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Journal of Chromatography B, 778 (2002) 131–145

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Evaluation of respiratory and cutaneous doses of chlorothalonil during re-entry in greenhouses

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Abstract

Five female workers were monitored for 5 consecutive days during re-entry into a greenhouse containing ornamental plants. Skin contamination (excluding hands) was evaluated with nine pads of filter paper placed on the skin. Hand contamination was assessed by washing with 95% ethanol. Respiratory exposure was evaluated by personal air sampling. The respiratory dose was based on a lung ventilation of 15 l/min. The doses absorbed were estimated assuming 10% skin absorption and 100% lung retention. Dislodgeable foliar residue was determined on days of re-entry to evaluate the decay of chlorothalonil. Chlorothalonil was analysed in the different matrices by GC–MS. Respiratory exposure was less than skin contamination, being 11.4±5.1% (mean±SD) of total exposure. The estimated total absorbed dose did not exceed the acceptable daily intake of 0.03 mg/kg body mass. The hands and unexposed skin of all workers were always found to be contaminated. Greater precautions are therefore needed to reduce skin exposure (clean gloves and suitable clean clothing every day).

Keywords: Respiratory exposure; Cutaneous exposure; Chlorothalonil

1. Introduction

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a contact fungicide used to control fungi that threaten various plants. It has moderate acute toxicity: the oral lethal dose, LD50, is more than 10 000 mg/kg body mass (b.w) in rats and 6000 mg/kg b.w. in mice; acute dermal LD50 is 10 000 mg/kg b.w. in albino rabbits and albino rats [1,2]. An acceptable daily intake (ADI) of 0.03 mg/kg b.w. was established [3], but no occupational exposure limits (OELs) for chlorothalonil (CHL) have been published. Skin contact with chlorothalonil may result in dermatitis or slight sensitivity: 14 out of 20 workers exposed to 0.5% chlorothalonil in a wood preservative developed dermatitis, all showed swelling and inflammation of the upper eyelids [4]. Allergic skin responses have also been noted in farm workers [5]. Different studies on animals indicate that chlorothalonil does not have reproductive, teratogenic and mutagenic effects [2,5,6]. Its carcinogenic potential is unclear: male and female rats fed chlorothalonil daily over a lifetime developed malignant and benign kidney tumors at the higher doses [5]. In another study in which mice were fed high daily doses of

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1570-0232/02/$ – see front matter
PII: S0378-4347(01)00541-2

chlorothalonil for 2 years, females developed tumours in the fore-stomach area and males developed malignant and benign kidney tumors [5]. Chronic studies on rats and dogs fed high dietary levels show that chlorothalonil is toxic to the kidney [7]. The IARC classifies chlorothalonil in Group 2B (possibly carcinogenic to humans) [8].

In mammals, CHL is metabolized via glutathione conjugation and enzymatic cleavage to cysteine–glycine, cysteine, *N*-acetylcysteine conjugates [9], and thiolated compounds, such as thiol and methylthio metabolites [10]. Mono-, di- and trithiol substituted derivatives (2,5,6-trichloro-4-thioisophthalonitrile, 2,5-dichloro-4,6-dithioisophthalonitrile and 5-chloro-2,4,6-trithioisophthalonitrile) have been found in animals [11]. Rats excrete about 6% of oral doses as metabolites in urine and dogs only excrete about 1% [12]. In experiments with orally dosed rats, a major part of CHL was recovered unchanged in the faeces, and nothing is known about the percentage of non-metabolized CHL excreted in urine of mammals [9]. The complex and varied pharmacokinetics of CHL makes biological monitoring of exposure difficult. The use of animal metabolic data may lead to failure to detect metabolites in human samples [13]. This result would not indicate low or no internal dose but simply that the wrong metabolite was monitored.

No studies on biological monitoring of occupational exposure to CHL have been published but there have been various reports in which respiratory and cutaneous exposure to this compound has been evaluated [14–17]. Procedures for sampling airborne CHL envisage use of glass fiber filters [14], glass fibre filters followed by an XAD-4 adsorbent tube [15], mixed cellulose ester filter [16–18], or polyurethane foam [19]. Skin exposure has been evaluated using long-sleeved cotton gloves [16,17], hands rinse and cotton gloves [18], and gauze pads, hands washing and long-sleeve cotton knit undershirts [15]. Dislodgeable chlorothalonil residues on leaves or fruits have also been determined [15,17,18]. Assay procedures for CHL in various matrices have usually been based on high-performance liquid chromatography (HPLC) analysis with UV detection [14,16–18]; gas chromatographic (GC) techniques with electron-capture detection (ECD) have been used in a few cases [15,19], and gas chromatography–mass spectrometry (GC–MS) is only used for verification [19].

The general aim of the present study was to evaluate exposure and occupational risk during manual operations performed in greenhouses after chlorothalonil treatment of ornamental plants. The specific focus of the research was evaluation of skin and respiratory doses and their contribution to estimated total actual dose. Respiratory exposure was evaluated by means of personal air samples obtained with mixed cellulose ester filters and OVS tubes containing XAD-2. Skin exposure was determined using skin pads and hand washing. Dislodgeable chlorothalonil residues on leaves were determined to check breakdown of the pesticide before re-entry into greenhouses. Since CHL metabolites in humans are not well known, unmodified CHL was assayed in urine of monitored workers. GC–MS was used to analyze chlorothalonil in the different matrices. A secondary aim of the study was to determine the effectiveness of protective clothing for minimizing occupational exposure.

2. Experimental

2.1. Subjects and exposure conditions

Five female workers (workers 1, 2, 3, 4, 5), aged 25 to 60 years (mean 37 ± 15 years) engaged in fixing runners of *Scindapsus* to a mossy support in a 25 500 m^3 greenhouse, were studied for a week. The workers did only one job during the week when monitoring took place: the plants were fixed manually with metal staples to the support to train growth upward. This was mainly carried out in sitting position. The plants had been treated with 750 g Daconil 75 WG (75% chlorothalonil) dispersed in 750 l water (equal to 0.75 g/l active ingredient) 38 h earlier (the spraying was carried out at 18.00 h on Saturday and the greenhouse was re-entered at 08.00 h the following Monday). The pesticide was sprayed manually on the plants using a spray rod attached to the pesticide tank. The operation lasted about 100 min. Every day of the week, water was nebulized into the greenhouse to maintain humidity. On the Friday, since all the plants had been stapled, a number were removed and replaced with other plants

treated in other greenhouses in the same manner and with the same calendar.

The women were healthy and had normal blood indicators of liver and kidney function. None of them was taking pharmaceuticals and alcohol consumption was limited (less than 250 ml wine/day). Three were non smokers; workers 2 and 3 smoked six cigarettes/day.

On the job, the workers wore cotton overalls, work shoes and two pairs of gloves (cotton in contact with the skin and latex on top). The 100% cotton overalls had no hood. They had long sleeves and legs, closed at the wrists and ankles with elastic, and a zip opening down the front. The gloves in contact with the skin were 100% cotton with elastic at the wrists. Under the overalls they wore undergarments, socks and a cotton T-shirt. During exposure, most areas of the body were therefore covered by one layer of cotton, with the following exceptions: the head and neck were fully exposed, the wrists and hands were covered by one layer of cotton and one layer of latex and the shoulders, back and chest which were covered by two layers of cotton.

The overalls were changed once a week, whereas the cotton gloves could be changed at the worker's discretion. The latex gloves were changed when they developed holes. The overalls were generally removed at the end of the work shift. Laundering of the overalls and cotton garments was done by the worker.

2.2. Ambient and biological monitoring

Biological monitoring was performed by assay of urinary chlorothalonil. A spot urine sample was obtained from each worker before the work shift on the Monday; 24-h urine samples were obtained every day of the working week. They were divided into two portions: during (08.00–17.00 h) and after (17.00–08.00 h next day) the work shift. The samples were used to determine diuresis and urinary creatinine. The results were expressed in micrograms of CHL/g creatinine and in total micrograms excreted in 24 h (during and after shift).

Every day of the working week from Monday to Friday, personal air sampling was carried out for the determination of the active ingredient in inhalable airborne particulate and vapour. Aerosol was sampled with portable SKC pumps fitted with mixed cellulose ester filters (37 mm in diameter, SKC), operating at an air flow of 2.9 l/min. Considering an air intake orifice 7 mm in diameter, this gave an air speed of 1.25 m/s, similar to that of breathing [20]. The vapour phase was sampled with portable SKC pumps fitted with amberlite XAD-2 tubes (SKC, OVS) at a flow of 1 l/min as recommended by the manufacturer. Air sampling was carried out throughout the 8-h work shift.

Every day of the working week from Monday to Friday, deposition of chlorothalonil on exposed skin (head and neck) was determined by placing a pad on the face of each worker. To evaluate contamination of unexposed skin, eight pads were placed under clothing in the following positions: anterior and posterior chest (representative of anterior shoulders and chest and posterior shoulders and back, respectively), right arm (representative of arms), left forearm (representative of forearms), left anterior thigh (representative of anterior thighs and hips), right posterior thigh (representative of posterior thighs and hips), left calf (representative of calves) and right shin (representative of shins and feet). The pads were squares of filter paper (49 cm^2 for unexposed skin and 20 cm^2 for the face) attached to the skin with sticking plasters.

Contamination of the skin of the hands was evaluated by washing with 150 ml 95% ethanol. The ethanol was poured slowly over the hands of the worker, who was instructed to rub her hands together. The wash liquid was collected in each worker's personal disposable aluminium tray. The worker then kept the hands and especially the nails in the alcohol for 30 s. The washing procedure was carried out before morning break, before lunch and at the end of the work shift (or whenever the worker wanted to wash her hands). The three hand wash samples were pooled.

Since chlorothalonil is reported to photodecompose in some solvents [21,22], all samples were protected from light with aluminium foil and stored in the freezer at −18°C.

2.3. Determination of dislodgeable foliar residues (DFRs)

To evaluate decay of the active ingredient, leaf

samples were obtained immediately before and after spraying with chlotothalonil and 41, 65, 89, 113 and 137 h after spraying. The samples were obtained with a punch [23], each sample consisting of 18 discs, 1.5 cm in diameter, from different leaves, making a total surface area of 31.8 cm^2, counting only one side of the discs. The sampling sites ($n=18$, nine on each half of the greenhouse) were the points of intersection of an imaginary grid dividing the two halves of the greenhouse into four equal quadrants. Sampling was from the same leaves up to 113 h after spraying. In subsequent sampling (Friday), the replacement of some of the plants made it impossible to use the same leaves, as the plants at the corners of the grid had changed. Sampling was therefore carried out in the same manner on different plants. The leaves chosen were at an intermediate stage of development. Samples were protected from light with aluminium foil and stored in the freezer at −18°C.

2.4. Sample analysis

2.4.1. Reagents and standards

The solvents used to prepare solutions of known titre and for extraction of samples were of the best commercially available analytical grade for pesticide analysis and were supplied by Merck (Bracco, Milan, Italy). Anhydrous sodium sulfate (>97% purity) used to dehydrate the urinary extracts was from Carlo Erba (Milan, Italy). Ultrapure water was obtained with a Millipore Milli-Q system (Milan, Italy). Chlorothalonil (certified assay: 97% purity) and the internal standard (I.S.) lindane (LIN) (certified assay: 99.9% purity), were obtained from Lab Service Analytica (Bologna, Italy). Standard chlorothalonil solution at a concentration of 131.92 μg/ml was prepared by dissolving appropriate quantities of standard in methanol. The solutions (working solutions) used to construct the calibration curves were prepared daily, diluting the standard solution with methanol. Standard lindane solution at a concentration of 1 mg/ml was prepared by dissolving appropriate quantities of I.S. in *n*-hexane. Working solution (2 μg/ml) was prepared when needed, diluting the standard solution with *n*-hexane.

2.4.2. Apparatus

Analysis was performed with a Shimadzu GC-17A chromatograph equipped with a split–splitless injector and an AOC-5000 autoinjector and fitted with a QP-5050A mass-selective detector (quadrupole) (Shimadzu Italia, Milan, Italy). The chromatographic capillary column was a Supelco MDN-12, 30 m× 0.25 mm I.D., film thickness 0.25 μm (Supelco, Milan, Italy). Temperatures were as follows: injector port and transfer line, 280°C; column, 50°C for 1.5 min, raised at 30°C/min to 170°C, isothermal for 0.1 min, raised at 5°C/min to 250°C, then isothermal for 10 min. Ultrapure (99.999% purity) helium carrier was used; the injection pressure was 100 kPa; injection (1–2 μl) was performed by the splitless technique.

MS conditions were as follows: ionization by electron impact (EI, 70 eV); detector 1.20 kV; selected ion monitoring (SIM) mode. Retention times and detected masses of the analytes were as follows: 13.2 min, m/z 181–183–219 for lindane (I.S.) and 15.4 min, m/z 264–266–268 for chlorothalonil.

Evaporation of sample extracts was performed in vacuo with a Strike 102 rotating evaporator (Steroglass, Italy). Vacuum was maintained with a Jet 1-Automatic Vacuum System recycled water pump (Genser Scientific Instruments, Rothenbourg, Germany).

2.4.3. Sample preparation

2.4.3.1. Urine. A 10-ml volume of sample, spiked with 100 μl I.S. working solution, was shaken and then was extracted three times with 5 ml dichloromethane in a mechanical shaker. The pooled extracts were dehydrated with anhydrous sodium sulfate and evaporated to dryness in a rotating vacuum evaporator at 30°C. The residue was made up with 0.5 ml *n*-hexane and injected into the GC–MS system.

2.4.3.2. Mixed cellulose ester filters and pads. The sample, spiked with 100 μl I.S. working solution, was allowed to dry for 60 min and was then extracted three times with 10 ml methanol in a mechanical shaker. The pooled extracts were evaporated to dryness in a rotating vacuum evaporator at 30°C; the residue, made up with 0.1–1 ml *n*-hexane, was injected into the GC–MS system.

2.4.3.3. XAD-2 tubes. The adsorbing phase, spiked with 100 μl I.S. working solution, was allowed to dry for 60 min. The sample was then kept in contact with 5 ml toluene for 30 min, before injection into the GC–MS system.

2.4.3.4. Hand wash liquid. A 10-ml volume of sample, spiked with 100 μl I.S. working solution, was evaporated to dryness in a rotating vacuum evaporator at 30°C. The residue was made up with 1 ml *n*-hexane and injected into the GC–MS system.

2.4.3.5. Leaves. Dislodgeable foliar residues were obtained by washing the sample twice with 25 ml 0.01% dioctyl sodium sulfosuccinate solution, then with 25 ml water. The pooled wash liquid, spiked with 100 μl I.S. working solution, was shaken and then extracted three times with 30 ml dichloromethane. The pooled extracts were dehydrated with anhydrous sodium sulfate and evaporated to dryness in a rotating vacuum evaporator at 30°C. The residue was made up with 1–2 ml *n*-hexane and injected into the GC–MS system.

2.4.4. Calibration curves, precision and recovery

Chlorothalonil was determined in all samples by calibration curves constructed by adding appropriate quantities of the working solutions to blank matrices. A sample of the same matrix not spiked with chlorothalonil (blank) was also prepared. The additions were in the range 1.65–105.56 μg/l for urine; 0.21–13.19 μg/sample for pads and mixed cellulose ester filters; 0.10–3.30 μg/sample for XAD-2 tubes; 0.16–10.56 μg/10 ml for hand wash liquid and 0.82–105.56 μg/sample for leaves. After addition of CHL standard, the spiked matrices (pads, XAD-2 tubes, mixed ester cellulose filters and leaves) were allowed to dry for 60 min. All spiked matrices underwent the same treatment as the samples.

Linear calibration curves (eight points in the range) were obtained by plotting the quotients of the peak area of CHL (m/z 266) and the I.S. (m/z 181) as a function of concentration.

To evaluate precision, 20 spiked samples were prepared for every matrix at three different concentrations: 10 of every concentration were analysed on the day of preparation to evaluate analytical repeatability; the others were stored in the freezer at −18°C to test stability and between-day precision. To evaluate recovery, the results obtained during evaluation of analytical repeatability were compared with those obtained with standard samples of CHL at the same nominal concentration.

2.5. Actual and absorbed doses

Concentrations of the pesticides in the air were used to obtain the actual respiratory dose for a lung ventilation of 15 l/min [24].

Daily skin contamination (except for hands) of each worker was the sum of the values for the different parts of the body, sampled by pads. Contamination of each part was obtained multiplying the concentration on the pads ($\mu g/cm^2$) by the surface area of the part, expressed as a percentage of total body surface area [25,26]. The total body surface area of each worker was obtained by the formula of Du Bois and Du Bois [27]. The sum of hand contamination, namely the quantity of pesticides in the hand wash liquid, and contamination of other skin areas were taken to be the total daily skin contamination.

To estimate the absorbed dose, we assumed 10% skin penetration [16,28] and 100% lung retention [16,29,30] of the active ingredient.

2.6. Statistical analysis

Kruskal–Wallis one-way analysis of variance (ANOVA) was used to compare the environmental monitoring data determined on the various days of sampling. This non parametric tests were chosen because the number of samples compared was so small that the type of distribution could not be identified. The significance levels were set at $\alpha = 0.05$. The analysis was carried out using the SPSS statistical software package (SPSS, Chicago, IL, USA).

3. Results

3.1. Analytical procedures

The precision, recovery and limits of detection for the various environmental and biological measurements are shown in Table 1. The limit of detection was calculated on the basis of a signal of the ion

Table 1
Detection limits (DLs), precision (RSD) and recovery of environmental and biological monitoring

Sample type	DL[a]	Concentration	No. replicates	Within-series imprecision (%)	Between-day imprecision (%)	Average recovery (%)
XAD-2 tube	0.11	0.41	10	4.8	13.2	98
(μg/sample)		1.65	10	4.1	13.4	97
		3.30	10	3.8	11.6	99
Mixed ester cellulose filters	0.012[b]	0.82	10	8.3	12.1	85
(μg/sample)		3.28	10	5.7	11.5	83
		13.19	10	4.5	11.7	88
Pads (μg/sample)	0.009[b]	0.82	10	6.5	13.6	97
		3.28	10	6.0	13.2	95
		13.19	10	5.9	10.7	93
Hand wash (μg/10 ml)	0.005	0.66	10	3.7	6.5	100
		2.64	10	3.5	5.5	99
		10.56	10	3.7	5.2	100
Leaves (μg/sample)	0.20	2.06	10	7.8	14.7	103
		8.24	10	7.7	13.8	95
		65.92	10	5.6	11.0	96
Urine (μg/l)	0.25	3.30	10	5.8	12.9	98
		13.20	10	5.0	11.1	97
		52.80	10	5.1	10.5	95

[a] The detection limit (DL) was calculated on the basis of a signal three times the background noise for an ion with m/z 266.
[b] Sample made up with 0.1 ml hexane.

with m/z 266 three times the background noise. The chromatograms obtained with three types of matrix (urine, mixed cellulose ester filters and leaves) are shown by way of example in Fig. 1. The mass spectrum of CHL is reported in Fig. 2. The spiked samples were stable at −18°C for at least 30 days.

The influence of matrices on the analytical results was examined. Aqueous standards were prepared in the same manner as the calibration standards in urine; methanol standards were prepared in the same manner as the calibration standard in pads, mixed cellulose ester filters and hand wash liquid; toluene standards were prepared in the same manner as the calibration standard in XAD-2 tubes; 0.01% dioctyl sodium sulfosuccinate–water standards were prepared in the same manner as the calibration standard in leaves. Analysis of these samples was carried out as described for calibration standards in matrices. The coefficients of correlation were greater than 0.998 for all matrices. Comparing the slopes of calibration curves obtained for matrices and aqueous or methanol or toluene or 0.01% dioctyl sodium sulfosuccinate–water standards did not reveal substantial differences. The slope for urine in relation to the slope for water was 105%, and for the other matrices 99% (XAD-2 tubes), 84% (mixed ester cellulose filters), 95% (pads), 99% (hand wash liquid) and 96% (leaves).

3.2. Ambient and biological monitoring

Table 2 shows the concentrations of CHL in personal air samples and pads, together with the quantities of the pesticide in the hand wash liquid of the five workers during the working week and on different days.

With regard to pads, the highest concentration of pesticide was found on the forearm, arm and anterior thigh, with ranges of 0.82–318.78, 0.20–66.53 and 3.88–75.10 μg/cm^2, respectively. Mean values de-

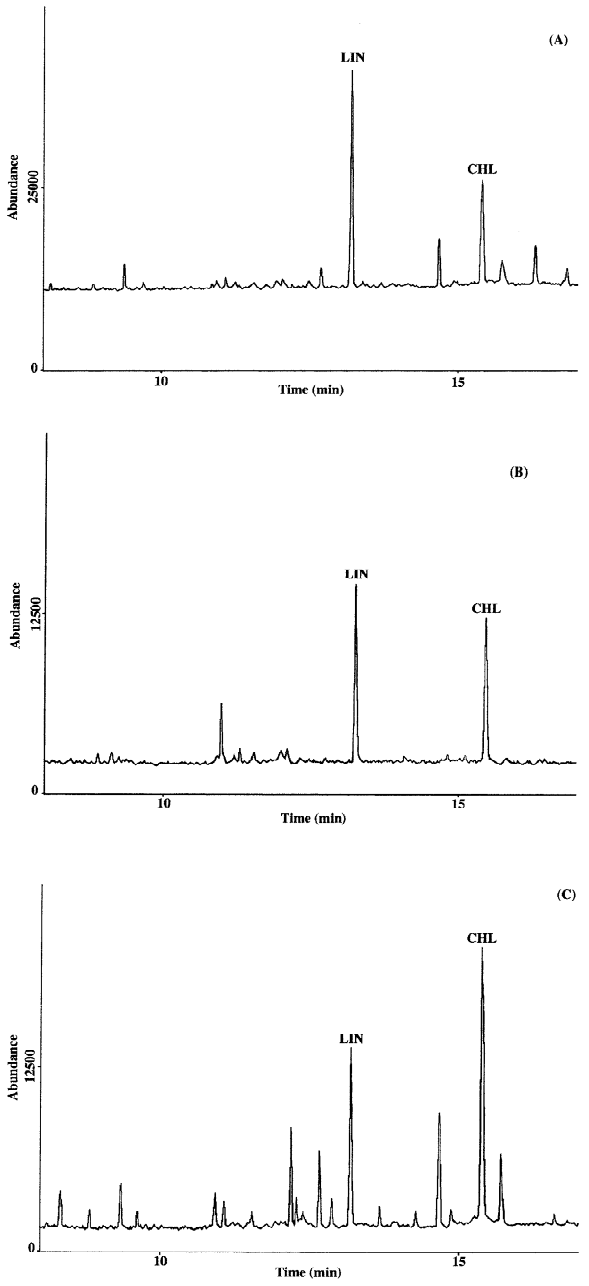

Fig. 1. Selected ion chromatogram of some of the samples analysed (A: urine sample containing 8.30 μg/l CHL; B mixed cellulose ester filter containing 0.82 μg CHL; C dislodgeable foliar residue at a concentration of 0.05 $\mu g/cm^2$). The detected masses were those of chlorothalonil (m/z 266) and I.S. (m/z 181).

tected in these areas (34.85±79.18, 10.64±16.47 and 19.07±16.19 $\mu g/cm^2$, respectively) were even greater than in pads on the face, a part of the body not covered by clothing. The posterior parts of the body (back and posterior thigh with ranges of 0.04–10.82 and 1.22–16.33 $\mu g/cm^2$, respectively) were less contaminated than the corresponding anterior parts (anterior chest 0.61–28.57 $\mu g/cm^2$) and contamination of inferior parts of the body, such as the shin and calf, was never negligible with ranges of 0.20–16.22 and 1.43–15.92 $\mu g/cm^2$, respectively. The chest was the least contaminated part of the body, presumably due to the fact that the workers had other garments under their overalls (e.g., a T-shirt) which reduced skin contamination in this area. Contamination seemed to have preferred parts of the body and the Kruskal–Wallis test (non parametric ANOVA) showed that the position of the pads was a significant variable ($\alpha=0.05$) in determining contamination of skin covered by clothing.

Table 2 also shows the variations in respiratory and cutaneous exposure on the different days of re-entry into the greenhouse. A gradual reduction of exposure was evident during the week for personal air samples and the Kruskal–Wallis one-way ANOVA showed that, on the whole, the concentration of CHL was related to the day of re-entry after spraying. A decreasing trend, at least over the first 3 days of re-entry, was also observed for face pads, whereas an increasing trend was observed for hand wash liquid. A random pattern of contamination was found for pads on skin covered by clothing.

The concentration of CHL in urine samples is shown in Table 3. Only 24% of samples were above the detection limit of CHL. The type of samples in which the compound was detected in each subject is shown in Table 3: there did not seem to be any systematic pattern of excretion during the working week. Concentrations of CHL in 30 urine samples from subjects not occupationally exposed to CHL, tested in our laboratory for occupational toxicology analysis, were undetectable in all cases.

Fig. 3 shows the DFRs of CHL immediately before and after spraying and on the days of re-entry into the greenhouse (the values are derived from analysis of 18 leaf discs sampled at different times from plants in different parts of the greenhouse). In the first 4 days of re-entry, decay showed a linear

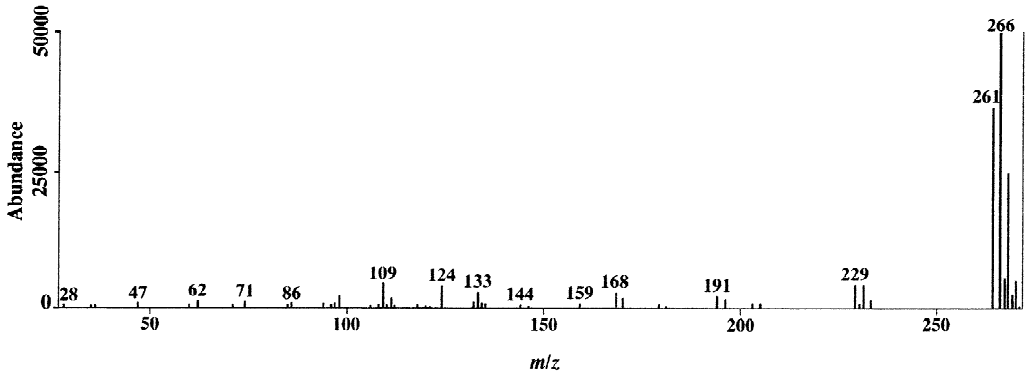

Fig. 2. Mass spectrum of chlorothalonil.

Table 2
Exposure values in five workers during the working week and on different days

Sample type	Mean±SD	Median	Geometric mean	Range
Personal air samples ($\mu g/m^3$)[a]				
All data	5.91±2.08	5.26	5.60	3.14–11.57
Monday	7.12±3.35	5.23	6.52	3.82–11.57
Tuesday	6.82±0.61	6.74	6.80	5.92–7.57
Wednesday	5.86±1.81	5.26	5.66	4.06–8.73
Thursday	5.79±1.77	5.37	5.61	4.36–8.75
Friday	3.95±0.62	4.27	3.91	3.14–4.49
Hand wash (μg)				
All data	166.54±68.53	164.64	148.12	31.64–289.56
Monday	125.81±67.63	137.50	107.14	36.30–208.07
Tuesday	147.09±95.99	146.64	117.20	31.64–289.56
Wednesday	180.23±56.09	145.86	173.65	135.68–252.20
Thursday	162.14±70.85	152.80	150.60	91.41–270.92
Friday	217.44±13.39	220.28	217.10	199.00–231.54
Face pads (ng/cm^2)				
All data	8.67±12.05	4.94	5.45	1.50–60.69
Monday	19.80±24.25	8.88	9.99	1.63–60.69
Tuesday	9.69±6.40	9.31	7.75	3.06–16.69
Wednesday	3.52±1.60	3.88	3.19	1.50–5.63
Thursday	4.54±3.49	2.38	3.60	1.75–9.69
Friday	5.81±2.59	5.75	5.40	3.44–9.94
Pads on unexposed skin (ng/cm^2)				
All data	11.42±30.29	5.71	4.72	0.04–318.78
Monday	21.19±62.95	4.39	4.02	0.04–318.78
Tuesday	8.43±10.25	4.90	4.07	0.18–52.24
Wednesday	10.44±15.63	6.33	5.26	0.20–75.10
Thursday	7.48±7.19	6.94	4.89	0.61–33.47
Friday	9.30±10.49	6.53	5.59	0.61–58.98

Kruskal–Wallis one-way ANOVA was significant for personal air samples and right arm and left forearm pads. Kruskal–Wallis one-way ANOVA was not significant for the other types of samples.

[a] Personal air sample values are the sum of vapour and particulate fractions.

Table 3
Urinary excretion of chlorothalonil by the five workers during the working week

Worker	Day	Sample type	CHL (μg/l)	CHL (μg/g creatinine)	CHL (μg excreted)
1	Thursday	During shift	0.50	0.31	0.10
	Friday	During shift	0.98	0.52	0.19
2	Monday	Basal	8.30	6.34	0.42
	Tuesday	During shift	0.53	0.54	0.15
	Friday	After shift	0.48	0.37	0.30
3	Monday	After shift	1.48	1.74	0.81
	Tuesday	After shift	2.32	3.31	1.62
4	Monday	During shift	0.45	0.37	0.25
	Tuesday	After shift	0.46	0.36	0.20
	Wednesday	During shift	2.00	1.33	0.70
	Wednesday	After shift	0.54	0.26	0.23
5	Monday	During shift	0.51	0.24	0.07
	Monday	After shift	2.01	1.09	0.70

(r=0.999) trend according to an equation of the type:

$$\text{CHL residue } (\mu g/cm^2) = -0.02 \cdot \text{time since treatment (h)} + 3.53$$

On Friday (137 h after treatment) an increase in DFR was found and was probably due to replacement of a number of plants with others treated in different greenhouses.

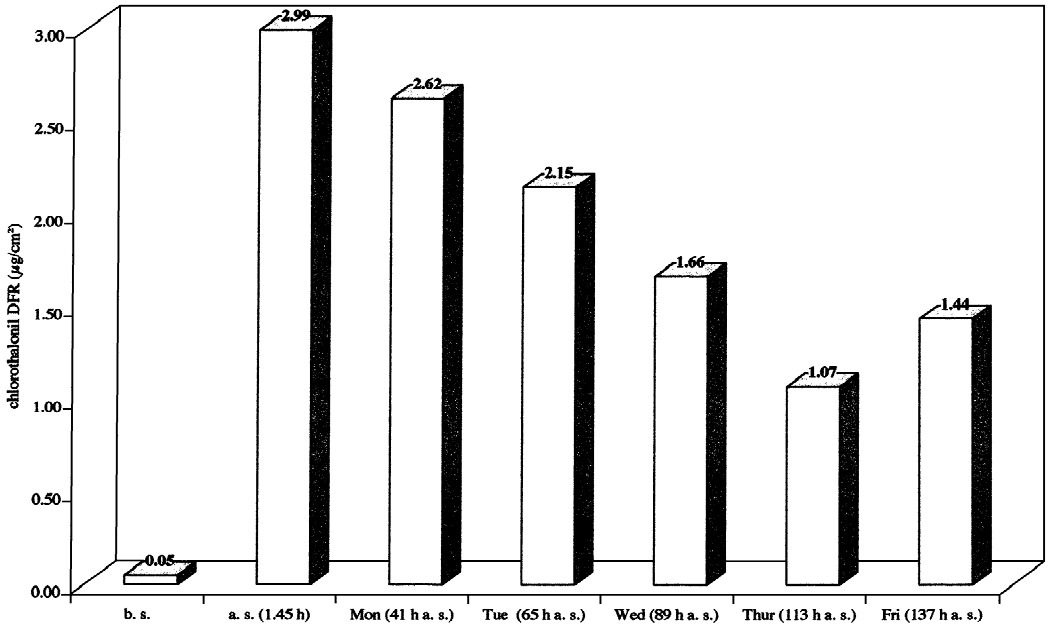

Fig. 3. Dislodgeable foliar residues (DFRs) of chlorothalonil before and after spraying and during the subsequent working days (b.s.=before spraying; a.s.=after spraying).

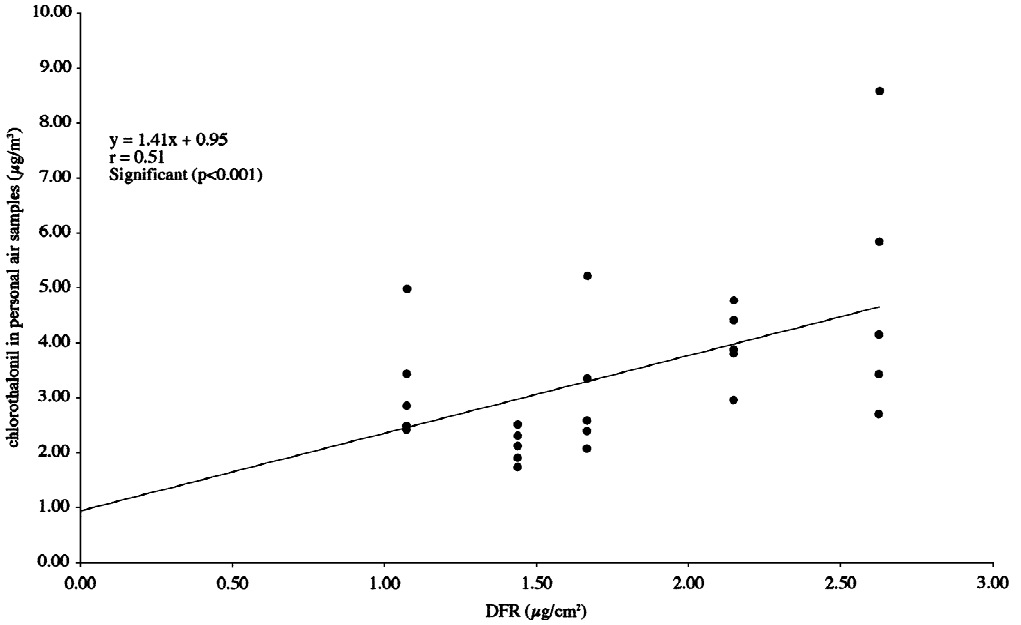

Fig. 4. Linear regression between concentration of chlorothalonil in personal air samples and dislodgeable foliar residues (DFRs).

Table 4
Estimate of actual and absorbed doses of chlorothalonil for workers engaged in manual operations on treated plants during the working week

		Respiratory dose (μg/day)	Skin actual dose (μg/day)	Total actual dose (μg/kg body mass)	Total absorbed dose (μg/kg body mass)
Monday	Mean±SD	40.74±20.45	386.25±295.91	6.67±4.02	1.25±0.58
	Median	34.58	408.28	7.39	1.27
	GM	37.56	297.22	5.47	1.12
	Range	23.72–76.34	121.74–847.41	1.82–11.55	0.45–2.01
Tuesday	Mean±SD	45.96±4.27	278.32±125.52	5.40±2.69	1.21±0.34
	Median	46.41	234.62	4.75	1.24
	GM	45.80	256.89	4.93	1.18
	Range	41.56–50.67	161.39–453.59	2.64–9.90	0.82–1.74
Wednesday	Mean±SD	38.09±11.72	341.93±55.74	6.20±1.40	1.17±0.29
	Median	32.02	352.78	6.60	1.18
	GM	36.80	338.39	6.05	1.14
	Range	27.64–56.53	289.12–423.03	4.01–7.74	0.76–1.53
Thursday	Mean±SD	37.89±11.78	281.39±66.42	5.35±1.93	1.09±0.30
	Median	34.38	274.10	5.19	1.14
	GM	36.63	274.72	5.05	1.05
	Range	29.04–57.72	189.52–357.29	2.80–8.05	0.67–1.38
Friday	Mean±SD	25.23±3.93	346.25±23.32	6.09±1.30	0.98±0.18
	Median	25.33	345.15	6.22	1.05
	GM	24.98	346.62	5.97	0.96
	Range	20.47–29.79	313.97–374.37	4.52–7.64	0.74–1.13

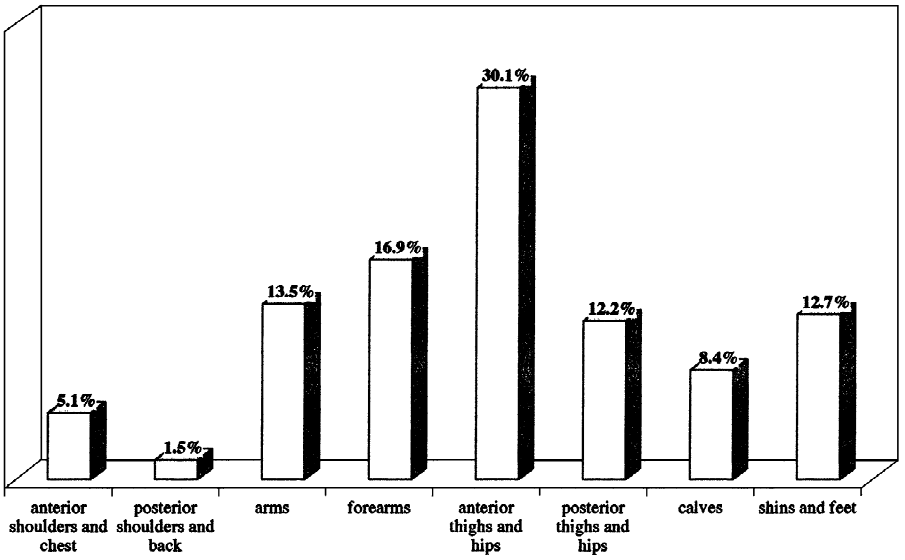

Fig. 5. Contamination of unexposed skin: percentages contributed by various parts of the body.

The DFR was significantly correlated with the concentrations of pesticide in personal air samples. The regression line equations and correlation coefficients are given in Fig. 4. There was no significant correlation with the quantity of pesticide in hand wash liquid and pads.

Estimates of actual and absorbed doses for the five workers were calculated and a summary is shown in Table 4. Fig. 5 shows the percentage contamination of the various parts of the body with respect to the dose found on unexposed skin.

4. Discussion

4.1. Sampling and analytical methods

The sampling procedures for airborne CHL (particulate and vapour) used in this study were validated previously by other authors [18]. Evaluation of skin exposure by means of filter paper pads and hand washing with 95% ethanol has also been reported in previous studies [31–33]. The efficacy of hand washing was not evaluated systematically but a second wash with 150 ml 95% ethanol by the five workers at the end of the Monday work shift, analysed separately, did not reveal detectable concentrations of pesticide in any sample. This shows that washing was almost total, at least for CHL not yet absorbed by the skin of the hands.

The methods used for extraction of samples are fast and give a good recovery of analyte and good precision within and between series. GC–MS was sufficiently specific to not require purification of the extract to remove interfering compounds. Detection limits were sufficiently low to enable detection of analyte in all environmental samples, even under conditions of low exposure, such as those observed in the present study. The sample storage conditions used ensured stability of the active ingredient in all matrices for the 30 days necessary for analysis.

4.2. Ambient and biological monitoring

In general, biological monitoring of exposure to pesticides by assay of the unmodified compound excreted in the urine is more specific for determining occupational absorption than assay of metabolites. This is because a given metabolite can be derived from different compounds, for example alkylphosphates can come from various organophosphorus insecticides [31–33], 3,5,6-trichloro-2-pyridinol from chlorpyrifos and chlorpyrifos-methyl [30,31], 3-phenoxybenzoic acid from various pyrethroids [34],

and so forth. The assay of unmodified compounds excreted in urine can also been falsified by dirty hands and/or contaminated clothing.

In the case of CHL, there have been no previous reports of biological monitoring of occupational exposure and thiol-substituted metabolites (2,5,6-trichloro-4-thioisophthalonitrile, 2,5-dichloro-4,6-dithioisophthalonitrile and 5-chloro-2,4,6-trithioisophthalonitrile) have only been detected in experimental animals [10,11]. In the absence of specific studies, we assayed CHL in urine excreted by workers during the working week. The results did not show a clear pattern and the presence of CHL in basal samples and samples collected during the Monday work shift suggest that contamination occurred during sample collection. No other considerations or comparisons with earlier data or environmental data are yet possible.

Personal respiratory exposure to CHL was monitored for airborne particulate and vapour, as the vapour pressure of 1.3 mPa at 40°C [1] did not exclude the presence of vapours of CHL in the work place. The sum of vapour and particulate samples measured during the working week was similar to those reported in previous studies [14–16]: during application to urban trees and ornamental shrubs, a concentration of 110 $\mu g/m^3$ was found in only 1/14 samples analysed [14], whereas during carnation cutting, the geometric mean was 90 $\mu g/m^3$ after dusting with wettable CHL powder and 10 $\mu g/m^3$ during sorting/bundling [16]. During mechanical tomato harvesting [15] mean concentrations were 5 $\mu g/m^3$ in two picking sites and 20 $\mu g/m^3$ in another particularly dusty work site.

In the present study, skin contamination was evaluated by a modification of the traditional pad technique proposed by Durham and Wolfe in 1961 [35]. Pads placed on various areas of exposed and unexposed skin were taken to be representative of various anatomical areas, on which contamination was assumed to be homogeneous.

A completely random pattern of contamination was observed for pads on unexposed skin. These pads presumably collected pesticide if overalls were not completely closed on the chest, if the sleeves were rolled up, through open parts (neck, ankles and wrists) and through the fabric itself or zips and buttoned openings. Examination of the data and greenhouse procedures indicates that in some cases, such as that of the forearms, high contamination levels observed on the Monday were probably due to rolled up sleeves and in all other cases, contamination was practically constant throughout the week, suggesting that CHL penetrated the fabric of the overalls.

Contamination of exposed skin can be due to deposition of airborne particulate as well as to contact with contaminated hands and clothes or plants. The concentrations of CHL measured in face pads showed a non significant correlation with those in personal air samples, probably because this parameter is determined by both deposition and contact.

The respiratory dose was much less than skin contamination under the present working conditions: it amounted to 11.4±5.1 (5.4–23.5)% [mean±SD (range)] of the total dose. The percentages of total skin dose represented by hands, exposed skin (head and neck) and unexposed skin were 51.9±15.5 (19.6–75.8), 3.1±3.6 (0.5–17.4) and 45.0±15.3 (22.3–42.7)% [mean±SD (range)], respectively. The main contributions to skin dose were contamination of hands and unexposed skin. It is interesting that the part of the body that contributed most to the latter percentage was the anterior thighs and hips (30%) evaluated by a pad on the anterior thigh. The reason for this high contribution was probably contamination by dirty hands and clothes occurring when the overalls are lowered to go to the toilet. The women mainly worked in sitting position (about 70% of work shift) and all of the anterior body was subject to contact with treated plants. Observation of the operations carried out showed that the plant was often taken from its stand and placed on the knees or the worker leaned over the plant, so that the anterior body and exposed skin became contaminated.

The hands were regularly found to be contaminated with CHL. The increasing trend found in hand wash liquid during the working week seems in contrast with the decrease in DFR, and is probably due to a build up of CHL in the cotton gloves which were only replaced at the worker's discretion. The high levels observed were probably due to internal contamination of gloves with pesticide. Observation of the operations carried out showed that latex gloves were often removed and not immediately replaced.

When the cotton gloves were removed, a gloved hand was used to remove the first glove but a naked hand to remove the other. In this way the skin of the hands became contaminated.

The significant correlation between DFR and CHL in personal air samples is interesting because it shows that respiratory exposure is proportional to residues on leaves and the dust containing CHL raised by handling of plants. Although the respiratory dose is only 11% of the total dose, it should not be ignored because respiratory absorption is usually 100% whereas skin absorption is usually only about 10%. The absence of correlation with skin doses depends on other problems, such as contamination of work clothes washed only once a week and internal contamination of cotton gloves, changed only at the discretion of the worker.

The estimated absorbed doses of CHL were compared with the ADI of 0.03 mg/kg b.w., that is, with the quantity of pesticide which can be absorbed daily throughout the lifetime, without toxic manifestations. Although the ADI is calculated for the general population, which is exposed through the diet (oral route), it is often used as a reference value, below which occupational risk is presumably negligible [31–33]. Absorbed and actual doses estimated for chlorothalonil, expressed in μg/kg b.w., were less than the respective ADI in all workers. It is therefore reasonable to conclude that although exposure occurs by cutaneous and respiratory but not oral routes, it should not create health risks.

Respiratory and skin doses estimated in the present study, expressed in μg/day, were always less by a factor of 25–40 than the indicative limit values (ILVs) of 1 and 10 mg/day reported by Brouwer et al. in 1992 [16]. The respiratory ILV (no-effect level·70 kg body mass/absorbed fraction·safety factor) was calculated on the basis of a respiratory retention and absorption of 100%, a safety factor of 30 and a no-effect level of 0.4 mg/kg/day. It represents the amount of pesticide presumed to be the highest level of exposure possible without adverse effects on health. The cutaneous ILV for chlorothalonil has been estimated for a cutaneous absorbed fraction of 10% [16]. Comparison with ILV also shows skin and respiratory exposure below levels which are risky for health and confirms the above observations regarding ADI.

The skin doses found in the present study cannot be compared with those obtained in previous studies in which potential rather than actual doses on skin under clothing were evaluated. Workers cutting carnations after spraying and after dusting chlorothalonil accumulated 14.4 and 4.4 mg/h (geometric mean), respectively; during sorting/bundling, the corresponding values were 27.0 and 3.5 mg/h,, respectively. Evaluation was carried out by means of long-sleeved cotton gloves which covered the hands and forearm [16]. In a further study regarding mechanical tomato harvesting, mean total skin exposure of the upper body, evaluated by means of gauze pads on top of the clothes, was 503.9 μg/h [15]. In the same study [15], long-sleeve cotton knit undershirts, worn under the workers' normal clothing, and hand washing after glove removal, gave mean skin contamination levels of 45.2 μg/h, quite similar to those obtained in the present study [skin contamination 40.85±17.83 μg/h (mean±SD), range 15.22–105.93 μg/h].

Transfer factors (cm^2/h), the ratio of skin exposure (μg/h) to dislodgeable foliar residue ($\mu g/cm^2$), have been developed to describe the rate of transfer of foliar pesticide residues to the skin during occupational activity [36–38]. DFRs obtained in the present study during the working week (mean±SD: 1.99±0.73 $\mu g/cm^2$) gave a mean transfer factor of 20.54±8.96 cm^2/h (range 7.65–53.26) for CHL. These values are similar to those obtained by other authors: during mechanical tomato harvesting a dislodgeable fruit residue of 1.13 $\mu g/cm^2$ and a transfer factor of 40 cm^2/h were reported from undershirt analysis, whereas a value of about 450 cm^2/h was obtained from potential skin exposure data [15]. In the cultivation of carnations, a transfer factor of 4500 cm^2/h of the pesticide from leaves to hands was obtained on the basis of potential hand exposure data using long-sleeved cotton gloves to collect pesticide [17].

5. Conclusions

The present study revealed low levels of exposure of workers engaged in fixing runners of *Scindapsus* to a mossy support in greenhouses treated with chlorothalonil. Absorbed and actual doses estimated

for chlorothalonil, expressed in μg/kg b.w., were less than the respective ADI in all workers. Although exposure did not reach alarming levels, CHL is suspected to be carcinogenic and exposure should therefore be kept as low as possible. The situation observed in the greenhouse can only be modified by education and supervision of the workers. Biological monitoring of some metabolites studied in experimental animals could aid the determination of internal doses.

Since actual skin doses were higher than respiratory doses, greater precautions should be taken to reduce contamination, especially of the hands and unexposed skin (clean gloves and cotton overalls). Contamination of unexposed skin suggests that the cotton overalls are unsuitable or incorrectly managed. Although the respiratory dose was only about 11% of the total, it should not be ignored because respiratory absorption is generally much more efficient than cutaneous absorption. A suggested improvement in worker protection would involve respiratory protection, for example with a face mask to filter out airborne particulate.

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