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## HCH ISOMERS AND HCB RESIDUES IN ROOT VEGETABLES AFTER THE APPLICATION OF LINDANE ( $\Gamma$ -HCH) TO THE SOIL

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### ABSTRACT

According to accepted agricultural practice, residue levels of lindane ( $\Gamma$ -HCH), other HCH isomers and HCB were determined in carrots and sugar beets grown under field conditions, where lindane had been applied to the soil as a sanitation agent. Samples of leaves and roots showed lindane and  $\alpha$ -HCH residues. Significant concentrations were observed in the carrot, especially in the peelings of the roots. High levels of lindane and  $\alpha$ -HCH residues were also observed in the seeds developed in growing plants, as well as  $\beta$ -HCH and  $\delta$ -HCH, which were not detected in growing plants, indicating specific accumulation of HCHs in the seeds.

### RESUMEN

Se determinaron los niveles de residuos de lindano ( $\Gamma$ -HCH) y de otros isómeros HCH y HCB en la zanahoria y en la remolacha azucarera cultivadas en el campo, de acuerdo con las prácticas agrícolas, donde el lindano fue aplicado al suelo como agente sanitario. En las muestras de las partes verdes y de las raíces de las plantas se determinaron lindano y  $\alpha$ -HCH. Las concentraciones mayores de los compuestos investigados se observaron en la zanahoria, especialmente en las cáscaras de las raíces. Los niveles significantes de lindano e isómeros  $\alpha$ -,  $\beta$ -,  $\delta$ -HCH se observaron en las semillas cosechadas. En las semillas sembradas se encontraron niveles menores, lo que indicó la acumulación específica de los isómeros HCH en las semillas cosechadas.

### INTRODUCTION

Lindane, the gamma isomer of hexachlorocyclohexane (HCH), has been used for decades as a foliar spray to control numerous insects in the protection of field crops, in soil applications and as a seed treatment (Ulmann 1973, Wegorek 1983, Worthing and Hance 1991). It is effective against a wide range of soil dwelling, phytophagous insects, other pests and some animal ectoparasites.

HCHs, like other organochlorine pesticides, can be absorbed by plants through sequential processes, beginning with surface retention, penetration from dead parts of the cell walls, active absorption by the plasmalemma in the protoplasts, and finally, transportation within the parenchyma to the conducting systems (Führ 1992, Scheunert 1992, Schlosserová 1992).

The absorption of pesticides follows two major pathways, through the roots and through the foliage. In certain cases, the axis and the base of roots, rhizomes and hypocotyl are the favored absorption points. Pesticide absorption

is also possible through the bark of trees and shrubs. Similarly, pesticides are sometimes absorbed through fruit peels and/or through seed coatings, as in the case of seed-dressing. All pathway processes may be followed by further transportation within the plants (Beall and Nash 1971, Trapp *et al.* 1990, 1991).

During the root uptake, lindane and its metabolites dissolved in water, can penetrate intercellular space and the cell walls of the root cortex. They are then taken up through the plasmalemma membranes of the cell of the root cortex and of the endodermis, partly by active processes and partly by diffusion (Nash and Beall 1970).

Foliar absorption of lindane and its metabolites can occur as a absorption from air or as vapor. Most of the pesticide remains fixed in the lipophilic cuticle, and only a small part of it penetrates the cuticle by diffusion (Cooper and Hall 1993). Lipophilic pesticides like HCH's, can penetrate the cellulose walls only through certain lipophilic interspaces. To penetrate the plasma membranes, HCH's need lipophilic pores in order to enter and activate further

transport forms, making accumulation processes possible (Barak *et al.* 1983, Chessells *et al.* 1988, Singh *et al.* 1991, Tanabe *et al.* 1991). The absorption of lipophilic pesticides by stomata is important primarily for pesticide vapors since they are able to penetrate the hydrophobic inner cuticle and are easily distributed among the cells (Peterson *et al.* 1990, Schroll and Scheunert 1992b, Bacci *et al.* 1992).

Temperature, humidity and light have been shown to be important environmental factors positively affecting the amount of absorption of foliar —adsorbed pesticides and their translocation from leaves to roots (Singh *et al.* 1989, 1992). Uptake from soil by roots is also positively affected by temperature and humidity, and total absorption from soil and translocation to leaves has been shown to be increased by light and temperature (Tanabe *et al.* 1991). These are the same conditions that increase the volatilization of lindane and HCH's from the soil (Peterson *et al.* 1990, Waliszewski 1993).

The aim of this project was to determine lindane ( $\Gamma$ -HCH), other HCH isomers and HCB residues distributed in root vegetables, specifically in the roots and leaves of carrot and sugar beets grown in contaminated soils under agricultural field conditions.

## MATERIALS AND METHODS

The experiments were performed in the Winnogóra Field Station of the Institute of Plant Protection in Poznan, Poland. In this project, fields of 50 m<sup>2</sup> were selected. Each field was sprayed with 350 grams of "Owadziak pylisty 2.4" (Zakłady Chemiczne Organika-Azot, Jaworzno, Poland) in aqueous suspension and then mixed with the soil using a tiller. The preparation was mixed thoroughly and repeatedly to a depth of 20 cm, the detailed dates on the soil conditions are presented in the previous paper (Waliszewski 1993). During the vegetation period there were no pesticides used and the weeds were combated manually. The contents of lindane and other HCH isomers in the applied preparations are presented in **table I**.

**TABLE I.** LINDANE, HCH ISOMERS AND HCB CONTENT (g/kg) IN THE PREPARATION Owadziak Pylisty 2.4 USED IN EXPERIMENTS

Compounds	First Application	Second Application
Lindane	20.0206	20.9624
$\alpha$ -HCH	0.0510	0.0509
$\beta$ -HCH	ND	ND
$\delta$ -HCH	0.0156	0.0186
$\epsilon$ -HCH	ND	ND
HCB	ND	ND

ND = not detected

For this research, lindane was applied twice. In spring of the first year, four weeks after application, the fields were sown with carrots and sugar beets. Another application of lindane was made after the autumn harvest. During the spring the roots were stored in a cellar at a temperature of +4°C  $\pm$  2°C and in the following spring were planted in the contaminated fields and left to seed.

## Sampling

Approximately four liters of soil each time, were collected with an Engler stick with a 5 cm id. to a depth of 10 cm using the cover method (Cochran 1963). In the field, the sample was mixed, and a part of the homogenous sample (500-700 grams) was put into a glass jar and taken to the laboratory. It was then dried for 2-3 days under laboratory environmental conditions and sifted through a 0.2 mm<sup>2</sup> sieve to obtain a homogenous dried sample. The sample was stored in a glass jar at a temperature of -20°C until the time of analysis.

For the analysis, the first samples of the soil were taken immediately before the lindane application and then one hour after the application. More soil samples were taken during the entire vegetation period, as reflected in **tables II and III**. After a lindane application in the autumn, subsequent samples of the soil were taken at the beginning of spring of the following year after the fields had thawed (Waliszewski 1993).

The samples of plants grown in the experimental fields were taken when their size made separation into green parts and roots their sampling possible. During the sampling about 30-40 carrots and 10-15 sugar beets were taken. They were subsequently taken in the periods shown in **tables IV, VI, VII and VIII**.

In the field the plant samples were packed in filter paper and transported to the laboratory. Subsequently, they were washed under a stream of tap water and dried with filter paper to remove all soil particles. They were then divided into leaves and roots, homogenized with a Hobart homogenizer and stored in glass jars in the freezer at -20°C until the analysis.

## Determination of HCH isomers and HCB

Solvents such as acetonitrile and petroleum ether (boiling point 40-50°C) used for analysis were fractionally distilled. Each time were gas chromatographically tested for the presence of interfering compounds. Acetic acid and sulfuric acid were of analytical grade. Anhydrous sodium sulfate and Celite 545 also of analytical grade, were heated overnight at 650°C.

In order to prevent contamination by undesirable compounds such as phthalate esters, all analytical equipment was made of glass. The glassware was washed with concentrated KOH solution and concentrated sulfuric acid, rinsed with distilled water and then with distilled acetone and petroleum ether.

TABLE II. FIRST YEAR. LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN SOIL AFTER SPRING APPLICATION

Compounds	Application		Weeks after the application					
	Before	1 hour after	3	8	10	13	17	21
Lindane	0.0019	0.6013	0.6042	0.3697	0.3344	0.2978	0.2686	0.1738
$\alpha$ -HCH	0.0003	0.0017	0.0020	0.0011	0.0011	0.0010	0.0010	0.0010
$\beta$ -HCH	ND	ND	0.0008	0.0005	0.0005	0.0008	0.0007	0.005
$\delta$ -HCH	0.0002	0.0012	0.0009	0.0006	0.0006	0.0005	0.0003	0.0004
$\epsilon$ -HCH	ND	ND	ND	ND	ND	ND	ND	ND
HCB	0.0004	0.0003	0.0002	0.0002	0.0002	0.0002	ND	0.0002

ND = not detected

Qualitative and quantitative determinations were performed with a gas chromatograph (Varian Model 2100) equipped with a  $^{63}\text{Ni}$  electron capture detector. The separation of HCH isomers was performed in a 360 cm x 2 mm glass column packed with 1.5% OV-17 + 1.95% QF-1 on Gas Chrom Q 80-100 mesh at a temperature of 185°C. Confirmation of identity of HCH isomers from selected samples was made using a Hewlett Packard Model 5992B gas chromatography / mass spectrometry. Each sample was analysed in duplicate and the results represent the arithmetic mean of the duplicate analyses.

The extraction procedure and cleanup of soil samples have been described in detail (Waliszewski and Szymczyński 1985, Waliszewski 1993). Plant samples were analyzed as follows:

Hundred grams of a previously ground sample were weighed into a wide-necked flask. Then 150 ml of acetonitrile were added, and the sample was left to macerate for 30 min. The sample was then homogenized with an

Ultra-turrax homogenizer, and the supernatant was vacuum filtered through a layer of Celite 545. The extraction was repeated one more time with 100 ml of acetonitrile and again vacuum filtered through the Celite 545 layer. The combined extracts were transferred to a 1000 ml separatory funnel and 500 ml of 5% sodium sulfate solution were added. The HCH residues were extracted with three 100 ml portions of petroleum ether, and the extracts were dried by filtering through a sodium sulfate layer. The combined extracts were then rotary evaporated to a few milliliters and quantitatively transferred to a 10 ml calibrated tube. The volume was adjusted with petroleum ether to 10.0 ml. One milliliter of concentrated sulfuric acid was added and the mixture vigorously shaken for half a minute and left for 2-3 minutes to obtain good phase separation. The petroleum ether layer was dried by passing it through the sodium sulfate layer into the other calibrated tube. The dried extract was then ready for GLC residue determination. The recovery study conduct-

TABLE III. SECOND YEAR. LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN SOIL AFTER AUTUMN APPLICATION

Compounds	Application		Weeks after the application					
	Before	1 hour after	24	28	32	34	44	46
Lindane	0.0018	0.6693	0.6683	0.3531	0.1548	0.1442	0.0788	0.0219
$\alpha$ -HCH	0.0002	0.0022	0.0026	0.0014	0.0007	0.0009	0.0006	0.0003
$\beta$ -HCH	ND	ND	ND	0.0013	0.0009	0.0011	0.0010	0.0009
$\delta$ -HCH	ND	0.0008	0.0010	0.0005	0.0006	0.0004	0.0005	0.0004
$\epsilon$ -HCH	ND	ND	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	0.0002	ND	ND	ND

ND = not detected

**TABLE IV.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE CARROT LEAVES AND ROOTS AFTER SOWING

Compounds	10 Weeks		13 Weeks		17 Weeks	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Lindane	0.1483	0.2482	0.0981	0.1500	0.0929	0.0986
$\alpha$ -HCH	0.0039	0.0017	0.0044	0.0014	0.0050	0.0011
$\beta$ -HCH	ND	ND	ND	ND	ND	ND
$\delta$ -HCH	ND	ND	ND	ND	ND	ND
$\epsilon$ -HCH	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND	ND

ND = not detected

ed with 10 replications for three fortification levels (0.1, 0.01 and 0.001 mg/kg) resulted in an excellent recovery of over 95% and a standard deviation and coefficient of variation below 10, which indicates good reproducibility and precision of method. The detection limits are established as 0.0001 mg/kg.

### RESULTS AND DISCUSSION

A two-year study was conducted on the selected 50 m<sup>2</sup> fields on which the lindane preparation, Owadziak pylisty 2.4 (Zakłady Chemiczne Organika-Azot, Jaworzno, Poland) was applied at the recommended dosage of 1.7 kg/ha by the Institute of Plant Protection, Poznan, Poland, considering a normal agricultural practice (Wegorek 1983). Lindane was applied in spring of the first year and after the first autumn harvest. The results obtained from the residue analyses of lindane, other HCH isomers and HCB

in the soil of the fields studied are presented in **tables II and III** (Waliszewski 1993). Four weeks after the spring application, taking into account the phytotoxicity period of lindane, the carrot and sugar beet seeds were sown. The first sugar beet samples were taken eight weeks later, and the carrot samples ten weeks later, according to the rate of plant growth. All plants were left to grow until the 17th week, and then the carrot and sugar beet roots were harvested and stored in a cellar during the winter. The weight of the harvested carrot roots varied about 30 grams, while that of the sugar beet varied about 750 grams  $\pm$  30%. During the spring the roots were stored in a cellar at a temperature of +4°C  $\pm$  2°C. After the spring visual changes in the root size were not observed, thus they were not weighed. In the spring of the following year, the stored roots were planted in the contaminated fields where the lindane had been applied the previous autumn. These plants were cultivated for the seeds from which the amount of lindane absorption and accumulation was determined.

**TABLE V.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE ELEMENTS OF CARROT ROOTS HARVESTED AT 17 WEEKS AFTER SOWING

Compounds	Whole roots	Peelings	Peeled roots
Lindane	0.0986	0.6290	0.0278
$\alpha$ -HCH	0.0011	0.0038	0.0002
$\beta$ -HCH	ND	ND	ND
$\delta$ -HCH	ND	ND	ND
$\epsilon$ -HCH	ND	ND	ND
HCB	ND	0.0001	ND

ND = not detected

**TABLE VI.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE CARROT LEAVES AND ROOTS PLANTED IN THE SECOND YEAR AND HARVESTED AFTER 22 WEEKS

Compounds	Carrot leaves	Carrot roots
Lindane	0.0708	0.2289
$\alpha$ -HCH	0.0062	0.0021
$\beta$ -HCH	ND	ND
$\delta$ -HCH	ND	ND
$\epsilon$ -HCH	ND	ND
HCB	ND	ND

ND = not detected

The residue levels of lindane, other HCH isomers and HCB found in the carrot, presented in **table IV**, revealed only the presence of lindane and  $\alpha$ -HCH, trace levels of HCB and the absence of the  $\beta$ -,  $\delta$ - and  $\epsilon$ -HCH isomers. Since climatic conditions were favorable for the volatilization of lindane and other HCH isomers from the soil, their vapor could be absorbed and incorporated into growing leaves. In time the residue levels decreased, with values below the 0.1 mg/kg, the limit value of accepted tolerance for vegetables.

At the last sampling, reflected in **table IV**, the lindane concentration in the carrot roots, was almost the same as that in the leaves and the  $\alpha$ -HCH was only detected from HCH isomers. There was 4.5 time less concentration in the roots. During the study period, the contamination of roots diminished with growth. By harvest time, it reached levels below the accepted tolerance of 0.1 mg/kg. The highest

concentration of pesticides in roots was always found at the beginning of the plant growth, and then it decreased. The reason for this is that the availability of pesticides decreases while the volume of the roots increases. This result in higher concentrations in young roots and in lower concentrations in mature ones, a fact that can be observed in **table IV** for carrots and **table VII** for sugar beets. If the plant growth is stopped and the roots still remain in the soil, an increase of residues can be observed again. The dilution of residues caused by plant growth stops, and a diffusion of pesticides into the roots takes place. These effects can be observed in **table VI**: higher residues were found in carrots of the second year when roots growth was stopped after they were planted. To estimate the gradient of lindane and HCH isomer concentrations in the carrot roots, presented in **table V**, the part of the root to be consumed (the peeled root) was separated from the discarded peeling. A higher concentration of lindane, 6.4 times higher, was observed in the peelings compared to the concentration in whole roots, and 3.5 times less in peeled roots compared to whole roots. It is well known in pesticide research that carrot roots have the greatest capacity among plants for absorbing the lipophilic pesticides from the soil (Schroll and Scheunert 1992a). Lindane and  $\alpha$ -HCH detected in the carrot roots studied showed notable accumulation in the peelings. It has been found that major concentrations of lipophilic compounds coming from organochlorine pesticides accumulate in the peelings. The whole roots showed less contamination with lindane due to the biological dilution of the sample. The lindane concentration determined in the peelings was 22.6 times greater than that found in the peeled roots used for consumption.

In the second year, the stored carrot roots were planted in the field to which lindane had been applied in autumn of the previous year. Twenty-two weeks after the roots were planted, the grown plants were harvested and separated into roots, leaves and seeds. The residue con-

**TABLE VII.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE SUGAR BEET LEAVES AND ROOTS AFTER SOOWING

Compounds	8 WEEKS		10 WEEKS		13 WEEKS		17 WEEKS	
	LEAVES	ROOTS	LEAVES	ROOTS	LEAVES	ROOTS	LEAVES	ROOTS
Lindane	0.0199	0.0633	0.0143	0.0119	0.0097	0.0036	0.0073	0.0037
$\alpha$ -HCH	0.0012	0.0022	0.0007	0.0014	0.006	0.0007	0.0008	ND
$\beta$ -HCH	ND	ND	ND	ND	ND	ND	ND	ND
$\delta$ -HCH	ND	ND	ND	ND	ND	ND	ND	ND
$\epsilon$ -HCH	ND	ND	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected

**TABLE VIII.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE SUGAR BEET LEAVES AND ROOTS, PLANTED IN THE SECOND YEAR AND HARVESTED AT THE 22TH WEEK

Compounds	Sugar beet leaves	Sugar beet roots
Lindane	0.0016	0.0008
$\alpha$ -HCH	0.0007	0.0001
$\beta$ -HCH	ND	ND
$\delta$ -HCH	ND	ND
$\epsilon$ -HCH	ND	ND
HCB	ND	ND

ND = not detected

tents found in the carrot roots and leaves are presented in **table VI**, and results of the seeds are in **table IX**. These results reveal a higher contamination of the carrot roots when compared to those of the previous year. This fact can be explained by the dilution of the residues caused by growth in the first year and the permanent diffusion of the pesticides into the roots where growth rate is low during the second year. The similarity of lindane concentration levels found in the carrot leaves for the two years can be explained by the new growth of the leaves in the second year, where the roots showed only a slight increase in size.

The contamination of sugar beet presented in **table VII** shows lindane and residue levels of  $\alpha$ -HCH. The quantity of lindane in the leaves decreased from 0.0199 to 0.0073 mg/kg between the 8th week and the moment

of harvest 17 weeks later. The  $\alpha$ -HCH however, maintained a low level almost without change. In the sugar beet roots, greater absorption of lindane and  $\alpha$ -HCH was observed in the younger roots than in the leaves. Subsequently concentration decreased rapidly by biological dilution as the roots grew.

In the second year, the sugar beet roots stored during the winter were planted in the contaminated field and left to grow for 22 weeks, the time necessary to produce seeds. At that moment, the harvested sugar beet plants were divided into leaves, roots and seeds. As indicated in **table VIII**, residue levels of lindane and  $\alpha$ -HCH, which were only detected in the roots, were lower than the levels observed the previous year. This phenomenon can be explained by increase in the size of the sugar beet roots. Accumulated compounds were transported from the roots to the leaves, making them grow at a greater rate than the previous year, which caused biological dilution of the pesticides absorbed.

The carrot seeds obtained from the plants studied, as indicated in **table IX**, showed a significant quantity of lindane and  $\alpha$ -,  $\beta$ - and  $\delta$ -HCH as well as the absence of  $\epsilon$ -HCH and HCB. By quantifying the total HCH levels in the seeds harvested to the sow, three times more HCH could be observed in these seeds. Moreover, significant levels of  $\beta$ - and  $\delta$ -HCH were observed in the seeds harvested. These levels could not be observed either in the leaf samples or in the root. Accumulation of HCH totals in sugar beet seeds, as presented in **table IX**, can also be observed with about 1.6 times higher value in the seeds harvested.

From this research it can be concluded that while using lindane in soil sanitation, in accordance with a good agricultural practice, it is likely to find lindane and  $\alpha$ -HCH residues in the growing plants. The carrot roots, as well as the sugar beet roots, absorb lindane and available  $\alpha$ -HCH from the polluted soil. These compounds are accumulated at higher concentrations in the peelings of the roots. This reflects the results obtained by

**TABLE IX.** LINDANE, HCH ISOMERS AND HCB RESIDUES (mg/kg) IN THE SEEDS SOWED AND HARVESTED FROM THE PLANT STUDIED

Compounds	Carrot		Sugar beet	
	Sowed	Harvested	Sowed	Harvested
Lindane	0.034	0.089	0.006	0.006
$\alpha$ -HCH	0.006	0.016	0.001	0.002
$\beta$ -HCH	0.002	0.025	0.001	0.004
$\delta$ -HCH	0.004	0.012	0.001	0.003
$\epsilon$ -HCH	ND	ND	ND	ND
HCB	ND	ND	ND	ND

ND = not detected

Schroll and Scheunert (1992a). The carrot and sugar beet leaves also contained lindane and  $\alpha$ -HCH, which originated from volatilized pesticides from the soil and this is concordant with the observations of Schroll and Scheunert (1992b). Since the loss of lindane and other HCH isomers from the soil depends mainly on its volatilization, the vapors were probably absorbed by the growing leaves. The carrot leaves as compared to the sugar beet leaves, grow near the surface of the soil and have greater lipophilicity which permits major absorption of pesticides as reflected in the residue analyses.

The residue analyses also reflected ubiquity of  $\alpha$ -HCH whose origin can only be explained by the bioisomerization of lindane to  $\alpha$ -HCH (Steinwandter 1976), but the rate of this process is not significant, as the quantities found in the leaves and roots always remained at low residue levels, near the detection limits.

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