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Iodine nutritional status and thyroid effects of exposure to ethylenebisdithiocarbamates

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ABSTRACT

Introduction: Italy is still characterized by a mild iodine deficiency and is among the most intensive users of chemical products for agriculture in Europe. The aim of this study was i) to evaluate thyroid effects of exposure to mancozeb, a fungicide widely used in agriculture, in a sample of Italian grapevine workers, and ii) to verify whether the iodine intake may modulate the risk of thyroid disruption due to the mancozeb metabolite ethylenthiourea (ETU).
Methods: One hundred seventy-seven occupationally exposed male workers (29 from Chianti, a mild iodine deficient area, and 148 from Bolzano an iodine sufficient province) and 74 non-occupationally exposed male controls (34 from Chianti and 40 from Bolzano) were enrolled in the study. Serum biomarkers of thyroid function, as well as urinary iodine and ETU concentrations were assessed. Moreover all the recruited subjects underwent clinical examination and thyroid ultrasound.
Results: Multivariate comparisons showed lower mean serum levels of FT4 in Chianti-workers as compared to Bolzano-workers. Moreover, an increased urinary iodine excretion (> 250 μg/L) was more frequently found among more exposed workers (ETU > 20 μg/L) than among less exposed ones and this effect was more pronounced in Chianti- than in Bolzano-workers. Chianti-workers also showed a significantly higher frequency of very low thyroid volume (≤6.0 ml) as compared to controls.
Conclusions: These findings showed a mild thyroid disrupting effect due to occupational exposure to mancozeb, more pronounced in workers residing in an area characterized by a mild to moderate iodine deficiency as compared to workers residing in an area covered by a long-lasting iodine prophylaxis program.

1. Introduction

Increasing evidence from *in vivo* and *in vitro* studies has demonstrated the vulnerability of the thyroid to endocrine disrupting effects due to environmental exposures. The most potent thyroid disruptor is iodine, a micronutrient essential for the thyroid hormone synthesis. Both insufficient dietary intake and excessive exposure to iodine have thyroid disrupting effects (Laurberg et al., 2010). Furthermore in the last two decades an increasing body of data have demonstrated that a wide range of chemicals with endocrine disrupting activities (endocrine disruptors, EDs) are able to interfere with the thyroid function (Brucker-Davis, 1998; De Angelis et al., 2007; Boas et al., 2009).

Abbreviations: SD, Standard Deviation; CH, Chianti; BZ, Bolzano; ETU, Ethylenethiourea; CI, Confidence Interval; NR, Normal Range; OR, Odds Ratio; GM, geometric mean; EDs, Endocrine Disruptors; EBDC, Ethylenebisdithiocarbamates; T3, Triiodothyronine; T4, Thyroxine; Tg, Thyroglobulin; TSH, Thyroid Stimulating Hormone; FT3, free T3; FT4, free T4; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; UIC, Urinary Iodine Concentration; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; US, Ultrasonography; RIA, Radioimmunological Assay

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Among EDs, pesticides are of particular concern due to their extensive worldwide utilization (EFSA, 2013), which leads to a potential widespread exposure involving occupational settings, residues in food (Mantovani 2015) and living environment in agricultural areas (Bradman et al., 2007; Fantke et al., 2011). Once these xenobiotics have entered the body, they usually undergo metabolic processes before excretion and only few compounds are excreted unmodified. Ethylenebisdithiocarbamates (EBDC), such as maneb and mancozeb, are a group of fungicides widely used in agriculture because of their low acute toxicity and short environmental persistence. In some countries EBDC are aerially sprayed by a light aircraft (van Wendel de Joode et al., 2014). Although EBDC have a low acute toxicity, this highly dispersive technique of application is of concern for people living near fields where EBDC are aerially sprayed as it can increase the EBDC environmental exposure.

EBDC are broken down into ethylenthiourea (ETU), which is both the main degradation product of EBDC and the most important metabolite from a toxicological perspective (Houeto et al., 1995). Furthermore, ETU is present as an impurity in several EBDC formulations and it is formed when cooking food with residues of mancozeb and other EBDC (US EPA, 2005). EBDC are used on grapes and tobacco crops. For this reason ETU can be present in wine and cigarette smoke (Aprea et al., 1996). As ETU is a specific EBDC metabolite its urine concentration has been suggested as a sensitive indicator for biological monitoring of occupational and environmental exposure to ETU and/or EBDC (Colosio et al., 2002; Fustinoni et al., 2008). In humans, the elimination half-life of ETU in urine has been estimated to be in the range of 19–100 h depending on the exposure routes (Kurttio and Savolainen, 1990; Lindh et al., 2008). Although neither EBDC nor ETU accumulate within the body, ETU can cross the placenta and can be secreted into breast milk (NTP, 1992). In a previous evaluation it was classified as possible carcinogenic to humans (group 2B) by IARC (IARC, 1987), but subsequently it has been re-classified in the group 3 due to an inadequate evidence of carcinogenicity (IARC, 2001). ETU is also known for its teratogenic properties (US EPA, 1996; NTP, 1992; Iwase et al., 1997), slight and transient immunomodulating capabilities (Colosio et al., 2007), and anti-thyroid activity. The latter is due to its ability to interfere with thyroid hormone biosynthesis by inhibition of thyroid peroxidase, an enzyme involved in the iodination of thyroglobulin which is the precursor of thyroid hormones triiodothyronine (T3) and thyroxine (T4) (Marinovich et al., 1997; Freyberger and Ahr, 2006). The capability to regulate the expression of genes involved in the thyroid function has also been reported in non-mammalian vertebrates (Opitz et al., 2009). Moreover alterations in thyroid weight, cells, hormones, iodine uptake, and thyroid tumors have been reported in chronic mancozeb and ETU exposed rats, mice, and dogs (Axelstad et al., 2011; Belpoggi et al., 2002; Chhabra et al., 1992; IARC, 2001).

Although in vivo studies have confirmed a thyreostatic effect of ETU at non teratogenic doses (Maranghi et al., 2013), only few studies have been conducted in humans. These have only revealed mild alterations in thyroid physiology and structure associated with mancozeb and ETU occupational exposure (Steenland et al., 1997; Panganiban et al., 2004). A cross-sectional study of EBDC-exposed Mexican backpack applicators has reported higher mean serum thyroid stimulating hormone (TSH) concentrations compared to non-exposed workers, as well as higher mean sister chromatic exchanges and chromosome translocations (Steenland et al., 1997). In another study conducted on mancozeb exposed Philippine banana plantation workers a positive correlation between ETU concentrations and size of solitary nodules measured with thyroid ultrasounds was observed (Panganiban et al., 2004). However, no information is available on whether an inadequate iodine intake, a condition still prevalent worldwide and still representing a major health concern in most countries (Zimmermann, 2013), may play a role in rendering the human thyroid more vulnerable to thyroid disrupting effects of EDs exposure.

Italy is still characterized by a mild iodine deficiency (Pastorelli et al., 2014). Our country is also among the most intensive users of chemical products for agriculture in Europe (EUROSTAT, 2012). Therefore the aim of this study was to evaluate thyroid effects of exposure to mancozeb in a sample of Italian grapevine workers, and to verify whether the iodine intake may modulate the risk of thyroid disruption due to the mancozeb metabolite ETU. To this end, workers and non-occupationally exposed controls were recruited in two areas with different iodine nutritional status: the autonomous province of Bolzano, which is iodine sufficient because of an efficient province-wide iodine prophylaxis program introduced in 1982 (Franzellin, 1998; Olivieri et al., 2015), and the Chianti area historically recognized as a mild iodine deficient area in the Tuscany, a region in the Central Italy (Aghini Lombardi et al., 1995; Maccherini et al., 1998).

2. Patients and methods

2.1. Study population

One hundred and seventy-seven occupationally exposed grapevine workers and 74 non-occupationally exposed controls were enrolled in the study. Specifically, 29 out of 177 grapevine workers resided in the Chianti area (CH-workers), whereas the remaining 148 were recruited in Bolzano province (BZ-workers). All the workers were male (age range: 21–71 years) and were randomly recruited from vineyard farms where mancozeb was systematically used. All of them had at least 1 year history of direct exposure to mancozeb. In all the recruited workers pesticide exposure occurred during preparation of pesticide mixture and its application in vineyards or during re-entry activities, i.e. activities involving workers' entry in the crops after pesticide application. Given the short half-life of ETU and the seasonal use of mancozeb between June and September, urine samples for the detection of exposure biomarkers (urinary iodine and ETU) were collected during the period of treatments (July–August). Spot urine samples were collected the day after the treatment in workers engaged in application of mancozeb (n=170) and the day after the re-entry in culture in workers engaged only in plant maintenance (n=7). Blood samples for thyroid function tests were collected after six weeks from the last treatment, in October, at the time of grape harvest. At this time all the recruited workers underwent a complete clinical examination and thyroid ultrasound.

The control group consisted of 74 non-occupationally exposed male subjects (age range: 29-59 years) recruited from the healthcare personnel of the Bolzano Hospital and the Local Health Unit of Florence. Specifically, 34 resided in the Chianti area (CH-controls) and 40 in the Bolzano province (BZ-controls).

None of the recruited subjects were known to be affected by thyroid diseases or were taking drugs interfering with thyroid function at the recruitment. In this study the sample size was the largest we could obtain taking into consideration available funds, availability of farmers where the use of mancozeb was exclusive or prevalent, and availability of workers and controls without thyroid diseases. Moreover, since the goal of the study was to evaluate thyroid effects of occupational exposure to mancozeb in iodine sufficient and iodine deficient areas, the study was designed with a larger number of workers.

A structured questionnaire was administered to all participants by medical personnel. Information on drinking and smoking habits, dietary pattern, additional pesticides besides mancozeb, pesticide use (occupational and non-occupational use), and individual protection devices use was collected. All the recruited subjects signed an informed consent form in acceptance of entering the study. This study was approved by the ethics committees of Bolzano Hospital and the Local Health Unit of Florence.

2.2. Evaluation of urinary biomarkers (iodine and ETU)

Urinary iodine concentration (UIC) has been indicated by WHO