

HUMAN EXPOSURE ASSESSMENT TO DIFFERENT ARSENIC SPECIES IN TEA

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ABSTRACT

Background. Inorganic forms of arsenic are much more highly toxic to humans than organic species. Their effects include being carcinogenic, genotoxic and neurotoxic, where in the latter case, above all, they affect nervous system development in the foetus, infants and children. The main foodstuffs contributing significantly to its total dietary intake are drinking water, rice (and its products), fish, seafood, cereals, seaweed, root vegetables, food supplements, mushrooms and tea. After water, tea is the second most popular beverage drunk in Poland with average consumption annually indicating that statistically every Polish inhabitant drinks at least one cup of tea daily.

Objectives. The aim of the study was to determine the total and inorganic content of arsenic in various black and green teas available on the market and thus to estimate consumer exposure to inorganic arsenic from this foodstuff.

Materials and Methods. Analyses of total and inorganic arsenic were performed on 23 samples of black and green teas that consisted of tea leaves, teas in bags and granules, from various sources. The analytical method was hydride generation atomic absorption spectrometry (HGAAS), after dry ashing of samples and reduction of arsenic to arsenic hydride using sodium borohydride. In order to isolate only the inorganic forms of arsenic prior to mineralisation, samples were subjected to concentrated HCl hydrolysis, followed by reduction with hydrobromic acid and hydrazine sulphate after which triple chloroform extractions and triple 1M HCl re-extractions were performed. Exposure of adults was estimated in relation to the Benchmark Dose Lower Confidence Limit (BMDL₀₅) as set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that resulted in a 0.5% increase in lung cancer (3.0 µg/kg body weight (b.w.) per day).

Results. Green teas were found to be more highly contaminated with both total and inorganic arsenic than black teas. Contamination of black teas total and inorganic arsenic was mean: 0.058 mg/kg (median: 0.042 mg/kg, 90th percentile: 0.114 mg/kg), and 0.030 mg/kg, (median: 0.025 mg/kg, 90th percentile: 0.030 mg/kg) respectively. Whilst for the green teas, these were correspondingly mean total arsenic content: 0.134 mg/kg (median: 0.114 mg/kg, 90th percentile: 0.234 mg/kg) and inorganic arsenic, mean: 0.100 mg/kg (median: 0.098 mg/kg, 90th percentile: 0.150 mg/kg). The estimated average adult exposures to inorganic arsenic in black and green tea were less than 1% of the BMDL₀₅. Green tea samples, with the highest measured inorganic arsenic, were found to cause an intake exceeding 0.5% of the BMDL₀₅ value. However when the drinking water is also accounted for when teas are prepared, then the exposure from black and green tea becomes exceeding 0.7% and 1.3% of the BMDL₀₅ value respectively.

Conclusions. Findings thus demonstrate that drinking black or green teas does not pose a significant health threat to consumers, even though contaminations in some individual samples were significant.

Key words: total arsenic, inorganic arsenic, tea consumption, exposure assessment

STRESZCZENIE

Wprowadzenie. Nieorganiczne związki arsenu charakteryzują się znacznie większą toksycznością w porównaniu z organicznymi połączeniami tego pierwiastka. Wykazują one działanie rakotwórcze, genotoksyczne oraz neurotoksyczne, przede wszystkim na rozwijający się ośrodkowy układ nerwowy płodu, niemowląt i małych dzieci. Do środków spożywczych mających istotny udział w całkowitym pobraniu arsenu nieorganicznego z diety należą: woda do picia, ryż i produkty na bazie ryżu, ryby, owoce morza, zboża, wodorosty morskie, warzywa korzeniowe, suplementy diety, grzyby, a także herbata. W Polsce herbata, po wodzie, jest drugim napojem, jeśli chodzi o wielkość spożycia. Średnie roczne spożycie herbaty na jednego mieszkańca w Polsce wskazuje, że statystyczny Polak wypija dziennie co najmniej jedną szklankę herbaty.

Cel. Celem badań było oznaczenie zawartości arsenu całkowitego i nieorganicznego w próbkach różnych rodzajów herbat czarnych i zielonych dostępnych w obrocie handlowym oraz ocena narażenia konsumentów na pobranie arsenu nieorganicznego z tym środkiem spożywczym.

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Material i metody. Analizie na zawartość arsenu całkowitego i nieorganicznego poddano 23 próbki herbat czarnych i zielonych - liściastych, ekspresowych i granulowanych, różnego pochodzenia. Zawartości arsenu całkowitego i nieorganicznego oznaczono po suchej mineralizacji próbek metodą absorpcyjnej spektrometrii atomowej z wykorzystaniem generacji wodorków (HGAAS), po uprzedniej redukcji arsenu do arsenowodoru za pomocą borowodoru sodu. W celu wydzielenia nieorganicznych form arsenu próbki przed mineralizacją poddano hydrolizie w środowisku stężonego HCl, a następnie redukcji w obecności kwasu bromowodorowego i siarczynu hydrazyny oraz 3-krotnej ekstrakcji chloroformem i reekstrakcji 1M HCl. Oszacowane narażenie w odniesieniu do osób dorosłych porównano z wartością najniższej dawki wyznaczającej (Benchmark Dose Lower Confidence Limit) $BMDL_{05}$ ustaloną przez the Joint FAO/WHO Expert Committee on Food Additives (JECFA) powodującą 0,5% wzrost zachorowań na raka płuc (3,0 $\mu\text{g}/\text{kg}$ m.c./dzień).

Wyniki. Zanieczyszczenie badanych herbat zielonych zarówno arsenem całkowitym jak i nieorganicznym było wyższe w porównaniu z zanieczyszczeniem herbat czarnych. W herbatach czarnych średnia zawartość arsenu całkowitego wyniosła 0,058 mg/kg (mediana: 0,042 mg/kg, 90-ty percentyl: 0,114 mg/kg), natomiast arsenu nieorganicznego 0,030 mg/kg, (mediana: 0,025 mg/kg, 90. percentyl: 0,030 mg/kg). W herbatach zielonych średnie zanieczyszczenie arsenem całkowitym wynosiło 0,134 mg/kg (mediana: 0,114 mg/kg, 90. parcentyl: 0,234 mg/kg) i arsenem nieorganicznym wynosiło 0,100 mg/kg (mediana: 0,098 mg/kg, 90. percentyl: 0,150 mg/kg). Oszacowane średnie narażenie na arsen nieorganiczny w odniesieniu do osób dorosłych wyniosło dla herbat czarnych i zielonych poniżej 1% wartości najniższej dawki wyznaczającej $BMDL_{05}$. Przy najwyższych stwierdzanych zawartościach arsenu nieorganicznego w przypadku herbaty zielonej przekroczyło 0,5% wartości $BMDL_{05}$. Natomiast uwzględniając dodatkowo wodę użytą do przygotowania napoju łączne narażenie dla herbat czarnych wyniosło 0,7%, a dla herbat zielonych 1,3% wartości $BMDL_{05}$.

Wnioski. Na podstawie uzyskanych wyników stwierdzono, że spożycie herbat zarówno czarnych jak i zielonych nie stanowi istotnego zagrożenia dla zdrowia konsumentów, pomimo iż zanieczyszczenie pojedynczych próbek było istotne.

Słowa kluczowe: arsen całkowity, aren nieorganiczny, herbaty, ocena narażenia

INTRODUCTION

Tea is one of the most common beverages drunk throughout the world. After drinking water, tea is the most popular drink in Poland. Its mean annual consumption for a single Polish inhabitant is 0.72 kg for 2012 [3] which, statistically speaking shows that each person drinks one cup of tea daily.

Tea brews are known to deliver substances beneficial to human health such as polyphenols, vitamins and essential elements (e.g. Ca, Mg, Zn, Cu, Fe), however they may also be the source of toxic metallic contaminants like Pb, Cd, As, Hg, Al, Cr, Ni, [1, 5, 7, 10, 11, 13, 23, 29, 31, 37]. Environment contamination of the soil and air is responsible for plants taking up these contaminants, including arsenic [13, 26]. In this respect, the most important sources of arsenic soil contamination are pesticides and the application of sewage sludge and phosphate fertilizers [27, 38]. Plants are able to accumulate arsenic and its compounds from the soil along with other heavy metals [25]. Degree of accumulation of arsenic and their compounds depends on acidity of the soil. Studies have demonstrated that plant roots contain much higher amounts of contaminants than stems or mature leaves, whereas young tea leaf shoots show relatively low levels of contaminants [11, 28].

It is estimated that 40% of metals pass into tea brews [30, 35] where the time brewed and temperature significantly affects by how much the contaminants, hazardous to health, are present in the final brew [23, 26]. According to Asian studies, the dominant form of arsenic in tea brews are inorganic compounds of As

(III) and As (V), [12, 38]. Quality of water used for preparation of infusion is equally important [30, 37]. Apart from lead, cadmium and mercury, arsenic is the fourth element whose intake pose a health concern [30].

The International Agency for Research on Cancer (IARC) has classified arsenic to Group I, as carcinogenic to humans. Arsenic has also demonstrated genotoxic and neurotoxic effects on humans mainly for the developing nervous systems of foetus, infants and children. It also causes cardiovascular disease, peripheral vascular disorders, anaemia, immune system disorders and impairs cellular metabolism in the liver and kidneys through enzyme inhibition. Such toxic effects are caused more by inorganic arsenic for which the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1989 has established a Provisional Tolerable Weekly Intake (PTWI) from all sources as being 0.015 mg/kg bodyweight [34]. In 2009, the European Food Safety Authority (EFSA) requested a revision to this value, and identified a range of doses for the Benchmark Dose Lower Confidence Limit ($BMDL_{01}$) of 0.3 – 8.0 $\mu\text{g}/\text{kg}$ b.w. per day [21]. Subsequently, in 2010 the JECFA proposed instead PTWI a $BMDL_{05}$ value of 3.0 $\mu\text{g}/\text{kg}$ b.w. per day for arsenic that causes a 0.5% rise in lung cancer incidence [35].

High amounts of organic arsenic compounds are mostly found in seafood (ie. arsenocholine, arsenobetaine, arsenosugars), but have much lower acute toxicity [30, 36]. Nevertheless, results from the European Commission Scientific Cooperation Project (SCOOP) show that seafood constitutes a significant to the dietary intake of arsenic [24]. Those foodstuffs that make

up a significant part of the dietary intake of inorganic arsenic are drinking water, rice and rice products. Other such sources are also fish, seafood, cereals, seaweed, root vegetables, food supplements, mushrooms and tea [6, 21]. For infants and children, significant sources of inorganic arsenic intake are in addition milk and dairy products [22]. The main source of human exposure to arsenic is through food. At the end of 2004, the SCOOP determined daily adult exposure of arsenic to be 37 – 66 µg of which 50% originated from seafood [36]. According to EFSA the average arsenic intake in EU countries ranges from 0.936 to 1.224 µg/kg b.w. per day [21].

Currently, binding EU legislation does not place any limits for arsenic contamination in foodstuffs. It does however provide limits for drinking water, standing at 0.01 mg/L for bottled and source waters [17, 18]. Furthermore, arsenic from pesticide residues found in foodstuffs should not exceed 0.01 mg/kg [20]. The use of pesticides containing arsenic is anyway banned by the legislation for EU countries, but they are still universally adopted in Asian countries which happen to be the main tea producers. The Standards of the Codex Alimentarius Commission of the FAO and WHO (Food and Agriculture Organisation & World Health Organisation) provides only maximum allowable levels of total arsenic in foodstuffs, but there are no established Maximum Levels (MLs) for inorganic arsenic compounds [8]. Legislation on food additives gives purity criteria including heavy metals, for arsenic at 1 – 3 mg/kg [4]. Work on establishing maximum levels for inorganic arsenic is currently in progress by the Committee on Environmental and Industrial Contaminants in Food. Among the foodstuffs under consideration for these MLs are rice and its products including rice destined for the production of food for infants and young children.

The study aims were thus to determine total and inorganic arsenic in various types of tea available on the market in Poland, taking into account their origin along with estimating consumer exposure to inorganic arsenic found in teas.

MATERIALS AND METHODS

Instruments & Equipment

An atomic absorption spectrometer, Philips Pye Unicam, model 9200 was used linked with a PU 9360 continuous hydride vapour generation system. Further equipment included a Vortex Multi Reax shaker (Heidolph, Germany), centrifuge (Eppendorf HG, Germany), Ceran 500 hotplate (Harry Gestigkeit GmbH, Germany), Mileston Pyro TC microwave oven (Italy), hydrophobic filters (Syringe type, Millipore, 0.45 µm pore diameter), class A laboratory glassware and FALCON test tubes made of polypropylene (PP).

Reagents

Hydrochloric acid (35 – 38% Avantor), nitric acid (65% Merck), hydrobromic acid (≥48% Sigma-Aldrich), hydrazine sulphate (Fluka), chloroform (99 – 99.4%, Sigma-Aldrich), magnesium nitrate hexahydrate (Merck), magnesium oxide (Chempur), potassium iodide (Avantor), ascorbic acid (Avantor), sodium hydroxide (Merck), sodium borohydride (Merck), a primary standard solution of arsenic (V) 1 g/L (Merck) and deionized water. The reagents were of a sufficiently high degree of purity.

Tea samples

Tea samples included 23 samples of black and green tea in leaf, bags or granular forms that are available on the Polish market. Most teas came from China, Sri-Lanka and India.

Sample preparation for total arsenic determination

Approximately 0.3 g tea samples were placed into a quartz vaporizer and 10 mL of ashing aid was added (20% w/v MgNO₃ · 6H₂O, 2% m/v MgO) followed by 5 ml of 50% v/v nitric acid. After evaporating to dryness on the hotplate, the residues were digested in a microwave oven using the manufacturer's recommended program to achieve mineralisation; with the final ashing temperature not exceeding 400°C. The resulting ash was dissolved in 5 ml 6M HCl, filtered and transferred to a flask and fill to 50 ml with 6M hydrochloric acid. Suitable blanks were prepared under the same conditions [2].

Sample preparation for determination of inorganic arsenic

Tea samples (0.5 g) were placed into polypropylene centrifuge tubes and 1 mL of deionised water was added followed by thorough shaking to ensure completely wetting. Concentrated HCL (20 mL) was then added and the mixture was left to stand overnight (12 – 15 hours). Reduction was next, performed with 2 mL of hydrobromic acid and 1ml hydrazine sulphate (1.5% m/m) followed by shaking by hand for 30 seconds. The mixture was three times extracted with chloroform (10 mL; with vortex-shaking for 3 minutes at 1400 revolutions/min (rpm)). The mixture was next centrifuged for 20 minutes at 4000 rpm after which the organic layer was transferred into clean polypropylene centrifuge tubes; this latter stage being so repeated twice more. The combined organic layers next underwent purification by being passed through hydrophobic membrane filters. The purified filtrate was extracted twice with 10 mL of 1M HCl. The mixture was shaken for 3 minutes and then centrifuged for 20 minutes at 4000 rpm.

The filtrates were transferred into flasks. The same procedure was used for sample blanks. Filtrates were then evaporated to dryness on a heating block and then

incinerated to ash in a microwave oven at 400 °C using progressively increasing the temperature to 400 °C at the rate of 50 °C/h. When 400 °C was achieved, the ash samples were maintained at this temperature for 8 hours after which a white ash was obtained. The remaining ash was moistened with water, dissolved in 6M HCl and transferred into 25 mL flasks [2].

Determination of total and inorganic arsenic

The analytical method used was an in-house one developed at the Department of Food Safety of the National Institute of Public Health - National Institute of Hygiene (NIZP-PZH), based on a method by Munoz et al. [16]. For total arsenic, determination appropriate volumes (10–20 mL) of the mineralised solutions were subjected to reduction in the presence of 6M HCl and a mixture of potassium iodide (5% m/v solution and 5% m/v solution of ascorbic acid, where the 6M HCl was used to make up the reaction mixture to a fixed volume. For determination of inorganic arsenic, the entire volume of mineralised solutions was used for the aforementioned reduction reaction. The final analyses of total arsenic and inorganic arsenic were performed by HGAAS before which the samples had been subjected again to reduction; this time with 1% sodium borohydride. Conditions used were optimised according to the instrument manufacturer's recommendations [2].

Standard curves

A standard curve was prepared ranging from 0 ng/mL to 5 ng/mL of standard solution of arsenic (V) containing 1 g As/L.

Quality control

Interlaboratory quality control was performed using crab meat containing known concentrations of inorganic arsenic which our laboratory obtained from its participation in the proficiency testing FAPAS round-0791 and reference material with a certified total arsenic content: Tea leaves INCT-TL-1 (Institute of Nuclear Chemistry and Technology - IChTJ).

RESULTS AND DISCUSSION

Total and inorganic arsenic were analysed in 23 tea samples, that included black tea (chiefly teas in bags) and green teas (most were leafy teas) originating from various world regions (China, Sri-Lanka and India). To enable comparison with data from other EU member States participating in the SCOOP (Scientific Cooperation on Questions Relating to Food) program, if the result was below detection limit (ie. 0.019 mg/kg inorganic arsenic and 0.012 mg/kg for total arsenic), calculations were made taking one-half of the range [9, 24].

Results of total arsenic and inorganic arsenic in teas are presented in Figure 1.

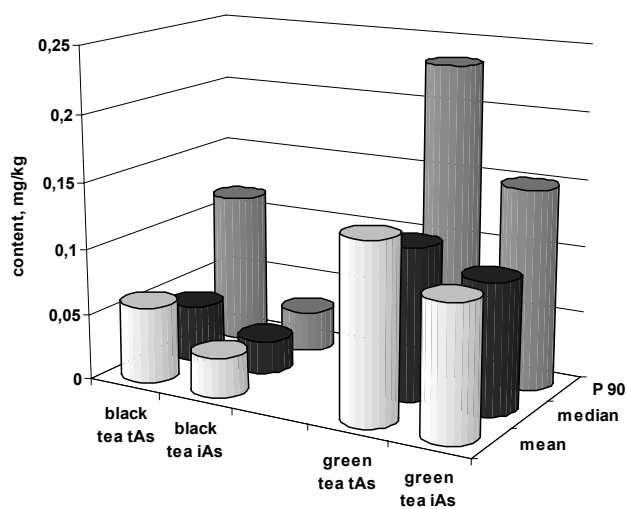


Figure 1. Contents of total (tAs) and inorganic arsenic (iAs) in tested samples of black and green teas, mg/kg

Arsenic contamination (both total and inorganic) was found to be higher in green teas than black teas. In the latter, the average concentration of total arsenic was 0.058 mg/kg and 0.030 mg/kg for inorganic arsenic, with corresponding medians and 90th percentiles being respectively 0.042 mg/kg and 0.025 mg/kg along with 0.114 mg/kg and 0.030 mg/kg. In black teas, the sample of black leafy tea from India was observed to have the highest inorganic arsenic content at 0.078 mg/kg.

In green teas, average values of total arsenic were 0.134 mg/kg (median and 90th percentile respectively being 0.114 mg/kg and 0.234 mg/kg). These results were comparable to other studies on Pu-erh [14]. Average levels of inorganic arsenic in these teas were however 0.100 mg/kg (with median 0.098 mg/kg and 90th percentile of 0.150 mg/kg). The highest inorganic arsenic contamination was seen for instant tea from China at 0.260 mg/kg. Total arsenic contamination in black teas were close to those in other EU countries, on average 0.066 mg/kg [21] but much lower than levels reported by Asian studies [12]. According to the SCOOP data, the mean total arsenic contamination was 0.144 mg/kg [24] which were twice the levels found in the presented study. The proportions of inorganic arsenic to total arsenic in the measured samples are shown in Figure 2.

Furthermore, the results of total arsenic determined in black teas were twice lower than those observed in monitoring studies during 2004-2008 [37]. Only a few studies however, have measured inorganic arsenic in teas which renders comparisons with other countries difficult. Most published studies has focused on determination of total arsenic, with omission of the more toxic inorganic arsenic compounds. According to the EU's Rapid Alert System for Food and Feed (RASFF) only a few notifications ap-

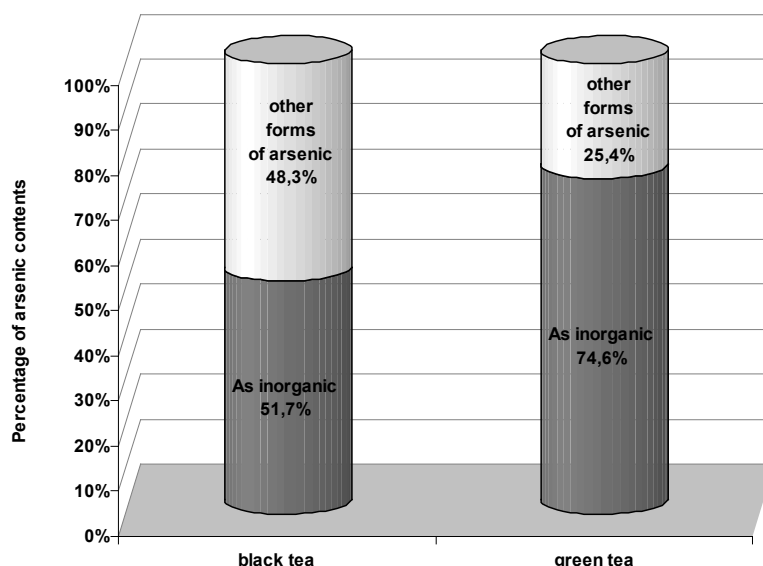


Figure 2. Percentage of different forms of arsenic in tested black and green teas

plied to raised levels of arsenic in teas compared to other foodstuffs. From 2006 there were two alert notifications on high arsenic levels in tea. One was in green tea from China (1.200 mg/kg), whilst the other in granulated black tea from India with 2.680 mg/kg of total arsenic [33].

Assuming that daily tea consumption is around 10 g (equivalent to 5 cups of tea) and that 40% of the arsenic content of tea is extracted into the brew [30, 37], then a single intake of inorganic arsenic from black or green teas will be below 1% of the $BMDL_{05}$ when based on mean levels of inorganic arsenic contamination found; likewise with the 90th percentile results. These estimations however, do not take into account any arsenic contamination of the brewing water that may additionally contribute to the intake of inorganic arsenic by up to 0.7% of the $BMDL_{05}$. From the highest of the above results in both teas, it appears that only the inorganic arsenic intake from green teas exceeds 0.5% of the $BMDL_{05}$. When considering however the arsenic contamination from drinking water in Poland [19], these intakes are 0.7% and 1.3% of the $BMDL_{05}$ for respectively black and green teas. The estimation of exposure from this study were lower than those reported in Asian countries [38].

Tea consumption data in Poland (from the Central Statistical Office) [3] as well as data from the Global Environment Monitoring System / Food Contamination Monitoring and Assessment Programme (GEMS/Food), of 2 g daily [32] are however significantly less than the assumed tea consumption made in the presented study. Thus, arsenic intake by the average adult person would be much lower than those estimated by this study.

CONCLUSIONS

1. Total and inorganic arsenic contamination in green tea was higher than black tea, however this does not

constitute a health risk.

2. Levels of contamination found were comparable to those reported in the EU but substantially lower than in Asian countries.
3. There were no clear-cut differences in inorganic arsenic contamination in tea from the various countries tested.
4. Introducing appropriate MLs into EU legislation for arsenic in teas, would seem to be suitable, to minimize health risk for consumers.

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Conflict of interest

The authors declare no conflict of interest.

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