

## Arsenic and Chromium in Canned and Non-Canned Beverages in Nigeria: A Potential Public Health Concern

J-M. U Maduabuchi, E. O. Adigba, C. N. Nzegwu, C. I. Oragwu, I. P. Okonkwo, Orish E. Orisakwe\*

ZETA-12, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, P.M.B. 5001, Nnewi, Anambra State, Nigeria.

\*Correspondence to Dr. Orish E. Orisakwe. E-mail: eorish@aol.com; greatzeta12@yahoo.com

Received: 28 April 2006 / Accepted: 21 February 2007 / Published: 31 March 2007

**Abstract:** Numerous studies have described environmental exposure of humans to heavy metals in African populations. Little is known about the exposure to heavy metal toxins from processed or unprocessed foods consumed in Africa, and no data exists on the food concentrations of arsenic and chromium, which are potential carcinogens and systemic toxicants. This study determined the concentrations of arsenic and chromium in beverages and fruit drinks commonly sold in Nigeria. Fifty samples of commonly consumed canned and non-canned beverages (imported and locally manufactured) purchased in Nigeria were digested in nitric acid and analyzed by atomic absorption spectrophotometry (AAS). 33.3% of the canned beverages had arsenic levels that exceeded the maximum contaminant level (MCL) of 0.01 mg/L set by U.S. EPA while 55.2% of non-canned beverages had their arsenic levels exceeding the MCL. The arsenic concentrations ranged from 0.003 to 0.161 mg/L for the canned and 0.002 to 0.261 mg/L for the non-canned beverages. Whereas 68.9% of the non-canned beverages showed chromium levels that exceeded the US EPA's MCL of 0.10 mg/L, 76.2% of the canned beverages had chromium levels that were greater than the MCL. The concentration range of total chromium in the canned beverages was 0.04 to 0.59 mg/L and 0.01 to 0.55 mg/L for the non-canned beverages. The sources of arsenic and chromium in the commercially available beverages are unclear and merit further investigation. This preliminary study highlights the need to study the toxicological implications of chronic low-level exposure to heavy metals from African markets.

**Keywords:** Arsenic, chromium, metals, poisoning, beverages, Nigeria.

### Introduction

The gastrointestinal tract (GIT) is exposed to various environmental pollutants including metals that contaminate food and water and may have toxic effects on the body. Arsenic and chromium are examples of such trace elements described as heavy metals because of their density. Recent studies indicate that some metals act as catalysts in the oxidative reactions of biological macromolecules, therefore the toxicities associated with these metals might be due to oxidative tissue damage [1]. Arsenic trioxide has been shown to decrease the activity of cholinesterase enzyme and to have clastogenic/genotoxic potential as measured by bone-marrow chromosomal aberrations and micronuclei tests in the Sprague-Dawley rats [2, 3].

Arsenic is an environmental toxicant, which contaminates drinking water in many countries like Bangladesh where up to 10 million tube wells that about 30 to 40 million people depend on are affected [4]. One of the

major mechanisms by which it exerts its toxic effect is through an impairment of cellular respiration by inhibition of various mitochondrial enzymes, and the uncoupling of oxidative phosphorylation [2].

Arsenic levels in food, with the exception of some seafood, are generally well below 1 mg/kg wet weight [5]. Wine may contain appreciable amounts of arsenic. Noble et al in 1976 found concentrations between 0.02 and 0.11 mg/L in 9 U.S. wines produced between 1949 and 1974. [6] Crecelius also investigated the levels and forms of arsenic in some US table wines in 1977 [7]. In over half of the samples, levels greatly exceeded 0.05 mg/L (provisional limit in the international drinking water standards: WHO). Elevated arsenic levels have been found in some bottled mineral waters. In 1976, Zoeteman and Brinkmann reported a mean arsenic concentration of 0.021 mg/L (range <0.001 to 0.19 mg/L) in bottled mineral waters sold in countries within the European Community [8]. In an investigation on lager beers from various

countries none of the samples contained more than 0.02 mg/L [9]. These findings were made several decades ago and as such contributed to the regulation of heavy metal levels in food in countries like U.S.A. High levels of arsenic in drinking water, food and sometimes air have been recognized for many decades in other regions of the world notably in China, India and some countries in Central and South America.

Although exposure may occur via the dermal and parenteral routes, the main pathways of exposure to arsenic include ingestion, and inhalation. The severity of adverse health effects is related to the chemical form of arsenic, and is also time- and dose-dependent. Recent reports have pointed out that arsenic poisoning appears to be one of the major public health problems of pandemic nature [10]. Millions of people are at the risk of cancer and other diseases because of chronic arsenic exposure [11]. A comprehensive analysis of published data indicates that arsenic exposure induces cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders, diabetes, hearing loss, hematologic disorders, and various types of cancer [10].

Long-term consumption of arsenic contaminated-water leads to serious health effects often referred to as arsenicosis. The symptoms of arsenicosis manifest themselves after several years initially as skin lesions, and progress to localized gangrene and eventually cancers of the skin, lung, bladder and kidneys [12, 13, 14]. Another potent source could be solubilized inorganic arsenic from outdated pesticide misidentified as spring water [15]. In a person who had ingested well water containing 0.2 mg of pentavalent arsenic, the inorganic arsenic (V) concentration in urine showed a marked increase (5-fold) the first 10 hours after exposure, indicating that some of the ingested arsenic was rapidly excreted unchanged in the urine [16]. The urinary levels of dimethylarsinic acid increased 5 to 10-fold between 10 and 70 hours after exposure in the case studied.

Chromium is a naturally occurring element found in rocks, soil, plants, animals, and in volcanic dust and gases. Chromium (III) is an essential nutrient in our diet, but we need only a very small amount; our bodies do not need other forms of chromium. It is a heavy metal found commonly in the environment in trivalent (Cr III) and hexavalent (Cr VI) forms. Cr (VI) compounds have been shown to be potent occupational carcinogens [17]. Chromium is also used in manufacturing chrome-steel or chrome-nickel-steel alloys (stainless steel) and other alloys, bricks in furnaces, and dyes and pigments, for greatly increasing resistance and durability of metals and chrome plating, leather tanning, and wood preserving. Manufacturing, disposal of products or chemicals containing chromium, or fossil fuel burning release chromium to the air, soil, and water. Fishes do not take up nor store it in their bodies.

Food is the main source of chromium intake by man. Chromium is fairly evenly distributed throughout the various food groups examined, but highest concentrations of chromium are found in the meat, fish, fruit and sugar groups. Potable water, fruit juices and soft drinks are some

of the most widespread beverages in the habitual diet, and they can contribute to chromium dietary intake. Chromium and other essential elements with antioxidant activity have been shown to be present in a total of 45 samples of different teas commercialized in Spain, and in wine samples of the very popular Italian Chianti Classico appellation, vintage 1997. [18, 19] Mean dietary chromium intakes in the UK (1976-78) were between 80 and 107 micrograms/day [20]. A study has been undertaken to determine the levels of chromium in the serum of apparently healthy exclusively breastfed infants at the sixth month of lactation. It was inferred that the exclusively breastfed infants are able to extract chromium from the maternal breast milk [21]. In 1999, Garcia et al determined the concentration of chromium in 90 different samples of beverages widely consumed in Spain [22]. In the analyzed samples, chromium values ranged from not detectable to 11.80 micrograms/L in potable water, from not detectable to 17.60 micrograms/L in fruit juices and from 3.60 to 60.50 micrograms/L in soft drinks.

Chromium poisoning can be acute or chronic. Death in acute chromium poisoning is usually due to uraemia. Chronic intoxication by inhalation or skin contact leads to incapacitating eczematous dermatitis, with oedema and ulceration. The reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates that, together with oxidative stress and oxidative tissue damage, and a cascade of cellular events including modulation of apoptosis regulatory gene p53 contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr (VI)-containing compounds. In the work of Upreti et al, it was suggested that the gut micro flora have a marked capacity to cope with the increased load of ingested metals and may contribute significantly in the protection against metal toxicity suggesting that the protection can be easily lost with any compromise to the gut epithelium [17].

Numerous studies have described environmental exposure of humans to heavy metals in African populations. Little is known about the exposure to heavy metal toxins from processed or unprocessed foods consumed in Africa, and no data exists on the food concentrations of arsenic and chromium, which are potential carcinogens and systemic toxicants. This study determined the concentrations of arsenic and chromium in beverages and fruit drinks commonly sold in Nigeria.

## Materials and Method

Fifty different samples of canned (21) and non-canned (29) beverages purchased in March 2005 in Nigeria were used in the study. The samples were digested in Teflon lab ware that had been cleaned in a high-efficiency particulate air – (HEPA) filtered (class 100), trace-metal-clean laboratory to minimize contamination. This protocol involved sequential cleaning of the lab ware in a series of baths in solutions (1 week each) and rinses (five per solution) in a three-step order namely a detergent solution and deionized water rinses, then 6-N HCl (reagent grade) solution and ultra-pure water rinses, finally, 7.5N HNO<sub>3</sub>

(trace metal grade) solution and ultra-pure water rinses. The lab ware was then air dried in a polypropylene laminar air flow-exhausting hood.

Five milliliters of each sample was used. The samples were digested by adding 15 ml of nitric acid and making it up to 50 ml with de-ionized water. This was heated until the solutions were fully digested and reduced to 10 ml. The solutions were allowed to cool and then filtered. Arsenic and chromium levels were analyzed using the Unicam Atomic Absorption Spectrophotometer (AAS) Model 929 with air acetylene flame. Arsenic level was analyzed at 193.7 nm wavelength while chromium level was analyzed at 358nm wavelength. Samples were analyzed in duplicates.

## Results

Table 1 shows the arsenic and chromium levels in the

canned beverages. The arsenic levels ranged from 0.003 to 0.161 mg/L for the canned and 0.002 to 0.261 mg/L for the non-canned beverages (Table 2). 33.3% of the canned beverages had arsenic levels that exceeded the maximum contaminant level (MCL) of 0.01 mg/L set by US EPA, while 55.2% of non-canned beverages had levels exceeding the MCL (Table 2).

The chromium levels in the canned beverages are also shown in Table 1. About 69% of the non-canned beverages showed chromium levels that exceeded the US EPA's MCL of 0.1mg/L (Table 2), while 76.2% of the canned beverages had chromium levels that were greater than the MCL (Table 1). The range of the chromium in the canned beverages was 0.04 to 0.59mg/L and 0.01 to 0.55 mg/L for the non-canned beverages. Taken together, 46% and 72% of the 50 beverages (canned and non- canned) purchased in 2005 in Nigeria failed to meet the U.S. EPA criteria for acceptable arsenic and chromium levels in consumer products.

**Table 1:** Arsenic and Chromium levels in canned beverages

<i>Products</i>	<i>As Level (Mg/L)</i>	<i>Cr Level (Mg/L)</i>	<i>Place Of Manufacture</i>
Picnic Soymilk (Maeil)	0.161	0.53	Seoul, South Korea
Remmy Rankky Orange	0.160	0.07	Wuging, Republic Of China
Sprite Soft Drink	0.051	<DL	Wadeville, South Africa
Star Pino Pineapple	0.030	0.25	Shariah, United Arab Emirates
Godys Malta Drink	0.023	0.17	"Imported From Germany"
Star Mango	0.020	0.05	Shariah, United Arab Emirates
Chinchin malt milk drink	0.011	0.39	Tianjin, China
Coca Cola	0.009	0.13	Wadeville, South Africa
Glorietta Lemonade Orange	0.005	0.05	Germany
Sagiko Pink Guava	0.004	0.59	Singapore
Original Precious Juice	0.003	0.04	Yunlin, Taiwan
Fanta Orange	<DL	0.18	Wadeville, South Africa
Sobela mixed fruit drink	<DL	0.18	Tianjin, China
Sweet Heart Mixed Fruit	<DL	0.41	Tianjin, China
Gold Quell Multivitamin	<DL	0.29	Gmbh, Germany
Luna Milk	<DL	0.48	Jedda, Saudi Arabia
Three Crowns Milk	<DL	0.51	Lagos, Nigeria
Peak milk	<DL	0.55	Leeuwarden, Holland
Lino Malt	<DL	0.20	Belgium.
Holsten Malta	<DL	0.23	Hamburg, Germany
Top Milk	<DL	0.55	Hamburg, Germany

<DL = value less than detection limit of 0.001 mg/L.

**Table 2:** Arsenic and Chromium levels in non-canned beverages

<i>Products</i>	<i>As Level (mg/L)</i>	<i>Cr Level (Mg/L)</i>	<i>Place of Manufacture</i>
La Casera Orange Drink	0.261	0.13	Lagos, Nigeria
Campina Yazzo Milk Drink	0.060	0.38	Aalter, Belgium
Mighty Nice Chocolate Drink	0.038	0.49	Cape Town, South Africa
Lucozade Boost	0.038	0.39	Ogun State, Nigeria
Sheeza Mango	0.034	0.27	Karachi, Pakistan
Vitamilk Soyamilk	0.030	0.35	Thailand
Grape Joy Of Health	0.027	0.16	Cansavay Bay, Hong Kong
Chivita Orange Juice	0.020	0.20	Lagos, Nigeria
Popcy Flavored Drink	0.017	0.41	Lagos, Nigeria
Sans Cream Soda	0.016	<DL	Ogun State, Nigeria
Ribena Black Currant	0.014	0.51	Ogun State, Nigeria
Lulu Apple Juice	0.014	<DL	Lagos, Nigeria
Chelsea Teezer Gin And Pinneapple	0.012	0.14	Lagos, Nigeria
V.Roovers Orange Drink	0.012	0.04	Ogidi, Nigeria
Fine Merit Yoghurt	0.011	0.48	Lagos, Nigeria
Delite Black Currant Drink	0.011	0.13	Lagos, Nigeria
Lactasoy Soymilk	0.009	0.41	Prachinburi, Thailand
Vina Orange Fresh	0.007	0.17	Lagos, Nigeria
Vitavite Orange Drink	0.007	0.12	Lagos, Nigeria
Savana Pineapple	0.004	0.55	Onitsha, Nigeria
V. Roovers Pineapple Cordial	0.002	0.20	Ogidi, Nigeria
Tico Orange Cordial	<DL	0.07	Lagos, Nigeria
La Casera apple drink	<DL	0.05	Lagos, Nigeria
Marigold Orange	<DL	0.07	Malaysia
Lucomalt	<DL	0.03	Ogun State, Nigeria
Vinamilk Yomilk	<DL	0.143	Vietnam
5-Alive citrus burst juice	<DL	<DL	Lagos, Nigeria
Mighty nice vanilla low fat	<DL	0.49	Cape Town, South Africa
Caprisonne pineapple drink	<DL	0.01	Lagos, Nigeria

<DL = value less than detection limit of 0.001 mg/L.

## Discussion

This study revealed that some beverage samples in Nigerian markets have arsenic and chromium levels above the recommended limits. Elevated arsenic levels have been found in some bottled mineral waters in the past. Zoeteman and Brinkmann reported a mean arsenic concentration of 0.021 mg/L (range <0.001 to 0.19 mg/L) in bottled mineral waters sold in countries within the European Community

several decades ago) [8]. In an investigation on lager beers from various countries, none of the samples contained more than 0.02 mg/L [9].

Two instances of mass poisoning by inorganic arsenic in Japan give a good picture of the diversity of symptoms associated with acute and sub-acute arsenic poisoning, though the nature of the clinical investigations on the victims makes it difficult to interpret some of the findings. The first episode occurred when over 12,000 infants were

poisoned with dried milk contaminated with inorganic arsenic [23, 24]. The milk powder contained 15 to 24 mg As/kg and the arsenic was reported to be in the pentavalent state, although no data exist on its form at the time of ingestion. It was estimated that the infants ingested 1.3 to 3.6 mg of arsenic daily depending on age, and 130 deaths were reported. Symptoms usually appeared after a few weeks of exposure and often included fever, insomnia, and anorexia. In the survey on more than 40,000 inhabitants in China, Province of Taiwan, Tseng, in 1977 established a positive dose-response relationship between the contents of arsenic in well water and the prevalence rate for skin cancer. The overall prevalence was 10.6 per 1000, and the male to female ratio, 2.9. Assuming a daily intake of 2 liters of water a total ingested dose of about 20 g of arsenic over a lifetime corresponds to a prevalence of roughly 6% [25].

In the present study, we have found that at least 46 % of the beverages in the Nigerian market contain arsenic quantities well over the prohibitive level as set by the US EPA guideline. The arsenic level ranged from 0.002 to 0.261mg/L. The upper limit of this range is feared to be too high at least considering habitual high/large volume intake of these beverages both in the young and the old especially during the dry seasons. Again another group that may be at risk is the pregnant women/unborn children considering the placental transfer of the arsenic and attendant health hazards to the unborn foetus.

The chromium levels encountered in the Spanish study were low compared to our findings in Nigeria. Moreover, the contribution of non-alcoholic beverages to dietary intake of this element, have been estimated to be 0.41 microgram/day in the common Spanish diet. [22] The range of values found in the 1991, Garcia et al study, is obviously lower than the findings in this present study. [22] This calls for serious public health concern on the part of both the Nigerian consumers and the regulatory agencies. Since as early as the 1950's, it has been known that chromium is essential for normal glucose metabolism. Too little chromium in the diet may lead to insulin resistance. However, there is still no standard against which chromium deficiency can be established. Nevertheless, chromium supplements are becoming increasingly popular. Various systematic reviews have been unable to demonstrate any effects of chromium on glycaemic regulation (possibly due partly to the low dosages used), but there is a slight reduction in body weight averaging 1 kg. In a double blind randomized placebo-controlled trial in a Chinese population with type-2 diabetes mellitus, supplementation with 1000 micrograms of chromium led to a fall in the glycosylated hemoglobin level (HbA1c) by 2%. Generally it was thought that toxic effects of chromium are seldom seen; recently, however, the safety of one of the dosage forms of chromium, chromium picolinate, has been questioned. One should be aware that individual patients with type-2 diabetes mellitus might have an increased risk of hypoglycemic episodes when taking chromium supplements as self-medication [26]. This speculation thus raises concern for people who may end up amassing toxic

levels of chromium from chronic low-level intake. Despite the popular opinion on increasing chromium intake as one of the essential elements, Nigerian consumers may be exposed to higher levels considering the relative contribution of the element to the diet by beverages alone. Further investigations into the level of exposure of humans to these heavy metals, the systemic availability of these contaminants upon consumption of foods and the short- and long-term toxicological implications and impact on consumer health are recommended among African populations.

**Acknowledgement:** The ZETA-12 (greatzeta12@yahoo.com), a group of undergraduate medical students of Nnamdi Azikiwe University, Nnewi Campus, Nigeria (Maduabuchi John-Moses Ugwuona, Alope Uchenna R, Oragwu Chikelue I., Adigba Ese O, Afuba Anthonia N, Nzegwu Christine N, Sr. Onubueze Ogochukwu A, Ezomike Chinonso N , Orji Tobecheukwu M, , Oguh Lawson N, Okose Paul N, and Okonkwo Ikemefuna P, Uneze Nkemakolam C) wish to express our gratitude to our lecturers and the Director of ACET Technologies Ltd, for their financial contributions to this work.

#### References:

1. Ercal, N.; Gurer-Orhan, H.; Aykin-Burns, N.: Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.*, **2001**, *1*(16), 529-539.
2. Patlolla, A. K.; Tchounwou, P. B.: Serum acetyl cholinesterase as a biomarker of arsenic induced neurotoxicity in sprague-dawley rats. *Int J Environ Res Public Health*. **2005**, *2*(1), 80-3.
3. Patlolla, A. K.; Tchounwou, P. B.: Cytogenetic evaluation of arsenic trioxide toxicity in Sprague-Dawley rats. *Mutat Res.*, **2005**, *10*; 587(1-2), 126-33. *Epub 2005 Oct 5*.
4. Wasserman, G. A.; Liu, X.; Parvez, F.; Ahsan, H.; Factor-Litvak, P.; van Geen, A.; et al.: Water Arsenic Exposure and Children's Intellectual Function in Arai hazar, Bangladesh. *Environ Health Perspect.*, **2004**, *112*, 1329-1333.
5. Westöö, G.; Rydälv, M.: Arsenic levels in foods. *Vår föda*, **1972**, *24*, 21-40.
6. Noble, A. C.; Orr, B. H.; Cook, W. B.; Campbell, J. L.: Trace element analysis of wine by proton-induced X-ray fluorescence spectrometry. *J. Agric. Food Chem.*, **1976**, *24*, 532-535.
7. Crecelius, E. A.: Arsenite and arsenate levels in wine. *Bull. Environ. Contam. Toxicol.*, **1977**, *18*, 227-230.
8. Zoeteman, B. C. J.; Brinkmann, F. J. J.: Human intake of minerals from drinking water in the European Communities. In: Amavis, R.; Hunter, W. J.; Smeets, J. G. P. M.: ed. Hardness of drinking water and public health, *Oxford, Pergamon Press*, **1976**, pp. 173-202.
9. Binns, F.; Ensor, R. J.; Macpherson, A. L.: Metal content of United Kingdom and overseas lager beers. *J. Sci. Food Agric.*, **1978**, *29*, 71-74.

10. Tchounwou, P. B.; Centeno, J. A.; Patlolla, A. K.: Arsenic toxicity, mutagenesis, and carcinogenesis—a health risk assessment and management approach. *Mol Cell Biochem.*, **2004**, *255*(1-2), 47-55.
11. National Research Council. Arsenic in Drinking Water. *National Academy Press, Washington, DC*, **2001** update.
12. Abernathy, C. O.; Liu, Y. P.; Longfellow, D.; Aposhian, H. V.; Beck, B.; Fowler, B.; et al.: Arsenic: Health effects, mechanisms of actions, and research issues. *Environ Health Perspect.*, **1999**, *107*, 593-597.
13. Kitchin, K. T.: Recent advances in carcinogenesis: models of action, animal model systems, and methylated arsenic metabolites. *Toxicol Appl Pharmacol.*, **2001**, *172*, 249-261.
14. Tchounwou, P. B.; Abdelghani, A. A.; Prammar, Y. V.; Heyer, L. R.; Steward, C. M.: Assessment of potential health risks associated with ingesting heavy metals in fish collected from a hazardous-waste contaminated wetland in Louisiana, USA. *Rev Environ Health*, **1996**, *11*(4), 191-203.
15. Lai, M. W.; Boyer, E. D.; Kleinman, M. E.; Rodig, N. M.; Ewald, M. B.: Acute arsenic poisoning in two siblings. *Pediatrics*, **2005**, *116*(1), 249-257.
16. Crecelius, E. A.: Changes in the chemical speciation of arsenic following ingestion by man, **1977**, *Environ. Health Perspect.*, *19*, 147-150.
17. Upreti, R. K.; Shrivastava, R.; Chaturvedi, U. C.: Gut microflora & toxic metals: chromium as a model. *Indian J Med Res.*, **2004** *119*(2), 49-59.
18. Cabrera, C.; Gimenez, R.; Lopez, MC.: Determination of tea components with antioxidant activity *J. Agric Food Chem.*, **2003**, *51*(15), 4427-35.
19. Monaci, F.; Bargagli, R.; Focardi, S.: Element concentrations in Chianti Classico appellation wines. *J. Trace Elem Med Biol.*, **2003**, *17* Suppl 1, 45-50.
20. Smart, G. A.; Sherlock, J. C.: Chromium in foods and the diet. *Food Addit Contam.*, **1985**, *2*(2), 139-47.
21. Okolo, N. S.; Okonji, M.; Ogbonna, C.; Ezeogu, A. F.; Onwuanaku, C.: Levels of calcium, aluminum and chromium in serum of exclusively breastfed infants at six months of age in Savannah region of Nigeria. *West Afr. J. Med.* **2001**, *20*(1), 13-6.
22. Garcia, E. M.; Cabrera, C.; Sanchez, J.; Lorenzo, M. L.; Lopez, M. C.: Chromium levels in potable water, fruit juices and soft drinks: influence on dietary intake. *Sci Total Environ.*, **1999**, *241*(1-3), 143-50.
23. Hamamoto, E.: Infant arsenic poisoning by powdered milk. *Nihon Iji Shimpo.*, **1955**, *1649*, 3-12.
24. Nakagawa, Y.; Iibuchi, Y.: On the follow-up investigation of Morinaga milk arsenic poisoning. *Igaku no Ayumi.*, **1970**, *74*, 1-3.
25. Tseng, W-P.: Effects and dose-response relationships of skin cancer and Blackfoot disease with arsenic. *Environ. Health Perspect.*, **1977**, *19*, 109-119.
26. Kleefstra, N.; Bilo, H. J.; Bakker, S. J.; Houweling, ST.: Chromium and insulin resistance. *Ned Tijdschr Geneesk.*, **2004**, *148*(5), 217-20.