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Exposure to mercury and thyroid function: Is there a connection?

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Abstract

Mercury (Hg) is one of the most important environmental pollutants with endocrine-disrupting properties. There is little data from epidemiological studies describing the dose-response relationship between toxic metal levels and hormone levels. The aim of this study was to use the nearest neighbor matching analysis to determine the difference in Hg concentration in healthy/sick subjects with thyroid disease and to use Benchmark modeling to determine the dose-response relationship between Hg levels in the blood and thyroid-stimulating hormone (TSH) and thyroid hormones in serum. Blood samples were collected and used for Hg measurement using the ICP-MS method, and separated serum was used for hormone analysis. The study showed the existence of a statistically significant difference in Hg levels measured in healthy and sick subjects and the existence of a dose-response relationship between Hg and all measured hormones, with a narrow interval obtained for the Hg-TSH pair. The results of this research support the use of the Benchmark dose approach for the purpose of analyzing data from human studies, and our further research will be focused on examining the impact of low doses on animal models in order to determine more precise effects of low doses on the organism.

Key words: endocrine disruption, mercury, thyroid function, BMD concept, nearest neighbor matching

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Introduction

Mercury (Hg) is one of the widespread metals and environmental pollutants that ranks among the top 10 substances of concern to human health (1). Mercury enters the environment from natural (earth crust, volcanic eruptions) and anthropogenic sources (alkali metal processing, coal, waste burning, gold mining) (2). Various forms of Hg are present in nature. Inorganic forms are released from various sources and reach the earth through atmospheric deposition, while the organic form of methyl-Hg is created as a result of the action of anaerobic microorganisms (3). Methyl-Hg bioaccumulates in the food chain and in products consumed by the general population (fish and seafood consumption). The most important route of Hg exposure for the human population is the gastrointestinal tract (consumption of bread - flour, meat of domestic animals and game birds, fish, maternal milk). In earlier years, due to anthropogenic activities and industrialization, massive and serious poisoning with this metal occurred in Japan and Iraq (4, 5). In order to improve and protect human health, it would be beneficial to reduce exposure to Hg and to be aware of the potential adverse effects it can cause.

Numerous toxic effects of Hg are well known. This toxic metal can cause adverse effects on the cardiovascular, hematological, nervous, digestive, and immune systems, can be harmful to the kidneys and lungs, and can cause developmental toxicity and adverse effects on the endocrine system (6–8). It is classified as a chemical that disrupts the endocrine system. Mercury exhibits harmful effects on reproductive health (9), contributes to the development of obesity (10), diabetes (11), affects the adrenal glands and the level of adrenal hormones in the plasma (12).

The normal functioning of the thyroid gland is essential for growth, development, and maintaining homeostasis in the human body. Thyroid hormones (TH) are significantly involved in metabolism, protein synthesis, and development of the nervous, cardiovascular and reproductive systems (13, 14). Today, thyroid diseases are a global problem that can affect the quality of life (15). In areas where there is no iodine deficiency, the prevalence of hypothyroidism (the most common cause is Hashimoto's thyroiditis) is between 1 and 2%, and the occurrence of the disease in women is 10 times more common than in men. Hyperthyroidism also occurs more often in women compared to men (the most common causes are Graves' disease and toxic multinodular goiter), and the prevalence of the disease in women is between 0.5 and 2%. It is considered that the prevalence of subclinical hypothyroidism [increased level of TSH and normal level of free thyroxine (fT4)] in the general population is between 4 and 10% (16). Various factors such as gender, age, and the level of iodine in the body can affect the prevalence of subclinical hyperthyroidism (low concentration of TSH and normal levels of free triiodothyronine (fT3) and fT4), and one of the estimates is that it is about 2.5% (17). Since the end of the 20th century, the incidence of thyroid cancer has been increasing, and it can be said that the diagnosis of these cancers has tripled in the last 30 years (18). The causes of thyroid gland dysfunction are not fully known, but it is known that numerous toxic metals present in the environment, including Hg, can contribute to the development of these disorders (19, 20).

Various studies have shown the influence of Hg on the thyroid gland and thyroid hormones. Mechanisms through which Hg disrupts TH are: binding to molecules containing SH groups in the gland, reduced production of TSH, inhibition of deiodination, damage to thyroid RNA. It is believed that Hg initially stimulates and later inhibits thyroid function (21, 22). In one in vitro study, methyl-Hg was shown to inhibit the activity of deiodinase type 2 (D2), the enzyme that converts T4 to T3. Since methyl-Hg is known to have a neurotoxic effect, it is thought that T3 deficiency due to D2 inhibition may contribute to the neurotoxicity of this compound (23). A study by Pamphlett et al. investigated the distribution of Hg in the thyroid gland. Appropriate techniques have confirmed that Hg accumulates in the follicular cells of the thyroid gland and that the percentage of the population with Hg in the follicular cells increases with age. As Hg can affect the appearance of genotoxicity, oxidative damage, and autoimmune reactions, its contribution to the development of hypothyroidism, autoimmune thyroiditis, and cancer is assumed (24). In a study by Rao et al., the effect of $HgCl_2$ on the antioxidant status of the thyroid gland of rats was monitored over a 60-day exposure period. Mercury had the effect of reducing the activity of antioxidant enzymes and increasing the level of lipid peroxidation (21). In a study that followed the exposure of workers to Hg vapors, it was observed that the serum concentration of reverse T3 was higher in the more exposed subjects. Moreover, the fT4/fT3 ratio was higher in those workers who were more exposed to Hg vapors (25).

The basic idea of the Benchmark dose (BMD) approach is to link the dose of the tested substance and the effect (response) being monitored. Previously, the No Observed Adverse Effect Level (NOAEL) concept was used for this purpose. If the NOAEL technique was used to determine the dose-response relationship, the maximum dosage administered in the experiment that did not cause adverse effects would be the outcome. Because of the shortcomings of the NOAEL approach, the BMD approach has recently been preferred for dose-response analysis. The BMD approach enables better use of data (better estimation compared to the NOAEL), avoids the influence of the experimental design on the study outcomes, and results in a dosing interval that is dependent on the reference response (BMR) – a previously established statistically significant effect change (26, 27). The European Food Safety Agency (EFSA) foresees the importance of applying this concept in epidemiological studies, but the number of such studies is small (26). In some of our previously published papers, this approach has been successfully used in the interpretation of results from human studies (28, 29). Examining the effects of low doses is of great importance because of the properties of endocrine disruptors (toxic metals such as mercury). In a study conducted by Goumenou et al., issues of low-dose exposure, long-term exposure, and non-monotonic dose-response were discussed (30).

The aim of this study was to analyze the thyroid-disrupting potential of Hg based on data obtained from the epidemiological study. It was investigated whether there was a statistically significant difference between the levels of Hg measured in the blood of subjects who were/were not suffering from various thyroid gland diseases. Furthermore,

the dose-response relationship was analyzed, where the dose represents the measured concentration of Hg in the blood of the subject, and the response is the level of hormones in the serum (TSH, fT4, fT3, T4, T3) in the general population using the BMD approach.

Materials and methods

Population characteristics

Samples of blood were obtained from subjects from the general population at the Clinical Center of Serbia and the Clinical Hospital Center "Bežanijska kosa" in Belgrade, Serbia. The study included 435 people (between 18 and 94 years old), 183 of whom were healthy and 252 of whom suffered from various disorders. 77 patients had various thyroid diseases (31 patients had hypothyroidism, 44 had hyperthyroidism, and 2 patients had thyroid cancer). There were 218 women and 217 males among those who responded. The study followed the Helsinki Declaration's ethical requirements. It was authorized by the University Hospital "Bežanijska kosa" Medical Center's Scientific and Ethical Committee (license number 9740/3), the Clinical Center of Serbia's Ethical Committee (license numbers 526/9, 31/8, and 579/19), the Medical Faculty of the University of Belgrade (license number 1322/XII-5) and the University of Belgrade's Ethical Commission for Biomedical Research (license numbers 650/2 and 288/2).

Sample preparation

After 12 hours of fasting, blood was collected from the anterior cubital vein. Each sample was clearly labeled with its identification number, date, and time of collection. The blood used for analysis was divided into two parts. An aliquot of blood was collected in Vacutainer tubes with K_2EDTA (BD Vacutainer® system) and used to determine the concentration of Hg. Serum was separated from the blood collected in test tubes by centrifugation at 3000 x g for 30 minutes after blood coagulation (Eppendorf centrifuge 5415 R, Eppendorf). This serum was later used to determine thyroid hormone levels. Until the moment of analysis, EDTA-blood and serum were stored at a temperature of -20°C.

Determination of Hg and thyroid hormone concentrations

Preparation of EDTA-blood sample for Hg determination involved digestion of 1ml of blood in Teflon cuvettes by adding 7ml of 65% HNO_3 and 1ml of 30% H_2O_2 in a microwave oven (Milestone START D, SK-10T, Milestone Srl, Sorisole, Italy). Along with the blood samples, blank samples consisting of 7ml of 65% HNO_3 and 1 ml of 30% H_2O_2 were subjected to digestion. The sample processing process includes heating (15 min at 180°C), digestion (15 min at 180°C), and cooling. After cooling, the samples were quantitatively transferred into normal vessels with a volume of 10ml. The ICP-MS method (ICP-MS 7700, Agilent Technologies, Santa Clara, CA, USA) was used for the Hg analysis. An external standard technique [multielement standard solution 1 g/L in diluted nitric acid (Merck, Darmstadt, Germany)] was applied for calibration. The accuracy of ICP-MS was validated with standard reference material (SRM) whole blood

Level 2 (Seronorm TM, Sero, Billingstad, Norway). For SRM preparation and analysis, the same procedure was applied to the EDTA-blood samples.

To determine the concentration of hormones (TSH, fT4, fT3, T4, T3) in the serum of subjects, representative of the general population, the chemiluminescent immunoassay (CLIA) technique was used on the Liason family of analyzers (DiaSorin Inc, USA) according to the principles of good laboratory practice. A chemiluminescent reaction with isoluminol derivatives was performed with bovine serum albumin and a specific monoclonal antibody to the hormone that binds to metal particles. A direct competitive chemiluminescence test was used to determine thyroid hormones.

Nearest neighbor matching

The "nearest neighbor" algorithm is the most commonly used technique for examining the matching of the results of a study. This technique allows, based on the results, to compare the "treated" subject with the closest "control", and if more controls are "closest" to the exposed subject, then the selection is made randomly. Matching is a method that makes it possible to unbiasedly estimate the effect of a treatment using the technique of matching the control and an exposed group. Nearest neighbor matching is often associated with the term greedy matching. "Greedy" refers to the fact that the matching for each subject is done independently of the matching of the others and therefore does not aim to optimize the criteria (31, 32).

This technique was used in our study to assess the Hg exposure of subjects with and without thyroid disease. Using R software, 77 subjects with diagnosed thyroid disease were matched with 76 subjects without the disease.

Dose-response modeling

PROAST software version 70.1 was used for dose-response modeling (the Dutch National Institute for Public Health and the Environment, RIVM). Quantal individual data on TSH, fT4, fT3, T4, T3, as well as Hg levels, were analyzed. As a covariant, the participants' gender was used. Hormone levels were assigned a value of 0 if they were within the reference range, and a value of 1 if they were outside of it. Reference values for TSH used are 0.270-4.20 μIU/ml, for fT4 12-22 pmol/L, for fT3 3.1-6.8 pmol/L, for T4 66-181 nmol/L and for T3 1.3-3.1 nmol/L. The BMD interval (BMDI) was determined using the model averaging method, which takes into account the results of all available models. The EFSA Scientific Committee has endorsed using this strategy since it responds to model and data uncertainties (26). Utilizing the Akaike information criterion (AIC), the model was assessed. The model with the lowest AIC value is thought to be the one to take into account when calculating the BMD interval, since it demonstrates how well the data fit into various models (26). The bootstrap method was used for model averaging. The BMR is described for quantal data as a specific increase in incidence over a background. A BMR with a 10% increased risk was applied.

Results

Table I shows the median, minimum, and maximum concentrations of Hg determined in the blood of the general population.

Table I Median, minimum and maximum concentrations of Hg determined in the blood of the general population

Tabela I Medijana, minimalna i maksimalna vrednost koncentracije Hg u krvi opšte populacije

	Hg concentration (μg/L)		
	women	men	all participants
median	3.444	5.219	3.986
min	0.022	0.022	0.022
max	361.077	118.151	361.077

The results of applying the nearest neighbor matching technique among the respondents from our study showed the existence of a statistically significant difference in the measured level of Hg between the respondents from the two matched groups of patients/controls ($p<0.001$). Median (min-max) values for the control group were 2.973 (0.022-120.311), and for patients 15.788 (0.022-118.151) μg/L (Figure 1).

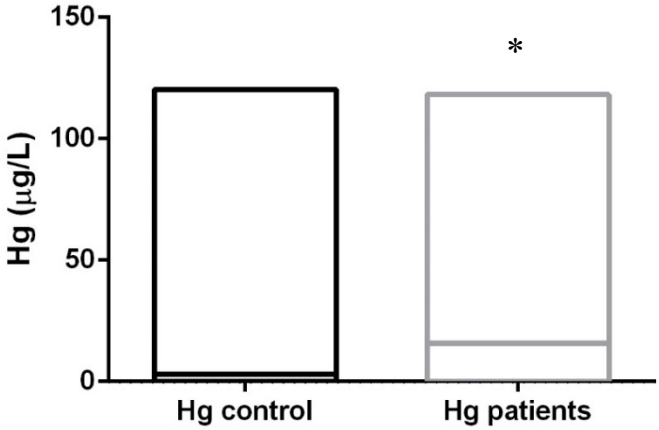

Figure 1. Median concentration of Hg measured in the blood of healthy subjects (Hg control) and subjects with thyroid gland diseases (Hg patients) (p<0.001)

Slika 1. Medijana koncentracija Hg izmerene u krvi zdravih ispitanika (*Hg control*) i ispitanika sa bolestima tiroidne žlezde (*Hg patients*) (p<0,001)

For Hg and all examined hormones, it was determined that there was a dose-response relationship. A bootstrap approach was used to average models resulting from combining several different models and models that fit the data better provide a better estimate. The assessment and evaluation of the used models was carried out with the AIC criteria. The AIC criteria are the basis on which model weights are usually based, and the results of individual models are combined using model weights with higher model weights providing a better fit to the data. Table II shows the list of models used for averaging and model weights.

Table II List of models used for averaging and model weights
Tabela II Lista modela korišćenih za *averaging* i težine modela

	model	**TSH**	**fT4**	**fT3**	**T4**	**T3**
women	two.stage	0.0016	0.0755	0.1189	0.024	0.0278
	log.logist	0.0372	0.1731	0.1424	0.179	0.2054
	Weibull	0.0327	0.0755	0.1225	0.1719	0.0247
	log.prob	0.0488	0.1783	0.141	0.21	0.241
	gamma	0.0281	0.1731	0.1605	0.1636	0.1698
	EXP	0.05321	0.1566	0.1573	0.1107	0.1648
	HILL	0.3195	0.168	0.1573	0.1408	0.1665
men	two.stage	0.1132	0.1666	0.1439	0.119	0.0761
	log.logist	0.1543	0.1666	0.1439	0.1527	0.0345
	Weibull	0.1528	1e-04	0.1439	0.1527	0.0345
	log.prob	0.1543	0.1666	0.1368	0.1558	0.0322
	gamma	0.1528	0.1666	0.1439	0.1527	0.0367
	EXP	0.1328	0.1666	0.1439	0.1302	0.4743
	HILL	0.1397	0.1666	0.1439	0.1368	0.3117

As a result of applying model averaging, BMDL (lower 95% confidence limit of the Benchmark dose) and BMDU (upper 95% confidence limit of the Benchmark dose) values were obtained for the dose-response relationship between Hg (dose) and thyroid hormones (effect). Table III shows these values. For the interpretation of the results, it is important to assess which of the monitored effects obtained the lowest BMDL value and which of the obtained BMDI intervals (the ratio between BMDU and BMDL) is narrow, which indicates high confidence in the estimates. The width of the interval affects the confidence of the model, and the width of the model is affected by the uncertainties of the model and data. BMDI can be considered narrow if it is smaller than the factor interval of 10 (33). In our study, the lowest BMDL was obtained for Hg-fT4 in men and is 0.000184 μg/L, but as this interval is quite wide, the estimate cannot be reliable. The narrowest BMDL-BMDU (BMDI) ratio was obtained for Hg-TSH in women (0.626-6.63 μg/L), where the width of the interval is 10.

Table III BMDL and BMDU values obtained for Hg-hormone pairs
Tabela III BMDL i BMDU vrednosti dobijene za Hg-hormoni parove

	women		**men**	
Hormone	**BMDL**	**BMDU**	**BMDL**	**BMDU**
TSH(μIU/ml)	**0.626**	**6.63**	1.2	132000
fT4(pmol/L)	0.294	236000	0.000184	82500
fT3(pmol/L)	1.81	319000	0.0206	238000
T4(nmol/L)	6.84	1850	34.7	58500
T3(nmol/L)	0.118	65.9	33.9	19700

Figures 2 and 3 show the dependence of hormone levels (TSH, fT4, fT3, T4, T3) on serum Hg concentrations.

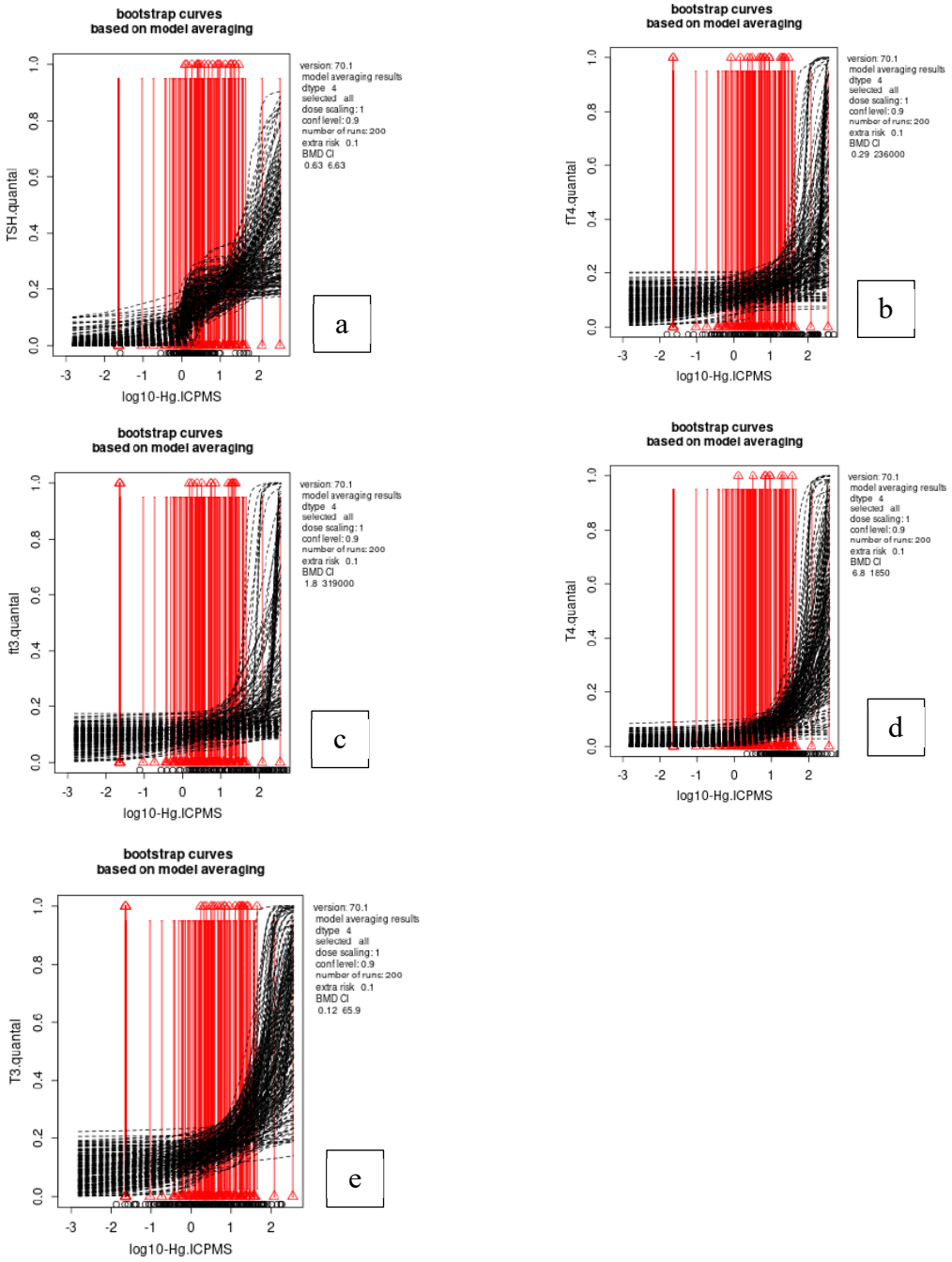

Figure 2. **Dependence of the levels of TSH (a), fT4 (b), fT3 (c), T4 (d) and T3 (e) on the concentration of Hg present in the serum of the general population of women based on Model averaging (bootstrap curves, 200 run). The X axis represents the log 10 concentration of Hg measured in the subjects' serum, and the Y axis represents hormone concentrations presented as quantal data.**

Slika 2. **Zavisnost nivoa TSH (a), fT4 (b), fT3 (c), T4 (d) i T3 (e) od koncentracije Hg prisutne u serumu opšte populacije žena na osnovu *Model averaging bootstrap curves, 200 run*). X osa predstavlja log 10 koncentracije Hg izmerene u serumu ispitanika, a Y osa predstavlja koncentracije hormona koje su predstavljene u vidu kvantalnih podataka.**

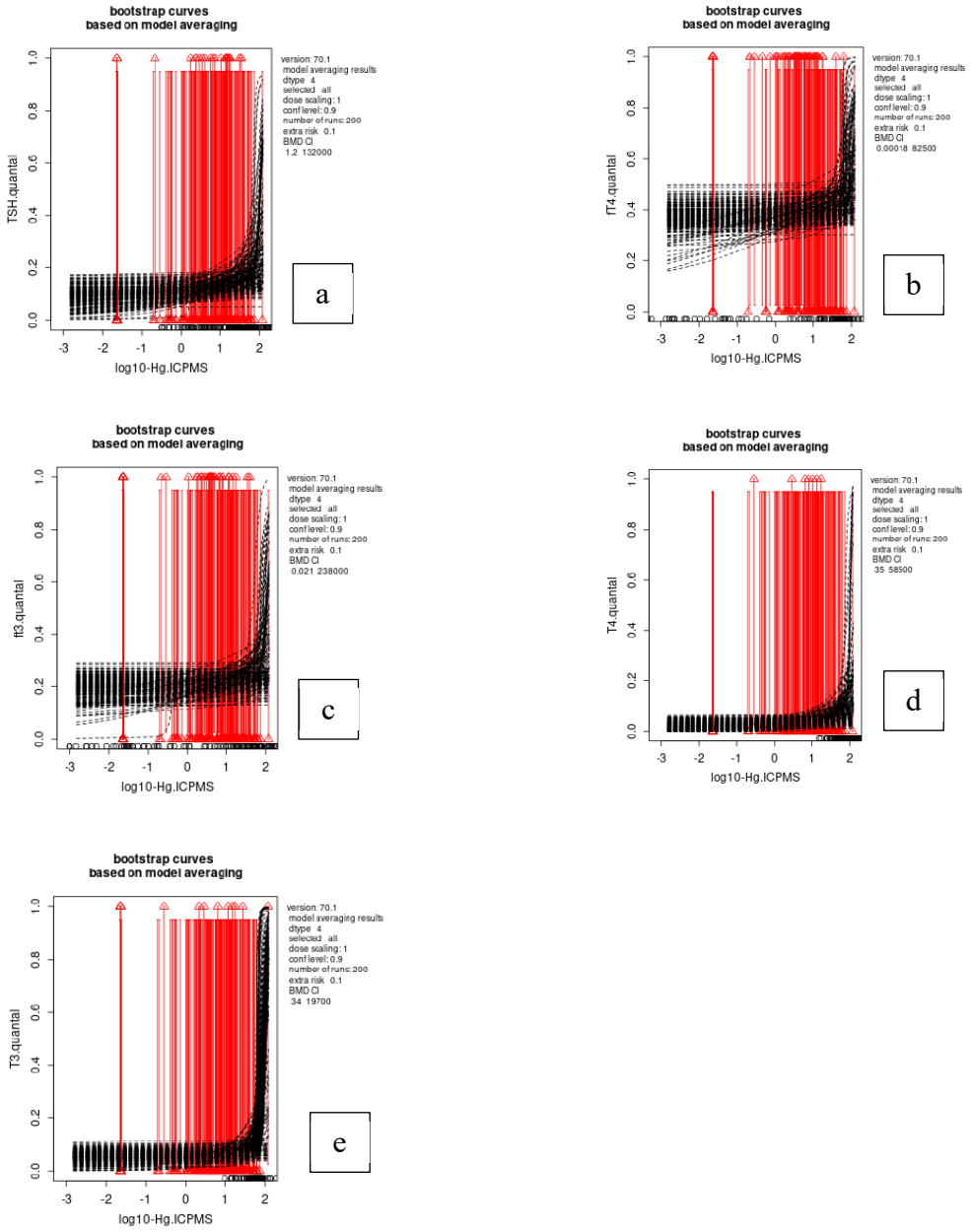

Figure 3. **Dependence of the levels of TSH (a), fT4 (b), fT3 (c), T4 (d) and T3 (e) on the concentration of Hg present in the serum of the general population of men based on Model averaging (bootstrap curves, 200 run). The X axis represents the log 10 concentration of Hg measured in the subjects' serum, and the Y axis represents hormone concentrations presented as quantal data.**

Slika 3. **Zavisnost nivoa TSH (a), fT4 (b), fT3 (c), T4 (d) i T3 (e) od koncentracije Hg prisutne u serumu opšte populacije muškaraca na osnovu *Model averaging* (*bootstrap curves, 200 run*). X osa predstavlja log 10 koncentracije Hg izmerene u serumu ispitanika, a Y osa predstavlja koncentracije hormona koje su predstavljene u vidu kvantalnih podataka.**

Discussion

Disrupting the synthesis or performance of thyroid hormones at any level can have a negative impact on the entire organism, and the patterns of the disorders are still not fully known. However, there is increasing evidence that metals, as environmental pollutants, can be significant factors that contribute to the development of the disorder (34, 35, 36) . Given that Hg is one of the most prevalent environmental pollutants and that the general population is exposed to it daily, the focus of our work was to examine whether Hg measured in the blood of the general population can be related to the disturbance of TH levels.

A study involving the population of Iran assessed the levels of bioelements and some toxic metals (Hg was also measured) in healthy patients and those suffering from various thyroid diseases (specifically, patients with hypothyroidism, hyperthyroidism, and cancers were included). Mercury levels in these populations were not statistically significantly different (37). This finding is not in accordance with our results, where the difference between the median concentrations was confirmed. The results of our study showed that the level of Hg was significantly different in subjects who had thyroid disease (hypothyroidism, hyperthyroidism, thyroid cancer) compared to those who were healthy ($p<0.001$). In the assessment, nearest-neighbor matching was used as an algorithm for examining the matching of study results.

Various epidemiologic studies have investigated the effects of Hg on TH levels in human blood, and the results of those studies have been contradictory. The results of a recent META analysis by Hu et al. showed that exposure to low blood Hg concentrations can be associated with altered TSH, fT4 (positively associated) and T4 (negatively associated). Moreover, META analysis suggests that Hg may significantly contribute to increased TSH and fT4 levels in children, adolescents, and pregnant women. This analysis found no association between Hg exposure and T3 and fT3 hormone levels (38). In contrast to the results obtained in this META analysis, the results of our analysis, which used the BMD approach, showed that a dose-response relationship could be established for Hg and all measured thyroid hormones in both male and female subjects, with the only significant Hg-TSH ratio in women. An earlier study of US residents examined the relationship between blood and urine levels of various metals and thyroid function. The data used in this research were obtained from the National Health and Nutrition Examination Survey (NHANES) for the 2007-2008 period. Subjects who suffered from thyroid gland disease, cancer, or who were using drugs for the treatment of thyroid gland disease were excluded from the study. In addition to cadmium and lead, Hg was also determined in the examinees' blood (the median was 0.9µg/L) (39). Factors such as race, gender, ethnicity, body mass index, serum lipid levels, and others were taken into consideration during the analysis. The relationship between metal levels and thyroid hormones was established using multivariate linear regression. The result of this study showed that Hg can lead to a decrease in the levels of T3, T4 and fT4, but also that it does not affect TSH (39), unlike our study where the effect of Hg on TSH was shown. In our study, unlike in the previously mentioned one, the population consisted of randomly

selected members of the general population of our country, among whom there were those with thyroid diseases. By randomly selecting study participants, the result obtained shows a more realistic state of exposure of the population. Scientists who investigated the effects of Hg on the population of pregnant women determined the concentration of Hg in the blood of the umbilical cord and TH hormones in the serum of the test subjects in a certain period of gestation. The test results showed the influence of Hg on T3, and the absence of influence on fT4 and TSH. This finding was explained by the influence of Hg on deiodination and the formation of T3 from T4 (23, 40). In contrast to the previously mentioned studies, there are also those in which no link between the influence of Hg on TH has been proven (41).

Especially in earlier research, there were studies related to occupational exposure to Hg. Compared to the general population, occupationally exposed persons come into contact with higher amounts of Hg. The effect of Hg on TH levels in miners who used Hg in gold production was examined in a study conducted by Afrifa et al. Hg and thyroid hormones were determined in the blood of 137 miners. A negative correlation was established between T3 and T4 and Hg ($p<0.001$), while the influence on TSH was not significant. This finding is explained by the still uncompensated feedback mechanism (42).

Using the BMD approach allows determining the dose that leads to certain changes based on a previously defined statistically significant change in effect that is usually 5 or 10% (BMR). The main advantage of applying this approach in the analysis is obtaining results in the form of intervals (BMDI), that is, the ratio between the lower (BMDL) and upper (BMDU) confidence limits of the dose that leads to a small, statistically significant change in effect. As previously mentioned, in addition to the BMDL value, which is important for exposure assessment and definition of the dose-response relationship, the width of the BMDL-BMDU interval is also important, because excessively wide intervals indicate the unreliability of the assessment carried out (26). Based on the results of our research, a narrow interval was obtained for the pair Hg-TSH in women, while fairly wide intervals were obtained for the ratio of Hg and other hormones in both women and men. The BMDI for Hg-TSH dose-response relationship is 0.626-6.63μg/L, which means that at a concentration of 0.626 μg/L of Hg in the blood, there may be a 10% increase in the risk of TSH level being out of reference range. As the median concentration of Hg in the blood of women is 3.444 μg/L, which is higher than the predicted BMDL value (0.626 μg/L), the risk of TSH level disorders in Serbian women population under the influence of Hg is rather worrying, especially having a mind that disturbances in TSH levels reflect on the functioning of the thyroid gland and the homeostasis of the entire organism.

In general, in the types of research where the relationship between metals and hormones is established, the application of multivariate linear regression is the most common case (39, 40, 43, 44), while there are few studies that apply the BMD approach for this purpose. In most cases so far, the use of the BMD approach has found application mostly in research conducted on animals. However, EFSA suggests the use of this approach also on data obtained in epidemiological studies, and it is considered that the

use of this approach can be of great importance for the interpretation of data obtained in human studies (26). Certainly, the use of BMD approaches to understand human data is limited and still underutilized. One of the few studies that applied the BMD approach to human data was concerned with determining the lead dose that can cause kidney damage from occupational lead exposure. Biomarkers of kidney damage were determined, which were measured in urine (e.g. β2 microglobulin), and of importance was the level of lead in the blood, which was also determined (45). Our previously published research confirmed the importance of the idea of using the BMD approach through the use of PROAST software to establish a relationship between the levels of toxic metals determined in the blood of the general male population (dose) and the levels of reproductive hormones: testosterone, follicle-stimulating (FSH) and luteinizing (LH) hormones in the serum of the subjects (response). The result showed the importance of the ratio of cadmium and testosterone and Hg and LH in terms of the risk of disturbances in the levels of the investigated hormones (29). As normal thyroid function is of great importance for the whole human organism (which was mentioned earlier), defining the amount of an agent that can cause a disorder can be useful in order for certain institutions to take measures to reduce exposure to chemicals and improve health. Practically, the application of the BMD approach allows for defining this dose as the amount that is already present in the subject's blood (and not from the external environment) and that directly interacts with the thyroid gland and hormones in different ways. Additionally, the application of nearest neighbor matching showed a statistically significant difference ($p<0.001$) in the Hg levels determined in the blood of subjects who were/were not diagnosed with thyroid disease, with the level of Hg being significantly higher in the affected subjects. This may additionally suggest the contribution of this metal in the development of diseases or disorders of the functioning of the gland in the studied population.

Some of the limitations of this study are: a 10% increase in the risk of changing the levels of the investigated hormones at the calculated Hg levels is a non-specific factor (genetic factors, as well as habits such as cigarette smoking and alcohol consumption can have an effect on hormone levels), it is not possible to determine the temporal relationship between the obtained results and exposure to this toxic metal, and other information about the subjects other than age and gender were not taken into consideration.

By applying the BMD concept, more reliable data were obtained in terms of the relationship between the metal concentration and the level of the examined hormones than if a correlation analysis was done.

Conclusion

This study revealed the thyroid-disrupting properties of Hg. It showed that a dose-response relationship can be established between Hg concentration and thyroid hormone levels (TSH, fT4, fT3, T4, T3). A narrow BMDI obtained for the Hg-TSH pair in women, along with the fact that BMD lower confidence limit is calculated to be lower than the medium levels measured in the Serbian population, indicates the importance of

environmental exposure to Hg in thyroid health. This research also encourages further use of the BMD approach in interpreting the results of epidemiological studies.

We aim to verify our hypothesis by using appropriate animal models treated with predetermined low doses of mercury that will reflect levels measured in humans. In that way, we hope to establish the precise effects and mechanistic pathways of such low doses of mercury on the endocrine system.

We are also planning to explore the thyroid-disrupting effects of multi-heavy metal low-level exposure in animal models, and to figure out the unique roles and characteristics of each metal in different pathways.

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Izloženost živi i funkcija štitaste žlezde: postoji li veza?

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Apstrakt

Živa (Hg) je jedan od najznačajnijih zagađivača životne sredine sa osobinama endokrinog ometača. Malo je podataka iz epidemioloških studija koji opisuju odnos doza-odgovor između nivoa toksičnih metala i nivoa hormona. Cilj ovog rada bio je da primenom *nearest neighbor matching* analize utvrdi razliku u koncentraciji Hg kod zdravih/obolelih ispitanika od bolesti štitaste žlezde i da primenom *Benchmark* modelovanja utvrdi odnos doza-odgovor između nivoa Hg u krvi i tireostimulišućeg hormona (TSH) i tiroidnih hormona u serumu. Uzorci krvi su sakupljeni i korišćeni za merenje Hg uz pomoć ICP-MS metode, a izdvojeni serum korišćen je za analizu hormona. Studija je pokazala postojanje statistički značajne razlike u nivoima Hg koji su izmereni kod zdravih i bolesnih ispitanika i postojanje odnosa doza-odgovor između Hg i svih merenih hormona, pri čemu je uzak interval dobijen za Hg-TSH par. Rezultati ovog istraživanja podržavaju upotrebu *Benchmark dose* pristupa u svrhu analize podataka iz humanih studija, a naša dalja istraživanja će biti usmerena na ispitivanje uticaja niskih doza na životinjskim modelima, u cilju utvrđivanja preciznijih efekata niskih doza na organizam.

Ključne reči: endokrini ometači, živa, funkcija štitaste žlezde, BMD koncept, *nearest neighbor matching*