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ORIGINAL PAPER

Levels of Ethylenethiourea (u-ETU) in a Population Living Near Vineyards

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Abstract

Dithiocarbamates (DTC) are the most commonly used synthetic fungicides in the Prosecco District (PD) of Treviso. The DTC biomarker is urinary ethylenethiourea (u-ETU). The aims of this biomonitoring study are to describe the level of u-ETU in residents in PD and to identify the factors associated with DTC exposure. In 2012 (baseline), we randomly extracted data for 126 children (3–5 years) and their families (260 adults), who were resident in 8 municipalities of PD and not occupationally exposed to DTC, from the local health database. For each subject, we obtained urine samples and socio-demographic, lifestyle and dietary information. In 2014 (after intervention), we evaluated the possible changes in u-ETU in 54 adults and 55 children at high risk of DTC exposure. The median baseline u-ETU was 0.35 µg/L. Approximately 5% of the samples had a u-ETU concentration > 5 µg/L. No u-ETU concentration exceeded 21 µg/L. Determinants of a higher u-ETU concentration were wine consumption (OR 2.04) and personal use of pesticides (OR 2.70) for adults; and living within 30 m from a vineyard (OR 9.51) and the pesticides use in the family (OR 6.25) for children. A significant u-ETU reduction in 49 adults and 25 children was observed from baseline to after intervention ($p = 0.01$). Wine consumption and production and DTC use in gardening influenced u-ETU concentrations in this population, although the levels were relatively low. The reduction in u-ETU from baseline to time after intervention probably reflects the effects of public health interventions.

Keywords Dithiocarbamates · Urinary · General population · Biomonitoring · Exposure · Risk factors

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Introduction

Dithiocarbamates (DTC) are organosulfur compounds widely used as fungicides for the treatment of grapes; these compounds contain SH- groups that promote reactive oxygen species with consequent oxidative stress, inflammation, and cellular metabolism alterations, leading to cell death (Rath et al. 2011).

The main biomarker of DTC, especially ethylenebisdithiocarbamates (EBDC), such as mancozeb and metiram, is urinary ethylenethiourea (u-ETU), which is formed by the hepatic degradation of DTC with a long half-life of 32–100 h (Houeto et al. 1995; Colosio et al. 2002; EPA 2005; van Wendel de Joode et al. 2014). Reference values for u-ETU have been established in Italian urban (Pavia, Torino, Trento, Verona) and rural populations (Rovescala) by Aprea et al., with the Italian Society for Reference Values (SIVR), and in studies on vineyard workers by Colosio et al. (Colosio et al. 2002, 2006; Aprea and Catenacci 2003). In 2017, the SIVR established 5 µg/L as the maximum value found in 95% of the general population not occupationally exposed to DTC (SIVR 2017). Nevertheless, there is no established threshold over which u-ETU determines adverse health effects.

The presence of DTC and u-ETU has been documented in several organs, especially in the thyroid gland; because of their rapid metabolism, DTC do not bio-accumulate and consequently have low acute toxicity (Kurttio and Savolainen 1990; Houeto et al. 1995; Colosio et al. 2002; van Wendel de Joode et al. 2014; Runkle et al. 2017).

DTC act as endocrine disruptors, particularly in the thyroid gland with an anti-thyroid action (Panganiban et al. 2004; EPA 2005; U.S. EPA 2005; Rath et al. 2011; Sugeng et al. 2013; Medda et al. 2017). In fact, the Agricultural Health Study (AHS) observed a significant increase in hypothyroidism in the wives of farmers who live in Iowa and North Carolina and are exposed to maneb/mancozeb (EBDC), with an OR adj. = 2.2 (95% CI 1.5–3.3) (Goldner et al. 2010). In contrast, regarding farmers enrolled in the AHS, EBDC exposure was not significantly associated with alterations in thyroid hormones (Lerro et al. 2017). The anti-thyroid effect seems to be due to the action of ETU, which inhibits thyroid peroxidase and may alter fetal neurodevelopment (Marinovich et al. 1997; Runkle et al. 2017).

Steenland et al. examined 49 backpack sprayers of EBDC living near Cuernavaca in central Mexico (an area with tomato crops) and found an increased level of thyroid-stimulating hormone (TSH) compared to controls, whereas thyroid hormone (T_4) was in the normal range (Steenland et al. 1997). These workers were highly exposed to EBDC and did not wear protective equipment; in fact, these workers had a mean concentration of u-ETU of 58 ± 26 µg/L (Steenland et al. 1997). Nevertheless, an Italian study by Medda et al., evaluating grapevine workers of the Chianti area and Bolzano Province, detected concentrations of thyroid hormones in the normal range, even if the occupationally exposed subjects had a high level of u-ETU (geometric mean of 12.2 µg/L) (Medda et al. 2017).

Different studies have shown the potential neurotoxicity of EBDC and ETU, including Parkinson-like symptoms and alterations in neurodevelopment of the fetus (Bjørling-Poulsen et al. 2008; Runkle et al. 2017). However, the potential teratogenic and carcinogenic effects of mancozeb and ETU are still debated (Runkle et al. 2013, 2017). A recent review by Runkle et al. affirmed that there is high to moderate evidence of the developmental and reproductive hazards of mancozeb from in vitro and animal studies, but moderate to low evidence in humans, derived by research indirectly measuring either occupational or residential exposure to mancozeb (Runkle et al. 2017).

IARC did not include DTC in the classification of carcinogenic agents; however, its metabolite, ETU, was classified as a possible carcinogen (group 2B) until 2001 and then defined as a non-classificable carcinogen (group 3) (IARC 2001, 2017). In contrast, the US Environmental Protection Agency (EPA) considers some DTC, e.g., mancozeb and metiram, as probable carcinogenic agents (group 2B) (U.S. EPA 2016).

In Europe, mancozeb has been classified with the R63 risk code (possible fetal damage) by UE directive 1999/45/CEE and with the code H361d by CLP classification (UE directive CE 1272/2008) (Unione Europea 2009).

It is also important to consider the possibility of mixture effects of different DTCs used in agriculture since these pesticides, especially EBDCs, have common effects on thyroid and nervous system due to common mechanisms caused by the metabolites carbon disulfide and ETU (U.S. EPA 2001). In fact, there is concern about possible risk for humans due to cumulative exposures to two or more DTC that can occur through diet (U.S. EPA 2001, 2005). NOAELs for neuropathology and thyroid effects were established, and they may be overcome in case of multiple and cumulative exposures (U.S. EPA 2001).

People are exposed to DTC through different pathways: skin, mucous membranes, respiratory and gastrointestinal tracts; and in different settings: occupational setting, environmental exposure (Houeto et al. 1995). The occupational exposure involves mainly the agricultural workers, especially appliers of DTC (National Toxicology Program 2011). In fact, use of DTC can affect air quality in agricultural areas during the period of use of DTC; farmers and even general population, living near these areas, may be exposed through inhalation (aerosol, dust) and skin. Inhalation of DTC can

provoke local irritation of respiratory mucosa, and dermal contact with these compounds can cause dermatitis, since DTC are sensitizing agents (IPCS 1988). Ethylenebisdithiocarbamates (EBDCs) are unstable in the presence of oxygen, moisture or biological systems (IPCS 1988). They are rapidly decomposed, especially by oxidative degradation, producing ETU, which is stable and is the major cause of toxicity (International Programme on Chemical Safety (IPCS 1988). In fact, ETU can be found in plants and in the environment (air, soil, water) following the agricultural use of DTC, as reported by Panganiban et al. (WHO 1988; Panganiban et al. 2004). Thus, ETU and DTC represent an hazard not only for human health but also for other living organisms, especially aquatic species (Houeto et al. 1995; U.S. EPA 2005).

Since EBDCs are used in agriculture not only for grapes protection but also for many other crops, consumption of fruits and vegetables, especially cooked vegetables, represents an important source of exposure to ETU among the general population (Houeto et al. 1995; EPA 2005).

Absorption of ETU from the digestive tract is very fast and ETU passed in blood rapidly, and it is excreted in urine, where it can be detected several days after exposure to EBDCs, since it has a long half-life (Houeto et al. 1995). Instead, EBCDs are absorbed slowly and they are metabolized by liver, producing ETU (Houeto et al. 1995).

Even the consumption of contaminated beverages, such as wine and beer, or drinking water can lead to an increase in u-ETU concentrations (Houeto et al. 1995; Colosio et al. 2002, 2006; Runkle et al. 2017). Additionally, tobacco smoking can cause alterations in ETU levels in urine since this compound is produced by tobacco combustion (Houeto et al. 1995; National Toxicology Program 2011).

Fungicides are pesticides widely used in viticulture as anti-grape blight, and these chemicals represent approximately 90% of the total amount of pesticides used in vineyards classified as controlled and guaranteed designation of origin (DOCG), such as Superior Prosecco of Conegliano-Valdobbiadene, (henceforth defined as 'The Prosecco District'), a hilly, rural area, in the Alpine foothills of the Treviso Province, on the left side of Piave river (Consorzio di tutela del vino Conegliano Valdobbiadene Prosecco 2016). The Prosecco District of approximately 20,000 hectares is located in the health district of Treviso, Northeastern Italy (Fig. 1) (Consiglio Comunale San Pietro di Feletto 2011). In 2012, vineyards covered over 4000 hectares, representing approximately 31% of the agricultural surface area (ULSS 7 2013).

According to an ARPAV (Regional Agency for Environmental Protection of Veneto) report, in the Prosecco District, during 2012, 88,699 kg of DTC (66,357 kg of mancozeb, 22,283 kg of metiram and 59 kg of ziram) were commercially sold (ARPAV 2012).

These products are mainly used during spring and early summer. During these seasons, the general population living near vineyards is indirectly exposed to DTC; however, the extent of exposure is unknown because of the lack of studies in this area.

The increasing extension of vineyards in the last decade, along with the rising public concern about the possible

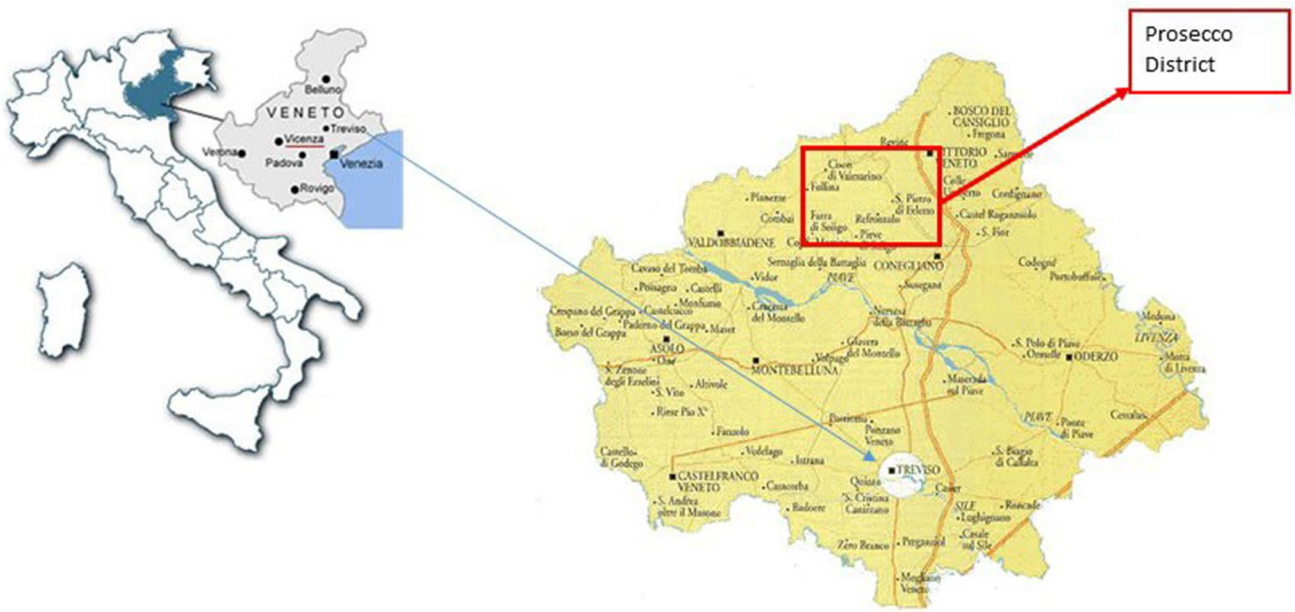

Fig. 1 Municipalities of the Prosecco District included in our study (Cison di Valmarino, Farra di Soligo, Follina, Pieve di Soligo, Refrontolo, San Pietro di Feletto, Susegana, Vittorio Veneto) and located in the Province of Treviso, Veneto Region of Italy, Europe

effects of DTC exposure on human health, especially in children, has been the stimulus for local health authorities of Treviso to conduct a biomonitoring study. In fact, children may be more exposed than adults to pesticides from several sources, especially air, soil, dust, in several setting, like green areas (e.g., parks, gardens, and playgrounds), since they commonly play outdoor and they can inhale, ingest (frequent hand-to-mouth activity) and/or enter in contact through skin with pesticides' residues (WHO 2008; Roberts and Karr 2012). The primary aim of the study was to estimate u-ETU concentrations as proxy of DTC exposure in people non-occupationally exposed who lived near the vineyards. As secondary aim we tried to identify other factors associated with u-ETU concentrations, such as the distance of homes and schools from the vineyards, outdoor activities, horticulture and gardening, dietary habits (consumption of vegetables, fruits, wine or other alcoholic beverages), smoking habit and use of certain medications.

Materials and Methods

Study Area

The study included 8 municipalities within the Prosecco District (Fig. 1). The municipalities were classified by the extent of viticulture (ha/km^2), which was available during the study period: the 2000 agricultural census of ISTAT (National Institute of Statistic) (ISTAT 2000). Farra di Soligo, S. Pietro di Feletto, Refrontolo were classified as potentially at high risk of DTC exposure (22–29 ha/km^2), Pieve di Soligo and Susegana (9–11 ha/km^2) at intermediate risk and Vittorio Veneto, Cison di Valmarino, and Follina (3–5 ha/km^2) at low risk.

Study Subjects

Eligible subjects were children aged 3–5 years in December 2011 and their parents who were residents of one of the eight municipalities. Families with at least one member who worked in agriculture and was occupationally exposed to DTC were excluded from the study.

The sample size was calculated on an estimated population prevalence of 50% over the reference limit of u-ETU in the urine (1 µg/L), as a precautionary principle, since there is an absence of recent similar studies. To obtain an absolute precision of ± 0.06, a sample of 267 adults was needed. Therefore, 160 children and their parents (480 subjects, including 20% missing individuals), who were residents in the area, were chosen. For each municipality, independent of population size, we randomly selected a sample of 20 eligible children and their corresponding parents from the local health registry.

Study Population

We mailed the selected subjects (160 children and 320 parents) and their pediatric and family specialist a letter explaining the aims of the study. Another letter was sent to the mayors of the eight municipalities. Subsequently, trained personnel from the local health agency Department of Prevention conducted a preliminary short, standardized phone survey related to the location and housing characteristics of the selected families. During the same phone call, the collection of a urinary sample, a self-administered questionnaire, and an informed consent form were planned. Self-administered questionnaires were sent by post to obtain information on diet, lifestyle, and other possible confounders. Finally, 269 adults and 138 children agreed to enrolment and satisfied the inclusion criteria.

The distance of the houses and schools from the vineyards (by cardinal points) and the surface area and altimetry of the vineyards were obtained by geocoding (Geomedia, Webgis).

When geocoded data were not available or if it was necessary to verify the data, technical personnel from the municipality obtained distances, surface areas, and altimetry, using municipal cartography and field measurements.

Questionnaires

Two questionnaires, one for adults and one for children, were created, and after pre-testing, these questionnaires were self-administered. The parents were responsible for also filling out the questionnaire for their child.

The questionnaires included information about age, gender, weight, height, address of the house, school and/or workplace, occupation, education degree, outdoor activities, consumption of water, fruit, vegetables, and other foods with specification of consumption patterns (household or local production), hobbies, such as gardening, use of pesticides in home gardening and horticulture activities, pets, smoking, alcohol consumption, and use of medications.

Exposure Assessment

Urinary ETU was quantified to assess the exposure to DTC during the period of highest utilization of these pesticides (June 2012, baseline). As recommended by the Condifesa of Treviso (Consortium for the Protection of Agriculture), a non-profit administrative authority of the Veneto region, urinary samples were collected from 11 to 22 June 2012. Each studied subject collected a spot sample of the second-morning urine, during the 24 h before the planned appointment with the health personnel, covered it with an aluminum film (to protect it from light) and kept it in the refrigerator. The health personnel get the urine samples at home of study subjects, transported them in a cool box to

the laboratory of the Conegliano Hospital, where finally the samples were frozen at a temperature of –20 °C. After the urine samples, kept in a cool box, were sent to the Industrial Hygiene Laboratory of the Department of Cardiac, Thoracic, and Vascular Sciences, Section of Public Health, University of Padova, who performed the analysis.

ETU concentrations in urinary samples were measured by gas chromatography and mass spectrometry (*Auto-System XL Gas Chromatograph-TurboMass Upgrade*, PerkinElmer Inc.) according to the method described by Fustinoni et al. with slight modifications (Fustinoni et al. 2005). Briefly, after the complete thawing of urine at room temperature, a 6-mL sample was spiked with NH_4Cl and KF in order to adjust the pH and ion strength, respectively, and then with internal standard (ETU-d_4), the analyte extracted with dichloromethane using a diatomaceous column and the organic layer dried in a vacuum centrifuge. After the dissolution of the residue, ETU and ETU-d_4 were derivatized, overnight, with the anhydrous acetonitrile, *N*-(tertbutyldimethylsilyl)-*N*-methyltrifluoroacetamide, tertbutyldimethyilsilyl chloride mixture 5:4:1 (v/v) to form the silyl derivatives. Subsequently, the mixture, containing the bis-silanized derivatives, was analyzed by gas chromatography–mass spectrometry. As recommended in the literature, we only considered valid samples with a creatinine level in the range of 0.3–3 g/L (Cocker et al. 2011). Thus, the analytical limit of detection was fixed at 0.3 µg/L for urinary samples.

After completion of the baseline study and publication of the early results on the website of the Health District of Conegliano-Pieve di Soligo, many municipalities, in succession, committed to banning mancozeb use for precautionary reasons. Additionally, the Prevention Department of Conegliano-Pieve di Soligo conducted a series of meetings with residents in the Prosecco District, during which health personnel revealed the early results of the study and explained useful precautions to reduce exposure to DTC.

To evaluate the possible effects induced by the public health actions conducted in 2013 and 2014, i.e., to demonstrate a decrease in the levels of exposure to DTC in the study population, additional data collection was conducted in the Prosecco District study area in 2014 (after intervention). The 20 original families with at least one member who had a u-ETU concentration over 5 µg/L in 2012 and another 20 families residing less than 30 m from large (> 6000 m^2) vineyards were reevaluated. This reevaluation led to the definition of a second study group that included 55 children and 54 adults. For each subject, two urinary samples were obtained: one at the beginning of the DTC spraying season (26 March–1 April 2014, pre-spraying season) and the second during the period of maximum exposure (from 3 to 6 June 2014, post-spraying season). Notably, only part (25 children and 49 adults) of the group identified after intervention was derived from the original group assessed at baseline.

For each subject enrolled in the study, we obtained informed consent, and our study was carried out in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Factors that may predict baseline u-ETU concentration were identified among the following independent variables as defined by the questionnaires: "risk associated with family residence in a specific municipality" (high, medium, and low); "distance between the house and the nearest vineyard (m)" stratified in 3 categories (≤ 30, 31–200, and > 200); "size (m^2) of the nearest vineyard" and "size (m^2) of the largest vineyard" both divided into 3 levels (≤ 6000; 6001–49,999; and $\geq 50{,}000$); "risk associated with attending an infant school located in a specific municipality" (high, intermediate, and low); wine and fruits/vegetables consumption (Yes/No); pesticide use in gardening (Yes/No); use of pesticides for pets (Yes/No); and medication use (Yes/No). The dependent variable, baseline u-ETU concentration, was analyzed as both continuous and dichotomous. Analyses based on u-ETU concentration as a continuous variable utilized multiple linear regression. However, its distribution was too skewed to allow the identification of a linear relation, despite attempts to logarithmically transform the continuous variable (data not shown). Therefore, the results of the study are presented using multiple logistic regression models, in which baseline u-ETU concentration was a dependent, categorical, dichotomous variable. We tested several cut-off points of the u-ETU distribution: ≥ 0.5 µg/L, > 1 µg/L, and > 5 µg/L. Although we acknowledge that there is no established threshold over which u-ETU determines adverse health effects, in the end, a cutoff u-ETU concentration of > 1 µg/L was chosen to obtain sufficient statistical precision in the multivariate analysis.

Descriptive statistical analyses were also conducted to compare the u-ETU values measured in this study population at baseline and after intervention.

All statistical analyses were performed using Microsoft Excel and SAS (version 9.3 SAS Institute INC., Cary, N.C., USA).

Results

Nine adults and twelve children were excluded from subsequent analyses for partial dropout, insufficient quantity of urine for sampling or urinary creatinine values out of the range of 0.3–3 g/L. Therefore, the final baseline study group included 260 adults (136 females and 124 males; mean age ± SD: 39.81 ± 5.39) and 126 children (60 females and

66 males; mean age ± SD: 4.46 ± 0.82) with valid urinary samples.

The u-ETU concentrations were not distributed normally, and u-ETU was measured at or above 0.5 µg/L in only 123 subjects (47.3%) (Tables 1 and 2).

Table 1 shows the distribution of the baseline u-ETU concentration among adults. Fifty-three subjects (20.4%) had a u-ETU concentration over 1 µg/L, while 14 (5.4%) had u-ETU concentrations higher than 5 µg/L. The maximum value of u-ETU in adults was 19.7 µg/L, and 95% of adults ($n = 247$) had u-ETU levels lower than 5.3 µg/L (Table 2). For children, we collected 126 valid urinary samples at baseline. Fifty-eight subjects (46.0%) had u-ETU concentrations greater than or equal to 0.5 µg/L. The median u-ETU and interquartile range were similar to those of adults (Table 3). Twenty-seven children (21.4%) had u-ETU levels > 1 µg/L, and only 7 children (5.6%) had a u-ETU concentration > 5 µg/L. Notably, 95% of the pediatric population had u-ETU levels lower than 8 µg/L, and the maximum value among children was 20.9 µg/L (Table 3).

The distributions of the baseline u-ETU concentrations for adults by gender, age, BMI, municipality of residence, distance from and size of the vineyard and other variables, obtained by questionnaires, are shown in Table 2. We found a higher proportion of adults with u-ETU levels ≥ 0.5 µg/L among wine drinkers (60.0%) than among non-drinkers (34.6%) ($p < 0.01$). A similar finding was also obtained for u-ETU concentrations above 1 µg/L: 26.9% vs. 13.9% ($p < 0.01$). There was a higher proportion of men (26.6%) with u-ETU concentrations above 1 µg/L than women with the same u-ETU concentration (14.7%) ($p = 0.02$) and a higher proportion of people with u-ETU levels above 1 µg/L among subjects older than 40 years than among people younger than 40 ($p = 0.04$).

The proportion of adults with u-ETU ≥ 0.5 µg/L was higher in those living within 30 m of the nearest vineyard (50.7%) than in those living farther than 200 m (48.4%) ($p = 0.72$). Additionally, the percentage of adults with u-ETU ≥ 0.5 µg/L was superior among those living in proximity (within 1.7 km) of a vineyard ≥ 50,000 m^2 (55.2%) than among residents in proximity of a vineyard < 6000 m^2 (44.4%), but this difference was not statistically significant ($p = 0.28$).

For children, the distribution of baseline u-ETU concentrations by gender, age, weight, municipality of residence, school risk area, distance home/school-vineyard and other characteristics is shown in Table 3. Similar to adults, the proportion of children with u-ETU ≥ 0.5 µg/L, stratified by home distance from the nearest vineyard, was greater in those living within 30 m of the nearest vineyard (61.1%) than in those living over 200 m from the nearest vineyard (40.6%, $p = 0.10$).

Considering the distance of the school from the nearest vineyard, a higher number of children attending a preschool located within 30 m of a vineyard had u-ETU value > 1 µg/L (31.3%) than those studying at a school situated at a greater distance: 31–200 m (22.6%) and over 200 m (20.8%) ($p = 0.63$), as shown in Table 3.

The proportion of children with baseline u-ETU concentrations over 1 µg/L was greater among residents in a high-risk area (29.2%) compared to those in a low-risk area (17.0%, $p = 0.25$) and for individuals attending a school located in a high-risk area (38.5%) than for those attending schools in a low-risk area (17.1%, $p = 0.10$) (Table 3). A higher proportion of subjects with u-ETU above 1 µg/L was found among children whose parents used pesticides in gardening than among those whose parents did not (31.3% vs. 18.1%, $p = 0.12$). In particular, there were more children with u-ETU ≥ 0.5 µg/L and > 1 µg/L if these compounds were used recently, i.e., from a few days to one week before (88.9% and 55.6%, respectively), with respect to less recent (more than one week before) garden treatment (43.5% and 21.7%, respectively) and no garden treatment (42.6% and 18.1%, respectively). These differences were statistically significant with $p = 0.03$ and $p = 0.03$, respectively.

For adults, from the multiple logistic regression models, an increased risk of having a baseline u-ETU concentration over 1 µg/L was found in subjects who conducted pesticide treatment in their home garden (OR 2.70; 95% CI 1.22–5.97) and in subjects who consumed wine daily (any amount) (OR 2.04; 95% CI 0.99–4.22) (Table 4). No significant association was found between baseline u-ETU values and home distance from the nearest vineyard and extension of the nearest vineyard (Table 4). Concerning medication use, we found a correlation with having u-ETU concentrations over 5 µg/L (OR 4.1; 95% CI 1.3–12.9), but these data were limited to 8 subjects exposed, and the medications were heterogeneous: NSAIDs, corticosteroids, antibiotics, oral contraceptives, protonic pump inhibitors, psychoactive drugs, and medications for asthma, hypercholesterolemia, antihistamines, and analgesics (results are not shown).

Instead, we observed a significant relationship between baseline u-ETU values > 1 µg/L in children and recent home

Table 1 Baseline urinary ETU concentration in adults and children

ETU concentration (µg/L)	Adults		Children	
	Number of urinary samples	Percent (%)	Number of urinary samples	Percent (%)
< 0.5	137	52.7	68	54.0
0.5–1	70	26.9	31	24.6
1.1–5	39	15.0	20	15.9
> 5	14	5.4	7	5.6
Total	260	100	126	100

Table 2 Distribution of baseline urinary ETU concentrations (μg/L) in adults by subject characteristics

Variables	Categories	*N*	*N* (%) ≥ 0.5 μ/L	*p* value	*N* (%) > 1 μ/L	*p* value	*N* (%) > 5 μ/L	*p* value	Mean ± SD	50th	75th	90th	95th	99th	Max	IQ
Parents	All	260	123 (47.3)	0.18[a]	53 (20.4)	0.02[a]	14 (5.4)	0.86[a]	1.20 ± 2.39	0.35	0.90	2.50	5.30	14.80	19.70	0.55
	Mothers	136	59 (43.4)		20 (14.7)		7 (5.2)		1.11 ± 2.31	0.35	0.80	1.60	5.40	12.20	16.20	0.45
	Fathers	124	64 (51.6)		33 (26.6)		7 (5.7)		1.29 ± 2.48	0.50	1.15	2.70	5.20	14.80	19.70	0.80
Age (years)	< 35	45	20 (44.4)	0.21[b]	10 (22.2)	0.04[b]	3 (6.7)	0.44[b]	1.12 ± 1.54	0.35	0.80	3.50	5.40	6.10	6.10	0.45
	35–39	93	37 (39.8)		11 (11.8)		3 (3.2)		0.91 ± 2.00	0.35	0.60	1.20	3.10	14.80	14.80	0.25
	40–44	82	44 (53.7)		19 (23.2)		4 (4.9)		1.28 ± 2.60	0.55	1.00	2.50	3.30	19.70	19.70	0.65
	≥ 45	40	22 (55.0)		13 (32.5)		4 (10.0)		1.81 ± 3.33	0.55	1.25	4.60	10.30	16.20	16.20	0.90
BMI	< 25	157	77 (49.0)	0.32[b]	33 (21.0)	0.53[b]	8 (5.1)	0.88[b]	1.11 ± 2.00	0.35	0.90	2.50	5.20	12.00	16.20	0.55
	25–29.99	75	32 (42.7)		14 (18.7)		5 (6.7)		1.44 ± 3.25	0.35	0.90	2.60	9.00	19.70	19.70	0.55
	≥ 30	16	10 (62.5)		5 (31.3)		1 (6.3)		1.41 ± 2.02	0.70	1.50	2.80	8.40	8.40	8.40	1.15
Residence risk	Low	97	47 (48.5)	0.32[b]	21 (21.7)	0.90[b]	3 (3.1)	0.45[b]	1.00 ± 1.75	0.35	0.90	2.50	3.50	14.80	14.80	0.55
	Medium	63	34 (54.0)		13 (20.6)		4 (6.4)		1.25 ± 2.29	0.50	0.90	1.70	6.10	12.20	12.20	0.55
	High	100	42 (42.0)		19 (19.0)		7 (7.0)		1.36 ± 2.93	0.35	0.95	2.90	7.15	17.95	19.70	0.60
Smoker	Yes	49	26 (53.1)	0.37[a]	14 (28.6)	0.11[a]	2 (4.1)	0.49[c]	1.12 ± 1.51	0.50	1.30	2.80	3.50	8.40	8.40	0.95
	No	211	97 (46.0)		39 (18.5)		12 (5.7)		1.22 ± 2.55	0.35	0.90	2.50	5.40	14.80	19.70	0.55
Wine consumption	Yes	130	78 (60.0)	< 0.01[a]	35 (26.9)	< 0.01[a]	9 (6.9)	0.27[a]	1.51 ± 2.93	0.60	1.10	2.80	6.50	16.20	19.70	0.75
	No	130	45 (34.6)		18 (13.9)		5 (3.9)		0.89 ± 1.63	0.35	0.60	1.45	3.10	9.50	12.20	0.25
Garden treatment	Yes	47	25 (53.2)	0.37[a]	16 (34.0)	0.01[a]	5 (10.6)	0.14[a]	1.95 ± 3.78	0.50	1.40	5.90	12.00	19.70	19.70	1.05
	No	213	98 (46.0)		37 (17.4)		9 (4.2)		1.03 ± 1.93	0.35	0.80	1.90	3.50	9.50	16.20	0.45
Garden treatment (time)[e]	Recent	16	8 (50.0)	0.54[b]	5 (31.3)	0.03[b]	3 (18.8)	0.05[b]	2.58 ± 4.22	0.43	1.95	12.00	12.20	12.20	12.20	1.60
	Not recent	30	17 (56.7)		11 (36.7)		2 (6.7)		1.67 ± 3.61	0.60	1.40	3.15	5.90	19.70	19.70	1.05
	No	213	98 (46.0)		37 (17.4)		9 (4.2)		1.03 ± 1.93	0.35	0.80	1.90	3.50	9.50	16.20	0.45
Vegetables /fruit consumption	≥2 servings	146	65 (44.5)	0.31[a]	31 (21.2)	0.70[a]	11 (7.5)	0.08[d]	1.36 ± 2.64	0.35	1.00	2.70	6.60	14.80	16.20	0.65
	< 2 servings	114	58 (50.9)		22 (19.3)		3 (2.6)		0.99 ± 2.01	0.50	0.80	2.40	3.00	6.50	19.70	0.45
Medications' use	≥2 drugs	75	35 (46.7)	0.81[a]	17 (22.7)	0.56[a]	8 (10.7)	0.03[c]	1.46 ± 2.74	0.35	1.00	5.20	6.60	16.20	16.20	0.65
	< 2 drugs	180	87 (48.3)		35 (19.4)		6 (3.3)		1.10 ± 2.25	0.35	0.90	2.30	3.40	14.80	19.70	0.55
Pesticides' use for pets	Yes	40	21 (52.5)	0.39[a]	14 (35.0)	0.03[a]	5 (12.5)	0.13[c]	2.05 ± 3.82	0.55	1.35	5.65	10.85	19.70	19.70	1.00
	No	132	59 (44.7)		25 (18.9)		6 (4.6)		1.04 ± 1.72	0.35	0.90	2.50	3.50	9.00	12.00	0.55
Distance of nearest vineyard (m)	≤ 30	71	36 (50.7)	0.72[b]	15 (21.1)	0.97[b]	7 (9.9)	0.07[b]	1.42 ± 2.65	0.50	1.00	2.60	8.40	16.20	16.20	0.65
	31–200	127	57 (44.9)		26 (20.5)		3 (2.4)		1.11 ± 2.42	0.35	0.80	2.50	3.10	14.80	19.70	0.45
	> 200	62	30 (48.4)		12 (19.4)		4 (6.5)		1.13 ± 1.98	0.35	0.80	2.40	6.10	12.20	12.20	0.45
Size of nearest vineyard (m^2)	≤ 6000	150	64 (42.7)	0.09[b]	25 (16.7)	0.36[b]	5 (3.3)	0.02[b]	0.87 ± 1.34	0.35	0.80	1.45	3.00	8.40	9.50	0.45
	6001–49,999	66	32 (48.5)		16 (24.2)		8 (12.1)		2.05 ± 4.09	0.35	1.00	6.10	12.20	19.70	19.70	0.65
	≥ 50,000	10	8 (80.0)		3 (30.0)		1 (10.0)		1.49 ± 1.90	0.75	1.30	4.65	6.50	6.50	6.50	0.80
	Unknown	34	19 (55.9)		9 (26.5)		0 (0.0)		0.92 ± 0.85	0.50	1.30	2.50	2.70	3.50	3.50	0.95
Size of largest vineyard (m^2)	≤ 6000	63	28 (44.4)	0.28[b]	11 (17.5)	0.06[b]	4 (6.4)	0.35[b]	1.01 ± 1.74	0.35	0.90	1.20	5.20	9.50	9.50	0.55
	6001–49,999	105	44 (41.9)		15 (14.3)		5 (4.8)		1.24 ± 2.94	0.35	0.70	1.90	4.00	16.20	19.70	0.35
	≥ 50,000	58	32 (55.2)		18 (31.0)		5 (8.6)		1.50 ± 2.49	0.60	1.40	3.60	6.60	14.80	14.80	1.05
	Unknown	34	19 (55.9)		9 (26.5)		0 (0.0)		0.92 ± 0.85	0.50	1.30	2.50	2.70	3.50	3.50	0.95
Distance between house and nearest vineyard (m)																
≤ 30	By size (m^2)	71														

Table 2 (continued)

Variables	Categories	*N*	*N* (%) ≥ 0.5 µ/L	*p* value	*N* (%) > 1 µ/L	*p* value	*N* (%) > 5 µ/L	*p* value	Mean ± SD	50th	75th	90th	95th	99th	Max	IQ
	≤ 6000	48	19 (39.6)	0.02[b]	8 (16.7)	0.42[b]	4 (8.3)	0.60[b]	1.13 ± 1.97	0.35	0.95	2.60	5.90	9.50	9.50	0.60
	6001–49,999	20	14 (70.0)		6 (30.0)		3 (15.0)		2.18 ± 3.92	0.65	1.50	7.20	12.60	16.20	16.20	1.15
	≥ 50,000	0	0		0		0		–	–	–	–	–	–	–	–
	Unknown	3	3 (100.0)		1 (33.3)		0		0.83 ± 0.49	0.60	1.40	1.40	1.40	1.40	1.40	0.90
31–200	By size (m^2)	127														
	≤ 6000	70	30 (42.9)	0.47[b]	10 (14.3)	0.28[b]	0	0.02[b]	0.67 ± 0.65	0.35	0.70	1.35	1.70	4.00	4.00	0.35
	6001–49,999	31	12 (38.7)		8 (25.8)		3 (9.7)		2.18 ± 4.61	0.35	1.30	3.30	14.80	19.70	19.70	0.95
	≥ 50,000	6	4 (66.7)		2 (33.3)		0		1.07 ± 0.93	0.80	1.30	2.80	2.80	2.80	2.80	0.95
	Unknown	20	11 (55.0)		6 (30.0)		0		1.03 ± 0.98	0.50	1.30	2.60	3.10	3.50	3.50	0.95
> 200	By size (m^2)	62														
	≤ 6000	32	15 (46.9)	0.20[b]	7 (21.9)	0.91[b]	1 (3.1)	0.19[b]	0.92 ± 1.25	0.35	0.80	1.60	3.60	6.60	6.60	0.45
	6001–49,999	15	6 (40.0)		2 (13.3)		2 (13.3)		1.60 ± 3.28	0.35	0.60	6.10	12.20	12.20	12.20	0.25
	≥ 50,000	4	4 (100.0)		1 (25.0)		1 (25.0)		2.13 ± 2.92	0.75	3.70	6.50	6.50	6.50	6.50	3.15
	Unknown	11	5 (45.5)		2 (18.2)		0		0.75 ± 0.67	0.35	0.90	1.40	2.50	2.50	2.50	0.55

[a] χ^2 statistical test

[b] Kruskal–Wallis statistical test

[c] Fisher statistical test

[d] Wilcoxon statistical test

[e] Recent: from few days to one week before collection of the urinary sample; not recent: more than one week before collection

Table 3 Distribution of baseline urinary ETU concentrations (μg/L) in children by subject characteristics

Variable	Categories	*N*	*N* (%) ≥ 0.5 μ/L	*p* value	*N* (%) > 1 μ/L	*p* value	*N* (%) > 5 μ/L	*p* value	Mean ± SD	50th	75th	90th	95th	99th	Max	IQ
Children	All	126	58 (46.0)	0.56[a]	27 (21.4)	0.42[a]	7 (5.6)	0.55[a]	1.34 ± 2.94	0.35	0.90	1.80	8.00	16.40	20.90	0.55
	Females	60	26 (43.3)		11 (18.3)		3 (5.0)		1.33 ± 3.14	0.35	0.80	2.10	6.15	20.90	20.90	0.45
	Males	66	32 (48.5)		16 (24.2)		4 (6.1)		1.34 ± 2.77	0.35	1.00	1.80	8.30	16.40	16.40	0.65
Age (years)	3	20	8 (40.0)	0.32[b]	3 (15.0)	0.38[b]	1 (5.0)	0.18[b]	1.04 ± 2.10	0.35	0.80	1.45	5.80	9.80	9.80	0.45
	4	43	24 (55.8)		13 (30.2)		5 (11.6)		2.26 ± 4.51	0.60	1.20	8.00	11.60	20.90	20.90	0.85
	5	50	19 (38.0)		9 (18.0)		1 (2.0)		0.77 ± 1.19	0.35	0.70	1.60	1.80	8.30	8.30	0.35
	6	13	7 (53.9)		2 (15.4)		0		0.90 ± 1.10	0.50	0.80	1.80	4.30	4.30	4.30	0.45
Weight (kg)	≤16.5	21	10 (47.6)	0.99[b]	3 (14.3)	0.26[b]	3 (14.3)	0.33[b]	2.41 ± 5.21	0.35	0.70	9.80	11.30	20.90	20.90	0.35
	16.6–19.2	37	18 (48.7)		6 (16.2)		1 (2.7)		0.87 ± 1.38	0.35	0.80	1.30	3.50	8.30	8.30	0.45
	19.3–21.5	26	12 (46.2)		9 (34.6)		1 (3.9)		1.22 ± 2.23	0.35	1.10	1.80	3.40	11.60	11.60	0.75
	> 21.5	31	14 (45.2)		6 (19.4)		2 (6.5)		1.39 ± 3.13	0.35	0.80	1.80	8.00	16.40	16.40	0.45
	Unknown	11	4 (36.4)		3 (27.3)		0		0.98 ± 1.21	0.35	1.40	1.80	4.30	4.30	4.30	1.05
Residence risk area	Low	47	16 (34.0)	0.11[b]	8 (17.0)	0.25[b]	1 (2.1)	0.41[b]	0.87 ± 1.69	0.35	0.70	1.40	1.80	11.30	11.30	0.35
	Medium	31	17 (54.8)		5 (16.1)		2 (6.5)		1.33 ± 2.58	0.50	0.80	1.80	9.80	11.60	11.60	0.45
	High	48	25 (52.1)		14 (29.2)		4 (8.3)		1.79 ± 3.93	0.50	1.20	3.40	8.30	20.90	20.90	0.85
School risk area	Low	35	12 (34.3)	0.19[b]	6 (17.1)	0.10[b]	1 (2.9)	0.40[b]	0.96 ± 1.94	0.35	0.70	1.40	4.30	11.30	11.30	0.35
	Medium	28	14 (50.0)		6 (21.4)		2 (7.1)		1.42 ± 2.71	0.43	0.85	3.50	9.80	11.60	11.60	0.50
	High	26	16 (61.5)		10 (38.5)		3 (11.5)		2.11 ± 4.35	0.60	1.50	8.00	8.30	20.90	20.90	1.15
	Unknown	37	16 (43.2)		5 (13.5)		1 (2.7)		1.09 ± 2.67	0.35	0.70	1.80	3.40	16.40	16.40	0.35
Distance home—vineyard (m)	≤ 30	36	22 (61.1)	0.10[b]	15 (41.7)	< 0.01[b]	4 (11.1)	0.23[b]	1.93 ± 3.37	0.65	1.60	8.00	9.80	16.40	16.40	1.25
	31–200	58	23 (39.7)		9 (15.5)		2 (3.5)		1.18 ± 3.07	0.35	0.80	1.40	3.50	20.90	20.90	0.45
	> 200	32	13 (40.6)		3 (9.4)		1 (3.1)		0.95 ± 2.02	0.35	0.60	1.00	4.30	11.30	11.30	0.25
Distance school—vineyard (m)	≤ 30	16	8 (50.0)	0.72[b]	5 (31.3)	0.63[b]	2 (12.5)	0.58[b]	2.11 ± 4.28	0.43	1.35	8.30	16.40	16.40	16.40	1.00
	31–200	53	24 (45.3)		12 (22.6)		3 (5.7)		1.50 ± 3.49	0.35	0.80	1.60	11.30	20.90	20.90	0.45
	> 200	24	13 (54.2)		5 (20.8)		1 (4.2)		1.12 ± 1.98	0.50	0.80	1.80	3.50	9.80	9.80	0.45
	Unknown	33	13 (39.4)		5 (15.2)		1 (3.0)		0.86 ± 1.39	0.35	0.80	1.80	2.60	8.00	8.00	0.45
Time spent indoor (hours)	0–5	53	26 (49.1)	0.30[b]	14 (26.4)	0.58[b]	4 (7.6)	0.75[b]	1.52 ± 3.05	0.35	1.10	1.80	9.80	16.40	16.40	0.75
	6–10	58	25 (43.1)		11 (19.0)		2 (3.5)		0.99 ± 1.82	0.35	0.80	1.60	4.30	11.30	11.30	0.45
	> 10	12	7 (58.3)		2 (16.7)		1 (8.3)		2.45 ± 5.87	0.55	0.80	3.40	20.90	20.90	20.90	0.45
	Unknown	3	0		0		0		0.35 ± 0.00	0.35	0.35	0.35	0.35	0.35	0.35	0
Vegetables consumption	< 2 servings	95	46 (48.4)	0.32[a]	22 (23.2)	0.50[a]	4 (4.2)	0.35[c]	1.23 ± 2.69	0.35	0.90	1.80	4.30	20.90	20.90	0.55
	≥ 2 servings	29	11 (37.9)		5 (17.2)		3 (10.3)		1.73 ± 3.73	0.35	0.80	8.30	11.30	16.40	16.40	0.45
Fruits consumption	< 2 servings	91	41 (45.1)	0.74[a]	21 (23.1)	0.56[a]	4 (4.4)	0.38[c]	1.24 ± 2.75	0.35	0.90	1.80	4.30	20.90	20.90	0.55
	≥ 2 servings	33	16 (48.5)		6 (18.2)		3 (9.1)		1.63 ± 3.50	0.35	0.80	1.80	11.30	16.40	16.40	0.45
Fruits/vegetables consumption	< 2 servings	111	49 (44.1)	0.23[a]	23 (20.7)	0.48[c]	4 (3.6)	0.03[c]	1.12 ± 2.51	0.35	0.80	1.60	3.50	11.60	20.90	0.45
	≥ 2 servings	13	8 (61.5)		4 (30.8)		3 (23.1)		3.28 ± 5.26	0.80	1.80	11.30	16.40	16.40	16.40	1.45
Garden treatment	Yes	32	18 (56.3)	0.18[a]	10 (31.3)	0.12[a]	2 (6.3)	0.75[c]	1.95 ± 4.47	0.60	1.40	1.80	16.40	20.90	20.90	1.05
	No	94	40 (42.6)		17 (18.1)		5 (5.3)		1.13 ± 2.18	0.35	0.80	1.60	8.00	11.60	11.60	0.45
Garden treatment (time)[d]	Recent	9	8 (88.9)	0.03[b]	5 (55.6)	0.03[b]	1 (11.1)	0.74[b]	3.50 ± 6.60	1.30	1.80	20.90	20.90	20.90	20.90	1.20
	Not recent	23	10 (43.5)		5 (21.7)		1 (4.4)		1.35 ± 3.31	0.35	1.00	1.60	1.80	16.40	16.40	0.65
	No treatment	94	40 (42.6)		17 (18.1)		5 (5.3)		1.13 ± 2.18	0.35	0.80	1.60	8.00	11.60	11.60	0.45

Table 3 (continued)

Variable	Categories	*N*	*N* (%) ≥ 0.5 µ/L	*p* value	*N* (%) > 1 µ/L	*p* value	*N* (%) > 5 µ/L	*p* value	Mean ± SD	50th	75th	90th	95th	99th	Max	IQ
Pesticide use for pets	Yes	19	8 (42.1)	0.54[a]	4 (21.1)	0.77[c]	0	0.58[c]	0.83 ± 0.93	0.35	1.00	1.60	4.30	4.30	4.30	0.65
	No	66	33 (50.0)		17 (25.8)		5 (7.6)		1.58 ± 3.38	0.43	1.10	1.80	9.80	20.90	20.90	0.75

[a] χ^2 statistical test

[b] Kruskal–Wallis statistical test

[c] Fisher statistical test

[d] Recent: from few days to one week before collection of the urinary sample; not recent: more than one week before collection

garden pesticide treatment by parents (OR 6.25; 95% CI 1.16–33.70), as shown in Table 4. Children who lived near (≤ 30 m) the vineyards had a higher risk of having u-ETU values > 1 µg/L compared with children living farther than 200 m (OR 9.51; 95% CI 1.87–48.46) from the nearest vineyard (Table 4).

Children did not seem to have a higher risk of having values of u-ETU over 1 µg/L based on the location of home and preschool, as shown in Table 4.

Comparing the results from time after intervention to those baselines for adults ($n = 49$) who participated in both time points of our study, we found a considerable decrease in the number of subjects with u-ETU concentrations over 5 µg/L: from 26.5 to 4.1% ($p < 0.01$), as shown in Table 5. Similarly, we observed a significant reduction in the percentage of adults with u-ETU values between 0.5 and 5 µg/L: from 32.7 to 22.4% ($p < 0.01$) (Table 5). We also obtained similar findings in children involved in both time points of the study ($n = 25$): the proportion of u-ETU concentrations between 0.5 and 5 µg/L decreased from 40% baseline to 32% after intervention, and no children had u-ETU values over 5 µg/L after intervention, compared to 20% at baseline (Table 5). The reductions in u-ETU concentrations were statistically significant ($p = 0.01$).

Among the 54 adults enrolled after intervention, 48 urinary samples were valid in both March (pre-spraying season) and June (post-spraying season). The percentage of subjects with u-ETU levels between 0.5 and 5 µg/L increased from 12.5% pre-spraying season to 20.8% post- spraying season ($p = 0.07$), and there were 2 subjects with post-spraying season u-ETU concentrations over 5 µg/L (Table 6).

For the 55 children of the population in 2014, 47 urinary samples were considered valid in both pre- and post-spraying season. The proportion of subjects with u-ETU levels between 0.5 and 5 µg/L increased from 14.9% pre-spraying season to 31.9% post-spraying season ($p = 0.03$), and there was one child with a post-spraying season u-ETU concentration over 5 µg/L (Table 6).

The increase in u-ETU concentration from pre- to post-spraying season, both in adults and in children, was probably due to a greater exposure to DTC in June 2014, which is the period of the maximum utilization of these pesticides.

Discussion

In our study, the proportion of adults with a concentration of u-ETU ≥ 1 µg/L at baseline was similar (23.9%) to that found by Aprea et al. in urban populations (24%) and lower than that in a wine-producing area located on the southern bank of the Po River (37%) (Aprea et al. 1996; Aprea and Catenacci 2003). The median value of baseline u-ETU in adults (0.35 µg/L) was well below the median reference value of

Table 4 Determinants of having values of baseline u-ETU > 1 µg/L in both adults and children, according to univariate and multiple logistic regression models

Variable	Univariate OR (CI 95%)	Adjusted OR (CI 95%)
Adults		
Wine consumption (any amount vs. no consumption)	2.29 (1.22–4.31)	2.04 (0.99–4.22)
Garden treatment (any treatment vs. no treatment)	2.46 (1.22–4.94)	2.70 (1.22–5.97)
Medications use (yes vs. no)	1.21 (0.63–2.34)	1.38 (0.65–2.91)
Pet pesticides (yes vs. no)	2.31 (1.05–5.04)	–
Home-vineyard distance (≤ 30 m vs. > 200 m)	1.12 (0.48–2.61)	0.89 (0.34–2.34)
Nearest vineyard surface (< 6000 m^2 vs. ≥ 50,000 m^2)	0.47 (0.11–1.93)	0.53 (0.12–2.38)
Children		
Residence risk area (high versus low)	2.01 (0.75–5.36)	0.97 (0.13–7.18)
Municipality of maternal school (high risk vs. low risk area)	3.02 (0.93–9.85)	1.07 (0.10–11.00)
Recent garden treatment (few days-max 1 week) vs. no treatment	5.66 (1.37–23.32)	6.25 (1.16–33.70)
Home distance from vineyard (30–200 m vs. > 200 m)	1.78 (0.45–7.09)	1.80 (0.40–8.04)
Home distance from vineyard (≤ 30 m vs. > 200 m)	6.90 (1.77–26.92)	9.51 (1.87–48.46)
School distance from vineyard (≤ 30 m vs. > 200 m)	1.73 (0.41–7.33)	–

Table 5 Comparison of the u-ETU levels between baseline (June 2012) and after intervention point (June 2014) in both adults and children

	June 2012	June 2014	*p* value
Adults (*n* = 49)			
< 0.5 µg/L	20 (40.8%)	36 (73.5%)	< 0.01
0.5–5 µg/L	16 (32.7%)	11 (22.4%)	
> 5 µg/L	13 (26.5%)	2 (4.1%)	
Children (*n* = 25)			
< 0.5 µg/L	10 (40.0%)	17 (68.0%)	0.01
0.5–5 µg/L	10 (40.0%)	8 (32.0%)	
> 5 µg/L	5 (20.0%)	0	

Table 6 Comparison of u-ETU levels between pre- (March) and post- (June) spraying season 2014 (after intervention) in both adults and children

	March 2014	June 2014	*p* value
Adults (*n* = 48)			
< 0.5 µg/L	42 (87.5%)	36 (75.0%)	0.07
0.5–5 µg/L	6 (12.5%)	10 (20.8%)	
> 5 µg/L	0	2 (4.17%)	
Children (*n* = 47)			
< 0.5 µg/L	40 (85.1%)	31 (66.0%)	0.03
0.5–5 µg/L	7 (14.9%)	15 (31.9%)	
> 5 µg/L	0	1 (2.1%)	

1 µg/L. The 95° percentile of baseline u-ETU (5.3 µg/L) in the Prosecco District was similar to the Italian reference (5 µg/L) and the English reference concentration (4.9 µg/L) but much lower than in the rural population of the study by Aprea et al. (16.5 µg/L) (Aprea et al. 1996; Bevan et al. 2013; SIVR 2017).

The differences between these two Italian studies conducted in wine-producing districts may be due to (a) different methods used to spray pesticides (in the areas evaluated by Aprea et al., pesticide spraying was performed by helicopter, whereas in the Prosecco District, ground-sprayers were preferred, and only copper sulfate was sprayed from the helicopter until 2016); (b) different proportions of wine-drinkers (50% in our population, 54% in the urban population of Aprea and 61% in the rural population of Rovescala); and (c) a higher proportion of smokers (36–38%) in the study by Aprea et al. compared to those (19%) in the Prosecco District.

To identify determinants of baseline u-ETU concentrations, we chose statistical methods (i.e., multivariate logistic regression) that estimated the probability to exceed a certain cutoff level above which the likelihood of a health risk might be increased. In the analyses that we conducted, baseline u-ETU values of 1 and 5 µg/L were considered as cutoffs because the former represented the median reference value and the latter coincided with the 95° percentile of the u-ETU reference concentration (Aprea et al. 1996; Colosio et al. 2006). In our study, at baseline, only 14 out of 260 adults (5.4%) exceeded this value, reflecting the frequency of the general population.

In our study, the distribution of baseline u-ETU concentrations was similar between adults and children. In fact, 46.0% of children and 47.3% of adults had u-ETU value over 0.5 µg/L. Among children, 5.6% showed a u-ETU value over 5 µg/L, similar to the value observed for adults and consistent with the reference value (SIVR 2017).

Moreover, in the children of our study at baseline, the median value of u-ETU was 0.35 µg/L and the 75° percentile

was 0.90 µg/L. These values were much lower than in a sample of children from Costa Rica (median = 1.6 µg/L; 75° percentile = 3.2 µg/L), who lived in villages surrounded by banana plantations, where mancozeb was used both by aerial spraying and ground-level application (van Wendel de Joode et al. 2016). Both children of our study and children from Costa Rica were environmentally exposed to mancozeb used in the agricultural setting. However, in Costa Rica, aerial spraying was more common than in the Prosecco District, where this technique was used only in otherwise inaccessible locations. No quantitative estimates of the amount of pesticide dispersed in the two areas were available.

Children may be more exposed than adults to pesticides in different settings (home and school) and from several sources: soil, dust, green areas (e.g., parks, gardens, and playgrounds), contaminated food and water, parents' clothing and pets (WHO 2008). The children can be exposed by inhalation, ingestion (frequent hand-to-mouth activity) or dermal contact (Roberts and Karr 2012). For these reasons, we expected higher levels of u-ETU among children in this study; however, we obtained a similar distribution of u-ETU concentrations as in adults, which reflected a similar level of exposure. The levels of baseline u-ETU in our study appeared much lower than those in agriculture workers and even in control groups in other studies (Colosio et al. 2003; Runkle et al. 2013). In fact, female farmworkers from Florida, who were occupationally exposed to mancozeb, had a median u-ETU value of 5.71 µg/g creatinine; 75°, 90° and 95° percentiles of 10.31, 15.41 and 26.78 µg/g creatinine, respectively (Runkle et al. 2013). In the Florida study, the local control group had a median value of 1.67 µg/g creatinine, and the 75° and 90° percentiles were 2.74 and 3.52 µg/g creatinine, respectively (Runkle et al. 2013). Furthermore, the concentrations found in 36 Ecuadorian flower-growers (median equal to 3.2 µg/g creatinine at the beginning of the work-shift and 6.2 µg/g creatinine at the end) and in 7 controls (0.7 µg/g creatinine) were also higher (Colosio et al. 2003). Moreover, Colosio et al. examined vineyard workers of a rural area of Northern Italy (Lombardy) who were exposed to mancozeb and reported u-ETU concentrations higher than those in our study at baseline. The corresponding values were as follows: median u-ETU level of 2.5 µg/g creatinine (range < 0.5–95.1) and mean concentration of 12.5 ± 25.9 µg/g (Colosio et al. 2002).

The differences between our findings and those presented in the literature suggest a lower exposure to DTC in our study sample compared to occupationally exposed subjects.

Subjects enrolled in our study also showed lower DCT exposure than the pregnant women studied by Handal et al. in Ecuador. In the latter group, 16 of these pregnant women were rose-growers and 10 were non-agricultural workers (Handal et al. 2016). Since the u-ETU concentrations were increased in both samples (median = 3.8 µg/L, 90° percentile = 10.41 µg/L), the authors suggested that DTC exposure may be caused not only by occupational exposure but also by diet.

In contrast, our findings were similar to those reported in the "Health Assessment of Mothers and Children of Salinas" (CHAMACOS) study, which was conducted on 342 pregnant women resident in Salinas Valley (California) (Castorina et al. 2010). In this wine region, DTC are widely utilized (more than 150,000 kg of maneb and mancozeb annually). In the CHAMACOS cohort, the 75°, 90° and 95° percentiles were < 0.1 µg/L, 0.7 µg/L and 1.5 µg/L, respectively (Castorina et al. 2010). Notably, u-ETU levels tend to decrease during pregnancy (Castorina et al. 2010).

Concerning home distance from vineyards, we did not find an association with baseline u-ETU levels in adults. However, in children, we observed a strong association between living less than 30 m from vineyards and baseline u-ETU concentrations over 1 µg/L (OR 9.51; 95% CI 1.87–48.46). The children may be more exposed than adults to pesticides since they commonly play outside (in home gardens) spending more time outdoor than adults and thus the distance of home from vineyards may be more relevant in children than in adults. These findings are partially in accordance with the results of the Infants' Environmental Health Study (ISA), which assessed u-ETU levels in pregnant women living near banana plantations in Costa Rica (where mancozeb was sprayed by airplane) (van Wendel de Joode et al. 2014). In ISA, women living within 40 m of the plantations had u-ETU concentrations 45% higher than those living a greater distance away (van Wendel de Joode et al. 2014).

Our study also found that a larger vineyard surface area is associated with a higher prevalence of adults with baseline u-ETU > 1 µg/L, although the association was not statistically significant ($p = 0.06$).

We observed higher baseline u-ETU concentrations in adults who treated their gardens with pesticides and in those who consumed wine. In fact, people can be exposed to ETU by food (in particular vegetables and fruits) and beverages, especially wine, which causes an increase in u-ETU values (Houeto et al. 1995; Colosio et al. 2002, 2006).

Concerning baseline u-ETU concentrations over 5 µg/L, we found a significant relationship only with medication use, but these data were limited to 8 exposed subjects.

Among children, recent pesticide treatment of home gardens by parents and a home-vineyard distance ≤ 30 m versus > 200 m were associated with higher u-ETU concentrations at baseline.

Comparing the findings at baseline with those obtained after intervention, we noted a relevant reduction in u-ETU concentrations among adults and children. As expected, the concentrations were higher in June (post-spraying season) than in March 2014 (pre-spraying season), as

vineyards are treated during the warmer months of grapes ripening on the vines.

Decreasing u-ETU levels from baseline to time after intervention may be due to several reasons, among which the important role played by public health actions was started in 2013, after the publication of early data from our study (ULSS 7 2013). As cited, the Consortium of Prosecco DOCG, together with many municipalities of the Prosecco District, committed to banning mancozeb from vineyard treatments (Comune di Conegliano 2014; Comune di Follina 2014; Consorzio di tutela del vino Conegliano Valdobbiadene Prosecco 2016). In fact, the use of mancozeb in the study district after intervention was much lower than that baseline. Indeed, a decrease of 35% (from 66,357 to 43,032 kg) was registered (ARPA 2012, 2014). Moreover, the use of metiram, an alternative DTC, increased (ULSS 7 2016). An important awareness-raising project of the Health Department of Prevention of Treviso, which was initiated in 2013, probably led the general population living near vineyards to more attentively control their domestic use of DTC for horticulture. However, along with lowering mancozeb use, alternative fungicides were used in the Prosecco District, including pesticides containing sulfur and copper, metiram, fosetyl-aluminum, dimethomorph and cymoxanil (ARPAV 2014).

Our study had some limitations. The questionnaires were self-reported; thus, there can be misreporting bias, recall bias and incomplete information, especially for the type of medications used. In addition, the number of subjects with detectable u-ETU concentrations was limited, and therefore, estimates may be statistically imprecise.

We did not collect environmental samples (air, soil). As already cited in this article, ethylenebisdithiocarbamates (EBDCs) are unstable in the presence of oxygen, moisture or biological systems (IPCS 1988). They are rapidly decomposed, producing ETU, which is stable to hydrolysis and is the major cause of toxicity (IPCS 1988). Thus, it is difficult to detect EBCDs in environmental samples (air, soil, food and water samples) for their short life, while ETU can be detected and in fact it is used as marker of EBCDs contamination (IPCS 1988). ETU can be metabolized by plants and soil microorganism and photo-oxidized in soil and water in the presence of photosensitizers. (IPCS 1988; Xu 2000). Moreover, temperature, humidity, wind and in general meteorological conditions may influence ETU levels in air. Also the time between spraying and samples' collection is important. The half-lives of ETU are: 8–9 days in air, 1–8 days in soil and 1–4 days in natural water (Xu 2000). In fact, the environmental concentrations of ETU may be influenced by rain and reactions with hydroxyl radicals in air, by photolysis in water, by field characteristics, humidity and metabolization by microorganisms in soil (Xu 2000). All the above-cited factors may affect detection of ETU in environmental samples, which resulted complex.

The study also had several strengths. We used a readily available exposure marker of established accuracy, for which reference values have been established by SIVR. We monitored the most exposed subjects after 2 years, after a drastic decrease in mancozeb use in the study area. We enrolled both adults and children in the Prosecco District, an area where there have been no previous studies on DTC exposure in the general population, especially in children.

Although we could not use geocoded information for all measurements, the home distance from the nearest vineyard and the vineyard surface area were accurate. In fact, when geocoded data were missing, the technical staff of the local municipalities conducted field measurements.

The questionnaires supplied information on diet, wine consumption and tobacco habit, which are all factors that may influence u-ETU values, and we included those data in multiple logistic regressions.

Conclusions

Our study found a relation between u-ETU levels and the use of DTC in home gardening in both adults and children and an inverse association between the u-ETU concentrations and the distance between the vineyards and the home in children. These findings suggest that the use of DTC both domestic and agricultural (for grapes' protection) may be a source of exposure for the study population. People can also be exposed to DTC environmentally through air by inhalation and by dermal contact with DTC residues in air and soil. The exposure by soil contact may be significant in children, who often play in lawns and residential turfs and have hand-to-mouth activities, with also the risk of ingestion of DTC residues. In adults, levels of u-ETU appeared to be related to wine consumption. In fact, DTC were widely used in vineyards and their metabolite ETU persists in grapes and in wine.

The proportions of subjects with u-ETU levels ≥ 0.5 µg/L decreased from the baseline to the post intervention evaluation in both adults and children. After intervention only two adults had u-ETU level over 5 µg/L. Even if we did not have health data of study population, we may suppose that the strong decrease in u-ETU concentrations under the precautionary level of 5 µg/L probably reduced the risk of effects on human health. This reduction in u-ETU was probably due to public health interventions, which probably led to decrease mancozeb use in vineyards and home gardens and an increased use of personal protective equipment. It seemed also important to organize educational meetings on the correct use of pesticides and of protective equipment, as we did during 2013. However, limiting further or banning

the use of more toxic DTC, regulating by deliberations of municipal councils, may be an effective public health action, as suggested by the reduction in u-ETU levels in 2014 after deliberations of some municipalities of the Prosecco District. Moreover, another possible strategy may involve the sustainable agriculture, like organic farming, that has been spreading in recent years.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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