THE JOINT INSECTICIDAL ACTION OF CYPERMETHRIN AND AMORPHOUS SILICA DUSTS AGAINST THE GRAIN WEEVIL, *SITOPHILUS GRANARIUS*

by

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Abstract

Bioassays of twenty sorptive amorphous silica dusts selected to cover a range of physico-chemical characteristics showed that only two characteristics had any appreciable effect on their toxicity to *Sitophilus granarius*. A hydrophobic surface increased toxicity, and porous silicas with pore diameters <2nm were insecticidally inactive. Four types of dust caused significantly different levels of water loss from the beetles: hydrophobic silicas > porous hydrophilic silicas > fumed hydrophilic silicas > porous hydrophilic silicas (pore diameter <2nm).

The extent to which dusts accumulated on insects moving through dust-treated wheat and the rate of turn-over on their cuticles were investigated. These experiments used dusts radio-labelled with ^{35}S-sodium sulphate, and a method of extracting the isotope and assaying it radiometrically was developed. The amount of dust (at a given sub-lethal concentration) that accumulated on the beetles reached or was close to reaching a maximum level within 24h. Three types of dust had different maximum pick-up levels: hydrophobic silicas > porous hydrophilic silicas > fumed hydrophilic silicas. The range of the rates of turn-over of silica dusts on the insects' cuticles was established.

To assess the joint action between cypermethrin and a sorptive dust, bioassays were performed on cypermethrin and the dust together at different ratios and the amount of each toxicant in the LC_{50} values were plotted as an isobologram. Isobolograms showed that hydrophobic silicas had greater potentiating action with cypermethrin than porous hydrophilic silicas, and that fumed hydrophilic silicas had either additive, sub-additive or antagonistic action with the cypermethrin.

The difference in the levels of water lost from beetles exposed to cypermethrin and a hydrophobic silica at different ratios, though significant, was small. This was interpreted as indicating that the potentiation observed between these toxicants was mainly due to optimal penetration of cypermethrin into the insects rather than optimal water loss.

DEDICATED TO MY PARENTS

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Section 1. Introduction

1.1 The insect cuticle as a barrier to water loss

The integument of an insect functions as an external skeleton and also serves to prevent water loss which would otherwise desiccate and kill terrestrial insects. In addition, it is the primary barrier to the entry of any topically applied compounds (including insecticides).

The integument comprises an epidermal cell layer together with an overlying cuticle which these cells have secreted. The cuticle is differentiated into two major regions: an inner region, the procuticle, which is up to 200 μm thick; and a thin outer region, the epicuticle, which is 1-4 μm thick. Details of the microscopic and molecular structure of the cuticle are available in a number of entomological texts (e.g. Richards 1 and Neville 2). Over the epicuticle there is a thin layer of wax or lipid secreted by the insect. The epicuticular lipid layer and the epidermis are involved in the passive and active prevention of water loss respectively from the insects.

The epicuticular lipids are thought to originate from oenocytes in the epidermis, and are transported to the surface of the insect through pore canals 0.15-1.0 μm in diameter which run through the cuticle at right angles to the surface. In the epicuticle each pore canal divides into a number of wax canals which radiate out and open onto the surface. There is a fairly rapid turn-over of the lipids which cover the cuticular surface. Nelson (3) found that C^{14}-labelled acetic and palmitic acid injected abdominally into *Periplaneta americana* were incorporated into the lipid layer within 3.5h.

Epicuticular lipid is generally a solid wax-like material, though in a small minority of species, including cockroaches, it is in the form

of a mobile grease. It consists mainly of mixtures of C_{25}-C_{31} hydrocarbons, with esters, alcohols, carboxylic acids, aldehydes and phospholipids. The composition of the lipids in a number of insect species have been identified (4, 5, 6, 7) and Bursell and Clements (6) point out that long-chain alcohols predominate in the "hard" waxes whereas the hydrocarbon content is high in "soft" waxes. Hydrocarbons form less than 7% of the "hard" cuticular lipids of two grain infesting beetles, *Tribolium castaneum* and *Tribolium confusum*. In some insects, a harder "cement" layer is secreted over the lipid layer to protect it, though little information is available about its composition. The cement layer of *Rhodnius prolixus* is thought to consist of proteins and polyphenols (8) while that of *Periplaneta americana* consists of tanned proteins and lipids (9).

There is some evidence that the lower layer of lipid immediately over the cuticle is the prime barrier to water loss (10, 11, 12). Some workers have suggested that this could be due to a particular stacking arrangement of the lower lipid molecules (13, 14, 15). Beament (13) proposed that the critical temperature observed in some insects above which the rate of water loss through the cuticle dramatically increases could be explained by the thermal agitation of this layer allowing the passage of water. Davis (16) however postulates that an increased rate of water loss is due to components of the waxy layer changing from a solid crystalline state to a liquid at the critical temperature.

The ability of the cuticle to reduce water loss from the insect does not entirely depend on the passive prevention of water loss by the epicuticular lipid layer. There is also evidence of an active component in the epidermis which not only contributes to the reduction in water loss

but is also partly responsible for the absorption of water into the insect (17, 18, 19). The pore canals may be involved in the latter process (20). This active component may be under endocrine and/or nervous control. Penzlin and Stolzner (21) found accelerated water loss from insects following removal of the frontal ganglion or severence of the frontal connectives. They suggested that this organ may receive signals from osmoreceptors and mediate the release of neurosecretory material. Treherne and Willmer (22) similarly observed accelerated water loss from decapitated *Periplaneta americana* but observed no such effect following severence of nervous connections in the neck, while injections of brain extract and to a lesser extent extracts of corpora cardiaca resulted in significant reductions in the rate of water loss from decapitated individuals. The latter results were interpreted as indicating neurohormonal control of cuticular transpiration. The possibility that a blood borne factor directly affects lipid secretion onto the epicuticle can be discounted, however, since Diehl (23) found that the rate by which ^{14}C-labelled acetate injected into cockroaches was incorporated into their cuticular lipid was not affected by decapitation.

Maddrell (24) has located neurosecretory axons supplying the abdominal epidermis of *Rhodnius prolixus*. The function of these axons is not known but they raise the possibility that the epidermis may come under more localised control than afforded by a blood-borne hormone. Evidence of localised nervous or neuroendocrine control of cuticular water loss comes from experiments involving insecticide-poisoned insects which are discussed in Section 1.3.3.

To summarise, it would appear that there are at least two ways in

which the capacity of the integument to prevent water loss from the insect can be reduced. The passive prevention of water loss by epicuticular lipids can be affected mechanically by sorptive dusts (Section 1.2.3) and an active component can be affected by direct or indirect action of neurotoxic insecticides (Section 1.3).

1.2 Amorphous silica dusts

1.2.1 Types of amorphous silica dusts and their physical characteristics

Silica dusts are usually classified according to their method of manufacture, although different types may be structurally similar.

The smallest recognisable amorphous silica particles within a dust are the ultimate particles. These may be regarded as enormous individual molecules of polymerised silica. Their size, the extent to which they are fused together (coalesced) and their spacial arrangement govern the specific surface area, pore size and pore volume of the dust. The ultimate particles are created by polymerising either silicic acid in an aqueous medium or silicon dioxide in a gaseous medium.

Silica dusts created in an aqueous medium:-

The types of silica whose ultimate particles are created in an aqueous medium are aerogels, xerogels and precipitated silicas. The diameter of their ultimate particles ranges from 10-100 nm.

Aerogels and xerogels are both made by grinding up a dried silica gel formed in an aqueous solution but differ in the way the gel is dried. They are manufactured by creating a saturated solution of $Si(OH)_4$ in aqueous solution which then polymerises to form the ultimate particles. Under certain conditions, the particles bond together through covalent siloxane linkages until they form a rigid network throughout the aqueous medium, a process called 'gelation'. By manipulating such variables as

the pH of the aqueous medium, its temperature, the time the gel is left in the medium (aged) and the concentration of all reactants, the size of the ultimate particles, the extent to which they are coalesced and their spacial arrangement can be controlled. The effect of varying these characteristics is illustrated in Fig.1.1. Generally, as the ultimate particle size is increased, specific surface area decreases while pore volume and pore diameter increase. Also, increasing the extent to which the ultimate particles are coalesced decreases specific surface area, pore volume and pore diameter. The different methods of creating saturated solutions of $Si(OH)_4$ and manipulating them to achieve the required types of gel are reviewed by Iler (25).

On completing its formation in the aqueous medium, a gel is dried and ground up to form a dust. The particles formed by grinding are referred to as the secondary particles. Xerogels are formed when the gel is dried by open heating or evaporation. The surface tension of the liquid drying from the pore structure causes the network to shrink, giving the dried gels (and dusts) a relatively small pore size and pore volume. Aerogels are formed by drying the liquid phase from the gel in such a way that no shrinkage of the gel network occurs. Kistler (26) prepared aerogels by replacing most of the water in the gel with alcohol, heating the gel in an autoclave above the critical temperature of the alcohol then venting the vapour. In this way, the liquid phase is removed without subjecting the gel to the compressive forces owing to the surface tension of the surface of the liquids.

Precipitated silicas are prepared in a similar fashion to the gels, however, instead of allowing the ultimate particles to form a network, they are either coagulated by the addition of salts or are aggregated

by floculating agents. The aggregates are then reinforced by allowing more silica to deposit on them, washed and dried. Precipitated silicas have a wide-pored open structure and are very similar to ground aerogels.

Silica dusts created in an gaseous medium:-

These types of dusts are known as the pyrogenic or fumed silicas. Hot SiO_2 vapour is cooled and condensed to form the ultimate particles. Different methods of forming the SiO_2 vapour are described by Iler (25). The method used by both the Cabot Corporation and Degussa to manufacture the 'Cab-O-Sil' and 'Aerosil' fumed silica products respectively involve the flame hydrolysis of $SiCl_4$. Silicon tetrachloride is burned in a hydrogen and oxygen flame at 1800^{o}C to form SiO_2 and HCl gas. The silica condenses to form particles 7-20 nm diameter which, while still in the molten state, collide and fuse to form branched, three dimensional chain-like aggregates. As the aggregates cool below the melting point of 1710^{o}C, further collisions result in the reversible mechanical entanglement of the aggregates which continues during the collecting and bagging process.

Barnby (27) investigated the structure of fumed silicas and concluded that the particles described by manufacturers as the smallest and initially formed particles (7-20 nm in diameter) are in fact made up of sub-particles approximately 1 nm in diameter, and the latter are the true ultimate particles. However, these sub-particles are so closely packed and the spaces between them so small that they evade detection by microscopic and nitrogen absorption techniques. So much surface area is lost between the points of contact between the sub-particles that it is the "secondary particles" (7-20 nm) that determine the specific surface area of the dust.

1.2.2 The chemistry of the silica surface

The chemistry of silica dusts is dependent on the chemical groups present on the surface of the silica particles. The chemical groups which have been identified on the silica surface are 1) hydroxyl groups, in this case called silanol groups, and 2) siloxane groups which are outwardly protruding oxygen atoms linking adjacent peripheral silicon atoms (Fig.1.3). The silanols are hydrogen bonded to each other and the silica surface becomes increasingly hydrophilic with increasing density of silanol groups. In contrast, siloxane groups are hydrophobic and hydrophobic patches also occur on the silica surface.

The polar nature of the silanol groups allows the silica dusts to hydrogen-bond atmospheric water. This is referred to as chemisorbed-water, as distinct from physically absorbed water drawn into and held in the dust by capillary action.

Lange (28) claims that physically absorbed water is lost from silicas when dried at 25°C-105°C and then chemisorbed water is lost at 105°C-180°C. From 110°C adjacent hydrogen-bonded silanol groups start to dehydrate forming a siloxane group for the loss of a water molecule (Fig.1.4) and thus making the silica surface more hydrophobic (29). This can be reversed by exposing the dusts to water-saturated air at room temperature.

Since the temperature at which chemi-sorbed water and silanol groups are lost overlap, it is impossible to calculate to percentage dry weight of a dust by heating it. The prevailing view is that low temperature drying under vacuum is the only way of removing absorbed water without disturbing silanol groups (25). This is a time-consuming process, so no allowance was made for water content when weighing out

dusts in the present work. The dusts were kept in sealed containers and were assumed to have had the water content estimated by the manufacturers on packaging.

Many types of commercially available silica dusts have had their surfaces chemically altered to make them more hydrophobic. There are several ways this can be achieved (25) but only two methods give dusts stable enough to be used in pesticide dust formulations. These are:-

(a) Esterification of the surface silanol groups with long chain alcohols so as to cover the silica surface with $\underline{Si}$-O-R groups. The resulting compounds are known as estersils.

(b) Reacting the surface silanols with organo-silicon intermediates to produce a surface of $\underline{Si}$-O-SiR_3 or similarly bonded groups. Examples of such reactions are:

$$\underline{Si}OH + ClSiR_3 \longrightarrow \underline{Si}OSiR_3 + HCl$$

$$(\underline{Si}OH)_2 + Cl_2SiR_2 \longrightarrow (\underline{Si}O)_2SiR_2 + 2HCl$$

$$(\underline{Si}OH)_3 + Cl_3SiR \longrightarrow (\underline{Si}O)_3SiR + 3HCl$$

$$2\underline{Si}OH + (CH_3)_3SiNHSi(CH_3)_3 \longrightarrow 2\underline{Si}OSi(CH_3)_3 + NH_3$$

In both (a) and (b) above, R represents an aliphatic hydrocarbon chain and $\underline{Si}$ represents a silicon atom which is part of the surface of the silica dust.

1.2.3 The mode of insecticidal action of silica dusts

It has long been accepted that desiccant dust insecticides kill insects by removing the epicuticular lipid layer and promoting a lethal level of water loss through the cuticle (30, 31, 32, 33, 34, 35, 36). Lipids are removed by either abrasion or adsorption depending on the characteristics of the dust used.

The fact that finely divided "hard" dusts such as carborundum and diamond cannot promote water loss from dead or motionless insects but only from moving insects (34, 35) suggests that these dusts act by abrading the cuticular wax. Alexander *et al* (33) using four hundred different dusts observed a clear correlation between hardness and effectiveness, and early work on the insecticidal efficacy of desiccant dusts in stored grain (37, 38, 39) showed that the susceptibility of grain-infesting beetles increased with increasing hardness of the dust particles.

Certain dusts which are "soft" and lack abrasive properties were found to be very effective insect desiccants (33) and could promote water loss even from motionless insects (40). It was considered that such dusts acted through the absorption of epicuticular lipid. Subsequent workers found that the most insecticidally active sorptive dusts were the synthetic amorphous silicas and acid-activated clays, and their early use and development has been reviewed by Ebeling (41).

Ebeling (42) characterised the physical properties most essential for conferring high insecticidal activity on a dust by assessing the toxicity to four species of insect of sixteen desiccant dusts ranging from highly abrasive non-sorptive carborundums to non-abrasive highly sorptive amorphous silicas. The sorptivity of the dusts was measured by their capacity to absorb beeswax, and their abrasiveness was assessed by rubbing the dusts against a piece of transparent vinyl plastic and measuring the increase in its opacity. It was found that high sorptivity most enhanced insecticidal activity, and the absorption of cuticular lipid from insects by the most highly sorptive dusts was visually observed. Consequently, most recent work on desiccant dust insecticides has centred on sorptive dusts rather than abrasive dusts (41).

1.2.4 Factors affecting the toxicity of desiccant dusts

(a) Physical characteristics of the dusts

In Ebeling's work on the mode of action of desiccant dusts (42), the toxicity of the dusts was found to be most correlated with their pore volume/wt. of dust and their specific surface areas (surface area/ wt. of dust) for the wide range of dusts used. Melichar and Willomitzer (43) also found a close correlation between the toxicity of seventeen silica and four silicate dusts and their specific surface areas (which ranged from 2-200 m^2/g dust) against the chicken mite, *Dermanyrsus gallinae*. Singh (44), however, found no such relationship with a greater selection of amorphous silica dusts all of which had specific surface areas over 110 m^2/g dust).

Ebeling (42) also found that dusts with a pore diameter of 2.2 nm or less were non-toxic, and postulated that narrow pores did not permit the entry of the larger molecules in epicuticular lipid.

A number of workers have found a relationship between the toxicity and the particle size of abrasive desiccant dusts, but no such relationship has been found for sorptive dusts. Both the toxicity of carborundums to *Sitophilus granarius* (33) and the capacity of china clay to cause accelerated water loss from bees (40) has been shown to increase as particle diameter decreased to 5 μm, below which dusts were less effective. Ebeling (42) also found that the toxicity of carborundum dusts to four species of insect decreased with increasing particle size. The degree of abrasion of an insect cuticle can be correlated with the intensity of staining with ammoniacal silver nitrate solution (45). Using this technique, David and Gardner (46) showed that dusts with coarse particles were less abrasive than those with fine particles.

Singh (44) however found no significant correlation between the particle size and toxicity of 15 highly sorptive amorphous silica dusts.

(b) The extent dusts are picked up by the insects

Singh (44) found that when flour beetles (*Tribolium castaneum*) were introduced to wheat treated with a radiolabelled sorptive dust, the amount of dust adhered to the beetles (assayed radiometrically) reached a maximum level which varied with the type and amount of dust used. When beetles which had already picked up a maximum amount of labelled dust were subsequently introduced to wheat similarly treated with unlabelled dust, the amount of labelled dust on the insects decreased until only a residual amount remained. From these results, Singh concluded that there was a turn-over of dusts on the insects cuticle and that the residual amount of labelled dust left on the insects represented a proportion that was irreversibly bound to the insects and not involved in turn-over. The maximum amount of dust picked-up was therefore a dynamic equilibrium level which was reached when the rate at which dust was picked up by the insects was equal to the rate at which it was rubbed off again.

It is most likely that as well as intrinsic toxicity, both the maximum amount of a dust picked up by the beetles and its rate of turn-over affect its ultimate insecticidal activity.

(c) The composition of the epicuticular lipid

Nair (47) investigated the effects of sorptive dusts on four species of grain-infesting insect and found that the weevil *Sitophilus oryzae* whose epicuticular lipids are protected by a cement layer was more resistant to the desiccant action of the dusts than those with unprotected lipids. Amongst the latter, resistance to the desiccant action of the dusts (which was in the order *Tribolium castaneum*>*Rhizopertha dominica*>*Bruchus chinensis*)

decreased with decreasing hardness and decreasing proportions of polar lipids.

Nair also found that inert dusts adhered better to insects with soft epicuticular lipid, and confirmed the observation of David and Gardner (46) that more dust adheres to rough coated or "hairy" insects than smooth coated insects.

(d) Humidity

As humidity increases, water is less easily lost to the atmosphere through the insect cuticle. Sorptive dust insecticides would therefore be expected to be less effective at high humidities. In addition, amorphous silica dusts have a moisture content which is in equilibrium with the surrounding atmospheric humidity and dusts gain moisture if humidity increases (25). Some dusts do this to the extent that they are no longer of any practical use as insecticides (42).

(e) Temperature

In an environment where the humidity could be kept constant, increasing the temperature would increase the water loss through the cuticle caused by sorptive dusts. Also, within limits, the activity of insects increases with temperature, thus increasing their contact with sorptive dusts. However, in stored grain an increase in temperature causes an increase in the humidity of the air between the grains (48). The net effect of temperature on the susceptibility of stored grain insects to desiccant dusts is therefore difficult to predict.

1.2.5 The field use of silica dusts as stored grain protectants

The usefulness of amorphous silica dusts for controlling insect pests in stored grain has long been realized and their successful field use has been well documented (49, 50, 51, 52, 53).

In more recent work, sorptive silica dusts generally compared unfavourably with conventional chemical insecticides (54, 55, 56). However, La Hue (57) found that Cab-O-Sil at 60 lb/1000 bushels (0.77 kg/1000 litres) of wheat was superior at protecting wheat from attack by *Rhizopertha dominica*, *Sitophilus oryzae* and *Cryptolestes pusillus* than both malathion and diazinon at 0.63 lb a.i and 0.25 lb a.i respectively per 1000 bushels (8.1 g.(a.i) and 3.2g.(a.i)/1000 litres).

White *et al* (58) found standard malathion treatment more effective in three year trials than two diatomaceous earths and two amorphous silicas (SG-68 and Cab-O-Sil), and pointed out several problems in the use of desiccant dust insecticides in stored grain. Firstly, dust blown into the air caused discomfort to workers handling the grain; and secondly dust coating the wheat decreased its flowability, hindered bulk handling and caused additional wear and tear on grain-handling equipment.

Although in many cases sorptive dusts have proved effective, the amounts of dust used are large and the level of dust in the grain may reduce its value in countries that have grain grading systems based on the purity of the grain such as the U.S.A. However, relatively inexpensive insecticidal dusts may be of use in underdeveloped countries where farmers cannot afford expensive chemical pesticides or where application of pesticides may be especially dangerous.

1.3 Cypermethrin

1.3.1 Nomenclature, development and properties

Cypermethrin ((RS)-α-cyano-3, phenoxybenzyl (1 RS)-*cis,trans*-3 (2,2-dichlorovinyl)-2, 2-dimethyl-cyclopropane carboxylate), also known as NRDC 149 is a synthetic pyrethroid insecticide first described by

Elliott *et al* (59). It is a mixture of eight steriosomeric forms. The ratio of the *cis* and *trans* ratios varies with the manufacturing process, but a *cis:trans* ratio of 2:3 w/w is commonly used in commercial formulations. The *cis* isomers are more toxic to *Musca domestica* by topical application and to *Spodoptera littoralis, Heliothis viriscens* and *Aphis fabae* as leaf residues (Shell Research Ltd., unpublished data). No information is available on the relative toxicities of the two steriosomers to *S.granarius*.

Technical grade cypermethrin is a viscous yellowish-brown gum which is liquid at 60°C. Its vapour pressure is 3.8×10^{-8} mm Hg. at 70°C (for pure cypermethrin). Its solubility in organic solvents at 20°C is: 103 g/l n-hexane; >450 g/l acetone, cyclohexane, ethanol, xylene and chloroform (60).

1.3.2 The primary mode of action of pyrethroids

Cypermethrin is active as a contact poison rather than as a stomach poison. Its primary mode of action is the same as that of pyrethrins and other synthetic pyrethroids.

Insects treated with a lethal dose of pyrethroid show typical symptoms of nervous poisoning. Generally, these symptoms include hyperactivity, ataxia, convulsions, paralysis and finally death. Like DDT, the pyrethroids show a negative coefficient of insecticidal activity with temperature (61).

The primary mode of action of pyrethroids is to suppress and eventually block the transmission of action potentials along insect nerve axons in a similar fashion to DDT (62). How this is brought about was discovered by Narashi (63) studying the action of the pyrethroid allethrin on crayfish giant axons. Allethrin causes suppression of action potentials

by blocking Na^+ and K^+ movement across the axon membrane during an action potential. The gradual strengthening of this effect is generally thought to be the primary lesion leading to the symptoms outlined above. Prior to the total blocking of action potentials, the gradual suppression of Na^+ and K^+ currents results in smaller action potentials. The Na^+ activation phase is also prolonged so that a 'negative after potential' follows action potentials in nerves. This makes the nerve more susceptible to stimulation, which may result in the hyperactivity and convulsions observed in the earlier stages of pyrethroid poisoning. It is thought that pyrethroid molecules directly plug the sites where ions pass through the nerve membrane during action potential.

Recently, Gammon *et al* (64) classified pyrethroids into two groups depending on the symptoms exhibited by poisoned cockroaches and their effect on cockroach sensory nerve preparations. Most pyrethroids fall into type I category. Symptoms of poisoning are restlessness, incoordination, prostration,paralysis and death. These pyrethroids induce repetitive firing in the cercal sensory nerves both *in vivo* and *in vitro*. Type II pyrethroids are the esters of α-cyanophenoxybenzyl alcohol, which includes cypermethrin, deltamethrin and fenvalerate. Symptoms of poisoning are ataxia and uncoordination but with periods of convulsion and intense hyperactivity sometimes with salivation. There are also sporadic sustained contractions and extensions of the legs. Eventually prostration, paralysis and death follow. No repetitive firing of the cercal sensory nerves occurs.

1.3.3 The effects of pyrethroids on the insects neuroendocrine system

It is generally accepted that the primary lesion at the nerve axon caused by pyrethroids is not directly responsible for the eventual death

of the insect. Death is more likely to occur as a result of the metabolic disruption and tissue damage caused by the uncontrolled release of neurohormones from the poisoned nervous system. This probably applies to all neurotoxic insecticides.

Pyrethroids have been shown to have an effect on the electrical activity of neurosecretory neurones, leading to the release of neurohormones. Orchard and Osborne (65) found that permethrin causes a massive increase in the number of spontaneous action potentials in neurosecretory axons of the stick insect *Carausius morosus*. This effect is transitory and much reduced with acetylcholine esterase inhibitors such as carbaryl and coroxon. Orchard (66) found that the synthetic pyrethroids permethrin, deltamethrin, bioresmethrin and bioallethrin have a similar effect on neurosecretory axons in *Rhodnius prolixus*. Since impulse conduction in neurosecretory cells is generally thought to control the release of neurosecretory material (67, 68) Orchard and Osborne proposed that the increase in electrical activity in pyrethroid treated neurosecretory axons causes the release of neurohormones. This was later confirmed by Singh and Orchard (69) who observed both increased electrical activity and the release of a hyperlipaemic hormone from the corpora cardiaca of bioresmethrin treated *Locusta migratoria*.

Pyrethroids as well as other types of insecticides are known to cause accelerated water loss from insects. Water loss may occur through the mouth, anus, spiracles or general cuticle:-

(1) Ingram (70) found that pyrethrins cause accelerated water loss both through the spiracles and cuticle of *Periplaneta americana* and *Musca domestica*. However, the water loss alone is not the cause of death since insects exposed to dry air until water loss reduced their body weight by

up to 15% (the range lost from insects killed with pyrethrins) did not die.

(2) Gerolt (71) found that pyrethroid, organochlorine, organophosphate and carbamate insecticides all caused accelerated water loss from *Musca domestica*. This is an active process since the water loss did not occur if the insects were transferred to an oxygen free atmosphere. Insecticides applied to the head and thorax caused water loss only from these parts, not the abdomen, and vice versa. The presence of the intact C.N.S. was required, however, since no water loss occurred from insecticide-treated isolated abdomens. These results suggest that the water retention mechanism in the integument is affected by neurosecretory neurones from or controlled by the C.N.S.

(3) Samanarayaka (72) observed insecticide-induced water loss from *Schistocerca gregaria* which accounted for up to 30% of their body weight and 60% of their haemolymph within 24 hours. Although most of the water loss is through the spiracles, there is considerable build up of fluid in the fore- and midgut which may be regurgitated, causing further water loss.

Accelerated water loss through the anus of pyrethroid poisoned insects may be due to the release of a diuretic hormone. Casida and Maddrell (73) observed the release of such a hormone from the nervous system of allethrin poisoned *Rhodnius prolixus*. The mechanism by which pyrethroids cause the build up of fluid in the mid and foregut (leading to oral water loss) is not known.

Accelerated loss of water induced by insecticides may be accompanied by prolonged opening (71, 72) and/or waterlogging of the spiracles when insecticides diffusing through the cuticular lipid layer reaches them. How these changes are mediated is not known.

Pyrethroids probably promote cuticular water loss by affecting the way the active water retention mechanism in the integument is regulated. This, however, may vary according to the species of insect. While Gerolt (above) demonstrated that the water retention mechanism in *M.domestica* is under localised control, experiments by Treherne and Willmer (22) suggested that in *Periplaneta americana* it is affected by a blood borne factor originating in the brain or corpora cardiaca which reduces water loss. Ingram (70) found that significantly more water was lost from *P.americana* treated with pyrethrins topically than by injection and suggested that the pyrethrins may act peripherally. Since pyrethroids promote cuticular transpiration both from insects in which the factor regulating the active water retention mechanism is released locally and from those where the factor is released from the C.N.S., it is possible that they act on peripheral sensory nerves which then affect the release of factor via the C.N.S.

1.3.4 The use of cypermethrin against stored grain pests

Much work has been published on the use of both pyrethrins and synthetic pyrethroids for protecting stored grain against insect attack, and generally both have compared favourably with other types of chemical insecticides (74, 75, 76, 77).

To date, however, no work on the use of cypermethrin has been published, although in an informal paper Berck (78) reports that cypermethrin was more effective in controlling insect pests in stored wheat than identical concentrations of permethrin (NRDC 143) over an eleven week period.

1.4 The joint action of insecticides

1.4.1 Assessing joint action

A number of workers have developed models to both qualify and quantify

the joint action between two compounds (neither, one or both of which may be individually active) and correspondingly a number of ways of classifying the joint action observed have been devised. These models have been reviewed by Hewlett and Plackett (79). In the present work, the joint action between sorptive dusts and cypermethrin has been assessed by a comparatively simple means which involves presenting the data from bioassays (Section 5) in the form of isobolograms.

Isobolograms have been used for over 100 years in pharmacology to describe the joint action produced by mixtures of drugs. They simplify the classification of joint action between mixtures of insecticides because the joint action is defined only by a specific final effect rather than any factors such as the number of physiological mechanisms affected. The terminology for the models described by isobolograms has not been fully standardised, so that used by Ariens *et al* (80) is used in this work. Fig.1.5 represents an isobologram for a mixture of two individually active drugs, A and B. Points on each isobole (I-V) represent the amounts of A and B in a formulation that are required to produce a given biological response, usually an LD_{50} (or LC_{50}). Points A and B represent the amounts of A and B respectively that individually produce the given biological response. Mixtures producing isobole I, a straight line, have additive action. Isobole II lying below the line A,B, represents potentiation or synergism. Isobole III represents sub-additive action, lying within the rectangle A,C,B,D and above line A,B. Isobole IV lying outside A,C,B,D represents antagonism . In isoboles I-IV, A and B are both separately active. Isobole V represents coalitive action where neither drug is separately active, but the mixture is.

For measurement of joint potencies of drug or insecticide mixtures Hewlett (81) proposed what he termed the "joint action ratio". This is applicable when there is potentiation, additive action or antagonism, but not when there is coalitive action. Figs.1.6 and 1.7 are isoblograms illustrating the derivation of the joint action ratio, R.

A point M on the isobole furthest from the line of additive action is taken. When potentiation (Fig.1.6) or antagonism (Fig.1.7) occurs, R=ON/OM. Thus for potentiation, R is the maximum ratio of the actual potency of the mixture of pesticides to what their potency would be if their action was additive; for antagonism it is the corresponding minimum ratio.

1.4.2 The joint insecticidal action between dusts and chemical toxicants

The insecticidal activity of chemical insecticides can be modified by either the carrier dust or the diluent dust used in a formulation. Walker and Anderson (82) showed that the potency of derris root varied with different carriers, while Turner (83) found that the order of efficacy of derris root formulated with different diluents was: pyrophillite > flaky talc > fibrous talc > clay diluents.

Varying the type of carrier dust used could modify both the pick-up of insecticide by the insects and its penetration. However, varying the diluent is less likely to affect the pick-up of the insecticide and such experiments are probably more valuable in determining how toxicity is enhanced.

Wigglesworth (35) found that *Rhodnius* nymphs were more susceptible to the toxic action of both rotenone and nicotine if their cuticles had been previously abraded with alumina dust. These results were interpreted as indicating that alumina enhanced the penetration of the insecticides into

the insect. However, the amount of water loss caused by the alumina was not measured, and the increased toxicity it caused might have been due to insects stressed by water loss being more susceptible to the toxic effects of the insecticides.

Ebeling and Wagner (84) made a comparative study of the effects of sorptive dusts on the toxicity of different types of insecticides. The effects of the dusts to two species of insect when applied to the insects both prior to the application of insecticides and as diluents to the insecticides were investigated.

For the pretreatment experiments, seven different insecticides were formulated on the the same type of non-sorptive carrier dust and their efficacy against insects which had previously been exposed to either sorptive dusts, non-sorptive dusts or were not pretreated was assessed. Generally, the efficacy of the insecticides to the insects previously treated with non-sorptive dusts was comparable to their efficacy against previously untreated insects. However, pretreatment with sorptive dusts decreased the efficacy of five organophosphorous compounds (naled, malathion, parathion, DDVP and trichlorfon) but increased the efficacy of lindane and carbaryl against *Drosophila pseudo-obscura*, and increased the efficacy of all seven insecticides against *Blatella germanica*. It was apparent, therefore, that the *independent* effect of the sorptive powders was either detrimental or beneficial to the efficacy of the insecticides depending both on the insect and the toxicant used.

In the experiment to assess the effects of sorptive dust diluents on the efficacy of different insecticides, Ebeling and Wagner (84) formulated the insecticides on a carrier dust. The insecticides were then applied to the insects both when mixed with a sorptive diluent and a non-sorptive diluent for comparison. When *D.pseudo-obscura* was used as the

test insect, the five organophosphorous compounds were comparatively less effective with the sorptive dusts, but lindane and carbaryl were equally effective with either dust. The same was true with *B.germanica*, except that carbaryl was more effective when mixed with a sorptive dust.

The effects of the sorptive dusts on the efficacy of insecticides when used independently clearly does not reflect their effects on insecticide efficacy when used as diluents. Ebeling and Wagner (84) suggested that the organophosphorous insecticides which are liquids (at R.T.) were strongly absorbed onto the sorptive dusts and were not readily available to the insect, but lindane and carbaryl which are crystalline were not absorbed.

Singh (44) investigated the joint insecticidal action between three synthetic pyrethroids (permethrin, cypermethrin and deltamethrin) with the fumed silica Cab-O-Sil M5^{R} against *Tribolium castaneum* in stored grain. Each insecticide was deposited at six different concentrations on the dust and the LC_{50} values of the formulations determined. Isobolograms derived from the data obtained showed that all three pyrethroids had potentiating joint action with the dust in the order deltamethrin > permethrin > cypermethrin.

A formulation containing both pyrethrins and a sorptive silica dust Dri-die (or SG-67), known as Drione ^{R} is in commercial use. Dri-die is an amorphous silica aerogel with an ammonium flusilicate monolayer over the particles added during manufacture and making up 4.7% of the dust by weight. Tarshis (85) found that a Drione formulation containing (w/w) 1.0% pyrethrins, 10% piperonyl butoxide, 38.12% amorphous silica, 1.88% ammonium flusilicate and 49% petrol base oil was more effective at

killing cockroaches than Dri-die, and Kamel *et al* (86) found that a "Drione dust" containing (w/w) 0.1% pyrethrins, 1% piperonyl butoxide, 1.9% petroleum base oil, 49.5% Dri-die and 47.5% inert filler was effective in controlling *Sitophilus granarius, S.oryzae, Rhizopertha dominica* and *Tribolium castaneun.*

There is no indication that Drione was formulated so as to optimise any potentiating joint action between pyrethrins and amorphous silica.

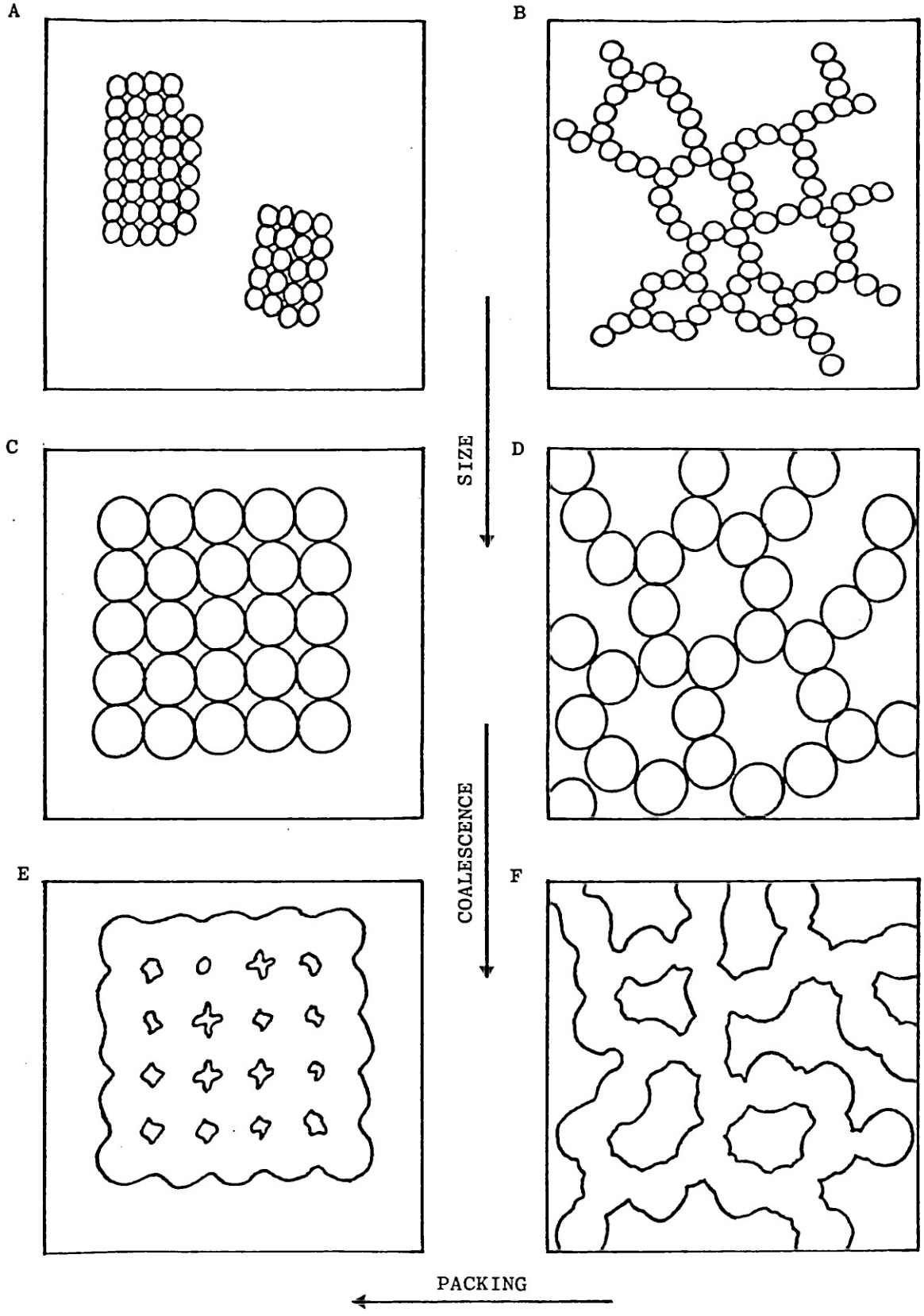
1.5 Aims of the thesis

There were two principal aims to the present work. The first was to determine which physico-chemical characteristics of amorphous silica dusts affected: (a) their insecticidal activity; (b) their capacity to cause water loss from insects; and (c) the extent to which they are picked-up and turned-over by the insects.

The second aim was to see which type of dusts (using a selection which encompassed different levels of (a), (b) and (c) above) most enhanced the insecticidal activity of the pyrethroid cypermethrin and to elucidate how joint insecticidal action was brought about.

The target insect was the grain weevil (*Sitophilus granarius*) in stored wheat.

Fig.1.1 Silica Gels and Powders. Schematic Representation of Cross-Sections of Different Structural Variations



A, small particles, close packed, low coalescence. B, small particles, open-packed, low coalescence. C, large particles, close-packed, low coalescence. D, large particles, open packed, low coalescence. E, large particles, close packed, highly coalesced. F, large particles open-packed, highly coalesced.

Fig.1.2 Schematic representation of the flame process manufacture of Cab-O-Sil (Cab-O-Sil handbook)

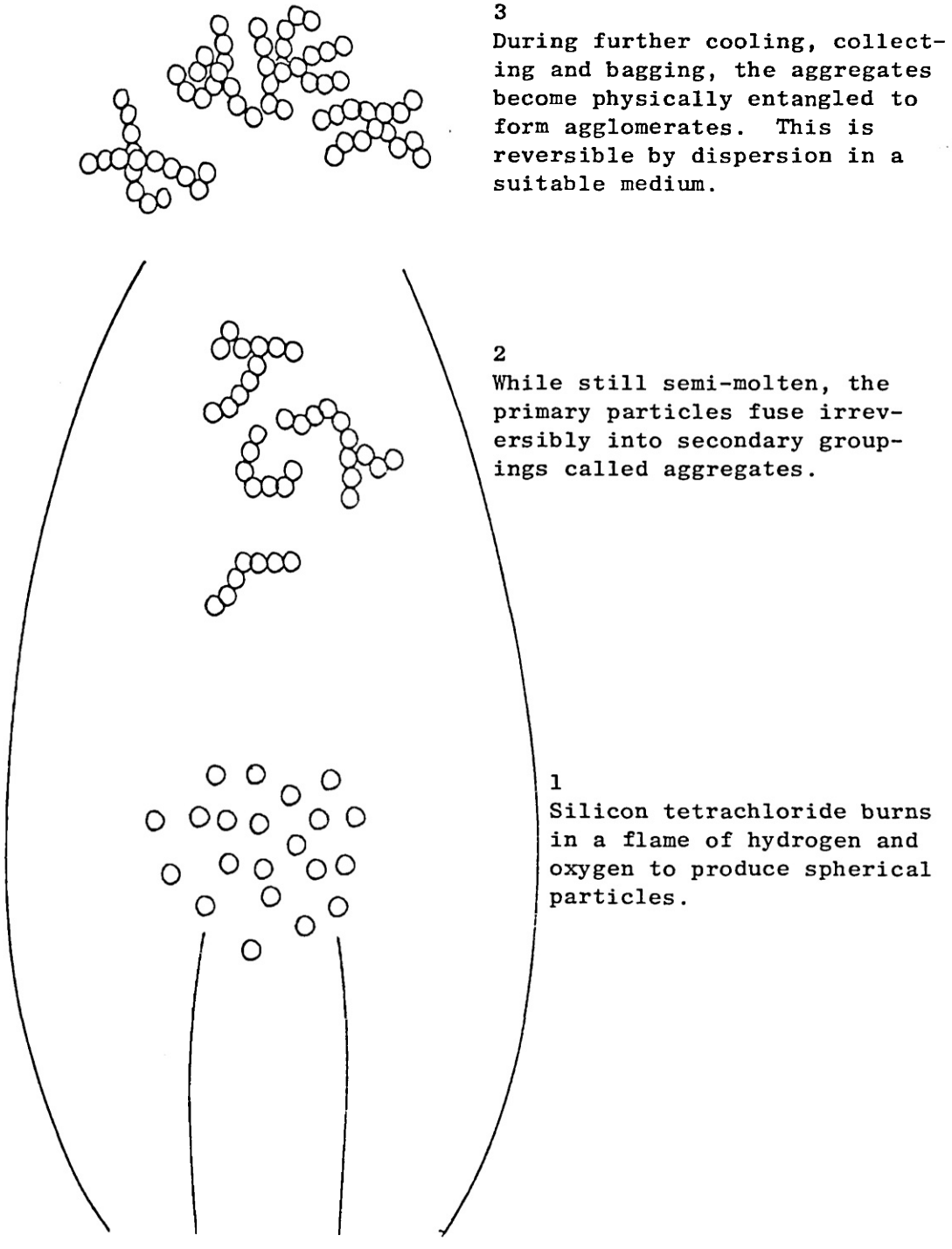


Fig.1.3 Typical groups which can occur on the surface of a amorphous silica dust, determining its chemistry

a = siloxane groups
b = silanol groups
c = H-bonded silanols

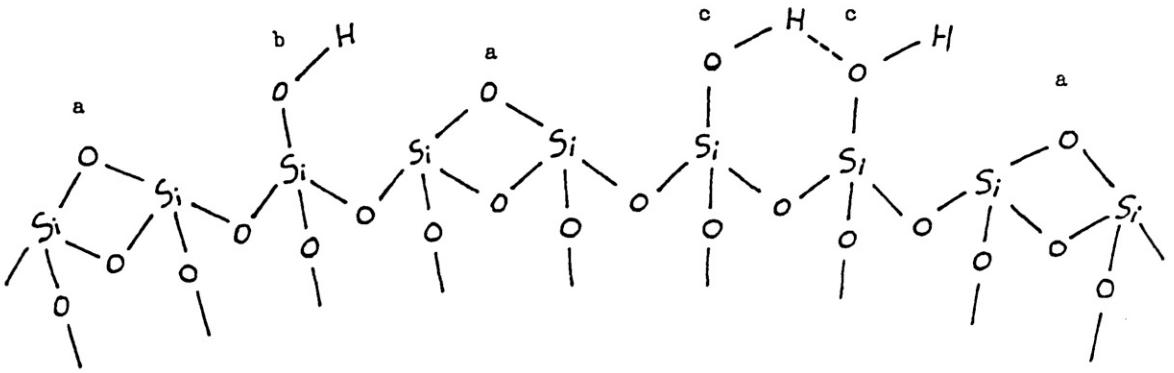


Fig.1.4 Reversible dehydration of the silica surface

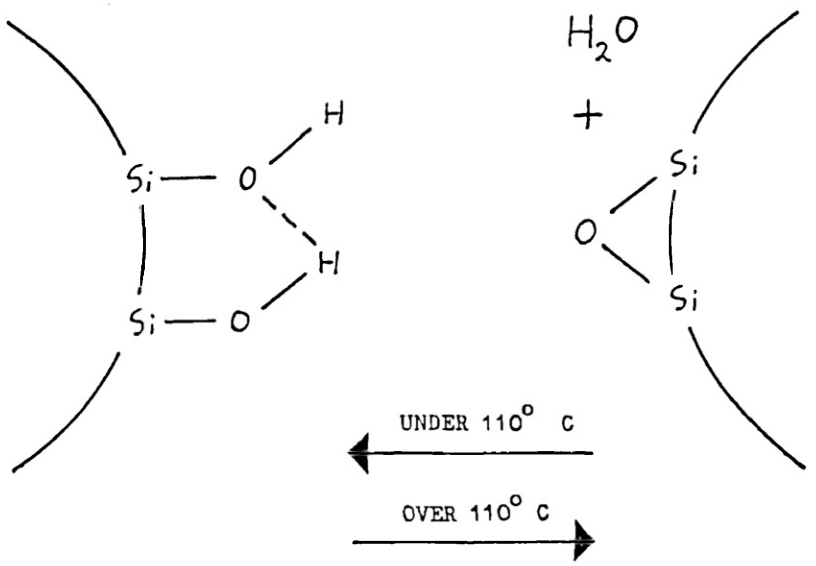
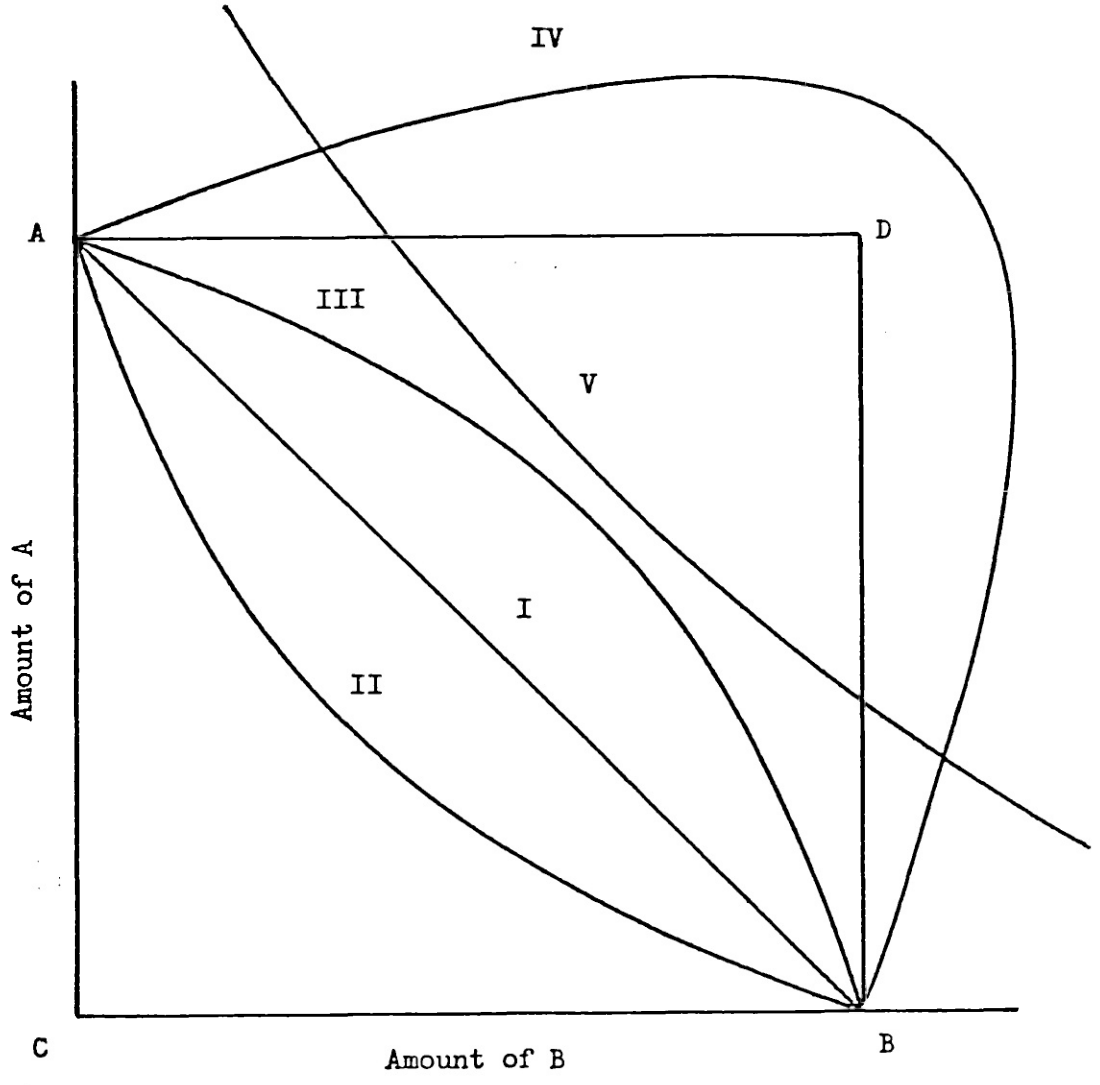


Fig.1.5 Isoboles where both compounds are separately active (I-IV), or where both are separately inactive (V). (After Hewlett and Plackett, 79).



See Section 1.4.1 for explanation.

Fig.1.6 Isobole for a pair of compounds separately active, showing potentiation. The joint action ratio is ON/OM (after Hewlett and Plackett, 79)

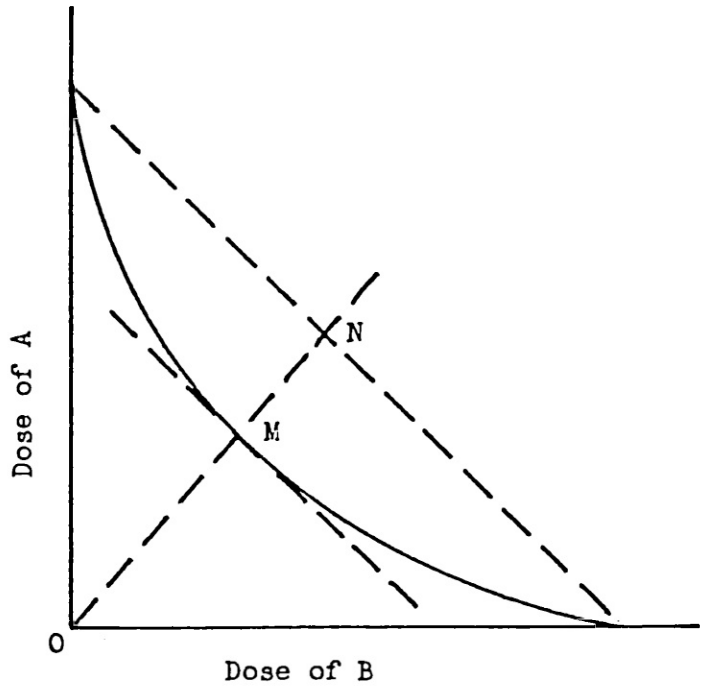
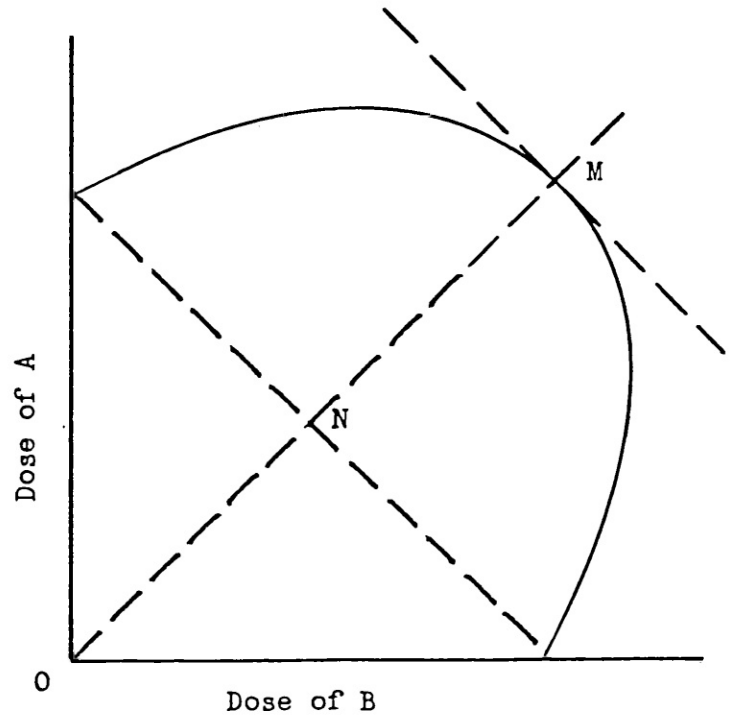


Fig.1.7 Isobole for a pair of compounds separately active, showing antagonism. The joint action ratio is ON/OM (after Hewlett and Plackett, 79)



Section 2

General Materials and Methods

2.1 Materials

2.1.1 Insects

A susceptible strain of *Sitophilus granarius*, "Windsor Normal", was obtained from the Pest Infestation Control Laboratory, Slough. Unsorted adult males and females 0-4½ weeks after emergence from the grain were used for experimental work.

2.1.2 Wheat

Pesticide-free wheat was obtained from J. Mayall & Sons, Shropshire. The wheat was a blend of Widgeon, Bounty and Flinor varieties in unknown proportions.

2.1.3 Sorptive Silica Dusts

Cab-O-Sil grades M5, H5 and EH5 were from the Cabot Corporation; Aerosil grades R972, 130 and 150, Wessalon S (now Sipernat 22S), Sipernat 22 and Sipernat D17 were from Degussa; HDK H20 was from Wacker Chemie; Gasil grades 23C, 23D, 23F, EBN, 114, AF, 35M, HP37, 200 and GM2 were from Crossfield Silicas Ltd. The physical properties and method of manufacture of these dusts are given in Appendix 1.

2.1.4 Cypermethrin

Technical grade cypermethrin (93% a.i) was obtained from Shell Research Ltd. The (*trans:cis*) ratio was 60:40.

2.1.5 ^{35}S-sodium sulphate

Crystalline sodium sulphate (18.9 mg) with specific activity 8.8 mCi (21.6.82) was obtained from Amersham International. The crystals were dissolved in distilled water (20 ml) in a syringe dispenser vial.

2.2 Methods

2.2.1 Insect culturing

A 10 lb preserve jar, wheat and filter papers were sterilized by heat at 70°C for two hours. Wheat was sterilized in a sealed container and allowed to cool and reabsorb water before use. Wheat was placed in the jar to a depth of 5 cm and two bands of fluon were painted around the inside of the jar above the wheat to prevent the insects escaping. 200-300 insects from a previous culture were added and the mouth of the jar sealed with filter paper stuck down with glue. After three weeks, the insects were sieved out of the wheat, discarded, and the wheat was resealed in the jar. Adult insects subsequently emerging from the wheat were used for experimental work. The cultures were terminated ten weeks after initiation.

Insects were reared at 25±1°C and 70±5% R.H. Under these conditions, the period from the egg being laid in the grain to the adult insect emerging from the grain was about 5½ weeks.

2.2.2 Admixing dusts with grain

Batches of wheat (100 g) at 14.5% moisture content were added to 1 lb jam jars. Dusts were separately weighed out onto filter paper which was inverted over the mouth of the jar and tapped to remove dust. The filter paper was then held firmly over the mouth of the jar and the dust mixed with the grain by holding the jar horizontally and rotating it around its vertical axis for 3 min. while shaking it vigorously for 5 seconds at the following intervals: 15 secs, 30 secs, 1 min, 1½ min, and 2 min. After mixing, the jars were kept sealed until the dust had settled on the wheat. A band of fluon was then painted around the inside of the jar above the wheat to prevent the beetles escaping.

2.2.3 Control of Wheat Moisture Content and Experimental Humidity

Stored grain reaches moisture equilibrium with the relative humidity of the atmosphere around it (87, 88, 89, 90). For all experiments in the present work, the wheat moisture content was controlled by adjusting the relative humidity of the surrounding air. Jars of treated wheat were sealed in a 12"x12"x8" plastic container with a crystallising dish containing potassium hydroxide solution at a concentration calculated to keep the relative humidity at 70% R.H (91). The container was stored at 25^{o}C for two weeks so that the wheat could reach equilibrium with the atmospheric moisture before insects were added to the jars. In order to speed up this process the wheat moisture content was previously adjusted to 14.5% by the addition of water (92), 14.5% being just below the equilibrium moisture content for 70% R.H (93).

2.2.4 Method of Assessing Bioassays

The treated wheat and beetles in each jar were tipped onto a tray (the vertical sides of which had been treated with fluon) and the beetles separated out. They were categorised as either alive or dead. Dead beetles were those that appeared brittle and did not move during a five minute observation period.

LC_{50} values and the slopes of the probit mortality/log dose lines were computed for each bioassay using a maximum likelihood program (94). The goodness of fit of observed mortalities to those calculated from the probit/log dose line were assessed using a Pearson chi-squared test.

2.3 Measurement of the Risella Oil and Beeswax Sorptivities of Sorptive Silica Dusts

2.3.1 Introduction

Since the lethal desiccant action of sorptive silica dusts is due to their ability to absorb the wax/lipid component of the insect cuticle

responsible for preventing water loss (Section 1.2.3), it is likely that the extent to which different dusts can adsorb cuticular lipid is one of the factors affecting their level of toxicity. To date, however, no method of assessing the amount of cuticular lipid that dusts absorb from insects has been devised, and other methods must therefore be used to predict this.

The sorptivity of porous silica dusts can be assessed using the oil absorption method (95). A non-volatile liquid which readily penetrates the pores of the silica is slowly added to the silica dust while the mixture is stirred until the pores are filled, at which point the dust loses its friable nature and can be moulded into a single mass held together by the surface tension of a thin film of liquid on the outer surface of the silica particles. Manufacturers estimates of the oil sorptivities of the porous silica dusts used in the present work are given in Appendix 1.

Singh (44) used an oil absorption method to assess the sorptivities of both porous and fumed sorptive silica dusts. Fumed silica dusts adsorb oil onto the surface of the aggregates rather than absorb oil into pores in the aggregates. However, oil can be held between the intricately tangled chain-like aggregates of fumed silicas (see Section 1.2.1).

Several criticisms can be levelled against the oil absorption test as a means of comparing the sorptivities of different silica dusts. Iler (25) suggests that the spaces between the aggregates of finely divided dusts become filled with oil, and gives an erroneously high estimate of the oil sorptivity compared with that of less finely divided dusts where this does not occur. Furthermore, the end point of the oil sorptivity test where the dust has absorbed all the oil it can is

subjective and is particularly difficult to judge with fumed silicas which have low bulk densities compared with the porous silicas (Appendix 1) and are not easily mixed with oil.

A different method of assessing the oil sorptivities of fumed silicas is described in the present work. The method involved finding the amount of fumed silica which could be stuck together by a given volume of Risella 17 oil. Porous silica dusts, however, absorb oil into their pores and do not stick together using the method described.

Sorptivity values for the dusts were also determined by a method which could be used for both the fumed and the porous silicas and thus compare the two types. The extent to which the dusts absorbed a beeswax coating off a glass surface was measured, a method similar to that used by Ebeling (42).

A premise of this investigation was that the ability of the dusts to absorb beeswax would reflect their ability to absorb insect cuticular lipid. Since the glands which secrete beeswax are specialised epidermal glands homologous to those that secrete a bee's epicuticular wax, it is reasonable to presume that the products are similar.

Warth (96) found that beeswax was composed of the same types of hydrocarbons, wax acids, esters and free alcohols present in varying proportions in other insect waxes, and that their concentrations in beeswax were hydrocarbons 10.5-13.5%, wax acids 13.5-14.5%, and esters 71%. The main ester present was myricyl palmitate, $C_{15}H_{31}CO.O.C_{26}H_{53}$, and the main hydrocarbon present was hentriacontane, $C_{31}H_{64}$. Unfortunately, no estimates for the contents of the cuticular lipids of *Sitophilus* spp. are available for comparison.

2.3.2 The Absorption of Risella Oil by Fumed Silica Dusts

Risella 17 oil (approx $1cm^3$) was weighed out onto an open plastic weighing pallet. The pallet was then completely covered with fumed silica shaken through a 1 mm sieve. The dust was left covering the pallet for 3 hours before being blown off with air at a set speed from an airline held 15 cm from the pallet. The pallet was reweighed and the amount of dust which adhered to the pallet was calculated. Three replicates were prepared for each fumed silica. The fumed silicas used had previously been exposed to air at 70% R.H for two weeks.

The amount of oil absorbed per wt of dust was calculated.

2.3.3 The Absorption of Beeswax by Sorptive Silica Dusts

Sixty-five glass vials (1"x2") were briefly dipped in melted yellow beeswax (B.D.H) so that the base and sides of each vial up to 3 cm from the base were coated in solidified beeswax and were weighed. Sixty vials were individually sealed inside 2 oz jars along with sorptive dust so that all of the wax-coated surface of the vials were covered in dust. Five remaining vials were not exposed to dust, as controls. All vials were then stored at $25^{o}C$.

After one week, all vials (including controls) were swabbed clean of dust with water-soaked cotton wool, rinsed in distilled water, air dried and weighed. The weight change of each vial was calculated.

2.3.4 Results and Discussion

The sorptivities of the fumed silica dusts calculated from the amounts of dust which could be stuck together by a given volume of Risella 17 oil are given in Table 2.1. The least sorptive dust was the hydrophobic silica Aerosil R972 and the most sorptive was Cab-O-Sil H5, however the range of sorptivities of the six dusts tested was small.

The oil sorptivities of the dusts derived from silica aerogels (Appendix 1) are indirectly dependent on the primary particle size of the dust. With increasing primary particle size, the specific surface area of the dusts decrease but the pore volume, pore diameter and consequently the sorptivity increase. The secondary particle size also affects the sorptivity of a dust to a small extent (Section 2.3.3). The above rules also apply to the precipitated silica dusts, however the high coalescence of their primary particles reduces pore size and pore volume so that the sorptivities of these dusts are lower than those of aerogels with the same primary particle size.

The oil sorptivities of the porous silica dusts used in the present work range from 280 g/100 g dust for Gasil HP37 (an aerogel) to 180g/100 g dust for Sipernat D17 (a hydrophobic silica), except for Gasil grades 200 and GM2 with oil sorptivities of 80 g/100 g dust.

The mean amounts of beeswax absorbed from the vials by both the fumed and porous dusts are given in Table 2.2.

The porous hydrophilic dusts absorbed more beeswax than the fumed hydrophilic and the hydrophobic silicas. However, the amount of wax absorbed/vial also reflects the bulk density of the dusts, since the amount of dust in close contact with (and able to absorb) the wax increases with increasing bulk density. To try and correct for this, the amount of wax removed/vial was divided by the bulk density of the dusts to give a relative weight for weight sorptivity value for each dust. These values are relative rather than absolute because the volume of dust into which the beeswax was absorbed was not known, and are only approximations since it was necessary to assume that the volume of each dust involved in absorption was the same.

For most of the hydrophilic sorptive dusts, the relative sorptivity values were between 0.75 and 1.30. There were three exceptions to this: Gasil grades 200 and GM2 gave values of 0.13 and 0.27 respectively, and Sipernat 22 gave a value of 0.36.

Ebeling (42) suggested that the low sorptivity of dusts with pore diameters of less than 2.2 nm may be because the larger beeswax molecules are unable to fit into such narrow pores. Gasil grades 200 and GM2 have pore diameters of less than 2 nm which is far narrower than the other dusts (Gasil handbook) and could account for their low sorptivity. Ebeling failed to note, however, that aerogels with narrow pores also have low pore volume, and both Gasil 200 and Gasil GM2 also have low oil sorptivities in comparison to other dusts.

The low beeswax sorptivity of Sipernat 22 is difficult to explain. It is possible that the large aggregates and large spaces between them inhibit the movement of beeswax.

The relative sorptivities of the three hydrophobic silicas tested were all low compared to those of the hydrophilic dusts (other than those mentioned above).

To summarise the three methods of assessing the sorptivity of silica dusts, the sorptivities of the fumed and of the porous dusts can be compared by using the method described in Section 2.3.2 and the oil absorption method (44, 95) respectively. The beeswax absorption method gave only approximate comparative estimates of the sorptivities of the dusts but was sufficient to establish that the fumed and porous dusts have comparable ranges of sorptivity (weight for weight). The hydrophobic dusts used in the present work were generally less sorptive than the hydrophilic dusts, with the exception of Gasil grades 200 and GM2.

Table 2.1 Risella 17 oil absorption of fumed silica dust

Dust	Mean oil absorption (g.)/g dust (± S.D.)
Aerosil R972	4.84 ± 0.13
Aerosil 130	4.99 ± 0.06
Aerosil 150	5.46 ± 0.06
Cab-O-Sil M5	5.77 ± 0.04
Cab-O-Sil H5	5.77 ± 0.06
Cab-O-Sil EH5	5.41 ± 0.04

Table 2.2 Amount of beeswax absorbed by silica dusts

Dust	Mean wt. change of vials (±S.D) (mg)	Relative Sorptivity
Aerosil 130	3.96 ± 0.11	1.07
Aerosil 150	2.78 ± 0.24	0.75
Cab-O-Sil M5	3.09 ± 0.19	0.84
Cab-O-Sil H5	4.41 ± 0.10	1.19
Cab-O-Sil EH5	4.33 ± 0.60	1.16
Sipernat 22	6.33 ± 0.74	0.35
Wessalon S	7.57 ± 1.41	0.84
Gasil HP37	12.51 ± 0.59	1.00
Gasil 23C	7.99 ± 1.78	1.00
Gasil 23D	8.04 ± 0.79	1.01
Gasil 23F	10.42 ± 0.37	1.30
Gasil AF	19.11 ± 3.20	0.96
Gasil 114	11.74 ± 0.47	0.78
Gasil EBN	16.30 ± 0.39	1.09
Gasil 35M	14.29 ± 0.29	1.10
Gasil 200	3.72 ± 0.44	0.13
Gasil GM2	8.61 ± 0.10	0.27
Aerosil R972	1.81 ± 0.51	0.36
Sipernat D17	2.09 ± 0.21	0.15
HDK H20	3.51 ± 0.27	0.59
Control	-0.03 ± 0.15	-

Section 3

The Toxicity and Sub-Lethal Effects of Sorptive Silica Dusts.

3.1 Introduction

The purpose of the experiments described in this Section was to determine which physico-chemical properties of amorphous silica dusts affect their insecticidal action. Bioassays were performed on twenty sorptive dusts listed in Section 2.1.3 which were selected to cover a range of physical properties (Appendix 1). The physical characteristics which varied were as follows:-

(i) Method of manufacture (i.e. fumed, aerogel or precipited)

(ii) Surface properties (hydrophilic or hydrophobic)

(iii) Mean secondary (aggregate) particle size.

(iv) Specific surface area

(v) Bulk density

(vi) Pore volume (porous dusts only)

(vii) Sorptivity

In the case of the porous dusts, physical characteristics such as specific surface area, pore volume and pore diameter are dependent on the size and extent of coelescence of the primary particles (Section 1). It is therefore not possible to vary any one of these characteristics without varying the others. Bulk density is largely dependent on the size and coelescence of the primary particles but also depends to some extent on the size of the secondary particles, since this affects packing density. The sorptivity of the dusts depends on all of the above characteristics.

Some of the dusts tested were made by grinding down the same aerogel or precipitate to different secondary particles sizes. These dusts share all physical characteristics except bulk density and sorptivity, neither

of which differ much if the difference in secondary particle size is small. Two series of dusts with these characteristics were included in the bioassays and it was therefore possible to assess the effect of different particle sizes alone on toxicity.

In the case of the fumed silica dusts, specific surface area decreases with increasing particle size, though the bulk density of the dusts does not change.

In order to determine whether or not the lethal action of sorptive silica dusts is due to accelerated water loss from insects, an experiment was performed to measure the amount of water lost from beetles exposed for 24 h to wheat treated with different types of dust.

Ten sorptive silica dusts were selected for this experiment. They represented all four types of dust used in the bioassays (hydrophobic, hydrophilic fumed, hydrophilic porous, and narrow pored/low sorptivity) and also all the different levels of toxicity, as determined in the bioassays. The extent to which the dusts caused water loss was then compared with their toxicity.

It was assumed that the weight lost by dust-treated insects was entirely due to water loss. The water loss after 24h exposure to dust treated wheat was found as follows:-

Water Loss = Weight difference + Weight of dust picked-up

The water loss experiment therefore comprised two parts; measurement of the weight difference and measurement of the amount of dust picked-up by beetles exposed to dust-treated wheat for 24h.

3.2 Methods.

3.2.1 Bioassays.

A calculated amount of dust was added and mixed with 10 jars of wheat (Section 2.2.2). Two additional jars of wheat were left free of dust as

controls. The jars were incubated at 25°C and 70% R.H (Section 2.2.3). After 14 days, 50 adult *S.granarius* (assorted sexes) were added to each jar.

The bioassays were assessed 10 days after the addition of beetles by the method described in Section 2.2.4.

3.2.2. Water loss in 24 hours

The sorptive dusts selected for this experiment were: Aerosil R972 and Sipernat D17 (hydrophobic); Cab-O-Sil M5, Cab-O-Sil H5 and Aerosil 150 (fumed hydrophilic); Gasil 35M, Wessalon S and Gasil 23F (porous hydrophilic); and Gasil 200 and Gasil GM2 (narrow pored/low sorptivity).

This experiment was in two parts:-

(i) Measurement of the amount of dust picked-up.

Dust (50 mg) radiolabelled with ^{35}S (Section 4.2.1) was added to and mixed with wheat (100g) in a preserve jar (Section 2.2.2). Three replicates were prepared for each dust. The jars were incubated at 25°C and 70% R.H in storage boxes (Section 2.2.3).

After 14 days, 50 adult *S.granarius* (unsorted sexes) were added to each jar. Twenty-four hours later, the contents of each jar were tipped onto a tray and the beetles removed with dry clean forceps. The amount of dust on the beetles was assessed radiometrically by the method described in Section 4.2.3.

(ii) Measurement of the weight change of the insects.

Dust (50 mg) was added to and mixed with wheat (100 g) in a preserve jar (Section 2.2.2). Ten replicates were prepared for each dust, and 10 jars of wheat were left free of dust as controls. All 110 jars were distributed at random into 10 storage boxes (eleven jars per box) and were incubated at 25°C and 70% R.H (Section 2.2.3).

After 14 days, a batch of 50 adult *S.granarius* (unsorted sexes) was weighed and added to each jar. Twenty-four hours later, the contents of each jar were tipped onto a tray. All 50 beetles were removed with dry clean forceps and the batch was reweighed. The weight change of each batch was calculated.

3.3 Results.

3.3.1 Bioassays.

Toxicity values for the twenty sorptive dusts tested are given in Table 3.1. These values were calculated and are expressed as described in Section 2.2.4.

All of the dusts investigated were less insecticidally active than the conventional chemical poisons used for stored grain protection (Section 1.2.5). The LC_{50} values for most of the dusts were in the region 50-150 mg.dust/100 g. wheat. Only two types of dust had toxicity levels conspicuously different from the rest.

Firstly, the three hydrophobic silica dusts used, HDK H20, Aerosil R972 and Sipernat D17 had the 2nd, 3rd and 4th lowest LC_{50} values respectively. In addition, the slopes of the probit/log. dose lines of these dusts are greater than those of all the other silica dusts. It would appear, therefore, that treatment resulting in a hydrophobic surface enhances the insecticidal activity of silica dusts.

Secondly, the two ground silica aerogels, Gasil grades GM2 and 200 were far less insecticidally active than the other sorptive dusts. Mortalities above 3% and 8% respectively could not be achieved even when the maximum amount of dust which could be admixed with the wheat without separation was used. The distinguishing feature of these two dusts was that their sorptivity was very low in comparison to the other hydrophilic dusts, which results from their low pore diameter and pore volume.

No physical characteristics other than having a hydrophobic surface and having low pore volume/low sorptivity appeared to affect the toxicity of the sorptive dusts.

Whether the dusts were fumed or porous (within the limits stated above) made no difference to their insecticidal activity. Both the hydrophilic fumed and hydrophilic porous silica dusts had similar levels of toxicity, and the dusts with enhanced toxicity due to having a hydrophobic surface included two fumed silicas and one porous silica.

The mean secondary particle size of the dusts also had no effect on insecticidal activity. In order of increasing secondary particle size, Gasil 35M, Gasil 114, Gasil EBN and Gasil AF were dusts originating from the same aerogel, as were Gasil 23D, Gasil 23C and Gasil 23F. Therefore each of the dusts in these two series differed only in their secondary particle sizes, and this did not affect insecticidal activity.

The bulk densities of the dusts ranged from 3.7g/100 ml to 20g/100 ml., but this had no noticeable effect on their insecticidal activities.

Specific surface area itself did not affect insecticidal activity. Most of the dusts tested had specific surface areas in the range 110-350 m^2/g. and specific surface area was not related to the toxicity of a dust within this range. However, Gasil grades 200 and GM2 with specific surface areas of 750 m^2/g were almost insecticidally inactive. This was due to a combination of physical characteristics resulting in low sorptivity (see above) rather than specific surface area.

3.3.2 Water Loss.

The amounts of each dust picked up by the beetles in 24 hours are given in Table 3.3 and the calculated amounts of water loss from the beetles caused by the dust are given in Table 3.2

To calculate the water loss caused by each dust, the mean amount of dust picked up by 50 beetles was added to the weight difference of each batch of 50 beetles before and after exposure to the wheat treated with sorptive dust. The water loss was then calculated as a percentage of the original weight of each batch of insects. The mean water loss was calculated for each dust.

Four different types of dust caused significantly different levels of water loss, all of which were significantly greater than the water loss from control insects. The levels of water loss were as follows:-

Hydrophobic dusts > Porous (hydrophilic) dusts > Fumed (hydrophilic) dusts >> Porous (low sorptivity) dusts

There were no mortalities in any of the treatment groups in the water loss experiment.

3.4 Discussion.

The amounts of water loss caused by each of the groups of dust identified in the previous Section does reflect their order of toxicity to *Sitophilus granarius*. However, the porous and fumed hydrophilic silicas caused different amounts of water loss despite having similar levels of insecticidal activity.

The hydrophobic silica dusts caused the highest level of water loss and correspondingly were the most toxic to the beetles. The sorptivities of these dusts, however, measured in terms of both oil sorptivity and beeswax sorptivity were lower than those of physically similar hydrophilic dusts. If the lethal action of the silica dusts is through absorption of epicuticular lipid, one might have expected the toxicity of the hydrophobic silicas to have been comparatively low. There are several possible explanations for this: (a) hydrophobic dusts have a lethal action which leads to water loss other than through the removal of epicuticular

lipids; (b) hydrophobic dusts may only absorb certain components of the insects epicuticular lipid, and these components may be particularly important in preventing water loss; and (c) hydrophobic dusts may be picked-up and turned-over on the surface of the beetles to a greater extent than the hydrophilic dusts.

Sorptive dusts are not known to promote water loss from insects by any means other than the removal of epicuticular lipid. It is unlikely that hydrophobic dusts could physically absorb water from the insects because they are water repellent. It is also most unlikely that any type of sorptive silica dust could interfere with a neuro-endocrine controlled water retention mechanism because there is no physical means by which the dust could enter the insects haemocoel.

The absorption of epicuticular lipid from insects by sorptive dusts has only been observed visibly (42) and consequently the type of lipids absorbed by the dusts have never been identified. However, since techniques for the identification and quantification of the components of the insects cuticular lipids have been developed (4, 5, 6, 7) it should be possible to investigate whether hydrophobic silica dusts do absorb specific components of the lipid barrier. The epicuticular lipid extracted from insects exposed to a hydrophobic dust and from those exposed to a hydrophilic dust could be compared with that extracted from control insects. Any specific type of epicuticular lipid removed by either of the two types of sorptive dusts could be identified.

The influence of the extent of pick-up and turn-over of different sorptive dusts on their insecticidal activity is discussed in Section 4.

The magnitude of the slopes of the probit/log dose lines derived from the results of the bioassays indicate that the range of susceptibility of the beetles to the hydrophobic dusts was narrower than their susceptibility

to the hydrophilic dusts. Consequently, the calculated LC_{95} values for the hydrophobic dusts were far lower than those of the hydrophilic dusts (Table 3.4). The LC_{95} values of the hydrophilic dusts were generally higher than the amount of dust which could be mixed with wheat without separation (approx 300 mg dust/100 g wheat). These dusts, therefore, could not be expected to give 100% kill under the experimental conditions of the bioassays.

Gasil grades 200 and GM2 caused comparatively little water loss and consequently had very low insecticidal activity. These two dusts have low oil and beeswax sorptivities in comparison to the other silica dusts tested (Section 2.3) and the likely reason for their low toxicity was that they were unable to absorb cuticular lipid rapidly enough to allow accelerated water loss through the insects cuticle.

Singh (44) also found Gasil 200 to be insecticidally inactive against *Tribolium castaneum* in stored wheat, whereas other more sorptive silica dusts (which also had wider pores or were fumed silicas) were insecticidally active.

It should be possible to determine whether the low toxicity of Gasil grades 200 and GM2 was due to their low sorptivities or due to having pores too narrow to allow the entry of cuticular lipids as suggested by Ebeling (42). Bioassays could be performed on a series of dusts whose pore volumes were gradually reduced to 0.4 cm^3/g and whose pore diameters reduced to 2nm, which are the specifications of Gasil 200 and Gasil GM2. Unfortunately no dusts with pore volumes between 0.4 cm^3/g and 1.6 cm^3/g could be obtained for inclusion in the present work. A gradual reduction in the toxicity of the dusts as the pore diameter approached 2 nm would indicate that the pore volume/sorptivity of the dust influenced insecticidal activity. If, however, the toxicity of

the dusts remained at approximately the same level as the pore diameter approached 2nm then dramatically decreased at 2nm diameter, this would indicate that there is a threshold pore diameter below which cuticular lipid molecules could not penetrate.

The hydrophilic dusts (other than Gasil grades 200 and GM2) all had similar levels of toxicity to *S.granarius*. However, the porous silicas caused more water loss from the beetles in 24 h than the fumed silicas. The different levels of water loss could be explained if the initial rates of pick-up and turn-over of dusts by the insects differed (Section 4). It is likely that both the rate of water loss and the eventual amount of water lost are responsible for the physiological stress lethal to an insect. It is most unlikely that either the fumed hydrophilic or the porous hydrophilic silica dusts kill insects other than by physical means because the two types of dust are chemically very similar.

Ideally the toxicity of different types of sorptive silica dusts should be compared with the water lost from dust-treated insects immediately prior to their death. This, however, is experimentally impossible with such a large number of beetles loose in stored grain. If the water loss from the beetles was measured after ten days exposure to wheat treated with dust, the same duration as the bioassays, the concentrations of dust involved would have caused mortalities amongst the beetles. This would invalidate the experiment because dead insects desiccate more quickly than live insects (Ingram 70), probably because dead insects have no active water retention mechanism.

Estimating the water loss from insects after 24 h exposure to dusts when no mortalities have occurred should be regarded as the best possible method of comparing the action of sorptive dusts. Other factors such as the pick-up and turn-over of the dust particles on the beetles also affect

their ultimate toxicity.

A number of workers have found relationships between the insecticidal activity of desiccant dusts and physical characteristics other than pore volume and whether the dusts have hydrophilic or hydrophobic surfaces (Section 1.2.4). In each case, however, the experiments described involved "abrasive" dusts either as well as or instead of "sorptive" dusts.

Although both Ebeling (42) and Mellichar and Willomitzer (43) found a positive correlation between the specific surface area and insecticidal activity of dusts, their experiments included "abrasive" dusts (with relatively low sorptivities and specific surface areas) as well as "sorptive" dusts. The present work confirms Singh's observations (44) that amongst truly "sorptive" dusts (with relatively high sorptivities and specific surface areas), there is no correlation between specific surface area and toxicity.

Correlations observed between the particle size and toxicity of abrasive dusts (33, 40, 42) did not hold true for the sorptive dusts used in the present work, even amongst dusts of different particle sizes derived from the same gel (and therefore all other physical characteristics were equal). However, in the case of fumed silica dusts, which have chain-like aggregates, particle size is not a well-defined concept and details of aggregate particle sizes (the "equivalent" to secondary particle size of porous dusts) are not supplied by dust manufacturers. The effects of particle size on the toxicity of fumed silicas cannot, therefore, be assessed.

Table 3.1 Results of the Bioassays

Name of dust	LD_{50} value mg/100 g wheat	95% confidence interval LD_{50} value	Slope	95% confidence interval of slope	χ^2	d.f.
Aerosil 130	101.1	91.1 - 112.3	2.67	2.16 - 3.18	13.54	8
Aerosil 150	86.6	77.4 - 96.6	2.43	1.94 - 2.92	12.33	8
Cab-O-Sil M5	79.1	66.6 - 93.9	1.53	1.06 - 2.01	9.28	8
Cab-O-Sil H5	108.7	94.7 - 124.6	2.03	1.55 - 2.51	10.75	8
Cab-O-Sil EH5	118.3	100.7 - 139.0	1.64	1.18 - 2.10	8.80	8
Sipernat 22	70.6	59.9 - 83.3	1.70	1.29 - 2.11	8.97	8
Wessalon S	91.6	82.2 - 102.1	2.69	2.15 - 3.23	13.62	8
Gasil HP37	49.2	40.8 - 59.3	1.94	1.49 - 2.39	10.14	8
Gasil 23C*	72.9	64.7 - 82.2	2.35	1.90 - 2.79	25.10	8
Gasil 23D*	92.8	77.5 - 110.9	1.43	1.03 - 1.84	32.20	8
Gasil 23F	55.9	46.0 - 67.8	1.68	1.29 - 2.07	12.70	8
Gasil 114	94.9	84.2 - 106.8	2.30	1.86 - 2.74	3.68	8
Gasil AF	149.0	126.6 - 175.2	1.91	1.49 - 2.33	9.68	8
Gasil EBN*	70.1	57.8 - 80.9	2.01	1.58 - 2.43	28.6	8
Gasil 35M	86.9	77.7 - 97.2	2.50	2.00 - 2.99	14.3	8
Gasil 200	only 8% mortality at 203 mg dust/100 g wheat					
Gasil GM2	only 3% mortality at 203 mg dust/100 g wheat					
Aerosil R972*	51.8	47.8 - 56.1	3.66	3.08 - 4.24	36.6	8
Wacker HDK H20	49.7	46.0 - 53.6	4.29	3.60 - 4.98	13.87	8
Sipernat D17	55.5	51.6 - 59.6	4.89	4.10 - 5.67	12.75	8

* On referring χ^2 value to tables, $p<0.05$, and therefore the observed probit mortalities are significantly different from those predicted by the probit/log dose line.

Table 3.2 The water loss after 24 h caused by sorptive dusts

Dust	Dust Type	Mean water loss %	Group mean water loss %	Transformed means (± S.D.)
Control	-	0.30		1.48±4.82
Sipernat D17	HP	7.75	8.00[a]	16.12±1.34
Aerosil R972	HF	8.25		16.63±1.52
Gasil 35M	P	6.66	6.23[ab]	14.90±1.50
Wessalon S	P	5.22		13.11±1.72
Gasil 23F	P	6.81		15.05±1.67
Cab-O-Sil H5	F	4.56	4.10[bc]	12.29±1.09
Cab-O-Sil M5	F	4.23		11.83±1.07
Aerosil 150	F	3.52		10.64±2.05
Gasil 200	PN	1.70	1.32[c]	7.39±1.23
Gasil GM2	PN	0.93		4.98±2.83

a,b,c: Two group means significantly different (single degree of freedom comparison; n=10, $p<0.001$)

H, hydrophobic. P, porous. F, fumed. N, narrow pore diameter

Table 3.3 Mean amounts of dust picked-up by beetles in 24 h from wheat treated with dust at 50 mg dust/100 g wheat

Name of dust	Mean wt. dust (µg)/insect (± S.D.)	Mean wt. dust (mg)/ 50 insects (± S.D.)
Aerosil R972	10.71±0.25	0.536±0.012
Sipernat D17	12.86±0.21	0.643±0.010
Gasil 35M	8.02±0.79	0.401±0.039
Gasil 23F	8.85±1.14	0.443±0.057
Wessalon S	7.42±0.62	0.371±0.031
Aerosil 150	5.05±0.11	0.253±0.005
Cab-O-Sil M5	5.68±0.26	0.284±0.013
Cab-O-Sil H5	5.18±0.52	0.259±0.026
Gasil 200	9.32±0.59	0.460±0.030
Gasil GM2	7.77±0.96	0.389±0.048

Table 3.4 Calculated LC_{95} values for the sorptive dusts

Dust	LC_{95} (mg dust/100g wheat	Dust	LC_{95} (mg dust/100g wheat
Aerosil R972	145	Wessalon S	373
HDK H20	120	Gasil HP37	345
Sipernat D17	120	Gasil 23C	364
Aerosil 130	416	Gasil 23D	1302
Aerosil 150	410	Gasil 23F	529
Cab-O-Sil M5	933	Gasil 114	490
Cab-O-Sil H5	698	Gasil AF	1076
Cab-O-Sil EH5	1183	Gasil EBN	459
Sipernat 22	650	Gasil 35M	393

Section 4

The Pick-up and Turn-over of Silica Dusts by Insects in Dust Treated Wheat

4.1 Introduction

It is most likely that the insecticidal activity of sorptive silica dusts depends on their rate of turn-over and equilibrium pick-up level on the surface of the insect (Section 1.2.4) as well as their capacity to absorb epicuticular lipids. The aim of the experiments in this Section was to see how the level of toxicity of the four groups of dusts identified in Section 3 was related to their rate of turn-over and equilibrium pick-up levels.

The experiments to determine the equilibrium pick-up levels and rates of turn-over involved assaying the amount of radio-labelled dust on the beetles for up to 24 h after their introduction to dust-treated wheat. Since no silica dust containing a radioactive isotope as part of its structure could be obtained, low concentrations of ^{35}S-labelled sodium sulphate were deposited on the dusts, a method similar to that used by Singh (44). Sodium sulphate was chosen to label the dusts because it was cheap, and being an ionic salt would dissolve in water but would not be transferred from the sorptive dusts into the insects epicuticular lipids.

If insects coated with a radioactive dust were added directly to a scintillant solution and the radioactivity assessed in a scintillation counter, the sodium sulphate would not dissolve and most would remain on the beetles. Consequently, approximately half of the β-particles from the radioactive decay of the ^{35}S would be adsorbed by the beetles. In addition, all of the beetles would lie together at the bottom of the scintillation vial which would result in further adsorption of β-particles

between the beetles.

In order to count radio-active disintegrations more efficiently, beetles were dropped into an aqueous solution to extract the sodium sulphate from the dusts and a scintillant solution added which formed a gel in which the beetles were suspended. The isotope in the vials was then assayed radiometrically. This method was quicker than taking aliquots of the aqueous solution with extracted sodium sulphate and adding them to a scintillant solution without the beetles, and obviated errors in measurement due to pipetting the extract.

Since the beetles were the main quenching agent in the scintillation vials, the quench curve was made by using different numbers of beetles to quench the ^{35}S standard.

Two additional experiments are described in this Section. The first was designed to show that: the ^{35}S in the dust was all extracted into aqueous solution and counted no matter how closely it adhered to the insects; no dust was lost transferring insects to the scintillation counter vials; and that the form of quench curve used provided reproducible corrected results.

The second experiment was designed to show that the sodium sulphate did not separate from the silica dust when mixed with the grain and that the presence of sodium sulphate on the surface of the dust did not affect its pick-up by the beetles.

4.2 Methods

4.2.1 Labelling the dusts

Sulphur -35 has a relatively short half-life (87.2 days). Consequently the concentration of $Na_2{}^{35}SO_4$ in the stock solution (Section 2.1.1) decreased considerably in the course of the present work and it was necessary to use higher quantities of the stock solution to prepare

radio-labelled dusts as the work progressed. Therefore only a qualitative description of the method used to label the dusts is given in this Section. The amount of sodium salt deposited on each silica dust never exceeded 0.06% w/w.

A calculated amount of ^{35}S-labelled salt from the stock solution was dried *in vacuo* in a round-bottomed flask over phosphorus pentoxide. The salt crystals were broken up and a calculated amount of Analar methanol added (the solubility of sodium sulphate in methanol is 2.43×10^{-4} g/g methanol, Seitell (97)). The flask contents were then magnetically stirred for 24h and the flask contents filtered through Whatman No.1 filter paper. The filtrate was added to the dust in a round-bottomed flask and evaporated to dryness. The amount of filtrate required was 10 cm^3/1000 mg silica dust. However, an additional 5 cm^3 of unlabelled methanol was required to cover the fumed silicas due to their low bulk densities.

4.2.2 Preparation of the quench curve

A quantity of ^{35}S-dioctyl sulphide of known specific radioactivity was weighed out into ten scintillation vials and a solution of scintillant (10 cm^3) was added to each. The scintillant solution used was "Cocktail T" (BDH) which contained (per litre): toluene, 660 cm^3; Triton X-100, 332 cm^3; 2,5-Diphenyloxazole (PPO), 5 g; and 1,4-Di-2-(5-phenyl-oxazolyl)-benzene (POPOP), 0.15 g.

Aqueous 0.05 M Na_2SO_4 (2 cm^3) and distilled water (3 cm^3) was added to each vial and a number of *S.granarius* (0-60) were added to the vials to quench the scintillant. The vials were then shaken and the contents formed a gel in which the beetles were suspended.

The vials were counted on a Beckman LS-250 scintillation counter which uses an external standard β-source to give an index of the counting

efficiency (expressed as an S-number) by the channels ratios method. The counting efficiency of the vials (%) was calculated (Appendix 2) and plotted against the respective S-number to give a quench curve (Appendix 3).

4.2.3 Radiometric assay of the labelled dust on the beetles

After having been removed from wheat treated with a radio-labelled dust, the beetles were dropped into a scintillation vial containing aqueous 0.05 M Na_2SO_4 (2 cm^3). The vials were left standing for 15 min before distilled water (3 cm^3) was added and the vials were sealed and rotated for 15 min. Insects coated with a hydrophobic dust were dropped into a vial containing Analar methanol (0.25 cm^3) and left for 5 min before the addition of aqueous Na_2SO_4. Scintillant solution (10 cm^3) was added to each of the vials which were then sealed and shaken so that the contents formed a gel in which the beetles were suspended. The vials were counted on a Beckman LS-250 scintillation counter. An S-number was also obtained as a measure of the counting efficiency.

For both the pick-up and the turn-over experiment, the beetles removed from each of the jars of wheat treated with labelled dust were all counted into the same scintillation vial.

4.2.4 Evidence that ^{35}S in dusts adhered to insects was reproducably extracted and counted

Samples of radio-labelled dust were weighed out into four groups (A, B, C and D) of 5-6 scintillation vials. Aqueous 0.05 M Na_2SO_4 (2 cm^3) was immediately added to groups A and B. A varying number of *S.granarius* (ranging from 5-45) were added to group A vials and 25 *S.granarius* were added to group B vials. The dust in both groups of vials was then assayed radiometrically as described in Section 4.2.3.

Twenty five *S.granarius* were added to the vials in groups C and D and left for 24 h. so that the insects could pick up the dust. Following this, the dust in group C vials was assayed radiometrically. A fifth group of 5-6 vials (group E) was prepared containing aq. 0.05 M Na_2SO_4 (2 cm^3). All the insects from each vial in group D were transferred to a corresponding vial in group E. Twenty five fresh *S.granarius* were added to group D vials and then the dust in both groups D and E vials were assayed radiometrically.

For the experiment involving Aerosil R972, Analar methanol (0.25 cm^3) was added to the insects (or vice versa) before the addition of aq. Na_2SO_4.

The d.p.m./mg dust (the specific activity) was calculated for all the vials in groups A, B and C. The d.p.m. obtained from corresponding vials in groups D and E were added together and the specific activity of the dust in each pair of vials was calculated. The figures from each group were compared using one-way analysis of variance.

4.2.5 Evidence that Na_2SO_4 is neither separated from nor affects the pick-up of silica dusts

Labelled Na_2SO_4 was deposited at two concentrations on different batches of Aerosil R972, Wessalon S and Cab-O-Sil M5 by the method described in Section 4.2.1. Dust (10 mg) was mixed with wheat in preserve jars (Section 2.2.2). Five-six replicates were prepared for both the high and low activity batches of each dust. Fifty adult *S.granarius* were added to each jar and the jars were incubated at 25°C.

After 24 h. as many beetles as possible were picked off the surface of the wheat with fine forceps and the amount of dust on them assayed radiometrically. The number of beetles/scintillation vial was recorded.

To calibrate each pick-up experiment, samples of the dust were weighed out into five scintillation vials. An aliquot of aqueous 0.05 M Na_2SO_4 (2 cm^3) followed by 20-30 untreated *S.granarius* were added to each. Methanol (0.25 cm^3) was added to Aerosil R972 before aqueous Na_2SO_4.

The dust content of the vials was assayed radiometrically (Section 4.2.3) and the specific activities (d.p.m./mg dust) of both the high and low activity batches of each dust were calculated.

4.2.6 The amount of dust accumulated on the beetles over 24 h. exposure to dust-treated wheat

Sixteen of the dusts listed in Section 2.1.3 which had a range of physico-chemical characteristics were included in this experiment.

Radio-labelled dust (10 mg) was added to each of 10 jars of wheat (100 g) and mixed (Section 2.2.2). The jars were incubated at 25°C and 70% R.H. (Section 2.2.3). After 14 days, 50 adult *S.granarius* (unsorted sexes) were added to each jar. The beetles were exposed to the dust for a given time before as many as possible were picked off the surface of the wheat and the amount of dust on them assayed radiometrically. The number of beetles/vial was recorded. The beetles were exposed to wheat treated with the dust for 1, 3, 6, 12 and 24 h., with two replicate jars for each time.

The specific activity of the dusts used in the pick-up experiment was calculated by the method described in Section 4.2.5.

4.2.7 The turn-over of sorptive dusts on the beetles at equilibrium level

The turn-over of two dusts from each of the groups identified as causing different levels of water loss from the beetles (Section 3.4.2) was investigated. The dusts were: Aerosil R972 and Sipernat D17 (hydro-

phobic silicas); Aerosil 150 and Cab-O-Sil M5 (fumed hydrophilic silicas): Wessalon S and Gasil 35M (porous hydrophilic silicas);and Gasil grades 200 and GM2 (low porosity/low sorptivity silicas).

Radio-labelled dust (10 mg) was admixed with wheat (100 g) in each of 12 jars, and unlabelled dust (10 mg) admixed with wheat in each of 10 jars (Section 2.2.2). The jars were incubated at 25°C and 70% R.H. (Section 2.2.3). After 14 days, 50 adult *S.granarius* (unsorted sexes) were added to each jar of wheat that had been treated with radio-labelled dust. Twenty-four hours later, after the insects had picked-up an equilibrium level of dust, the contents of each jar were gently tipped onto a tray. The beetles from two jars were immediately collected and the amount of dust on them was assayed radiometrically. The beetles from each of the remaining jars were collected and each batch of beetles was transferred to a jar of wheat treated with un-labelled dust. The beetles were exposed to the unlabelled dust for 1, 3, 6, 12 or 24 h., with two replicate jars for each time. After exposure the beetles were picked off the surface of the wheat and the amount of radiolabelled dust still left on them was assayed radio-metrically. The number of insects/scintillation vial was recorded.

The specific activity of the dusts used in this experiment was calculated by the method described in Section 4.2.5.

4.3 Results and Discussion

4.3.1 Evidence that ^{35}S in dust adhered to the beetles was reproducibly extracted and counted

There was no significant difference in the mean specific activities of the dusts calculated from the vials in groups A, B, C and (D+E) for either Wessalon S, Cab-O-Sil M5 or Aerosil R972 (in each case, $p>0.05$, One-Way analysis of variance). The results of these experiments are shown

in Table 4.1.

Although the vials in group A contained different numbers of beetles, this did not significantly affect the calculated specific activity of the dust in these vials. This indicates that the novel form of quench curve used (where beetles were used to quench the scintillant) was able to correctly convert c.p.m. to d.p.m.

The difference in the preparation of the vials in groups B and C was that the insects in group C vials were allowed to pick-up the dust for 24 h. before the amount of dust was assayed radiometrically, whereas the insects in group B vials were not given time to pick-up the dust. However, there was no significant difference in the calculated specific activity of the dusts in either of these two groups of vials. This demonstrates that the method of radiometrically assaying the dust on the beetles described in Section 4.2.3 extracted the ^{35}S from the dust whether it was closely adhered to the beetles or not.

The mean specific activity of the dusts calculated by adding together the d.p.m. obtained from corresponding vials in groups D and E did not significantly differ from the values obtained from groups A, B and C. This shows that no dust was lost when the beetles were transferred from the dust-treated wheat to the scintillation vials.

The technique for extracting and radiometrically assaying $Na_2{}^{35}SO_4$ described in the present work was designed to assay dust only on the surface of the beetles. Sulphur-35 on dusts ingested by the beetles would not have been extracted and counted. It is unlikely, however, that ingestion of the dusts would have had any effect on their toxicity to the beetles since Ebeling *et al* (98) found that the toxicity of a silica aerogel dust to *Periplaneta americana* was the same whether or not the insects had had their mouths sealed. Failure to detect $Na_2{}^{35}SO_4$

on dust ingested by the beetles was therefore an advantage of the assay method described in this work.

4.3.2 Evidence that Na_2SO_4 was neither separated from nor affected the pick-up of silica dusts

For all three types of dust tested, there was no significant difference between the mean amounts of high and low activity dusts (indicating high and low Na_2SO_4 content respectively) picked up by the beetles ($p>0.05$, two way analysis of variance). The results of these experiments are shown in Table 4.2.

The results demonstrated that the Na_2SO_4 did not separate from the silica dust in the wheat. If the Na_2SO_4 had separated out, insects exposed to the high activity silica dust would have apparently picked-up more dust than those exposed to the low activity silica dust.

Secondly the results demonstrated that the amount of Na_2SO_4 on a dust (within the concentration levels used) did not significantly affect the extent to which a dust could be picked-up by the beetles. It is most likely therefore, that the amounts of radiolabelled dust on the beetles in the pick-up and turn-over experiments does reflect the amounts of unlabelled dust on the insects.

The above findings apply to all three types of silica surface used in the present work:- a hydrophobic dust (Aerosil R972); a hydrophilic porous surface (Wessalon S); and a hydrophilic fumed silica (Cab-O-Sil M5).

4.3.3 The amount of dust accumulated on the beetles over a 24 h. exposure period to dust treated wheat

Figures 4.1-4.8 show the mean weight of dust/insect adhering to *S.granarius* at various times (0-24 h) after the beetles had been introduced into wheat treated with different types of silica dust at 10 mg/100g.

In each case, the dust concentration was less than the calculated LD_{50} for a 10 day bioassay, and no mortality was observed in the test populations over the 24 h exposure period. The original data for these experiments are given in Appendix 4.

The amounts of most of the dusts which adhered to the beetles reached an equilibrium level within 24 h. of the beetles having been introduced to the wheat. The remaining dusts were all close to equilibrium after this period of exposure, and it is likely that all would have reached equilibrium level within 36 h. exposure. Gasil grades GM2 and 200 reached equilibrium level within 1 h. of the beetles having been introduced to the wheat, faster than any of the other dusts.

The equilibrium pick-up levels of the two hydrophobic dusts tested, Aerosil R972 and Sipernat D17 were far higher than those of the other dusts. The equilibrium level of both of these dusts was approximately 3.5 µg/insect.

There was little difference in the equilibrium pick-up levels of the hydrophilic porous and hydrophilic fumed silica dusts tested; all were within the range 1.0-2.2 µg dust/insect. A rank test, however, shows that the median equilibrium pick-up levels of the porous silicas and the fumed silicas were significantly different ($p = 0.018$, Mann-Whitney test), the porous silica dusts having been picked-up more than the fumed silicas.

When the insects were exposed to the dusts at 50 mg dust/100 g. wheat (Section 3.2.2) the amounts of the two hydrophobic dusts on the beetles 24 h. after their introduction to the wheat were again higher than the other types of dust. There was also a distinct difference between the amounts of hydrophilic porous and hydrophilic fumed silicas picked-up by the beetles. The porous silicas were picked-up to a

greater extent than the fumed silicas. This experiment, however, gave no indication of whether the amounts of dust on the beetles represented equilibrium levels or not.

Three groups of dust can therefore be distinguished by their different equilibrium pick-up levels: hydrophobic silicas > hydrophilic porous silicas > hydrophilic fumed silicas. Narrow pore diameter or low porosity made no difference to the equilibrium pick-up levels of the porous silica dusts since the equilibrium levels of Gasil grades 200 and GM2 were in the same range as the pick-up levels of the other porous silica dusts.

No characteristics other than a hydrophobic surface and whether the dust was a fumed or a porous silica appeared to affect the extent to which the dusts were picked-up by the beetles. Although various authors have made casual observations of the extent to which a target insect picked-up a particular dust insecticide, no previous work has been performed to find which characteristics of dust insecticides affect the extent to which insects pick them up. As part of another experiment, however, Alexander *et al.* (33) observed that particles of carborundum above 15 μm in diameter adhered poorly to insects and that adhesion increased progressively with reduction of particle size from 10 μm to 5 μm diameter. No such effect was observed in the present work. The equilibrium pick-up level of the precipitated silica Sipernat 22 was similar to that of the other porous hydrophilic dusts despite its comparatively large particle size ($\sim$80 μm diameter).

Using a similar experimental procedure to that described in the present work (Section 4.2.6), Singh (44) found that the equilibrium pick-up levels by *T.castaneum* of four sorptive silica dusts from wheat were in the order: Aerosil R972 > Gasil 200 > Wessalon S $\simeq$ Cab-O-Sil M5.

In the present work the rank of the pick-up levels of these four dusts by *S.granarius* was similar: Aerosil R972 > Gasil 200 = Wessalon S > Cab-O-Sil M5. The difference in the relative levels of pick-up of these four dusts by *T.castaneum* and *S.granarius* may have been due to the different method used by Singh to radiometrically assay the amount of radiolabelled dust on the beetles. Singh (44) added the beetles directly to the scintillant solution so that the $Na_2{}^{35}SO_4$ would not have dissolved off the dust which coated the beetles. Consequently, many of the β-particles (from the radioactive decay of the sulphur-35) would have been absorbed by the insects. If the different dusts (which contained Sulphur-35) had been washed off the beetles into the scintillant solution to different extents, the counting efficiency would have been higher the more the dust was washed off the beetles. Therefore the apparent relative equilibrium pick-up levels of the dusts by *T.castaneum* may have been incorrect.

4.3.4 The turn-over of sorptive dusts on the beetles at equilibrium level

Figures 4.9-4.12 show the mean amounts of radiolabelled dust on the beetles over a 24 h. period after their introduction to wheat treated with non-radiolabelled dust. As with the above experiment, no mortality in the test populations was observed. The original data for these experiments are given in Appendix 5.

The figures show that the amount of radiolabelled dust on the beetles declined towards a residual equilibrium level. It must be stressed, however, that the total amount of dust on the beetles (labelled plus unlabelled dust) remained the same. These results therefore confirm Singh's observation (44) that there is a turn-over of dust on the surface of the beetles and that a proportion of the original radioactive dust apparently remains on the beetles.

Seven of the radiolabelled dusts tested were close to reaching a residual equilibrium level on the beetles 24 h. after their introduction to wheat that had been treated with unlabelled dust, and would probably have reached equilibrium within 36 h. The amount of Aerosil 150 on the beetles was not as close to equilibrium as the other dusts, but would probably have reached it after 48 h.

The equilibrium amount of dust on the insects (Section 4.3.3) is reached when the rate at which the beetles pick-up fresh dust from the wheat equals that with which it is rubbed off again. Ideally, therefore, the amount of labelled dust on the beetles at any time after their introduction to wheat treated with unlabelled dust will depend on the rate of loss of labelled dust from the beetles as well as the rate at which it is regained from the wheat.

In the present work, however, two assumptions were made about the nature of turn-over which simplified the calculation of rate of turn-over:-

(a) The amount of dust on the beetles at equilibrium level was at the most only 1.75% of the amount still on the wheat (Section 4.3.3). Therefore when beetles coated with an equilibrium level of labelled dust are transferred to wheat treated with unlabelled dust, the amount of labelled dust returned to the beetles from the wheat would be negligible and therefore the rate of reduction of labelled dust on the beetles would be approximately first order.

(b) The amount of dust on the beetles at equilibrium pick-up level comprises a proportion that is irreversibly bound to the beetles and is not exchangeable and a proportion that is "loose" and exchangeable (Singh, 44). The former is equivalent to the new residual equilibrium level of labelled dust on the beetles after their transfer to wheat

treated with unlabelled dust.

The rate of reduction in the amount of labelled dust on the beetles would be described by the following equation:-

$$a_1 = a_o e^{-kt}$$

$$\text{or} \quad \ln a_1 = \ln a_o - kt$$

where t = time after introduction of beetles to wheat treated with unlabelled dust (h)

k = rate constant (h^{-1})

a_o = amount of the "loose" component of the radiolabelled dust on the beetles ($a_t - a_\infty$) at t=0 (µg/insect)

a_1 = amount of the "loose" component of the radiolabelled dust on the beetles ($a_t - a_\infty$) at time t (µg/insect)

a_t = total amount of labelled dust on the beetles at time t (µg/insect)

a_∞ = the residual level of radiolabelled dust on the beetles (µg/insect).

Although none of the labelled dusts had quite reached equilibrium levels, values were estimated visually from Figs.4.9-4.12 and are given in Table 4.3. The rate of turn-over of a dust is calculated from k x a_o, using values obtained from the slope and intercept of the line fitted to plots of $\ln a_1$ against time.

The plots of $\ln a_1$/time were significantly non-linear for Gasil grades 200 and GM2 but were not so for the remaining dusts (Appendix 6). The calculated rates of turn-over of the latter and the respective regression equations are given in Table 4.4. Non-linearity was determined using standard methods (99), partitioning the residual sum of squares from the regression into components due to pure sampling error

(from one-way ANOVA) and lack of fit to the linear model.

Visually the plots of $\ln a_1$/time appeared curvilinear. This was most pronounced for Gasil grades 200 and GM2 and was least noticable for the two hydrophobic dusts. The most likely explanation for the curvilinearity is that the particles of dust on the beetles are not all exchangeable with equal ease. Dust particles in exposed places might be exchanged more rapidly than those in protected places on the insect, as indicated by the initially steep and later shallow slopes respectively of the plots of $\ln a_1$/time. Why this should be more pronounced as the toxicity of the groups of dusts decreases is unclear. It is possible that the two non-toxic dusts, Gasil grades 200 and GM2 have relatively poor adhesion to the epicuticular lipid owing to their low sorptivities.

The first order model was sufficient to establish the range of the rates of turn-over of sorptive silica dusts on *S.granarius*, but could not be used to accurately compare the rates of turn-over of different silica dusts because the 95% confidence intervals of the calculated rates were too wide. More accurate estimates of the rates of turn-over might be made using rate constants calculated from both the initial and later loss of labelled dust from the beetles, however more points on the plots of $\ln a_1$/time would be required.

Statistical comparisons of both the slopes and intercepts of the regression lines for all six insecticidally active silica dusts (Table 4.5) show that the rate constant (- slope of the regression line) for the turn-over of Aerosil 150 was significantly lower than those of all the other dusts, and the intercept (ln of the amount of "loose" dust on the beetles) for Gasil 35M was significantly lower than those for Sipernat D17, Aerosil R972 and Aerosil 150. This indicates that the rate of turn-over of Aerosil 150 was significantly lower than those of

all the other dusts except Gasil 35M, and that the rate of turn-over of Gasil 35M was significantly lower than those of Sipernat D17 and Aerosil R972. No other significant differences in the rates of turn-over of the dusts could be distinguished.

The amounts of the two hydrophobic dusts that were irreversibly bound to the insects and not exchangeable were greater than those of the hydrophilic dusts. Better estimates of the non-exchangeable levels of dust could be obtained if insects which had picked up an equilibrium level of labelled dust were exposed to two batches of wheat similarly treated with unlabelled dust, each for 24 h. This would allow the labelled dust on the insects to reach residual equilibrium level and would also considerably reduce the amount of labelled dust rubbed back onto the insects from the wheat.

Table 4.1 Data for counting method experiment

Dust	Wessalon S	Aerosil R972	Cab-O-Sil M5
	1226230	147898	103276
	1206380	149242	102141
Group A	1280865	152245	105302
dpm/mg dust	1248675	146721	105072
	1289711	158294	106212
	-	146201	-
Mean	1250372	150100	104401
Standard deviation	35350	4560	1652
	1272961	163847	106802
	1340655	144571	106183
Group B	1223541	150668	102174
dpm/mg dust	1251124	150307	105005
	1272017	156570	106485
	-	147192	-
Mean	1272060	152193	105310
Standard deviation	43297	6985	1932
	1248094	149979	107044
	1272180	142201	104488
Group C	1137256	147563	103336
dpm/mg dust	1239015	158499	105941
	1318572	153844	103222
	-	145939	-
Mean	1243023	149871	104806
Standard deviation	66682	5824	1663
	1315307	157743	100958
	1155131	152822	102648
Group D + E	1227492	151657	107337
dpm/mg dust	1251055	154396	103387
	1313123	148275	105981
	-	161395	-
Mean	1252422	154381	104062
Standard deviation	66573	4640	2574
F-ratio (n,d)	0.26 (3,16)	0.90 (3,20)	0.36 (3,16)

Table 4.2 The amounts of high and low level activity dusts picked-up by the beetles

Dust	Wessalon S	Aerosil R972	Cab-O-Sil M5
Pick-up of high activity dust (µg/insect)	2.63 2.63 2.31 2.59 2.61 2.86	4.09 4.31 3.99 4.57 4.07 4.17	2.26 1.99 2.26 2.72 2.16 2.05
Mean Standard deviation	2.61 ± 0.18	4.20 ± 0.21	2.24 ± 0.26
Pick-up of low activity dust (µg/insect)	2.42 2.16 2.58 2.32 2.59 2.10	4.41 4.43 4.83 4.70 3.84 4.21	2.02 2.08 2.05 2.05 1.90 2.35
Mean Standard deviation	2.36 ± 0.21	4.40 ± 0.35	2.08 ± 0.15
Activity ratio $\left(\frac{\text{high}}{\text{low}}\right)$ of dusts	5.30	6.12	4.29

Table 4.3 Estimated residual amounts of radio labelled dust on the beetles 24 h. after their introduction to wheat treated with unlabelled dust

Sorptive dust	Wt. of dust/insect (µg)
Sipernat D17	1.50
Aerosil R972	1.60
Gasil 35M	~ 0.50
Wessalon S	0.90
Aerosil 150	~ 0.20
Cab-O-Sil M5	0.90
Gasil GM2	~ 0.20
Gasil 200	0.22

Table 4.4 Equations of the regression lines fitted to $\ln a_1$/time for each dust, and the calculated rates of turn-over of the dusts on the beetles

Dust	Regression equation ($\ln a_1$/time)	R^2 value	Rate of turn-over with 95% C.I. (µg dust/insect/hour)
Sipernat D17	y=0.240-0.132x	0.94	0.168(0.111-0.249)
Aerosil R972	y=0.341-0.113x	0.93	0.159(0.103-0.188)
Gasil 35M	y=-0.155-0.113x	0.88	0.097(0.050-0.173)
Wessalon S	y=0.0565-0.132x	0.91	0.140(0.079-0.234)
Cab-O-Sil M5	y=-0.008-0.119x	0.83	0.118(0.055-0.232)
Aerosil 150	y=0.258-0.069x	0.89	0.089(0.057-0.131)

Table 4.5 Comparisons between the intercepts ($lna_1(t=0)$) and the slopes (-rate constants) of the regression equations fitted to lna_1/time for six insecticidally active silica dusts (Students T-test).

Comparisons of -k

	Sipernat D17	Aerosil R972	Wessalon S	Gasil 35M	Cab-O-Sil M5	Aerosil 150
Sipernat D17	-					
Aerosil R972	NS	-				
Wessalon S	NS	NS	-			
Gasil 35M	NS	NS	NS	-		
Cab-O-Sil M5	NS	NS	NS	NS	-	
Aerosil 150	***	**	***	**	**	-

Comparisons of $lna_1(t=0)$

	Sipernat D17	Aerosil R972	Wessalon S	Gasil 35M	Cab-O-Sil M5	Aerosil 150
Sipernat D17	-					
Aerosil R972	NS	-				
Wessalon S	NS	NS	-			
Gasil 35M	*	*	NS	-		
Cab-O-Sil MS	NS	NS	NS	NS	-	
Aerosil 150	NS	NS	NS	*	NS	-

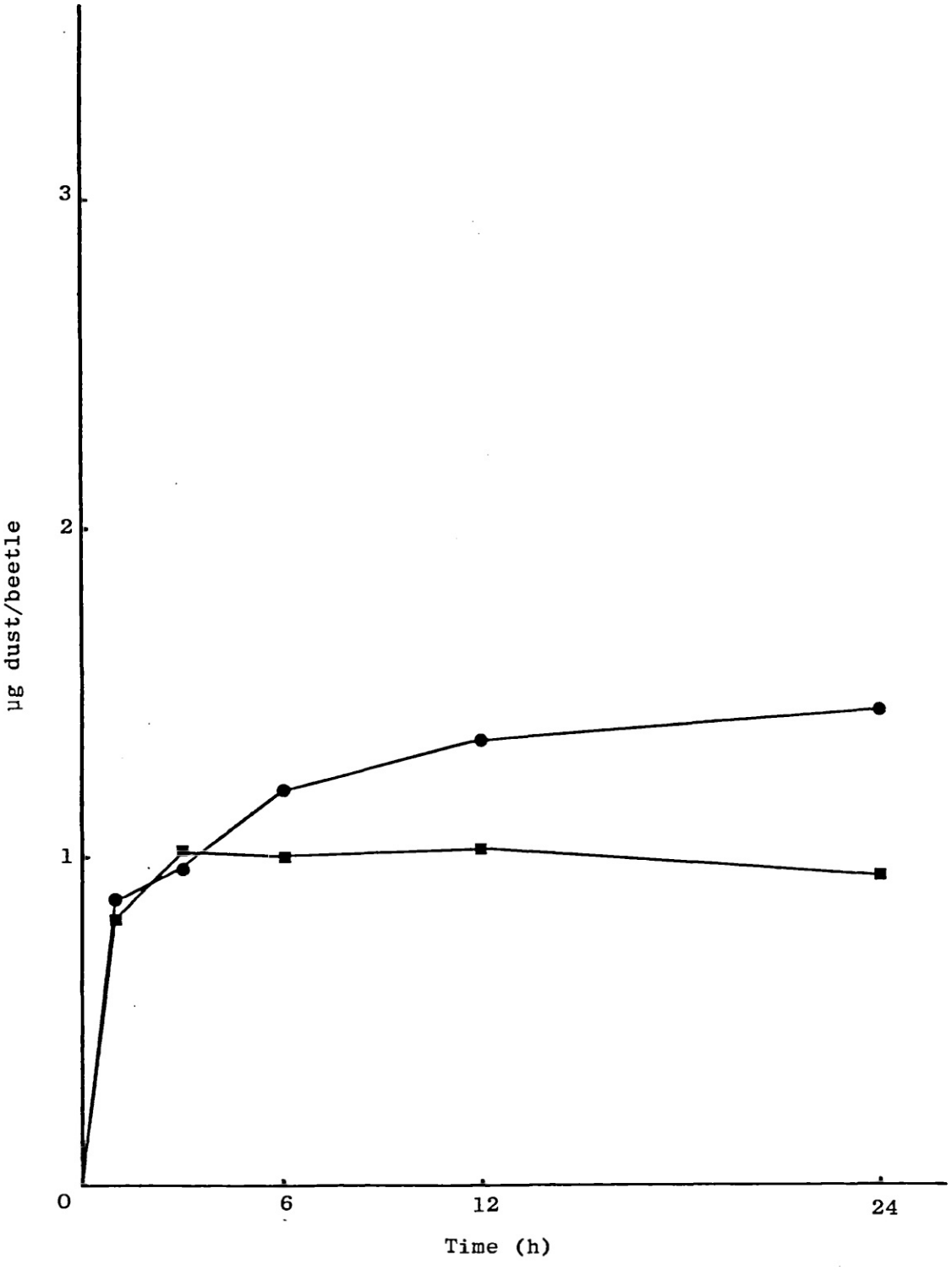
NS = not significant. * $p<0.05$

** = $P<0.01$. *** $p<0.001$

Figs.4.1-4.8

The mean amounts of radio-labelled dust which accumulated on *S.granarius* for 24h. after their introduction to wheat treated with dust at 10 mg/100g wheat.

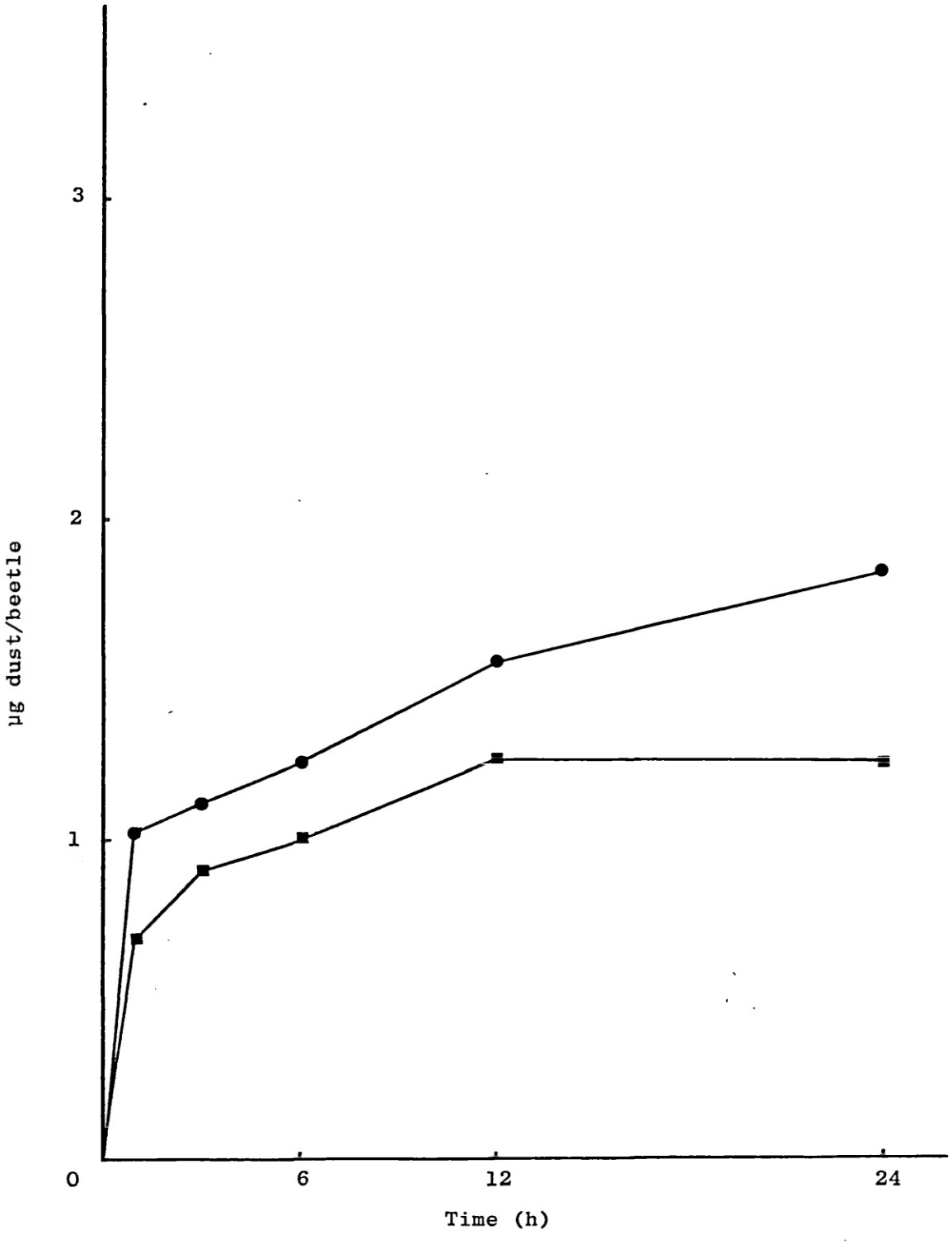
Fig.4.1 Pick-up of Cab-O-Sil M5 and Cab-O-Sil EH5



■ Cab-O-Sil EH5

● Cab-O-Sil M5

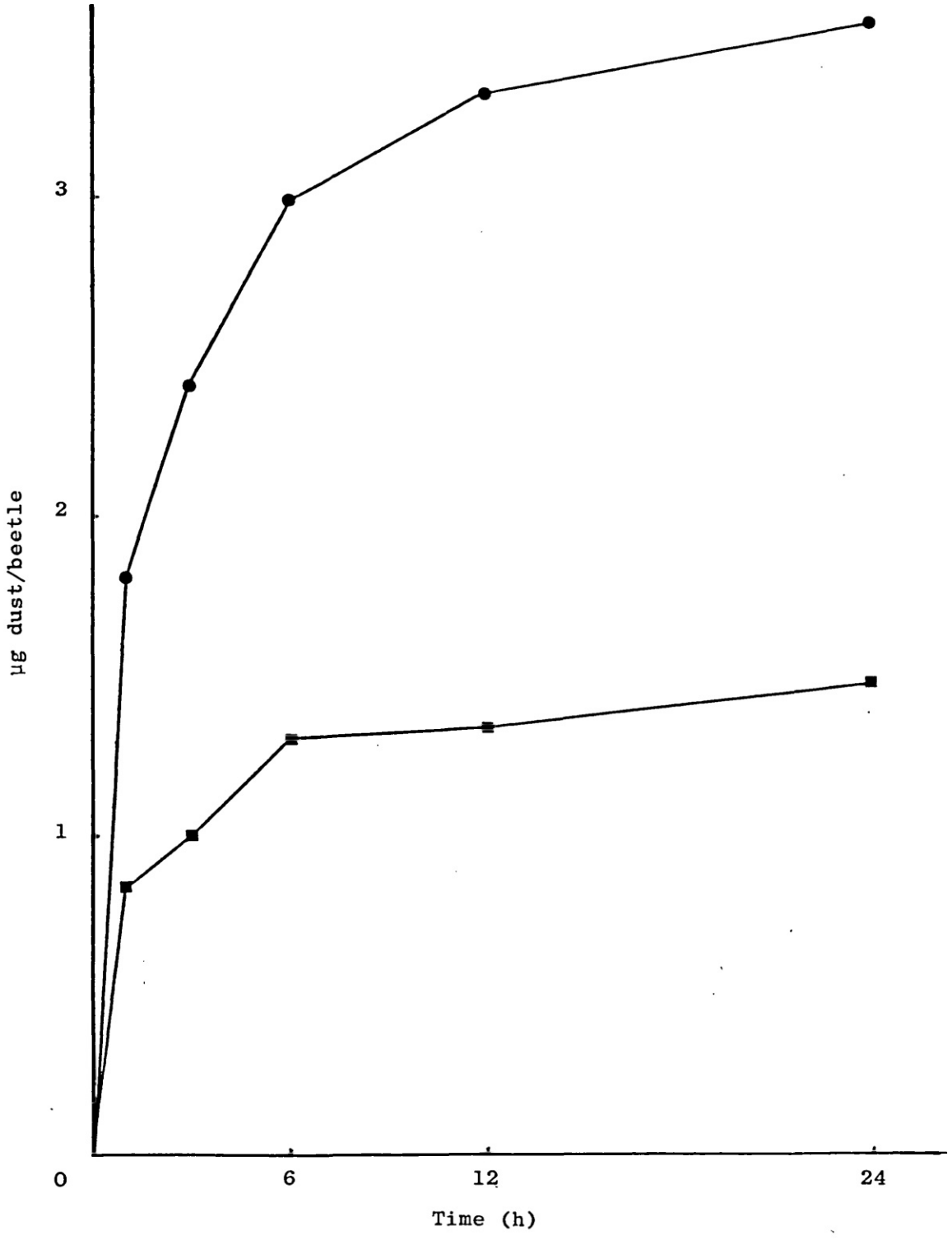
Fig.4.2 Pick-up of Aerosil 130 and Cab-O-Sil H5



■ Cab-O-Sil H5

● Aerosil 130

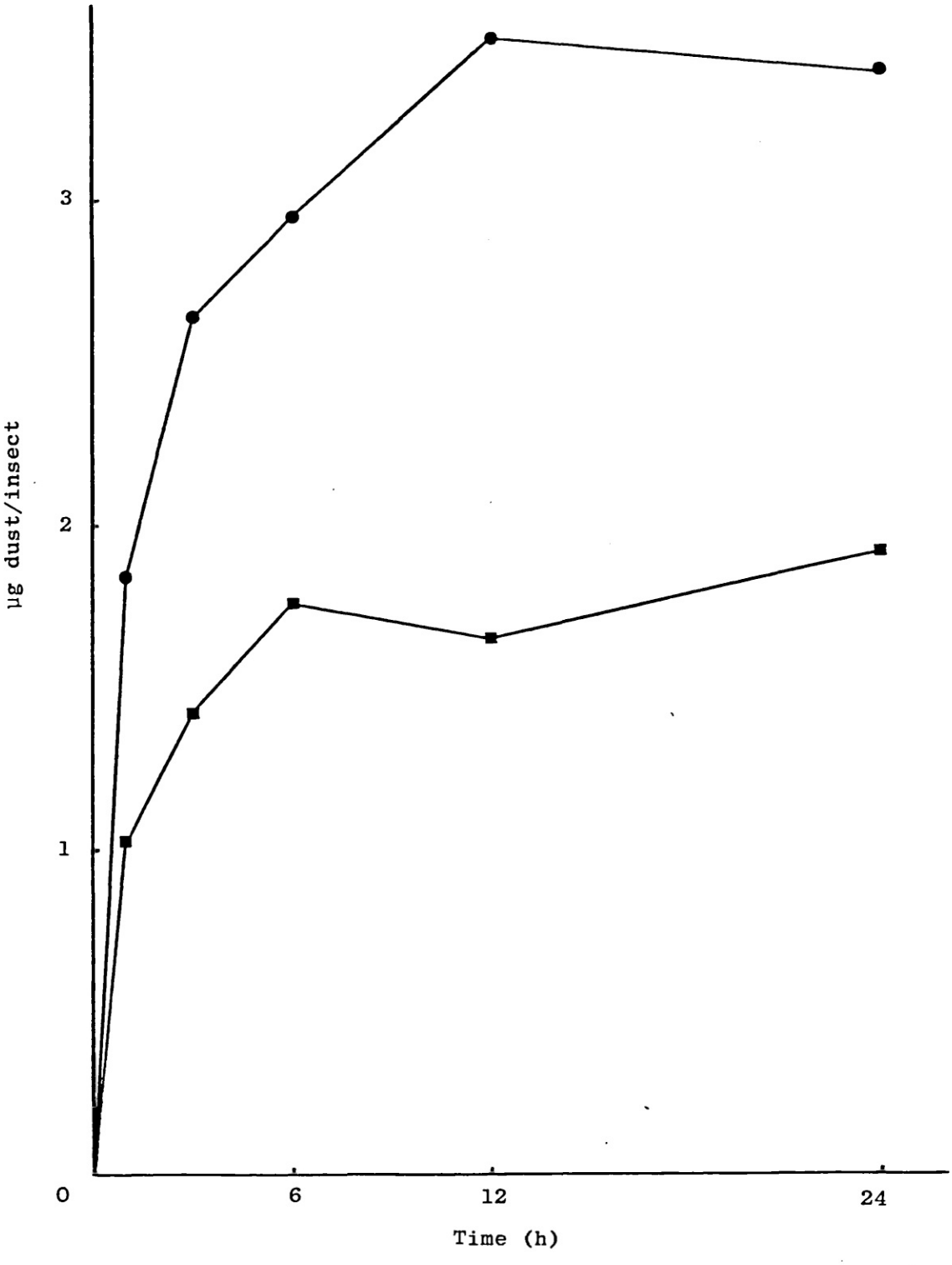
Fig.4.3 Pick-up of Aerosil R972 and Aerosil 150



● Aerosil R972

■ Aerosil 150

Fig.4.4 Pick-up of Sipernat D17 and Gasil 23D



● Sipernat D17

■ Gasil 23D

Fig.4.5 Pick-up of Gasil 23C and Gasil 35M

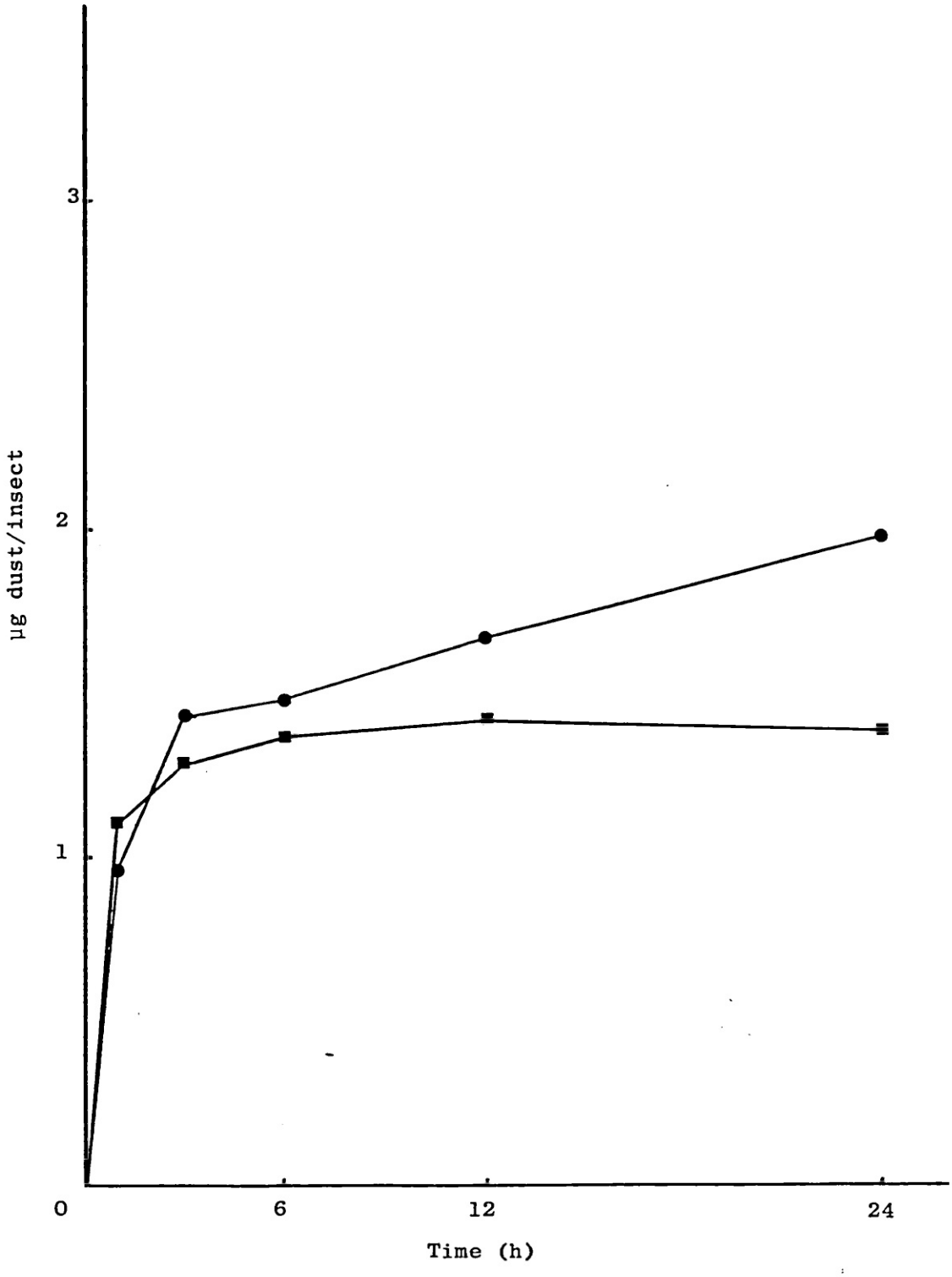


Fig.4.6 Pick-up of Wessalon S and Sipernat 22

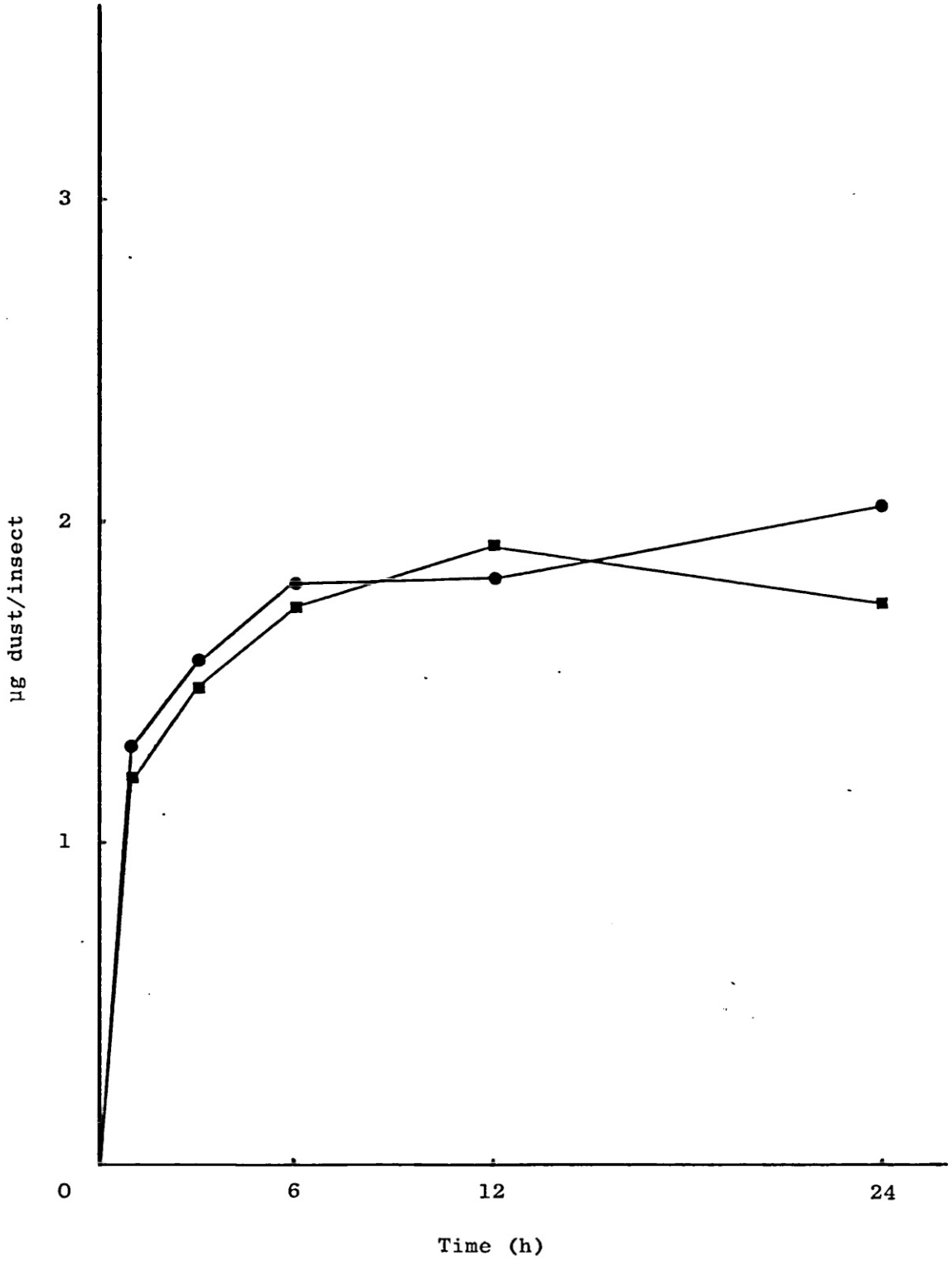


Fig.4.7 Pick-up of Gasil 23F and Gasil HP37

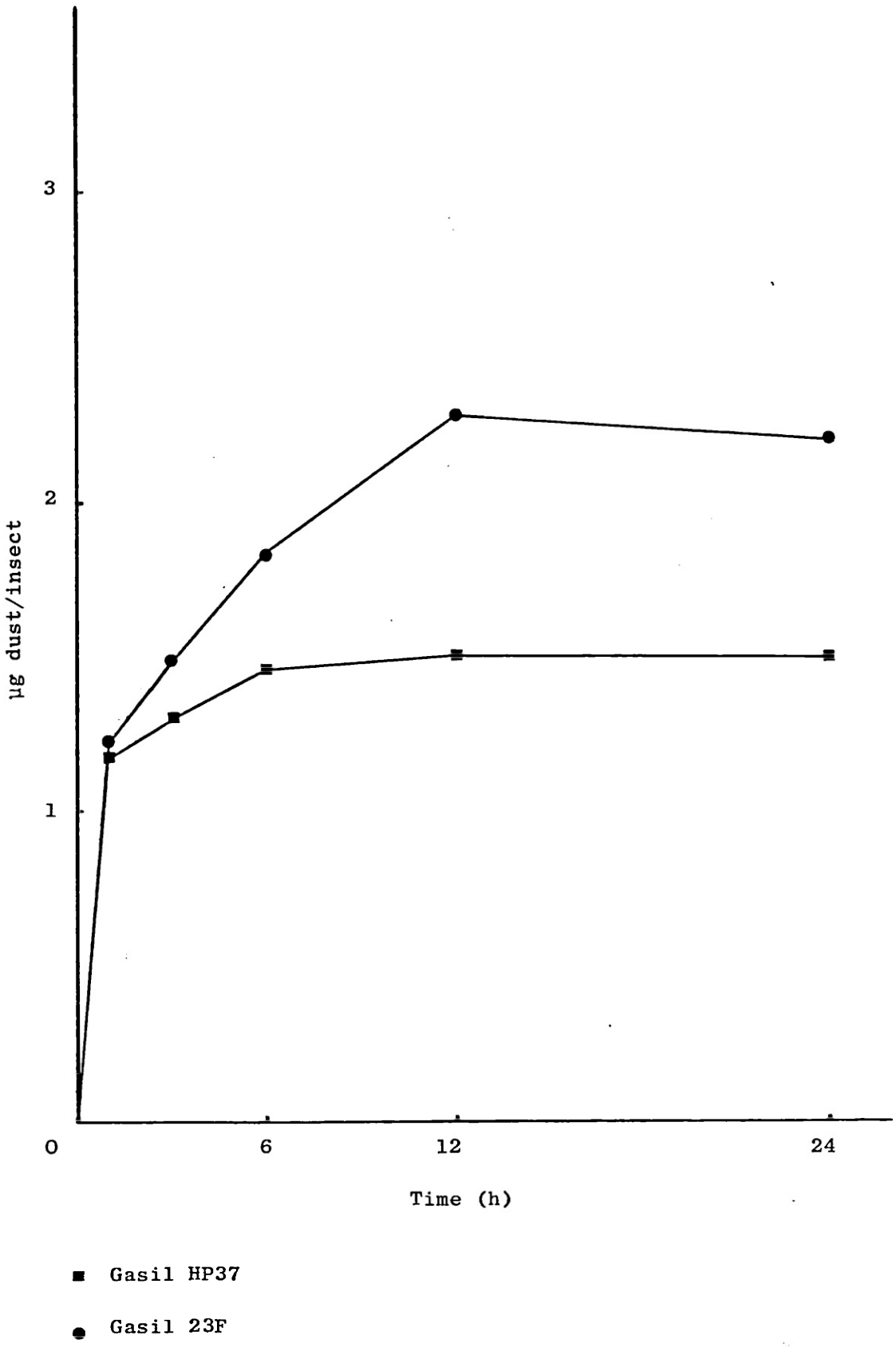


Fig.4.8 Pick-up of Gasil 200 and Gasil GM2

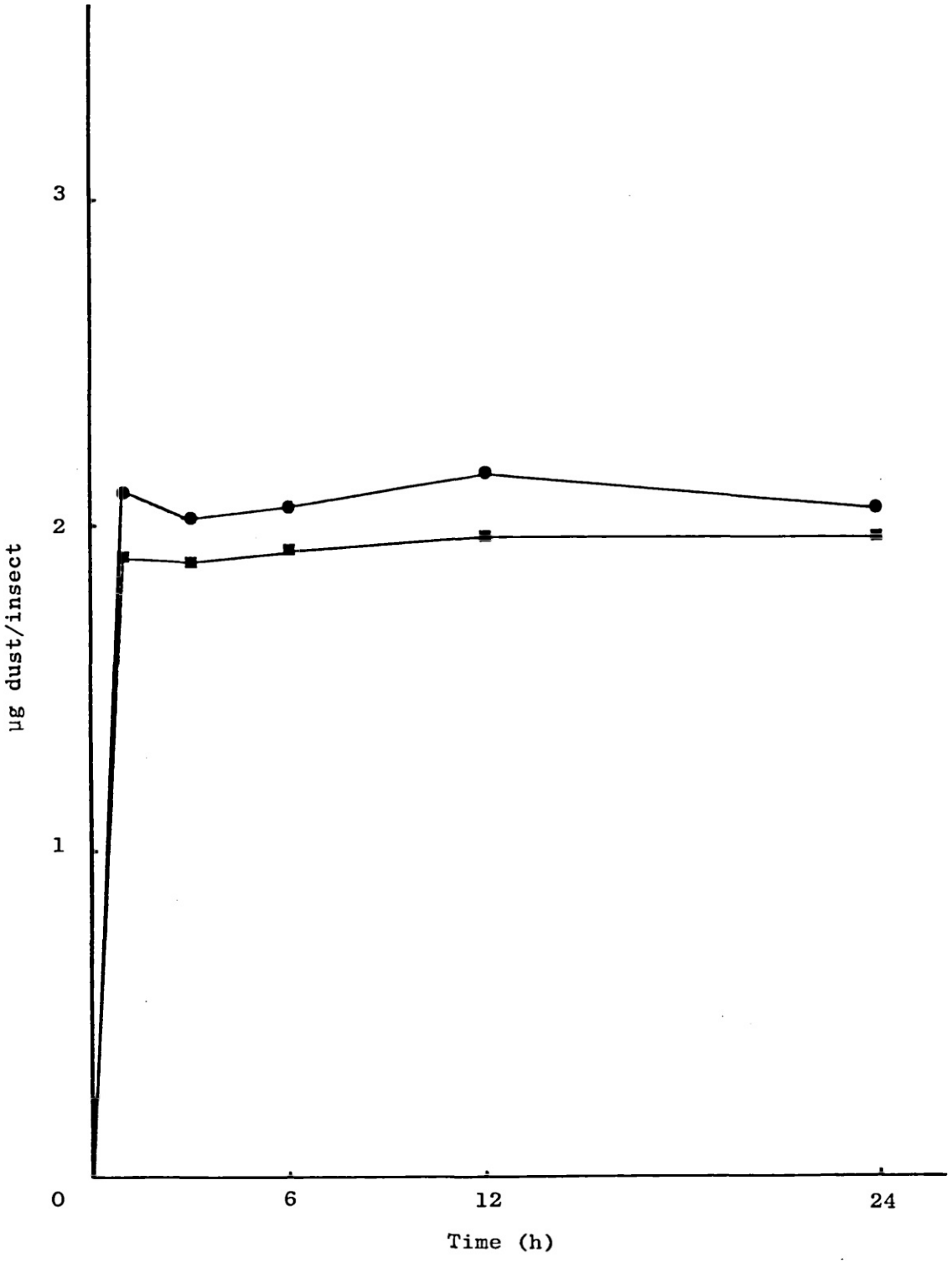


Fig.4.9-4.12

The mean amount of radiolabelled dust on the insects for 24h. after their introduction to wheat treated with non-labelled dust at 10 mg/100g wheat. (The beetles had previously been allowed to pick-up the equilibrium maximum level of radiolabelled dust from wheat treated with 10 mg labelled dust/100g wheat.

Fig.4.9 Sipernat D17 and Gasil 200

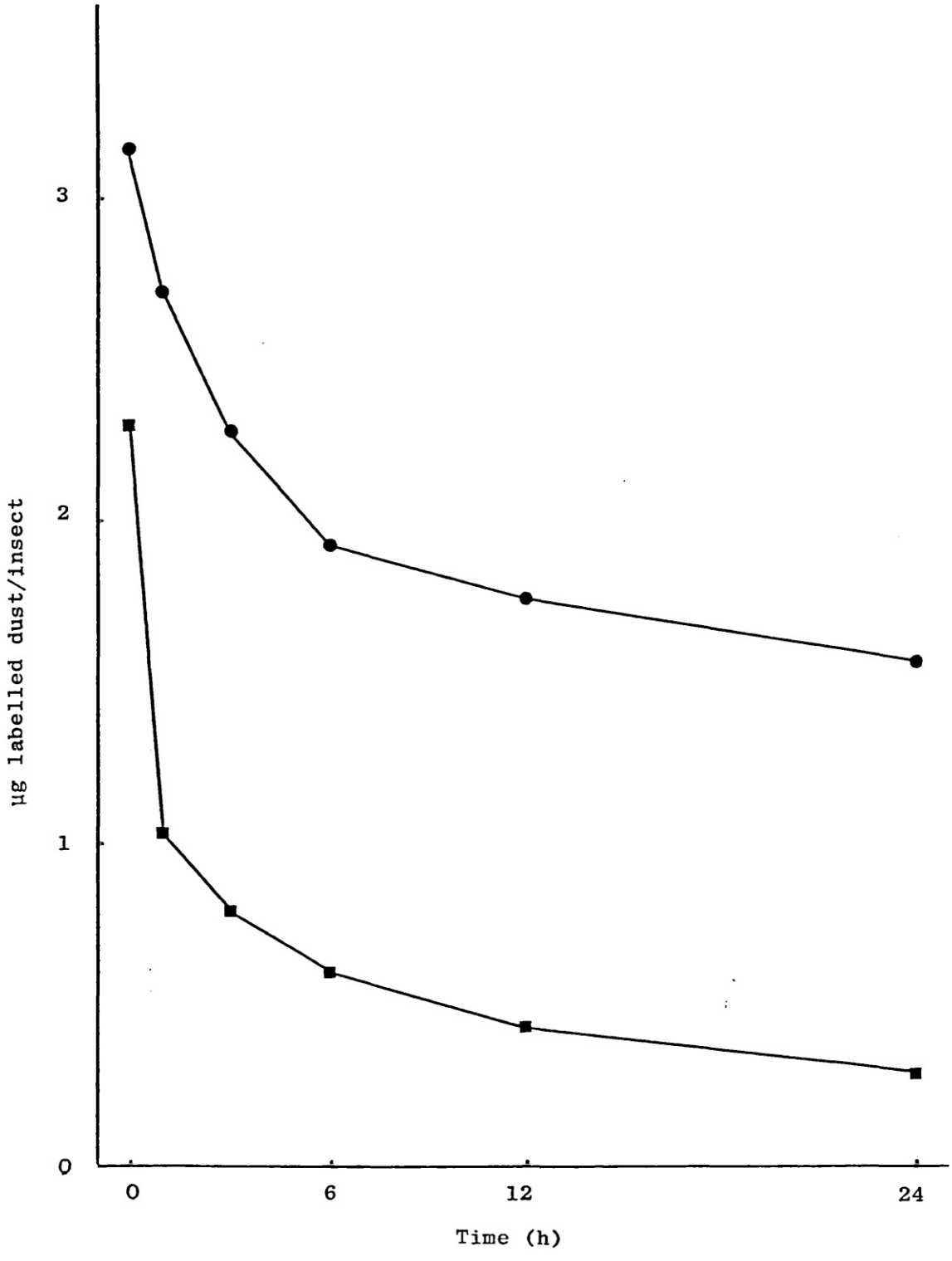
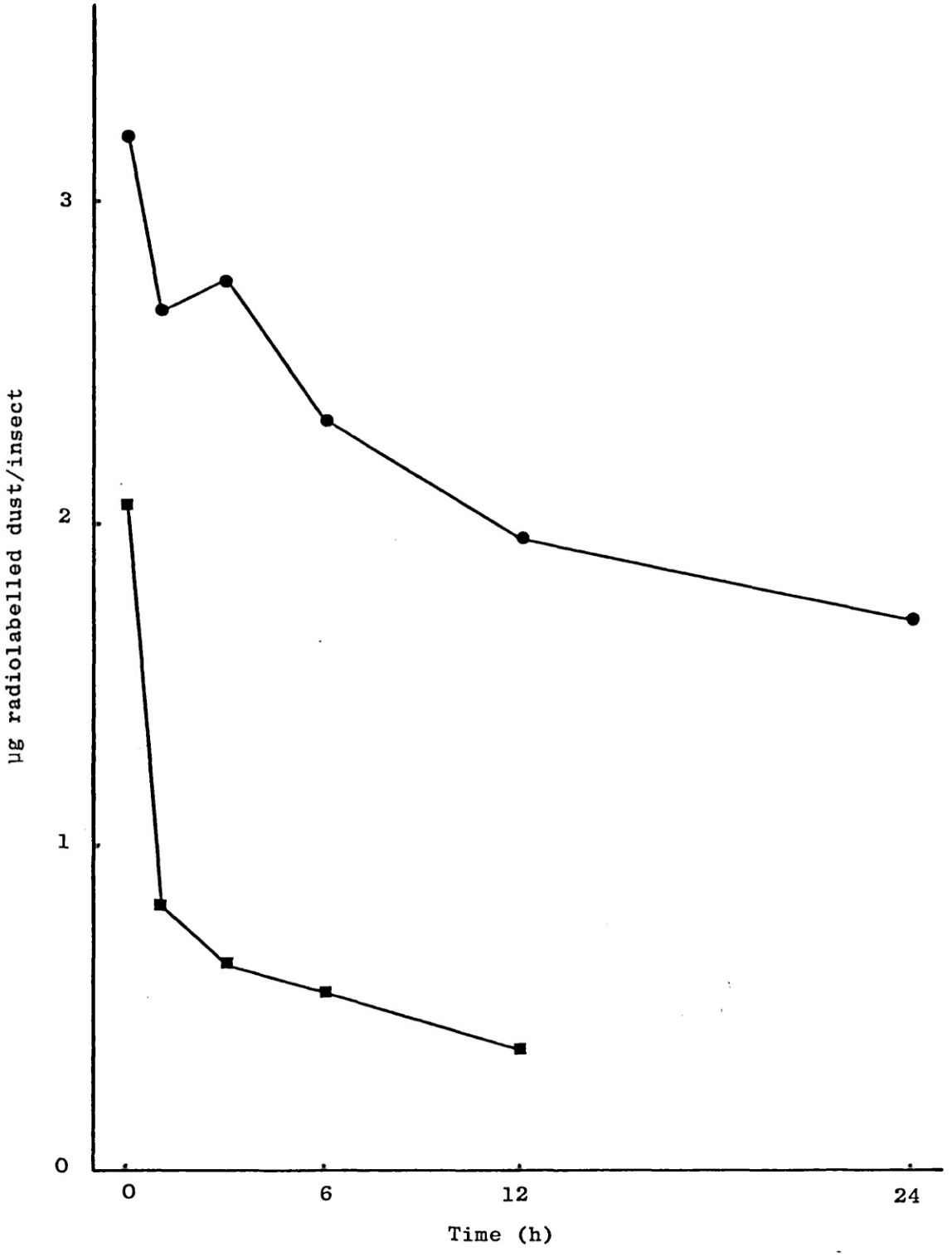


Fig.4.10 Aerosil R972 and Gasil GM2



● Aerosil R972

■ Gasil GM2

Fig.4.11 Cab-O-Sil M5 and Aerosil 150

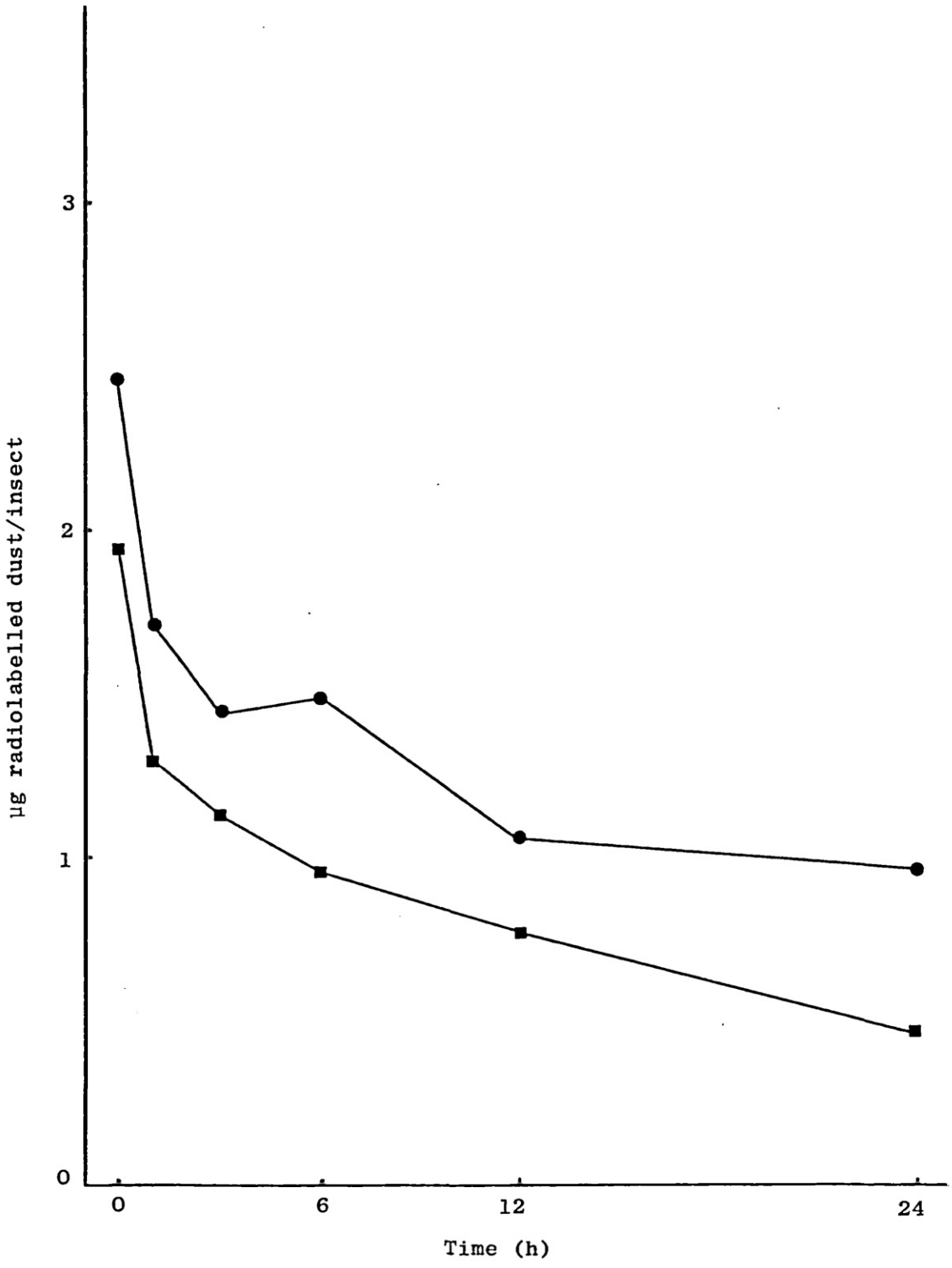
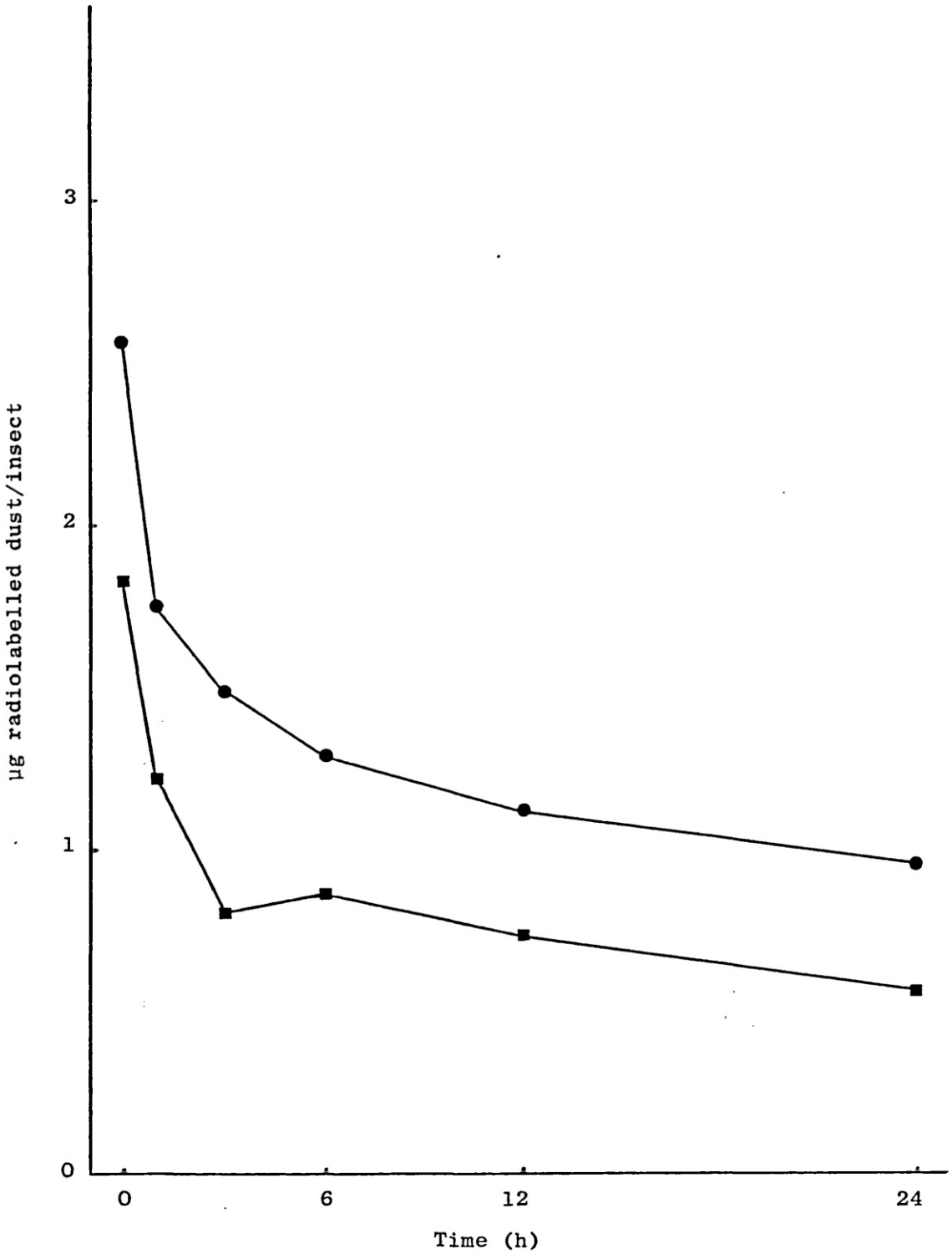


Fig.4.12 Wessalon S and Gasil 35M



● Wessalon S

■ Gasil 35M

Section 5

The Joint Insecticidal Action of Sorptive Silica Dusts and Cypermethrin

5.1 Introduction

A series of bioassays was performed to assess and compare the joint insecticidal action between cypermethrin and a selection of eight sorptive silica dusts, which included hydrophobic, porous hydrophilic and fumed hydrophilic dusts. These dusts could also, therefore, be grouped according to the level of water loss they caused from *S.granarius* (Section 3), their level of insecticidal activity (Section 3), and their equilibrium pick-up levels (Section 4). The dusts used were: Aerosil R972 and Sipernat D17 (hydrophobic); Cab-O-Sil M5, Cab-O-Sil H5 and Aerosil 150 (fumed hydrophilic); and Wessalon S, Gasil 23F and Gasil 35M (porous hydrophilic).

The cypermethrin was formulated on Gasil GM2 at a concentration of 0.1% a.i(w/w). Gasil GM2 was selected as the carrier dust because it caused minimal water loss from the beetles and was non-insecticidal (Section 3). Formulating the cypermethrin at only 0.1% w/w on the dust sufficiently bulked out the pyrethroid to facilitate weighing out small quantities.

The bioassays were performed on the cypermethrin dust with a sorptive dust at six different ratios. The amounts of cypermethrin and sorptive dust in the LC_{50} values of each ratio of the two toxicants were plotted as isobolograms (Section 1.4.1). The shape of the isobolograms obtained indicated the nature of joint action between the two toxicants. The cypermethrin:sorptive dust ratios tested were: 5:1, 2:1, 1:1, 1:2·5, 1:5 and 1:10.

A further experiment was performed to try and determine the cause of the potentiating joint action between cypermethrin dust and a particular

sorptive silica dust. Since water loss is the primary lethal lesion caused by sorptive silica dusts and is a secondary feature of pyrethroid poisoning, the role of water loss in the joint action between the two toxicants was investigated.

The water loss was determined from beetles exposed for 24h to wheat treated with the LC_{50} value of cypermethrin dust alone, the LC_{50} of Sipernat D17 alone, and six different ratios of the two at levels which fall on the line for additive joint action on the isobologram for the joint action between these two toxicants (Fig.5.2). The cypermethrin dust: Sipernat D17 ratios were those used for the toxicity experiments. Sipernat D17 was selected as the sorptive dust for the experiment firstly because it showed the greatest potentiation with the cypermethrin dust, and secondly because the 95% confidence limits of its LC_{50} value were narrower than those of all the other dusts tested (Section 3) and thus the estimates of the amounts of the two toxicants required for each formulation were the most reliable.

As with the investigation into the water loss from *S.granarius* caused by the sorptive dusts alone (Section 3), the mode of action study comprised two parts. Firstly the amount of each dust picked-up by the insects from each formulation was assessed, and secondly the weight loss from beetles exposed to each formulation was measured.

5.2 Methods

5.2.1 Preparation of cypermethrin dust

Technical cypermethrin (10.7 mg, 93.5% a.i) dissolved in n-hexane (25 cm^3) was washed into a round-bottomed flask containing Gasil GM2 (10 g) with excess n-hexane (100 cm^3) and the mixture rotary evaporated to dryness. The dust was stored in a stoppered bottle in the dark at 5^oC when not in use.

5.2.2 Preparation of labelled cypermethrin dust

Cypermethrin at 0.1% w/w on Gasil GM2 was prepared as above. Sodium sulphate (containing sulphur-35) was dissolved in methanol, added to the cypermethrin dust in a round-bottomed flask (10 cm^3 methanol/ mg dust) and the dust rotary evaporated to dryness.

5.2.3 Bioassay of cypermethrin dust/sorptive dust mixtures

The toxicity of the cypermethrin dust alone was assessed by the method described in Section 3.2.1.

To assess the toxicity of different formulations of cypermethrin dust and a sorptive dust, calculated amounts of each component were separately weighed into a jar of wheat and mixed (Section 2.2.2). Five-six dose levels were prepared for each pyrethroid dust/sorptive dust ratio, and 4-6 jars were left free of dust as controls to make a total of 36 jars. The jars were distributed at random into 3 storage boxes (12/box) and incubated at 25°C and 70% R.H. (Section 2.2.3). After 14 days, 50 adult *S.granarius* (unsorted sexes) were added to each jar. The 10-day LC_{50} values to *S.granarius* of all the formulations were assessed by the method described in Section 2.2.4.

5.2.4 Mode of action (water loss) experiment

The amounts of cypermethrin dust and Sipernat D17 for each ratio of the two toxicants on the line for additive action in Fig.5.2 are given in Table 5.1.

The experiment was in two parts:-

(i) Measurement of the amount of each dust picked-up

The amounts of the cypermethrin dust and Sipernat D17 on the insects 24 h. after their introduction to dust-treated wheat were assessed separately.

The calculated amount of each dust was added to and mixed with wheat

(Section 2.2.2). Three replicate jars of wheat for each ratio of cypermethrin dust and Sipernat D17 were prepared using radiolabelled cypermethrin dust, and three replicate jars were prepared using radiolabelled Sipernat D17 (Section 4.2.1). The jars were incubated at 25°C and 70% R.H. (Section 2.2.3).

After 14 days, 50 adult *S.granarius* (unsorted sexes) were added to each jar. Twenty-four hours later the contents of each jar were gently tipped onto a tray, the beetles removed with clean dry forceps and the amount of dust adhered to the beetles assayed radiometrically (Section 4.2.3).

(ii) Measurement of the weight change of the beetles

The calculated amount of each dust was added to and mixed with wheat (Section 2.2.2). Ten replicate jars were prepared for each cypermethrin dust/Sipernat D17 ratio, and ten jars were left free of dust as controls. All ninety jars were distributed at random into eight storage boxes (11-12 per box) and were incubated at 25°C and 70% R.H. (Section 2.2.3).

After 14 days, a batch of 50 adult *S.granarius* (unsorted sexes) was weighed and added to each jar. The weight loss of each batch after 24h. exposure to the wheat was calculated as in Section 3.2.2.

5.3 Results and Discussion

5.3.1 Bioassays

The amounts of cypermethrin and sorptive dust in the LC_{50} values of each of the cypermethrin dust/sorptive dust formulations are given in Tables 5.2-5.9 and are presented as isobolograms in Figs.5.1-5.8.

The result of the bioassay of the cypermethrin dust alone is included in Table 5.2.

All of the points on the isobolograms for cypermethrin formulated with the hydrophobic dusts and with the porous hydrophilic dusts fell below the line for additive joint action. This shows that there was potentiation between cypermethrin and these two types of silicas. The joint action ratios derived from the isobolograms are given in Table 5:10, and are highest for the two hydrophobic dusts. The potentiation was generally greater for the higher cypermethrin dust : sorptive dust ratio (5:1-1:1), though this was more apparent with the hydrophobic dusts.

Of the fumed hydrophilic dusts tested both Cab-O-Sil M5 and Aerosil 150 showed a small degree of potentiation at the highest cypermethrin dust : sorptive dust ratios. However, points on the isobolograms representing the low cypermethrin dust : sorptive dust ratios were above the line for additive joint action which shows that either sub-additive or antagonistic joint action occurred. The antagonism was such that at the cypermethrin dust : sorptive dust ratio of 1:10, the amounts of sorptive dust in the LC_{50} values were greater than the LC_{50} values of the dusts alone.

Since mortalities of less than 50% were obtained for the highest dose levels of cypermethrin dust/Cab-O-Sil M5 at ratios of 1:5 and 1:10, no LC_{50} values could be calculated. However, the highest dose levels of the 1:5 and 1:10 ratios contained 50 mg and 70 mg of Cab-O-Sil respectively, which indicates that both ratios must have at least been sub-additive and the 1:10 ratio probably antagonistic.

Cab-O-Sil H5 was the only fumed hydrophilic silica which did not show either antagonistic or sub-additive joint action with the cypermethrin dust. The points on the isobolograms either fell on or below the line for additive joint action.

The joint action ratios derived from the isobolograms for the action between cypermethrin dust and the fumed hydrophilic silicas are also given in Table 5.10. However, the values only represent the highest value for the points that were below the line for additive action, i.e. only the maximum potentiation was measured.

Although the two hydrophobic dusts showed the greatest degree of potentiation with the cypermethrin dust as well as the highest toxicity when used alone, the different levels of potentiation shown by the fumed hydrophilic and the porous hydrophilic dusts with the cypermethrin dust did not reflect the similar toxicities of these two types of dust when used alone. However, the order of magnitude of the joint action of the three different types of dust with cypermethrin dust is the same as that for the equilibrium pick-up levels of the dusts (Section 4) and the water loss they cause from the beetles (Section 3), i.e. hydrophobic silicas > porous hydrophilic silicas > fumed hydrophilic silicas.

It is unlikely that the very low potentiation or antagonism between the fumed hydrophilic silicas and the cypermethrin dust was due to fumed silicas picked-up by the beetles inhibiting pick-up of the cypermethrin dust because the hydrophobic and the porous hydrophilic silicas which had the highest equilibrium pick-up levels also had the highest level of potentiation with the cypermethrin dust.

The different amounts of water loss from the beetles caused by the three different types of dust in a 24 h. period of exposure is a reflection of the ability of the dusts to remove the beetles' cuticular lipid barrier. It is possible, therefore, that the removal of epicuticular lipid influences joint action in two ways.

Firstly, the dust which most effectively removed epicuticular lipid would cause the highest level of water loss. This would result in greater

physiological stress on the beetles and probably leads to a stronger potentiation with the cypermethrin dust.

Secondly, if the removal of epicuticular lipid allowed the cypermethrin to penetrate into the insect more easily, the dusts which removed the epicuticular lipid most efficiently would most enhance insecticide penetration which might lead to the greater potentiation with the cypermethrin dust.

These two factors may explain the agreement in the level of water loss and level of potentiation caused by the three groups of silica dust.

There is no clear reason for the antagonistic joint action between both Cab-O-Sil M5 and Aerosil 150 and the cypermethrin dust. However, it is possible that very slow penetration of cypermethrin into beetles exposed to these formulations is not sufficient to kill them within the duration of the bioassays.

Although in the present work no clearly defined potentiation between fumed hydrophilic silica dusts and cypermethrin dust was found, Singh (44) demonstrated that there was potentiating joint action between Cab-O-Sil M5 and three synthetic pyrethroids (permethrin, cypermethrin and deltamethrin). Singh's method of assessing joint action, however, differed from that described in the present work in three ways: the test insect used was *Tribolium castaneun*; the moisture content of the wheat used in the bioassays was 10% (in equilibrium with a relative humidity of 35%, le Patourel, unpublished data); and the pyrethroids were formulated on the insecticidal sorptive dust rather than a non-insecticidal carrier dust.

Firstly, unlike *S.granarius*, *T.castaneum* does not have a cement layer over its epicuticular lipids which affords some protection against

the action of desiccant dusts (Nair, 47). It is possible, therefore, that water is lost more rapidly through the cuticles of *T.castaneum* treated with sorptive dusts and this leads to greater potentiation with pyrethroids.

Secondly, the lower humidity/wheat moisture content of Singh's bioassays would have increased the susceptibility of the beetles to desiccation. This might also have enhanced potentiation between the sorptive dust and the pyrethroid.

In Singh's work, the pick-up of the pyrethroids by the insects would have been dependent on the pick-up of the sorptive dusts, whereas in the present work the two toxicants were picked-up independently of each other. It is possible, therefore, that depositing the pyrethroid actually on the sorptive dust might improve potentiation, though no direct comparison between Singh's work and the present work can be made since two other experimental conditions were different (above).

One advantage of formulating the pyrethroid on the sorptive dust was that the two toxicants did not have to be weighed out separately and consequently the bioassays took less time to prepare. A disadvantage of Singh's method, however, was that in order to obtain a point on the isobologram for the pyrethroid alone the pyrethroid had to be formulated on a non-insecticidal carrier dust (in this case, talc was used). This may have had completely different pick-up characteristics than the Cab-O-Sil M5 and thus given rise to an incorrect calculation of the joint action ratio.

5.3.2 The mode of action of cypermethrin/sorptive dust formulations

The amounts of water loss caused by each cypermethrin dust/Sipernat D17 formulation from the beetles in 24 h. are given in Table 5.11. Although no mortalities were observed in any of the test populations,

nearly all of the beetles (except for those in the control groups) were incapacitated after 24 h. The amounts of each toxicant on the beetles 24 h. after their introduction to dust-treated wheat are given in Table 5.12.

The results show that different ratios of cypermethrin dust and Sipernat D17 did cause significantly different amounts of water loss from the beetles over a 24 h. exposure period ($p=0.001$, One-way analysis of variance). Apart from the 1:10 ratio, the formulations of cypermethrin dust and Sipernat D17 caused more water loss from the beetles than either of the two components when used alone. Water loss was highest from beetles exposed to 5:1 and 2:1 ratios where potentiation was greatest.

The mean amounts of water loss caused by each formulation, however, did not differ greatly. All dust treatments caused between 6.71% and 8.22% weight loss. The difference between these two figures is less than the difference in water loss from beetles exposed for 24 h. to the porous hydrophilic and the fumed hydrophilic silicas (Section 3) both of which had similar levels of toxicity to *S.granarius*. This indicates that water loss from the beetles was not the major factor responsible for potentiation between cypermethrin dust and Sipernat D17. It is likely, therefore, that potentiation was mainly caused by the optimum penetration of pyrethroid into the insect. Singh (44) found that there was no significant difference in the rate of water loss from *Tribolium castaneum* exposed to Cab-O-Sil M5 and to the pyrethroid permethrin at 0.5% and 10% w/w on Cab-O-Sil M5 when the beetles were exposed to the loose dusts in a beaker for 24h. As with the present work, these results were interpreted as indicating that water loss played little part in the potentiating joint action between pyrethroids and sorptive dusts, and that enhanced penetration of the pyrethroid into the insect was probably the important factor.

Ideally, the rate of penetration of cypermethrin into beetles exposed to different cypermethrin dust/sorptive dust ratios should have been measured directly in order to determine the importance of insecticide penetration in potentiating joint action. It would have been necessary to measure the amount of cypermethrin that had penetrated through the cuticle and into the living tissue of beetles exposed to different ratios of cypermethrin dust and Sipernat D17. The cypermethrin could either have been assayed using G.L.C. analysis, or radiolabelled cypermethrin could have been assayed radiometrically.

Several difficulties arising from the use of insecticides formulated on dusts and the choice of experimental insect, however, made such experiments practically impossible.

Firstly, the cypermethrin picked-up by the insects would have been distributed between the carrier dust on the insect surface, the epicuticular lipid layer, and the insects living tissue below the cuticle. It would have been very difficult to separate these three components without affecting the latter.

Secondly, the small size of *S.granarius* and its hard cuticle would have made surgical removal of pieces of tissue or the extraction of haemoplasm for the reproducible assay of cypermethrin content very difficult.

A comparison can be made between the extent of the potentiation at each cypermethrin dust/Sipernat D17 ratio and the amount of toxicant picked-up by the beetles in 24 h. It is first necessary to explain two numerical terms used to explain each:-

(i) The "joint action ratio" proposed by Hewlett (81) to quantify joint action is only derived from the maximum deviation from the line for additive action on an isobologram (Section 1.4.1). In the present work, a similar ratio was found for each point on the isobologram for the

joint action between cypermethrin dust and Sipernat D17. These ratios were termed "potentiation ratios", the maximum "potentiation ratio" being the same as Hewlett's joint action ratio.

(ii) As part of the experiment to assess the water loss caused by each ratio of cypermethrin dust and Sipernat D17, the amounts of each component picked-up by beetles exposed to each ratio were calculated. The amount of each toxicant picked-up could be expressed as a proportion of its individual LC_{50} value. The proportions of both toxicants picked-up from each ratio of the two were added together. Since by definition the LC_{50} levels of each toxicant have an equal effect, the sum of the proportions of each toxicant gives a measure of the "total toxicant" picked-up by the beetles from wheat treated with a formulation of cypermethrin dust and Sipernat D17 (the proportions are multiplied by 10^4 to be on the same scale as "potentiation ratio").

The amount of "total toxicant" picked up by beetles exposed to each formulation of cypermethrin dust and Sipernat D17 along with the potentiation ratio of each formulation are shown in Fig.5.9 and are also given in Table 5.13.

The shape of the plots for "total toxicant" picked-up and the potentiation ratio for the eight formulations are clearly similar. The most striking result is that the amount of "total toxicant" on beetles exposed to wheat treated with the LC_{50} value of Sipernat D17 alone and with the LC_{50} value of cypermethrin dust alone after 24 h. was almost identical. The highest pick-up of "total toxicant" was by beetles exposed to the 5:1 and 2:1 ratios of cypermethrin dust and Sipernat D17, the ratios with the highest "potentiation ratios".

The fact that nearly all the beetles(other than those in the control groups) were incapacitated by the end of the water loss experiment and

the similarity between the plots for "total toxicant" and "potentiation ratio" indicate that the 10-day toxicity of the cypermethrin dust/Sipernat D17 formulations and the extent of the potentiation between these two toxicants depend upon the amount of dust picked-up and turned-over on the beetles within 24 h. of their introduction to treated wheat. Furthermore, the amount of dust on the beetles at 24 h. would be close to the total amount which affected them over 10-days.

Taking into consideration the results of the bioassays of the cypermethrin dust/sorptive dust formulations and the levels of water loss the different formulations caused from the beetles, a possible mode of joint action between cypermethrin and sorptive dusts can be hypothesised.

Pyrethroid poisoning is a relatively rapid process and involves the penetration of the insecticide into the beetles which causes nervous and neuro-endocrine lesions leading to lethal metabolic disorganisation. In contrast, the action of sorptive dusts is relatively slow and involves the turn-over of dusts on the insect and the adsorption of epicuticular lipid. Starting with cypermethrin dust alone, as the proportion of sorptive dust in the formulation is increased, epicuticular lipid is removed more rapidly leading to faster water loss and facilitating the penetration of cypermethrin into the insect. Eventually a formulation is reached where the rate of removal of epicuticular lipid, the rate and amount of water loss and the rate and amount of cypermethrin penetration into the insect causes a level of toxicity reflecting optimum joint action. Until this point, the ease with which the cypermethrin could penetrate into the insects was the dominant influence on the joint action, and the speed of knock-down probably increased. As the proportion of pyrethroid in the formulations is further decreased and the proportion of sorptive dust increased, the availability of cypermethrin most influences the joint action, and smaller joint action ratios occur.

Table 5.1 The amounts of cypermethrin dust and Sipernat D17 in each formulation used in the water loss experiment

Ratio	Amount Cyp. dust (mg.)	Amount sorptive dust (mg.)
Cypermethrin dust	25.1	-
5:1	23.0	4.60
2:1	20.4	10.2
1:1	17.4	17.4
1:2.5	11.8	29.5
1:5	7.50	39.0
1:10	4.56	45.6
Sipernat D17	-	55.5

Table 5.2 LC_{50} values for formulations of Aerosil R972 and Cypermethrin dust and for Cypermethrin dust alone

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	13.89(13.03-14.82)	2.78(2.61-2.96)	6.03(4.52-7.54)	1.94	3
2:1	13.24(12.39-14.15)	6.62(6.20-7.08)	6.20(4.30-7.59)	0.62	3
1:1	10.49(9.62-11.45)	10.49(9.62-11.45)	4.49(3.00-6.00)	4.01	3
1:2.5	9.34(8.77-9.94)	23.35(21.93-24.85)	7.24(5.75-8.73)	1.02	3
1:5	4.99(4.39-5.67)	24.95(21.95-28.35)	3.40(1.85-4.95)	4.40	3
1:10	3.80(3.54-4.07)	38.00(35.40-40.70)	5.20(4.15-6.25)	7.03	4
Cypermethrin dust only	25.11(23.82-26.49)	———	6.80(5.70-7.90)	11.79	6

* mg of cypermethrin dust/100g wheat or µg cypermethrin/100g wheat

** mg of sorptive dust/100g wheat

Table 5.3 LC_{50} values for formulations of Sipernat D17 and Cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc.of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	14.83(14.16-15.53)	2.97(2.83-3.11)	10.09(7.64-12.54)	3.83	2
2:1	12.08(10.32-14.14)	6.04(5.16-7.07)	4.90(2.61-7.19)	9.78^S	3
1:1	12.01(11.28-12.82)	12.01(11.28-12.82)	6.05(4.48-7.62)	3.80	3
1:2.5	9.82(9.17-10.51)	24.55(22.93-26.28)	6.58(4.88-8.28)	4.78	2
1:5	6.69(6.27-7.15)	33.45(31.35-35.75)	6.41(4.62-8.20)	5.81	3
1:10	4.25(3.83-4.71)	42.50(38.30-47.10)	3.82(2.72-4.92)	3.51	3

* mg cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg sorptive dust/100g wheat

S On referring χ^2 value to tables, $p<0.05$, and therefore the observed probit mortalities are significantly different from those predicted by the probit/log dose line.

Table 5.4 LC_{50} values for formulations of Gasil 35M and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc.of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc.of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	19.89(17.79-22.37)	3.98(3.56-4.47)	6.91(5.09-8.73)	8.38^S	3
2:1	17.09(15.99-18.29)	8.55(8.00-9.15)	7.35(5.74-8.96)	5.21	3
1:1	13.73(12.07-15.65)	13.73(12.07-15.65)	5.28(3.41-7.15)	11.72^S	4
1:2.5	11.38(9.96-12.99)	28.45(24.90-32.48)	2.96(2.16-3.76)	3.05	3
1:5	8.05(7.07-9.17)	40.25(35.35-45.85)	2.77(1.87-3.87)	5.12	4
1:10	5.76(4.91-6.75)	57.60(49.10-67.50)	2.72(1.71-3.73)	0.82	3

* mg cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg sorptive dust/100g wheat

S see Table 5.3

Table 5.5 LC_{50} values for formulations of Wessalon S and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	17.07(16.02-18.17)	3.41(3.20-3.63)	9.07(6.79-11.35)	0.12	1
2:1	15.06(14.50-16.78)	7.80(7.25-8.39)	6.39(5.07-7.71)	4.89	3
1:1	13.26(12.32-14.23)	13.26(12.32-14.23)	6.21(4.94-7.48)	5.70	3
1:2.5	10.92(9.88-12.06)	27.30(24.70-30.15)	4.04(2.28-5.80)	5.59	3
1:5	8.08(7.16-9.13)	40.40(35.80-45.65)	3.83(2.70-4.96)	1.65	3
1:10	5.33(4.67-6.08)	53.28(46.72-60.75)	3.58(2.41-4.75)	5.13	3

* mg cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg sorptive dust/100g wheat

Table 5.6 LC_{50} values for formulations of Gasil 23F and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	18.69(17.60-19.88)	3.74(3.52-3.98)	7.25(5.77-8.73)	3.71	3
2:1	15.92(15.11-16.78)	7.96(7.56-8.39)	8.97(6.83-11.11)	4.89	2
1:1	12.86(11.85-13.95)	12.86(11.85-13.95)	5.55(4.13-6.97)	2.08	3
1:2.5	9.78(8.70-11.00)	24.45(21.75-27.50)	3.31(2.48-4.13)	7.37	3
1:5	7.51(6.82-8.29)	37.55(34.10-41.45)	3.66(2.70-4.62)	5.28	4
1:10	4.59(4.17-5.05)	45.90(41.70-50.50)	3.89(2.66-5.19)	4.87	3

* mg of cypermethrin dust/100 g wheat OR µg cypermethrin/100g wheat

** mg of sorptive dust/100g wheat

Table 5.7 LC_{50} values for formulations of Aerosil 150 and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degrees of freedom
5:1	17.29(16.03-18.66)	3.46(3.21-3.73)	6.19(4.83-7.55)	0.83	3
2:1	18.59(16.53-20.88)	9.30(8.27-10.44)	6.01(4.11-7.91)	13.80^S	3
1:1	18.51(15.30-22.35)	18.51(15.30-22.35)	2.90(1.88-3.92)	1.94	3
1:2.5	17.95(16.63-19.36)	44.90(41.58-48.40)	5.66(4.43-6.89)	4.92	3
1:5	14.34(11.50-17.92)	71.70(57.50-89.60)	3.36(1.64-5.08)	12.77^S	4
1:10	9.99(8.34-11.97)	99.90(83.4-119.7)	2.57(1.65-3.49)	6.56	4

* mg cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg sorptive dust/100g wheat

S see Table 5.3

Table 5.8 LC_{50} values for formulations of Cab-O-Sil M5 and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degree of freedom
5:1	17.16(15.96-18.49)	3.43(3.19-3.70)	6.90(5.29-8.51)	2.39	2
2:1	16.21(15.18-17.32)	8.11(7.59-8.66)	7.06(5.65-8.47)	3.10	3
1:1	15.00(13.26-16.97)	15.00(13.26-16.97)	5.39(3.13-7.65)	10.58^S	3
1:2.5	13.98(12.62-15.52)	34.95(31.55-38.80)	4.48(3.43-5.53)	3.44	3
1:5	> 10	> 50	-	-	-
1:10	> 7	> 70	-	-	-

* mg cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg sorptive dust/100g wheat

S see Table 5.3

Table 5.9 LC_{50} values for formulations of Cab-O-Sil H5 and cypermethrin dust

Cypermethrin dust:sorptive dust ratio	Conc. of cypermethrin* in LC_{50} value (and 95% confidence limits)	Conc. of sorptive dust** in LC_{50} value (and 95% confidence limits)	Slope of Probit/log dose curve (and 95% confidence limits)	χ^2	Degree of freedom
5:1	20.66(19.21-22.20)	4.13(3.84-4.44)	5.61(4.49-6.73)	3.61	3
2:1	19.81(17.32-22.67)	9.91(8.66-11.34)	7.43(5.12-9.74)	10.24^S	4
1:1	19.75(18.41-21.13)	19.75(18.41-21.13)	6.41(5.14-7.68)	1.75	4
1:2.5	12.75(11.58-14.07)	31.88(28.95-35.18)	3.97(2.99-4.95)	5.91	3
1:5	11.42(10.03-13.02)	57.10(50.15-65.10)	2.71(1.78-3.64)	7.97	4
1:10	6.73(6.13 -7.38)	67.30(61.30-73.80)	3.74(2.81-4.71)	5.23	4

* mg of cypermethrin dust/100g wheat OR µg cypermethrin/100g wheat

** mg of sorptive dust/100g wheat

S See Table 5.3

Table 5.10 Joint action ratios between cypermethrin dust and sorptive dusts

Sorptive dust	Joint action ratio
Aerosil R972	1.61
Sipernat D17	1.66
Wessalon S	1.49
Gasil 23F	1.36
Gasil 35M	1.41
Aerosil 150	1.35
Cab-O-Sil M5	1.37
Cab-O-Sil H5	1.25

Table 5.11 Water loss in 24h. from beetles exposed to Sipernat D17:cyp. dust formulations

Cyp. dust:sorptive dust ratio	Water loss (% original wt.)	Transformed mean % water loss (± standard dev.)
Cyp. dust only	6.71	15.00±0.44
5:1	8.12	16.54±0.31
2:1	8.22	16.65±0.34
1:1	7.95	16.27±0.85
1:2.5	7.87	16.28±0.89
1:5	7.37	15.75±0.68
1:10	6.77	15.07±0.71
Sipernat D17 only	7.34	15.70±0.80
Control	0.57	3.22±0.67

Table 5.12 Amounts of each toxicant picked up in 24h. by beetles exposed to Sipernat D17:cyp. dust formulations

Cyp.dust:sorptive dust ratio	Amount of Sipernat D17/insect (µg)	Amount of cyp. dust/insect (µg)	Total dust/ insect (µg)
Cyp. dust only	-	5.83	5.83
5:1	1.19	6.65	7.84
2:1	3.03	6.12	9.19
1:1	4.73	4.42	9.15
1:2.5	7.76	3.07	10.83
1:5	10.01	1.81	11.82
1:10	11.11	1.02	12.13
Sipernat D17 only	12.97	-	12.97

Table 5.13 The amounts of Sipernat D17 and cypermethrin dust/ beetle expressed as a proportion of the respective LC_{50} values, and the amount of "total toxicant"/ beetle

Cyp. dust:sorptive dust ratio	Proportion of LC_{50} of cyp. dust (x 10^4)	Proportion of LC_{50} of Sipernat D17 (x10^4)	Total toxicant
Cyp. dust only	2.32	-	2.32
5:1	2.65	0.21	2.86
2:1	2.44	0.55	2.99
1:1	1.76	0.85	2.61
1:2.5	1.22	1.40	2.62
1:5	0.72	1.80	2.52
1:10	0.41	2.00	2.41
Sipernat D17 only	-	2.34	2.34

Fig.5.1 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Aerosil R972

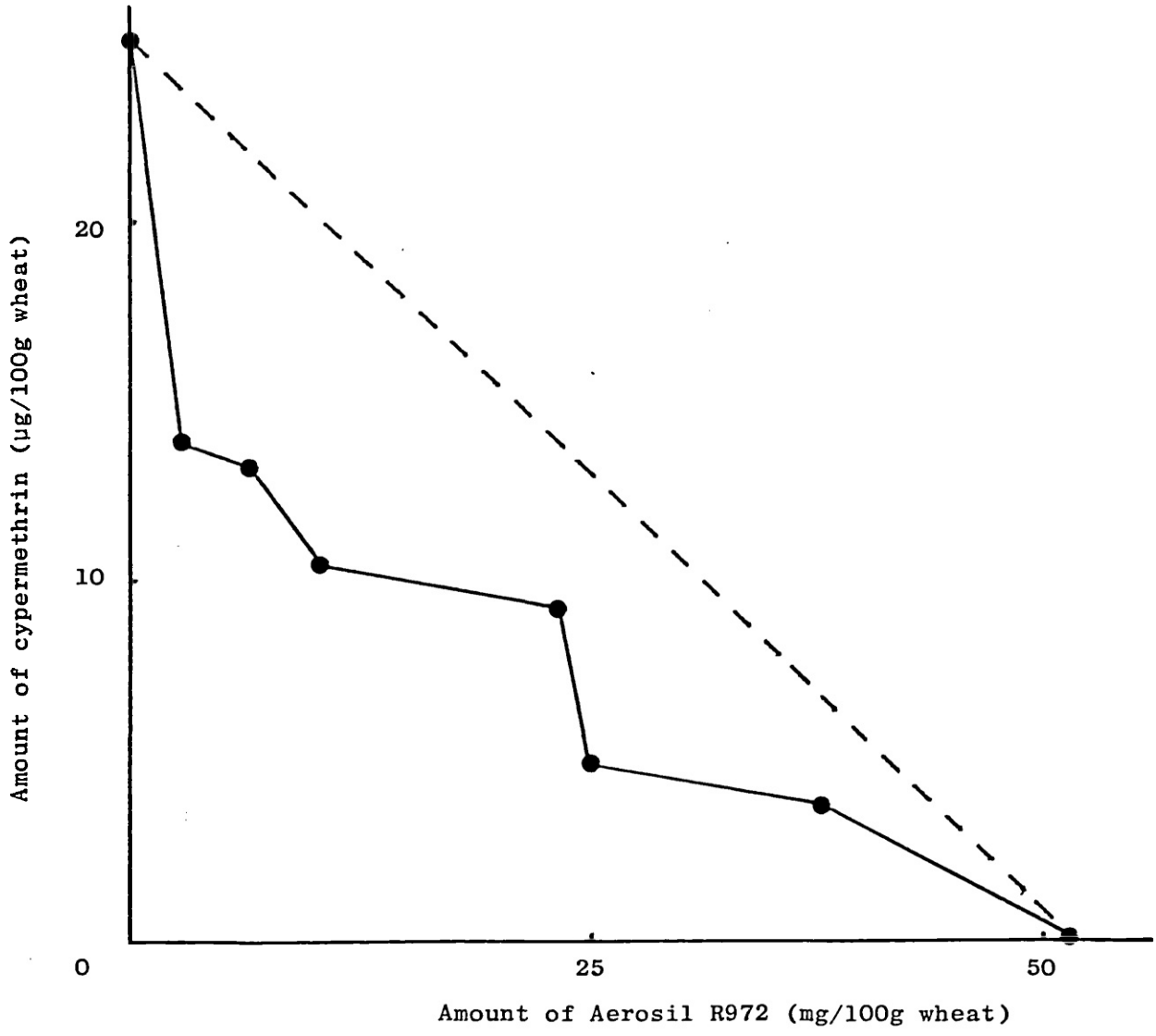


Fig.5.2 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Sipernat D17

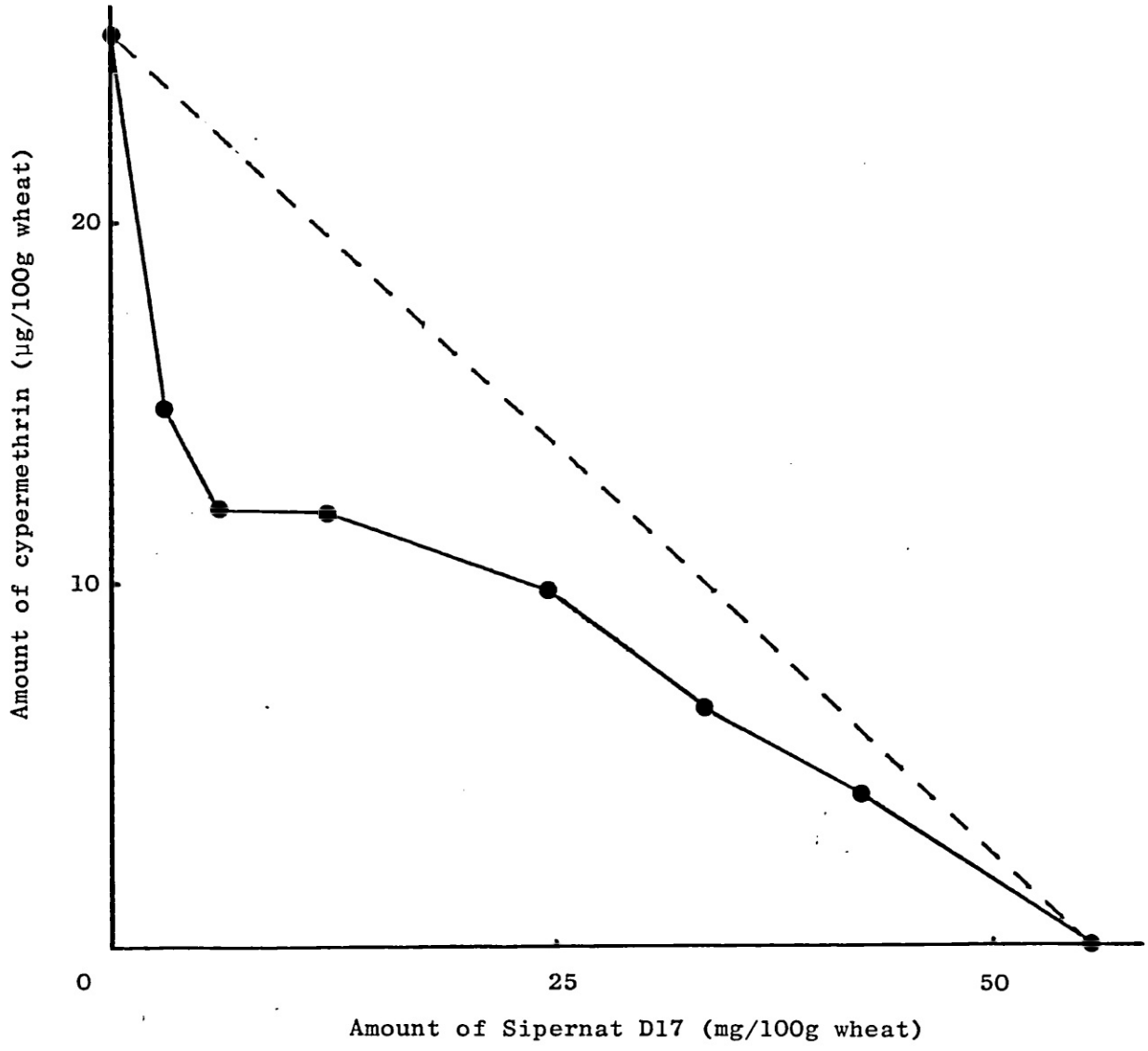


Fig.5.3 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Gasil 35M

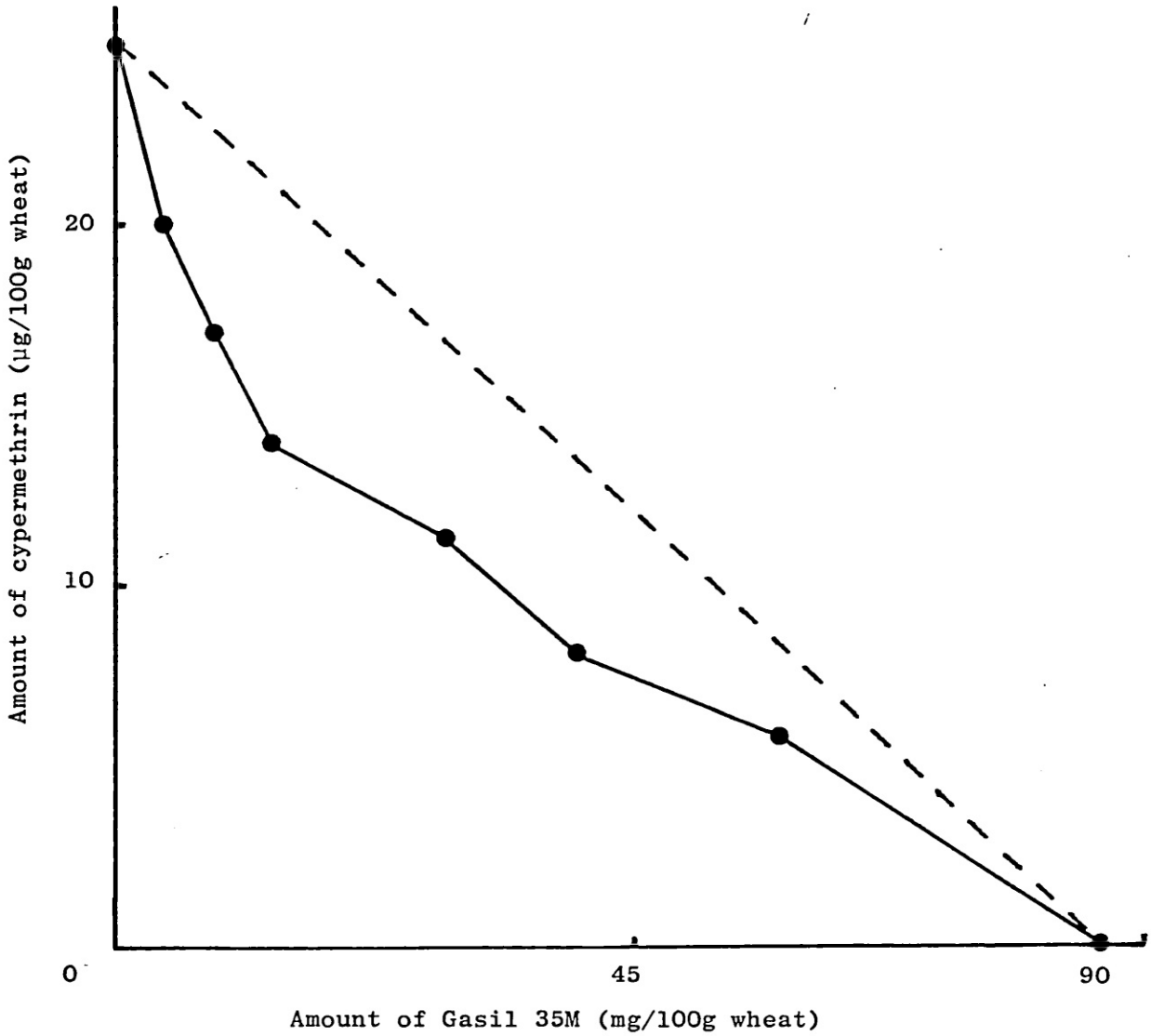


Fig.5.4 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Wessalon S

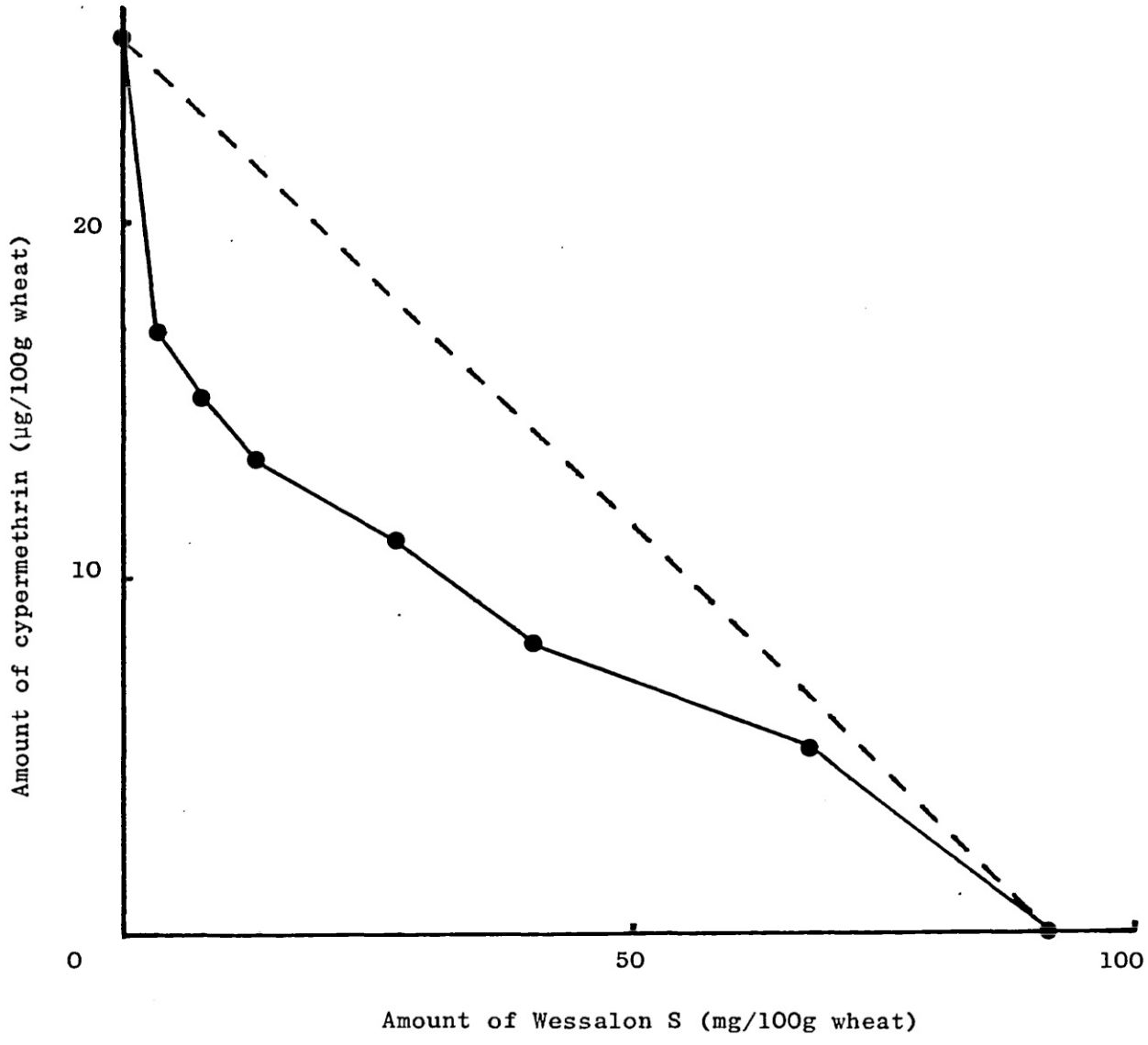


Fig.5.5 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Gasil 23F

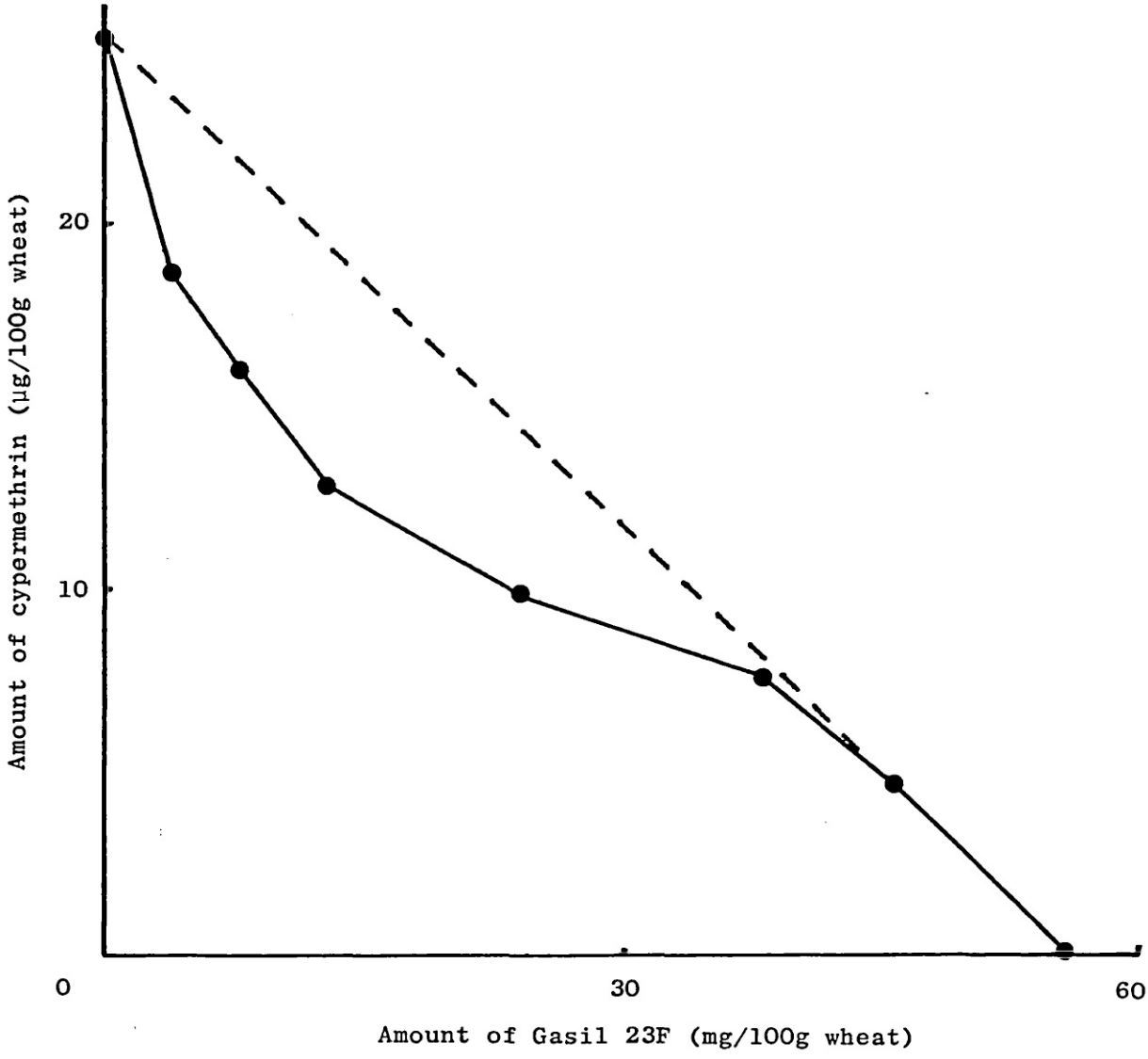


Fig.5.6 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Aerosil 150

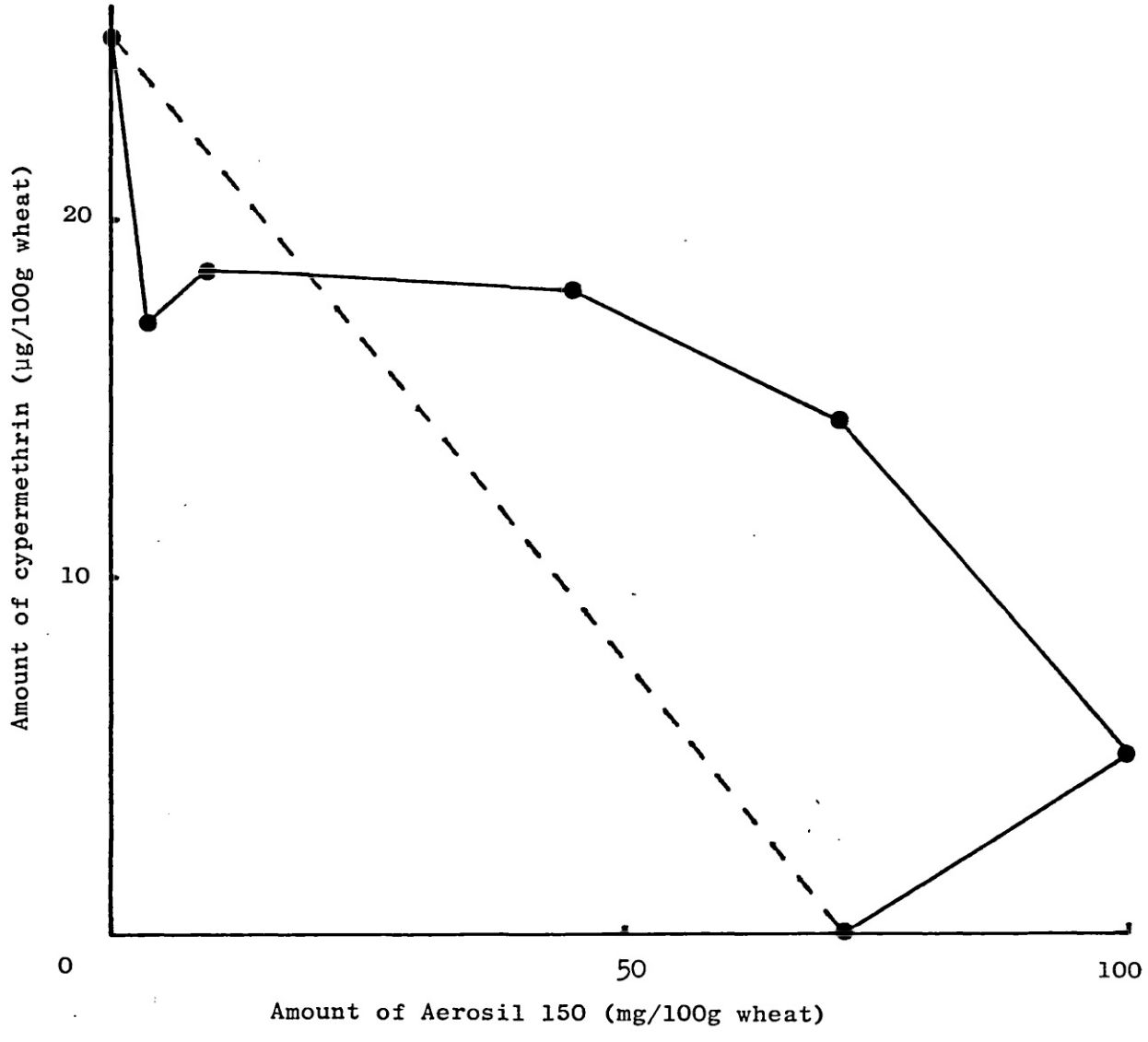


Fig.5.7 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Cab-O-Sil M5

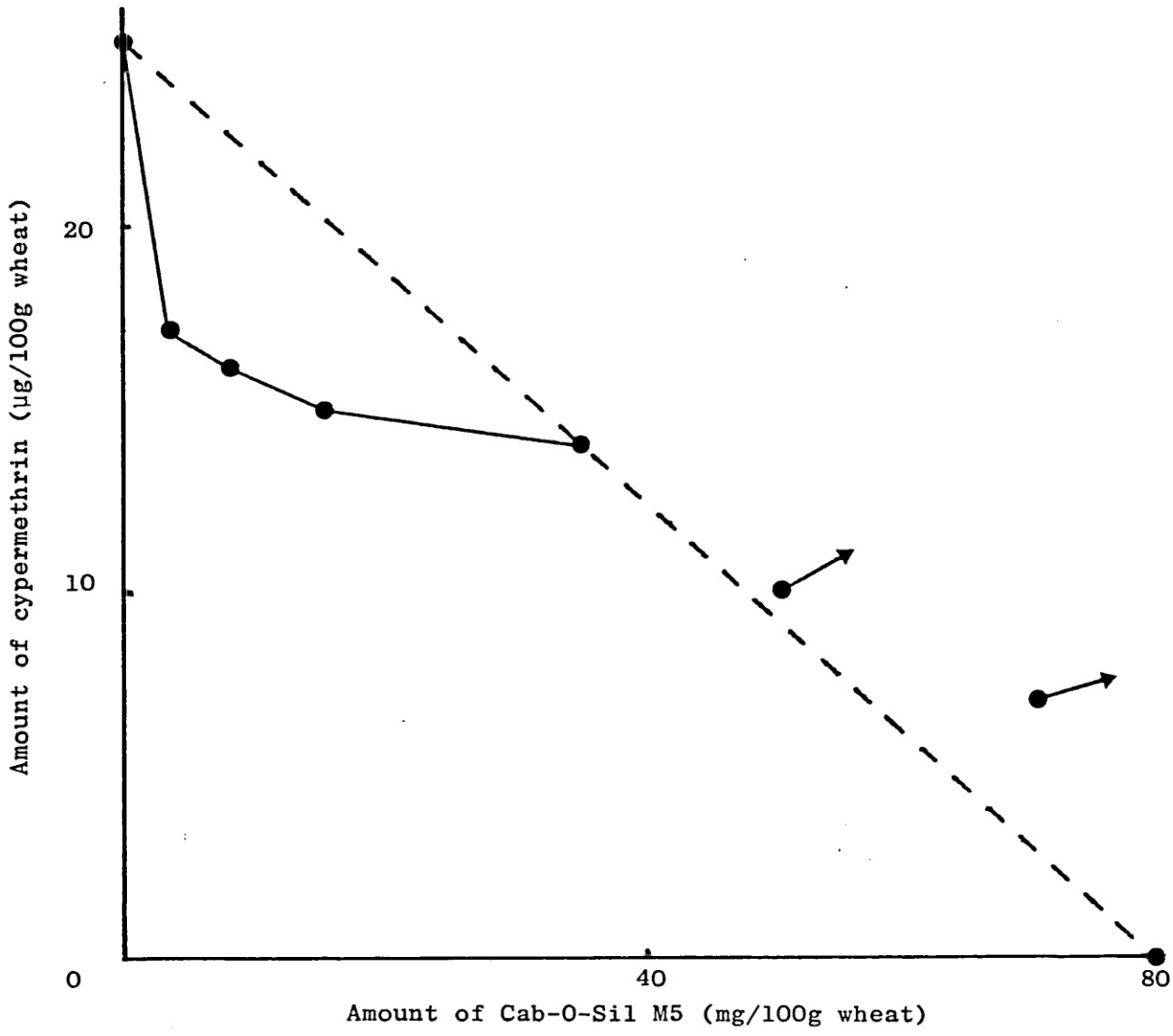


Fig.5.8 Isobologram depicting the amount of each toxicant in the LC_{50} values of formulations of cypermethrin dust and Cab-O-Sil H5

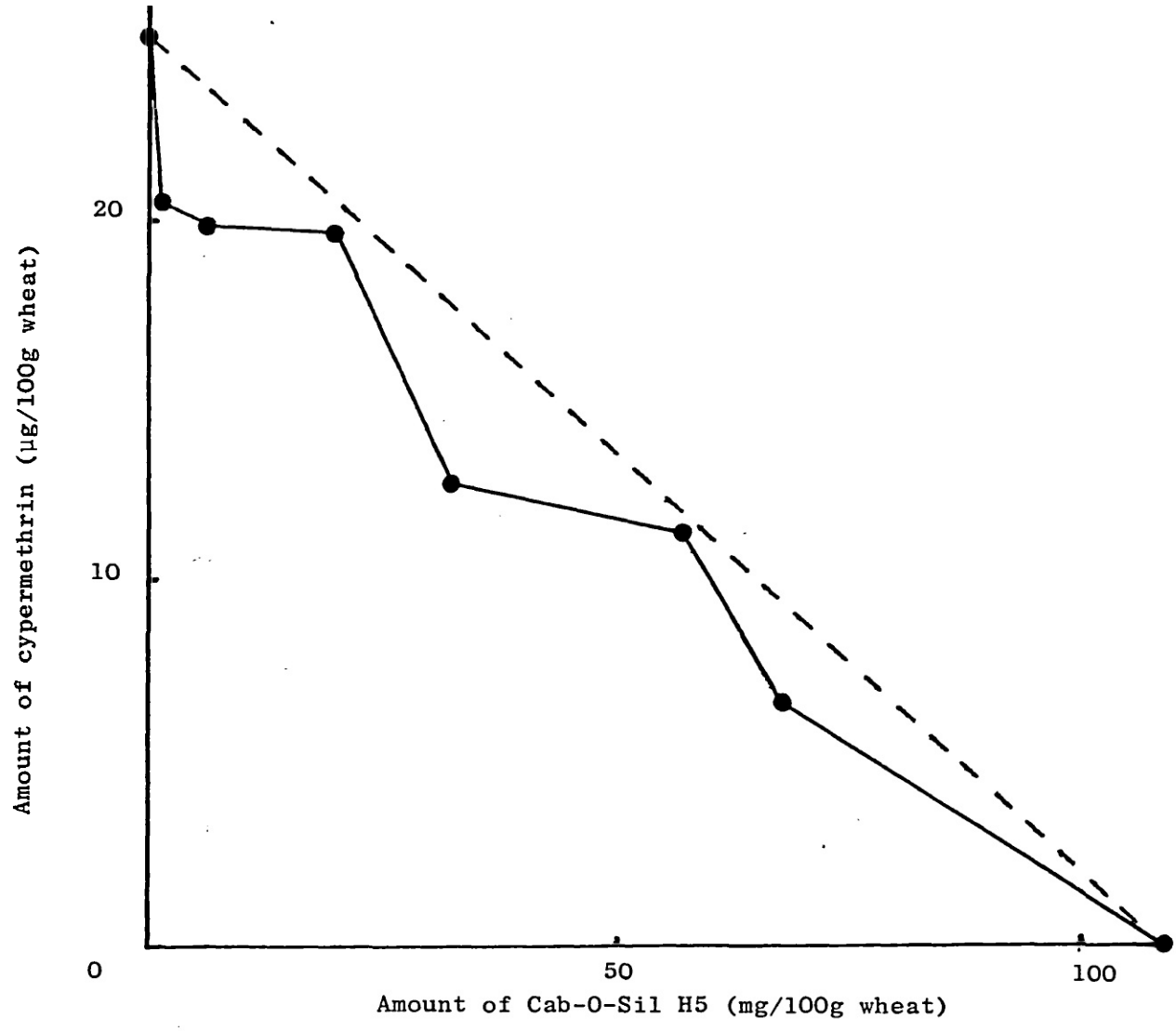
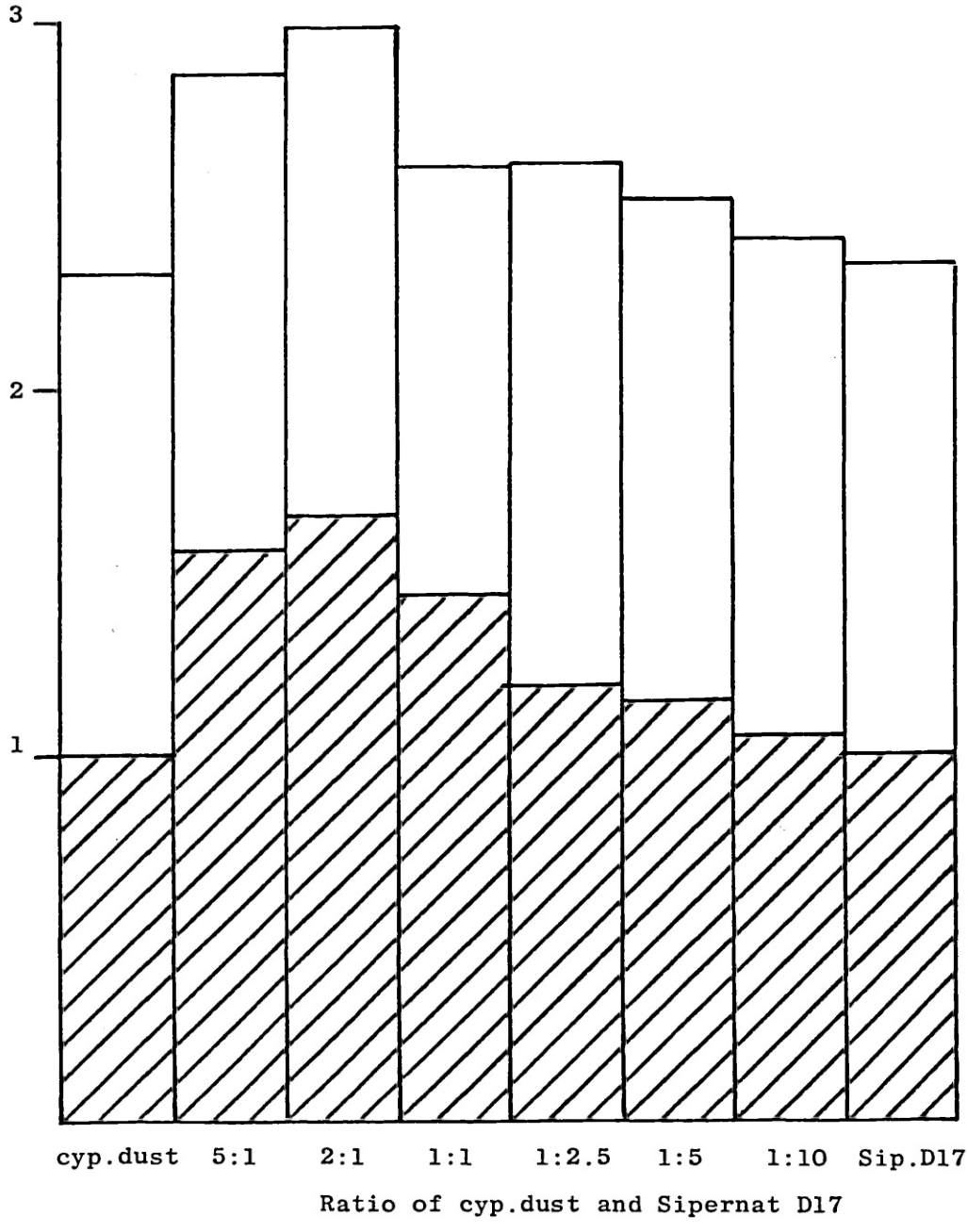


Fig.5.9 The amount of "total toxicant" on beetles exposed to different ratios of Sipernat D17 and cypermethrin dust after 24h. and the potentiation ratio of each formulation



Unshaded scale: Amount of "total toxicant"/beetle ($x10^4$)

Shaded scale (superimposed): Potentiation ratio

Section 6

Summary of results and General Discussion

6.1 Summary of results

(1) At the LC_{50} level, the toxicity of most of the sorptive dusts tested to *S.granarius* were in the range 50-150 mg/100g. wheat. Only two physico-chemical charactistics had any appreciable effect on toxicity: surface treatment resulting in hydrophobicity enhanced toxicity; and porous silicas with pore diameter <2nm were insecticidally inactive.

(2) At the LC_{95} level, the hydrophobic silica dusts are far more insecticidally active than hydrophilic silica dusts. The calculated LC_{95}'s of the three hydrophobic silicas tested are in the range 100-150 mg/100g wheat. The LC_{95}'s for the hydrophilic dusts are all over 300 mg/100g wheat, which is the maximum concentration of dust which can be mixed with the wheat without it separating out.

(3) When *S.granarius* were exposed for 24h. to wheat treated with sorptive silica dust at 50 mg/100g wheat, four different types of dust caused significantly different levels of water loss. These were:-

Hydrophobic dusts > Porous hydrophilic dusts > fumed hydrophilic dusts >> Porous dusts (pore diameter <2nm)

(4) When *S.granarius* were introduced to wheat treated with radio-labelled dust at 10 mg/100g wheat, the amount of dust on the beetles (assayed radiometrically) either reached or was close to reaching an equilibrium level within 24h. Three groups of dust can be distinguished by having different maximum pick-up levels:-

Hydrophobic dusts >> Porous hydrophilic dusts > fumed hydrophilic dusts

The dusts can also be grouped as above according to the amounts on the beetles after 24h. exposure to wheat treated with dusts at 50 mg/100g wheat.

(5) The turn-over experiment confirmed Singh's observation (44) that there is a turn-over of dust on the insects cuticle and that a proportion of the dust is irreversibly bound to the insects and not exchangeable. A first order model was sufficient to establish the range of the rates of turn-over of the insecticidally active dusts, however, plots of $\ln a_1$/time were significantly non-linear for Gasil grades 200 and GM2 and their rates of turn-over could not be calculated. The lack of linearity was thought to indicate different rates of turn-over on different regions of the insect.

(6) Isobolograms derived from the LC_{50} values of different ratios of two toxicants show that cypermethrin dust (cypermethrin at 0.1% w/w on Gasil GM2) has a potentiating joint action with hydrophobic silica dusts and with porous hydrophilic dusts. Potentiation was greatest with the hydrophobic dusts. Of the fumed hydrophilic dusts, Cab-O-Sil H5 had slight potentiating or additive joint action with cypermethrin dust, while both Aerosil 150 and Cab-O-Sil M5 had slight potentiation at high cypermethrin dust: sorptive dust ratios and sub-additive or antagonistic joint action at low ratios.

(7) The water loss was determined from *S.granarius* exposed for 24h. to wheat treated with the LC_{50} value of cypermethrin dust alone, the LC_{50} value of Sipernat D17 alone, and six combinations which fall on the line for additive joint action between the two toxicants (Fig.5.2). Although different dust treatments did cause significantly different levels of water loss, the difference was small. This was interpreted

as indicating that optimun cypermethrin penetration into the insect rather than optimum water loss is the major cause of potentiation.

(8) In the above experiment, the amount of "total toxicant" (Section 5.3.2) on the beetles after 24h. exposure to the different ratios of Sipernat D17 and cypermethrin dust reflected the potentiation shown by the different ratios.

Two additional experiments were performed to validate the method used to radiolabel the sorptive dusts and to reproducibly extract and radiometrically assay the isotope on the dust picked up by the beetles:-

(a) The first experiment demonstrated that: the ^{35}S in the dust is all extracted into aqueous solution (for radiometric assay) no matter how closely it is adhered to the beetles; no dust is lost transferring beetles from dust treated wheat to scintillation counting vials; and that the novel form of quench curve used in the present work provides reproducible corrected results.

(b) The second experiment demonstrated that sodium ^{35}sulphate used to label the dusts does not separate from the dust when admixed with wheat, and that the presence of sodium sulphate on the surface of the dust does not affect the extent to which beetles can pick it up.

6.2 General Discussion

The factors which influence the ultimate insecticidal activity of sorptive silica dusts are their sorptivity (which can be regarded as their intrinsic toxicity), maximum pick-up level, and their rate of turnover on the insect. All of these factors are related to the dusts physico-chemical characteristics, and a hypothesised mechanism by which they

combine to influence the ultimate insecticidal activity of the dust is shown in Fig.6.1.

The sorptivity, maximum pick-up level and rate of turn-over of the dusts influence the removal of epicuticular lipid which allows the desiccation of the insect and leads to incapacitation and death. The influence of the turn-over of the dust is complex, since once the insect is incapacitated, the turn-over of dust no longer occurs. In addition, the extent to which the dust particles become saturated with lipid while in transit on the insects cuticle is not known.

The hydrophobic silica dusts caused a higher level of water loss from the beetles in 24h. than the hydrophilic dusts at similar concentrations (Section 3.3.1), thus showing that the higher maximum pick-up levels of the former were sufficient to make up for their low sorptivities and cause the most rapid removal of epicuticular lipid. Beetles exposed to hydrophobic dusts would therefore be incapacitated more quickly than those exposed to hydrophilic dusts. Once incapacitated, however, the turn-over of dust on the beetles ceases so that only dust already present on their cuticles can affect them further. It is likely, therefore, that the initial rapid water loss caused by the hydrophobic dusts and their high maximum pick-up levels are mainly responsible for the high 10-day toxicities of these dusts.

The fact that the fumed and porous hydrophilic silicas have similar 10-day toxicities (Section 3.3.1) but caused different levels of water loss from the beetles in 24h. (Section 3.3.2) can be explained by the length of time that the turn-over of dust on the beetles continues. The porous dusts have a higher maximum pick-up level and cause more water loss from the beetles in 24h, than the fumed dusts. Consequently, the porous dusts incapacitate the beetles more quickly but are picked-up

and turned-over for a shorter time. However, the beetles were evidently less susceptible to the slower water loss caused by the hydrophilic dusts than to that caused by the hydrophobic dusts, and the differences in rates of water loss, maximum pick-up level and sorptivity of the porous and fumed hydrophilic silicas were not sufficient to cause dissimilar levels of toxicity over 10 days.

The porous hydrophilic dusts with low pore diameters (Gasil 200 and Gasil GM2) have low sorptivities, but unlike the hydrophobic dusts their maximum pick-up levels are not high enough to compensate for this which results in low insecticidal activity.

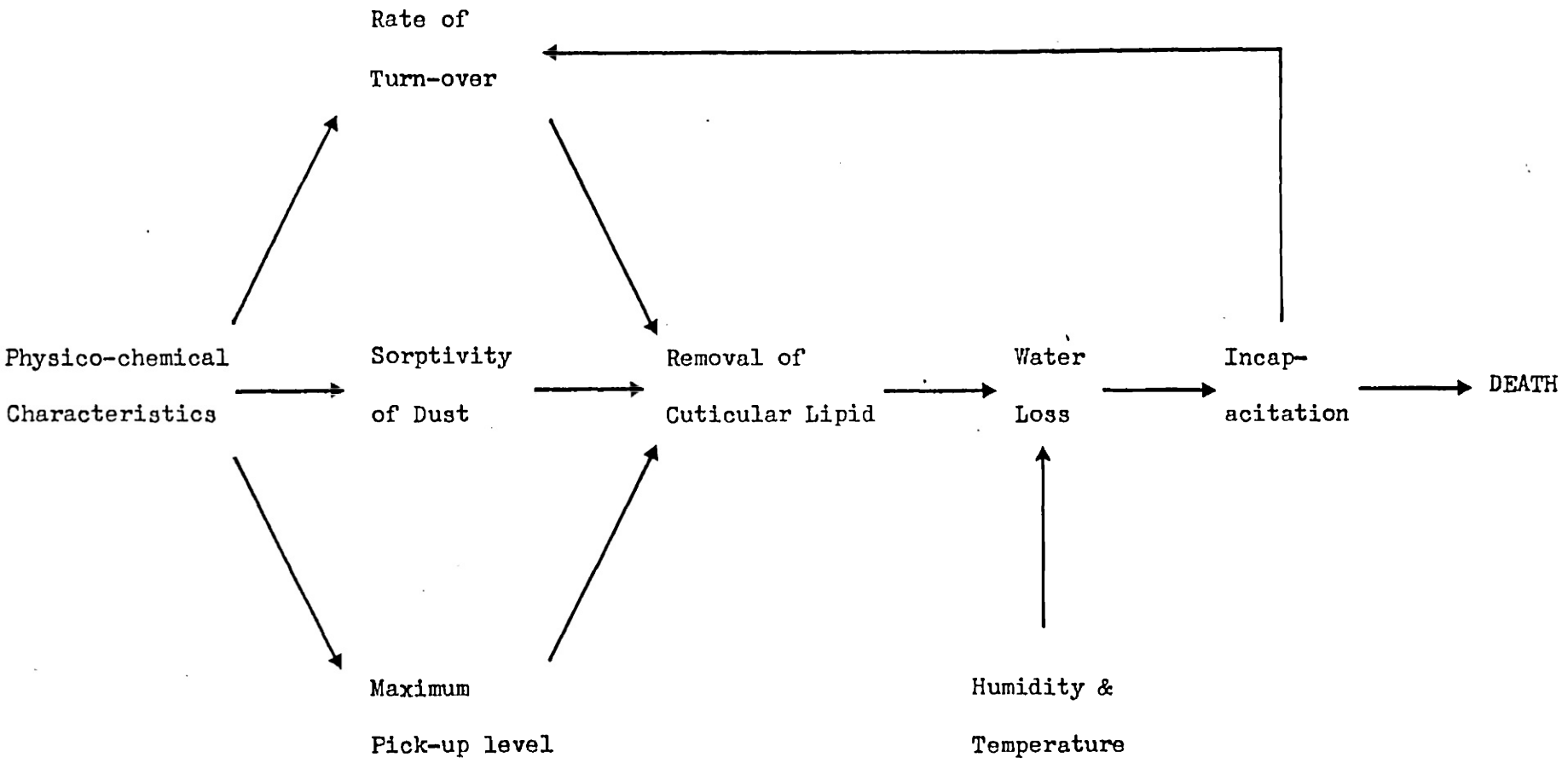
The factor which best reflects the extent to which a sorptive dust potentiates cypermethrin dust is its capacity to cause water loss from the beetles in 24h. rather than its 10-day toxicity. However, the water loss from the beetles is caused by the removal of epicuticular lipid, and this possibly enhances the penetration of cypermethrin into the insects and causes potentiation. The mode of action experiment (Section 5.2.4) indicated that potentiation is mainly due to optimum penetration of cypermethrin into the insect rather than optimum water loss, since the difference in the levels of water loss from insects exposed to each formulation of the two toxicants, though significant, was small.

A fortuitous result of the mode of action experiment was that the amount of "total toxicant" on the beetles after 24h. exposure to each ratio of Sipernat D17 and cypermethrin dust reflected the potentiation for that formulation. Since the beetles exposed to each ratio were all knocked-down within this period, the amount of toxicant on the beetles was probably close to that which was responsible for the 10-day toxicity of that ratio.

Clearly the best way to determine whether optimal penetration of cypermethrin into the beetles was the major cause of potentiation would have been to assay the amount of pesticide which penetrated into the beetles in the mode of action experiment. However, owing to the problems outlined in Section 5.3.2 this would have been practically impossible. These problems could be obviated if a larger experimental insect was used from which samples of either tissue or haemoplasm could be drawn without contamination from dust on the outside of the insect. An ideal test insect would be the adult Mealworm beetle, *Tenebrio molitar*.

To summarise the results of the present work, most of the aims outlined in Section 1.5 were achieved. The characteristics which most enhanced the toxicity, capacity to cause water loss, and the maximum pick-up level of the dusts were identified. The range of the rates of turn-over of the dusts on the beetles (at sub-lethal levels) was established, however the 95% confidence intervals of these values were too wide for the characteristics which most affected rate of turn-over to be identified. The role of insecticide penetration in the potentiating joint action between cypermethrin and sorptive dusts was investigated only by indirect means, however experiments which might overcome this difficulty have been suggested.

Figure 6.1 The factors which influence the insecticidal activity of amorphous silica dusts to beetles in stored grain.



Appendix 1. Physical properties of the dusts used in the present work (manufacturers estimates)

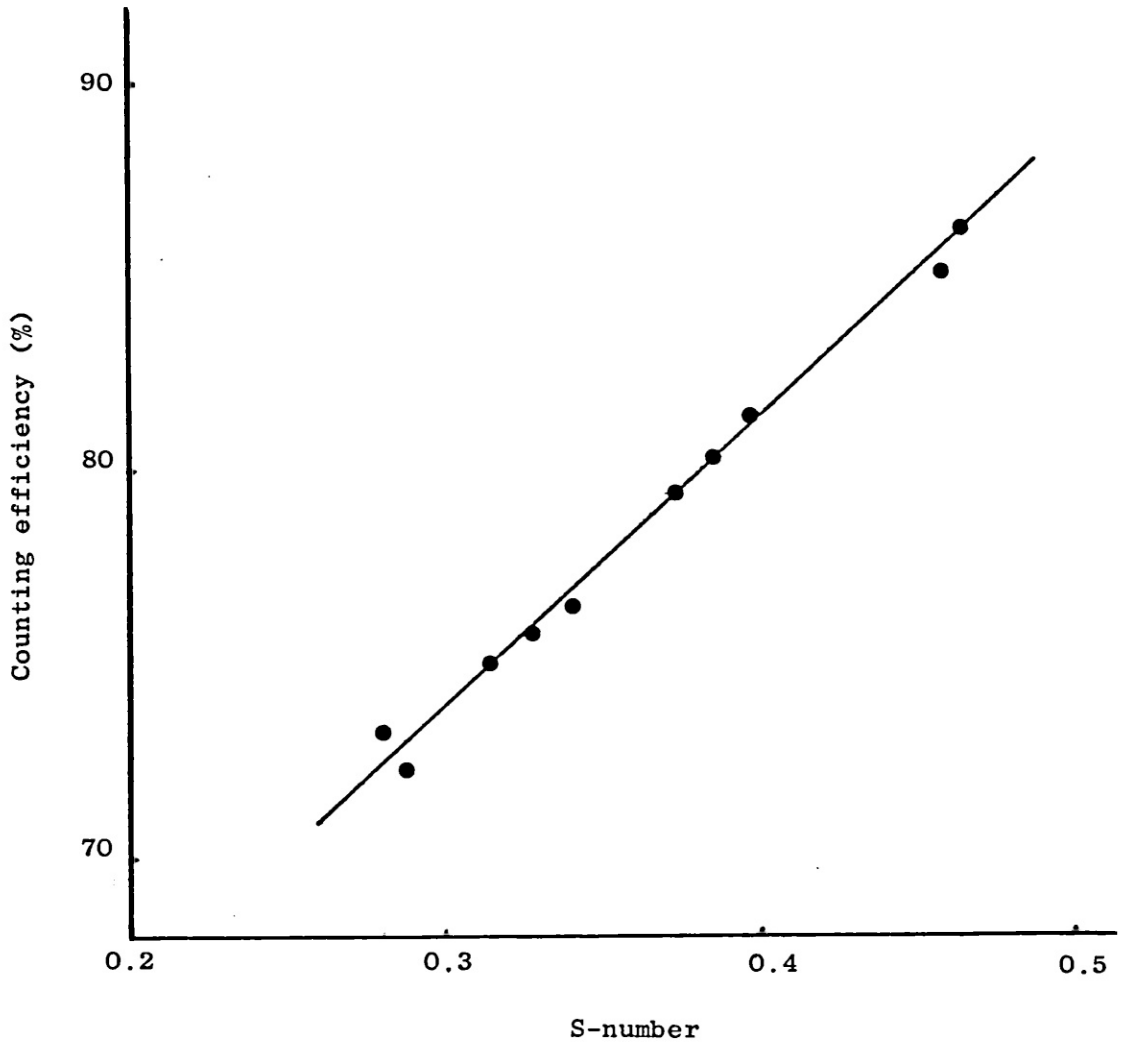
	Method of Manufacture	Primary particle size (nm)	Secondary particle size (μm)	Specific surface area(m^2/g)	Pore volume (cm^3/g)	Bulk density ($g/100cm^3$)	Oil absorption (g/100g dust)
Aerosil 130	F	16	-	130±25	-	3.7	-
Aerosil 150	F	14	-	150±15	-	3.7	-
Cab-O-Sil M5	F	14	-	200±25	-	3.7	-
Cab-O-Sil H5	F	7	-	325±25	-	3.7	-
Cab-O-Sil EH5	F	7	-	390±40	-	3.7	-
Aerosil R972	HF	16	-	120±30	-	5	-
Wacker HDK H20	HF	NA	-	170±30	-	5	-
Sipernat D17	HP	28	N.A	110	NA	14	180
Sipernat 22	P	18	80.0	190	NA	18	230
Wessalon S	P	18	5.0	190	NA	9	240
Gasil 35M	A	NA	3.2	320	1.2	15	200
Gasil 114	A	NA	5.7	320	1.2	15	200
Gasil EBN	A	NA	8.0	320	1.2	15	200
Gasil AF	A	NA	17.0	320	1.2	20	180
Gasil 23D	A	NA	2.0	290	1.6	8	260
Gasil 23C	A	NA	2.8	290	1.6	8	260
Gasil 23F	A	NA	3.5	290	1.6	8	260
Gasil HP37	A	NA	6.5	280	1.6	12.5	280
Gasil 200	A	NA	4.5	750	0.4	28	80
Gasil GM2	A	NA	10.0	750	0.4	32	80

F = fumed. P = precipitated. A = aerogel. H = hydrophobic. N.A = no information available

Appendix 2. Data for Quench curve

Wt. standard (mg)	calculated d.p.m.	c.p.m.	% efficiency	S-number	No. insects
74.3	8157.2	5887.9	72.3	0.287	60
145.0	15919.3	11660.4	73.2	0.279	55
79.0	8673.3	6507.0	75.0	0.314	50
76.3	8376.8	6349.4	75.8	0.328	45
84.8	9310.0	7126.2	76.5	0.341	40
81.6	8958.7	7114.8	79.4	0.373	30
131.1	14393.2	11717.5	81.4	0.398	20
99.4	10912.9	8763.2	80.3	0.385	10
70.1	7696.1	6631.9	86.2	0.464	0
106.8	11725.4	9899.5	85.0	0.457	0

Appendix 3 Quench curve for ^{35}S in aqueous Cocktail T gel using beetles as a source of quench



Appendix 4 Data for the amount of dust on *S.granarius* over a 24h period

Dust	Aerosil 130		Aerosil 150		Cab-O-Sil H5		Cab-O-Sil M5	
	µg dust/insect		µg dust/insect		µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result	each replicate	mean result	each replicate	mean result
1	1.03 1.00	1.02	0.83 0.82	0.83	0.69 0.69	0.69	0.89 0.82	0.86
3	1.07 1.15	1.11	0.99 1.00	1.00	0.87 0.93	0.90	0.99 0.96	0.97
6	1.37 1.10	1.24	1.41 1.20	1.30	0.99 1.01	1.00	1.15 1.25	1.20
12	1.52 1.58	1.55	1.39 1.29	1.34	1.23 1.26	1.25	1.36 1.35	1.36
24	1.77 1.90	1.83	1.52 1.42	1.47	1.32 1.15	1.24	1.49 1.41	1.45

Appendix 4(cont.) Data for the amount of dust on *S.granarius* over a 24h period

Dust	Cab-O-Sil EH5		Aerosil R972		Sipernat D17		Gasil 35M	
	µg dust/insect		µg dust/insect		µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result	each replicate	mean result	each result	mean result
1	0.82 0.80	0.81	1.76 1.88	1.82	1.75 1.92	1.84	1.01 1.18	1.10
3	1.02 1.00	1.01	2.43 2.41	2.42	2.83 2.44	2.64	1.20 1.35	1.28
6	0.95 1.05	1.00	2.99	2.99	3.22 2.69	2.96	1.35 1.39	1.37
12	1.03 1.01	1.02	3.31 3.35	3.33	3.82 3.17	3.50	1.43 1.41	1.42
24	0.87 1.00	0.94	3.52 3.55	3.54	3.44 3.35	3.40	1.40 1.37	1.39

Appendix 4 (cont.) Data for the amount of dust on *S.granarius* over a 24h period

Dust	Gasil HP37		Gasil 23C		Gasil 23D		Gasil 23F	
	µg dust/insect		µg dust/insect		µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result	each replicate	mean result	each replicate	mean result
1	1.14 1.19	1.17	0.78 1.01 0.99 1.07	0.96	0.89 1.15 1.09 0.96	1.02	1.21 1.23	1.22
3	1.33 1.26	1.30	1.35 1.62 1.32 -	1.43	1.42 1.68 1.37 1.20	1.42	1.49 1.49	1.49
6	1.38 1.54	1.46	1.39 1.75 1.29 1.47	1.48	2.03 1.62 1.48 1.90	1.76	1.74 1.93	1.84
12	1.50 1.50	1.50	1.77 1.73 1.55 1.64	1.67	1.78 1.52 1.63 1.68	1.65	2.39 2.17	2.28
24	1.44 1.56	1.50	2.39 1.72 1.80 2.01	1.98	1.95 1.96 1.71 2.04	1.92	2.17 2.22	2.20

Appendix 4 (cont.) Data for the amount of dust on *S.granarius* over a 24h period

Dust	Gasil 200		Gasil GM2		Sipernat 22		Wessalon S	
	µg dust/insect		µg dust/insect		µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result	each replicate	mean result	each replicate	mean result
1	2.11	2.11	1.85 1.95	1.90	1.22 1.15	1.19	1.30 1.29	1.30
3	2.14 1.89	2.02	1.80 1.97	1.89	1.55 1.42	1.49	1.50 1.61	1.56
6	2.19 1.93	2.06	1.99 1.84	1.92	1.75 1.73	1.74	1.90 1.72	1.81
12	2.25 2.06	2.16	2.03 1.88	1.96	1.88 1.98	1.93	1.77 1.89	1.83
24	2.09 2.00	2.05	2.08 1.83	1.96	1.81 1.69	1.75	1.86 2.24	2.05

Appendix 5

The amount of radiolabelled dust left on the beetles after their introduction to wheat treated with unlabelled dust

Dust	Cab-O-Sil M5		Aerosil 150	
	µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result
0	2.39 2.52	2.46	1.83 2.07	1.95
1	1.82 1.62	1.72	1.37 1.23	1.30
3	1.37 1.51	1.44	1.15 1.11	1.13
6	1.62 1.35	1.49	0.91 1.00	0.96
12	1.14 0.98	1.06	0.84 0.71	0.78
24	0.96 0.99	0.98	0.53 0.41	0.47

Appendix 5 (cont.)

Dust	Gasil 200		Gasil GM2	
	µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result
0	2.36 2.22	2.29	1.90 2.21	2.06
1	1.12 0.93	1.03	0.84 0.79	0.82
3	0.84 0.74	0.79	0.68 0.58	0.63
6	0.52 0.67	0.60	0.54 0.55	0.55
12	0.41 0.44	0.43	0.42 0.32	0.37
24	0.29 0.29	0.29	-	- -

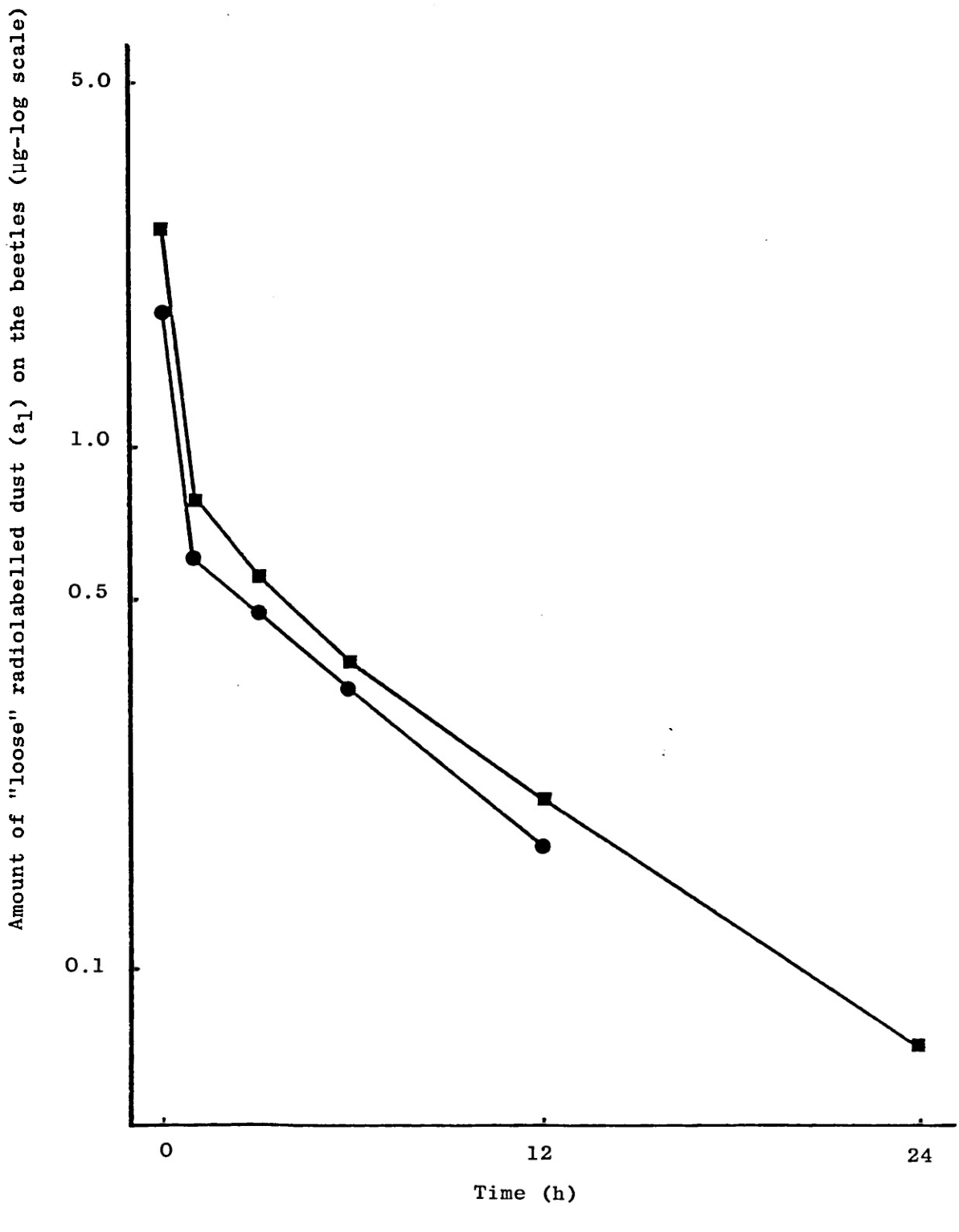
Appendix 5 (cont.)

Time	Gasil 35M		Wessalon S	
	µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result
0	1.86 1.78	1.82	2.49 2.62	2.56
1	1.29 1.17	1.23	1.78 1.72	1.75
3	0.81 -	0.81	1.58 1.40	1.49
6	0.90 0.84	0.87	1.38 1.20	1.29
12	0.72 0.76	0.74	1.07 1.16	1.12
24	0.59 0.54	0.57	0.98 0.93	0.96

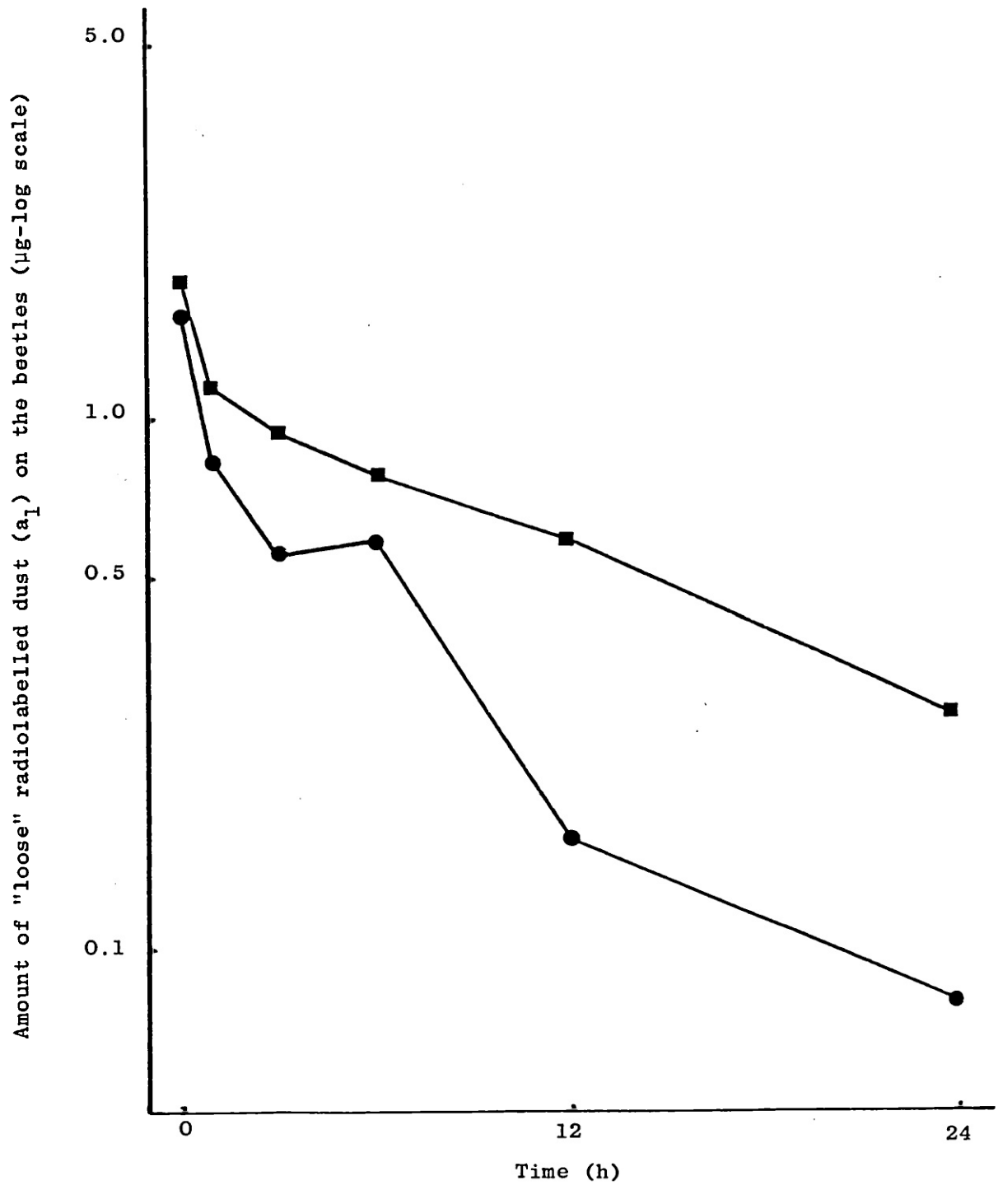
Appendix 5 (cont.)

Dust	Sipernat D17		Aerosil R972	
	µg dust/insect		µg dust/insect	
exposure time (h)	each replicate	mean result	each replicate	mean result
0	3.24 3.05	3.15	3.10 3.30	3.20
1	2.70 2.71	2.71	2.60 2.72	2.66
3	2.31 2.25	2.28	2.33 3.19	2.76
6	1.94 1.92	1.93	2.20 2.44	2.32
12	1.80 1.71	1.76	1.89 2.00	1.95
24	1.59 1.54	1.57	1.73 1.67	1.70

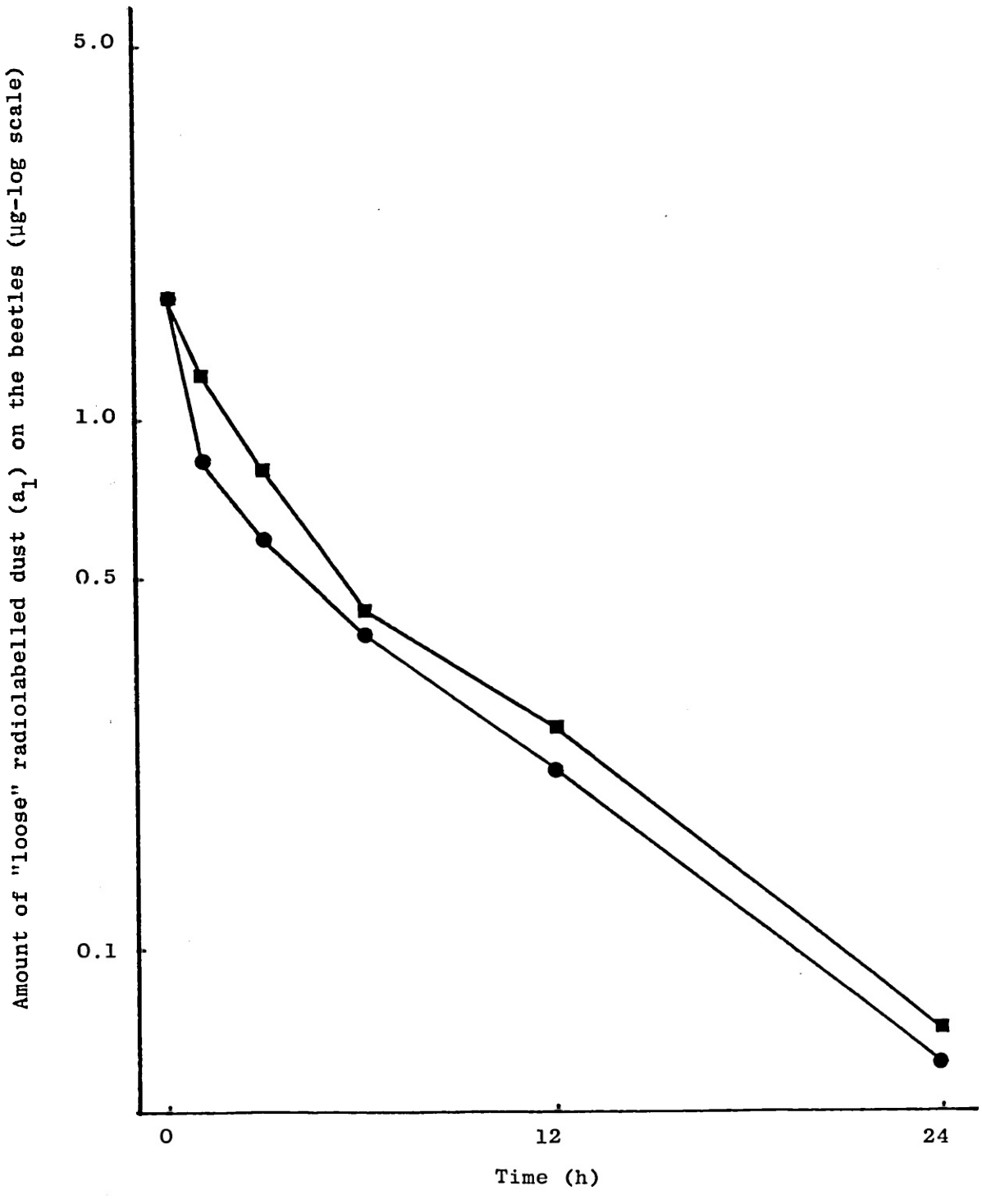
Appendix 6 Mean amounts of "loose" radiolabelled Gasil 200 and Gasil GM2 on the beetles (log scale) after their introduction to wheat treated with unlabelled dust



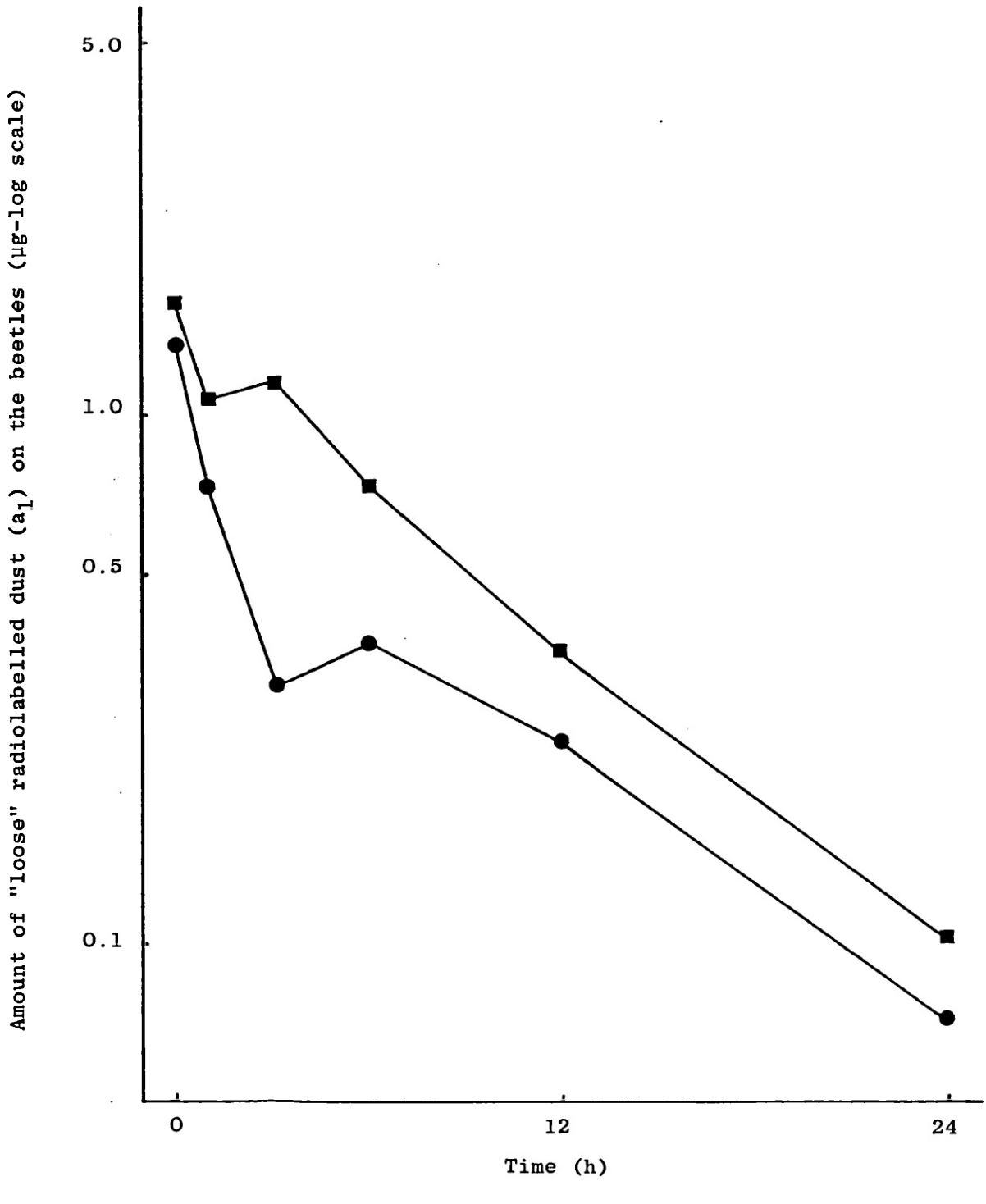
Appendix 6(cont). Mean amounts of "loose" radiolabelled Wessalon S and Cab-O-Sil M5 on the beetles (log scale) after their introduction to wheat treated with unlabelled dust



Appendix 6 (cont). Mean amounts of "loose" radiolabelled Sipernat D17 and Aerosil 150 on the beetles (log scale) after their introduction to wheat treated with unlabelled dust



Appendix 6(cont). Mean amounts of "loose" radiolabelled Aerosil R972 and Gasil 35M on the beetles (log scale) after their introduction to wheat treated with unlabelled dust



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