

Long-Term Studies of Fungicide Concentrations in Greenhouses. 3. Exposure Risks after Spraying in Greenhouses

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The study presented concerns the dermal exposure risks during harvest work in greenhouses after fungicide application. The contamination of the fungicide vinclozolin on clothes, overalls and gloves, worn during harvest after application was investigated. The fungicide was extracted from the cloth with 1-chlorobutane and finally determined with HPLC. The average amount of fungicide found on the clothes, the potential exposure, was 25 mg after 8 h of work in the greenhouse. It was estimated that most of the fungicide amount was found on gloves. Assuming a maximum uptake of vinclozolin (on bare skin) of 10%, the value of the dermal exposure was estimated to be 2.5 mg/day. In the absence of threshold values for dermal, exposure a comparison was made with recommended values for acceptable daily intake of foodstuffs. This comparison reveals that the assumed dermal exposure is higher than the value set by the U.S. Environmental Protection Agency but lower than the value given by the World Health Organization. Thus, the use of protective garments during harvesting, especially on the hands, is recommended.

Keywords: *Exposure; health risks; fungicides; vinclozolin; cucumber; greenhouse*

INTRODUCTION

In previous greenhouse studies it has been found that air concentrations of vinclozolin and triadimefon declined rapidly after fungicide application (Nilsson et al., 1996). However, the amount of residues on surfaces in greenhouses and climate chambers was found to be substantial a long time after application. The amount of vinclozolin on cucumber leaves 7 days after application was found to be 50% of the primary value (Nilsson et al., 1996). In a similar investigation, concerning benomyl and carbendazim with an original deposit of 0.95–1.2 μg of benomyl/cm² leaf area, half-lives of benomyl and carbendazim were found to be 44 and 53 h, respectively (Liesivuori et al., 1988).

Greenhouse work in cucumber cultivations normally starts 3 days after application of pesticides. Sometimes an earlier re-entry is necessary the day after application. Under these conditions health risks due to air exposure are negligible, but the risks of dermal exposure certainly need to be taken into consideration.

The main objective of this paper was to estimate the exposure risk by determining the amounts of fungicide on clothing. This study is as far as we know the first one concerning dermal exposure to vinclozolin in greenhouses.

EXPERIMENTAL PROCEDURES

Equipment. A high-performance liquid chromatographic system (HPLC) was used for the final analysis, including a pump and a variable-wavelength detector operated at 240 nm (LKB 2150 and LKB 2151, LKB, Bromma, Sweden), an integrator (HP3388, Hewlett-Packard, Avondale, PA), and a

column (Spherisorb S10W 10 μm , 20 cm length, 4.6 mm i.d., Hichrom, Reading, U.K.), operated at a flow rate of 0.5 mL/min. The injection volume was 20 μL .

Chemicals. Vinclozolin [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolinedione, CAS Registry No. 50471-44-8 and IUPAC name (*RS*)-3-[3,5-(dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolinedione-2,4-dione], was obtained from BASF, Solna, Sweden. The fungicide formulation used was Ronilan FL (BASF, Limburgerhof, Germany) consisting of 500 g of vinclozolin/L. The LD₅₀ value for Ronilan FL, oral and dermal, is 4 mg/kg of body weight and for inhalation the LD₅₀ is 3 mg/L for 7 h of exposure (Fransson, 1990). Vinclozolin is sensitizing and weakly skin irritating (Bergkvist et al., 1991). 1-Chlorobutane AR (LabScan, Dublin, Ireland) of HPLC grade was used for extraction and as mobile phase in HPLC.

Sampling Procedure for the Estimation of Dermal Exposure. Studies concerning exposure to Ronilan FL in cucumber cultivation were performed during 1993 and 1994. In the first study harvesters A and B were equipped with cotton overalls and cotton gloves. On the basis of information obtained in these first experiments, the second investigation was restricted to a study of the exposure on arms and hands. In these experiments harvesters A–C wore cotton gloves, two on each hand, and sleeves attached on their shirt by safety pins. The cotton material was chosen since its texture permits a good trapping efficiency.

Dermal exposure was measured during harvesting, 3 days after spraying. Further information concerning experimental conditions is given in Table 1.

After sampling, the overalls and gloves used were turned inside out, put in plastic bags, and stored cold at 8 °C until analysis was performed, normally within a week.

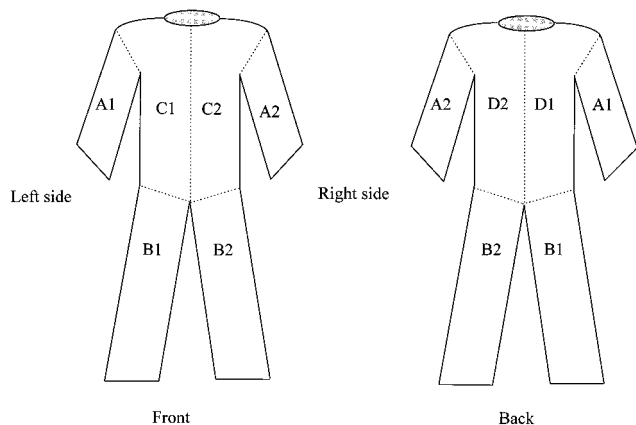
Analysis Procedure. The overalls were cut in pieces as shown in Figure 1. The pieces were placed in glass jars, and 1-chlorobutane (500–700 mL), enough to cover the cloth, was added and the sample ultrasonicated for 2 h. Then the 1-chlorobutane solution was decanted and evaporated to 10 mL before final analysis with HPLC as previously described (Papantoni et al., 1995). The total volume of 1-chlorobutane

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Table 1. Spraying Events and Determination of Vinclozolin on Clothes [Three Persons Participated in the Study (A–C)]

expt date	sprayer	expt (worker)	dosage of vinclozolin applied (ratio) ^a (mg/m ²)
May 1993	low volume ^b	1(A)	0.18 (1.32)
May 1993	high volume ^b	2(B)	0.14 (1.5)
April 1994	high volume ^c	3(B)	0.14 (1.0)
April 1994	high volume ^c	4(C)	0.14 (1.0)
April 1994	high volume ^c	5(B)	0.14 (1.0)
April 1994	high volume ^c	6(C)	0.14 (1.0)
April 1994	low volume ^c	7(A)	0.145 (1.04)
April 1994	low volume ^c	8(B)	0.145 (1.04)

^a The ratio is calculated as the quotient between the actual dosage and the lowest dosage. ^b The workers wore overalls and single gloves. ^c The workers wore sleeves on their arms and two pairs of gloves.

**Figure 1.** Scheme of how the overalls were cut. (A) represents the sleeves, (B) the legs, (C) the trunk and (D) the back.

and the volume of the decanted 1-chlorobutane solution were measured, allowing the entire amount of fungicide on the cloth pieces to be calculated. The concentration of vinclozolin in the unrecovered portion of the 1-chlorobutane is assumed to be the same as that in the recovered portion. The same procedure was repeated for gloves (left, right, inner, and outer) and sleeves using extraction volumes of 100 and 500 mL respectively.

The extraction efficiency was evaluated by applying Ronilan FL emulsion (containing 180 mg of vinclozolin) on five cotton cloth pieces (ca. 1260 cm² surface area). After drying, the cloth pieces were put in glass jars, 200 mL of 1-chlorobutane was added, and extraction proceeded as described above.

RESULTS AND DISCUSSION

A recovery of 85% with an RSD value of 7% was obtained in the determination of the extraction recovery described under Analysis Procedure. The amounts of vinclozolin found were corrected for extraction losses with the recovery value obtained. The detection limit concerning vinclozolin for the analytical method (20 μ L injection volume) is 0.3 mg/mL, calculated as 3 times the noise (Papantoni et al., 1995), which gives a detection limit for the dosimetry matrix of 0.2 μ g per body part sampled.

Results concerning the amounts found on the inner and outer gloves in experiments 3–8 are listed in Table 2. Evaluation with the paired *t*-test at the 95% confidence level revealed significantly lower amounts of vinclozolin on inner gloves compared to outer gloves. The average percentage reaching the inner gloves was 30% (SD = 12%).

Results of the amounts of fungicide trapped on cotton clothes in experiments 1–8 are presented in Table 3.

Table 2. Amount of Vinclozolin, Corrected Regarding Extraction Losses (15%), That Penetrated into Gloves

expt	vinclozolin on left glove (μ g)			vinclozolin on right glove (μ g)		
	inner	outer	penetration percentage ^a	inner	outer	penetration percentage ^a
3	370	951	28	289	623	32
4	282	726	28	260	1452	15
5	105	1603	6	287	574	33
6	272	607	31	133	487	22
7	46	56	45	48	60	44
8	99	164	38	76	98	44
av ^b			29			32

^a Glove penetration = inner/(inner + outer) \times 100. ^b The grand average for left and right inner glove is 30%.

The values in Table 3 regarding gloves in experiments 3–8 are obtained by adding the amounts of vinclozolin collected on both the inner and the outer glove.

When the results of exposure on different parts of the body were compared, the largest amounts of vinclozolin were found on hands and arms (Table 3).

The amount of fungicide that might penetrate the skin after passing through the cloth was not considered, resulting in an underestimation. The underestimation depends on the body part considered and can be estimated from the absorption values obtained by Maibach et al. (1971), who performed an investigation on malathion and parathion applied with acetone on human skin. On the forearm the absorption was found to be 10%, and on the palm of the hand it was 11.8% of the applied dosage of parathion. After malathion was applied, the absorption was 6.8% on the forearm and 5.8% on the palm of the hand. In double cotton layer experiments, with an average amount on the inner glove of 30% (SD = 12%), the underestimation of vinclozolin on the forearm should be ca. 3% using Maibach's absorption value. However, since the calculations for percentage on inner gloves are relatively uncertain, the values in Table 3 are given without any correction for the amount of fungicide that might have passed to the skin.

Average values on cotton garment corresponding to an exposure period of 8 h are presented in Table 4. There are great differences in exposure between occasions and between workers. This may depend on differences in working methods, but when glove dosimetry is considered, the duration of the sampling period also seems to be important. In longer sampling periods, experiments 1, 2, 7, and 8, exposure values per hour are remarkably lower than in shorter sampling periods. The explanation may be that when the pores in the gloves are filled with particles, new particles are prevented from entering. This, together with loss of pesticide due to penetration of the gloves to the skin, points at the importance in glove dosimetry of changing the gloves frequently. Preferably it should be done at least each hour to prevent underestimation of the potential dermal exposure. The average value of exposure on gloves in experiments 3–6 is 3.6 times higher (880 μ g/h) than the average value on all gloves in experiments 1–8 (250 μ g/h). Regarding the estimation of dermal exposure on hands, the values obtained in experiments 3–6, which are of short duration, are considered most reliable and were thus chosen for calculation of the total exposure. This approach gave for an 8 h working day a total average exposure of 22.9 mg of vinclozolin on clothes, estimated from values on gloves in experiments 3–6 and values on the other parts of the body in experiments 1–8.

Table 3. Amounts of Vinclozolin Found, Corrected Regarding Extraction Losses (15%), on Gloves and Overalls in Experiments 1 and 2 and on Gloves and Sleeves in Experiments 3–8 in Greenhouse 1

body part sampled	amount of vinclozolin ($\mu\text{g/h}$)							
	expt 1 ^a	expt 2 ^a	expt 3 ^a	expt 4 ^a	expt 5 ^a	expt 6 ^a	expt 7 ^a	expt 8 ^a
trunk, right front	46	33						
trunk, left front	50	44						
back, right	44	47						
back, left	41	48						
leg, right	49	73						
leg, left	34	341						
right sleeve	106	44	85	57	171	— ^c	111	— ^c
left sleeve	58	34	— ^c	94	106	— ^c	151	— ^c
hand, right	107 ^b	165 ^b	912	1712	861	620	108	174
hand, left	377 ^b	259 ^b	1321	1008	1708	879	102	263

^a Sampling times were 270 min in experiment 1, 180 min in experiment 2, 55 min in experiment 3, 65 min in experiment 4, 60 min in experiment 5, 45 min in experiment 6, 330 min in experiment 7, and 120 min in experiment 8, respectively. ^b The hand exposure is here underestimated since the workers in these experiments only wore a single glove on each hand, in the other experiments, 3–8, the workers wore two gloves on each hand. ^c These samples were lost.

Table 4. Calculated Average Exposure Values for 8 h on Gloves and Overalls Worn by Harvesters in a Vinclozolin-Treated Greenhouse (Calculated from Results in Experiments 1–8 in Table 2)

part sampled	amount of vinclozolin (SD) ^a (μg)	no. of observations
right sleeve	719 (362)	6
left sleeve	709 (362)	5
trunk, right front	318 (71)	2
trunk, left front	378 (37)	2
back, right	368 (16)	2
back, left	362 (26)	2
leg, right	487 (138)	2
leg, left	1504 (1736)	2
hand, right	8210 (3797)	4
hand, left	9832 (2955)	4
sum	22887 (2449)	31

^a The values within parentheses represent the standard deviation (SD).

CONCLUDING DISCUSSION

Since most of the exposure during harvesting was found on gloves and sleeves, an absorption value of 10% of the total calculated exposure amount has been used in this study. This approximation has also been used by Kolmodin et al. (1995) when estimating the risks regarding handling of permethrin-treated plants by forestry workers. In their investigation the exposure was also mainly on hands and the concentrations of the pesticide were similar to the concentrations in our study.

The amount found in the inner gloves in experiments 3–8 was on average 30% of the detected amount. For the other parts of the body a single cotton layer, made of the same material as the glove material, was used. An addition of 30% to the amount obtained for other parts of the body was made to correct for the underestimation. This gives an exposure value of 2.5 mg after correction, assuming 10% absorption.

Absorption on cotton fiber might be larger compared to bare skin since the cloth is more porous resulting in a larger surface. In the estimation above we have not considered the risk for overestimation depending on larger surface.

On the other hand, the amount of fungicide which might have penetrated the cloth into the skin during the sampling period leading to an underestimation has not been taken into account.

An alternative could instead have been to use a hand wash procedure in the determination of the amount accessible for dermal exposure. However, this is questionable from health considerations, and this procedure

will give an underestimation if the amount penetrating the skin is taken into account.

In the absence of threshold values of dermal exposure a comparison was made with recommended values for acceptable daily intake of foodstuffs. Regarding vinclozolin this is 0.07 mg/kg of body weight according to the WHO (1991) and 0.025 mg/kg of body weight according to the EPA. Using body weight as the basis of calculation, 60 kg corresponds to 4.2 mg using WHO's limit during 1 day of exposure. According to the EPA, 54 kg corresponds to 1.35 mg for women and 70 kg corresponds to 1.75 mg for men during one day of exposure.

The estimated exposure value of 2.5 mg lies between the limits of the EPA and the WHO.

Leaf sampling was also performed in connection to experiments 1, 2, 7, and 8 (Nilsson et al., 1996). Previous measurements showed that after 3 days ca. 80% of the fungicide is left on the leaves, after 1 week about 50% remains, and after 4 weeks the concentration is close to 0. Dermal exposure is dependent on the amount of fungicide on leaves and is expected to follow the decline curves of the fungicide.

When considering the health risks of vinclozolin it should be regarded that in Sweden 4 h of harvest work in fungicide-treated cucumber cultivations is more common than 8 h. Harvest of cucumber in greenhouses is performed from the third day after application and thereafter every second day for at least 2 weeks. The workers are exposed to a decreasing amount of fungicide during this period until the next spraying event. Furthermore, a normal working week is 5 days, and the acceptable daily intake values are based on daily exposure. Taking these facts into consideration, the health risks after vinclozolin application are estimated to be small during normal harvesting on a yearly basis. The risks can be further reduced if the workers wear efficient protective clothes, especially gloves.

ACKNOWLEDGMENT

We thank all members of the Department of Agricultural Engineering who participated in the experimental work, especially Miss Susanne Ljungberg for working with the final analysis.

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Received for review May 30, 1995. Accepted June 12, 1996.® This study was mainly financed by the Swedish Working Environment Fund, which is gratefully acknowledged.

JF950325C

® Abstract published in *Advance ACS Abstracts*, August 1, 1996.