LETTER TO THE EDITOR

EVALUATION BY ENVIRONMENTAL MONITORING OF PESTICIDE ABSORPTION IN FARM WORKERS OF 18 ITALIAN TOMATO CULTIVATIONS

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Tomato cultivation farms of Southern Italy were investigated in order to evaluate the general working conditions and the levels of exposure of farm workers to pesticides, during the mixing/loading and the application of pesticides on fields. Information on working modalities, personal protective equipment, etc. was collected using a questionnaire. Inhaling and cutaneous exposure levels were measured, and the estimated pesticide total absorbed dose was compared with Admissible Daily Intakes (ADIs). Field treatments were mainly carried out by using sprayers with open cab tractors, and, in 57.9% of cases, the pesticide mixture was manually prepared by mixing pesticides in a pail, often without using gloves (59.5%). The estimated pesticides absorbed doses varied in the range 0.56-2630.31 mg (mean value, 46.9 mg), and 20% of the measured absorbed doses exceeded ADIs. The findings obtained in the 18 examined farms show a worrying situation, suggesting the investigation of many more farms, so that a statistically significant picture of tomato cultivations in Southern Italy could be formed. Besides, the planning of training courses aimed to increase workers consciousness about health risks and how they can be prevented is advisable.

Tomato culture is widespread all over the world and represents one of the main industrial cultivations both for economic relevance and for diffusion. After the United States, Italy is the main tomato producing country, and in particular, some areas of Southern Italy (Puglia, Campania and Basilicata regions) account for more than 40% of cultivated surfaces, having a remarkable importance in the entire national production volume. Tomato cultivations are infested by numerous parasites and, as a consequence, the fields are treated with numerous pesticides, the absorption of which by humans affects the health in various ways according to the chemical properties of different pesticides. These effects range from respiratory and cutaneous irritations to serious nephropathy and death due to carcinogenic diseases (1, 2).

To date, many efforts have been made to protect

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consumers and the general population by controlling pesticides in food, air, water and soils (3-5). On the contrary, studies regarding occupational exposure are mainly focused either on the evaluation of health effects, or on the individuation of factors influencing exposure, or on the definition of predictive algorithms (6-7). Furthermore, research studies reporting measurements to evaluate the actual exposure of the workers to pesticides have been mainly carried out by biological monitoring investigations rather than by measuring environmental pesticide exposure levels (8).

TheAuthors evaluated health risks of farm workers on 18 tomato farms. General -information regarding working modalities, use of personal protective equipment (PPEs) etc. were collected. Inhaling and cutaneous exposure levels were measured by using personal samplers and the cutaneous surrogate technique, respectively. Samples were analysed by GC/MS-SIM (Gas Chromatography/Mass Spectrometry-Selected Ion Monitoring).

The results obtained are here reported in order to bring on the international scene a case-study of the actual working conditions and exposure levels of workers in some tomato cultivations of Southern Italy.

MATERIALS AND METHODS

Tomato farms were investigated by collecting general information through a questionnaire and by the personal environmental monitoring of workers, during the preparation of pesticide mixtures and their application on fields (field treatment).

Questionnaire

The questionnaire was divided into three parts, based on the collection of the following information: Part I) farms: number of workers, field extension, frequency of fields treatments with pesticides, pesticides containers disposal modality; Part II) subjects: sex, age, weight, height, task (mixing/loading and application of pesticides on fields); Part III) working conditions (on the day of sampling): meteorological conditions (temperature, cloudiness, wind), pesticides used (physical state of formulations, chemical nature and amount of active principle), protective personal equipment (boots, gloves, respirators, face-shields, overalls), clothes (short/long sleeves and pants), hours spent for the field treatment and/ or the mixing/loading step (exposure time), application techniques/machines (atomizer, pressurized cab, sprayer with open cab tractor, sprayer with enclosed cab tractor, boom, backpack), mixing system (enclosed and open).

Environmental monitoring

The sampling for the evaluation of inhaled exposure was carried out by using a combined captation system, constituted by XAD-2 resins and 2 µm, 37 mm, PTFE membranes (SKC Inc. PA, USA). The combined system was connected to a pump with a flow rate of 2 L/min. For cutaneous exposure evaluation, 9 Whatman 2 fiber paper pads (Whatman International Ltd., Maidstone, England) were applied directly onto the workers' skin (below clothing), in different locations over the body: face (4x4 cm² pad), front and back chest, right back forearm, left front arm, left front thigh, right back thigh, right shin-bone, left calf (7x7 cm² pads) Pad locations were established as suggested by the United States Environmental Protection Agency (9). Combined captation system and pads were applied to each examined worker before the preparation of pesticide mixtures and/or the field treatment with pesticides, and they were removed soon after the exposure. Samples were transported to the analysis lab inside refrigerated bags, and stored at -20°C, before analysis.

Analytes were quantified by GC/MS-SIM (Focus GC/single quadrupole mass spectrometer DSQ, Xcalibur software version 1.2, Thermo-Electron Corporation, Waltham, MA, United States). With regard to the cutaneous matrix, the adopted analytical procedure was recently validated by the Authors, and is reported in detail in literature, together with validation parameters (10). In the case of resins and membranes, quantification and detection limits, and analyte stability conditions, analytical precision and accuracy were evaluated according to EPA Guidelines (11).

Calculus of the inhaling/cutaneous exposure and of the total absorbed dose

Inhaling exposure was calculated by dividing the amount of pesticide found in resins and membranes in respect to the sampled air volume. The respiratory absorbed dose due to inhaling exposure was calculated taking into account the time of exposure, lung ventilation of 20 L/ min and lung retention of 100% in absence of protective personal equipment. Lung retention of 8% was assumed when respirators with P2 filters were used, in agreement with the criteria for the evaluation of filter efficacy, published by the Italian Organization for Standardization, appointed by the Italian Government and the European Union to develop and approve technical standards (12). Dermal exposure for each pad was calculated by multiplying the measured pesticide concentrations and the surface of the anatomic zone represented by the pad. This surface was obtained by using the anatomic model of Popendorf (13), which gives, for each anatomic zone, a coefficient representing the skin surface percentage. In detail, coefficients were multiplied by the Total Body Surface (TBS), that was calculated for each investigated subject, according to the formula introduced by Du Bois and Du Bois (14):

TBS $(cm^2)=71,84$ •Weight(Kg)^{0.425}•Height(cm)0.725. The total dermal exposure (skin contamination) was obtained by multiplying the sum of pesticide amounts found on each anatomic area by a skin penetration coefficient. Since skin penetration coefficients are not well-defined for all the examined pesticides, a skin penetration of 10% was assumed as an average value for all the considered pesticides. The total absorbed dose (TAD) was given by the sum of respiratory and cutaneous absorbed dose.

Statistical analysis

The statistical description of data was carried out by using SPSS software, version 15.0 for Windows (SPSS ITALIA s.r.l., Bologna, Italy). Quantitative data were transformed in logarithms, and the normality of distribution functions was evaluated by the Kolmogorov-Smirnov test. When quantitative data were divided into subdistributions (defined by the kind of pesticide, the adopted PPEs, etc.), normality tests were applied again by using the Kolmogorov-Smirnov test if the number of data in the examined subdistribution was higher than 30, otherwise the Shapiro-Wilk test was used. When normality occurred, mean values, means standard errors (SEs) and standard deviations (SDs) are reported in the text, otherwise only the median value is shown. Normal subdistributions were compared either by the Student t-test for unpaired data (in the case of two subdistributions) or by the univariate ANOVA and post hoc Scheffè tests (in the case of more than two subdistributions).

Quantitative results shown below were obtained in logarithmic functions, nevertheless, for an easier data interpretation, they are reported in μg instead of logarithms.

RESULTS

General information and working conditions

The evaluation of pesticide absorption was performed on farm workers of 18 tomato cultivation farms (fields extension: $1300-40000 \text{ m}^2$) in Southern Italy (Puglia, Campania and Basilicata regions) during the mixing/loading and the application of pesticides on fields. In each investigated farm, 1-2 subjects were involved in pesticide mixing/loading and field treatments, for a total of 22 workers, 100% men, aged from 25 to 40, except for one 65-year-old worker. Generally, workers were charged both to prepare the pesticide mixtures and to apply them on fields, only in 9 cases (24.3%), the two tasks were carried out by different subjects. The exposure time ranged from 0.5 to 3.1 h.

Field treatment frequency ranged from 7 to 18 per year (from April to September), and formulas (liquid, 19.0%; granular, 22.2%; powder, 16.7%; powder contained in water soluble bags, 8.3%) of various concentrations of active principles belonging to different chemical classes, were used.

Empty pesticides containers were discharged in three different ways: through specialized firms (23.0%), by washing (with water) and then burning containers (30.8%), or by washing and putting them into common rubbish (46.2%).

On the 18 investigated farms, various modalities





and machines were used to distribute pesticides on fields: atomizers and sprayers with an open cab tractor, 5.3%; pressurized cabs, 10.5%; backpacks, 15.8%; booms, 26.3%. Also different mixing/ loading techniques were adopted: open system (casks), 15.8%; enclosed system (mixers), 26.3% and manual, with "pails", 57.9%. The manual technique with "pail" means that pesticides mixture was manually prepared in a pail and then poured into and diluted in a cask for the application on fields.

Fig. 1 (left panel) shows the PPEs used (as percentages) on the days of sampling. Since PPEs were often not used (59.5%), clothing was also investigated and the obtained findings are shown in the right panel of Fig. 1 (percentages refer to cases in which PPEs and pressurized cabs were not used).

Pesticide handled amount and total absorbed dose

Twenty-four samplings (days of sampling), from May to July, were performed. Some farms produced different kinds of tomato (classic and S. Marzano DOP tomatoes) and, in this case, they were examined more times. A total of 38 resins and membrane samples (inhaling exposure), together with 342 pads (cutaneous exposure) were collected. During each treatment, 1-4 different pesticides (chosen from abamectin, chlorpyriphos, chlorpyriphos methyl, λ -cyhalothrin, cypermethrin, deltamethrin, dimethoate, dimethomorph, fenbutatin oxide, hexaconazole, hexythiazox, imidacloprid, metalaxyl, propargite, tolylfluanid) were used, and the amount of active principle handled by farm workers ranged from 1.7 to 2630.3 g (mean, 114.0 g; SD, 4.9 g). The absorption was estimated by environmental monitoring analyses using resins and membranes for the measurement of inhaling exposure and pads for dermal exposure. The measurement of the pesticide concentration in the air inhaled by workers and on their cutis estimates only the level of contamination. Since the research was focused on the estimation of the dose of pesticide actually absorbed by workers, lung ventilation, lung retention and skin penetration coefficients were taken into account, so that the total absorbed dose (TAD, given by the sum of the respiratory dose and the cutaneous absorbed dose) was calculated, obtaining results ranging from 0.56 to 2630.31 µg. The respiratory dose was not detectable in 38.1% of analysed samples, in contrast with the 14.8% of cutaneous samples, and the mean value of the dermal absorbed dose was 45.19 µg, against a value of 4.42 μ g for the inhaling one.

Influence of PPEs, clothing, application and mixing/ loading techniques on pesticides total absorbed dose

As expected, a significant difference (p=0.018) was found between TAD mean values measured

Table I. Pesticide total absorbed dose normalized in respect to the pesticide handled amount, according to the application

 technique: range of variation, standard deviation, mean/median value and standard error.

Min-max (µg)	SD (µg)	Mean ± SE / Median (µg)
1 1	<i>.</i>	ND
0.01-0.02		0.01 (median)
		0.09
0.04-1.82	3.25	0.16±1.41
0.02-19.06	5.00	0.42±1.24
0.39-257.04	15.34	5.21±3.29
	Min-max (μg) 0.01-0.02 0.04-1.82 0.02-19.06 0.39-257.04	Min-max (μg) SD (μg) 0.01-0.02 0.04-1.82 3.25 0.02-19.06 5.00 0.39-257.04 15.34

ND: not detectable

"Total absorbed dose was measurable only in 1 sample.

^b Total absorbed doses related to these application techniques were normally distributed.

Percentage of using	Pesticide	TAD range	TAD/kg ^c	ADI ^d
		Min-Max ^b (µg)	(mg/kg)	(mg/kg)
18.2	metalaxyl ^a	2.29-446.68	0.0005	0.03
17.0	cypermethrin ^a	5.49-323.60	0.0051	0.05
15.6	dimethomorph ^a	3.16-707.95	0.0005	- 0.06
9.1	chlorpyriphos ^a	18.20-1202.31	0.0028	0.003
8.0	λ-cyhalothrin	3.1-47.7	0.0002	0.02
5.7	hexaconazole ^a	8.32-112.20	0.0005	0.005
5.7	tolylfluanid	1107.4-9233.5	0.0630	0.10
4.5	fenbutatin oxide	1286.1-3634.4	0.0350	0.01
3.4	chlorpyriphos methyl	857.4-271.4	0.0080	0.01
3.4	propargite	6056.8-7670.8	0.4900	0.002
2.3	dimethoate	4711.0-6171.0	0.0775	0.02
1.1	hexythiazox	51.0	0.0007	0.03
1.1	imidacloprid	5.6	0.0001	0.06
1.1	abamectin	330.2	0.0050	0.0005

Table II. Total absorbed doses of specific pesticides in comparison with ADIs.

^aNormal distributions, according to Shapiro-Wilk normality test

^b Variation ranges of the total absorbed dose

^e Total absorbed dose mean values with respect to body weight expressed in mg/kg, for an easier comparison with ADI. ^d Acceptable Daily Intake (Australian Government, 2011).

when workers used PPEs (12.81 ± 1.54 µg; range of variation, 2.29-44.70 µg; SD, 0.50 µg) and when they did not use any PPE (70.82 ± 1.28 µg; range of variation, 0.56-2630.01 µg; SD, 0.77 µg).

With regard to clothing, field treatments were carried out in summer, hence farm workers usually wore short-sleeved shirts and short pants, and much higher levels of the pesticide absorbed dose were found in the case of short clothing (104.45 ± 1.55 µg) in respect to long one (44.82 ± 2.06 µg). This difference was mainly due to the length of pants

rather than sleeves. In fact, in the case of short sleeves, when long pants were used, a mean TAD of $52.32\pm1.36 \ \mu g$ was found, much lower in respect to 142.79 $\ \mu g$ (median) obtained in the case of short pants.

The influence of using different application techniques on pesticide absorption was investigated independently from the amount of pesticides used in the day of sampling, by normalizing the measured TAD in respect to the amounts of pesticides handled. The data were then divided into subdistributions, according to the adopted application technique. The obtained results are reported in Table I. The influence of mixing techniques on absorption was also investigated, but no difference in absorbed doses of pesticides was found.

DISCUSSION

The results of this study reveal that on the 18 farms investigated, farm workers do not use any personal protective equipment when handling pesticides, and either do not know, or completely neglect, the potential risks related to an incorrect disposal of pesticide containers. In particular, the use of "pails", the absence of PPEs and the disposal of pesticide containers without any particular caution, showed a worrying situation, suggesting that workers were not aware of the risks.

This observation is confirmed by the obtained findings concerning the measured pesticide total absorbed dose (TAD). As expected, the pesticide total absorbed dose was mainly due to the cutaneous exposure rather than to inhaling, because of pesticide low vapour pressure, leading to low airborne pesticide concentrations (6).

The importance of clothing, especially when no PPEs were used, was confirmed by the amount of pesticides found on pads located on particular zones, such as forearms, shin-bones and calves (data not shown). When examined workers wore long sleeves, forearms were "protected", and the mean amount of pesticide found on the corresponding pad was lower than the one found in the case of short sleeves, with a significant difference, confirmed by the *t*-test (t = 4.379, p = 0.001). The same findings were obtained for short and long pants with regard to pads located on shin-bones and calves.

The results obtained on the influence on TAD due to the use of different techniques/machines during pesticide mixing/loading and application on fields, indicate that absorption was higher when a backpack was used. It must be noted that only few workers were in charge of preparing formulates without applying them on fields, therefore the TADs reported in Table I actually also took into account the pesticide absorption during the mixing step.

In order to evaluate the health risk of the investigated workers, the pesticide total absorbed

doses were compared with the Acceptable Daily Intakes (ADIs). Table II reports the obtained mean results in respect to the absorption of each specific investigated pesticide, together with the corresponding ADIs. The same findings, calculated on individuals, show that 20% of the measured absorbed doses exceeded ADI values. This is possibly due to the fact that fortunately, tomato farms in Southern Italy are characterized by low extension fields, so the time of exposure to pesticides during the application on fields is limited, and, as a consequence, despite the general working conditions described above, the amount of pesticides actually absorbed by workers is low.

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