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THE MODE OF ACTION OF INSECT REPELLENTS

by

© MALCOLM JOHNSTON REDDY

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled THE MODE OF ACTION OF INSECT REPELLENTS submitted by Malcolm Johnston Reddy in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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A B S T R A C T

The sites of action of insect repellents on German roaches and mealworms were identified from behavioural choice chamber experiments. Electrophysiological recordings of American cockroach nerve impulses verified these sites and qualified the types of neural response. The liquid and vapour phases of repellents were examined separately. Insect repellents act mainly through the chemoreceptor organs on insect legs and antennae. These organs all appear to respond in the same way to both the liquid and vapour phases of repellents and other irritant chemicals such as benzene. In practice, the vapour phase is more important since the antennae have more receptors and do not normally come into contact with liquid repellents. There is a difference between the electrophysiological responses of roaches to attractant vapour and repellent vapour. The detection of attractants is thought to be through a specific olfactory mechanism, and the detection of irritant chemicals through the common chemical sense which is a property of all nervous tissue.

Human olfactory responses were considered in terms of the stereochemical theory of odour (Amoore, 1962), and the conclusion was that the theory is too simple to account for all the observed facts of chemical stimulation, particularly the action of repellents.

A C K N O W L E D G M E N T S

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C O N T E N T S

L I S T O F T A B L E S

L I S T O F F I G U R E S

1. INTRODUCTION

Most modern insect repellents have been developed by empirical testing. In these tests compounds were applied to a surface such as the human forearm, which was then placed inside an insect cage. Counts were then taken of the numbers of insects settling in unit time on such a treated surface. This research was stimulated by the need for better protection against biting insects during the Far Eastern campaigns of the Second World War (1939-1945). Further mass testing using similar methods have since produced a variety of repellents for a variety of insects, mites and ticks, and suitable for use on domestic animals, plants, and stored materials, as well as better and longer lasting repellents for human use. The mode of action of these repellents remains mostly unknown and consequently it is not yet possible to postulate the characteristics of the ideal repellent or formulation based on physiological requirements. It is probable that different insect species will respond optimally to different repellents, and also that no chemical repellent will ever be completely effective for as long a period as may sometimes be called for.

This thesis deals with the means by which insects respond to known repellents and the work was done concurrently with work done in this laboratory by Khan (1965) and presented in a paper entitled "Effects of Repellents on

Mosquito Behaviour". These are only two limited approaches to the problem, many others are possible. One important approach involves the isolation of probable repellent factors by analyzing physical and chemical properties which are common to the many repellents e.g. many repellents are plasticizers but not all plasticizers are repellents, at least to any marked degree. In short, no definite physical or chemical requirements have been unearthed by this approach, although some useful information has emerged, such as the discovery by Dethier (1951) that the taste sensitivity of blow flies to an homologous series of aliphatic glycols increases with the chain length. Applying these findings to repellents, it would be assumed that of two repellents with similar chemical properties, the one with the larger molecular weight would be more effective.

Many compounds have repellent properties, including many insecticides and the organic solvents such as benzene and toluene used in formulating these (see section 4.). Other compounds may affect or prevent normal behaviour, and by breaking a chain of responses involved in directional behaviour, appear to act as repellents. The problem of distinction between interference and repellency is part of this thesis. True insect repellents are herein treated as compounds which elicit an avoiding response in insects. Many repellents have no practical application for such

reasons as toxicity, irritancy, repellency to man or lack of persistance. Commercial repellents have been selected from a large group of repellent chemicals and have many other properties pertaining to their practicality which must not be confused with properties pertaining to their repellency. Repellents may act in different ways. An insect repellent like butyric anhydride (see section 5.) has such a nauseating odour to humans, that its repellency to bees should surprise nobody, but dimethyl phthalate and diethyl toluamide are rarely repulsive to man, which makes them commercially valuable. Human senses are not homologous with insect senses, and it is that area of insect sensitivity which does not overlap with human sensitivity that is most important in studying insect repellents. It seems as important to use differential sensitivity in the designing of repellents as it is to use differential toxicity in the development of insecticides. Analogy with human senses can help understanding, but only when applied to the interpretation of direct observations of insects.

There are three senses most likely involved in the perception by insects of repellent chemicals, smell, taste, and the common chemical sense. Smell, or olfaction, is generally associated with vapours; and taste, or gustation, with liquids; although the physiological basis of neither is fully understood. Aquatic insects, for instance, can smell under water (Hodgson, 1951). The sense of smell, normally

mediated in insects by receptors located on the antennae, operates at much lower concentrations than the sense of taste, (taste receptors are found on the tarsi and palpi). Smell is a much more versatile sense than taste, responding to a greater variety of substances. Sensitivity, both qualitative and quantitative, is a function related to the rate of molecular turnover at the nerve ending adsorption sites, and seems to be the best distinction between smell and taste. This will be further discussed in the next section. The common chemical sense is best defined as the ability of all living tissue to respond to irritant chemical stimuli. It can be assumed that nervous tissue is more sensitive than other tissue to irritant chemicals, and is necessary to mediate a response in insects (Kalmus and Hocking, 1960).

The original purpose of this study was to determine in the insect the sites of action of insect repellents and to determine the senses involved, with particular reference to the part played by the common chemical sense.

2. STATE OF KNOWLEDGE

2.1 Introduction

By earlier definition (page 13), repellency is itself instrumental in evoking an avoiding response. The physiological pathway involved is thus: repellent stimulus - sensory receptors - central nervous system - effectors - behavioural response. The simplest form this pathway could take is the reflex arc, but although a feeding mosquito would seem to remove a leg when it was brushed with repellent (Khan, 1965) this is not true repellency, since the insect remained feeding. More complicated patterns involving movement responses have been classified by Fraenkel and Gunn (1940) into taxes and kineses, the prefix negative being used to denote that the direction concerned is away from the stimulus source. A taxis means movement straight to or from the stimulus source and must involve either two groups of receptors or the continued movement of one group, so that a gradient or directional stimulus can be sensed. Increased speed, or rate of turning, will result in an undirected response known as a kinesis. Both taxes and kineses of course depend upon the ability to detect changes in the intensity (or concentration) of the stimulus. Békésy (1964) has described how olfaction can lead to a true directional response or taxis, and Wright (1962a) discussed mosquito attraction and repulsion.

Commercial repellents may also act by interrupting the

chain of events leading to some other form of behaviour, such as host selection or blood feeding by biting flies. Such action is not strictly repellent, but a form of interference. This could be the result of an interaction away from the insect with an attractant substance but it is more likely that the interaction will be at the receptor sites of the insect, possibly by a form of competition for those sites.

All known changes in behaviour after repellent treatment, could however be explained in terms of conflict between a repellent stimulus and stimuli leading to other behaviour. That is to suggest that the conflicting stimuli act on different receptors, or independently on the same receptors. Variations in stimulus intensity, individual sensitivity, and physiological state could result in great variations in behaviour, and explain why few repellents are 100% effective in practice. A very hungry insect is less likely to be put off its meal by an unpleasant odour. The site of interaction in such a case would be the central nervous system.

The most unattractive of all possibilities is that all the mechanisms described can be combined in the action of insect repellents.

Insects are capable of responding to temperature change, sound, light, touch, and chemicals. All of these can evoke repellent responses in insects, but at normal temperatures

repellents do not emit sound or light of optical wave lengths nor do they reflect light of any unusual nature. Repellents could interfere with the reception of such stimuli, but that again is not active repellency. Touch is also regarded as unimportant in the action of commercial repellents, since the acting quantities are too small to have much effect on the texture of the substrate; although the sticky nature of insect repellents may contribute to their effect.

2.2 Insect chemoreceptors

Because of the thick, relatively impervious nature of the insect cuticle, chemoreceptors are fairly easily identified as thin walled sensilla or nerve end organs, occurring on the antennae, tarsi, and labial palps of most insects. The possibility that repellents can penetrate the cuticle elsewhere and thus act on nervous tissue not connected to the chemoreceptors is considered in sections 2.3 and 4., but since chemoreceptors are the primary sites for the perception of chemical stimuli, they may be considered of primary importance.

Taste receptors, or contact chemoreceptors of insects have been identified on the legs and labellae (Frings and Frings, 1949; Peters, 1961; Adams, 1961; Owen, 1963). Dethier (1955) described thin walled hairs and thin walled cones on the blowfly Phormia regina Meigen. These contained three neurons, one responding to mechanical stimuli, one exclusively to sugars, and the third responding to solutions of salts and other substances, perhaps including repellents. Recent work involving the electrophysiology of these organs (Hodgson and Roeder, 1956; Hodgson, 1957) has verified and extended these discoveries. Because the technique used involved a current carrying fluid-filled glass electrode placed over the organs (only the tip is sensitive) the research was limited to water soluble substances. Most commercial repellents are hardly soluble in water at all,

and less direct methods are required to determine how these organs respond to repellents.

Insect olfactory organs have been described by Dostal (1958), Schneider (1961), and Slifer (1961). Begg and Hogben (1946) identified the olfactory nature of such sense organs in Drosophila melanogaster Linnaeus, by comparing the responses of normal flies with those of mutants in which the organs in question (figure 31) were reduced. The olfactory receptors on the antennae of insects are capable of responding to a wide variety of substances, often at extremely low concentrations. Structurally, there is little to differentiate between olfactory and gustatory receptors, apart from their different locations on the insect. The differences between gustation and olfaction have to be studied from the biochemical and physiological aspects, the morphology of the structures concerned is of little help.

Roys (1954) reported that the isolated nerve cord of a cockroach responds to the vapours of certain chemicals such as benzene and toluene. Since there are no specific receptors concerned here, he attributed this to the common or general chemical sense. He went on to state "if chemoreception of this sort is a fundamental property of nerve tissue then no special receptor is needed to translate chemical action into nerve impulses". This raises the possibility that the reception of irritant chemicals is not confined to the gustatory and olfactory chemoreceptors.

2.3 Physiology of chemoreception

The perception of repellent chemicals by insects is mostly if not wholly a function of the insect's chemosensory organs. The study of repellent action must be integrated with current knowledge and theory pertaining to the larger category of chemoreception. The mechanisms involved in this are not well understood, and the field abounds in theories, many of which have little basis in fact. There is a quantitative difference between olfaction and gustation, olfactory thresholds are much lower than gustatory thresholds. The qualitative difference between olfaction and gustation, that is the ability of the organs of smell to respond to a much greater variety of chemicals, can be explained in terms of varying thresholds of sensitivity at a series of receptor sites. Whether this is the case or whether there are receptor sites which are specific for certain chemicals or groups of chemicals depends upon further understanding of the basic physiology of the sense of smell. Although there are dangers in interpolating data obtained from the study of human chemosensory mechanisms and applying them to insects, this approach is very helpful for basing hypotheses. Humans have the great advantage of being able to record their sensations directly, whereas in dealing with other animals, sensations reaching the brain have to be assumed from either behavioural or electrophysiological observations.

In insects the taste receptors are located mainly on

the legs and labial palps (section 2.2). Chemoreception in insects has been reviewed by Dethier (1956a, 1963) and by Hodgson (1958). An electrophysiological method for recording the nerve impulses from single tarsal taste receptors was described by Hodgson, Lettvin and Roeder (1955), and the method continued by Hodgson and Roeder (1956), and Hodgson (1957). The taste receptors were shown histologically to contain three fibres, one responding to mechanical stimuli, one (the S-fibre) highly specifically to various sugars, and the third (the L-fibre) responding to salts and electrolytes. Sometimes a fourth fibre is found which responds to water (Mellon and Evans, 1961). The fibres could be identified in the impulse recordings by characteristic amplitudes and frequencies (the latter of course varying with stimulus concentration). Interaction was noted, strong stimulation of the S-fibre would inhibit the response to the L-fibre and vice versa. Since the S-fibre is stimulated only by certain sugars and is associated with the feeding response, and the L-fibre is stimulated not only by salts but also by a wide variety of inorganic and organic compounds and is associated with rejection, interaction between the two is of considerable importance in considering the action of insect repellents. Dethier (1951, 1952a, 1953, 1955) has done considerable work on acceptance and rejection thresholds, and has found that for an homologuous series of aliphatic organic compounds, molecular rejection thresholds

decrease with an increase in chain length. This should be explained by any chemoreception theory. Dethier also correlated threshold values with water solubility and oil solubility, but failed to draw from this any concrete relation which would indicate a suitable hypothesis for the mechanism of nerve end stimulation by chemicals.

In mammals, the taste receptors on the tongue present a more confused picture, four or five physiologically different fibres have been identified by electrophysiological means which were less exact than those used by Hodgson in insects. These fibres are sometimes capable of reacting to more than one substance (Cohen, Hagiwara and Zotterman, 1955), e.g. acid and salt, whereas others react only to the one substance e.g. salt. It may be that the true differences are threshold variations between the different fibres, effected perhaps through the associated ions or hydrogen molecules. Specific sugar receptors appear to be present and there may also be isolated receptors of the kind found more often in the olfactory region of the head. In terms of the mechanism by which nerve endings respond to chemical stimulation, the general physiology of chemoreception in mammals and indeed in most animals is probably similar to that of insects.

Olfaction in insects has been reviewed by Dethier (1954, 1963), and a bibliography compiled by Hocking (1960). A large number of authors have measured the sensitivity of

insects to odours, using behavioural thresholds. The behavioural threshold is that concentration of the stimulus which evokes a given response from the insect. The electrophysiological threshold, that concentration of stimulus which evokes nervous impulses in the sensory neurons, has only recently been measured (Schneider, 1962). There is evidence that olfactory acuity is some function of the number of sensilla involved (Dethier, 1952b; Ribbands, 1955; Dostal, 1958). An important aspect of odour sensitivity is that an attractive odour may become repellent at higher concentrations, and a normally repellent odour may be attractive at very low concentrations (Dethier, 1947). This factor may be of great importance in the action of repellents (see sections 3.3 and 5.4). Insects respond to a wide variety of chemical vapours, and some of these responses appear to be very specific (Schneider, 1962). In man, stereoisomers of the same substance often have different smells (Beidler, 1952). Such specificity is similar to that observed in the S-fibre (sugar) of the insect contact chemoreceptors, but it would be unreasonable to suggest that there is a different olfactory receptor for each class of compound. Boeckh, Kaissling and Schneider (1966) have stated that there are two types of olfactory receptor cells, often occurring in the same receptor end organ. These are the 'generalists' which have a wide spectrum of response and the 'specialists' which have a narrow spectrum of response. The 'generalists' can be regarded

as analogous to the L-fibres of the contact chemoreceptors, and the 'specialists' as somewhat analogous to the S-fibres. However, the narrow range of compounds for which the specialist cells respond does not seem to have any chemical, physical or structural homogeneity. The picture is complicated by the occurrence of summation and inhibition between these two types of cells, similar to that found in the contact chemoreceptors. Stimulation of one cell may cause inhibition of the other cells, or it may cause sensitization. This has not been worked out in detail.

In recent years, a great deal of work has been done on the nerve action potentials resulting from the olfactory stimulation of insects (Boistel and Coraboeuf, 1953; Roys, 1954; Boistel, Lecompte and Coraboeuf, 1956; Schneider and Hecker, 1956; Schneider, 1957a; Morita and Yamashita, 1961; Schneider and Boeckh, 1962; Schneider, Lacher and Kaissling, 1964; Lacher, 1964). Some of this work will be discussed in section 4.1. Wohlbarsht (1966) has stated that there are two action potentials when a nerve ending is stimulated by an odour. These are the receptor and impulse potentials. The receptor potential is a low amplitude potential originating in the nerve ending which is exposed at the tip of the chemosensory hairs. The impulse potential is of high amplitude, and originates near the cell body. The receptor potential in one neuron can cause sensitization or desensitization in neighbouring neurons, thus accounting for summation and

inhibition. All this does not explain how the receptor potential is initiated in the tip of the neuron. In considering the ways in which odorous molecules could initiate receptor potentials, we may consider other animals as well as insects, for although the basic physiological mechanism involved may differ between species, this is unlikely. Ehrensvärd (1942) and Davies (1953) theorized that odorous molecules adsorbed on to the plasma membrane of the neuron dislocate that membrane and initiate nerve impulses. Such theory fits well with the best of the recent olfactory theories, that of Amoore (1962, 1964). Since groups of chemicals having the same smell do not appear to have any common chemical properties, Amoore postulated that it is the shape of the molecule that counts. Molecules of similar shape, regardless of their chemical constitution can fit into a number of pre-shaped adsorption sites on the nerve endings. How this affects the polarity of the membrane is still a mystery. Amoore's work is discussed in detail in section 5. Wright (1962b, 1964a) postulated a theory by which the low frequency molecular vibrations of odorous molecules stimulate the receptor nerve endings. According to this infra-red adsorption theory, the action of repellent chemicals and high concentrations of water vapour should be similar and repellents should be attractive at very low humidities; this does not appear to be the case (Hocking and Khan, 1966).

If, as suggested by Roys (1954), the common chemical sense is a fundamental property of nerve tissue in response to irritant chemical stimuli, then the basic mechanism of chemoreception could be common to all nervous tissue. Specialization of certain nerve endings, during the process of evolution, could have resulted in the increased sensitivity of these nerves to the basic process of chemical stimulation. Given a complete range of sensory neurons, with varying degrees of chemical sensitivity, one is left with the problem of selectivity. Roys (1954) proposed a series of filters, each one selective for a particular group of chemicals. I prefer the idea that the development of increased sensitivity is restricted to groups of chemicals. As the sensitivity of a neuron increases, the number of compounds to which it responds at this increased sensitivity decreases. This is not incompatible with Amoore's stereochemical theory. Although by this process 'specialist' receptors (Boeckh, Kaissling and Schneider, 1965) would respond to a very low concentration of certain restricted compounds they could still respond to higher concentrations of general chemical stimuli, as do the less specialized chemosensory neurons. Inhibition, occurring among groups of neurons (Hodgson, 1957; Wohlbarsht, 1966) would complete the discriminatory process. A low concentration of a specific chemical could stimulate a 'specialist' neuron, but not an associated 'generalist' neuron; a higher

concentration of a more general irritant stimulus could stimulate both neurons, but the receptor potential of the 'generalist' neuron would inhibit the impulse potential of the 'specialist' neuron so that the impulses reaching the brain would be different in the two instances.

Because of the cuticle, insects do not have free nerve endings other than those of the chemosensory organs themselves, where the common chemical sense could be said to reside. Other animals do have other free nerve endings, and Beidler (1966) has compared gustatory receptors, olfactory receptors and free nerve endings. Anyone who has accidently got insect repellent on his lips knows that nerve endings in this region are sensitive to general chemical stimuli.

2.4 Behaviour in response to repellents

Repellent action has been reviewed by Dethier (1956b). Many people tend to think of chemical stimuli in terms of attractants and repellents. In reality, the effects of chemical stimulation on insect behaviour are far more complicated. Dethier, Brown and Smith (1960) have designated chemicals under five basic terms, attractants, repellents, arrestants, stimulants and deterrents. An attractant causes insects to make orientated movements towards its source, a repellent causes insects to make orientated movements away from its source, an arrestant causes a reduction of speed or an increase in turning rate resulting in little or no net movement, a stimulant elicits some particular form of behaviour such as locomotion, feeding, mating or oviposition, a deterrent inhibits such behaviour. Practically all these responses, excepting the stimulation of feeding, mating or oviposition, have been reported in insects as a result of exposure to insect repellents. Evans (1961) stated that repellents do not effect the sugar feeding of blow flies except in high concentration, whereas Khan (1965) stated that repellents inhibited the feeding of mosquitoes on both blood and sugars, reduced the mating rate and caused the rejection of oviposition sites. Repellents also affected orientation to gravity and centrifugal force, and a visual response to black stripes. He also stated that mosquitoes became quiescent and less active when repellents were

applied to them. Wright (1964a), disagreed with this last statement, stating that dimethyl phthalate initially increases the activity of resting mosquitoes before narcosis ensues. He also observed that mosquitoes in a stream of repellent vapour turned more frequently, which would cause aggregation outside the stream (see arrestant above). Wright (1962a) stated that a warm wet surface, normally attractive to mosquitoes, does not appear to be attractive in the presence of repellent vapour. From this he concluded that repellents affect other sensory systems as well as the chemosensory. Kalmus and Hocking, (1960) also reported on mosquito behaviour in conjunction with repellents and reported that some attractive factors operated in the presence of a repellent but that the chain of responses leading to the completion of certain types of behaviour was interrupted. Peters and Kemper (1958) attributed much of the effect of insect repellents to a disturbance of the general physiological state of the insect, mediated through the general or common chemical sense. For a frustrated scientist working on repellents, it is tempting to suggest that this is indeed the case, both in insects and in man.

Bar-Zeev and Smith (1959) and Bar-Zeev and Schmidt (1959) have stated that it is the vapour phase of repellents which is important. Dethier (1952b) and Dethier and Yost (1952) showed that removal of organs bearing olfactory receptors from Phormia regina causes insensitivity of the

flies to repellent vapour. However, Glynne-Jones, (1952) has shown that phenol and acetic acid act on the olfactory sense organs of bees at low concentrations, but at higher concentrations seem to act on the common chemical sense. Kalmus and Hocking (1960) sum this up by saying that a good repellent would seem to require three attributes, a bad smell, gustatory or contact repellency and a general irritant action through the common chemical sense.

3. EXPERIMENTAL - CHOICE CHAMBER EXPERIMENTS

3.1 Introduction

There are many empirical methods of evaluating insect repellents (Shepard, 1960). Most of these are designed to test the repellents under the conditions in which they will be used. For example, mosquito repellents are often tested for protection time and degree of protection whilst applied to the human skin under field conditions. In many such tests the repellent is being tested in the presence of attractive factors, and as a preventative against both normal and specialized behaviour, such as blood feeding. Such methods cannot be comparably applied to all insects, nor can they differentiate between compounds which interfere with some behavioural pattern and those compounds which induce active repellency. Since I wished to consider simple repellency, to determine the sites of action on insects and the part played by the liquid and vapour phases of repellents, I chose a method of repellent evaluation that would allow the repellent effect to be tested in the absence of all other known attractive or repellent stimuli.

The simple binary-choice test chamber is a commonly used method of testing insect behaviour. Originally, the chamber was used to determine the humidity preferences of insects (Gunn and Cosway, 1938) and has been repeatedly used for that purpose since (Willis and Roth, 1950; Bar-Zeev, 1960). The use of this type of chamber to screen repellents

was suggested by Bar-Zeev (1962). The long neglect of the simple choice chamber for testing repellents is not an oversight on the part of repellent workers, but merely because for practical purposes more severe and demanding tests are usually desired for repellent evaluation. The binary-choice chamber is divided into two parts, identical in all ways except for the experimentally introduced variable. Other factors such as temperature and illumination must be the same on both sides of the chamber, so that any deviation from an expected distribution of the insects placed in the chamber can be attributed to the introduced factor.

I designed a variation of this type of chamber to test repellents separately in their two phases, liquid and vapour. By this method, I hoped to separate the repellent effect into contact and olfactory repellency. By using, in this test chamber, insects with some of their appendages painted with nail varnish to block the sense organs, it was hoped to discover which groups of sense organs mediated the response to each of the two phases of the repellent, and to what extent.

3.2 Preliminary Experiments

Associated with a layer of liquid is a layer of vapour above it, emanating from the liquid. If a liquid repellent is applied to a porous material, sufficient flow of air down through the material will effectively remove this vapour layer. A circular binary choice testing chamber 15 cm in diameter was constructed with wire mesh in a large glass funnel. The funnel was supported in a tripod stand, and the neck of the funnel was connected with rubber hose to the bench vacuum. The wire mesh floor of the test chamber was covered with glass fibre cloth, two unconnected halves joined with cellophane tape. One half was treated with repellent by soaking in acetone with a known concentration of repellent in solution, the other half was untreated, soaked merely in pure acetone. Glass fibre cloth has a loose porous construction as well as being insoluble in most organic solvents (most repellents are plasticizers and soften rayon and acetate fibres). The vapour layer associated with the treated cloth could be sucked down by turning the vacuum on, which maintained a flow through the cloth's surface of about 25 cm/sec. Test insects were prevented from leaving the floor of the chamber by treating the smooth glass walls with polytetrafluorethylene which provides a surface too smooth for the insects to climb.

Four arrangements of the test cage set up were possible:

(1) a single layer of cloth in the cage, half treated half

untreated, the vacuum off; test conditions for total repellency.

(2) a single cloth layer as (1), but the suction fan on; test conditions for contact repellency with a liquid phase only.

(3) two layers of cloth; the lower half treated and half untreated, the upper layer entirely untreated, and separated from the lower layer by a 1 mm thick non-absorbent monofilament mesh, of the type used in insect window screens, made of glass fibre 12x12 mesh; test conditions for vapour repellency only, since the test insects were kept from contact with the liquid but still exposed to the vapour layer.

(4) two cloth layers as (3) but with the vacuum on; test conditions for the total efficiency of the set up. If the apparatus works properly, the insects are not in contact with either liquid or vapour and there should be no repellency.

The experiments were run in the dark in a basement room which had an almost constant temperature. Insects in each half of the chamber were counted at regular intervals and an index of repellency was calculated for each count, using the formula:

$$\text{index of repellency} = \frac{100\,(\text{number on untreated side} - \text{number on treated side})}{\text{total number of insects}}$$

Counts were made visually, a light being switched on for the purpose. The insects were disturbed by the light, but subsequently resettled in the dark. This redistribution for each separate reading is necessary if the results are to be treated statistically, since observations have to be independent for the statistical formulae to be valid. Readings were made at hourly intervals.

Tests were started using german roaches (Blattella germanica Linnaeus) and the roach repellent MGK R-874 (2-hydroxyethyl-n-octyl sulphide), since this is an extremely efficient repellent to roaches (Goodhue, 1960). The logic behind the preference for a repellent known to be almost entirely effective is that such a material may be supposed to possess all the characteristics of a 'total' repellent, any less efficient material may be deficient in some aspect of repellency.

Although an encouraging loss of repellency was noted when the vacuum was turned on (figure 1), the control experiments (see page 35) with two cloth layers and the vacuum on did not produce a total loss of repellency. From this I reasoned that the apparatus was working, but not working well enough. The bench vacuum was not powerful enough to entirely remove the layer of vapour above the cloth. The results were however, sufficient to warrant the construction of more elaborate apparatus.

Because readings were taken at hourly intervals, the

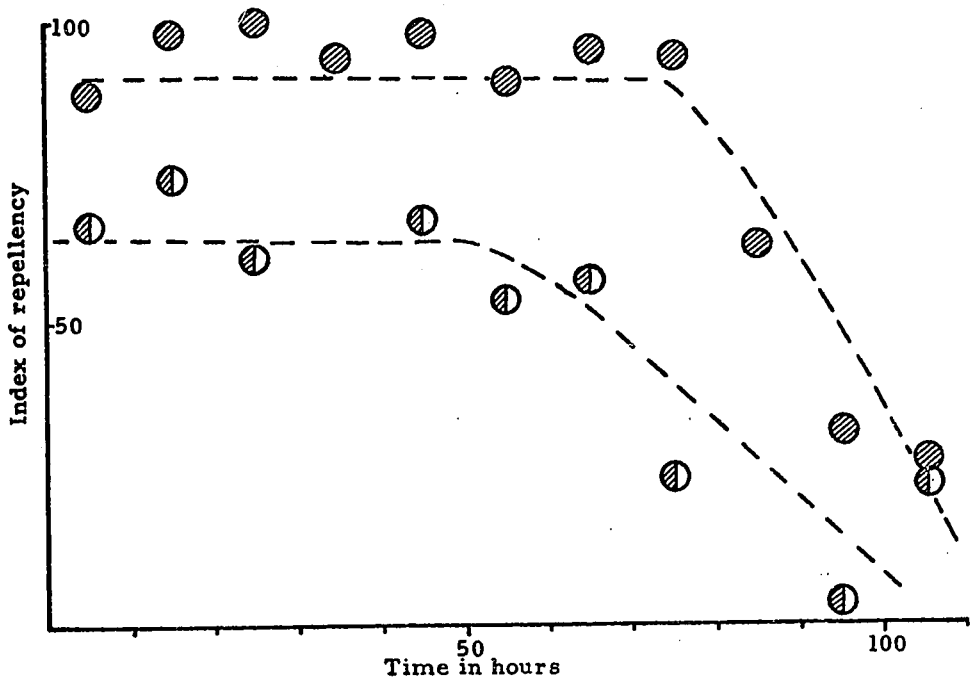


Figure 1. Blattella germanica, indices of repellency plotted against time. The repellent is MGK R-874.
◐ contact repellency, vapour removed by suction.
● total repellency, liquid and vapour phases present.
Counts were made visually every hour, each circle represents the mean of 10 hourly readings.
The indices for contact repellency are considerably higher here than they are in figure 4. This indicates that in these preliminary experiments the suction apparatus was not powerful enough.
The graph does show that the indices of repellency remain fairly steady for about 70 hours before they start to fall off. Subsequent experiments were run for periods of time considerably shorter than 70 hours.

tests naturally took a long period of time. It was noted that after about 70 hours the indices of repellency began to fall sharply (figure 1). This loss of repellency occurred slightly earlier with the vacuum on (figure 1), which suggests some loss of the repellent due to evaporation. The major factor in this drop in repellency was probably the physiological state of the insects, which received no nourishment throughout the test. Subsequent experiments were run over a much shorter time, keeping well within the period where the repellent indices were shown to remain fairly constant (figures 3 and 4).

Improved apparatus working on the same principle as that described at the beginning of section 3.2 was made. The binary choice test chamber 12 cm in diameter was constructed in the inlet port of a 1.5 kw centrifugal blower (figure 2). The floor of the test chamber was again glass fibre cloth, but needed no mesh support, being held by a removable flange, which also acted as the chamber wall. The wall was painted with polytetrafluorethylene to make it slippery. The four experimental arrangements were the same as described on pages 34 and 35.

Readings were taken by camera (figure 5), to avoid any bias from visual observations and any premature disturbance of the insects by human presence (figure 2). The camera was triggered to take a single frame every 10 minutes by a switch on a slow moving kymograph. 100 frames were

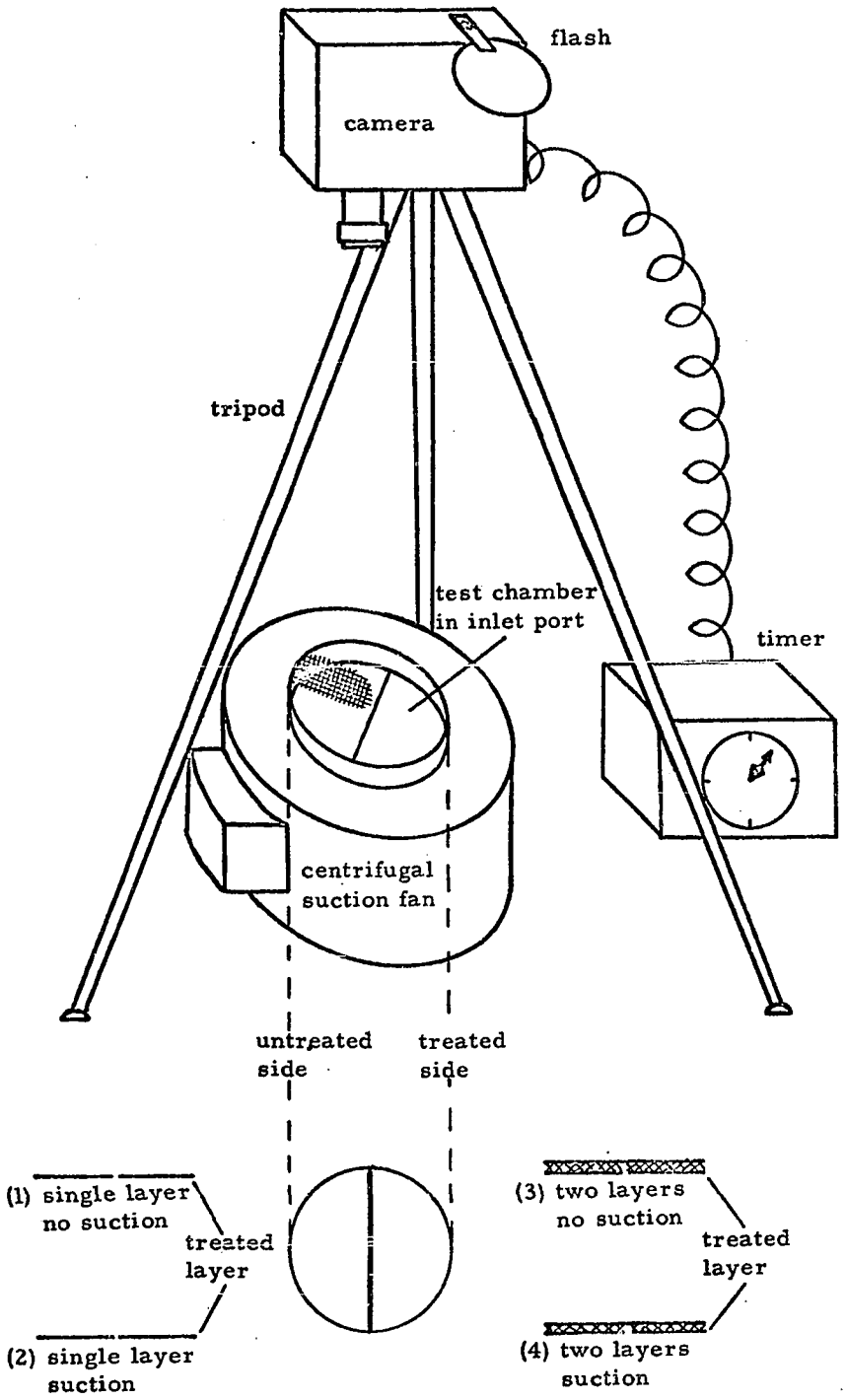


Figure 2. Sketch of the choice-chamber apparatus, and chamber floor arrangements for separating the repellent phases:
(1) liquid and vapour present
(2) liquid only present
(3) vapour only present
(4) neither phase present (control).

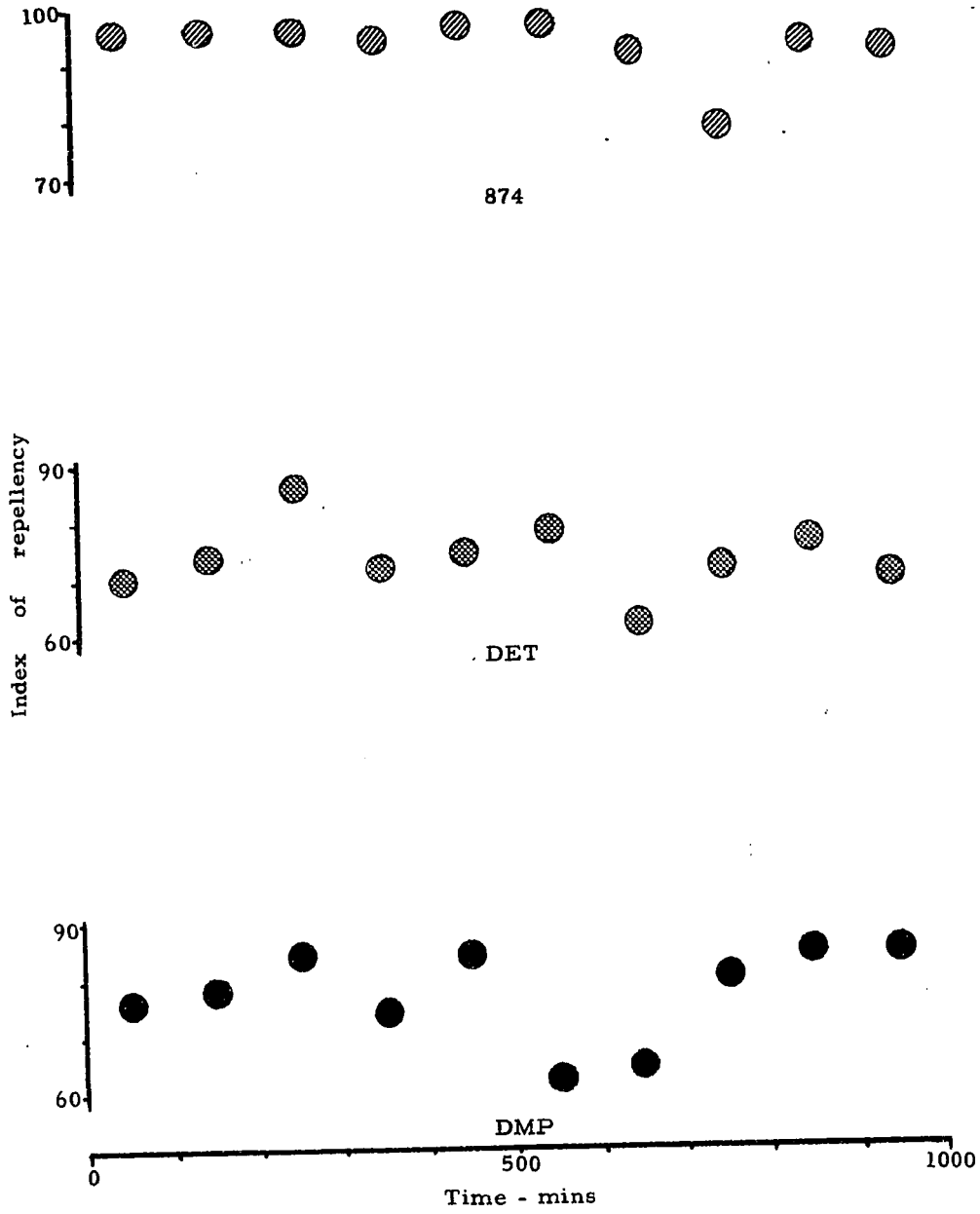


Figure 3. Blattella germanica, untreated insects, both phases of repellent present, for 3 repellents. Indices of repellency averaged at intervals of 10 readings, showing that there is no consistent decrease in the repellent effect over the period of time the tests were run.

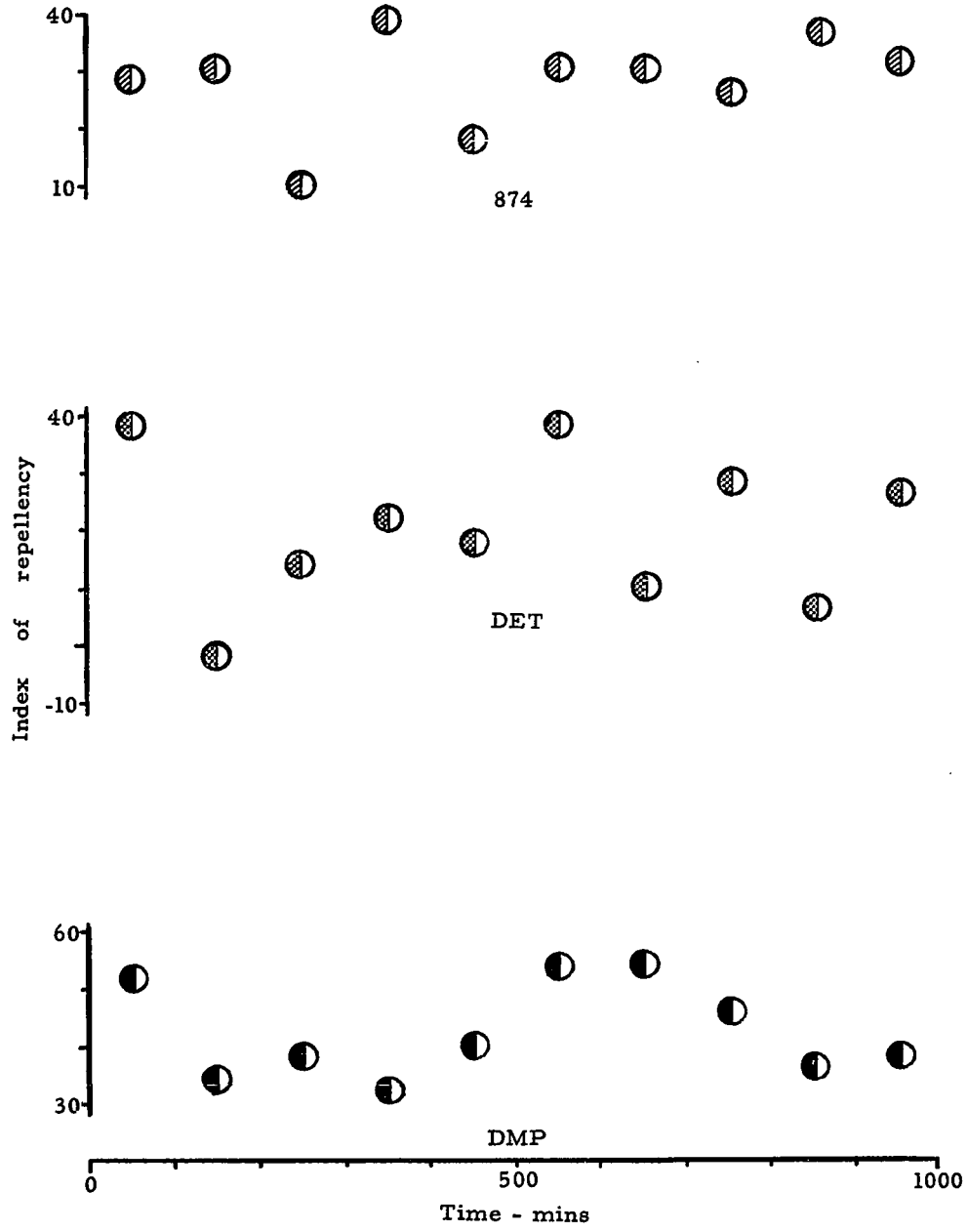
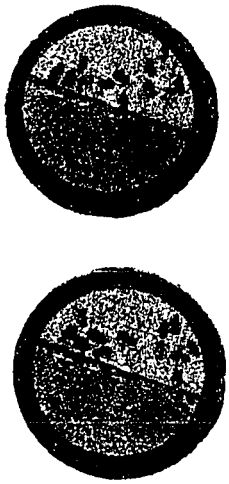
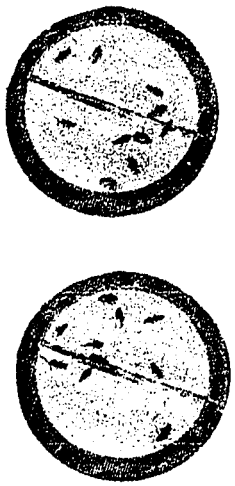


Figure 4. Blattella germanica, untreated insects, repellent liquid only (suction fan on), for 3 repellents. Indices of repellency averaged at intervals of 10 readings, showing that there is no consistent decrease in the repellent effect over the period of time that the tests were run.

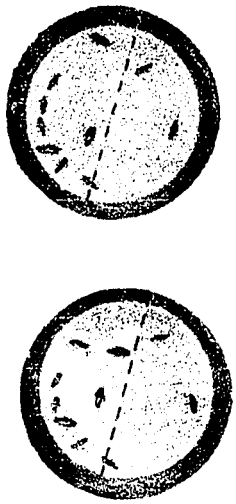


a. repellent 874 (below), 1 layer no suction. Total repellent indices (100, 100).



b. repellent DET (below), 1 layer suction. Liquid phase indices (-10, 30)

c. repellent 874 (right), 2 layers no suction. Vapour phase indices (80, 50).



d. repellent DMP (right), 2 layers no suction. Vapour phase indices (50, 50).

Figure 5. Four pairs of successive frames from the camera readings of the binary-choice test chamber. The insects are German roaches, and can be seen to have moved between frames, though often not very much. For purposes of calculation, insects straddling the centre line are counted as half on the treated side and half on the untreated.

taken on each test, which at 10 minutes per frame, took about 17 hours, well within the period of effective repellency (figures 3 and 4).

The entire apparatus was erected in an abandoned refrigerator room, which was virtually soundproof and had a constant temperature (25C). The insects were left alone in the dark throughout the recording period.

The centrifugal blower sucked air through the test chamber at about 60 cm/sec. This improved suction reduced the observed repellency in the control tests, where two layers of cloth are used conjointly with suction (figures 2 and 7). It also reduced the contact repellent effect considerably (figure 1, cf. figure 4), where one cloth layer is used with the suction on (figure 2).

In experiments to determine the sense organs involved in responses to each of the repellent phases, insects with their legs, palps, or antennae painted with nail varnish were used in conjunction with the apparatus described above. German roaches (Blattella germanica) and mealworm beetles (Tenebrio molitor Linnaeus) were mostly used for their convenient size, smaller insects such as silverfish being too small to paint easily with nail varnish. Flying insects would, of course, flee the floor of the test cage. Some tests were run with horse flies (Tabanus frontalis Walker) with their wings clipped, but these insects did not survive well under experimental conditions.

Three repellents were used; roach repellent MGK R-874, for reasons mentioned on page 36, and the two most widely used commercial insect repellents dimethyl phthalate and N,N-diethyl m-toluamide.

3.3 Preliminary results

The results of the preliminary experiments are shown graphically in figures 6-11. The means only are used, since any measure of variability has little statistical significance with this method of testing. If the binary-choice apparatus is used with batches of insects, and a number of successive readings taken, only one estimate of the repellent effect (measured by the index, page 35) is obtained. No measure of the variability within the batch of insects itself can be obtained. This, and other considerations will be discussed in section 3.4. The results given in figures 6-11, are similar to those that would be obtained from rapid screening techniques used for quick testing of repellent compounds (Bar-Zeev, 1962). The statistically supported significance of the figures resulting from these tests is severely limited, and strict allocation of the total repellent effect into the two phases (liquid and vapour) of the repellent, is not possible. Neither is it possible to differentiate on the insects the receptor areas mediating the responses to these two phases, with any degree of accuracy. Rough estimates are possible, and obvious trends can be seen and may be useful guides, particularly in the more practical aspects of insect repellency.

In the control tests where the vapour phase of the repellent is removed by suction, and the liquid phase by a

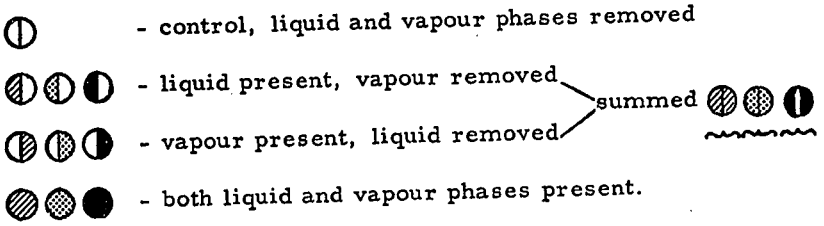
Figures 4-9. Indices of repellency; means at circle centres.

For 3 insects : figure 4, Tabanus frontalis
figures 5-9; a, b, c, (above), Blattella germanica
figures 5-9; d, e, f, (below), Tenebrio molitor.

With 5 treatments: figures 4 & 5, untreated, all receptors active
figure 6, legs and palps active, antennae painted
figure 7, antennae and palps active, legs painted
figure 8, antennae active, legs and palps painted
figure 9, palps active, legs and antennae painted.

With 3 repellents : left and obliquely shaded, MGK R-874
centre and stippled, N, N-diethyl m-toluamide
right and solid, dimethyl phthalate.

In 4 repellent phase combinations; left halves of circles, liquid phase; right halves, vapour phase; thus from left to right:



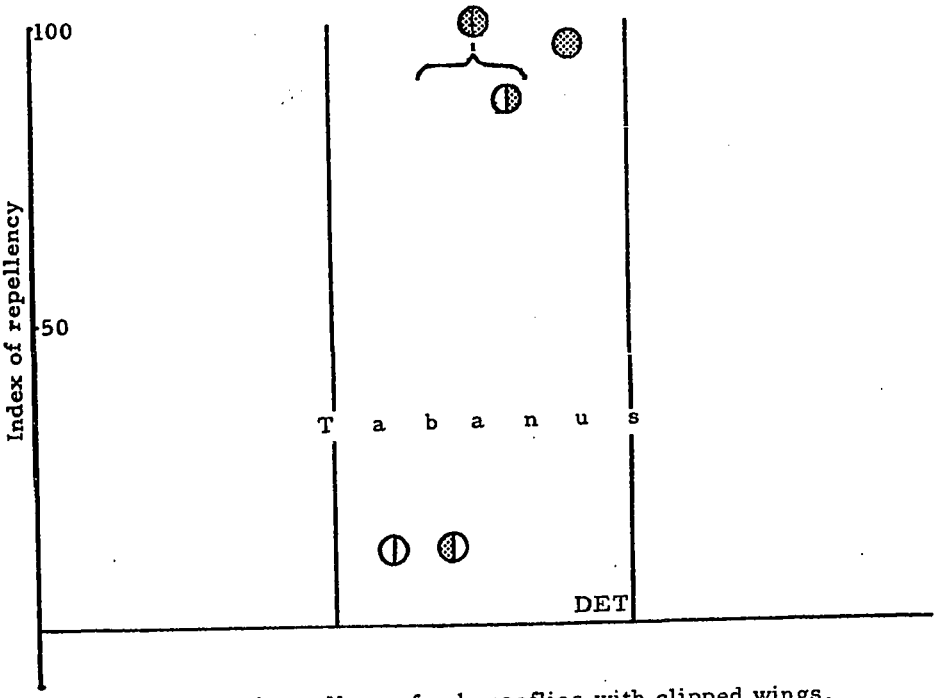


Figure 6. Indices of repellency for horseflies with clipped wings, otherwise untreated. With the repellent diethyl toluamide only.

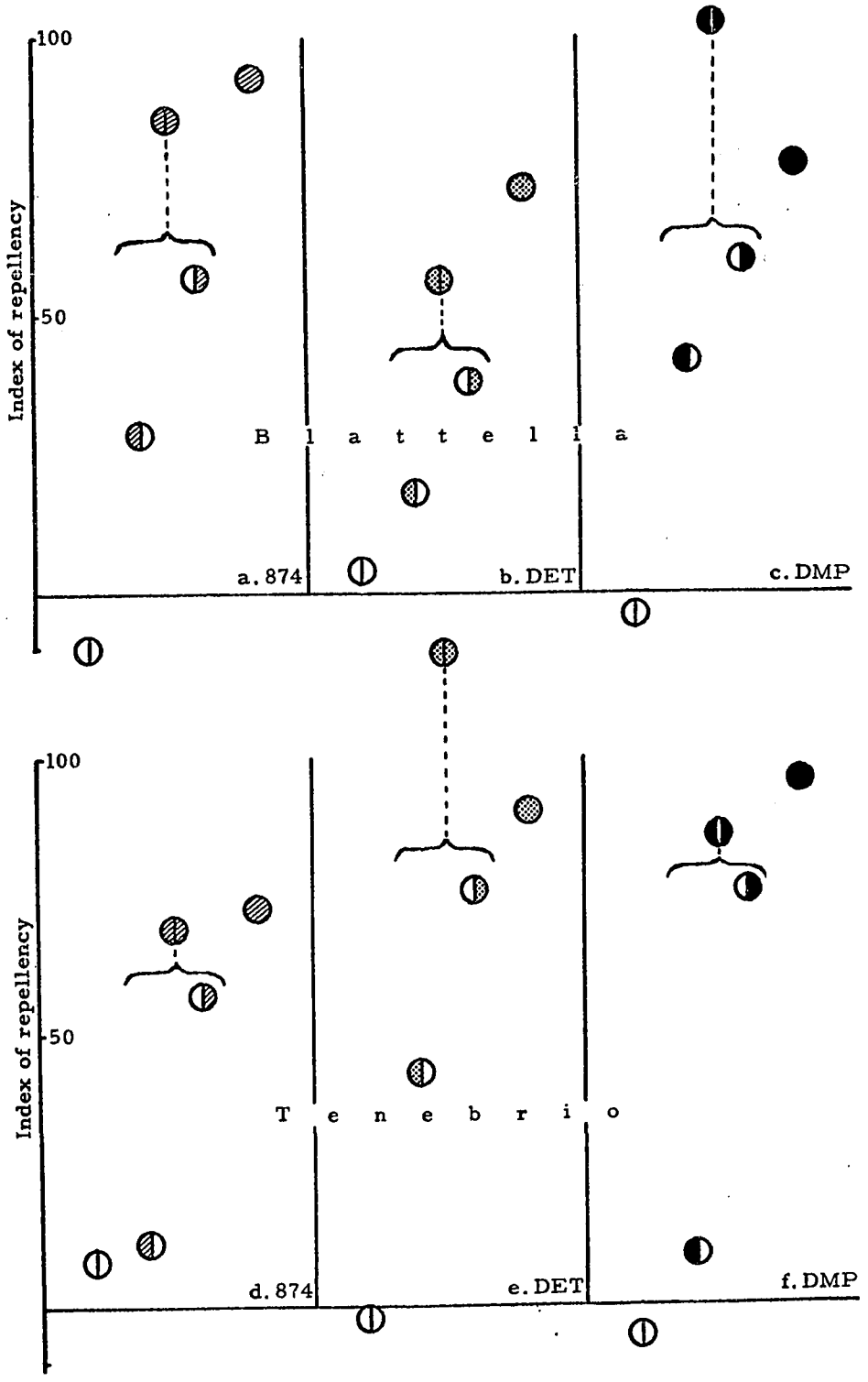


Figure 7. Indices of repellency, untreated insects.

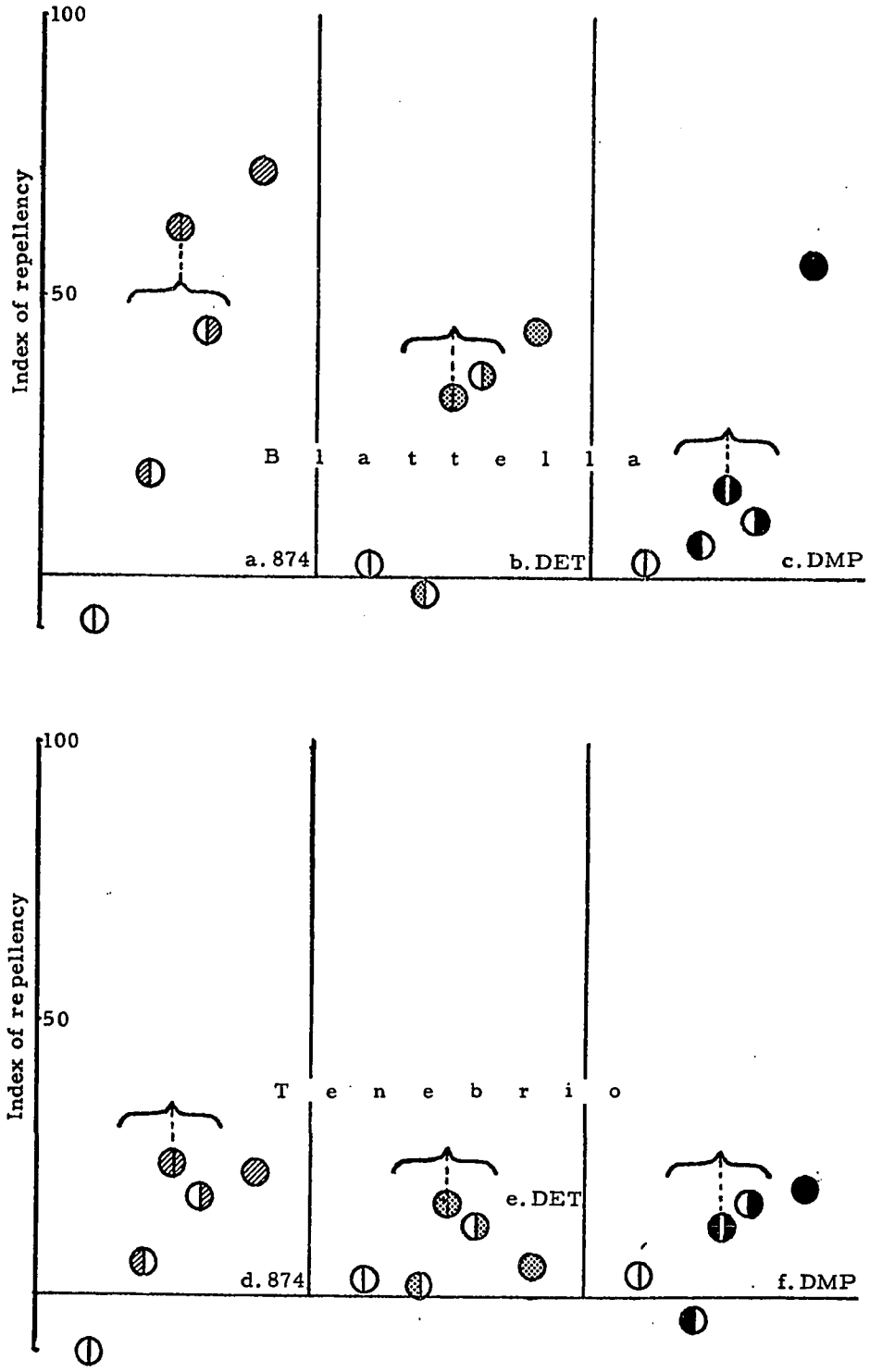


Figure 8. Indices of repellency, antennae painted, legs and palps active.

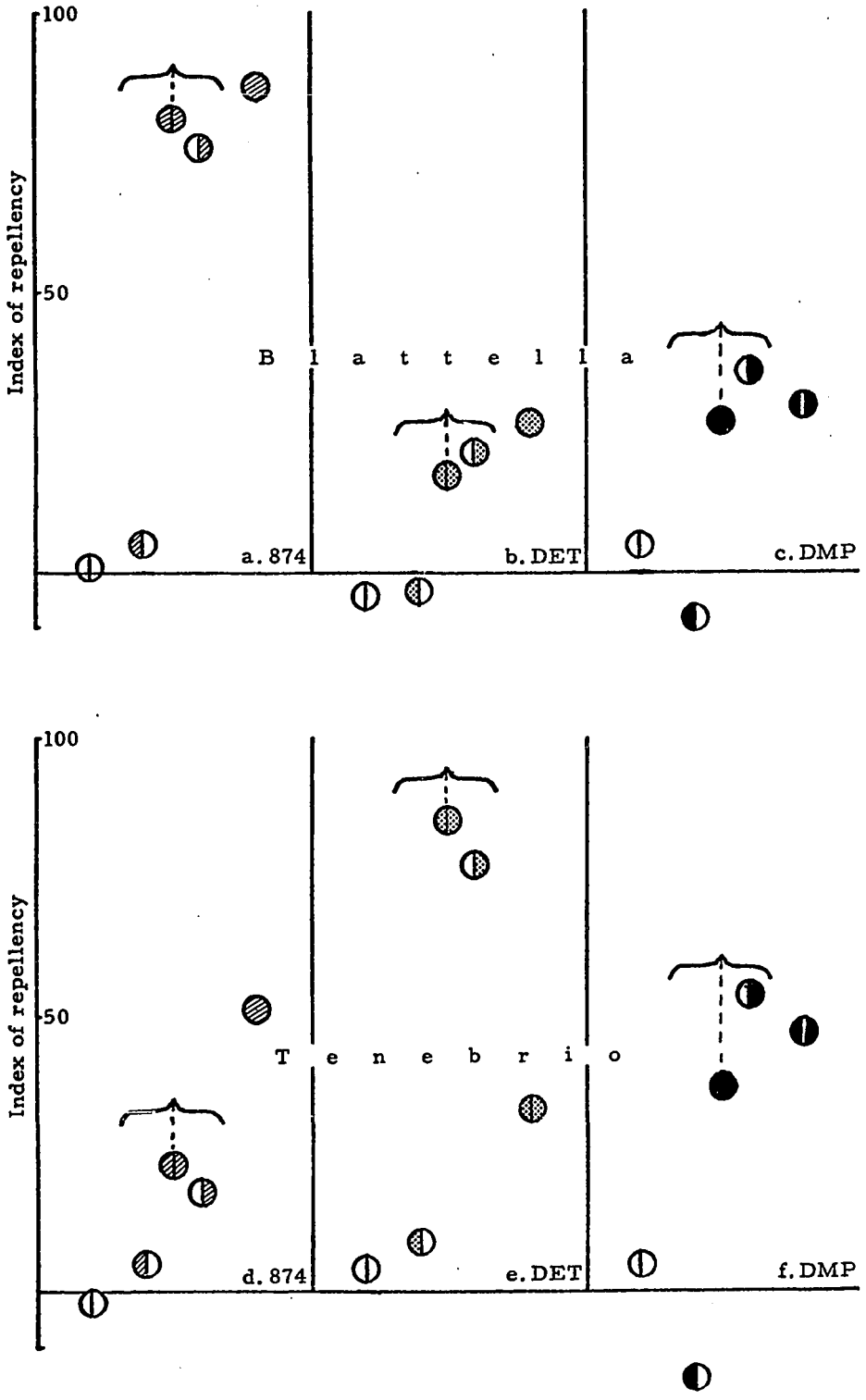


Figure 9. Indices of repellency, legs painted, antennae and palps active.

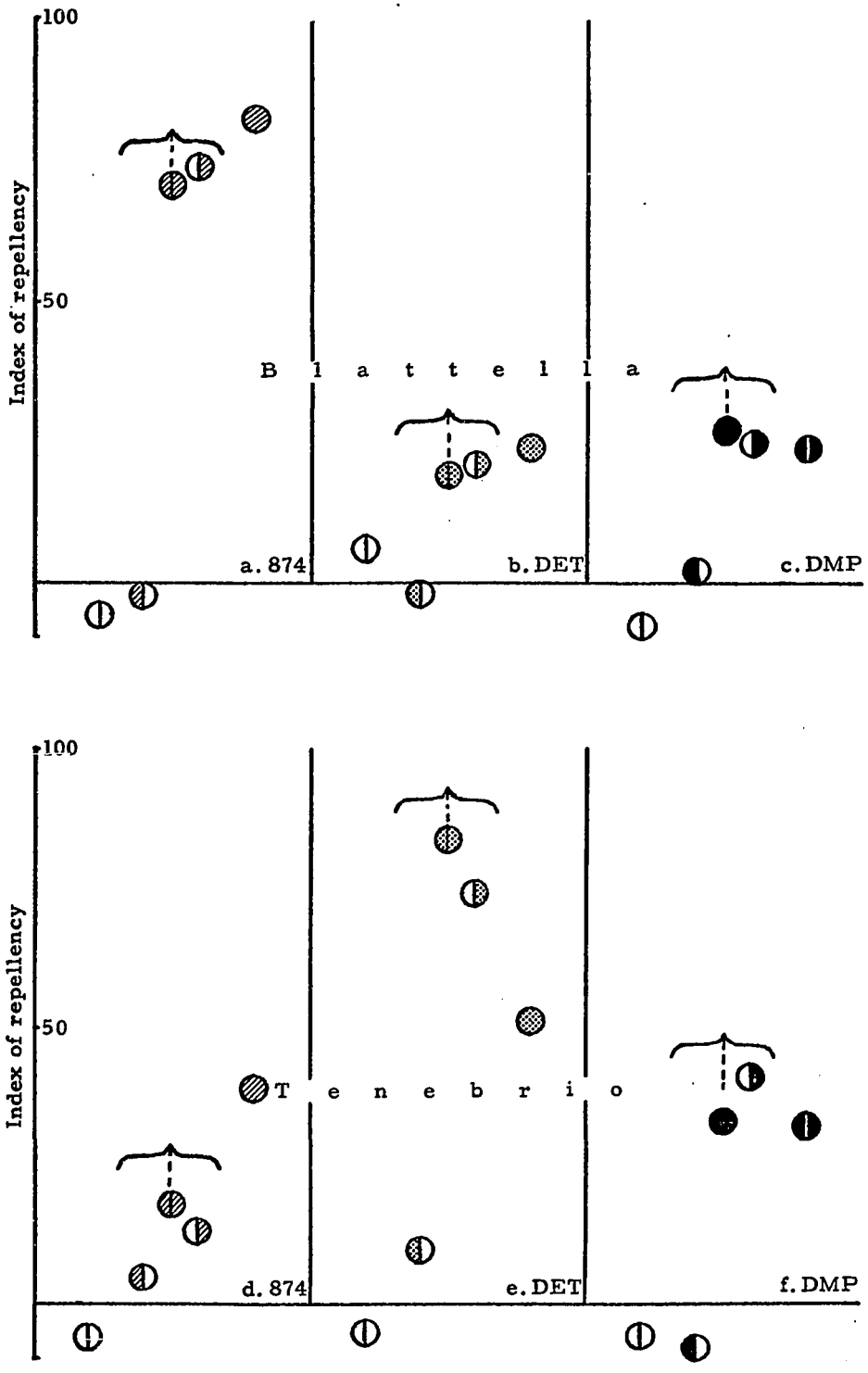


Figure 10. Indices of repellency, legs and palps painted, antennae active.

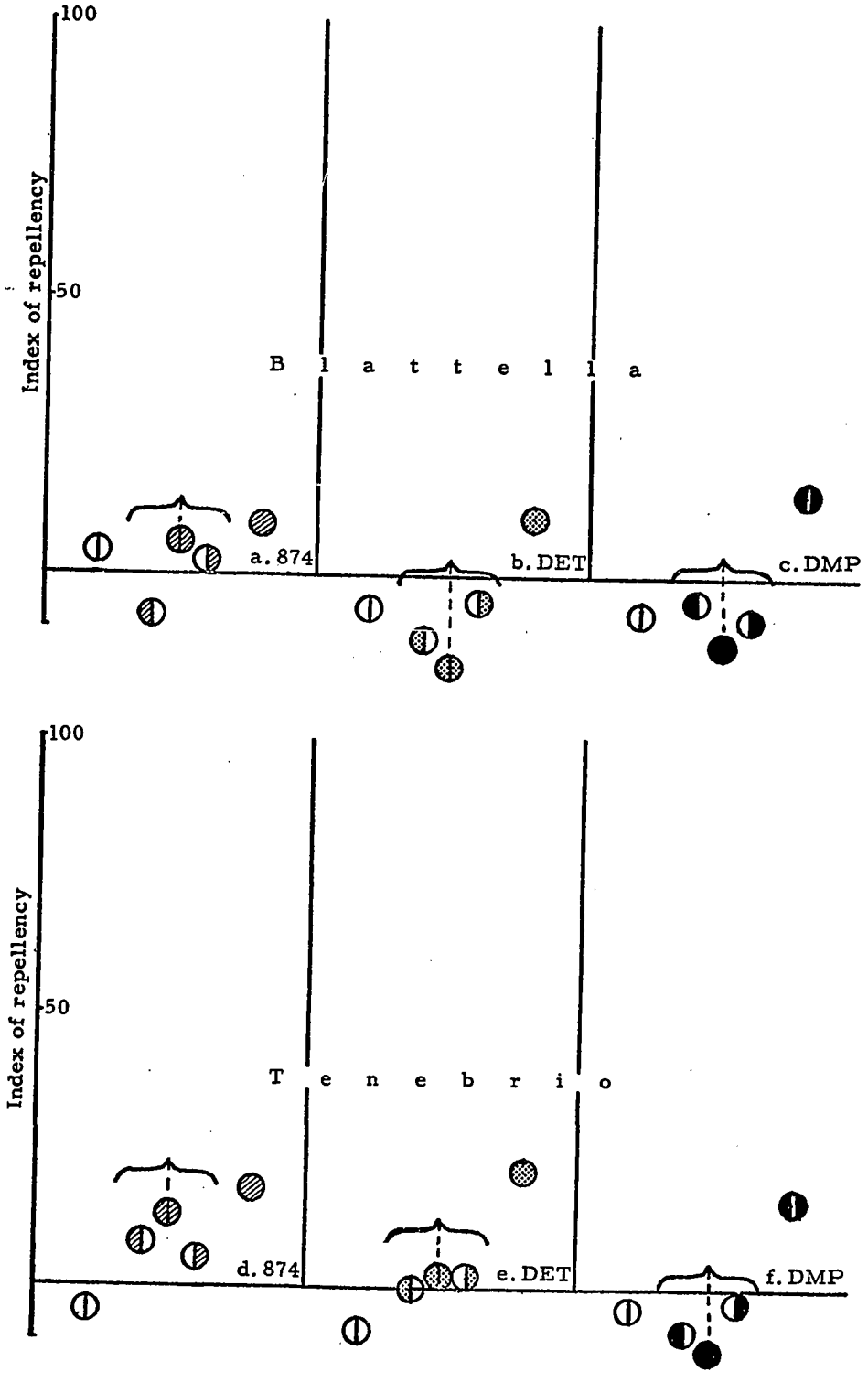


Figure 11. Indices of repellency, legs and antennae painted, palps active.

second layer of untreated cloth, all the indices of repellency are gratifyingly low. Many indices are even negative, which suggests the possibility of attraction which insects have been shown to display at very low concentrations of repellents (Dethier, 1956b). Other negative indices appear in experimental observations where they might well not be expected (figure 9), and this too might be explained as attraction at low concentration. The only honest evaluation is that the repellent effect is little or absent in cases where the index is on or below zero. The low repellent indices for the controls with the untreated insects (figures 6 and 7) indicate that the apparatus was functioning as intended, since when the two systems designed to split the repellent effect into liquid and vapour phases were run together, almost total removal of the effect was achieved.

With all three repellents used and all insects, the vapour phase of the repellents seems to be more important. In all tests run with untreated insects (figures 6 and 7), the vapour effect is greater than the liquid effect, the ratio varying from about 2:1 for the roach and the beetle on DET (figures 7a, b, c, e), to about 6-8:1 for the beetle (figures 7a, f) and the horse fly (figure 6b). Apart from Tenebrio on DET (discussed on page 54), the clear implication from these observations is that the contact effect is much more important with the roach than with either the

beetle or the horse fly. This contact effect is associated with the receptors in the legs, since it is removed if the legs are painted with nail varnish to block the receptors (figures 9, 10). However, blocking the antennae also reduces the contact effect, entirely with the roach on DET and partially with the roach on R-874 and DMP (figures 8a, c). Roaches are known to beat the substrate with their long antennae. The contact effect could also then involve the antennae in the roach.

In all figures, the indices obtained from the separate repellent phases have been summed (above the brace) so that then can be compared with the index of total repellency. If the apparatus is capable of separating the two phases completely, and if contact chemoreception in insects is quite distinct from olfaction, then the sum of the separate indices should add up exactly to the total index. The summed indices are less than the total index in 22 out of 31 cases (figures 7a, b, d, f; 6a, b, c, f; 9a, b, c, d, f; 10a, b, d; 4a, b, c, d, e, f), significantly less in nine of these (figures 7b; 8c; 9d; 10a, d; 11b, c, e, f). In these latter significant nine there is no predominance of any one repellent or insect, but the treatment does stand out. Treatment in figure 11 is the blocking of both legs and antennae with nail varnish, and all the indices of repellency obtained are so low that little importance can be attached to the values, particularly since many of the

indices are negative in this figure. As has been previously stated (pages 24 and 52), this may indicate a vary low but perceptible concentration of repellent. The summed indices for vapour and liquid are greater than the total index in 9 out of the 31 cases (figures 6b; 7c, e; 8d, e; 9e; 10c, e, f), and significantly greater in 4 of these cases (figures 7c, e; 9e; 10e). The particular case of Tenebrio on DET predominates among these latter significant four. DET is the only repellent which shows any appreciable contact effect with Tenebrio (figure 7e). Figures 7e and 10e are remarkable in that the response to vapour alone is considerably greater than the total index itself. For this I have no explanation. The overall pattern from all this discrepancy in additivity is clear; a separation of repellency into two separate parts, contact chemoreception or gustation associated with the liquid phase, and olfaction associated with the vapour phase, cannot be accurately made.

The tests run with treated insects (insects with various groups of receptors blocked with nail varnish) showed that although there is some association between the liquid phase and tarsal receptors, and between the vapour phase and antennal receptors, there is no clear cut distinction. Very little of the repellent effect can be attributed to the palps; figure 10 is very little different from figure 9. Figure 11, which sould show any repellent effect due to the

palps and any other chemoreceptors except those of the legs and antennae, indicates that these other receptors are relatively unimportant as far as repellency goes. The receptors of the legs and antennae, then, are the chief sites for the perception of repellents. The tarsal receptors in the roach seem to be far more important than they are in the beetle (figure 8). In both species, however, the tarsal receptors appear to be sensitive to vapours to some degree (figure 8). The antennae of neither the beetle nor the roach seem to respond to the liquid repellent phase (figure 10), but are quite sensitive to vapours, particularly in Tenebrio which seems to have little sensitivity for liquids alone (figure 7).

3.4 Choice chamber statistics and results

The following experiments were designed to eliminate certain errors associated with the tests as described in sections 3.2 and 3.3, and to allow valid statistical comparisons to be made.

The apparatus was essentially the same as that described in section 3.2 (figure 2), but was vented to the outside to remove any repellent vapour in the room. Certain differences in procedure were adopted: only adult male German roaches were used, avoiding the possibility of introducing sex attractants into the chamber from female insects; the insects were reared at 23 C in a culture room and the tests conducted in a darkened room at 23 C and relative humidity of 30% - 40%; all insects used were first anaesthetized with carbon dioxide, transferred to individual vials and allowed to recover in the test room for 2 hours, whether they had been treated with nail varnish to block their sense receptors or not; the insects were adults between 3 and 10 days old, and were not used more than once. Since roaches have a tendency to congregate or clump, readings were taken with only one roach in the chamber at a time. For each reading, a roach was dropped on the centre line of the test chamber, allowed to settle for exactly 3 minutes, and a photograph taken of its position. The roach was removed and dropped again for a second reading, and so on through the 10 readings. The roach was then discarded. Thus after

each reading the insect was thoroughly disturbed, and to this extent the readings may be said to be independent.

There is another reason why it was decided to switch to the more laborious process of taking readings on single insects rather than using a batch of insects. If several readings are taken of the distribution of a single insect in the test chamber, the data produced must follow a binomial pattern since the insect can only be counted as on the treated side of the chamber or not. The resulting ratio of readings on the treated side versus readings on the untreated side will give an estimate of the probability of the insect being on the untreated side, which is a measure of the degree of repellency; for that insect alone. If the experiment is repeated using different insects, a measure can be obtained of the variability of this degree of repellency within the insect population. This variation may not be binomial, indeed, it is more likely to follow a normal pattern, since it is the variation shown by a natural population in response to a repellent substance. If a batch of insects is used, say 10 at a time, and an average of 3 counted on the repellent treated side, unless each insect is marked and counted separately we have no way of knowing whether each insect spent 3 tenths of its time on the treated side, or whether 3 insects spent all their time on the treated side and 7 insects spent all their time on the untreated side. Thus we have no measure of the variability

of the repellent effect within the insect population.
Experiment I.

To show that there is no difference between the behaviour of untreated male German roaches when they are placed in a binary-choice test chamber with:

1. no repellent present and no air flow down through the chamber, both halves of the chamber being untreated cloth;
2. no repellent present, identical untreated halves to the chamber floor, but with the suction fan on;
3. two layers of cloth separated by glass fibre mesh, half the lower layer treated with repellent (MGK R-874), suction fan on. Conditions for testing the efficiency of the apparatus (see figure 2).

For each test, 10 separate readings were taken on each of 20 roaches. For every reading, a roach was placed on the centre line of the chamber, allowed to settle in the dark, and a photograph taken of the insects position. The results of each test were tabulated (tables 1, 2 and 3) and tested statistically against the following null hypothesis: there is no difference between the observed distribution of the experimental data and an expected binomial distribution, with a proposed probability of 0.5 that an insect will be on either side of the chamber.

The results are given in tables 1, 2 and 3. In none of these tests is χ^2 significant at the 0.05 probability level, and therefore in no case can the null hypothesis be

rejected. In the absence of the experimentally introduced repellent stimulus, the test insects chose their side of the test chamber at random, with the expected probability of 0.05 (table 1). This distribution was binomial (tables 1 and 2), and was not affected by air flow down through the chamber (table 2). With the apparatus set to remove both the liquid and vapour phases of a repellent present in one side of the test chamber, the test insects showed no significant preference for either side of the chamber, indicating that the two repellent phases had been effectively removed (table 3).

Experiment II.

This experiment was designed to test the response of treated and untreated German roaches to various phases of the repellent MGK R-874 (purity 96.4%). It was hoped to answer the following questions:

Can the repellent effect be partitioned into a vapour effect and a liquid effect?

Which receptor sites on the insect respond to repellent, and to what extent?

Is there an association between the receptor sites and the repellent phases; ie. do the legs mostly respond to liquid and the antennae to vapour?

The experimental conditions were the same as described at the beginning of section 3.4 (page 34) and 10 readings were taken for each of the 20 separate insects used in each

N = number of times each insect recorded on the right hand side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	0.20
8	1	0.0439	0.88	0.16
7	1	0.1172	2.34	0.77
6	6	0.2051	4.10	0.88
5	6	0.2500	5.00	0.20
4	4	0.2051	4.10	0.00
3	0	0.1172	2.34	2.34
2	2	0.0439	0.88	1.42
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20	1.000		$\chi^2 = 6.21$

$p = 0.5$, $1-p = 0.5$

10 degrees of freedom (only one degree of freedom is lost, since p was not estimated)

Table 1. Binomial distribution fit for 20 Blattella germanica adult males, no repellent, no treatment, no airflow in the test chamber. For the binomial fit calculations see Steel and Torrie (1960).

N = number of times each insect recorded on the right hand side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	0.20
8	2	0.0439	0.88	0.16
7	2	0.1172	2.34	0.05
6	4	0.2051	4.10	0.00
5	5	0.2500	5.00	0.00
4	4	0.2051	4.10	0.00
3	1	0.1172	2.34	0.77
2	2	0.0439	0.88	1.42
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20			$\chi^2 = 2.84$

$p = 0.5$, $1-p = 0.5$

10 degrees of freedom (only one degree of freedom is lost, since p was not estimated)

Table 2. Binomial distribution fit for 20 adult male Blattella germanica, no repellent, no treatment, but with the suction fan on; ie. airflow down through the chamber.

N = number of times each insect recorded on the untreated side	O = observed insect frequency	Expected probability $p^N(1-p)^{10-N}$ times the binomial coefficients	E = expected frequency = expected probability times 20	$\frac{(O-E)^2}{E}$
10	0	0.00098	0.02	0.02
9	0	0.0098	0.20	3.20
8	1	0.0439	0.88	0.16
7	1	0.1172	2.34	0.77
6	2	0.2051	4.10	0.88
5	6	0.2500	5.00	0.20
4	4	0.2051	4.10	0.00
3	4	0.1172	2.34	0.05
2	1	0.0439	0.88	0.16
1	0	0.0098	0.20	0.20
0	0	0.00098	0.02	0.02
	20	1.000		$\chi^2 = \underline{5.86}$

p = 0.5, 1-p = 0.5

10 degrees of freedom (only one degree of freedom is lost, since p was not estimated)

Table 3. Binomial distribution fit for 20 adult male Blattella germanica, repellent MGK R-874 present in the lower left layer of the choice chamber floor. Suction fan on, and the insects separated from the repellent by a layer of fibre mesh and a second layer of cloth. Control conditions for the removal of both the liquid and vapour phases of repellent.

treatment combination. The experiment was designed as a 3 x 4 factorial, and analysed by standard analysis of variance procedures. The controls for the experimental design were not included in the main analysis, but treated separately (experiment I) because the analysis of variance presumes a common error variance. In the experimental readings there were two sources of error variation, the binomial variation present in the test chamber readings on each insect (sampling error), and the variation in the response of different insects from the population to the repellent stimulus. An assumption of the analysis is that all measured variables are normally independently distributed. Since the basic readings were binomial, they were transformed by the arcsin $\sqrt{X}$ transformation (Steel and Torrie, 1960), giving data which is approximately normal (see table 4).

A randomization procedure was carried out on the treatment combinations to minimize error, and the design was as follows.

A: repellent phase treatments. a=3.

A liquid repellent only

A vapour repellent only

A liquid plus vapour repellent

B: insect treatments. b=4.

B palps exposed (legs and antennae blocked)

B legs exposed (palps and antennae blocked)

B antennae exposed (legs and palps blocked)

B untreated, all sensory areas exposed.

N = number of times each insect recorded on the untreated side	X = recordings of each insect on untreated side as a proportion N/10	Recordings of each insect on untreated side as %	Index of repellency	$y = \sin^{-1}\sqrt{X}$
10	1.0	100	100	90.0
9	0.9	90	80	71.6
8	0.8	80	60	63.4
7	0.7	70	40	56.8
6	0.6	60	20	50.8
5	0.5	50	0	45.0
4	0.4	40	-20	39.2
3	0.3	30	-40	33.2
2	0.2	20	-60	25.6
1	0.1	10	-80	18.0
0	0.0	0	-100	0.0

Table 4. Arcsin$\sqrt{X}$ transformation. For all statistical procedures, the basic readings which follow a binomial pattern are transformed into angles of equal information. The transformed readings are approximately normally distributed.

R: 20 male roaches used per treatment. r=20.

10 readings taken on each insect, $y = \sin^{-1}\sqrt{X}$

where X = recordings of each insect on untreated side as a proportion.

The gross data and results are summarized in table 5. Since interaction was statistically significant when compared with the error term, the main effects were compared with the interaction, showing that overall only factor A repellent phase was significant. The interaction means that in this experiment the two factors, repellent phase and insect treatment did not act independently of each other; and that for meaningful interpretation of the data, the effect of each treatment must be examined separately; such effects are known as simple effects.

Before going on to the simple effects, the nature of the interaction was examined to see if its meaning could be understood in terms of the experiment. An interaction can be expressed as a function of the regression characteristics of the treatment means. Tukey (1959) has dealt with this type of problem and devised an approach, even though the levels of each factor are not orthogonal. If the treatment means for each level of a factor are averaged over all levels of the other factor, factor level means are obtained, ($\bar{A}$ and $\bar{B}$ in table 6). These are estimates of proportions, and for ease of calculation were transferred into deviations from the overall treatment mean, giving the x_A and x_B values

	A_1 liquid repellent	A_2 vapour repellent	A_3 liquid and vapour repellent
B_1 palps only exposed	$\sum y$ = 929.0 $\sum y$ = 46494.64	968.4 51035.12	993.6 55583.24
B_2 legs only exposed	980.0 53073.04	1090.0 63864.88	1172.0 72020.24
B_3 antennae only exposed	1067.0 60857.56	1243.6 80918.32	1395.8 99569.96
B_4 legs, palps and antennae exposed (untreated)	1036.0 56448.20	1098.6 61180.56	1581.2 127078.16

Source	degrees of freedom	sums of squares	mean square	F
Treatments	(ab-1) = 11	(20591.5)		
A	a-1 = 2	8246.8	4123.4	5.63*
B	b-1 = 3	7949.3	2650.0	3.62
AB	(a-1) (b-1) = 6	4395.4	732.6	3.99*
Error	ab(r-1) = 228	41909.2	183.8	
Total	rab-1 = 239	62500.7		

*significant at 0.05 probability level.

Table 5. Analysis of variance table for experiment II, and a summary of the results. Values shown are based on transformed data. Raw data are presented in appendix A.

	A_1	A_2	A_3	$\bar{B}$	x_B
B_1	46.46 (52.55)	48.42 (55.95)	49.68 (58.13)	48.19 (55.56)	-8.29
B_2	49.00 (56.96)	54.50 (66.28)	58.60 (72.86)	54.03 (65.50)	-2.45
B_3	53.35 (64.36)	62.18 (78.21)	69.79 (88.06)	61.77 (77.63)	5.29
B_4	51.80 (61.75)	54.93 (66.98)	79.06 (96.40)	61.93 (77.86)	5.45
$\bar{A}$	50.15 (58.94)	55.01 (67.12)	64.28 (81.17)	56.48 (69.50) overall mean	
x_A	-6.33	-1.47	7.80		

Table 6. Transformed treatment means (denoted as $\bar{y}$) for 3 levels of repellent factor, A_1 liquid, A_2 vapour, A_3 liquid plus vapour; and 4 levels of sense organ treatment, B_1 palps only, B_2 legs only, B_3 antennae only, B_4 all sense organs exposed. Average effects of a factor at each level of the other factor are shown under $\bar{A}$ and $\bar{B}$, x_A and x_B are the deviations of $\bar{A}$ and $\bar{B}$ from the overall mean 56.48. Untransformed values for these means ie. % of insects on the untreated side are given in brackets.

in table 6. The experimental treatment means are denoted as $\bar{y}$ values. Using the x values as the basis for linear regression equations, theoretical sums of squares can be calculated for the linear regression of A on B, B on A, and for the A-linear B-linear interaction, which is a measure of the extent to which the two regressions are not additive but multiplicative. The A-linear B-linear sum of squares comes to 2853.8, which is significant. A multiple regression equation based on the linear additive and linear multiplicative sums of squares was estimated as:

$$\hat{y} = x_A + x_B + 0.1x_Ax_B + 56.48$$

(all figures in the transformed range). The $\hat{y}$'s are estimates of the treatment means $\bar{y}$ (see table 7). Table 7 also shows the residues $(\bar{y} - \hat{y})$ of the treatment means not attributable to linear additive and multiplicative regression. These residues would include any effect due a particular association between two specific levels of the main factors, such as between the vapour phase of repellent and the antennae. These residues are all non-significant, both individually and collectively. This indicates that there is no significant correlation between any particular group of sense organs and any particular phase of repellent.

The simple effects are a measure of the effect of each level of each factor examined separately over all levels of the other factor. Table 8 shows these effects. Factor A, repellent phase had a significant effect when the test

		A_1 liquid repellent	A_2 vapour repellent	A_3 liquid and vapour repellent	x_B
B_1 palps only exposed	$\hat{y}$	47.11	47.94	50.01	-8.29
	$\bar{y}-\hat{y}$	-0.65	0.48	-0.33	
B_2 legs only exposed	$\hat{y}$	49.16	52.92	59.92	-2.45
	$\bar{y}-\hat{y}$	-0.16	1.58	-1.32	
B_3 antennae only exposed	$\hat{y}$	52.09	59.52	73.70	5.29
	$\bar{y}-\hat{y}$	1.26	2.66	-3.91	
B_4 untreated	$\hat{y}$	52.15	59.66	73.98	5.45
	$\bar{y}-\hat{y}$	-0.35	-4.73	5.08	
	x_A	-6.33	-1.47	7.80	

Table 7. Estimates ($\hat{y}$) of the transformed experimental means ($\bar{y}$, see table 6), based on the multiple regression equation $\hat{y} = x_A + x_B + 0.1 x_A x_B + 56.48$. This equation was estimated from the experimental sums of squares. The non-significant residues ($\bar{y}-\hat{y}$) include the contributions due to any particular association between a repellent phase A, and an insect treatment B. The term $0.1 x_A x_B$ accounts for most of the significant interaction noted in the main analysis (table 5). The increase in the repellent effect due to insect treatment is greater if accompanied by an increase in the repellent effect due to repellent phase.

Source	degrees of freedom	sums of squares	mean square	F
A in B_1	2	105.3	52.7	0.29
A in B_2	2	928.2	464.1	2.53
A in B_3	2	2707.8	1353.9	7.37*
A in B_4	2	8901.1	4450.5	24.21*
A + AB	8	12642.4		
B in A_1	3	558.1	186.0	1.01
B in A_2	3	1902.1	634.0	3.45*
B in A_3	3	9883.9	3294.6	17.92*
B + AB	9	12344.1		
Error			183.8	

*significant at 0.05 level.

Table 8. Simple treatment effects. Figures in the transformed range. The effect of differing repellent phases A is significant for B_3 (insects with the antennae exposed) and B_4 (untreated insects). The effect of the differing insect treatments B is significant for A_2 (exposure to repellent vapour) and A_3 (liquid plus vapour together).

insects were untreated or had their antennae exposed. The repellent phase was not significant when the insects used had only the legs or palps exposed. Factor B, insect treatment, had a significant effect when the insects were exposed to the vapour phase of repellent or to both phases together. The insect treatment was not significant when the insects were exposed to liquid alone. In table 9, the treatment means are arranged in order of magnitude and classified according to levels of significance, based on Duncan's multiple range test.

In addition to the main analysis, the simple effects of repellent phase were analysed separately, including the control from table 1, (table 10). Duncan's multiple range test was also applied to these treatment means.

The significant differences between the treatment means for a factor at fixed levels of the other factor, based on Duncan's test, are summarized in table 11. This completes the analysis. The conclusions are as follows.

The effect of different repellent phases.

(1) With untreated insects, the shown repellent effect was greatest for both phases of repellent together. There was no significant difference between the effect produced by liquid repellent alone and vapour repellent alone, but all repellent treatments caused a significantly greater effect than the control without repellent.

Treatment	average % insects on untreated side	transformed treatment means	significance levels
A_3 B_4	96.40	79.06	(1)
A_3 B_3	88.06	69.79	(2)
A_2 B_3	78.21	62.18	(2) (3)
A_3 B_2	72.86	58.60	(3) (4)
A_2 B_4	66.98	54.93	(3) (4) (5)
A_2 B_2	66.28	54.50	(3) (4) (5)
A_1 B_3	64.36	53.35	(3) (4) (5)
A_1 B_4	61.75	51.80	(4) (5)
A_3 B_1	58.13	49.68	(4) (5)
A_1 B_2	56.96	49.00	(5)
A_2 B_1	55.95	48.42	(5)
A_1 B_1	52.55	46.46	(5)

Table 9. Significance levels for the 12 treatment means:

A_1 liquid repellent

A_2 vapour repellent

A_3 liquid and vapour repellent

B_1 palps exposed

B_2 legs exposed

B_3 antennae exposed

B_4 untreated insects

Treatments which are not significantly different from each other have the same number opposite.

	mean % of insects on untreated side	$\bar{y}$ transformed treatment means	Σy	Σy^2
A_0 no repellent	49.48	44.70	894.0	41489.12
A_1 liquid repellent	61.75	51.80	1036.0	56448.20
A_2 vapour repellent	66.98	54.93	1098.6	61180.56
A_3 liquid and vapour repellent	96.40	79.06	1581.2	127078.16

Source	degrees of freedom	sums of squares	mean square	F
repellent phase treatments	3	13354.2	4451.4	46.9*
error	76	7210.6	94.9	
	79	20564.8		

*significant at 0.05 probability level.

Table 10. Analysis of variance for untreated insects. Repellent phase treatments are the same as in the main analysis in experiment II, but include also the no repellent control.

Treatment	average % insects on untreated side	transformed treatment means	significance levels			
A_3 B_4	96.40	79.40	(1)			
A_2 B_4	66.98	54.93		(2)		
A_1 B_4	61.75	51.80		(2)		
A_0 B_4	49.48	44.70			(3)	
4^0 repellent phase treatments, untreated insects						
A_3 B_3	88.06	69.79	(1)			
A_2 B_3	78.21	62.18	(1)	(2)		
A_1 B_3	64.36	53.35		(2)		
3^1 repellent phase treatments, insects with antennae exposed						
A_3 B_4	96.40	79.06	(1)			
A_3 B_3	88.06	69.79		(2)		
A_3 B_2	72.86	58.60			(3)	
A_3 B_1	58.13	49.68				(4)
4^3 insect treatments, liquid and vapour repellent present						
A_2 B_3	78.21	62.18	(1)			
A_2 B_4	66.98	54.93	(1)			
A_2 B_4	66.28	54.50	(1)			
A_2 B_2	55.95	48.42		(2)		
4^2 insect treatments, vapour repellent only present						

Table 11. Duncan's multiple range significance levels for all significant simple effects from the main analysis (table 8) and the separate analysis with control (table 10). Treatments which are not significantly different from each other share the same number opposite.

factor A, repellent phases	factor B, insect treatment
A_0 no repellent	B_1 palps active
A_1 liquid	B_2 legs active
A_2 vapour	B_3 antennae active
A_3 liquid and vapour	B_4 all groups active

(2) For insects with only the antennae exposed and the legs and palps covered, the effect of both repellent phases together was significantly greater than for liquid alone, but not than for vapour alone. There was no significant difference between the effect of the liquid and vapour phases of repellent.

(3) For insects with either the legs only exposed or palps only exposed, differences in repellent phase treatment had no significant effect.

The effect of insect treatment (blocking groups of sense organs with nail varnish).

(4) With both phases of repellent present together, all 4 insect treatments were significantly different from each other. In order of descending repellent effect, they were: untreated insects; antennae exposed; legs exposed; palps exposed.

(5) With repellent vapour only present, insects with the palps only exposed were significantly less repelled than insects with legs, antennae, or all sense organs exposed. There was no significant difference between these last 3 treatments.

(6) With repellent liquid only present, there was no significant difference shown due to insect treatment.

Some answers may be made to the questions posed on page 59. The removal by the experimental apparatus of either the liquid or vapour phase of repellent did reduce the repellent

effect, but still left it greater than the control. The vapour phase seemed more effective than the liquid, but in no case could this be declared significant. The legs and antennae were both shown to be capable of responding to repellent, but the palps were not. The response produced by the antennae was greater than that shown by the legs, although this was only significant with both phases of repellent present. No qualitative differences could be shown between the responses of the various groups of sense organs to the two phases of repellent. All observed differences could be explained in quantitative terms; ie. as the repellent effect connected with repellent phase increased from liquid to vapour to both phases, the importance of the sensitivity of the sense organ groups was increased geometrically as well as arithmetically. This fits well with the simple morphological observation that there are more sense organs on the antennae of German roaches than on the legs, and more on the legs than on the palps. It also indicates that there is little qualitative difference between these groups of receptors. If there are separate receptors involved in the perception of repellent vapours and repellent liquids, they do not seem to be confined to separate areas.

4. EXPERIMENTAL - ELECTROPHYSIOLOGY

4.1 Introduction

Although behavioural studies remain the most important methods in evaluating insect repellents, and in finding out the ways repellents act on insects, such experiments are essentially subject to the physiological state and individual variation of the insects used. Statistical comparisons can be made when a batch of insects is tested under identical conditions for the presence or absence of a single given stimulus. It is not possible in practice to exclude all other stimuli. This can result in complicated interactions, since any behavioural response other than the simple reflex arc is the outcome of a complex processing in the brain. The tests in section 3. provide circumstantial evidence of the ways in which the various chemoreceptor groups respond to insect repellents.

Electrophysiology provides a more objective approach. If probes are placed on the sensory nerves, electrical nerve potentials recorded with suitable equipment may be regarded as basic sensory information passing to the brain. By comparing such nerve responses after stimulation of the sense organs with attractant and repellent chemicals, to the resting potentials obtained in the absence of known stimuli, one is decoding raw information put out by the sensory receptors before it has been interpreted by the brain. Any obvious and consistant differences observed in these nerve responses

can be interpreted to give direct evidence of the sites of action of insect repellents. By comparing the nerve responses to other stimuli such as mechanical ones, with the responses to a combination of repellent and mechanical stimulation, one can also show whether repellents interfere with the reception of other stimuli at the sites of action or whether they act independently at these sites. Should the various stimuli act independently at the receptor sites, any effect by the repellent on the normal responses to other stimuli is the result of interaction in the brain.

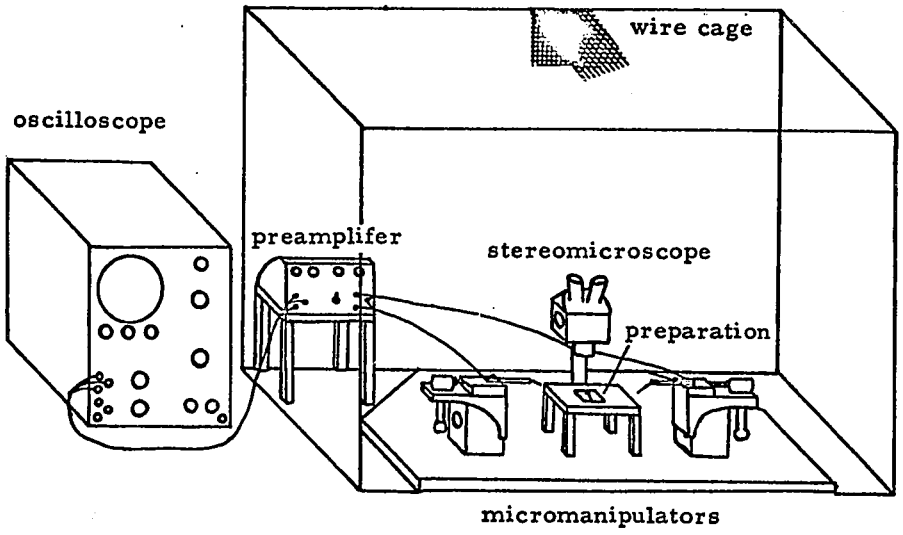
No really satisfactory method has been devised for recording the electrical responses of single sense organs being stimulated by vapours. Some success was achieved by Morita and Yamashita (1961) but the method used is quite inexact when compared with Hodgson's (1957) method for recording the electrical responses from labial chemosensory hairs to water soluble chemical stimuli. General antennal preparations have been used by a great number of authors to characterize odour responses (Boistel, Lecompte and Coraboeuf, 1953; Roys, 1954; Smith and Roys, 1955; Schneider, 1957b; Roessler, 1961; Schneider and Boeckh, 1962; Boeckh, 1962; Schneider, Lacher and Kaissling, 1964; Lacher, 1964; Schneider, Block, Boeckh and Priesner, 1967). Probes placed generally in the antennae of insects and recording from the antennal nerve do not record potential spikes of the frequency that would be expected from a single nerve fibre, but

record overall potential changes resulting from the stimulation of a large number of sensory neurons. Such a slow overall change in electrical potential as a result of olfactory stimulation has been christened by Schneider (1957b) an electroantennogram. It is such a preparation that I proposed to use to compare the antennal responses of cockroaches to various attractants and repellents, in the vapour phase and in the liquid phase. I also proposed to use a similar preparation for the cockroach leg so that the results would be comparable with those obtained from the antennae.

4.2 Material and methods

The recording apparatus (figure 12): all recording equipment except the oscilloscope and camera was housed in a wire mesh cage to reduce extraneous induced interference, particularly 60 cycle mains noise. All metal apparatus was grounded to a steel base plate beneath the preparation, and this in turn was grounded by a single heavy copper wire to a cold water pipe. If a single ground is not used, ground eddy currents can result.

Electrodes: the electrodes used in most experiments were tungsten wire. The wire was sharpened to a point by dipping in molten sodium nitrite and then polished electrolytically after a method described by Hubel (1957). Since tungsten does not solder easily, the tungsten needles produced were crimped in the evacuated cores of short lengths of solder wire and the lead wires were crimped in the other end of the solder. These quickly replaceable electrodes could then be tied to insulated rods fixed in the micromanipulator clamps. The tungsten needles were insulated to the tip by dipping in Insulex, a vinyl lacquer (Donaldson, 1958). The impedance of tungsten electrodes is quite high, depending on the size of the uninsulated tip, but decreases as the frequency of an applied varying potential increases (Donaldson, 1958). This impedance variation in tungsten and other polarizable electrodes makes them unsuitable for the measurement of static potentials such as the cell membrane



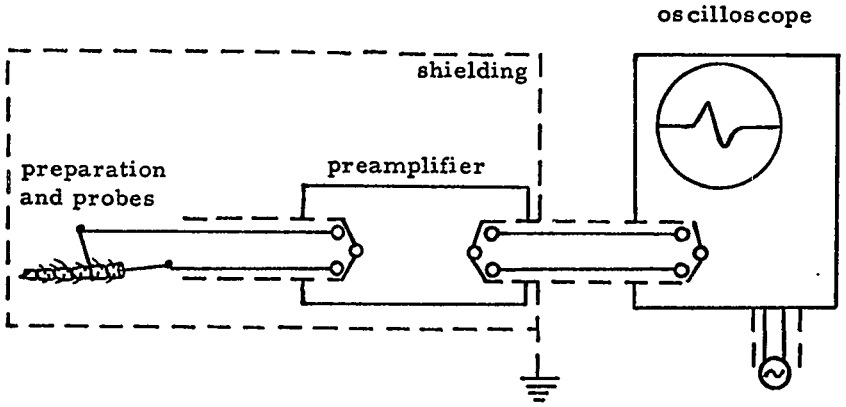


Figure 12. Sketch of the electrophysiological recording apparatus, and a block diagram of the circuit involved.

resting potential, but they are quite suitable for comparative studies of nerve action potentials, providing two identical electrodes are used. They have the advantage of being mechanically robust compared with the fragile glass micro-pipettes of the non-polarizable silver - silver chloride electrodes. Such standard silver - silver chloride electrodes (Donaldson, 1958) were also used in a few preparations to check the results obtained with the tungsten electrodes. These were quite comparable, both tungsten and glass electrodes had an impedance of one to two megohms.

The Preamplifier: shielded leads from the electrodes were connected to the input (push-pull) stage of a battery operated Grass p-8 d-c preamplifier. The intrinsic noise level of this model is rated at 20 microvolts at the maximum amplification of 2000, and is a little greater than this in practice. This means that any detectable spike must be greater than 30 microvolts. High sensitivity of this order is necessary for a number of reasons. Although the action potential across the membrane of a single nerve fibre is of the order of 80 millivolts (Hodgkin, 1951), the full potential can only be detected by electrodes placed directly on the membrane, and the recorded potential drops rapidly as the electrode distance from the nerve fibre increases. This drop in potential is most often the case with recordings from whole nerves or bundles of fibres, as in these experiments. Types of response are identified more by frequency

than by amplitude. Spike amplitudes can only be considered when the same preparation is being used undisturbed. Another factor reducing the observed potential in these experiments is the high impedance of the probes (1 or 2 megohms) as compared to the input impedance of the preamplifier (10 megohms). The recorded potential only approaches the electrode potential if the electrode impedance is much lower than the input impedance of the amplifiers since:

$$\text{recorded potential} = \frac{(\text{actual potential})(\text{amplifier impedance})}{\text{electrode impedance} + \text{amplifier impedance}}$$

This was not the case in the set up used, and the recorded potentials were therefore much lower than the actual potentials.

The oscilloscope: the output leads from the preamplifier were connected to the d-c difference input terminals of a Tektronix 502 dual-beam oscilloscope. With these connections, the oscilloscope records only the difference in absolute potential between the two input leads and potential fluctuations affecting both probes are not registered. The lower beam of the oscilloscope was not used, since a time base could be obtained from the grid lines on the screen and the time base on the oscilloscope (accurate to 3%).

Permanent recordings were made with a Polaroid Land Camera fixed on the bezel mount flange of the screen. High contrast positive transparency film was used. The single shot nature of the camera and the cost of film severely

limited the number of recordings that could be made. Most of the results are based on written notes taken during visual observations of the screen.

Insect preparations: American roaches Periplaneta americana Linnaeus were used for this work because their size made them convenient for operation. They were readily available from laboratory cultures, and their nervous anatomy is well known.

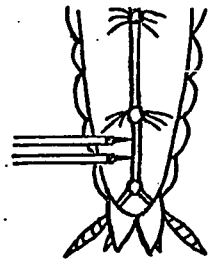
Probes were placed with the aid of two Leitz micromanipulator units mounted on a firm cast steel base. The operation was observed through a Zeiss binocular stereomicroscope. Four basic insect preparations were used:

Cercal preparation (figure 13a).

Decapitated roaches were dissected from the dorsal side, revealing the ventral nerve cord. Probes were placed under the ventral nerve cord about 1 mm apart and the cord lifted slightly off the underlying tissue. The abdominal cavity was then filled with mineral oil, which prevented desiccation as well as stimulation of the cord itself due to the presence of repellent vapour (Roys, 1954).

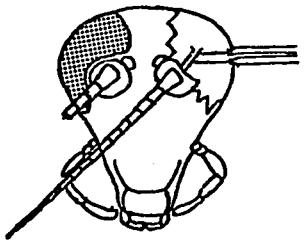
Leg preparation (figure 13b).

The decapitated cockroach was dissected in the coxal region of the foreleg. Two probes were placed on the main nerve leading from the leg, and the exposed region covered in mineral oil.

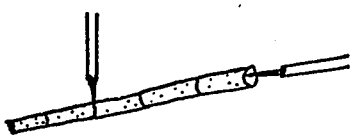


a. cercal preparation, probes on the ventral nerve cord.

b. leg preparation, probes on the main nerve in the coxa.



c. complete antennal preparation, probes on the intact antennal nerve.

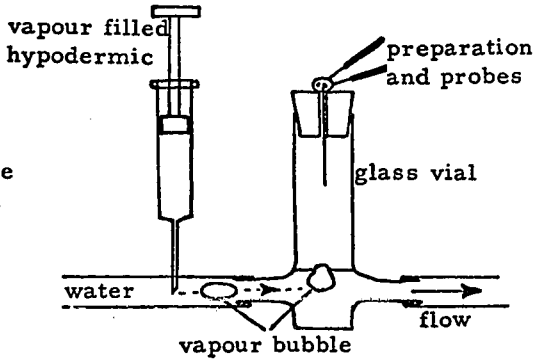


d. detached antennal preparation, indifferent probe in the cut end of the antenna, different probe between two segments.

e. eye dropper for stimulating the cerci with puffs of clean air, and with puffs of repellent vapour.

f. glass rod for applying vapour stimulation by dipping in liquid chemicals and holding near the preparation.

g. brush for painting liquids on the preparation.



h. apparatus for delivering a bubble of vapour to a leg or antennal preparation without much mechanical disturbance.

Figure 13. Insect preparations and probe placements (a, b, c, d). Methods of stimulus application (e, f, g, h).

Complete antennal preparation (figure 13c).

The head of the roach was removed from the body and dissected in the eye region to reveal the antennal nerve. Two probes were placed on the nerve and the exposed preparation was covered with mineral oil. Since, in this preparation, the brain was still intact and connected to the antennal nerve, the recordings were apt to be confused with signals going from the brain through the motor neurons.

Detached antennal preparation (figure 13d).

A cockroach antenna was cut off near the base and a reference electrode inserted well into the antenna lumen. Fluid soon congealed in the space between the electrode and the antennal walls, preventing desiccation of the interior of the antenna. A very fine recording electrode was inserted at a joint in the antenna, usually between segments 5 and 6 (Roys, 1954). Beyond the first antennal segments, there are no muscles in a cockroach antenna, and any signals received from this type of preparation can safely be said to be of sensory origin.

Four methods of stimulation were used:

Bursts of electrical activity in the ventral nerve cord of a cockroach are produced when the cerci are stimulated with a puff of clean air from an eye dropper (figure 11e). This is mechanical stimulation. A similar eye dropper could be filled with a repellent or attractant vapour and any of the preparations could be subjected to a puff of treated air.

Vapour stimulation without the accompanying puff was achieved by merely holding a glass rod (figure 13f) which had been dipped in the liquid repellent or attractant, close to the preparation.

Repellents were applied in liquid form with a squirrel hair paint brush (figure 13g).

The most common method of delivering a vapour stimulus to electrophysiological preparations is by adding the chemical stimulus to a continuous stream of vapour blown over the preparation. This tends to produce mechanical vibrations of the preparation which are transduced into electrical pulses (Schneider, 1957a). A more gentle method of delivering a vapour stimulus was devised (figure 13h). The leg or antennal preparation was set up on a cork stopper in a glass vial so that the leg or antenna protruded through a hole in the stopper into the lumen of the vial. Two pieces of glass tubing were welded into the bottom of the vial and rubber hose attached to these. A slow water flow was maintained through these tubes across the bottom of the vial. A hypodermic syringe could be filled with saturated chemical vapour, and a bubble of known volume injected into the rubber tubing. The bubble would travel slowly along the tubing and pop up into the vial exposing the preparation to whatever vapour the bubble contained, without causing any violent mechanical artifacts. This preparation was only good for vapours insoluble in water, and for single

stimulations or combined effects, since there was no way of removing the vapour once it had been delivered.

4.3 Results

Cercal preparation.

Mechanical stimulation of cockroach cerci either by a puff of air or by touching with a needle produces easily recorded electrical activity in the ventral nerve cord. This electrical activity takes the form of spikes similar to those seen in figure 14. Spikes of various amplitudes may be present and in general it can be said that spikes of different amplitudes represent recordings from different nerve fibres. Since the action potential of all nerve tissue is about 80 millivolts (Hodgkin, 1951) two main factors are responsible for different amplitudes being recorded. These are: the distance of the probes from the various nerve fibres, since recorded potential drops rapidly as the distance of the probe from the neuron membrane increases; the size of the neuron in question, since although the action potential of all neurons is similar the current is not, and a large current from a large fibre records as a higher potential than a small current from a small fibre, because there is less drain by the recording apparatus (section 4.2). The frequency of the recorded spikes from the cercal preparation depends on the intensity of the stimulation, the greater the stimulation the greater the frequency. This relationship between stimulus intensity and action potential frequency is a basic law of sensory physiology. Action potentials recorded from the cercal

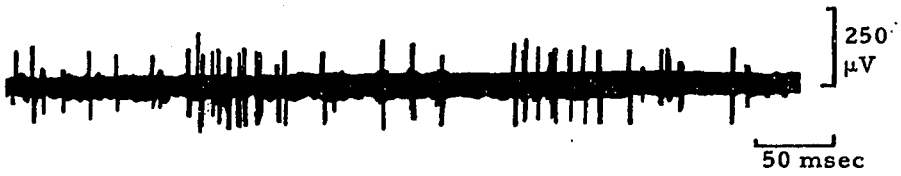


Figure 14. Complete antennal preparation. Nerve-muscle potentials whose presence makes this type of preparation unsuitable for investigations into chemosensory responses. Note the frequency of the responses from the time base.

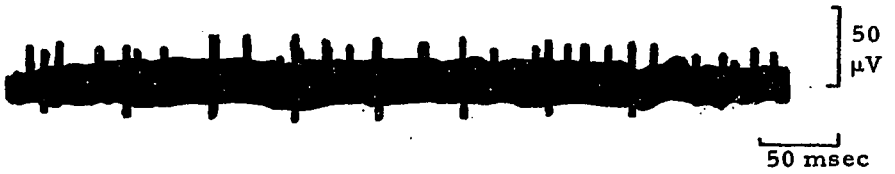


Figure 15. Detached antennal preparation. Mechano-vibrations recorded from an isolated antenna in still clean air. These weak potentials were the only noted steady state responses from the preparation. The baseline of about 20μV shows the limit of amplification of the recording apparatus.

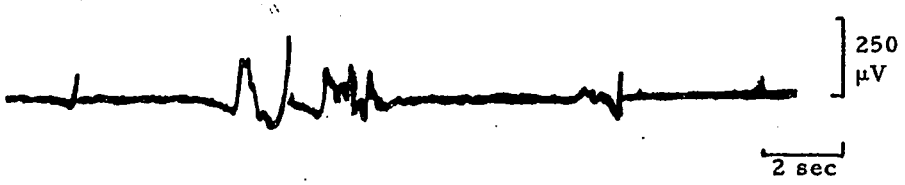


Figure 16. Detached antennal preparation. Electroantennogram from an antenna stimulated with an attractant, banana vapour. Stimulus applied by holding a glass rod which had been dipped in crushed banana, near the preparation. Note the time base, this is a very slow-changing potential.

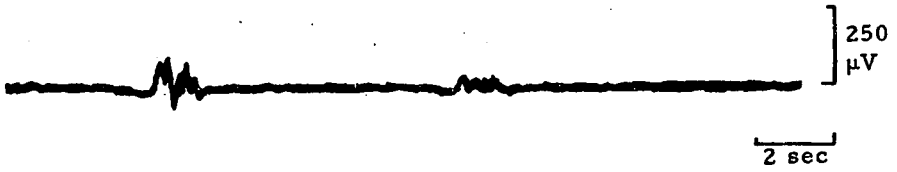


Figure 17. Detached antennal preparation. Responses to stimulation with banana vapour (left) and repellent vapour (right), 5 minutes after exposing the preparation to repellent vapour for 1 minute. The repellent was MGK R-874, and the stimuli were applied with glass rods. The decreased amplitude of the attractant response (cf. figure 16) shows the very slow recovery of the preparation from the effects of repellents.

preparation of a cockroach to a burst of stimulation such as a puff of air records as bursts of high frequency spikes which gradually slow down to an occasional spike, the normal resting activity of the nerve. No difference in the amplitude or frequency of the recordings was observed between a cercal preparation stimulated with clean air and the same preparation stimulated by a puff of air containing any chemical whatsoever, repellent or attractant. Furthermore, the mechanical response was not affected by painting the cerci with liquid repellent. It should be noted here again that the ventral nerve cord was covered with mineral oil to prevent it being directly affected by chemical vapours. Refined mineral oil itself does not appear to affect the preparation in any way other than to increase its longevity by preventing desiccation. Repellents used were MGK R-874, dimethyl phthalate and diethyl toluamide; other chemicals used included benzene, toluene, and ether; the attractant used was banana vapour. Ripe bananas produce a vapour extremely attractive to cockroaches, although the active ingredients have not been determined.

Antennal preparations.

Antennal preparations, where the antennal nerve was still attached to the brain, produced spikes which could be associated with the antennal muscles (figure 14). These spikes were of a frequency range normally associated with nerve - muscle preparations, about 100 cycles per second.

These unwanted signals disappeared in preparations where the antenna was removed from the head, and the only recordings obtained from such preparations in still clean air, were from weak mechanical vibrations (figure 15). These mechano-vibrations just showed above the 20 microvolt limit of amplification of the apparatus. Neither the muscle potentials nor the mechano-vibrations could be confused with the 40 times slower potentials of the electroantennograms. Slow potential changes of the electroantennogram are thought to be the summed potentials coming from the many receptors on the antenna. The cockroach antenna responded quite violently to stimulation by banana vapour (figure 16). The response to repellent vapours was much less marked (figure 17 right) and closely resembled in amplitude the continuous stimulation produced when the antenna was painted with liquid repellent (figure 18). Breaks appeared in the response to continuous repellent application (figure 18). Such breaks were also noted by Roys (1954). After stimulation with repellent vapour the response of the antenna to stimulation by attractant vapour was considerably reduced for several minutes (figure 17). Full recovery was effected after about 20 to 30 minutes. Benzene, toluene and ether vapours acted very similarly to the vapours of repellents such as MGK R-874, diethyl toluamide, and dimethyl phthalate (figure 19).

Figure 18. Detached antennal preparation. Response to liquid repellent (874) painted on the antenna. The amplitude of the response is similar to that obtained from repellent vapour (figure 17). The breaks seen in the response to continuous stimulation were noted by Roys (1954).

Figure 19. Detached antennal preparation. Response to benzene vapour delivered by the bubble apparatus. Allowing for the greater scale of this recording compared with that of figures 16, 17 and 18, this response is similar to that produced by insect repellents.

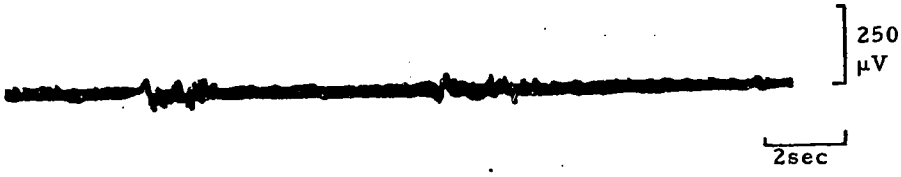


Figure 20. Leg preparation. Response to repellent vapour, dimethyl phthalate. Two separate stimulations from a treated glass rod. The leg is not quite as sensitive as the antenna, but the form of the response is very similar.

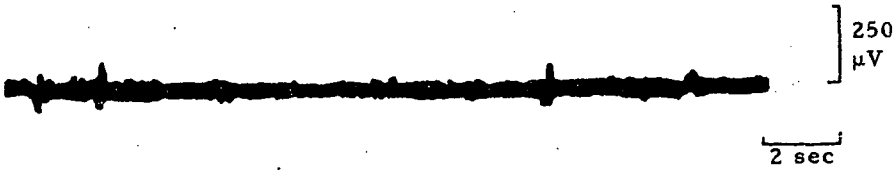


Figure 21. Leg preparation. Response to painting with liquid DMP 90 minutes after the onset of stimulation. The breaks between the bursts of activity are much longer now, and the response is very ragged but still recognizable.

Leg preparations.

The cockroach leg did not respond to the attractant vapour at all. The leg did respond to repellent vapours in very much the same way as the antenna (figure 17 right). Repellent liquids painted on the leg produced the same sort of response as vapours except that the duration of response was much greater. I could detect no difference in the response of the legs to stimulation by liquid repellents from the response of the antennae (figure 18). The presence of repellent liquid on the leg caused stimulation for a great length of time, breaks in the stimulation gradually becoming longer and longer. However, activity was still noted even after an hour and a half (figure 18). Benzene, toluene, and ether all acted similarly to repellents when applied to the leg in either liquid or vapour form although the response, particularly to ether, was slightly more pronounced.

4.4 Electrophysiological responses of Blattella germanica to MGK R-874

Although most of the electrophysiological experiments were done using Periplaneta americana because of its large size, some tests were carried out with the German roach so that the results could be compared with the behavioural findings presented in section 3.4. More sophisticated apparatus was available for the Blattella work than that used with Periplaneta, but the methods and techniques used were basically the same. Silver - silver chloride electrodes were used, the reference electrode being placed generally in the body of the roach and the recording electrode placed by micromanipulator on the desired nerve via a fluid - filled microcapillary. The signals went through a Medistor A-35 electrometer amplifier (single - sided input) and a Tektronix Type 122 amplifier to a Tektronix 502 oscilloscope. Permanent records were taken with a Grass C4 camera, which inverts the traces (positive is down in figures 22 - 29). The apparatus was capable of making continuous recordings of greater sensitivity than the apparatus described in section 4.2. The nerve activity recorded ranged from 8 - 1000 Hz, in contrast to the slow d-c shifts described in section 4.3.

Adult male German roaches were used for all these experiments. The insects were between 3 and 10 days old and had been reared at 23 C. Each specimen was anaesthet-

ized with carbon dioxide, attached to a wax coated slide and allowed to recover for 30 minutes. The probes were placed in the roach and the recordings made in the dark. The repellent stimuli, both vapour and liquid were applied by means of a fine glass rod which had been dipped in MGK R-874. A flashlight was used during stimulus application, since the bench light caused 60 cycle interference. The light itself did not appear to affect the preparation.

Cercal preparation.

The recording electrode was placed on one of the two cercal nerves posterior to the last abdominal ganglion, and the exposed preparation covered in mineral oil. Although this preparation responded well to mechanical stimuli, no response was obtained when a glass rod dipped in MGK R-874 was held near the cercus (figure 22). When liquid MGK R-874 was applied to the cercus with a glass rod there was a short initial response to the mechanical stimulation, but no further response to the repellent itself (figure 23).

Antennal preparation.

The recording electrode was placed in the antenna in the region of the sixth antennal segment. When a glass rod dipped in MGK R-874 was held near the antenna, a sharp burst of electrical activity lasting about one second was recorded (figure 24). When the antenna was painted with liquid MGK R-874, activity of similar amplitude and dura-

Figure 22. B. germanica, recording from the left cercal nerve. Lack of response to stimulation by MGK R-874 vapour.

Figure 23. B. germanica, recording from the left cercal nerve. After the slight initial response to mechanical stimulation, no activity was produced by MGK R-874 liquid.

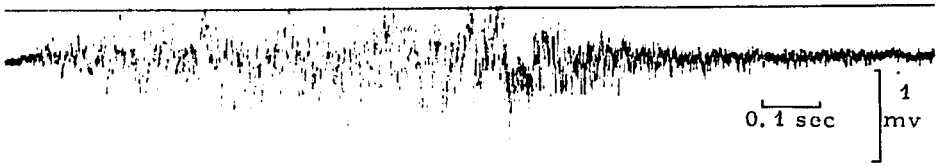


Figure 24. B. germanica, response of the right antennal nerve to stimulation by MGK R-874 vapour.

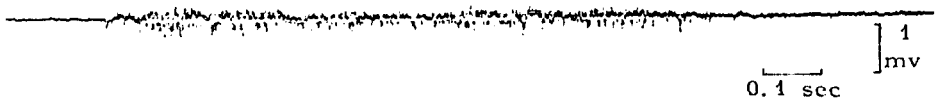


Figure 25. B. germanica, response of the right antennal nerve to stimulation with MGK R-874 liquid. The vertical recording scale is 1/2 that of figure 24, which makes both these responses of the same order of amplitude. This response was followed by alternating resting periods and further activity.

tion was noted (figure 25). This was followed by a resting period of about 2 seconds, another burst of activity, a resting period of 3 seconds, more activity and so on, with the periods of inactivity getting longer. Similar patterns of activity were observed when the preparation was subjected to continuous stimulation by MGK R-874 vapour. This pattern was also noted by Roys (1954). The antennal responses to MGK R-874 were obtained in 9 out of 10 attempts.

Leg preparation.

With the recording electrode placed in the tibial region of the roach foreleg, responses could only be obtained in 5 out 10 attempts, probably due to the greater difficulty in placing the probe on the nerve. The response of the leg to MGK R-874 vapour was similar to that of the antennae, but of shorter duration (about ½ second, figure 26). The response of the leg to MGK R-874 liquid was ½ second bursts of activity with intermittent resting periods similar to those noted for the antennae (figure 27).

Palp preparation.

The recording electrode was placed in the first segment of a labial palp, and the rest of the mouthparts sealed with wax to prevent undue mechanical activity. No response to MGK R-874 vapour could be obtained (figure 28). In 4 out of 6 attempts the palps responded to MGK R-874 liquid with irregular bursts of activity and resting periods (figure 29). The resting periods were shorter than those

Figure 26. B. germanica, response from the right foreleg after stimulation with MGK R-874 vapour.

Figure 27. B. germanica, response from the right foreleg after stimulation with MGK R-874 liquid. This response was followed by alternating resting periods and further activity.

Figure 28. B. germanica, recording from the left labial palp. Lack of response to stimulation by MGK R-874 vapour.

Figure 29. B. germanica, recording from the left labial palp. Response to MGK R-874 liquid. This response was followed by irregular resting periods and activity.

observed in the legs and antennae.

The electrophysiological experiments with Blattella confirmed the general conclusions reached with Periplaneta. Both the legs and antennae can respond to both the vapour and liquid phases of repellent, which confirms the findings of section 3.4. The type of nervous response was similar for both liquid and vapour with the legs and antennae of Blattella. The bursts of activity produced by the legs were of shorter duration than those produced by the antennae, which I think may be a function of the sensitivity range of the sense organs involved. The situation with the palps is not clear; I could detect a response to liquid MGK R-874 but not to vapour. No statistical significance was obtained in section 3.4 for the ability of the palps of Blattella to detect MGK R-874. It is possible that the small number of chemosensory organs present on the palps are contact chemoreceptors in the sense that they respond only to very high concentrations of chemical stimuli.

4.5 Significance of results

It is not surprising that repellents do not directly affect mechanoreception in insects. Even if the response to an irritant chemical stimulus is a fundamental property of all nervous tissue, that tissue must be exposed to the chemical for any response to be noted. In man insect repellents can be applied to the skin without any discomfort, but if the same materials are applied to the lips, where the cutaneous sense organs (sensitive to touch and heat not smell or taste) lack the protection afforded by the skin on the rest of the body, a sharp tingling or burning sensation is experienced. The sensory nerves of insects' mechanoreceptors are not exposed to the surface (figure 30), but are protected by the insect cuticle which is a good barrier to most chemicals. For chemoreception to be possible, chemicals must have access to sensory tissue, and insect chemoreceptors have pores or openings which make this possible, (Dethier, 1955; Slifer and Sekhon, 1962), (figure 31). Indeed, the chemoreceptors and possibly the hygroreceptors seem to be the only locations on insects where nervous tissue is exposed to stimulation by chemicals, and for this reason are the sites of action of insect repellents.

The similarity in the two species of roach between the responses of the legs and antennae to repellents, in both the liquid and vapour phases, suggests that either both the legs and antennae are liberally endowed with chemosensory neurons

capable of responding to repellents or that all chemosensory neurons are capable of responding to repellents. Since there is a pronounced qualitative difference between the legs and antennae in their response to attractants, the antennae responding very strongly and the legs not at all, we may modify the previous statement. Either both the legs and antennae are liberally endowed with receptors capable of responding to repellents; or all chemosensory tissue is capable of responding to repellents, but the mechanism is different from that whereby insects smell specific attractants or taste the presence of stimulants like sugar (Hodgson, Lettvin and Roeder, 1955). Now the response of the American roach antenna to banana vapour was considerably reduced after the preparation had been exposed to repellents. If the olfactory receptors for attractants are capable of responding to repellents by a separate mechanism, then this reduction in attractant response must be due to nervous adaptation and not to competition at the site of action. On the other hand, if the neurons responsible for the reception of repellent are separate from those responsible for the perception of attractants, they cannot act independently else repellents would not cause a reduction in the attractant response.

The similarity in the electrophysiological responses of insects to both repellents and irritant chemicals such as benzene, toluene and ether is not surprising. Benzene for

instance, is a known insect repellent, but it has no commercial application since it is so volatile. In man this general class of irritant chemical has the same effect as repellents, when applied to the lips. The contact repellent effect of both insect repellent and general irritant chemicals could be readily explained in terms of our own experience, by the common chemical sense, were it not for the fact that insects respond to the vapours of these compounds, whereas we do not. That is not to say we cannot smell these substances, but that they do not repel us.

5. HUMAN RESPONSES TO ODOURS

5.1 Introduction

Since olfaction appears to play such a large part in the mode of action of insect repellents, the fundamental study of repellent action is dependent on knowledge of the basic olfactory processes. Of the theories concerning these processes, the stereochemical theory of Amoore is the currently popular one.

Amoore (1962) and Amoore, Johnston and Rubin, (1964) followed up a suggestion by Moncrieff (1951) that odorous molecules fit into complementory receptor sites in the sensory endings of the olfactory system. If molecules fit the sites, a nerve impulse is initiated. Substances with molecules of the same size and shape will then have the same smell, regardless of chemical composition. A molecular analysis by Amoore of a wide variety of compounds having similar smells showed that they did indeed have similar shapes. Amoore theorized that there were a limited number of primary odour sites into which molecules of primary odour type fit. Large complex molecules may have groupings fitting more than one site, and the resulting complex smell should be duplicable by a suitable admixture of small primary molecules. To identify these sites, Amoore listed the most commonly occurring types of smell mentioned in the literature, along with the chemicals having these smells. He postulated 7 basic classes, with a possible extra 3.

They are:

probable primary odours;

I camphor (aceous)
II pungent
III ethereal
IV floral
V peppermint (y)
VI musky
VII putrid

primary or complex;

VIII almond
IX aromatic
X aniseed

probably complex odours;

XI lemon
XII cedar
XIII garlic
XIV rancid.

Although Amoore's theory seems to fit most of the known facts of olfaction, it is open to criticism on the grounds that the selection of odorous categories (above) presumes that most people are able to consistently ascribe a given adjective to a given smell, and that different people will apply the same adjective to the same smell. A simple test for this was incorporated into an experiment originally designed to test other human reactions to odours.

While giving a departmental seminar, I attempted to illustrate the effect of combining attractive and repellent stimuli by presenting the audience with three vials, one containing an attractive scent (Chanel #5), one a repulsive bee repellent (butyric anhydride) and one a mixture of these

two. My thesis was simply that there is no reduction of the repellent effect when an attractant is presented similtaneously, and that repellents work more or less independently of the presence or absence of attractive stimuli. To my consternation, one member of the audience vehemently proclaimed a dislike for the perfume and a liking for the repellent. Although this did not disqualify the point I was trying to make, merely reversing the presumed classification of the two odours, (he asserted that the noxious perfume ruined the delightful rancid butter smell of the bee repellent), grave doubts were raised about the universality of an understanding of odours. Since the possessor of this eccentric nose was from India, his response could have been the result of environmental conditioning, of racial origin, or merely an example of extreme variation in a total population response.

Subsequently a much larger controlled experiment was designed to test some human responses to odours, including the immediate effect of exposure to insect repellents. This followed a suggestion from Dr. B. Hocking that repellent action may be partly the result of a lowered sensitivity to all chemical stimuli following the exposure of chemoreceptors to repellents. This followed observation of the slow recovery time of repellent treated electrophysiological preparations. The odorous compounds used in this experiment were selected so that some critique of Amoore's hypothesis

could also be made.

5.2 Material and methods

The subjects for the smell experiments were guests of Dr. B. Hocking at two consecutive soirees, and included staff and graduate students from the Department of Entomology, their spouses, fiancees and friends. The sexes were in roughly equal proportions, and there was a large minority from countries other than those of Europe and North America. Each person was tested twice during the evening, once before supper, and once after supper (that is after their senses had been exposed to alcohol, tobacco smoke, and a surfeit of excellent food). Each subject was given a mimeographed sheet (appendix B, page 149) and asked to smell ten numbered vials from a rack of 32, in the order listed on their sheet. Four questions were asked about each vial:

Reaction; pleasant, no reaction, unpleasant.

Strength of smell; no smell (0), weak (1), medium (2), strong (3).

Identification; subjects were asked to identify the smell, if possible.

Comment and describe; subjects were asked to choose descriptive adjectives from a provided list, and invited to comment further if they wished.

The stoppered vials containing the test compounds soaked in cotton wool were covered with numbered labels and presented on a tray in numerical order for ease of selection. The numbers were changed before each session to prevent

recognition by this means. No vial number appeared twice on any sheet, although a subject could often sample the same vial twice during the two sessions, but under a different number. The vial numbers were scrambled randomly except for the vials containing repellents, which appeared first or last by design on all but a few sheets.

Odorous substances used in the tests were obtained from the Department of Chemistry at the University of Alberta, and were selected to represent as many of Amoore's (1962) classifications as possible. The 32 substances were:

			Amoore class	
(a) repellents;	1	dimethyl phthalate		
	2	butyric anhydride		
	3	diethyl toluamide		
(b) 'type' compounds, from which Amoore classes are named;	4	camphor	I	Camphoraceous
	5	'Bellogia' (carnation)	IV	Floral
	6	peppermint oil	V	Pepperminty
	7	almond oil	VIII	Almond
	8	aniseed oil	X	Aniseed
	9	citron oil	XI	Lemon
	10	cedar oil	XII	Cedar
	11	garlic	XIII	Garlic
(c) 'sample' compounds from Amoore classes (Amoore, 1962);	12	cyclohexanol	I	Camphoraceous
	13	acetic acid	II	Pungent
	14	chloroform	III	Ethereal
	15	anisole	IV	Floral
	16	cyclohexanone	V	Pepperminty
	17	piperitone	V	Pepperminty
	18	phenylacetic acid	VI	Musky
	19	trimethylamine	VII	Putrid
	20	benzaldehyde	VIII	Almond
	21	chlorobenzene	IX	Aromatic
	22	quinolene	X	Aniseed
	23	limonene	XI	Lemon
	24	valeric acid	XIV	Rancid

(d)	25	triethylamine
miscel-	26	D-carvone
laneous;	27	'Lotus' (perfume)
	28	'Vert-vert' (perfume)
	29	human sweat (from foot)
(e)	30	water
controls;	31	water
	32	water

Each substance was analyzed separately for the following:

Overall reaction (pleasant, no reaction, unpleasant).

The expected ratio by the null hypothesis being 1:1:1. The observed ratio here is the expected ratio for the reaction sub-categories.

Strength of smell.

The overall strength and the mean (scale 0-3) provided another variable response.

Reaction and strength.

The following sub-categories of the reaction and strength figures were tested for significant deviations from the expected ratio (see appendix B).

Repellents first against repellents after: this test was built into the experimental set up and was the prime reason for the experiment (see page 106).

Sex male against sex female.

White Caucasian against other race groups: this regrettably crude ethnic or cultural sub-division was the only one possible with the limited number of subjects present. The reasons for its inclusion are mentioned on page 106.

Before supper against after supper (see page 108).

Identification.

The ability of the subjects to identify the test substances is given as a measure of olfactory 'education'.

Adjective selection.

Subjects were asked to choose up to three descriptive adjectives and were provided with a list of 22 to choose from. This list was compiled from the 14 odour classifications of Amoore, the 6 of Henning (1915) (translated from the German) and the 4 of Crocker and Henderson (1927). Two of these adjectives appear in both Amoore's and Henning's lists. Since Amoore's categories are based on the predominance in the literature of his listed descriptive adjectives then the adjective selection in the experiment should show a favouring for Amoore's adjectives, otherwise the very basis of Amoore's categories is suspect.

Amoore_class_selection.

Where applicable, that is for those compounds representing one of Amoore's 14 listed classes, the number of subjects choosing the correct adjective is given.

5.3 Results

The complete results are given in appendix B. In addition to the figures for each individual compound, figures are given for groups of compounds (pages 151 - 157), they are:

(a) compounds no. 1-3, repellents.

(b) compounds no. 4-11, Amoore 'types', (see page 109).

(c) compounds no. 12-24, Amoore 'samples', (see page 109).

(b + c) compounds no. 4-24, all compounds with a known Amoore classification.

(d) compounds no. 25-29, miscellaneous, (see page 110).

(e) compounds no. 30-32, controls (water).

(a + b + c + d + e) compounds no. 1-32, all compounds tested.

Very few statistically significant results were obtained for the sub-categories of reaction and strength. Those results which were significant are marked with an asterisk in appendix B and are as follows:

Reaction (pleasant, no reaction, unpleasant).

A gradually accumulated difference in reaction between white Caucasians and others was noted in groups (b + c), all compounds with a known Amoore classification, and groups (a + b + c + d + e), all compounds tested. Overall, white Caucasians marked more compounds as pleasant than did other race groups. This gradually accumulated difference in reaction showed significance for one compound alone, no. 8,

aniseed oil.

Strength (scale 0-3).

Males marked all three repellents, group (a) compounds no. 1-3 stronger than did females, significantly so for compound no. 2 (butyric anhydride) and compound no. 3 (diethyl toluamide).

Compound no. 2 (butyric anhydride) was marked as significantly stronger when a repellent was smelled first than when a repellent was not smelled first. It should be pointed out here that owing to the design of the experiment, the repellent smelled first here would be in most cases butyric anhydride itself. This type of relationship applied to all three repellents but not to any of the other compounds, where 'repellent first' included all three repellents in approximately equal ratio.

Compound no. 30, water was marked significantly stronger after repellent than before repellent. The numbers involved here are very small, and cannot be used with confidence for the Chi^2 test.

White Caucasians marked group (b), Amoore 'types'; group (c) Amoore 'samples'; group (b + c), all compounds with a known Amoore class; and group (a + b + c + d + e), all compounds tested; as significantly stronger in smell than did other races.

Compound no. 12 (cyclohexanol) was marked significantly stronger after supper than before supper.

Identification.

The ability of the subjects to identify the compounds was overall very poor. The best identified single compound was peppermint oil with 60% of the subjects getting it correct.

Adjective selection (from a list provided, appendix B, page 150).

As a critique of Amoore's selection of smell groups by the frequency of descriptive adjectives, the absence of statistical preference for Amoore's adjectives over the adjectives used by other authors is more meaningful than a preference for Amoore's. In appendix B, independent selections and cases where the subjects made no choice are listed for completeness. By the null hypothesis, the expected ratio of adjectives selected from the entire list given the subjects was, 14 (Amoore): 6 (Henning): 4 (Crocker and Henderson).

No significant deviation from the expected ratio was noted for the following groups:

(a) compounds no. 1-3, repellents.

(d) compounds no. 25-29, miscellaneous.

(e) compounds no. 30-32, controls water.

A significant deviation from the expected ratio of selected adjectives was noted in the following groups:

(b) compounds no. 4-11, Amoore 'types'.

(c) compounds no. 12-24, Amoore 'samples'.

(b + c) compounds no. 4-24, all compounds with a known Amoore classification.

(a + b + c + d + e) compounds no. 1-32, all tested compounds added together.

The Chi^2 test serves to indicate when the observed ratio is significantly different from the expected ratio. When the adjectives are divided into three lists, a significant deviation from the expected ratio could be due to a pronounced preference for one list, or pronounced lack of preference for a list. After observing the figures, I noted that in many cases the subjects showed a distinct lack of preference for Henning's 6 adjectives, possibly due to the fact that they were literally translated from the German and lacked the meaning to individuals associated with common idiom. Amoore's adjectives, therefore, are separately compared with Crocker and Henderson's. The expected ratio is 14 (Amoore): 4 (Crocker and Henderson).

No significant deviation from this expected ratio was noted for the following groups:

(a) compounds no. 1-3, repellents.

(c) compounds no. 12-24, Amoore 'samples'.

(d) compounds no. 25-29, miscellaneous.

(e) compounds no. 30-32, controls (water).

A significant deviation from the expected ratio was noted for the following groups:

(b) compounds no. 4-11, Amoore 'types'.

(b + c) compounds no. 4-24, all compounds with a known Amoore classification.

(a + b + c + d + e) compounds no. 1-32, all tested compounds added together.

Amoore_class_selection.

In group (b + c), compounds no. 4-24, which can be assigned to an Amoore group either as a 'type' or 'sample', the number of correct adjectives is compared with the expected number of 1/14 of the total number of Amoore adjectives selected. The number of correct adjectives out of the total number of Amoore adjectives is also given as a percentage. The deviation from the expected ratio was significant in all groups concerned:

(b) compounds no. 4-11, Amoore 'types', 70.1% correct

(c) compounds no. 12-24, Amoore 'samples', 27.1% correct

(b + c) compound no. 4-24, all compounds with a known Amoore classification, 44.4% correct.

5.4 Significance of results

In only two cases was any significant difference noted between tests in which a repellent was smelled first and tests in which a repellent was smelled after. Butyric anhydride smelled stronger if smelled first than if smelled after repellents (any of the three repellents). This case alone out of all compounds tested supported the suggestion by Hocking (page 106) that repellents tend to anaesthetize the sense of smell and subsequent response to olfactory stimuli. However, since butyric anhydride is the only compound for which this effect was noted and furthermore it is a repulsive not an attractive stimulus, a better explanation can be given. Butyric anhydride has a very revolting and powerful smell to most people, and most people when presented it initially may be tempted to mark it as very strong. After having smelled some of the other compounds in the test such as triethylamine and trimethylamine, which are even more powerful, butyric anhydride would not seem so strong. This is then probably a case of subjective relativity. The other significant case where water, compound no. 30, was marked as stronger after repellent than before, might be put down to a confusing effect of repellents. Water should have no smell at all, although the vials may have acquired odours through handling. However, similar results were not obtained with vial no. 29, which contained human foot odour. The numbers involved in this strength test for compound no. 30

(water) were so small that the Chi^2 test could not be regarded as valid.

Males found repellents significantly stronger smelling than did females. Smell thresholds have been shown to vary between the sexes, the male having a lower threshold than the female (Nichols and Bailey, 1887). In the tests the repellent concentration was probably well above the threshold level for both sexes, and this phenomenon was not noted for any other compound. When such a variation in response can be shown between the sexes in one species it is not surprising that there would be a pronounced difference in response between two different species. Indeed, such a variation in sensitivity to an insect repellent is a prime requisite of such material.

White Caucasians generally showed a marked liking for more compounds than did others. They also marked them as stronger. Since most of the test compounds were unknown to all of the subjects, this cannot be the result of familiarity. Besides, when strength of smell was plotted against identification (that is familiarity), only a low correlation was obtained. Whilst such a difference in preference is probably the result of conditioning rather than race, I cannot postulate what this conditioning could be. The original observation that led to the inclusion of this test (see page 106) was exactly the opposite to the overall observed picture and must be regarded as a freak instance.

Only one substance, cyclohexanol, showed any significance between having been smelled before supper or after supper. It was listed as stronger after supper. The difference was not very great, being just significant and I think that in view of the fairly small numbers involved it does not justify any conclusions. One thing seems fairly clear; satiation with food, and short term exposure to smoke and liquor do not seem to affect the sense of smell unduly, at least as regards smells other than those to which the subjects were continuously exposed, (adaptation to a particular smell like H_2S is a well known phenomenon).

The poor showing of the subjects in identifying the compounds, even very familiar ones, indicates that people in general are badly educated as far as smell goes (cf. Wright, 1964b, p. 105).

As stated in the previous section, significant deviations from the expected ratio of adjective selection appear partly due to a lack of preference for Henning's list. When Amoore's list is compared with Crocker and Henderson's, a preference for Amoore's list is found in group (b), Amoore 'type' compounds, and an overall preference if all compounds are considered, group (a + b + c + d + e). There is no significant preference if all other compounds except group (b), Amoore 'types', are considered together. Thus the overall preference may be considered to be due to a strong preference for Amoore's list in group (b). Since group (b)

contains compounds for which Amoore's classes are named, it is not surprising that people should select the corresponding adjective e.g. pepperminty for peppermint oil. With other compounds which do not have such an obvious descriptive adjective, Amoore's list is not significantly preferred. This is of particular interest in section (c), Amoore 'samples', which are substances that have been assigned by Amoore to certain smell classes. The preference for Amoore's adjectives holds well with familiar compounds but not well at all with unfamiliar substances. Amoore based his smell groups on the frequency of descriptions in the literature which likely bears a close correlation with substances that people are most familiar with. In other words, people refer more frequently to smells they know well. There is a great step from this to correlating all smells with a few dozen familiar ones. The results from my tests seem to indicate that when presented with an unknown or unfamiliar smell, people do not tend to select Amoore's smell group adjectives in preference to Crocker and Henderson's.

There was a significant preference for the 'correct' Amoore class adjectives in both group (b) Amoore 'types', and group (c) Amoore 'samples'; 70.1% correct in group (b) and 27.1% correct in group (c). This is quite impressive for the Amoore's 'types', as it should be for reasons previously stated. It is not so impressive for the Amoore 'samples' although this frequency of selection would be

sufficient to confirm a compound's presence in a class, if one assumes that the classes are completely reliable. It is by no means so overwhelming as to suggest that such a compound could in no circumstances fit better in some other class not named by Amoore.

The results are best summarized as follows:

In man insect repellents do not anaesthetize the sense of smell or appreciably change the response to other odours. The sense of smell is not noticeably affected by tobacco smoke, liquor and gastronomic satiation.

Men probably have a lower smell threshold than women for insect repellents. Extreme variation in repellent smell thresholds between man and insects could be an important property of insect repellents (see section 6., Discussion).

There is a difference both in response and in sensitivity to odours between people with different ethnic and cultural backgrounds. The sense of smell is affected by learning and association as are other senses.

The extent of olfactory 'education' in man is very low. People have difficulty in correctly identifying all but the commonest smells and have difficulty associating them consistantly with descriptive adjectives.

Amoore used trained observers to assign his compounds to his fourteen classes. Even untrained observers do this fairly well (Amoore class selection), when choosing from a prescribed list.

Amoore's selection of classes was based on the frequency of the appearance of descriptive adjectives in the literature; that is by untrained observers. Since most people do not seem to have a very good idea of what they are talking about as regards smell, the basis of Amoore's smell categories is suspect.

6. DISCUSSION

When the conclusions from section 3. the choice chamber experiments and section 4. electrophysiological experiments are added together they produce this picture. The principal sites of action of insect repellents are on the legs and antennae, and to a lesser extent on the labial palps. These areas possess thin walled chemoreceptors which are the only parts of insects where chemicals have access through the cuticle to the sensory tissue. The chemoreceptors on both the antennae and legs can respond to both vapours and liquids, and do so in a similar electrophysiological way. In practice insect antennae are rarely in contact with the substrate and therefore play little part in the behavioural response to contact repellency. In practice the vapour phase of repellents is more important than the liquid phase because both the antennae and legs are exposed to vapours and the antennae have more chemoreceptors than do the legs. Antennae respond to attractant vapours but the legs do not, therefore, either repellents and attractants act through different receptor neurons or they act on the same neurons but through different mechanisms, these two mechanisms being olfaction for attractants and the common chemical sense for repellents. Since there is more evidence for the dual mechanism theory, let us consider it first.

(1) The exposed ventral nerve cord of insects can be stimulated by irritant chemicals (Roys, 1954).

(2) Man is sensitive to repellents and other irritant chemicals, where he has free nerve endings. Human lips contain touch and heat receptors but not olfactory or gustatory receptors.

(3) There is a decrease in the antennal response of insects to attractants after the antenna has been exposed to repellent (section 4.3). This could be due to competition at the receptor sites of attractants and repellents or due to neurophysiological adaptation which is the reduction of nervous impulse frequency during prolonged stimulus application. Adaptation is a common feature of nerve physiology.

(4) Repellent treated mosquitoes do not display their normal response to humidity and carbon dioxide (Wright, 1962a). The receptors for both humidity and carbon dioxide are found on the antennae of insects and closely resemble other chemoreceptors in structure, many of them having pores opening to the surface (figure 31). Some of these receptors are also sensitive to heat (Lacher, 1964).

If repellents can stimulate olfactory, gustatory, hygro- and carbon dioxide receptors in insects, and fail to stimulate the mechanoreceptors only because the concerned neurons are not exposed (figure 30), then the confused behaviour of repellent treated insects noted by Khan (1965) is not surprising. I cannot believe that the sensory mechanisms of

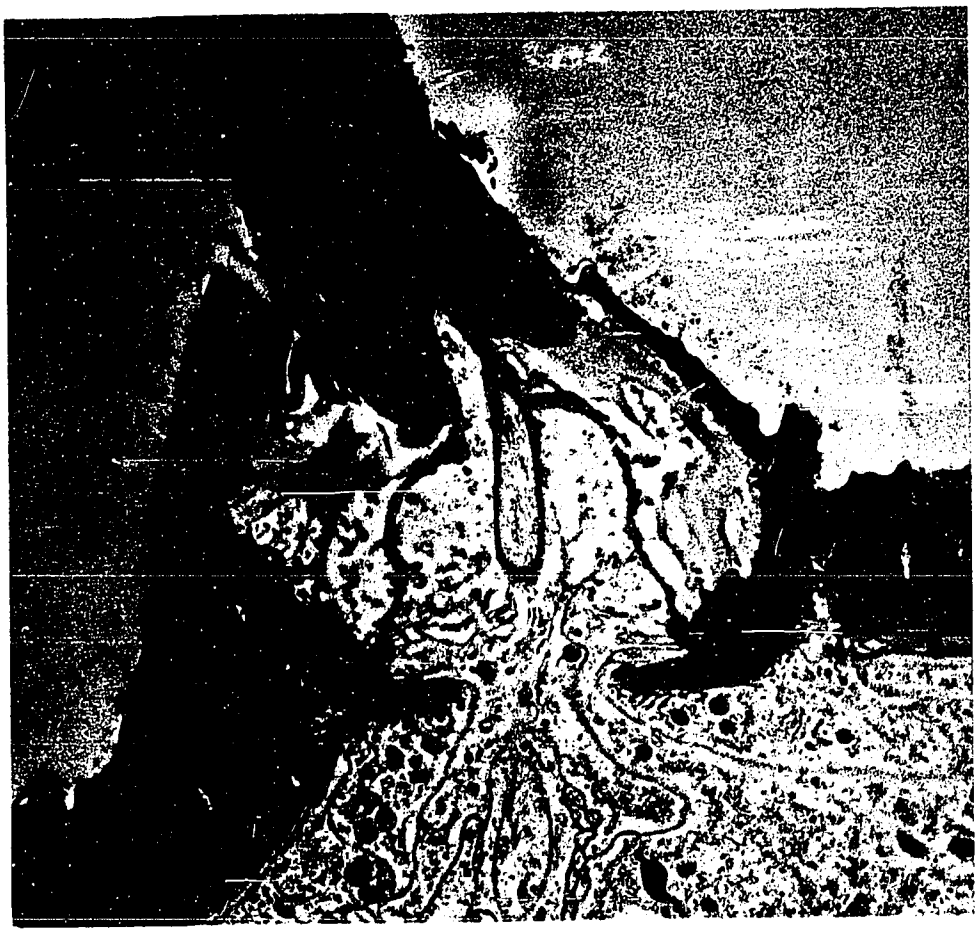


Figure 30. Electron micrograph of a tactile seta on the antenna of Drosophila melanogaster. Note the thick cuticle. Total magnification, 10,000.
By courtesy of Miss M. M. Perry.

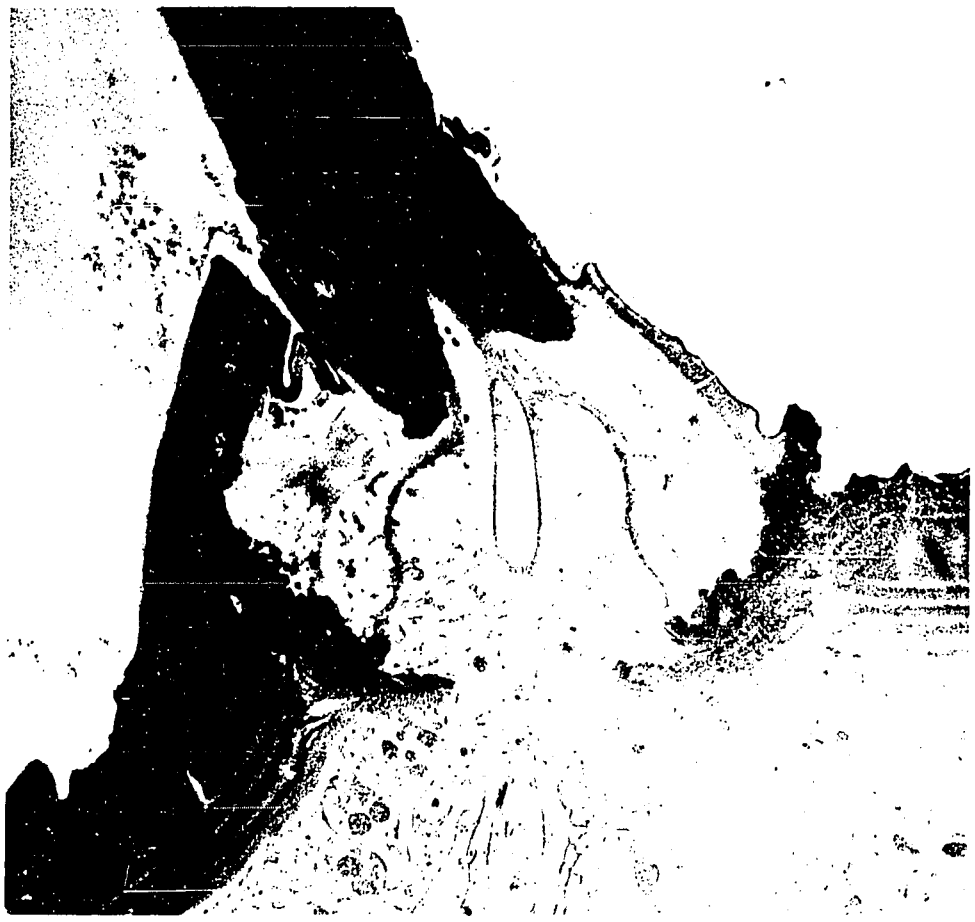


Figure 30. Electron micrograph of a tactile seta on the antenna of Drosophila melanogaster. Note the thick cuticle. Total magnification, 10,000.
By courtesy of Miss M. M. Perry.

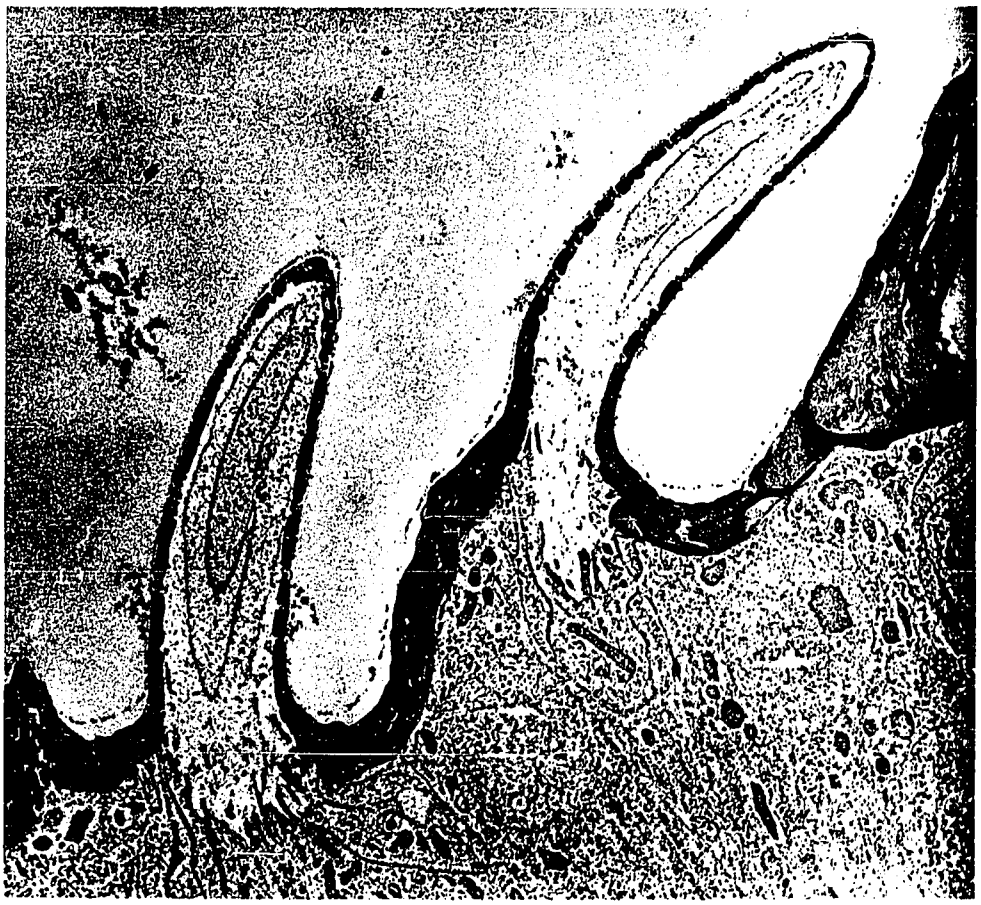


Figure 31. Electron micrograph of a chemosensory cone on the antenna of Drosophila melanogaster. These cones were identified as hygroreceptors by Begg and Hogben (1946). Note the pores opening to the exterior, and the presence of at least 2 neurons.
Total magnification, 7,700.
By courtesy of Miss M. M. Perry.

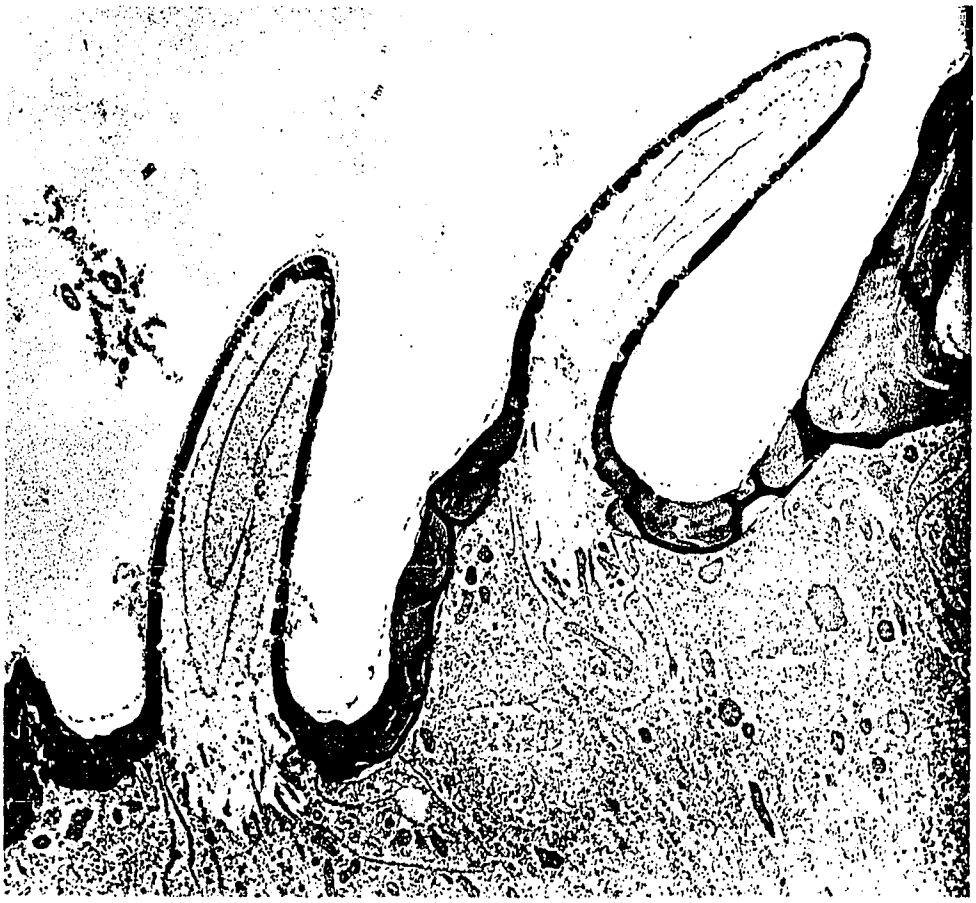


Figure 31. Electron micrograph of a chemosensory cone on the antenna of Drosophila melanogaster. These cones were identified as hygroreceptors by Begg and Hogben (1946). Note the pores opening to the exterior, and the presence of at least 2 neurons.
Total magnification, 7,700.
By courtesy of Miss M. M. Perry.

insects are so inelegant in function as to allow total disruption of their powers of discrimination by repellents, especially since the standard insect response to high concentration of repellent is orientated repulsion.

There is convincing electrophysiological evidence for the presence of separate neurons which respond to general or irritant chemical stimuli (Hodgson, 1957; Boeckh, Kaissling and Schneider, 1965). The 'generalist' neurons are commonly associated in the receptor end organs with 'specialist' neurons which respond to a more restricted class of chemicals such as sugar (Hodgson, 1957), or attractants (Schneider, 1964). Even hygroreceptors contain at least two neurons (figure 31) and carbon dioxide sensors are similar in structure to other chemoreceptors (Lacher, 1964). The 'specialist' neurons of chemosensory organs respond to attractants or stimulants such as sugar, and the 'generalist' neurons respond to a wider range of chemical stimuli. Inhibition of the 'specialist' impulse potentials by 'generalist' receptor potentials could account for the observations (3) and (4) above. Inhibition could not account however, for (1) and (2) above (page 124).

I think that the known facts of repellency are best accounted for by combining the two mechanisms and two neurons systems. The situation would be as follows:
All chemoreceptors in insects (including hygroreceptors and carbon dioxide receptors) contain at least two types of

sensory neurons, the 'generalists' which respond to a wide spectrum of chemical stimuli,and the 'specialists' which respond selectively to a narrow range of chemical stimuli at a much greater sensitivity.

Both types of receptor can respond to general irritant chemicals such as repellents, through the common chemical sense. At very low concentrations of repellent stimulus, only the 'specialist' receptors would respond, resulting in attraction.

At higher concentrations of repellent, the 'specialist' receptors for all types of attractant stimuli including water, carbon dioxide and heat are stimulated and some of the 'generalist' neurons are stimulated, causing slight inhibition of the 'specialist' impulse potentials. The result is confusion, disorientation and various types of abnormal behaviour discussed in section 2.4.

At a very high concentration of repellents, total stimulation of the 'generalist' neurons produces total inhibition of the 'specialist' impulse potential resulting in active repellency.

The difference between the pronounced response of insects to repellent vapours and the poor response of man can perhaps best be accounted for by different sensory morphologies, and different sensory needs. The olfactory receptors of man are not grouped together in separate end organs and are spread over a sensitive area of the nasal

epithelium. Although human olfactory neurons can undoubtedly by classed also as 'generalists' and 'specialists', these two types of cell probably do not form the sort of discrete association that they do in insects. Although summation and inhibition occur in the olfactory region of man, it is probably a more gradual and less definitive process than it is in insects. Since the environment of insects is greatly restricted compared with the human environment and since there is a restricted amount of surface on an insect available for sensory perception, sensory information received by an insect from its chemoreceptors would have to be simple and unambiguous to allow a clear and unequivocal behavioural response.

There is now the basic problem of distinguishing between olfaction and gustation and the common chemical sense. If all nervous tissue is capable of responding to general chemical stimuli then it is probable that the common chemical sense is a more basic and primitive form of chemoreception than are either gustation or olfaction. This is reasonable in evolutionary terms, even amoebae are capable of responding to unsuitable changes in the chemical constitution of their environment. The basic mechanism whereby a chemical substance causes depolarization of the nerve membrane is still a mystery, but given such a basic mechanism, it is likely that specialized chemosensory systems such as those of olfaction and gustation are modifications of the

basic mechanism not innovations. If such modifications were to take the form of specifically shaped stereochemical adsorption sites such as those of Amoore (1962) it is possible that the neurons concerned would still retain the primitive ability to respond to any irritant chemical with a molecule small enough to fit those sites. In section 5. it is pointed out that Amoore's theory is based more on semantics than on biochemistry, and it is quite possible that the process of olfaction is far more complicated than he would have us believe. Amoore (1964) himself was obliged with two of his smell categories (putrid and pungent), to discard the stereochemical shape of the molecules and suggest that the important factor was the electrochemical charge. Now if repellents can stimulate the same sites as attractants, and as I have suggested by the same basic transducing mechanism; why then is the electrophysiological response to repellents smaller in amplitude than that to attractants (section 4.3)? Why also are insects more sensitive to larger molecules than to smaller molecules of a similar chemical structure (Dethier, 1955; section 2.3, page 22)? There is an aspect of chemical stimulation which we have overlooked, and that is the mechanism by which molecules of stimulant chemicals are removed from or neutralized at the receptor sites. Nothing is known about this mechanism, but it is obvious that it must exist. If this mechanism involves the removal of stimulant chemical molecules

from the receptor sites, then it is reasonable that a small molecule would be more easily dislodged than a larger one, and that a large molecule which fits exactly into the stereochemical shape of the site would be very difficult to dislodge indeed. Thus the intensity of the stimulation would be a function of the length of time that a stimulant molecule spends in an adsorption site. Insects are so sensitive to minute quantities of certain specific attractants such as sex attractants (Schneider, 1962) that a slow rate of turnover at the adsorption sites would seem to be necessary to achieve this high intensity of stimulation. Note that high electrophysiological stimulation is involved (Schneider, Lacher and Kaissling, 1964). If a large variety of assorted chemical molecules can stimulate at the stereochemical adsorption sites; and if the intensity of the stimulation depends on the length of time the molecules remain in the sites, hence on molecular size (the better the fit the slower to be removed); there should be a cut off point in molecular size above which general chemical molecules can not fit into the adsorption sites unless of the exact stereochemical shape. This was actually noted by Dethier (1955).

I am therefore suggesting that the stereochemical adsorption sites are important as regards the turnover rate of stimulant chemical molecules and that the better these molecules fit into these sites, the slower they are

displaced and the longer they stimulate. I am also suggesting that the basic mechanism by which a chemical causes membrane depolarization at these sites has nothing whatever to do with the shape of the molecules. Until this transducing mechanism is determined, chemoreception and the mode of action of insect repellents will remain a mystery. In looking for this mechanism I think that the intensity of induced stimulation should be disregarded since that may well be a function of molecular size and shape. Rather should all chemicals be classified into two groups, those which can be detected by the chemical senses and those which cannot, and their properties examined accordingly.

The suggestions that I have made concerning the size and shape of generally irritant chemicals, and the types of neuron that are concerned in the perception of these chemicals, do have practical bearing on the mode of action of insect repellents. Although repellents were shown to reduce the response to attractants by insects, (section 4.5), repellent vapour did not seem to affect the human sense of smell unduly (section 5.4). I have stated that I believe most chemicals can stimulate at any adsorption site, provided the molecules are small enough to fit it. I have also stated that I think that the intensity of the stimulation depends upon the length of time the molecules remain in the adsorption sites. In the case of badly fitting molecules I think that the time the molecules remain in the

adsorption sites is very short indeed, as is the case with repellent molecules and the 'specialist' (attractant) receptor sites. The reduced response to attractants by insects after exposure to repellents is due to inhibition of the 'specialist' reception potentials by the 'generalist' receptor potentials; and not due to competition between the attractant and repellent for the 'specialist' receptor sites. The stereochemical adsorption sites on the 'generalist' neurons are probably smaller, more numerous, and less specifically shaped than the sites on the 'specialist' neurons. There are probably several kinds of sites on the 'generalist' neurons and it is the different numbers and types of these 'generalist' sites, as well as the total numbers of 'generalist' neurons and their close apposition with 'specialist' neurons, which provides the difference between the responses of insects to repellent vapours, and the much weaker human responses. An effective insect repellent should fit into as many of the receptor sites as possible on the insect's 'generalist' neurons, but yet should have the molecular size and shape to resist removal from the sites, and thus remain a long time. Clearly, there is some sort of compromise involved here, between a small repellent molecule which would fit all of the sites but only remain a short time, and a larger molecule which would fit fewer of the sites but remain for a long time. A single repellent molecule may have a number of stereochemical configurations

on its surface, which can fit well into a number of stereochemical sites. Such a molecule should be a very effective repellent. Determination of the size and shape of the stereochemical adsorption sites on the insect's 'generalist' neurons and the ways these sites differ from human stereochemical sites is the first step in designing the 'ideal' repellent. From this information the stereochemical shape of the ideal repellent can be determined.

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8. APPENDICES

8.1 Appendix A, experimental observations upon which the analysis of variance in section 3.4 experiment II, is based.

A_1 liquid phase of repellent MGK R-874

number of times each insect recorded on untreated side	frequency of observations			
	B_1 palps only	B_2 legs only	B_3 antennae only	B_4 all groups
10	0	0	1	0
9	1	2	1	2
8	2	3	4	3
7	3	3	4	4
6	3	3	3	4
5	4	3	3	3
4	3	2	2	2
3	2	2	1	1
2	1	1	1	1
1	1	1	0	0
0	0	0	0	0
	20	20	20	20

A_2 vapour phase of repellent MGK R-874

number of times each insect recorded on untreated side	frequency of observations			
	B_1 palps only	B_2 legs only	B_3 antennae only	B_4 all groups
10	0	1	2	0
9	2	3	5	3
8	3	3	4	4
7	3	3	3	4
6	2	3	3	4
5	3	3	2	3
4	3	2	1	1
3	2	1	0	1
2	1	1	0	0
1	1	0	0	0
0	0	0	0	0
	20	20	20	20

A_3 liquid plus vapour phases of MGK R-874

number of times each insect recorded on untreated side	frequency of observations			
	B_1 palps only	B_2 legs only	B_3 antennae only	B_4 all groups
10	1	1	3	9
9	2	4	9	9
8	3	4	5	2
7	2	4	2	0
6	3	3	1	0
5	2	2	0	0
4	2	1	0	0
3	2	1	0	0
2	2	0	0	0
1	1	0	0	0
0	0	0	0	0
	20	20	20	20

8.2 Appendix B, odour response results

The following pages contain tabulated results from section 5., Human responses to odours. A specimen sheet of the questionaire presented the subjects is shown on page 150.

The results for each compound tested are given on separate sheets (pages 158-189), as well as the summed results for groups of compounds:

(a) compounds no. 1-3, repellents (page 151).

(b) compounds no. 4-11, Amoore 'types' (page 152).

(c) compounds no. 12-24, Amoore 'samples' (page 153).

(d) compounds no. 25-29, miscellaneous (page 154).

(e) compounds no. 30-32, controls (water) (page 155).

(b + c) compounds no. 4-24, all compounds with a known Amoore classification (page 156).

(a + b + c + d + e) compounds no. 1-32, all compounds tested (page 157).

Since the Chi^2 test for a significant deviation from an expected ratio was used throughout in the analysis of these results, where applicable, both the observed and expected numbers are given. These appear as fractions, observed numbers/expected numbers. Chi^2 is obtained by the following general formula (Steel and Torrie, 1960):

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

Any obtained Chi^2 value is statistically significant if it is greater than the value at the desired probability level

given in the Chi^2 tables, with the relevant degrees of freedom. By the null hypothesis a significant Chi^2 at $P \leq 0.05$ means a 5% probability that the observed distribution is the same as the expected; or conversely a 95% probability that the observed distribution is not the same as the expected.

The questionaire given the subjects was similar to this:

Name ..... Sex ..... Date .....

Please read questionaire throughly, and answer as honestly as possible. SNIFF THE VIALS IN THE ORDER INDICATED ON THIS SHEET, from the top down. Do not collaborate, cross sniff, or back sniff.

VIAL no.	REACTION Pleasant - Mark + Indifferent - Mark 0 Unpleasant - Mark -	STRENGTH No smell - mark 0 Weak - mark 1 Medium - mark 2 Strong - mark 3	IDENTIFY if possible Leave blank if you do not know.	COMMENT AND DESCRIBE Choose one to three of the following adjectives or use your own to describe the type of smell. Comment further if you wish. Acid, almond, aniseed, aromatic, burnt, camphoraceous, caprylic (goaty), cedar, ethereal, floral, fragrant, fruity, garlic like, lemon like, musky, pepperminty, pungent, putrid, rancid, resinous, spicy, tarry.
2				
27				

10 vials in all.

Compound no. 1-3 Group (a) repellents

Amoore class - Number of tests, 64

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	9/-	30/-	25/-	73	(mean 1.14)
repellent first	6/4.7	17/15.6	10/13.0	46/38.0	
repellent not first	3/4.3	13/14.4	15/12.0	27/35.0	
sex, male	5/4.7	16/15.6	12/13.0	57/38.0*	
sex, female	4/4.3	14/14.4	13/12.0	16/35.0	
white Caucasian	8/6.5	18/21.6	20/18.0	59/52.6	
other races	1/2.5	12/8.4	5/7.0	14/20.4	
before supper	3/4.3	15/14.4	13/12.0	41/35.0	
after supper	6/4.7	15/15.6	12/13.0	32/38.0	

Identification: 1, 1.6%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
21/22.2	11/9.5	6/6.3	5	31

Amoore	Crocker & Henderson
21/21.0	6/6.0

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 4-11 Group (b) Amoore 'types'

Amoore class - Number of tests, 169

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	115/-	38/-	16/-	316	(mean 1.87)
repellent first	52/48.3	15/16.0	4/6.7	143/132.7	
repellent not first	63/66.7	23/22.0	12/9.3	173/183.3	
sex, male	50/52.9	22/17.5	6/7.4	134/145.4	
sex, female	65/62.1	16/20.5	10/8.6	182/170.6	
white Caucasian	90/86.3	27/28.5	9/12.0	257/237.0*	
other races	25/28.7	11/9.5	7/4.0	59/79.0	
before supper	55/62.1	27/20.5	10/8.6	161/170.6	
after supper	60/52.9	11/17.5	6/7.4	155/145.4	

Identification: 62, 36.7%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
127/99.8*	22/42.8	22/28.5	6	37

Amoore	Crocker & Henderson
127/115.9*	22/33.1

Amoore class selection: 89/9.07* 70.1%

* statistically significant ($P \leq 0.05$).

Compound no. 12-24 Group (c) Amoore 'samples'

Amoore class - Number of tests, 280

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	98/-	64/-	118/-	599	(mean 2.14)
repellent first	38/43.1	32/28.2	54/51.9	266/263.6	
repellent not first	60/54.9	32/35.8	64/66.1	333/335.4	
sex, male	46/51.0	38/33.3	61/61.4	300/311.5	
sex, female	52/47.0	26/30.7	57/56.6	299/287.5	
white Caucasian	78/69.6	41/45.4	80/83.8	455/425.3*	
other races	20/28.4	23/18.6	38/34.2	144/173.7	
before supper	51/50.0	37/32.6	54/60.2	314/305.5	
after supper	47/48.0	27/31.4	64/47.8	285/293.5	

Identification: 9, 3.2%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
188/152.8	33/65.5	41/43.7	18	61

Amoore	Crocker & Henderson
188/178.1	41/50.9

Amoore class selection: 51/13.43* 27.1%

* statistically significant ($P \leq 0.05$).

Compound no. 25-29 Group (d) miscellaneous

Amoore class - Number of tests, 105

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	47/-	30/-	28/-	177	(mean 1.69)
repellent first	23/17.4	10/11.1	6/10.4	63/65.5	
repellent not first	24/29.6	20/18.9	22/17.6	114/111.5	
sex, male	23/21.6	13/13.8	12/12.9	79/81.4	
sex, female	24/25.4	17/16.2	16/15.1	98/95.6	
white Caucasian	38/34.3	20/21.9	19/20.4	131/129.2	
other races	9/12.7	10/8.1	9/7.6	46/47.8	
before supper	27/26.3	19/16.8	13/15.7	93/99.1	
after supper	20/20.7	11/13.2	15/12.3	94/77.9	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
59/54.3	21/23.3	13/15.5	5	37
Amoore		Crocker & Henderson		
59/56.0		13/16.0		

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 30-32 Group (e) controls (water)

Amoore class - Number of tests, 61

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	3/-	54/-	4/-	29	(mean 0.48)
repellent first	2/1.4	26/24.8	0/1.8	9/13.3	
repellent not first	1/1.6	28/29.2	4/2.2	20/15.7	
sex, male	0/1.4	27/24.8	1/1.8	9/13.3	
sex, female	3/1.6	27/29.2	3/2.2	20/15.7	
white Caucasian	3/2.3	41/41.6	3/3.1	26/22.3	
other races	0/0.7	13/12.4	1/0.9	3/6.7	
before supper	0/1.5	28/27.5	3/2.0	13/14.8	
after supper	3/1.5	26/26.5	1/1.0	16/14.2	

Identification: 1, 1.6%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
12/12.3	5/5.3	4/3.5	9	37

Amoore	Crocker & Henderson
12/12.4	4/3.6

Amoore class selection: -

* statistically significant ($p \leq 0.05$).

Compound no. 4-24

Amoore class -

Group (b + c) Amoore 'types' and 'samples'
Number of tests, 449

Reaction to smell:

Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	213/-	102/-	134/-	915	(mean 2.04)
repellent first	90/91.6	47/43.9	58/57.6	409/393.5	
repellent not first	123/121.4	55/58.1	76/76.4	506/521.5	
sex, male	96/106.5	60/51.0	67/67.0	434/457.5	
sex, female	117/106.5	42/51.0	67/67.0	481/457.5	
white Caucasian	168/153.3	68/73.4	89/96.5*	712/658.8*	
other races	45/59.7	34/28.6	45/37.5	203/256.2	
before supper	106/110.8	64/53.0	64/69.7	475/475.8	
after supper	107/102.2	38/49.0	70/64.3	440/439.2	

Identification: 71, 15.8%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
315/252.6	55/108.2	63/72.2*	24	98

Amoore	Crocker & Henderson
315/294.0	63/84.2*

Amoore class selection: 140/22.5* 44.4%

* statistically significant ($P \leq 0.05$).

Compound no. 1-32

Amoore class -

Group (a + b + c + d + e) all compounds

Number of tests, 679

Reaction to smell:

Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	272/-	216/-	191/-	1194	(mean 1.76)
repellent first	121/117.0	100/92.9	74/82.1	527/513.4	
repellent not first	151/155.0	116/123.1	117/108.9	667/680.6	
sex, male	118/130.6	116/103.7	92/91.7	579/573.1	
sex, female	154/141.4	100/112.3	99/99.3	615/620.9	
white Caucasian	217/198.6	147/157.7	131/139.4	*928/871.6*	
other races	55/73.4	69/58.3	60/51.6	266/322.4	
before supper	136/141.4	126/112.3	93/99.3	622/620.9	
after supper	136/130.6	90/103.7	98/91.7	572/573.1	

Identification: 73, 10.8%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
407/341.3	92/146.3	86/97.5*	43	203

Amoore	Crocker & Henderson
407/383.4	86/109.6*

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 1 Dimethyl phthalate Group (a) repellent

Amoore class - Number of tests, 24

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	2/8	19/8	3/8*	13	(mean 0.54)
repellent first	0/1	9/9	2/1	7/6	
repellent not first	2/1	10/10	1/2	6/7	
sex, male	1/1	9/9	2/2	8/6	
sex, female	1/1	10/10	1/1	5/7	
white Caucasian	2/1	11/12	2/2	8/8	
other races	0/1	8/7	1/1	5/5	
before supper	1/1	10/9	1/2	8/7	
after supper	1/1	9/10	2/1	5/6	

Identification: 1, 4.2%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
3/2.1	0/1	1/0.67	1	19

Amoore	Crocker & Henderson
3/3.1	1/0.89

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 2 Butyric anhydride Group (a) repellent

Amoore class - Number of tests, 20

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	3/6.7	3/6.7	14/6.7 *	41	(mean 2.05)
repellent first	2/1.7	3/1.7	6/7.7	32/22.6 *	
repellent not first	1/1.4	0/1.4	8/6.3	9/18.5 *	
sex, male	2/1.7	2/1.7	7/7.7	33/22.6 *	
sex, female	1/1.4	1/1.4	7/6.3	8/18.5 *	
white Caucasian	3/2.6	3/2.6	11/11.9	34/34.9	
other races	0/0.5	0/0.5	3/2.1	7/6.2	
before supper	1/1.8	2/1.8	9/8.4	26/24.6	
after supper	2/1.2	1/1.2	5/5.6	15/16.4	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
7/9.9	7/4.2	3/2.8*	3	4

Amoore	Crocker & Henderson
7/7.8	3/2.2

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 3 Diethyl toluamide Group (a) repellent

Amoore class - Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	4/6.7	8/6.7	8/6.7	19	(mean 0.95)
repellent	4/2.2	5/4.4	2/4.4	7/10.5	
first repellent not first	0/1.8	3/3.6	6/3.6	12/8.6	
sex, male	2/2.0	5/4.0	3/4.0	16/9.5 *	
sex, female	2/2.0	3/4.0	5/4.0	3/9.5	
white Caucasian	3/2.8	4/5.6	7/5.6	17/13.3	
other races	1/1.2	4/2.4	1/2.4	2/5.7	
before supper	1/1.4	3/2.8	3/2.8	7/6.7	
after supper	3/2.6	5/5.2	5/5.2	12/12.4	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/9.9	4/4.2	2/2.8	1	8

Amoore	Crocker & Henderson
11/10.1	2/2.9

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 4 Camphor — Group (b) Amoore 'type'

Amoore class I Camphoraceous — Number of tests, 24

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	14/8	6/8	4/8	49	(mean 2.04)
repellent first	6/5.3	1/2.3	2/1.5	22/18.4	
repellent not first	8/8.7	5/3.7	2/2.5	27/30.6	
sex, male	6/6.4	3/2.8	2/1.8	19/22.5	
sex, female	8/7.6	3/3.2	2/2.2	30/26.5	
white Caucasian	9/9.9	4/4.3	4/2.8	40/34.8	
other races	5/4.1	2/1.7	0/1.2	9/14.2	
before supper	7/9.4	5/4.0	4/2.7	32/32.8	
after supper	7/4.6	1/2.0	0/1.3	17/16.2	

Identification: 6 — 25.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
22/14.0	2/6.0	0/4.0*	0	7

Amoore	Crocker & Henderson
22/17.1	0/4.9*

Amoore class selection: 12/1.6* — 54.5%

* statistically significant ($P \leq 0.05$).

Compound no. 5 'Bellogia' Group (b) Amoore 'type'

Amoore class IV Floral Number of tests, 18

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	15/6.0	2/6.0	1/6.0*	34	(mean 1.89)
repellent first	7/6.7	1/0.9	0/0.4	17/15.1	
repellent not first	8/8.3	1/1.1	1/0.6	17/18.9	
sex, male	7/5.9	0/0.8	0/0.4	11/13.3	
sex, female	8/9.1	2/1.2	1/0.6	23/20.7	
white Caucasian	12/11.7	2/1.6	0/0.8	28/26.5	
other races	3/3.3	0/0.4	1/0.2	6/7.5	
before supper	5/6.7	2/0.9	1/0.4	14/15.1	
after supper	10/8.3	0/1.1	0/0.6	20/18.9	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/18.6	7/8.0	14/5.4*	1	1

Amoore	Crocker & Henderson
11/19.5	14/5.5*

Amoore class selection: 6/0.8* 54.5%

* statistically significant ($P \leq 0.05$).

Compound no. 6 Peppermint oil Group (b) Amoore 'type'

Amoore class V Pepperminty Number of tests, 20

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)
overall	18/6.7	2/6.7	0/6.7 *	45 (mean 2.25)
repellent first	5/5.4	1/0.6	0/0.0	16/13.5
repellent not first	13/12.6	1/1.4	0/0.0	29/31.5
sex, male	6/7.2	2/0.8	0/0.0	16/18.0
sex, female	12/10.8	0/1.2	0/0.0	29/27.0
white Caucasian	15/14.4	1/1.6	0/0.0	39/36.0
other races	3/3.6	1/0.4	0/0.0	6/9.0
before supper	10/9.9	1/1.1	0/0.0	23/24.8
after supper	8/8.1	1/0.9	0/0.0	22/20.2

Identification: 12, 60.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
19/12.8	2/5.5	1/3.7*	0	5

Amoore	Crocker & Henderson
19/15.5	1/4.5

Amoore class selection: 15/1.4* 78.9%

* statistically significant ($P \leq 0.05$).

Compound no. 7 Almond oil — Group (b) Amoore 'type'

Amoore class VIII Almond — Number of tests, 23

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	18/7.7	4/7.7	1/7.7*	49	(mean 2.13)
repellent first	8/7.8	1/1.7	1/0.4	23/21.3	
repellent not first	10/10.2	3/2.3	0/0.6	26/27.7	
sex, male	8/9.4	3/2.1	1/0.5	26/25.5	
sex, female	10/8.6	1/1.9	0/0.5	23/23.5	
white Caucasian	13/12.6	3/2.8	0/0.7	37/34.3	
other races	5/5.4	1/1.2	1/0.3	12/14.7	
before supper	7/7.8	3/1.7	0/0.4	18/21.3	
after supper	11/10.2	1/2.3	1/0.6	31/27.7	

Identification: 12 52.2%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
20/13.4	1/5.8	2/3.8*	0	3

Amoore	Crocker & Henderson
20/17.1	2/4.9

Amoore class selection: 15/1.4* 75.0%

* statistically significant ($P \leq 0.05$).

Compound no. 8 Aniseed oil — Group (b) Amoore 'type'

Amoore class X Aniseed — Number of tests, 19

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	14/6.3	1/6.3	4/6.3*	44	(mean 2.32)
repellent first	5/4.5	1/0.3	0/1.3	13/14.1	
repellent not first	9/9.5	0/0.7	4/2.7	31/29.9	
sex, male	5/5.2	1/0.4	1/1.5	16/16.3	
sex, female	9/8.8	0/0.6	3/2.5	28/27.7	
white Caucasian	31/11.1	1/0.8	1/3.2*	35/34.8	
other races	1/2.9	0/0.2	3/0.8	9/9.2	
before supper	7/6.6	0/0.5	2/1.9	18/20.7	
after supper	7/7.4	1/0.5	2/2.1	26/23.3	

Identification: 7, 36.8%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/9.3	3/4.0	2/2.7	3	5

Amoore	Crocker & Henderson
11/10.1	2/2.9

Amoore class selection: 9/0.79* 81.8%

* statistically significant ($P \leq 0.05$).

Compound no. 9 Citron oil — Group (b) Amoore 'type'

Amoore class XI Lemon — Number of tests, 21

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)
overall	21/7	0/7	0/7*	42 (mean 2.00)
repellent first	12/12	0/0	0/0	25/23.9
repellent not first	9/9	0/0	0/0	17/18.1
sex, male	9/9	0/0	0/0	19/18.1
sex, female	12/12	0/0	0/0	23/23.9
white Caucasian	16/16	0/0	0/0	34/31.9
other races	5/5	0/0	0/0	8/10.1
before supper	11/11	0/0	0/0	24/21.8
after supper	10/10	0/0	0/0	18/20.2

Identification: 14 66.7%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
20/13.4	1/5.8	2/3.8*	1	1

Amoore	Crocker & Henderson
20/17.1	2/4.9

Amoore class selection: 17/1.4* 85.0%

* statistically significant ($P \leq 0.05$).

Compound no. 10 Cedar oil — Group (b) Amoore 'type'

Amoore class XII Cedar — Number of tests, 21

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	13/7.0	7/7.0	1/7.0*	32	(mean 1.52)
repellent first	8/6.2	2/3.4	0/0.5	16/15.4	
repellent not first	5/6.8	5/3.6	1/0.5	16/16.6	
sex, male	7/6.8	4/3.6	0/0.5	16/16.6	
sex, female	6/6.2	3/3.4	1/0.5	16/15.4	
white Caucasian	10/9.2	4/5.0	1/0.7	26/22.7	
other races	3/3.8	3/2.0	0/0.3	6/9.3	
before supper	7/8.1	6/4.3	0/0.6	19/19.8	
after supper	6/4.9	1/2.7	1/0.4	13/12.2	

Identification: 7 — 33.3%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
16/10.5	1/4.5	1/3.0*	1	4

Amoore	Crocker & Henderson
16/13.2	1/3.8

Amoore class selection: 9/1.1* 56.3%

* statistically significant ($P \leq 0.05$).

Compound no. 11 Garlic — Group (b) Amoore 'type'

Amoore class XIII Garlic — Number of tests, 23

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	2/7.7	16/7.7	5/7.7*	21	(mean 0.91)
repellent first	1/0.9	8/6.9	1/2.2	11/9.0	
repellent not first	1/1.1	8/9.1	4/2.8	10/12.0	
sex, male	2/1.1	9/9.1	2/2.8	11/12.0	
sex, female	0/0.9	7/6.9	3/2.2	10/9.0	
white Caucasian	2/1.5	12/11.8	3/3.7	18/15.5	
other races	0/0.5	4/4.2	2/1.3	3/5.5	
before supper	1/1.2	10/9.8	3/3.1	13/12.8	
after supper	1/0.8	6/6.2	2/1.9	8/8.2	

Identification: 4 17.4%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
8/7.6	5/3.2	0/2.2	0	11

Amoore	Crocker & Henderson
8/6.2	0/1.8

Amoore class selection: 6/0.6* 75.0%

* statistically significant ($P \leq 0.05$).

Compound no. 12 Cyclohexanol Group (c) Amoore 'sample'

Amoore class I Camphoraceous Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	4/6.7	5/6.7	11/6.7*	48	(mean 2.40)
repellent	2/2.4	5/3.0	5/6.6	26/28.8	
first repellent not first	2/1.6	0/2.0	6/4.4	22/19.2	
sex, male	1/2.0	2/2.5	7/6.5	25/24.0	
sex, female	3/2.0	3/2.5	4/6.5	23/24.0	
white Caucasian	3/3.2	5/4.0	8/8.8	41/38.4	
other races	1/0.8	0/1.0	3/2.2	7/9.6	
before supper	0/1.0	1/1.3	4/2.8	18/12.0*	
after supper	4/3.0	4/3.7	7/8.2	30/36.0	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
19/15.1	4/6.5	3/4.3	0	3

Amoore	Crocker & Henderson
19/17.1	3/4.2

Amoore class selection: 2/1.4 10.5%

* statistically significant ($P \leq 0.05$).

Compound no. 13 Acetic Acid Group (c) Amoore 'sample'

Amoore class II Pungent Number of tests, 23

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	6/7.7	1/7.7	16/7.7*	66	(mean 2.87)
repellent first	3/2.1	0/0.3	5/5.6	23/23.1	
repellent not first	3/3.9	1/0.7	11/10.4	43/42.9	
sex, male	5/2.6	0/0.4	5/6.9	29/28.4	
sex, female	1/3.4	1/0.6	11/9.1	37/37.6	
white Caucasian	6/5.0	0/0.8	13/13.3	54/54.8	
other races	0/1.0	1/0.2	3/2.7	12/11.2	
before supper	3/2.9	1/0.5	7/7.8	32/31.7	
after supper	3/3.1	0/0.5	9/8.2	34/34.3	

Identification: 8, 34.8%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
5/14.0	1/6.0	18/4.0*	0	2

Amoore	Crocker & Henderson
5/14.8	18/4.2*

Amoore class selection: 4/0.36 80.0%

* statistically significant ($P \leq 0.05$).

Compound no. 14 Chloroform Group (c) Amoore 'sample'

Amoore class III Ethereal Number of tests, 21

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	6/7.0	9/7.0	6/7.0	43	(mean 2.05)
repellent first	3/3.1	5/4.7	3/3.1	23/22.4	
repellent not first	3/2.9	4/4.3	3/2.9	20/20.6	
sex, male	2/3.1	6/4.7	3/3.1	21/22.4	
sex, female	4/2.9	3/4.3	3/2.9	22/20.6	
white Caucasian	5/3.7	5/5.6	3/3.7	27/26.7	
other races	1/2.3	4/3.4	3/2.3	16/16.3	
before supper	2/3.4	6/5.1	4/3.4	30/24.5	
after supper	4/2.6	3/3.9	2/2.6	13/18.5	

Identification: 1, 4.8%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
12/8.2	0/3.5	2/2.3	2	7

Amoore	Crocker & Henderson
12/10.9	2/3.1

Amoore class selection: 4/0.86* 33.3%

* statistically significant ($P \leq 0.05$).

Compound no. 15 Anisole — Group (c) Amoore 'sample'

Amoore class IV Floral — Number of tests, 18

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	8/6.0	6/6.0	4/6.0	40	(mean 2.22)
repellent first	4/2.6	2/2.0	0/1.3	13/13.2	
repellent not first	4/5.4	4/4.0	4/2.7	27/26.8	
sex, male	5/4.9	5/3.7	1/2.4	22/24.4	
sex, female	3/3.1	1/2.3	3/1.6	18/15.6	
white Caucasian	6/5.8	4/4.3	3/2.9	32/28.8	
other races	2/2.2	2/1.7	1/1.1	8/11.2	
before supper	3/4.5	5/3.4	2/2.2	23/22.4	
after supper	5/3.5	1/2.6	2/1.8	17/17.6	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
13/8.8	0/3.8	2/2.5*	3	3

Amoore	Crocker & Henderson
13/11.7	2/3.3

Amoore class selection: 0/0.93 0.0%

* statistically significant ($P \leq 0.05$).

Compound no. 16 Cyclohexanone Group (c) Amoore 'sample'

Amoore class V Pepperminty Number of tests, 18

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	3/6.0	9/6.0	6/6.0	28	(mean 1.56)
repellent first	1/3.5	5/4.5	3/3.0	14/14.0	
repellent not first	2/1.5	4/4.5	3/3.0	14/14.0	
sex, male	1/1.5	4/4.5	4/3.0	13/14.0	
sex, female	2/1.5	5/4.5	2/3.0	15/14.0	
white Caucasian	2/2.3	8/7.0	4/4.7	23/21.8	
other races	1/0.7	1/2.0	2/1.3	5/6.2	
before supper	1/1.2	5/3.5	1/2.4	14/10.9	
after supper	2/1.8	4/5.5	5/3.2	14/17.1	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/13.4	10/5.7	2/3.8	1	4

Amoore	Crocker & Henderson
11/10.1	2/2.9

Amoore class selection: 1/0.79 9.1%

* statistically significant ($P \leq 0.05$).

Compound no. 17 Piperitone — Group (c) Amoore 'sample'

Amoore class V Pepperminty — Number of tests, 22

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	18/7.3	3/7.3	1/7.3*	41	(mean 1.86)
repellent first	2/2.5	1/0.4	0/0.1	6/5.7	
repellent not first	16/15.5	2/2.6	1/0.9	35/35.3	
sex, male	7/9.0	3/1.5	1/0.5	19/20.5	
sex, female	11/9.0	0/1.5	0/0.5	22/20.5	
white Caucasian	14/13.1	1/2.2	1/0.7	34/29.9	
other races	4/4.9	2/0.8	0/0.3	7/11.1	
before supper	8/9.0	2/1.5	1/0.5	18/20.5	
after supper	10/9.0	1/1.5	0/0.5	23/20.5	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
19/11.7	1/5.0	0/3.3*	1	3

Amoore	Crocker & Henderson
19/14.8	0/4.2

Amoore class selection: 9/1.36* 47.4%

* statistically significant ($P \leq 0.05$).

Compound no. 18 Phenylacetic acid Group (c) Amoore 'sample'

Amoore class VI Musky Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	5/6.7	10/6.7	5/6.7	21	(mean 1.05)
repellent first	3/2.5	4/5.0	3/2.5	11/10.5	
repellent not first	2/2.5	6/5.0	2/2.5	10/10/5	
sex, male	3/3.0	5/6.0	4/3.0	14/12.6	
sex, female	2/2.0	5/4.0	1/2.0	7/8.4	
white Caucasian	4/3.5	8/7.0	2/3.5	14/14.7	
other races	1/1.5	2/3.0	3/1.5	7/6.3	
before supper	5/2.8	4/5.5	2/2.8	15/11.6	
after supper	0/2.2	6/4.5	3/2.2	6/9.4	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
5/4.7	1/2.0	2/1.3	3	11

Amoore	Crocker & Henderson
5/5.4	2/1.6

Amoore class selection: 1/0.36 20.0%

* statistically significant ($P \leq 0.05$).

Compound no. 19 Trimethylamine Group (c) Amoore 'sample'

Amoore class VII Putrid Number of tests, 27

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	0/9.0	0/9.0	27/9.0*	78	(mean 2.89)
repellent first	0/0.0	0/0.0	16/16.0	46/46.0	
repellent not first	0/0.0	0/0.0	11/11.0	32/32.0	
sex, male	0/0.0	0/0.0	15/15.0	44/43.7	
sex, female	0/0.0	0/0.0	12/12.0	34/34.3	
white Caucasian	0/0.0	0/0.0	18/18.0	56/54.9	
other races	0/0.0	0/0.0	9/9.0	22/23.1	
before supper	0/0.0	0/0.0	15/15.0	43/43.7	
after supper	0/0.0	0/0.0	12/12.0	35/34.3	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
16/12.3	1/5.3	4/3.5	1	6

Amoore	Crocker & Henderson
16/15.6	4/4.4

Amoore class selection: 1/1.14 6.3%

* statistically significant ($P \leq 0.05$).

Compound no. 20 Benzaldehyde Group (c) Amoore 'sample'

Amoore class VIII Almond Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	15/6.7	2/6.7	3/6.7*	40	(mean 2.00)
repellent first	5/6.0	1/0.8	2/1.2	17/16.0	
repellent not first	10/9.0	1/1.2	1/1.8	23/24.0	
sex, male	8/8.3	1/1.1	2/1.7	20/22.0	
sex, female	7/6.7	1/0.9	1/1.3	20/18.0	
white Caucasian	11/8.3	0/1.1	2/1.7	25/22.0	
other races	4/6.7	2/0.9	3/1.3	15/18.0	
before supper	8/8.3	2/1.1	1/1.7	21/22.0	
after supper	7/6.7	0/9.0	2/1.3	19/18.0	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
15/9.9	2/4.2	0/2.8*	0	5

Amoore	Crocker & Henderson
15/11.7	0/3.3*

Amoore class selection: 10/1.07* 66.7%

* statistically significant ($P \leq 0.05$).

Compound no. 21 Chlorobenzene Group (c) Amoore 'sample'

Amoore class IX Aromatic Number of tests, 23

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	10/7.7	8/7.7	5/7.7	48	(mean 2.09)
repellent first	5/4.3	3/3.4	2/2.2	20/20.6	
repellent not first	5/5.7	5/4.6	3/2.8	28/27.4	
sex, male	4/4.8	5/3.8	2/2.4	22/23.0	
sex, female	6/5.2	3/4.2	3/2.6	26/25.0	
white Caucasian	9/6.1	3/4.9	2/3.0	35/29.3	
other races	1/3.9	5/3.1	3/2.0	13/18.7	
before supper	6/5.7	4/4.6	3/2.8	28/27.4	
after supper	4/4.3	4/3.4	2/2.2	20/20.6	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
17/11.7	1/5.0	2/3.3*	0	7

Amoore	Crocker & Henderson
17/14.8	2/4.2

Amoore class selection: 5/1.21* 29.4%

* statistically significant ($P \leq 0.05$).

Compound no. 22 Quinolene — Group (c) Amoore 'sample'

Amoore class X Aniseed — Number of tests, 21

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	4/7.0	6/7.0	11/7.0	42	(mean 2.00)
repellent first	2/1.7	3/2.6	4/4.7	17/18.1	
repellent not first	2/2.3	3/3.4	7/6.3	25/23.9	
sex, male	2/1.9	3/2.9	5/5.3	20/20.2	
sex, female	2/2.1	3/3.1	6/5.7	22/21.8	
white Caucasian	2/2.8	5/4.3	8/7.8	33/29.8	
other races	2/1.2	1/1.7	3/3.2	9/12.2	
before supper	3/1.7	3/2.6	3/4.7	14/18.1	
after supper	1/2.3	3/3.4	8/6.3	28/23.9	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
12/8.2	1/3.5	1/2.3	1	8

Amoore	Crocker & Henderson
12/10.1	1/2.9

Amoore class selection: 1/0.86 8.3%

* statistically significant ($P \leq 0.05$).

Compound no. 23 Limonene — Group (c) Amoore 'sample'

Amoore class XI Lemon — Number of tests, 24

Reaction to smell:	pleasant	no reaction	unpleasant	Strength of smell: 0 (min) - 3 (max)	
overall	18/8.0	4/8.0	2/8.0	45	(mean 1.88)
repellent first	8/8.3	3/1.8	0/0.9	21/20.7	
repellent not first	10/9.7	1/2.2	2/1.1	24/24.3	
sex, male	8/9.0	3/2.0	1/1.0	19/22.5	
sex, female	10/9.0	1/2.0	1/1.0	26/22.5	
white Caucasian	15/12.8	1/2.8	1/1.4	33/32.0	
other races	3/5.2	3/1.2	1/0.6	12/13.0	
before supper	12/11.3	3/2.5	0/1.3	27/28.4	
after supper	6/6.7	1/1.5	2/0.7	18/16.6	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
22/15.2	4/6.5	0/4.3*	1	2

Amoore	Crocker & Henderson
22/17.1	0/4.9

Amoore class selection: 7/1.57* 31.8%

* statistically significant ($P \leq 0.05$).

Compound no. 24 Valeric acid Group (c) Amoore 'sample'

Amoore class XIV Rancid Number of tests, 23

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	1/7.7	1/7.7	21/7.7*	59	(mean 2.57)
repellent first	0/0.5	0/0.5	11/10.1	29/28.3	
repellent not first	1/0.5	1/0.5	10/10.9	30/30.7	
sex, male	0/0.5	1/0.5	11/10.9	32/30.7	
sex, female	1/0.5	0/0.5	10/10.1	27/28.3	
white Caucasian	1/0.7	1/0.7	17/15.5	48/43.7	
other races	0/0.3	0/0.3	4/5.5	11/15.3	
before supper	0/0.5	1/0.5	11/10.9	31/30.7	
after supper	1/0.5	0/0.5	10/10.1	28/28.3	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
22/19.8	7/8.5	5/5.7	5	0

Amoore	Crocker & Henderson
22/21.0	5/6.0

Amoore class selection: 6/1.57* 27.3%

* statistically significant ($P \leq 0.05$).

Compound no. 25 Triethylamine Group (d) miscellaneous

Amoore class - Number of tests, 21

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	0/7.0	1/7.0	20/7.0*	62	(mean 2.95)
repellent first	0/0.0	0/0.2	5/4.8	15/14.9	
repellent not first	0/0.0	1/0.8	15/15.2	47/47.1	
sex, male	0/0.0	1/0.5	10/10.4	32/32.2	
sex, female	0/0.0	0/0.5	10/9.6	30/29.8	
white Caucasian	0/0.0	1/0.7	14/14.2	43/44.0	
other races	0/0.0	0/0.3	6/5.8	19/18.0	
before supper	0/0.0	1/0.5	9/9.6	30/29.8	
after supper	0/0.0	0/0.5	11/10.4	32/32.2	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/8.2	0/3.5	3/2.3	3	4

Amoore	Crocker & Henderson
11/10.9	3/3.1

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 26 D-Carvone — Group (d) miscellaneous

Amoore class - — Number of tests, 22

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	12/7.3	4/7.3	6/7.3*	48	(mean 2.18)
repellent first	5/3.8	2/1.3	0/1.9	16/15.4	
repellent not first	7/8.2	2/2.7	6/4.1	32/32.6	
sex, male	7/4.9	1/1.6	1/2.5	17/19.7	
sex, female	5/7.1	3/2.4	5/3.5	31/28.3	
white Caucasian	9/8.2	3/2.7	3/4.1	36/32.6	
other races	3/3.8	1/1.3	3/1.9	12/15.4	
before supper	6/7.1	4/2.4	3/3.5	25/28.3	
after supper	6/4.9	0/1.6	3/2.5	23/19.7	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
19/14.0	5/6.0	0/4.0	0	4

Amoore	Crocker & Henderson
19/14.8	0/4.2

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 27 'Lotus' Group (d) miscellaneous

Amoore class - Number of tests, 22

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	18/7.3	4/7.3	0/7.3*	41	(mean 1.86)
repellent first	9/8.1	1/1.8	0/0/0	21/18.5	
repellent not first	9/9.9	3/2.2	0/0.0	20/22.5	
sex, male	11/9.0	0/2.0	0/0.0	23/20.5	
sex, female	7/9.0	4/2.0	0/0.0	18/20.5	
white Caucasian	13/12.2	2/2.7	0/0.0	28/27.9	
other races	5/5.8	2/1.3	0/0.0	13/13.1	
before supper	9/9.0	2/2.0	0/0.0	22/20.5	
after supper	9/9.0	2/2.0	0/0.0	19/20.5	

Identification: 0 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
16/18.1	9/7.8	6/5.1	2	3

Amoore	Crocker & Henderson
16/17.1	6/4.9

Amoore class selection:

* statistically significant ($P \leq 0.05$).

Compound no. 28 'Vert-Vert' Group (d) miscellaneous

Amoore class - Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	15/6.7	5/6.7	0/6.7*	23	(mean 1.15)
repellent first	7/6.0	1/2.0	0/0.0	8/9.2	
repellent not first	8/9.0	4/3.0	0/0.0	15/13.8	
sex, male	4/6.0	4/2.0	0/0.0	5/9.2	
sex, female	11/9.0	1/3.0	0/0.0	18/13.8	
white Caucasian	14/11.3	1/3.8	0/0.0	21/17.3	
other races	1/3.7	4/1.2	0/0.0	2/5.7	
before supper	10/8.3	1/2.8	0/0.0	13/12.7	
after supper	5/6.7	4/2.2	0/0.0	10/10.3	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
11/12.8	7/5.5	4/3.7	0	8

Amoore	Crocker & Henderson
11/11.7	4/3.3

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 29 Human foot sweat Group (d) miscellaneous

Amoore class - Number of tests, 20

Reaction to smell: Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	2/6.7	16/6.7	2/6.7*	3	(mean 0.15)
repellent first	2/0.9	6/7.2	1/0.9	3/1.4	
repellent not first	0/1.1	10/8.8	1/1.1	0/1.6	
sex, male	1/0.9	7/7.2	1/0.9	2/1.4	
sex, female	1/1.1	9/8.8	1/1.1	1/1.6	
white Caucasian	2/1.7	13/13.6	2/1.7	3/2.6	
other races	0/0.3	3/2.4	0/0.3	0/0.4	
before supper	2/1.4	11/11.2	1/1.4	3/2.1	
after supper	0/0.6	5/4.8	1/0.6	0/0.9	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
2/1.2	0/0.5	0/0.3	0	18

Amoore	Crocker & Henderson
2/1.6	0/0.4

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 30 Water — Group (e) control

Amoore class - — Number of tests, 20

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	1/6.7	17/6.7	2/6.7*	8	(mean 0.40)
repellent first	1/0.5	9/8.5	0/1.0	1/4.0*	
repellent not first	0/0.5	8/8.5	2/1.0	7/4.0	
sex, male	0/0.5	9/8.5	1/1.0	3/4.0	
sex, female	1/0.5	8/8.5	1/1.0	5/4.0	
white Caucasian	1/0.7	12/11.9	1/1.4	6/5.6	
other races	0/0.3	5/5.1	1/0.6	2/2.4	
before supper	0/0.5	9/8.5	1/1.0	3/4.0	
after supper	1/0.5	8/8.5	1/1.0	5/4.0	

Identification: 1, 5.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
2/2.9	1/1.2	2/0.8	5	13

Amoore	Crocker & Henderson
2/3.1	2/0.9

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 31 Water — Group (e) control

Amoore class - — Number of tests, 21

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	2/7.0	19/7.0	0/7.0*	10	(mean 0.48)
repellent first	1/0.6	5/5.5	0/0.0	4/2.9	
repellent not first	1/1.4	14/13.5	0/0.0	6/7.1	
sex, male	0/0.7	7/6.3	0/0.0	3/3.3	
sex, female	2/1.3	12/12.7	0/0.0	7/6.7	
white Caucasian	2/1.5	14/14.4	0/0.0	10/7.6	
other races	0/0.5	5/4.6	0/0.0	0/2.4	
before supper	0/1.0	11/9.9	0/0.0	3/5.2	
after supper	2/1.0	8/9.1	0/0.0	7/4.8	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
6/5.8	2/2.5	2/1.7	1	12

Amoore	Crocker & Henderson
6/6.2	2/1.8

Amoore class selection: -

* statistically significant ($P \leq 0.05$).

Compound no. 32 Water — Group (e) control

Amoore class - — Number of tests, 20

Reaction to smell: — Strength of smell: 0 (min) - 3 (max)

	pleasant	no reaction	unpleasant		
overall	0/6.7	18/6.7	2/6.7*	11	(mean 0.55)
repellent first	0/0.0	12/10.8	0/1.2	4/6.6	
repellent not first	0/0.0	6/7.2	2/0.8	7/4.4	
sex, male	0/0.0	11/9.9	0/1.1	3/6.1	
sex, female	0/0.0	7/8.1	2/0.9	8/4.9	
white Caucasian	0/0.0	15/15.3	2/1.7	10/9.4	
other races	0/0.0	3/2.7	0/0.3	1/1.6	
before supper	0/0.0	8/9.0	2/1.0	7/5.5	
after supper	0/0.0	10/9.0	0/1.0	4/5.5	

Identification: 0, 0.0%

Adjective selection:

Amoore	Henning	Crocker & Henderson	Independent	no choice
4/3.5	2/1.5	0/1.0	3	12

Amoore	Crocker & Henderson
4/3.1	0/0.9

Amoore class selection: -

* statistically significant ($P \leq 0.05$).