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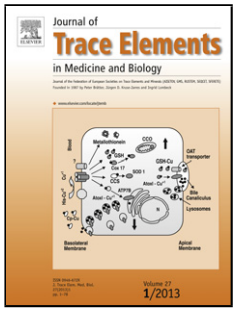

Title: The toxicological analysis of Cu, Mn and Zn as elemental impurities in pharmaceutical herbal products for teething available in pharmacies in Poland

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The toxicological analysis of Cu, Mn and Zn as elemental impurities in pharmaceutical herbal products for teething available in pharmacies in Poland

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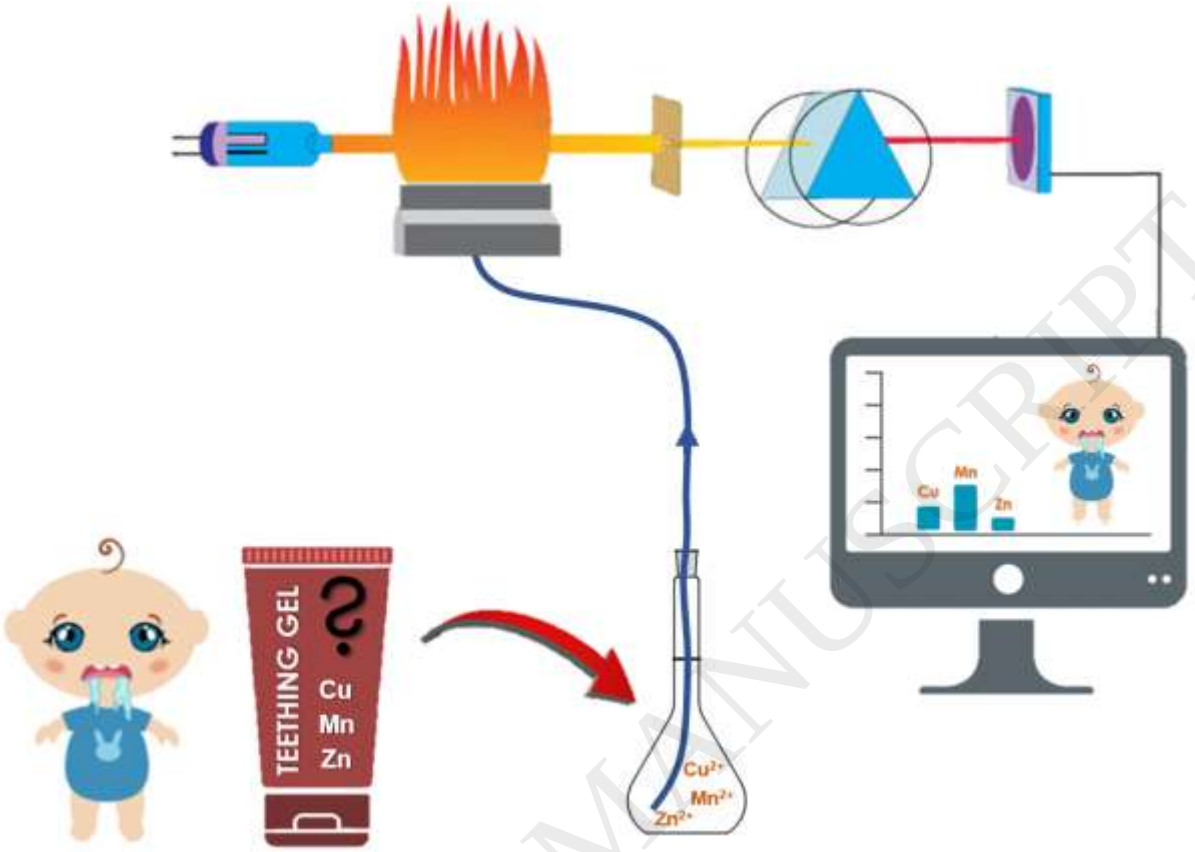
Graphical abstract



Highlights:

- Impurity profile of a five teething gels based on herbs available in Polish pharmacies including Cu, Mn and Zn impurities;
- The level of elemental impurities in a single administration is not a threat to babies in infancy period;
- The results of the daily intake of EIs through teething gel (ng/day) are satisfactory and confirm the safety of all teething gels;
- All investigated teething gels meet the standards of directive ICH Q3D;
- Each of the teething gels do not represent a health hazard to the infants.

Abstract:

The monitoring of elemental impurities (EIs) in pharmaceutical materials is often not adequately treated, although it is a very important topic because the directive ICH Q3D requires a wide range of elements, often at low concentrations, to be monitored. This article describes the quantitative toxicological analysis of copper, manganese and zinc as EIs in the pharmaceutical gels for teething containing herbs available in Poland. The levels of EIs were measured to evaluate whether the intake of these metals through the gels was within recommended levels. The flame absorption spectrometry (FAAS) following microwave induced digestion (concentrated nitric acid) was applied to determine the levels of Cu, Mn and Zn in the products. This article was motivated especially by the facts that: (i) herbs can be a potential source of EIs; (ii) Cu, Mn and Zn are essential trace elements in the infancy period; (iii) there is a general lack of data around the risk assessment associated with exposure to these EIs in this kind of pharmaceutical. Our safety assessment is based on triple approach including: (1) profile of EIs in gels; (2) the actual amount of EIs in the appropriate amount of gel applied with a single administration (one drop) and (3) the daily exposure of EIs in analysed teething pharmaceuticals due to the maximum daily dose. Our results show that all EI levels meet the standards of directive ICH Q3D. It can be concluded that all of the teething gels investigated, based on herbs, available in Polish pharmacies do not represents a health hazard to babies.

Abbreviations:

AAS – atomic absorption spectrometry technique, EIs – elemental impurities, ICH Q3D – International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use, PDE – permitted daily exposure, SD – standard deviation, US EPA – the United States Environmental Protection Agency, WHO – World Health Organisation.

Keywords:

Copper; Manganese; Zinc; Elemental impurities; Atomic absorption spectrometry; Pharmaceutical for teething; Toxicological analysis; Infants

1. Introduction

The safety assessment of pharmaceutical products and ingredients is very important because potentially toxic and harmful contaminants like elemental impurities (EIs) must be identified, and limits defined, for the maximum levels that a patient should be exposed to. This is an important topic in modern toxicological analysis because new procedures for the analysis of EIs in pharmaceutical products and ingredients are being developed and at present, control of EIs in pharmaceutical products and ingredients is currently undergoing a transition from control based on levels in components of pharmaceuticals to control based on permitted daily exposures (PDE) in pharmaceutical products [1]. EIs may arise from various sources, including:

(1) catalyst elements, (2) inorganic contaminants that may enter a drug product from raw materials, (3) the manufacturing process, (4) the environment and (5) packaging and container closure systems. Directive ICH Q3D (International Council for Harmonization of Technical

Requirements for Pharmaceuticals for Human Use) provides guidelines around these EIs. ICH Q3D aims to limit the presence of EIs in pharmaceutical products and ingredients intended for human use [2-3]. For this purpose, this directive [2] includes three classes of metals based on their toxicity, i.e. around PDE and the likelihood of occurrence in the drug product. While routine control analyses usually focus on elements belonging to the main groups, the elements considered as essential trace elements are rarely topic of studies.

Copper, manganese and zinc are essential trace elements and have a different and important biochemical function in living organisms. However, the lack of homeostasis of these elements is important from toxicological point of view. For example, an excess of copper has been recorded and shown to cause problems only under certain specific conditions, notably genetic disorders such as Wilson disease [3]. On the other hand, the symptoms of manganese toxicity can result in a permanent neurological disorder known as manganism [4]. Chronicly high zinc intake can result in severe neurological diseases attributable to copper deficiency as the results of antagonism of both elements [5]. Hence, toxicological analysis of these trace elements are very important topic according to level of these metals in oral pharmaceuticals.

The aim of this article was the toxicological analysis of copper, manganese and zinc as EIs in pharmaceutical for teething containing herbs. Our studies included the five most available teething gels based on herbs available in Polish pharmacies.

2. Material and methods

2.1. Apparatus

A CEM MDS-2000 microwave digestion system (CEM, Matthews, NC, USA) was applied for the digestion of teething gels. All measurements for the determination of copper, manganese and zinc were carried out using a Perkin-Elmer 5100 ZL atomic absorption

spectrometer (Perkin-Elmer, Norwalk, CT, USA) with flame atomisation. Cu, Mn and Zn hollow cathode lamps were used as the emission sources. Argon (99.999%) was applied as a purge gas. Background corrections were performed by Zeeman background correction. Further information about instrumentation and detailed parameters are described in Supplementary materials 1 (SM1).

2.2. Chemicals, reagents and solutions

All the chemicals and reagents applied in our studies were of high purity grade – SupraPur. Demineralised water from a Milli-Q water purification system (Millipore, Bedford, MA, USA) with the resistivity of 18.3 M-ohm-cm was used for the preparation of standard and sample solutions. Concentrated (65%) nitric acid for digestion was of spectroscopic grade from Merck (Darmstadt, Germany). Argon at purity 99.99% was used as the purge gas. Cu, Mn and and Zn standard stock solutions of 1000 µg/L were obtained from Merck (Darmstadt, Germany). The certified reference material was Corn Flour (INCT-CF-3) obtained from the Institute of Nuclear Chemistry and Technology Department of Analytical Chemistry (Warsaw, Poland). Additional information about the certified reference material are in the Supplementary materials 2 (SM2).

2.3. Samples and treatment of samples

In our studies the five most available teething gels based on herbs available in Polish pharmacies were analysed. The choice of products was justified by

- availability of gels in pharmacies in Lesser Poland Voivodeship (Kraków and Niepołomice);
- the results of paediatricians' opinions (n = 10) from Kraków;
- interview about popularity among parents (n = 15; 27–36 years old) from Niepołomice (Poland);

- literature overview including parenting and pharmaceutical blogs in Poland.

To maintain the highest methodological standards, all samples was coded (A, B, etc.) before studies. The manufacturer's declared ingredients are presented in the Supplementary materials 3 (SM3).

To minimize any potential impurities from other sources, all steps during the sampling procedure were carried out in plastic equipment.

Because all of the samples had an aluminium lid which could act as a source of EIs, the first few centimetres of each gel from the tube was discarded. Homogenisation was used before weighing each of the samples.. Approximately 0.3 g of each gel was used for each analysis. A microwave-assisted digestion was used for samples preparation because is suitable to bring EIs into solution for further analysis. In all cases, 5.0 mL of concentrated nitric acid was used for digestion of the gel. The closed bombs were microwaved for two hours. After cooling, the resulting solutions were diluted up to a final volume of 20 mL in volumetric flasks with demineralized water and kept as stock sample solutions room temperature (20-25 °C) until analysis.

2.4. Determination of metals and analytical figures of merit

All metals were determined in the digested gel samples using a flame atomic absorption spectrometry technique (F AAS). All instrumentation and detailed parameters are described in Supplementary Materials 1 (SM1). All steps of our research have been schematically summarised in Fig. 1.

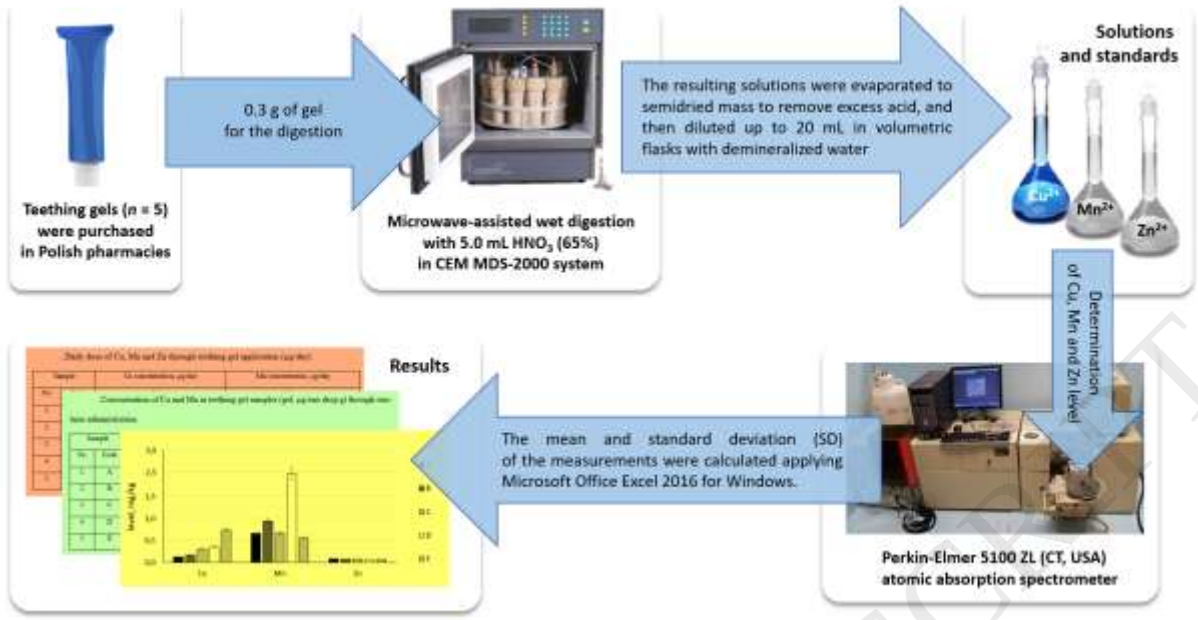


Figure 1. Schematically represented all toxicological analysis steps.

In each case, very good linearity with good correlation coefficients (0.999 for Cu, 0.998 for Mn and 0.998 for Zn) were obtained. The values of the correlation coefficients confirms the linearity of the AAS instrument for precision and accuracy of results. The recoveries, LOD and LOQ values were acceptable. The details about analytical figures are described briefly in Supplementary materials 4 (SM4). The confirmation of the quality control and validation are previously described for Cu [6], Mn [7] and Zn [8]. The SD were calculated from the three replicate measurements of lowest concentrations in each calibration plot. All calculations were made using Microsoft Office Excel 2016 for Windows.

3. Results

The routine toxicological studies for monitoring of EIs in pharmaceutical materials as raw results are important because the results allow a check against fulfillment of standards. However, appropriate safety assessment of teething gels dedicated for infants based on herbs can not only include the measuring EIs. Hence, taking into account also the toxicological

aspects of Cu, Mn and Zn impurities in the analysed teething gels, additional points of view should be also considered. In our studies two aspects were also considered:

- the level of EIs in the drop of pea-sized gel (about one drop of teething gel applied in one administration) - to check the actual amount of metal consumed in one portion (single exposure);
- the daily exposure of applied teething gels – to calculate the daily dose taking in to account the maximum daily dose.

3.1. The impurity profile of a teething gels including Cu, Mn and Zn impurities

The raw results as EIs profile including Cu, Mn and Zn levels in the teething gels are given in Figure 2. The results show that Cu, Mn and Zn were present in all samples. The level of Cu was in the range of 0.125 to 0.733 µg/g. The Mn level was in the range of 0.558 to 2.960 µg/g. On the other hand, the Zn level in all samples was below 1 µg/g (0.065 – 0.083 µg/g).

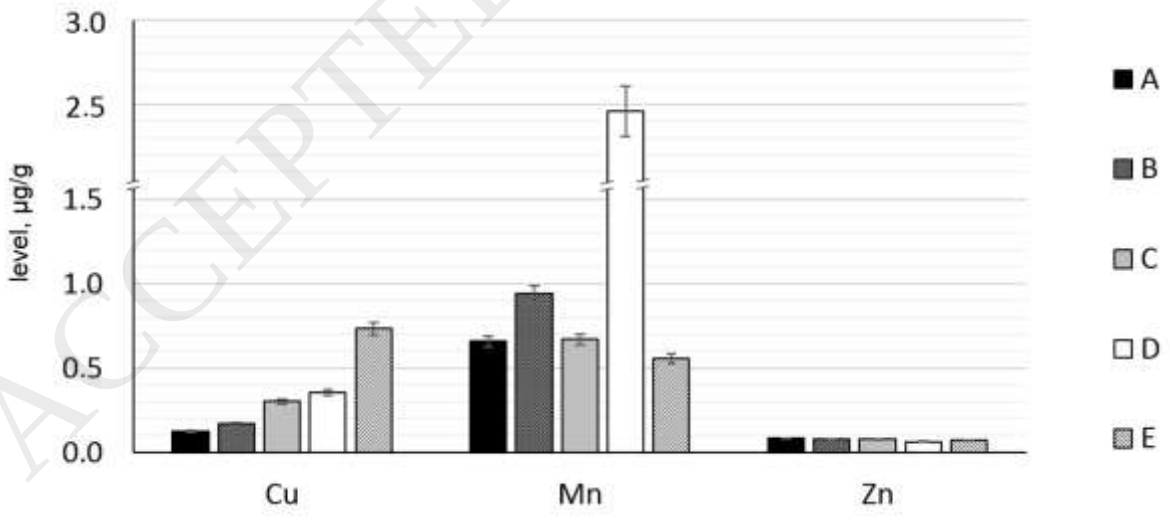


Fig. 2. The impurity profile of a teething gels including Cu, Mn and Zn impurities - EIs level per g of teething gel.

3.2. Level of Cu and Mn impurities in the drop of pea-sized gel (one-time administration of each teething gel)

The EIs profile including copper, manganese and zinc levels in the teething gels are valuable especially for the safety assessment of teething gels around EI's. However, more important information from the toxicological point of view comes from the level of each of the EIs in the drop of pea-sized gel because this amount of gel should be applied into the painful area with a clean finger. The mass of one drop was measured as 0.15 g. Considering the Zn level in samples (<1 µg/g), only the Cu and Mn levels were considered. The calculated levels of Cu and Mn impurities in the drop of pea-sized gel are presented in Table 1.

Table 1. Level of Cu and Mn impurities in the drop of pea-sized gel (gel, µg/one drop) through one-time administration.

3.3. Daily intake of Cu and Mn impurities through teething gel (level of EIs per day)

There is no doubt that the frequency of use of teething gel depends on many different factors. However, usually the application of each pharmaceutical can be repeated up to a maximum of six times per day. Hence, the daily intake of Cu and Mn impurities were calculated – Table 2.

Table 2. Daily intake of Cu and Mn impurities through teething gel (µg/day).

4. Discussion

Copper is an essential trace element in humans because this element plays a vital role in

the functioning of several crucial enzymes. It is also closely related to normal haematopoiesis and cellular metabolism. A general, a review of relevant safety data for humans shows that this element can produce adverse effects to the gastrointestinal tract, liver, and kidney upon ingestion of toxic doses [4]. Moreover, toxic levels of this element are related to Wilson disease – an autosomal, recessively inherited, inborn error involving abnormal copper handling by the liver [11]. Because Cu has a permitted concentration in drugs (oral concentration) of 300 μg/g [9], our results (0.125 to 0.733 μg/g) confirm the safety of Cu levels in the samples. Additionally, based on PDE for Cu in drug products (oral concentration) recommended by directive ICH Q3D (3400 μg/day [9]), all of the samples meet the guidelines because each sample is characterised by level of < 1 μg/day.

It can be supposed that probably, manganese is an essential trace element for infants due to the fact that is an essential dietary mineral for mammals. Manganism, a neurological disorder caused by toxic levels of Mn can occur. However, the level of manganese responsible for manganism has not yet been established [10].

On the other hand, zinc take part in many physiological functions and is ubiquitous within every cell in the body. The deficit of this element is connected to depression disorders [11], against – chronic high zinc intake can result in severe neurological diseases attributable to copper deficiency [5].

Manganese and zinc are classified by ICH Q3D [9] as other metals, i.e. "*elemental impurities for which PDEs have not been established due to their low inherent toxicity and/or differences in regional regulations which are not addressed in this guideline*". In this situation, ICH Q3D points out that appropriate sources of information about safety assessment of pharmaceuticals (acceptable levels of EIs) should be other guidelines and/or regional regulations. However, there is a lack of guidelines and/or regional regulations and practices

related to these elements, especially in teething gels in Poland and other EU countries. Hence, it is not possible to check or compare our results with permitted concentrations of elemental impurities [9], PDE, limits or other published studies. Notwithstanding, Mn and Zn impurities levels *via* oral administration are important information for patients with compromised hepatic function [12] because levels of these essential trace elements in teething gels can be very valuable information for infants with compromised hepatic function [13]. This study also provides a set of data points against which other researchers can study.

5. Conclusions

The obtained results show that all analysed teething gels avaiable in Polish pharmacies contain Cu (0.125 – 0.733 µg/g), Mn (0.558 – 2.960 µg/g) and Zn (0.065 – 0.083 µg/g). impurities at a very low level. All of the teething gels investigated meet the standards of directive

ICH Q3D. There is no doubt that level of Cu, Mn and Zn impurities in a single administration is not a threat to babies in the infancy period. Considering the daily intake of EIs through teething gel (ng/day), our results confirm the safety of all teething gels. It can be concluded that each of the teething gels do not represent a health hazard to the infants.

Our results may be very valuable for other researchers around the levels of essential trace element impurities in pharmaceuticals based on herbs. Moreover, it would be valuable to carry out a broader study considering other EIs and different teething medicaments containing herbs (for example from other countries) as in previously published studies [14-16].

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 1. Level of Cu and Mn impurities in the drop of pea-sized gel (gel, μg/one drop) through one-time administration.

Sample		Level of impurities, μg/one drop	
		Cu	Mn
No.	Code	Mean	Mean
1.	A	0.019	0.099
2.	B	0.025	0.141
3.	C	0.045	0.100
4.	D	0.053	0.444
5.	E	0.110	0.084

Table 2. Daily intake of Cu and Mn impurities through teething gel (µg/day).

Sample		Daily intake, µg/day	
		Cu	Mn
No.	Code	Mean	Mean
1.	A	0.112	0.592
2.	B	0.153	0.848
3.	C	0.272	0.602
4.	D	0.320	2.664
5.	E	0.660	0.502