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Multiroute Exposure Assessment and Excretion of Urinary Metabolites of Fenitrothion During Manual Operations on Treated Ornamental Plants in Greenhouses

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Abstract. The results of environmental and biological (five subjects) monitoring of exposure to fenitrothion during manual operations on treated ornamental plants in greenhouses are reported. Urinary excretion [GM (GSD)] of alkylphosphates [dimethylphosphate (DMP) + dimethylthiophosphate (DMTP)] (nmol/g creat) was 244.8 (1.8), 174.0 (2.0), and 354.4 (1.6) respectively, on the first (Monday), third (Wednesday), and fifth (Friday) days of work. These levels were not significantly higher than those recorded in a control group (21 subjects) in which urinary excretion [GM (GSD)] of DMP + DMTP was 102.8 (4.2) nmol/g creat. Air concentrations of fenitrothion (nmol/m^3) ranged from 45.5 to 81.2 on Monday, 17.3 to 27.1 on Wednesday, and 9.7 to 19.1 on Friday. Dose estimates showed that the respiratory-absorbed doses of fenitrothion accounted, on the average (GM), for 94.7%, 93.1%, and 91.5% of the total absorbed dose on Monday, Wednesday, and Friday, respectively. Multiple regression analysis showed a significant correlation ($r^2 = 0.595$) between urinary excretion of DMP + DMTP, respiratory-absorbed dose, and skin-absorbed dose, estimated on Monday and Wednesday. Total estimated absorbed doses did not exceed the acceptable daily intake for fenitrothion. Serum and erythrocyte cholinesterase activities were not significantly different before and after exposure.

Fenitrothion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorodithioate) is an organophosphorus insecticide used in agriculture to treat fruit trees, vegetables, ornamental plants, cereals, stored grains, cotton, and other crops. Fenitrothion has anticholinesterase activity and moderate acute toxicity with oral LD50 values in rats and mice ranging from 330 to 1,416 mg/kg body weight. Acute dermal toxicity in rodents is reported to range from 890 to more than 2,500 mg/kg body weight (WHO 1992). The LC50 value in rats exposed for 8 h is estimated to be more than 186 mg/m^3 (WHO 1992). In short-term studies on rats and dogs, no-observed-adverse-effect levels (NOAELs) based on brain-ChE activity were 10 mg/kg diet and 50 mg/kg diet, respectively. Long-term studies on rats and mice indicated a NOAEL of 10 mg/kg diet (WHO 1992). An acceptable daily intake (ADI) of 0.003 mg/kg body weight was established in 1984 (WHO 1986), but no occupational exposure limit (OEL) has even been published. No carcinogenic effects were found in any of the long-term studies (WHO 1992). Fenitrothion was not found to be mutagenic in *in vitro* and *in vivo* studies or teratogenic at doses of up to 30 mg/kg body weight in rabbits and up to 25 mg/kg body weight in rats (WHO 1992).

After intravenous injection of 15 and 40 mg/kg ^{32}P-fenitrothion in rats and guinea pigs, fenitrothion disappeared rapidly from the blood and tissues (Miyamoto 1964, 1969). The radioactivity was mostly excreted in urine within 2–4 days (85–97%), up to 10% was eliminated in the feces, making a total recovery of nearly 100%. The metabolites in excreta were demethyl fenitrothion, demethyl fenitrooxon, dimethylphosphorothioic acid, and dimethylphosphoric acid. Fenitroxon was detected only after intravenous administration of a large dose of fenitrothion and not after oral administration (Miyamoto *et al.* 1963; Miyamoto 1964). Hollingworth *et al.* (1967) found that ^{32}P-fenitrothion administered orally to mice at doses of 3, 17, 200, and 850 mg/kg was rapidly eliminated in the urine and feces with a recovery >90% 72 h after treatment. The metabolites isolated indicated that both the P-O-alkyl and P-O-aryl bonds of fenitrothion and fenitroxon were hydrolyzed. There was no evidence of reduction of the nitro group to aminofenitrothion or oxidation of the ring methyl group. At the lowest dose, demethyl fenitrothion, demethyl fenitroxon, dimethylphosphorothioic acid, and dimethylphosphoric acid were the major metabolites. At 200 mg/kg body weight, the amounts of dimethylphosphorothioic acid and dimethylphosphoric acid decreased and demethyl fenitrothion increased. ^{14}C-fenitrothion administered orally at a dose of 15 mg/kg to rats, mice, rabbits, and dogs was readily absorbed from the gastrointestinal tract and distributed to various tissues within 1–3 h of treatment, and most of the radiocarbon was rapidly eliminated in the urine (Miyamoto *et al.* 1976). Chromatographic analysis of the urinary metabolites showed the absence of intact fenitrothion and the presence of as many as 18 metabolites. The differences in composition of the metabolites in the various animals were mostly quantitative. Thus, fenitrothion was metabolized by two

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major pathways namely, O-demethylation and hydrolysis of the P-O-aryl bond (Miyamoto *et al.* 1976).

The dermal penetration of ^{14}C-ring-labeled fenitrothion was determined in rhesus monkeys and rats. In monkeys, 49 ± 4% of the fenitrothion (urinary excretion half-life = 14 h) was absorbed from the forehead, and 21 ± 10% (half-life = 17 h) from the ventral forearm. In rats 84 ± 12% (half-life = 20 h) was absorbed from the mid-dorsal region (Moody and Franklin 1987).

Dimethylphosphorothioic acid (DMTP) and dimethylphosphoric acid (DMP) are among the main metabolites reported for fenitrothion in mammals. These metabolites, which are excreted in the urine as sodium and potassium salts, may be used as biological indicators of exposure to fenitrothion and other phosphoric esters with ester groups subject to hydrolysis (WHO 1986; Maroni 1986).

The aim of the present study was to evaluate exposure and occupational risk during manual operations to fenitrothion-treated ornamental plants in greenhouses. We endeavored to evaluate the contributions of skin and respiratory exposure to the total estimated dose, using the alkylphosphates DMTP and DMP as biological indicators of exposure to the pesticide. A secondary aim of the study was to determine means to minimize occupational exposure.

Materials and Methods

Subjects and Exposure Conditions

Five female workers (workers 1, 2, 3, 4, 5), aged 20 to 49 years (28 ± 12 years) engaged in fixing runners of Scindapsus to a mossy support in a 24,570-m^3 greenhouse, were studied for a week. The plants had been treated with 2 kg Bayer Folithion (48.5% fenitrothion) dispersed in 20 L water (equal to 514.1 g/L active ingredient) 38 h earlier (the spraying was carried out at 6pm on Saturday and the greenhouse was re-entered 8am the following Monday). The pesticide was distributed by a nebulizer, which functioned by remote control. The device was installed in the central corridor of the greenhouse so that all plants received fairly homogeneous treatment. Plants near the nozzles were protected from an excessive dose by appropriately disposed plastic sheets. On the Monday and the other days that the greenhouse was entered, drops of liquid were visible on the leaves; this was not pesticide but water nebulized into the greenhouse to maintain humidity. On the Thursday, a number of stapled plants were removed and replaced with new plants.

The women did seasonal work in the greenhouse. The study period was the beginning of a work period after an interruption of 6 months. Hence, the women did not have prior occupational exposure to phosphoric esters or other pesticides.

The women were healthy and had normal blood indicators of liver and kidney function. None of them were taking pharmaceuticals. As far as drinking and smoking habits were concerned, worker 1 reported drinking 250 ml of wine per day and worker 4 reported smoking 7 cigarettes/day.

A control group consisting of 21 women who lived in the same residential area as the workers, was selected. The controls, however, worked in the offices of the local health unit and were not occupationally exposed to organophosphorus pesticides. None of the controls were taking medicines; four were smokers of more than 10 cigarettes/day and five, including the four smokers, drank 250–500 ml wine per day.

On the job, the workers wore cotton overalls, a cotton apron, work shoes, and two pairs of gloves (cotton in contact with the skin and latex on top). Under the overalls they wore undergarments, socks, and a cotton T-shirt. 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.

Biological Monitoring

Biological monitoring was performed by assaying urinary alkylphosphates (DMP and DMTP). A spot urine sample (basal) was obtained from each of the five workers on Monday before the work shift. Twenty-four-hour urine samples were obtained on Monday, Wednesday, and Friday of the working week. For the control group, an extemporary morning urine sample was obtained. Urine samples were also analyzed for creatinine, and the results were expressed in nmoles of DMP + DMTP/g creatinine.

Alkylphosphates were assayed by GLC with flame photometric detection after derivatization with pentafluorobenzylbromide (Aprea *et al.* 1996a). The analytical detection limit was 12 nmol/L for DMTP and 18 nmol/L for DMP (the detection limit was calculated on the basis of a signal three times the background noise). The percentage coefficient of variation (CV%) was 9.1 for DMTP and 11.4 for DMP (10 replicates).

Respiratory Exposure

On Monday, Wednesday, and Friday, personal air sampling was performed in breathing zone for all workers by means of portable Du Pont Alpha 1 samplers. Fenitrothion inhalable particulate was sampled using glass fiber membranes, 37 mm in diameter (Sartorius). The sampling flow through the membranes was 2.8 L/min, equal to an air velocity of 1.25 m/s through the 7-mm sampling opening. Air sampling was continued for the whole of the 8-h work shift. The fenitrothion in the membranes was assayed by GLC with alkaline flame detection. The membranes were first extracted with acetone in an ultrasound bath. The detection limit was 0.07 nmol/membrane (the detection limit was calculated on the basis of a signal three times the background noise). The CV% was 5% (10 replicates) and recovery 97.2%.

Skin Contamination

On Monday, Wednesday, and Friday, deposition of fenitrothion on exposed skin (the neck and head) was determined. This was done by securing a pad, consisting of a 20-cm^2 square of filter paper, directly on the skin of the face (cheek) with a sticking plaster.

In order to evaluate contamination of skin covered by work clothing, pads were placed in the chest and anterior abdominal regions of the workers, under their clothing. The choice of only two pads was motivated by the fact that the anterior part of the body was certainly the part most subject to contamination by contact during the fixing operations. The pads were squares of filter paper measuring 49 cm^2, fixed to the skin with sticking plaster.

The assay of fenitrothion in the pads was the same as for the glass fiber membranes. The detection limit was 0.7 pmol/cm^2 (the detection limit was calculated on the basis of a signal three times the background noise). The the CV% was 8% (10 replicates) and recovery 96.8%.

Contamination of the skin of the hands was evaluated by washing: 200 ml of 95% ethanol was slowly poured over each worker's hands while she rubbed them together. The ethanol was collected in the worker's personal disposable aluminum basin. After this, the worker allowed her hands and especially nails to soak in the solution for 30 s. The operation was repeated twice. The procedure was performed before the lunch break and at the end of the work shift. The ethanol from the two washes, at lunch break and at the end of the work shift, was pooled in a single container. Fenitrothion in the hand-wash liquid

was assayed as for the glass fiber membranes after evaporation of the solvent. The cotton gloves of the workers were collected at the end of the work shift on the 3 days. Fenitrothion was extracted with acetone and assayed.

Estimate of Doses

Air concentrations of pesticides were used to calculate the actual respiratory dose on the basis of a lung ventilation of 15 L/min (Zhuang *et al.* 1993). Contamination of exposed skin (head and neck) was calculated multiplying the concentrations detected on the face pads (pmol/cm^2) by the total surface area of skin exposed. This area was about 6.8% of the total body surface area (Popendorf and Leffingwell 1982), which was calculated for each worker by the formula of Du Bois and Du Bois (1916). Estimated total skin contamination was taken to be the sum of the contamination of head and hands, since the results obtained with the pads placed under the protective clothing showed that contamination of other areas of skin was negligible.

To estimate the quantity of fenitrothion absorbed by the workers, it is necessary to hypothesize the fraction absorbed via the respiratory system and the skin. We assumed (overestimated) that 100% of the respiratory dose was absorbed. We also assumed, on the basis of studies with rhesus monkeys (Moody and Franklin 1987) that 35% of the skin dose of fenitrothion was absorbed (mean absorption from the forehead and from the ventral forearm).

Assay of Serum and Erythrocyte Cholinesterase Activities

Blood samples were obtained from the five workers to evaluate serum and erythrocyte cholinesterase activities. A basal blood sample was taken before the Monday work shift and at the end of the Friday work shift. The two values were compared.

Serum and erythrocyte cholinesterase activity was assayed by a modified version of the method of Ellman *et al.* (1961) described by Alcini *et al.* (1988); the coefficient of variation of the whole analytical procedure was 5% for both activities.

Determination of Dislodgeable Foliar Residue (DFR)

To evaluate decay of the active principal, leaf samples were obtained immediately before and after spraying with fenitrothion and 41, 65, 89, 113, and 137 h after spraying. The samples were obtained with a punch (Iwata *et al.* 1977), 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 two halves 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 89 h after spraying. In subsequent sampling (Thursday and 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.

Analysis was performed by GLC with alkaline flame detection (NPD). The dislodgeable residue was extracted from the leaves with an aqueous solution of detergent, from which the active principal was re-extracted with ethyl acetate before gas chromatography (Goh *et al.* 1986). The detection limit of the method was 1.8 pmol/cm^2 (the detection limit was calculated on the basis of a signal three times the background noise); analytical reproducibility (CV%) was 6% (10 replicates) and recovery 82.5%.

Statistical Analysis

Kruskal-Wallis one-way ANOVA was used to compare the biological and environmental monitoring data and the doses determined on the various days of sampling. The significance of pairwise comparisons for each day was evaluated by the Mann Whitney U test. The same test was used to compare urinary excretion of metabolites by the workers and controls. These nonparametric 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 $\alpha = 0.05$ for Kruskal-Wallis one-way ANOVA and for the Mann Whitney U test. The analysis was carried out using the SPSS statistical software package (SPSS Inc., Chicago, IL).

Results

The concentrations of alkylphosphates (DMP + DMTP) in worker and control urine samples grouped according to day of sampling are shown in Table 1. Kruskal-Wallis one-way ANOVA showed that on the whole, urinary excretion of alkylphosphates was not related to the day of the week on which the urine sample was obtained. The Mann Whitney U test showed that the urinary excretion of alkylphosphates on the days of reentry into the greenhouse, was not significantly different from basal values or from the values of the control group.

Table 2 shows the concentrations of fenitrothion in personal air samples and face pads, together with the quantities of the pesticide in the cotton gloves and hand wash liquid. The concentrations of the pesticide in the air ranged from 45.5 to 81.2 nmol/m^3 on Monday and dropped to 17.3–27.1 and 9.7–19.1 nmol/m^3 on Wednesday and Friday, respectively. A similar decreasing trend between the first and fifth working day was also found for fenitrothion concentrations in face pads, which dropped from 29.2 and 58.5 pmol/cm^2 on Monday to 7.2 and 36.4 pmol/cm^2 on Wednesday and Friday. Kruskal-Wallis one-way ANOVA showed that on the whole, the concentration of fenitrothion in personal air samples was related to the day of sampling. The Mann Whitney U test showed that the differences between Monday, Wednesday, and Friday and between Wednesday and Friday were significant. The same test applied to the face pad concentrations did not show any significant influence of day of sampling. Significant differences (Mann Whitney U test) were only found between the samples of Monday and Friday.

Pesticide contamination on the skin of the hands ranged from 4.0 to 17.4 nmol, 1.6 to 11.9 nmol, and 0.8 to 11.3 nmol on Monday, Wednesday, and Friday, respectively. The quantities of active ingredient found in the cotton gloves worn by the workers ranged from 7.9 to 17.2 nmol, 3.6 to 11.4 nmol, and 6.4 to 13.0 nmol on the same three days, respectively. Kruskal-Wallis one-way ANOVA showed that on the whole, the concentrations of fenitrothion found in hand wash liquid and gloves were not related to the day of sampling. The Mann Whitney U test did not reveal any significant difference in pairwise comparisons of the data for all the days of sampling.

The concentrations of pesticide found in the pads placed under the clothing on the chest and anterior abdomen are not shown in the table because they were always below the analytical detection limit.

Concentrations of fenitrothion in personal air samples were significantly correlated ($p < 0.05$) with the rate of deposition of

Table 1. Urinary excretion of DMP + DMTP (nmol/g creat) in controls (n = 21) and in workers engaged in manual operations on plants (n = 5) treated with fenitrothion

Sample	Mean ± SD	GM (GSD)	Median	Range
Workers[a,b]				
Basal	261.2 ± 262.5	182.6 (2.5)	119.4	71.9–704.1
Monday	278.8 ± 143.5	244.8 (1.8)	327.5	80.0–270.0
Wednesday	206.4 ± 117.2	174.0 (2.0)	230.9	128.0–444.7
Friday	387.4 ± 178.9	354.4 (1.6)	365.1	219.8–629.9
Controls[b]	221.4 ± 247.6	102.8 (4.2)	132.6	8.5–788.4

[a] Kruskal-Wallis one-way ANOVA was not significant for urinary concentrations of alkylphosphates in workers
[b] Urinary excretion of alkylphosphates by workers and controls did not differ significantly (Mann Whitney U test)

Table 2. Exposure of workers engaged in manual operations on treated plants on different days

	Gloves[a] (nmol)	Face Pads[a] (pmol/cm^2)	Hand Wash[a] (nmol)	Personal Air Sample[a] (nmol/m^3)
Monday				
GM (GSD)	13.00 (1.39)	38.33 (1.29)	6.3 (1.78)	53.62 (1.27)
Range	7.90–17.21	29.2–58.5	4.0–17.4	45.5–81.2
Wednesday				
GM (GSD)	7.24 (1.53)	18.95 (1.90)	3.5 (2.2)	21.57 (1.17)
Range	3.64–11.36	7.2–36.4	1.6–11.9	17.3–27.1
Friday				
GM (GSD)	9.12 (1.37)	14.33 (2.03)	2.2 (2.8)	13.54 (1.33)
Range	6.39–12.99	7.2–36.4	0.8–11.3	9.7–19.1

[a] Kruskal-Wallis one-way ANOVA was significant for personal air samples; the differences in concentrations were significant (Mann Whitney U test) between the different days. Kruskal-Wallis one-way ANOVA was not significant for hand wash, gloves or face pads; the differences in face pad concentrations between Monday and Friday were significant (Mann Whitney U test)

the active ingredient per unit surface area of face pad. The regression line equation was $y = 0.0012x + 0.0284$ and the correlation coefficient $r^2 = 0.819$ (Figure 1).

Table 3 shows the DFR of fenitriothion immediately before and after spraying and on the days of re-entry into the greenhouse. Leaf concentrations dropped sharply in the first 40 h after spraying, from 534.3 to 75.4 pmol/cm^2, and more gradually thereafter until Wednesday (89 h). On Thursday (113 h) an increase in DFR was found, probably due to the replacement of some plants. The results obtained on Friday (137 h) were compatible with those of the previous day. The DFR was significantly correlated with the quantities of pesticide found under the latex gloves, namely on the cotton gloves, and with the concentrations of pesticide in personal air samples. There was no significant correlation with the quantity of pesticide in hand wash liquid. The regression line equations and correlation coefficients are given in the captions of Figures 2, 3, and 4.

The respiratory dose (nmol), estimated skin contamination (nmol), total actual dose, and total estimated absorbed dose (nmol/kg body weight) of the five workers are shown in Table 4. Kruskal-Wallis one-way ANOVA showed that on the whole, the respiratory dose and the total doses (actual and absorbed) of fenitrothion were related to the day of the week on which the samples were obtained. The Mann Whitney U test revealed significant differences for pairwise comparisons of all sampling days. On the other hand, Kruskal-Wallis one-way ANOVA did not show any significant relation between total skin dose or contamination of exposed skin (head and neck) and day of sampling. The Mann Whitney U test revealed significant differences between exposed skin contamination on Monday and Wednesday, and between total skin contamination on Monday and Wednesday and on Monday and Friday.

Respiratory dose expressed as a percentage of total actual dose was [GM (SGM)] 86.3 (1.0)%, 82.7 (1.1)%, and 79.6 (1.2)%, respectively, on Monday, Wednesday, and Friday. The main contribution of skin contamination to total actual dose was by exposed skin (head and neck); the quantity of fenitrothion on the hands, in fact, was only [GM (SGM)] 1.7 (2.0)%, 2.2 (2.0)%, and 2.0 (2.7)% of the total dose, respectively, on the three days of sampling. The difference between total dose and total estimated absorbed dose (nmol/kg body weight) of the five workers was very small because we assumed that 100% of the respiratory dose and 35% of the skin dose was absorbed (Moody and Franklin 1987). Respiratory-absorbed dose expressed as a percentage of total absorbed dose was [GM (SGM)] 94.7 (1.1)%, 93.1 (1.0)%, and 91.5 (1.2)%, respectively, on Monday, Wednesday, and Friday.

Multiple regression analysis for urinary excretion of DMP + DMTP (total nmol excreted in 24 h)(y) versus respiratory dose (nmol)(x_1) and total skin-absorbed dose (nmol)(x_2) on Monday and Wednesday showed statistically significant ($r^2 = 0.595$, significance level $p < 0.05$) fitting of the data to the equation $y = -0.585x_1 + 27.930x_2 + 19.246$.

Serum and erythrocyte cholinesterase activities at the end of the working week (Friday) did not differ from preexposure activities by more than 10%. The results are shown in Table 5.

Discussion

Urinary excretion of alkylphosphates was not significantly higher than in the control group of subjects not occupationally exposed to pesticides. The ranges of concentration observed were much wider among controls (8.5–788.4 nmol/g creat). Urinary excretion of metabolites in controls is probably derived from residues of organophosphorus pesticides in food. This wide range may therefore be related to the presumably wide range of residues in different foods. In a previous study at least one alkylphosphate was detected in all 124 members of a general population group with concentrations of DMP + DMTP in the range of 5.5–1,145.1 nmol/g creat [GM (GSD) 126.9 (2.7) nmol/g creat] (Aprea *et al.* 1996b). The range observed in the exposed workers was slightly less than that of controls, probably because the workers ate one meal a day in the company canteen, which would have made their diets more uniform.

Significant differences were not even found between urinary excretion of alkylphosphates before (basal) and during reentry of the greenhouse (Monday, Wednesday, and Friday). However, the highest urinary excretion was observed on the Friday, when levels of airborne pesticide (which was responsible for more than 90% of the dose absorbed) were significantly lower than on the other days. This unexpected result could be related to anomalous cutaneous absorption on the Thursday, when moni-

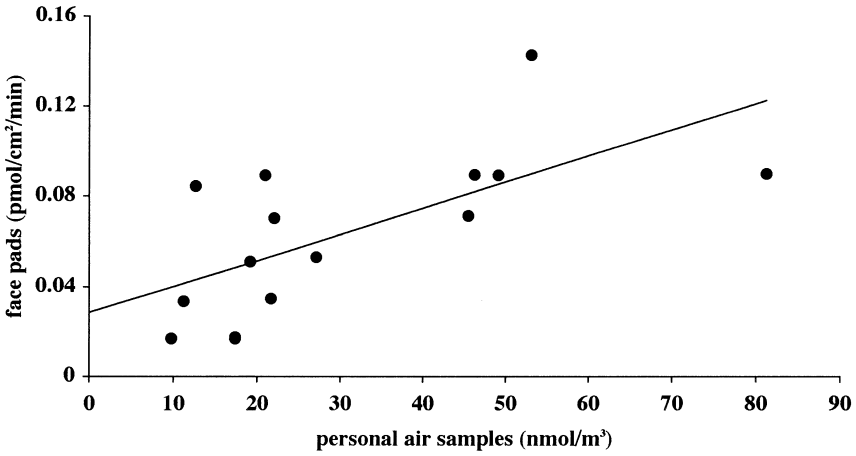

Fig. 1. Linear regression line ($y = 0.0012x + 0.0284$) between concentration of fenitrothion in personal air samples and in face pads ($r^2 = 0.427$; significant $p < 0.05$)

Table 3. Dislodgeable foliar residues (DFR) of fenitrothion before (hour 0) and after treatment (hour 1.45), and during the subsequent working week

Hours	DFR (pmol/cm²)
0	<1.8
1.45	534.3
41	75.4
65	59.5
89	45.1
113	71.8
137	41.5

toring was not performed. Skin absorption is a slow process and could be the reason for the increase in excretion of metabolites the next day. The increased exposure could have been due to stapling the new plants brought to the greenhouse on the Thursday, possibly without correct use of gloves. This explanation is supported by the higher DFR detected on Thursday (the Friday observation is in line with that of the previous day accounting for decay) and with a (nonsignificant) increase in pesticide levels found on gloves on Friday. The hand wash data of Friday had a wider dispersion than on previous days, without an increase in the geometric mean.

The results indicate that if the workers used the gloves correctly, absorption of fenitrothion remained at very low levels which were no higher than the exposure associated with a normal lifestyle (general population). On the other hand, other occupational activities associated with exposure to phosphoric esters, studied by the present authors, have shown significantly higher urinary excretion of alkylphosphates than in an appropriately selected control group. During reentry into a peach orchard previously treated with chlorpyrifos-methyl and azinphos-methyl for thinning of juvenile fruits, performed with various types of skin and respiratory protection, urinary excretion of alkylphosphates was in the range of 66.0–8,833.0 nmol/g creat (Aprea *et al.* 1994). In workers exposed to chlorpyrifos-methyl during vine spraying and manual leaf thinning, urinary excretion of alkylphosphates was in the range of 49.0–5,089.6 and 161.1–1,512.7 nmol/g creat, respectively (Aprea *et al.* 1997). In workers engaged in preparing commercial formulations of dimethoate (formulation, bottling, and packing), urinary excretion of alkylphosphates was in the range 103.5–22,968.5 nmol/g creat (Aprea *et al.* 1998).

No published occupational exposure limit (OEL) is available for fenitrothion, but if we take the value of 0.2 mg/m^3 of the ACGIH (1998) for various organophosphorus insecticides (azinphos-methyl, chlorpyrifos, methylparathion, fenthion, sulfotep), the concentrations found in the air samples on the 3 days of sampling were $\frac{1}{10}$ to $\frac{1}{67}$ of this limit. The highest level observed on Monday (81.2 nmol/m^3, 0.02 mg/m^3) was an order of magnitude below this limit. The concentrations detected indicate actual exposure because the workers did not wear a mask to protect the airways during the work shift, and the absorbed dose because we assume 100% lung retention. The concentration of 0.2 mg/m^3 has a safety factor of more than 1,000 with respect to the LC50 (> 186 mg/m^3) estimated for fenitrothion in rats exposed for 8 h (WHO 1992).

Pesticides in the form of airborne particulate are a source of respiratory exposure and contamination of unprotected skin. Since fenitrothion concentrations in face pads were significantly correlated with those in personal air samples, contamination of exposed skin can be attributed to deposition of airborne pesticide rather than to contact with the hands or contaminated clothing. In the latter case, in fact, contamination of the face pads would be incidental rather than related to the concentrations of pesticide in the personal air samples.

Contamination of protected skin (under protective clothing) may indicate penetration of pesticides through the overalls (unsuitable fabric, penetration through zip fasteners, closures, etc.) or contact with the hands or contaminated clothing. In the occupational task in question, there were few opportunites for contamination. The additional protection of the cotton apron may have been a contributing factor.

The gloves (latex and cotton) used during the study provided good protection, since hand contamination was much less than that of exposed skin and than the respiratory dose. The fenitrothion found on the cotton gloves and on the hands could be due to incorrect use of the two types of gloves or to poor barrier properties of the gloves. The highest values in the ranges of contamination observed on the hands in the 3 days of sampling always belonged to worker 5, suggesting that the cause was incorrect use. The nonsignificant correlation observed between DFR and quantity of pesticide in hand wash liquid confirms that contamination of the hands is not systematic but incidental and hence probably due to incorrect use of gloves.

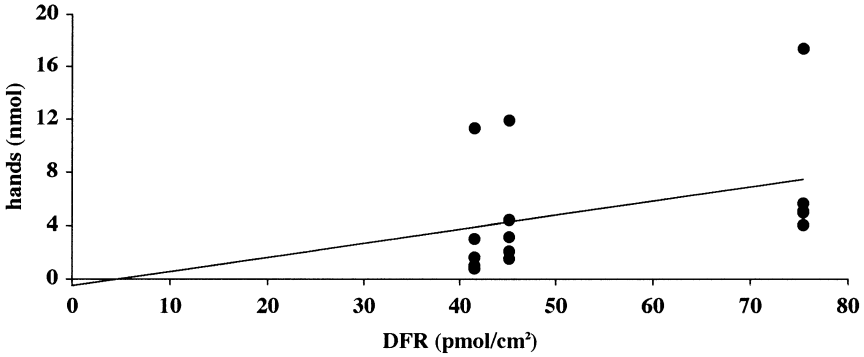

Fig 2. Linear regression line ($y = 0.105x - 0.467$) between dislodgeable foliar residues of fenitrothion and quantities of fenitrothion on hands ($r^2 = 0.122$, not significant)

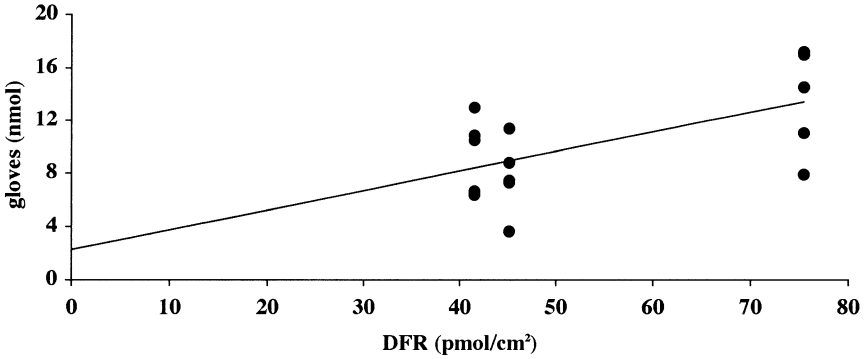

Fig. 3. Linear regression line ($y = 0.148x + 2.262$) between dislodgeable foliar residues of fenitrothion and quantities of fenitrothion on hands ($r^2 = 0.348$; significant $p < 0.05$)

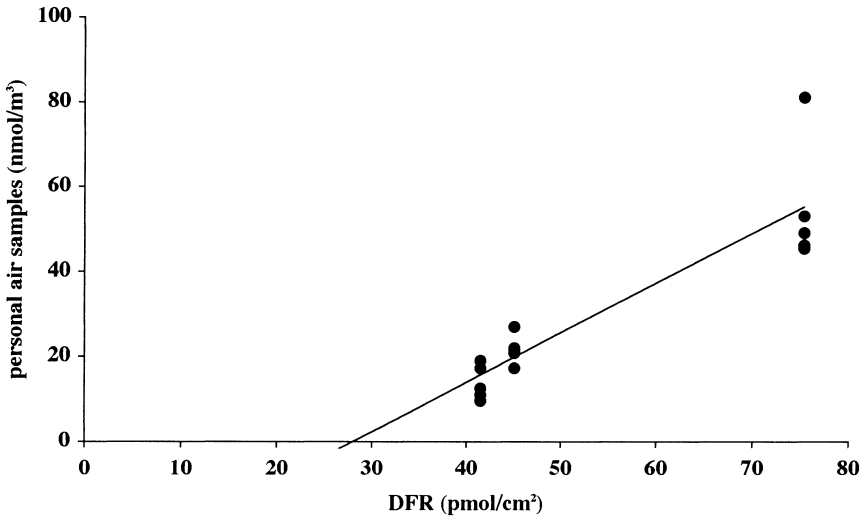

Fig. 4. Linear regression line ($y = 1.164x - 32.618$) between dislodgeable foliar residues of fenitrothion and concentration of fenitrothion in personal air samples ($r^2 = 0.819$; significant $p < 0.05$)

Exposure levels of the workers are related to DFR or that part of the active principal that did not penetrate the leaf tissue and could thus be removed by contact. In fact, the DFR of fenitrothion was significantly correlated with the amounts of pesticide detected under the latex gloves (cotton gloves) and in personal air samples. These results suggest that the DFR contributes both to concentrations of pesticide on cotton gloves and respiratory exposure. The active principal dispersed as airborne particulate in turn induces exposed skin contamination (face pads) as shown by the significant correlation in Figure 1.

In the present study, contamination of exposed skin was evaluated by a modification of the traditional pad technique proposed by Durham and Wolfe (1961). The pad was placed on the face of the workers and the material it collected was considered to represent skin contamination of the head and neck. This assumption is based on the fact that contamination of exposed skin is attributed substantially to deposition of airborne pesticides rather than to direct contact with the pesticide. Deposition on the whole of the exposed surface area (albeit small) can therefore be regarded as homogeneous. Total skin contamination was calculated as the sum of that of exposed skin and hands.

The multiple regression analysis of urinary excretion of alkylphosphates versus respiratory and skin absorbed doses showed significant fitting of the data for Monday and Wednesday. This is further confirmation that urinary alkylphosphates

Table 4. Estimate of potential respiratory dose and skin contamination by fenitrothion for workers engaged in manual operations on treated plants during the working week

	Respiratory Dose[a] (nmol)	Head + Neck[a] (nmol)	Hands[a] (nmol)	Total Skin Contamination[a] (nmol)	Total Actual Dose[a] (nmol/kg b.w.)	Total Absorbed Dose[a] (nmol/kg b.w.)
Monday						
worker 1	494.3	39.6	4.0	43.6	9.3	8.8
worker 2	278.9	31.2	5.1	36.3	5.4	5.0
worker 3	281.9	41.0	5.7	46.7	5.2	4.7
worker 4	326.1	65.2	5.0	70.2	6.3	5.6
worker 5	298.1	42.4	17.4	59.8	6.0	5.3
Wednesday						
worker 1	134.7	15.7	1.6	17.3	2.6	2.4
worker 2	108.1	7.7	3.2	10.9	2.1	1.9
worker 3	137.0	32.9	4.4	37.3	2.8	2.4
worker 4	128.1	40.7	2.1	42.8	2.7	2.3
worker 5	167.2	25.6	11.9	37.5	3.4	3.0
Friday						
worker 1	72.5	15.7	3.0	18.7	1.6	1.4
worker 2	111.7	7.7	0.8	8.5	2.1	2.0
worker 3	62.7	8.1	1.0	9.1	1.1	1.0
worker 4	122.7	24.6	1.7	26.3	2.4	2.1
worker 5	81.8	42.4	11.3	53.7	2.3	1.7

[a] Kruskal-Wallis one-way ANOVA was significant for respiratory dose, for total actual dose, and for total absorbed dose; differences between values on the 3 days were significant (Mann Whitney U test). Kruskal-Wallis one-way ANOVA was not significant for total skin contamination and head + neck; differences in total skin contamination and head + neck between Monday and Wednesday and in total skin contamination between Monday and Friday were significant (Mann Whitney U test)

Table 5. Preexposure erythrocyte (AChE) and serum (BuChE) cholinesterase activity (IU/ml) in all subjects monitored[a]

	Preexposure Samples		Postexposure Samples	
	AChE	BuChE	AChE	BuChE
Worker 1	3.87	1.96	96.4%	99.3%
Worker 2	4.34	3.01	103.4%	99.6%
Worker 3	4.50	3.60	102.6%	101.3%
Worker 4	5.83	4.18	98.4%	101.2%
Worker 5	4.26	3.56	95.5%	99.0%

[a] Postexposure activity at the end of the working week (Friday) is expressed as a percentage of preexposure values

are good biological indicators even at low exposure levels. The levels of urinary excretion on Friday, indicative of very low exposure levels, may have been confounded by skin absorption the previous day.

In our study, absorption occurred prevalently (> 90%) by inhalation, which includes the quantity absorbed by swallowing pesticide-bearing particulate deposited in the upper airways. In the absence of specific short- and long-term studies to evaluate inhaled doses that do not cause toxic effects in humans and experimental animals, the estimated absorbed doses of fenitrothion were compared with the ADI, which is the quantity of the pesticide that can be absorbed daily over a lifetime without giving rise to toxic manifestations. Although ADI is calculated for the general population, exposed by the oral route, it is a useful reference value, below which occupational risk is presumably negligible, even if the routes are different. The total absorbed doses of individual workers never exceeded the ADI of 10.8 nmol/kg body weight (3 μg/kg body weight) for fenitrothion (WHO 1986). The values observed exceeded half the ADI only on the first day of the working week, dropping to even lower values on subsequent days. Mean weekly values [GM (GSD)] were 2.8 (1.8) nmol/kg body weight. Adoption of a safety factor of 3 to allow for absorption routes other than oral would mean that the interval for reentry of the greenhouse (38 h after treatment) used in this occupational situation is appropriate, combined with the protective clothing worn, for preventing exposure of workers to fenitrothion.

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