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Review Article

A review on thimerosal: an irreplaceable element of long-term immunisation strategy in low income countries

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ABSTRACT

Thimerosal, an organic-mercury (Hg) compound containing 49.55% Hg by weight, is added to vaccines as a preservative permitting formulation of multi-dose vaccine vials. Being a derivative of ethylmercury, it has been linked with autism as a possible risk factor based on the assumption that exposure to ethylmercury would have similar neurotoxic effects as another mercurial compound, methylmercury. In 1999, AAP issued a joint statement emphasising the removal of thimerosal from vaccines. Subsequently, several studies have been conducted; those showing positive association between thimerosal exposure and autism have been recognised to be fraught with methodological flaws. On the other hand, many well controlled studies have failed to find any such causal relation and there are others that have clearly demonstrated a much favourable kinetic profile of ethylmercury as compared to methylmercury. Owing to the lack of data, AAP retired its original statement in 2002. Recently, thimerosal has been exempted from regulation by Minamata Convention on Mercury resulting in the continued use of low cost thimerosal containing vaccines in low income countries which cannot afford to run their immunisation program using single dose thimerosal free vaccines, that comparatively cost much higher, as is the case in high income countries. Some bodies view this as a discrimination on the basis of wealth of a nation and have opposed this decision. This review presents various studies regarding the causal association between thimerosal containing vaccines and autism. The current evidence fails to support any such association. Hence this review supports the exemption of thimerosal from regulation and also justifies its use in LICs for uninterrupted vaccination of the most vulnerable population.

Keywords: Ethylmercury, Low income countries, Methylmercury, Thimerosal, Thimerosal containing vaccines, Thimerosal free vaccines

INTRODUCTION

One of the most efficient methods to halt the occurrence of infectious diseases and deaths globally is vaccination. Universal immunization programmes for children have achieved significant reductions in common childhood illnesses by building herd immunity against most of the infectious agents.[1] Immunization is not without risks so it is essential that safety concerns receive utmost attention.[2]

Repeated puncture of multidose vials can lead to accidental contamination of vaccines leading to microbial growth. Preservatives i.e. the compounds that prevent the growth of or kill various bacterial and fungal microorganisms, can prevent this deleterious event if added to the multidose formulations.[3] The use of preservatives in multidose preparations was further stressed upon by a few tragic incidences in the early 20th century following the use of multidose vials that did not contain a preservative. One such incident occurred in Bundaberg, Australia in 1928 when 21 children received an injection from a multidose formulation of diphtheria toxin-antitoxin mixture which had been formulated without a preservative. Multiple puncturing had led to staphylococcal contamination of the vial. Of these 21 children, 12 died. Following this incident, it was recommended that biological products meant for repeated

use should include preservatives to inhibit bacterial growth.[4]

The United States Code of Federal Regulations (CFR) finally incorporated the addition of preservatives in multidose vaccines in January 1968, although, prior to this, many biological products had contained preservatives. Various preservatives being used in the US licensed vaccines include Thimerosal (in Influenza multidose formulations); Benzethonium chloride/ Phemerol (Anthrax vaccine named Biothrax by Emergent BioDefense Operations Lansing Inc.), Phenol (in Typhoid Vi Polysaccharide vaccine named Typhim Vi by Sanofi Pasteur, SA; Pneumococcal Polysaccharide vaccine named Pneumovax 23 by Merck and Co, Inc.); and 2-phenoxyethanol (IPV named IPOL by Sanofi Pasteur, SA). The Food and Drug Administration (FDA) licenses the product containing a preservative and not that particular preservative.[3]

FDA also recommends the addition of preservatives in multidose vaccine vial with the exception of certain live viral vaccines, so as to prevent their bacterial and fungal contamination on repeated puncture.[4]

In contrast, products formulated in single-dose vials do not require preservatives. However, some physicians and health clinics prefer multidose vials as these are less expensive and require less storage space along the cold chain. But withdrawal of vaccine from these preparations should be done by a highly aspetic technique since preservatives alone cannot be relied upon for the elimination of the risk of contamination.[4]

Thimerosal, also known as merthiolate, thiomersal, timerosal and tiomersal is one such preservative and is chemically a ethylmercurithiosalicylate.[5] It is an organic-mercury (Hg) compound containing 49.55% Hg by weight that is added to vaccines as a preservative, at concentrations from 0.005% to 0.01% (i.e. 12.5µg Hg or 25µg Hg per 0.5mL vaccine dose). It rapidly dissociates in saline solutions into ethylmercury chloride, ethylmercury hydroxide, and sodium thiosalicylate.[6] It was introduced in 1930's and since then, several studies have been conducted on it.[3]

At concentrations of 0.001% (1 part in 100,000) to 0.01% (1 part in 10,000), it has been shown to exhibit broad spectrum antimicrobial activity. The presence of 0.01% thimerosal in a vaccine means 25µg of Hg or 50µg of thimerosal per 0.5mL dose of vaccine.[3]

Not only vaccines, its properties have also been utilised in other preparations like immune globulin preparations, ophthalmic and nasal products, antivenins and skin test antigens. Also, no major adverse effect has been reported so far that can be directly linked to the use of thiomerosal, but only minor local injection site hypersensitivity reactions with local swelling and redness.[3]

THIMEROSAL AND AUTISM: A TALE FROM THIMEROSAL CONTAINING VACCINES TO THIMEROSAL FREE VACCINES

Thimerosal has been linked with autism as a possible risk factor. Autism is a developmental disorder marked by impaired communication skills, deficient social interaction, and restricted or repetitive patterns of behaviors. It is classified under the broad category of "pervasive developmental disorders" (PDDs) also including Rett's syndrome, childhood disintegrative disorder, Asperger's syndrome and PDD not otherwise specified.[2] Possible environmental triggers for autism include lack of breastfeeding, lack of supplementation of arachidonic acid and docosahexaenoic acid with use of infant formula, vaccinations in childhood, analgesic use like acetaminophen and others, viral infections, and others. A compound also derived mainly from environmental sources particularly dental amalgam and pollution from coal burning power plants, i.e. mercury has been determined as a neurotoxic compound. It has been explored as a potential causal factor of autism in children who are additionally exposed to mercury from thimerosal used as a preservative in vaccines.[7]

The neurotoxic properties of mercury have been mainly attributed to an organic mercurial compound, methylmercury, which has been determined to produce neurologic and renal damage on cumulative exposure. It has a long half life. It accumulates in the brain by crossing the blood brain barrier and gets converted to inorganic mercury. Whereas a related organic mercury compound, ethylmercury, is a constituent of thimerosal. It gets rapidly excreted via stools and has a half life much shorter than methylmercury, reaching to baseline value within 30 days of administration. Its effect on a child's neurodevelopment and health has not been determined yet.[8]

In the late 1980s and 1990s, the Centers for Disease Control and Prevention (CDC) gradually expanded the routine childhood immunization schedule thus increasing the number of doses of Thimerosal-containing vaccines (TCVs) to be administered to the infants i.e. three doses of Thimerosal-containing hepatitis B vaccine, and four doses of Thimerosal-containing Haemophilus Influenzae type b (Hib) vaccine in addition to the routinely administered five doses of Diptheria-Tetanus-whole-cell-Pertussis (DTP) vaccine containing thimerosal. Some infants were additionally administered three doses of Thimerosal-containing influenza vaccine. With this immunisation schedule, the total mercury exposure was estimated to be as high as 200µg of mercury during the first six months of life.[9] These estimates suggested that the infants could have received a total dose of ethylmercury (through thimerosal) in excess of the exposure limit for methylmercury set by the Environmental Protection Agency (EPA).[10] That means the data on methylmercury had been used to determine the safety limits for exposure to thimerosal i.e. a limit of 0.1µg/kg/day as set by EPA and 0.47µg/kg/day by World Health Organisation (WHO).[11]

Early 1990s also witnessed an increased awareness of the harmful effects of even a low dose of mercury, which along with these theoretical estimates of exposure to another compound of mercury, i.e. ethylmercury, prompted FDA to conduct a risk assessment of thimerosal use in vaccines. On the basis of the risk assessment from the FDA, the American Academy of Pediatrics (AAP) and the United States Public Health Service (USPHS), on July 7, 1999, issued a joint statement emphasising the removal of thimerosal from vaccines.[12] It was a precautionary measure based on the assumption that exposure to ethylmercury would have a similar effect on children's neurodevelopment as the exposure to methylmercury. Although the effect of intermittent exposure to low dose of ethylmercury had not been assessed sufficiently, it was assumed to have harmful consequences.[8]

Though it was not clear whether the guidelines established for cumulative exposure to methylmercury by a number of in vivo and in vitro studies, were similarly applicable to intermittent exposure to low dose of ethylmercury, their risk profile was considered equivalent by FDA owing to the lack of definite data comparing the toxicity profile of the two compounds, in spite of them being two different chemical entities.[3,13] Hence, the initial risk assessments for ethylmercury were actually based on the studies of toxicity profile of oral methylmercury but subsequent studies have shown that the two differ substantially in the tissue disposition and metabolism kinetics.[14] In accordance, much progress has been made in formulating vaccines containing trace or no thimerosal, till date. FDA licensed two new pediatric formulations of hepatitis B vaccines: RecombivaxHB (Merck, thimerosal free) in August 1999 and EngerixB (Glaxo SmithKline, thimerosal free) in January 2007. The FDA also approved a second Diptheria-Tetanus- acellular-Pertussis (DTaP) vaccine formulated without thimerosal (Aventis Pasteur's Tripedia, trace thimerosal) in March 2001 and also approved Aventis Pasteur, Ltd to manufacture a thimerosal free DTaP vaccine, Daptacel, in 2002.[3]

Chiron/Evans was approved in September 2001 for manufacturing a formulation of their influenza vaccine, Fluvirin, containing trace thimerosal. Similarly, Aventis Pasteur, Inc was approved in September 2002 approved to manufacture a preservative free formulation of their influenza vaccine, Fluzone, containing trace thimerosal which was later approved in December 2004. Td vaccines, Aventis Pasteur Inc's Decavac, Aventis Pasteur Inc's DT vaccine and Aventis Pasteur, Ltd's Td vaccine, are also available in preservative free formulations.[3]

Measles, mumps, and rubella (MMR), Varicella (chickenpox), inactivated polio (IPV), and pneumococcal conjugate vaccines have never contained thimerosal and Influenza (flu) vaccines are currently available in both thimerosal-free and thimerosal-containing formulations.[15]

From 1996, infants were immunised with a thimerosal free pentavaccine combining the polio vaccine and the Hib vaccine with DTP, at 2,4, 6 and 18 months of age, with a booster of polio, pertussis (cellular), tetanus booster (thimerosal-free) at 4 to 6 years. The cellular pertussis vaccine was replaced by the acellular vaccine in the combined vaccine in 1998. Hence, children were exposed to almost a nil cumulative dose of ethylmercury from 1996 onwards.[16] Later other vaccines were approved in a Thimerosal free formulation as mentioned earlier. With these advances, all vaccines for use in children as per the recommended immunization schedule are now available free of thimerosal as a preservative and an infant can now be exposed to a maximum amount of mercury of <3µg by the age of 6 months down from 187.5µg in 1999 from vaccines on the recommended childhood immunization schedule.[2] Inactivated influenza vaccine is available in both formulations, thimerosal containing and the one with either no thimerosal or only a trace of it (≤1 than mcg mercury per dose). But the formulation of preservative free preparations has been made possible by manufacturing single dose vials of vaccines rather than multi-dose vials.[3]

Since formulation of multi-dose vials costs much cheaper than single dose vials, TCVs are still being used outside the US in low income countries (LICs). The high-income countries (HICs) are able to maintain the demand supply ratio with single dose vaccines which is not the case with LICs where the advantage of using multi-dose vaccines for immunisation of all the children takes precedence over perceived hazards to mercury exposure.[14]

In Oct 2013, the Minamata Convention on Mercury formulated by the United Nations Environmental Programme (UNEP) exempted thimerosal from regulations applied to mercury.[5] Thimerosal hence, continues to be a part of vaccine supply in few parts of the globe leading to an uneven distribution of TCVs. It continues to be administered to children in LICs but not in HICs, a practice previously followed due to lack of awareness and economic issues but as a part of a global policy after the Minamata convention in 2013. This has been claimed to be unethical by some, that the future of the growing children be decided by the wealth of the nation.

LITERATURE REVIEW

Several studies, both in vivo and in vitro, have been conducted till date, of which many found a positive association between thimerosal and neurodevelopmental disorders (NDs) (Table 1 and Table 2) but many others could not found any such association (Table 3 and Table 4). The association between thimerosal and autism still remains controversial.

DISCUSSION

Despite the use of thimerosal free vaccines, autism prevalence has been on a continuous high and has seen no decline. Fombonne et al, surveyed a birth cohort of 27749 children born between 1987 to 1998 and identified children with PDD and its link with cumulative thimerosal

exposure by age of 2 years. The author reported a statistically significant linear increase in the prevalence of PDDs during the study period in spite of the differing ethylmercury exposure among the children. Also, prevalence was seen to be significantly higher in thimerosal free birth cohorts than that in thimerosal-exposed cohorts suggesting no causal relation between thimerosal exposure and increasing trend in PDDs.[16]

Table 1: In-vivo studies showing positive association of TCV with NDs.

Study	Sample Size	Findings	Conducted by
Ecological study	DTaP vaccines analysed from 1992 to 2000	Thimerosal-containing DTaP vaccine (T-DTaP) is associated with increased incidence of NDs than thimerosal-free DTaP vaccine.	Geier MR et al[17]
Case-control study	221 cases and 18 controls	TCV vaccinated children with autistic spectrum disorders (ASD) have increased urinary mercury concentrations than did vaccinated controls but it was similar in both matched vaccinated and unvaccinated controls.	Bradstreet J et al[18]
Ecological study	-	The increases in prevalence of NDs correlates linearly with increasing exposure to mercury from TCVs.	Geier DA[19]
Ecological study	-	Significantly increased odds ratios for NDs following T-DTaP in comparison to thimerosal-free DTaP vaccines.	Geier DA[20]
Two- phased ecological study	-	Phase 1- increased risks of NDs following T-DTaP than thimerosal-free DTaP vaccines. Phase 2 - cumulative exposures to thimerosal is significantly associated with NDs	Geier DA[21]
Meta-analysis	-	Significantly increased risk of NDs were associated with TCV exposure.	Geier DA et al[9]
Case control examination of database	-	Significantly increased odds ratios for NDs following T-DTaP in comparison to thimerosal-free DTaP vaccines.	Geier DA et al[22]
Two- phased ecological study	-	Newly diagnosed NDs prevalence increased in 1994-mid 2002 and decreased from mid 2002 to 2005. i.e. the trends correspond directly to the expansion and subsequent reduction of cumulative mercury exposure from TCVs	Geier DA et al[23]
Case series	9 children with NDs diagnosed from 2005-2006	8 of 9 patients with ASD had increased exposure to mercury during their in utero or childhood developmental periods, from Thimerosal-containing biologics/vaccines	Geier DA et al[24]
Cohort study	1824 children aged 1-9 years	Boys vaccinated with Thimerosal-containing Hepatitis B vaccine were seen to be more susceptible than unvaccinated boys to developmental disability.	Gallagher C et al[25]
Cohort study	Children vaccinated between 1995-2001	The higher the proportion of children receiving recommended vaccinations, the higher was the prevalence of AUT or SLI.	DeLong G[26]
Cohort study	33, 166 and 82 infants from three distinct sociocultural communities	A higher score of neurological development at 6 months in kids born to mothers exposed to different levels of fish-Methylmercury, was negatively associated with exposure to additional TCV-Ethylmercury.	Dorea JG et al[27]
Cohort study	196 infants born between January 2001 and March 2003	The deficit in the psychomotor development index (PDI) was significantly higher in TCV group	Budzyn DM et al[28]
Two- phased study (I^{st} phase: cohort; II^{nd} phase: case control study)	-	Phase 1- significantly increased incidence of ASD following the Thimerosal-containing DTaP vaccine than Thimerosal-free DTaP vaccine; Phase 2 - cases with an ASD tend to receive increased Hg from thimerosal containing hepatitis B vaccine.	Geier DA et al[6]
Case control study	-	ND cases were significantly more likely than controls to receive increased organic-Hg exposure	Geier DA et al[29]

Table 2: Pre-clinical (in-vitro and in-vivo) studies showing positive association of TCV with NDs.

Study	Findings	Conducted by
In vitro study on human neurons and fibroblasts	Thimerosal induced DNA and membrane damage and activated caspase-3–dependent apoptosis at micromolar concentrations in human neurons and fibroblasts	Baskin DS et al[30]
Experimental, prospective, bioetapic in-vivo study on 45 postnatal hamsters	The exposure to thimerosal in doses equivalent to those through vaccines was associated with smaller stature, low encephalon weight and low corporal weight in postnatal hamsters.	Laurente et al[31]
In-vitro study conducted on blood lymphocytes from 3 healthy, non-smoking males, aged 20, 21 and 23 years	Thimerosal has genotoxic (increase in sister chromatid exchanges frequency) and cytotoxic effect (decrease in mitotic index and proliferation ındex) in cultured human peripheral blood lymphocytes	Eke D et al[32]
In-vivo study on the cerebellum and cerebrum of mice	Thimerosal injection induced metallothionein (MT) messenger RNA (mRNA) and protein expression in cerebellum and cerebrum showing the association of TCVs with autism.	Minami T et al[33]
In-vitro study on human neuronal and fetal cells	Thimerosal induced significant cellular toxicity similar to that observed in studies on autism at low nanomolar (nM) concentrations in in-vitro human neuronal and fetal cells.	Geier DA et al[34]
In-vivo study on rat brain	Thimerosal exposure during embryonic development produces impairment of brain monoaminergic system i.e. increase in hippocampal serotonin and dopamine in rat brain	Ida-Eto M et al[35]

In its 26th meeting in Geneva, Switzerland, on 6-7 June 2012, the Global Advisory Committee on Vaccine Safety (GACVS) reviewed all the recently published studies and confirmed that the half life of ethylmercury in blood is 3-7 days. It also reviewed the quantitative risk assessment model used by FDA for cumulative toxicity of thimerosal in humans and concluded that the blood and brain ethyl mercury levels attained from cumulative vaccine doses do not reach toxic levels, as suggested by various animal and human studies, thus negating any relation between thimerosal exposure through vaccines and neurological toxicity. It also identified methodological flaws in the three ecological studies suggesting such positive association.[54]

The Immunisation Safety Review Committee (ISRC), in its eighth and final report in 2005 concluded after reviewing all published and unpublished epidemiological studies, that these evidences do not support any causal relation between thimerosal containing vaccines and autism and the ones supporting the association did not follow a proper methodology. Also, it reported that the potential biological mechanism underlying the occurrence of vaccine induced autism that have been put forward till date are theoretical only.[2] A review of the risk assessment by FDA by Ball LK et al in 2001 also revealed no evidence of any serious adverse effect by thimerosal exposure through vaccines, except for minor local hypersensitivity reactions.[4]

The existing safety guidelines for methylmercury were applied to ethylmercury as a precautionary measure in 1999 due to the lack of data available for kinetic profile of ethylmercury. But subsequent in vivo studies by Pichichero ME et al, and Barregard L et al, and in vitro study by Burbacher et al, have proved a favourable kinetic profile of ethylmercury i.e. shorter half life, rapid excretion in stools and no accumulation in the brain.[36,49,52] These clearly indicate that methylmercury is not suitable as a reference compound for ethylmercury and the applicability of similar guidelines to both is unjustified. Nevertheless, methylmercury safety guidelines still remain translated to ethylmercury.

Most of the studies concluding a causal relation between exposure to thimerosal and neurological outcomes have been conducted by one author either alone or in association with other authors.[6,9,17-24,29] Most of the studies by Geier and Geier have used Vaccine Adverse Events Reporting System (VAERS) as the source of data.[6,9,17,19,20-23] VAERS is established as a surveillance tool to evaluate vaccine safety. Though it is simple to use and flexible in design, its usage is limited by various potential limitations like underreporting, erroneous reporting, multiple exposures and outcomes.[23] Anyone can report to VAERS since it is a passive reporting system. Also, reporting patterns at VAERS are highly influenced by the publicity of the adverse events. Goodman and Nordin, in 2006, showed that many of the adverse events due to thimerosal reported at VAERS were related to litigation for vaccine injury. They reported VAERS as a possible source of bias in longitudinal studies.[55] Hence VAERS is not suitable for this purpose. This makes the conclusions of these studies unreliable, invalid and non-interpretable. Also, these studies are fraught with many methodological flaws which were also recognised by GACVS and ISRC as mentioned above and also pointed by Parker SK et al, and were considered non-contributory in the analyses of the association between thimerosal and autism.[2,41,54]

Table 3: In-vivo studies showing no causal association of TCV with NDs.

Study	Sample Size	Findings	Conducted by
Descriptive	-	Administration of TCVs does not raise mercury blood levels above safety limits in infants. Ethylmercury gets eliminated from blood rapidly in the stools after administration of TCVs (half-life of 7 days).	Pichichero ME et al[36]
Population-based cohort study	467450 children born between 1990 and 1996 in Denmark	Children vaccinated with TCV had similar risk of ASD as those vaccinated with thimerosal-free vaccine.	Hviid A et al[37]
Ecological study	956 children 2 - 10 years old diagnosed with autism between 1971-2000 in Denmark	The incidence of autism did not increase till 1990 when thimerosal was used but the incidence increased from 1991-2000 and continued to rise when thimerosal was removed from vaccines.	Madsen KM et al[38]
2-phased retrospective cohort study	Phase I- 124170 infants born between 1992-99; Phase II- 16717 children born between 1991-97	TCVs and NDs show no significant associations consistently with conflicting results at different health maintenance organizations (HMO).	Verstraeten T et al[39]
Ecological study	-	Increased exposure to TCVs is not causally related to the increased prevalence of autism in children.	Stehr-Green P et al[40]
Systematic review	-	Studies reviewed do not establish an association between TCVs and ASD, and those that do have methodological flaws rendering their results invalid. Also, the pharmacokinetics of ethylmercury significantly differs from that of methylmercury.	Parker SK et al[41]
Population–based cohort study	>14000 children born between 1991-92 in UK	No evidence suggestive of any harmful effect of early exposure to thimerosal on neurologic or psychological development could be found.	Heron J et al[11]
Retrospective cohort study	109863 children born from 1988 to 1997	There was no evidence of any association of thimerosal exposure through DTP/DT vaccines with NDs, With the possible exception of tics.	Andrews N et al[42]
Literature review and interpretation	-	No scientific evidence suggestive of an etiologic role of TCVs or MMR in autism found, even in children with autism.	Taylor B[43]
Retrospective study	27749 children born from 1987 to 1998 in Montreal, Canada	Prevalence of PDD increased during the study period with higher rate in thimerosal free birth cohorts than in thimerosal exposed cohorts indicating that increasing trend in PDD was unrelated to thimerosal exposure.	Fombonne E et al[16]
Retrospective cohort study	1047 children between the ages of 7 and 10 years	No causal association was found between early exposure to mercury from TCVs and neuropsychological function deficits at the age of 7 to 10 years.	Thompson et al[44]
Cross-sectional study	214 mothers of children diagnosed with NDs between 1995 and 2005.	Analysis of complete records including the blood group status and RhIg exposure of 214 families showed that Rh status was not found to be higher in mothers of autistic children than in the general population and exposure to thimerosal containing antepartum RhIg, was also not higher for children with autism.	Miles JH et al[45]
Ecological study	Department of Developmental Services (DDS) data from Jan 1, 1995 to March 31, 2007	No recent decrease in autism was shown by DDS data in California despite the removal of thimerosal from almost all childhood vaccines.	Schechter R et al[46]
Case-control study	256 children with ASD and 752 controls	Increased risk of ASDs was not related to in utero or early-life exposure to TCVs and immunoglobulin preparations.	Price CS et al[47]
Retrospective cohort study	96 cases diagnosed with autism and 192 controls in Poland	Odds ratios for the risk of autism in infants vaccinated with TCVs were 1.52 for doses 12.5-87.5 µg, 2.78 for 100-137.5 µg and 1.97 for ≥150 µg suggesting no association between TCVs and autism.	Budzyn DM et al[48]
Case-control study	15 patients receiving thimerosal containing *Staphylococcus* toxoid vaccine in a clinical trial	Ethylmercury from thimerosal does not get accumulated in blood in adults.	Barregard L et al[49]
Cohort study	1,047 children	No statistically significant association was found between early thimerosal exposure from vaccines and neuropsychological factors except tics in boys.	Barile JP et al[50]
Meta-analysis of published literature before April 2014	-	No association was found between thimerosal exposures and ASD but significant association was found between environmental exposures and ASD.	Yoshimasu K et al[51]
Prospective cohort study	318 children	No association was found between early TCV exposure and neurological development in each stage of life up to the 9yrs of age.	Budzyn DM et al[8]

Table 4: Pre-clinical (in-vitro and in-vivo) studies showing no causal association of TCV with NDs.

Study	Findings	Conducted by
In-vivo study on infant monkeys	The elimination half-life of ethylmercury after thimerosal exposure, in blood was much shorter than that of methymercury. Thimerosal exposed monkeys had much lower brain concentrations of total mercury than methylmercury infants, indicating different toxicokinetics of both compounds.	Burbacher et al[52]
In-vitro study on lymphocytes from thymic glands of young rats	Incubation with Thimerosal at 3-30microM caused increase in [Ca^{2+}] and depolarisation of membranes; induced apoptosis at 30microM; and cell death on prolonged incubation. But also concluded that this is unlikely to be seen in infants' lymphocytes since vaccination results in submicromolar blood concentration of thimerosal.	Ueha-Ishibashi T et al[53]

Studies by Geier and Geier, DeLong G and Gallagher C et al, have linked the increased prevalence of ASD or impaired neurological development in children to the cumulative exposure to thimerosal containing vaccines during routine immunisation.[6,9,17,19-22,25,26,29] Most of these studies were conducted in children immunised in the 1990s when the vaccines were being administered according to the expanded immunisation schedule. But the prevalence of NDs has continued to increase (Fombonne et al, Madsen KM et al,) even with the use of thimerosal free vaccines after the joint statement regarding thimerosal use in 1999, as must have been expected according to the results of these studies.[16,38] This observation diminishes the likelihood of any association of an increase in neurological abnormalities in children with exposure to thimerosal.

In-vitro studies on human neurons and fibroblasts, cultured peripheral lymphocytes and neuronal and fetal cells show the cytotoxic effect of thimerosal at low concentrations but do not show in any way that these effects form the underlying basis of expression of autistic features; the relevant mechanisms for which still remain unknown.[30,32,34]

The cohort studies by Budzyn DM et al, Hviid A et al, Andrews N et al, Heron J et al, and Thompson et al, do not support any causal relation between thimerosal and autism.[8,11,37,42,44] A cohort study by Verstraeten T et al, and Barile et al, also found no such significant association of thimerosal with autism but did show significant association of thimerosal containing vaccines to tics and language delay and recommended additional research into the conflicting results of their study.[39,50] A meta-analysis conducted by Yoshimasu K et al, did not show any significant association of thimerosal exposures to an increased risk of ASD or ADHD but reported significant association for environmental mercury exposures in both ASD and ADHD.[51] Most of the studies (Verstraeten T et al, Hviid A et al, had controlled for potential confounding variables such as sex, birth order, maternal factors like age, race, education, etc.[37,39] These studies were conducted in different set of populations utilising different methodology.

Verstraeten T et al, found a positive association between TCVs and tics and language delay but this finding was not consistently observed at all the three health maintenance organization (HMO) databases and also suggested further research with different designs in this area.[39]

Based on the epidemiological evidences, the AAP, in May 2002, retired its original joint statement with USPHS on use of thimerosal.[12,56]

The exemption of thimerosal by Minamata convention on mercury is considered unjustified by non governmental organisations (NGO) like Coalition for Mercury Free Drugs (CoMeD), arguing that it has led to a double standard in vaccine safety with the children in wealthier nations being exempted from thimerosal exposure (i.e. a reduced or no-thimerosal standard) whereas those in LICs are being exposed to thimerosal at a higher rate and frequency (i.e. a predominantly thimerosal containing standard) thus compromising their national development.[5] This argument is not correctly laid.

As mentioned above, thimerosal when added as a preservative to vaccines, allows them to be formulated in multi-dose vials which form a critical part of immunisation programmes in LICs that already share a huge burden of infectious diseases in the most vulnerable part of their population. Shifting from the multidose vaccines to single dose formulations will need extensive additional resources for time consuming and increased manufacturing, cold chain storage, transport, administration and increased infrastructure to handle the waste. This would put an additional huge economic burden on LICs, which if not met, will leave many of the children unimmunised and prone to a host of deadly and otherwise vaccine-preventable diseases. This poses a much greater threat to their national development than being intermittently exposed to a compound whose toxicity concerns still remain unproven and theoretical.

Moreover, thimerosal as a preservative in vaccines for preventing bacterial and fungal contamination of vaccines, remains unbeatable by other preservatives available in the market.[57] The vaccine stability, safety and efficacy could

potentially be altered if at all the multi-dose formulations are provided with other preservatives which would further require a time consuming and extensive testing of the vaccines. Hence, thimerosal still remains the preservative of choice in resource poor countries. It can also play a critical role in dealing with emergencies such as a pandemic of influenza, in HICs when there is a need to increase the vaccine manufacturing, supply and delivery rapidly.[1]

At present, nearly 84 million children are being immunised with TCVs annually.[57] Treating different people according to their needs and requirements is not unethical until and unless the practice itself has been proven to be associated with some avoidable harms. Thimerosal has a long record of safe use in vaccines with the recent toxicity concerns which still remain unfounded. Moreover, banning thimerosal will ultimately lead to an interruption in the supply of vaccines to the children in LICs, weakening the herd immunity developed so far by vaccination, disrupting the immunisation programme and thus reversing the achievements gained through the intensive immunisation, till date, in halting the spread of infectious diseases. Hence the continued use of thimerosal in vaccines in few subsets of population is not unjustified or unethical as claimed by few NGOs.

Such claims mainly come from CoMeD, which is a US based organisation run by Mark and David Geiers, the authors of most of the studies finding a positive association between TCVs and autism. The results of many of them have been refuted by the premier committees involved in evaluation of vaccine safety.[2,54] Moreover, banning thimerosal altogether from vaccines is not going to affect the routine immunisation of children in their country.

CONCLUSION

Thimerosal continues to be a safe and effective preservative for use in vaccines and permits the formulation of multi-dose vials which cost much cheaper than the single dose vials and do not put an additional economic burden especially on the developing nations. The toxicity concerns associated with thimerosal are only theoretical and are actually based on the increased awareness regarding the adverse effects of exposure to even a low dose of methylmercury, despite the fact that thimerosal is a derivative of ethylmercury only. Both the compounds have an entirely different kinetic profile and the guidelines of methylmercury should not be applicable to ethylmercury and hence, thimerosal. Moreover, the use of thimerosal allows the immunisation program to run at a much cheaper cost than with the use of thimerosal free single dose vaccines. This especially holds importance in LICs where the benefits of using thimerosal far outweigh the yet unsubstantiated risks associated with its use. Hence, the review supports the exemption of thimerosal from the UNEP treaty and justifies its continued use in LICs for smooth and uninterrupted running of their immunisation programme.

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