International Archives of Occupational and Environmental Health (2020) 93:491–502
https://doi.org/10.1007/s00420-019-01499-0

ORIGINAL ARTICLE

Evaluation of the effects of chronic occupational exposure to metallic mercury on the thyroid parenchyma and hormonal function

M. M. Correia[1] · M. C. Chammas[2] · J. D. Zavariz[2] · A. Arata[3] · L. C. Martins[4] · S. Marui[5] · L. A. A. Pereira[6]

Received: 17 April 2019 / Accepted: 28 November 2019 / Published online: 12 December 2019

Abstract

Introduction Experiments in animals exposed to mercury (Hg) in different chemical states have shown thyroid parenchymal and hormone alterations. However, these experiments did not allow the establishment of dose–response curves or provide an understanding of whether these Hg effects on the thyroid parenchyma occur in humans.

Objective To evaluate the association between chronic occupational exposure to metallic Hg and alterations in thyroid hormones and gland parenchyma 14 years after the last exposure.

Methods A cross-sectional study including 55 males exposed in the past to metallic Hg and 55 non-exposed males, paired by age, was conducted in the Hospital das Clínicas (Brazil) from 2016 to 2017. Serum concentrations of total and free triiodothyronine (TT3 and FT3), free thyroxine (FT4), thyrotropin (TSH), reverse T3 (RT3), selenium and antithyroid antibody titers were obtained. The Hg and iodine concentrations were measured in urine. The thyroid parenchyma was evaluated by B-mode ultrasonography with Doppler. The nodules with aspects suspicious for malignancy were submitted to aspiration puncture with a thin needle, and the cytology assessment was classified by the Bethesda system. The *t* test or Mann–Whitney test, Chi-square test and Spearman correlation were used to compare the exposed and non-exposed groups and examine the relationships between the variables. Univariate and multivariate logistic regression models were used to trace determinants of the risk of thyroid hormone alteration. Statistical significance was defined by $p < 0.05$.

Results The urinary Hg average was significantly higher in the exposed group than in the non-exposed group ($p < 0.01$). The mean TSH serum concentration in the exposed group was higher, with a statistically significant difference between the groups ($p = 0.03$). Serum concentrations of TSH exceeded the normality limit (4.20 µIU/ml) in 13 exposed individuals (27.3%) and 4 non-exposed individuals (7.3%), with a statistically significant association between the hormonal increase and exposure to Hg ($p = 0.02$). In the logistic regression model, exposure to Hg (yes or no) showed an odds ratio = 4.86 associated with an increase of TSH above the normal limit ($p = 0.04$). The serum concentrations of RT3 showed a statistically borderline difference between the groups ($p = 0.06$). There was no statistically significant difference between the mean TT3, FT3 and FT4 serum concentrations in the Hg-exposed group compared to the non-exposed group. The proportions of the echogenicity alterations were higher in the exposed group compared to the non-exposed group (27.3% versus 9.1%; $p = 0.03$). Papillary carcinomas were documented in three exposed individuals and one non-exposed individual. A follicular carcinoma was recorded in one non-exposed individual.

Conclusions Due to the higher serum TSH concentration and the prevalence of parenchymal alterations in the Hg-exposed group, even after cessation of exposure, it is recommended that the thyroid status of exposed workers be followed for a long period.

Keywords Metallic mercury · Chronic mercury intoxication · Thyroxine · Selenium · Thyroid ultrasound

Introduction

Mercury (Hg) can be present in different chemical states, and its important toxic actions are determined by its various forms. The clinical condition and the entry pathways into the organism vary according to its chemical structures

✉ M. M. Correia
m.correia@hc.fm.usp.br

Extended author information available on the last page of the article

(Holmes et al. 2009; Guzzi and La Porta 2008). Intoxication can occur in all age groups; however, the risk is greater for workers exposed to mercury vapor (Faria 2003; Holmes et al. 2009). Activities such as mining; chlorine–alkali production; manufacturing of precision instruments, thermometers and fluorescent lamps, recycling lamps; and preparing dental amalgams are recognized sources of occupational exposure (Faria 2003; Magos and Clarkson 2006; Iavicoli et al. 2009).

In the metallic form, Hg has a high vapor pressure, and it is highly volatile, favoring its entrance via the airway. In the lungs, approximately 80% of the metal is absorbed into the alveoli and then transported through the blood to various organs, such as the thyroid, pituitary, kidneys, pancreas, gonads and especially the central nervous system, in which Hg exerts toxic effects (Ellingsen et al. 2000; Magos and Clarkson 2006).

According to the World Health Organization (2003), the urinary mercury concentrations expected in an asymptomatic population would be up to 10 µg/l. The American Conference of Governmental Industrial Hygienists recommends 20 µg/g of creatinine as an index for the maximum occupational biological exposure (ACGIH 2013).

Biological exposure indices were established from epidemiological and toxicological studies to prevent neurotoxic effects or renal damage in exposed workers. However, in addition to the neurotoxic and nephrotoxic actions, Hg can lead to endocrine changes (Zhu et al. 2000; Iavicoli et al. 2009; Tan et al. 2009; Rana et al. 2014).

Metals can induce toxicity in the endocrine gland by direct action, altering the synthesis, transporting protein, or interacting with hormone receptors, resulting in hormonal dysfunction. Nevertheless, the mechanisms of action are still not entirely known (Iavicoli et al., 2009; Rana et al. 2014).

Experiments in animals using organic and inorganic forms of Hg showed evidence of disorders of the iodine organification in the thyroid that were associated with functional and morphological changes of the gland (Zhu et al. 2000; Amorim et al. 2000; Tan et al. 2009). However, these experiments still do not allow the establishment of a dose–response curve or the extrapolation of the results for humans.

Studies in workers exposed to metallic Hg observed that the ratio of free thyroxine (FT4) to free triiodothyronine (FT3) was higher in the exposed group and there was a significant increase in reverse T3 serum (RT3) concentrations compared to the non-exposed group (Barregard et al. 1994; Ellingsen et al. 2000).

The high affinity of Hg for sulfhydryl groups can lead to the formation of complex bonds and result in inhibition of the function of selenoenzymes such as deiodinases (Sin et al. 1990; Ellingsen et al. 2000; Soldin et al. 2008). These enzymes play an important role in the conversion of thyroxine (T4) to triiodothyronine (T3), and selenium is an essential component of its structure (Choi et al. 2008).

In addition to effects on thyroid hormone function, some studies have evaluated the probability of the relationships between Hg exposure and the increase in thyroid nodules (Baccarelli et al. 2000; Bevenga et al. 2015).

Autopsy studies demonstrated high concentrations of Hg in the thyroids of retired mineworkers (Nylander and Weiner 1991; Björkman et al. 2007). However, evaluation of the thyroid parenchyma has not been analyzed in these studies.

The aim of this study was to evaluate the thyroid function and thyroid parenchyma aspects in workers chronically exposed to metallic mercury, on average, 14 years after their last exposure.

Materials and methods

1. Study design: A cross-sectional epidemiological study including 55 males who were submitted to an industrial exposure to Hg for a period longer than 1 year. The participants exposed to Hg were selected from the "Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo"—Brazil. The non-exposed group included only males who were paired to those in the exposed group by age; this group was composed of employees and relatives of the other patients of the Hospital. All participants underwent a structured interview, which emphasized sociodemographic context, medical and occupational histories, between 2016 and 2017. The interview included information about tobacco smoking, alcohol consumption, drug abuse, the number of amalgam dental fillings and previous personal or familial diseases.

The exclusion criteria were the use of medications such as amiodarone, lithium carbonate and corticosteroids; treatment with radioactive iodine therapy or other forms of radiation; treatment for thyroid disease; and a previous history of nephropathy or severe hepatopathy. Moreover, individuals with occupational exposure to other metals or pesticides, exposure to ionizing radiation, consumption of cocaine or cocaine derivatives, or alcohol abuse were excluded. Women were not included in the study because thyroid disorders are more frequent in females. The sample size was selected according to the studies of Ellingsen et al. (2000), considering a difference of 12% in the RT3 concentrations between the exposed and the non-exposed groups. The sample was calculated for a δ of 10%, a study power of 80% and an error type α equal to 5%.

A total of 78 male participants with past occupational exposure to Hg for more than 1 year fulfilled the study criteria. Among these participants, two progressed to death,

ten discontinued medical follow-up before starting the study, and 11 individuals were excluded due to thyroxine treatment for hypothyroidism (Fig. 1).

The non-exposed group was composed of 72 individuals. Among these participants, four were excluded due to alcohol and cocaine abuse, four due to pesticide and metal exposure, and two due to thyroxine treatment. In addition, seven interrupted their participation before completing the tests. The non-exposed group consisted of 55 individuals matched to the exposed group by age (Fig. 1).

A total of 110 individuals agreed to participate in the study and signed the informed consent form.

2. Laboratory analyses: The blood samples were obtained by a cubital venepuncture between 7:00 am and 9:00 am for measurement of the hormonal concentration, anti-thyroid peroxidase antibody (TPO Ab), anti-thyroglobulin antibody (Tg Ab) and selenium levels. For this respective analysis, the normality ranges were thyrotropin (TSH) (electrochemiluminescence assay) 0.27–4.20 µIU/ml; FT4 (electrochemical immune analysis), 0.93–1.70 ng/dl; T3 (electrochemical immune analysis), 80–200 ng/dl; FT3 (competitive chemiluminescent immunoassay), 0.24–0.37 ng/dl; RT3 (liquid chromatography coupled to tandem mass spectrometry), 8–25 ng/dl; TPO Ab [immunochemiluminometric assay (ICMA)], < 4 IU/ml; Tg Ab (ICMA), < 9 IU/ml; and selenium (graphite furnace atomic absorption spectrometry), 46–143 µg/l. The intra-assay and inter-assay coefficient (CV %) were as follows: TSH (6.4% and 7.5%); RT3 (level: 23.2 ng/dl: 4.9% and 7.2%); FT4 (2.8%, 2.7%); FT3 (level: 0.14 ng/dl: 6.6% and 8%; level 0.26: 2.6% and 5.1%, level 0.96: 5.1% and 1.3%); TPO Ab (5.7% and 5.1%); Tg Ab (7.6%,16.9%); and selenium (3.4% and 6.7%).

For the analysis of the iodine concentrations, isolated samples of urine were collected in a polyethylene container. The indirect detection method was used for the Sandell–Kolthoff reaction, with 100–299 µg/l as the range of sufficiency.

For the analysis of Hg, urine samples were collected in the morning in a polyethylene container, and they were kept under refrigeration until the time of analysis using the atomic absorption spectrophotometry method. The reference values for the non-exposed group were up to 5 µg/g of creatinine or up to 10 µg/l Hg. The limit of detection for this method was 0.4589 µg/l, the quantification limit adopted was 1.0 µg/l, and the intra-assay coefficient of variation (CV) was 3.77% (5.0 µg/l). Concentrations of urinary Hg during the period of work exposure were obtained from the hospital medical records. The weight and height of the individuals were verified on a calibrated scale (Filizola) that had a minimum load capacity of 2.5 kg, a maximum load capacity of 150 kg and intervals of 0.1 kg. Height (m) was verified in individuals without footwear. The body mass index (BMI) was obtained by the relation between weight (kg) and height in meters square (kg/m^2).

3. Ultrasonography: The ultrasound was performed in B-mode (brightness) with Doppler using the Phillips IU-22, Toshiba Aplio 500 and General Electric E9, with a high-frequency linear transducer (7–15 MHz). The examining physician, who had expertise in ultrasonog-

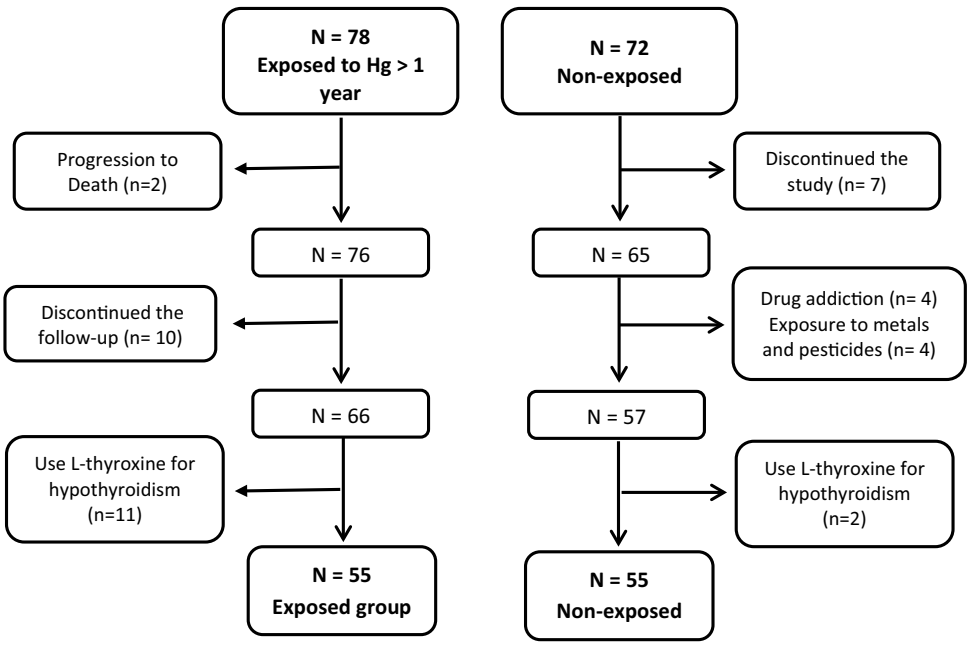

Fig. 1 Selection of the exposed and non-exposed individuals

raphy, did not know if the individual belonged to the exposed or non-exposed group. The evaluated aspects of the thyroid were location, dimensions (normal limits of 6–15 cm^3), contour of the gland, echogenicity, echotexture, vascularization of the parenchyma and identification of nodules. The echogenicity of the gland was analyzed by comparing it with the muscular tissues and with the submandibular gland, which was considered a normal standard gland that is slightly more echogenic than the adjacent structures. The echotexture was classified according to the homogeneous (normal), heterogeneous and finely heterogeneous aspects. The Doppler evaluated the thyroid vascularization. The analysis was subjective; it was considered normal when it was more limited to the points of the main arteries and glandular poles, and it was considered enlarged when it assumed a more diffuse distribution with accentuation of the vessels reflecting an inflammatory involvement of the parenchyma (Höfling et al. 2012). The characteristics of the nodules observed in the study were as follows: dimensions (longitudinal, transversal and anteroposterior; the largest axis was analyzed); volume (cm^3); location (right and left lobes, subdivided into upper third, middle third and lower third, and isthmus); type (solid, mixed and cystic); echogenicity (hypoechogenic, isoechogenic and hyperechogenic); contour (regular, or irregular); presence of halo; calcifications; vascularization; and the resistivity index (RI). Thyroid nodules were classified according to the Chammas et al. (2005) criteria. The criteria described by Haugen et al. (2016) and Chammas et al. (2005) suggested that the presence of predominantly central vascularization, a high resistivity index, the presence of microcalcifications, hypoechogenicity, and irregularity of the nodule margins were considered as suspicious characteristics of malignancy. All nodules with suspicious characteristics of malignancy were submitted to an ultrasound-guided fine-needle aspiration puncture (FNA) for cytological evaluation of the lesion. The analysis of the aspirated material was classified according to the Bethesda System (Cibas and Ali 2009).

4. The statistical analysis was performed with IBM SPSS Statistics software (IBM Corp., version 23.0, 2014, Armonk, New York, USA). The significance level assumed for the tests was $p < 0.05$. For the continuous variables, the results were expressed as the mean, standard deviation (± SD) and minimum and maximum values. Adherence to the normal curve was assessed through the Kolmogorov–Smirnov test. When the adherence was confirmed, the comparison of the means of the variables in the exposed and non-exposed groups was performed using the *t* test, and if the variables did not follow the Gaussian distribution, the Mann–Whitney *U* test was used. For the qualitative variables, the results were expressed in numbers and proportions using Pearson's Chi-square test (with a correction of continuity) or Fisher's exact test. The relationship between the pairs of variables was evaluated using Spearman's correlation method. The urinary Hg concentrations included in the Spearman's correlation analysis were those obtained during the occupational period in the exposed group and those of the non-exposed group ($N = 107$). For the risk quantification of the TSH increase, the univariate and multiple logistic regression models were used with the hormonal alteration status as a dependent variable. Each independent variable was added to the model separately, and when statistical significance was verified ($p < 0.05$), it was included in the multivariate model. The independent variables tested in the model were the condition of exposure to Hg (yes or no), iodine and selenium concentrations (grouped into quartiles), elevated titres of ATPO and ATG antibodies (yes or no), and grouped BMI values (≤ 25 kg/m^2 or higher). The urinary Hg concentrations were grouped into two classes (≤ 10 µg/l or > 10 µg/l) according to the value recommended by the World Health Organization (2003). Confounding variables were smoking habit (yes or no), alcohol consumption (servings per week) and age range. This regression model was also applied to analyze the odds ratio (OR) for the risk of elevated RT3 (above the third quartile). The confounding variables were age range, alcoholism and tobacco consumption. The independent variables included work duration, exposure to Hg (yes or no), urinary Hg (≤ 10 µg/l or 10 µg/l), iodine and selenium concentration (grouped into quartiles), Tg Ab and TPO Ab elevated titers (yes or no). The confidence interval analysis (CI) was 95%.

Results

The exposed group included 55 individuals with past industrial exposure to Hg for longer than 1 year. The average age of the participants was 55.2 years (SD ± 6.6 years) and ranged from 41 to 68 years. The majority of the employees in the exposed group (96.4%) worked in lamp manufacturing; the other individuals (3.6%) worked in the chlorine and alkali industry and thermometer manufacturing. The average work length period in the individuals exposed to Hg was 14.5 years (SD ± 7.26 years) and ranged from two to 36 years. The exposure to Hg in the exposed group had ceased an average of 14.2 years (SD ± 7.37 years) prior to when workers were evaluated in the study.

The non-exposed group consisted of 55 individuals who were matched to the exposed group by age. The average age in the non-exposed group was 53.2 years (SD ± 6.8 years)

and ranged from 41 to 70 years. There was no statistically significant difference in the average age between the groups ($p = 0.158$; Table 1).

The degree of schooling degree was not significantly associated with exposure according to Pearson's Chi-square test ($p = 0.08$). Smoking habits (current or past) were reported more often by the exposed individuals (56.4% versus 36.4%); however, the difference between the groups was not statistically significant ($\lambda^2 = 1.41$; $p = 0.23$). The average alcohol consumption (number of servings per week) was 4.50 (SD: ± 4.04) in the non-exposed group and 4.63 (SD: ± 3.66) in the exposed group; a statistically significant difference was not observed between the average weekly alcohol consumption ($p = 0.91$).

The BMI was elevated in both groups; the exposed group presented a mean BMI of 29 kg/m^2 (SD: ± 4.6 kg/m^2) and the non-exposed group presented a mean BMI of 27.8 kg/m^2 (SD: ± 4.4 kg/m^2). The *t* test did not show a significant difference between the groups ($p = 0.15$; Table 1).

Table 1 shows the urinary Hg concentrations obtained during the exposure period from medical records. After cessation of exposure, the urinary Hg concentrations were obtained between 2016 and 2017, along with the non-exposed group.

In the hospital medical records, results of urinary Hg analyses performed during the period of occupational exposure were found for 52 individuals. The comparison between the urinary Hg concentration means showed a statistically significant difference between exposed and non-exposed group ($p < 0.01$; Table 1).

After cessation of exposure, there was a significant reduction in the urinary Hg mean, reaching the value of 2.2 µg/l (SD 2.75 µg/l). In this period, urinary Hg concentrations were similar to those observed in the control group ($p = 0.82$; Fig. 2).

The comparison of the urinary Hg means during the exposure period and after the resignation from labor

Table 1 Mean and standard deviation (± SD) of age, body mass index (BMI), urinary mercury, thyroid hormones, selenium and iodine concentrations, stratified by exposure groups

Variables	Mean ± SD Range		*p* value
	Exposed to Hg ($N = 55$)	Non-exposed ($N = 55$)	
Age (years)	55.2 ± 6.61 41–68	53.4 ± 6.80 41–70	0.16[a]
BMI (kg/m^2)	29 ± 4.61 19.7–40.4	27.8 ± 4.37 17–38.9	0.15[a]
Hg (µg/l) work period	51.9 ± 40.13 2–180	1.8 ± 1.62 0.11–8.2	0.00[b]
Hg (µg/l) after exposition ceased	2.2 ± 2.75 0.22–17.3	–	–
TSH (µIU/ml)	3.31 ± 2.31 0.6–11.5	2.31 ± 1.07 0.9–5.6	0.03[b]
T3 (ng/dl)	122.3 ± 18.53 72–159	123.7 ± 19.36 86–188	0.69[a]
RT3 (ng/dl)	19.4 ± 4.59 12–33	17.6 ± 4.01 9–26	0.06[b]
FT3 (ng/dl)	0.35 ± 0.04 0.3–0.4	0.36 ± 0.04 0.3–0.5	0.18[a]
FT4 (ng/dl)	1.18 ± 0.2 0.8–1.6	1.20 ± 0.2 0.8–1.8	0.69[a]
FT4/FT3	3.33 ± 0.66 0.8–1.6	3.36 ± 0.65 2.2–5.4	0.79[a]
Iodine (µg/l)	203.8 ± 72.86 33–315	199.8 ± 81.77 60–446	0.79[a]
Selenium (µg/l)	77.4 ± 19.83 46–139	72.7 ± 15.02 33–109	0.45[b]

TSH thyrotropin, *T3* triiodothyronine, *RT3* reverse triiodothyronine, *FT3* free triiodothyronine, *FT4* free thyroxine, *FT4/FT3* ratio of free thyroxine:free triiodothyronine

[a]*p* value from *t* test

[b]*p* value from Mann–Whitney *U* test

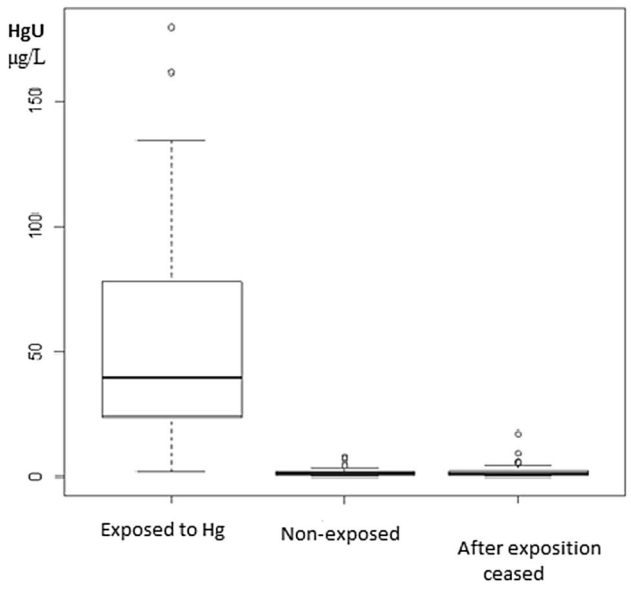

Fig. 2 Comparison of urinary mercury concentrations (µg/l) between exposed, non-exposed and after cessation of exposure

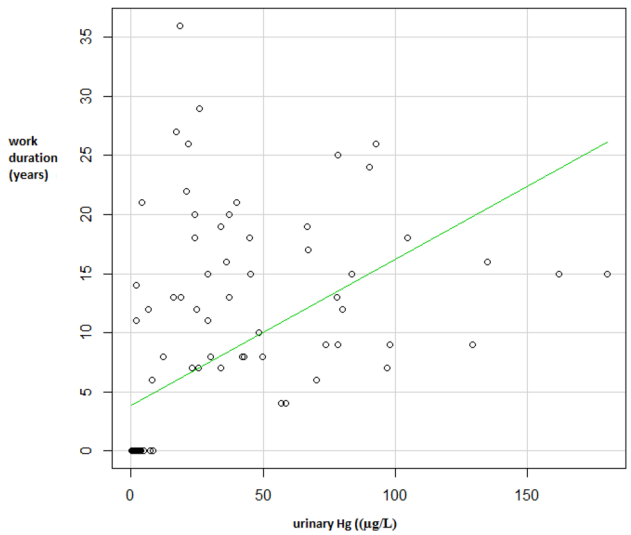

Fig. 3 Spearman correlation coefficient between urinary mercury (µg/l) and work duration (years)

activities also showed significant differences between the periods ($p < 0.001$).

Work duration showed a positive correlation with urinary Hg concentrations ($\rho = 0.776$; $p < 0.001$; Fig. 3).

The mean serum TSH concentration was significantly higher in the group exposed to Hg than in the non-exposed group ($p = 0.03$; Table 1). The hormone concentrations exceeded the limit of normality (4.20 µIU/ml) in 13 individuals exposed to Hg (27.3%) and in four non-exposed individuals (7.3%). Fisher's exact test showed an association that was statistically significant between the increase in TSH and the exposure to Hg ($p = 0.02$).

Spearman's correlation coefficients between serum TSH concentration and work duration showed a significant positive relationship ($\rho = 0.219$; $p = 0.01$). Regarding the variables age, BMI, tobacco consumption, alcohol consumption, elevated TPO Ab titres, iodine and selenium concentrations, the correlation coefficients were not statistically significant.

The RT3 concentration mean was slightly higher in the exposed group than in the non-exposed group; however, the significance of the difference between the groups was borderline ($p = 0.06$; Table 1). The serum RT3 concentrations showed a statistically significant positive Spearman correlation with urinary Hg concentration obtained during the exposure period ($\rho = 0.210$, $p < 0.05$).

There were no statistically significant differences between the means of the T3, FT3, and FT4 concentrations between the exposed and non-exposed groups. Only one individual exposed to Hg presented with T3 levels lower than the limit of normality (80 ng/dl).

The FT4 concentrations showed a significant negative correlation with the duration of work ($\rho = -0.336$, $p = 0.01$) and with the iodine concentrations ($\rho = -0.420$; $p = 0.02$).

There were no statistically significant differences between the means of blood selenium concentrations or urinary iodine in the exposed and non-exposed groups.

The proportion of participants with an elevated Tg Ab titre corresponded to 5.5% ($n = 3$) in the Hg-exposed group and 3.6% ($n = 2$) in the non-exposed group. The TPO Ab titre was altered in six individuals exposed to Hg (10.9%) and in two non-exposed individuals (3.6%). The proportion of elevated antibody titres did not show a statistically significant difference between the groups according to Fisher's exact test ($p = 0.27$).

The number of teeth with amalgam fillings did not show a statistically significant correlation with the concentrations of thyroid hormones, selenium, TPO Ab or elevated Tg Ab titres.

The logistic regression model was used to identify the risk of a serum TSH concentration elevated above 4.20 µIU/ml. The univariate model indicated that the elevated antibody titres (Tg Ab and TPO Ab) and urinary iodine independent variables presented statistically significant ORs. The variables associated with exposure, urinary Hg concentration (≤ 10 µg/l or > 10 µg/l), Hg exposure status (yes or no) and work duration (years) presented statistically significant ORs. In the multiple logistic regression model, the variables urinary Hg and Hg exposure presented a statistically significant OR ($p < 0.05$; Table 2). The other variables did not have predictive value in the multivariate model (Table 2). The Nagelkerke R^2 corresponded to 0.17.

The univariate logistic regression model for RT3 concentrations above the third quartile (21 ng/dl) showed

Table 2 Odds ratios for the risk of an elevated TSH concentration (> 4.20 µIU/ml) associated with urinary mercury, antibodies, tobacco consumption, work duration and exposure to mercury

Model	Variables	OR	95% CI		*p* value
			Inferior	Superior	
1	Urinary Hg	5.35	1.46	19.57	0.01
	Tg Ab	7.99	0.77	82.65	0.08
	TPO Ab	3.34	0.56	19.93	0.63
	Tobacco consumption (yes or no)	2.22	0.67	7.42	0.19
	Constant	0.03	–	–	0.00
2	Hg exposure (yes or no)	4.77	1.17	19.48	0.03
	Work duration (years)	0.62	0.16	2.39	0.49
	Tg Ab	6.11	0.66	56.93	0.11
	TPO Ab	3.34	0.56	19.93	0.19
	Constant	0.06	–	–	0.00

Urinary Hg: ≤ 10 µg/l or > 10 µg/l

OR odds ratio, *CI* 95% confidence interval, antibodies: *Tg Ab* anti-thyroglobulin antibody, *TPO Ab* anti-thyroid peroxidase antibody

Table 3 Odds ratio (OR) for the risk of an elevated RT3 concentration (above the third quartile; 21 ng/dl) associated with tobacco consumption, urinary mercury, work duration and autoimmunity markers

Variables	OR	95% CI		*p* value
		Inferior	Superior	
Tobacco consumption (yes or no)	1.52	0.58	3.96	0.39
Urinary Hg	3.34	1.03	10.82	0.04
TPO Ab	2.39	0.27	20.75	0.43
Tg Ab	0.00	0.00	–	0.10
Work duration (years)	0.53	0.16	1.75	0.30
Constant	0.18	–	–	0.00

Urinary Hg: ≤ 10 µg/l or > 10 µg/l

CI 95% confidence interval, *Tg Ab* anti-thyroglobulin antibody, *TPO Ab* anti-thyroid peroxidase antibody urinary

that the variables tobacco consumption, elevated TPO Ab and Tg Ab titres, urinary Hg concentrations (≤ 10 µg/l or > 10 µg/l), and work duration (years) presented statistically significant ORs.

In the multivariate model, only the urinary Hg variable had a statistically significant odds ratio (OR = 3.34, $p = 0.044$; Table 3). The Nagelkerke R^2 corresponded to 0.13.

All participants presented with thyroid enlargement at a topical location (Table 4). The proportion of thyroid enlargement was the same between exposed and non-exposed participants.

Reduced dimensions of the gland were observed in four exposed individuals (7.3%), and this alteration was not observed in the non-exposed group. In these subjects, the US Doppler showed a parenchyma with heterogeneous echotexture, the presence of echogenic crossbars and reduced vascularization.

The reduction of parenchyma echogenicity was observed in 15 exposed individuals (27.3%) and 5 non-exposed individuals (9.1%). Pearson's Chi-square test showed a statistically significant association between the reduction of echogenicity and exposure to Hg ($p = 0.026$; Table 4). Among the participants exposed to Hg with reduced echogenicity, only one presented with positive TPO Ab.

There was no statistically significant association between the exposure to Hg and the thyroid echotexture pattern or increased parenchyma vascularization (Table 4).

The presence of nodules was similar between the Hg exposed group and the non-exposed group; nodules were observed in 15 Hg exposed individuals and 16 non-exposed individuals. The dimensions of the nodules varied from 0.2 to 7.7 cm. A participant in the non-exposed group had the nodule with the largest dimensions and volume. The majority of the nodules presented with dimensions smaller than 1 cm (67.7%) in both groups.

Considering the echography aspects of the nodules suggesting malignancy, in accordance with the criteria described by Haugen et al. (2016) and Chammas et al. (2005), four individuals who were exposed to Hg (7.2%) and two non-exposed individuals (3.6%) presented with characteristics suspicious for malignancy.

A fine-needle aspiration puncture (FNA) under US guidance was indicated as a complementary investigation for these suspicious nodules. The cytological analysis of the nodule samples indicated Bethesda class V (lesion suspicious for malignancy) in three individuals exposed to Hg and one non-exposed individual. In addition, in one individual exposed to Hg and one non-exposed individual, the cytological analysis revealed Bethesda class II (benign nodule). All the participants who presented with a Bethesda class V, as well as the non-exposed individual who presented a nodule with a 7.7-cm dimension, were referred to surgery.

Table 4 Characteristics of the thyroid ultrasound in the exposed and non-exposed groups

Characteristics	Exposed		Non-exposed		Total		*p* value
	N	%	*N*	%	*N*	%	
Topical location	55	100	55	100	55	100	–
Regular dimension	38	69.1	42	76.4	80	72.7	–
Enlarged dimension	13	23.6	13	23.6	26	23.6	
Reduced dimension	4	7.3	–	–	4	3.6	
Irregular contour	34	61.8	38	69.1	72	65.5	0.42
Irregular contour	21	38.2	17	30.9	38	34.5	
Regular echogenicity	40	72.7	50	90.9	90	81.8	0.03
Reduced echogenicity	15	27.3	5	9.1	20	18.2	
Regular echotexture	12	21.8	14	25.5	26	23.6	0.30
Finely heterogeneous echotexture	31	56.4	35	63.6	66	60.0	
Heterogeneous echotexture	12	21.8	6	10.9	18	16.4	
Regular vascularization	32	58.2	34	61.8	66	60.0	0.85
Increased vascularization	23	41.8	21	38.2	44	40.0	
Reduced vascularization	2	3.6	–	–	2	1.8	–
Total	55	100	55	100	110	100	–

p value: Pearson's Chi-square test

The thyroid anatomopathological assessment demonstrated the presence of papillary thyroid carcinoma in all nodules classified as Bethesda V-rated lesions on FNA. The pathology of the 7.7 cm nodule revealed the presence of invasive follicular carcinoma.

Considering the small number of cases, it was not possible to apply statistical tests for comparisons.

Discussion

In addition to deposits in the central nervous system, metallic Hg may also accumulate in the thyroid gland for a long time, even after exposure has ceased (Falnoga et al. 2006).

This study evaluated thyroid hormones and echographic aspects of the thyroid gland in workers exposed to Hg many years after the end of occupational exposure activity. The hypothesis is that the toxic effects of the metal may be delayed by its deposition in the thyroid gland for a long time (Nylander and Weiner 1991; Björkman et al. 2007).

The exposed and non-exposed groups presented with similar age, BMI, degree of schooling, alcohol consumption and tobacco consumption.

Labor activities in the lamp, chlorine–alkali and thermometer manufacturing industries, such as those observed in this study, are recognized for their risk of exposure to Hg (Holmes et al. 2009).

Studies on lamp manufacturing in São Paulo, State of Brazil, described occupational risks that contributed to Hg exposure (Zavariz and Glina 1993). The authors reported frequent lamp fragmentation with consequent release of Hg in the manufacturing area. In addition, the accumulation of drops of Hg on the floor under the machines was associated with elevated temperatures favoring Hg evaporation into the atmosphere (Zavariz and Glina 1993). The measured environmental Hg in the manufacturing area was not in accordance with the occupational hygiene recommendation. Most of the individuals exposed to Hg who were evaluated in the present study (96%) worked in these lamp factories of São Paulo.

In this study, the average urinary Hg concentration was significantly higher in the exposed group compared to the non-exposed group ($p < 0.01$). In addition to the elevated urinary Hg concentration, the length of the average time of exposure was considerably long (14.5 years), similar to the observations in previous studies (Barregard et al. 1994; Ellingsen et al. 2000). Supporting the exposure to Hg, there was a positive correlation between urinary Hg and work duration ($p < 0.05$). The long period of exposure to Hg may favor the deposition of the metal in the thyroid gland.

The thyroid has high concentrations of selenium due to its role in the constitution of deiodinases and in the synthesis of hormones (Köhrle and Gärtner 2009). The high affinity of Hg for selenium may favor its deposition in the gland (Hansen 1988; Bulato et al. 2007; Zemolin et al. 2012).

No statistically significant difference was observed in selenium concentrations between the exposed and non-exposed groups ($p > 0.05$). However, the workers had not been subjected to Hg exposure for, on average, 14.2 years. Although the selenium concentration was considered to be in the normal range, the formation of selenium and mercury complexes may affect the bioavailability and function of the micronutrient and thus alter the function of deiodinases (Bulato et al. 2007; Soldin et al. 2008; Mulder et al. 2012).

The high mean TSH concentrations in the Hg-exposed individuals were one of the main findings of this study.

The proportion of individuals with TSH concentrations above the normal limit was also significantly higher in the exposed group ($p < 0.05$). In addition, serum concentrations of TSH and duration of work exhibited a subtle but statistically significant positive correlation ($\rho = 0.219$; $p = 0.012$).

During the group selection process, it was observed that the proportion of individuals with T4 treatment was higher in the exposed group (14% versus 2.8%), adding evidence on the possible impact of Hg exposure on the thyroid function.

Afrifa et al. (2018) observed a slightly elevated TSH among gold miners, but this association was not statistically significant. However, because all participants worked in the mining area, it was not possible to obtain a pure non-exposed group. Abdelouahab et al. (2008) observed a significant positive relationship between serum TSH and blood Hg concentration in individuals with elevated fish intake. However, fish consumers could be exposed to an organic Hg form.

Metallic Hg might have an effect on the thyroid gland through the inhibition of deiodinases (Ellingsen et al. 2000). Barregard et al. (1994) observed an alteration in the FT4:FT3 ratio due to a decrease in FT3 levels. These enzymes play an important role in the conversion of T4 to T3 in organs and in the anterior pituitary (Barregard et al. 1994; Ellingsen et al. 2000; Mori et al. 2007). Reducing T3 may lead to pituitary stimulation and increased TSH secretion (Benvenga et al. 2015).

In addition to this effect on deiodinases, studies in rainbow trout (*Oncorhynchus mykiss*) exposed to sub-lethal concentrations of inorganic and organic Hg verified that the metal can stimulate the pituitary–thyroid axis (Bleau et al. 1996).

Sun et al. (2018) found a significant increase in TSHβ and mRNA expression in zebrafish larvae treated with mercury chloride ($HgCl_2$) and considered that the metal may have a disruptive effect on the hypothalamic–pituitary axis. The authors observed changes in the expression of genes linked to the synthesis of hormones. Nevertheless, these studies still do not support conclusions regarding whether these effects of Hg may occur in the pituitary gland in humans. Further studies may contribute to understanding whether the metallic form of Hg could cause these alterations in humans.

Other possible explanations for the TSH elevation include autoimmune thyroiditis leading to functional impairment of the gland and an increase of the TSH (Höfling et al. 2012). However, in this study, the proportion of positive antibodies did not show a statistically significant difference between the groups. Additionally, the multiple logistic regression model did not present a statistically significant OR for this variable.

The action of Hg on the deiodinases may lead to a reduction in the conversion of T4 to T3, with a consequent increase in RT3 (Ellingsen et al., 2000). These authors observed a significant increase of RT3 that was associated with the cumulative concentration of Hg among the workers exposed to Hg.

The present study showed a borderline significant difference in the mean RT3 concentration between the groups. However, Spearman's correlation coefficient showed a positive relationship between RT3 and urinary Hg concentrations ($p < 0.05$). In addition, the multiple logistic regression model showed that the urinary Hg variable showed predictive value for the RT3 concentration being above the third quartile ($p < 0.05$). These results corroborate the possibility that the tendency of RT3 being higher in exposed individuals is related to the effects of Hg.

The means of the T3, FT3 and FT4 concentrations were similar between the exposed and non-exposed groups. Different results were observed in a study with mothers and newborns that compared groups with a higher or lower number of amalgam restorations and the distance from the residences to the industrial areas (Ursinyova et al. 2012; Takser et al. 2005). The authors concluded that, even at low levels of Hg exposure, thyroid hormones could be affected (Ursinyova et al. 2012; Takser et al. 2005). However, these surveys involved larger samples.

The contribution of this study was the addition of Doppler US to evaluate the characteristics of the thyroid parenchyma and the nodules. Previous studies did not include evaluation of the thyroid gland parenchyma. Doppler US offers high accuracy in the morphological evaluation of the gland, and it is associated with a low-cost advantage.

The proportion of the echogenicity alteration was higher in the Hg-exposed group, with a statistically significant association with the exposure ($p < 0.05$).

The echogenicity reduction aspect can be related to the inflammatory process, usually observed in autoimmune thyroiditis (Höfling et al. 2012). However, a statistically significant correlation between the TPO Ab and the hypoechogenicity pattern was not observed in the Hg-exposed group ($p > 0.05$).

In experimental studies, Shi et al. (2018) observed that the amphibian thyroid *(Bufo gargarizans)*, treated with $HgCl_2$ presented with a loss of follicular cell layers, cellular disarrangement and follicular hyperplasia of the thyroid. At doses above 18 µg/l, gland deformity and a reduction of deiodinases and mRNA levels of α and β hormone receptors were observed. Further studies are necessary to investigate whether the deposition of Hg in metallic form could lead to these structural changes in the human gland.

Nylander and Weiner (1991), in necropsy studies of dentistry staff, observed a very high concentration of Hg in the thyroid, even after the professionals stopped working. These findings reinforce the importance of evaluating the thyroid parenchyma.

The presence of thyroid nodules identified by US was similar between the groups. Moreover, it was not possible to establish an association between the presence of malignant nodules and Hg exposure in the present study.

According to Benvenga et al. (2015), the increased incidence of thyroid nodules in the Beijing and Guangzhou populations could be related to increased environmental pollution. Among the pollutants, Hg was identified as one of the possible agents related to thyroid alterations.

Chung et al. (2016) did not observe an association between Hg concentrations and thyroid malignant nodules in anatomopathological examination from patients submitted to surgery. However, these individuals were not occupationally exposed, and Hg concentrations tended to be higher in exposed workers.

Malandrino et al. (2016) reported an increased incidence of papillary cancer in volcanic region residents. The authors observed that metal concentrations, including Hg, were several times higher in residents of these regions. The authors discuss the possibility of these metals being related to the increased incidence of papillary cancer.

The limitations of this present study were related to the Hg concentrations acquired from the period of occupational exposure in the hospital's medical records; it was not possible to access all the results of this period. According to the Brazilian labor legislation, this examination must be performed every 6 months during the period of occupational exposure. However, no 6-month results were available. In addition, the examinations were performed in different laboratories, and no standardization was possible.

Beyond these issues, it was also not possible to investigate the Hg concentrations in the air of the work environments.

The increase of TSH concentrations in exposed individuals is a new finding.

Review studies reported that the level of TSH was an independent predictor of malignancy in thyroid nodules (Popoveniuc and Jonklaas 2012). Chronic high TSH secretion predisposes the thyroid gland to develop hyperplastic and neoplastic lesions by epigenetic mechanisms associated with hormonal disequilibrium. (Rana 2014). These results indicate the relevance of evaluating thyroid function in exposed workers for a long period. Current knowledge about metal endocrine disruptor effects (Iavicoli et al. 2009) is still limited, and there are few studies on occupational exposure to establish the biological limits for this purpose.

Conclusions

The study revealed that individuals with chronic occupational exposure to Hg presented hormonal and thyroid parenchyma alterations, even after the work activity ceased. In three exposed individuals, malignant nodules were identified. These findings show the importance of evaluating thyroid hormones in workers exposed to Hg for a long time, even after the cessation of exposure. Additional studies are needed to establish non-toxic biological indices for the thyroid gland of exposed workers.

Acknowledgements The study presented here was financed by the Ministério Público do Trabalho (Brazilian Public Ministry Labor). The funding organization did not participate in the study design, collection and analysis of the manuscript data. We would like to thank Professor Marcilia Medrado Araujo Faria (in memory) for her care with the participants of this study and her contribution to the development of the research at Serviço de Saúde Ocupacional do Hospital das Clínicas.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest.

Ethics approval and consent to participate All participants entered the study on a voluntary basis. Participants were fully informed about the purpose, procedures, risks, and benefits of participating in this study. Informed consent was obtained from all individual participants included in the study. The research project was approved by the Ethics and Research Committee from Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (inscription no. 12.438/HC-FMUSP).

References

Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M et al (2008) Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of Quebec (Canada). Environ Res 107(3):380–392

Afrifa J, Ogbordjor WD, Duku-Takyi R (2018) Variation in thyroid hormone levels is associated with elevated blood mercury levels among artisanal small-scale miners in Ghana. Plos One 13(8):e0203335. https://doi.org/10.1371/journal.pone.0203335

American Conference of Governmental Industrial Hygienists (2013) Mercury, elemental. documentation of the threshold limit values and biological exposure indices, vol 7. ACGIH®, Cincinnati

Amorim MIM, Mergler D, Bahia MO, Dubeau H, Miranda D, Lebel J et al (2000) Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An Acad Bras Ciênc 72(4):497–507

Baccarelli A, Pesatori AC, Bertazzi PA (2000) Occupational and environmental agents as endocrine disruptors: experimental and human evidence. J Endocrinol Investig 23(11):771–781

Barregård L, Lindstedt G, Schütz A, Sällsten G (1994) Endocrine function in mercury exposed chloralkali workers. Occup Environ Med 51(8):536–540

Benvenga S, Antonelli A, Vita R (2015) Thyroid nodules and thyroid autoimmunity in the context of environmental pollution. Rev Endocr Metab Disord 16(4):319–340

Björkman L, Lundekvam BF, Laegreid T, Bertelsen BI, Morild I, Lilleng P et al (2007) Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study. Environ Health 6:30

Bleau H, Daniel C, Chevalier G, van Tra H, Hontela A (1996) Effects of acute exposure to mercury chloride and methylmercury on plasma cortisol, T3, T4, glucose and liver glycogen in rainbow trout (Oncorhynchus mykiss). Aquat Toxicol 34(3):221–235

Bulato C, Bosello V, Ursini F, Maiorino M (2007) Effect of mercury on selenium utilization and selenoperoxidase activity in LNCaP cells. Free Radic Biol Med 42(1):118–123
Chammas MC, Gerhard R, de Oliveira IR, Widman A, de Barros N, Durazzo M et al (2005) Thyroid nodules: evaluation with power Doppler and duplex Doppler ultrasound. Otolaryngol Head Neck Surg 132(6):874–882
Choi AL, Budtz-Jørgensen E, Jørgensen PJ, Steuerwald U, Debes F, Weihe P et al (2008) Selenium as a potential protective factor against mercury developmental neurotoxicity. Environ Res 107(1):45–52
Chung HK, Nam JS, Ahn CW, Lee YS, Kim KR (2016) Some elements in thyroid tissue are associated with more advanced stage of thyroid cancer in Korean women. Biol Trace Elem Res 171(1):54–62
Cibas ES, Ali SZ (2009) The Bethesda system for reporting thyroid cytopathology. Thyroid 19(11):1159–1165
Ellingsen DG, Efskind J, Haug E, Thomassen Y, Martinsen I, Gaarder PI (2000) Effects of low mercury vapour exposure on the thyroid function in chloralkali workers. J Appl Toxicol 20(6):483–489
Falnoga I, Tusek-Znidaric M, Stegnar P (2006) The influence of long-term mercury exposure on selenium availability in tissues: an evaluation of data. Biometals 19(3):283–294
Faria MA (2003) Chronic occupational metallic mercurialism. Rev Saude Publica 37(1):116–127
Guzzi G, La Porta CA (2008) Molecular mechanisms triggered by mercury. Toxicology 244(1):1–12
Hansen JC (1988) Has selenium a beneficial role in human exposure to inorganic mercury? Med Hypotheses 25(1):45–53
Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE et al (2016) 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 26(1):1133
Höfling DB, Chavantes MC, Juliano AG, Cerri GG, Knobel M, Yoshimura EM et al (2012) Assessment of the effects of low-level laser therapy on the thyroid vascularization of patients with autoimmune hypothyroidism by color Doppler ultrasound. Endocrinology 2012:126720
Holmes P, James KA, Levy LS (2009) Is low-level environmental mercury exposure of concern to human health? Sci Total Environ 408(2):171–182
Iavicoli I, Fontana L, Bergamaschi A (2009) The effects of metals as endocrine disruptors. J Toxicol Environ Health B Crit Rev 12(3):206–236
Köhrle J, Gärtner R (2009) Selenium and thyroid. Best Pract Res Clin Endocrinol Metab 23(6):815–827
Magos L, Clarkson TW (2006) Overview of the clinical toxicity of mercury. Ann Clin Biochem 43(Pt 4):257–268
Malandrino P, Russo M, Ronchi A, Minoia C, Cataldo D, Regalbuto C et al (2016) Increased thyroid cancer incidence in a basaltic volcanic area is associated with non-anthropogenic pollution and biocontamination. Endocrine 53(2):471–479
Mori K, Yoshida K, Nakagawa Y, Hoshikawa S, Ozaki H, Ito S (2007) Methylmercury inhibition of type II 5'-deiodinase activity resulting in a decrease in growth hormone production in GH3 cells. Toxicol 237(1–3):203–209
Mulder PJ, Lie E, Eggen GS, Ciesielski TM, Berg T, Skaare JU et al (2012) Mercury in molar excess of selenium interferes with thyroid hormone function in free-ranging freshwater fish. Environ Sci Technol 46(16):9027–9037
Nylander M, Weiner J (1991) Mercury and selenium concentrations and their interrelations in organs from dental staff and the general population. Br J Ind Med 48(11):729–734
Popoveniuc G, Jonklaas J (2012) Thyroid nodules. Med Clin N Am 96(2):329–349
Rana SV (2014) Perspectives in endocrine toxicity of heavy metals—a review. Biol Trace Elem Res 160(1):1–14
Shi Q, Sun N, Kou H, Wang H, Zhao H (2018) Chronic effects of mercury on *Bufo gargarizans* larvae: thyroid disruption, liver damage, oxidative stress and lipid metabolism disorder. Ecotoxicol Environ Saf 164:500–509
Sin YM, Teh WF, Wong MK, Reddy PK (1990) Effect of mercury on glutathione and thyroid hormones. Bull Environ Contam Toxicol 44(4):616–622
Soldin OP, O'Mara DM, Aschner M (2008) Thyroid hormones and methylmercury toxicity. Biol Trace Elem Res 126(1–3):1–12
Sun YL, Li YW, Liu ZH, Chen QL (2018) Environmentally relevant concentrations of mercury exposure alter thyroid hormone levels and gene expression in the hypothalamic-pituitary thyroid axis of zebrafish larvae. Fish Physiol Biochem 44(4):1175–1183
Takser L, Mergler D, Baldwin M, de Grosbois S, Smargiassi A, Lafond J (2005) Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environ Health Perspect 113(8):1039–1045
Tan SW, Meiller JC, Mahaffey KR (2009) The endocrine effects of mercury in humans and wildlife. Crit Rev Toxicol 39(3):228–269
Ursinyova M, Uhnakova I, Serbin R, Masanova V, Husekova Z, Wsolova L (2012) The relation between human exposure to mercury and thyroid hormone status. Biol Trace Elem Res 148(3):281–291
World Health Organization (2003) Elemental mercury and inorganic mercury compounds: human health aspects. WHO, Geneva
Zavariz C, Glina DMR (1993) Effects of occupational exposure to mercury in workers at a light bulb factory in Santo Amaro, São Paulo. Brazil. Cad Saúde Pública (Rio de Janeiro) 9(2):117–129
Zemolin AP, Meinerz DF, de Paula MT, Mariano DO, Rocha JB, Pereira AB et al (2012) Evidences for a role of glutathione peroxidase 4 (GPx4) in methylmercury induced neurotoxicity in vivo. Toxicology 302(1):60–67
Zhu X, Kusaka Y, Sato K, Zhang Q (2000) The endocrine disruptive effects of mercury. Environ Health Prev Med 4(4):174–183

Affiliations

M. M. Correia[1] · M. C. Chammas[2] · J. D. Zavariz[2] · A. Arata[3] · L. C. Martins[4] · S. Marui[5] · L. A. A. Pereira[6]

M. C. Chammas
cristina.chammas@hc.fm.usp.br

J. D. Zavariz
juliazavariz@hotmail.com

A. Arata
ane.arata@gmail.com

L. C. Martins
lourdesc@unisantos.br

S. Marui
suemimar@usp.br; suemi.marui@hc.fm.usp.br

L. A. A. Pereira
pereira.aa@gmail.com

[1] Serviço de Saúde Ocupacional do Departamento de Medicina Legal, Ética Médica, Medicina Social e do Trabalho da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

[2] Serviço de Ultrassom do Instituto de Radiologia da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

[3] Nuclear and Energy Research Institute, Department of Materials Science and Technology Center (CCTM), São Paulo, Brazil

[4] Programa de Pós-graduação da Universidade de Santos (UNISA), Santos, São Paulo, Brazil

[5] Disciplina de Endocrinologia e Metabologia da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

[6] Programa de Pós-graduação da Disciplina de Patologia da Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil