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Behaviour of γ -Hexachlorocyclohexane in Stored *Vicia Faba* Beans

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INTRODUCTION

Lindane (γ -1,2,3,4,5,6-hexachlorocyclohexane) has been widely used in agriculture for nearly four decades as a soil insecticide, foliar spray on crops, seed dressing and also for the protection of stored grains. It is considered as a persistent insecticide although it is sufficiently volatile to have a fumigant action. Much work has already been done concerning the transformation and degradation of this important chemical.¹⁻⁹⁾

Screening of the available literature indicates that only little is known about the fate and degradation of lindane in stored grains.

The aim of the present work is to study the rate of penetration and degradation of the insecticide inside *Vicia Faba* beans.

MATERIALS AND METHODS

1. Chemical

[U-¹⁴C] γ -Hexachlorocyclohexane of specific activity 0.5 mCi/g (obtained by dilution of 250 μ Ci purchased from Amersham International plc, England) was used in this investigation.

2. Grains

Vicia Faba beans (var. Giza 1) were used with a moisture content of 11%. During the 26-week experimental storage period, the moisture content remained almost unaltered. This was monitored during the experiment by drying weighed samples at 120°C, every 4 weeks until constant weight.

3. Treatment

Faba beans were treated in a similar way described by Zayed and Farghaly.¹⁰⁾ In a dish covered with polyethylene sheeting, the grains were sprayed with the radioactive insecticide.

Two dose levels were used: 30 and 60 mg/kg grains. The lower dose exceeds the level recommended for practice (20–26 ppm), in order to compensate the losses which normally occur during spraying. To 1 kg beans, the dose was applied in 10 ml of 2% Tween 80 in water. The sprayed grains were rolled in a polyethylene container for 15–30 min to ensure good mixing and distribution of the insecticide. Then the treated beans were left to dry for 5 hr at room temperature (25°C) with occasional shaking.

4. Sampling

For determination of the actual concentration of the insecticide on the grains, usually 10 samples, each weighing 50 g, were taken at random from the seeds after 24 hr of the treatment. The samples were taken for analysis at different periods of time over 26 weeks, ground and assayed for radioactivity (compare Table 1).

5. Extraction

The samples (50 g each) were washed three times with 30 ml of a mixture of ethyl alcohol and water (1:3) at room temperature to remove the insecticide on the outer surface of the grains (external extract). The beans were then crushed in a mortar and extracted by Soxhlet apparatus for 24 hr with 95% methanolic solution (internal extract). The radioactivity in the extracts was directly determined by liquid scintillation counting (LSC). A definite weight of the extracted grains, after drying under reduced pressure, was combusted in a Packard Tri-Carb sample oxidizer (Model 306), and then assayed for unextracted radioactivity by LSC. Radioactivity levels were corrected for quenching using an internal standard.

6. Analysis

The external and internal extracts were analyzed by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). The ¹⁴C-residues in the internal extract were separated by a fractionation procedure. After evaporation of the alcoholic extract, the residue obtained was partitioned between chloroform and water (chloroform 1). The aqueous layer was acidified with 2 N HCl, then heated for 2 hr, and the water phase was re-extracted with chloroform (chloroform 2). The water and chloroform extracts were

Table 1 Residues of ^{14}C -lindane in treated bean grains (expressed as μg insecticide/kg grains).

Treatment period (weeks)	Actual dose ^{a)}	¹⁴ C-Residues in						% Recovered ¹⁴ C-activity
		External extract		Internal extract		Non-extractable form		
		μg	% ^{b)}	μg	% ^{b)}	μg	% ^{b)}	
4	A	8950	34.1	14,500	55.3	490	1.9	91.3
	B	27,240	54.4	18,380	36.7	790	1.6	92.7
9	A	6380	24.3	16,790	63.9	700	2.7	90.9
	B	20,050	40.0	23,700	47.3	930	1.9	89.2
13	A	4360	16.6	18,750	71.5	840	3.2	91.3
	B	16,400	32.7	26,860	53.6	1360	2.7	89.0
17	A	4570	17.4	20,950	79.8	540	2.1	99.3
	B	15,500	30.9	29,890	59.7	1500	3.0	93.6
22	A	3880	14.8	21,410	81.6	470	1.8	98.2
	B	14,450	28.8	32,780	65.4	1520	3.0	97.2
26	A	3450	13.2	20,760	79.1	440	1.7	94.0
	B	12,300	24.6	31,960	63.8	1260	2.5	90.9

^{a)} A=26.2 mg/kg, B=50.1 mg/kg.

^{b)} As % of actual dose on seeds.

analyzed by TLC on silica gel HF₂₅₄: aluminium oxide (42: 18) using isooctane (2,2,4-trimethylpentane): dioxane: *N,N*-dimethylformamide (98: 1: 1, developing system 1)¹¹⁾ and silica gel G using benzene: ethyl acetate (7: 3, system 2). GLC analyses were performed on a Varian 1400 apparatus equipped with an electron-capture detector (e.c.d.) and the 6 feet $\times$ 1/8 inch stainless steel column packed with 3% OV-101 was maintained at 190°C with a 30 ml/min carrier (N_2) flow. The injection part and detector were kept at 220°C and 250°C, respectively.

RESULTS AND DISCUSSION

Table 1 shows the residues of ^{14}C -lindane in treated *Faba* beans stored for different periods of time. Recoveries of radioactivity were satisfactory, being generally over 89% of the applied dose. The decrease in the balance of radioactivity may be attributed, at least partially, to the volatility of γ -hexachlorocyclohexane.

The obtained data indicate that lindane penetrates into the seed coat fairly rapidly. Thus, about over 55% of dose A (Table 1) could be detected inside the grains 4 weeks after application. The amount of residues inside the beans was found to increase with the lapse of time over the first 22 weeks. It was observed that the penetration of the toxicant does not parallel the dose applied (Table 1).

The results also show that only 1.6–3.2% of the applied dose was in a non-extractable form which

could only be detected and determined by combusting the extracted beans followed by LSC. The observed percentages of binding of γ -hexachlorocyclohexane residues are relatively small in comparison with organophosphorus insecticides such as tetrachlorvinphos, in case of which 20–30% of the total dose binds firmly to *Faba* beans.¹⁰⁾ In this connection, it is worthy to mention that the binding capacity of ^{14}C -lindane to other matrixes was shown to be negligible. Mirna *et al.*¹¹⁾ indicated that only around 3% of [^{14}C] γ -hexachlorocyclohexane binds to the meat of treated rabbits.

Following the fractionation procedure mentioned, all ^{14}C -activity present in the internal extract could be removed in chloroform-1 extract. The water and chloroform-2 extract, on the other hand, proved to contain negligible radioactivity, so that metabolites are present mainly in a free form.

The results obtained from TLC and GLC analyses show that lindane was not degraded, to an appreciable extent, in stored beans. Over 98% of the radioactivity was recovered as unchanged insecticide 26 weeks after treatment. At least four substances could be detected, which contributed to about 2% of the extractable residues. The *R_f*-values and relative percentages of these transformation products are shown in Table 2. 2,4,5-Trichlorophenol and dichlorophenols could be differentiated by repeated elution in system 1 using authentic samples as

Table 2 *Rf*-values of γ -hexachlorocyclohexane and some of its degradation products in stored *Faba* beans.

Compound	<i>Rf</i> -value in TLC		% ^{c)}
	System 1 ^{a)}	System 2 ^{b)}	
Lindane	0.1	0.93	98.2
Pentachlorobenzene	0.73	0.86	1.0
2,4,5-Trichlorophenol	0.01	0.90	0.4
Mixture of dichlorophenols	0.04-0.06	0.87-0.89	0.2

^{a)} Isooctane : dioxane : *N,N*-dimethylformamide (98 : 1 : 1), adsorbent silica gel HF₂₅₄ : aluminium oxide (42 : 18).

^{b)} Benzene : ethyl acetate (7 : 3), adsorbent silica gel G.

^{c)} Related to metabolites in chloroform-1.

references. Spots were then located by Gibb's reagent¹²⁾ which immediately gives a blue colour with a dichlorophenol mixture whereas trichlorophenol slowly gives a blue colour. For identification purposes, each spot was separately analyzed by GLC after methylation. The dichlorophenol fraction gave a definite peak, corresponding to the mixture of the methyl ethers of 2,4- and 2,5-dichlorophenols (*Rt* for each was 3.6 and 4 min). Trichloroanisole was identical with an authentic methyl ether of 2,4,5-trichlorophenol (*Rt*=8.8 min). These data are in accordance with those previously reported for similar columns.^{13,14)}

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要 約

γ -Hexachlorocyclohexane の貯蔵ソラマメ中での挙動

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[¹⁴C] γ -Hexachlorocyclohexane の乳濁液を、貯蔵状態のソラマメに噴霧し、26 週間にわたってその浸透経過を調べたところ、4 週間で全薬量の 37~55% が、また 22 週間で 65~82% が浸透した。95% メタノールで抽出されない部分は全薬量の 1.6~3.2% であった。抽出物中に見いだされる基質以外の ¹⁴C 量は 2% 以下であり、ペンタクロロベンゼン、2,4,5-トリクロロフェノールならびにジクロロフェノール類を含んでいた。