La Medicina del Lavoro

Med Lav 2015; *106*, 4: 294-315

Dermal exposure and risk assessment of tebuconazole applicators in vineyards

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Key words

Conazoles; dermal dosimetry; pesticide application

Parole chiave

Conazoli; dose cutanea; applicazione di pesticidi

Summary

Introduction: *Models used in the pre-marketing evaluation do not cover all work scenarios and may over- or underestimate exposure.* **Objectives:** *Uncertainties present in the extrapolation from pre-marketing to the post-marketing warrant exposure and risk assessment in real-life working conditions.* **Methods:** *Seven vineyard pesticide applicators were followed for a total of 12 work-days. A data collection sheet was developed specifically for this study. Workers' body exposure, hands, and head exposure were measured. Tebuconazole was analyzed using LC-MS/MS.* **Results:** *Median potential and actual body exposures were 22.41 mg/kg and 0.49 mg/kg of active substance applied, respectively. The median protection factor provided by the coverall was 98% (range: 90-99%). Hand exposure was responsible for 61% of total actual exposure, and was reduced by more than 50% in workers using gloves. The German Model underestimated the exposure in one work-day, and grossly overestimated it in 3 work-days.* **Conclusions:** *High levels of potential body exposure were efficiently controlled by the cotton coverall. Use of personal protective devices, especially chemically-resistant gloves and head cover is the main determinant of skin protection. Field studies on pesticide exposure in real-life conditions and development of methods and tools for easier risk assessment are necessary to complement and confirm the risk assessment done in the authorization process.*

Riassunto

«Esposizione cutanea ed asseverazione del rischio nell'applicazione di tebuconazolo in viticoltura». **Introduzione:** *I modelli predittivi che sono impiegati per la valutazione in sede autorizzativa (pre-marketing) del rischio chimico da pesticidi non considerano tutti i possibili scenari e possono, di conseguenza, condurre a una sovra-stima o*

Pervenuto il 14.5.2015 - Revisione pervenuta il 12.6.2015 - Accettato il 15.6.2015

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This research has been supported by the grant of the 7th Framework European Union Project ACROPOLIS (grant agreement KBBE- 245163)

a una sotto-stima della reale esposizione. **Obiettivi:** *I fattori di incertezza che ad essa si sommano nell'estrapolazione dagli scenari pre-marketing all'uso in campo sono tali da rendere necessaria la valutazione del rischio tramite misura diretta nelle reali condizioni di impiego.* **Metodi:** *Sette viticoltori che hanno applicato il fungicida tebuconazolo sono stati esaminati per un totale di 12 giorni-uomo, attraverso la misura dell'esposizione cutanea sul corpo, sulle mani e sul capo. Le informazioni complementari sono state raccolte con un questionario specificamente elaborato. Il tebuconazolo è stato misurato con un sensibile metodo che impiega la cromatografia liquida ad elevata prestazione accoppiata alla spettrometria di massa tandem.* **Risultati:** *I livelli di esposizione potenziale e attuale sono risultati rispettivamente pari a 22.41 e a 0.49 mg/kg (valori mediani, milligrammi per kg di sostanza attiva applicata). Il valore mediano del fattore di protezione garantito dal vestiario indossato è risultato pari al 98% (90-99%). L'esposizione sulle mani è risultata contribuire per il 61% circa all'esposizione complessiva, e l'uso dei guanti ha contribuito a ridurla di oltre la metà. I calcoli predittivi svolti attraverso il German Model hanno condotto in 3/12 giornate-uomo a una sovra-stima rispetto alla reale esposizione.* **Conclusioni:** *L'uso di presidi personali di protezione, in particolare di guanti resistenti agli agenti chimici e di un copricapo sono risultati i principali determinanti responsabili per la protezione dall'eccesiva esposizione cutanea. È risultato evidente che, a complemento e a conferma dell'esito delle valutazioni condotte in sede autorizzativa, sono tuttora necessari sia studi in campo sia un affinamento di metodi semplificati per la valutazione del rischio da applicazione di pesticidi in agricoltura.*

Introduction

Cultivation of vines and winemaking is an agricultural activity that is both an important cultural tradition and a source of income for farmers, their families, as well as market-oriented estates. Grapevine is a delicate crop that can be infested by many pests, among which are pathogenic molds and fungi. In this scenario, fungicides are among the most frequently used PPPs in vineyards. Conazoles are a very selective class of fungicides developed as inhibitors of fungal sterol 14 -demethylase, and they are currently among the most frequently used compounds and in this class tebuconazole (TEB) represents one of the most widely employed active ingredients.

Since pre-marketing assessment of TEB identified safe uses under controlled application, the compound was authorized for the use in European Union. In laboratory animals, TEB can cause malformations (10, 24) and interferes with steroid biosynthesis (29, 41, 54), however, at doses higher than those used to set the reference values (EFSA 2008).

Risk assessment for pesticide operators, workers, residents and bystanders is a necessary step defined by the European Union (EU) regulation (7, 16) for authorization of the use of any active substance. For this purpose, models such as the German model (34), EUROPOEM (51) and PHED (42), have been widely used. The first two, used in EU, are based on field studies conducted for the most part in the 90s in northern European conditions. They estimate workers' exposure (*i.e.*: dermal deposition) based on a set of variables such as *e.g.* the amount of active substance used, the area treated, and the use of personal protective equipment in the mixing and loading and/or application phase. The assessed exposure is used to estimate the internal dose (kg/body weight), using a dermal absorption coefficient usually derived from studies in animals (in vitro and/or in vivo) or with in vitro human skin. The estimated absorbed dose is then compared to the Acceptable Operator Exposure Level (AOEL), which is the maximum amount of active substance to which an operator may be exposed without any adverse health effects. If the absorbed dose is lower, the active substance is authorized. The regulation of the EU Parliament (16) also expects the regulators and risk assessors who authorize pesticides to take into account the possible differences in environmental conditions and application technique between different zones of EU. Some field studies on pesticide exposure and risk have shown that the models can under- and overestimate exposure (36, 45, 49), as well as that they do not cover all of the work scenarios, such as use of closed tractors, and not even all the typical phases of work with pesticides, such as maintenance and cleaning of the equipment (9).

In our previous work we reported basic elements for the bio-monitoring of exposure to TEB in vineyard workers (20). TEB-OH was proposed as a representative biomarker of TEB exposure, and the best sampling time was work-shift, as well as post-24 hours, based on the good correlation between the urine biomarker levels and the actual dermal exposure. However, the lack of biological health-based limit values, even for pesticides with well-known metabolites, asks for risk assessment based on personal exposure monitoring.

To perform risk assessment in the post marketing phase, it is necessary to take in due account the fact that in the open field the major route of exposure is the skin (2, 19). In addition, the limit value set in the authorization process is the AOEL, which is an internal dose, and the risk assessment can be carried out only by conducting dermal exposure studies. These field studies may also be used for the development of user-friendly tools for exposure and risk assessment in the field (9). Other characteristics of the scenario are the modality of work (4, 8) and the use of personal protective devices (PPDs), most importantly hand protection (5, 27). Therefore, it is necessary to evaluate the factors that, together with PPDs, influence the exposure of the skin.

The importance of azole fungicides in crop protection (17) justifies the need of carrying out exposure and risk assessment in actual working conditions. This study was designed to assess the workers' dermal exposure to TEB and to collect and explore potential determinants of pesticide exposure during routine application in vineyards. Measured dermal exposure was then compared with that estimated by the German model (34). Furthermore, protection offered by the cotton coverall in preventing dermal exposure to pesticides was evaluated.

Methods

The setting

The study was run from May to July 2011 in the area of Monferrato, Piedmont, Northern Italy. Monferrato is a world-famous wine-producing area, where the local cultivars are the source of commercially prized wine brands. Due to the hilly landscape, small vineyards, ranging from 2 up to 20 hectares are most common and their uphill laying and irregular size command the use of small, mainly open-cockpit tractors for towing small-volume spraying tanks and for manual spraying of smaller or physically unattainable garden vineyards. Seven study subjects (workers) were recruited based on their use of TEB and willingness to participate in our study. The workers were asked to avoid changing their normal work-day routine, and were offered feedback on personal exposure and risk assessment, and suggestions to improve their work routine. It was required that they do not use any conazole two weeks prior to our monitoring. The weather conditions were always standard, with little or no wind and without any rain.

This study was performed within the EU-funded ACROPOLIS (Aggregate and Cumulative Risk Of Pesticides: an On-Line Integrated Strategy) research project (1). All study participants read and signed the informed consent form approved, together with the study plan, by the Ethical Committee of the University of Milan.

Study protocol

The study protocol is defined by two coordinated levels of data collection:

1) Data collection sheet consisting of questions regarding the characteristics of the farm, farmer, and the work-day;

2) Assessment of potential and actual dermal exposure, i.e. monitoring head, body, and hands exposure during all phases of work with pesticides;

Data collection sheet

Based on our previous studies, and published literature search, a data collection sheet was developed to collect determinants and modifiers of exposure during the investigated work-days. It was divided into several parts including: information about the enterprise, the worker, each working phase (mixing and loading, application, cleaning and maintenance of machineries), and the use of personal protective devices during each phase of the work. Trained members of our research team filled in the questionnaire. A version in English of the data collection sheet used in this study is reported as Supplementary material S1.

Personal dermal exposure monitoring

Measurements of personal dermal exposure were used to quantify potential and actual dermal exposure of workers on each work-day. Potential dermal exposure (in brief potential exposure) is defined as the amount of pesticide coming into contact with the working clothes and personal protective devices (32, 47). Actual dermal exposure (in brief actual exposure) is defined as the amount of pesticide coming into contact with the workers' skin, available for absorption (32, 47)

On each application day the farmer wore a standardized outfit supplied by the investigators and composed of underwear (a cotton t-shirt and cotton boxers), a cotton coverall as the working suit and a hospital-type non-woven fabric head cover. Dermal exposure assessment was performed according to the Organization for Economic Co-operation and Development guidelines (39).

To collect samples for hand exposure assessment, farmers were required to notify a study team member when they wished to wash their hands, so that the sampling procedure could be performed before. Their hands were washed with a total volume of 100 mL of a 20% v/v mixture of isopropyl alcohol in water, which was poured in four to five aliquots on the subject's hands and collected in an underneath basin. The hand wash was also performed at the end of work activities, just before the worker disrobed.

The farmers used their own gloves, therefore their contamination (potential hand exposure) was not taken into consideration since their possible prior contamination with pesticide residues or permeability could not be assessed in quantitative terms.

At the end of the work-day, the farmer disrobed the work outfit, which was cut on-site by the field investigators. The coverall was cut in 12 sections, eight from the four limbs and four from the torso, the underwear t-shirt was cut in 2 sections and the boxers were taken as a whole. An additional sample was obtained from the head cover.

For each work-day 12 coverall cuts, 3 underwear sections, one head cover, one to five hand wash samples were collected and analyzed, for a total number exceeding 230 specimens for the entire study. All specimens were kept at the temperature of +4° in a dark place before shipping to the laboratory, where they were processed and the analytical samples were frozen until the analysis.

Sample preparation and measurement

Specimens were coded before the preparation and analysis were performed. TEB on dermal samplers was determined after desorption by an aqueous/alcoholic solution in the presence of tebuconazole-D6 as internal standard, by liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS).

Coverall and underwear. The sections of the coverall/underwear or the head cover were individually stored into food-grade polyethylene bags at the moment of cutting. Individual bags were weighted to obtain the net weight of canvas. A desorption solution of aqueous methanol (25% v/v) containing tebuconazole-D6 (Dr. Ehrenstorfer, LabService, Anzola Emila, Italy) at the concentration of 100 µg/L was prepared. For every 20 g of fabric a 100 ml volume of desorption solution was added and desorption was operated shaking the samples for 2 h at room temperature. The recovery of the procedure, estimated spiking 10 and 100 µg of TEB to each sample, ranged from 82 to 111% (CV% 6.9).

Handwash. The handwash liquid (about 100 mL) was spiked with tebuconazole-D6 to a final concentration of 100 µg/L.

Analysis. A sub-sample of each solution was filtered and analyzed by a high performance liquid chromatography system (Surveyor, Thermo Scientific, Rodano, Italy) equipped with a Betasil C18 column (150 mm length, 2.1 mm internal diameter and 5 m particle size; Thermo Scientific, Rodano, Italy) kept at room temperature, using a isocratic mixture of aqueous formic acid (0.5%) and methanol (30:70) at 0.25 ml/min as eluent. The liquid chromatograph was interfaced with a LC-MS/MS (TSQ Quantum Access, Thermo Scientific, Rodano, Italy) equipped with a heated-electro spray ionization source. The ionization source parameters were: spray voltage 4500 V, ion transfer tube temperature 350°C, vaporization temperature 300°C, nitrogen as sheath gas and auxiliary gas operating at the pressure of 50 and 5 units (arbitrary scale), tube lens offset 76 V. Collision-inducted dissociation was performed using Ar as the collision gas at a pressure of 1.5 mTorr. TEB and tebuconazole-D6 were detected in the positive ion mode and quantification was based on multiple reaction monitoring (MRM) following the transition m/z 308 → 70 + 308 → 25 + 308 → 151 for TEB and m/z 314 → 72 + 314 → 25 + 314 → 154 for TEB-D6. Retention times were 10.32 min and 10.17 min, respectively, for TEB and tebuconazole-D6. The method had a precision of less than 10%, evaluated as the coefficient of variation, with accuracy between 95 and 103%. The limits of quantification were 0.6 μg/L for TEB in the coverall, underwear or head cover solutions and 1.1 μg/L for TEB in hand-wash solutions.

Risk assessment of dermal exposure

The absorbed dose (mg/kg body weight) was estimated by multiplying the sum of actual body, head and hand exposure by the dermal absorption factor of Tebuconazole, and then dividing it by the body weight of each worker. Risk assessment was then performed by comparing the absorbed dose to the AOEL, and expressed as the percentage of AOEL (AOEL saturation). Both the dermal absorption factor (13% based on a study in monkeys) and the AOEL (0.03 mg/kg bw) of TEB are defined in the European authorization process, and available in the authorization document (10).

Risk assessment for each worker/work-day was also performed using German model Microsoft Excel® tool downloaded from the United Kingdom Health and Safety Executive (50) website. In this case, also the inhalatory exposure was taken into account, in order to compare field measurements with the scenario assumed by the risk assessors in the authorization phase.

To generalize our results to a group of conazole pesticides commonly used in vineyards, risk assessment was also done for penconazole, triticonazole, ciproconazole, bromuconazole and epoxiconazole, using tebuconazole as a tracer substance (21, 31, 32) and calculating proportional exposure to other conazoles at recommended use rates (40). Data on recommended use rate for vineyards, dermal absorption factor and AOEL for these active substances were retrieved from their European authorization documents (11-15), and are available in Supplementary table 1. This method is based on the same principle as the models currently used for exposure assessment of all active substances in the authorization process, which is based on the use of the AOEL, the dermal absorption coefficient and the use rate.

Data management and statistical analysis

The potential body exposure was calculated as the sum of sampler section exposures, which were measured from the cuts (cut ID from 1 to 12) of the coverall.

$$Potential\ exposure_{body}\ (mg) = \\ = \sum_{coverall\ section=1}^{12} TEB_{i\ coverall\ section}\ (mg)$$

The actual body exposure was calculated from the amount of TEB measured in the t-shirts (sampler section ID 13 and 14) and boxers (sampler section ID 15), plus the extrapolation from these samples to the surface not covered by underwear (see formula below). The extrapolation was based on the body surfaces calculated using the Mosteller formula (37), considering the proportions of body areas of a normal healthy male.

$$Actual\ exposure_{body}(mg) = \sum_{underwear\ section=13}^{15} TEB_{i\ underwear\ section}\ (mg) + \frac{TEB_{boxer}\ (mg)}{boxer\ area\ (dm^2)} \times uncovered\ leg\ area\ (dm^2) + \frac{TEB_{t-shirt}\ (mg)}{t-shirt\ area\ (dm^2)} \times uncovered\ arm\ area\ (dm^2)$$

The actual total exposure was calculated summing actual body exposure with the amount of TEB on hand and head according to the formula:

$$Actual\ exposure_{total}(mg) = Actual\ exposure_{body}\ (mg) + Actual\ exposure_{hand}\ (mg) + Actual\ exposure_{head}(mg)$$

Protection factor is the fraction of pesticide retained by the barrier of the work clothing layer (33), and was calculated as:

$$Protection\ Factor\ (\%) = \frac{Potential\ exposure_{body}}{Potential\ exposure_{body} + Actual\ exposure_{body}} \times 100\%$$

Data management and statistical analyses were performed in custom Microsoft Excel® Worksheets and in the R Language and Environment for Statistical Computing (46, 53). Since the sample was small, and the continuous variables were not normally distributed, medians, minimum and maximum values, as well as non-parametric statistical tests (Mann-Whitney-Wilcoxon test) were used in the description of results and in the statistical analyses.

Results

Study subjects

A total of 7 healthy male workers, all right-handed, were followed during their normal working activities, which include the preparation of the mixture and filling the tank of the tractor-mounted or hand-held sprayer (mixing and loading), spraying the pesticide (application) and in some cases routine after-work cleaning of the equipment (cleaning). Five were independent farmers, one was an employee and the other was an independent specialized hired professional. Three workers worked for 1 day each, three worked for 2 days (two workers for two consecutive days and one other for two non-consecutive days, with a break of three weeks), and one worked for 3 consecutive days. All personal exposure measures were considered as independent, and are reported per work-day. There were a total of 12 work-days, which are chronologically coded from A to L in tables and text. The main relevant characteristics of the subjects are shown in table 1.

The vineyard owners, who supplied real estate maps and authorized photo- and video recording, reported the estate size and position. The brand of TEB fungicide used, its composition and applied amounts were disclosed by the farmers to the investigators in the field based on the official records kept at the estate.

Characteristics of work-days

The work conditions during the examined work-days are reported in table 2. In all work-days when vineyard treatment was performed, meteoro-

Table 1 - Main personal characteristics of the participating farmers

Worker ID	Work-day ID[(1)]	Age (years)	Height (cm)	Weight (kg)	Body Surface [(2)] (dm^2)
1	A, E	49	180	100	250
2	B	50	180	95	238
3	C, D	51	178	91	225
5	F, G, H	40	168	57	133
6	I, J	41	185	90	231
7	K	47	170	78	184
8	L	36	180	90	225
Minimum	-	36	168	57	133
Median	-	47	180	90	225
Maximum	-	51	185	100	250

[(1)] Four workers perform work on more than one work-day, therefore work-days are marked by letters A to L in tables and text

[(2)] Calculated according to Mosteller (37)

Table 2 - Synopsis of application conditions in the examined work-days.

			Application and Cleaning						General working conditions	
Worker ID	Work-day ID	Mixing (n)	Application Mode	Treated Area (ha)	Amount of TEB used (g)	Tank Capacity (L)	Interventions	Cleaning	Conditions of machineries	Total work time (h)
1	A	4	Open tractor	5.0	198.0	400	Yes	Yes	Clean	5
2	B	3	Open tractor	6.0	594.0	600	No	No	Clean	6
3	C	2	Open tractor	2.0	99.0	300	No	No	Clean	5
3	D	3	Hand-held hose	4.0	67.5	300	No	No	Clean	6
1	E	5	Open tractor	6.0	594.0	300	Yes	No	Clean	8
5	F	4	Closed tractor	5.0	148.5	400	No	No	Dirty	10
5	G	3	Closed and open tractor	4.0	148.5	400	No	No	Dirty	9
5	H	1 + 4 (1)	Open tractor and back-pack	2.5	148.5	400 + 16[2]	No	No	Dirty	3
6	I	4	Open tractor	17.0	1,530.0	1400	Yes	Yes	Clean	10
6	J	2	Open tractor	10.0	900.0	1400	Yes	Yes	Clean	10
7	K	4	Open tractor	1.8	117.0	300	No	Yes	Clean	7
8	L	4 (WG)	Closed tractor	3.0	1260.0	800	No	Yes	Clean	8
% positive	-	-	-	-	-	-	33%	42%	-	-
Minimum	-	2	-	1.8	67.5	300	-	-	-	3
Median	-	4	-	4.5	173.3	400	-	-	-	6
Maximum	-	5	-	17.0	1,530.0	1400	-	-	-	10

(WG) Wettable granules (in all other cases the workers used Wettable powder)

(1) Backpack sprayer

logical conditions were deemed adequate by the farmers, with little if any wind or rain, and external temperature and humidity within seasonal variability.

The first phase of every work-day was mixing and loading. This phase was repeated often during the day, depending on the size of the vineyard, the size of the tank, and the application modality. The median number of mixing and loadings was 4 (from 2 to 5). In the majority of cases (11 work-days) the product was in the form of hydro-soluble or wettable powder, while only in 1 occasion (work day L) the worker used wettable granules. All commercial products contained at the same concentration of 4.5% w/w TEB. Dispersible sulphur was added to the sprayed mixture.

The second phase of work was application. On 7 work-days an open tractor, on 2 work-days a closed tractor, on 1 work-day a hand-held sprayer, on 1 work-day a combination of an open tractor and a hand-held sprayer, and on 1 work-day a combination of an open and a closed tractor were used by the farmers. The treated area ranged from 1.8 to 17 hectares per day (the usual unit for acreage in Italy; 1 hectare = 0.01 square kilometer or approximately 4 acres), with a median of 4.5 hectares, depending on the application modality and the nature of the estate's landscape, which ultimately dictates application speed. The workers used a median of 3.85 kg of formulation (range 1.5 - 34 kg). Taking into account the concentration of TEB in the product, the median amount of TEB sprayed during a typical work-day was 173 (range 67-1530) g. The median tank capacity was 400 L (from 16 L for the back-pack sprayer, to 1400 L for a big tractor-mounted sprayer in a larger estate).

Cleaning was performed during five work-days, and consisted in washing the equipment and the interior of the tank with a water hose. In general, the work place and the equipment were considered to be in clean conditions on 9 work-days out of 12. Total work time varied between 3 and 10 h.

Personal Protective Devices

All workers wore cotton coveralls, and underneath white cotton t-shirt and boxers, which were specifically supplied for the investigation. The other personal protective equipment, such as working boots, gloves and masks, was not supplied by the study team, and large differences were noticed among farmers regarding their availability and use. Only 2 workers (5 work-days) wore protective shoes, while 4 workers (5 work-days) wore generic shoes, and one worker wore open shoes (slippers) on 2 work-days. Most of workers (6 out of 7, and 10 work-days out of 12) had work gloves available, but the gloves' material and condition varied. In particular, five workers (5 work-days) wore new professional gloves (neoprene), while there was a worker (2 work-days) who wore no gloves. Six workers had a face mask with a filter available (11 out of 12 workdays). Information regarding the use of personal protective devices is shown in table 3.

Potential and actual dermal exposure, and risk assessment

Table 4 summarizes the potential and actual exposure for each work-day. The median potential body exposure was 6.18 (range 1.68 - 21.50) mg and the median actual body exposure was 0.20 (range 0.01 - 0.80) mg. Median head exposure was 0.10 (range 0.02 - 1.67) mg. The workers washed their hands from 1 to 5 times during the work-day. In hand wash a median level of 0.38 (range 0.11 - 2.02) mg was found. Median total actual exposure was 1.02 (range 0.16 - 3.68) mg. Body exposure contributed to the total actual exposure with a median value of 18%, while the head contributed with 16%, and the hands with 61%. When the amount of active substance used during the work day is taken into account, the median potential body exposure was 22.41 (range 2.72 - 318.57) mg/kg of

Table 3 - Personal protection devices used during the work-days

		Characteristics of Personal Protection Devices (PPDs)					Use of personal protection devices in different phases of work					
							Mixing and Loading		Application		Cleaning of machines	
Worker ID	Work-day ID	Feet protection	Gloves available	Material of gloves	Quality of gloves	Mask available	Gloves	Mask	Gloves	Mask	Gloves	Mask
1	A	Protection shoes	Yes	Neoprene	Used	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	B	Generic closed shoes	Yes	Neoprene	Used	Yes	Yes	Yes	No	No	-	-
3	C	Generic closed shoes	Yes	Neoprene	New	Yes	Yes	Yes	No	Yes	-	-
3	D	Generic closed shoes	Yes	Rubber	Used	Yes	Yes	Yes	No	No	-	-
1	E	Protection shoes	Yes	Neoprene	New	Yes	Yes	Yes	Yes	Yes	-	-
5	F	Protection shoes	Yes	Neoprene	New	Yes	Yes	Yes	No	No	-	-
5	G	Protection shoes	Yes	Neoprene	Used	Yes	Yes	Yes	No	No	-	-
5	H	Protection shoes	Yes	Neoprene	Used	Yes	No	Yes	Yes	Yes	-	-
6	I	Generic open shoes	No	-	-	Yes	No	Yes	No	Yes	No	Yes
6	J	Generic open shoes	No	-	-	Yes	No	Yes	No	Yes	No	Yes
7	K	Generic closed shoes	Yes	Neoprene	New	Yes	Yes	Yes	Yes	Yes	Yes	No
8	L	Generic closed shoes	Yes	Neoprene	New	No	Yes	No	No	No	Yes	No
% positive	-	-	83%	-	-	92%	75%	92%	33%	58%	60%	60%

Table 4 - TEB potential and actual dermal exposures

Worker ID	Work-day ID	Application Mode	TEB potential exposure		TEB actual exposure					
			Body [(1)] mg	Normalized body [(2)] mg/kg of a.s.	Body [(1)] mg (% of total)	Normalized body [(2)] mg/kg of a.s.	Head mg (% of total)	Hands mg (% of total)	Total mg (% of total)	Normalized total [(3)] mg/kg of a.s.
1	A	Open tractor	1.68	8.47	0.05 (19)	0.24	0.02 (8)	0.18 (73)	0.25 (100)	1.25
2	B	Open tractor	6.51	10.96	0.42 (16)	0.71	0.13 (5)	2.02 (79)	2.57 (100)	4.33
3	C	Open tractor	12.56	126.85	0.33 (9)	3.31	1.67 (45)	1.68 (46)	3.68 (100)	37.19
3	D	Hand-held hose	21.50	318.57	0.42 (30)	6.22	0.52 (37)	0.47 (33)	1.41 (100)	20.92
1	E	Open tractor	5.58	9.39	0.61 (71)	1.03	0.10 (11)	0.15 (18)	0.86 (100)	1.45
5	F	Closed tractor	6.20	41.78	0.04 (10)	0.28	0.10 (23)	0.28 (67)	0.42 (100)	2.83
5	G	Closed and open tractor	4.32	29.09	0.07 (21)	0.45	0.07 (22)	0.18 (57)	0.32 (100)	2.13
5	H	Open tractor and back-pack	5.52	37.20	0.06 (5)	0.38	0.10 (9)	1.01 (86)	1.17 (100)	7.87
6	I	Open tractor	10.84	7.08	0.80 (31)	0.52	0.09 (3)	1.66 (66)	2.54 (100)	1.66
6	J	Open tractor	14.15	15.73	0.68 (44)	0.76	0.04 (3)	0.82 (53)	1.54 (100)	1.71
7	K	Open tractor	6.15	52.54	0.01 (2)	0.08	0.45 (80)	0.11 (18)	0.56 (100)	4.80
8	L	Closed tractor	3.43	2.72	0.01 (7)	0.01	0.03 (20)	0.11 (73)	0.16 (100)	0.12
Minimum	-		1.68	2.72	0.01 (2)	0.01	0.02 (3)	0.11 (17)	0.16	0.12
Median	-		6.18	22.41	0.20 (18)	0.49	0.10 (16)	0.38 (61)	1.02	2.48
Maximum	-		21.50	318.57	0.80 (71)	6.22	1.67 (80)	2.02 (86)	3.68	37.19

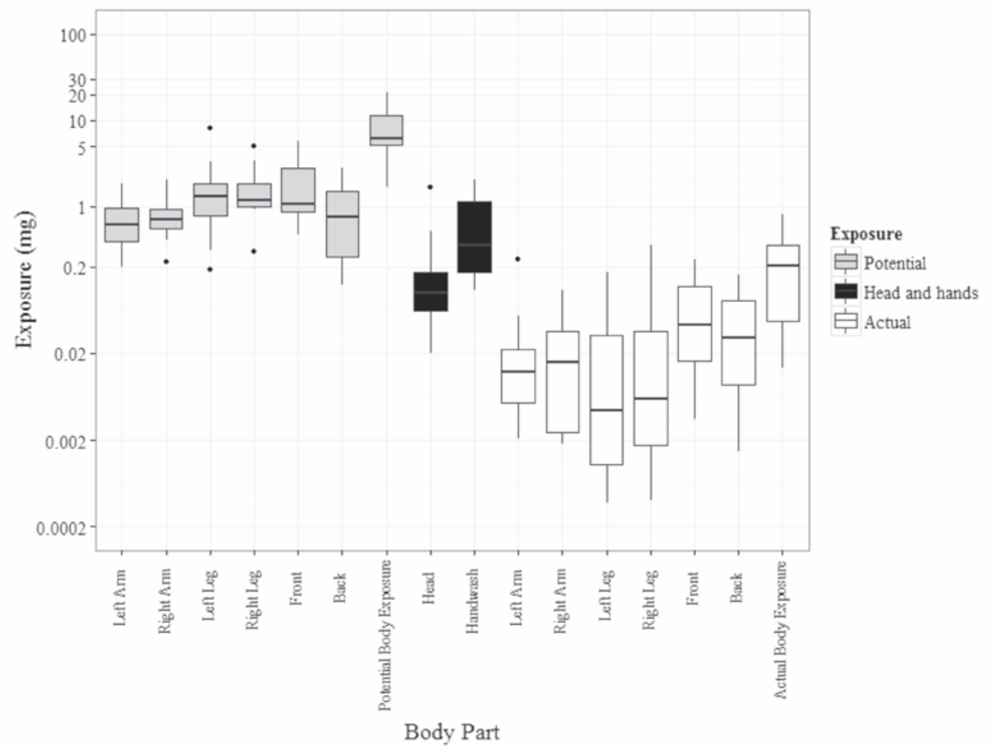
[(1)] Body: torso + limbs (without hand and head exposure)
[(2)] Normalized body: Body exposure (mg) per kilogram of active substance applied during the work-day
[(3)] Normalized total: TEB total actual exposure (mg) per kilogram of active substance applied during the work-day

active substance applied, while the median actual body exposure was 0.49 (range 0.01 - 6.22) mg/kg of active substance applied; by adding head and hands exposure, the median total actual exposure was 2.48 (range 0.12 - 37.19) mg/kg of active substance applied. Cotton coverall provided the workers with a median protection factor of 98% (from 90% to 99%).

Potential and actual exposure of different body parts is shown in figure 1. The potential exposure box plots suggest that the most exposed regions of the body are the legs, followed by the front, back and arms (contamination measured on the clothes). Left and right parts of the body are exposed almost equally, while the front is slightly more exposed than the back. The actual exposure box plots paint a different image, with front and back exposure being the highest, followed by the exposure of the legs and of the arms (contamination measured on the skin).

To explore the differences in the contamination of different body regions independently of their surface, we have standardized the exposure of each cut by its surface area (table 5). On the coverall, the most contaminated regions are the chest, back and the abdomen, with the median contamination of 0.49 mcg/cm^2, 0.37 mcg/cm^2 and 0.36 mcg/cm^2, respectively, followed by the forearms, thighs and shins. The boxers were the most contaminated underwear, followed by the front of the t-shirt and the back of the t-shirt, with contaminations of 0.03, 0.02 and 0.01 mcg/cm^2 respectively.

The spider-plots of figure 2 show a comparison of the distribution of pesticide deposition on the



Arm (left and right: upper arm + forearm; ***Leg*** (left and right): thigh + shin; ***Front*** chest + abdomen; ***Back*** upper back + lower back

Figure 1 - TEB potential and actual exposure by region of the body

coverall of a worker who sprayed from an open tractor (left), and of a worker who used a hand-held sprayer (right). The two plots not only show a much higher median contamination of the worker working with hand-held sprayer, but also a different distribution of exposure.

We explored the influence of the use of gloves on the actual exposure of hands in different phases of work. Figure 3 shows the levels of hand contamination depending on the use of gloves during the two phases known to give the major contribution to the total daily exposure, namely mixing and loading, and application. Although not statistically significant (Mann-Whitney U test, U=21, p=0.21), probably due to the small size of the examined group, the use of gloves, especially during the mixing and loading phase, may lower the exposure of hands by more than 50%.

Table 6 contains the risk assessment information for tebuconazole for each work-day, using the field measures as well as using the German model (34) with different settings (see Materials and Methods). The median AOEL saturation calculated from the field measurements was 4.73% (range 0.76 - 17.38%). Hands had the highest contribution to the overall risk, with a median risk of 2.19%, the body (median 1.20%, range 0.07 - 3.74%) and the head (median 0.57%, range 0.09 - 7.97%) followed. The median risk calculated using the German model was 6.94% (range 1.12 - 77.42%), when using the dermal absorption specified for tebuconazole in the authorization documents (10). In one case (work-day C) the German model underestimated the risk of our worker more than 10 times, while in 3 cases (work-days I, J and L) it overestimated the risk 7 to 25 times.

Table 5 - Contamination (microg) measured on coverall and underwear cuts per area (cm^2)

Cut/Location	Area (1) Median (cm^2)	TEB contamination per area Minimum (mcg/cm^2)	Median (mcg/cm^2)	Maximum (mcg/cm^2)
Chest	1560	0.03	0.49	1.73
Abdomen	1730	0.17	0.36	2.76
Upper and lower back	1960	0.08	0.37	1.39
Left upper arm	980	0.04	0.20	0.98
Right upper arm	960	0.02	0.23	0.98
Left forearm	1180	0.09	0.36	0.92
Right forearm	1320	0.15	0.34	1.08
Limbs	3090	0.01	0.07	0.53
Left thigh	1660	0.09	0.31	1.92
Right thigh	1960	0.08	0.31	0.92
Left shin	2720	0.04	0.32	1.78
Right shin	2480	0.01	0.25	1.37
Head cover (2)	1000	0.01	0.05	0.84
T-shirt front	3940	0.004	0.014	0.058
T-shirt back	4200	0.002	0.007	0.031
Boxers	3640	0.001	0.026	0.043

(1) Coverall and underwear size varied from the international standard S to XXL and was appropriate to workers stature
(2) The head cover was of a standard size for all workers

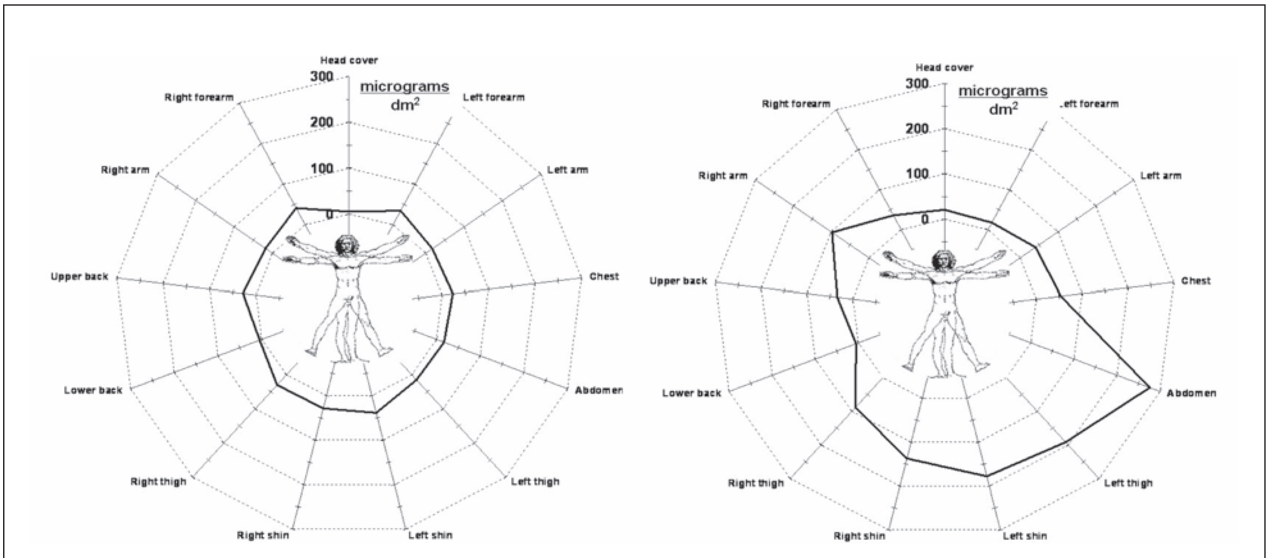


Figure 2 - Spider plot of tebuconazole contamination on farmer's coveralls. (A) one who sprayed from a closed-cocpit tractor; (B) one who sprayed manually from a hose (passing by the left hip) hand-sprayer

Table 7 summarizes the risk assessment estimated for a group of conazole fungicides, considering the ratio between the recommended use rate of tebuconazole and each of the other conazoles in vineyards (see Supplementary table 1). For penconazole, triticonazole and cyproconazole, the median AOEL saturations were 0.35%, 0.51% and 2.99% respectively. For bromuconazole, the median AOEL saturation was 21.76%, and for epoxiconazole the median AOEL saturation was 85.01%. It

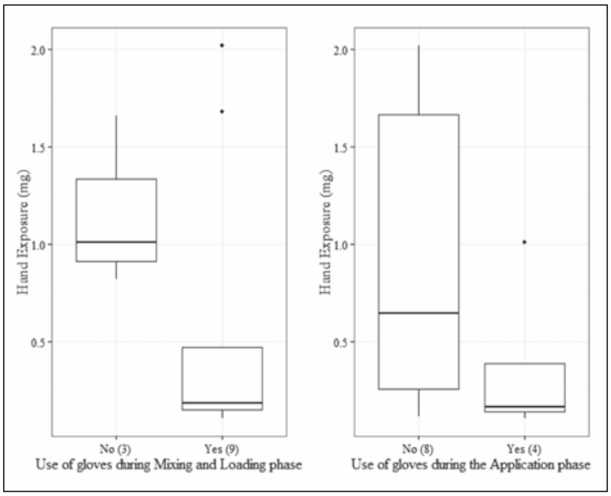


Figure 3 - Hand exposure on the use of gloves during mixing and loading and during application

is worth noting that for all the conazoles, except epoxiconazole, the absorbed dose was lower than the AOEL in all work scenarios. For epoxiconazole, the limit was exceeded on 6 out of 12 work-days, out of which on 4 occasions an open tractor was used, in one occasion a hand-held pressure hose was used, and in one occasion a combination of an open tractor and a back-pack method.

Discussion

Health risk related with pesticide use in small and middle-size farms is rarely assessed during application of a registered commercial product. In this study, we explored the exposure and risk in different scenarios of TEB use, and attempted to shed more light on the characteristics and determinants of exposure during routine pesticide spraying in vineyards.

Work was carried out with hand-held equipment, open and closed tractors, and combinations of tractors and hand-held equipment during the same work-day (see table 2). This is explained by the characteristics of the terrain, as well as the different sizes of vineyards, which ranged from very small to larger ones, and accounts for the differences in working hours recorded in our study. Different working conditions, especially application modalities, are known to entail different levels of

Table 6 - Risk assessment for each work-day for TEB using the field measures and the German Model. Risk is expressed as AOEL saturation (Exposure/AOEL)

Worker ID	Work-day ID	Weight (kg)	Risk Body (AOEL Saturation)	Risk Head (AOEL Saturation)	Risk Hands (AOEL Saturation)	Risk Tebuconazole[(1)] (AOEL Saturation)	German Model (AOEL Saturation)
1	A	100	0.22%	0.09%	0.78%	1.09%	1.89%
2	B	95	3.74%	0.59%	9.21%	13.55%	9.58%
3	C	91	1.41%	7.97%	8.00%	17.38%	1.37%
3	D	91	1.45%	2.48%	2.24%	6.17%	8.20%
1	E	100	1.34%	0.43%	0.65%	2.42%	5.67%
5	F	57	0.38%	0.75%	2.13%	3.26%	2.39%
5	G	57	1.06%	0.54%	1.37%	2.97%	2.39%
5	H	57	0.29%	0.78%	7.68%	8.75%	10.66%
6	I	90	2.81%	0.42%	7.99%	11.22%	77.42%
6	J	90	3.09%	0.21%	3.94%	7.24%	45.54%
7	K	78	0.10%	2.48%	0.59%	3.17%	1.12%
8	L	90	0.07%	0.15%	0.55%	0.76%	24.31%
Min		57	0.07%	0.09%	0.55%	0.76%	1.12%
Median		90	1.20%	0.57%	2.19%	4.72%	6.94%
Max		100	3.74%	7.97%	9.21%	17.38%	77.42%

(1) Sum of body, head and hands risk

Table 7 - Risk assessment for each work-day for TEB and characteristic conazoles registered in the European Union

Worker ID	Work-Day ID	Application mode	Tebuconazole (AOEL Saturation)	Penconazole (AOEL) Saturation	Triticonazole (AOEL Saturation)	Cyproconazole (AOEL Saturation)	Bromuconazole (AOEL Saturation)	Epoxiconazole (AOEL Saturation)
1	A	Open tractor	1.09%	0.08%	0.12%	0.69%	5.03%	19.65%
2	B	Open tractor	13.55%	1.02%	1.48%	8.60%	62.54%	244.29%
3	C	Open tractor	17.38%	1.30%	1.89%	11.03%	80.22%	313.34%
3	D	Hand-held hose	6.17%	0.46%	0.67%	3.92%	28.48%	111.24%
1	E	Open tractor	2.42%	0.18%	0.26%	1.54%	11.17%	43.63%
5	F	Closed tractor	3.26%	0.24%	0.36%	2.07%	15.05%	58.77%
5	G	Closed and open tractor	2.97%	0.22%	0.32%	1.88%	13.71%	53.55%
5	H	Open tractor and back-pack	8.75%	0.66%	0.95%	5.55%	40.38%	157.75%
6	I	Open tractor	11.22%	0.84%	1.22%	7.12%	51.78%	202.28%
6	J	Open tractor	7.24%	0.54%	0.79%	4.59%	33.42%	130.53%
7	K	Open tractor	3.17%	0.24%	0.35%	2.01%	14.63%	57.15%
8	L	Closed tractor	0.76%	0.06%	0.08%	0.48%	3.51%	13.70%
Min	-	-	0.76%	0.06%	0.08%	0.48%	3.51%	13.70%
Median	-	-	4.72%	0.35%	0.51%	2.99%	21.76%	85.01%
Max	-	-	17.38%	1.30%	1.89%	11.03%	80.22%	313.34%

exposure to workers, being highest for hand-held and lowest for the use of air-conditioned tractor with carbon filters (23, 38). Our data, although obtained from a small sample population, confirm this difference in both absolute values and the distribution of exposure (figure 2). The use of hand-held equipment led to a much higher contamination of lower parts of the body (abdomen, back, legs), and especially a peak on the left side of our right-handed worker. This was likely because the worker had the hose of the sprayer passing and in close contact with the left part of his body. The spider graphs (figure 2) and the box-plots showing how exposure depends on the body region (figure 1) may be a good risk communication method when giving feedback to the workers regarding their work practices.

The potential and actual body exposure of our workers (table 4) fall in the same range of those measured in open-field pesticide applicators exposed to isoproturon (30), procymidone (3) and terbuthylazine (52). The median potential body exposure of our workers is lower than the minimal potential body exposure reported by the only study available for TEB (49), but the authors explored only the exposure of hand-held applicators in low vineyards. Our sole hand-held applicator had the potential body exposure (normalized per quantity of active substance) higher than their highest exposed subject, but the total actual exposure at the level of their least exposed subject. It should be noted (figure 1) that the highest contribution to the potential exposure was that of the legs, while the highest contribution to actual exposure was that of the front and back. Assuming that the cotton coverall worn by the workers provide the same level of protection in all body regions, these data may be explained by the closest contact of clothes with skin in the trunk zone, which is not the case with the clothes over arms and legs. To our knowledge, this is the first report underlying this point. The cotton coverall used by the workers provided them with a high protection factor (98%), comparable to that provided by cotton and Resist Spills® coveralls during hand-held application of TEB (49). Nevertheless, the protection provided by the cotton coveralls in our study is higher than that reported by other authors for standard cotton garments (reportedly 73% to 88%) and in the range of the protection provided by Tyvek® coveralls (2, 18, 52).

The use of personal protective equipment is one of the most important determinants of protection in open field farming. Our study showed a high variability in the access to the basic personal protective equipment (shoes, gloves and masks), and an even larger variability in their use in the different work phases (table 3). It is worth noting that one of our workers (work-days I and J) used slippers during his two work-days, but since our study was not designed to measure feet exposure, the contribution of this determinant cannot be quantified. Most workers did not have new and adequate (chemically protective neoprene) gloves. The minimum set of personal protective devices that the worker must wear when handling pesticides and the interpretation of the label's instructions most commonly is left to the workers and/or their employers.

Several studies have identified hand exposure as the main contributor to the total skin exposure (2, 5, 30), and our study has confirmed this finding by showing a median contribution of 61% to total actual exposure. However, this is much higher than the contribution of hand exposure in the only available study on hand-held TEB application (49), where the contribution of hands to total actual dermal exposure was 4%. In particular, workers who use gloves during mixing and loading and application phases have much lower hand exposure (figure 3). However it should be noted that re-use of gloves is known to increase the exposure of the hands, because of damages to the gloves or their internal contamination, and because workers often do not wash their hands after removing the gloves and wearing them again (6, 22, 25, 35).

Head exposure contribution is mentioned only rarely in studies of pesticide exposure. This route of exposure was identified as one of the major contributors of actual dermal exposure by a study of TEB exposure in hand-held applicators (49). In our study, head exposure gave a median contribution of 16% to total actual exposure (table 4). This can present a significant addition to the absorbed dose, although the physical barrier represented by the hair to penetration through the scalp should be taken into account (43). In fact, a lower absorption in this region was indicated by the poor correlation between head exposure and urinary excretion of metabolites in our study (20).

Looking at the difference of hand contamination between the workers who did or did not use gloves in the mixing and loading and in the application phases, and considering the contribution of head exposure to the total actual exposure, it can be concluded that hand and head protection should always be worn while working with pesticides. New neoprene gloves on the hands and a disposable cover as head protection may be the most efficient solution.

In this study, we also used the German model for Risk Assessment of TEB exposure. The results (table 6) suggest that the pre-authorization models underestimate the fraction of active substance which reaches the skin when small amounts are used. On the other side, the German model overestimated this fraction when large amounts of pesticides were used. The first scenario reflects the case of small and family-based farms, which are also traditionally less covered by health and risk assessment services while the second describes the case of large farms, that often use more protected tractors and more efficient PPDs. Other authors have described similar findings (45, 48), and some tried to explain it by a high overestimation of head and respiratory exposure by the German model (49). In our study, we have considered the inhalatory exposure to be negligible, based on the findings of other authors (2, 19), and this might represent one part of the explanation of this difference. It should be noted that the models are based on exposure studies that show a high variability (1-3 orders of magnitude) and usually take the 50-75th percentiles because variability is averaged over the 90 days of exposure on which the AOEL is based. The variability of our study falls in the same interval. The good correlation of excreted TEB metabolite and actual skin exposure measured in our study (20) supports the confidence we have in the exposure and risk assessment presented.

It was possible to use the information from the authorization process of Penconazole, Triticonazole, Cyproconazole, Bromuconazole and Epoxyconazole, to perform theoretical risk assessment, considering Tebuconazole as a tracer. Only for Epoxiconazole, in 6 out of 12 work-days, the exposure would have been higher than the AOEL. This information could be explained by the low AOEL of this active substance (0.008 mg/kg bw/day) and higher dermal absorption ratio (50%) (44), compared to Tebuconazole (10). It is necessary to underline the importance of defining accurate dermal absorption coefficients in the authorization process, since it greatly influences the risk assessment results. E.g. median urine metabolite excretion rate in our study indicates an absorption coefficient of 17 - 21% for TEB (20), that is consistent with official authorization documents, that put it between 13% and 37% (10, 26).

This field study has some limitations, most of which are intrinsic to the unavoidable compromise between the extent of information to be gained and the interference of the study on the farmers' daily work. Although we tried to minimize it by asking farmers not to change their usual working conditions, it is possible that the workers will have somewhat modified their practices because of the presence of external observers. For example, unattended farmers would have most likely worn shirts and jeans, or a Tyvek® coverall over their normal clothes, as we observed in previous studies (48, 52). These facts could have resulted in an exposure lower than normally occurring.

Another limitation of the present study is the relatively small number of participants, but this is a common condition, especially in small-size enterprises, where the standard is having 2 to 10 participants working on several work-days (3, 48, 49, 52). Additionally, it is difficult to account for the potential error in the calculation of the actual skin exposure based on the extrapolation from t-shirts and boxers to the uncovered skin area, but the above mentioned correlation between the actual skin exposure and urine metabolite levels (20) reduces the probability of this kind of error.

We can conclude from the present study that our farmers had a relatively high potential body exposure to TEB, but the body PPDs (cotton coverall) was very efficient in preventing contamination of the skin. Hand and head exposures account for the major part of actual dermal exposure; therefore wearing gloves during the mixing and loading and the application phase can reduce hand exposure by more than 50%. Educational and preventive actions should be focused on better use of personal protective equipment, especially chemically-resistant gloves and head protection, since these interventions could greatly increase the safety of workers (28).

Considering the differences between the results of this study and the predictions risk by the German model, as well as the results of risk assessment for epoxiconazole in suggested use-rate scenario, it is conceivable that further field studies on pesticide exposure and risk assessment in real-life field conditions are necessary to complement and confirm the risk assessment done in the authorization process, and that methods and tools for easier risk assessment in the field need to be developed.

No potential conflict of interest relevant to this article was reported

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Supplementary Information (S1): Data collection sheet used for the recording of field conditions during the study of exposure to TEB. Version in English language

Company Information Company ID:______________

Name of the company: ____________________________________

Address: ____________________________________

Town: ____________________________________

Province: ____________________________________

Region: ____________________________

Company stamp

Name of the responsible person in the company: ____________________________________

Contact phone: ____________________________ or ____________________________

Total surface under fields: __________ (ha) Surface of vineyards: _________ (ha)

Number of workers engaged in spraying pesticides: ______________

Comments: ____

____

____

Worker information Worker ID: ______________

Last name: ______________________________ First name: ______________________________

Date of birth: _ _ / _ _ / _ _ _ _

Telephone number: _______________________ Mobile phone number: _____________________

Has the worker signed the consent form?

1) YES **2) NO**

Province of birth (Country if foreigner): __________ Provine of living: ___________________

Region of living: ______________________

Sex: ________ Age: ___________ Height: ______ (cm) Weight: _______ (kg)

Primary hand: 1) Right 2) Left

Years of education (school): _______________

Knowledge of Italian (only foreigners):
1) Bad 2) Medium 3) Good

Smoking status:
1) Non smoker 2) Smoker 3) Ex-smoker

Does he consume alcohol regurarly:
1) Yes 2) No

Does he suffer from any chronic disease:
1) Yes 2) No

Does he have any dermatological problems:
1) Yes 2) No

Does he use any dermatological medications:
1) Yes 2) No

Local or systemic?
1) Local 2) Systemic

Which medication? ________________________

Does he spray pesticides in some other company?
1) Yes 2) No

When is the last time he sprayed pesticides?
Date: _ _ / _ _ / _ _ _ _
How many days ago? _____________

Working day information Working day ID: _____________

Date of work: _ _ / _ _ / _ _ _ _

Study name: _________________________

Name of the product: _______________________

Active substance: __________________________

Concentration of the active substance in the product:

_____________________________ (%)

Formulation:
1) Powder
2) Granules
3) Bags
4) Liquid

Size of the tank: __________ (litres or hectolitres)

Amount of the product per ONE tank: _______ (kg)

Amount of product per hectare: ________ (g/ha)

Amount of mixture per hectare: _________ (l/ha)

Wind:
1) No wind 2) Light wind 3) Strong wind

Job title of the worker (usual job):

____

Phases of work that he does (circle all):
1) Mixing and loading
2) Spraying (Application)
3) Cleaning and maintenance
4) Re-entry (After how many days? __________)

Personal protective devices

Body protection:
1) None (normal clothes)
2) Mono-use coverall
3) Multy-use coverall

Coveral material:
1) Cotton
2) Tyvek
3) __________________

What is he wearing under the coverall:
1) Normal clothes 2) Underwear

Does he have gloves:
1) Yes 2) No

Material of the gloves:
1) Latex 2) Rubber 3) Neoprene (profess.)

Condition of the gloves:
1) Used 2) New

What does he wear on his feet:
1) Normal shoes 2) Boots 3) Protective shoes (antiinfor.)

Inhalatory protection (mask):
1) None 2) Paper 3) Filter

Head protection:
1) None 2) Hat 3) Hood of the coverall

Personal protective devices in different phases of work (check):

Phase of work	Coverall	Gloves	Mask	Head prot.
Mix and load				
Application				
Maintenance				

Comments:
____
____
____
____
____
____
____
____

Mixing and loading

Where is mixing done?
1) Pre-mixture container 2) Directly in the tank

Number of mixing and loading for that working day:
____

Avarage time for a mixing and loading:
_______(min)

Comments:
____
____
____
____
____

Did any incidents happen during mixing and loading (e.g. splash or spill)?
1) Yes 2) No

Application

Application mode:
1) Hand-sprayer 2) Tractor sprayer

How old is the sprayer equipment? _______ (years)

How old is the tractor (if any)? _______ (years)

Sprayer type:
1) Atomisator
2) Nebulisator
3) Sprayer withut air assistance
4) Sprayer with air assistance
5) Backpack pump

Culture type:
1) Herb 2)Tree

Distance between rows: _____________ (m)

Area treated during the day: ________________ (ha)

Liters of mixture per hectare: ___________ (l/ha)

Number of applications during the day? _________

Avarage duration of one application: ________(min)

Total duration of application during the day? ___ (h)

Working pressure: __________ (bar)

Did the worker exit the tractor during application?
1) Yes 2) No

Did the worker spray on himself or the tractor?
1) Yes 2) No

Did the tractor have problems during the spraying?
1) Yes 2) No

How many times? ______________

How long (avarage) did it last? ____________(min)

Comments:

Incidents druing application? 1) Yes 2) No	______________________________ ______________________________ ______________________________ ______________________________ ______________________________
Maintenance	
Does the worker wash the tank after the treatment? 1) Yes 2) No	How much time? ____________ (min)
Does the worker wash the tractor after the treatment? 1) Yes 2) No	How much time? ____________ (min)
Where does the water go? 1) Ground 2) Container	Comments: ______________________________
Incidents during this phase? 1) Yes 2) No	______________________________ ______________________________ ______________________________ ______________________________
Reduction factors	
Type of tractor: 1) Open 2) Closed 3) Closed with filters	Are filters changed reguralrly (every 2000 hours)? 1) Yes 2) No
Is the tractor and the sprayer maintained regularly? 1) Yes 2) No	How many years of experience does the worker have? ______________ (years)
What kind of education (diploma) does he have? ______________________________	Does he have any kind of agricultural education? 1) Yes 2) No
Does he have the licence to spray pesticides? 1) Yes 2) No	Comments: ______________________________
How would he rate his skill (1 = bad; 10 = great)? _______	______________________________ ______________________________ ______________________________
How would he rate the toxicity of the substance (1-10)? _______	______________________________ ______________________________
How would he rate his exposure of the day (1-10)? _______	

Supplementary Information (S2)

Supplementary Table 1. Use rate, AOEL and dermal absorption of tebuconazole, penconazole, triticonazole, ciproconazole, bromuconazole and epoxiconazole (from the authorisation process).

Active substance	Use rate (g/ha)	AOEL (mg/kg bw/day)	Dermal absorption (%)
Tebuconazole	100	0.030	13
Penconazole	25-40	0.030	1-5
Triticonazole	9-12.5	0.025	11
Ciproconazole	100	0.020	1-10
Bromuconazole	200	0.025	5-45
Epoxiconazole	125	0.008	50