

Probabilistic risk assessment of exposure to fluoride in most consumed brands of tea in the Middle East



Mohammad Miri^a, Amit Bhatnagar^b, Yousef Mahdavi^c, Leila Basiri^d, Alireza Nakhaei^e, Rasoul Khosravi^f, Hadi Eslami^g, Seyed Mehdi Ghasemi^h, Davoud Balarakⁱ, Ahad Alizadeh^j, Amir Mohammadi^k, Zahra Derakhshan^l, Reza Ali Fallahzadeh^k, Mahmoud Taghavi^{m,*}

^a Cellular and Molecular Research Center, Department of Environmental Health Engineering, School of Public Health, Sabzevar University of Medical Sciences, Sabzevar, Iran

^b Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211, Kuopio, Finland

^c Department of Environmental Health Engineering, Student Research Committee, Mazandaran University of Medical Sciences, Mazandaran, Iran

^d Department of Environmental Health Engineering, Zabol University of Medical Sciences, Zabol, Iran

^e Department of Clinical Biochemistry, Zahedan University of Medical Sciences, Zahedan, Iran

^f Social Determinant of Health Research Center, Department of Environmental Health Engineering, Faculty of Health, Birjand University of Medical Sciences, Birjand, Iran

^g Department of Environmental Health Engineering, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

^h Student Research Committee, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ⁱ Department of Environmental Health Engineering, Zahedan University of Medical Sciences, Zahedan, Iran

^j Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

^k Environmental Science and Technology Research Center, Department of Environmental Health Engineering, Student Research Committee, Faculty of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^l Department of Environmental Health, School of Health, Larestan University of Medical Sciences, Larestan, Iran

^m Department of Environmental Health Engineering, Gonabad University of Medical Sciences, Gonabad, Iran

ARTICLE INFO

Keywords:

Tea
Fluoride
Monte Carlo simulation
Probabilistic risk assessment
Chronic daily intake
Hazard quotient

ABSTRACT

The aim of this study was to evaluate the fluoride concentration in seven most consumed brands of tea in the Middle East which was imported to Iran through Zabol County. In the present study, the health risk of exposure to fluoride was estimated using a probabilistic approach. Monte Carlo simulation and sensitivity analysis were applied to quantify uncertainties in risk estimation. The highest mean and 95th percentile of chronic daily intake (CDI) was observed for children group. Iranian tea and Kenya tea had maximum CDI and target hazard quotient (THQ) values among studied brands of tea which followed by Green tea, Taksetare tea, Ceylan tea, Red tea, and White tea. These result indicated that there were significant risks of exposure to fluoride in most of studied brands of tea for children (THQ > 1). Sensitivity analysis showed that fluoride concentration and daily intake were the most influential variables in three exposed populations. In conclusion, the fluoride concentration in some studied brands of tea is high and it put children at risk risks of exposure to fluoride.

1. Introduction

Fluoride (F⁻) is an element, which has both advantages and disadvantages for human health. At appropriate concentration, fluoride plays an essential role in the health of teeth and bones but elevated concentration of fluoride (more than standard levels set by World Health Organization (1.5 mg/l)) is responsible for various diseases and health problems in humans such as dental and skeletal fluorosis, as well as damage to the parathyroid, kidney and liver (Fallahzadeh et al., 2018; Khosravi et al., 2017; Zarrabi et al., 2015). Fluoride ion is mainly absorbed into the body through the diet as compared to other sources.

Tea, a non-alcoholic beverage, has gained significant popularity and its consumption has increased all over the world in recent decades (Pehrsson et al., 2011; Simpson et al., 2001). The consumption of black tea has been estimated to be about 1.5 kg per capita in Iran and about 4.5% of whole tea consumption in the world is related to this country. It indicates that there is high tendency to drink tea in Iran and it has now become an integral part of Iranian diets (Asgari et al., 2008; Malakootian et al., 2011). Some characteristics of tea such as anti-mutagenic, anti-carcinogenic, and antioxidant activities have made it a healthy drink (Hossenli et al., 2013; Sharma et al., 2007). Despite its benefits, it has been reported that tea has high fluoride content which

* Corresponding author.

E-mail address: taghavi66@yahoo.com (M. Taghavi).

can be harmful (Morés et al., 2011; Zhang et al., 2015).

A Monte Carlo simulation is an effective method to identify uncertainties of each parameter. In this technique, each value of parameters distribution inserted to exposure equation randomly and this process completed many times, until a distribution of predicted results, which indicate overall uncertainty of input parameters, are obtained (USEPA, 1992).

Sistan and Baluchestan is one of the frontier provinces of Iran. Zabol is one of the major cities of the province and the city center of Sistan County (Miri et al., 2017). Because of shared border with Afghanistan and Pakistan, this city is one of the most important centers for importing tea to Iran. The imported tea from this region is from different countries like India, Sri Lanka, Italy, Kenya, and China. The remarkable thing in relation to the imported tea is that they have no label of quality and health characteristic, country of origin, date of packing and expiry. Given that the imported tea is first entered to Afghanistan and Pakistan, it seems that tea is consumed in most of Middle East countries. Therefore, it was decided to study the amount of fluoride in imported tea by sampling at the point of entry to Iran. The aim of this study was to evaluate the fluoride concentration in seven most consumed brands of tea in the Middle East (Kenya, Ceylon, Iranian, Taksetare, Red, White, and Green tea), which were imported to Iran through Zabol County. Besides, the effect of brewing time of tea was evaluated to examine the trend of fluoride concentration. The chronic daily intake (CDI) and target hazard quotient (THQ) were calculated using a probabilistic approach for children, teens, and adults. Finally, Monte Carlo simulation and sensitivity analysis were applied to quantify uncertainties in risk estimation.

2. Materials and methods

2.1. Sample collection

This study was undertaken to evaluate the fluoride concentration in 7 brands of tea infusions (Kenya, Ceylon, Iranian, Taksetare, Red, White, and Green tea). These brands of tea were received in woody/plastic packages and with no information about the place of cultivation, manufacturer, and details about their processing. Also, there was no information about technical procedures, environmental conditions on the site, and agronomical practices used by the food companies. In the present study, a total of 105 tea samples (250 g) were collected from 5 wholesales of tea during 3 months (April, August, and October). Then, the fluoride content was measured in 7 different brands of tea available in Zabol markets including black tea (Kenya, Ceylon, Iranian, and Taksetare) and also Red, White, and Green tea.

2.2. Materials and analytical procedure

All the reagents including NaF, $C_2H_3NaO_2$, NaCl, NaOH, and

cyclohexylene dinitro tetra acetic acid (CDTA) were of analytical grade and were purchased from Merck Company. Double-distilled water was used for the preparation of fluoride standards, solutions, and tea infusions. Total ionic strength adjuster buffer (TISAB) was prepared by dissolving 4 g of CDTA, 57 mL glacial acetic acid, and 58 g NaCl in about 800 mL double-distilled water. Then, pH was adjusted to 5.5 by adding 5 M NaOH and diluted to 1 L with double-distilled water (Mereta, 2017; Mondal and George, 2015).

For each brand of tea, 1 g of tea leaves were added to 50 mL boiling double-distilled water to prepare the tea infusion. Then, 15 mL of tea infusion from each brand was collected after 5, 10 and 15 min to evaluate the effect of time on the amount of released fluoride.

The fluoride content of tea infusions was measured by a potentiometric method using a fluoride ion selective electrode (Model 7102, Shanghai Rousull Technology Co. Ltd, China). The limit of detection for this electrode was 0.02 ppm (Pyschik et al., 2017). In order to measure the potential of each sample by a potentiometer (Denver Instrument Ultra Basic pH/mV meter UB-10), 15 mL of TISAB II was added to 15 mL of each tea infusion. Serial dilution of a 100 mg/l of NaF solution was used to plot the calibration curve. The fluoride content of each infusion was determined using standard curve (Malinowska et al., 2008a). All experiments were performed in duplicate.

2.3. Health risk assessment

As mentioned earlier, tea is one of the main sources of fluoride intake; therefore, in this study, CDI and non-carcinogenic risks of this element in tea were estimated using a probabilistic technique. In our study health risk assessment were conducted for three age groups (0–10 years old as children, 11–20 years old as teens, and 21–72 years old as adults) because of the physical and behavioral differences. For this purpose, the following equations were used based on USEPA (1989) (USEPA, 1989):

$$\text{Chronic Daily Intake: CDI} = \frac{C \times DI \times EF \times ED}{BW \times AT} \quad (1)$$

$$\text{Target Hazard Quotient: THQ} = \frac{CDI}{RfD} \quad (2)$$

Where C is the fluoride concentration in tea infusion (mg/l), DI is the average daily intake rate of tea (l/day), EF is exposure frequency (day/year), ED is the exposure duration (year), BW is body weight (kg) and AT is the averaging time (days); CDI is the chronic daily intake (mg/kg/day), and RfD is the reference dose (mg/kg/day). The result of THQ and CDI were reported as a mean and 95th percentile, and the criteria of safety level for THQ was 95th percentile.

Many uncertainties might occur during calculation of health risk assessment. High uncertainty was observed when single-point values were used to estimate health risk of exposure to pollutants such as fluoride. Therefore, in the present research, Monte Carlo simulations as

Table 1
Parameters used in risk assessment and their probabilistic distribution.

Parameter	Unit	Distribution type	Value	Reference
daily intake (DI)	L/day	Uniform	Children: (Min = 0.3;Max = 0.5) Teens: (Min = 0.5;Max = 1) Adults: (Min = 1;Max = 1.5)	
Exposure frequency (EF)	day/year	Triangular	(Min = 180, Mode = 345, Max = 365)	(Smith, 1994)
Exposure duration (ED)	year	Fixed value	6	(Huang et al., 2017)
Body weight (BW)	kg	Lognormal	Children: (Mean = 16.68, SD = 1.48) ^a Teens: (Mean = 46.25, SD = 1.18) ^a Adults: (Mean = 57.03, SD = 1.10) ^a	(Wu et al., 2011)
Averaging time (AT)	days	Fixed value	Children: 2190 Teens: 2190 Adults: 9125	(Huang et al., 2017)
Oral reference dose	(mg/kg/day)	Fixed value	0.06	(Fallahzadeh et al., 2018)

^a Geometric mean and geometric standard deviation.

a probabilistic approach were employed to reduce the uncertainty of estimations. Table 1 was presented the parameters that used in risk estimate and their probabilistic distributions for three age groups. The health risk assessment of exposure to fluoride was calculated based on values of the presented parameters. To identify the significant impact of these parameters on the model output, a sensitivity analysis was conducted. Crystal Ball software (version 11.1.2.4, Oracle, Inc. USA) with 10,000 iterations was employed for Monte Carlo simulations and sensitivity analysis.

2.4. Statistical analysis

Statistical results were conducted using R software (version 3.0.0). The distributions of fluoride concentration and other quantitative variables were evaluated by the Shapiro-Wilks test. According to the normality of variables, nonparametric or parametric methods were used. The difference of fluoride concentration in different teas was analyzed using Kruskal–Wallis, and Bonferroni post-hoc tests. The effect of brewing time on the amount of released fluoride concentration to infusions from different teas was analyzed using repeated-measurement ANOVA. P-values less than 0.05 were accepted as statistically significant.

3. Results and discussion

3.1. Fluoride concentration in different tea brands

The infusions of black tea have higher fluoride content than other types of tea. The fluoride concentration of Kenya tea was equal to 3.332 ± 1.341 mg/l and higher than the Iranian tea in April. However, the fluoride concentration of the Iranian tea was equal to 4.244 ± 0.437 mg/l and 5.185 ± 0.873 mg/l in August and October, respectively. Also, it is clear that the lowest concentration of fluoride was related to White tea infusion. Fig. 1 shows that the mean fluoride concentration in infusions of each brand of teas is as follows:

Iranian tea > Kenya tea > Taksetare tea > Green tea > Ceylan tea > Red tea > White tea.

Various studies have reported that black tea has high fluoride content. For example, Chan et al. reported that fluoride concentration in a blend of black tea infusion in the UK ranged from 0.63 to 5.26 mg/l

which were in accordance with the present study (Chan et al., 2013). Malinowska et al. conducted a study to evaluate the fluoride concentration in tea infusions and its daily intake by humans. They found that the concentration of fluoride in black tea infusions after 10 min of brewing was higher than other kinds of tea. The fluoride concentration in the black tea infusions was found to be 0.56–6.13 mg/l (Malinowska et al., 2008a). Mahvi et al. showed the high fluoride concentration in the infusions of black tea (Mahvi et al., 2006). They examined 10 brands of tea and observed that the concentration of fluoride was in the range of 0.53–2.60 mg/l. In another study, Fung et al. assessed the releasing level of fluoride into tea liquor from 17 brands of green tea, black tea, oolong tea, Pureh, Brick, and Black-brick tea during infusion and they observed that there was extremely high concentration of fluoride in Brick tea liquor (7.34 mg/l) in comparison with black tea (1.89 mg/l) and green tea (1.6 mg/l) liquors. The highest fluoride concentration in tea leaves was for brick tea leaves due to the use of old and fallen leaves and even branches (Fung et al., 1999). Also, the fluoride concentration of Iranian green tea infusion with different additives was ranged from 0.162 to 3.29 mg/l as reported by Maleki et al. which was comparable with fluoride concentration in green tea infusions (ranging from 0.71 to 3.71 mg/l) in the present study. They also reported that fluoride concentration of infusions was not significantly affected by additives (Maleki et al., 2016). Yuwono evaluated the fluoride concentration in black, green, and herbal tea and reported that the maximum fluoride concentration was related to black tea and its concentration in black, green, and herbal tea ranged from 0.95 to 4.73 mg/l, 0.70–1.00 mg/l, and 0.26–0.27 mg/l after 5 min, respectively. He also reported that the fluoride concentration might change depending on the type of tea. In addition, the difference of the sample sources such as leaf age, maturity, genetics of the plant, and type of soil could be regarded as some of the reasons for the variation of fluoride concentration (Yuwono, 2005).

3.2. Effect of brewing time

The fluoride concentration (mg/l) in infusions at different brewing time has been shown in Table 2. As seen in Table 2, the concentration of fluoride was increased by increasing brewing time in all brands. In this case, the highest variations of fluoride concentration in infusion were observed in the Kenya tea. The concentration of fluoride was 3.075 ± 0.696 mg/l, 4.794 ± 1.085 mg/l, and 5.380 ± 0.917 mg/l

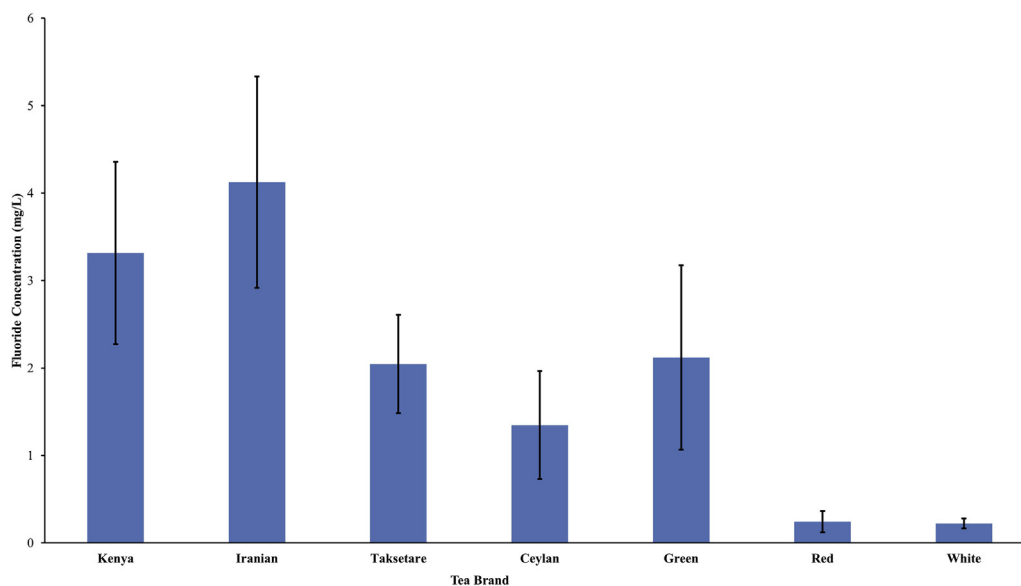


Fig. 1. Mean fluoride concentration in studied tea brands (Kenya, Iranian, Taksetare, Ceylan, Green, Red and White tea) after 10 min brewing. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The effect of brewing time on F⁻ concentration (mg/l) in infusions.

Tea Brand	F ⁻ content (mg/l)	brewing time (min)		
		5	10	15
Kenya tea	3.07 ± 0.69 ^a	4.79 ± 1.08 ^a	5.38 ± 0.91 ^a	
Red tea	0.33 ± 0.25	0.34 ± 0.27	0.34 ± 0.27	
White tea	0.22 ± 0.0	0.23 ± 0.05	0.22 ± 0.06	
Green tea	1.35 ± 0.15	1.80 ± 0.30	1.98 ± 0.55	
Iranian tea	3.76 ± 0.85 ^{ab}	4.63 ± 0.13 ^a	4.93 ± 0.28 ^b	
Taksetare tea	2.19 ± 0.12	2.43 ± 0.07	2.58 ± 0.0	
Ceylan tea	1.38 ± 0.11	1.48 ± 0.04	1.62 ± 0.04	

Data were expressed as mean ± standard deviation of three replicates. Means with different superscripts (a, b) in the same row indicates a statistical difference ($p < 0.05$).

for this brand at 5, 10 and 15 min, respectively. The lowest variation of fluoride concentration with time was related to Red and White tea. The fluoride concentrations in these brands were 0.224 ± 0.006 mg/l, 0.232 ± 0.052 mg/l and 0.224 ± 0.063 mg/l at 5, 10 and 15 min, respectively. Also, the most fluoride contents were released into infusions at the first 5 min. Zerabruk et al. evaluated the effect of brewing time on the release of fluoride into tea infusions and obtained similar results (Zerabruk et al., 2010). In another study, the effect of brewing time on the concentration of released fluoride in tea infusions was investigated and observed a direct relationship between brewing time and fluoride level in the tea infusions (ŞükrüKalaycı and Somera, 2003).

3.3. Statistical variations

The results of Kruskal–Wallis test showed that fluoride concentration in infusion had a significant difference between different brands of tea (overall $p < 0.001$). A Bonferroni post-hoc test revealed that the fluoride concentration in Kenya tea (3.31 ± 1.04 mg/l) was significantly higher than red (0.24 ± 0.12 mg/l, $p < 0.001$), white (0.22 ± 0.06 mg/l, $p < 0.001$), and Ceylan tea (1.35 ± 0.62 mg/l, $p < 0.001$). Significant differences were observed between fluoride concentration in infusion of red tea, green tea ($p = 0.001$), Iranian tea ($p < 0.001$) and Taksetare tea ($p = 0.001$). There were no significant differences between the Kenya tea, Iranian tea (4.12 ± 1.2 mg/l), green tea (2.12 ± 1.05 mg/l), and Taksetare tea (2.04 ± 0.56 mg/l).

Repeated-measures ANOVA demonstrated a significant main effect of brewing time ($F = 28.693$, $p = 0.001$). This study showed that the main effect of tea brands was significant ($F = 39.615$, $p < 0.001$). The most important analysis was the significance of interaction effect of brewing time and type of tea. This effect showed that fluoride concentration in an infusion of different tea brands and different brewing times had a significant difference ($F = 6.224$, $p = 0.011$).

3.4. Health risk assessment

The non-carcinogenic risk of exposure to fluoride concentration in each brand of tea was evaluated using Monte Carlo simulations technique. Tables 3 and 4 indicated the mean and 95th percentile CDI and THQ for each brand of tea. The highest mean and 95th percentile of CDI was observed in children group. Iranian tea (mean: $8.09 \text{ E-}2$ and 95th percentile: $1.38 \text{ E-}1$) and Kenya tea (mean: $6.52 \text{ E-}2$ and 95th percentile: $1.12 \text{ E-}1$) had maximum CDI values among studied tea brands. Peng et al. estimated the CDI of fluoride from black tea, green tea, oolong tea, white tea, Pureh tea, and reprocessed tea which was 0.02, 0.01, 0.03, 0.01, 0.02 and 0.02 mg/kg/day, respectively (Peng et al., 2016). When, the THQ values for an element is higher than 1, it can be considered as an element with health risk (Sofuoglu and Kavcar, 2008). In the present study, the 95th percentile of THQ values of Kenya tea, green tea, Iranian tea, and Taksetare tea in children group was higher than 1 (Kenya tea = 1.87, Green tea = 1.44, Iranian tea = 2.30, Taksetare

Table 3
Mean and 95th percentile of CDI for different age groups.

Tea Brand	Children		Teens		Adults	
	Mean	95th	Mean	95th	Mean	95th
Kenya tea	6.52E-2	1.12E-1	4.73E-2	7.63E-2	1.41E-2	2.39E-2
Red tea	4.69E-3	9.48E-3	3.20E-3	6.64E-3	1.02E-3	2.04E-3
White tea	4.31E-3	7.15E-3	2.89E-3	4.88E-3	9.40E-4	1.52E-3
Green tea	4.19E-2	8.63E-2	2.80E-2	5.83E-2	9.15E-3	1.86E-2
Iranian tea	8.09E-2	1.38E-1	5.40E-2	9.31E-2	1.75E-2	2.89E-2
Taksetare tea	4.02E-2	6.66E-2	2.71E-2	4.52E-2	8.87E-3	1.42E-2
Ceylan tea	2.65E-2	5.16E-2	1.76E-2	3.48E-2	5.68E-3	1.11E-2

Table 4
Mean and 95th percentile of HQ values for different age groups.

Tea	Children		Teens		Adults	
	Mean	95th percentile	Mean	95th percentile	Mean	95th percentile
Kenya tea	1.09	1.87	7.29E-1	1.27	2.35E-1	3.98E-1
Red tea	7.82E-2	1.58E-1	5.33E-2	1.11E-1	1.70E-2	3.40E-2
White tea	7.19E-2	1.19E-1	4.82E-2	8.13E-2	1.57E-2	2.53E-2
Green tea	6.98E-1	1.44	4.67E-1	9.71E-1	1.52E-1	3.10E-1
Iranian tea	1.35	2.30	9.01E-1	1.55	2.92E-1	4.82E-1
Taksetare tea	6.70E-1	1.11	4.52E-1	7.53E-1	1.46E-1	2.36E-1
Ceylan tea	4.42E-1	8.61E-1	2.94E-1	5.80E-1	9.47E-2	1.85E-1

tea = 1.11). Also for teens group, the 95th percentile of THQ in Iranian tea was higher than 1 (Iranian tea = 1.55). These result indicated that there were significant risks of exposure to fluoride in most of the studied brands of tea for children due to the lower body weight of children than other studied groups (Huang et al., 2017).

The fluoride has both detrimental and beneficial effect on human health. Various standards have been established to prevent its hazardous effect. The World Health Organization (WHO) has recommended a daily fluoride intake of 2 and 2–4 mg for children and adults, respectively; however, the threshold level of 2.5 for children and 4 for adults is suggested by USEPA (Cao et al., 2006). Other studies reported that black tea might increase the risk of dental fluorosis due to the large level of fluoride concentration (Emekli-Alturfan et al., 2009). Waugh et al. (2016) investigated the fluoride concentration in some brands of black tea infusions in the Republic of Ireland. Based on the results of risk assessment, the majority of the population in the Republic of Ireland were at a high risk of chronic fluoride exposure and associated adverse health effects (Waugh et al., 2016).

3.5. Sensitivity analysis

Sensitivity analysis is a process that can determine the effect of every variable on the output. The results of sensitivity analysis help to find the parameters that have the most effect on estimated risks and can help focus on these variables in future studies (USEPA, 1992).

Qualitative sensitivity analysis of non-carcinogenic risk assessment for children, teens, and adults are presented in Fig. 2. Contribution to variance for fluoride concentration (C), daily intake (DI) and exposure frequency (EF) was ranged from 52.8 to 87.5%, 47 to 31.5% and 6.2–19.8%, indicated these parameters have positive effect on risk estimates for three exposed groups. Also, body weight (BW) had a weak negative effect (range from -0.1 to -6.7%) on estimated risk of exposure to fluoride. In overall, C and DI were the most influential variables in three exposed populations.

3.6. Uncertainty analysis

To quantify uncertainties during risk assessment, in addition to

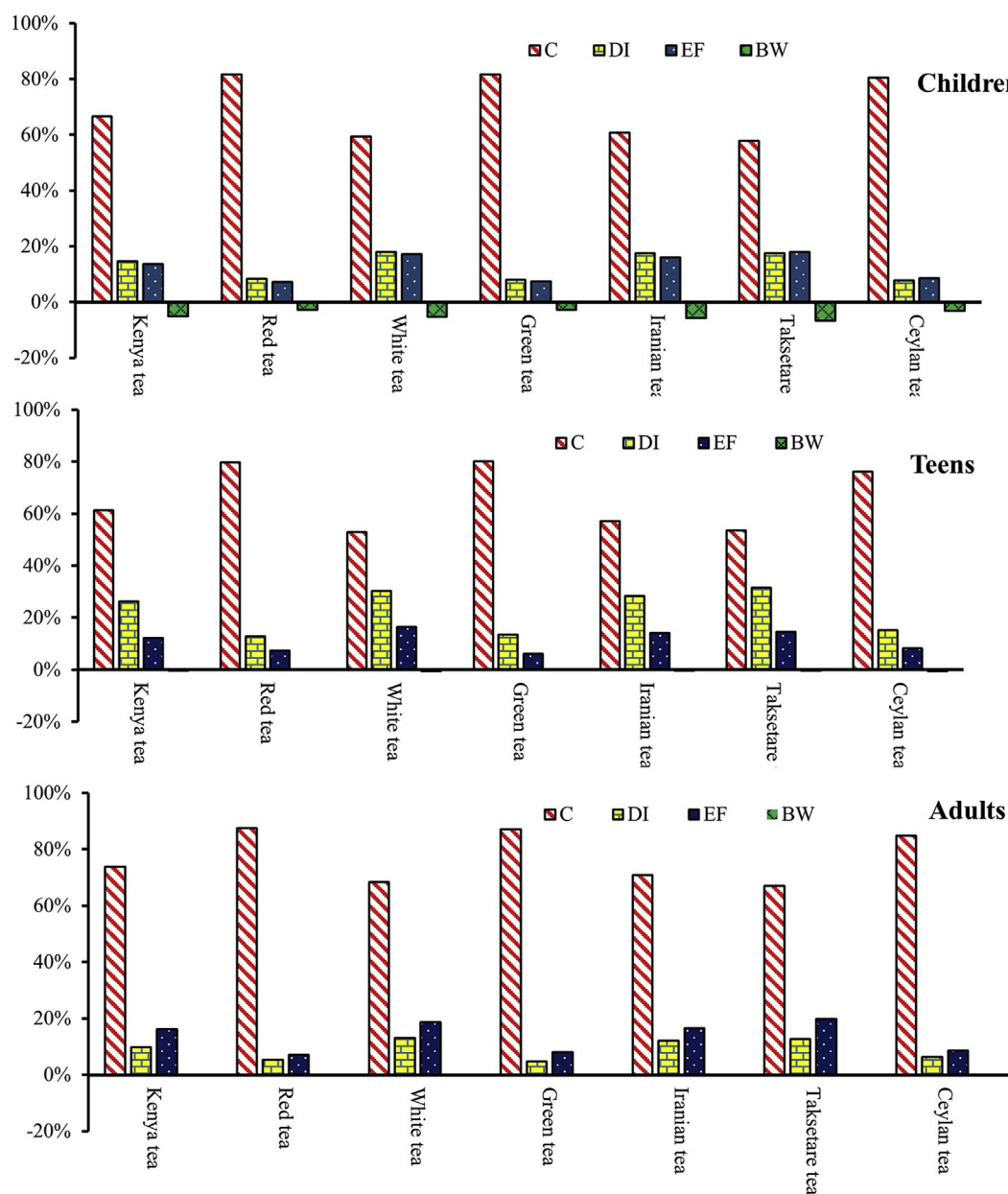


Fig. 2. Sensitivity analysis of target hazard quotient (THQ) model for exposure to fluoride in three studied groups (C: fluoride concentration, DI: daily intake, EF: exposure duration and BW: body weight).

Monte Carlo simulations, other uncertainties of input variables were remained, especially the parameters that identified by sensitivity analysis. For instance, fluoride concentration was measured based on the appropriate method and all experiments were performed duplicate to increase the accuracy of measurements and decrease the uncertainties. The consumption rate of tea in a different climate is variable, however, personal habits and cultural conditions are also affected the daily intake of fluoride (Craig et al., 2015; Malinowska et al., 2008b).

Some other sources of fluoride such as drinking water or foods and fluoride supplements also can increase the daily intake of fluoride (Erdal and Buchanan, 2005; Huang et al., 2017; Li et al., 2009). Our probabilistic health risk assessment for studied inhabitants could be underestimated because other sources of fluoride were not considered. Thus, in future studies, for accurate and precise estimate risks of exposure to fluoride, additional data of different exposure pathway and different sources should be considered.

4. Conclusions

In the present work, the fluoride concentration of 7 brands of tea was measured and the effect of brewing time was evaluated and finally, the health risk of the obtained fluoride concentration in each brand of tea was calculated. The results revealed that Iranian tea (4.125 mg/l) and Kenya tea (3.314 mg/l) had higher fluoride concentration. The lowest fluoride concentration was related to Red (0.244 mg/l) and White (0.223 mg/l) tea. The fluoride concentration was increased by increasing the brewing time. In addition, it was observed that time had a significant effect on releasing fluoride in tea infusion of Iranian tea and Kenya tea. The maximum variation of fluoride concentration with time was observed in Kenya tea. Therefore, it could be concluded that 5 min was adequate brewing time for Iranian tea and Kenya tea in order to prevent the release of a high amount of fluoride. Iranian tea and Kenya tea had maximum CDI and target hazard quotient (THQ) values among studied brands of tea which followed by Green tea, Taksetare tea, Ceylan tea, Red tea, and White tea. Most brands of tea had THQ

more than 1 for children, which indicated that this group was in the high risk of non-carcinogen hazards from these brands of tea with high fluoride level that needs more attention.

Acknowledgment

The authors would like to thank the laboratory staff of the Department of Environmental Health Engineering, Faculty of Health, for their collaboration and the Research Deputy of Zabol University of Medical Sciences for financially supporting this study.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.fct.2018.03.023>.

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