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# Interconversion of Stereoisomers of Endosulfan on Chickpea Crop under Field Conditions

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**Abstract:** The pure individual stereoisomers of endosulfan, alpha-endosulfan and beta-endosulfan, were applied as emulsifiable concentrates to chickpea crop at the rate of 175 g a.i. $ha^{-1}$ and 50 g a.i. $ha^{-1}$, respectively. The dissipation rate of these isomers revealed that alpha-endosulfan interconverted to beta-endosulfan in minor quantities, while it was converted into endosulfan sulfate on chickpea leaves in significant amounts. On application, beta-endosulfan was converted to endosulfan sulfate and alpha-endosulfan in relatively smaller amounts. The study indicates that the beta stereoisomer of endosulfan is more persistent because it is resistant to interconversion and metabolic change.

## 1 INTRODUCTION

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a hexahydro -6,9-methano-2,4,3-benzodioxathiepin 3-oxide) is one of the few remaining organochlorine insecticides currently being used worldwide.[1,2] It controls a relatively large number of pests which infest a wide spectrum of field crops. Since the stereoisomers, alpha- and beta-endosulfan, differ in their toxicity towards pests as well as towards mammals ($LD_{50}$ housefly 5·5 mg $kg^{-1}$ for alpha-endosulfan, 9·0 mg $kg^{-1}$ for beta-endosulfan; rat (oral), 76 mg $kg^{-1}$ for alpha-endosulfan, 240 mg $kg^{-1}$ for beta-endosulfan) the relative rate of dissipation of these isomers, their interconversion on a field crop and the rate of formation of the toxic metabolite, endosulfan sulfate, are important in the context of its satisfactory use.

Most of the work[2,3] on endosulfan has been carried out using commercial endosulfan, which is a mixture of alpha and beta stereoisomers. The behaviour of the individual stereoisomers has not yet been studied under field conditions in detail, except in tobacco plants.[4] The phototransformation of alpha-endosulfan, when coated with triethylamine on the leaf of a plant, to beta-endosulfan has been reported.[5] Another study on the photolysis of endosulfan showed that beta-endosulfan degraded faster than alpha-endosulfan in hexane solution.[6]

This paper deals with the characterisation of the metabolites, estimation and interconversion of stereoisomers of endosulfan after applying emulsifiable concentrates of the individual isomers separately to chickpea (*Cicer arietinum* L.) crop. This report is a continuation of our earlier work[2] on endosulfan.

## 2 MATERIALS AND METHODS

### 2.1 Chemicals

All the chemicals used were of analytical reagent (AR) quality. All the solvents were glass distilled before use. Hexane used had a boiling range of 60–80°C. Acetone was refluxed over potassium permanganate for one hour and distilled before use. Charcoal (Darco G-60) was used for cleanup. Thin-layer chromatography (TLC) was performed using silica gel GF-254. Iodine vapours were used as the visualising agent in TLC.

### 2.2 Chromatography

Endosulfan (technical grade) was obtained from Excel Industries Limited, Bombay, India. Analytical samples of pure alpha-endosulfan and beta-endosulfan were obtained by column chromatography of technical endosulfan over silica gel (60–80 mesh). The column was eluted with hexane followed by benzene + hexane (5 + 95 by volume). The compounds eluted were recrystallised from hexane. Purities of the samples were established



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by determining their mp (alpha-endosulfan 108°C, beta-endosulfan 208°C), TLC (hexane + benzene, 1 + 1 by volume; alpha-isomer $R_F$ = 0·57, beta-isomer $R_F$ = 0·32) and by GLC.

### 2.3 Preparation of endosulfan sulfate

Endosulfan sulfate was prepared by oxidising alpha-endosulfan (1 g, 2·45 mmol) with an excess of potassium permanganate (0·35 g, 22·15 mmol) in the presence of glacial acetic acid (15 ml). The reaction mixture was stirred at room temperature for 5 h and then worked up. The product was separated by column chromatography over silica gel, the compound eluted from the column with benzene + hexane (2 + 9, by volume) and then recrystallised from benzene; m.p. 181°C.

### 2.4 Preparation of emulsifiable concentrates of pure isomers

Emulsifiable concentrates of the pure alpha-endosulfan and beta-endosulfan isomers were prepared by dissolving 210 mg of alpha-endosulfan or 60 mg of beta-endosulfan in cyclohexane (15 ml) containing 'Tween 80' (0·5 ml); this provided sufficient amount for a single plot. The formulation was mixed with 600 ml water and applied by high volume spray (500 litre $ha^{-1}$) using a hand sprayer, which was thoroughly washed after applying each isomer.

### 2.5 Field layout

Chickpea (CV Pusa 203) was raised at the farm of Indian Agricultural Research Institute, New Delhi during the Winter Season of 1990. The plot size was 4 m × 3 m. Three such plots were used for each isomer. The stereoisomers, alpha- and beta-endosulfan, were applied separately at the pod formation stage as described in Section 2.4. The application rate of alpha-endosulfan was 175 g a.i. $ha^{-1}$ and that of beta-endosulfan was 50 g a.i. $ha^{-1}$. A control experiment (without pesticide) was also conducted under similar condition where only 'Tween 80' and water were sprayed.

### 2.6 Sampling

A representative sample of chickpea leaves was collected randomly from each of the three plots for each isomer at different time intervals.

### 2.7 Extraction and cleanup

Two-hundred-gram leaf samples were chopped and sub-samples (25 g) were homogenised with propan-2-ol + hexane (50 ml; 1 + 3 by volume). The residual matter after filtration was again blended with propan-2-ol + hexane (2 × 50 ml; 1 + 3 by volume) in a Waring laboratory blender for 2 min and the contents filtered. The hexane layer was separated and stored. To the propan-2-ol portion, saline water (20 g $litre^{-1}$, 100 ml) was added and the pesticide was partitioned into hexane (3 × 30 ml). Cleanup was effected by charcoal following the procedure of Gopal and Mukherjee.[2]

The harvest-time grains (25 g) and pod covers (10 g) were extracted separately in a Soxhlet for 6 h with hexane + acetone (250 ml, 1 + 1 by volume). The yellow solvent mixture was reduced in volume by rotary evaporation. The pesticide was transferred to hexane (3 × 30 ml) after addition of saline water (20 g $litre^{-1}$). An appropriate amount of acetone was added to the hexane to make up the relative volume of the two solvents to acetone + hexane, 1 + 9 by volume. The solution was decolorised by allowing it to stand for 5 min after swirling with charcoal (Darco G-60, 0·25 g). The colourless solution thus obtained was subjected to gas-liquid chromatography.

### 2.8 Weather

The maximum and minimum temperatures during the experiment were 24·8°C and 10·7°C, respectively. The average sunshine hours were 6·4 per day and the relative humidity was 78·3 %. There was 8·3 mm total rainfall during this period.

### 2.9 Recovery

The average percent recoveries of alpha-endosulfan, beta-endosulfan and endosulfan sulfate from each sample of separately fortified chickpea leaves were 93, 92 and 85, respectively, at 0·5 and 0·1 $\mu g\ g^{-1}$ levels.

### 2.10 Analytical method/gas chromatographic analysis

The samples were analysed for the residues of alpha- and beta-endosulfan and the metabolite endosulfan sulfate using a Varian 3400 GLC fitted with 63 Ni electron capture detector. The column (glass, 2 m × 2 mm i.d.) was packed with 1·5 % OV-17 + 1·95 % OV-210 on chromosorb WHP (80–100 mesh). The nitrogen flow rate was maintained at 30 ml $min^{-1}$. The column temperature was set at 210°C, injector port at 250°C and detector at 275°C. The retention times of alpha- and beta-endosulfan and endosulfan sulfate were 3·12, 5·69 and 7·53 min, respectively. Endosulfan ether and endosulfan lactone eluted at 1·98 and 4·50 min, respectively.

To check the identities of the compounds and eliminate any error resulting from co-extractives or artifacts, the analysis was carried out on a GLC fitted with a column of different polarity, i.e. a megabore column (HP 1, US Patent No. 4 293 415 methyl silicone gum) of 10 m × 0·53 mm i.d. and 2·65 $\mu$m film thickness. The temperatures maintained were column 210°C, injector port 250°C and detector 300°C. The carrier gas flow was set at 20 ml $min^{-1}$. The retention times of endosulfan

ether, alpha-endosulfan, endosulfan lactone, beta-endosulfan and endosulfan sulfate were 0·80, 1·91, 2·0, 2·41 and 3·20 min, respectively.

Identities of the isomers were also confirmed by TLC (solvent system: hexane + benzene, 1 + 1 by volume, $R_F$ values as given in Section 2.2), and by the micro-alkali derivatisation method.[7]

The analysis was carried on 1·5 % OV-17 + 1·95 % OV-210 for the present experiment.

## 3 RESULTS AND DISCUSSION

The formulations of pure alpha-endosulfan and beta-endosulfan were prepared by weighing appropriate amounts of each isomer, so that they were present in the same ratio as found in the commercial formulation i.e. 78 % alpha-endosulfan and 22 % beta-endosulfan.

The average extractable residue of alpha-endosulfan was 12·56 $\mu g\, g^{-1}$ 1 h after application of alpha-endosulfan to chickpea leaves (Table 1). By day 4, 63 % of the applied alpha isomer had dissipated. The appearance of beta isomer and endosulfan sulfate on the fourth day after treatment indicated the possibility of interconversion of alpha- to beta-endosulfan and its oxidation to endosulfan sulfate on the field crop (Table 1). The percentage ratios of alpha, beta and endosulfan sulfate were 88·0, 3·4 and 8·5, respectively, showing that alpha-endosulfan was converted to its stereoisomer, beta-endosulfan in about 1 % and to endosulfan sulfate in about 4 %. This is in contrast to field trials with alpha-HCH and gamma-HCH carried out on the same crop, where no such interconversion of stereoisomers was observed.[8]

Dividing the total residues on day 4 by its residues recorded on day 0, it can be shown that, in the day 4 sample, the loss of alpha-endosulfan due to interconversion or oxidation accounts for only 42·0 % of the alpha-endosulfan applied initially on day 0. The rest, 58·0 % remains unaccounted for. The non-toxic ether and lactone were formed (*vide* GLC). These were, however, not quantified. It is documented in the FAO/WHO[9] bulletin that only the parent pesticide, namely, alpha-endosulfan + beta-endosulfan and the toxic metabolite endosulfan sulfate need to be estimated for pesticide persistence experiments. The other degradative/metabolic products, endosulfan diol, endosulfan lactone, endosulfan ether and the hydroxy ether of endosulfan need not be determined for residue studies, as they have relatively lower mammalian toxicity and are also safe towards beneficial insects.

A major part of endosulfan lactone, ether and hydroxy ether might have been converted to endosulfan diol which could not have been directly estimated by GLC without derivatisation,[10] due to its relatively low response towards the electron capture detector in gas-liquid chromatography.

By day 7 after application, while alpha-endosulfan was 16·6 % of the initial sample, the total endosulfan, namely alpha-endosulfan, beta-endosulfan and endosulfan sulfate, was 36·0 % of the alpha isomer applied. After day 7, endosulfan sulfate decreased gradually and was not

**TABLE 1**
Residues of Stereoisomers of Endosulfan on Chickpea Leaves

| *Sampling (day)* | *Residue[ab] (mg g$^{-1}$)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Application of alpha-endosulfan* | | | | *Application of beta-endosulfan* | | | |
| | *alpha* | *beta* | *endosulfan sulfate* | *Total* | *alpha* | *beta* | *endosulfan sulfate* | *Total* |
| 0 | 12·56 | — | — | 12·56 | — | 2·40 | — | 2·40 |
| 4 | 4·64 | 0·18 | 0·45 | 5·27 | 0·08 | 1·52 | — | 1·60 |
| | (63·0) | | | (58·0) | | (36·7) | | (33·3) |
| 7 | 2·08 | 0·28 | 2·16 | 4·52 | 0·19 | 1·16 | 0·99 | 2·34 |
| | (83·4) | | | (64·0) | | (51·7) | | (2·5) |
| 9 | 1·84 | 0·13 | 0·08 | 2·05 | 0·07 | 0·81 | 0·58 | 1·46 |
| | (85·4) | | | (83·7) | | (66·3) | | (39·1) |
| 15 | 0·19 | 0·016 | 0·12 | 0·33 | — | 0·61 | 0·21 | 0·82 |
| | (98·4) | | | (97·3) | | (74·6) | | (65·8) |
| Harvest | | | | | | | | |
| Pod Covers | — | — | 0.28 | | — | 0·03 | 0·035 | |
| Grain | — | — | — | | — | — | — | — |

[a] Average of three replicates.
[b] % dissipation is given in parenthesis.
[c] —: Not detectable (<0·01 mg g$^{-1}$).

detected ($<0{\cdot}01$ $\mu g\ g^{-1}$) in harvest grains. The endosulfan sulfate was, however, present in pod covers. Alpha-endosulfan dissipated with time, with 98·4 % lost in 15 days and was not found in harvest grains or pod covers. Beta-endosulfan level reached its maximum in day 7 samples, but it constituted only 6·19 % of the total endosulfan recorded for that day.

On applying an EC formulation of beta-endosulfan, the parent compound dissipated relatively slowly (half-life 8 days), as also reported in our earlier communications[2,3] when a commercial formulation of endosulfan was applied on different crops.

The parent compound (beta-endosulfan) remaining on the crop in day 15 samples was only 25·4 % of the initial residues recorded on day 0. The rest can be accounted for by loss due to (i) weathering (ii) conversion to either alpha-endosulfan or to endosulfan sulfate or to non-toxic endosulfan lactone and endosulfan ether and (iii) unextractable or non-detectable residues including endosulfan diol. By day 4, beta-endosulfan had dissipated by only 36·7 % as compared to 63·0 % in the case of alpha-endosulfan. The relative residues of beta-endosulfan and alpha-endosulfan in the day 4 sample were 95 and 5 %, respectively. Unlike the case of alpha-endosulfan, no endosulfan sulfate was formed during this period. The sulfate was first detected in day 7 samples. The proportions of endosulfan sulfate, beta-and alpha-endosulfan changed to 42·3, 49·5 and 8·1 % respectively, in day 7 samples. Similarly, the proportion of beta-endosulfan further changed to 55·5 % at the cost of formation of endosulfan sulfate and other unrecorded metabolites in day 9 samples. Alpha-endosulfan still constituted 4·8 % of the total residues recorded on day 9, showing a slower conversion from beta-endosulfan to alpha-endosulfan. Alpha-endosulfan and endosulfan sulfate were formed on application of beta-endosulfan but in lower amounts and endosulfan sulfate slowly decreased with time. Applying an emulsifiable concentrate formulation of pure endosulfan sulfate in a field study[11] showed its rate of dissipation to be very slow, when applied at the rate of 1000 g $a^{1}$ $ha^{-1}$ on pigeonpea leaves. Although endosulfan sulfate was present in the same amount as beta-endosulfan in harvest pod covers, no alpha-isomer was detected in harvest pod cover or grain samples. Harvest grains contained no residues of the parent compound, beta-endosulfan, its isomer alpha-endosulfan or its metabolite, endosulfan sulfate.

This study illustrates that alpha-endosulfan is converted more readily to the sulfate in significant amounts, whereas interconversion to beta-endosulfan is slower. Beta-endosulfan was found to be resistant to both interconversion and oxidation to the sulfate on the chickpea crop, as expected from the thermodynamically more stable stereoisomer.

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