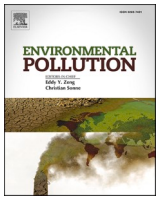
Environmental Pollution 316 (2023) 120428

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Exploring the endocrine disrupting potential of lead through benchmark modelling – Study in humans

Dragana Javorac [a], Katarina Baralić [a], Đurđica Marić [a], Stefan Mandić-Rajčević [b], Danijela Đukić-Ćosić [a], Zorica Bulat [a], Aleksandra Buha Djordjevic [a,*]

[a] Department of Toxicology "Akademik Danilo Soldatović", University of Belgrade – Faculty of Pharmacy, 11221, Belgrade, Serbia
[b] Institute of Social Medicine and School of Public Health and Health Management, Faculty of Medicine, University of Belgrade, Dr Subotića 15, Belgrade, Serbia

ARTICLE INFO

Keywords:
BLL
Endocrine effects
General population
BMD

ABSTRACT

Exposure to low levels of a toxic metal lead (Pb) affects human health, and its effect as an endocrine disruptor has been reported. However, the precise role of Pb in endocrine health is still unclear because no dose-response relationship has been established for such an effect. The present study aimed to examine blood Pb levels (BLLs) in relation to serum levels of free triiodothyronine (fT3), free thyroxine (fT4), thyroid stimulating hormone (TSH), and insulin in 435 nonoccupationally exposed Serbian subjects (218 women, 217 men, 18–94 years of age, mean age 48). In addition, benchmark dose (BMD) values were calculated for these endocrine endpoints using the PROAST 70.1 software. An explicit dose-response dependency between BLL and TSH, fT3, fT4, testosterone, and insulin serum levels was evident from BMD modelling. The results support the positive association between BLLs and serum insulin levels, with observed dose-response and calculated BMD values of 1.49 and 0.74 μg Pb/dL in males and females, respectively. Collectively, our findings reported potential endocrine-disrupting effects of Pb at the environmental exposure levels experienced by current Serbian population. They also strengthen the notion that the blood Pb threshold level for an endocrine effect is low.

1. Introduction

Endocrine-related noncommunicable diseases are of public health concern, with the increasing number of cases (Manasova, 2021). The development of such diseases is thought to primarily depends on the complex interaction of human genome and exposome (Iglesias-González et al., 2021). Exposome is a relatively new paradigm in environmental science research that includes all lifelong environmental factors contributing to disease development (Rappaport and Smith, 2010; Wild, 2012). Among these factors, exposure to endocrine-disrupting chemicals, including toxic metals, have a significant impact on human endocrine health (Schug et al., 2011; Kahn et al., 2020; Fróes-Asmus et al., 2021).

Lead (Pb) is a toxic metal widely spread in the environment and has been used for many years in the industry (Almonacid Garrido et al., 2021; Huang et al., 2021). Although significant efforts have been made to diminish Pb levels in the environment (ban of Pb gasoline, Pb paint, etc.), as a nondegradable toxicant, Pb is still present, and exposure to this metal persists (Rocha and Trujillo, 2019; Heusinkveld et al., 2021). In human and animal studies, Pb affects almost all systems: cardiovascular (Shah et al., 2010; Javorac et al., 2021, 2022), endocrine (El-Sayed and El-Neweshy, 2010; Kahn et al., 2014; Balachandar et al., 2020), nervous (Bokara et al., 2008; Javorac et al., 2020), immune and many others (Mitra et al., 2017). The primary biomarker of Pb exposure is measurement of blood Pb levels (BLL). The BLLin adults that is less than 5 μg/dL is considered to be acceptable by regulatory bodies (World Health Organization, 2021). However, some research data have suggested that there is no safe level for Pb exposure and that even nanograms of Pb can be neurotoxic (EFSA, 2010; Vorvolakos et al., 2016).

Evidence shows that Pb can act as an endocrine disruptor in humans and animals, affecting hormones' physiological values and endocrine disbalance (Doumouchtsis et al., 2009). Several epidemiological studies evaluated Pb's effects on thyroid hormones, cortisol, vitamin D, insulin, and testosterone levels (Ng et al., 1991; Fortin et al., 2012; Kahn et al., 2014; Wang et al., 2021). The most frequently investigated endpoint is the effect on thyroid hormones, while only a few studies have documented for other endpoints. Both increase and decrease in hormones were reported, while data are missing on dose-response analysis.

* Corresponding author.
E-mail address: aleksandra.buha@pharmacy.bg.ac.rs (A.B. Djordjevic).

https://doi.org/10.1016/j.envpol.2022.120428
Received 9 June 2022; Received in revised form 1 October 2022; Accepted 10 October 2022
Available online 13 October 2022

Researchers have also reported sex differences in BLLs that affect hormone levels (Luo and Hendryx, 2014; Jr, 2019).

To develop health-based guidelines for chemicals in human health risk assessment, scientists used a regulatory accepted approach for dose-response analysis – the benchmark dose (BMD) approach. It has been proposed as a replacement of the no observed adverse effect level (NOAEL). The BMD method relies on dose-response modelling of chemicals to estimate the size of a possible adverse health effect in the investigated population given a particular exposure level. Based on the last European Food Safety Authority (EFSA) guideline, the BMD approach could be used in animals. However, researchers are strongly encouraged to use it to analyse the epidemiological data sets (Hardy et al., 2017). The World Health Organisation (WHO) has recommended applying BMD models to examine the associations of exposure with health outcomes using human epidemiological data, which was detailed in updated chapter 5 of the WHO publication Environmental Health Criteria 240 (FAO/WHO, 2020).

The exact role of Pb in endocrine health is still unclear and the dose-response relationship for these effects has not been derived yet. Therefore, the present study aims to characterize the relationship between BLLs and serum hormonal levels using data obtained from human biomonitoring study of the Serbian population and to determine a BMD for selected endocrine endpoints.

2. Materials and methods

2.1. Study population

The study has been conducted at three sites: Clinical Hospital Centre *Bezanijska kosa*, Clinical Centre of Serbia, and the University of Belgrade – Faculty of Pharmacy. The study population was chosen to represent the general population of Serbia and included 435 participants, 218 women and 217 men from five cohorts: prostate and testes cancer patients (104), breast cancer and benign breast dysplasia patients (96), pancreatic cancer patient (22), thyroid and metabolic disorders patients (77) and healthy volunteers (136). To analyse potential correlations and dose-response interactions between BLL and serum hormone levels in the total study population, all cohorts were considered collectively representing the general population. The participants were not exposed to Pb at workplace. The average age was 48 years, ranging from 18 to 94. Participation in the study was voluntary, and informed consent was collected from each participant after providing each subject with detailed information. The study protocol was approved by the Scientific and Ethical Committee of the Clinical Hospital Centre, Bezanijska kosa (Ethical License No. 9740/3), Clinical Centre of Serbia (License No. 526/9, 579/19, 31/8), and University of Belgrade – Faculty of Pharmacy (License No. 650/2, 288/2). The study was undertaken in accordance with the principles laid down in the Oviedo Bioethics Convention and EU Regulation No 536/2014 on clinical trials on medicinal products for human use and fully complies with ethical guidelines given in Helsinki Declaration.

2.2. Sample preparation

After a 12 h fasting period, venous blood samples were collected from the cubital vein in metal-free tubes containing K_2EDTA anticoagulant. Another aliquot of the blood was collected in serum tubes and stored at 2–8 °C until hormone analyses were performed. After coagulation, serum was prepared, and hormone analyses were performed immediately. The blood samples with anticoagulant were used for Pb levels determination and they were labelled correctly and stored at −20 °C until Pb measurement was conducted.

2.3. Hormone analyses

Free triiodothyronine (fT3) and free thyroxine (fT4), thyroid-stimulating hormone (TSH), and insulin in the serum samples were measured for all participants. Serum testosterone levels were measured for males only. Serum levels of these hormones were assayed by commercial reagents using standard accredited procedures. All procedures were conducted following the principles of Good Laboratory Practice (GLP).

2.4. Measurement of blood Pb levels

The whole blood samples (1 mL) were digested using microwave-assisted digestion (Milestone, Start D SK-10 T, Milestone Srl, Sorisole, Italy), with 1 mL of H_2O_2 (30%) and 7 mL HNO_3 (68%). After cooling at room temperature, samples were restored to 25 mL volume with deionized water. Levels of Pb were determined by graphite furnace method using AAS GTA 120 graphite tube atomizer, 200 series AA, Agilent Technologies, Santa Clara, CA, USA. The accuracy of AAS analyses was verified using certified reference material (RM) Seronorm Trace Elements Whole Blood L-2 (SeronormTM, Sero, Billingstad, Norway). Metal analyses were carried out following the manufacturer's specifications, with a low probability of contamination. All chemicals for metal analyses were purchased from Fisher Scientific (Germany) and were of pure analytical grade.

2.5. Statistical analyses

All data were analyzed with SPSS 23.0 (IBM, Armonk, NY, USA) and Graph Pad Prism8 software (GraphPad Software Inc., San Diego, USA). After tests for normal distribution, homogeneity, and variance, data were analyzed by parametric or nonparametric tests as appropriate. Data were presented as median and ranges (minimum-maximum) or mean ± standard deviation. The differences between groups were tested using the Mann-Whitney *U* test. The associations between BLLs in each group, fT3, fT4, TSH, testosterone, and insulin, were assessed by multiple linear regression. The regression coefficient (β) and its 95% confidence interval (CI) were calculated for the related parameters. BLLs were modelled as continuous data in multiple linear regression.

2.6. Benchmark dose modelling

Benchmark dose modelling was performed using PROAST web software version 70.1 developed by Dutch National Institute for Public Health and the Environment, RIVM. BLLs data were set as internal dose and they were analyzed as continuous variable for 425 subjects. Hormone levels were analyzed as quantal data (0 – within the normal range, or - 1 out of normal range). A Benchmark response (BMR) of 10% was used for quantal data signifying a 10% extra risk of hormone levels being outside the normal range. The model averaging method was used to calculate the Benchmark dose (BMD) interval as an approach that considers the results obtained by applying all available models. The number of bootstrap runs was set as 200. It determines how often new datasets are generated (from the responses according to the average model) to calculate the BMD confidence interval. Experience shows that 200 runs generally give results that are adequate for risk assessment purposes, i.e., the lower BMD's confident bound (BMDL) and the upper BMD's confident bound (BMDU) will probably be within around 5% or 10% of the values that would be found with a vast number of runs (Hardy et al., 2017; Jensen et al., 2019). Akaike information criterion (AIC) was set as default (2 units), and it is a measure of how well the data fit into specific models (Hardy et al., 2017). Gender is set as a covariate. As a result of a conducted analysis, a lower level of BMD reliability (BMDL) and upper level of BMD reliability (BMDU) values were obtained with 95% confidence intervals.

3. Results

3.1. Characteristics of study subjects

The study subjects were grouped based on their BLLs and gender. Among 435 cohort participants, there was an almost equivalent distribution of males (49.88%) and females (50.12%) (Table 1). Among males, 67.75% had BLLs lower than 5 μg/dL. Among females, 71.55% had BLLs lower than 5 μg/dL.

3.2. Hormone levels

When all subjects were included in an analysis, statistically significant higher insulin levels were observed in the group with BLLs higher than 5 μg/dL than those with BLLs lower than 5 μg/dL, $P < 0.0001$ (Table 2). Also, insulin level was higher in the female and male group, with BLLs higher than 5 μg/dL. Testosterone level was higher in males who had BLLs ≥5 μg/dL ($P = 0.0379$). There were no statistically significant differences in fT3, fT4, and TSH levels between the group with BLLs higher than 5 μg/dL and the group with BLLs lower than 5 μg/dL.

3.3. Multivariate regression analyses

Multivariate analysis was examined in all subjects and in subgroups (male and female). The covariates were set as smoking status and presence of disease. As data in Table 3 indicate, serum insulin levels were positively associated with BLLs in all subjects, males and females with p-values <0.001 for all groups ($P < 0.0001$) (Table 3). For all other hormones (fT4, fT3, TSH and testosterone) there were no significant association with BLLs.

3.4. Benchmark dose modelling

The BMDs were determined for all hormones measured and the calculated values for BMDs are presented in Table 4, while graphical outputs are provided in Fig. 1. The narrowest BMDL-BMDU interval was calculated for the insulin levels indicating the well-fitting data. The lowest observed BMD was derived from testosterone and fT_3 levels in males. However, the calculated intervals for these hormones were the widest, indicating statistical uncertainties of the fitted models or data (Hardy et al., 2017). The selected BMR 10 indicates 10% extra risk of abnormal hormone values due to calculated BLL.

4. Discussion

Although numerous studies report adverse effects of Pb at high exposure levels, there are limited data on effects of Pb at low levels, especially on endocrine health. Furthermore, the dose-response relationship for the Pb effect on an endocrine endpoint has never been determined. In the presented study, we have observed a clear dose-response relationship between serum levels of various hormones and BLLs. The data indicates to a potential link between Pb exposure and insulin levels, however more research is required to establish how Pb exposure affects other hormones.

Although the most intensively studied toxic effect of Pb on the endocrine system is thyroid dysfunction, there are limited data on specific mechanisms of Pb thyroid disrupting effects. Lead was found to affect many organs and systems, including the thyroid gland itself. The general mechanisms of toxic actions, including oxidative stress, induction of apoptosis and inflammation, and epigenetic mechanism, are likely involved (Flora et al., 2012; Matović et al., 2015; Wallace et al., 2020; Javorac et al., 2021). Furthermore, some researchers suggested that induction of autoimmunity and the increase of thyroid peroxidase antibodies in women could be involved (Nie et al., 2017). Prior studies in humans have implicated an effect of Pb exposure on the hypothalamic-pituitary-thyroid axis (Singh et al., 2000). The possible mechanism by which Pb reduced T3 levels was through a reduction of hepatic type I iodothyronine deiodinase (Chaurasia and Kar, 1997). Pb may also have an ability to affect the homeostasis of essential ions involved in thyroid function such as calcium, iodine, selenium (Zimmermann and Boelaert, 2015; Benvenga et al., 2019; Javorac et al., 2020; Stojsavljević and Rovčanin, 2021). In epidemiological studies, the published data are inconclusive; there are reports of positive, negative, or non-correlation between BLLs and thyroid hormone levels (ATSDR, 2020).

In the present study, a dose-response relationship was observed between BLLs and thyroid hormone status, but the calculated intervals for the thyroid hormones were wide, indicating statistical uncertainties of the fitted models or data. Furthermore, multivariate regression analysis showed no significant association between blood lead levels and thyroid hormones. Having in mind that we obtained inconsistent results using different statistical methods, further studies need to be carried out in order to explain the potential association between lead exposure and thyroid hormones. For TSH hormone, calculated BMDLs were 0.702 μg Pb/dL for males and 0.686 μg Pb/dL for females, while for fT3, BMDLs were 0.268 μg Pb/dL for males and 1.8 μg Pb/dL for females. For fT4, 0.736 μg Pb/dL and 0.478 μg Pb/dL were calculated as BMDLs for males and females, respectively. In the Korean National Health and Nutrition Examination Survey (n = 1688), BLLs (mean: 1.96 μg/dL) were found to be positively correlated with TSH levels in the Korean general population (mean TSH: 2.17 μIU/mL) (Choi et al., 2020). In the Yugoslavia Prospective Study, a positive correlation was seen between BLL and thyroid peroxidase antibodies, while a negative association was seen between BLL and fT3. Similarly, a study of Turkey adolescents showed that fT4 levels were negatively associated with BLLs, while TSH and T3 levels were unrelated (Koyuncu et al., 2006). The sex differences in thyroid hormone levels were evident from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994), where T4 levels decreased as the BLLs increased in women only (Jr, 2019). In NHANES 2007–2010, including 6231 adult participants, blood Pb was not associated with TSH, but it was positively associated with fT3 in all subjects and in males, while the negative association of BLL and total T4 was seen in males (Luo and Hendryx, 2014).

Experimental studies reported effects of Pb on male reproduction. For example, Pb was found to decrease semen quality, and sperm motility and cause histological alterations of Leydig cells (El-Sayed and El-Neweshy, 2010; El-Magd et al., 2017). Hypothalamic–pituitary-gonadal axis impairment was an essential mechanism of Pb effect on sex hormonal levels (Rotter et al., 2016). Because Pb can pass through the blood-brain barrier, it may interference with Ca-dependent second messenger signalling, involved in pituitary hormone release. (Fullmer, 1992; Doumouchtsis et al., 2009). Also, Pb can accumulate in reproductive organs and may have a direct effect on steroidogenesis by inhibiting the expression of steroidogenesis acute regulatory protein (Liu et al., 2003). Our results indicate the differences in testosterone levels between two subgroups of males (Table 2). Benchmark modelling showed a higher incidence of abnormal (out of range) testosterone levels in a subgroup with higher BLLs. Some

Table 1
Study subjects based on gender and BLLs.

	All subjects	BLLs		
		< 5 μg/dL	**≥ 5 μg/dL**	
Total	435			
Male, n (%)	217 (49.88)	147 (67.75)	70 (32.25)	
Female, n (%)	218 (50.12)	156 (71.55)	62 (28.45)	
			≥ 5 μg/dL (subgroups)	
			5–10 μg/dL	**10–30 μg/dL**
Male, n (%)			42 (19.35)	28 (12.95)
Female, n (%)			39 (17.9)	23 (10.55)

Blood lead levels (BLLs).

Table 2
Comparing serum hormone levels in study subjects grouped by gender and blood lead levels.

Hormone	BLLs								
	All subjects			Male			Female		
	<5 μg/dL	≥5 μg/dL	P value	<5 μg/dL	≥5 μg/dL	P value	<5 μg/dL	≥5 μg/dL	P value
fT_3 (pmol/L)	4.89	4.73	NS	4.94	4.87	NS	4.81	4.68	NS
	1.30–60.34	1.95–27.37		1.30–60.34	1.95–27.37		1.89–19.90	2.33–18.02	
fT_4 (pmol/L)	15.96	16.06	NS	14.38	16.76	NS	16,76	17.01	NS
	4.35–98.2	3.00–62.2		4.35–25.29	3.00–26.60		11.75–98.20	9.30–62.20	
TSH (μIU/ml)	1.76	1.92	NS	1.76	1,96	NS	1.75	1.91	NS
	0.01–75.29	0.01–88.59		0.04–10.28	0.01–20.51		0.01–75.29	0.01–88.59	
Insulin (μIU/ml)	11.56	20.60	**<0.001**	14.65	16.11	**0.006**	9.52	40.91	**<0.001**
	1.37–203.5	0.2–777.8		2.02–192	3.05–472.1		1.37–203.5	0.20–777.8	
Testosterone [a] (ng/mL)	/	/		4.915	5.250	**0.038**	/	/	
	/	/		0.28–50.20	0.36–67.55		/	/	

Results are presented as median and ranges (minimum-maximum). Triiodothyronine (fT3), thyroxine (fT4), thyroid stimulating hormone (TSH), not significant (NS), statistical significance (P). [a] Obtained only in males; Mann-Whitney U statistic test was performed.

Table 3
Multivariate linear regression analyses of BLLs (Pb μg/dL) and investigated hormones.

Overall BLLs					
Model					
Parameter estimates	Variable	Estimate	Standard error	95% confidence interval	P value
β0	Intercept	5050	0,7842	3508 to 6593	<0,0001
β1	B: fT3	−0,03464	0,05250	−0,1379 to 0,06860	0,5098
β2	C: fT4	−0,07838	0,04324	−0,1634 to 0,006650	0,0707
β3	**D: Insulin**	**0,02815**	**0,003241**	**0,02178 to 0,03452**	**<0,0001**
β4	E: TSH	−0,001385	0,03462	−0,06947 to 0,06670	0,9681
β5	G: Smoking	0,4569	0,4704	−0,4681 to 1382	0,3320
β6	H: Disease	−0,5325	0,4751	−1467 to 0,4019	0,2631
Male BLLs					
Model					
Parameter estimates	Variable	Estimate	Standard error	95% confidence interval	P value
β0	Intercept	4355	1164	2058 to 6652	0,0002
β1	B: fT3	−0,04513	0,05764	−0,1589 to 0,06867	0,4348
β2	C: fT4	−0,01364	0,07837	−0,1684 to 0,1411	0,8620
β3	**D: Insulin**	**0,03012**	**0,005703**	**0,01886 to 0,04138**	**<0,0001**
β4	E: TSH	0,05518	0,1710	−0,2823 to 0,3927	0,7473
β5	F: Testosteron	0,08063	0,04551	−0,009214 to 0,1705	0,0783
β6	G: Smoking	1166	0,7940	−0,4015 to 2733	0,1438
β7	H: Disease	−1853	1022	−3870 to 0,1647	0,0716
Female BLLs					
Model					
Parameter estimates	Variable	Estimate	Standard error	95% confidence interval	P value
β0	Intercept	3603	1714	0,2224 to 6984	0,0368
β1	B: fT4	0,01303	0,08623	−0,1571 to 0,1831	0,8801
β2	C: fT3	−0,1153	0,2757	−0,6591 to 0,4284	0,6761
β3	**D: Insulin**	**0,02673**	**0,003869**	**0,01910 to 0,03436**	**<0,0001**
β4	E: TSH	−0,003375	0,03515	−0,07272 to 0,06598	0,9236
β5	G: Smoking	0,1043	0,5796	−1039 to 1248	0,8573
β6	H: Disease	−0,2941	0,5925	−1463 to 0,8747	0,6203

Triiodothyronine (fT3), thyroxine (fT4), thyroid stimulating hormone (TSH), blood lead levels (BLLs), statistical significance (P), linear regression coefficient (β).

epidemiological studies suggested that Pb exposure impacts testosterone levels, but the results are inconclusive (Luo et al., 2020). Findings similar to the ones observed in our study were reported in a large epidemiological study of 2286 Chinese men where BLLs were positively correlated with testosterone levels (P for trend = 0.001). Also, in the U.S. NHANES 1999–2004, BLLs were positively correlated with testosterone levels in the adult males (Kresovich et al., 2015). In another study of 150 men, higher Pb levels and a slight decrease in testosterone were found in the infertile group compared to the fertile one (Ali, 2019).

A reduction in testosterone levels was observed in some occupational Pb exposed population studies (Balachandar et al., 2020). However, the low-level Pb exposure experienced by the general population was associated with higher levels of serum testosterone levels in the study of 240 Croatian men (Pizent et al., 2007). BMD modelling performed in our research, showed a positive incidence of abnormal testosterone values among participants with higher BLLs, with BMDL: 0.005 μg/dL. However, the observed BMDI is wide, indicating certain uncertainties in tested models.

There is evidence from epidemiological studies that Pb may be implicated in the pathogenesis of metabolic diseases. In the study of Pakistani mothers and infant pairs, higher blood, urine, and hair Pb levels were found in diabetic compared to non-diabetic (Kolachi et al., 2011). Data from experimental animal models of diabetes suggested that Pb-induction of oxidative stress and production of free radicals could inhibit the main components of the insulin signalling pathway leading to insulin resistance (Fridlyand and Philipson, 2006). Hyperglycaemia and glucose intolerance were observed in rats exposed to 0.05% w/v Pb in drinking water for 24 weeks (Tyrrell et al., 2017). Our results

Table 4
Benchmark dose intervals for BLLs (Pb μg/dL) based on hormonal serum levels obtained by PROAST 70.1 software and model averaging method with BMR of 10%.

Hormone	Gender	$BMDL_{10}$ (μg/dL)	$BMDU_{10}$ (μg/dL)
TSH			
	male	0.702	19.3
	female	0.686	13
fT3			
	male	0.268	21800
	female	1.8	474
fT4			
	male	0.736	22.8
	female	0.478	13.5
Testosterone			
	male	0.0053	3650
	female	/	/
Insulin			
	male	1.49	6.78
	female	0.74	1.78

Lower level of BMD reliability (BMDL), upper level of BMD reliability (BMDU), triiodothyronine (fT3), thyroxine (fT4), thyroid stimulating hormone (TSH).

demonstrated the significantly higher insulin levels in a group with BLL higher than 5 μg/dL, and dose-response modelling showed a positive incidence of abnormal (out of normal range) insulin levels with BMDL in males 1.49 μg/dL and 0.74 μg/dL in females. The differences between male and female BMDLs values might be due to sex differences in insulin sensitivity. Degrees of insulin resistance, body composition, and energy balance vary significantly between men and women. A key factor in the emergence of insulin resistance and obesity-related problems is the distribution of adipose tissue, particularly the presence of enhanced visceral and hepatic adiposity (Geer and Shen, 2009). In comparison to obese women, obese males have worse insulin sensitivity, higher blood glucose levels, and lower levels of adiponectin, all of which contribute to intraabdominal obesity and insulin resistance (Chang et al., 2018). Our data indicate higher circulating insulin levels among in those with higher BLLs. The published epidemiological data on insulin levels and the BLL association are scarce. Significant positive associations between BLL and fasting blood glucose were identified in an occupationally exposed population in the United Arab Emirates, suggesting a probable relationship between Pb exposure and diabetes (Bener et al., 2001).

In the present study, we applied the Benchmark approach to derive BMDL values and sex was entered as a covariate. In males, the lowest BMD value for fT3 was 0.268 μg Pb/dL and it was 0.053 μg Pb/dL for testosterone. In females the lowest calculated BMD for fT4 was 0.478 μg Pb/dL and it was 0.68 μg Pb/dL for TSH. To the best of our knowledge, there is no published data on internal BMD based on BLLs in humans considering an endocrine endpoint. According to results from occupationally exposed lead-acid battery workers in China, the BMR was set at a level of 5% (continuous data) and the BMDL of 13.5 μg Pb/dL for red blood cells concentration or 10.5 μg Pb/dL for hemoglobin levels, based on hematological toxicity, and even the tougher threshold of 6.6 μg Pb/dL for micronuclei or 3.5 μg Pb/dL for telomere length, based on genotoxicity were derived (Wang et al., 2020). Presently, it is considered that most Pb toxic mechanisms occur at BLL of 5 μg/dL. For this reason, the Center for Disease Control and Prevention (CDC) is considering lowering the reference values of BLL, in children from 5 μg/dL to 3 μg/dL (CDC, 2017; Rocha and Trujillo, 2019). Also, in 2012, the WHO withdrew a tolerable intake level of Pb because such intake level was associated with decreased intelligence in children (Joint Expert Committee on Food Additives (JECFA) 2012). Our results support these considerations keeping in mind that the derived BMDLs for Pb that increase risk of developing abnormal hormonal levels for 10% are lower than 5 μg/dL (recommended by CDC for the general population), especially when it comes to insulin for which the observed BMDI was narrow suggesting certainty of the calculated values. Our calculated BMDLs results were lower than BMDLs, derived by EFSA, 3.6 μg/dL BLL for a 1 mm mercury change in systolic blood pressure and 1.5 μg/dL for chronic renal disease (EFSA CONTAM Panel, 2010).

A key strength of the present study is that we used a regulatory accepted BMD approach which is proposed by EFSA. The EFSA has recognized as one of the needs, to evaluate the potential application of the BMD method in the human data analysis (Hardy et al., 2017). Furthermore, by using a nonoccupationally exposed subjects we investigated an effect of the real-life low environmental exposure to Pb on our hormonal system (Goumenou et al., 2021). There are several limitations which include a cross-sectional design, further subjects with levels higher than clinical range and subjects with levels lower than clinical range were categorized into the same group when performing BMD analyses and lastly, clinical reference ranges of serum hormones remain controversial and might not always represent "normal" range. However, observed low levels of BLL responsible for 10% additional risk of developing an imbalance in certain hormone levels represents a fruitful area for further work. More research into the effects of Pb in these dose levels should be undertaken. The measured internal Pb dose levels will be mathematically transferred into external doses for future animal studies to further test if these dose levels reproduce toxic endocrine effects.

5. Conclusion

The study set out to establish if exposure to Pb in real life situations can affect various endocrine endpoints. Presented observations suggested that measured BLLs of general, nonoccupationally exposed populations are related to hormonal status. The BMD modelling has shown a clear dose-response relationship with a positive trend between BLLs, as a biomarker of Pb exposure and circulating levels of hormones, TSH, fT3, fT4, testosterone, and insulin. Further investigation is required to assess lead's endocrine potential with regard to its effects on other hormones. . The weight of data strongly suggested the positive association between Pb exposure and higher insulin levels, with observed dose-response and calculated BMDLs: 1.49 μg Pb/dL and 0.74 μg Pb/dL in males and females, respectively. More than 30% participants (BLL higher than 5 μg/dL), due to BLL develop 10% extra risk of having insulin levels out of normal range. Our results provide new insight into assessing human health risk assessment of low Pb exposure, especially with regards to its endocrine-disrupting effects. Although this study focuses on the role of Pb in endocrine health, its results may well have bearing on the possibility of using the BMD approach when analysing data obtained from human datasets. Overall, this study clearly strengthens the idea that Pb threshold established for its endocrine effects is very low and in line with real-life exposures.

Funding

This research was supported by the Science Fund of the Republic of Serbia, PROMIS, Grant No 6066532, "Decoding the role of exposome in endocrine health" – DecodExpo project.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dragana Javorac, Katarina Baralic and Aleksandra Buha Djordjevic. The first draft of the manuscript was written by Dragana Javorac and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Scientific and Ethical

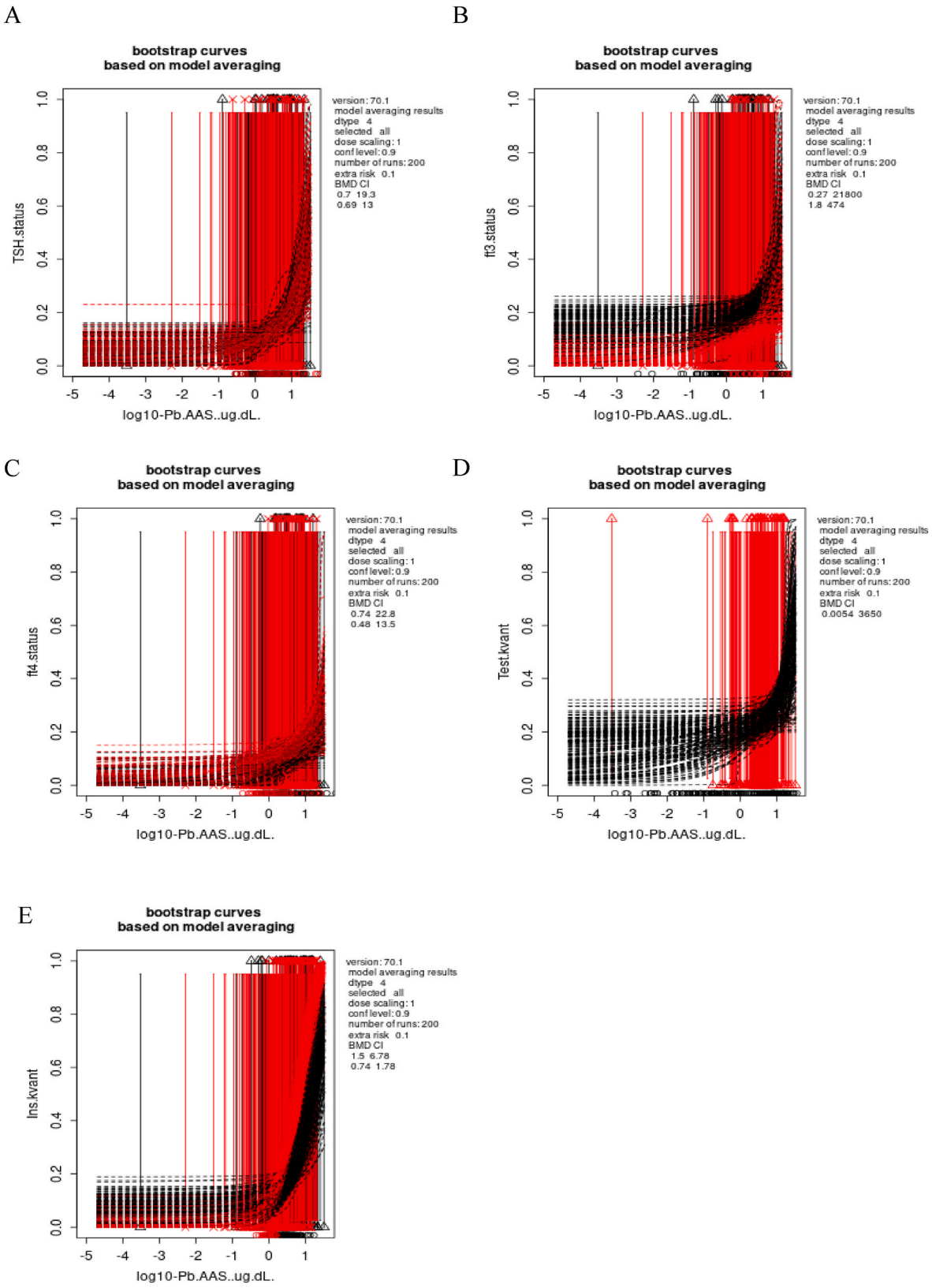


Fig. 1. BMD modelling for observed TSH (A), fT3 (B), fT4 (C), testosterone (D), insulin (E), (0 - value is in a normal range, 1 value is out of normal range) in study subjects as a function of blood Pb concentrations (μg/dL) with gender as covariate based on model averaging. The red triangles represent the medians of data, while the black circles represent the BMD derived after each model averaging bootstrap run. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Committee of the Clinical Hospital Centre, Bezanijska kosa (Ethical License No. 9740/3), Clinical Centre of Serbia (License No. 526/9, 579/19, 31/8) and University of Belgrade – Faculty of Pharmacy (License No. 650/2, 288/2).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Author statement

Dragana Javorac: Conceptualisation, Formal analysis, Investigations, Writing-original draft; *Katarina Baralic*: Conceptualisation, Formal analysis, Investigations; **Đurđica Marić**: Conceptualisation, formal analysis, Investigations; **Stefan Mandić-Rajčević**: Data curation, Methodology, Investigation; **Danijela Đukić-Ćosić**: Conceptualisation, Supervision, Investigation; **Zorica Bulat**: Conceptualisation, Supervision, Investigation; **Aleksandra Buha Djordjevic**: Conceptualisation, Visualization, Investigation, Methodology, Formal analysis, Supervision. Funding acquisition, Project administration, Writing –review & editing. All authors have red and approved the final manuscript version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have shared the link to my data at the Attach File step.

References

Ali, H.M., 2019. Effect of some heavy metals on testosterone hormone in infertile men. J. Univ. Babylon Pure Appl. Sci. 368–377.

Almonacid Garrido, M.C., Jiménez Navarro, P., Peinador Asensio, J., et al., 2021. Tap water lead levels in Madrid (Spain): degree of compliance and health risk assessment. Expo. Heal. 13, 207–218. https://doi.org/10.1007/s12403-020-00374-5.

ATSDR, 2020. Toxicological Profile for Lead. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Balachandar, R., Bagepally, B.S., Kalahasthi, R., Haridoss, M., 2020. Blood lead levels and male reproductive hormones: a systematic review and meta-analysis. Toxicology 443, 152574. https://doi.org/10.1016/j.tox.2020.152574.

Bener, A., Obineche, E., Gillett, M., et al., 2001. Association between blood levels of lead, blood pressure and risk of diabetes and heart disease in workers. Int. Arch. Occup. Environ. Health 74, 375–378. https://doi.org/10.1007/s004200100231.

Benvenga, S., Feldt-Rasmussen, U., Bonofiglio, D., Asamoah, E., 2019. Nutraceutical supplements in the thyroid setting: health benefits beyond basic nutrition. Nutrients 11, 2214. https://doi.org/10.3390/nu11092214.

Bokara, K.K., Brown, E., McCormick, R., et al., 2008. Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. Biometals 21, 9–16. https://doi.org/10.1007/s10534-007-9088-5.

CDC, 2017. What Do Parents Need to Know to Protect Their Children? Retrieved from. http://www.cdc.gov/nceh/lead/ACCLPP/%20blood_lead_levels.htm.

Chaurasia, S.S., Kar, A., 1997. Protective effects of vitamin E against lead-induced deterioration of membrane associated type-I iodothyronine 5'-monodeiodinase (5'D-I) activity in male mice. Toxicology 124, 203–209. https://doi.org/10.1016/S0300-483X(97)00155-8.

Chang, E., Varghese, M., Singer, K., 2018. Gender and sex differences in adipose tissue. Curr. Diabetes Rep. 18 https://doi.org/10.1007/s11892-018-1031-3.

Choi, J.Y., Huh, D., Whan, K., Id, M., 2020. Association between blood lead levels and metabolic syndrome considering the effect of the thyroid-stimulating hormone based on the 2013 Korea National health and. nutrition examination survey 53, 1–14. https://doi.org/10.1371/journal.pone.0244821.

Doumouchtsis, K.K., Doumouchtsis, S.K., Doumouchtsis, E.K., Perrea, D.N., 2009. The effect of lead intoxication on endocrine functions. J. Endocrinol. Invest. 32, 175–183. https://doi.org/10.1007/BF03345710.

EFSA, 2010. Scientific opinion on lead in food. EFSA J. 8 (1–146), 1570.

EFSA CONTAM Panel, 2010. Scientific opinion on lead in food. EFSA J. 8 (1–146), 1570. https://doi.org/10.2903/j.efsa.2010.1570.

El-Magd, M.A., Kahilo, K.A., Nasr, N.E., et al., 2017. A potential mechanism associated with lead-induced testicular toxicity in rats. Andrologia 49. https://doi.org/10.1111/and.12750.

El-Sayed, Y.S., El-Neweshy, M.S., 2010. Impact of lead toxicity on male rat reproduction at "hormonal and histopathological levels. Toxicol. Environ. Chem. 92, 765–774. https://doi.org/10.1080/02772240902984453.

FAO/WHO, 2020. Dose-response assessment and derivation of health-based guidance values, 5-151. In: Principles and Methods for the Risk Assessment of Chemicals in Food, p. 1.

Flora, G., Gupta, D., Tiwari, A., 2012. Toxicity of lead: a review with recent updates. Interdiscipl. Toxicol. 5, 47–58. https://doi.org/10.2478/v10102-012-0009-2.

Fortin, M.C., Cory-Slechta, D.A., Ohman-Strickland, P., et al., 2012. Increased lead biomarker levels are associated with changes in hormonal response to stress in occupationally exposed male participants. Environ. Health Perspect. 120, 278–283. https://doi.org/10.1289/ehp.1103873.

Fridlyand, L.E., Philipson, L.H., 2006. Reactive species and early manifestation of insulin resistance in type 2 diabetes. Diabetes Obes. Metabol. 8, 136–145. https://doi.org/10.1111/j.1463-1326.2005.00496.x.

Fróes-Asmus, C.I.R., Meyer, A., da Cunha, A.J.L.A., et al., 2021. Multiple environmental exposure in pregnant women and their children in the city of Rio de Janeiro, Brazil, Rio birth cohort study: PIPA project. Expo. Heal. 13, 431–445. https://doi.org/10.1007/s12403-021-00394-9.

Fullmer, C.S., 1992. Intestinal interactions of lead and calcium. Neurotoxicology 13, 799–808.

Geer, E.B., Shen, W., 2009. Gender differences in insulin resistance, body composition, and energy balance. Gend. Med. 6, 60–75. https://doi.org/10.1016/j.genm.2009.02.002.

Goumenou, M., Djordjevic, A.B., Vassilopoulou, L., Tsatsakis, A.M., 2021. Endocrine disruption and human health risk assessment in the light of real-life risk simulation. In: Toxicological Risk Assessment and Multi-System Health Impacts from Exposure. Elsevier, pp. 147–162.

Hardy, A., Benford, D., Halldorsson, T., et al., 2017. Update: use of the benchmark dose approach in risk assessment. EFSA J. 15, 1–72. https://doi.org/10.2903/j.efsa.2017.4658.

Heusinkveld, D., Ramírez-Andreotta, M.D., Rodríguez-Chávez, T., et al., 2021. Assessing children's lead exposure in an active mining community using the integrated exposure uptake biokinetic model. Expo. Heal. 13, 517–533. https://doi.org/10.1007/s12403-021-00400-0.

Huang, Z., chen, Yin X., Chen, M., et al., 2021. Relationships between Dietary Patterns and Low-Level Lead Exposure Among Children from Hunan Province of China. Expo Heal. https://doi.org/10.1007/s12403-021-00432-6.

Iglesias-González, A., Schaeffer, C., Dahm, G., et al., 2021. Comprehensive assessment of local population chemical exposome by combination of organic pollutant- and metal-Multi-Residue analysis in hair. Expo. Heal. https://doi.org/10.1007/s12403-021-00444-2.

Javorac, D., Antonijević, B., Anđelković, M., et al., 2021. Oxidative stress, metallomics and blood toxicity after subacute low-level lead exposure in Wistar rats: benchmark dose analyses. Environ. Pollut. 291, 118103 https://doi.org/10.1016/J.ENVPOL.2021.118103.

Javorac, D., Đorđević, A.B., Anđelković, M., et al., 2020. Redox and essential metal status in the brain of Wistar rats acutely exposed to a cadmium and lead mixture. Arh. Hig. Rada. Toksikol. 71, 197–204. https://doi.org/10.2478/aiht-2020-71-3425.

Javorac, D., Tatović, S., Anđelković, M., et al., 2022. Low-lead doses induce oxidative damage in cardiac tissue: subacute toxicity study in Wistar rats and Benchmark dose modelling. Food Chem. Toxicol. 161, 112825 https://doi.org/10.1016/j.fct.2022.112825.

Jensen, S.M., Kluxen, F.M., Ritz, C., 2019. A review of recent advances in benchmark dose methodology. Risk Anal. 39, 2295–2315. https://doi.org/10.1111/risa.13324.

Joint Expert Committee on Food Additives (JECFA), 2012. Safety Evaluation of Certain Food Additives: Seventy-Sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives.

Jr, E.F.K., 2019. Journal of Trace Elements in Medicine and Biology the relationships between blood lead levels and serum thyroid stimulating hormone and total thyroxine in the third National Health and Nutrition Examination Survey. J. Trace Elem. Med. Biol. 51, 130–137. https://doi.org/10.1016/j.jtemb.2018.10.010.

Kahn, L.G., Liu, X., Rajovic, B., et al., 2014. Research | Children ' S Health Blood Lead Concentration and Thyroid Function during Pregnancy : Results from the Yugoslavia Prospective Study of Environmental Lead Exposure, pp. 1134–1141.

Kahn, L.G., Philippat, C., Nakayama, S.F., et al., 2020. Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol. 8, 703–718. https://doi.org/10.1016/S2213-8587(20)30129-7.

Kolachi, N.F., Kazi, T.G., Afridi, H.I., et al., 2011. Status of toxic metals in biological samples of diabetic mothers and their neonates. Biol. Trace Elem. Res. 143, 196–212. https://doi.org/10.1007/s12011-010-8879-7.

Koyuncu, M., Delibas, N., Dundar, B., et al., 2006. The effect of long-term low-dose lead exposure on thyroid function in adolescents 101, 140–145. https://doi.org/10.1016/j.envres.2005.10.002.

Kresovich, J.K., Argos, M., Turyk, M.E., 2015. Associations of lead and cadmium with sex hormones in adult males. Environ. Res. 142, 25–33. https://doi.org/10.1016/j.envres.2015.05.026.

Liu, M.Y., Leu, S.F., Yang, H.Y., Huang, B.M., 2003. Inhibitory mechanisms of lead on steroidogenesis in MA-10 mouse Leydig tumor cells. Arch. Androl. 49, 29–38. https://doi.org/10.1080/225-01485010290031556.

Luo, J., Hendryx, M., 2014. Relationship between blood cadmium, lead, and serum thyroid measures in US adults - the National Health and Nutrition Examination

Survey (NHANES) 2007-2010. Int. J. Environ. Health Res. 24, 125–136. https://doi.org/10.1080/09603123.2013.800962.
Luo, Q., Zhao, H., Jiang, Y., et al., 2020. Association of Blood Metal Exposure with Testosterone and Hemoglobin : A Cross-Sectional Study in Hangzhou Birth Cohort Study, vol. 136. https://doi.org/10.1016/j.envint.2019.105451.
Manasova, I.S., 2021. Feature of Endocrine Diseases 18, 164–168.
Matović, V., Buha, A., Dukić-Ćosić, D., Bulat, Z., 2015. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food Chem. Toxicol. 78, 130–140.
Mitra, P., Sharma, S., Purohit, P., Sharma, P., 2017. Clinical and molecular aspects of lead toxicity: an update. Crit. Rev. Clin. Lab Sci. 54, 506–528. https://doi.org/10.1080/10408363.2017.1408562.
Ng, T.P., Goh, H.H., Ng, Y.L., et al., 1991. Male endocrine functions in workers with moderate exposure to lead. Br. J. Ind. Med. 48, 485–491. https://doi.org/10.1136/oem.48.7.485.
Nie, X., Chen, Y., Chen, Y., et al., 2017. Lead and cadmium exposure, higher thyroid antibodies and thyroid dysfunction in Chinese women. Environ. Pollut. 230, 320–328. https://doi.org/10.1016/j.envpol.2017.06.052.
Pizent, A., Jurasovic, J., Cvitkovic, P., Teliš, S., 2007. Reproductive toxicity of low-level lead exposure in men 105, 256–266. https://doi.org/10.1016/j.envres.2007.05.011.
Rappaport, S.M., Smith, M.T., 2010. Epidemiology. Environment and disease risks. Science 330 (6003), 460–461.
Rocha, A., Trujillo, K.A., 2019. Neurotoxicity of low-level lead exposure: history, mechanisms of action, and behavioral effects in humans and preclinical models. Neurotoxicology 73, 58–80. https://doi.org/10.1016/j.neuro.2019.02.021.
Rotter, I., Kosik-Bogacka, D.I., Dołęgowska, B., et al., 2016. Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging. Environ. Geochem. Health 38, 749–761. https://doi.org/10.1007/s10653-015-9758-0.
Schug, T.T., Janesick, A., Blumberg, B., Heindel, J.J., 2011. Endocrine disrupting chemicals and disease susceptibility. J. Steroid Biochem. Mol. Biol. 127, 204–215. https://doi.org/10.1016/j.jsbmb.2011.08.007.
Shah, F., Kazi, T.G., Afridi, H.I., et al., 2010. Environmental exposure of lead and iron deficit anemia in children age ranged 1-5years: a cross sectional study. Sci. Total Environ. 408, 5325–5330. https://doi.org/10.1016/j.scitotenv.2010.07.091.
Singh, B., Chandran, V., Bandhu, H.K., et al., 2000. Impact of lead exposure on pituitary-thyroid axis in humans. Biometals 13, 187–192. https://doi.org/10.1023/A:1009201426184.
Stojsavljević, A., Rovčanin, B., 2021. Impact of essential and toxic Trace metals on thyroid health and cancer: a review. Expo. Heal. 13, 613–627. https://doi.org/10.1007/s12403-021-00406-8.
Tyrrell, J.B., Hafida, S., Stemmer, P., et al., 2017. Lead (Pb) exposure promotes diabetes in obese rodents. J. Trace Elem. Med. Biol. 39, 221–226. https://doi.org/10.1016/j.jtemb.2016.10.007.
Vorvolakos, T., Arseniou, S., Samakouri, M., 2016. There is no safe threshold for lead exposure: A literature review. Psychiatrike 27, 204–214. https://doi.org/10.22365/jpsych.2016.273.204.
Wallace, D.R., Taalab, Y.M., Heinze, S., et al., 2020. Toxic-metal-Induced alteration in miRNA expression profile as a proposed mechanism for disease development. Cells 9. https://doi.org/10.3390/cells9040901.
Wang, B., Wan, H., Cheng, J., et al., 2021. Blood lead, vitamin D status, and albuminuria in patients with type 2 diabetes. Environ. Pollut. 276, 116653 https://doi.org/10.1016/j.envpol.2021.116653.
Wang, T.T., Tu, Y., Zhang, G., et al., 2020. Development of a benchmark dose for lead-exposure based on its induction of micronuclei, telomere length changes and hematological toxicity. Environ. Int. 145, 106129 https://doi.org/10.1016/j.envint.2020.106129.
Wild, C.P., 2012. The exposome: from concept to utility. Int. J. Epidemiol. 41, 24–32. https://doi.org/10.1093/ije/dyr236.
World Health Organization, 2021. WHO Guideline for the Clinical Management of Exposure to Lead, 2021. Licence: CC BY-NC-SA 3.0 IGO. Cataloguing-in-Publication. World Health Organization, Geneva.
Zimmermann, M.B., Boelaert, K., 2015. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 3, 286–295. https://doi.org/10.1016/S2213-8587(14)70225-6.