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ESTIMATE OF DIETARY EXPOSURE TO SULFITES IN CHILD POPULATION FROM BENI MELLAL REGION (MOROCCO)

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Abstract

Sulfites are commonly used as chemical preservatives in the food industry. The aim of this study was to estimate the dietary exposure of children to sulfites in Morocco and assesses the potential health risks associated with their consumption. Exposure was estimated by combining the maximum permitted levels (MPLs) and mean analytical levels of sulfites in four processed foods frequently consumed by children, along with consumption data obtained from a food frequency questionnaire completed by 187 school children. The results were compared with the acceptable daily intake (ADI) set for sulfites to determine the potential health risk. Using MPLs, the mean dietary exposure to sulfites was found to be 0.35 mg/kg of bw/day for the entire population, representing 50% of the ADI. In the high-intake scenario, it was 0.45 mg/kg of bw/day, which accounted for 64% of the ADI. Based on analytical levels in tested foods, the mean dietary exposure was estimated to be 0.20 mg/kg of bw/day for the entire population, corresponding to 29% of the ADI. In the high-intake scenario, it was 0.34 mg/kg of bw/day, corresponding to 49% of the ADI. Processed potato chips were identified as the major contributor to sulfite intake, exceeding the national MPL. The results showed that the dietary exposure of children to sulfites from four processed foods in Morocco is within safe limits. However, cumulative sulfite intake from different food sources should be taken into consideration.

Key words*: sulfites, foods, exposure, children, health risk.*

INTRODUCTION

Sulfites, including sulfur dioxide (SO_2) and sulfating agents, such as sodium metabisulfite ($Na_2S_2O_5$) and potassium metabisulfite ($K_2S_2O_5$), are widely utilized preservatives in the food industry. However, it is known that exposure to these compounds can lead to sensitivities and adverse clinical effects. For instance, some individuals with asthma may experience symptoms of allergic response such as bronchoconstriction and bronchospasm (Gad, 2014). Other reported adverse effects include hives, diarrhea, abdominal pain and cramps, nausea and vomiting, pruritus, fainting, headache, and skin rashes (Wilson & Bahna, 2005).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established an Acceptable Daily Intake (ADI) for sulfites at 0.7 mg/kg body weight, expressed as sulfur dioxide (SO_2) (JECFA, 1974). Assessing dietary exposure to sulfites can provide valuable information regarding whether the ADI for sulfites is being surpassed and which population groups are most at risk of exceeding this limit. This information is crucial for regulatory agencies, food manufacturers, and consumers to ensure the safety of food additives and protect public health.

Foods in which the use of sulfites is permitted in Morocco and the corresponding maximum permitted levels (MPLs) are specified in the Regulation no. 2750-22 **(**Ministry of Agriculture and Ministry of Health, Morocco, 2022).

Assessments of dietary exposure to sulfites in the child population have been conducted in several countries, employing various methodologies such as MPLs, analysis of sulfite levels in selected foods, and analytical data provided by national competent authorities (WHO, 2009). Overall, the majority of these studies have shown that dietary exposure to

sulfites only exceeds the ADI in high intake scenarios (WHO, 2009).

The objective of the present study was to estimate children's dietary exposure to sulfites in Morocco by considering the maximum permitted levels (MPLs) and mean analytical levels of sulfites in four processed foods that are commonly consumed as snacks by children, along with consumption data derived from a food frequency questionnaire. The estimated dietary exposure was then compared to the acceptable daily intake (ADI) set for sulfites to assess potential health risks.

MATERIALS AND METHODS

To estimate dietary exposure to sulfites (and other food additives), two types of data are required: selected food consumption data and the sulfite levels in those foods. The sulfite levels in foods can be obtained from the maximum permitted levels (MPLs) set in national regulations, allowing the calculation of the theoretical maximum daily intake (TMDI) as the initial step. Subsequently, in a second step, the sulfite levels are analytically determined in each food type to generate a more precise estimate of dietary exposure.

Four types of processed foods, which are potentially consumed in high quantities by children (potato chips, biscuits and crackers, fruit juice, confectionery), were selected from the list of foods in which sulfites may be added as a food additive, as outlined in the Moroccan Regulation no. 2750-22. Food consumption data were collected using a food frequency questionnaire specifically designed to gather information on the frequency of consumption (daily, weekly, monthly) and the amounts consumed of selected foods. Simple calculations were conducted to approximate the food consumption for each respondent and for each food type (in kg/day). The individual values were then aggregated, and the average consumption was calculated for the entire population, as well as for consumers and regular consumers specifically.

Respondents to the food frequency questionnaire were students aged between 8 and 12 years old from three schools in the Beni Mellal region, Morocco: two public schools (one in an urban area and one in a rural area) and one private school. All respondents were assisted by their teachers while filling out the questionnaire, and the final aim of the study was not disclosed to the students in order to avoid biasing the results. In total, 187 students successfully completed the survey.

The food frequency questionnaire consists of two sections: the first section covers general information such as gender, area of residence, and measured body weight. The second section focuses on consumption data for four processed foods, where respondents are asked to answer three questions with predefined answer options. First, respondents were asked whether or not they consumed the food at least once a month (consumer status). If the respondent indicated being a consumer, they were further asked whether or not they consumed the food type at least once a week (regular consumer status). For regular consumers, the third question aimed to understand the frequency of consumption (once a week, twice, three times, four times, or more). The final question aimed to determine the amount of food consumed each time, with specific response options based on the food type. Additionally, photos (without brand names) illustrating each food type were presented to the students to assist them in identifying the food in question.

In the first step, the calculation of the theoretical maximum daily intake (TMDI) was carried out, as recommended by the Codex Alimentarius Commission for food additives (CAC, 1989), to assess theoretically the dietary exposure of school children to sulfites from four commonly consumed processed snack foods (potato chips, biscuits and crackers, fruit juice, and confectionery). The TMDI (in mg) was calculated by combining the maximum permitted levels (MPLs) of sulfites in processed foods set by national regulations, with the consumption data obtained from the food frequency questionnaire. Furthermore, the theoretical exposure to sulfites (in mg/kg bw/day) and the corresponding ADI percentage were derived from the TMDI calculations.

The MPLs of sulfites, expressed in mg/kg of SO_2 (or mg/liter), and the food groups in which sulfites are allowed are sourced from Moroccan Regulation no. 2750-22. According to this regulation, sulfites can be legally used as a food additive in over 100 food items, including

potato chips, biscuits, fruit juice, and confectionery.

For comparison purposes, MPLs for sulfites in selected foods set in the Codex Alimentarius standard (CAC, 1995) are different from those established by the Moroccan Regulation, while MPLs set in the European Union by Regulation no. 1333/2008 (European Commission, 2008) are the same as those set in Moroccan Regulation (Table 1). However, only national MPLs were used to estimate the TMDI of sulfites in children.

Food samples were collected from local grocery stores and supermarkets in the Beni Mellal region, located in north-central Morocco, between August and November 2021. A total of 57 samples of potato chips, confectionery, biscuits and crackers, and fruit juice were tested to determine their sulfite levels.

Table 1. MPLs for sulfites according to Moroccan Regulation (MR), European Union Regulation (EUR) and Codex Alimentarius Standard (CAS)

Food type	MR MPLs (mg/kg)	EUR MPLs (mg/kg)	CAS MPLs (mg/kg)
Potato chips	100	100	50
Biscuits and crackers	50	50	50
Fruit juice	250	250	50
Confectionery	50	50	Not Permitted

MPLs: Maximum Permitted Levels

Sulfite levels were determined using the optimized Monier-Williams method, which is an approved method for measuring sulfites in foods, with a limit of quantification (LOQ) of 10 mg/kg (AOAC, 2000). A 50 g portion (or ml for liquid samples) of the sample was ground in a blender with a mixture of ethanol and water (5%/95%). The resulting ethanol/water solution was transferred to a round-bottom flask and heated under reflux with a hydrochloric acid solution (90 ml) for 105 minutes, converting the sulfites into sulfur dioxide (SO_2). A stream of nitrogen was injected into the refluxing solution to carry the SO_2 to a neutral solution of hydrogen peroxide (3%) in a bubbler. Upon reaching the hydrogen peroxide solution, the SO_2 was oxidized and transformed into sulfuric acid, which was then titrated using a standard sodium hydroxide solution. All samples were analyzed twice.

Exposure to sulfites in Moroccan children was estimated by combining the mean analytical levels of sulfites in various foods, including potato chips, dry biscuits and crackers, fruit juice, and confectionery. This estimation was based on the food consumption data derived from the food frequency questionnaire. To calculate the daily intake of sulfites from each food type, the daily consumption of each food type (in kg) was multiplied by the mean analytical level (in mg of SO_2/kg). The results for each food type were then summed up to estimate the total daily intake of sulfites (in mg of SO_2/kg).

The dietary exposure to sulfites (in mg/kg body weight/day) was calculated by dividing the total daily intake values by the individual body weight of the respondents. In order to assess the risk, the estimated dietary exposure of children to sulfites was compared to the ADI value of 0.7 mg/kg body weight/day as established by JECFA in 1974. The ratio between the ADI and the dietary exposure was also calculated. Mean dietary exposure was calculated for the entire population of respondents (whole population), as well as for consumers (at least once per month) and regular consumers (at least once per week).

Data entry and statistical processing were carried out using Microsoft Excel (Microsoft Corporation, USA, 2010) and Minitab Statistical Software (version 16, Minitab Inc. USA). The results obtained are expressed as "mean ± standard deviation". The range of concentration values as well as the standard error are also mentioned. Significant differences between means were determined using a one-way analysis of variance (ANOVA test) combined with a Tukey's test with a significance level of ≤ 0.05.

RESULTS AND DISCUSSIONS

Consumption data of selected foods were collected in March 2023 through the food frequency questionnaire (FFQ) carried out in primary schools. A total of 187 students including 85 boys (45%) and 102 girls (55%) aged between 8 and 12 years were able to complete the FFQ. The average measured body weight was around 25 kg (girls and boys combined).

Table 2 presents food consumption data for all population categories, including the whole population, consumers only, and regular consumers only. Among the entire student population, the average daily consumption of potato chips is 7 grams. Of these students, 81% (151 individuals) are consumers of potato chips, with an average daily consumption of 9 grams. Moreover, 74% of the students (138 individuals) are categorized as regular consumers, consuming an average of 12 grams per day. In comparison, the consumption of confectionery within the same population was slightly higher. The whole population had an average daily consumption of 9 grams of confectionery. Out of these individuals, 92% (172 students) were consumers of confectionery, with an average consumption of 10 grams per day. Furthermore, 84% (157 people) of the population were identified as regular consumers of confectionery, consuming an average of 14 grams per day.

An average of 25 grams/day of processed biscuits is consumed by the entire population. Among the consumers (170 students, 91% of the population), the average consumption reaches 28 grams/day. Interestingly, the regular consumers exhibit the same average consumption of 28 grams per day. The whole population of primary school students consumes an average of 26 grams of processed fruit juice per day. Among the consumers (164 students, 88% of the population), the average consumption increases to 29 grams per day. Among the regular consumers (149 students, 80% of the population), the average consumption remains at 32 grams per day.

These findings provide insights into the consumption habits of primary school students regarding the four processed items, emphasizing the prevalence and frequency of consumption within this population category. It is worth noting that the daily consumption of all processed foods showed a remarkable similarity between boys and girls, as both genders exhibited nearly identical levels of intake.

Table 3 presents the number of samples and mean concentrations of sulfites for each food type, along with the National MPLs provided for comparison. All samples of processed potato chips and confectionery tested positive, with mean concentrations of 680 and 32 mg/kg, respectively. The coefficient of variation between samples was below 10%. Fruit juice and biscuit samples from local brands had sulfite levels below the Limit of Quantification (LOQ). The MPL set for potato chips was widely exceeded in the tested samples.

Theoretical daily intake of sulfites, assuming compliance with national MPLs, and corresponding dietary exposure for each population category are provided in Table 4. The ratio between the ADI and theoretical exposure is also included in Table 4. The results indicate that the ADI is not exceeded for all population categories, ranging from 50% (for the total population) to 64% (for regular consumers). Therefore, the health risk level is considered low regarding children's exposure to sulfites from snack-type processed foods.

In a study conducted in Lebanon by Soubra et al. (2006) using MPLs and consumption data for various food types, the TMDI of sulfites for children and teenagers aged between 8 and 18 years old was found to be significantly higher than the ADI. However, in a study conducted in the Basque Country by Urtiaga et al. (2013), the ADI was not exceeded at the mean level (8.8%). It was only significantly exceeded in the high-intake scenario (99th percentile) for children aged between 4 and 6 years old, reaching 120%. It is worth noting that these studies employed an extended list of food items, including as many as possible from children's diets.

Table 2. Consumption data of processed foods for primary schools students (187 samples)

Processed food	Mean consumption (g/day)			% of consumers	% of regular consumers
	Whole population	Consumers	Regular consumers		
Potato chips	7	9	12	81	74
Confectionery	9	10	14	92	84
Biscuits and crackers	25	28	28	91	91
Fruit juice	26	29	32	88	80
Total daily consumption	67	76	86	-	-

Table 3. Mean analytical levels of sulfites in selected processed foods

Processed food	No. of samples	Mean concentration (mg SO_2/kg)	Range (mg SO_2/kg)	Standard error (mg SO_2/kg)	National MPLs (mg SO_2/kg)
Potato chips	18	680±33	650-725	7.89	100
Biscuits and crackers	10	< LOQ	< LOQ	-	50
fruit juice	9	< LOQ	< LOQ	-	250
Confectionery	20	32±4	24-36	0.98	50

Table 4. Theoretical exposure to sulfites (TMDI) assuming compliance with national MPLs

Processed food	National MPLs (mg SO_2/kg)	Theoretical intake of sulfites assuming compliance with national MPLs (mg SO_2/day)		
		Whole population	Consumers	Regular consumers
Potato chips	100	0.7	0.9	1.2
Confectionery	50	0.45	0.5	0.7
Biscuits and crackers	50	1.25	1.4	1.4
Fruit juice	250	6.5	7.25	8
Total intake of SO_2 (mg/day)		8.9	10	11.3
Exposure to SO_2 (mg/kg bw/day)[a]		0.35	0.4	0.45
% of the ADI[b]		50%	57%	64%

[a]Average body weight = 25 kg
[b]Acceptable Daily Intake (ADI) for sulfites = 0.7 mg/kg of body weight (JECFA, 1974).

Table 5. Exposure estimate to sulfites based on analytical levels

Processed food	Mean concentration (mg SO_2/kg)	Intake of sulfites using analytical levels (mg SO_2/day)		
		Whole population	Consumers	Regular consumers
Potato chips	680±33	4.76	6.12	8.16
Confectionery	32±4	0.30	0.32	0.45
Biscuits and crackers	0	0	0	0
Fruit juice	0	0	0	0
Total intake of SO_2 (mg/day)		5	6.44	8.61
Exposure to SO_2 (mg/kg bw/day)[a]		0.2	0.26	0.34
% of the ADI[b]		29%	37%	49%

[a]Average body weight = 25 kg
[b]Acceptable Daily Intake (ADI) for sulfites = 0.7 mg/kg of body weight (JECFA, 1974).

The dietary exposure estimate to sulfites, based on the mean analytical concentrations in food samples, was found to be well below the ADI for all population categories, as shown in Table 5. The mean estimated daily exposure to sulfites for the whole population of children was 0.20 mg/kg bw/day, which represents 29% of the ADI. Among consumers, the mean dietary exposure to sulfites was 0.26 mg/kg bw/day, corresponding to 37% of the ADI. In the high-intake scenario (regular consumers), the estimated exposure was 0.34 mg/kg bw/day, accounting for 49% of the ADI. The absence of sulfites in biscuit and fruit juice samples played a significant role in facilitating low sulfite exposure in children. Potato chip samples, which had sulfite levels exceeding the national MPL, were the major contributor to the daily intake, accounting for nearly 95% of the exposure.

However, the estimated daily intake for regular consumers was approximately 50% of the ADI, solely from two food types containing sulfites (potato chips and confectionery).

This percentage remains a concerning finding when considering the overall exposure from various potentially sulfite-containing foods that are part of children's diets. Further comprehensive studies, utilizing an expanded list of processed foods, are necessary to assess the total daily exposure of children in a more detailed and refined manner.

The average body weight of 25 kg, which was used to calculate the daily exposure for both girls and boys, as well as for the entire subject population (aged between 8 and 12 years), may

potentially lead to overestimation or underestimation of the dietary exposure to sulfites in this study.

In a study carried out in Lebanon (Soubra et al., 2006) the average daily exposure to sulfites of the total population of student was 0.4 mg/kg bw/day, representing 57% of the ADI. In the high-intake scenario (95th percentile of consumers only), the daily exposure to sulfites represented 215% of the ADI. In France (Bemrah et al., 2009), the mean exposure to sulfites was 0.102 mg/kg bw/day for children, corresponding to only 15% of the ADI. At the 97.5th percentile, daily exposure to sulfites represents 46% of the ADI. Potatoes products and dried fruits were the major contributors. In a study from Ireland using analytical data provided by the food industry (Vin et al., 2013), the mean exposure of children aged between 5 and 12 years old was 0.292 mg/kg bw/day (42% of the ADI). In the high-intake scenario (97.5th percentile), the ADI was well exceeded (130%) (Vin et al., 2013).

CONCLUSIONS

In this study, the dietary exposure of children to sulfites, which are used as food additives in processed foods, was estimated using a stepwise approach. Firstly, the exposure to sulfites was estimated by combining the maximum permitted levels (MPLs) set in national regulations with food consumption data, specifically the TMDI. In the second step, the estimate of dietary exposure to sulfites was conducted by substituting MPLs with analytical levels. In both steps, the average daily exposure to sulfites was found to be well below the acceptable daily intake (ADI), even for children who regularly consumed the selected foods (high-intake scenario). The high concentration of sulfites in processed potato chips, exceeding the national MPL, significantly contributed to the daily intake of sulfites.

It can be concluded that the health risk for children is low, considering that the ADI represents a lifelong exposure without any risk. However, concerns may arise regarding children who consume multiple processed foods treated with preservatives, given the limited number of processed foods included in this study. Further research is needed to assess the total daily exposure of children to sulfites from various potentially sulfite-containing foods.

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