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REVIEW PAPER

Abating Mercury Exposure in Young Children Should Include Thimerosal-Free Vaccines

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Abstract Pediatric immunization is essential to prevent, control and eradicate children`s infectious diseases. Newborns and infants in less developed countries have a concentrated schedule of Thimerosal-containing vaccines (TCVs); pregnant mothers are also immunized with TCVs. Metabolic changes during early development are demonstrably an important risk factor for ethylmercury (EtHg) effects on neurodevelopment, while exposure to Thimerosal sensitizes susceptible individuals to life-long contact dermatitis. Concerns regarding toxicity of Hg have moved rich nations to withdraw it from medicines and, in particular, Thimerosal from pediatric vaccines; it has been more than 20 years since rich countries started using Thimerosal-free vaccines. TCVs and Thimerosal-free vaccines show dissimilar profiles of adverse effects. Thimerosal-free vaccines have shown a decrease in contact dermatitis, while TCVs showed a significant association with increased risk of tic disorders; in some circumstances, EtHg in combination with other neurotoxic substances negatively impacted neurobehavioral tests. In studies that explored vaccines and risk of tics, Thimerosal was a necessary factor. However, when the binary exposure to organic Hg forms (TCV–EtHg and fish-MeHg) was considered, effects on neurobehavioral tests were inconsistent. Conclusions: (a) The indiscriminate use of pediatric-TCVs in less developed countries carries an unjustifiable and excessive EtHg exposure with an unnecessary risk of neurotoxicity to the developing brain; (b) measurable benefits (of Thimerosal-free) and measurable risks of tic disorders have been associated with the (Thimerosal-containing) type of vaccine; (c) Thimerosal-free vaccines are clinically and toxicologically justifiable and they should be available to children in less developed countries.

Keywords Thimerosal-free vaccines · Ethylmercury · Infants · Contact dermatitis · Tic disorders

Introduction

The pathogenesis of Hg toxicity has received input from a wide range of in vitro and in vivo experimental studies. These have identified molecular and genetic factors driving the heterogeneity of immunological and neurobehavioral outcomes and the respective risks of exposure to all chemical-Hg forms [1]. Biochemical, clinical and epidemiologic studies indicate that small amounts of Thimerosal can lead to adverse effects [2]. Thimerosal, a preservative/adjuvant commonly used in vaccines is associated with an increased toxicological risk from pediatric Thimerosal-containing vaccines (TCVs). Therefore, concerns regarding the toxicity of Hg have found different solutions in regards to pediatric vaccines. Compared to the USA, which had Thimerosal in approximately 30 different childhood vaccines, France only had it in two (in 1999), and these two were also available in a Thimerosal-free formulation [3]. Nevertheless, the most developed nations have withdrawn Hg from medicines and, in particular Thimerosal from pediatric vaccines; it has been more than 20 years since developed countries started using Thimerosal-free vaccines [4].

Thimerosal has a limited role in immunogenicity (of intended antigens) but has a use in some vaccine manufacturing processes [4]. Thimerosal is a very active compound with a potential to act on the immunological and

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neurological systems of young children at the levels in which it is used as preservative in pediatric vaccines [4]. Indeed, there are no evidence-base safety criteria to guide decisions supporting/recommending TCVs in the current pediatric immunization calendar; data concerning the safety of ethylmercury (EtHg) in pediatric-TCVs are limited. The realization that there are plausible adverse effects of pediatric TCVs has led to measures being taken in modern vaccine development (single Thimerosal-free monodose vials) to diminish children's exposure to EtHg [5]. Since the inception of Thimerosal-free vaccines, measurable advantages have been accumulated in the international medical literature.

Our understanding of the adverse effects of Thimerosal in pediatric vaccines has received inputs from studies addressing different endpoints: diagnostic conditions and molecular effects on neurobehavioral tests. Additionally, developmental (physiological, neurological and immunological) changes that occur in early life (fetus, infants and young children) are central to understanding the heightened susceptibility to Hg toxicity.

It is noteworthy that economically advanced countries have discontinued the use of TCV in children <5 years of age. Despite all scientific evidence, the Minamata Convention exempted TCVs from regulation/restriction [6]. However, TCV for children of less developed countries persist. Sykes et al. 2014 [6] discussed the discrepancy between Thimerosal exposure in the low- and middle-income countries and high-income countries in regards to level of Thimerosal exposure from the vaccines used in pregnant mothers and young children. Sykes et al. 2014 [6] called attention for the existing double standard regarding TCV use in low- and middle-income countries and the global impact of the Minamata Convention on Hg exposure.

This paper puts into perspective the main reasons for implementing Hg-free vaccines for all children. The objective of this study is to summarize the existing scientific evidence on the adverse effects associated with pediatric-TCVs and the benefits of Thimerosal-free vaccines.

Early Life Vulnerability to Components in TCVs (EtHg and Adjuvant-Al)

Infant's pre- and post-natal exposures to toxicants are common and depend on the maternal exposure during pregnancy and lactation. However, children immunized with TCVs are also exposed to EtHg and adjuvant-Al. Among vaccine ingredients that are both immunotoxic and neurotoxic (Thimerosal–EtHg and adjuvant-Al), the mode of exposure as well as biologic activities are starkly different between young children and adults. Indeed, age of infant, and a variety of other factors related to the chemical mixture (EtHg and adjuvant-Al) of the TCV are scarcely studied; these factors include differences in adjuvant-Al chemical forms [7]. Nevertheless, the universal recommendation of TCV (in less developed countries) does not take into account infant susceptibility to early EtHg exposure. Furthermore, there are no prognostic scoring systems developed to screen risks of cumulative exposure (EtHg and adjuvant-Al) with other neurotoxic substances frequently occurring in disadvantaged environments. Additionally, clinical factors related to the difference between infants and adults are not incorporated in study protocols to test for the toxicity of TCV–EtHg. Despite a clear indication of adverse effects related to neurodevelopment and immunotoxicity [4], children receiving TCVs are not scrutinized for risks of known adverse effects.

Compared to adults, children (in less developed countries) on the current vaccine schedule are more exposed to EtHg (on a body weight basis) and metabolize it differently (Table 1). Metabolic differences related to body weight are likely to rise in neonates due to water loss and blood–brain-barrier (BBB) immaturity. Indeed, measured EtHg half-life seems higher in younger children than in older children and adults (Table 1). Additionally, comparing young and older infants (newborns versus 6 months), blood and urine Hg clearance are starkly dissimilar [8, 9]. Moreover, children are prone to certain infectious diseases and have genetic differences that may contribute to variability in Hg metabolism and toxic response. Preterms and newborns are likely to have immature organs that are sensitive to Hg toxicity (BBB, hepatic and renal clearance) and naive immunological systems [9].

Studies of Hg metabolism in infants (0–6 months) receiving TCVs showed age-related differences in blood-Hg levels concentrations; additionally, Hg in feces and in urine of young children is metabolized differently [9]. The excretion of Hg is faster in older children. Post vaccination with TCV in newborns provokes a rise in blood Hg, as shown in studies from different countries [10–13]. In Iranian infants, 33% showed blood Hg concentrations were above safe limits (5.9 μg/L) in samples taken from 2 to 28 days after immunization with TCVs [12]. Stajich et al. [10] also showed that blood Hg was higher in preterm than in term babies; overall, compared to younger infants and to immature newborns, it is demonstrable that older infants clear blood TCV–Hg faster [9]. The age variation in pharmacokinetics of Thimerosal increases the risk of side effects in infants and young children (Table 1).

Acceptable daily intake of neurotoxic elements exists for adults in regards to orally taken food or drinking water; for children however, there are uncertainties regarding short- and long-term health risks [14]. In the specific case of TCVs, when it is injected in newborns or infants, there are no acceptable Hg (or adjuvant-Al) body burdens. Table 1

Table 1 Summary of comparative aspects between the young and adult human in relation to exposure and metabolism of low ethylmercury doses and susceptibility to its effects

Selected aspects	Fetus and infants	Older children/adults	References
TCV uptake	Mandatory	Optional	
Likelihood of exceeding blood Hg threshold	Yes	No	Dórea [9]
6 month cumulative	150 µg	25 µg	Dórea [97]
Dose (reference) µg/kg	8.33 (premature 16)	0.36	Dórea [97]
Blood clearance	Slower	Faster	Dórea [9]
Agravating factor			
Newborn water-loss	Yes	No	Dórea [9]
BBB penetration	Likely	Unlikely	Dórea [9]
Co-exposure (MeHg)	High in breastfed	Likely to be lower	
Co-exposure (Al)	Higher	Lower	Dórea [9]
Effects			
Brain residency	Likely to affect neurochemistry	Less likely	Dórea [4]
Biomarkers	Hair, blood	Hair, blood	Dórea [9]
Half-life in blood	6.3[a] and 7 days[b]	5.6 days	Pichichero et al., [98, 99]; Barregard et al. [100]
Contact dermatitis	Increased incidence	Unknown	Table 2
Tic disorders	Increased incidence	Unknown	Table 3
Neurobehavioral	Possibly	Unlikely	Table 4

TCVs Thimerosal-containing vaccines, also contain adjuvant-Al, *BBB* blood–brain barrier

[a]Premature, [b]Term infants

summarizes some of the unique considerations between infants and adults regarding safety issues that should concern vaccine formulators and pediatricians regarding Thimerosal. Indeed, during pregnancy the expected cord blood Al:Hg ratio is 2.5 [15] while Al:Hg ratio absorbed from TCV is 50 [16].

Adverse Effects of Thimerosal in Vaccines

Immunological: Contact Dermatitis

Exposure to the injectable form of Thimerosal is critical for contact dermatitis and the amount of EtHg in this acute exposure elicits an immunologic response quite different from chronic (oral) Hg exposure from maternal milk. Furthermore, Wantke et al. [17] tested Thimerosal and its molecular components (ethyl mercuric chloride and thiosalicylic acid) concluding that EtHg is the causative agent of the hypersensitivity. Indeed, when compared to EtHg, MeHg-derivatives reacted similarly [18]. Therefore, local reactions resulting from TCVs are expected in Thimerosal-sensitive patients, justifying the recommendation to use other preservatives [19].

Sensitization to Thimerosal is not a contraindication to immunization with TCVs [20]. However, contact dermatitis is usually a lifelong reaction but is considered to be without clinical significance [20]. Contact allergy or sensitization used to be prevalent in both unselected children and adults before Thimerosal was withdrawn from vaccines [21]. With the increase in pediatric TCVs, many countries have reported an increase in children's sensitization to Thimerosal. Indeed, there are consistencies across studies summarized in Table 2 indicating a clear trend for contact dermatitis to increase with TCV use and a decreasing trend in countries that started using Thimerosal-free vaccines.

The introduction of tick-borne encephalitis vaccine in Austria was followed by an increase in the number of allergies to merthiolate [22]. In the USA Thimerosal (secondary to vaccine exposure) was considered the most prevalent allergen [23]. A 15-year meta-analysis by Krob et al. [24] reported Thimerosal as the second most common allergen. At the height of TCVs in pediatric vaccines, sensitization to this allergen was reported from 1 to 37% in different countries [25]. Milingou et al. [26] reported a significant increase in positive reactions to Thimerosal between the studied periods (period A: 1980–1993, 1.8%; period B: 1994–2007; 18.0%) coinciding with changes in TCV use in Greece [27]. Indeed, after using Thimerosal-free vaccines, noticeable changes in Thimerosal sensitivity were measured in several countries. Benefits of withdrawing Thimerosal from pediatric vaccines were observed as decreased patch-test reactions in several countries (Table 2). After withdrawing Thimerosal from pediatric vaccines a significant change in frequency of sensitization to this compound was measured in Poland [28, 29], Denmark [30, 31], Italy

Table 2 Summary of studies showing/attributing changes in allergic contact dermatitis related to Thimerosal in vaccines

Reference	Year	Country	Key conclusions
Lindemayr and Becerano [22]	1985	Austria	"The slightly increased number of allergies to merthiolate in 1984 might be attributed to the widespread use of merthiolate-preserved FSME vaccines"
Wohrl et al. [101]	2003	Austria	"Sensitization to Thimerosal, presumably resulting from Thimerosal-containing vaccines, depended on age"
Fernández Vozmediano and Hita [102]	2005	Spain	This high incidence of sensitization to Thimerosal is due to wide range of uses (including vaccines). The majority of the patients testing positive to Thimerosal also presented a positive reaction to Hg
Goon and Goh [103]	2006	Singapore	A relatively high rate of positive patch tests to Thimerosal may be related to use of vaccines and antiseptics containing this substance
Zug et al. [104]	2008	USA	The development of an allergy to Thimerosal is likely a result of routine vaccination
Jacob et al. [23]	2008	USA	Positive reactions to Thimerosal were probably secondary to vaccine exposure, which is expected to decrease over time as fewer vaccines are being preserved with this agent
Kuljanac et al. [105]	2011	Croatia	The high rate of sensitization to Thimerosal in children was attributed to widespread use as a preservative in a variety of compounds including vaccines
Czarnobilska et al. [28, 29]	2011	Poland	"A limitation of exposure to the preservatives thimerosal resulted in decreased rates of sensitization to these haptens"
Thyssen et al. [30, 31]	2007	Denmark	Decrease in allergy in the general population of Denmark was attributed to Thimerosal-free vaccine
Warshaw et al. [106]	2010	USA	Thimerosal reaction rates were significantly lower in older individuals
Fortina et al. [32]	2016	Italy	Contact sensitization to Thimerosal was very low in very young children and higher in older age groups, probably as a result of the recent limitation of its use as a preservative in vaccines

[32], and the USA (Table 2). In Italian children reacting positive (4.2%) to Thimerosal, 11.8% were considered relevant reactions [32].

In countries still using TCVs, allergic contact dermatitis still has a high prevalence for Thimerosal in Brazil (18.4%) [33], Thailand (10.6%) [34], and in China (8.9%) also as photoallergic contact dermatitis [35]. In countries that measured patch testing according to age, the older population (reflecting less exposure to pediatric TCV) showed a decrease in Thimerosal sensitivity in adults of South Korea [36] and Germany [37]. Additionally, the use of Thimerosal-free vaccines can diminish the Hg burden of neonates and young children.

Diagnosed Neurological Disorders

The plausibility that TCV–EtHg could be associated with neurological disorders in young children has been intensely investigated. A summary of studies from different countries showing significant association between TCV–EtHg and diagnosed tic disorders is illustrated in Table 3. Different statistical models were employed on cohorts of children from the USA, UK, France, and Italy. Tic disorders are significantly associated with TCVs in all studies done in the USA. These epidemiological studies were conducted by independent teams in different databases—Vaccine Safety Datalink (VSD) and Health Maintenance Organization (HMO). Tozzi et al. [38] in Italy reported that phonic tics were lower in the lower TCV intake group but not statistically significant. However, studies done in the UK showed contradictory outcomes: while Andrews et al. [39] working with the General Practice Research Database (n = 109,683) reported a significant positive association between number of shots up to 6 months and risk of tic disorders, the Avon Longitudinal Study of Parents and Children (ALSPAC) study reported quite the opposite [40]: children seemed to benefit from TCVs in respect to measured outcomes.

It is worth mentioning that Iqbal et al. [41] studied the total number of antigens in vaccines and reported no increased risk of tic disorder related to all vaccines in the schedule after adjusting for TCVs; this is the same CDC cohort where others reported an increased risk of tics [42, 43]. Therefore, it seems that in studies that explored vaccines and risk of tics, Thimerosal was a necessary factor. Indeed, tic disorder associated with Hg poisoning resulting from traditional Chinese medicines has been reported [44]. In the ALSPAC cohort, higher socioeconomic status was positively associated with blood Hg [45] and negatively associated with tic disorders [46]; further analysis also showed a role of maternal morbidity as a risk factor for tic disorders in the offspring [47]. It is noteworthy that most children with tic disorders (2/3) showed other neurological and neurobehavioral comorbidities [48].

Table 3 Summary of studies exploring a link between tics and TCV in children

Reference	Country	n	Data base	Outcomes
Verstraeten et al. 2003 [58]	USA	110,883	VSD	Cumulative TCV by 1, 3, and 7 m. Increasing exposure to Hg was associated with a greater likelihood of tics in one HMO population
Geier and Geier 2005 [53]	USA	NG	VSD	Exposure at 3 months of age increased risk of tics
Young et al. 2008 [54]	USA	278,624	VSD	Birth to 7 month period, the rate of tics was approximately 3.4 times higher given a 100 µg increase in Hg exposure in TCVs
Thompson et al. [42]	USA	1047	CDC	Among boys, higher exposure to Thimerosal associated with higher likelihood of motor and phonic tics
Geier et al. 2017 [107]	USA	1.95 million	VSD	"TCV-Haemophilus influenzae type b administrated within the first 15 months of life was significantly ($p < 0.001$) more likely than controls to have a diagnosis of tic disorder"
Geier et al. 2014 [55]	USA	1 million	VSD	"Male tic-disorder cases were significantly more likely than male controls to have received increased organic-Hg from TCV-HepB administered within the first month-of-life (OR 1.65, $p < 0.0001$), first 2-months-of-life (OR 1.64, $p < 0.0001$), and first 6 months-of-life (OR 2.47, $p < 0.05$), whereas female tic-disorder were significantly more likely than female controls to have received increased organic-Hg from TM-HepB administered within the first 6-months-of-life (OR 4.97, $p < 0.05$)"
Geier et al. 2015 [108]	USA	1.9 million	VSD	On a µg of EtHg basis, tic disorder cases were significantly (OR 1.034) more likely than controls to receive increased EtHg exposure from TCV–HBV
Barile et al. 2012 [43]	USA	1047	CDC	Significant association between early Thimerosal exposure and the presence of tics in boys
Iqbal et al. 2013 [41]	USA	1047	CDC	After correction for TCVs, no increased risk of tics with cumulative antigen exposures from all vaccines
Leslie et al. 2017 [109]	USA	2547	MarketScan® commercial claims and encounters	Children with tic disorder were more likely to have had a vaccination (influenza and meningitis) in the preceding 6, and 12 months than matched controls; there was a higher proportion of males with tic disorders**
Andrews et al. 2004 [39]	UK	109,683	GPRD	Higher risk for tics with increasing Thimerosal doses
Tozzi et al. 2009 [38]	Italy	1403	Comissioned study	Phonic tics were lower (0.43% of patients) in the lower group intake compared to the higher intake group (0.87% of patients)
Heron and Golding, 2004 [40]	UK	>14,000	ALSPAC	The more exposed the infant, the more beneficial the outcome: doses by 4 months and reported tics at 91 months ($p < 0.027$); tics at 91 months ($p < 0.025$)
Lloret et al. 2013 [110]	France	NG	FPVD	Drugs (including influenza vaccine and HBV) related to motor and/or vocal tics were found in reports of adverse drug reactions from 1st January 1984 to 31st December 2010

ALSPAC Avon Longitudinal Study of Parents and Children, *GPRD* General Practice Research Database, *HBV* hepatitis B vaccine, *VSD* Vaccine Safety Datalink, *CDC* Center for Disease Control (2003–2004 study), *FPVD* French Pharmacovigilance Database, *NG* not given (years 1984 to 2010)

**Vaccines likely to contain Thimerosal [111]

Besides tic disorders, TCVs in young children have been associated with other neurodevelopmental issues; the most controversial has been autism spectrum disorders (ASD). The underlying etiology of autism is unknown but its current rates are a research topic of exceptional importance. Indeed, TCV and ASD is a controversial issue associated with pediatric immunization. Recent reviews have addressed environmental factors associated with its development that incudes Hg and TCVs as risk factors for ASD [49, 50]. Studies that examined the link between TCVs and ASD are not in agreement. While methodologies and populations may show contradictory outcomes, a recent review indicated that the majority of studies linked Hg and TCVs with the risk of an ASD diagnosis [51]. Some uncertainties may be related to statistical models. A recent report suggested an increased ASD risk among

children whose mothers received an influenza vaccination (between 2000 and 2010) in their first trimester; however the association was not statistically significant after adjusting for multiple comparisons [52].

The Geier research team and associates have contributed much of the work showing TCVs and increased risk/association with several disorders such as attention deficit/hyperactivity disorder [53–57], language delay [53, 58], memory deficits [38], childhood obesity [59], emotional disturbances [58, 60], ataxia [61], premature puberty [62], developmental delay [63–66], speech disorder [42, 61, 65, 67–69]. Studies in Japan considered the association of Thimerosal with attention deficit and hyperactivity disorder negligible [70].

Trends in Risk of Neurological Disorders After Discontinuation of TCV

After discontinuation of TCV in young children most studies in the USA have shown a trend in decreasing neurological outcomes [61, 67]. In Dennmark there was a decline in the rate of ASD in the 2002–2004 birth cohort (1.0%) compared to the 1994–1995 birth cohort (1.5%) [71]. However, other groups have not seen significant increase in risk of pervasive developmental disorders in Canada [72, 73] and in the UK [40]; in the USA most of the risks of deficits in neurophysiological functions was not significant [42].However, discontinuation of TCV in Canada did not modify the risk of pervasive developmental disorders [74].

Co-exposure of TCV-EtHg and Other Neurotoxic Substances: Neurobehavioral Tests

Recently, the plausibility that small doses of Hg in TCVs could be associated with neurological disorders or neurocognitive delays has been investigated. Research done in young children (from different countries) exposed to vaccine-EtHg alone and in combination (co-exposure with other neurotoxicants) supports this assumption.

Young children are exposed to small doses of different types of toxic substances, some acutely (like Thimerosal–EtHg and adjuvant-Al) and orally in maternal milk; indeed, multiple toxic metal exposure through breastfeeding can occur depending on the maternal diet/lifestyle [75, 76]. Furthermore, Claus Henn et al. [77] have addressed exposure to a chemical mixture of metals as a class of toxic substances with the ability to cause neurological effects. They identified metal-mixture components of environmental origin (air pollution or toxic waste) and stressed that it was challenging to account for the correlated structure of the mixtures in epidemiological studies [78]. Therefore, early life EtHg co-exposure with other neurotoxicants represents a potential increased risk of neurotoxic effects. Albeit of common occurrence, studies that factor out individual components' effects are scarce.

Thus children immunized with TCVs are routinely exposed to a defined chemical mixture of adjuvant-Al and Thimerosal–EtHg in a binary [79] and highly correlated Al:Hg ratio [16]. Exposure to both forms of Hg (MeHg and EtHg) can occur simultaneously in populations with habitually high fish-consumption and taking TCVs [80]. These organic Hg (EtHg and MeHg) forms are equally toxic to the central nervous system with the most sensitive outcomes [1]. There are already several studies that provide information on concurrent exposure to multiple neurotoxicants (that include TCV–EtHg) during early life (pregnancy and infancy) and interaction with neurorobehavioral tests. These studies are summarized in Table 4, indicating a potential risk of neurobehavioral delays depending on attenuating or aggravating co-measured variables.

When the main source of the combined Hg exposure is acute intramuscular injection with vaccine-EtHg and fish-MeHg (chronic enteral) co-exposure, results show deviation from additivity for neurobehavioral test outcomes. Acute exposure (TCV–EtHg) does not equal chronic exposure (in maternal milk), and fish-MeHg ends up dominating the Hg levels in mothers' and infants' hair [81]. The estimates of total hair-Hg levels in infants reflect maternal fish-MeHg. Indeed, it is challenging to factor out adverse effects (of Hg) and benefits of certain practices (breastfeeding) or lifestyle (fish consumption) when dealing with biomarkers such as blood and hair-Hg. In some studies, eating fish showed that biomarkers (blood and/or hair-Hg) were positively associated with neuro-behavioral tests [40, 82].

In communities with high fish intake, changing fish consumption habits (hair-Hg) did not seem to influence the response to neurodevelopmental tests among children that had received TCVs [83]. However, principal component analysis (PCA) showed variability associated with pre- and post-natal Hg exposure; the first component represented explained 57% of variance related to birth weight and TCV use [84]. In studies where additional TCV–EtHg was compared during pregnancy [85] or at perinatal time [86], there were no significant differences. Comparing hospital versus home delivery resulted in no significant differences in neurobehavioral tests [87]; in hospital-delivered babies, besides a higher exposure to TCV, there was additional stress related to hospitalization and to a high rate of cesareans [87]. Furthermore, when children with extended lactation (>6 months) were compared to children breastfed for 6 months there were no significant interactions (positive or negative) of TCVs with neurobehavioral tests [88]. Overall it seems that when only the cumulative occurrence of organic Hg forms (TCV–EtHg and fish-MeHg) was considered, EtHg was not sufficient to influence neurobehavioral tests.

Table 4 Studies of co-exposures of TCV–Hg with other neurotoxicants and neurodevelopment tests in children (adapted and expanded from Dórea 2017)

References (country)	Comparisons	EtHg and co-occurring neurotoxicant	Neuro-behavior tests (ages)	Outcomes
Heron and Golding 2004 [40] (UK)	Exposure to high and low combined organic Hg forms	Maternal fish consumption (MeHg) during gestation	Denver scale (6, 18, 30 m)	Both children Thimerosal and fish consumption provided beneficial effects
Marques et al. 2007 [86] (Brazil)	Time of HBV shots: <24 h x >24 h after birth	Maternal fish consumption (hair-Hg) during gestation	GDS (6 m)	Infants immunized 2–4 days after hospital discharge showed no significant difference in neurodevelopment compared to the group immunized within 24 h
Marques et al. 2008 [84] (Brazil)	PCA screening of variables linked to pre- and post-natal Hg exposure	Exclusive breast-feeding for 6 m and maternal fish consumption	GDS (6 m)	Principal component analysis discriminated variability of neurodevelopment outcomes associated with variables that included pre- and post-natal Hg exposure; the first component represented birth weight and vaccine TCV vaccines variability and explained 57% of variance
Marques et al. 2009 [89] (Brazil)	Pre- and post-natal Hg exposure	Breast-milk Hg intake, and maternal high fish consumption (hair-Hg)	GDS (6, 36, 60 m)	Infant hair-Hg (at birth and 6 m) and length of lactation (beyond 6 m) were each significantly correlated with GDS, but in opposite ways: length of lactation was positive correlated with GDS at 60 m; hair-Hg concentrations were negative correlated with GDS at 6 m ($r=0.333$; $p=0.002$) and 60 m ($r=0.803$; $p=0.010$), but not at 36 m)
Marques et al. 2010 [92] (Brazil)	Maternal TCV (Td) during pregnancy	Maternal fish-MeHg (hair-Hg) breast-feeding 6 m	GDS (6 m)	Infants exposed to maternal TCV had no significant difference in GDS at 6 m
Marques et al. 2010 [83] (Brazil)	Changes in traditional lifestyle involving family fish consumption	Chidren fish consumption (hair-Hg)	GDS (0–5 y)	No significant association with total TCV–EtHg exposure at time of test
Mrozek-Budzyn et al. 2012 [90] (Poland)	Neonatal TCV exposure	GLM adjustments: total-Hg and Pb in cord blood	BSID-II (12 and 24 m)	TCV exposure adversely affected PDI only in the 12 and 24 m tests. The overall deficit in the PDI was significantly higher in TCV group ($\beta=-4.42$, $p=0.001$); The MDI scores were not significantly affected. clinically assessed developmental delay (PDI and MDI <85)
Lee and Ha 2012 [95] (S. Korea)	Differences in TCV history	Second hand smoke exposure during pregnancy	BSID-II (6 m)	When vaccines were considered as a covariate, the effect size increased slightly and the significance level remained marginal

Table 4 (continued)

References (country)	Comparisons	EtHg and co-occurring neurotoxicant	Neuro-behavior tests (ages)	Outcomes
Marques et al. 2012 [93] (Brazil)	Children with vaccination history	Fish-MeHg (hair-Hg) and unspecified metals from tin-mining pollution	GDS (1–59 m)	The multivariate model showed significant variables affecting the GDS: breastfeeding, HHg, maternal education, and children's age; TCVs in the multivariate model did not show any significant association with neurodevelopment
Dórea et al. 2012 [91] (Brazil)	Children TCV from urban, fishing and mining areas	Fish-MeHg (hair-Hg) and unspecified tin-mining pollution	GDS (6 m)	Differences in vaccine coverage among (EtHg) was significantly higher ($p = 0.0001$) in urban infants (150 µg) than in infants from either village (41.67 µg, Itapuã; 42.39 µg, Bom Futuro). A higher score of neurodevelopment was negatively associated with exposure to additional TCV–EtHg
Marques et al. 2014 [16] (Brazil)	Children with full vaccination schedule and exposure to neurotoxicants during the perinatal period	Pb, MeHg, TCVs (EtHg, and Al)	BSID-II (6, 24 m)	MDI and PDI were statistically significant lower only at 24 m of age for children living in a multi-exposure environment that included higher EtHg exposure. Multivariate regression analysis showed that MDI was negatively affected by breast-milk Pb and by HHg. PDI was positively affected by breastfeeding and negatively affected by EtHg
Dórea et al. 2014 [92] (Brazil)	Toddlers with vaccination history in different rural environments	Fish-MeHg (hair-Hg) and unspecified tin-mining pollution	GDS (12–24 m)	Fishing village toddlers showed significantly higher exposure to both fish-MeHg and TCV–EtHg; significant differences were seen only in the proportions of most severely affected toddlers (GDS <70) in the more exposed group
Marques et al. 2015 [94] (Brazil)	Children with vaccination history in rural mining area	Maternal fish-MeHg (hair-Hg) during pregnancy and breastfeeding and tin-mining pollution	BSID-II (6, 24 m)	No significant association of BSID with total TCV–EtHg exposure. There was a significant sex difference with boys showing more sensitivity related to BSID delays
Mrozek-Budzyn et al. 2015 [82] (Poland)	Early exposure to TCV–EtHg (HBV, DTP)	Shorter and longer periods after TCV (first 9 y); adjust confounders: cord blood-Hg and blood-Hg at the 5 y of life	Fagan (6 m); BSID-II (12, 24, 36 m); WISC (6, 7, 9 y)	Developmental test results in children exposed to TCVs up to the 6 m did not depend on thimerosal dose. However, BSID-II at 36 m and WISC-R at 9 year were significantly higher for those exposed to TCVs

Table 4 (continued)

References (country)	Comparisons	EtHg and co-occurring neurotoxicant	Neuro-behavior tests (ages)	Outcomes
Marques et al. 2016 [88] (Brazil)	Toddlers with TCV history and breast-feeding (6 m and up to 2 y)	Maternal exposure to fish-MeHg (hair-Hg) and infant TCV–EtHg	BSID-II (6, 24 m)	Fish-MeHg exposure (combined with TCV-EtHg) did not show a significant dose effect (related to length of breastfeeding) on neurodevelopment among groups
Marques et al. 2016 [80] (Brazil)	Children exposed to high and low combined organic Hg forms	Perinatal exposure to organic-Hg forms: maternal fish-MeHg (hair-Hg) and EtHg from maternal and infant TCVs	BSID-II (6, 24 m)	There was a statistically significant increase in BSID delay related to the combined exposure to Hg (MeHg > EtHg). Neurodevelopment delays due to low-doses of organic Hg are undiscernible and unpredictable
Marques et al. 2016 [87] (Brazil)	Home versus hospital delivery and perinatal organic-Hg exposures	Maternal fish consumption (hair-Hg) and infant's TCV–EtHg taken at delivery	BSID-II (24 m); Stanford-Binet IQ test (60 m)	In spite of the differences in HHg and EtHg levels between hospital-born and home-born children, the percentage of hospital-born children with BSID (MDI or PDI) scores <80 was not significantly different from those born at home

BSID-II The Bayley Scales of Infant Development, second edition, *EtHg* ethylmercury, *GDS* Gesell Development Scores, *GLM* general linear model, *HBV* hepatitis B vaccine, *IQ* intelligence quotient, *LM* limited effect, *MDI* mental development index, *MeHg* methylmercury, *m* months, *NS* not significant, *PDI* Psychomotor Development Index, *y* years, *TCV* Thimerosal containing vaccines, *WISC-R* Wechsler Intelligence Scale for Children-Revised, *Td* tetanus-diphtheria vaccine

Negative effects associated with TCV–EtHg (and MeHg) were observed in Amazonian rural and urban children with a family background of habitual fish consumption. In urban children, hair-Hg was negatively correlated with neurodevelopment (GDS) at 6 and 60 months but not at 36 months [89]. In mostly rural communities with high fish consumption, neurodevelopmental delays increased with increased total Hg exposure (from both fish-MeHg and TCV–EtHg) but were undiscernible [80].

In most studies of combined exposures (that included TCV–EtHg) with other pollutants, there were significant negative associations with neurobehavioral tests. There is an increased risk of untoward neurologic events resulting from the co-occurring exposures of TCV–EtHg with Pb [90], or with a combination of Pb, MeHg, and Al [16], fish-MeHg and mining pollution [91–94], or environmental smoke [95].

The toxicity of the binary (adjuvant-Al and EtHg) mixture, containing two well-documented neurotoxicants, showed a significant association with neurodevelopmental delays in some studies in the presence of other toxic metals, but not in others. The modifying factors driving the heterogeneity of the responses and risk of environmental interactions remain elusive. Bellinger [96] has pointed out that when test scores are associated with clinical diagnostic results, the former (continuous variables) are statistically more powerful than the latter as a measure of neurological outcome.

Section Summary

1. There are unpredictable adverse (immunological and neurological) effects linked to TCV–EtHg exposure. Given the consistency of occurrence of adverse effects associated with pediatric TCVs, it is reasonable to anticipate that Thimerosal-free vaccines will abate Hg burden in the very young and decrease the risk of neurological adverse effects.
2. In studies that explored vaccines and risk of tics, Thimerosal was a necessary factor. However when only the cumulative occurrence of organic Hg forms (TCV–EtHg and fish-MeHg) was considered, EtHg was not sufficient to influence neurobehavioral tests.
3. In early developmental stages, cumulative (or additive) co-exposure of EtHg with other neurotoxic agents (or socioeconomic-related adversities) can be associated with diminished neurobehavioral test scores while clinical diagnosis can be related to TCVs. Despite that, children in less developed countries continue to receive TCVs.
4. The lack of proper evaluation of TCVs and/or EtHg exposures remains a source of concern for young children in less developed countries, thus justifying ethical (and equitable) policies regarding Thimerosal-free vaccines for all children.

Concluding Remarks

- End points such as tics and dermatitis are disproportionately more common in children immunized with TCVs.
- The use of TCVs during pregnancy and early life carries an unnecessary risk of both, immunologic and neurologic effects.
- Policies to protect young children from exposure to TCV–EtHg have been implemented in developed countries and shown benefits related to contact dermatitis.
- Health authorities should recognize the need for equal policies (to reach all children) regarding Thimerosal-free vaccines.
- The use of pediatric-TCVs in less developed countries is ethically unjustifiable and questionable to ignore scientific evidence that demonstrate benefits of withdrawing Thimerosal from pediatric vaccines.
- Thimerosal-free vaccines (for pregnant mothers and the very young) should be part of the international compliance to abate Hg exposure.

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Compliance with Ethical Standards

Competing interest The authors declare that they have no competing interests.

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