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STUDY OF THE TOTAL MERCURY CONTENT IN VARIOUS MEDIA RELEVANT TO HUMAN HEALTH AND THE ENVIRONMENT

DISSERTATION SUMMARY
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The dissertation contains 121 pages and is illustrated with 39 tables, 15 figures and a bibliography of 214 references.

CONTENTS

LIST OF ABBREVIATIONS

EFSA	European Food Safety Authority
MPC	Maximum permissible concentration
WWTP	Waste water treatment plant
EQS	Environmental Quality Standard
CRM	Certified reference material
CTS	Certified test sample
DMA	Direct mercury analyzer
EPA	Environmental Protection Agency (USA)
Hg	Mercury
HNO_3	Nitric acid
IMEP	International Measurement Evaluation Programme
IRMM, IRMM-JRC-EC	Institute for Reference Materials and Measurements
$K_2Cr_2O_7$	Potassium dichromate
LOD	Limit of detection
LOQ	Limit of quantification
MV	Maximum value
PE	Polyethylene
PP	Polypropylene
PS	Polystyrene
PT	Proficiency testing
PVC	Polyvinyl chloride
RSD_r	Coefficient of variation in repeatability conditions
RSD_R	Coefficient of variation under reproducibility conditions
UNEP	United Nations Environment Programme
WHO	World Health Organization

I. INTRODUCTION

Mercury (Hg) is an extremely cytotoxic, neurotoxic, immunotoxic, endocrine, inflammatory and reproductive toxin causing chronic neurological, immune and autoimmune, cardiovascular, hormonal, oral and reproductive diseases. Mercury is a risk factor for a number of diseases according to some authors: Alzheimer's, autism, neurological diseases and epilepsy, thyroid disorders, multiple sclerosis, etc.

Since 2003, when mercury was declared a global pollutant by the United Nations Environment Programme (UNEP) Governing Council due to its adverse effects on human health and ecosystems, a process of international cooperation to reduce its harmful effects has been underway. The main problem for humans is related to the transboundary nature of Hg contamination, which requires mandatory action at local, regional, national and international levels.

Mercury is a non-essential element identified by UNEP and the World Health Organisation (WHO) as one of the ten most toxic chemicals of particular concern to human health, for which there are no safe levels of exposure. Due to its widespread use, mobility and high toxicity, mercury causes serious environmental pollution. When it enters the environment, it participates in processes of biotransformation, migration and bioaccumulation, that's why it is classified by the European legislationas a priority pollutant and requirements for monitoring and control of the concentrations of this element in various environments are imposed.

To address the issue of mercury, the Minamata Convention was adopted in October 2013 and entered into force in 2017 after being ratified by 50 countries, incl. Bulgaria, to ensure the protection of human health and the environment from emissions of anthropogenic origin and releases of mercury and mercury compounds into the air, water and soil. The Convention regulates the entire life cycle of mercury by introducing restrictions and requirements for the primary extraction, production and trade of mercury and mercury-added products and the environmentally sound management of mercury waste. To control the risk from the use of mercury and its compounds worldwide, harmonised measures were introduced to prevent and limit human and environmental exposure and coordinated action to address mercury use and trade at global level.

To guarantee a high level of protection of human health and the environment from anthropogenic emissions and discharges of mercury and mercury compounds, Regulation (EU) 2017/852 of the European Parliament and of the Council on mercury was adopted in 2017, aligning EU legislation with the international rules set out in the Minamata Convention.

The quantification of mercury in different media (cosmetic products, food and food supplements, water, soil, sludge, polymeric materials and articles, etc.) and their use data are the basis for the assessment of the health risk arising from the intake of this toxic element.

II. AIM AND TASKS

The aim of the study is to optimize and validate methods for determining the total mercury content by means of a direct mercury analyzer of solid and liquid samples and to apply the obtained results to the assessment of the safety of various media relevant to human health and the environment**.**

Tasks

1. Optimization and validation ot methods for the determination of total mercury content by means of a direct mercury analyzer DMA-80 in cosmetics, food, waters, soil and sludges, and polymeric materials.

2. Study of the total mercury content in different matrices relevant to human health and the environment:

2.1. Mercury content in cosmetic products.

2.2. Mercury content in food, food supplements and food additives.

2.3. Mercury content in waters.

2.4. Mercury content in soils.

2.5. Mercury content of sewage sludges.

2.6. Mercury content in polymeric products.

3. Summarising, systematising and evaluating the data obtained on the level of mercury contamination of the various media and assessing their safety in terms of human health and the environment.

III. MATERIALS AND METHODS

Environments, tested for mercury content

2583 samples from different media were analysed:

- Cosmetics - 1051 samples;
- Food, food supplements and food additives - 227 samples;
- Drinking water - 998 samples;
- Soils - 104 samples;
- Sludges - 109 samples;
- Articles of polymer materials - 94 samples.

Certified Reference Materials (CRMs) and participation in JRC IRMM Proficiency testings (PTs) were used for calibration and quality control of analyses for the optimization, validation and application of methods for the determination of mercury in various matrices.

Methods used

EPA 7473 'Method for the direct determination of mercury in solid and liquid samples' by DMA-80, Milestone

The method was developed specifically for the DMA-80 and is based on controlled heating in an oxygen environment of solid and liquid samples in a quartz tube. The sample is dried and then thermally and chemically decomposed. The decomposition products are carried by an oxygen stream to the catalytic section of the quartz tube. Halogens, nitrogen and sulfur oxides are captured by a hot catalyst bed. The remaining decomposition products are transferred to an amalgamator that selectively captures Hg. After the system is purged with oxygen to remove any remaining gases or decomposition products, the amalgamator is heated rapidly, releasing mercury vapor. The oxygen flow carries the mercury vapors through absorption cells located in the light path of a fixed wavelength atomic absorption spectrometer. Absorbance (peak height or peak area) is measured at 253.7 nm as a function of mercury concentration.

The total calibration range of the DMA-80 is (0.08 - 600) ng Hg, composed of a low (0.08 - 40) ng Hg and a high (40 ÷ 600) ng Hg range.
A calibration curve is created for each of the two cuvettes connected in series. The first (long) cuvette is for measuring low levels of mercury (up to about 40 ng), the second (short) cuvette is for measuring high levels of mercury (up to about 600 ng). The measurement is performed in both cuvettes. If the maximum of the first signal is above 0.8 A, the second signal is automatically used to calculate the result.

Calibration of the DMA-80 provides long-term reliability due to the stability of the system and the relatively long life of the catalyst tube and amalgamator, eliminating the daily calibrations often required by conventional instruments.
With the application of EPA 7473 "Method for the Direct Determination of Mercury in Solid and Liquid Samples" by DMA-80, Milestone, accurate and reproducible results were obtained, in a short time and without prior sample preparation.
The Descriptive Statistics application in Microsoft Excel was used for statistical processing and analyses of the results.

Apparatus

For the determination of mercury concentrations in different samples, a direct analyzer of solid, liquid and gas samples DMA 80, Milestone, an integrated system based on the principle of atomic absorption spectrometry was used. The instrument contains two measuring cells (for low and for high concentration range), a mercury lamp and a detector. The measurement is performed at 253.7 nm resonance line for mercury. Oxygen as the carrier gas is used, with an inlet pressure of 4 bar and a flow rate of 200 ml/min.

IV. RESULTS AND DISCUSSION

Optimization and validation of EPA 7473 method for mercury determination in various media with direct mercury analyzer DMA-80

The analytical conditions of DMA-80 for the determination of mercury in different matrices were adapted and modified according to the specificity of the investigated samples.
When optimising the analytical conditions for the determination of mercury in different media with the DMA-80, those conditions were selected where the results obtained were in best match with the mercury values of the respective CRMs with which the optimisation was performed.
The validation of the methods for the determination of total mercury in different media with the direct mercury analyser DMA-80 was performed in accordance with BDS EN ISO/IEC 17025 on the following parameters:

- *limit of detection (LOD) and limit of quantification (LOQ)*

The method blank was measured 90 times (10 % nitric acid, empty sample boat, DMA-80) with a calibration curve in the range (0.08 - 40) ng Hg. LOD (3б) and LOQ (6б) were 0.04 ng and 0.08 ng, respectively.
The LOD and LOQ for individual sample types from various media are different, depending on the amount of sample being digested.

- *measurement interval*

The working range of the method is (0.08 - 600) ng Hg and includes two ranges: low (0.08 - 40) ng Hg and high (40 ÷ 600) ng Hg.

- *linearity*

Working calibration curves were obtained with working standard solutions in one of the two concentration ranges: low (0.0008 - 0.40) mg Hg/kg and high (0.40 - 6.0) mg Hg/kg by appropriate dilution from the CRM for mercury with a concentration of 1000 mg Hg/l (Figure 1). The correlation coefficient of the resulting standard straight line should be r > 0.995. There is no need to recalibrate the device each time, but the calibration curve should be checked before starting operation. The check shall be performed by measuring working standard solutions. The deviation of the concentrations of the control standard solutions for checking the stability of the instrument calibration shall be within ± 10 %.

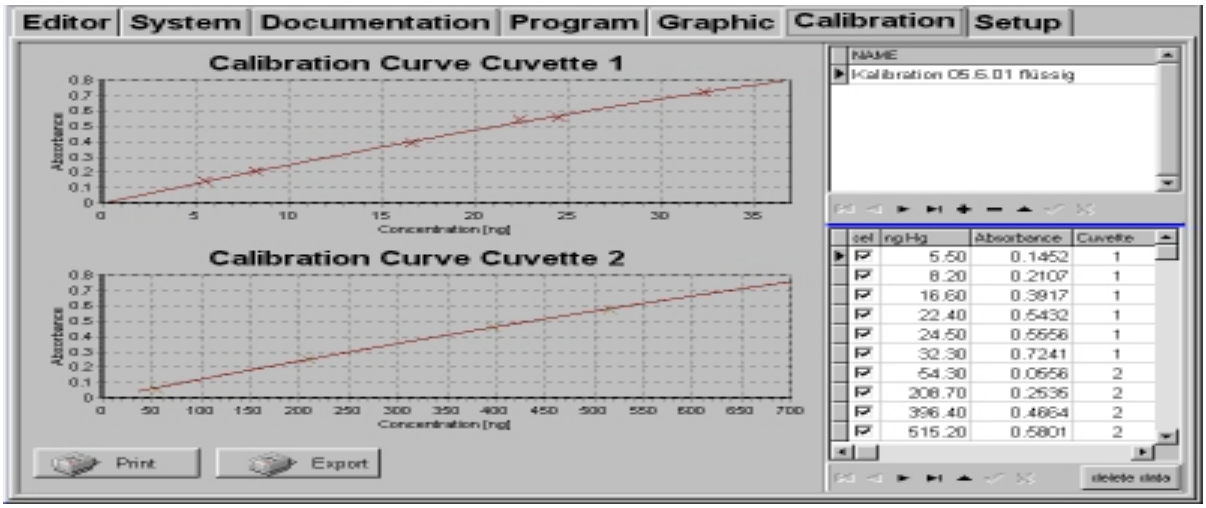

Fig. 1. Calibration curves for the first and second cuvettes installed in the DMA-80

The peak of the mercury absorption signal, in real time is shown in Figure 2 and allows direct tracking of the measurement.The two cuvettes are arranged in series in the DMA-80. Two different peaks come out for each sample due to their different length.

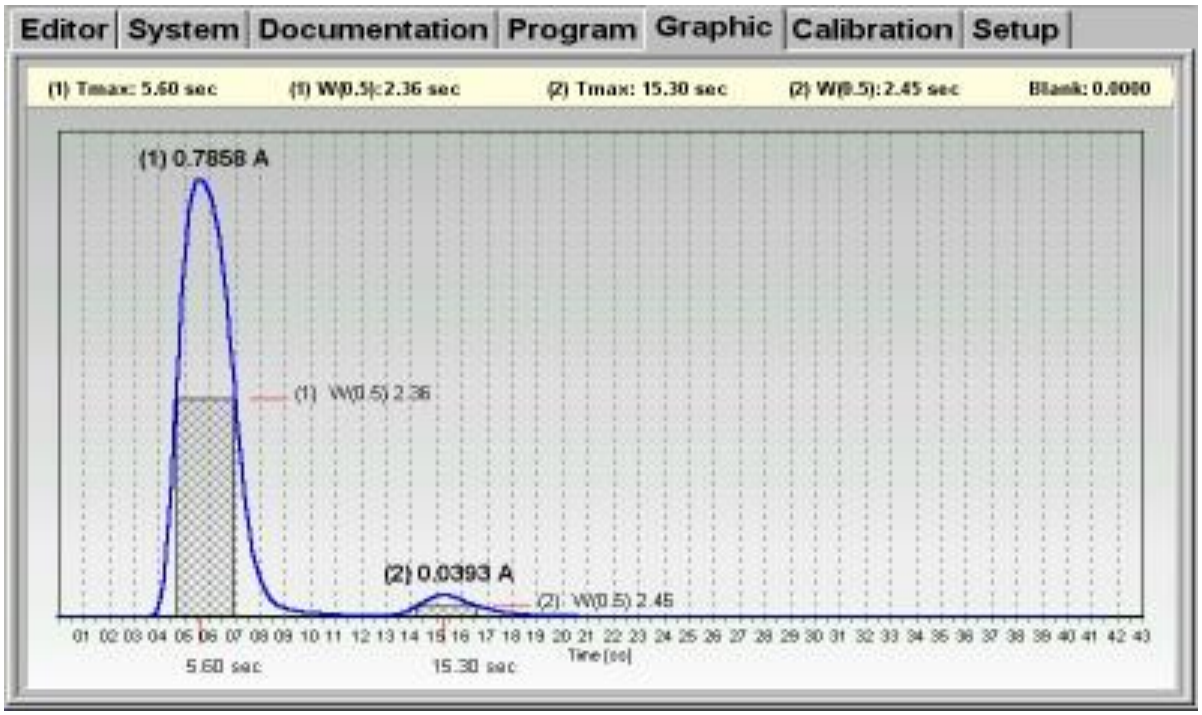

Fig. 2. Absorption signals for mercury in the two cuvettes connected in series

Optimization and validation of the *EPA 7473 method* for the determination of mercury in cosmetic products.

A green tea food supplement (IMEP - 28: Total Cd, Pb, As and Hg in food supplements) was used to optimise the analytical conditions and validate the method for the determination of mercury in cosmetic products. As no CRM is available for cosmetics, a CRM similar composition (e.g. for henna) is used. The method for the direct determination of Hg in solid and liquid samples by DMA-80 is matrix independent and there is no need for matrix matching of the CRM for method validation, but due to the requirements of BDS EN ISO/IEC 17025, we have tried to find the most suitable matrix CRM from those available on the market.

Drying times (60 s or 100 s) and decomposition times (150 s or 180 s), drying temperatures (200°C or 300°C) as well as combinations between them were varied to optimize the analytical conditions. The results obtained for mercury content deviated from the reference value (0.0129 ± 0.0026) mg/kg from - 24.6 % to 15.1 % in the different variants.

The optimised analytical conditions for the quantification of mercury in the cosmetic samples by means of DMA-80 are presented in Table 1.

The analytical characteristics of the developed method for the determination of mercury in cosmetic products by DMA-80 are presented in Table 2.

Table 1. **A**nalytical conditions optimised for the determination of Hg in cosmetic products on the DMA-80

Parameter	DMA-80
Sample quantity	0.010 – 0.100 g
Drying temperature	200 °C
Drying time	100 s
Decomposition temperature	850 °C
Decomposition time	180 s
Wait time	60 s

Table 2. Analytical characteristics of the DMA-80 method for the determination of mercury in cosmetic products

Parameter	Value
Limit of detection (LOD)	0.002 mg/kg*; 0.004 mg/kg **
Limit of quantification (LOQ)	0.004 mg/kg*; 0.008 mg/kg**
Measurement interval	(0.08 ÷ 40) ng Hg и (40 ÷ 600) ng Hg
Recovery	94.24 %
SD_r	0.0007 mg/kg
SD_R	0.0010 mg/kg
Coefficient of variation under repeatability conditions	6.10 %
Coefficient of variation under reproducibility conditions	8.92 %
Bias	- 5.43 %
Uncertainty	0.00068 mg/kg (5.83 %)

SD_r and SD_R - standard deviations under repeatability and reproducibility conditions
*LOD and LOQ for cosmetic oral hygiene products
**LOD and LOQ for cosmetic products for face, hair and body

The result obtained from participation in the interlaboratory comparison test of the Institute for Reference Materials and Measurements (IRMM-JRC-EC, Geel, Belgium) "IMEP - 28: Total Cd, Pb, As and Hg in food supplements" with a reference value for mercury, was assessed as very good by the z-score value (- 0.3) and is presented in Table 3.

Table 3. Hg concentration detected in certified reference material "Food supplements"

	Reference values		Reported values		
CRM	Value, mg/kg	Expanded uncertainty, mg/kg	Value, mg/kg	Expanded uncertainty, mg/kg	Bias, %
"Food supplements"	0.0129	0.0026	0.0122	0.0012	- 5.43

The adapted and validated *EPA 7473 method* for the determination of mercury in cosmetic products has good analytical performance as confirmed by the interlaboratory test evaluation.

Optimization and validation of the *EPA 7473 method* for the determination of mercury in food.

The Total diet CRM No ARC/CL (HDP) was used to optimise the analytical conditions for the determination of mercury in food. Drying times (10 s, 30 s, 60 s or 120 s) and decomposition times (150 s or 180 s) were varied, as well as combinations in between. The results obtained for mercury content deviated from the certified value of (6.6 ± 3.6) µg/kg from -15.5 % to 14.4 % in the different variants.
The optimised analytical conditions for the quantification of mercury in the analysed food samples with DMA-80 are presented in Table 4.

Table 4. Analytical conditions optimized for the determination of Hg in food, food supplements and food additives by means of DMA-80

Parameter	DMA-80
Sample quantity	0.010 – 0.100 g
Drying temperature	300 °C
Drying time	120 s
Decomposition temperature	850 °C
Decomposition time	150 s
Wait time	60 s

The analytical parameters of the adapted method were validated by analysis of a Certified Tuna Test Sample (CTS) in the framework of participation in the International Measurement Evaluation Programme (IMEP-20) organized by IRMM, JRC, EC. The analytical characteristics of the developed method for the determination of mercury in food, food supplements and food additives with DMA-80 are presented in Table 5.
The results of the CTS "Tuna fish" analyses compared to the certified mercury value are presented in Table 6.

Table 5. Analytical characteristics of the DMA-80 method for the determination of mercury in food, food supplements and food additives

Parameter	Value
Limit of detection (LOD)	(0.00005 ÷ 0.002) mg/kg*
Limit of quantification (LOQ)	(0.0001 ÷ 0.004) mg/kg*
Measurement interval	(0.08 ÷ 40) ng Hg и (40 ÷ 600) ng Hg
Recovery	105.5 %
SD_r	0.03 mg/kg
SD_R	0.14 mg/kg
Coefficient of variation under repeatability conditions	0.7 %
Coefficient of variation under reproducibility conditions	3.5 %
Bias	3.47 %
Uncertainty	0.14 mg/kg (3.13 %)

SD_r and SD_R - standard deviations under repeatability and reproducibility conditions
*LOD and LOQ are recalculated for the different groups of media studied, according to the amount of sample studied

Table 6. Hg concentration detected in certified test sample "Tuna fish"

	Certified values		Reported values		
CTS	Reference value, mg/kg	Extended uncertainty, mg/kg	Value, mg/kg	Extended uncertainty, mg/kg	Bias, %
"Tuna fish"	4.32	0.16	4.47	0.14	3.47

The result obtained for mercury concentration in the CTS shows a very good match with the certified value. The optimised and validated *EPA 7473 method* for the determination of mercury in food has a good analytical performance.

Optimization and validation of *EPA 7473 method* for mercury determination in water.

Sample preparation for the analyses of waters for mercury content is carried out at the time of sampling. Waters are sampled and stored in acid-washed borosilicate glass containers or in quartz containers, as mercury passes through the walls of plastic containers. Samples for mercury are stabilised with a defined amount of $K_2Cr_2O_7$ solution and conc. HNO_3 (1 cm^3 stabilising solution per 100 cm^3 of water).

Stabilising solution: to 0.5 g of potassium dichromate were added 50 cm3 of nitric acid and make up to 100 cm3 with bidistilled water. Preservation of the water samples is carried out at the time of sampling, the preserved sample is stored in a refrigerator and is suitable for analysis for 1 month.

Achieving lower LODs and LOQs is possible after preconcentration - analysing a larger sample spread over several sample boats since the maximum sample volume/weight is limited by the size of the sample boats (up to 0.5 ml or 0.5 g). A preconcentration mode is used in water testing for mercury where three parallel samples are analysed in three sample boats and the measurement is performed according to a set procedure by the manufacturer. Each sample boat is introduced into the catalyst furnace and the sample is thermally decomposed and the mercury released is accumulated successively in the amalgamator. The accumulated mercury from the three parallel samples is thermally desorbed and the total mercury content of the sample is measured.

The instrumental parameters of the DMA-80 for mercury analysis in waters have been optimized with the CRM for Hg (Fluka Analytical TM, 1000 mg/L). A 50.0 µg/l solution was prepared (2.5 ng of Hg was imported) and 0.5 cm^3 of $K_2Cr_2O_7$ stabilizing solution was added. Drying times (30 s or 60 s) and decomposition times (150 s or 180 s) were varied, as well as combinations in between. The results obtained for mercury content shifted from the expected value of (50.0 ± 0.05) mg/l from - 9.5 % to 8.0 % in the different variants.

The quantification of mercury in the analysed water samples was performed with DMA-80 under the optimised analytical conditions (Table 7) with the analytical characteristics of the method shown in Table 8.

Tab. 7. Analytical conditions optimized for Hg determination in DMA-80 waters

Parameter	**DMA-80**
Sample quantity	1.5 ml (3x500 µl)
Drying temperature	300 °C
Drying time	60 s
Decomposition temperature	850 °C
Decomposition time	180 s
Wait time	60 s

Table 8. Analytical characteristics of the DMA-80 method for mercury determination in water

Parameter	Value
Limit of detection (LOD)	0.00003 mg/l
Limit of quantification (LOQ)	0.00005 mg/l
Measurement interval	(0.08 ÷ 40) ng Hg и (40 ÷ 600) ng Hg
Recovery	99.98 %
SD_r	1.37 µg/l
SD_R	1.46 µg/l
Coefficient of variation under repeatability conditions	2.75 %
Coefficient of variation under reproducibility conditions	2.93 %
Bias	0.016 %
Uncertainty	1.12 µg/l (2.24)

SD_r and SD_R – standard deviations in repeatability and reproducibility conditions with DMA-80

The results obtained from the analysis of the solution prepared by CRM for Hg (Fluka Analytical TM, 1000 mg/l) with a concentration of 50.0 µg/l (2.5 ng of Hg was imported) are presented in Table 9.

Table 9. Hg concentration detected in certified reference material „Fluka Analytical™"

	Expected values		Received values		
CRM	Value, µg/l	Expanded uncertainty, µg/l	Value, µg/l	Extended uncertainty, µg/l	Bias, %
CRM for mercury „Fluka Analytical™"	50.0	5.0	49.992	1.12	0.016 %

The adapted and validated *EPA 7473 method* for the determination of mercury in waters has very good analytical performance, demonstrated by the match between the obtained and expected mercury concentration values for the certified reference material.

Optimization and validation of *EPA 7473 method* for mercury determination in soils and sludges.

To optimize the analytical conditions and validate a method for the determination of mercury in soils and sludges, the "Typical Black Soil Composition" CRM Nos 2507 to 2509-83 was used. Drying times (10 s or 60 s) and decomposition times (150 s or 180 s) varied, as well as combinations in between. The results obtained for mercury content deviated from the certified value of (210 ± 70) µg/kg from -7.6 % to 8.5 % in the different variants.
The quantification of mercury in the soil and sludge samples tested was performed with DMA-80 under the optimized analytical conditions shown in Table 10 with the analytical method characteristics shown in Table 11.

Tab. 10. Analytical conditions optimized for the determination of Hg in soils and sludges on DMA-80

Parameter	DMA-80
Sample quantity	0.050 g
Drying temperature	300 °C
Drying time	10 s
Decomposition temperature	850 °C
Decomposition time	180 s
Wait time	60 s

Tab. 11. Analytical characteristics of the DMA-80 method for mercury determination in soils and sludges

Parameter	Value
Limit of detection (LOD)	0.0008 mg/kg
Limit of quantification (LOQ)	0.0016 mg/kg
Measurement interval	(0.08 ÷ 40) ng Hg и (40 ÷ 600) ng Hg
Recovery	94.01 %
SD_r	7.69 µg/kg
SD_R	11.37 µg/kg
Coefficient of variation under repeatability conditions	3.90 %
Coefficient of variation under reproducibility conditions	5,74 %
Bias	– 5,6 %
Uncertainty	5.35 µg/kg (2.6 %)

SD_r and SD_R – standard deviations under repeatability and reproducibility conditions

The results of the CRM "Typical Black Soil Composition" analyses to validate a method for determining mercury in soils and sludges compared to the certified mercury value are presented in Table 12.

Tab. 12. Hg concentration detected in certified reference material "Typical Black Soil Composition" Nos 2507-83 - 2509-83, ASSO

	Certified values		Received values		
CRM	Value, µg/kg	Expanded uncertainty, µg/kg	Value, µg/kg	Expanded uncertainty, µg/kg	Bias, %
"Typical Black Soil Composition"	210	70	198.22	5.35	- 5.6

The result obtained for the mercury concentration in the CRM shows good match with the certified value. The optimised and validated *EPA 7473 method* for the determination of mercury in soils and sludges has good analytical performance.

Optimization and validation of the *EPA 7473 method* for the determination of mercury in polymeric materials.

The instrumental parameters of the DMA-80 for the analysis of mercury in polymeric materials have been optimized with the CRM Polyethylene ERM - EC681. Drying times (10 s or 60 s) and decomposition times (150 s or 180 s) as well as combinations in between are varied. The results obtained for mercury content deviated from the certified value (4.50 ± 0.15) mg/kg from - 1.6 % to 10.9 % in the different variants.
The quantification of mercury in the investigated samples of polymeric materials was performed with DMA-80 under optimized analytical conditions shown in Table 13.

Tab. 13. Analytical conditions optimized for the determination of Hg in polymeric materials by DMA-80

Parameter	DMA-80
Sample quantity	0.010 g
Drying temperature	300 °C
Drying time	60 s
Decomposition temperature	850 °C
Decomposition time	150 s
Wait time	60 s

The determined analytical parameters of the validated method for the determination of mercury in polymeric materials by analysis of a certified reference material BCR EC 681 Polyethylene with the certified value for mercury (4.50 ± 0.15) mg/kg are presented in Table 14.
The results of the analyses of CRM BCR EC 681 Polyethylene to validate a method for the determination of mercury in polymeric materials compared to the certified mercury value are presented in Table 15.

Tab. 14. Analytical characteristics of the method for the determination of mercury in polymeric materials with DMA-80

Parameter	Value
Limit of detection (LOD)	0.004 mg/kg
Limit of quantification (LOQ)	0.008 mg/kg
Measurement interval	(0.08 ÷ 40) ng Hg и (40 ÷ 600) ng Hg
Recovery	101.58 %
SD_r	63.18 µg/kg
SD_R	144.86 µg/kg
Coefficient of variation under repeatability conditions	1.38 %
Coefficient of variation under reproducibility conditions	3.19 %
Bias	1.59 %
Uncertainty	87.47 µg/kg (1.91 %)

SD_r and SD_R – standard deviations under repeatability and reproducibility conditions

Tab. 15. Hg concentration detected in certified reference material BCR EC 681 Polyethylene

	Certified values		Received values		
CRM	Value, µg/kg	Expanded uncertainty, µg/kg	Value, µg/kg	Expanded uncertainty, µg/kg	Bias, %
BCR EC 681 Polyethylene	4500	150	4571	87.47	1.58

The adapted and validated EPA 7473 method for the determination of mercury in polymeric materials has good analytical performance. Its validity is demonstrated by the very good match of the value obtained with the certified value.

Summary discussion of the influence of matrix in optimizing analytical conditions for mercury determination by DMA-80

The matrix of the tested samples has a significant influence in optimizing the analytical conditions for mercury determination by DMA-80. 'Difficult' matrices, such as samples with high organic content (cosmetics, food, polymeric materials, etc.), when present in larger quantities, can 'explode', causing problems in the catalyst or amalgamator and consequently reducing the sensitivity of the system. Drying and decomposition temperatures and times should be selected to obtain complete combustion of the sample and avoid memory effects. In optimizing the analytical conditions, instrument parameters recommended by the DMA-80 manufacturer and specified in EPA Method 7473 were initially selected.

Drying temperature

The drying of the sample is intended to remove the liquid content without boiling and partially decompose the volatile organic compounds. The recommended drying temperature of 300 °C is applied to most of the samples, at which the maximum drying rate is achieved. When optimising the conditions for matrices containing liquid ingredients with boiling points below 100°C (cosmetic products), it was found that at a lower drying temperature (200°C) the results obtained were in better agreement with the value of the certified reference material compared to those obtained at a drying temperature of 300°C.

Drying time

The duration of the drying stage varies according to the volume of the sample or according to the percentage of water in the sample. Longer drying times allow the sample to be partially ashed beforehand, which removes most of the matrix. This is particularly important for samples with a high organic content before the high temperature decomposition step, thereby reducing the intensity of the exothermic reaction.

The following drying times were determined in the optimization: cosmetics - 100 s, food, food supplements and food additives - 120 s, polymeric materials - 60 s, water - 60 s, soils and sludges - 10 s.

Decomposition temperature

In the present work, the temperature at which the different samples were pyrolysed was 850 °C. At this temperature, complete ashing occurs and the available mercury is released from the matrix. At lower thermal decomposition temperatures it is likely that some of the bonds in the matrix will not break and some of the mercury will be retained in the rest of the sample.

Decomposition time

In optimising the time for which the sample is exposed to high temperature, two options (150 s and 180 s) have been applied. Samples with high organic matrix content degraded relatively faster. At a decomposition time of 150 s, the best results were found for food, food

supplements and food additives and polymeric materials; 180 s was optimal for cosmetics, water, soil and sludge.

Purge time

The time required to purge all pyrolysis products out of the system before the Hg measurement begins is 60 s for all media, as is the standard setting.

Sample quantity

When selecting the optimum sample quantity, it was found that samples with larger mass (>100 mg) were not completely ashed and decomposed, resulting in residual contamination and the need to extend the purge time to reduce the "memory" effect. On the other hand, smaller sample mass results in lower reproducibility and precision, which is probably due to insufficient sample representativeness and therefore inhomogeneity. Other factors taken into account were the organic content of the sample, the expected Hg concentration in the sample, and the sample boat capacity of the DMA-80.

In order to achieve lower detection and determination limits, there is the possibility to analyse a larger sample spread over several sample boat sizes by re-concentration. The mercury released after thermal decomposition of the sample from each sample boat is accumulated successively in the amalgamator, desorbed thermally and the total mercury content of the sample is measured.

The optimum amount of sample that degrades as completely as possible, causes minimal residual contamination and provides the ability to determine the mercury concentration within the operating range of the instrument with good sensitivity, accuracy and reproducibility is in the range of 10 - 100 mg for solid samples (cosmetics - 10-100 mg, food, food supplements and food additives - 10-100 mg, soils and sludges - 50 mg, polymeric materials - 10 mg) and 1.5 ml (distributed 500 µl in three sample boats) for aqueous samples.

STUDY OF THE MERCURY CONTENT IN DIFFERENT MEDIA RELEVANT TO HUMAN HEALTH AND THE ENVIRONMENT

Study of mercury content in cosmetic products

Cosmetic products for hair, face and body

823 samples were tested: hair cosmetics (n=237), face cosmetics (n=234) and body cosmetics (n=352). The samples were Bulgarian commercial products and were taken randomly.

The data on the mercury content in hair, face and body care products tested are presented in Table 16 and Table 17.

In most of the analysed samples, the levels of the element (Hg) were below the limit of quantification of the analytical procedure (LOQ 0.008 mg/kg). Hg at concentrations of 0.0083 ÷ 0.040 mg/kg were detected in only 24 pcs. of the 823 samples, which is 2.9 % of all analysed products (Table 16).

Tab. 16. Mercury content above LOQ detected in individual cosmetic products

№	Product	$C_{Hg} \pm U$, mg/kg
1	Henna, natural-1	0.0267 ± 0.0016
2	Henna, natural -2	0.0284 ± 0.0017
3	Henna, natural -3	0.0210 ± 0.0012
4	Henna, natural -4	0.0179 ± 0.0010
5	Henna, red-1	0.0193 ± 0.0011
6	Henna, red -2	0.0131 ± 0.0008
7	Henna, black -1	0.0193 ± 0.0011
8	Henna, black -2	0.0139 ± 0.0008
9	Henna, chestnut	0.0144 ± 0.0008
10	Henna, mahogany	0.0211 ± 0.0012
11	Hair mask with Dead Sea mud	0.0140 ± 0.0008
12	Face cream, whitening	0.0128 ± 0.0007
13	Face cream, herbal	0.0100 ± 0.0006
14	Face cream, natural	0.0165 ± 0.0010
15	Anti-wrinkle face cream	0.0097 ± 0.0006
16	Face rejuvenating cream with collagen	0.0129 ± 0.0007
17	Eye cream	0.0192 ± 0.0011
18	Body cream, cosmetic	0.0094 ± 0.0005
19	Shower gel with raspberry scent	0.0398 ± 0.0023
20	Toilet soap with grapeseed oil	0.0092 ± 0.0005
21	Toilet soap with cocoa butter	0.0110 ± 0.0006
22	Toilet soap with shea butter	0.0083 ± 0.0005
23	Massage oil with rose extract	0.0129 ± 0.0007
24	Massage oil for dry skin	0.0135 ± 0.0008
Guide 'Indicators and permissible levels of microbiological and chemical purity of cosmetic products and methods for verifying compliance with these indicators'.		***1.0***

The concentrations of mercury found in the 24 cosmetic products did not exceed the permissible level as technical contamination (1.0 mg/kg), according to the Guide „Indicators and permissible levels for microbiological and chemical purity of cosmetic products and methods for verifying compliance with these indicators“ (the Guide – in the text below) and Art. 17 of Regulation (EC) 1223/2009, according to which a small amount of a prohibited

substance which is due to impurities of natural or artificial ingredients, to the manufacturing process, to storage or to migration from the packaging, which is technically unavoidable in good manufacturing practice, is allowed provided that this presence is consistent with the safety of the product. It is noteworthy that a large proportion of the products (17/24) in which mercury content has been determined – henna, hair mask with Dead Sea mud, herbal and natural face cream, soaps with grapeseed, cocoa and shea oils, massage oil with rose extract – used raw materials of natural origin. Almost all henna samples (10/11) contained Hg, although at very low levels (0.013 ÷ 0.028) mg/kg.

The Hg content in all three groups of Bulgarian cosmetic products examined was very low and varied within a narrow range. Relatively, the highest proportion of items in the hair cosmetic group (4.7 % of the samples tested) was found to contain mercury levels ranging from 0.013 mg/kg to 0.028 mg/kg, followed by face cosmetics (2.6 %, C_{Hg} from 0.0097 mg/kg to 0.019 mg/kg) and body cosmetics (only 2 % of the group showed mercury concentrations ranging from 0.0083 mg/kg to 0.040 mg/kg) (Table 17).

Table. 17. Proportion of items with mercury content above the LOQ in the hair, face and body cosmetic product groups studied

Product groups	**Total number samples**	**Samples with C_{Hg} > LOQ, Number (%)**	**C_{Hg} > LOQ, mg/kg min – max**
Cosmetic products for hair	237	11 (4.7)	0.013 ÷ 0.028
Cosmetic products for the face	234	6 (2.6)	0.0097 ÷ 0.019
Cosmetic products for the body	352	7 (2.0)	0.0083 ÷ 0.040
Guide 'Indicators and permissible levels of microbiological and chemical purity of cosmetic products and methods for verifying compliance with these indicators'.			***1.0***

The study shows that the tested Bulgarian cosmetic products for hair, face and body, in terms of mercury content, comply with the requirements of the European legislation and the above mentioned Guide. Only in a few cases (24 of the 823 samples analysed) the Hg concentrations were above the limit of quantification of the analytical procedure (LOQ 0.008 mg/kg). The presence of Hg was mainly observed in products containing raw materials of natural origin, but at very low levels that do not pose a health risk to consumers.

Cosmetic products for oral hygiene

A total of 228 cosmetic products for oral hygiene were tested, including 202 toothpastes (carbonate and silicate) and 26 mouthwashes. Depending on the abrasive used in the toothpastes, they were subdivided into 31 carbonate toothpastes with calcium carbonate abrasive and 171 silicate toothpastes with precipitated silica abrasive.

Mercury content above the limit of quantification of the analytical procedure (LOQ 0.004 mg/kg) was found in 105 toothpastes tested, which is 52 % of all samples analysed (Figure 3). Concentration of 0.0044 mg/kg to 0.133 mg/kg of mercury was determined in 48% (82 pieces) of the silicate toothpastes. Mercury levels ranging from 0.0050 mg/kg to 0.079 mg/kg were observed in 74.2 % of the carbonate toothpastes.

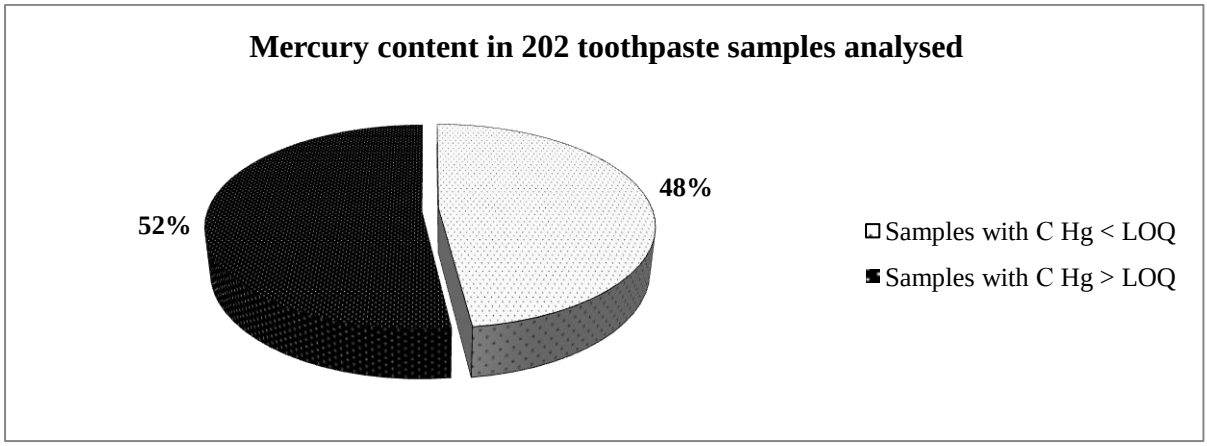

Fig. 3. Results of mercury content analysis in toothpastes

Mercury content above the limit of quantification of the analytical procedure (LOQ 0.004 mg/kg) was detected in 3 samples mouthwash (11.5% of the samples tested) (Figure 4). The values obtained ranged from 0.0042 mg/kg to 0.070 mg/kg.

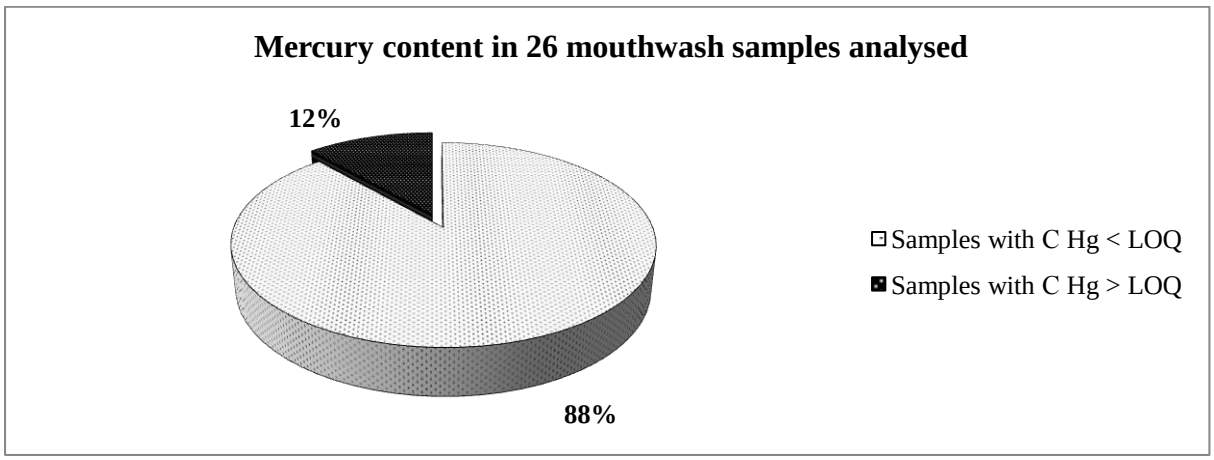

Fig. 4. Results of mercury content analysis in mouthwashes

The data for mercury content above the LOQ in the toothpastes and mouthwashes analysed are presented in Table 18.

Table 18. Proportion of items with mercury content above the LOQ in the groups of oral hygiene cosmetic products tested

Product groups	**Total number samples**	**Samples with C_{Hg} > LOQ, Number (%)**	**C_{Hg} > LOQ, mg/kg min – max**
Toothpastes	202	105 (52.0)	0.0044 ÷ 0.133
▪ silica	171	82 (48.0)	0.0044 ÷ 0.133
▪ carbonate	31	23 (74.2)	0.0050 ÷ 0.079
Mouthwashes	26	3 (11.5)	0.0042 ÷ 0.070
Guide 'Indicators and permissible levels of microbiological and chemical purity of cosmetic products and methods for verifying compliance with these indicators'.			***0.2***

On the basis of the results of the study, it can be concluded that mercury was detected in almost half of the products analysed (47.4 %), but at concentrations below the acceptable level of technical contamination (0.2 mg/kg for oral cosmetic products) according to the Guide and Article 17 of Regulation (EC) 1223/2009, which does not imply an increased health risk for consumers.

24 hair, face and body products and 108 oral hygiene products of the 823 samples of cosmetic products tested contained Hg above the limit of quantification of the analytical procedure. The average Hg levels found in the hair, face and body cosmetic products were many times below the permissible levels, and in the oral care cosmetic products about four times below the permissible levels, as required by the Guide. Due to the frequency of use, the application of several layers, and the large body surface area being treated, the Hg content of these products should be monitored, since on the one hand, values close to the LOQ are considered to mean that the cosmetics are safe, but on the other hand, Hg is a toxic element that accumulates, so that exposure to even small amounts could be dangerous to human health. There is no theoretical safe level for this highly toxic element - any concentration above the LOQ is dangerous.

Study of mercury content in foods, food supplements and food additives

The mercury content of different food groups has been studied:

- Fish and fish products - 8 fish samples (river (n=5) and marine (n=3));
- Cereals and their products - 16 samples (white flour (n=10), bread mix (n=3), wheat (n=1), bran (n=1), corn (n=1));
- Dairy - 8 samples (cheese (n=5) and yellow cheese (n=3));
- Vegetables and their products - 7 canned vegetable samples;
- Fruits and their products - 5 samples of plum mousse;
- Meat, poultry and their products - 5 canned meat samples;
- Other - 89 samples of wine (red (n=58) and white (n=31)); 36 samples of sugar (refined (n=22) and raw (n=14)) and 4 samples of sweeteners;
- Food supplements - 24 samples (vitamins, minerals, herbs, amino acids, hormones and combinations thereof);
- Algae-based food supplements - 8 samples (natural aluminosilicates and natural products);
- Food additives 17 samples (flour stabilizer (n=3); flour improver (n=6); lyophilized starter cultures for dairy products (n=8)).

Fish and fish products

Fish is a major source for mercury intake in humans with the diet. Mercury accumulates in fish and zooplankton through bioaccumulation processes.

The mercury content of five species of fish from a dam in north-western Bulgaria (Perca fluviatilis, Abramis brama, Silurus glanis, Carassius auratus gibelio, Chondrostoma nasus) and three species of marine fish (Dicentrarchus labrax, Scophthalmus maeoticus, Sparus aurata) was investigated. The sampling was carried out randomly. The results obtained are presented in Table 19 and Table 20.

Tab. 19. Mercury content in fish from a reservoir in northwestern Bulgaria

Type of fish	**C_{Hg} ± U, mg/kg**
Silurus glanis	0.115 ± 0.003
Carassius auratus gibelio	0.110 ± 0.003
Perca fluviatilis	0.187 ± 0.005
Abramis brama	0.140 ± 0.004
Chondrostoma nasus	0.030 ± 0.001
Regulation (EU) 2023/915	***0.50***

The levels of Hg found in the freshwater fish species were relatively low, ranging from 0.030 mg/kg to 0.187 mg/kg and were in accordance with the requirements of Regulation (EU) 2023/915 on maximum levels of certain contaminants in food and repealing Regulation (EC) No 1881/2006, which sets the maximum level of Hg in fish products and fish muscle meat at 0.30 mg/kg to 1.0 mg/kg. The lowest Hg content was found in the Chondrostoma nasus sample (0.030 ± 0.001 mg/kg) and the highest in the Perca fluviatilis sample (0.187 ± 0.005 mg/kg). The Hg content of the different fish species was between three (Perca fluviatilis) and seventeen times (Chondrostoma nasus) lower than the maximum level defined by the Regulation.

Table 25. Mercury content in marine fish

Type of fish	**C_{Hg} ± U, mg/kg**
Dicentrarchus labrax	0.127 ± 0.004
Scophthalmus maeoticus	0.091 ± 0.003
Sparus aurata	0.114 ± 0.003
Regulation (EU) 2023/915	***0.50***

The Hg concentrations obtained in the marine fish species studied were relatively low, with similar values ranging from 0.091 mg/kg to 0.127 mg/kg. They are below the maximum level specified in Regulation (EU) 2023/915 (0.50 mg/kg).
The detected values for freshwater and marine fish in comparison with the maximum level of Regulation (EU) 2023/915, are visually presented in Figure 5.
No significant differences in Hg content were found between freshwater and marine fish. Although the determined Hg content is below the maximum level, daily consumption of fish may lead to bioaccumulation of this metal in tissues and organs, therefore prolonged intake may lead to health disorders. Children, in particular, could be affected as their organs are in a developing stage.

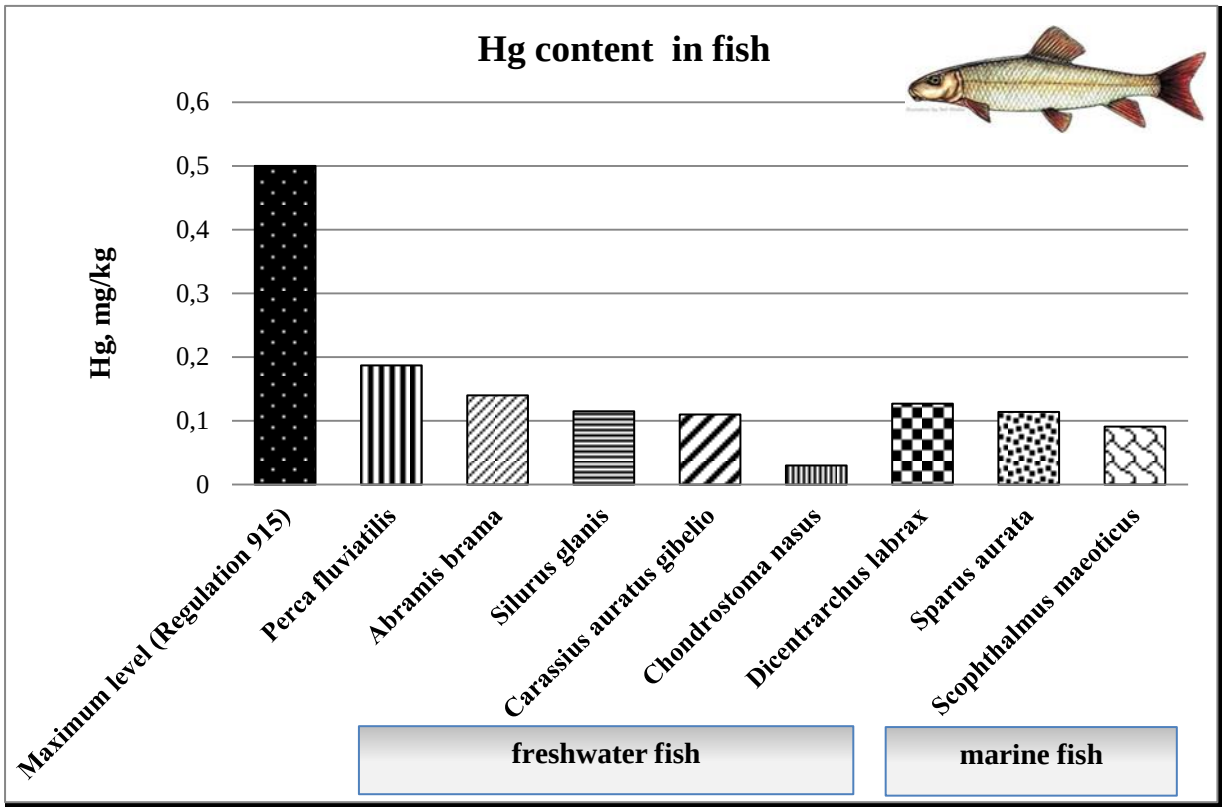

Fig. 5. Comparison of Hg concentrations detected in fish with the maximum level of Regulation (EU) 2023/915

The European Food Safety Authority (EFSA), 2012, adopted an opinion on mercury and methylmercury in food, which established a tolerable weekly intake for inorganic mercury of 4 μg/kg bw and for methylmercury of 1.3 μg/kg bw (both expressed as mercury). According to the opinion, exposure to methylmercury above the acceptable weekly intake is of concern, but the beneficial effects of fish consumption in reducing methylmercury exposure should also be taken into account. This requires consideration of the role of fish and seafood in European diets and an assessment of the beneficial effects of their consumption on health outcomes, including the effects of fish consumption during pregnancy on neurodevelopment in children and the effects of seafood consumption on the risk of cardiovascular disease in adults. EFSA concluded consumption of about 1-2 portions of fish and seafood per week and up to 3-4 portions per week during pregnancy is associated with better functional outcomes in children's neurodevelopment compared to no seafood consumption. Such amounts are also associated with lower coronary heart disease mortality in adults.

Mercury is one of the substances that is capable of long-range transport and is largely ubiquitous in the environment. Such substances can be detected for decades in the aquatic environment at levels posing a significant risk (Directive 2013/39/EU).

Fish are a suitable bioindicator of environmental mercury contamination as they accumulate it in their tissues (highest concentrations in muscle) directly from the water but also through their diet, thus mercury transport through the food chain can be assessed. Mercury can be transported through successive levels of the food chain and its concentration increases in each successive trophic level.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy sets out a strategy against water pollution, including the identification of priority substances of concern to the

aquatic environment. Directive 2013/39/EU of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy introduces environmental quality standards (EQS) for priority hazardous substances, including mercury, which surface waters must meet to be in good chemical status ensuring an adequate level of protection of the environment and human health. Certain substances accumulate in biota and are therefore subject to an EQS for biota in addition to the EQS for surface waters, such as Hg (20 µg/kg).
If the mean Hg concentrations found in the freshwater and marine fish species studied are compared with the Hg EQS for biota (20 µg/kg), they are higher by a factor of 1.5 (Chondrostoma nasus) to 25 (Perca fluviatilis) (Table 19, Table 20 and Figure 11). These values, although only 8 in number, are an indication of a possible deterioration in the chemical status of the relevant aquatic ecosystems concerned and of a potential risk of exposure to or through the aquatic environment. In order to adequately assess the chemical status of surface water bodies in accordance with Directive 2013/39/EU, the competent authorities of the Member States shall plan and monitor priority substances that tend to accumulate in sediment and/or biota (including mercury) and take measures to reduce or eliminate substances that are toxic, persistent and bioaccumulative.

Cereals and cereal products
Mercury levels in 16 cereals and their products were tested. Data on mercury concentrations in the samples analysed are presented in Table 21. The Hg content of the cereal samples was below the limit of quantification of the analytical procedure (LOQ 0.0008 mg/kg).
The presence of Hg in the flours studied was observed but at very low concentrations ranging between < 0.0008 mg/kg and 0.0065 mg/kg.

Table 21. Hg content detected in the cereals and their products tested

№	Product	Number of samples	$C_{Hg} \pm U$, mg/kg min - max
1	Wheat	1	< 0.0008
2	Bran	1	< 0.0008
3	Maize	1	< 0.0008
4	White flour	10	< 0.0008 ÷ 0.0065
5	Bread mix	3	0.0021 ± 0.0001 0.0014 ÷ 0.0027

Milk and dairy products
5 cheese samples (sheep's milk white brine, buffalo milk white brine, cow's milk white brine, goat's milk white brine) and 3 samples of yellow cheese (from sheep's, buffalo and cow's milk) were analysed for mercury content. In all samples the mercury concentrations were below the limit of quantification of the analytical procedure (LOQ 0.0005 mg/kg).

Vegetables and their products
Seven samples of canned vegetables were tested for mercury. The levels found were slightly above the limit of quantification of the analytical procedure (LOQ 0.0002 mg/kg) and varied within a very narrow range: from 0.0004 mg/kg to 0.0011 mg/kg. The mercury concentration data for the samples analysed are presented in Table 22.

Table 22. Hg content detected in the tested canned vegetables

№	Product	C_{Hg} ± U, mg/kg
1	Lutenica	0.0006 ± 0.00002
2	Fried eggplant with tomato sauce	0.0007 ± 0.00002
3	Pickled bell peppers	0.0008 ± 0.00003
4	Eggplant salad	0.0004 ± 0.00001
5	Ivar homemade	0.0011 ± 0.00003
6	Fried eggplant with vegetables	0.0004 ± 0.00001
7	Stuffed vine leaves	0.0004 ± 0.00001

Fruits and their products

5 samples of plum mousse were analysed for mercury. In all samples the mercury concentrations were below the limit of quantification of the analytical procedure (LOQ 0.004 mg/kg).

Meat, poultry and their products

The mercury content of 5 samples of canned meat was determined. In three of the samples the Hg concentration was below the limit of quantification of the analytical procedure (LOQ 0.0008 mg/kg). In the other two samples the concentration found was 0.0014 mg/kg, slightly above the LOQ. The Hg concentration data for the samples analysed are presented in Table 23.

Table 23. Hg content detected in the canned meats tested

№	Product	C_{Hg} ± U, mg/kg
1	canned meat (beef)	< 0.0008
2	canned meat (beef)	0.0014 ± 0.00004
3	canned meat (beef and heart)	0.0014 ± 0.00004
4	canned meat (beef and heart)	< 0.0008
5	canned meat (beef and heart)	< 0.0008

According to the results obtained for Hg content, the consumption of food from the groups of "Milk and dairy products", "Vegetables and their products", "Fruits and their products" and "Meat, poultry and their products" does not lead to an increased intake of Hg and adverse effects on human health, resp.

Other:

Wine

89 samples were tested: 58 red wines and 31 white wines. The Hg concentrations determined in the wines examined were below the limit of quantification of the analytical procedure (LOQ 0.0008 mg/l). In accordance with the results obtained on Hg contamination, the consumption of Bulgarian white and red wine does not contribute to an increased Hg intake and does not pose a health risk to consumers.

Sugar and sweeteners
36 samples of sugar and 4 samples of sweeteners were analysed, 1 synthetic (cyclamate) and 3 natural (stevia). The mercury concentrations in the sugar and sweetener samples are shown in Table 24.

Table 24. Hg concentrations detected in sugar and sweetener samples tested

Product	Number of samples	C_{Hg}, mg/kg, min - max
Refined sugar	22	< 0.0013 ÷ 0.0045
Raw sugar	14	< 0.0013 ÷ 0.0034
Synthetic sweeteners	1	< 0.004
Natural sweeteners	3	< 0.0016

The mercury content of refined and raw sugar is in approximately the same range. There are no regulated maximum levels for Hg in sugar in European legislation. In assessing the content of this element in the samples examined, a comparison was made with established standards in Brazil and Vietnam. Compared with the maximum limits set by Brazilian legislation (0.01 mg/kg) and the Vietnamese National Technical Regulation (0.05 mg/kg), the results obtained for the presence of Hg in the sugar samples tested were orders of magnitude lower.
The Hg concentrations for synthetic and natural sweeteners were below the LOQ of the analytical procedures (LOQ 0.004 mg/kg and LOQ 0.0016 mg/kg, respectively). Regarding Hg contamination, there is no purity criterion for most of the sweeteners. For comparison, the purity criterion for Acesulfame K in Regulation (EU) No 231/2012 (1 mg/kg) was used to assess its content in the sweeteners analysed, against which they are in compliance with European legislation.
The results of the study indicate that the use of sugar and sweeteners does not pose a health hazard to consumers in terms of mercury contamination.

Food supplements and food additives
Mercury levels in plant-based food supplements (dry herbal tablets, herbal extracts, dried algal biomass), vitamins and minerals, etc., as well as in some food additives have been studied. Twenty-four samples of food supplements (vitamins, minerals, herbs, amino acids, hormones and combinations thereof); 8 samples of algae-based food supplements, natural aluminosilicates and natural products); 17 samples of food additives (lyophilized starter cultures for lactic acid products (n=8); flour stabilizer (n=3); flour improver (n=6)) were investigated.
Mercury concentrations in the analyzed food supplements samples are shown in Table 25, Table 26 and Figure 12.
From the data reported in Table 25 and Table 26, it can be seen that the results obtained for mercury are below the maximum levels (0.10 mg/kg) specified in Regulation (EU) 2023/915.
Figure 6 clearly shows the ratio of mercury concentrations found in the different food supplements to the maximum level in Regulation (EU) 2023/915. The mercury content of food supplements based on natural aluminosilicates is about twice the maximum level lower, for vitamins and minerals it is twenty times lower.

Table 25. Hg concentrations detected in food supplements (vitamins, minerals, herbs, amino acids, hormones and combinations thereof)

№	Product	$C_{Hg} \pm U$, mg/kg
Vitamins, minerals		
1	Vitamin C - Long, tablets	0.0082 ± 0.0003
2	Vitamin C - Long + Zn, tablets	0.0043 ± 0.0001
3	Vitamin C + Mg, vials	0.0032 ± 0.0001
4	Polyvitamin syrup with fruit and orange flavour, liquid	< 0.003
5	Selenium, tablets	< 0.0016
6	Zinc, tablets	< 0.0016
Combined food supplements: vitamins, minerals, herbs		
7	Vitamin C + Zn + acerola, echinacea, rue and propolis, tablets	0.004 ± 0.0001
8	Vitamin C + flavonoids, capsules	0.080 ± 0.003
9	Vitamin C + propolis and echinacea, tablets	0.006 ± 0.0002
10	Vitamin E, Vitamin B6 + Mg + Avram tree extract, large-flowered mugwort oil, capsules	< 0.0016
Herbal extracts, herbal tablets and capsules		
11	Golden root/ Rhodiola rosea: ▪ water-alcohol extract of the roots ▪ tablets	 0.0015 ± 0.00005 0.004 ± 0.0001
12	Herbal water-based extract "Elron"	< 0.0016
13	Combined herbal complex: Hawthorn leaf and flower, Rosehip fruit, St. John's wort stalk, Valerian roots, Lime flower, Peppermint leaf, tablets	0.0021 ± 0.0001
14	Baikal shlemnik, tablets	0.005 ± 0.0002
15	Valerian, tablets	0.005 ± 0.0002
16	Combined herbal complex: Hawthorn, Peppermint, Valerian, tablets	0.0095 ± 0.0003
17	Grandma's teeth/Tribulus terrestris, tablets	0.0057 ± 0.0002
18	Hoodia gordonii - cactus-like plant, capsules	0.018 ± 0.0006
19	Combined herbal complex: Senna leaf, Zurnestetc bark, tablets	0.0092 ± 0.0003
20	Combined herbal complex: Chinese Angelica, Indian Bread, Ginseng, capsules	0.0224 ± 0.0007
Amino acids, hormones		
21	Amino acid complex: Lactalbumin, Wheat and whey hydrolysate + Glutamine + Leucine + Potassium , ampoules	0.003 ± 0.0001
22	Beef protein containing a broad spectrum of amino acids, ampoules	< 0.003
23	Animal protein - glycine, hydroxyproline, proline and alanine + turmeric and cinnamon, powdered	0.0040 ± 0.0002
24	Melatonin, tablets	0.023 ± 0.0007
Regulation (EU)2023/915		***0.10***

Table 26. Hg concentrations detected in algae-based food supplements, natural aluminosilicates and natural products

№	Product	$C_{Hg} \pm U$, mg/kg
Food supplements based on algae		
1	Seaweed Spirulina/Atthrospira maxima: ▪ capsules ▪ tablets	 0.0016 ± 0.00005 0.009 ± 0.0003
2	Algae AFA/Aphanizomenon flos-aquae, capsules	0.0161 ± 0.0005
Food supplements based on natural aluminosilicates		
3	Huma, powder	0.086 ± 0.003
4	Natural zeolite with high mineral content Clinoptilolite, tablets	0.0043 ± 0.0001
Food supplements based on natural products		
5	Brown salt enriched with herbs	< 0.0008
6	Propolis tablets for throat	0.0032 ± 0.0001
7	Klee tincture	0.0043 ± 0.0001
8	Rose oil, capsules	0.0212 ± 0.0007
Regulation (EU) 2023/915		***0.10***

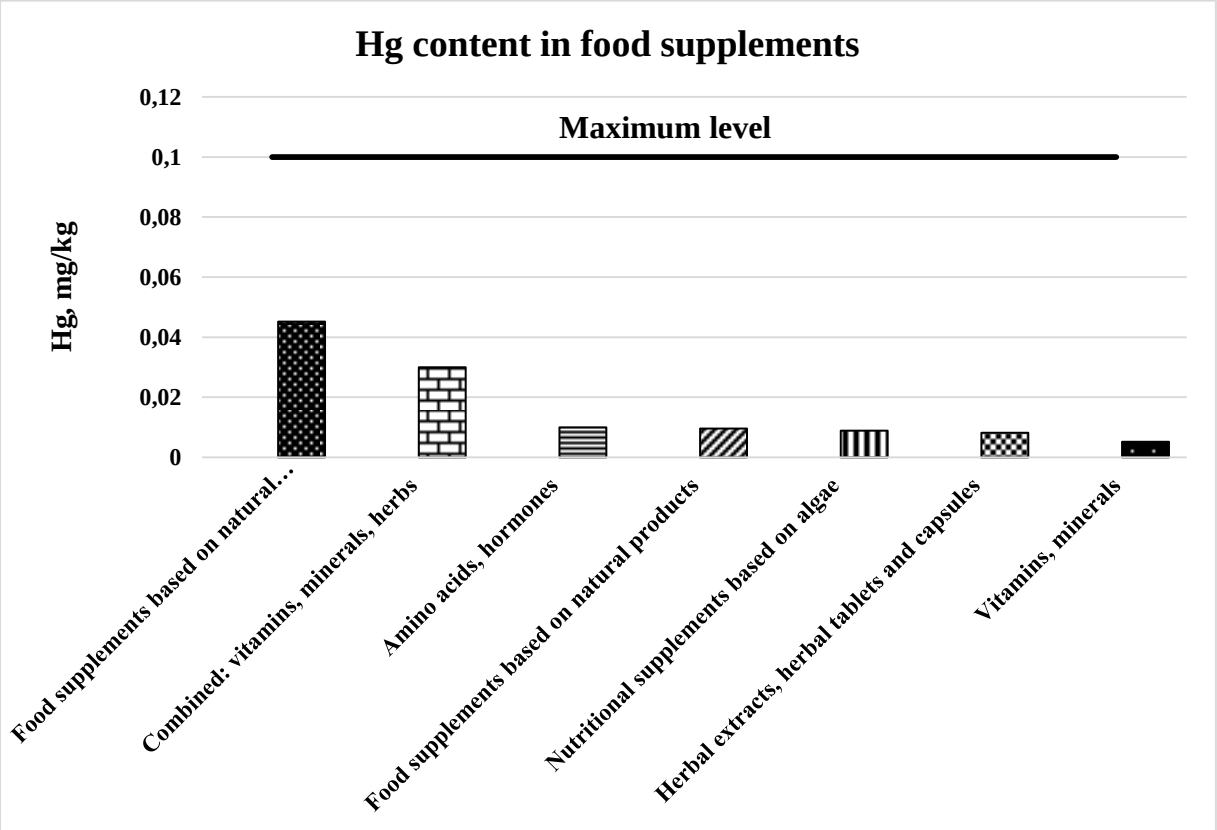

Fig. 6. Comparison of Hg concentrations detected in different types food supplements with the maximum level in Regulation 2023/915

In the food additives investigated, the Hg concentrations detected were very close and many times below the specific purity criteria in Regulation (EU) 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Table 27).

Table 27. Hg concentrations detected in food additives

№	Product	Number of samples	C_{Hg} ± U, mg/kg min – max
1	Lyophilised starter cultures for lactic acid products	8	0.0010 ± 0.00003 0.0005 – 0.0015
2	Improvers for flour	6	0.0017 ± 0.00005 0.001 – 0.0024
3	Stabilisers, flour correctors	3	0.0015 ± 0.00005 0.001 – 0.002
Regulation (EU) 231/2012			***1***

In conclusion, with regard to mercury content, the tested samples of food supplements and food additives meet the requirements of European legislation and are safe for consumers.

Study of mercury content in waters

998 water samples were analysed to assess the safety of drinking water and its compliance with the national legislation on mercury:

- 863 samples from the water supply networks of 17 regions in Bulgaria;
- 75 samples from three categories of bottled water: natural mineral (n=50), spring (n=14) and table (n=11) from 8 brands of bottled mineral water, 2 brands of bottled spring water and 3 brands of table water;
- 60 mineral water samples from 3 deposits.

Data on mercury content of mains water samples; bottled mineral, spring and table waters and mineral waters from deposits are presented in Table 28, Table 29 and Table 30, respectively.

Table 28. Hg concentrations detected in the mains waters tested of 17 districts in Bulgaria

District	Total number of samples	Number of samples with C_{Hg} > LOQ	C_{Hg}, µg/l min – max
District 1	422	17	< 0.05 – 0.9
District 2	115	3	< 0.05 – 0.3
District 3	84	1	< 0.05 – 0.1
District 4	55	-	< 0.05
District 5	47	1	< 0.05 – 0.1
District 6	36	-	< 0.05
District 7	22	5	< 0.05 – 0.8
District 8	22	-	< 0.05
District 9	15	-	< 0.05
District 10	12	-	< 0.05
District 11	10	-	< 0.05
District 12	8	-	< 0.05
District 13	6	-	< 0.05
District 14	5	-	< 0.05
District 15	4	-	< 0.05
District 16	3	-	< 0.05
District 17	2	-	< 0.05
MV for Hg, Ordinance No. 9/2001 on the quality of water intended for drinking purposes			***1.0***

Mercury levels in 836 of the 863 water samples tested from the mains of 17 districts in Bulgaria were below the limit of quantification of the analytical procedure (LOQ 0.05 µg/l). In five of the districts, mercury was present in a total of 27 samples (3%), at levels close to the LOQ (Figure 7) and many times lower than the maximum value (MV) for mercury in Regulation No 9 on the quality of water intended for drinking purposes (1.0 µg/l).

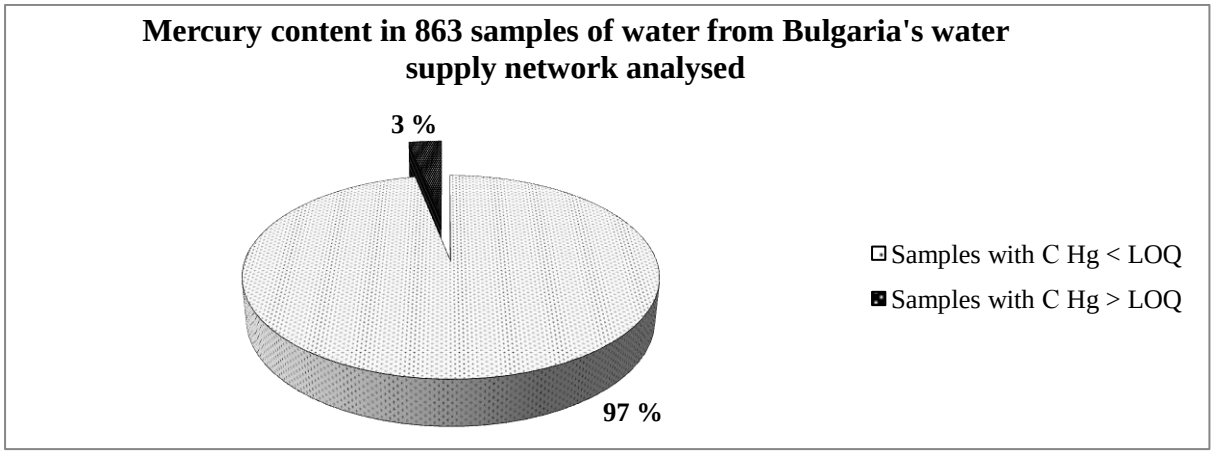

Fig. 7. Results of Hg content analysis in water from the water supply network of Bulgaria

Table 29. Hg concentrations detected in the three categories of bottled water tested

Product	**Number of samples**	**C_{Hg}, mg/l**
Natural mineral water	50	< 0.00005
Spring water	14	< 0.00005
Table water	11	< 0.00005
MV for Hg, Ordinance on the requirements for bottled natural mineral, spring and table waters intended for drinking purposes		***0.0010***
MV for Hg, Ordinance No. 9/2001 on the quality of water intended for drinking purposes		***1.0 µg/l (0.0010 mg/l)***

Table 30. Hg concentrations detected in the mineral waters investigated from three deposits

Deposit	**Number of samples**	**C_{Hg}, µg/l**
Deposit 1	30	< 0.05
Deposit 2	25	< 0.05
Deposit 3	5	< 0.05
Groundwater Quality Standard for Hg, Groundwater Exploration, Use and Conservation Ordinance No. 1/2007		***1.0***
MV for Hg, Ordinance No. 9/2001 on the quality of water intended for drinking and domestic purposes		***1.0***

The results of the study showed that in all 75 bottled water samples (natural mineral, spring and table water) the mercury concentrations were below the limit of quantification of the analytical procedure (LOQ 0.00005 mg/l).
The detected concentrations for mercury in the 60 mineral water samples analysed from three deposits were below the limit of quantification of the analytical procedure (LOQ 0.05 µg/l).
The study shows the samples of mains water, bottled water (mineral, spring and table water) and mineral water from mineral deposits tested are clean and safe in compliance with national and European legislation with regard to mercury content and do not pose a risk to public health.

Study of mercury content in soils

104 soil samples from areas affected by anthropogenic factors were analyzed for mercury content:

- Area 1 – opencast mining and beneficiation of copper and other ores, (n=15);
- Area 2 - cement production, (n=4);
- Area 3 - pharmaceutical manufacturing, (n=29);
- Area 4 - copper deposit, opencast mining and beneficiation of copper ores (n=39);
- Area 5 - cement and asbestos products manufacturing (n=3);
- Area 6 - metallurgical production (n=14).

Data from the survey are shown in Table 31 and Figure 8.

Table 31. Hg concentrations detected in the soils studied from six areas affected by anthropogenic factors

Area	**Number of samples**	**C_{Hg}, mg/kg dry soil min – max**	**$C_{Hg} \pm U$, mg/kg dry soil**
Area 1	15	0.0205 ÷ 0.4570	0.0965 ± 0.0193
Area 2	4	0.0329 ÷ 0.0954	0.0573 ± 0.0115
Area 3	29	0.0170 ÷ 0.1737	0.0534 ± 0.0107
Area 4	39	0.0118 ÷ 0.1465	0.0427 ± 0.0085
Area 5	3	0.0467 ÷ 0.0782	0.0593 ± 0.0119
Area 6	14	0.0282 ÷ 0.2491	0.1038 ± 0.0208
Ordinance No. 3/2008 on the permissible content of harmful substances *in soils of industrial/production sites* *of arable land and permanent grassland*			***10*** ***1.5***
National average background concentrations according to Ordinance 3/2008			***0.03***

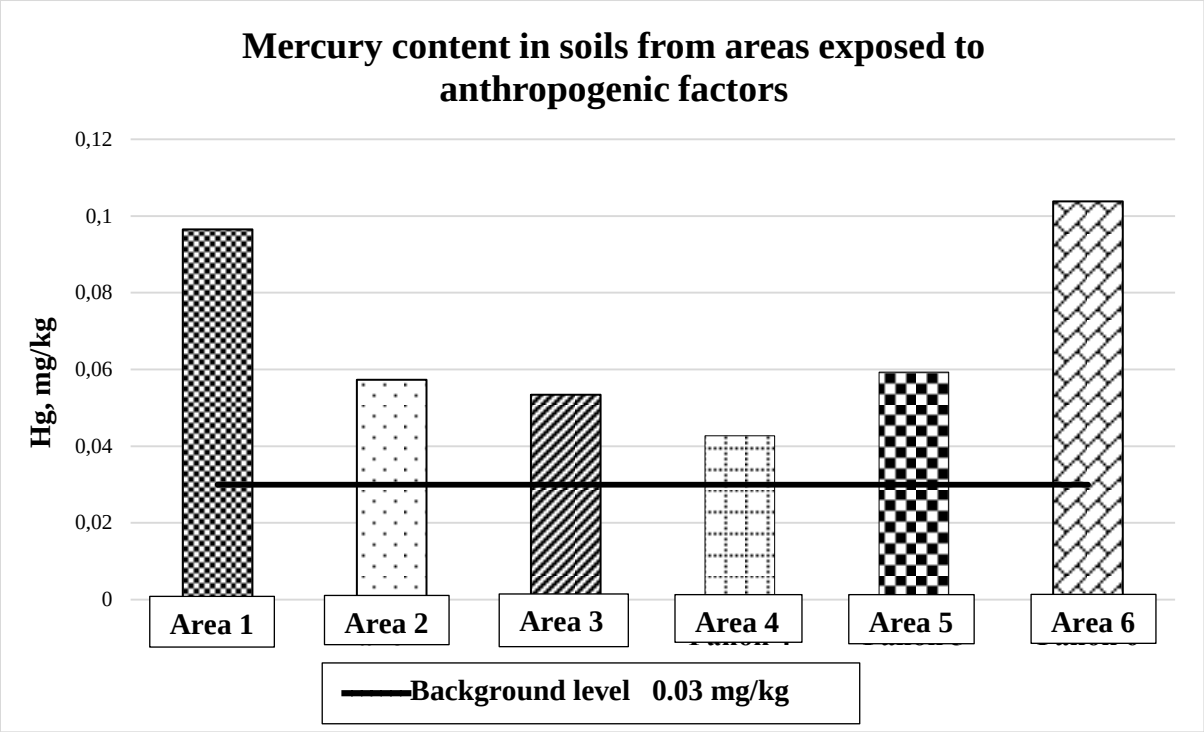

Fig. 8. Mercury levels in soils from six production areas in Bulgaria

Mercury is present as a natural impurity in the soil depending on the source rock substrate or may be artificially introduced from anthropogenic activities. Anthropogenic Hg emissions have a huge impact on the environment and a cause of increased risk to humans.

The highest average Hg content in the investigated soils was observed in Area 6 (metallurgical production), followed by Area 1 (opencast mining and beneficiation of copper and other ores). The average mercury levels in the soils of Area 2 (cement production), Area 3 (pharmaceutical production) and Area 5 (cement and asbestos products production) are similar. Area 4 (copper deposit, opencast mining and beneficiation of copper ores) has the lowest mercury concentrations (Figure 8).

The average mercury concentrations in soil samples from areas affected by anthropogenic activities are above the regulatory background concentration for Bulgaria (0.03 mg/kg). The detected exceedances of the background concentration are most likely due to the cumulative impact of anthropogenic loading. The mercury concentration in soil samples in four of the six areas commented, exceeded their respective background concentration by about a factor of two, and in the other two areas by more than a factor of three (Figure 8).

The results of the analysed soils from the six production areas show that the mercury content of the soil is below the maximum permissible concentration for arable land and permanent grassland (1.5 mg/kg) and the MPC for mercury in soils for industrial/production sites (10 mg/kg). The data are in accordance with the requirements of the national legislation.

Study of mercury content in sludges

109 sludges from 19 urban WWTPs were tested for mercury content over a period of 6 years. Table 32 and Figure 9 show the results for mercury content of the investigated WWTP sludges by year.

Table 32. Mercury content in the WWTP sludge samples tested

Year	Number of samples	C_{Hg}, mg/kg min-max	$C_{Hg} \pm U$ mg/kg	MPC, mg/kg*
I	13	0.42 ÷ 2.70	1.03 ± 0.21	**16**
II	10	0.21 ÷ 2.98	1.32 ± 0.26	
III	9	0.02 ÷ 7.02	1.72 ± 0.34	
IV	30	0.44 ÷ 6.39	1.68 ± 0.34	
V	33	0.27 ÷ 3.50	1.60 ± 0.32	
VI	14	0.38 ÷ 4.99	1.99 ± 0.40	

** Ordinance on the procedure and method for the recovery of sludge from wastewater treatment through its use in agriculture (promulgated by SG No. 63 of 12. 08. 2016)*
NB: Council Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture sets in Annex IB limit values for the concentration of mercury in sewage sludge for use in agriculture of 16 to 25 mg/kg dry matter.

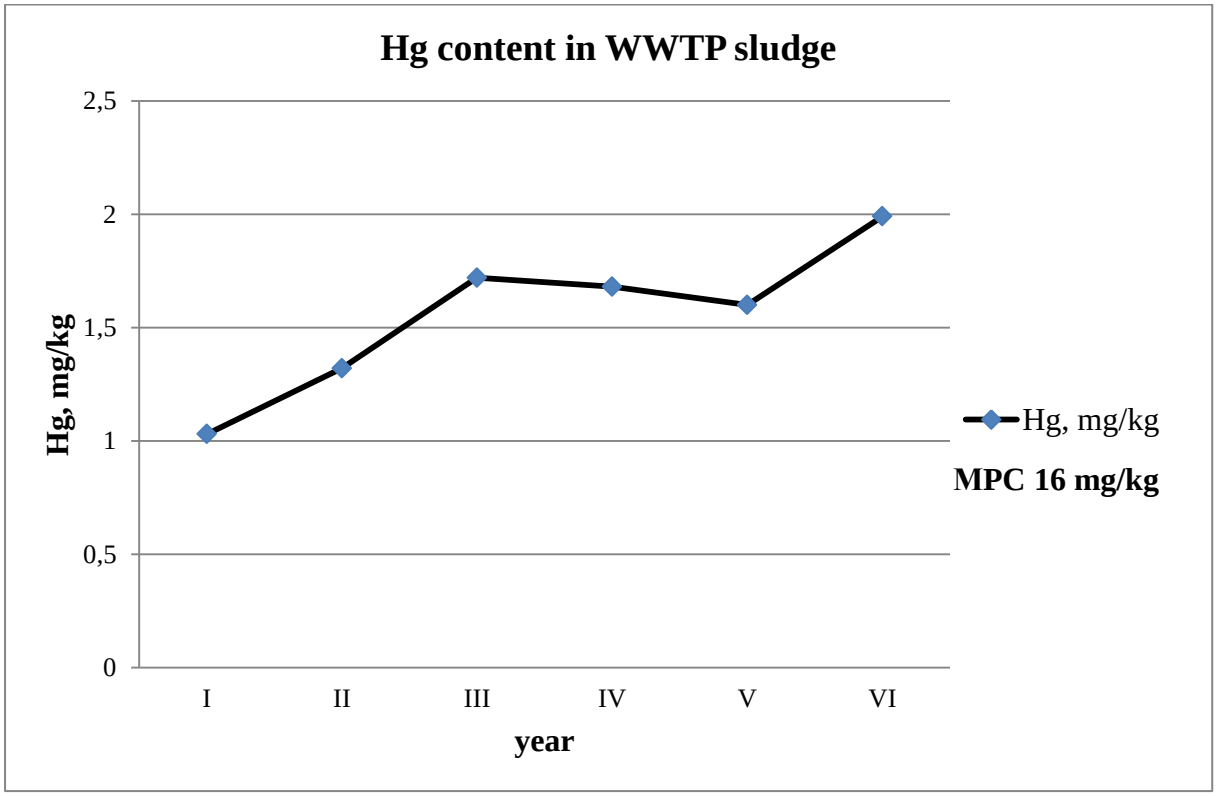

Fig. 9. Average mercury content of the WWTP sludge tested by year

Mercury was detected in all samples tested. Figure 9 shows the increase in the mercury content of the sludge from the first to the third year (by 28-30 %), followed by a two-year slight decrease and in the last year there is again an increase in the average concentration. In general, the average values found for mercury in sewage sludge are very low, varying within

a narrow dynamic range, many times below the maximum permissible concentration of 16 mg/kg according to the Ordinance on the procedure for the recovery of sewage sludge by agricultural use.
From the results obtained it can be concluded that the investigated sewage sludge, in terms of mercury content, can be used in agriculture without posing a health and environmental risk.

Study of mercury content in polymeric material products

The mercury content was determined in 94 samples of four groups of polymeric materials: polystyrene (n=44), polypropylene (n=28), polyethylene (n=14) and polyvinyl chloride (n=8). The Hg concentrations detected in the samples analyzed were very low and varied within a narrow range for polystyrene, polypropylene, polyvinyl chloride and below the limit of quantification of the analytical procedure (LOQ 0.008 mg/kg) for polyethylene (Table 33).

Table 33. Hg concentrations detected in the products tested from four groups of polymeric materials

Products	**Number of samples**	**C_{Hg}, mg/kg min-max**
Polystyrene	44	< 0.008 ÷ 0.18
Polypropylene	28	< 0.008 ÷ 0.025
Polyethylene	14	< 0.008
Polyvinyl chloride	8	< 0.008 ÷ 0.012

The results obtained from the mercury analyses in polymeric materials are in accordance with European and national requirements and indicate the presence of mercury at safely low concentrations.

The results obtained from the study on mercury content in different media of relevance for human health and the environment by optimising and validating methods for the determination of the element concentration in cosmetic products, food, waters, soils and sludge and polymeric materials, their safety assessment in compliance with the European and national requirements give the basis for the following

V. CONCLUSIONS

1. Methods for the determination of mercury in cosmetic products, food, waters, soils and sludge and polymeric materials based on EPA 7473 „Method for the Direct Determination of Mercury in Solid and Liquid Samples“ have been optimized and validated with a very good analytical performance:

- limit of detection LOD 0.04 ng Hg and limit of quantification LOQ 0.08 ng Hg of the method, ranging from 0.00003 mg/kg to 0.004 mg/kg and from 0.00005 mg/kg to 0.008 mg/kg, respectively, recalculated for the different groups of the individual media according to the amount of sample tested;
- coefficients of variation in repeatability conditions RSD_r from 0.7 % to 6.1 % and reproducibility RSD_R from 2.9 % to 8.9 %;
- bias -5.6 % to 3.5 %;
- uncertainty from 1.9 % to 5.8 %,

making them suitable for application in the study of mercury content in components of the living environment.

2. The mercury content of various media relevant to human health and the environment has been investigated and their safety assessed. In most of the samples analyzed, Hg concentrations were below the LOQ of the analytical procedures. The presence of Hg above the LOQ was detected in 493 of the 2583 samples (19 % of all products analysed).

3. Cosmetic products

- mercury content was detected in 12.6 % of the total 1051 cosmetic products analysed, in the ranges (0.008 ÷ 0.040) mg/kg for hair, face and body cosmetic products and (0.004 ÷ 0.133) mg/kg for oral hygiene cosmetic products; these concentrations are below the acceptable level of technical contamination (1.0 mg/kg and 0.2 mg/kg, respectively);
- the presence of Hg is mainly determined in products containing raw materials of natural origin, but at very low concentrations that do not pose a risk to the health of consumers.

4. Food, food supplements and food additives

- Hg content is below or slightly above the LOQ of the respective analytical procedures in the investigated food groups: Cereals and their products, Milk and dairy products, Fruits and their products, Vegetables and their products, Meat, poultry and their products, Wine, Sugar and sweeteners and Food additives;
- Hg concentrations found in the examined freshwater and marine fish species were in the range (0.030 ÷ 0.187) mg/kg and below the maximum level of 0.50 mg/kg set in Regulation (EU) 2023/915;
- the presence of mercury was determined in the tested food supplements at concentrations of (0.0015 ÷ 0.086) mg/kg, which are below the maximum level (0.10 mg/kg) in Regulation (EU) 2023/915.
- the tested food, food supplements and food additives comply with the requirements of European and Bulgarian legislation and are safe for consumers in terms of mercury content.

5. Water

- the mercury content of drinking waters (tap, bottled and mineral waters from deposits) is in the range (< 0.05 ÷ 0.9) µg/l;
- the presence of mercury was detected in 3.1 % of the 863 tap water samples at concentrations close to and above the LOQ (0.05 µg/l) but below the specified maximum value (1.0 µg/l);
- the Hg concentrations in all tested bottled and mineral water samples from deposits were below the LOQ;
- the tested drinking water samples are clean and safe, in compliance with national and European legislation regarding mercury content and do not pose a risk to public health.

6. Soils

- the average mercury content determined in soil samples from six areas affected by anthropogenic factors is in the range (0.0427 ÷ 0.1038) mg/kg;
- mercury levels found in soils from these areas are above the regulatory background concentration for Bulgaria (0.03 mg/kg), do not exceed the MPC for mercury in soils of arable land and permanent grassland (1.5 mg/kg) and are in compliance with the national legislation as they are many times lower than the MPC for soils of industrial/production sites (10 mg/kg).

7. Sewage sludge

- The average mercury content of the investigated WWTP sludges, in six consecutive years, was in the range (1.03 ÷ 1.99) mg/kg;
- the results show the absence of mercury contamination of the sewage sludge in amounts above the MPC (16 mg/kg); it can be recovered by agricultural use without generating health and environmental risks with respect to this element.

8. Products of polymeric materials

- The determined mercury content of the analysed polymeric materials is in the range (< 0.008 ÷ 0.18) mg/kg;
- the results obtained are in accordance with the European and national requirements and indicate the presence of mercury at safe low concentrations.

9. The results of the study of the total mercury content in various media relevant to human health and the environment indicate that the investigated objects are safe in terms of the presence of mercury and do not pose a risk of increased uptake of this toxic element when used.

VI. CONTRIBUTIONS

- For the first time in Bulgaria, comprehensive studies of mercury content in various media relevant to human health and the environment have been carried out: cosmetic products, water, food, soil, sludge for agricultural use, products of polymeric materials, by applying an optimized and validated EPA 7473 "Method for direct determination of mercury in solid and liquid samples".

- A large number of data have been obtained to establish mercury contamination levels of cosmetics, water, food, soil, sludge for agricultural use, products of polymeric materials. Compliance with the requirements of European and national legislation on mercury content in the media studied has been assessed.

- The summarised and systematised information on the presence of mercury in the investigated media can be used in the fulfilling Bulgaria's commitments to implement Regulation (EU) 2017/852 and the Minamata Convention on Mercury (in relation to research activities, in accordance with Article 19(1)(b) of the Convention).

- Collected data on mercury content in various media important for human health and the environment can serve to create and build a database and take preventive action.

- The mercury data obtained provide quantitative information on the actual population exposure to mercury and can be used for exposure and health risk assessment.

VII. LIST OF PUBLICATIONS
on the topic of the dissertation:

1. D. Stankova, G. Paunova, L. Mechkueva, R. Georgieva. Lead, arsenic, mercury and cadmium content in Bulgarian wines. Nutritional science in the prevention and treatment of modern diseases, 70-72 (2019).

2. D. Stankova, G. Paunova, R. Georgieva. Contents of lead, cadmium, mercury and chromium in articles of polymeric materials. Proceedings of the Annual University Scientific Conference, National University of Defence, V. Tarnovo, 410-417 (2018).

3. A. Tachev, M. Sidjimov, L. Mechkueva, G. Paunova, D. Stankova, N. Vassileva, A. Kamburova. Collection, analytical study and health assessment of a database on the content of chemical contaminants (toxic elements) in oral hygiene cosmetic products. Bulgarian National Association, Essential Oils, Perfumery and Cosmetics, Scientific Information Bulletin, no. 62 (2014).

4. L. Mechkueva, D. Stankova, I. Karadzhova, R. Georgieva, G. Paunova. Analytical procedure for the determination of total mercury in fish. Bulgarian Journal of Public Health, 5 (1), 32-38 (2013).

5. D. Stankova, L. Mechkueva, R. Georgieva. Total mercury content in foods and wines. "Nutrition science faces new opportunities and challenges," ed. B. Popov, Bulgarian Society of Nutrition and Dietetics, Sofia, 76-78 (2008).

VIII. PARTICIPATION IN SCIENTIFIC EVENTS
in relation with thedissertation:

1. Symposium on Toxicology and Annual Meeting of the Bulgarian Society of Toxicology, Sofia, 12.11.2016, D. Stankova, G. Paunova, L. Mechkueva, R. Georgieva, "Mercury content in Bulgarian cosmetic products" (poster).

2. VIII National Conference on Nutrition (01-03.06.2017), D. Stankova, G. Paunova, L. Mechkueva, R. Georgieva, "Lead, arsenic, mercury and cadmium content in Bulgarian wines" (report).

3. Seminar "Contemporary Aspects of Public Health and Ecology" (17.10.2017), D. Stankova, R. Georgieva, L. Mechkueva, "Study of lead, cadmium, arsenic and mercury content in cereals and wheat flour" (poster).

4. Annual University Scientific Conference, National University of Defence, Sofia V. Tarnovo, 14-15.06.2018, D. Stankova, G. Paunova, R. Georgieva, "Lead, cadmium, mercury and chromium content in polymer products" (report).

5. IX National Conference on Nutrition (31.05-02.06.2018), D. Stankova, G. Paunova, R. Georgieva, L. Mechkueva, "Comparative study of lead, cadmium, mercury and arsenic content in sugar and sweeteners" (report).

6. Second Annual Seminar for PhD Students and Young Scientists "Contemporary Aspects of Public Health and Ecology" (19.10.2018), D. Stankova, "Toxic Elements (Pb, Cd, Hg, Cr) in Polymer Products" (report).

7. X Jubilee National Conference on Nutrition (30.05-01.06.2019), D. Stankova, R. Georgieva, L. Mechkueva, "Toxic elements in cereals and cereal products - analytical determination and safe assessment" (poster).

8. XI National Congress on Nutrition with International Participation "Nutrition Science with an Assessment of the Present and a Look into the Future" (26-29.05.2022), D. Stankova, R. Georgieva, L. Mechkueva, "Toxic Elements in Bottled Waters - Mineral, Spring and Table Waters Produced in Bulgaria (poster).