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Terbuthylazine in hair as a biomarker of exposure

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ABSTRACT

Terbuthylazine (TBA) is an herbicide widely used in corn cultivation. Herein we evaluate the measurement of hair TBA as biomarkers of exposure. Five Sprague Dawley rats were gavaged with TBA for 3 days, and then the back hair was shaved and analyzed for TBA. In addition, head hair samples from 10 corn farmers, 9 rural residents, and 6 urban residents were collected at the end of the application season. Hair TBA was detected by liquid chromatography triple quadrupole mass spectrometry after solvent extraction. TBA was quantifiable in all rat samples with a mean concentration of 0.92 (±0.26) ng/mg, which corresponds to a 0.12% incorporation rate. TBA was quantifiable in all farmer samples (median: 0.67 ng/mg), in 75% of rural resident samples (0.01 ng/mg) and in none of the urban resident samples (<0.01 ng/mg), with a statistical difference among groups ($P < 0.01$). Our results suggest that TBA is incorporated in hair and prompt further investigation on the use of hair TBA as a potential biomarker of cumulative exposure.

1. Introduction

Terbuthylazine (TBA; Fig. 1) belongs to the chloro-triazine family of powerful herbicides that act as inhibitors of plant photosynthesis and is the active ingredient of many different formulations on the market. TBA is applied to corn, sorghum, potatoes, peas, sugar cane, vines, fruit trees, citrus, coffee, palm oil, cocoa, olives, rubber, and other trees in tree nurseries and new plantings. After the application, TBA is absorbed by roots and leaves and is distributed throughout the plant. It can be used in both pre- and post-emergency treatments and is particularly suitable for weed control of annual dicots (WHO, 1998). In Italy, TBA is largely applied to corn, which is cultivated over an area of about 1.1 millions of hectares, largely in the North, including 365,000 ha in Lombardy (ISTAT, 2009). The sowing period starts in early February and lasts until early May (Rapparini, 2009).

Human exposure studies have shown that TBA is mildly to moderately irritating to the eyes, and slightly irritating to the skin, but it is not a skin sensitizer (Health and Safety Database, 2011). The US Environmental Protection Agency classifies TBA in Group D as "not classifiable as to human carcinogenicity" (EPA, 2006). Ecotoxicology studies shown that TBA has strong soil sorption (WHO, 1998), so it may be transported to both ground and surface waters and can reach the atmosphere (Otto et al., 2007; Irace-Guigand and Aaron, 2003). TBA is slowly degraded in the soil so, after repeated treatments, it is enriched in the top soil and can exert direct toxic effects on different soil animals (Salminem et al., 1996).

Human exposure to TBA may occur in agriculture settings during mixing and/or loading of herbicide formulation, crop application, re-entry activities, and cleaning and maintenance of the equipment (Arbuckle et al., 2002). Additionally, individuals in the general population residing in a rural area may be exposed (EPA, 2006). Exposure assessment in agriculture includes several critical issues, including: the use of plant protective products that follows a calendar based on atmospheric conditions and crop needs, rather than predefined time tables, different routes of exposure, different application techniques and equipment, and variable use of personal protective devices.

Biomonitoring is a valid tool to perform exposure assessment as it integrates all sources and routes; however, very often conventional matrices such as blood and urine only allow the investigation of recent exposures (Maroni et al., 2000). To overcome this limitation and assess cumulative exposure to toxicants, the use of head hair has been proposed as a matrix for biological monitoring. The use of hair began in the 1960s to assess exposure to heavy metals; over the years, improved analytical methods, both in terms of sensitivity and specificity, have allowed the detection of organic substances. Currently, hair analysis is applied to the routine measurements of drugs of abuse in forensic sciences, and has other research applications in clinical medicine, occupational and environmental toxicology (Villain et al., 2004; Tsatsakis et al., 2008).

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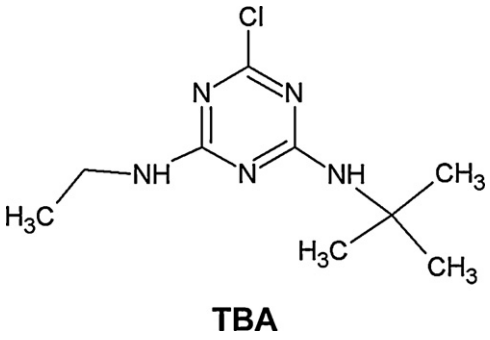

Fig. 1. Molecular structures of terbuthylazine (TBA).

Mechanisms of incorporation of chemicals in hair are not completely understood, but it is believed that adsorbed chemicals are transported by blood to capillaries located in the proximity of the hair follicle where they enter hair growing cells by passive diffusion. Other mechanisms to explain the presence of chemicals in hair include incorporation from deep skin compartment during hair shaft formation, deposition by diffusion from sweat or sebum secretions into the completed hair shaft, and environmental contamination after the hair has emerged from skin (Pragst and Balikova, 2006). In deep skin incorporation and, to a lesser extent, in deposition by diffusion from sweat or sebum secretions, chemicals are into hair shaft, but not into hair cells. In both cases they were associated to cumulative exposure and are believed to be stronger linked to hair in comparison with chemicals deposited by environmental contamination (Pragst and Balikova, 2006; Boumba et al., 2006).

The aim of the present work was to evaluate the use of TBA in head hair as a potential biomarker for assessing cumulative TBA exposure. In this work, we verified the presence of TBA in the keratin matrix of rats after parenteral administration of the herbicide. We also evaluated the level of TBA in the hair of individuals exposed in their work and/or living environments.

2. Materials and methods

2.1. Animals

Five Sprague Dawley male rats (Crl:CD Charles River Laboratories, Calco, Italy), 7–8 weeks old, were used for the experiment. Before starting treatment, a pre-treatment hair sample (T0) from the dorsal region (about 10 cm^2 of area) between shoulder blades was obtained by shaving. This hair sample served as negative control. The animals were kept in single cages in 12 h dark/light cycles and were fed with food pellets (Italiana Mangimi) *ad libitum*. Animals received TBA dissolved in ethanol (5 mg/kg body weight) by gavage once a day for 3 days, for a total administered TBA of 2.25 mg/rat. The administered dose was chosen as a reasonable compromise between low toxicity [no observable effects level (NOEL): 2.1 mg/kg body weight per day; WHO, 1998], and sufficient levels to observe a significant uptake in rat hair. On the fourth day, when the hair regrowth was apparently almost complete, but the shaved area was still identifiable, the rats were sacrificed and post-treatment hair samples were collected both from the previously shaved area (T1) and from a proximal unshaved dorsal area (T2). For each hair sample, the weight and shaved area were registered.

2.2. Study population

The field survey was conducted in 2009 and involved 25 subjects belonging to three groups with different potential exposures: 10 corn farmers (agricultural workers; AW) living in a village in Cremona province, Lombardy, Italy involved in applying TBA in the pre-emergency treatment of crops; 9 rural residents (RR) from the same village, without family ties with the AW; and 6 subjects living and working in the urban area of Milan (urban residents; UR), the Lombardy capital, located 40 km from the rural area. From March to May, the farmers treat the sown fields. Hair samples were collected at the end of the treatment season (June) by cutting a lock of hair as close as possible to the root in the occipital region of the head. A typical sample weighed 100 mg, and was 5 cm long (ranging from 2 to 10 cm). Each sample was attached with paper masking tape on a paper sheet with a mark to indicate the direction of the root and was stored at room temperature in the dark.

A questionnaire administrated by an industrial hygienist was used to collect information about personal characteristics, including hair color, smoking habit, proximity of residence to corn fields, and consumption of green garden vegetables produced in a personal garden. Farmers provided additional information about the use of plant protection products, i.e. name and trademark of the formulation, the concentration of TBA in the formulation, mixing and loading operations, treated area, amount of formulation applied per area, application techniques and equipment, machinery maintenance activities, and use of personal protective devices. All subjects were informed about the aim of the study and gave their written informed consent.

2.3. Detection of TBA in hair

TBA in hair was detected using a liquid chromatography-triple quadrupole mass detector in the presence of terbuthylazine-D5 (d_5-TBA, Dr. Ehrenstorfer, LabService, Anzola Emila, Italy) as isotopically labeled standard. Approximately 50 mg human hair, obtained by selecting 3 cm of hair starting from the root, or 14–47 mg rat hair, were rinsed with 2.5 mL of deionized H_2O, vortexed for a few seconds in a glass vial, and then the water was removed and transferred to a separate vial. Methanol (MeOH, 2.5 mL) was added to the washed hair sample, which was then extracted in an ultrasound bath at 59 kHz for 5 h at 50 °C. Aliquots (0.5 mL) of both the rinsing solutions (RS) and the extracts (E) were added to 25 μL isotopically labeled standard (IS) solution and submitted to analysis. Analysis was performed on a Surveyor high performance liquid chromatography system (Thermo Scientific, Rodano, Italy) equipped with a Betasil C18 column (150 mm length, 2.1 mm internal diameter and 5 μm particle size; Thermo Scientific) kept at room temperature, using an isocratic mixture of aqueous formic acid (0.5%) and MeOH (30:70) at 0.3 mL/min as eluent. The liquid chromatography instrument was interfaced with a triple quadrupole mass spectrometer equipped with a hot-electro spray ionization source (TSQ Quantum Access with H-ESI; Thermo Scientific). TBA and the IS were detected in the positive ion mode and quantification was based on single reaction monitoring (SRM) following the transition m/z 230 → 174 for TBA and m/z 235 → 179 for d_5-TBA. The TBA retention times were 3.91 min (coefficient of variation <0.3%). The method had a linearity up to 25 ng/mg hair, precision of less than 10%, evaluated as the coefficient of variation, with accuracy between 91 and 107% and a limit of quantification (LOQ) of 0.01 ng/mg hair.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS 17.0 package for Windows (SPSS Inc., Chicago, IL, USA). Information from questionnaires was analyzed using descriptive statistics (for continues variables) or frequency analysis (for categorized variables). Differences in frequency distributions were evaluated using Fisher χ^2 test. A value corresponding to one-half of the quantification limit (LOQ) was assigned to measurements of TBA below analytical quantification (Hornung and Reed, 1990). Non-parametric statistic was used to compare RR and AW (Kruskal–Wallis test) and to correlate variables (Spearman's correlations). A P value of 0.05 was considered statistically significant.

3. Results

3.1. TBA absorption in rats

No TBA was found in pre-treatment samples, while TBA was found in both RS and E, in both the T1 and T2 samples (Table 1). Given the similarity of the T1 and T2 values, these data were pooled to estimate a mean TBA content of 0.92 (±0.27) ng/mg in post-treatment hair. On the basis of hair weight and the corresponding shaved area, a hair density of 10 mg/cm^2 was estimated. Calculating a total surface area of about 200 cm^2, according to Diack's formula: $(7.47 \times \text{body weight})^{0.66}$ (Diack, 1930), a total of about 2000 mg hair/rat was estimated. Considering the average TBA level in the samples, it was calculated that, in the hair from the entire rat, there would be about 1843 (±533) ng TBA, which corresponds to an incorporation rate of about 0.12% (±0.036%).

3.2. Study population characteristics

The main characteristics of the study population are reported in Table 2. The groups differed in several characteristics: gender was not uniformly distribute among groups, with 100% AW males in and only 67% in both RR and UR; AW were older than UR, their body weight was higher and the percentage of participants with dark hair was lower than in the other groups. Smoking habits was similar in