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Risk assessment and monitoring programme of nitrates through vegetables in the Region of Valencia (Spain)

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A B S T R A C T

This study was carried out to determine current levels of nitrate in vegetables marketed in the Region of Valencia (Spain) and to estimate the toxicological risk associated with their intake. A total of 533 samples of seven vegetable species were studied. Nitrate levels were derived from the Valencia Region monitoring programme carried out from 2009 to 2013 and food consumption levels were taken from the first Valencia Food Consumption Survey, conducted in 2010. The exposure was estimated using a probabilistic approach and two scenarios were assumed for left-censored data: the lower-bound scenario, in which unquantified results (below the limit of quantification) were set to zero and the upper-bound scenario, in which unquantified results were set to the limit of quantification value. The exposure of the Valencia consumers to nitrate through the consumption of vegetable products appears to be relatively low. In the adult population (16–95 years) the P99.9 was 3.13 mg kg^{-1} body weight day^{-1} and 3.15 mg kg^{-1} body weight day^{-1} in the lower bound and upper bound scenario, respectively. On the other hand, for young people (6–15 years) the P99.9 of the exposure was 4.20 mg kg^{-1} body weight day^{-1} and 4.40 mg kg^{-1} body weight day^{-1} in the lower bound and upper bound scenario, respectively. The risk characterisation indicates that, under the upper bound scenario, 0.79% of adults and 1.39% of young people can exceed the Acceptable Daily Intake of nitrate. This percentage could join the vegetable extreme consumers (such as vegetarians) of vegetables. Overall, the estimated exposures to nitrate from vegetables are unlikely to result in appreciable health risks.

1. Introduction

Nitrate is a common compound in nature that is part of the nitrogen cycle. It can be found in soil, surface and underground water as well as in biomass. In addition, nitrate is currently used as a fertilizer in agriculture, which has allowed a significant increase in world food production (Mosier et al., 2005). As a consequence, high levels of nitrate can be accumulated in plants-vegetables (European Food Safety Authority (EFSA), 2013). Furthermore, nitrates are also authorised as additives in the food industry identified by the European codes E-251 and E-252 (European Commission, 2011a).

Human exposure to nitrate is mainly exogenous through the consumption of vegetables, and to a lesser extent through water and authorised additives used in the preservation of meat products. For nitrates, the major part of the daily intake in foodstuffs is related to vegetable consumption (EFSA, 2008) due to the elevated nitrate accumulation capacity of these products. Higher levels of nitrate tend to be found in leaves whereas lower levels occur in seeds or tubers. As a consequence, leafy crops such as lettuce and spinach generally have higher nitrate concentrations (EFSA, 2013).

Although nitrate is relatively non-toxic below maximum levels (MLs), its metabolites such as nitrite, nitric oxide and N-nitroso compounds, make nitrate of regulatory importance because of their potentially adverse health implications such as

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methaemoglobinemia and carcinogenesis (EFSA, 2008). It has been widely reported that young infants (0–3 months) are the most sensitive group to methaemoglobinemia, probably due to the consumption of high amounts of nitrates from vegetables (mainly spinach) and inappropriate storage of cooked vegetables (EFSA, 2010a).

Several studies have recently been carried out attempting to prove the association between consumption of nitrate and nitrite with different types of cancer, including brain, oesophagus, stomach or colorectal cancer. While the evidence obtained so far is inconclusive (Bryan et al., 2012; Milkowski et al., 2010; Gumanova et al., 2016), the International Agency for Research on Cancer (IARC, 2010) maintains the classification of nitrate within the group 2A, considering that "It is probably carcinogenic to humans" and a recent recommendation by IARC, has classified the consumption of red meat as probably carcinogenic to humans (group 2A) and processed meat as carcinogenic to humans (group 1) (IARC/ WHO, 2015).

In order to prevent potential adverse effects on human health, the European Commission has established maximum levels for nitrates in certain leafy vegetables (European Commission, 2011b).

The Region of Valencia is a vulnerable area to high nitrate concentrations as a consequence of an intensive agricultural activity. Furthermore, as nitrate concentrations can vary greatly among regions depending on factors such as farming practices, climate, soil quality, and manufacturing processes (EFSA, 2013), the evaluation of nitrate exposure through vegetables grown in this "local area" is therefore important in order to assess their safety for the local inhabitants of the Region of Valencia.

Regarding exposure assessment to contaminants through diet, the application of probabilistic techniques has been gaining increasing interest internationally. In contrast with the deterministic methodology, these techniques allow the estimation of the distribution of intakes amongst multiple individuals in a specified population, taking into consideration the variability in food consumption between and within individuals and in occurrence of residues in food commodities (EFSA, 2012).

The aim of this study is to present the results of the monitoring of nitrate in vegetables marketed in the region of Valencia during the years 2009–2013, to estimate exposure under a probabilistic approach and to characterise the resultant risk by comparing with the Acceptable Daily Intake (ADI) value established by the former Scientific Committee on Food (SCF) and reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 (FAO/WHO, 2003).

2. Materials and methods

2.1. Food sampling and nitrate data

Nitrate data were obtained from monitoring programme of the Region of Valencia, carried out from 2009 to 2013. A total of 533 samples of seven vegetables species: Lettuce (*Lactuca sativa*), iceberg-type lettuce (*Lactuca sativa* var. *capitata*), spinach (*Spinacia oleracea*), potato (*Solanum tuberosum*), chard (*Beta vulgaris* var. *cicla*), artichoke (*Cybara scolymus*) and carrot (*Daucus carota*) were collected in different geographical areas and seasons to take into account potential variability. Furthermore, lettuces were collected grown both undercover or in the open air. These seven vegetable species were selected either because the legislation establishes a maximum limit (European Commission, 2011b) (as is the case for lettuce and spinach) or because these vegetables are consumed in high proportion in the Region of Valencia (as is the case for potato, chard, artichoke and carrot).

Sampling was carried out randomly by inspectors from the Public Health Department, according to the European Commission Regulation (EC) No.1882/2006 (European Commission, 2006b) in local markets, large supermarkets and grocery stores of the Region of Valencia. The vegetable samples were labelled and chilled at 4 °C in plastic bags after collection. Just before analysis, inedible parts were removed and the rest were cut into small pieces and homogenized in a blender.

For intake estimates, left-censored results (data below the limit of quantification (LOQ), corresponding to 80 mg kg^{-1}) were processed using the substitution method, recommended by international organizations (EFSA, 2010b; GEMS/Food- Euro, 1995). For each vegetable, considering a censoring rate higher than 60%, two scenarios were assumed for left-censored results: a lower-bound (LB) scenario, in which unquantified results (below the LOQ) were set to zero and an upper-bound (UB) scenario, in which unquantified results were set to the LOQ value. Regarding vegetables for which more than 40% of results were quantified, the unquantified results, lower and upper bounds were replaced by an estimated "middle bound" assuming that undetected results are equal to half the detection limit. Although it has been widely recognised that the substitution method has some disadvantages (El-Shaarawi and Esterby, 1992; Helsel, 2005), it is still widely used, mainly with the justification that it is easy to implement, it is widely understood and that the upper bound practice leads to conservative estimates for exposure assessment calculations, i.e. over-estimation of the mean and under-estimation of the variability (EFSA, 2010b). In addition, considering that the number of left-censored data of nitrates in vegetables is low and that it is described that the performance of the substitution-based method improves as the censoring percentage decreases (Shoari et al., 2015), the substitution method was considered appropriate for the intake estimates in a first attempt. If the intake estimated by the UB scenario, which could lead to an overestimation, indicates a possible health risk, the risk assessor could progress to a different methodology to manage left-censored data.

2.2. Analysis of nitrate

Analyses were conducted by the Public Health Laboratory of Valencia, accredited following the ISO/IEC 17025 standard. The content of nitrate was determined by high performance liquid chromatography (HPLC) using a Photo Diode Array detector. Reagent and standard solutions, sample treatment, instrumental, method validation and analytical quality assurance can be found in the supplementary data associated to this article (see Appendix).

2.3. Consumption data

Intake estimates were based on the consumption data obtained from a questionnaire-based dietary survey that was conducted and validated in 2010–2011 by the Valencia Public Health Directorate. Dietary data were collected through a 24-h recall in which 1476 subjects (195 young people from 6 to 15 years of age and mean 43.5 kg body weight, and 1281 adults from 16 to 95 years of age and 71.2 kg mean body weight) were asked in a face-to-face interview to recall and describe the kinds and amounts of all foods and beverages ingested during the previous 24-h period.

The initial sample of individuals was divided in three waves or groups in order to take into account variations in consumption patterns according to season; the first wave was conducted between the months of June and July 2010, the second between September and November, and the third between November and February of 2011. Self- reported body weight was also collected in the face-to-face interview and used in exposure calculations.