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# Tailoring LC approaches to address the chemical biology of toxic metals in humans

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The global contamination of ~15% of agricultural soils with toxic metal (loid) species (TMS) including arsenic, cadmium, mercury, and nickel compromises food safety and chronically exposes millions of people to these inorganic pollutants which are absorbed into the bloodstream to various degrees. The health ramifications that pertain to this arguably biggest problem in the postgenomic world remain poorly defined, particularly with regard to babies, children, pregnant women and industrial workers. To better understand the underlying complex exposure-response relationship, it is crucial to address chemical biology related research questions unfolding within the blood-organ nexus. Since the associated complexity makes it particularly challenging to causally link human exposure to exceedingly small daily TMS doses with adverse health effects and environmental diseases, the use of appropriate analytical tools is paramount. One research tool whose full potential to probe dynamic bioinorganic processes has not yet been fully recognized is liquid chromatography (LC). Recent results will be used to highlight how LC methods can be tailored to address chemical biology questions pertaining to toxic mercury and cadmium species at near physiological conditions. It will be argued that the inherent versatility of LC methods makes it eminently suitable to tackle questions regarding the exposure-response relationship of other emerging TMS to more effectively deal with this emerging public health crisis.

## KEYWORDS

cadmium, mercury, nickel, manganese, element-specific detection, separation mechanism, mechanism of chronic toxicity

## 1 Introduction

It is estimated that 9 million people died of pollution related causes in 2015 (Fuller et al., 2022). Since 14–17% of agricultural soils globally are contaminated with toxic metal species (TMS), such as Ni, Cd, Hg and Pb (Hou et al., 2025), the ingestion of contaminated food affects millions of people worldwide (Ugulu et al., 2025). The subsequent influx of TMS from the gastrointestinal tract into the bloodstream urgently requires a better understanding of the associated adverse health ramifications that will eventually unfold at the organ level. To this end, the biomolecular mechanisms which explain why any given toxic metal species (TMS) causes organ damage and/or a disease were well posed, but unsolved problems 50 years ago (Passow et al., 1961), and largely remain so today. Even though immense progress has been made in terms of elucidating relevant biomolecular mechanisms of action (Aschner et al., 2022), we are still unable to reasonably predict how much of an orally ingested TMS dose—in form of contaminated food and/or drinking water - actually reaches toxicological target organs to disrupt vital cellular processes therein (Doroudian and Gailer, 2022). In addition, there is no strategy about how we should address



which makes them particularly useful to study interactions between TMS and ligands at near physiological conditions. These capabilities include their intrinsic ability to observe a) the on-column formation of complexes between metal (loid)s that are injected and ligands that are dissolved in the mobile phase (Liska et al., 1979; Pourzadi and Gailer, 2024), b) the temporal stability of molecular constituents in liquid formulations [e.g., the organomercury bactericidal agent thimerosal in vaccines to establish their shelf-life; (Reader and Lines, 1983)], c) the stability of metal-based compounds in biological fluids [e.g., thimerosal in red blood cell cytosol (Gibson et al., 2017)] as well as metal-based anticancer drugs (e.g., cisplatin and carboplatin) in blood plasma (Sooriyaarachchi et al., 2011) and – perhaps most importantly - d) a shift of the retention time of an on-column formed TMS species based on employing different LC-based separation mechanisms, namely, size exclusion chromatography (SEC), anion-exchange chromatography (AEX) and/or reversed phase chromatography (RPC). Thus, the utilization of physiological buffers in conjunction with LC-methods and appropriate element-specific detectors (e.g., flame atomic absorption spectrometry or inductively coupled plasma atomic emission spectroscopy) should therefore allow to obtain new insight into the toxicological chemistry of TMS species at near physiological conditions.

### 3 Top-down and bottom-up LC approaches to probe the bioinorganic chemistry of TMS

The interaction of TMS with biomolecules can be probed using top-down or bottom-up approaches (Gailer, 2013). The top-down approach refers to the direct LC-analysis of biological fluids to which a TMS has been added using mobile phases that mimic its background electrolyte using appropriate buffer salts (Jahromi et al., 2010). SEC, for example, tolerates the injection of blood plasma when PBS-buffer is used (Manley et al., 2009), while RBC cytosol was analyzed using a 0.1 M Tris buffer which contained 2.5 mM GSH (Gibson et al., 2017) to simulate the RBC cytosol concentration. Conversely, the bottom-up approach can be employed to simulate a specific bioinorganic chemistry reaction, such as the binding of an injected TMS onto a column with a ligand that is dissolved in physiological buffer as the mobile phase (Pourzadi and Gailer, 2024).

## 4 Application of LC-methods to address bioinorganic TMS problems

To date, SEC, AEX and RPC have been successfully employed to probe bioinorganic processes of TMS at the bloodstream-organ nexus in a top-down (TD) and a bottom-up (BU) manner.

### 4.1 Formation of organ available TMS metabolites in plasma

#### 4.1.1 SEC reveals a MeHg<sup>+</sup> metabolite that is delivered to the blood brain barrier (TD)

While MeHg<sup>+</sup> has long been known to be neurotoxic, the structure of the organ available metabolite that is formed in

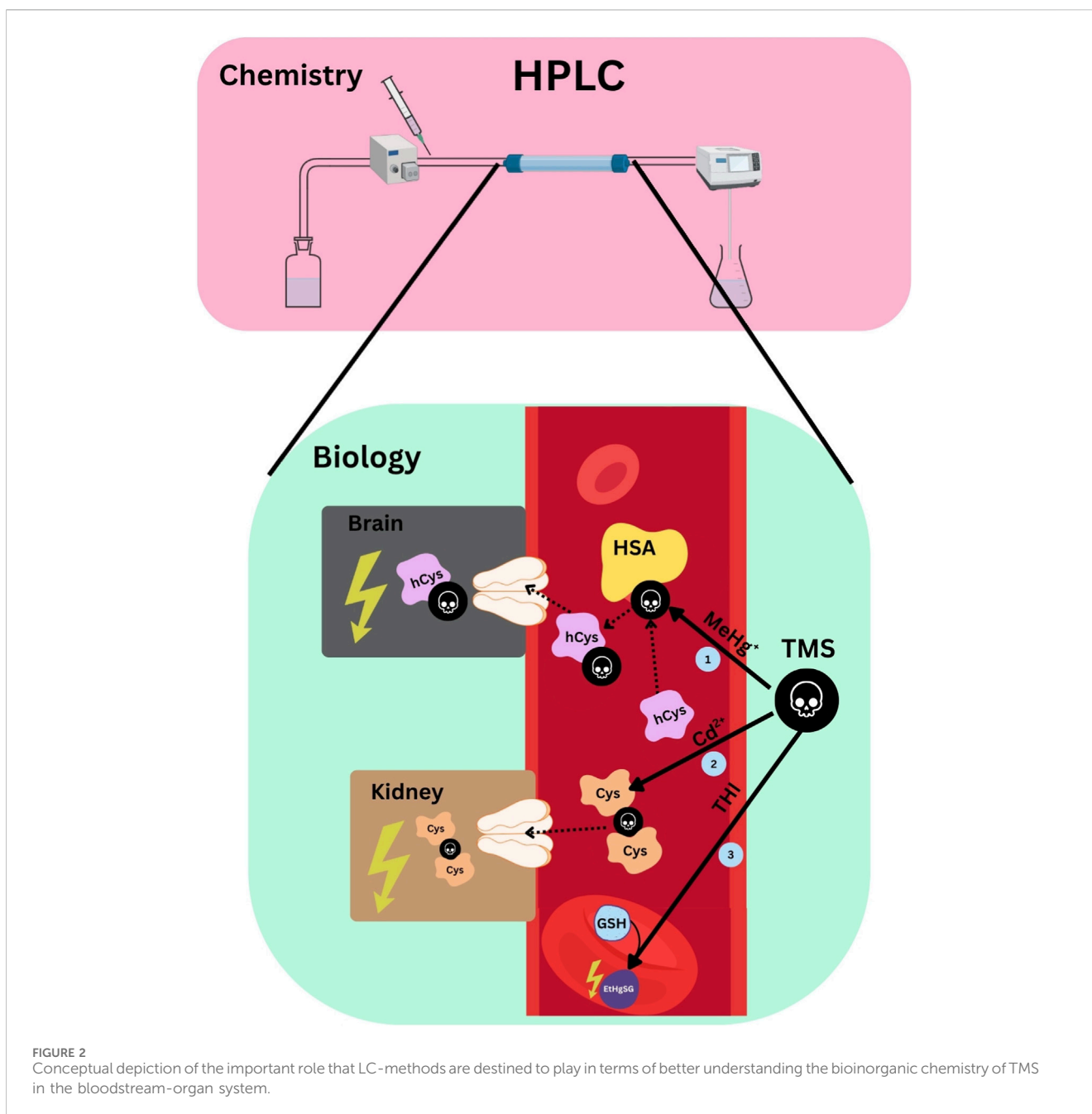
plasma and enters the brain has been elusive. To gain insight, the utilization of a PBS-buffer mobile phase and the analysis of rabbit plasma that had been spiked with MeHg<sup>+</sup> revealed the elution of a rabbit serum albumin (RSA)-MeHg complex (Bridle et al., 2022). Since small molecular weight (SMW) thiols, such as homo-L-cysteine (hCys) are present in blood plasma at 10–15 μM, the mobilization of MeHg<sup>+</sup> from its RSA binding sites was investigated by gradually increasing the hCys mobile phase concentration from 50 to 300 μM and injecting MeHg-spiked rabbit plasma. The results revealed that the 50 μM hCys containing mobile phase shifted the retention time of MeHg from the protein elution range to the SMW elution range, implying the on-column formation of a MeHg-hCys complex. The formation of the latter complex was confirmed by electrospray ionization-mass spectrometry (ESI-MS). The presence of L-amino acid transporters 1 (LAT-1/2) at the blood-brain-barrier was previously demonstrated to recognize structurally closely related MeHg-Cys complexes. Thus, the observed formation of MeHg-hCys complexes at near physiological conditions of blood plasma provides a feasible pathway by which MeHg<sup>+</sup> is delivered to the brain (Figure 2), where adverse biochemical processes including the inhibition of the selenoprotein glutathione peroxidase 4 (GPx) will then unfold (Chen et al., 2023).

#### 4.1.2 AEX identifies Cd<sup>2+</sup> metabolites that are delivered to the kidneys (BU)

Although Cd<sup>2+</sup> has long been known to be a nephrotoxin, the organ available metabolite(s) that is/are formed in plasma has/have remained elusive, but likely involves the formation of Cys-complexes (Bridges and Zalups, 2017). The application of AEX in conjunction with a mobile phase comprised of 100 mM NaCl and 5.0 mM Tris-buffer (pH 7.4) allowed to observe the elution of the injected Cd<sup>2+</sup> species. Since Cys is implicated in the translocation of Cd<sup>2+</sup> from blood plasma to the kidneys, Cd<sup>2+</sup> was then chromatographed with mobile phases containing increasing Cys concentrations between 0.1 and 10 mM (Gautam et al., 2023). The Cd-species that eluted with the 100 and 200 μM Cys mobile phase were then structurally characterized by X-ray absorption spectroscopy. The results revealed a mixture of Cd species with tetrahedral coordination suggesting the formation of [Cd(Cys)<sub>1/2</sub>]<sup>+1</sup> complexes which may represent the nephrotoxic species that are then uptaken by the kidneys (Figure 2) to induce cellular damage by processes that are still incompletely understood (Thevenod and Lee, 2024).

### 4.2 RPC reveals the GSH mediated degradation of a pharmaceutical TMS at pH 7.4 (BU)

The bactericidal organomercurial thimerosal (THI) has been used as a vaccine additive since the 1950s. Since vaccines are intramuscularly injected, THI will therefore interact with mammalian cells (e.g., RBCs) and the biological thiols therein. The donation of the ethylmercury (EtHg<sup>+</sup>) moiety from THI to mammalian proteins that contain surface accessible contain thiol groups (Geri et al., 2024), however, has not been observed at physiologically relevant conditions. Since GSH is present in RBC cytosol at ~2.5 mM, a LC-method was developed (Le et al., 2025) which allowed to observe the GSH-mediated degradation of THI



(Degorge et al., 2025). In brief, the addition of increasing GSH concentrations to the mobile phase (2.5–15.0 mM) resulted in a decrease of the Hg peak corresponding to THI and the elution of a new Hg-peak with a shorter retention time. With the 15 mM GSH mobile phase, two Hg-peaks were detected which displayed equal intensities. The elucidation of the molecular structure of the unknown Hg peak by ESI-MS revealed a GS-HgEt adduct, which allowed to propose a degradation mechanism for THI at pH 7.4 (Degorge et al., 2025), which may unfold in RBCs (Figure 2). While the GSH-mediated degradation of THI was observed in the presence of 30% of acetonitrile, these results serve as an important starting point to better understand the side-effects of THI, such as the transfer of the EtHg-moiety from the GS-EtHg adduct to other cytosolic proteins.

### 4.3 Discussion

The application of SEC and AEX allowed the structural characterization of organ available  $\text{MeHg}^+$  and  $\text{Cd}^{2+}$  metabolites that are formed in plasma, while the application of RPC revealed the mechanism by which GSH degrades the vaccine additive thimerosal. Taken together these findings are highly relevant to better understand the exposure-response relationship related to the exposure of humans to TMS (Zhang et al., 2025). The emerging exposure of human populations to other metals of high technological relevance, such as  $\text{Ni}^{2+}$  (Parveen et al., 2025) and  $\text{Mn}^{2+}$  (Deng et al., 2024) will similarly require a better understanding of the corresponding bioinorganic processes to develop cheap palliative measures to mitigate their impact (Parveen et al., 2025)

as they already compromise food security on a global scale (Ugulu et al., 2025). The application of LC-methods in conjunction with other advanced instrumental analytical methods (Weng et al., 2024) and metalloproteomic approaches (Zhou et al., 2022) thus represent a useful addition to the existing analytical toolbox to uncover the mechanism-integrated framework which links human exposure to multiple TMS with the etiology of environmental diseases (Gailer, 2024; Leung et al., 2024) and are destined to play an important role to foster the development of more effective strategies to globally reduce their adverse impact on human health (Gulma, 2025).

## Ethics statement

The withdrawal of blood from rabbits to obtain blood plasma and red blood cells was approved by the Conjoint Health Ethics Board of the University of Calgary. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

JG: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review and editing.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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