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1 Forensic toxicology

2 Q2 A case of thallium intoxication by walking in a field

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ABSTRACT

Thallium intoxications are a rare occurrence in forensic sciences. This paper reports a case of chronic thallium poisoning in a couple hospitalized in Milan, Italy, in which toxicological analyses were performed by the Institute of Legal Medicine of Milan on the request of the Judicial Authority. Preliminary analyses confirmed the presence of thallium in the blood and urine samples of the couple. After positive results were obtained from the biological samples, the Judicial Authority learned that the son of the couple used thallium powder illegally in his field and that the father helped his son in that field almost every day. Therefore, the Judicial Authority suspected that the man had accidentally contaminated the house environment, thus intoxicating his wife. Consequently, they requested the seizing of multiple items from their residency to verify this hypothesis. Each object was sampled internally (content or internal surface) and externally (surface of the container) and the concentrations of thallium were evaluated using ICP-MS analyses. Positive results of items indicate a thallium contamination caused by an external vector. Indeed, they suggest that none of the elements analyzed was the contaminant responsible for the intoxication but the examination of the soles of the man's shoes argued in favor of an external contamination (the son's field), suggesting that he was the accidental mean of transportation of the heavy metal into the house. This paper presents a rare case of chronic thallium intoxication in a domestic setting where contamination occurred from an illegal usage of thallium in a field.

13 Introduction

14 Heavy metals are compounds that are normally present in nature [1].
 15 Their toxicity can give rise to important morbidity and mortality in
 16 humans both in cases of acute and chronic exposures. The clinical
 17 diagnosis of heavy metal poisoning can be delayed due to the wide
 18 spectrum of symptoms and the difficult identification of some heavy
 19 metals because of their colorless, odorless and tasteless features [2–7].
 20 Examples of heavy metals that satisfy the characteristics described above
 21 include antimony, arsenic, chromium, lead, mercury and thallium.

22 Thallium salts (mainly sulfates) are still used as insecticides and
 23 rodenticides, although it is banned in the United States of America, Italy
 24 and other countries [5,8,9]. These products have been responsible for
 25 intentional poisoning events as well as accidental contaminations of
 26 agricultural food products, mainly grain and rice [10]. Thallium
 27 poisoning can be accidental, criminal, suicidal, occupational or
 28 therapeutic [8,11–14].

29 In the present paper, the authors report a case of chronic thallium
 30 poisoning of a couple, brought to the Institute of Legal Medicine of Milan
 31 and investigated by the Judicial Authority. The aim of the toxicological
 32 analyses performed was to verify the hypothesis of the Judicial Authority,
 33 namely, that the man had been accidentally intoxicated in the son's field
 34 (where powder of thallium was used as insecticide and rodenticide) and

then accidentally contaminated the house environment and his wife. 35

Material and methods 36

2.1. Circumstantial data 37

38 This paper reports the case of a married couple, who were brought to
 39 the Emergency Room of a hospital in Milan, following the onset of various
 40 symptoms including nausea, abdominal pain and alopecia. In fact, the
 41 couple went to the hospital three times in the course of a single month; the
 42 last time, the doctors decided to hospitalize them suspecting a poisoning,
 43 given the aggravation of their symptoms. To verify the diagnosis,
 44 biological samples of the couple (specifically, blood and urine) were
 45 analyzed.

Material 46

47 Biological samples (blood and urine of the couple) were analyzed to
 48 verify the hypothesis (based on the symptoms) of thallium intoxication.
 49 Urine samples were creatinine corrected, because creatinine is an index of
 50 renal function and heavy metals cause kidney malfunction. Given the
 51 positive results obtained from biological samples, the Judicial Authority
 52 was alerted by the hospital to investigate the mean of contamination of

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Table 1
Items tested for presence of Thallium – include amounts detected.

Sample	Positivity	Thallium concentration
Man's blood	Thallium	56.5 ng/mL
Woman's blood	Thallium	16.2 ng/mL
Man's urine	Thallium	329.7 ng/g of creatinine
Woman's urine	Thallium	168.0 ng/ g of creatinine
External washing of sugar container	Thallium	3.7 ng/mL
External washing of bicarbonate container	Thallium	15.4 ng/mL
External washing of biscuit box	Thallium	6.2 ng/mL
Filter of the vacuum cleaner	Thallium	232.1 ng/g
External washing of ornament 1	Thallium	34.7 ng/mL
External washing of ornament 2	Thallium	21.2 ng/mL
External washing of ornament 3	Thallium	7.4 ng/mL
External washing measuring cup 3	Thallium	12.6 ng/mL
External washing of the coffee box	Thallium	1.2 ng/mL
Surface of the soap	Thallium	18.7 ng/g
External washing of the toothpaste tube	Thallium	8.8 ng/mL
External washing of the soles of the man's shoes	Thallium	1440 ng/mL

Negative results: sugar; bicarbonate; wine vinegar; liquid coffee; crackers; biscuits; wine; packaging of the dishwashing detergent; dishwashing detergent; external washing of wine bottle; external washing of hypodermic syringe 1 and 2; measuring cup 1 and 2; fine salt; big salt; pepper; insecticide; potato peel; inside of potato; rice; pasta; coffee powder; surface of bread; inside of bread; chocolates; candies; candy paper; inside of the soap; inside of the toothpaste; bath foam; washing of the container of the bath foam; Washing of the soles of the woman's shoes.

the couple. For this reason, objects and food were seized from the house of the couple (listed in Table 1).

The external washing of items (i.e. when the surface of an item cannot be sampled, toxicological analyses are performed after the external washing of the items), external and internal surfaces and the contents of different items of the house were investigated, as listed in Table 1.

Methods

Toxicological analyses were performed on biological samples and on different items of the house (i.e. external washing, external and internal surfaces, contents), as listed in Table 1.

Table 2
The acceptance criteria for accuracy.

Certified value (ppb)	Measure 1	Recovery %	Measure 2	Recovery %	Measure 3	Recovery %
0.1	0.103	103	0.098	98	0.101	101
0.2	0.201	101	0.198	99	0.203	101.5
0.5	0.497	99.4	0.507	101	0.501	100
1	1.04	104	1.01	101	0.98	98
2	1.987	99.3	2.01	100	2.04	102
4	4.03	100.7	4.07	101.8	3.97	9.3

Table 3
Six calibrations points spiked at different concentrations.

Replicates (ppb)	1	2	3	4	5	6	SD	% RSD
0.1	0.0056	0.0055	0.0058	0.0055	0.0061	0.0053	0.00028	4.97
0.2	0.0074	0.0079	0.0078	0.007	0.0073	0.0075	0.000331	4.43
0.5	0.0081	0.0078	0.0084	0.008	0.0076	0.0078	0.000281	3.53
1	0.0098	0.0101	0.0099	0.01	0.0102	0.0101	0.000147	1.47
2	0.016	0.0158	0.0162	0.0161	0.0159	0.0157	0.000187	1.17
4	0.0271	0.027	0.0268	0.0269	0.0271	0.0272	0.00792	0.54

The samples were mineralized in microwave with 6 mL of HNO₃ Ultrapur and 2 mL of H₂O₂ Ultrarex II [15]. The preliminary analyses were performed in semiquantitative mode using the Agilent ICP MS 7500 series instrumentation and automatic sampler at Asx-510 Autosampler.

Validation data

A dosage of the concentrations of thallium was performed on all elements which tested positive. For this purpose, a calibration line was prepared using a standard solution (Backer Instra) at a concentration of 10 ppm. Appropriate dilutions of this solution were carried out and different samples were prepared in order to set up 6 calibration points of a concentration of 0.1–0.2–0.5–1–2–4 ppb. A solution of yttrium (Backer Instra) at a concentration of 10 ppb was used as an internal standard.

Method Detection Limit (MDL) was calculated from three times the standard deviation of 10 external measurements of unspiked samples. The endogenous content in the unspiked samples was subtracted before determining accuracy and precision. For accuracy, the recovery of six preparations spiked at different concentrations (0.1–0.2–0.5–1–2–4 ppb) was maintained in a range between 90%–110%, thus meeting the acceptance criteria for accuracy (Table 2). The same requirements were met in calculating precision (repeatability) for six preparations spiked at different concentrations meeting the % Relative Standard Deviation (RSD) criteria with a value $\leq 20\%$ (Table 3).

Limit of Detection (LOD) for thallium was calculated and evaluated to be less than 25 ppt. In order to evaluate the ruggedness, a second operator, on a different day, prepared and analyzed six samples spiked at different concentrations with freshly made solutions. Ruggedness was calculated as operator/day to operator/day precision change in the mean. For the element in topic, the second analyst/day mean for all six samples was within $\pm 16\%$ of the mean result of the previous day and first analyst. The %RSD for all 12 preparations was $\leq 20\%$.

Stability was calculated by running the six spiked solutions made by the second analyst after storing them at room temperature for approximately 48 h. Concentrations were calculated, and the mean values were compared to the samples previously analyzed in order to establish solution stability of the spiked samples. The mean change was maintained within $\pm 20\%$ from initial results and the solutions was therefore considered as stable.

Specificity was assessed by quantifying the spiked samples against the calibration standard solution and meeting the accuracy requirements.

Quality control (QC), expressed as trueness, was performed by analyzing the certified reference multi-element synthetic urine SERO-NORM TRACE ELEMENTS URINE L-1 (rif.210605 lotto 1011644) digested with the same protocol of the samples. Variability of the QC was never greater than 6%.

Results

The toxicological analyses reported positive results for the biological samples of the couple and for the external surface of different items, as detailed in Table 3. In particular, the man's blood and urine samples reported 56.5 ng/mL and 329.7 ng/g of thallium and creatinine, respectively. The woman's biological samples noted 16.2 ng/mL of thallium in blood samples and 168.0 ng/g of creatinine in urine samples.

The contents and the internal and external surface of the items seized from the house of the couple were analyzed to evaluate the presence of thallium in the house environment. The external washing and external surface of the majority of the items (including the sugar container, bicarbonate container, biscuit box, vacuum cleaner, ornaments 1, 2 and 3, measuring cup 3, coffee box, surface of the soap, toothpaste tube and soles of the man's shoes) gave positive results. All internal surfaces and contents of items gave negative results, whereas only some external washings or surfaces were negative (such as packaging of dishwashing detergent, hypodermic syringes 1 and 2, candy wrappers, wine bottle, potato peels, surface of bread, container of the bath foam, soles of the woman's shoes). The highest concentration of thallium (1440 ng/mL) was detected on the soles of the man's shoes.

Discussion

After the onset of the same symptoms (including nausea, abdominal pain and alopecia) in a married couple, the doctors suspected an intoxication. The couple went to the Emergency Room three times in a month and they were hospitalized the last time, after the aggravation of their symptoms. Therefore, analyses on blood and urine samples were performed to determine which substance caused the symptoms.

As the results of the biological samples revealed a positivity to thallium, the Judicial Authority suspected that the couple had been chronically intoxicated by the metal. As a result of our investigations, chronic intoxication was confirmed. Indeed, normal levels of thallium in blood samples remain below 8 ng/mL [16], whereas after a lethal dose, levels are reported at 1000–8000 ng/mL [17]. Therefore, the thallium levels of the couple (56.5 ng/mL in blood samples of the man and 16.2 ng/mL in the woman's sample) are higher than the normal levels of thallium and below the lethal dose in serum. Urine is the most commonly used sample for the detection of chronic thallium exposure [18]. The levels of thallium in urine in cases of non-exposed individuals are below 1.1 mcg/g of creatinine (creatinine corrected) [18–21] or below 1 mcg/L (uncorrected) [21,22], while the concentration of thallium after an acute intoxication are reported at 1800–2000 mcg/L [17]. Urinary thallium levels in cases of prolonged exposure to thallium due to environmental contamination [22,23], poisoning of a population that lived near a cement plant [24] or workers population exposed to thallium [19,25] report concentrations of the metal included in the ranges between non-exposed individuals and acute poisoning individuals which corresponds to the values of the couple under investigation: 3.29 mcg/g of creatinine in the man's urine sample and 1.68 mcg/g of creatinine in the woman's urinary sample. Furthermore, the couple arrived at the Emergency Room with severe symptoms, including nausea, abdominal pain and alopecia. These symptoms are consistent with early and intermediate stages of thallium poisoning, suggesting an exposure of up to two weeks [26–32]. Therefore, given the characteristic symptoms of thallium poisoning presented by the couple and the positive results obtained from their biological samples, the Judicial Authority confirmed the chronic thallium intoxication of the couple. However, further

investigations were requested to understand how the couple had been exposed to thallium.

The Judicial Authority learned that the son of the couple illegally used thallium powder in his field, as rodenticide and insecticide, and that the father would go to work in that field almost every day. Thus, the hypothesis was that the man had been intoxicated in the field and then accidentally contaminated the house, inadvertently poisoning his wife. To verify this hypothesis, toxicological analyses were requested on various items of the house of the couple as well as on the soles of the man's and woman's shoes.

As a result, not all items resulted positive to thallium. In fact, the internal surface and the contents of all items gave negative results. From the results obtained, it can be inferred that none of the items analyzed was directly responsible for the poisoning. Indeed, the presence of the metal was only detected on the outer surface of the items or on the containers of the specimens tested, suggesting that the items came into contact with thallium solely through their external manipulation. It is therefore arguable that none of the elements inside the house acted as a contaminant, but that they were all contaminated by an external source. Furthermore, these positive results were obtained on materials of everyday use, such as the surface of the soap, biscuit box, toothpaste container (among others), indicating a chronic exposure to the metal instead of an occasional contamination.

Higher levels of thallium were detected in the plasma and urine samples of the man (56.5 ng/mL and 329.7 ng/g of thallium and creatinine, respectively) with respect to the biological analyses of his wife (16.2 ng/mL of thallium in blood samples and 168.0 ng/g of creatinine in urine samples) suggesting that the man was more exposed to the metal source. Moreover, the highest concentration of thallium on items was detected under the soles of the man's shoes (1400 ng/mL), whereas the soles of woman's shoes gave negative results. The soles of the woman's shoes were analyzed to exclude that the intoxication was delivered from a source different from the son's field. Indeed, given that the woman did not work in that field, if some specific personal objects of the woman (such as the shoes, that are directly in contact with the environment) were contaminated, the source of contamination would be different from the son's field. The lower levels of thallium in the biological samples of the woman and the negative results obtained on the soles of the woman's shoes suggest that the source of contamination was not a common environment of the couple.

Consequently, the highest concentration of thallium obtained from the soles of the man's shoes, which he used to work in his son's field every day, are consistent with a source of contamination in the son's field where thallium powder was used as insecticide and rodenticide. Therefore, the positive results obtained solely on the external washing or surface of items and on the soles of the man's shoes corroborate the suspicions of the Judicial Authority: the man, contaminated working in the son's field, was the mean of transportation of the metal to the residence, which resulted in the accidental intoxication of his wife.

Conclusion

The high toxicity of thallium is often associated with an acute or chronic intoxication with a fatal outcome. In this paper, we report a case of chronic intoxication of a couple, caused by environmental and accidental intoxications, in which biological samples were analyzed with ICP-MS analyses. The complexity and specificity of the analytical techniques suitable for the identification of the metal rendered these poisonings difficult to diagnose; indeed, in the present case, the hypothesis of intoxication was considered only after the aggravation of the symptoms. However, after the positivity obtained from the biological samples of the couple, the hypothesis of intoxication by a metal was confirmed, allowing the doctors to treat the couple for chronic thallium intoxication and save them.

Ethical approval

This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

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

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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