CRYSTAL FORMS AND AVAILABILITY OF LINDANE RESIDUES TO AN AMBROSIA BEETLE

by

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ABSTRACT

Some of the factors affecting the form and size of crystals produced by the solvent exchange method are described for lindane. The effect of different crystal forms on the availability of this toxicant to the ambrosia beetle Trypodendron lineatum (Oliv.) was evaluated from gross functional effects.

The beetles were brought into contact for known periods of time with deposits of a prescribed dosage of lindane crystals of two forms. Mortality was used as an index of the availability of the different deposits. The loss in weight with time was followed for deposits of different dosages of lindane crystals of the two forms, as a possible factor contributing to differences in residual toxicity.

With dosages corresponding to those used for preventing damaging attacks on logs by this ambrosia beetle, viz. 180 mg./ft.^{2}, no difference in effect was demonstrated between crystals of the two forms. No striking difference in rate of residue loss was demonstrated when deposits "aged" in a draught free cupboard. The minimum and maximum temperature recorded in the cupboard during the storage period was 14.5^o and 23.5^{o}C.

The concept of availability of insecticidal deposits and the factors affecting it are discussed.

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INTRODUCTION

In economic entomology the aim in studying the sequence, - insecticidal deposit - insecticide picked up - insecticide "inside the insect", is to be able to apply a deposit of a residual insecticide to a surface that will give the most effective control per unit weight of insecticide for the longest period of time. For ambrosia beetles attacking logs, the physical state of the insecticide should be such that it is available throughout the attack period in a form in which the beetles during contact pick up enough insecticide to prevent them from boring into the wood of the log, and ultimately to kill them. The availability of a deposit is especially important when an insect is in contact with it for only a brief time or when the deposit has "weathered", been "worn down" or is applied at a low dosage. These conditions often exist in the chemical protection of logs from ambrosia beetles.

The toxic effect of a residual insecticide to insects walking on treated surfaces depends on two broad factors; one pertains to the inherent toxicity of the substance attributable to its molecular configuration and its reactivity with the specific insect, and the other to the transfer and entry of the substance from the treated surface to points in the insect where interactions occur.

This study of the availability of lindane concerns the problems associated with the latter of these factors. The transfer of a residual insecticide from a treated surface to the points of effect involves transfer from the residue to the insect surface, followed by passage into the interior of its body. The facility and amount of transfer from the surface deposit to the points of functional effect are embraced by the term "availability".

Availability depends upon factors pertaining to the residue and to the insect. The residue factors include the quantity of toxicant per unit area, - this soon reaches a maximum. The quantity of toxicant per unit area is governed by the dosage applied and the loss with time by sublimation. The topography of the deposit also affects its availability; this topography is governed by the method of application of the deposit, the degree of subdivision of the material into particles and the form of these particles. The ease of dislodgement of the residue from the surface or the insect will influence its availability. This susceptibility to dislodgement depends on particle size, shape, absorption into the surface, adhesive and cohesive forces. The rate of transfer of vapour by sublimation, which will affect the availability of a residue, is governed by the vapour pressure of the substance, the particle size and shape, the number of particles per unit area, the topography of the deposit, the temperature and wind velocity.

Lindane and commercial practice: the problem.

Benzene hexachloride (BHC), or correctly 1, 2, 3, 4, 5, 6 - hexachlorocyclohexane (HCH), is the insecticide used to prevent damaging attacks of logs by ambrosia beetles. HCH exists as a number of stereoisomers only one of which, the gamma-isomer, is effectively insecticidal, acting by its residual toxicity not its repellency to ambrosia beetles. HCH containing not less than 99% of the gamma-isomer is known as Lindane.

Commercially, a spray containing 0.4% of the gamma-isomer by weight is applied to logs at the rate of about 1 Imperial gallon per 100 sq. ft. of log surface, this corresponding to 181 mg. of gamma-isomer per sq. ft. This treatment of logs has not given consistent results and in looking for the causes of this inconsistency, a number of possibilities present themselves. Commercial formulations of HCH consist of varying proportions of the different isomers together with other chlorinated hydrocarbons and additives and it is possible that these other substances may influence the uptake of the gamma-isomer. Further, in commercial practice the dosage of the gamma-isomer may vary and each formulation of HCH may produce a crystal deposit of different character on a surface, the surface itself influencing the type of deposit formed. This deposit also varies in its persistence, this persistence perhaps being a function of the differential aging of the varying deposits. The problem considered herein was associated with the availability of lindane residues to insects. Deposits of lindane consist of various types of crystal, and since these crystals differ in form, size, surface area and aging, their ease of pick up may vary and so be of importance in the availability of this insecticide to insects.

MATERIAL AND METHODS

HCH of 99.9% gamma-isomer content was used and applied in the form of crystals to a uniform surface at prescribed dosages. In the experimental testing of these crystals it was necessary to investigate and standardize the production of these crystals, the methods of application of prescribed dosages to test surfaces, and the bio-assay of the insecticide.

1. The preparation of, and factors affecting, lindane crystal forms.

One of the most effective means of pre-forming crystals of predictable character, and the one adopted in the present investigation, is the method of solvent exchange. For a water-insoluble substance such as lindane this consists of dissolving the material in a water-soluble solvent, then mixing the solution with water. The consequent dilution of the solvent results in its super-saturation with respect to the solute and crystallization of the solute occurs.

A number of factors influencing solvent exchange had to be considered. These were:- the nature of the solvent, the concentration of lindane in it, the proportion of lindane solution in the final water/lindane solution mixture, the presence or absence of surfactant in the lindane solution or in the water, the chemical nature and concentration of the surfactant employed, the direction of addition of concentrate and water, the rate of addition, the rate of stirring of the mixture, the temperature during admixture and the temperature of storage of the suspension after admixture.

In investigating the influence of these factors on the size and shape of lindane crystals formed by the solvent-exchange method the following procedure was adopted.

A known weight of lindane was dissolved in a known volume of a water-miscible solvent. The solution was kept in a water-bath at a constant temperature.

A suspension of lindane crystals formed when a known volume of the solution was admixed with a known volume of water. When the admixture was completed the suspension was divided into two equal parts; one part was examined immediately then stored at a constant temperature, the other was centrifuged for five minutes at about 1000 r.p.m. or until the supernatant was clear. This clear supernatant was decanted and the crystals resuspended in distilled water and centrifuged again. After this second spin the supernatant was decanted and the crystals examined and stored in a known volume of distilled water at a constant temperature. Slade (1945) gives the solubility of the gamma-isomer of HCH in distilled water as 1 part per million by weight.

The effect of varying some of the conditions was investigated using saturated solutions of lindane in formic acid, ethanol, methanol, acetic acid, methyl cellosolve, acetone, and tetrahydrofurfuryl alcohol. Only in the case of acetic acid was the effect of varying all the possible factors, one at a time, investigated: acetic acid was selected because lindane is intermediate in its solubility in this acid. For the other solvents the effect of varying the direction of addition in the presence and absence of a surfactant was observed. Availability comparisons were made between deposits of (a) rhomboid and (b) needle-like crystals of lindane. These were chosen because of their dissimilarity in shape, their relative stability and ease of preparation from one lindane solution.

The rhomboid crystals were prepared by adding the lindane solution to water. 5 ml. of a solution made by dissolving 1.0 gram of lindane in 20 ml. of acetic acid was added to 45 ml. of distilled water in which 0.05 ml. of the surfactant Triton X-155 had been dissolved. The rate of stirring was 60 r.p.m. and the time for addition 2 minutes. The suspension initially was made up of snow-flake like crystals, (Figure 1), but after standing for 48 hours at 25°C.

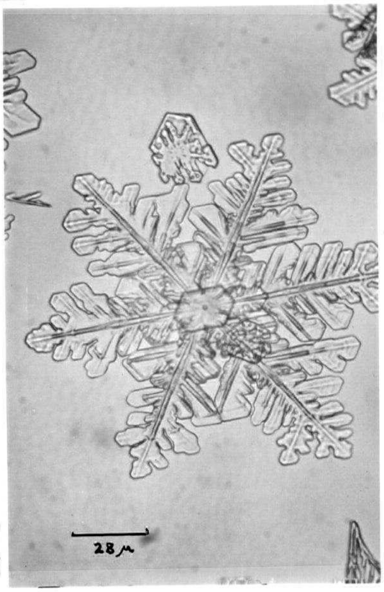

Figure 1. "Snowflake" crystal of lindane

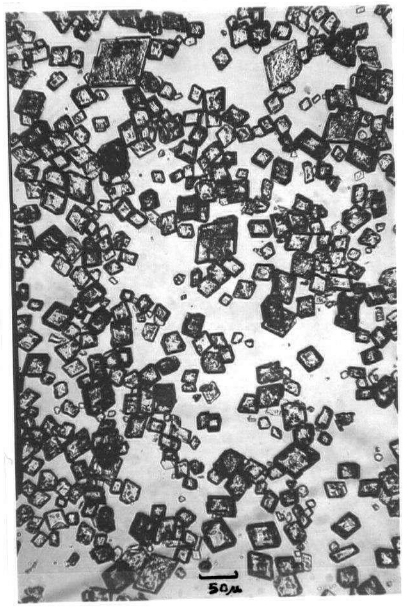

Figure 2. Rhomboid crystals of lindane

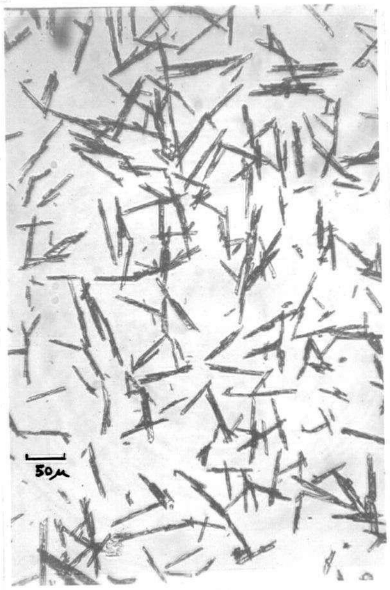

Figure 3. Needle-like crystals of lindane

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these completely transformed to rhomboid shaped crystals. This suspension of rhomboid shaped crystals, (Figure 2), was centrifuged, washed with distilled water and centrifuged again. The supernatant was poured off until only 2 ml. of the suspension remained. This was the material which was loaded into the spray gun and applied to the test surface.

The needle-like crystals were prepared by running water into a solution of lindane. 100 ml. of distilled water were run into 2 ml. of a lindane solution in which 0.05 ml. of Triton X-155 had been dissolved. The lindane solution was prepared by dissolving 1.0 gram of lindane in 20 ml. of acetic acid. The time for addition was 5 minutes and the rate of stirring 60 r.p.m. Immediately after preparation the suspension was centrifuged, washed with distilled water, centrifuged again and then all but 2 ml. of the supernatant decanted. This 2 ml. of suspension of the needle-like crystals (Figure 3), was sprayed on to the test surface.

The most important factor affecting crystal formation was found to be the direction of addition. Other factors vary in importance according to the direction of addition.

When the lindane solution is added to water, lindane crystals precipitate in consecutive small amounts as each drop of lindane solution reaches the water. Precipitation continues throughout the period of admixture. Under these conditions the factors governing crystal size are the solvent itself, the proportion of solvent in the final mixture and the temperature of storage of the suspension.

The presence of surfactant even in low concentration, (0.1% by volume or less), results in a greater degree of uniformity of crystal size in the suspension. The three non-ionic surfactants used - Triton X-155, Igepal CA Extra, and Atlas 1045 A,* - each resulted in different shaped lindane

* Triton X-155 is an alkyl aryl polyether alcohol.
Igepal CA Extra is an alkyl aryl poly glycol ether.
Atlas 1045 is a polyoxyethylene sorbitol oleate laureate.

crystals. Whether the surfactant was added to the lindane solution or to the water made no difference to the resulting crystal size or shape. Small variations in the concentration of lindane and surfactant around 0.1% are unimportant in their effect on crystal size and shape.

The addition of a surfactant, increasing the amount of surfactant, increasing the proportion of lindane solvent in the final mixture, using a solvent in which lindane has a high solubility or increasing the temperature of formation and storage, increase the rate of growth and hence the mean dimensions of the crystals of the suspension. This follows as a corollary of von Weimarn's "laws" of precipitation: the rate at which first formed crystals grow increases with their solubility in the medium. When the lindane solution is added to water no other factors affect the size and shape of the lindane crystals produced.

When the water is added to the lindane solution a completely different set of conditions prevail. As water is added to the lindane solution no precipitation takes place at first. When the lindane dissolvent power of the solvent-water mixture has been reduced to a certain value, precipitation of lindane crystals occurs rapidly and is completed after the addition of a relatively small amount of water. There is little or no supersaturation. The crystals grow rapidly and there is no aftergrowth on storage. The temperature of precipitation or storage and the proportion of solvent in the final mixture are unimportant in determining crystal size. The rate of addition and stirring are here the two important factors affecting crystal size. When the rates of stirring and addition are low, long needle-like crystals are formed. If the rate of stirring and addition are increased shorter needle-like crystals result. A poor lindane solvent with high rates of addition and stirring produces even shorter needles.

2. <u>The application of lindane crystals of different shapes to the test surface.</u>

Surface residues of insecticides may be prepared in a variety of ways, the number depending on the properties of the substance. Lindane can be applied as a powder of pulverized crystals, as an aqueous suspension of crystals, as a solution in a suitable organic solvent, as an emulsion with water carrying droplets of lindane dissolved in a water-insoluble solvent, or from vapour.

The intent of this part of the study was to prepare deposits of distinct crystal forms of lindane. A crystalline deposit can be prepared by condensing vapour directly on a surface, by applying the lindane in a volatile solvent to the surface or by applying dusts or suspensions prepared by crystallization under other conditions. The most convenient method was that of spraying a suspension of lindane crystals onto the surface to be treated.

There are many difficulties in preparing deposits of known crystal shapes of lindane. On surfaces of any one material it is difficult to cause and control the deposition of crystals of known shape from lindane vapour. When a solution of lindane in a volatile solvent is applied to an absorptive surface, it is difficult to predict the dosage and shape of the crystals which form at the surface; if the surface is non-absorptive the sequence of events during evaporation of the solvent is very haphazard and practically impossible to predict with certainty.

The original intention to employ natural bark surfaces was discarded. The natural surfaces are far too variable. Instead, a hard fibreboard was selected as a uniform absorptive surface somewhat resembling bark.

The fibreboard used was blocks 15 by 15 mm. by 1/8 inch of PV weatherproof hardboard.

A measured dosage of lindane crystals was applied to a square of 100 test blocks (10 by 10) by the following procedure. The suspension of lindane crystals of rhomboid or of needle-like shape was transferred by pipette to the reservoir of an artist's air-brush* and sprayed as uniformly as possible on to the square of test blocks using a pressure of 30 p.s.i. (Figure 4). To check on the weight of lindane applied to each block, 10 of the blocks, each with a clean weighed cover glass 15 by 15 mm. clipped to its upper face were spaced among the blocks being sprayed. If the cover-slips received the same weight of lindane as the uncovered blocks they could be weighed at intervals and their increase in weight taken as indicating the weight of lindane being applied to each block. In practice the spray gun gave an uneven spray pattern: one cover slip might receive twice the weight of lindane as an adjacent one and there was thus reason to suppose similar variation to be occurring among the fibreboard blocks. Revolving the blocks on a turntable did not improve the result because the rather viscous crystal suspensions would not flow evenly through the sprayer. A dilute suspension could not be used because too large a volume of water carrying the crystals, when applied to the blocks and the glass surfaces, caused run-off resulting in uneven distribution of crystals on the surface of each. The dosage on the fibreboard blocks used in preliminary tests was estimated by visual comparison with the known weight of lindane on the glass cover slips.

After preliminary tests using fibreboard blocks it was decided that in order to know the exact dosage of lindane on a surface, the weighed glass cover slips would be used as the test surfaces.

For this, 25 blocks each with a weighed glass cover slip clipped on to its upper surface were sprayed with a suspension of lindane crystals of the

* Manufactured by Thayer & Chandler, Chicago 7.

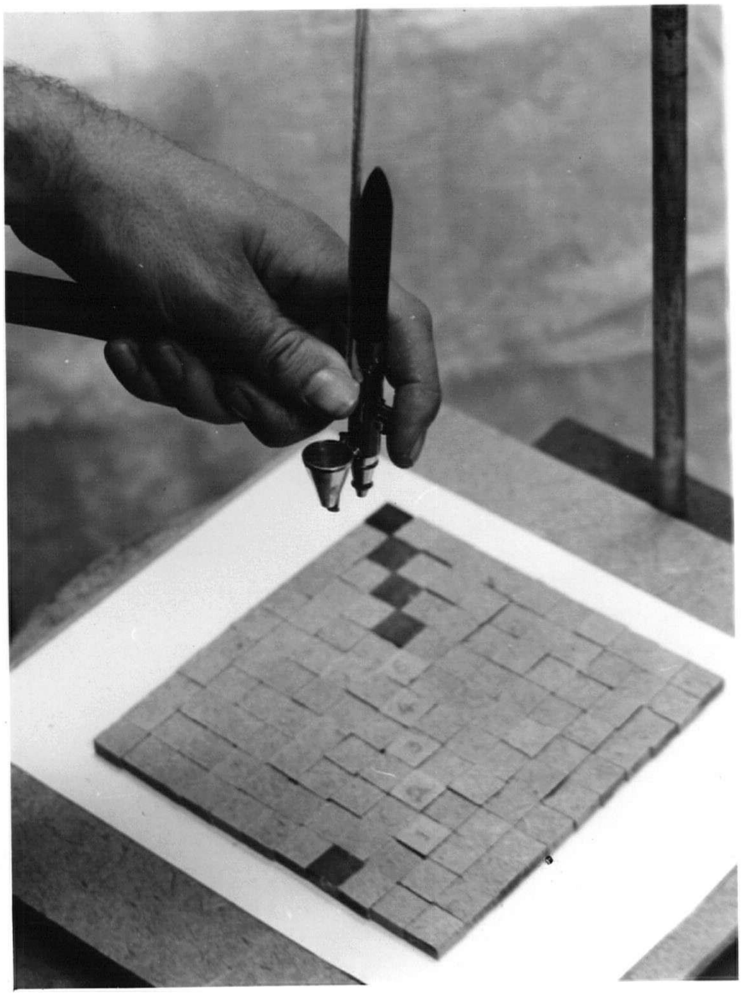

Figure 4. Spraying the test blocks

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rhomboid or needle-like shape. The area of each cover slip was 225 sq. mm. The weight of lindane to be applied to give a dosage equivalent of, for example 180 mg. per $ft.^2$, was calculated for 223 sq. mm., 2 sq. mm. being the area of the cover slip covered by the two clips holding the slip to the block. For 180 mg. per sq. ft. each cover slip should receive 0.00043 grams of lindane. All those cover slips receiving from 0.00041 to 0.00045 grams of lindane, that is, a dosage equivalent to 173 to 187 mg. per sq. ft. were accepted as test surfaces for the beetles.

Persistence of deposits.

A deposit of needle-like crystals has more crystals per unit area than does a deposit of the same dosage of the rhomboid crystals of lindane: it might therefore be expected to lose weight by sublimation more rapidly than a deposit of the same dosage of rhomboid crystals. Furthermore, differences in the packing of the crystals at higher dosages might lead to marked differences in the persistence and availability of deposits of crystals of the two shapes. This was studied by following the loss in weight of known dosages of crystals of the two shapes. Dosages were calculated from the weight of lindane sprayed onto clean weighed glass cover slips. All deposits were prepared on the same day, stored in a closed dark cupboard, and shielded from dust by a glass pane placed 1 cm. above them. The minimum and maximum temperatures recorded in the cupboard during the storage period was 14.5°C. and 23.5°C. The deposits were weighed at weekly intervals.

A deposit of 180 mg. per sq. ft. of both the rhomboid (Figure 5), and the needle-like crystals (Figure 6), was completely dissipated by sublimation during the eighth week after preparation. There was no appreciable difference in the rate of loss from deposits of crystals of the two different shapes.

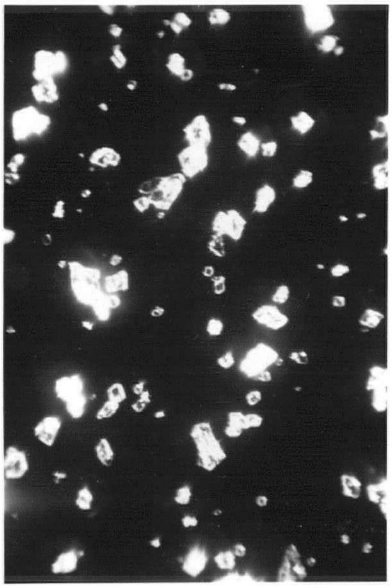

Figure 5. 180 mg./ft.^{2} - rhomboids

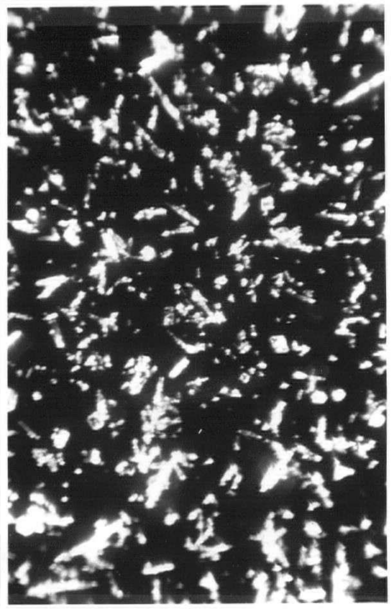

Figure 6. 180 mg./ft.^{2} - needles

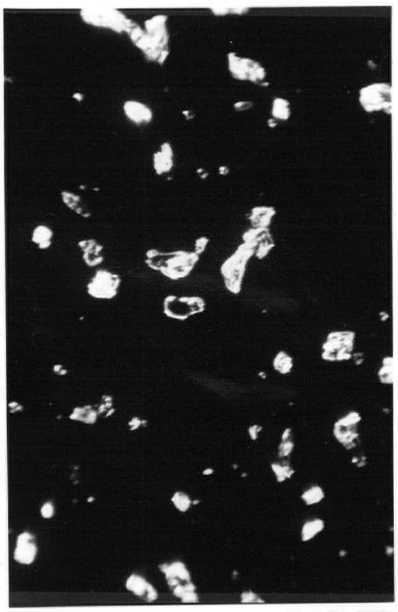

Figure 7. 50 mg./ft.^{2} of rapidly "aged" rhomboids

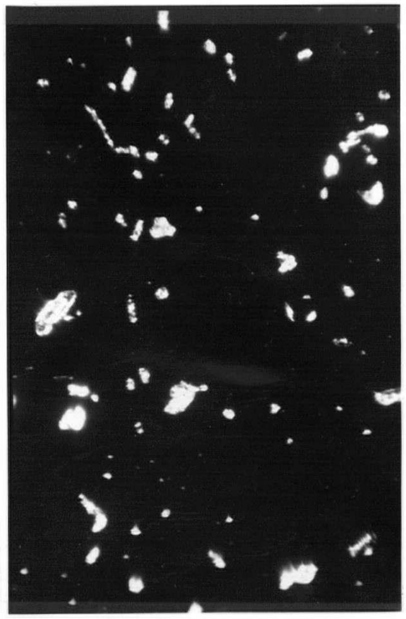

Figure 8. 50 mg./ft.^{2} of rapidly "aged" needles

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Higher dosages disappeared at a slower rate.

Deposits of 180 mg./sq. ft. of crystals of the rhomboid and of the needle-like crystals of lindane were placed in a stream of warm air and caused to lose weight until they corresponded to a dosage of 50 mg. per sq. ft. (Figures 7 and 8). They were then tested for differences in availability to beetles.

3. Bioassay of deposits.

The availability of lindane crystals was assessed from the mortality of beetles exposed to a deposit of both needle-like or rhomboid-shaped crystals sprayed onto a test surface. A difference in mortality of beetles contacting deposits of the same dosage for the same period of time was held to indicate a difference in availability of lindane from the two crystal shapes.

The beetles used throughout the tests were adults of the lined ambrosia beetle *Trypodendron lineatum* (Oliv.). These were collected as over-wintering adults in duff from the Harris Creek drainage in the Cowichan Lake Land district of Vancouver Island. The duff was collected and concentrated into a fraction containing most of the beetles by passing it through a graded series of vibrating screens. This duff concentrate was stored in polyethylene bags at $0^{\circ}C$. Beetles were collected from this duff as they were required for testing. Three or four handfuls of duff were removed at a time and spread to form a layer about 1 to 3 cms. deep over the bottom of a metal tray floating in a sink of warm water at $30^{\circ} - 35^{\circ}C$. Three 60 watt bulbs placed about 50cms. above the duff surface provided a source of light. As the duff warmed, the beetles crawled to the surface where they were picked up with fine forceps, sexed and transferred singly to shell vials stoppered with a plug of cotton wadding. Teneral beetles and beetles unable to walk evenly were not used for test purposes.

The aim of the tests was to detect any difference in the availability of the deposits to beetles standing or walking over the insecticidal surface. Initially beetles were confined on a deposit by a circular slotted cage, (Figure 9). The cage had to be as open as possible to avoid any build-up in fumigant effect from the deposit and its walls were to provide no footholds which might allow the beetle to walk up and over them; by placing a split iron ring on its outer ledge the cage was made too heavy for the beetle to nudge off the deposit. Using this method a deposit was bioassayed once by one beetle only then discarded. The beetle was placed on the deposit for a set period of time. After each beetle had been on the deposit for its allotted time it was transferred to an unsprayed fibreboard block for observation then to a shell vial and kept at 25°C. and 70% Relative Humidity. The beetles were examined 12 and 24 hours later. For examination the beetles were removed from the vials, placed on a clean surface and observed for five minutes; if during this time they showed no movement in response to gentle prodding, they were classed as dead. Those beetles which moved were grouped according to whether they showed themselves capable of normal walking or not and classed as normal or abnormal. Control beetles were treated in the same manner except that they walked over an unsprayed surface.

These preliminary tests indicated that a method of exposing beetles to deposits for periods of less than one minute would have to be devised, for all the beetles died when the exposure period was greater. For tests involving exposure times of less than one minute, beetles were mounted on cardboard wedges affixed to a pin using partially thickened "Hyrax" mounting medium to attach the beetles by their thoracic nota. By mounting a beetle on a pin it could be allowed to contact a deposit for a period of one second, (Figure 10). After initially "feigning death" a beetle would usually begin cautiously to extend

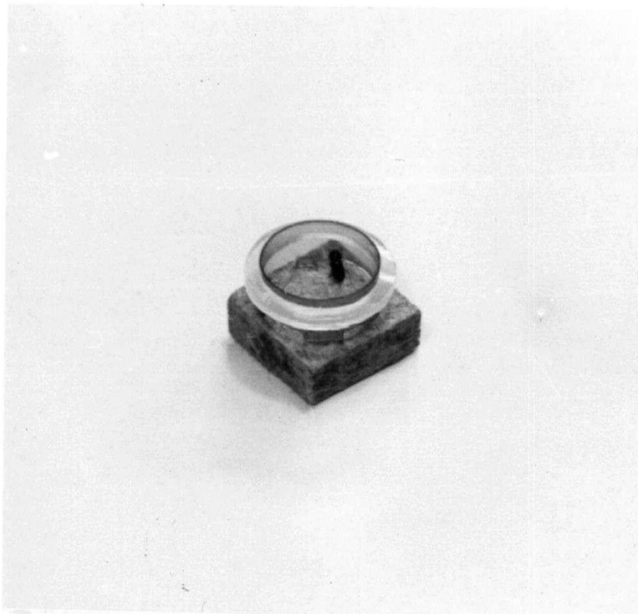

Figure 9. Method of exposing beetle to deposit for more than one minute

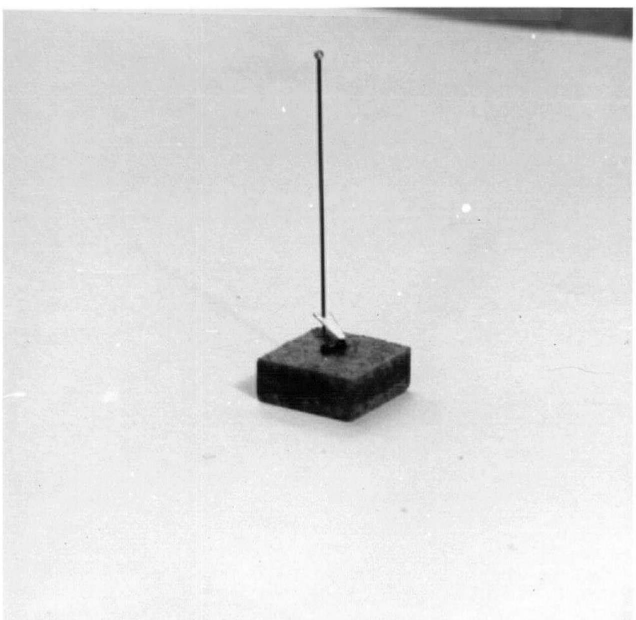

Figure 10. Method used to expose beetle to deposit for less than one minute

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its legs from under the body. If the beetle was now firmly touched onto the deposit and removed after the timed contact, a distinct grip of the legs of the beetle could be felt as it was removed from the surface. Care was taken to ensure that each beetle did not keep its legs folded under the body during exposure, but contacted the insecticide with its legs in the standing position. Using this method five beetles were tested consecutively on the deposit on one cover slip. Beetles were touched down, one in the centre and one at each of the four corner areas of a deposit. Beetles for controls were mounted but touched onto a clean glass surface instead of an insecticidal one. The results of these tests are shown in Tables I-III.

Table I. Results of bioassay of needle-like crystals.

DATE OF TESTING	TYPE OF DEPOSIT	NUMBER OF BEETLES USED	PERIOD OF EXPOSURE IN SECONDS	CONDITION OF BEETLES 12 hrs. after exposure Normal	Abnormal	Dead	24 hrs. after exposure Normal	Abnormal	Dead
17-V-59	1280 mg/ft.^{2} on fibre-board	6	600	0	0	6	0	0	6
		5	300	0	0	5	0	0	5
		5	150	0	0	5	0	0	5
		10	75	0	3	7	0	0	10
		10	40	0	1	9	0	0	10
		10	20	0	7	3	0	0	10
		10	10	0	7	3	0	0	10
		10	Controls	8	1	1	8	1	1
12-VI-59	1280 mg/ft.^{2} on glass	50	1	0	45	5	0	39	11
		10	Controls	6	4	0	6	3	1
15-VI-59	1280 mg/ft.^{2} on glass; assayed 4 days after preparation	5	10	0	5	0	0	2	3
		5	5	0	4	1	0	1	4
		5	2	0	5	0	0	3	2
		5	2	* 0	4	1	0	1	4
		5	Controls	4	1	0	0	4	1
16-VI-59	1280 mg/ft.^{2} on glass; assayed 5 days after preparation	5	40	0	4	1	0	2	3
		5	40	* 0	4	1	0	2	3
		5	30	0	5	0	0	5	0
		5	Controls	5	0	0	5	0	0
		5	Controls	* 3	2	0	3	2	0

* Beetles kept in contact with plug of tube.

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Table II. Results of bioassay of needle-like crystals.

DATE OF TESTING	TYPE OF DEPOSIT	NUMBER OF BEETLES USED	PERIOD OF EXPOSURE IN SECONDS	CONDITION OF BEETLES: 12 hrs. after exposure			CONDITION OF BEETLES: 24 hrs. after exposure		
				Normal	Abnormal	Dead	Normal	Abnormal	Dead
16-VII-59	180 mg/ft.^{2} on glass; assayed 24 hrs. after preparation	10	1	0	6	4	0	0	10
15-VII-59	180 mg/ft.^{2} on glass	5	1	0	1	4	0	0	5
21-VI-59	180 mg/ft.^{2} on glass	30	1	0	16	14	0	7	23
		5	Controls	3	0	2	3	0	2
21-V-59	160 mg/ft.^{2} on fibre-board	10	600	0	0	10	0	0	10
		10	300	0	0	10	0	0	10
		10	150	0	0	10	0	0	10
		10	75	0	0	10	0	0	10
		10	40	0	0	10	0	0	10
		10	20	0	1	9	0	0	10
		10	10	0	5	5	0	2	8
		10	5	0	1	9	0	1	9
		10	Controls	8	0	2	6	2	2
20-VII-59	50 mg/ft.^{2} "aged" needles on glass	10	1	0	8	2	0	6	4

Table III. Results of bioassay of rhomboid crystals.

DATE OF TESTING	TYPE OF DEPOSIT	NUMBER OF BEETLES USED	PERIOD OF EXPOSURE IN SECONDS	CONDITION OF BEETLES 12 hrs. after exposure Normal	Abnormal	Dead	24 hrs. after exposure Normal	Abnormal	Dead
20-VI-59	180 mg/ft.^{2} on glass	5	30	0	0	5	0	0	5
		5	20	0	0	5	0	0	5
		10	10	0	0	10	0	0	10
		5	5	0	0	5	0	0	5
		5	Controls	4	1	0	4	1	0
20-VI-59	180 mg/ft.^{2} on glass	5	10	0	0	5	0	0	5
		5	5	0	0	5	0	0	5
		5	3	0	1	4	0	0	5
		10	1	0	1	9	0	1	9
		5	Controls	5	0	0	5	0	0
23-VI-59	180 mg/ft.^{2} on glass	35	1	0	5	30	0	2	33
		10	Controls	5	4	1	5	3	2
16-VII-59	180 mg/ft.^{2} on glass; assayed 24 hrs. after preparation	10	1	0	4	6	0	0	10
		5	Controls	2	2	1	2	1	2
26-V-59	160 mg/ft.^{2} on fibre-board	10	2	0	10	0	0	2	8
		20	1	2	18	0	2	5	13
		10	Controls	7	3	0	7	2	1
20-VII-59	50 mg/ft.^{2} "aged" rhomboids on glass	10	1	0	8	2	0	2	8

DISCUSSION

This discussion not only considers the work described herein, but also includes a review of the literature relevant to understanding the great variability and inconsistent results found in the commercial use of HCH, together with a general discussion on availability of insecticides, especially lindane and other chlorinated hydrocarbons.

1. Method of Application of lindane to a surface.

Apart from its intrinsic properties, the physical state of an insecticide is one of the most important factors affecting its availability. The state of a deposit of lindane depends upon the form in which it is applied, that is as a solution, emulsion, suspension or dust, and the surface to which it is applied. In this section the deposits produced from different formulations on different surfaces are considered, then the effect on availability of different crystal size, form, disposition and different surfaces is discussed.

Solution of HCH applied to non-absorbent surfaces.

The outer layer of the insect cuticle contains lipoids and is easily wetted by oils so that an oil solution of a contact insecticide would be expected to be readily available. Oil films containing HCH on non-absorbent materials such as glass or metal are extremely toxic initially; contamination of the tarsi and penetration occur readily. If the solvent itself does not enter the organism, the effectiveness of the insecticide solution will vary according to the ease with which the solution contacts and wets the cuticle and the solubility of the insecticide in the solvent. For a given concentration of a toxic solute in a series of solvents, the system in which the solute is least soluble will be the most toxic, since the toxicant will pass into the organism more readily (Ferguson, 1939). Alternatively, the solvent itself may penetrate into the insect and exert a toxic action or act as a carrier for the toxicant, or the solvent may increase the permeability of the insect to the insecticide. In general, lighter mineral oils penetrate more rapidly than heavier ones which in turn penetrate more rapidly than vegetable oils.

On a non-absorbent surface the solvent evaporates to give, under normal laboratory conditions, a mixture of crystals and supersaturated or supercooled droplets. The more volatile the solvent the greater is the proportion of liquid droplets. These droplets eventually crystallize on exposure. On glass the sequence of events during evaporation is haphazard. The condition of the resulting deposit varies from one application to another (Barlow and Hadaway 1952, Vickers 1947, Bruce 1949). On a glass surface crystallization occurs more rapidly from a continuous oil film than from discrete droplets, and gives a smaller proportion of supersaturated droplets.

The change from a "wet" to a "dry" deposit is often characterized by a marked reduction in insecticidal effectiveness as the toxicant becomes less available. The high fumigant action of lindane is a complicating factor. There will be a drop in effectiveness on crystallization of lindane solutions only if the availability of the lindane is greater from the solution than from the crystals. This will occur if the vapour pressure of lindane is greater in solution, or if larger amounts of lindane or more rapid penetration of the lindane occurs from the solution. Crystallization can be induced by mechanical stimulation or by insects walking over the surface. If the insecticidal solution is more effective, then this period of effectiveness could be prolonged by the inclusion of a non-volatile oil or of a substance inhibiting crystallization. Crystallization probably occurs at a different rate for a solution of a mixture of the isomers of HCH than for the gamma isomer alone.

Solutions of HCH on absorbent surfaces.

When oil solutions are applied to absorbent materials such as bark the high initial toxicity of non-absorbent surfaces is not generally observed because the oil penetrates the substrate. Only a small proportion of the insecticide dosage applied may be available at the surface. Schmitz and Goette (1948) found that only 30% of the DDT applied to poplar wood as a kerosene solution remained within 0.001 inch of the top surface. The penetration of an insecticide with its solvent into certain absorbent materials does not result in permanent loss for crystallization may bring the insecticide back to the surface. Parkin and Green (1947) discovered that wall-board, sprayed with a solution of DDT in kerosene, showed a marked increase in toxicity to houseflies between the first exposure and the second 4 weeks later. Movement of the flies on the surface during the first exposure induced crystallization so that a dense mass of crystals appeared. This "blooming" does not occur on all absorptive surfaces. It occurs most readily on fibrous materials. Barlow and Hadaway (1952) find that for DDT the best examples of induced crystallization occur from 5% - 10% concentrations in solvents of medium boiling point range (120° - 200°C.). The physical state of the "bloom" varies according to the insecticide and the volatility and solvent properties of the oil. For highly volatile solvents such as acetone, evaporation takes place before the solution has soaked into the substrate and a solid incrustation is immediately left on the surface. Lyon (1956) reports that the shapes of the crystals of many insecticides differ according to the dosage applied in acetone solution. The toxic plate-like crystals of lindane decreased in number per unit area compared with small, less available, granular crystals, when the dosage was increased or decreased from 320 mg. per sq. ft. The crystals of a spontaneous "bloom" may differ from those produced after mechanical stimulation.

Emulsions of HCH on absorbent and non-absorbent surfaces.

The observations concerning oil solutions, apply to a large extent to emulsions, which, on many surfaces behave as oil solutions with probably some additional complications due to the presence of sticky and hygroscopic materials such as emulsifiers and stabilizers which increase adherence. Bruce (1949) has described the deposit on wood from emulsions of DDT in different solvents. On glass and less noticeably on wood, the size of the DDT crystals depended on the speed of crystallization which in turn depended on the physical properties of the solvent; rapid crystallization produced crystals of small size. Toxicity tests with houseflies showed that a loss of toxicity occurred on glass as the emulsion dried. On wood, crystallization of DDT on the surface resulted in an increase in toxicity similar to that occurring with a kerosene solution on wall-board.

Aqueous suspensions on non-absorbent surfaces.

When aqueous suspensions are sprayed onto non-absorbent materials such as glass, metal and some woods, the wetting agent solution is often unable to drain away. It remains on the surface with the insecticide particles, and on drying causes them to strongly adhere to the substrate and be less available.

On hydrophobic absorptive surfaces the droplets of an aqueous suspension may remain distinct and the crystals of the insecticide aggregate instead of remaining as discrete particles as the wetting agent dries.

Aqueous suspensions on absorptive surfaces.

When aqueous suspensions are sprayed on to absorptive materials such as bark, the water penetrates into the material and leaves the particles on the surface. Surface deposits from aqueous suspensions of wettable powders are greater than those from kerosene solutions or emulsions and differences in availability to insects making contact with the surface are shown by differences in kills

(Hadaway and Barlow 1949). Further studies (Hadaway and Barlow 1951) show that the effectiveness of surface deposits from aqueous suspensions of insecticides against mosquitoes exposed for short contact periods is related to the size and shape of the insecticidal particles.

The availability of the particles from a deposit is determined by the adhesion of the particles to the substrate and the insect cuticle, and the extent to which insects walking over the substrate come into contact with the particles. The more important forces tending to dislodge particles are very dependent on size; gravity is proportional to size cubed and so is the inertial force invoked by vibration. It requires an acceleration of about 4000 times gravity to shake off a 100 micron particle and as much as 500,000 times gravity to shift a 10 micron one (Busvine 1957). Insects therefore, tend to become more and more effectively contaminated by smaller and smaller particles. The optimum size is of the order of 1 - 10 microns. Below this range cohesion of the separate particles is so strong that aggregations are formed and these become the effective particles of the system.

Size of particles and availability.

The size of particles of an insecticide as a factor affecting its availability varies with the disposition of the particles at the surface, the conformation of the surface and the dimensions and morphology of the insect contacting the surface. A flaw in the experiments described in the present work is the considerable range in the size of the crystals in the suspensions applied. A preparation may owe almost all of its insecticidal activity to a comparitively small percentage of effective particles. Differences in availability of crystals of different sizes are attributable to differences in the amounts of insecticide removed from the surface by and retained on, the insect. Generally, fewer large crystals will be picked up than small ones and the few large crystals that are taken up will be more readily detached from the insect by cleaning and other movements. The rate of penetration of the cuticle might be less from a large attached crystal than from many smaller ones of the same total mass. For the same dosage of insecticide there will be a smaller total number of crystals when these are large, than for smaller crystals. If the movement of an insect is at random with respect to the location of the crystals on that surface, then it is more likely to contact crystals when these are small. This is not a factor to be considered at the dosage levels used in the tests described which leave more of the surface covered with insecticide than uncovered. The size of the crystals of a deposit of lindane undoubtedly has an important bearing on the persistent toxicity of a deposit.

A deposit of lindane of small-sized crystals will be readily available but it will rapidly disappear as the crystals sublime. A deposit of larger crystals might not be so readily available initially but it will become so as the crystal size decreases by sublimation. For initial and residual toxicity a mixture of smaller and larger sized crystals is preferable.

Form of crystals and availability.

The form of the insecticide crystals will affect its availability if the rate of pick-up is critical and affected by changing shapes of crystal. Crystals of shapes with a large surface area will sublime more rapidly than those having a smaller surface area relative to their mass.

Disposition of crystals and availability.

The disposition of the insecticide crystals on a surface affects its availability. On fibrous materials lindane commonly forms needle-like or rectangular plates with only one end attached to the fibrous substrate and the other end projecting away. These crystals will be more available to an alighting insect than crystals which encrust and closely adpress the surface. Long fragile needles may be more available than shorter needles if the insect breaks off small pieces of the long needles in walking among them. Those crystals which "bloom" in response to mechanical stimulation differ in some instances from those "blooming" spontaneously. Crystals blooming spontaneously are usually less easily picked up from the surface than crystals of the same shape which have been sprayed on as an aqueous suspension.

Additives and availability.

In the tests described, concentrated suspensions of lindane crystals were applied. In commercial practice a suspension would also contain inert diluents and additives. These will affect the availability of the insecticide to an insect at the surface. Unless the diluent particles aid the pick-up, penetration or retention of the insecticide, the diluent will decrease the availability of the insecticide. The diluent may increase the persistence of the insecticide by either masking it from sublimation or by reducing its vapour pressure. Rainshowers easily wash off deposits from suspensions of wettable powders. Inclusion of an adhesive increases the resistance to wash-off but may decrease the contact availability to insects.

Nature of surface and availability.

The nature of the surface to which the insecticide is applied can greatly affect its availability. Sorption of the insecticidal particles may occur. Thus Hadaway and Barlow (1952) found that particles of 10 - 20 microns of lindane at a dosage of 25 mg. per sq. ft. on mud blocks were no longer visible after 3 - 4 hours at 26°C. The rate of sorption into the block increased as the temperature increased and the particle size decreased. Kills of mosquitoes were obtained for several weeks from blocks treated with lindane due to the sorbed insecticide. Insecticides having little fumigant action, for example DDT or dieldrin, completely lose their effectiveness after sorption because they are no longer on the surface and available to the insect.

The surface may cause changes in the crystals of the insecticide. In this work it was found that the needle-like crystals formed when a solution of lindane in methanol was admixed with water, rapidly transformed to crystals of rhomboid shape when sprayed onto glass, mica, and cellulose acetate surfaces. This transformation was completed in 30 minutes. When acetic acid was used as the lindane solvent, the change in form of the needle-like crystals occurred at a much slower rate.

Topography of the surface and availability.

The topography of the surface in relation to the size of the crystals and the dimensions and structures of the insect parts which contact the surface, affect the availability of the insecticide. Hadaway and Barlow (1952) found that there was an optimum availability to the mosquito Aëdes aegypti (Linn.) of DDT crystals in the size range 10 - 20 microns on plaster blocks. Above this size range there was an inverse relation between particle size and availability. On mud blocks the inverse relation is continuous, and crystals in the 0 - 10 micron range are most effective. It is thought that this difference is due to the nature of the plaster surface which interferes to some extent with the availability of the very small particles. This effect was less well shown for lindane crystals. Hadaway and Barlow also found that the crystals in the optimum size range were also more effective as an insecticide even when equal numbers and therefore different dosages were compared. In the tests here described for ambrosia beetles the crystals of lindane were all larger than the surface irregularities of the glass on which they were deposited. On a bark surface with its fissures, outgrowths and variable texture, crystals might congregate and persist longer in the crevices, especially if the spray were applied till "run-off". The ambrosia beetles tend to start their tunneling in these crevices.

2. Discussion of bioassay.

No marked difference in the availability of the two crystal forms of lindane was shown by the method of bioassay used in this work. The beetles were always receiving sufficient lindane to kill them; the amount of lindane available was never low enough to show any marked differential effect of the two crystal shapes. Failure to demonstrate any difference in availability between the two crystal forms could conceivably occur if penetration of the lindane through the cuticle is so rapid that a lethal dose could be absorbed in a contact period of one second; retention of the lindane crystals would then be superfluous. No difference would be demonstrable if both crystal shapes offered the same surface area for vaporization, or offered the same area for contact with the cuticle of the insect. It is unlikely that an exposure of one second to the vapour alone from the dosages used in the tests would be lethal to the ambrosia beetle. If this were so no differences would be detectable, using mortalities, from equal dosages of the two crystal shapes providing these both vaporized at the same rate.

An insecticide is said to be available in the sense that it is capable of "being used by" the insect. The word availability is used in two distinct ways. Available insecticide may signify that part of a residual deposit which is picked up by, remains in contact with, and is available for absorption into the insect cuticle.

Alternatively, the available insecticide may denote the amount of insecticide transferred from the deposit to the point of functional effect. In this work the latter usage is the one adopted. The reason for this decision will become apparent from the ensuing paragraphs. Mortality was used as a gross index of availability.

Armstrong *et al* (1951) adopt a usage of available which is closer to that part of a residual deposit picked up by remaining in contact with and available for absorption into the insect cuticle. These workers placed granary weevils on filter paper to which 11 micrograms per sq. cm. of the alpha, beta, gamma, or delta isomer of hexachlorocyclohexane (HCH) dissolved in acetone had been applied. The deposit formed is a flat encrusting layer of not readily detached crystals. The amount of each isomer picked up by the insects was determined as two fractions, an "outside" fraction representing that which could be removed by cold methanol washings and which was from the superficial layers of the cuticle, and an "inside" fraction which had penetrated deeper and was recovered after decomposing the insects with nitric acid. After 7 hours exposure the amounts of isomer dissolved in the waxy layer of the epicuticle are found to be proportional to the solubility of each isomer in this wax. Only in the case of the gamma-isomer is there also extensive penetration during the first 7 hours into regions below the outer layers of the cuticle. This results in toxic symptoms; 50% of the beetles were dead after 7 hours exposure and 84% after 22 hours exposure. Those remaining alive after 7 hours exposure were relatively immobile. In the period from 12 to 22 hours exposure the "outside" fraction of gamma-isomer picked up was reduced; it is possible that the rate of solution in the wax layer is reduced owing to the immobility of the insects, whilst the rate of penetration, - as shown by the amount of "inside" fraction, - remains constant or increases. The fact that the "inside" fraction of gamma-isomer did not increase until all or almost all of the insects were dead, - after 22 hours exposure - and then showed a rapid increase, cannot be explained at present. It may be that the developing toxic action or effects of the small amount of gamma-isomer penetrating in the first few hours of exposure causes or effects an increased permeability to lindane. The delta-isomer is the only one other than the gamma to show any considerable degree of penetration of the epicuticle. Its "outside" fraction is at all times greater in amount than that of the gamma-isomer. No toxic effects are apparent from the delta-isomer and the equilibrium condition between "outside" and "inside" fractions reached after 24 hours exposure is not much changed at 48 and 72 hours exposure.

Such a distinction between "outside" and "inside" insecticide is arbitrary. A lindane crystal on an insect's leg is outside the insect in position, but as soon as lindane has dissolved in the epicuticle and penetrated protoplasm it is as much "inside" the insect as it is anywhere else. The fumigant action of lindane makes it difficult to regard "available" lindane as that in contact with and available for absorption into the insect. Armstrong et al found that lindane was not picked up to any less extent when direct contact with the deposit is prevented.

From the practical viewpoint, interest in the availability of residual deposits of insecticide centres around the amount of insecticide needed to rapidly inactivate and ultimately kill the insect. Not enough is known about the physiological processes affected by lindane to be able to measure the amount of insecticide transferred from the surface deposit to the point of functional effect, for the point of functional effect is not known.

Conceivably, lindane could act in one or all of the following ways: it could disrupt the permeability of the protoplasm of the insect. Its site of action would be general and death due to the effects of this disruption of permeability. Mullins (1955) suggests that lindane is toxic because it is adsorbed on an interface and there disrupts permeability. Only lindane is able to orientate within the interstices of a putated membrane lattice structure of protoplasm proposed by Mullins.

Lindane may enter the cell and interfere physically with its processes. Here the site of action could be general or specific depending on whether such entry and interference occurred in all or only some cells of the insect. Slade

(1945) suggested that lindane is used as a metabolite instead of meso-inositol. The elucidation of the molecular configuration of the isomers of HCH has since shown that the delta-isomer not the gamma is akin to the structure of meso-inositol.

Lindane may react with a substance of general or specific cells of the insect to produce substances which bring about disruption of cell functions.

Bot (1952) concludes that both DDT and lindane move in the insect nervous system but considered this secondary to spread through the haemolymph. Penetration of DDT and lindane occurs in the absence of normally functioning sense organs. To determine whether lindane penetrates the cuticle only because of absorption by it, Bot excised the dorsal cup-shaped portion of the thorax of a fly. Another fly was placed inside the mouth of a glass tube and the cuticular cup fixed over it in the mouth of the tube, the concave side outwards. The latter was filled with lindane in various solvents. After 12 hours contact the imprisoned fly was set free and allowed to pass its legs over its body. No poisoning was observed either then or when the wing of an insect was used instead of the cuticular cup. This raises the question why spread of the toxicant did not occur in this experiment, whereas it did in an experiment in which the cuticle was excised, kept out of place for one minute then replaced, the wound edges being sealed with beeswax. Bot presumes that the pertinent difference was that in this latter experiment haemolymph was present and able to penetrate into the cuticle through the dead hypodermal cells. It is cogent to suggest that perhaps lindane can penetrate the cuticle only from the epicuticle inwards not from the hypodermis outwards. This would provide an alternative explanation of the failure of lindane to penetrate from the inside of the thoracic bowl or the wing. Bot reports that in contrast with DDT which locally can stimulate the motor nerve in such a way that an amputated leg shows independent contractions, lindane appears to restrict its action, or its effects, to the sensory nerve system or to the ganglia. The toxicity of DDT cannot in all cases be attributed to the dose administered, for if spreading over the body of locally applied poison is prevented, it appears that the place and area of application play a part in the onset of symptoms. This was not observed for lindane and chlordane, - this may have been due to the greater toxicity of these latter insecticides, - but it was also not observed for toxaphene which is of the same order of toxicity to houseflies as DDT. Both the roach and the housefly are able to withdraw and excrete conversion products of lindane by the Malpighian tubules (Bot 1952).

In the absence of knowledge of the site of action of lindane, yet bearing in mind the practical importance of the availability of residual deposits, it was decided to use mortality as a gross index of availability.

Theorising, the following sequence is postulated: when an insect alights and walks over a lindane deposit, lindane vapour enters the epicuticle; lindane from crystals in contact with the cuticle also dissolves in the epicuticle. This then acts as a reservoir for supply to aqueous, fatty and proteinaceous tissues. The insect may be regarded as a series of physical phases between which the lindane becomes distributed. It is probable that only in one or part of one of these phases does the lindane concentration become critical in disturbing the physiological processes of the insect. In this critical phase a high concentration for a short time, or a low concentration for a long time may bring about inactivation or death of the insect. Detoxication mechanisms will possibly also be operating within and across these phases. In using mortality as an index of availability, we are actually concerned with how the amount, rate and perhaps the location of lindane absorbed affects mortality. In the prevention of damaging attacks of logs by ambrosia beetles, the shape of lindane crystals might well affect the ease of pick-up during a short contact period on a sparse deposit and might also affect the rate of penetration into the beetle. To kill the beetle before it tunnels into the wood of a log, a larger amount of lindane picked up will, by causing a large concentration of toxicant in a critical phase, be more effective than a smaller dose which will kill the beetle, but take longer to do so.

Insect behaviour and insecticide availability.

The behaviour of the insect on an insecticidal surface will affect the availability. An insecticide will be more available to an insect alighting and running over a surface than to a stationary insect. On bark the ambrosia beetle tends to penetrate crevices - it is in these crevices that crystals of an insecticide would collect and persist and hence be more available than from a similar dosage applied to a plane surface.

Hadaway and Barlow (1952) report that females of Anopheles stephensi (Liston) are activated by DDT deposits and fly from a treated surface after a few minutes contact. This occurs whether the particles are large, small, easily detached or stuck on to the surface. A deposit is lethal only when it consists of small readily available particles, otherwise the mosquito is stimulated to fly from the surface before acquiring a lethal dose. A. stephensi is stimulated to flight by contact with deposits of lindane just as rapidly as it is from DDT deposits. Lindane is so much more toxic than DDT to a mosquito that the insect invariably acquires a lethal dose during its brief contact period. Insects are not stimulated to fly when lindane acts soley by means of its fumigant action, for example after sorption has occurred. In general the amount of insecticide picked up during a given contact period varies slightly from species to species according to tarsal structure, stance and behaviour. Particle size appears to be more critical with an anopheline which remains stationary than with Aedes aegypti which tends to walk over a surface. This relationship between particle size and effectiveness is less marked for lindane than for DDT because of its greater toxicity than DDT - the pick-up of only a few particles of lindane is lethal, - and also perhaps because of its high fumigant action. No repellent effect on ambrosia beetles of HCH treatments of logs has been noted.

Lewis and Hughes (1957) found that the rate of uptake of particles of a lipophilic dye from a loose deposit by the blowfly Phormia terraenovae (R.-D.) decreases rapidly after the first 60 seconds of walking. The collecting efficiency diminishes by about 30% immediately after initial contact with the deposit, and by 90% after 15 seconds of continuous walking over a light deposit. The quantity of particles picked up in the act of settling or alighting on the deposit considerably exceeded that picked up in subsequent steps.

Insect stance and structure, and insecticide availability.

The stance of an insect on an insecticidal surface will affect the availability of an insecticide. Gratwick (1957) found from measurement of the uptake of lipophilic dyestuff particles picked up by five different species of insect, that the uptake per step by insects of different size and leg structure differed only slightly. The total uptake was greater for the larger insects but the smaller insects picked up a greater weight of particles per unit body weight at each step. It was also found that the ratios of the actual amounts picked up by the different pairs of legs was similar for the insects studied. In all cases the hind legs were shown to pick up an amount equal to the sum picked up by the fore and middle legs. The first segments of the hind tarsi are more important accumulators of particles than the fore or middle tarsi. From observations of the position of particles picked up by the tarsal segments Gratwick concluded that the tarsal setae help to keep particles away from the main surface of the cuticle, which is not easily contaminated where the setae are dense.

Insect cleaning movements and insecticide availability.

A study of the effect of redistribution and loss of particles on an insect which performs vigorous cleaning movements has shown that particles are lost from head, thorax and abdomen in the same proportions in which they are acquired and that cleaning movements transfer particles from one region to another. Lewis and Hughes (1957) report that the head of a fly at all times retains about 7% of the total weight of particles picked up, the legs 48%, wings 13% and abdomen 28%. The pulvilli are not important sites of accumulation - they retain about 2% of the total particles; nor are the pulvilli preferred sites of entry of DDT. The proportions of particles on each of the three pairs of legs also remain constant but the distribution on the tarsal segments changes. The rate at which fine particles are discarded by a contaminated fly depends largely on the rate at which cleaning movements deliver particles back to the tarsal spines, from which they are returned to the substrate. The rate of loss is rapid until the quantity retained by the fly falls to 20 micrograms but is slow beyond 10 micrograms. Gratwick concludes than an insecticidal treatment is likely to be less effective on insects possessing tarsi that are densely clothed with setae than on those with fewer tarsal setae, and more effective on insects that clean themselves frequently than on those that do so rarely. The legs of the lined ambrosia beetle, (Figures 11, 12, 13), are sparsely endowed with setae and the beetles do not indulge in frequent cleaning movements.

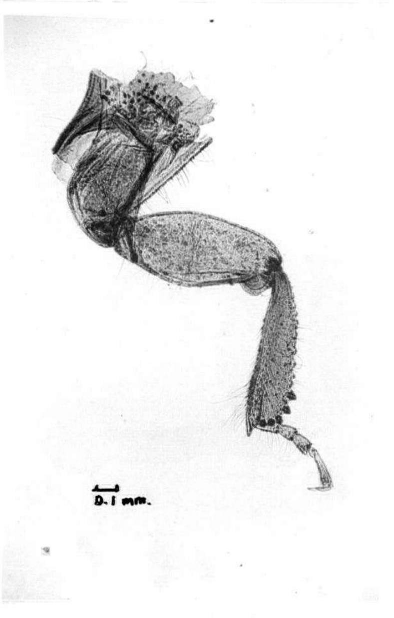

Figure 11. Fore-leg of ambrosia beetle

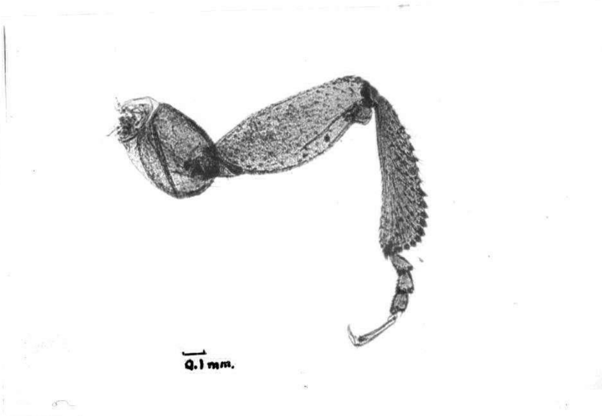

Figure 12. Mid-leg of ambrosia beetle

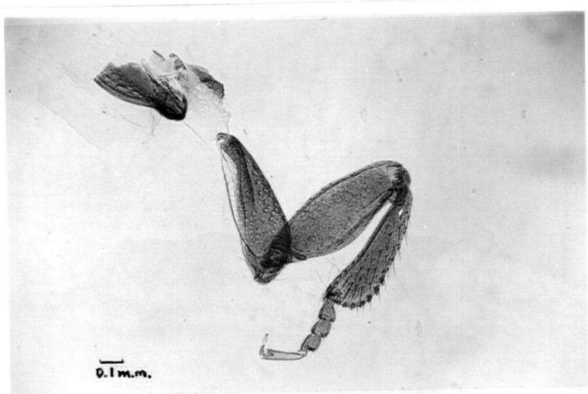

Figure 13. Hind-leg of ambrosia beetle

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SUMMARY

Conditions influencing the configurations and sizes of gamma-hexachlorocyclohexane crystals formed by solvent exchange were studied. The conditions studied included differences in solvents, concentrations, emulsifiers, direction and rates of addition, rates of stirring and the temperature of formation and storage of the crystals.

Two of the most distinctly different crystal forms, viz. needles and rhomboids, were chosen for biological testing on the ambrosia beetle Trypodendron lineatum (Oliv.). Functional availability of residues was measured by the gross lethal effect on the beetles brought momentarily into podal contact with treated surfaces.

CONCLUSIONS

1. The most important factor affecting the crystal formation of lindane by the solvent exchange method was the direction of addition. When the lindane solution is added to water the factors affecting crystal form are the type and concentration of surfactant present, the proportion and type of lindane solvent in the final mixture and the temperature of formation and storage of the suspension. When water is added to the lindane solution, the rate of addition and stirring are the two important factors affecting crystal size. The type of surfactant present has some effect on crystal form.

2. Under the conditions of experimentation, using mortality as a gross index, no difference could be demonstrated in the availability of deposits of lindane of two crystal forms, viz needles and rhomboids.

3. Deposits of 180 mg. per sq. ft. of either the needle-like or the rhomboid shaped crystals of lindane were completely dissipated during the eighth week after storage under known conditions. Deposits of higher dosage disappeared at a slower rate, as indicated by percentage loss per unit time.

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