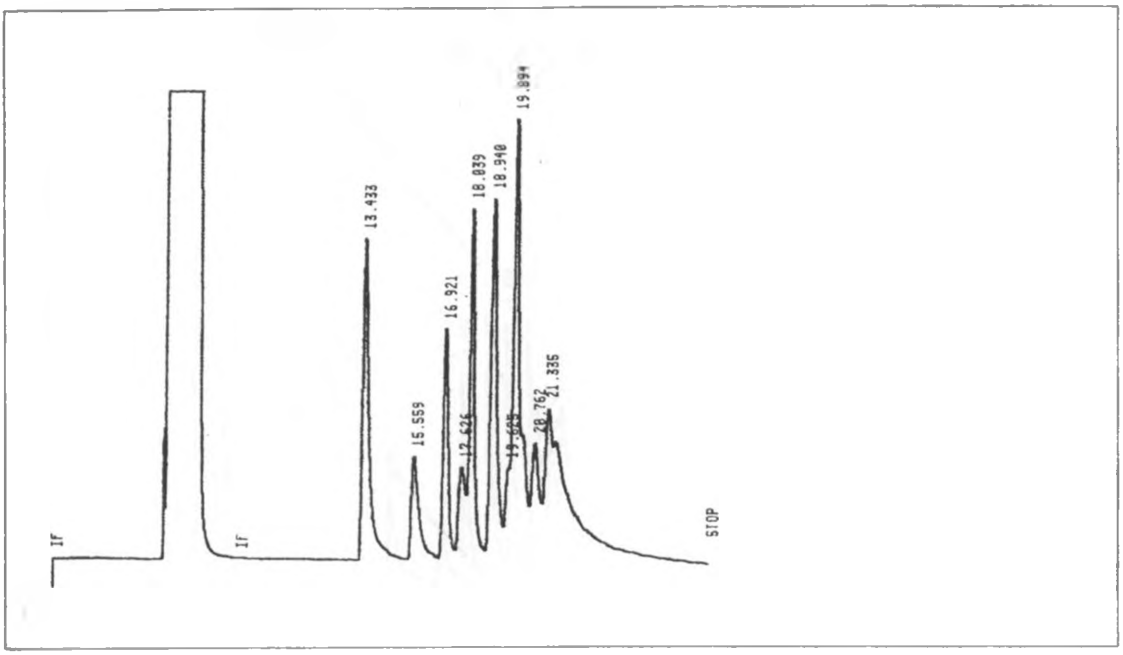
Appendix 12: guaiacol sample standards and 2-methyl-4-methoxy phenol internal standard

Concentration (μg/ml)	guaiacol peak area	2-methyl-4-methoxy phenol (200μg/ml) peak area	Normalised peak area
400	631889	303240	2.0838
300	283581	198695	1.4272
200	181660	196595	0.9240
100	74606	231910	0.3217

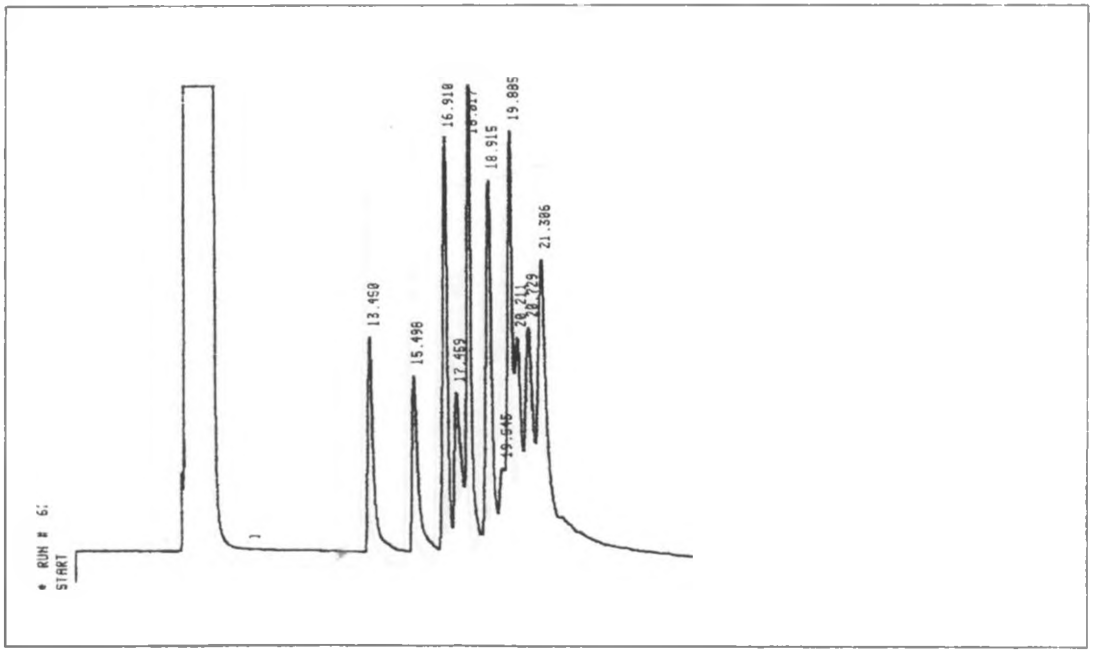


guaiacol calibration plot

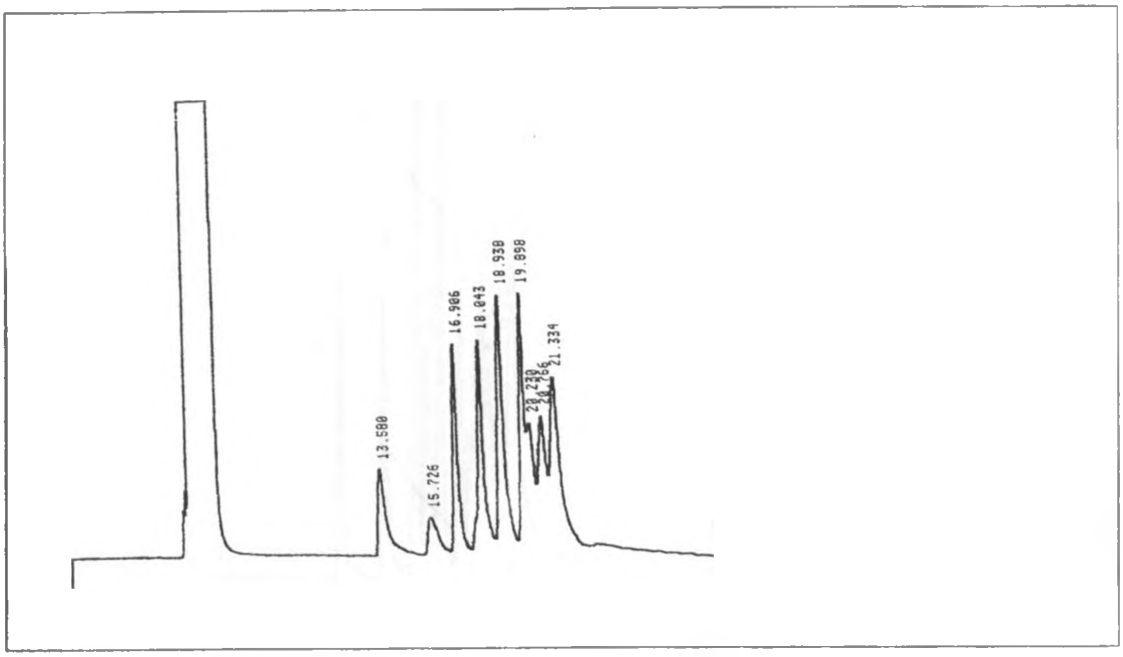
Appendix 13: Gas chromatogram of the sample collected on day 1 from a dispenser in the wind tunnel



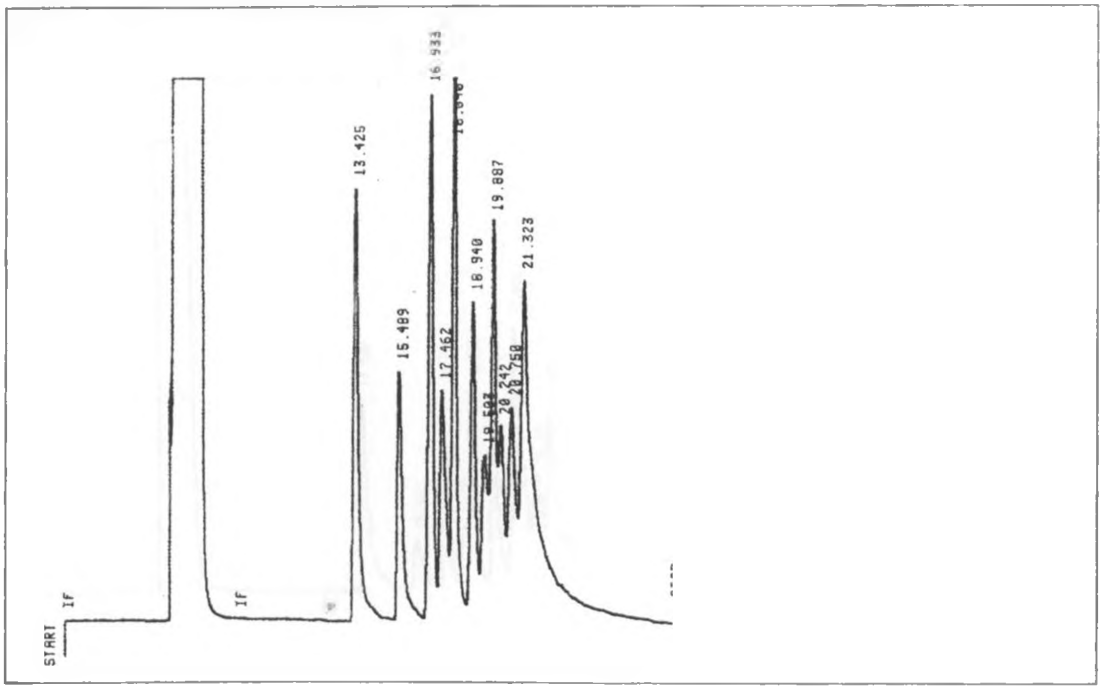
Appendix 14: Gas chromatogram of the sample collected on day 4 from a dispenser in the wind tunnel



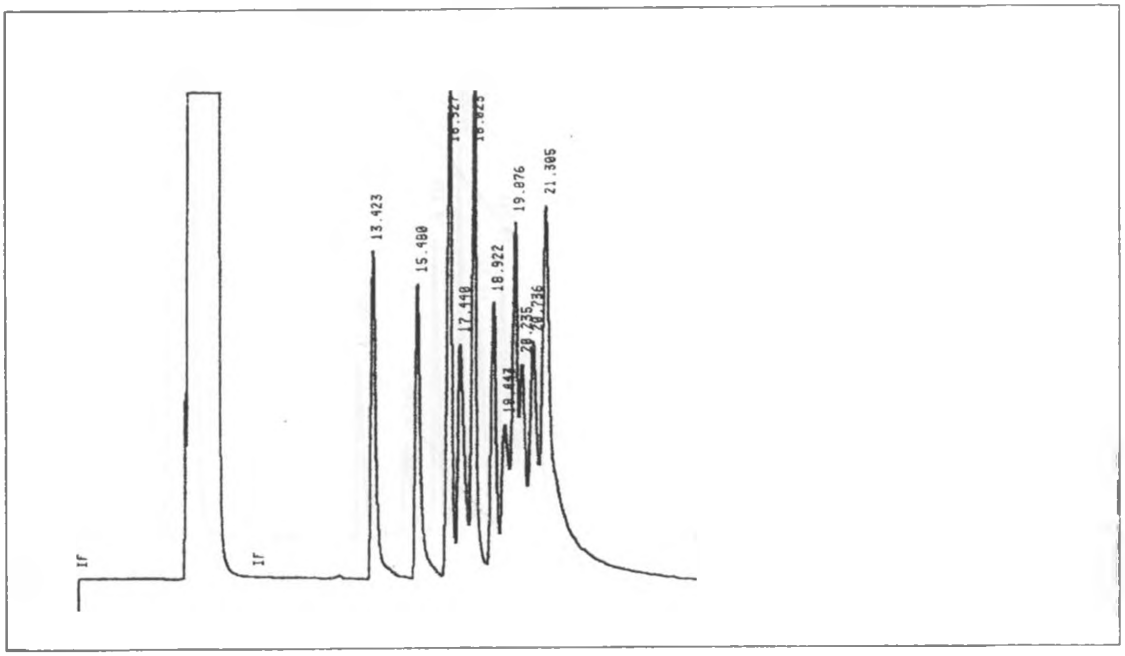
Appendix 15: Gas chromatogram of the sample collected on day 7 from a dispenser in the wind tunnel



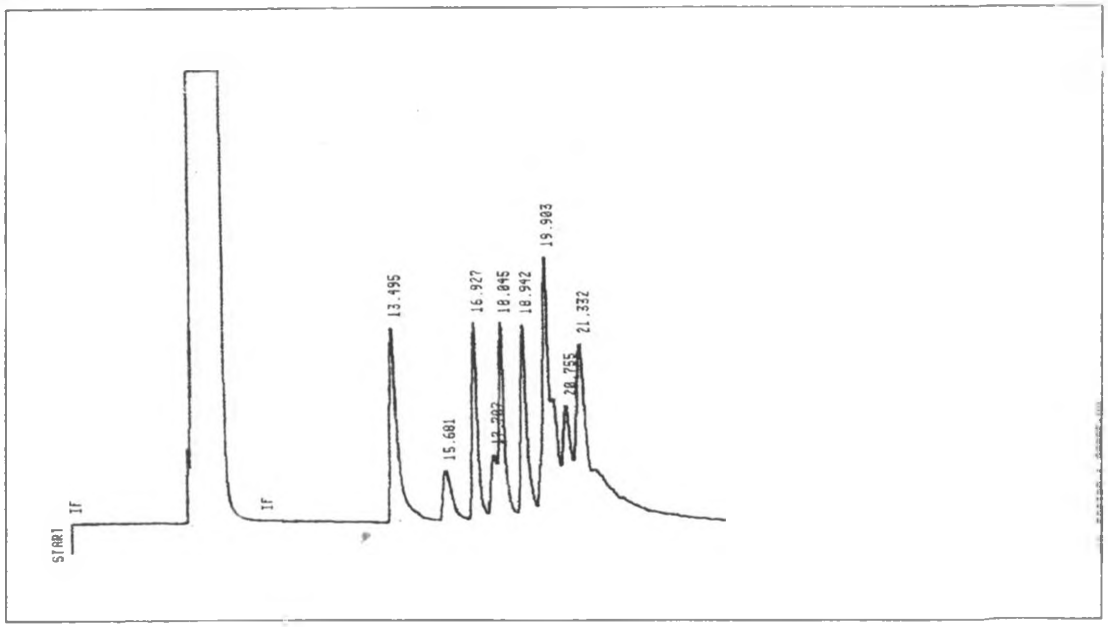
Appendix 16: Gas chromatogram of the sample collected on day 1 from a dispenser exposed to the sun under semifield conditions



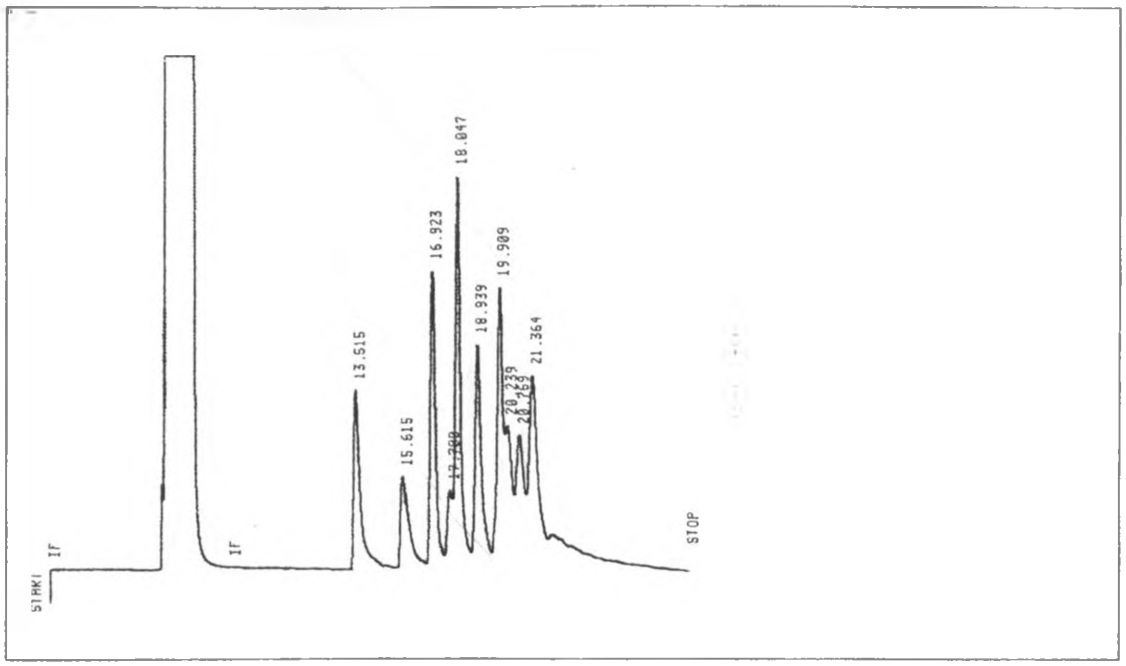
Appendix 17: Gas chromatogram of the sample collected on day 4 from a dispenser exposed to the sun under semifield conditions



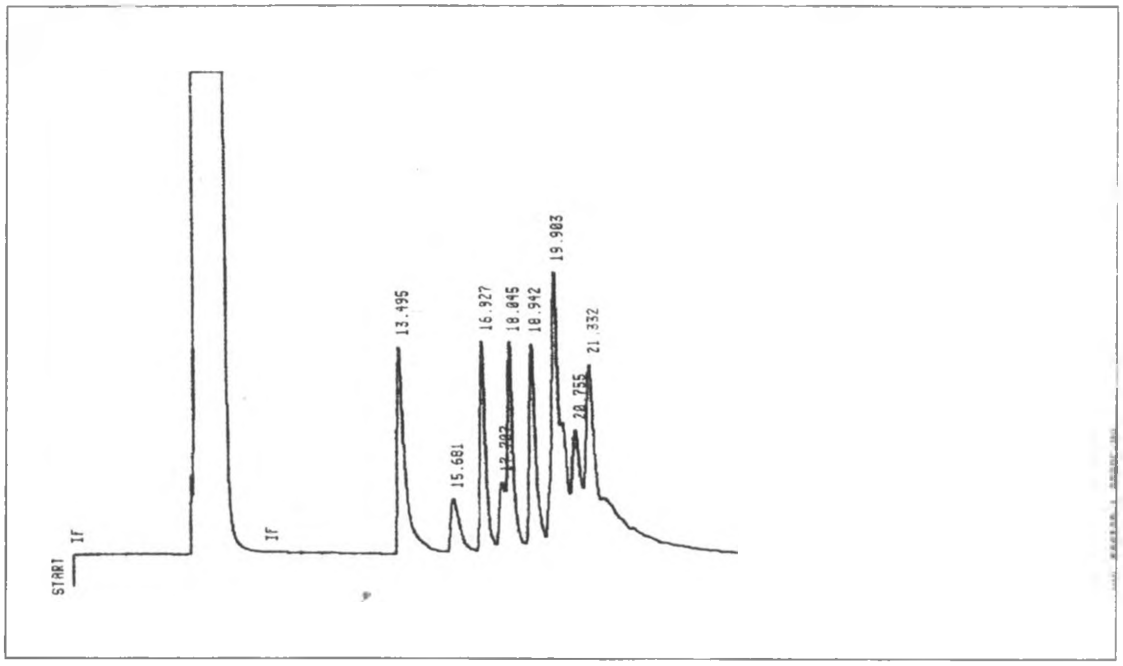
Appendix 18: Gas chromatogram of the sample collected on day 7 from a dispenser exposed to the sun under semifield conditions



Appendix 19: Gas chromatogram of the sample collected on day 1 from a dispenser exposed to the shade under semifield conditions



Appendix 20: Gas chromatogram of the sample collected on day 4 from a dispenser exposed to the shade under semifield conditions



Appendix 21: Gas chromatogram of the sample collected on day 7 from a dispenser exposed to the shade under semifield conditions