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ROTHAMSTED RESEARCH

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Poisoning of Honeybees by Pesticides: Investigations of the Changing Pattern in Britain over 20 Years

J. H. STEVENSON, P. H. NEEDHAM and J. WALKER*

Introduction

Much of the research programme of the Insecticides and Fungicides Department concerns the safer and more efficient use of pesticides. This includes the development of improved control agents and of better methods of managing their use to optimise efficacy and minimise effects on unintended recipients. Because of the importance of the honeybee (*Apis mellifera*) in Britain in food production as a pollinator and also as a source of honey, investigation of the hazard to bees from modern insecticides falls naturally into this programme.

Honeybees are a particularly useful species for studying side effects of pesticides because most colonies are under fairly constant observation and their death or changes in behaviour are likely to be readily noticed. Although estimates of colony numbers are approximate, they are often more accurate than those of other insects that may be equally beneficial and that may be studied. Many conclusions from studies of honeybees may apply to other pollinating insects.

At Rothamsted we have used three techniques to obtain information about pesticide poisoning of honeybees and its avoidance:

1. Laboratory assessment of acute toxicity of unformulated pesticides.
2. Field evaluation of the toxicity of formulations applied to crops when bees are foraging and therefore at risk.
3. Investigation of samples of bees thought by bee-keepers to have been poisoned.

Investigations under the first two headings have yielded much valuable information and enabled us to devise standard test procedures which are now incorporated in the Pesticides Approval Scheme of the British Ministry of Agriculture, Fisheries and Food (MAFF) for the registration of pesticide products (see Appendices 1 and 2). However, the third technique has proved the most useful for assessing actual hazard in practice.

Laboratory assessment of toxicity

Much laboratory and field work was undertaken during and immediately after the war on the toxicity of newly developed pesticides as they were introduced (*Rothamsted Reports for 1939–45*, 197, 225; *for 1946*, 72, 77; *for 1947*, 61, 73; *for 1948*, 72, 82; *for 1950*, 109; *for 1951*, 124; *for 1952*, 111). A few examples will illustrate the work done during this early phase. Median lethal doses (LD50) for acute oral toxicity of seven compounds to worker honeybees were determined (Table 1). In addition HCH (BHC) and lead arsenate were found to be very toxic to honeybees, while DDT was comparatively safe (Way and Synge, 1948). As a stomach poison, colloidal DDT was about four times more toxic than a crystalline suspension. The herbicide DNOC and its sodium and ammonium salts were toxic and reported to be a potential danger to bees, while the 'hormone'

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TABLE 1

Acute oral toxicity of pesticides to worker honeybees (μg per bee), determined before 1963

Aldrin	0·27
Chlordane	1·2
Parathion	0·04
Schradan	10
Sodium DNOC	2–5
TEPP	4
Toxaphene	40

weedkillers MCPA and chlorthial were safer. The possibility of incorporating honeybee repellants in spray formulations was also investigated but with little success.

The need to standardise methods became apparent because honeybee toxicity data submitted to the MAFF by firms seeking approval for new pesticides were obtained by a number of different techniques and were not readily comparable. Investigations started in 1963, at the request of MAFF, led to the establishment of standard techniques to assess acute oral and contact toxicity of unformulated pesticides to worker honeybees (Stevenson, 1968; MAFF, 1971, see Appendix 1). It was important to know the exact amount of insecticide received by each bee in these tests, and techniques involving sprays or dusts or contact with prepared surfaces were therefore avoided. Interference with the behaviour patterns of social insects during bioassay can cause high control mortality. For example keeping the bees in cages with wire mesh sides reduced fighting, possibly because communication between cages was maintained. It was also essential to ensure complete anaesthesia with carbon dioxide during handling.

Values for contact toxicity were obtained by applying a one-microlitre drop of the pesticide dissolved in the appropriate quantity of acetone to each anaesthetised worker bee. Acetone solutions dissolved or suspended in 20% sucrose in water and fed (0·2 ml) to groups of ten bees were used to determine oral toxicities. Median lethal doses (LD50) were obtained by the probit method. Pesticides used were generally at least 95% pure, with two exceptions: natural pyrethrum extract (20% pyrethrins) and demephion (70% active ingredients).

TABLE 2

Acute contact and oral toxicity to worker honeybees (Apis mellifera) *of technical samples of pesticide*

n = number of determinations on which estimate is based, see text

	Contact		Oral	
	LD50 μg per bee	*n*	LD50 μg per bee	*n*
Allethrin	3·4	4	9·1 4·6	2 3
Azinphos-methyl	0·063	2	0·15	3
Benomyl	>10		>10	
Bioresmethrin	0·0057	3	0·055	1
Captan	>10		—	
Carbaryl	1·3	2	0·14	2
Carbophenothion	1·4	2	5·2	2
Chlordane	1·4	3	—	
Chlorfenethol	>50		>100	
Chlorfenvinphos	4·1	1	0·55	1
Chlorpyrifos	0·059	1	0·25	3
Dazomet	>50		>10	
DDT	3·9	3	3·7	5
Decamethrin	0·051	3	0·079	1
Demephion	0·36	2	0·22	2

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	Contact		Oral	
	LD50 µg per bee	*n*	LD50 µg per bee	*n*
Demeton-S-methyl	0·26	3	0·21	4
Demeton-S-methyl sulphone	0·20	2	0·19	1
Dialifos	9·5	1	29·2	2
	28·6	1		
Diazinon	0·22	2	0·20	2
Dicamba	>100		>10	
Dichlofluanid	—		>70	
Dicofol	>50		>10	
Dicrotophos	0·076	1	0·068	3
Dieldrin	0·16	8	0·32	6
Diflubenzuron	>30		>30	
Dimethoate	0·12	3	0·15	8
Disulfoton	4·3	4	16	2
			39	1
Endosulfan	7·1	4	6·9	3
Endrin	1·2	3	1·4	2
	0·65	2	0·46	2
Ethiofencarb	2·3	2	1·5	1
Ethylmercury chloride	22	2	13	1
Fenazaflor	12·2	2	2·9	1
Fenitrothion	0·018	3	0·019	3
Fonofos	3·3	3	8·4	1
gamma-HCH (BHC)	0·46	3	0·45	1
	0·20	6	0·76	1
Malathion	0·27	2	0·38	3
MCPA	>100		>10	
Mecoprop	>100		>10	
Menazon	4·3	3	0·46	2
Mevinphos	0·070	6	0·027	3
Omethoate	0·083	1	0·048	2
Oxamyl	0·31	2	0·094	2
Oxydemeton-methyl	0·54	5	0·31	3
Paraquat dichloride	>48		>25	
Permethrin	0·11	2	0·28	3
Phenylmercury acetate	—		10	2
Phorate	0·32	3	0·44	4
Pirimicarb	>54		3·2	1
Pirimiphos-ethyl	<0·5		0·39	2
Pirimiphos-methyl	0·39	2	0·36	1
Pyrethrins	0·29	4	0·15	2
	0·13	4		
Quinomethionate	—		>80	
Resmethrin	0·015	3	0·069	1
Rotenone	>60		>30	
Thiofanox	0·058	1	0·062	1
Thiometon	0·55	2	0·56	2
Tolyfluanid	>100		43	1
Triadimefon	>25		>25	
Triazophos	0·055	3	0·074	2
Trifluralin	>100		>50	

Standard errors for single tests are small but variation between individual tests is larger and it seems realistic to take this into account. In the three years 1964 to 1966 standard deviations for all tests were 27, 21 and 20% (mean 23%) for contact tests, and 35, 22 and 43% (mean 33%) for oral tests, and we have no reason to think this level of accuracy has changed subsequently. The higher error in the feeding tests is to be expected because doses are presented to ten bees as a competing group, rather than as individuals. After a series of preliminary experiments, each estimate is based on a small number of probit regressions indicated in Table 2 under '*n*'. A good measure of the percentage

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standard error for an LD50 can be obtained by dividing the above standard deviation by $\sqrt{n}$. Thus for allethrin by contact the value would be $23/\sqrt{4} = 11{\cdot}5\%$.

Results of tests using these techniques are given in Table 2.

Field evaluation of toxicity

Laboratory evaluations (Tables 1 and 2) give a useful indication of potential hazards to honeybees of pesticides but full scale field trials with many foraging honeybees present are essential to test the safety of any pesticide application procedure which may put bees at risk. Factors such as foraging behaviour, formulation and method of insecticide application can then be studied.

Smaller scale trials in glasshouses or in tents can give valuable, although limited, information (Davis *et al.*, 1974), but honeybees restricted in this way rarely behave as in the field, often spending more time trying to escape than in foraging. However, it may be possible to demonstrate that a treatment is dangerous without the necessity of an expensive full scale experiment.

In early field trials Butler *et al.* (1943) (*Rothamsted Report for 1939–45*, 197) observed the effects of foraging on sprayed orchards including collection of contaminated water. They confirmed the toxicity of arsenicals and reported repellent effects of lime sulphur, nicotine sulphate and copper sulphate. Way and Synge (1948) showed that DDT sprays and dusts on open blossom caused negligible damage to honeybees, bumble bees and the solitary bee *Andrena*, while HCH was very toxic (*Rothamsted Report for 1946*, 72, 77). Later (*Rothamsted Report for 1948*, 72) the danger of using DNOC on flowering charlock was demonstrated, confirming laboratory work.

Following the work on laboratory toxicity the Insecticides and Fungicides Department was also asked to recommend a standard field procedure for assessing the toxicity of formulated pesticide applications to foraging honeybees in normal agricultural practice. A series of field trials showed that granular formulations of systemic organophosphate insecticides applied to flowering field beans were very much safer than sprays (Free *et al.*, 1967) and that endosulfan sprays were safer than azinphos-methyl and malathion formulations (cf. Table 1) (Needham & Stevenson, 1973). The techniques used were summarised as a Working Document of the Pesticides Safety Precautions Scheme (MAAF, 1974) which is reproduced in full in Appendix 2.

These tests are expensive and time consuming. They are only relevant if a manufacturer intends to recommend application when there is a potential risk to bees and they will therefore not be required for every new formulation.

The formulation under test is applied to a large area of the flowering crop for which it is to be recommended. Colonies must be brought specially to the site and the sprays applied when weather conditions are suitable for maximum foraging. Frequent observations of the colonies and foraging bees must be made. A treatment known to be toxic to honeybees is always included to confirm the validity of the particular trial.

Examination of allegedly poisoned bees

For one year (1948) (*Rothamsted Report for 1948*, 71; G. J. Glynne-Jones, private communication) and continuously since 1956 (Needham *et al.*, 1966; Stevenson & Walker, 1974) samples of honeybees, thought by beekeepers to have been poisoned by pesticides, have been submitted to the Bee Advisory Unit of MAFF. After examination for disease, the samples are forwarded to Rothamsted for analysis to establish whether poison can be detected. Meanwhile Bee Advisory Unit workers investigate the circumstances and obtain as much field evidence as possible. By combining this information and studying

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the results for each year as a whole, the principal causes of honeybee poisoning and their extent can be identified and distinguished from more isolated incidents. This system enables recommendations to be made for the safer use of insecticides and also acts as a sensitive indicator of serious new hazards. Obviously not all incidents are reported to us, but we obtain sufficient information to give a representative picture.

Analytical methods. When the problem of confirming poisoning in dead bees was first posed methods for residue analysis were insensitive and identification of an insecticide was difficult. Early work depended entirely on biological assay of extracts using the fruit fly (*Drosophila melanogaster*) and yellow fever mosquito larvae (*Aedes aegypti*) but did not of course identify the toxicant involved. Some classification was possible by paper chromatography or solvent fractionation of the extracts which separated DDT and cycldiene compounds from HCH and any organophosphate material that had not broken down to non-toxic products. The development of gas-liquid chromatography (GLC) enabled organochlorine compounds to be identified much more reliably.

Organophosphate insecticides act by inhibiting the activity of acetyl cholinesterase. We were therefore able to develop a technique in which measurement of the residual cholinesterase in dead bees was used as evidence of poisoning.

Later advances in GLC enabled some organophosphate insecticides and their metabolites to be identified, but since procedures needed to be modified for each compound, thereby extending analysis time unrewardingly, the general cholinesterase inhibition method is normally used (Table 3). Methods used up to 1966 have been reviewed (Needham *et al.*, 1966).

Organochlorine insecticides. The procedures currently used for extracting bees, for biological assay and gas chromatography are essentially those described by Needham *et al.* (1966). After maceration in diethyl ether, the resulting extract is adsorbed on to celite and the solvent evaporated. This is eluted with aqueous acetone leaving excess beeswax and other interfering substances on the celite, and then partitioned from the aqueous acetone into petroleum ether. This solution is then analysed by gas–liquid chromatography or evaporated on to the inside surface of glass vials, for contact biological assay with *Drosophila melanogaster* adults. Biological assay is valuable for confirming that a toxic material is present, especially on occasions when gas chromatography has detected no known toxicant. Alternatively, it may indicate that unusual GLC peaks do not represent toxic substances. Occasionally, when small peaks are observed at the retention times of HCH and dieldrin, combined gas chromatography/mass spectrometry is useful in confirming the presence of particular insecticides.

Organophosphate compounds. The reduction in cholinesterase activity due to poisoning with these compounds was originally measured by a Warburg technique (Needham *et al.*, 1966) but now a spectrophotometric method is used (Ellman *et al.*, 1961).

Bee heads are homogenized in buffer and cholinesterase activity measured by incubating with acetylthiocholine chloride. The hydrolysis product thiocholine reacts with 5,5-dithiobis-2-nitrobenzoic acid to form a coloured product which is assayed at 412 nm. After extensive tests on the effects of partial decomposition of bees in transit to the laboratory, we concluded that a cholinesterase level below one third of that in an unpoisoned control could be reported as 'probably poisoned by an organophosphate insecticide'.

Carbamate compounds. Inhibition of cholinesterase by carbamates is reversible after death and is therefore not detected by the above technique so another type of assay is

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TABLE 3

Number of reported bee poisoning incidents and insecticides implicated

	1948*	'56*	'57	'58*	'59	'60*	'61*	'62	'63	'64	'65	'66	'67*	'68	'69	'70*	'71	'72*	'73	'74	'75*	'76*	'77*
No. of incidents	63	9	11	8	25	34	44	24	35	30	66	31	53	39	41	63	65	80	81	138	112	135	119
Negative	33	1	5	3	5	10	9	8	3	8	24	11	16	14	13	18	25	37	22	43	26	31	48
Organophosphate			2	3	13	17	22	9	20	16	30	12	32	17	27	35	28	35	52	80	80	85	43
Organochlorine																							
HCH		3	3		2	1	3	1	1	1	4	1	1	5		1	10	3	1	4	3	7	6
Dieldrin		1			4	3	7	3	8	3	5	3				4	1	2				1	6
HCH + dieldrin											2	1	2		1								2
OP + dieldrin													1			2				1			
OP + HCH														1		1		1	1	3		5	
Carbaryl								3	3	2		2		2		1		1	5	6		4	
Unidentified	9	3	1		1	1	1				1	1					1			1		1	2

* Also found: 1948: arsenic 14, arsenic + DDT 1, DDT + lime sulphur 4, nicotine 1, DNOC 1; 1956 DNOC 1; 1958 DDT 1, arsenic 1; 1960 DDT 1, arsenic 1; 1961 mercury 1, aldrin 1; 1967 aldrin 1; 1970 HCH + DDT 1; 1972 aldrin 1; 1975 HCH + organophosphate 3; 1976 carbaryl + organophosphate 1; 1977 organophosphate + DDT 1.

TABLE 4

Bee poisoning situations and the number of incidents attributed to them

Aerial incidents may also be included under specific crops. Incidents where cause of poisoning was not discovered are not included

	1948	'56	'57	'58	'59	'60	'61	'62	'63	'64	'65	'66	'67	'68	'69	'70	'71	'72	'73	'74	'75*	'76	'77
Aerial						1			11	9	1	6	12	1	7	3		19	16	11	11	27	30
Field Beans			1	1	8	10	8	4	14	3	15	7	21	5	13	7	3	19	21	33	6	7	5
Brassica		1	2	2	3		2	2		1	2			1	2	4	3	5	4	12	30	24	8
Cereals		2																			22	28	30
Wasp baits						1	3		4			1											
Orchards	19	3	2	1												2	3	1	5	11	3	5	
Blossom thinning														2				1	5				
Soft fruit	1			1								1		1				2	5		1		1
Pea						1				6			1	2		5	5	3			3		
Potatoes																8	1		1				3
Contamination			1								2	1		1	3	2	6	2	2	3			4
Malicious													1	1		2	1	1					4

* Also five cases concerning carrots in 1975

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used. Bees are macerated in methylene dichloride and the extract passed through a Florisil column to separate insecticides from interfering substances before identification by high pressure liquid chromatography using a Permaphase ETH column and methanol-water as the mobile phase. Carbaryl and pirimicarb can be detected by this method (*Rothamsted Reports for 1971*, 195; and *for 1973*, 186).

Test for starvation. Early in the flight season, we often receive a large proportion of bee samples which show no evidence of poisoning, and have suspected that death may be due to starvation. In connection with other work on sugars in honeybees (*Rothamsted Reports for 1976*, 133; and *for 1977*), thoraces of healthy bees were shown by thin layer chromatographic analysis to contain appreciable amounts of glucose (5–10 μg per bee) and fructose (1·5–2·5 μg) while bees that had been deliberately starved had very little ($<$0·5 and $<$0·25 μg per bee respectively) and bees poisoned in the laboratory had intermediate levels. It is therefore possible to detect whether bees 'might have starved'.

Results. Results are classified according to crop involved (Table 4) and cause of poisoning (Table 3). The total number of samples received has steadily increased as the scheme has become better known among beekeepers. The principal causes of poisoning are discussed below.

Discussion

The accumulation of data from the three sources described has enabled us to identify the principal dangers to honeybees from the use of modern pesticides in England and Wales, to monitor the effects of changes in agricultural practice and to make recommendations for the protection of honeybees.

Laboratory and field tests. Results of the laboratory and field studies agree well with data obtained from poisoning incidents; the former therefore seem valuable for a preliminary assessment of the potential hazard of proposed treatments with new pesticides in situations where bees may be at risk.

Experience has shown that honeybees from different sources vary little in their response to insecticides. In 1967 we compared the toxicity of dimethoate to five different strains of worker bees (*A. mellifera*) from Buckfast Abbey. None was significantly more resistant or more susceptible than our bees (*Rothamsted Report for 1967*, 175). Collaborative work with other laboratories throughout the country using the method described in Appendix 1 has shown little variation in LD50.

Patterns of bee poisoning. Honeybee poisoning and the suggested preventive measures are best discussed separately for each type of application.

Field beans. The use of organophosphate systemic insecticides to control black bean aphid (*Aphis fabae*) has been a major cause of bee poisoning (Table 4). As with most other poisoning situations, the danger to bees is almost entirely confined to the flowering period when all bees foraging on the crop are at risk if pesticides lethal to bees are applied. Although some pesticides exhibit residual toxicity for a few days after application, by far the greatest hazard occurs during actual spraying and is due to foraging bees being hit by insecticide droplets. This risk could be easily avoided by recommendations that (1) insecticides be applied before flowering, (2) compounds with low toxicity to bees such as menazon (or more recently pirimicarb) should be used, or (3) that the safer granular formulations recommended by us (Free *et al.*, 1967) be applied. It was particularly dis-

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appointing therefore, that widespread bee poisoning associated with field bean spraying again occurred in 1974 after the encouragingly small numbers of incidents in immediately preceding years. These were probably due to smaller populations of aphids and consequent reduced use of insecticides rather than improved methods of treatment.

Aerial application. The large proportion of poisoning incidents associated with aerial spraying confirms that this method is more hazardous to bees than treatment by ground machines. Air space above and alongside the crop is contaminated and spray drift is more likely to reach neighbouring flowering crops, flowering weeds and hedgerows. Weather conditions and mechanical breakdowns limit the value of any prior notification given to beekeepers, and many consider the closing or moving of their colonies during spraying to be quite impractical.

A ban on aerial treatment of crops in flower and more precise application of pesticides should do much to reduce the risk but it is also important that the public relations aspect of aerial spraying is not allowed to deteriorate further.

Brassica crops. The earlier poisoning incidents associated with Brassica crops were mostly on mustard, while later cases reflect the increase in area of oilseed rape which is exceptionally attractive to foraging bees (Table 5). The number of bee poisoning incidents associated with field beans depends at least partly on the level of *A. fabae* infestation and is not related simply to annual area; unfortunately there is a much closer relation between the area of oilseed rape grown and incidence of bee poisoning.

TABLE 5

Area of field beans and oilseed rape (ha) grown in England, 1971–77, and number of reported bee poisoning incidents associated with these crops

	Field beans		Oilseed rape	
	Area	No. of incidents	Area	No. of incidents
1971	61 600	3	5 100	3
1972	52 700	19	6 900	5
1973	59 700	21	13 700	4
1974	66 300	35	24 500	13
1975	39 900	2	39 000	23
1976	44 400	7	48 800	24
1977	37 000*	5	55 000*	8

*Provisional

It should often be possible to control pollen beetle (*Meligethes aeneus*) and seed weevil (*Ceuthorhynchus assimilis*) on rape by spray application before the crop flowers. However, on winter rape in particular, it has sometimes been necessary to treat fields several times in a season against weevil and pod midge (*Dasyneura brassicae*) including some spraying during the flowering period with consequent risk to pollinating insects (Alford & Gould, 1975).

Closure or movement of bee hives during spraying is seldom practical and the principal means of protecting bees in this situation has been the use of selective insecticides (Stevenson & Walker, 1975; Needham & Stevenson, 1973) such as endosulfan or phosalone. To establish suitable techniques for application, field trials of the type described are essential.

Seed weevil and midge damage is heavier on farms where rape has been grown for several years and solutions which minimise risk to bees are being sought by several manufacturers. In particular application after flowering and greater use of selective insecticides are being studied.

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Orchards. Results for 1948 revealed that spraying fruit trees in flower was then the main hazard to honeybees. Fruit growers who pay for honeybees to be brought to their orchards for pollination are well aware of their value and the hazards of pesticide sprays. Changes in the insecticides used and spray programmes that carefully avoid flowering periods were rapidly introduced and have greatly reduced the hazard to honeybees that arose from orchard spraying.

Since 1968 carbaryl has been implicated in losses of bees in orchards, both as an insecticide and as a fruit thinner, and its use when orchards are attractive to foraging bees is a serious hazard.

Cereal sprays. Apart from 1956 cereal spraying was not a recorded cause of bee poisoning until 1975 when there were extensive insecticide applications to control cereal aphids, because such applications had become economically attractive. We thought at first the resultant increase in poisoning was entirely due to bees flying over cereal fields en route from colony to forage sites, but we now have evidence that in some fields they are attracted to the honeydew produced by the aphids and in others to flowering weeds in the crop. The use of aphicides with low toxicity to bees or of granular formulations may solve this unexpected problem.

Contamination. Bee poisoning resulting from the use of paints pre-treated woods and wood preservatives containing insecticides to construct and maintain hives can only be avoided by publicising the problem and labelling products well. We know of several incidents where dichlorvos resin strips were used to 'protect' stored comb against wax moth, especially during the winter. The insecticide was taken up by the wax, killing all bees to which it was subsequently introduced. HCH has caused similar trouble and continuing publicity in beekeepers' journals is needed.

Malicious damage. Bees are not popular with everyone, especially when colonies are sited in small suburban gardens and a few incidents have been reported where bees were deliberately poisoned. However, this is not as serious a problem as it is in Germany (Stute, 1967).

Starvation. The accumulation of dead bees at the hive entrance can result from a variety of causes but is usually attributed by beekeepers to poisoning. A large proportion of 'negative' samples, in which no evidence of poisoning can be found, are received early in the season and we have concluded that death in such cases may be due to the spring removal of collective winter deaths or starvation following a winter which exhausts inadequate food reserves. The recent development of a test for starvation confirmed this in some cases, although some beekeepers still do not recognise this as a possible cause of death.

Geographical distribution. Poisoning incidents in Wales and north and west England are much less common than in East Anglia, reflecting primarily the distribution of the field bean and oilseed rape crops and the nature of the farming, rather than the distribution of honeybee colonies.

Other beneficial insects. The extent to which our results apply to bumblebees and solitary bees, and their relevance to the protection of parasitic and predatory species is of interest.

HCH was shown to be much more toxic to bumblebees as well as honeybees than DDT (*Rothamsted Report for 1946*, 72, 77), and it was also very toxic to the solitary bee,

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Andrena. It is often difficult to collect sufficient individuals to obtain accurate toxicity figures, but tests with two species of bumblebee (*Bombus lucorum* L. and *B. agrorum* Fab.), in which a range of concentrations of each of four insecticides were applied to groups of three to six insects, established the limits within which contact LD50 values lie (Table 6) (*Rothamsted Report for 1966*, 176).

TABLE 6

Contact toxicity of four insecticides to bumblebees

Dose range (μg per bee) within which LD50 lies. The number of tests for each compound and the total numbers of insects used are also given

	Demeton-methyl (μg)	Dimethoate (μg)	Disulfoton (μg)	Phorate (μg)	Total no. of insects used
Bombus lucorum					
Queens	6–24 (4)*	5–20 (4)	Over 40 (3)	6–23 (4)	99
Workers/drones	1–2 (5)	2–5 (4)	2–10 (5)	1–2 (5)	181
Bombus agrorum					
Queens	10–24 (3)	1–5 (3)	5–10 (3)	1–5 (2)	94
Workers/drones	1–3 (4)	0·5–2 (4)	1–4 (4)	1–2 (5)	283

* No. of tests given in parentheses.

On field beans in flower observations on foraging behaviour of bumblebees and Syrphidae showed a reduction of activity after spraying with demeton-S-methyl (Free *et al.*, 1967) and recommendations to include observations on bumblebee behaviour in field trials with new insecticides are included in Appendix 2. They are particularly important because bumblebees will forage earlier and later in the day and under poorer weather conditions than honeybees. Hence it is difficult to spray when they are not at risk.

Precautions to avoid application of pesticides in flower are unlikely to benefit parasites and predators greatly, but the use of selective insecticides may well protect other Hymenoptera including parasites.

General recommendations

The injudicious use of insecticides can cause financial loss to beekeepers, but insecticides are an essential part of maximum food production and their absence would make some crops uneconomic. Unless beekeepers accept this fact and contractors and farmers recognise their responsibility to preserve beneficial insects, attempts to establish co-operation between them will be counter-productive. As well as attempting to bring farmer and beekeeper closer together, the aim must be either to keep the insecticide and the bee apart or to use a selective chemical with low toxicity to honeybees.

Keeping the insecticide and foraging bees apart. There are several ways, some obvious, to achieve this.

1. Insecticides are often applied on a preventive routine basis and may sometimes be avoided altogether if an informed inspection shows that the pest is not present in economically important numbers. Insect population monitoring in East Anglia suggested that many oilseed rape crops were sprayed unnecessarily in 1974 and 1975 (Alford & Gould, 1975).

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2. Spray application when the crop is not flowering and has no other attractive features in it like flowering weeds should ensure that there is normally little risk to foraging bees. Insecticides that are highly toxic to bees but otherwise desirable can then reasonably be used although the contamination of air space used as a flight line to a more distant attractive crop, and the recent problem with cereals reported above, underline the possibility of unexpected hazards arising.
3. Some formulations or application techniques greatly reduce direct contact and so minimise the hazard for bees. The very low mortality when granular formulations are applied to flowering field beans is probably achieved because pesticides which hit foraging bees do not adhere, in contrast to spray droplets. Even safer are seed dressings which may allow the use of highly toxic compounds such as aldicarb.
4. Spraying early or late in the day, or in dull weather when bees are less active, is often advocated, but in practice this can involve considerable organisational problems even in favourable weather. It is not an absolute solution and takes no account of the longer period of activity of wild beneficial insects.
5. Closure and/or movement of colonies in advance of spraying are also frequently offered as partial solutions, but may not be practical because the beekeeper cannot always reach all colonies at short notice or at notified times. Delays in the spraying programme leading to prolonged closure, as well as inadequate ventilation, lack of shade or particularly hot weather, can cause losses through suffocation which may be severe and are often disastrous. Removal may take the bees to another area where spraying is in progress. Although co-operative schemes between beekeepers and spray contractors have been operated effectively, the most successful are usually in counties with a low level of insecticidal application, and further research on safe methods of closure might benefit beekeepers with ready access to their colonies.

Use of chemicals with low toxicity to honeybees. Table 2 indicates insecticides that might be or are used with least risk to honeybees, notably menazon and pirimicarb as aphicides, endosulfan and phosalone on oilseed rape and even DDT. Such selectivity is clearly a desirable feature which should be actively sought in new insecticides and should benefit not only bees but also other beneficial insects. Compounds with low toxicity to bees may not be very toxic to parasitic Hymenoptera, but parasites and predators from other orders must also be considered. In the continuing programme on ways of minimising adverse side effects of crop protection practices in the Insecticides and Fungicides Department, increasing attention is being directed at the protection of these other species.

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APPENDIX 1

Laboratory Testing of Pesticide Products for Toxicity to Honeybees*

I. Introduction

1. All pesticide products notified for use in agriculture, horticulture, home gardening, or in forestry which might be used where bees are at risk, should normally be tested for toxicity to honeybees. It will not be necessary to submit bee toxicity data for every notification.

2. The Rothamsted Experimental Station, Harpenden, Hertfordshire has developed a method for measuring the toxicity of pesticides and other substances to honeybees (Stevenson, 1968) to enable manufacturers, formulators and others to do their own tests. The techniques are outlined in this working document.

3. These tests are designed to compare the toxicity of technical, unformulated compounds and to provide a basis for identifying those potentially hazardous to honeybees. The bioassay of unformulated compounds is preferred but results based on formulated material may be acceptable. It should be appreciated that methods of formulation and of application can greatly affect toxicity in practice and similar tests with formulated material, for which the methods can be modified, may also be necessary.

II. Supply of worker honeybees for the tests

4. It is essential to have an experienced beekeeper in charge of the honeybee colonies. Beekeepers will appreciate that colonies kept primarily for this work should not be expected to yield honey.

5. Bees should only be removed from hives on the advice of the beekeeper. It is recommended that this is done between May and the beginning of November, when as a

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guide up to 1000 bees per week have been taken from five colonies and many more than this number could probably be taken without ill effect. It may be possible to extend the season from the end of March to the beginning of December in southern England, but the number of bees taken may have to be fewer at the beginning and end of the season than during the middle. Hives should never be opened in very cold weather.

III. Contact toxicity tests

6. Before opening a hive, the colony may, if necessary, be partly subdued with a minimum use of smoke—a standard beekeeping practice. Worker bees may then be carefully shaken or gently swept, with a soft brush from the combs into a plastic bucket, wide enough to accept the end of the frame and fitted with a lid. Care should be taken not to include the queen. Bees should preferably be taken from above a queen excluder. Up to 1000 bees may be kept without harm in a two-gallon bucket for at least an hour, provided several layers of filter paper are placed in the bottom to absorb moisture.

7. Groups of bees should be placed in cages. If anaesthesia is employed to facilitate this it should be kept to a minimum and only carbon dioxide or carbon dioxide/air mixtures used. A suitable cylindrical cage for this purpose is 4½ in deep and 1½ in in diameter, made of ⅛-in tinned wire mesh and closed at both ends by corks. Ten bees are the optimum number for this size of cage. The cages should be stored upright and the bees fed 20% sucrose in water from 2 in × ⅜ in glass tubes, with their open ends restricted to about ⅛-in orifices and inserted through the top cork. If excessive control mortalities occur it is suggested that a water feeder is provided in addition to the sucrose. Worker honeybees should be kept in these cages at 26–27°C. The bees should be kept under relatively constant conditions and away from draughts but adequately ventilated. Under these conditions the bees will usually survive for at least seven days.

8. At least two lots of ten bees should be tested at each of five or six dosage rates to obtain regression lines but more bees may be used if desired. The test pesticide should be dissolved in acetone where possible, but if another solvent is necessary, its toxicity to honeybees must be determined. Bees used for controls should be treated with the test material less the active ingredient(s). Where toxic solvents cannot be avoided, it may be advisable to reduce the volume applied. Counts of mortality should be made at 24 h, 48 h, and if necessary over a longer period.

9. The bees should again be anaesthetised with carbon dioxide before applying the test chemical. The anaesthetised bees are laid, ventral surface up, on filter paper in a petri dish and 1·0 μl drops (or smaller if necessary—see above) of the test material dissolved in acetone placed on the ventral thorax using a micro-applicator (Arnold, 1965, 1967). The treated bees should be returned to the cages and kept for 24 h or longer when the number of bees killed is estimated. Median lethal dose (LD50) values (expressed as active ingredient) can then be read from graphs plotted on logarithm/probability paper or calculated using the probit method (Finney, 1952). Corrections should be made for any bees in the controls that die, but tests in which the control losses exceed 10% should be treated with suspicion and repeated if at all possible.

IV. Oral toxicity test

10. To determine the oral toxicity to honeybees the pesticide is presented to groups of ten bees in the wire cages already described. A solution of the appropriate concentration of the test material in acetone (1 part) is mixed with 20% sucrose in water (19 parts) to give the most concentrated of a series of doses; dilutions are made with a mixture containing 5% acetone and 20% sucrose. A satisfactory solution or suspension of the test material is usually obtained in this way but sometimes another solvent may be needed

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instead of acetone. Each group is fed 0·2 ml of these mixtures, i.e. 20 μl per bee. Tubes with smaller orifices (*c.* $\frac{1}{16}$-in diameter) than those used to feed syrup to the bees (*c.* $\frac{1}{8}$-in diameter) should be used to administer the insecticide and the doses can be measured into the tubes with a hypodermic syringe. The tubes are placed in the corks before the ten bees are put in the cage. Control treatments, given the solvent, sugar and water mixture only, should always be included.

11. The bees will share the 0·2 ml of test material preparation between themselves and so receive similar doses; this technique is simpler than feeding the bees individually.

12. When the bees have taken all the test material solution (after $\frac{1}{2}$ to 5 h), they are given unlimited 20% sucrose as in the contact toxicity tests, and those killed are counted 24 h after initial dosage and at longer intervals if necessary. If excessive control mortalities occur it is suggested that a water feeder is provided in addition to the sucrose.

V. Further information

13. Further information may be obtained from Dr. J. H. Stevenson, Insecticides Department, Rothamsted Experimental Station, Harpenden, Hertfordshire.

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APPENDIX 2

Testing Pesticide Products for Toxicity to Foraging Bees under Field Conditions*

I. Introduction

1. In Working Document No. 13, a procedure is given for the laboratory testing of pesticide products for toxicity to honeybees. If such products are to be used on or near flowering crops or plants where bees might be at risk, it may be necessary to establish toxicity under field conditions. The presence of flowering weeds in a crop also provides another circumstance where foraging bees might be at risk.

2. A method has been developed at the Rothamsted Experimental Station, Harpenden, which, if care is taken, will enable manufacturers, formulators and others to perform their own field tests on pesticidal products and obtain worthwhile results. It is recognised that the cost of such tests will be high but in practice they will only rarely be required.

3. The test procedures outlined in this working document are designed to compare a test formulation, a formulation which is known to kill bees, and a control. In a trial to demonstrate that a formulation is not hazardous to bees, the treatment known to kill them is obviously essential but it may be possible to dispense with the control (see Fig. 2).

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Each treatment is applied to a specified area of a flowering crop on which bees from the test colonies are actively foraging. Care is taken to ensure foraging bees are visiting the experimental plot and that none are visiting areas treated with other pesticides. Experiments using this method are described by Free *et al.* (1967). Because of the large size of the plots and the difficulties of this type of experiment it is appreciated that replication will seldom be possible.

II. The trial procedure

Hives

4. It is essential to have an experienced beekeeper in charge of the honeybee colonies.
5. Bees used at the trial site must be moved at least two miles from their previous location otherwise they may return to their old foraging grounds. Hives should be placed in or on the edge of the crop to be sprayed and the flight path should be unobstructed. At least four colonies are needed at each plot to ensure an adequate number of bees visiting each treatment. Bees should be at the test site not more than two days before insecticide application because they tend to begin foraging in areas immediately adjacent to their hives. Some observations of the hives should be made before the pesticides are applied (see Section III, 14).

Test plots

6. The plots should be sufficiently large to ensure that a high proportion of foraging bees will visit the treated area and sufficiently far apart to reduce the likelihood of bees visiting adjacent plots. The treated crop must extend at least 200 yards from the hives. These requirements could be met by spraying each pesticide on 7–10-acre areas separated from each other by a minimum of 300 yards.
7. Examples of the arrangement of test plots used in trials to determine toxicity of sprays to foraging honeybees are shown diagrammatically in Figs. 1–2.

Test conditions

8. The bees must genuinely be at risk at the time of the application and the flowering crop must therefore be really attractive; not, for example, in the later stages of petal fall. The application should be made between 10.00 and 16.00 hours in fine weather. How fine depends on the previous few days; after a period of bad weather bees will forage in weather conditions they would avoid if the preceding period had been exceptionally fine.
9. Adjacent areas should not be treated with pesticides during the course of the trial.
10. A minimum of three plots will normally be required for each trial, i.e. one each for the control, the test material and for an insecticide which is known to kill bees, for example dimethoate or demeton methyl sprays. The use of a material known to be highly toxic to bees ensures that the test bees were at risk under the conditions of the trial. A negative result from the candidate material would be very suspect if there was also a negative result from the treatment known to be toxic.
11. An attempt should be made to estimate the population of each hive before treatment if only very roughly and subjectively, and any colonies not seen to be in a healthy condition should be rejected.

Duration of trial

12. The trial should continue for two to three weeks to allow any delayed effects to become evident.

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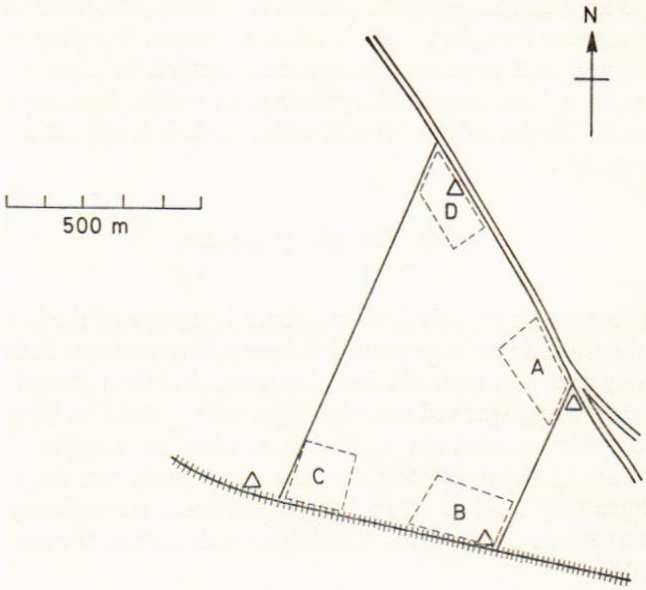

FIG. 1. Example of trial to determine the toxicity of sprays to foraging honeybees. Siting of four plots in a 100-acre field of flowering rape. △—Position of colonies.

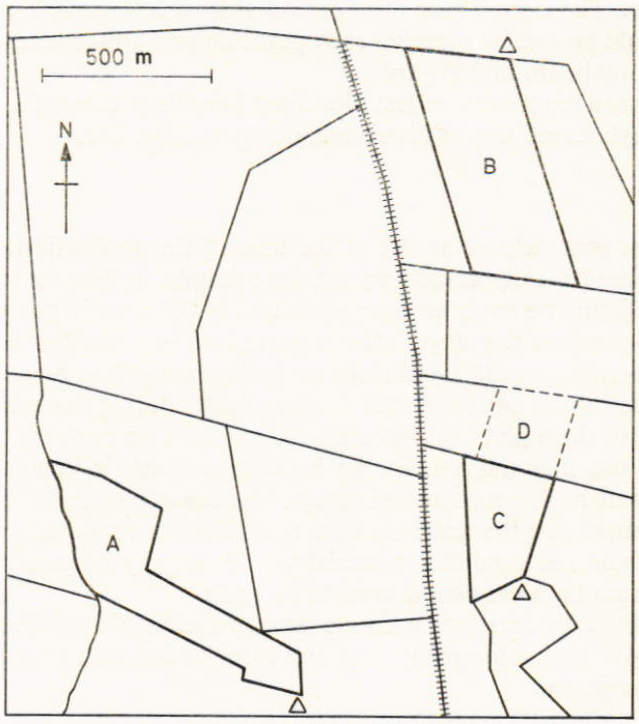

FIG. 2. The plan of an experiment to test the toxicity of pesticides to foraging honeybees in which three separate fields of beans were used as plots. Field D, also beans, was not part of the experiment. The remaining fields were planted with other crops. △—Position of colonies; A—Phorate, 29 acres; B—Disulfoton, 25 acres; C—Oxydemeton-methyl, 11·5 acres; D—A field of beans NOT part of the experiment.

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III. Assessment of results

13. The choice of observations made depends to some extent on the type of application and on the expertise of the available staff. The toxicity of the test chemical will almost certainly be shown by the number of dead bees thrown out of the hive. A decline in the hive population is more difficult to detect; it is not very accurate but it will indicate catastrophic losses. The first of the following methods for assessing results should always be used and as many of the other methods as is practicable.

Dead bee traps

14. Suitable traps are square boxes, the width of the hive, with a fine wire netting bottom which will not permit the passage of bees, and a coarse galvanised wire (1-in mesh) top. They are placed so that one of the top edges is immediately below the hive entrance and the coarse wire is extended over the entrance. If bees die in the hive the survivors will remove them, and they should be collected regularly from the dead bee traps for counting. These traps thus provide a good indication of poisoning. A large number of bees affected by insecticides return to the colony to die. Dead bees should therefore be collected from the traps for some weeks after the test treatment to provide a record of delayed mortality.

Hive populations

15. The method of Jeffree (1951) is suggested in which the colony is carefully dismantled and the bees on each comb are scored after comparison with a set of photographs of comb covered with known numbers of bees. By summing the results of these scores, an estimate of the total population is obtained. These counts also serve as a check that large numbers of bees have not died away from the hives. Such bees would not appear in the dead bee traps and therefore could be overlooked.

State of the colony

16. An examination of the general state of the colonies by a very experienced beekeeper before and after treatment may provide useful data. Such a person will be able to assess possible effects on the developing brood, the rate at which the queen is laying eggs and the significance of the number of queen or drone cells.

Activity at the hive

17. The frequency and direction of foraging is best recorded as the number of landings on return from flights. This, preferably combined with the use of pollen traps (para. 20), will indicate the test crop is being worked and any fall in activity may be due to the toxic or repellent effect of the pesticide.

Foraging activity on the test crop

18. Foraging activity varies with fluctuation in cloud cover, wind and rain; but worthwhile estimates of this activity are made by observers walking slowly through the crop for a set distance and time, while noting all foraging bees seen. For example, in one experiment a 220 yd strip, 6 ft wide was covered in 15 min. Three or four counts should be made at each plot, as near as possible at the same time. Comparative figures obtained before and after treatment will indicate changes in the foraging population.

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Honey crop

19. The honey crop can be estimated by weighing the hives at intervals during the observation period. This should give further evidence of the activity and strength of the colonies.

Pollen traps

20. Pollen traps are wire grids fitted to hive entrances through which the bees have to crawl to enter their hive (Free, 1959). The grids scrape the legs of bees so that their pollen loads fall into a collecting trap. Examination of the pollen will reveal the proportion of foraging bees working the test crop. Because different types of pollen loads vary in size and ease of detachment from the legs, the method is not an exact one, but is very useful for comparing colonies, and for indicating changes in foraging after spraying.

Analysis of dead bees, honey, wax and pollen

21. Biological and chemical assay for pesticide residues can produce valuable data. The methods of Needham *et al.* (1966) may be found useful.

Other observations

22. Observations of bumblebees and other beneficial insects will be of considerable value in assessing overall effects of pesticide applications. They may usefully be made during this type of experiment, for example when estimating foraging activity or by placing traps in the plots.

Further information

23. Further information may be obtained from Dr. J. H. Stevenson, Insecticides Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, who will be pleased to advise particularly when these tests are being planned.

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