Online publication ahead of print, AJMHS Vol 59, 2022

Thiomersal and Mercury Derivatives: a Review of Epidemiological, Pharmacological and Analytical Studies

Irsida Mehmeti*, Malvina Hoxha

Catholic University "Our Lady of Good Counsel", Tirana, Albania

Abstract

The simultaneous exposure to different sources of mercury should be taken into consideration, due to potential additive effects. This review summarizes recent data on the effects of thiomersal and related mercury species from the chemical, pharmacological, toxicological and epidemiological point of view. The final objective is to observe if any connection between chemical properties of thiomersal and mercury derivatives in neurodevelopmental disorders can be stated from the literature. Studies on thiomersal and its degradation products toxicity, as well as additive effects from other sources of mercury were searched in PubMed, Scopus, and SciFinder Scholar. The publications considered relevant to the topic were selected, reported and commented. Studies reporting thiomersal in other forms and dosages from those found in vaccines (0.005% - 0.01%p/v in liquid dosage forms), were not taken in consideration. In our study were included only publications in English language published after 2013. The toxicological mechanisms of thiomersal in neurodevelopmental disorders are various, such as the GSH depletion, induction of oxidative stress, increase in reactive oxygen species and reactive nitrogen species. Despite the similar toxicities of EtHg and MeHg in vitro, these results cannot be inferred to in vivo due to different pathways of the two molecules. Although there are a lot of preclinical studies which assess the mechanism and effects of thiomersal in animals, there are many differences between human biological patterns and animal anatomy. As far as the other studies are

Address for correspondence: Irsida Mehmeti*, Rr.Dritan Hoxha, Tirane, Albania, 1000. Email: i.mehmeti@unizkm.al

concerned, six epidemiological studies reported no association between vaccines and neurodevelopmental outcomes However, the limitations of in vitro, in vivo, clinical studies and epidemiological studies make their conclusions inadequate to generalize the results about the relationship between thiomersal and neurodevelopmental disorders. Moreover, although the cytotoxicity of mercury significantly depends on its chemical form, most of the scientific studies are focused on the determination of the total amount of mercury in cells, mostly because of the lack of efficient speciation analytical methods and adequate samples.

Keywords: thiomersal, toxicity, epidemiological studies, mercury speciation analysis

INTRODUCTION

Thiomersal (also known as thimerosal) gained public attention in 1999. Thiomersal, an organic-mercury compound containing 49.6% Hg by weight, has been used since 1930 as a preservative in multi-dose vials biological products due to its fungicidal and bactericidal effects (1). It is present in biological products at concentrations that vary from 0.005% to 0.01% and generates ethylmercury (EtHg) in aqueous solutions (2). A concentration of 0.01% corresponds to 50 μg of thiomersal per 0.5 mL dose or approximately 25 μg of mercury per 0.5 mL dose. In 1999, the FDA removed thiomersal from U.S licensed childhood vaccines as a precautionary measure (2).

Besides thiomersal containing biological products, human populations are exposed to different forms of low chronic levels of mercury derivatives, which could have an additive or synergistic effect on their health. In particular, mercury vapor (from dental amalgams), inorganic mercury (from cosmetics, diuretics, and laxatives) and methylmercury (MeHg, coming from fish ingestion) should be considered. It should be noted that all the different mercury forms are able to generate Hg++. The permissible level of inorganic mercury in drinking water is 6 μg L^{-1} (3), while normal level of mercury concentration in human whole blood is <10 μg L^{-1}. Concentrations > 50 μg L^{-1} and 200 μg L^{-1} indicate a significant exposure to alkyl and inorganic mercury, respectively (4).

The international treaty Minamata Convention on Mercury was signed in October 2013 with the objective “to protect the human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds”. All signing nations agreed to reduce the emission and use of Hg to protect human and environmental health (5). In September 2018, 97 States and 1 regional economic integration organization had deposited their instruments of ratification, acceptance, approval of or accession to the Convention with the Depositary, the Secretary-General of the United Nations (6). Due to the high toxicity and bioaccumulation factor, mercury determination is very important in environmental and biological samples. However, toxicity of mercury is not strictly correlated with its total content but is much more dependent on its chemical form. Therefore, speciation analysis of mercury is extremely important (7).

A comprehensive survey on the impact of mercury on the US population is given by the Fourth Report on Human Exposure to Environmental Chemicals, where mercury and its metabolites were measured in blood, serum, and urine samples from random subsamples of the National Health and Nutrition Survey (NHANES) (8).

Although mercury toxicity depends on the route and dose of administration, the worldwide goal is to reduce environmental mercury exposure. Since March 2001 no vaccine for routine use in children contains thiomersal in Canada, with the exception of some influenza vaccines (9). Moreover,

manufacturers removed thiomersal from all vaccines in Australia's National Immunization Program in 2000 (10). Thiomersal was also removed from United Kingdom vaccines between 2003 and 2005 (11). Although thiomersal free vaccines are commercialized in the majority of European countries, the European Commission and the European Medicines Agency consider that immunization with vaccines containing thiomersal should continue to be used. In a thorough safety review conducted by the Institute of Medicine in 2001 and further in 2004, the body of evidence favored the rejection of a potential relationship between the mercury in biological products and neurodevelopmental disorders (11). Different studies have shown the toxic effect of thiomersal in the embryonic tissue cells, retaining to be more toxic to leukocytes than bacteria (12). Hg^{2+}, EtHg and MeHg species were found in the rat brain after the injection of thiomersal solution, instead of low Hg^{2+} species level in blood (13). EtHg compounds can cause disorders of the central nervous system, arrhythmia, kidney, heart and liver lesions, lesions of cerebral cortex, paralysis, spasms (14). In addition, mercuric Hg has been reported to cause immunological reactions (15). Neurodevelopmental disorders are deficits in the neurological functioning and brain development of children that affect the emotions and behavior, language and communication skills, motor skills, memory and learning capacities. According to Diagnostic and Statistical Manual of Mental Disorders (DSM-5), they include autism spectrum disorder (ASD), tic disorder (TD), attention deficit/hyperactivity disorder (ADD/ADHD), communication disorders, intellectual developmental disorder, and specific learning disorders (16). Hooker et al. quoted 6 studies conducted by different groups indicating that the exposure to thiomersal is not associated with autism, or in some cases it may even decrease the risk of autism (17-23). FDA supports the safety of thiomersal' containing vaccines based on robust body of peer' reviewed scientific studies conducted over the past 15 years (24). Global Advisory Committee on Vaccine Safety reviewed several pharmacokinetic and epidemiological studies concerning thiomersal conducted between 2003 and 2008 and concluded that the available evidence strongly supports the safety of the use of thiomersal as a preservative for inactivated vaccines (25). While the use of mercury-containing vaccines has declined in recent years due to the development of single-dose formulations, thimerosal has been used in some immune globulin preparations, anti-venins, skin test antigens, and ophthalmic and nasal products, in addition to some vaccines. In this review we report a summary of the preclinical, clinical and epidemiological studies concerning the potential relationship between mercury derivatives, in particular thiomersal and neurodevelopmental disorders. We aim to ascertain if these studies tend to support or to exclude a link between thiomersal and several neurological disorders.

MATERIAL AND METHODS

This review was conducted rigorously following the preferred reporting items for systematic reviews (PRISMA-P) guidelines. (PRISMA (prisma-statement.org)

Literature search

A bibliographic-research was done using the databases of PubMed, Scopus, and SciFinder Scholar. Only articles in English language were selected and were filtered the articles published after 2013. The following keywords were used in the Scopus database "thiomersal, thimerosal, epidemiological studies, speciation analysis, neurodevelopmental disorders", in the PubMed database were used ((((((neurodevelopmental disorders) AND (thiomersal)) AND (epidemiological)) OR (observational)) OR (cohort studies)) OR (cross sectional)) OR (ecological) ((thiomersal) AND (pharmacological properties) AND (toxicology) AND (chemical properties)), in the SciFinder Scholar the following keywords were used: thiomersal, neurodevelopmental disorders, degradation products, vaccines, speciation analysis.

All authors independently reviewed the full texts to further assess if the selected studies fulfilled the eligibility criteria. Fig. 1. reports a schematic diagram of the literature search procedure.

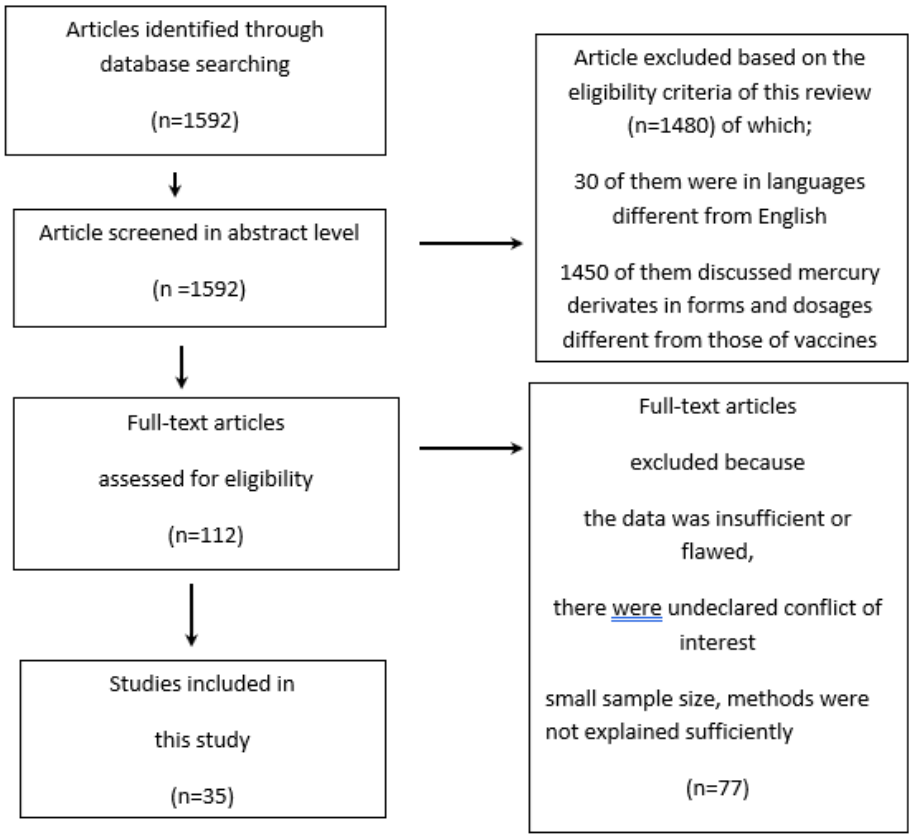

Figure 1. Prisma flow diagram: schematic diagram of literature searches and selection for articles included in this systematic review.

Inclusion and Exclusion criteria

Inclusion criteria:

Review articles, meta-analysis, original articles, studies on thiomersal found in biological products, follow up studies were included in this review.

Exclusion criteria

Studies reporting thiomersal in other forms and dosages from those found in vaccines (0.005% - 0.01%p/v in liquid dosage forms), were not taken in consideration. Studies published in languages other than English were excluded. Studies published before 2013 were excluded.

Data extraction

The data extracted from epidemiological studies were: the year of publication, the type of the study, the dose of Hg from thiomersal exposure considered in the study, the sample size and the outcomes reporting the possible relationship between thiomersal and neurodevelopmental disorders, the database used for the study. Each of the variables is an indicator of the strengths and limitations of the related study considered for this review, which influences the final conclusions obtained. To obtain valid results from the epidemiological studies, the study design and methods used are very important.

Data extracted from pre-clinical and clinical studies were: dosage of thiomersal used, dosage form, plasma half-life of thiomersal, plasma concentration, form of exposure, duration of exposure, the mechanisms of action, and toxicity.

Data extracted from analytical studies: Original articles, focusing on mercury species specific analysis, were investigated. Total determination of mercury was not considered. Studies addressed to the determination of mercury different species in biological samples were given special attention. Novel biomarkers were considered of great interest.

Data Analysis

The outcomes retrieved from epidemiological studies were pondered according to the sample size and dosage of thiomersal considered. If the sample size was small or the dosage of thiomersal was far from those used in vaccines, the outcomes considered were less relevant. The type of database used for the study was considered to evaluate the credibility of the results.

Pre-clinical studies were analysed considering the type of animal or sample cells used to study the effects of mercury derivatives, types of exposure to the mercury sources and forms of mercury. Biological, metabolic and anatomic patterns of animals were confronted with human ones to evaluate the inference of outcomes retrieved from animal studies to humans.

Clinical studies were evaluated according to the type of population considered for the purposes of the study, dosage and form of thiomersal studied, their metabolism in the body, half-life, deposition in various tissues and elimination from the organism. Pharmacokinetic and pharmacodynamic parameters were discussed and considered to evaluate their influence in the deposition of mercury in organism. Moreover, the source of exposure to mercury derivates was considered to evaluate whether it is similar to

natural sources of exposure and especially to vaccines exposure.

While the data extracted from the analytical studies such as biomarkers, types of exposure, degradation process of each form of mercury derivatives were used to analyze the impact of each of them in the concentration of mercury in different parts of organism, especially central nervous system.

RESULTS

The 35 selected studies were categorized into each group according to their type and design

Pre-clinical studies (n= 10); Clinical Studies (n=7); Epidemiological studies (n=10); Meta-analysis (n=2); Review (n=1); Speciation analysis (n=5).

1. Studies on Thiomersal Containing Biological Products

Many articles in literature report either the toxicology of MeHg or compare the toxicology of EtHg and MeHg from various toxicokinetic and toxicodynamic points of view, using various biomonitoring parameters. It is already established that all forms of Hg are toxic (1). Some studies pretend that EtHg toxicity is almost similar to MeHg and extrapolate the neurological outcomes of MeHg exposure to TCV-EtHg exposure (26). Mercury derived from both MeHg and EtHg, can reach neural cells, where it can deplete glutathione and induce apoptosis even at low doses (27). Moreover, it has been shown that MeHg and EtHg have additive or synergistic effects in the brain (28). Hempel et al. showed that the chemical properties of organic mercury substances influence their ability to permeate the cell membrane of various tissues resulting in toxicological differences between organic mercury substances (29), therefore, the neurotoxic effects of the two organomercurial species supposed of having a similar toxicity depend on their capacity to penetrate the central nervous system (CNS). Studies evaluating the toxicity of mercury assess only one chemical form and way of exposure at a time, with environmental studies addressing the toxicology of MeHg and epidemiological studies addressing EtHg toxicology. The thiomersal dosage in a single thiomersal containing biological product varies from 12 μg to 25 μg dependent on the manufacturer (29). Although it is supposed that these doses exceed the safety limits set by the US Environmental Protection Agency (EPA) (0.7 μg Hg/Kg/body weight/week), it is important to highlight that EPA guidelines were designed for oral ingestion of MeHg, while EtHg with a safety factor of 10, is administered intramuscularly (29). Studies which evaluate the combination of various forms of exposure and all forms of mercury should be further developed. The physicochemical properties of various Hg species influence their metabolic destination which in turn is a crucial factor in determining the toxicity of mercurial compounds (30). In particular, when considering the comparison of the two compounds, their metabolism in the body, half-life, deposition in various tissues and affinity for

various molecules should be taken into consideration. A recent review discusses this comparison (25).

The absence of established criteria for neurological outcomes for low doses thiomersal exposure has incited studies evaluating the association between thiomersal and neurodevelopmental outcomes (31).

The studies have been categorized in three groups, namely pre-clinical studies, clinical studies, and epidemiological studies.

1.1. Pre-Clinical studies

1.1.1 In vitro studies

Zimmermann et al. showed that MeHg and EtHg induce cell death and glutathione depletion in rat glioma cell cultures to the same extent (32). Wehe et al., found that thiomersal and MeHg have similar time-dependent uptake by human astrocytes (33). Recent studies have further clarified that at nanomolar concentrations (50 nM) thiomersal disturbed the poly-(ADP-ribosyl)-ation reaction that is induced by DNA strand breaks in human astrocytes (34). Despite the similar toxicities of EtHg and MeHg in vitro, these results cannot be inferred to in vivo due to different pathways of the two molecules (35).

1.1.2 In vivo studies

Studies in rats showed that thiomersal exposure in early life caused alterations of serotonin and dopamine systems. Besides rats, other animal models such as hamsters, rhesus macaques, and mice have been used to assess neurotoxic effects of mercury. Early studies in animals showed that the concentration of inorganic mercury in the brain and kidneys of rats is higher in animals exposed to EtHg compared to equivalent doses of MeHg, and that Hg remains in the blood longer in animals treated with MeHg (36). Zareba et al. found that the organic mercury levels in the brain and kidneys of neonatal mice (10 days postnatal) treated with single intramuscular injections of EtHg and MeHg were lower for mice treated with thiomersal, while the inorganic Hg concentrations were higher in the MeHg group (37). Hurry et al. showed that EtHg and thiomersal were more concentrated in the blood, brain and kidneys of immature mice compared to adult animals (38). Duszczyk-Budhathoki et al. demonstrated that there was an increase in cerebral extracellular concentration of glutamate, which is an important indicator of excitoxicity, in rats exposed to thiomersal (39). Studies in mice, rats and monkeys showed that the brain/ blood ratio of inorganic Hg was higher after EtHg exposure compared to MeHg exposure and that thiomersal is metabolized to inorganic Hg faster than MeHg (26). Lohren et al. showed that thiomersal is more toxic than inorganic Hg and is able to penetrate the blood brain barrier (36). Olczak et al. reported that treatment of neonatal brain with thiomersal lead to changes in the brain dopaminergic system and impaired social interactions which persist in adult life (37). Recently, Yoshida et al. showed that the toxicity of MeHg in suppressing the movement of ventricular ependymal cilia, which drives the flow of cerebrospinal fluid to support neuronal functions, was slightly greater than that of EtHg

(38). The authors suggested that the difference in neurotoxicity depends on the blood-brain barrier permeability of the two organic compounds and that thiomersal is neurotoxic also at low levels. A study conducted in China by Li et al. using doses of thiomersal-mercury 20-fold higher than those used in Chinese infants in the first 4 months of life, showed long-lasting alterations of neurodevelopment, synaptic function, and endocrine system in neonatal mice (39). These alterations were supposed to be responsible for autistic like behavior in mice (40). However, it should be noted that the dosage at which these alterations were observed was 20 times higher than the dosage at which infants are exposed. Therefore, the results cannot be transferred to vaccines.

Magos et al., revealed that an equimolar dose of EtHg was less neurotoxic than MeHg, but a 20% increase in the dose of EtHg was enough to raise the sum of coordination disorder scores slightly and ganglion damage significantly above those in MeHg -treated rats. Interestingly, three or 10 days after the last treatment, rats given 8.0 or 9.6 mg Hg kg-1 EtHg had higher total or organic mercury concentrations in blood, and lower concentrations in kidneys and brain than MeHg -treated rats (41, 42).

Other studies conducted in animals demonstrated the toxic effects of thiomersal on tissue structure and function, as well as in neuro-function and behavior: e.g. 240 μg Hg kg-1 b.w evoked a rapid increase in glutamate overflow in rats (43); 12, 240, 1440 and 3000μg Hg kg-1 b.w. showed a marked decline in the density of striatal D2 receptors, suggesting alterations of the brain dopaminergic system in rats, and an impairment of locomotor activity, an increase of anxiety/neophobia, and reduction of pro-social interactions in Suckling Wistar rats (38). In line with these findings, other studies conducted in mice demonstrated that 5.6-14.2 μg kg-1 EtHg b.w. reduced the locomotion in mice and altered the glutamate receptors and transporters (44).

Oliveira et al. through a proteomic analysis of the brains from animals administered organic mercury compounds showed that EtHg did not elevate superoxide dismutase, in confront to MeHg (45). Chen et al. observed negative effects on neurodevelopment of premature rats by using a dose of thiomersal about 10-fold higher than the respective to newborns (460. However adverse events were reported only after administration of doses of thiomersal that exceeded by 100 folds the dose found in human vaccines. Moreover, the route of administration influenced the rate of adverse events due to toxicokinetic differences. Although rodent model has helped a lot to design experimental studies for the neurotoxicology of thiomersal, the small body size of these animals limits the interpretation of the findings. Considering these limitations, animal models, such as primates which share the same evolutionary patterns and developmental characteristics with humans, and respond similarly to the toxic insults, have been used to address neurobehavioral and other neurological outcomes after thiomersal administration (47).

Recent studies demonstrated that there were no differences in neuronal cellular or protein changes in the cerebellum, hippocampus, or amygdale between the exposed and control group of rhesus macaques (48). The authors replicated in rhesus macaques the vaccination schedule used to immunize infants in USA from 1990s and 2008 and observed that there were no neuropathological abnormalities like those observed in ASD. Other authors replicated the infant vaccination schedule used in USA between 1994-1999 in rhesus macaques and concluded that there was no plausible evidence for neurodevelopmental deficits, learning difficulties, acquisition of neonatal reflexes, development of object permanence, and assessment of social behavior in vaccinated animals (45).

1.2. Clinical studies

Biomonitoring studies are used as a key tool for monitoring the occurrence and extent of the mercury exposure in human organisms (26). Measuring the chemical molecule, weather it is the molecule itself or its metabolites in a biological matrix is a useful tool to assess the development of adverse effects. Clinical studies are important to study the link between homeostasis alteration in humans and adverse health outcomes. The results obtained by these studies will help to assess the population exposure to mercurial compounds and develop public health policies to prevent the exposure and resulting risks. The aim of clinical studies is to show the different pathways in the organism of various chemical structures of mercury.

It has been shown that EtHg blood half-life after TCV exposures varies from 3 to 7 days (49), and it is excreted more rapidly than MeHg as it is more easily converted into inorganic mercury, which is eliminated through kidneys. In regard to the average concentration of total mercury in samples of whole blood, Stajich et al. measured the blood levels in pre and full-term infant on average 60 h after vaccination. The amount of mercury in the blood compartment corresponded to 3.5 % and 5.1% of the injected dose, with an average concentration of total mercury in samples of 7.36 and 2.24 μg Hg L-1 for pre and full-term infants, respectively (50). A preliminary report by Pichichero et al. demonstrated the blood levels of mercury in two groups of infants, of 2 and 6 months respectively, after receiving vaccines containing thiomersal. The blood samples were collected one week or more after the last vaccination. The highest recorded blood level was 5.1 μg Hg L-1 in a 2-month old infant, almost 5 days after the last vaccination. The authors found that most blood levels were below 2 μg Hg L-1 (51, 52).

The Hg concentration in the brain and the velocity of its accumulation regulate its toxicity. These two parameters are in turn influenced by the mode of exposure. It should be noted that, despite the numerous studies evaluating the concentration of Hg in the hair as a form of exposure to thiomersal, it has been shown that EtHg conversion to inorganic Hg occurs before

inorganic Hg gets to the hair, therefore hair concentration of mercury cannot be used as reliable biological matrix for thiomersal exposure (53).

Evaluating the toxicokinetic parameters and toxicodynamic mechanisms in vivo is essential to give a final answer regarding the toxicity of thiomersal. Barregard et al. found that the blood concentrations of MeHg assumed from fish were much higher than those of EtHg administered from TCVs (54). In an early study Magos et al. demonstrated that there were no significant differences between MeHg and EtHg toxicity on the dorsal root ganglia or on coordination disorders; however, at equimolar bases EtHg was less neurotoxic than MeHg (45). Recently, in a randomized, single blinded clinical trial, Hiew et al. studied the safety and immunogenicity of two Hepatitis B vaccine formulations (thiomersal-free and thiomersal-containing) in 408 healthy Vietnamese infants. The authors concluded that the thiomersal-free vaccine was associated with fewer local adverse events (55), however this study has important limitation such as follow up period, which is only 6 months. A follow up period of more than 2 years is minimally required.

1.3. Epidemiological studies

The results obtained by the studies which evaluate the toxicity of EtHg in animal models cannot be inferred directly to the human situations, due to metabolic differences (45), hence studies in humans are necessary.

We searched the epidemiological studies evaluating the Hg exposure from biological products and the neurodevelopment outcomes in humans, focusing on studies published from 2013 onwards. However, we also included some other earlier studies to confront the conclusions of each of them about the association between thiomersal and neurodevelopment outcomes, in order to highlight the progress made throughout years in terms of methodologies used, study design, data set, font of information and new diagnoses identified. Along with original studies we also selected two meta-analysis which included original studies conducted before 2012 in order to have a general view of the relationship between thiomersal and neurodevelopment outcomes (56, 57).

From each study we extracted the following variables: the year of publication, the type of the study, the dose of Hg from thiomersal exposure considered in the study, the sample size and the conclusions reported.

As far as the other studies are concerned, 6 studies (56-61), reported no association between vaccines and neurodevelopmental outcomes.

A meta-analysis by Taylor et al. quantitatively estimated the available data from case-control and cohort studies which studied the possible link between autism and childhood vaccination (56). Four of the five cohort studies included in the Taylor et al. meta-analysis were conducted in large populations and were therefore of sound methodology. The authors reached the conclusion that vaccination was not related with the risk of neurodevelopment disorder. Another meta-analysis by Yoshimasu et al. stated that the effects

of thiomersal containing vaccines on ASD occurrence were negligible (57).

The result of the two meta-analysis by Taylor et al. and Yoshimasu et al. reported no link between vaccination and thiomersal exposure (56, 57). Mrozek et. al. evaluated whether there is an association between early TCV exposure and mental delays of milder intensity using specific mental development test (59). Uno et al., conducted a case control study in Japanese people and reported that MMR vaccination and increased exposure to TCV did not increase the risk for autism spectrum disorder (60). The authors studied all children already diagnosed with ASD who were treated in the Yokohama Psycho-Developmental Clinic. The selected population was homogenous on biological characteristics, which minimized the effect of population-specific risk factors that might interact with environmental exposures and lead to physiologic alterations. Studies investigating thiomersal exposure to Amazonian children encountered many difficulties to reach a precise conclusion on the neurodevelopmental outcome, due to the multi-level exposure to organic mercury from fish consuming and tin-ore places. Prospective cohort studies should be conducted with accurate follow-up methods since individuals with neurodevelopmental disorders are not uniform throughout their life. However, in three studies conducted by Marques et al., the recruitment of all villagers prevented the sampling biases (58, 62). Dorea et al. reported that in the Itapuã population, the proportion of severely compromised (GDS < 70) toddlers was higher than in the other areas and suggested that there might be a gene–environment interaction for Hg (52).

In addition, a Cochrane review in 2012 which assessed the effectiveness and safety of MMR vaccine considering five randomized controlled trials, one controlled clinical trial, 27 cohort studies, 17 case-control studies, five time-series trials, one case cross-over trial, two ecological studies, and six self-controlled case series studies, concluded that the exposure to the MMR vaccine was unlikely to be associated with autism (63). Moreover, after removal of thiomersal containing vaccines, some countries like Canada did not observe a decreased risk of pervasive neurodevelopment outcomes.

2. Mercury Derivatives Toxicity Mechanisms

Hunter et al. was the first one to report the toxic effects of EtHg compounds in animal experiments in the early 1870s, which were confirmed a century later in human outbreaks of poisoning (64), such as the poisoning that took place in Iraq in 1950 (65). The strong affinity of sulfhydryl (-SH) groups for mercury has often been considered to be involved in the mechanism of toxicity of mercury and its compounds (67). Due to the strong affinity of mercury towards sulphur and the almost universal presence of this chalcogen in the human body in the form of thiols and disulphides in peptides, proteins and DNA, sulphur is the major binding partner of mercury

compounds under physiological conditions. Mercury unfolds its neurotoxic effects by binding to thiols or disulphides in the nervous system, thus inhibiting enzyme activities distorting protein structure or blocking biologically active thiols. In addition, the high mobility of organic mercury in the body is attributed to the formation of small molecular weight thiol complexes that are readily transported across cell membranes. Studies indicate that EtHg half-life is shorter than MeHg half-life in the human body, with pharmacokinetics and pharmacodynamics properties being different. In addition, EtHg is actively excreted into the gut (68, 69). When thiomersal breaks down in the human body into Hg2+ species, other than the alteration of the normal cellular function, it can bring to the loss of function of different proteins, enzymes, membranes, or to the bioaccumulation particularly in the brain (70). In addition, the loss of endothelial cell membrane integrity or the leaky membranes are a result of the effects of thiomersal. Magos et al. suggested that EtHg is less potent in producing neurologic signs and symptoms than MeHg, where the threshold for neurologic effects has been estimated at about 200 μg Hg L-1. Severe intoxication was associated with blood levels in excess of 2,000 μg Hg L-1, with milder intoxication at 1,000 μg Hg L-1 (710. There are a number of mechanisms through which mercury compounds cause toxic action in the body. Some of them are commented below.

2.1. GSH levels

Glutathione is a co-factor for the glutathione peroxidase selenoenzymes and can induce a protective effect (72). GSH antioxidant system is a target of TM, which decreases the GSH levels in different human cells, such as in dendritic cells (73), in human neuroblastoma and glioblastoma cells, and also is responsible for the neurotoxicity. In this regard, James et al. revealed that in ASD patients, thiomersal exposure highly decreased the reduced glutathione to oxidized glutathione ratio and increased the free radical generation (74). Olczak et al. demonstrated that thiols play a critical role in mitigating the level of toxicity from thiomersal and the effects of thiomersal are also dose dependent. The toxicity equation is as follows: Exposure (Dose) + Susceptibility (Thiol Content/Availability) = Outcomes (Level of Insult) (75).

Glutamine/amino acid (ASCT2) transporter has a physiological function in the amino-acid substrates transport and is essential in intracellular glutathione synthesis. MeHg is responsible for the inhibition of ASCT2, due to the binding of Cys residues in the transporter (76). Other than the alteration of the amino acid transport, Hg can also alter the energy metabolism and induce the phospholipid breakdown (72). Becucci et al. revealed that the lipodepsipeptide syringomycin E (SR-E) interacts with two mercury-supported biomimetic membranes, which contain a self-assembled phospholipid monolayer (SAM) and a tethered bilayer lipid membrane (tBLM). They are both

separated from the mercury surface by a hydrophilic tetraethyleneoxy (TEO) spacer that acts as an Hg2+ ionic reservoir (77).

2.2. Oxidative stress

Induction of the oxidative stress after thiomersal exposure, followed by GSH depletion and cellular lysis, had been related to its toxicity by Anundi et al. four decades ago (78). However, subsequent studies evidenced that MeHg, responsible of an elevated generation of reactive oxygen species (ROS), could either reduce GSH levels or start off an adaptive response to oxidative stress by increasing GSH levels (79). Later on, Sharpe et al. found that cells that were hypersensitive to thiomersal had higher levels of oxidative stress markers (80). More recently, the roles of mitochondrial dysfunction and endoplasmic reticulum (ER) stress on EtHg-induced cytotoxicity, was studied by Choi et al. in HK-2 cells and in an in vivo mouse model (81). Cells were treated with EtHg at concentrations ranging from 0.25 to 4 μM (exposure times between 6 and 48h). EtHg resulted cytotoxic to HK-2 cells in a dose- and time-dependent manner, with IC50 values of 9.6, 9.5, 2.2 and 1.1 μM at 6, 12, 24, and 48h, respectively. Subsequently, EtHg-induced kidney toxicity via autophagy in mice was investigated. EtHg was administered by intraperitoneal injection at doses of 0, 1, 2, 5, and 10 mg kg-1 bw/day for 3 consecutive days. Grp78, Xbp1, and Chop were evaluated at the mRNA and protein levels. The results indicated that even sub-toxic doses of EtHg can induce ER stress and autophagy. Since oxidative stress is known to be closely related to ER stress, the authors measured reactive oxygen species production in HK-2 cells after treatment with various concentrations of EtHg (from 0.1 to 2 μM) for 3 h and quantified the production of reactive oxygen species by FACS analysis. A dose-dependently increase of reactive oxygen species production after EtHg treatment was observed. In a parallel experiment, HK-2 cells were simultaneously treated with N-acetyl cysteine (NAC, as antioxidant) and EtHg in comparison with cells treated only with EtHg. The results supported the hypothesis that EtHg-induced ER stress is partly mediated by oxidative stress in human renal proximal tubular HK-2 cells. Together ER stress and reactive oxygen species generation induced by EtHg promote autophagic signaling in HK-2 cells. On the basis of their results, the authors suggest that autophagy may play a dual role in EtHg-induced kidney cell toxicity, being protective at low doses and deleterious at high doses. NADPH oxidase-independent production of reactive oxygen species, which are likely to be involved in mercurial-induced NET formation, was reported by Haase et al (78). Oxidative stress caused by mercury derivatives was recently investigated by Cariccio et al. (80), and Gukovskaya et al. (81), who related the increase in reactive oxygen species and reactive nitrogen species (RNS) levels to several aspects of organic mercury-induced neurotoxicity.

2.3. Mobilization of Intracellular Ca++

Mercurials interact with Ca++ signaling, triggering its release from intracellular stores, subsequently followed by influx of extracellular Ca++ and activation of many cellular functions (78). A broad spectrum of evidence indicate that calcium dysregulation may be central to the neuronal vulnerability. Under physiological conditions, neuronal calcium homeostasis is maintained by an equilibrium between calcium influx and releasing mechanisms (Ca2+-channels), and calcium efflux mechanisms (Ca2+-pumps and -exchangers) (82). Changes in ion transport by thiomersal were evidenced in rat thymocytes by Gukovskaya et al. (81). In particular, they reported a significant increase of the cytosolic free Ca++ concentration. Inhibition of Ca++ -ATPase was not proved at that time. By contrast, evidence was given that arachidonate metabolites could mediate the ion transport. These data where then confirmed by Nabemoto et al. in rat pheochromocytoma PC12 cells (83). The pivotal role of the Plasma Membrane Calcium ATPases (PMCAs) in the etiology of neurodegeneration has been recently underlined (82). Alkyl mercury toxicity related to alteration of calcium homeostasis was reported by Risher et al (84). Elemental, organic and inorganic forms of mercury are also able to stimulate the intracellular calcium ion mediated activation of the mitogen-activated protein kinase (MAPK/P38 system), leading to toxic levels of reactive oxygen species and reactive nitrogen species production, which can eventually result in cellular apoptosis and necrosis (82). Blocking calcium binding proteins (e.g. calmodulin) by mercury derivatives, can also lead to toxic effects (85). The effects of organic mercury on Ca++ mobilization have recently been reviewed by Risher (84).

2.4. Immune-Inflammatory Pathways

In high doses, mercurials are immunosuppressive while lower doses stimulate the immune system (78). The extent of immune suppression and immune stimulation varies between species and is influenced by genetic susceptibility, levels and duration of exposure, gender and age. Loison et al. demonstrated that the exposure to non-toxic concentrations of thiomersal induced cell cycle arrest in G0/G1 phase of TCR-activated T cells, and inhibited the release of proinflammatory cytokines such as interferon gamma (IFN-γ), interleukin-1 beta (IL-1ß), TNFα, interleukin-2 (IL-2) (86). It should be noted that while methylmercury initially induces a similar level of immunosuppression to ethylmercury, the subsequent immunostimulatory effect is significantly attenuated, compared with ethylmercury. This could be attributed to differences in their rates and extent of conversion into inorganic mercury (87). Immunological reactions related to thiomersal exposure have been reported since the eighties (88, 89). The hypothesis was then raised that the autoimmunogen effect of EtHg might be entirely due to Hg2+ formed in the body (90). More recently, the formation of neutrophil extracellular traps (NETs) in response to thiomersal and its metabolites (EtHg, thiosalicylic acid, Hg++) was

investigated, concluding that only EtHg and Hg++ triggered NETosis (78). Immunological effects of thiomersal and Al-adjuvants were reported by Dorea who highlights that these effects are often detected in concomitance with co-exposure to methylmercury (MeHg) or other neurotoxicants (91).

2.5. Epigenetic changes and effects on cytoskeletal proteins

Exposure to Hg compounds may also be associated with epigenetic changes and with reduced protein synthesis both in vivo and in vitro (92,93) and methylation processes (69). From the other hand, MeHg can also have adverse effects on cytoskeletal proteins (microtubules) and cytoskeleton- regulating protein (Rho family proteins) bringing to disturbances in neuronal migration and differentiation (36, 94). Microtubules are important elements for intracellular transport, made of polymers of tubulin that contain TSH groups, which have high affinity for MeHg and can bring to depolymerization of cerebral microtubules (36, 95). Moreover, other than tubulin polymers, microtubules are also composed of different microtubule associated proteins (MAPs), in particular MAP-2, whose expression is attenuated in neurons, bringing to hyperphosphorilation of tau (36). Tian et al. have also demonstrated that the inhibition of the development of dentate gyrus neurons can lead to deficits in learning and memory capacity (96). After binding to the tubulin/microtubule structure of the axon, Hg can cause the axon to degenerate and it induces the neuronal excitotoxicity, by causing excessive accumulation of extracellular glutamate (69). This is also accompanied with an hyperactivation of N-methyl-D-aspartate receptors, which can bring to cytoskeleton inactivation (97). Stankovic et al. revealed that large, long-range neurons/axons are selectively vulnerable to Hg, and their loss is irreparable (98).

2.5.1. Gender differences

Different studies have revealed that males, infant and fetal tissue are found to be more vulnerable to thiomersal exposure, in respect to females, children and adults (99,100). Branch et al. found that thiomersal has a differential maximum tolerated dose depending on whether the mouse was male or female (100). He showed a 3-fold increased toxicity in male mice compared to female mice with a maximum tolerated dose of thiomersal in males of 25.6 mg kg-1, and a maximum tolerated dose in females of 76.8 mg/kg (100, 101). These findings were further supported by other studies (71). Moreover, Khan et al., stated that perinatal thiomersal exposure in male and female rat neonates, brings to an alteration of thyroid-hormone dependent gene expression, with males found to be more vulnerable (98,99). In line with this study, Sulkowski et al. reported that the local intra brain conversion of thyroxine to 3',3,5 triiodothyronine (T3), was decreased by 60.9% in thiomersal exposed male rat pups (74, 101). Studies suggest that males are more vulnerable because the neuroinflammatory response is higher in males than females, the glutathione levels are lower in

males than females, the sulfate-based detoxification capacity is lower in males than females, the vulnerability to oxidative stress is higher in males, and because female hormones (estrogen and progesterone) have a neuroprotective effect (101).

3. Mercury Derivatives and Their Speciation Analysis in Biological Samples

one or two carbon atoms. A constant flux between these three forms occurs (104). Contrary to elemental mercury, ingestion is the most common way of exposure for inorganic and MeHg, an organomercury which originates from the metabolism of mercury by microorganisms in aquatic sediments. It accumulates predominantly in fish and other seafood and constitutes the main source of dietary mercury exposure in the general population (36). Differently from MeHg, EtHg is

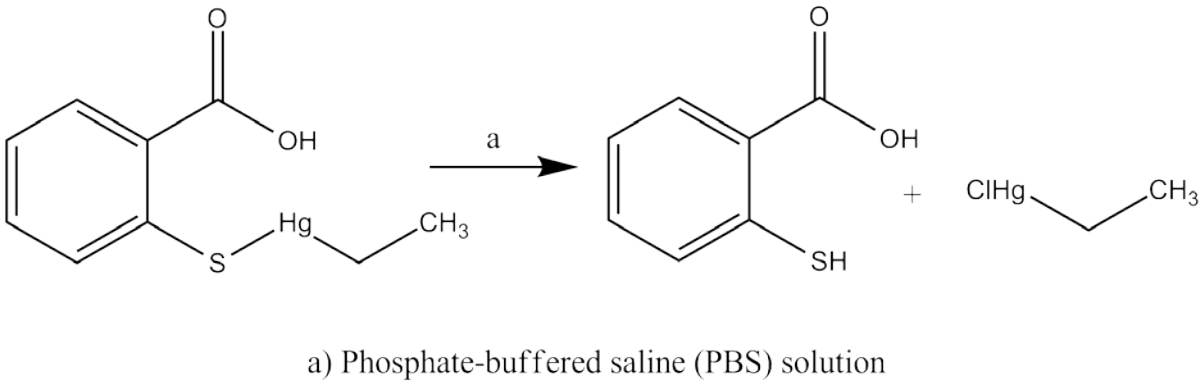

Figure 2. Decomposition of thiomersal to thiosalicylic acid and EtHg in buffered solution (Tan, M, and Parkin, J.E. 2000)

Mercury is present in the environment, both by natural occurrence, and as a result of human activities. It can appear as elemental mercury (Hg°), inorganic mercury (mercurous and mercuric) or organomercury (102). Elemental mercury is a highly volatile, easily diffusible and lipid soluble form, which is generally assumed by humans through inhalation, either during occupational activities or from dental amalgam release (103). The mercurous cation (Hg-Hg++) contains two atoms of mercury and readily dissociates in the body to Hg° and Hg++. Organomercury represents a class of compounds in which bivalent mercury is covalently bound to

strictly anthropogenic, since ethylation processes are not known in nature. The main source of EtHg is thiomersal (o-carboxyphenyl-thio-ethyl-sodium salt, THI), a fungicidal and bactericidal agent present in multi-dose vaccine vials, whose structure has been determined by single crystal X-ray diffraction (104). Thiomersal is hydrolyzed in aqueous media according to figure 2 (105).

At high exposure levels, all mercury compounds can be toxic, though by various degree, since they have considerably different kinetics. After inhalation, almost 80% of elemental mercury diffuses from the alveolar regions of the lung to the blood compartment. It easily passes through

cell membranes, including the blood brain barrier and placenta, by cell diffusion. Recently it has been suggested that membrane transport proteins could also be involved (106). The concentration in the blood initially declines rapidly (1-3 days half-life), followed by a 1-3 weeks half-life. After absorption, it is oxidized to inorganic forms, mainly through hydrogen-peroxide-catalase pathway. However, it has been reported that animals given mercuric chloride exhale mercury vapor, therefore a cycle of oxidation/reduction exists in the cells (107). It has been suggested that this cycle, whose significance is not fully understood, could play a role in the mobility of inorganic mercury, as the reduced form of mercury is highly mobile whereas the oxidized form is not (107). Kidneys play a significant role in accumulation and excretion of elemental mercury (107, 108). Mercuric mercury is poorly absorbed by the human gastrointestinal tract (< 15%) and penetrates the blood-brain barrier to a much lower extent with respect to either elemental or MeHg (109). The half-life of mercuric mercury in blood is similar to the slow-phase half-life of elemental mercury. Excretion occurs by renal and faecal routes. Mercuric mercury is secreted into bile as a complex with glutathione (GS-Hg-SG), whose structure resembles that of oxidized glutathione (GS-SG); the endogenous carriers of GS-SG could therefore recognize it (110). The organo-compound MeHg is absorbed from the gastrointestinal tract and penetrates into the brain, placenta and other tissues (109), where undergoes slow dealkylation to inorganic mercury. Several hypotheses suggest the involvement of selenium (Se) and free radical attack in this process, but the precise mechanism has yet to be determined (111). MeHg declines in human blood with a half-life of approximately 50 days. The compound accumulates into growing hair, which represents a useful marker of exposure in epidemiologic studies (112). It is mostly eliminated through feces (113). The high mobility of MeHg in the body is due to the formation of small molecular weight thiol complexes. When MeHg binds cysteine (or omocysteine), the resulting complex mimics methionine and can therefore be recognized by neutral aminoacid carriers. The enzymes gamma-glutamyltranspeptidase and dipeptidase are involved in the process. By contrast, the transport out of the cell seems to occur through a glutathione complex using glutathione carriers (114). EtHg, derived from thiomersal, is closely related to MeHg, and very mobile in the body, probably through the same mechanisms. The degradation of thiomersal has been investigated, amongst others by Parkin (115), in an attempt to justify the results of Caraballo, who reported that in degraded samples of THI, the initially formed thiosalicylic acid (TSA) was absent (116). The mechanism reported in figure 3 was proposed, where the rate determining step should be the metal catalyzed oxidation of TSA to dithiosalicylic acid (DTSA), which in turn reacts with EtHg to reform THI and sulfenobenzoic acid (SEBA). Recycling of this

reaction would ultimately lead to disappearance of THI.

bioavailability and mobility), therefore species-specific Hg analysis is requested. In addition, the

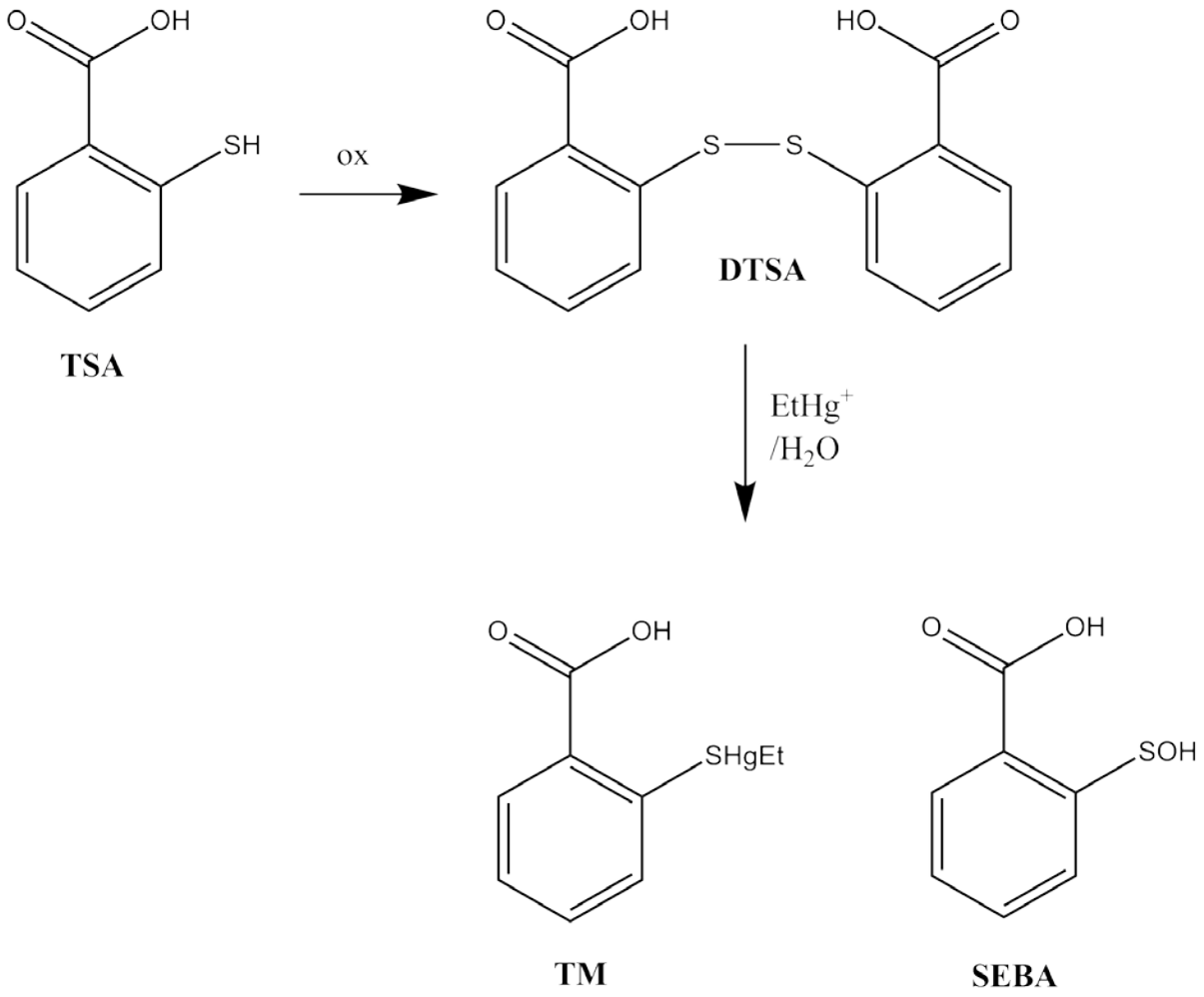

Figure 3. Principal degradation products of thiomersal from thiosalicylic acid and EtHg (Tan, M, and Parkin, J.E. 2000) (TSA: thiosalicylic acid, DTSA: dithiosalicylic acid, TM: thiomersal; SEBA:sulfenobenzoic acid)

Since EtHg is considered to be the antibacterial species in THI, this mechanism would also justify the greater activity of THI degraded solutions with respect to fresh ones (117). Contrary to MeHg, EtHg is readily eliminated by the body (101). As previously stated, the toxicity of single forms of mercury depends on their respective chemical characteristics (e.g. solubility, choice of indicator media is crucial.

Different methods have been reported for the determination of mercury compounds (118). Amongst others, high performance liquid chromatography (HPLC) (119), gas chromatography (GC) (120), and capillary electrophoresis (CE) (121) have been applied for analysis of mercury in food, environmental and

biological samples. With respect to GC and CE, HPLC has many advantages, e.g. ease of sample derivatization, ease of interface to analytical atomic spectrometries [ultraviolet-visible spectroscopy (UV), atomic absorption spectrometry(AAS), atomic fluorescent spectrometry(AFS),inductively coupled plasma mass spectrometry (ICP-MS)], and the ability to analyze both inorganic and organo-metallic species. In particular, reversed phase high performance liquid chromatography (RP-HPLC), using mobile phases comprising of high salts and moderate amounts of organic solvents, is commonly used. However, the adverse effects on the environment from the use of large amounts of mobile phases, due to the long separation times, should be taken into account (116,119-122). The potential of ion-pairing (IP) RPC coupled with ICP-MS detection, using an aqueous mobile phase containing small amounts of reagents, in mercury speciation has been recently reported. Detection limits were 0.015, 0.014, 0.028 μg L-1 for Hg++, MeHg, and EtHg, respectively. Good agreement with certified values as well as good recoveries were obtained (123). Ionic liquids were also used as mobile phase modifiers in the separation of Hg2+, MeHg and EtHg species in food by reversed-phase high-performance liquid chromatography coupled to UV-cold vapor atomic fluorescence spectrometry (RP-HPLC-UV-CV-AFS), by varying different parameters (124). Cold vapor atomic absorption spectrometry (CV-AAS) has also been widely employed due to its simplicity and high sensitivity, though it often requires a separation and preconcentration step. To this aim, different nanomaterials (carbon nanotubes, carbon nanohorns, carbon nanocones/disks, carbon dots, and graphene), which possess a large surface area and short diffusion route, thus resulting in high extraction efficiency and rapid extraction dynamics, are often used. In this respect, a novel microextraction technique, dispersive-micro-solid phase extraction (D-μ-SPE), has been reported (125).

A common analytical method such as ethylation/gas chromatography is able to accurately measure MeHg, but it fails to measure EtHg and Hg2+, which give the same diethylated analogue. Different alkylation, e.g. propylation (1260 and butylation (127) coupled to gas chromatography GC/ICP-MS or to GC/atomic fluorescence spectrometry (AFS) allow separate and precise determination of organo (MeHg and EtHg) and inorganic (Hg++), but the procedures are more laborious and difficult to be scaled. In addition, preliminary solvent extraction is needed, which further extends the procedure time. Burbacher et al. and Zareba et al. employed selective reduction techniques to measure organic mercury as the difference between the measured total and inorganic Hg concentrations (128). However, this procedure does not allow to distinguish between MeHg and EtHg, which is essential in the case of thiomersal containing vaccines (TCV). In addition, the approach requires sample > 10mg. Several methods using Hg-thiourea complex liquid chromatography

have been described as useful for a scalable, automated analysis of MeHg and Hg++ (129,130). Recently, Dorea J.G. reported a modification of the Shade's procedure, which allowed ion-pairing reversed phase chromatographic separation of MeHg, EtHg, and Hg++. Atomic fluorescence detection was used. Detection limits are valued 0.050, 0.10, and 0.10 ng g−1 for MeHg, Hg++, and EtHg, respectively, for a 20-mg sample. This method allows the use of very low samples and is particularly useful when applied to TCV (131). The simultaneous determination of MeHg, EtHg, and Hg++ in human blood, hair and urine has recently been reported (132). The required sample mass was 0.15 g, 0.5 g , and 0.1 g, respectively.

Recently, Karst highlighted the importance to study the possible chemical binding between EtHg and protein in vaccines (133). The seasonal tetravalent influenza vaccine (IV) Vaxigrip tetra (Sanofi, Paris, France), containing inactivated split virions of two influenza A and two influenza B strains (each 30 μgmL−1 hemagglutinin (HA), propagated in chicken eggs), was diluted 1:20 and incubated with THI in concentrations of 0.35, 0.7, 3.5, 7 and 14 μg L−1 Hg. Mixtures were stored under storage conditions of vaccines. By ultrafiltration and subsequent total reflection x-ray fluorescence (TXRF) they preliminary detected the binding of EtHg to the protein fraction of TCV. Further information was obtained through SEC/ICP-MS. They reported that mercury binds on a protein larger than 133 kDa, thus suggesting adduct formation with HA. However, most HgEt remains free in solution (133).

DISCUSSION

It should be emphasized that the toxicological mechanisms of thiomersal in animals can be evaluated only at cellular level and from a toxicokinetic or toxicodynamic point of view. Factors that might influence the toxicokinetics and toxicodynamics of thiomersal in humans such as genetics, sex, birth weight, gestational age, maternal health, and chemical co-exposures cannot be evaluated in animals or in vitro studies. Although there are a lot of preclinical studies which assess the mechanism and effects of thiomersal in animals, there are many differences between human biological patterns and animal anatomy. Janzen et al. demonstrated that there are different binding capacities and affinities of mercurial compounds to hemoglobin when comparing the hemoglobin of humans and rats (83). Moreover, phagocytic cells of various animals, which dealkylate and degrade the organic mercurial compounds, demonstrate different dealkylating capacity compared to humans (84). The most important factor to be considered when comparing the toxicity of thiomersal in animals with that in humans is the impossibility to have an animal model of the normal human mind, due to the lack of language in animals, which is a crucial indicator of logical thinking of humans' minds (85). As it is already known, neurodevelopmental disorders are mostly characterized and diagnosed estimating

parameters linked to social behavior, logical thinking and acting, and intellectual capacity. Moreover, in experimental conditions where there is only a minimal perturbance of normal homeostasis of neuronal setting, only selected aspects of neuronal performance and behavior alterations can be observed (85). Thus, only a limited set of signs and symptoms characterizing human neurodevelopmental disorders can be observed in animal studies and the total metabolic effects of Hg from thiomersal exposure in animals cannot be inferred to humans. Moreover, there are no animal models to reflect different homeostatic and metabolic conditions pertaining to human organism such as preterm infants, small gestational weight, prenatal exposure and genetic alterations, which influence the inference of animal experiments to human organism.

The controlled trials are considered the highest standard to evaluate the association between cause and effect, but in the context of established vaccination programs and practices in children, controlled trials are not possible. Unique methods to test the association between thiomersal and neurodevelopment outcomes are the epidemiological studies.

Although the usage of thiomersal in vaccines has decreased, the prevalence of neurodevelopmental disorders has increased in the recent years. This observation disfavors a possible link between TCV and neurodevelopmental disorders. However, the prevalence of autism and autistic spectrum disorders is increasing as a result of many factors, such as increasing pollution of the environment and exposure risk, improved detection of the syndrome due to changes in case definitions and improved diagnostics methods.

Epidemiological studies have several inherent limitations and biases, due to differences between study population and confounding factors that affect the final results. Therefore, it is necessary to approach the causal association between thiomersal and neurodevelopment outcomes through additional methods. It should also be noted that the number of thiomersal–containing vaccines are decreasing world-wide, but the prevalence of ASD is increasing.

It should be underlined that, although the cytotoxicity of mercury significantly depends on its chemical form, most of the scientific studies are focused on the determination of the total amount of mercury in cells, mostly because of the lack of efficient speciation analytical methods and adequate samples. Subtracting methods have often been proposed, but they could bring to inaccurate results, due to possible untargeted mercury species in cell system. In addition, the most common biomarkers, e.g. hair, urine, feces or blood Hg levels, are strongly affected by the intra-population variability. Therefore, the need for new analytical methods provided with high accuracy and sensitivity for mercury species in a small number of cells as well as novel potential biomarkers should be taken into account.

CONCLUSION

Thiomersal, an antiseptic and antifungal organic mercury compound present in various biological

products, decomposes in aqueous media generating several mercuric derivatives, characterized by different distribution, transportation, metabolism, and excretion from human organisms.

The toxicological mechanism of thiomersal in neurodevelopmental disorders are various, such as the GSH depletion, induction of oxidative stress, increase in reactive oxygen species and reactive nitrogen species, calcium dysregulation which can bring to neuronal vulnerability, TM immunosuppressive properties, epigenetic changes, reduced protein synthesis both in vivo and in vitro, methylation processes. Moreover, other studies indicate that Hg can also trigger brain inflammation by causing the release of pieces of damages neurons (debris). Mercury exerts its neurotoxic effect by binding to thiols in the nervous system and inhibiting the enzyme activities.

However, the limitations of in vitro, in vivo, clinical studies and epidemiological studies make their conclusions inadequate to generalize the results about the relationship between thiomersal and neurodevelopmental disorders. Although thiomersal has a long record of safe and effective use preventing bacterial and fungal contamination of biological products, more knowledge on its possible side effects is needed, together with the awareness that human beings are exposed to several sources of mercury derivatives, which could exert additive effects, mostly on fetuses and newborn infants. In this respect, issues related to co-exposure, modifying factors, genetic polymorphism, and cumulative doses of thiomersal have not exhaustively been addressed in literature.

Acknowledgements: None declared.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

REFERENCES

1. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. Pediatrics 2001; 107(5):1147-1154. doi: 10.1542/peds.107.5.1147.

2. US Food and Drug Administration.Thimerosal in vaccines. 2018 http://www.fda.gov/biologicsbloodvaccines/safetyavailability/vaccinesafety/ucm096228.htm#tox [Accessed 26 October 2018].

3. Adlnasab L, Ebrahimzadeh H, Asgharinezhad AA, Aghdam MN, Dehghani A, Esmaeilpour S. A preconcentration procedure for determination of ultra-trace mercury (II) in environmental samples employing continuous-flow cold vapor atomic absorption spectrometry. Food Anal Methods 2014; 7: 616–628.doi: 10.1007/s11270-014-2086-7.

4. Schober SE, Sinks TH, Jones RL, Bolger PM, McDowell M, Osterloh J, et al. Blood mercury levels in US children and women of childbearing age, 1999-2000. JAMA 2003; 289: 1667–1674. doi: 10.1001/jama.289.13.1667.

5. Ha E, Basu N, Bose-O'Reilly S, Dorea JG, McSorley E, Sakamoto M, et al. Current progress on understanding the impact of mercury on human health. Environ. Res 2017; 152: 419-433: doi: 10.1016/j.envres.2016.06.042.

6. UNEP, Status of ratification of the Minamata Convention on Mercury as at 18 September 2018.

7.Rice KM, Walker EM Jr, Wu M, Gillette C, Blough ER. Environmental mercury and its toxic effects. J Prev Med Public Health 2014; 47(2):74-83. doi: 10.3961/jpmph.2014.47.2.74

8. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. http://www.cdc.gov/nchs/nhanes.htm. [Accessed 10 November 2018].

9. Immunization Canada. https://immunize.ca/questions-and-answers. [Accessed September 2019]

10. Australian Department of Health. Are vaccines safe? https://beta.health.gov.au/health-topics/immunisation/about-immunisation/are-vaccines-safe.[Accessed September 2019]

11. NHS. Why vaccination is safe and important? .2019.https://www.nhs.uk/conditions/vaccinations/vaccine-ingredients/. [Accessed September 2019]

12.Morton HE, North LL Jr, Engley FB Jr. The bacteriostatic and bactericidal actions of some mercurial compounds on hemolytic streptococci. J Am Med Assoc 1948; 136(1):37-41. doi:10.1001/jama.1948.72890180010008.

13. Rodrigues JL, Serpeloni JM, Batista BL, Souza SS, Barbosa FJr. Identification and distribution of mercury species in rat tissues following administration of thimerosal or methylmercury. Arch Toxico L 2010; 84(11): 891-896. doi:10.1007/s00204-010-0538-4.

14. Ramanauskayte MB, Baublis PP. Clinical picture and treatment of organomercurial pesticide poisoning in children. Pediatr Moscow 1973; 32(2): 56-60.

15. Bjorklund G, Dadar M, Mutter J, Aaseth J. The toxicology of mercury: Current research and emerging trends. Environ Res 2017; 159: 545-554. doi: 10.1016/j.envres.2017.08.051.

16. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM). https://dsm.psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596. [Accessed October 2018]

17. Hooker B, Kern J, Geier D, Boyd H, Sykes L, King P, et al. Methodological Issues and Evidence of Malfeasance in Research Purporting to Show Thimerosal in Vaccines Is Safe. BioMed Res International 2014; 2014: 1-8.

18. Madsen KM, Lauritsen MB, Pedersen CB, Thorsen P, Plesner AM, Andersen PH, et al. Thimerosal and the occurrence of autism: negative ecological evidence from Danish population-based data. Pediatrics 2003; 112(3 Pt 1): 604-606. doi: 10.1542/peds.112.3.604.

19. Stehr-Green P, Tull P, Stellfeld M, Mortenson PB, Simpson D. Autism and thimerosal-containing vaccines: lack of consistent evidence for an association. Am J Prev Med 2003; 25(2): 101-106. doi: 10.1016/S0749-3797(03)00113-2.

20. Hviid A, Stellfeld M, Wohlfahrt J, Melbye M, Association between thimerosal-containing vaccine and autism. JAMA 2003; 290(13): 1763-1766. doi:10.1001/jama.290.13.1763.

21. Andrews N, Miller E, Grant A, Stowe J, Osborne V, Taylor B. Thimerosal exposure in infants and developmental disorders: a retrospective cohort study in the United Kingdom does not support a causal association. Pediatrics 2004; 114(3): 584-591.doi:10.1542/peds.2003-1177-L.

22. Verstraeten T, Davis RL, DeStefano F, Lieu TA, Rhodes PH, Black SB. Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. Pediatrics 2003; 112(5): 1039-1048.

23. Price CS, Thompson WW, Goodson B, Weintraub ES, Croen LA, Hinrichsen VL, et al. Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism. Pediatrics 2010; 126(4), 656-664. doi:10.1542/peds.2010-0309.

24. U.S. Food and Drug Administration. FDA Actions Pertaining to Thimerosal in Vaccines. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/thimerosal-and-vaccines#action. [Accessed November 2019]

25. World Health Organization.Thiomersal in vaccines. 2012.https://www.who.int/vaccine_safety/committee/topics/thiomersal/Jun_2012/en/. [Accessed October 2019]

26. Dorea JG, Farina M, Rocha JB. Toxicity of ethylmercury (and Thimerosal): a comparison with methylmercury. J Appl Toxicol 2013;33(8): 700-711. doi:10.1002/jat.2855

27. Dorea JG. Integrating experimental (in vitro and in vivo) neurotoxicity studies of low-dose thimerosal relevant to vaccines. Neurochem Res 2011; 36(6):927-938. doi:10.1007/s11064-011-0427-0

28. Suzuki K, Nakai K, Sugawara T, Nakamura T, Ohba T, Shimada M, et al. Neurobehavioral effects of prenatal exposure to methylmercury and PCBs, and seafood intake: neonatal behavioural assessment scale results of Tohoku study of child development. Environ Res 2010;110(7): 699-704.
doi: 10.1016/j.envres.2010.07.001

29. Hempel M, Chau YK, Dutka BJ, McInnis R, Kwan KK, Liu D. Toxicity of organomercury compounds: bioassay results as a basis for risk assessment. Analyst 1995;120(3): 721-724. doi:10.1039/an9952000721

30. Ruggieri F, Majorani C, Domanico F, Alimonti A. Mercury in Children: Current State on Exposure through Human Biomonitoring Studies. Int J Environ Res Public Health 2017; 12;14(5):519. doi:10.3390/ijerph14050519.

31. Dorea JG. Low-dose Thimerosal (ethyl-mercury) is still used in infants` vaccines: Should we be concerned with this form of exposure? J Trace Elem Med Biol 2018; 49: 134-139. doi: 10.1016/j.jtemb.2018.05.010

32. Zimmermann LT, Santos DB, Naime AA, Leal RB, Dórea JG, Barbosa F, Jr Farina M.

Comparative study on methyl- and ethylmercury-induced toxicity in C6 glioma cells and the potential role of LAT-1 in mediating mercurial-thiol complexes uptake. Neurotoxicology 2013; 38:1–8. doi: 10.1016/j.neuro.2013.05.015

33. Wehe CA, Pieper I, Holtkamp M, Thyssen GM, Sperling M, Schwerdtle T, et al. On-line species-unspecific isotope dilution analysis in the picomolar range reveals the time- and species-depending mercury uptake in human astrocytes. Anal Bioanal Chem 2014;406(7): 1909-1916. doi:10.1007/s00216-013-7608-4

34. Pieper I, Wehe CA, Bornhorst J, Ebert F, Leffers L, Holtkamp M, et al. Mechanisms of Hg species induced toxicity in cultured human astrocytes: genotoxicity and DNA-damage response. Metallomics 2014;6(3): 662-671. doi:10.1039/c3mt00337j

35. Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. Environ Health Perspect 2005; 113(8):1015-1021. doi:10.1289/ehp.7712

36. Lohren H, Blagojevic L, Fitkau R, Ebert F, Schildknecht S, Leist M, et al. Toxicity of organic and inorganic mercury species in differentiated human neurons and human astrocytes. J Trace Elem Med Biol 2015;32: 200-208. doi: 10.1016/j.jtemb.2015.06.008

37. Olczak M, Duszczyk M, Mierzejewski P, MeyzaK, Majewska MD. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats. Behav Brain Res 2011;223(1): 107-118. doi: 10.1016/j.bbr.2011.04.026

38. Yoshida S, Matsumoto S, Kanchika T, Hagiwara T, Minami T. The organic mercury compounds, methylmercury and ethylmercury, inhibited ciliary movement of ventricular ependymal cells in the mouse brain around the concentrations reported for human poisoning. Neurotoxicology 2016;57: 69-74. doi: 10.1016/j.neuro.2016.08.007

39. Li X, Qu F, Xie W, Wang F, Liu H, Song S, et al. Transcriptomic analyses of neurotoxic effects in mouse brain after intermittent neonatal administration of thimerosal. Toxicol Sci 2014;139(2): 452-465. doi:10.1093/toxsci/kfu049

40.Sulkowski ZL, Chen T, Midha S, Zavacki AM, Sajdel-Sulkowska EM. Maternal thimerosal exposure results in aberrant cerebellar oxidative stress, thyroid hormone metabolism, and motor behavior in rat pups; sex- and strain-dependent effects. Cerebellum 2012; 11(2): 575-586. doi:10.1007/s12311-011-0319-5

41. Magos L, Brown AW, Sparrow S, Bailey E, Snowden RT, Skipp WR. The comparative toxicology of ethyl- and methylmercury. Arch Toxicol. 1985; 57(4): 260-267. doi:10.1007/BF00324789[42] Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Critical reviews in toxicology 2006; 36(8): 609-662. doi:10.1080/10408440600845619

43. Duszczyk-Budhathoki M, Olczak M, Lehner M, Majewska MD. Administration of thimerosal

to infant rats increases overflow of glutamate and aspartate in the prefrontal cortex: protective role of dehydroepiandrosterone sulfate. Neurochem Res 2012; 37(2): 436-447. doi:10.1007/s11064-011-0630-z

44. Hornig M, Chian D, Lipkin WI. Neurotoxic effects of postnatal thimerosal are mouse strain dependent. Mol Psychiatry 2004; 9(9):833-45. doi:10.1038/sj.mp.4001529.

45. de Oliveira Souza VC, de Marco KC, Laure HJ, Rosa JC, Barbosa F, Jr. A brain proteome profile in rats exposed to methylmercury or thimerosal (ethylmercury). J Toxicol Environ Health A 2016;79(12): 502-512. doi:10.1080/15287394.2016.1182003

46. Chen YN, Wang J, Zhang J, Li SJ, He L, Shao DD, et al. Effect of thimerosal on the neurodevelopment of premature rats. World J Pediatr 2013;9(4): 356-360. doi:10.1007/s12519-013-0443-z

47. Curtis B, Liberato N, Rulien M, Morrisroe K, Kenney C, Yutuc V, et al. Examination of the safety of pediatric vaccine schedules in a non-human primate model: assessments of neurodevelopment, learning, and social behavior. Environ Health Perspect 2015;123(6): 579-589. doi:10.1289/ehp.1408257

48. Gadad BS, Li W, Yazdani U, Grady S, Johnson T, Hammond J, et al. Administration of thimerosal-containing vaccines to infant rhesus macaques does not result in autism-like behavior or neuropathology. Proc Natl Acad Sci USA 2015;112(40): 12498-12503. doi:10.1073/pnas.1500968112

49. Pichichero ME, Gentile A, Giglio N, Umido V, Clarkson T, Cernichiari E, et al. Mercury levels in new-borns and infants after receipt of thimerosal-containing vaccines. Pediatrics 2008;121(2): e208-214. doi:10.1542/peds.2006-3363

50. Stajich GV, Lopez GP, Harry SW, and Sexson WR. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. J Pediatr 2000; 136(5):679-81. doi:10.1067/mpd.2000.105133

51. Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and, metabolism in infants receiving vaccines containing thiomersal: a descriptive study. Lancet 2002; 360(9347):1737–41. doi: 10.1016/S0140-6736(02)11682-5.

52. Rooney JP. The retention time of inorganic mercury in the brain--a systematic review of the evidence. Toxicol Appl Pharmacol 2014;274(3): 425-435. doi: 10.1016/j.taap.2013.12.011

53. Dorea JG, Wimer W, Marques RC, Shade C. Automated speciation of mercury in the hair of breastfed infants exposed to ethylmercury from thimerosal-containing vaccines. Biol Trace Elem Res 2011;140(3): 262-271. doi:10.1007/s12011-010-8695-0

54. Barregard L, Rekic D, Horvat M, Elmberg L, Lundh T, Zachrisson O. Toxicokinetics of mercury after long-term repeated exposure to thimerosal-containing vaccine. Toxicol Sci 2011;120(2): 499-506. doi:10.1093/toxsci/kfr009

55. Hieu NT, Sarnecki M, Tolboom J. The safety and immunogenicity of two hepatitis B vaccine

formulations (thiomersal-free and thiomersal-containing) in healthy vietnamese infants: a phase III, prospective, single-blinded, randomized, controlled trial. Pediatr Infect Dis J 2015;34(1): 79-83. doi:10.1097/INF.0000000000000479

56. Taylor LE, Swerdfeger AL, Eslick GD. Vaccines are not associated with autism:an evidence-based meta-analysis of case-control and cohort studies. Vaccine 2014; 32(29): 3623-3629. doi: 10.1016/j.vaccine.2014.04.085

57. Yoshimasu K, Kiyohara C, Takemura S, Nakai, K.A meta-analysis of the evidence on the impact of prenatal and early infancy exposures to mercury on autism and attention deficit/hyperactivity disorder in the childhood. Neurotoxicology 2014; 44: 121-131. doi: 10.1016/j.neuro.2014.06.007.

58. Marques R C, Bernardi JV, Cunha, MP, Dorea JG. Impact of organic mercury exposure and home delivery on neurodevelopment of Amazonian children. Int J Hyg Environ Health 2016;219(6): 498-502. doi: 10.1016/j.ijheh.2016.05.002

59. Mrozek-Budzyn D, Majewska R, Kieltyka A. Early exposure to thimerosal-containing vaccines and children's cognitive development. A 9-year prospective birth cohort study in Poland. Eur J Pediatr 2015;174(3): 383-391. doi:10.1007/s00431-014-2412-5

60. Uno Y, Uchiyama T, Kurosawa M, Aleksic B, Ozaki N. Early exposure to the combined measles-mumps-rubella vaccine and thimerosal-containing vaccines and risk of autism spectrum disorder. Vaccine 2015;33(21): 2511-2516. doi: 10.1016/j.vaccine.2014.12.036

61. Dorea JG, Marques RC, Abreu L. Milestone achievement and neurodevelopment of rural Amazonian toddlers (12 to 24 months) with different methylmercury and ethylmercury exposure. J Toxicol Environ Health A 2014;77:1-3, 1-13. doi:10.1080/15287394.2014.861335

62. Marques RC, Bernardi JV, Abreu L, Dorea JG. Neurodevelopment outcomes in children exposed to organic mercury from multiple sources in a tin-ore mine environment in Brazil. Arch Environ ContamToxicol 2015;68(3): 432-441. doi:10.1007/s00244-014-0103-x

63. Demicheli V, Rivetti A, Debalini M G, Di Pietrantonj C. Vaccines for measles, mumps and rubella in children. Cochrane Database Syst 2012.(2):CD004407. doi: 10.1002/14651858.CD004407.pub3.

64. Hunter D. Diseases of occupations. London: Little, Brown; 1969.

65. Jalil MA, Abbasi AH. Poisoning by Ethyl Mercury Toluene Sulphonanilide. Br J Ind Med 1961; 8(4):303-8. doi:10.1136/oem.18.4.303.

67. Risher JF, Tucker P. Alkyl Mercury-Induced Toxicity: Multiple Mechanisms of Action. Rev Environ Contam Toxicol 2017; 240:105-149. doi: 10.1007/398_2016_1.

68. Gołoś A, Lutyńska A. Thiomersal-containing Vaccines - A Review of the Current State of Knowledge. Przegl Epidemiol 2015; 69(1):59-64, 157-61. PMID: 25862449.

69. Clements CJ. The evidence for the safety of thiomersal in newborn and infant vaccines.

Vaccine 2004; 22(15-16):1854-61. doi: 10.1016/j.vaccine.2003.11.017.

70. Stringari J, Nunes AK, Franco JL, Bohrer D, Garcia SC, Dafre AL, et al. Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. Toxicol Appl Pharmacol 2008; 227(1):147-54. doi: 10.1016/j.taap.2007.10.010.

71. Magos L. Review on the toxicity of ethyl mercury including its presence as a preservative in biological and pharmaceutical preparations. J Appl Toxicol 2001; 21(1):1-5. doi:10.1002/jat.721.

72. Bjorklund G, DadarM. MutterJ, Aaseth J. The toxicology of mercury: Current research and emerging trends. Environ Res 2017; 159:545-554. doi: 10.1016/j.envres.2017.08.051.

73. Agrawal A, Kaushal P, Agrawal S, Gollapudi S, Gupta S. Thimerosal Induces TH2 Responses via Influencing Cytokine Secretion by Human Dendritic Cells. J Leukoc Biol 2007; 81(2):474-82. doi:10.1189/jlb.0706467.

74. James SJ, Rose S, Melnyk S. Jernigan S. Blossom S, Pavliv O, et al. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. The FASEB J 2009; 23(8):2374-83. doi:10.1096/fj.08-128926

75. Olczak M, Duszczyk M, Mierzejewski P,Bobrowicz T, Majewska MD. Neonatal administration of thimerosal causes persistent changes in mu opioid receptors in the rat brain. Neurochem Res 2010; 35(11):1840-7. doi:10.1007/s11064-010-0250-z.

76. Gliddon CM, Shao Z, LeMaistreJL, Anderson CM. Cellular distribution of the neutral amino acid transporter subtype ASCT2 in mouse brain. J Neurochem 2009; 108(2):372-83. doi:10.1111/j.1471-4159.2008.05767.x.

77. Becucci L, Tramonti V, Fiore A, Fogliano V, Scaloni A, GuidelliR. Channel-forming Activity of Syringomycin E in Two Mercury-Supported Biomimetic Membranes. Biochim Biophys Acta 2015; 1848(4):932-41. doi: 10.1016/j.bbamem.2014.12.007.

78. Haase H, Hebel S, Engelhardt G, Rink L. Ethylmercury and Hg2+ induce the formation of neutrophil extracellular traps (NETs) by human neutrophil granulocytes. Arch Toxicol 2016; 90(3):543-50. doi:10.1007/s00204-015-1484-y.

79. Grotto D, Valentini J, Fillion M, Passos CJS, Garcia SC, Mergler D, et al. Mercury exposure and oxidative stress in communities of the Brazilian Amazon. Sci Total Environ 2010; 408(4):806-11. doi: 10.1016/j.scitotenv.2009.10.053.

80. CariccioVL,Sama A, Bramanti P, Mazzon E. Mercury involvement in neuronal damage and in neurodegenerative diseases. Biol. Trace Elem Res 2019; 187(2):341-356. doi:10.1007/s12011-018-1380-4.

81. Gukovskaya AS, Trepakova ES, Zinchenko VP, Korystov YN, Bezuglov VV. Effect of the sulfhydryl reagent thimerosal on cytosolic free calcium and membrane potential of thymocytes.

Biochim Biophys Acta 1992; 1111(1):65-74. doi:10.1016/0005-2736(92)90275-q.

82. Hajieva P, Baeken MW, Moosmann B. The role of Plasma Membrane Calcium ATPases (PMCAs) in neurodegenerative disorders. Neurosci Lett 2018;663:29-38.doi:10.1016/j.neulet.2017.09.033.

83. Nabemoto M, Ohsawa K, Nakamura H, Hirabayashi T, Saito T, Okuma Y, et al. Reversible activation of secretory phospholipase A2 by sulfhydryl reagents. Arch Biochem Biophys 2005; 436(1):145-53. doi: 10.1016/j.abb.2005.02.003.

84. Risher JF, Tucker P. Alkyl mercury-induced toxicity: multiple mechanisms of action. Rev Environ Contam Toxicol 2017; 240:105-149. doi: 10.1007/398_2016_1.

85. Dopp E, DoppUvon Recklinghausen, Hippler, J. Toxicity of volatile methylated species of bismuth, arsenic, tin, and mercury in mammalian cells in vitro. J Toxicol 2011; 2011:503576. doi:10.1155/2011/503576.

86. Loison E, Poirier-Beaudouin B, Seffer V, Paoletti A, Abitbol V, Tartour E, et al. Suppression by thimerosal of ex-vivo CD4+ T cell response to influenza vaccine and induction of apoptosis in primary memory T cells. PloS one 2014; 9(4), e92705.

87. Morris G, Puri BK, Frye RE, Maes M. The Putative Role of Environmental Mercury in the Pathogenesis and Pathophysiology of Autism Spectrum Disorders and Subtypes. Mol Neurobiol 2018; 55(6):4834-4856. doi:10.1007/s12035-017-0692-2.

88. Cai F, Backman HA, Baines MG. Thimerosal: an ophthalmic preservative which acts as a hapten to elicit specific antibodies and cell mediated immunity. Curr Eye Res 1988; 7(4):341-51. doi:10.3109/02713688809031783.

89. Mondino BJ, Brawman-Mintzer O, Boothe WA. Immunological complications of soft contact lenses. J Am Optometric Ass 1987; 58(10), 832-5. PMID: 3316352

90. Havarinasab S, Bjoern E, Ekstrand J. Hultman P. Dose and Hg species determine the T-helper cell activation in murine autoimmunity. Toxicology 2007; 229(1-2), 23-32. doi: 10.1016/j.tox.2006.09.006

91. Dorea JG. Low-dose Thimerosal in pediatric vaccines: Adverse effects in perspective. Environ Res 2017; 152:280-293. doi: 10.1016/j.envres.2016.10.028.

92. Goodrich JM, Basu N, Franzblau A, Dolinoy DC. Mercury biomarkers and DNA methylation among Michigan dental professionals. Environ Mol Mutagen 2013; 54(3):195-203. doi:10.1002/em.21763.

93. Yoshino Y, Mozai T, Nakao K. Biochemical changes in the brain in rats poisoned with an alkylmercury compound, with special reference to the inhibition of protein synthesis in brain cortex slices. J Neurochem 1956;13(11):1223-30. doi:10.1111/j.1471-4159.1966.tb04281.x.

94. dos Santos AA, Hort MA, Culbreth M, López-Granero C, Farina M, Rocha JB, et al. Methylmercury and Brain development: A review of recent literature. J Trace Elem Med Biol 2016; 38:99-107. doi: 10.1016/j.jtemb.2016.03.001.

95. Carratù MR, Signorile, A. Methyl mercury injury to CNS: mitochondria at the core of the matter. Open Acc Toxicol 2015; 1(2), 1-6.

96. Tian J, Luo Y, Chen W, Yang S, Wang H, Cui J, et al. MeHg Suppressed neuronal potency of hippocampal NSCs contributing to the puberal spatial memory deficits. Biol Trace Element Res 2016; 172(2):424-436. doi:10.1007/s12011-015-0609-8.

97. Xu F., Farkas S., Kortbeek S., Zhang F.X, Chen L, ZamponiGW, et al. Mercury-induced toxicity of rat cortical neurons is mediated through N-methyl-D-Aspartate receptors. Mol Brain 2012; 5:30. doi:10.1186/1756-6606-5-30.

98. Stankovic RK, Shingde M, Cullen KM. The experimental toxicology of metallic mercury on the murine peripheral motor system: a novel method of assessing axon calibre spectra using the phrenic nerve. J Neurosci Methods 2005; 147(2):114-25. doi: 10.1016/j.jneumeth.2005.03.009.

99. Migliarini S, Pacini G, Pelosi B, Lunardi G, Pasqualetti M. Lack of Brain Serotonin Affects Postnatal Development and Serotonergic Neuronal Circuitry Formation. Mol Psychiatry 2013; 18(10):1106-18. doi:10.1038/mp.2012.128.

100. Branch DR. Gender-selective toxicity of thimerosal. Exp Toxicol Pathol 2009; 61(2):133-6. doi:10.1016/j.etp.2008.07.002.

101. Kernn JK, Geier DA, Homme KG, King PG, Bjorklund G, Chirumbolo S, et al. Developmental neurotoxicants and the vulnerable male brain: a systematic review of suspected neurotoxicants that disproportionally affect males. Acta Neurobiol Exp 2017; 77(4): 269-96.

102. Cariccio VL, Sama A, Bramanti P, Mazzon E. Mercury involvement in neuronal damage and in neurodegenerative diseases.Biol. Trace Elem Res 2018; 187: 341-356. https://doi.org/10.1007/s12011-018-1380-4

103. Woods JS, Martin MD, Leroux BG, DeRouen TA, Leitao JG, Bernardo MF, et al. The contribution of dental amalgam to urinary mercury excretion in children. Environ Health Perspect 2007; 115(10): 1527-1531.doi: 10.1289/ehp.10249.

104. Fourth National Report on Human Exposure to Environmental Chemicals. March 2018. Vol 1.

105. Melnick JG, Yurkerwich K, Buccella D, Sattler W, Parkin G. Molecular structures of thimerosal (Merthiolate) and other arylthiolate mercury alkyl compounds. Inor Chem 2008; 47(14): 6421-6426.doi: 10.1021/ic8005426.

106. Arudra P, Nguiffo-Podie Y, Frost E, Dubowski JJ. Decomposition of thimerosal and dynamics of thiosalicylic acid attachment on GaAs(001) surface observed with in situ photoluminescence. J Physical Chem C 2010;114(32): 13657-13662. doi: 10.1021/jp102192j.

107. Clarkson TW, Vyas JB, Ballatori, N. Mechanism of mercury disposition in the body. Am J Ind Med 2007; 50(10): 757-764. doi:10.1002/ajim.20476.

108. Sandborgh-Englund G, Elinder CG, Langworth S, Schutz A, Ekstrand J. Mercury in

biological fluids after amalgam removal. J Dent Res 1998; 77(4): 615-624. doi:10.1177/00220345980770041501.

109. Vahter M, Mottet NK, Friberg L, Lind B, Shen DD, Burbacher T. Speciation of mercury in the primate blood and brain following long-term exposure to methyl mercury. Toxicol Appl Pharmacol 1994; 124: 221-229.doi:10.1006/taap.1994.1026.

110. Ballatori N, Hammond CL, CunninhgamJB, Krance SM, MarchanR. Molecular mechanisms of reduced glutathione transport: Role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins. Toxicol Appl Pharmacol 2005; 204:238–255. doi: 10.1016/j.taap.2004.09.008.

111. Branco V, Caito S, Farina M, Teixeira da Rocha J, Aschner M, Carvalho C. Biomarkers of mercury toxicity: Past, present, and future trends. J Toxicol Environ Health Part B 2017; 20(3): 119-154.doi: https://doi.org/10.1080/10937404.2017.1289834

112.Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari, E, et al. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 2003; 361:1686-92.doi:10.1016/S0140-6736(03)13371-5.

113. Smith JC, Farris FF. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol 1996; 37: 245-252.doi:10.1006/taap.1996.0078.

114. Kajiwara Y, Yasutake A, Adachi T, Hirayama K. Methylmercury transport across the placenta via neutral amino acid carrier. Arch Toxicol 1996; 70(5), 310-4. doi:10.1007/s002040050279.

115. Tan M, Parkin JE. Route of decomposition of thiomersal (thimerosal). Int J Pharm 2000; 208: 23-34. doi:10.1016/S0378-5173(00)00514-7.

116. Caraballo I, Rabasco AM, Fernandez-Arevalo M. Study of thimerosal degradation mechanism. Int J Pharm 1993; 89: 213–221. doi: 10.1016/0378-5173(93)90246-C.

117. Davies DJG, Anthony Y, Meakin BJ. The antimicrobial activity of degraded thiomersal solutions. Expo Congr Int Technol Pharm 1983; 4: 238–242.

118. Gibson MA, Sarpong-Kumankomah S, Nehzati S, George GN, Gailer J. Remarkable difrefences in the biochemical fate of Cd2+, Hg2+,CH3Hg+ and thimerosal in red blood cell lysate. Metallomics 2017; 9: 1060–1072. doi:10.1039/c7mt00069c.

119. Liu Y-M, Zhang F-P, Jiao B-Y, Rao J-Y, Leng G. Automated dispersive liquid-liquid microextraction coupled to high performance liquid chromatography - cold vapour atomic fluorescence spectroscopy for the determination of mercury species in natural water samples. J Chromatogr A 2017; 1493: 1-9. doi: 10.1016/j.chroma.2017.03.002.

120. Shigeta K, Tao H, Nakagawa K, Kondo T, Nakazato T. A simple and robust method for determination of alkylmercury in seawater and industrial wastewater by phenylation pretreatment combined with GC-MS. Analytical Sci 2018; 34(2), 227-233.

doi:10.2116/analsci.34.227.

121. Chen YQ, Cheng X, Mo F, Huang LM, Wu Z, Wu Y, et al. Ultra-sensitive speciation analysis of mercury by CE-ICP-MS together with field-amplified sample stacking injection and dispersive solid-phase extraction. Electrophoresis 2016;37(7-8): 1055-1062. https://doi-org.pros.lib.unimi.it:2050/10.1002/elps.201500460

122. Cheng H, Wu C, ShenL, Liu J, Xu Z. Online anion exchange column preconcentration and high-performance liquid chromatographic separation with inductively coupled plasma mass spectrometry detection for mercury speciation analysis. Anal Chim Acta 2014; 828: 9–16. doi: 10.1016/j.aca.2014.04.042.

123. Cheng H, Chen X, Shen L, Wang Y, Xu Z, Liu J. Ion-pairing reversed-phase chromatography coupled to inductively coupled plasma mass spectrometry as a tool to determine mercurial species in freshwater fish. J Chromatogr A 2018; 1531: 104-111.
doi: 10.1016/j.chroma.2017.11.029.

124. Castro Grijalba A, Quintas PY, Fiorentini EF, Wuilloud RG. Usefulness of ionic liquids as mobile phase modifiers in HPLC-CV-AFS for mercury speciation analysis in food. J Anal At Spectrom 2018; 33(5): 822-834.
doi: 10.1039/C8JA00059J.

125. Khaligh A, Mousavi HZ, Shirkhanloo H, Rashidi A. Speciation and determination of inorganic arsenic species in water and biological samples by ultrasound assisted-dispersive-micro-solid phase extraction on carboxylatednanoporous grapheme coupled with flow injection-hydride generation atomic absorption spectrometry. RSC Adv 2015; 5: 93347–93359. doi: 10.1039/C5RA17229B.

126. Gibicar D, Logar M, Horvat N, Marn-Pernat A, Ponikvar, R, Horvat M. Simultaneous determination of trace levels of ethylmercury and methylmercury in biological samples and vaccines using sodium tetra(n-propyl) borate as derivatizing agent. Anal Bioanal Chem 2007; 388: 329–340. doi: 10.1007/s00216-007-1208-0.

127. Qvarnstrom J, Lambertsson L, Havarinasab S, Hultman P, Frech W. Determination of methylmercury, ethylmercury and inorganic mercury in mouse tissues, following administration of thimerosal, by species-specific isotope dilution GC-inductively coupled plasma-MS. Anal Chem 2003; 75: 4120–4124. doi: 10.1021/ac0342370.

128. Zareba G, Cernichiari E, Hojo R, Nitt SM, Weiss B, Mumtaz MM, Clarkson TW. Thimerosal distribution and metabolism in neonatal mice: comparison with methyl mercury. J Appl Toxicol 2007; 27(5): 511-518. doi:10.1002/jat.1272.

129. Shade CW, Hudson RJ. Determination of MeHg in environmental sample matrices using Hg-thiourea complex ion chromatography with on-line cold vapor generation and atomic fluorescence spectrometric detection. Environ Sci Technol 2005; 39: 4974–4982. doi:10.1021/es0483645

130. Vermillion BV, Hudson RJM. Thiourea catalysis of MeHg ligand exchange between

natural dissolved organic matter and a thiol-functionalized resin: a novel method of matrix removal and MeHg preconcentration for ultratrace Hg speciation analysis in freshwaters. Anal Bioanal Chem 2007; 388: 341–352. doi:10.1007/s00216-007-1207-1

131. Dorea JG, Bezerra VL, Fajon V, Horvat M. Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. Clin Chim Acta 2011; 412(17-18): 1563-1566. doi: 10.1016/j.cca.2011.05.003.

132. Queipo AS, Rodriguez-Gonzalez P, Davis WC, Garcia AJI. Development of a common procedure for the determination of methylmercury, ethylmercury, and inorganic mercury in human whole blood, hair, and urine by triple spike species-specific isotope dilution mass spectrometry. Anal Chem 2017; 89(12): 6731-6739. doi: 10.1021/acs.analchem.7b00966.

133. Strohmidel P, Sperling M, Karst U. Investigation on the binding of ethylmercury from thiomersal to proteins in influenza vaccines. J Trace Elem Med Biol 2018; 50: 100-104. doi: 10.1016/j.jtemb.2018.06.011.