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Biomonitoring short- and long-term exposure to the herbicide terbuthylazine in agriculture workers and in the general population using urine and hair specimens

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ABSTRACT

The aim of this work was to evaluate short-term and long-term exposure to terbuthylazine (TBA) in agriculture workers (AW), rural residents (RR), and urban residents (UR) using urine and hair specimens. Twelve AW, 13 RR, and 17 UR were included in the study. Urine spot samples were collected with two different protocols. AW urine samples were collected before the application season (February, U_0), at bedtime on the day of TBA application (March–May, U_1), and prior to the next shift on the day after TBA application (U_2). RR and UR urine samples were collected on any day during the application season (U_e). Hair samples were collected for all subjects before the application season (February, H_0) and at the end of the season (June, H_1). TBA and its metabolite desethylterbuthylazine (DET) were measured by liquid chromatography coupled with triple quadrupole mass spectrometry detection. DET was exclusively found in urine, while TBA was mostly found in the hair. In the AW, the urinary levels of DET were not detected in the U_0 samples, and they increased to median levels of 1.81 and 2.94 μg/L in the U_1 and U_2 samples, respectively ($p < 0.001$). In the RR and UR, DET was not detected in the U_e samples. In the UR, TBA was not detected in the H_0 samples, and the median levels of TBA were 0.01 ng/mg hair in both the AW and RR. In the H_1 samples, the median TBA levels were not detected, 0.01, and 0.08 ng/mg hair in the UR, RR, and AW, respectively ($p < 0.001$). Urinary DET and hair TBA are promising candidates for biomonitoring short- and long-term exposure to TBA. The use of this herbicide in agriculture leads to exposure in rural residents.

1. Introduction

Terbuthylazine (TBA, CASRN: 5915-41-3, Fig. 1), atrazine, simazine, and propazine are chlorotriazine herbicides, which are very effective inhibitors of photosynthesis. These chemicals are selective herbicides that are applied to several crops including maize, which is the major cultivated crop of the Po Valley in Northern Italy. In the past atrazine was the most popular herbicide, but due to its environmental persistence it was banned in Italy since 1986; as a consequence it has been replaced with TBA that has become the most used herbicide for the pre-emergency treatment of maize (Bozzo et al., 2013). According to the Eurostat, in 2003, TBA was ranked sixth among the most used herbicides (4.3% of total herbicides used), with an annual consumption of 3624 tons (Eurostat, 2007).

Since 2008, restrictions on the use of TBA were introduced to protect water bodies. Indeed, TBA and its degradation product desethylterbuthylazine (DET) are the most frequently detected pesticide residues in both surface and underground waters in several regions of Northern Italy. The percent of TBA detection is up to 90% for concentrations greater than 0.1 mg/L in the region with extensive maize cultivation (ISPRA, 2010).

The acute toxicity of TBA in experimental animals is low, and it has been classified in Toxicity Category III for acute effects caused by oral, dermal, and inhalation exposure (USEPA, 1995). TBA is moderately irritating to the eyes and slightly irritating to the skin, but it is not a sensitizer (Health and Safety Database, 2013). The environmental persistence of TBA is a major concern, as it has a strong soil sorption (WHO, 1998) and it is slowly degraded. Therefore, after repeated treatments, TBA is enriched in the top soil and can exert direct toxic effects on different soil animals (Salminem et al., 1996).

In mammals, the metabolic degradation of TBA is dependent on the cytochrome P-450 oxidative system, through oxidative dealkylation and oxidation of the methyl group of the tert-butyl alcohol (WHO, 1998). Studies in rats and cattles that were administered ^{14}C-labeled TBA showed its complete metabolism and excretion in urine and feces with a half-life of 16–17 h (WHO, 1998). In humans, a preliminary study on workers involved in TBA production found that 1–2% of TBA was excreted unchanged, and approximately 50–60% of its urinary metabolites were DET, 30–35% were diaminochlorotriazine (DACT), and approximately 5% were desisopropyl atrazine (DIA) (Fig. 1)

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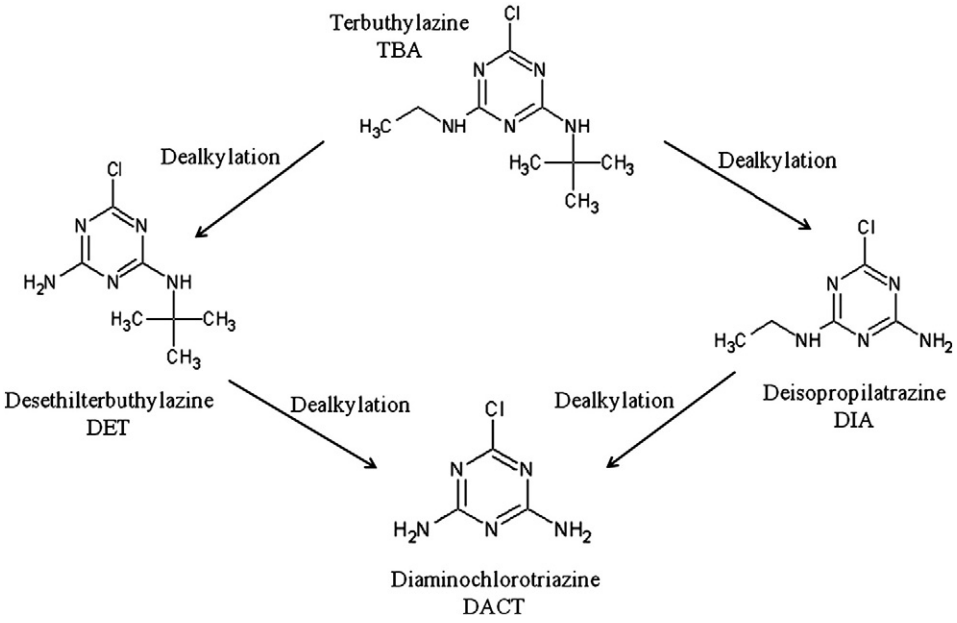

Fig. 1. Terbuthylazine (TBA) metabolic pathway.

(Catenacci et al., 1998). These data show that the metabolic pathway of TBA is very similar to that of atrazine (Barr et al., 2007).

In agriculture, occupational exposure to TBA may occur through inhalation and dermal contact during its use as an herbicide (Health and Safety Database, 2013). In addition, the general population may be exposed to TBA via ingestion of contaminated drinking water (Health and Safety Database, 2013).

In the last decade hair was investigated as a biological matrix for assessing exposure to pesticides and other persistent organic pollutants (reviewed in Appenzeller and Tsatsakis, 2012 and in Schramm, 2008). Indeed, the hair may be used to detect cumulative exposure in a similar manner to what is routinely performed in the field of drugs of abuse. We recently investigated TBA exposure through hair analysis: our study found that TBA is incorporated in the hair of rats following oral administration (Mercadante et al., 2012a). Moreover, TBA is incorporated in both the hair of AW applying TBA to maize crops and the hair of subjects living in rural areas next to the crops (Mercadante et al., 2012a).

The purpose of the present study was to further evaluate the measurement of TBA and DET in hair as a marker of cumulative exposure. In addition, the use of urine as a method for the biological monitoring of TBA exposure was simultaneously evaluated, and the results of these two approaches were compared. Before establishing the protocol for urine collection, a small preliminary kinetics study was performed to determine the best sampling times. With these aims, we investigated AW applying TBA on maize, residents of the same rural area, and residents of an urban area 40 km away from the maize crops.

2. Material and methods

2.1. Study subjects and sampling protocol

The study was conducted in 2010 in a small village of the province of Cremona, Lombardy, Italy. It involved 12 AW residing in the village who applied TBA to maize crops in pre- and post-emergence treatments, 14 rural residents (RR) of the same village without family ties to the AW, and 17 urban residents (UR) who lived and worked in the urban area of Milan, the capital of Lombardy, located 40 km away from the village. Ten of these AW were previously investigated in 2009 (Mercadante et al., 2012a).

Between March and May the maize fields were treated with TBA-based formulations for the control of weeds and annual dicots. The following formulations were used: Bolero Micromix by Monsanto (166.5 g/L TBA coformulated with acetochlor), Sulcotrek by Agan Chemical Manufacturers Ltd. (327 g/L TBA coformulated with sulcotrione), and Lumax by Syngenta Crop Protection (187.5 g/L TBA coformulated with S-metolachlor). A single subject applied Myrtos Agrosol Duo (glyphosate with residual TBA content) to treat the edges of ditches.

In a preliminary study to evaluate the kinetics of TBA excretion, two AW were asked to collect individual spot samples of each void starting before their shift on the first day of TBA application, then during TBA application, and continuing for approximately 48 h. For each void, the time of collection and the volume of urine were self-recorded.

The sampling strategy for the main study is shown in Fig. 2. Urine collection was performed using two different protocols: one for the AW, and another for the RR and UR. The AW collected three spot samples. The first was a pre-exposure sample (U_0) collected from the second void of the day in February before the beginning of the application season. The second was a post-exposure sample (U_1) collected at bedtime on the day of the TBA application. The third was a post-exposure sample (U_2) collected prior to the next shift on the day after the application. The RR and UR collected one spot samples from the second void of any day (U_e) after the beginning of the application season (March–May).

Hair collection was performed following the same protocol for all subjects. Two samples were obtained. The first was a pre-exposure sample (H_0) collected in February, and the second was a post-exposure sample collected at the end of the application season (H_1) in June.

Study subjects were individually visited by the investigators before the beginning of the study. The subjects received information on the aim and modalities of the study and signed the informed consent. Each subject was provided with instructions for urine collection and a sampling kit consisting of sampling tubes and a collection sheet. The subjects were instructed to collect urine autonomously and to record the sampling times. The collected samples were then chilled and stored at 4 °C, and picked up by the investigators within 48 h. The samples were delivered to the laboratory and stored at −20 °C until analysis.

Hair samples were taken by the investigators, who selected a strand from the occipital area of the head, cutting as close as possible to the root. A typical hair sample was 5 cm in length (from 2 to 10 cm) and weighted 100 mg. The strand was attached with masking tape to a sheet on which the direction of the hair (root tip) was indicated. Hairs were stored at room temperature in the dark until analysis.