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DETERMINATION OF TOXIC DIETHYLENE GLYCOL IN TOOTHPASTES IN DAR ES SALAAM BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AND COLORIMETRIC METHODS

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ABSTRACT

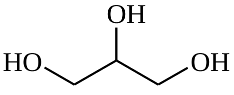
The aim of this study was to determine the presence and the levels of diethylene glycol (DEG) in toothpaste products commonly used in Dar es Salaam. Forty five samples of different brands of imported and locally manufactured toothpastes were collected and processed prior to analysis. High performance thin layer chromatography (HPTLC) method was used for the qualitative detection of diethylene glycol in the toothpastes. In quantitative analysis, a colorimetric method that involved ultraviolet–visible (UV-Vis) spectrophotometric measurements of absorbances of prepared coloured samples and standards was used. DEG was found in 37.8% of the tested samples. The concentrations of the DEG detected in the samples varied from 0.106 to 7.71 mg/g. The results indicated that 88.2% of the samples found with DEG were obtained from imported toothpastes and 11.8% of the samples found with DEG were from local pharmaceutical industries. The findings indicate public health risks because, according to the WHO standards, toothpastes should have no traces of DEG as it is toxic. There is a need for regular checking of the composition of both imported and locally manufactured toothpastes by the regulatory authorities.

Keywords: Diethylene glycol, Toothpaste, TLC, UV-Vis Spectrophotometry, Tanzania

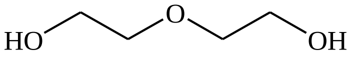
INTRODUCTION

Toothpastes are used to clean and protect teeth from decay. The toothpastes are composed of active ingredients such as sodium fluoride (anticavity) and triclosan (antibacterial agents) which help to fight cavities and reduce the risks of gum diseases and inactive ingredients such as glycerin, flavour, cellulose gum, sodium lauryl sulphate, sorbitol, mica, sodium hydroxide, water and titanium dioxide which give the toothpastes their sweet tastes, binders and texture (humectants) (BP 2005). Glycerin, also commonly called glycerol or glycerine with the IUPAC name propane-1,2,3-triol, is a colourless and viscous liquid that is widely used in pharmaceutical formulations. Glycerin is a sugar alcohol that has a sweet taste and low toxicity. It has three hydrophilic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Glycerin is used in medical pharmaceutical and personal care preparations, mainly for improving smoothness, providing sweet tasting, and is used for lubrication and as a humectant. It is found in wide products like cough syrups and expectorants, toothpastes, mouthwashes, skin care products, shaving cream, hair care products and soaps (Kenyon et al. 1998, The Chemical Company 2018).

Diethylene glycol (DEG) (IUPAC name: 2-(2-hydroxyethoxy)ethanol) is illegally used as counterfeit glycerin and sold as a component of toothpastes (Bogdanich and McLean 2007, Medsafe 2007). Glycerin and diethylene glycol are similar in appearance, smell and taste (WHO 1999). Diethylene glycol is a clear, hygroscopic and odourless liquid. It is miscible with water and polar organic solvents such as alcohols and ethers (Schep et al. 2009). The structures of glycerin and diethylene glycol are shown below.



Glycerin



Diethylene glycol

Glycerin is more viscous and has a higher melting point (17.8 °C) than that of diethylene glycol (–10.45 °C). The boiling points of glycerin and diethylene glycol are 290 °C and 245 °C, respectively. A solution of diethylene glycol and water is used as a coolant. It lowers the freezing point of a solution and elevates its boiling point. Diethylene glycol is also a building block in organic synthesis. It is a solvent for nitrocellulose, resins, dyes, oil, and other organic compounds. It is a humectant for tobacco, cork, printing ink and glue. It can also be found in some hydraulic fluids and brake fluids (Rebsdat and Mayer 2000, Carlson and Carlson 2005, O’Neil et al. 2006, Schep et al. 2009).

Glycerin costs about three times the price of diethylene glycol. Because of its toxicity, diethylene glycol is not allowed for use in foods and drugs. Diethylene glycol is toxic/ poisonous to humans and animals, and death can occur by renal failure. Several poisonings occurred when diethylene glycol was substituted for the non-toxic naturally occurring “triol” glycerin in foodstuff and pharmaceuticals (O'brien et al. 1998). If ingested, DEG can cause nausea, abdominal pain, urinary problems, kidney failure, breathing problems, lethargy, convulsions, dizziness, coma and on occasions death (O'brien et al. 1998, WHO 1999, Schep et al. 2009). Ingestion or application of diethylene glycol contaminated products, such as toothpastes and medicinal syrups, can cause systemic alcohol intoxication, acidosis and subsequent multiorgan failure, leading to hundreds of deaths (WHO 1999).

Due to rapid economical developments and economic challenges in various countries and increasing rates of counterfeit products, toothpastes used in Tanzania are likely to be adulterated. Adulteration of products containing glycerin with the less expensive and highly toxic diethylene glycol has been reported in some countries such as New Zealand, Panama and Iraq (O'brien et al. 1998, Bogdanich and McLean 2007, Medsafe 2007). To the best of our knowledge, no study has been conducted in Tanzania to assess diethylene glycol in toothpastes. It was suspected that toothpastes are most likely counterfeited with diethylene glycol, hence the need for conducting the present study. The main objective of this study was to determine the presence and the levels of diethylene glycol in the toothpastes commonly used in Dar es Salaam, Tanzania as well as to assess the variations in the levels of the diethylene glycol among different brands of the toothpastes.

Various methods have been employed for the analysis of diethylene glycol in pharmaceutical products, such as toothpastes, and other samples. The methods include thin layer chromatography (TLC), which is a screening method (qualitative test) that is capable of detecting diethylene glycol in toothpaste qualitatively at the levels from 1.0 to 2.0% visually, while levels below 1.0% can be detected through treatment with chemicals such as potassium permanganate or iodine vapour (Kenyon et al. 1998). TLC is inexpensive, simple and easy to use, but it has limitations and cannot be used for the quantification of DEG. Other applicable methods used for the detection and quantification of diethylene glycol are gas chromatography (Baffi et al. 2000, Ferrari and Giannuzzi 2005, Kamimura et al. 2008, ASTM International 2018), high performance liquid chromatography (HPLC) (Zhou et al. 2007), ultra-performance liquid chromatography–mass spectrometry (Hernández et al. 2008), capillary electrophoresis with electrochemical detection (Xing et al. 2009), mass spectrometry (Ding et al. 2009) and gas chromatography-mass spectrometry (Fu et al. 2011). These methods are generally expensive and have technical challenges in terms of applications, especially in institutions and countries which do not have modern equipment for the detection of DEG. Standard colorimetric methods for the quantitative determinations of different types of glycols (e.g., monoethylene glycol or ethylene glycol and propylene glycol) are available in literature (Jabbar and Faizullah 2013, Ling et al. 2013, ASTM International 2016, ASTM International 2018) and have been successfully applied for the determination of the glycols in various samples such as in antifreeze samples and for other applications (e.g., for the molecular weight determination). However, no study was found to investigate diethylene glycol in toothpastes by colorimetric methods.

Therefore, this study applied a combination of high performance thin layer chromatography (HPTLC) technique and colorimetric method to analyse diethylene glycol in the toothpaste samples. The colorimetric method can be used to quantify the diethylene glycol contents in toothpaste samples. Colorimetric method is based on the change in the intensity of the colour of a solution with variations in concentrations (Davidson 1988, Davidson 2007). Sensitivity and accuracy of the method increase when a spectrophotometer is used to measure the colour intensity. Basically, it measures an absorbance of a sample at a specific wavelength. Blank readings are made first for the purpose of calibrating the spectrophotometer so that any absorbance attributable to the solvent and/or glass cuvette can be compensated so to read zero. For any given concentration, the amount of light absorbed depends upon the concentration according to the Beer-Lambert's law.

MATERIALS AND METHODS

Sample collection and storage

Nine different brands of toothpastes (Whitedent triple action, Whitedent gel, Whitedent herbal, Colgate, Aloe, Glister, Sensodyne, Smokers and Angola) samples were collected. A total of forty five (45) samples were collected. The brands were labelled A to I, and for each type, five sample pieces were purchased from different shops in Dar es Salaam and transported to the Chemistry department, University of Dar es Salaam where they were stored according to the specified conditions as per manufacturers' instructions prior to laboratory preparation and analysis. The details of the samples are presented in Table 1.

Chemicals, equipment and analytical materials

The chemicals (solvents, reagents and standard) used included acetone, acetonitrile (analytical grade, 99.5% purity, Merck, Germany), ammonium hydroxide (25%, analytical grade), potassium permanganate, phosphoric acid, sodium bisulphate, iron (III) chloride ($FeCl_3$), sulphuric acid (Fischer Scientific, UK), toluene (analytical grade, 99.8% purity, Scharlau, EU) and diethylene glycol (DEG) standard (GC grade, 99% purity, Prolabo). The HPTLC plates were purchased from Merck, Darmstadt, Germany. The instruments used included a high performance thin layer chromatography (HPTLC) system. Scanning in HPTLC was performed using a CAMAG TLC scanner, operated by winCATS software version 1.4.3 (CAMAG). Absorption spectra were measured using a double beam ultraviolet–visible (UV-Vis) spectrophotometer (JENWAY 6850).

Table 1: Toothpaste samples collected from different shops

Toothpaste brand	**Manufacturer's location (Country)**	**Sample codes**	**Number of samples collected**
A	Tanzania	S3, S8, S13, S18, S23	5
B	Tanzania	S4, S10, S15, S20, S25	5
C	Tanzania	S5, S9, S14, S19, S24	5
D	Thailand	S2, S7, S12, S17, S22	5
E	China	S1, S6, S11, S16, S21	5
F	India	S26, S31, S32, S33, S34	5
G	India	S27, S35, S36, S37, S38	5
H	China	S28, S30, S39, S40, S41	5
I	China	S29, S42, S43, S44, S45	5

Sample preparation

A sub-sample (1 g) of toothpaste (equivalent to one application on toothbrush) was weighed into a centrifuge tube and 5 mL of distilled water were added and mixed well. Acetonitrile (5 mL) was added to reduce the foam that appeared, and then, centrifugation was done for 10 minutes. The supernatant of the resultant extract was transferred to a test tube. 1 mL of the supernatant extract from the test tube was diluted with 2 mL of methanol into a 5-mL volumetric flask followed by thorough shaking (Kenyon et al. 1998). The solution was ready for qualitative analysis test.

Standard preparation

Diethylene glycol (100%) as a standard solution was used to prepare 0.1% diethylene glycol working standard solution by diluting 1.0 mL of 100% diethylene glycol to 10 mL of methanol in a 10 mL volumetric flask followed by thorough shaking.

HPTLC test procedures

Three spots of 10 µL volume each were applied on a 5 cm × 10 cm size HPTLC plate with 250 µm thickness silica gel coated aluminium sheet by 10 µL capillary tubes. The standard solution spot was at the middle and the sample solution spots at the ends of the HPTLC plate. The HPTLC plate was developed in the mobile phase having the mixture of acetone, 5 M ammonium hydroxide and toluene (17:2:1), until the mobile phase reached three quarters of the plate. Visualization of dried plate was done either by staining with potassium permanganate dissolved in the developing solvent or iodine vapour and the scanner was used at the wavelength of 325 nm (Kenyon and Layloff 2008, Ghanem et al. 2012). Yellow spots started to appear on the plate 1 minute later while diethylene glycol took up to 10 minutes. The R_f (retardation factor) values of the analytes in samples and standard were calculated according to the equation below and compared in order to confirm the presence or absence of diethylene glycol in the analysed samples.

$$R_f = \frac{\text{Distance travelled by the solute (DEG)}}{\text{Distance travelled by solvent (mobile phase)}}$$

Colorimetric method

After the HPTLC tests, the toothpaste samples were further subjected to quantitative tests for the determination of diethylene glycol and confirming the adulteration using colorimetric method (ultraviolet-visible spectrophotometry). Colorimetric method was used to determine the levels of the diethylene glycol that reacted to produce colour. The intensity of the colour from the reaction is proportional to the concentration of the diethylene glycol being tested, and a UV-visible spectrophotometer is used to measure the colour intensity. Basically, it measures absorbance using glass cuvette. In order to calculate the concentration of diethylene glycol present in the samples, first a calibration curve was prepared which was used to quantify the DEG contents in the toothpaste samples. The compositions of the standard solutions used for the preparation of the calibration curves are presented in Table 2. A series of diethylene glycol standard solutions at concentrations of 0.001%, 0.01%, 0.1%, 1%, 10%, and 20% were prepared in 100-mL volumetric flasks. To a portion (5 mL) of the final standard solution in 100-mL volumetric flask, 1.0 mL of 2% potassium permanganate solution and 0.5 mL of phosphoric acid were added. The solution was mixed well and kept at room temperature for 10 minutes. The solution was then decolourized by adding 1.6 mL of 2.5% sodium bisulphate solution followed by addition of 10 mL of acetonitrile and incubated at room temperature for 10 minutes. A mixed solution of 1% $FeCl_3$ and 1.6% sulphuric acid (1:1 v/v, 10 mL) was added. The mixture was left for 20 minutes at room temperature; then, the absorbances of the coloured solutions were measured at an excitation wavelength of 620 nm using a spectrophotometer. A fresh calibration curve was prepared for every day of the analysis. These procedures were applied for the preparations of the sample solutions for spectrophotometric determinations. The spectrophotometer was calibrated using blank solutions and was set at a wavelength of 620 nm. The absorbance of each solution of the calibrator was determined systematically using cuvettes rinsed with the respective calibrator levels to be tested for all the six calibrators as well as the samples.

Table 2: Concentrations of standard solutions and other details used for generating calibration curves

Concentration of standard (%)	Concentration of standard (mg/mL)	Standard DEG volume taken (mL)	Methanol added (mL)	Total volume (mL)
20	200	20 from 100% standard	80	100
10	100	50 from 20% level	50	100
1.0	10	10 from 10% level	90	100
0.1	1.0	10 from 1% level	90	100
0.01	0.1	10 from 0.1% level	90	100
0.001	0.01	10 from 0.01% level	90	100

The absorbances of the standard solutions and sample solutions were measured as shown in the data summarised in Table 3 and used for the quantification of the diethylene glycol contents in the toothpaste samples. All the concentrations of the standards were within a linear range as shown in the calibration curves presented in Figure 1 a-c. The calculations were done using the calibration curve drawn and provided the relationship between the unknown concentration and absorbance. The absorbance of each solution that corresponded to the concentration of DEG was determined using the linear equation:

$$y = mx + y_0,$$

where y = absorbance, m = slope, x = analyte concentration in samples that was calculated and y_0 was a constant that described the background. The concentrations obtained from the calibration curves were converted by multiplying by the ratio of the final volume/initial amount of sample (mL/g) to obtain the concentrations of the diethylene glycol in the samples. The method was validated in toothpaste samples spiked at levels of 0.001, 0.01, 0.1, 10 and 20% (0.01, 0.1, 1, 10, 100 and 200 mg/mL, respectively). Reagent (procedural) blanks were also tested.

Table 3: Raw data for analysis of diethylene glycol standard and samples of toothpastes

Calibration description	Standard conc. (%)	Standard conc. (mg/mL)	Absorbance of standard	Samples analysed (codes)	Absorbance of sample
1st calibration, for brands A, B and C	20	200	1.874	S3, S4, S5, S8,	0
	10	100	0.939	S9, S10, S13,	0
	1	10	0.124	S14, S18, S19,	0
	0.1	1	0.099	S20, S23, S24	0
	0.01	0.1	0.035	S15	0.039
	0.001	0.01	0.011	S25	0.037
2nd calibration, for brands D, E, F and G	20	200	1.863	S1, S2, S6, S7,	0
	10	100	0.944	S11, S12, S16	0
	1	10	0.119	S26, S31–S34	0.099–0.112
	0.1	1	0.101	S27, S35–S38	0.088–0.105
	0.01	0.1	0.041	S17, S21, S22	0
	0.001	0.01	0.013		
3rd calibration, for brands H and I	20	200	1.868		
	10	100	0.951		
	1	10	0.115		
	0.1	1	0.099	S28, S30, S39–S41	0.059–0.067
	0.01	0.1	0.029		
	0.001	0.01	0.008	S29, S42–S45	0

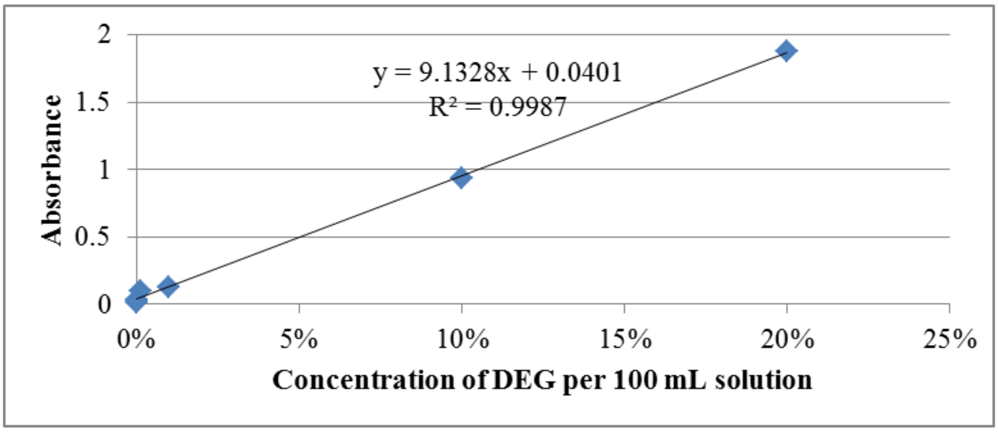


Figure 1 a: Calibration curve used to find levels of DEG in toothpaste brands A to C

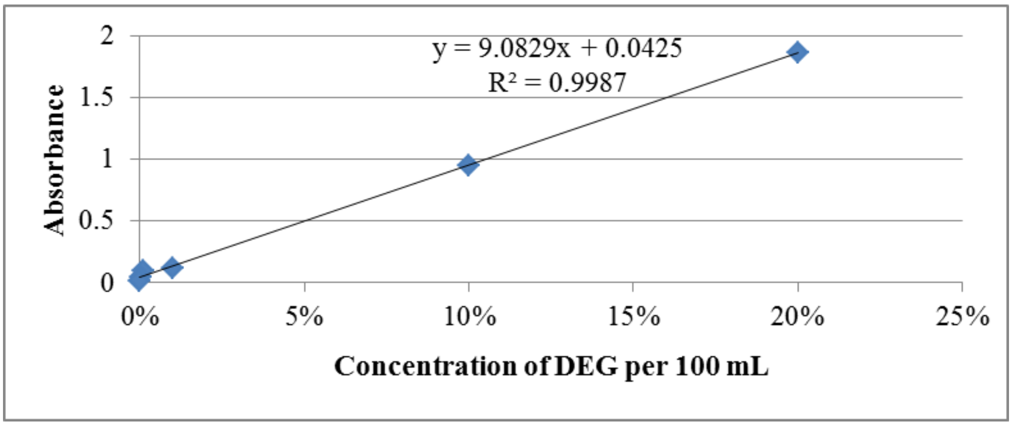


Figure 1 b: Calibration curve used to find levels of DEG in toothpaste brands D to G

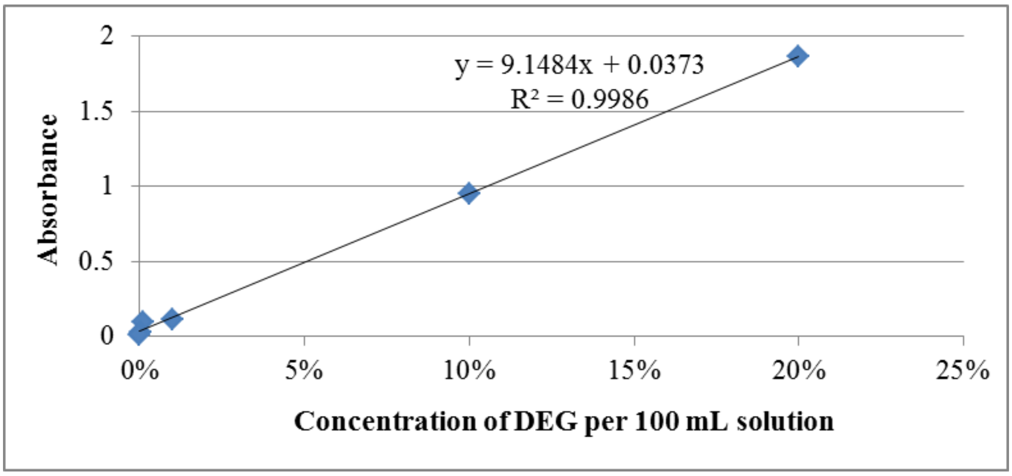


Figure 1 c: Calibration curve used to find levels of DEG in toothpaste brands H and I

RESULTS AND DISCUSSION

Qualitative test results from the screening technique

The results of the qualitative analysis for diethylene glycol are summarised in Table 4. Diethylene glycol (DEG) was detected in four out of the nine brands of the toothpastes. The toothpaste samples that were found with DEG in the HPTLC tests were from brands B, F, G and H, suggesting that they were adulterated. DEG was not detected in the toothpaste samples from brands A, C, D, E and I, suggesting that they were not adulterated.

Concentrations of diethylene glycol in toothpastes determined by colorimetric method

In the colorimetric method, the concentrations of diethylene glycol in toothpastes were tested and quantified using the calibration curves. Linearity of the method for the tested concentrations (R^2 values) ranged from 0.9986 to 0.9987. Satisfactory recoveries ranging from 95 to

99% and precision (relative standard deviations of < 5%) were obtained. DEG was not detected in the blank samples.

The concentrations of diethylene glycol determined by colorimetric method varied from 0.106 to 7.71 mg/g (Table 5). Among the toothpaste samples analysed, 37.8% were found containing diethylene glycol. Most of the samples found with diethylene glycol were from the imported toothpastes. The highest concentrations obtained were in samples S26 (7.71 mg/g) and S27 (6.98 mg/g) of the toothpaste brands F and G, respectively, both being imported products. The lowest concentrations of diethylene glycol were obtained in samples S15 (0.109 mg/g) and S25 (0.106 mg/g), both from toothpaste brand B manufactured in Tanzania. The results showed that among the samples found with diethylene glycol, 88.2% of them were from imported toothpaste brands while 11.8% of them were from local manufacturing industries. The levels of diethylene glycol found in the locally manufactured toothpastes (ranged from 0.106 to 0.109 mg/g) were generally lower than the levels of DEG found in the imported toothpastes (ranged from 2.40 to 7.71 mg/g).

According to the WHO (1999), the amount of diethylene glycol in toothpastes must be zero, which means that any products of tooth pastes are required not to have any amounts or traces of diethylene glycol. Therefore, the levels of diethylene glycol found in some samples are not acceptable. There could be significantly high health risks from exposure of human beings to the concentrations of DEG found in the toothpastes, particularly for children who sometimes swallow the toothpastes during oral hygiene, i.e., during brushing of their teeth.

Table 4: Summary of the results for the qualitative analyses/tests

Toothpaste Brand	**Number of samples analysed**	**R_f for sample**	**R_f for standard**	**Number of samples found with DEG**	**Number of negative samples (DEG not detected)**
A	5	0.00	0.56	0 (Nil)	5 (All)
B	5	0.56	0.56	2 (S15, S25)	0 (Nil)
		0.00	0.56	0 (Nil)	3 (S4, S10, S20)
C	5	0.00	0.56	0 (Nil)	5 (All)
D	5	0.25	0.56	0 (Nil)	5 (All)
E	5	0.00	0.56	0 (Nil)	5 (All)
F	5	0.56	0.56	5 (All)	0 (Nil)
G	5	0.56	0.56	5 (All)	0 (Nil)
H	5	0.56	0.56	5 (All)	0 (Nil)
I	5	0.17	0.56	0 (Nil)	5 (All)
Total	**45**	**0.00-0.56**	**0.56**	**17**	**28**

Table 5: Results for the quantitative analyses

Toothpaste brand	Sample codes	*n*	Samples with DEG	Concentration of DEG (mg/g)	Detection frequency (%)
A	S3, S8, S13, S18, S23	5	Nil	ND	0
B	S4, S10, S15, S20, S25	5	S15, S25	0.106 to 0.109	40.0
C	S5, S9, S14, S19, S24	5	Nil	ND	0
D	S2, S7, S12, S17, S22	5	Nil	ND	0
E	S1, S6, S11, S16, S21	5	Nil	ND	0
F	S26, S31–S34	5	All	6.80 to 7.71	100
G	S27, S35–S38	5	All	5.87 to 6.98	100
H	S28, S30, S39–S41	5	All	2.40 to 3.28	100
I	S29, S42–S45	5	Nil	ND	0

ND = Not Detected; *n* = Number of samples

Comparison of the levels of diethylene glycol with previous findings in other countries

The concentrations of DEG found in this study were lower than the DEG levels in the toothpaste brands that were found in New Zealand, which included Excel, Maxam, Evafresh, Tian Qi, Hei Mei Cpp, Mr Cool, Mr Fresh, Dr Cool, Smile, Crescent and Tri Leaf Spearmint. Their levels of DEG ranged from 2.43 to 11.83% (24.3 to 118.3 mg/g) (Medsafe 2007). The concentrations of diethylene glycol found in this study were comparable to the concentrations of DEG found in toothpastes in Panama. DEG was found in 6,000 tubes of toothpastes in Panama in the products that appeared to have originated from China. The DEG was found in brands of toothpastes labelled Excel and Mr. Cool at concentrations ranging between 1.7 and 4.6% (Bogdanich and McLean 2007). Diethylene glycol was also found in samples of different types of toothpastes in the local markets of Baghdad, Iraq analysed using Gas Chromatography-Flame Ionization Detector (GC-FID) and the concentrations of DEG in the samples ranged between 101 and 839 ppm [0.101 and 0.839 mg/g] (Almosawi and Alobaidi 2011). The toothpaste brands found containing diethylene glycol in Iraq and their origins were Crest1 and Crest2 (Germany), Sign, Chuzi, Sinan, QYZ, Dentakleen and Crust (China), Aim (USA), Amber (Iraq), Colgate1 (Saudi Arabia), Signal (France), Megadent (Bulgaria), Formula (Indonesia), Mediann (Korea), Sanino (Turkey), Sensodyne, Dentamint and Colgate2 (unidentified origins); while DEG was not detected in the samples of the brands Everfrest from China, Brushup and Miswak from the United Arab Emirates (Almosawi and Alobaidi 2011). The concentrations of diethylene glycol found in the toothpaste samples in Baghdad, Iraq are comparable to the concentrations found in the adulterated toothpastes of brand B in the present study but are generally lower than the concentrations found in most of the adulterated toothpastes in the present study (samples from brands F, G and H).

CONCLUSIONS

The findings indicate that adulterating of toothpastes with diethylene glycol could be a problem in our country especially with the imported ones. Generally the results suggest that there is a need for thorough testing of the imported and locally manufactured toothpaste products. The Tanzanian regulatory authorities such as the Tanzania Food and Drugs Authority (TFDA) and Tanzania Bureau of Standards (TBS) should strictly check the compositions of the toothpastes and other products (such as cough syrups) that are likely to be adulterated with diethylene glycol so as to limit the adulterating rates and reduce or prevent the health risks to the people.

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