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POINT OF VIEW

Thimerosal, an organic mercury compound used as a vaccine preservative: A real necessity or regulatory inertia?

Contributions from analytical chemistry

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Before beginning this text, one point must be made very clear. Vaccination is essential for preventing serious diseases and avoiding outbreaks that threaten public health. Vaccines protect individuals and the community through herd immunity, reducing mortality, hospital costs, and permanent sequelae associated with infections. Vaccination is an act of caring for yourself and others.

Thimerosal (TM) is a mercury-containing organic compound widely used as a preservative in various biological and pharmaceutical products, including many vaccines, to prevent the growth of harmful microbes inadvertently introduced into the vaccine during its use. The documented antimicrobial properties of TM contribute to the safe use of vaccines in multi-dose vials, which are less expensive, easier to store, and help reduce waste. TM, which is approximately 50% mercury by weight, has been one of the most widely used preservatives in vaccines. It is metabolized or degraded to ethylmercury (EtHg) and thiosalicylate. In general, a vaccine containing 0.01% (m/v) TM as a preservative contains 50 µg of TM per 0.5 mL dose, corresponding to approximately 25 µg of mercury per 0.5 mL dose.[1] The use of TM as a preservative in multi-dose vaccines is controversial because this compound has been abolished in the United States and the European Union, either due to its replacement with other preservatives (free mercury) or the adoption of single-dose formulations. In Brazil, it is somewhat surprising that of the use of TM in cosmetics (topical use) has been suspended,[2] partly related to allergic contact dermatitis, but its use in vaccines is still permitted. The World Health Organization (WHO) supports this decision, stating that “ethylmercury is present in thiomersal as a preservative in some vaccines and does not pose a health risk.”[3] However, a growing body of scientific evidence has increasingly challenged this assertion.

Experimental models for assessing the toxicity of a given species are particularly decisive. Regardless of the model (simple or complex) tested with TM, evidence of this compound’s toxicity consistently emerges to varying degrees (Figure 1). TM has demonstrated the ability to form adducts with cysteine, glutathione, and especially with carrier proteins, binding to free thiol groups and thereby being transported throughout the body, reaching other proteins, enzymes, and organs.[4] In this sense, the effect of TM on proteins has been associated with its ability to induce protein fibrillation,[4,5] impair hemoglobin’s capacity to bind oxygen, and increase protein glycation.[6] The use of electrospray ionization–mass spectrometry (ESI-MS) has confirmed TM’s high affinity for proteins containing free thiol groups, leading to metalation and the formation of stable adducts with cytochrome *c*, ribonuclease A, carbonic anhydrase I, and superoxide dismutase, thereby

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compromising the natural activity of these enzymes.[7] When used as a cellular model, erythrocytes exposed to TM show alterations in essential functions, particularly in oxygen transport capacity, along with changes in cellular morphology.[8] Across different cellular models, TM has consistently demonstrated toxicity, indicating that the doses used to achieve antimicrobial activity cannot be considered safe.[9]

Different animal models (flies, fish, and rodents) have consistently demonstrated that TM is a toxic compound, even at sublethal doses.[10–12] In mouse models, TM compromises vaccine potency through thiol modification, affecting the antigenicity and immunogenicity of the formulation by reducing the binding activity between antigens and antibodies.[13] In contrast, a Wistar rat model mimicking TM exposure in infants following childhood vaccination revealed significant damage to bioenergetic pathways within the nervous system, particularly the brain.[14] Moreover, in baby monkeys exposed to TM-containing vaccines, researchers found that the fraction of inorganic mercury in the brain ranges from 21% to 86% of total mercury measured, with an average of ≈ 70%.[15]

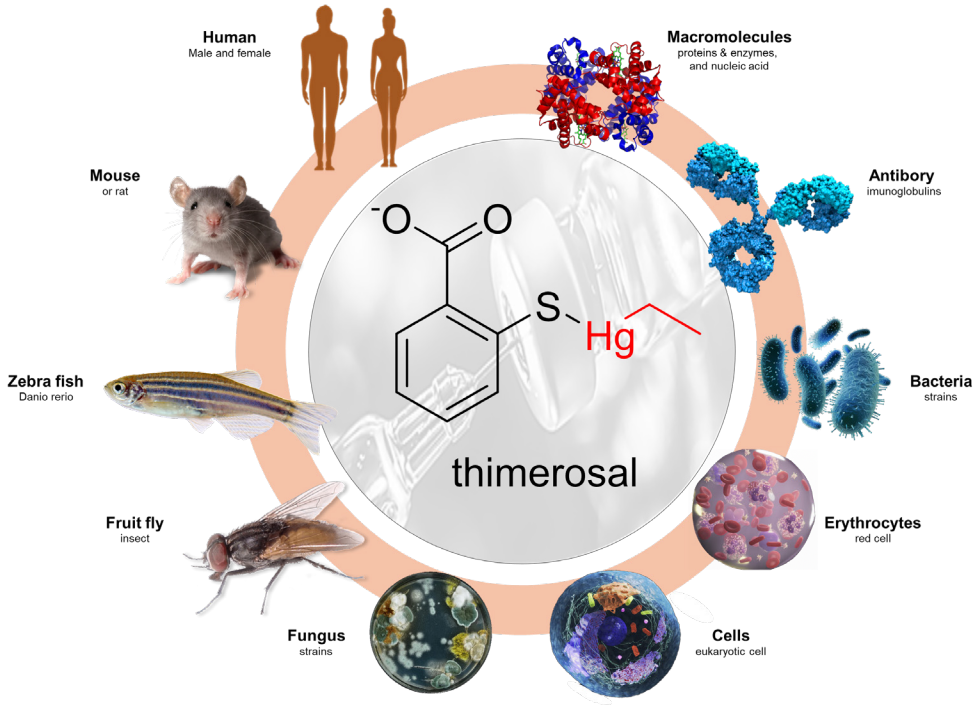

Figure 1. The chemical structure of thimerosal and examples of different experimental models for toxicity assessment.

In vitro studies comparing EtHg with methyl mercury (MeHg) have shown similar outcomes in cardiovascular, neural, and immune cells. However, under *in vivo* conditions, evidence indicates distinct toxicokinetic profiles between MeHg and EtHg, with the latter exhibiting a shorter blood half-life, different compartment distribution, and faster elimination. EtHg's toxicity profile, therefore, differs markedly from that of MeHg, leading to distinct patterns of exposure and associated toxicity risks.[16] From another perspective, studies on the environmental fate and risk of mercury have mostly focused on total mercury and the toxic species MeHg. However, EtHg has long been overlooked, partly due to analytical limitations. The occurrence of EtHg and its possible natural sources in the environment provide essential background information and valuable clues for understanding its natural presence and environmental behavior.[17] Thus, the distribution and toxicological aspects of EtHg are not solely associated with TM use; they are also related to other environmental and chemical pathways. Therefore, expanding research on TM and EtHg is a strategic priority to achieve a more comprehensive understanding of their biological effects and associated impacts.[18]

In this context, the continued use of TM in some vaccines reflects less an unavoidable scientific necessity and more a set of regulatory, logistical, and economic barriers. The proven stability of these formulations, the low rate of serious adverse events, and the reduced cost of multidose vials create a scenario in which regulatory agencies are reluctant to require reformulations that would necessitate new stability, safety, and immunogenicity studies. From an industry perspective, the lack of economic incentives to modify products intended primarily for low-return markets reinforces institutional inertia, even in the face of technically feasible alternatives consistent with global efforts to reduce mercury use.

In this context, analytical chemistry plays a crucial role in providing evidence that extends beyond traditional safety indicators. Sensitive chemical speciation methods, for example, enable the distinction between EtHg, MeHg, and their inorganic forms, thereby revealing metabolic pathways that in the past could not be assessed with conventional toxicological approaches. These advances enable the characterization not only of the kinetics of systemic elimination, but also of the formation and accumulation of inorganic species in target tissues, providing a more accurate basis for reassessing risks in vulnerable subpopulations. In addition, microbiological monitoring techniques and chemical stability analyses provide robust data to validate formulations without TM or with alternative preservatives, demonstrating that microbiological safety can be preserved through optimized packaging systems or the adoption of single-dose presentations.

Based on this evidence, a central conclusion can be drawn: The maintenance of TM today is more a consequence of a regulatory and productive framework that is insufficiently dynamic than it is a result of real scientific limitations. The data generated by analytical chemistry, speciation studies, kinetic analyses, stability evaluations, and post-use surveillance not only allow for a more detailed characterization of the toxicological profile of EtHg, but also provide technical support for transitioning to safer and scientifically sound alternatives. Thus, analytical advances cease to function merely as evaluative tools and become true catalysts for change, providing the scientific basis required for regulators and manufacturers to adopt policies and formulations that progressively reduce dependence on mercury compounds in vaccines.

Finally, it is crucial to emphasize that, regardless of whether TM is present, vaccination remains essential. For adolescents and adults, TM-associated risks are typically minimal; however, for infants and newborns, existing uncertainties deserve more careful consideration. Nevertheless, the choice between a TM-containing vaccine and no vaccination at all is unequivocal: Vaccination unquestionably remains the safer and more responsible option.

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