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Risk assessment of exposure to pesticides through dietary intake of vegetables typical of the Mediterranean diet in the Basque Country

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ABSTRACT

This study examined estimated dietary exposure among the Basque Country Autonomous Community (northern Spain) to pesticides resulting from dietary intake of unprocessed vegetables. Samples were collected according to a sampling plan established previously, which was performed taking into account statistical factors, such as the population distribution, the point of sale, (local shops or supermarkets), the season and the consumption frequency of each vegetable. A total of 221 samples were analyzed using gas chromatography tandem mass spectrometry (GC–MS/MS), and liquid chromatography tandem mass spectrometry (LC–MS/MS). Results showed that 48.0% of the samples contained no pesticide residues, while 52.0% contained pesticides, and 6.8% of all samples showed residues above the maximum residue limit (MRL). As for the pesticides detected, 56 different active substances were detected, including fungicides and insecticides as the main pesticide types. All of the positive samples were collected in local-area shops. The potential risk to the consumers through vegetable intake was estimated by calculating the Hazard Quotient (HQ), showing ranges between 0.001–0.214%. These results indicate that the exposure to pesticides from vegetable intake among Basque consumers did not raise health concerns.

1. Introduction

The perception of consumer risks related to pesticide use is a subjective issue. The Food-Related Risk Eurobarometer places pesticide residues as the main risk for consumers (European Food Safety Authority, 2010). Pesticides are increasingly viewed as non-organic and industrially synthesized chemicals and the laws that govern their use are considered to be unreliable and controlled by the agrochemical industry. Studies on risk perception (Harris et al., 2002; Melnyk et al., 2011; Peres et al., 2006) often show that consumers view pesticides as a high risk, whereas the actual risk turns out to be low when examined scientifically, as has been demonstrated by numerous institutions and global agencies (European Food Safety Authority, 2014a).

Dietary exposure to pesticide residues is assumed to be five orders of magnitude higher than other exposure routes, such as from breathing air or drinking water (Juraske et al., 2009). According to the World Health Organization (WHO), on average 30% of the diet consists of fruits and vegetables (Martin Cerdeño, 2009), and fruits and vegetables are the most frequently consumed food group. Because fruits and vegetables are consumed mostly raw or semi-processed, it is expected that they contain higher pesticide residue levels in comparison with other groups of food with plant origin. The FAO/WHO recommends including this food group in the diet since their active compounds may decrease the risk of cardiovascular diseases (Ivey et al., 2015; Woodside et al., 2013). On the other hand, vegetables act as contaminant vehicles between nature and humans, and they can also have potentially negative health effects (Alavanja and Bonner, 2012; George and Shukla, 2011; Parron et al., 2014). Since the ingestion of pesticides from vegetable intake presents potential risks for humans (Melnyk et al., 2011), widespread concerns about controlling and assessing vegetable food safety has arisen in recent years.

In addition, vegetable consumers can be simultaneously exposed to several active substances that may potentially contribute to a cumulative effect in the individual. The study of cumulative and synergistic effects of pesticides is a recommendation of the European Commission (European Community, 2005) and organizations such as EFSA propose possible methodologies for assessing the cumulative effects from exposure through food to

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different selected groups of pesticides (European Food Safety Authority, 2014a; Jensen et al., 2013). However, taking into account the current state of affairs, with more than 400 active pesticide substances permitted in the EU, it is practically impossible to quantify the risk of pesticide intake considering all of the cumulative and synergistic effects. The most widely used method for long-term risk assessment requires a comparison between the exposure calculated by means of the individual residue levels consumed and the acceptable daily intake (ADI).

An evaluation of the exposure to pesticides by means of vegetable intake seems to be essential to assess the risk caused by an elevated consumption of vegetables in the Mediterranean diet and because vegetables are often consumed raw. Therefore, members of the EU are committed to obtaining accurate results on food safety. Since the diet standards of Mediterranean countries depend on the consumption of specific foods (Tavani and La Vecchia, 1995), the so-called "Mediterranean diet" makes it necessary to take into account local diets that affect homogenous populations. In this sense, several studies have been carried out in countries and regions from the Mediterranean area bringing to light the specific characteristics of their population (Shen et al., 2015). In the case of the Basque Country (northern Spain), a study was carried out in 2007 that showed a higher vegetable intake than for the rest of the country and neighboring countries in terms of unprocessed or raw food (Elika, 2008).

Thus, the objective of the present study, which has been applied to a community of about 2.5 million inhabitants, was to propose a model showing how to estimate the risks of pesticide intake from a diet with high vegetable content. In this study, pesticide residues were monitored in nine of the most-consumed vegetables in the Basque Country. Analytical techniques based on chromatography coupled to mass spectrometry were used to determine pesticide residues during 2012–2014. The risk assessment was calculated according to the guideline provided by EFSA (European Food Safety Authority, 2014b).

2. Material and methods

2.1. Vegetable samples

A total of 221 samples from several supermarkets and local markets in the Basque Country were collected according to a sampling protocol established beforehand. The sampling consisted of 8 sampling times between 2012 and 2014: June 2012, October 2012, February 2013 and April 2013 for the first campaign; and June 2013, October 2013, February 2014 and April 2014 for the second campaign. The plan took into account the following criteria: (1) the particular province of the Basque Country (Araba, Biscay or Gipuzkoa); (2) the population within each province; (3) season; and (4) frequent consumption of individual vegetables. It was necessary to include the season as a factor because of seasonal differences in quantities and types of vegetables consumed. In spring and summer, mainly uncooked lettuce, tomatoes and peppers are consumed, while in autumn and winter boiled leeks, carrots, chard and courgettes are widely consumed and, consequently, oil intake also is higher. Thus, 9 different vegetables were collected, including chard, courgettes, onions, beans, lettuces, peppers, leeks, tomatoes and carrots, representing the most-consumed vegetables in the Basque Country community. The commodities that were analyzed represent 82.3% of the vegetable intake of the Basque community diet (Elika, 2008). Samples were collected using the following criteria: (1) the origin and (2) the point of sale of the vegetable, noting whether collected in a large supermarket or a local shop. With respect to origin, only 11.7% of the collected samples were produced in the Basque Country (local origin); three-quarters of them, 74.9%, originated from outside of any of the production areas mentioned above. For 13.5% of the samples it was not possible to establish the origin.

The sampling procedure was in accordance with European regulation of sampling for official control for pesticide residues (European Community, 2002). Briefly, a sample size of 2 kg was taken, homogenized with a blender (Minipimer 7, Braun, Aschaffenburg, Germany) and then aliquots of 10 g were added to 50-mL Falcon tubes and stored in a freezer at −42 °C until the analysis.

2.2. Chemicals and pesticide standards

Reagent-grade anhydrous sodium chloride (99.5%) and magnesium sulfate (97%) were purchased from Panreac (Barcelona, Spain). Primary secondary amine (PSA)-bonded silica (100 g, bulk) was supplied by Supelco (Bellefonte, PA). The mobile phase was prepared with HPLC–MS grade acetonitrile purchased from Scharlab (Barcelona, Spain) and with ammonium formate (99%) and ammonia solution (25%) for the pH adjustment from Sigma-Aldrich (Steinheim, Germany). All solutions were prepared in ultra-high purity water (UHP) obtained from tap water pre-treated using Elix reverse osmosis cartridges and filtered by a Milli-Q system from Millipore (Bedford, MA).

Pesticides were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions were prepared in acetonitrile and stored at 4 °C. Standard working solutions were prepared by dilution of stock solutions in acetonitrile. Caffeine was used as an internal standard for a correct quantification in GC–MS.

2.3. Instrumentation

The liquid chromatographic analysis was performed on a 1200 Series HPLC system coupled to a 6410 triple quadrupole mass spectrometer from Agilent Technologies (Wilmington, DE) operating in dynamic multiple reaction monitoring mode (dMRM) and equipped with an electrospray ionization source (ESI) operating in positive ion mode. The data acquisition and analysis was carried out using Agilent MassHunter Workstation Data Acquisition software, B.02.01 version. The column used in the chromatographic separation was a Zorbax (Agilent) SB-C18 narrow bore column (2.1 mm × 100 mm, 3.5 μm) with a guard column with the same packing material, maintained at 60 °C. The mobile phase consisted of a mixture of ammonium formate (5 mM)/formic acid 0.01% (eluent **A**) and acetonitrile/water (ammonium formate (5 mM)/formic acid 0.01%) (95:5) (eluent **B**). Gradient separation of the analytes was performed, using a flow rate of 0.5 mL/min.

GC–MS analyses were performed with a Varian 3800 (Varian Instruments, Sunnyvale, CA) gas chromatograph (GC). A Varian 1200L triple quadrupole mass spectrometer (QqQ) was coupled to the GC. A FactorFour VF–5 ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Varian was used. The QqQ mass spectrometer was operated using electron ionization (EI, 70 eV); and the MS/MS conditions were fixed for each compound, using an EI-MS/MS library specially created for the target analytes under the experimental conditions, trying to select as a precursor ion that with the highest m/z ratio (greater selectivity) and abundance (greater sensitivity).

The systems used to centrifuge the samples were a 5415 Eppendorf (Madrid, Spain) and Allegra™ X-22R centrifuge (Beckman Coulter, Pasadena, CA).

2.4. Pesticide analysis

The analysis of pesticide residues was performed at the Analytical Bioclinical Laboratory (LAB, SL., Almeria, Spain). Validated and accredited in accordance with UNE-EN ISO/IEC