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REVIEWS

Thimerosal and Children's Neurodevelopmental Disorders

Luis Maya MD, Flora Luna MD.
Medicine Faculty of San Fernando.
Mayor National University of San Marcos.
Lima, Peru.

Abstract: The causal relationship between thimerosal (ethylmercury), preservative in pediatric vaccines, and the increase of children's neurodevelopmental disorders as a result of the extension in immunization schemes is evaluated. The scientific information was reviewed for relate thimerosal and evidences to allow an assessment of possible causal association among them; the evidence found in epidemiological, ecological, biomolecular, toxicology, biosafety, fetal toxicology and reproductive health studies signal the possible causal association of thimerosal exposure and neurodevelopmental disorders of the child. Such neurotoxicity occurs in infants and fetuses of vaccinated pregnant women due to cumulative doses of mercury. The various types of evidence imply thimerosal as the causal agent, aggravating or triggering neurodevelopmental disorders of the child. Mercury toxicity forced progressive thimerosal withdrawal. Unfortunately, in the vaccines there was a substantial delay in demonstrating the negative impact of thimerosal. Currently there exist vaccines without thimerosal, whose use is causing a lower incidence of children's neurodevelopmental disorders.

Key words: Thimerosal; autism; nervous system diseases; child development; vaccines.

INTRODUCTION

In 2004, the American Academy of Pediatrics (AAP) and the Department of Health and Human Services of the United States of North America (U.S.), launched an epidemic alert, impressed by the number, each time more alarming of cases of autism and other diffuse disorders of child development, indicating at that time 1 of every 6 North American children had a developmental or behavioural disorder and that 1 in every 166 children had an autistic spectrum disorder (ASD).

These entities currently form a heterogeneous group of illnesses, in which are included, besides autism, strictly speaking, Rett syndrome, Asperger syndrome, and generalized development disorder. ^(1,2)

Multiple studies have shown an extraordinary increase in cases of ASD since the middle of the 1980s. ⁽³⁻¹⁵⁾ In agreement was an investigation carried out by the U.S.

House of Representatives. In that country, the epidemic showed an annual growth rate of 10 to 17%, until the year 2003. ⁽¹⁶⁾

The statistical data of the U.S. Department of Education 2002 show that, at the end of the 1960s, only 919 new cases of autism had been reported in the U.S. territory. However, since the decade of the 1980s, the growth continued and adopted an exponential curve, such that by the beginning of the 1990s the number of new cases had risen to 6 785 and arrived at almost 100 000 in 2002, which represented an increase of 700% in scarcely 10 years. ⁽¹⁷⁾ In what is referred to as estimations of prevalence, during the 1940s, in that country, the recorded rate of autism was 1 in every 10 000 children. In 1977 it was 1 for every 2 500; in 1985, it increased to 1 in every 1 133; in 1998, it grew to 1 in every 323, and in 2004, it reached the shocking figure of 1 in every 166 children. ^(15, 18)

The above mentioned statistics changed ASD into the largest epidemic of mental diseases ever observed in the history of the U.S., meaning more cases than diseases such as Down syndrome, Diabetes Mellitus Type 1 and pediatric cancer. ⁽⁸⁾

Even though in our midst we do not yet have available statistical data that estimate the magnitude of this problem, various countries worldwide have found similar increases. In China, for example, where ASD was practically unknown, coincidental with the introduction of North American vaccine producing companies since the year 1999, it was reported that over 1,8 million children were recently diagnosed with autism; in addition, ASD has also significantly risen in India, Nicaragua, Argentina and in other developing countries during the latest years. ⁽¹⁹⁾

The fact of having seen such a striking increase in cases, as described, definitively ruled out the hypothesis that these illnesses were due exclusively to a genetic disorder, since such conditions take many years to develop and they never reach epidemic numbers, except ionization exposures and nuclear catastrophes.

Some investigators proposed that such a phenomenon could be due to changes and improvements in the diagnostic criteria. However, despite the fact that since 1994 the DSM IV (*Diagnostic and Statistical Manual 4th Ed.*) criteria began to be used in the U.S. and throughout the world, as a required source for diagnosis of mental diseases, the number of new cases identified as ASD continued growing significantly until the year 2001. ⁽⁶⁾ In addition, multiple studies have found that factors such as migratory phenomena or improvements in diagnostic tools do not explain the increase in cases and that the epidemic is genuine. ^(8, 11, 13, 15, 16, 20, 21)

ROLE OF VACCINES IN THE EPIDEMIC

Historically, until before the year 1980, at least two-thirds of autistic children showed serious neurological problems shortly after birth. Nevertheless, during the period from 1980-1985, while the incidence of ASD clearly rose throughout the world, a new form of autism simultaneously began to be described, so-called delayed or regression, in which the affected children showed notable changes in the loss of their social relationships, language and behaviour, between the first and second year of life. For 1985, the occurrence of regression autism had already equalled that of autism since

birth, suggesting that this change acquired from the disease went beyond those types due to genetic problems or innate metabolic defects. ⁽¹⁸⁾

Furthermore, a recent investigation shows from the evaluation of home videotapes, that the pathological changes in communication, sensitivity, social relationships, repetitive behaviour and forms of child games, clearly manifest themselves between 12 and 24 months of age in children who are subsequently diagnosed with ASD. ⁽²³⁾

In accordance with the studies of the Autism Research Institute (ARI), for the year 1997, autism of delayed or regression in initiation had reached at least 80% of the total of cases. Despite the changes in the diagnostic criteria previously pointed out, the exponential growth of the disease suggests that this phenomenon is not due to innate metabolic defects but rather to an acquired condition. ⁽²⁴⁾

In addition, various studies noted that the curves of increase of ASD cases coincide with the rise in the number of pediatric vaccines and their respective boosters, being directly related to the dose of total exposure to thimerosal, a preservative and antiseptic composed of 49,6% of a form of organic mercury (Hg), so-called ethylmercury, contained in the majority of pediatric vaccines. ⁽²⁵⁻²⁷⁾

Thimerosal, or [(ethylmercury)tio] 2 benzoate of sodium (ethylmercuric salicylic acid), whose molecular formula is $C_9H_9HgNaO_2S$ (Figure 1), separates in the organism in ethylmercury and thiosalicylate, being a highly unstable chemical composition. Due to its great liposolubility, it can easily cross the blood-brain and placental barriers, exhibiting a short time of half-life in the blood. It can deposit itself in the central nervous system, where subsequently it is transformed into inorganic Hg, which accumulates in the human and animal brain, showing a half-life between 227 and 540 days. Organic Hg can also bind to glutathione and other plasma proteins, such as metallothioneins, proteins that play a protective role in preventing the extracellular transport of Hg. On the base of limited available information, it has been concluded that the noxious effects of ethylmercury can be similar (or even worse) to those of methylmercury, another organic mercurial with documented neurotoxicity.

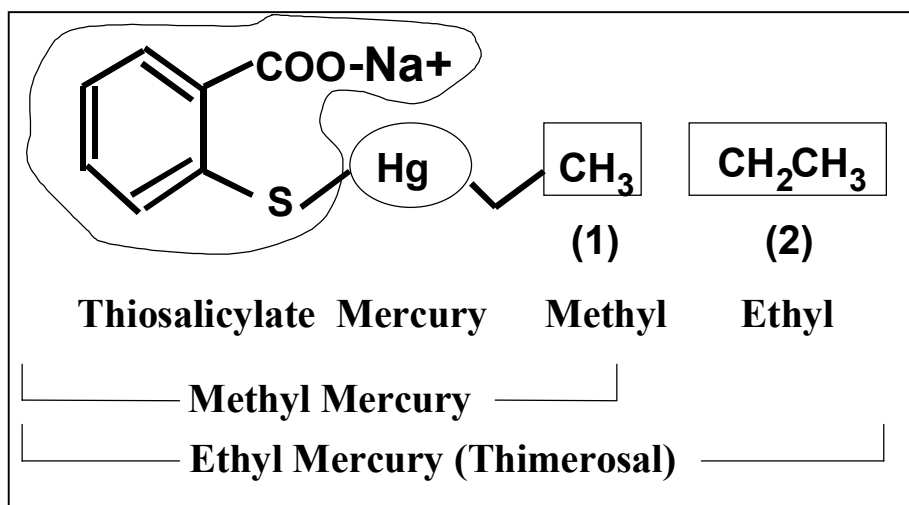


Figure 1. Chemical structure of organic mercurials.

It has been estimated that if a child born in the U.S. received all of the established doses of vaccination within his first 6 months of age, the values of exposure to Hg would have been increased in a significant manner as time goes on: ⁽²⁸⁾

Born 1950 – 1970:	50 µg of Hg.
Born 1971 – 1975:	75 µg of Hg.
Born after 1992:	187,5 µg of Hg.

In other words, the dose of Hg received just in the first six months of life would have been elevated more than 3 times in less than 20 years, due to the rise in the number of immunizations approved by the AAP and so accepted by the U.S. Centers for Disease Control and Prevention (CDC), as well as the U.S. Food and Drug Administration (FDA). Even more, at 18 months of age, the mercurial exposure rises to 237,5 micrograms (µg) and, if three additional vaccines are administered against the flu up to 1 and a half years of life, such as was suggested in some population groups, then the total exposure to Hg could have been as high as 275 µg. ^(29, 30)

It is given that the observational studies showed a direct correlation between the incidence rates of ASD and the mercurial exposures from vaccines, along with multiple clinical evidence, and similar laboratory evidence was found between these illnesses and mercury intoxication by S. Bernard and colleagues ⁽³¹⁾ were the first to formally establish a causal relationship.

On the other hand, it has been pointed out that the elevated ranges of frequency of ASD in urban zones, in relationship to rural ones, also are consistent with an environmental geographical association ^(32, 33); furthermore, considering that concomitantly with the larger exposure to thimerosal from vaccines, globally, a larger environmental exposure has been reported to heavy metals, especially to Hg. ^(34, 35) In Peru, this environmental contamination could be larger in rural mining areas located in the country's mountain range; while in children on the coast, the largest mercurial exposure would be associated with the vaccines containing ethylmercury.

Recently, even though one accepts that they can present overlapping cases on both sides, two principle types of autism have been described: ⁽²⁴⁾

* **Type I (classic or since birth):** occasioned by a severe metabolic disorder, due to an innate metabolic defect, with genetic associations. In affected children, the autistic characteristics appear quickly in life, accompanied many times by other clinical alterations that can even be more dramatic. There have been mentioned in the literature at least two dozen conditions: *Le cri-du-chat* syndrome, Prader-Willi syndrome, Angelman syndrome, Rubenstein-Taybi syndrome, Smith-Magenis syndrome, Rett syndrome, histidinemia, Lesch-Nyhan disorder, fragility of X chromosome syndrome, sclerosis tuberosa, neurofibromatosis type 1, and phenylketonuria, among others. ⁽³⁶⁻³⁸⁾

* **Type II (acquired or regression):** relatively new form, primarily responsible for the exponential increase in the cases of autism in the world. This variant is presumed to be a genetic predisposition, but it is certain in toxic exposures (heavy metals) and immunologic (vaccinations with live weakened virus). Although it is certain that it was always present in some measure, it has changed into a more prevalent form, due to the increase and severity of the above-mentioned factors of exposure. ⁽³⁹⁾

It has been pointed out that these two classifications could become arbitrary, when metabolic disorders of genetic origin are almost of equal severity to the effects of acquired disease. But, it is very clear that the cases of Autism Type I now constitute a small minority and that the large part of cases, even 19 out of every 20, would not have shown manifestation of autism had there not been exposures to one or more triggering risk factors. In agreement with this opinion – large toxic and/or immunologic exposure – major defects will be observed in neurodevelopment, having checked individual variations determined by genetic differences, immunological state, physical conditions and nutritional status. ⁽²⁴⁾

Based on more than 30 years of investigation, the ARI has determined that establishing such injuries of neurodevelopmental disorders are: ⁽²⁴⁾

1. The use of organic Hg (thimerosal) as a preservative in pediatric vaccines, which increased in number and frequency during the appearance of the epidemic of ASD and other infant neurodevelopmental disorders in the U.S., as well as its interaction with other toxins contained in the vaccines, such as aluminium and formaldehyde.

2. The increase in the number of vaccinations and their respective booster shots, via multivalent shots, especially the vaccine against measles contained in the triple viral vaccine combination measles–mumps–rubella (MMR). ⁽⁴⁰⁻⁵³⁾

3. Multiple immunizations given early in infancy to newborns and breastfeeding infants less than 6 months (as well as the vaccine against viral hepatitis B), administered before they have acquired sufficient metabolic maturity and immunological capability. ⁽⁵⁴⁾

4. The increase in environmental pollution, given the presence of heavy metals, pesticides, herbicides, fungicides, perchlorates, chemicals of military use, etc.; conditioned on major exposure to heavy metals and toxic chemical substances. ⁽⁵⁵⁻⁶⁰⁾

5. The use of antimony as a nonflammable agent in children's clothing in some areas of the U.S., where houses are made of wood and American law requires the use of pyjamas with this material.

6. The diminution of quality of nutrition during pregnancy and first infancy, especially from the decrease in breastfeeding and its substitution by artificial milk formulas.

7. The use of substances of abuse before and during gestation. ^(61, 62)

CONTENTS OF ORGANIC MERCURY IN PERUVIAN VACCINATION PLANS

Various international health agencies have developed guides to establish the maximum exposure levels permitted for methylmercury (another form of organic Hg contained basically in water and foods, previously better and more extensively studied and which historically has served as comparison with ethylmercury from thimerosal). These include the U.S. Environmental Protection Agency (EPA 1997), the U.S. Agency for Toxic Substances and Disease Registry (ATSDR 1999), the U.S. FDA (Federal Register 1979), and the World Health Organization (WHO 1996). These levels of maximum exposure varied from 0,1 micrograms per kilogram of daily weight ($\mu\text{g}/\text{kg}/\text{day}$) from the EPA, up to 0,47 $\mu\text{g}/\text{kg}/\text{day}$ established by the WHO ^(28, 63), according to what is shown in Table 1.

Table 1: Maximum permitted levels of oral exposure to methylmercury.*

OFFICE	MAXIMUM PERMITTED ORAL DAILY EXPOSURE (µg/kg.)	LIMITS OF ORAL EXPOSURE ACCUMULATED AVERAGE, CALCULATED FOR CHILDREN YOUNGER THAN 6 MONTHS ⁽¹⁾ (µg)
EPA	0.1	65 – 106
ATSDR	0.3	194 - 319
FDA	0.43	259 - 425
OMS	0.47	305 - 501

* *Ball L. et al., Pediatrics 2001.*

⁽¹⁾ *Percentiles of body weight 5th – 95th.*

EPA (U.S. Environmental protection Agency), FDA (U.S. Food and Drug Administration), WHO (World Health Organization), ATSDR (Agency for Toxic Substances and Disease Registry).

The wide recommended range is due to variations in margins of safety, differences in the emphasis placed on the sources of data, the different objectives of the agencies and the type of population to whom the guide is directed to protect. All of the guides, however, fall in the same order of magnitude. Although it is certain that these guides can be used as references to evaluate the maximum permitted exposure to organic Hg, they are not necessarily valid limits on those that show toxicity. ⁽²⁸⁾ Nevertheless, if the levels of exposure surpass the various limits of these guides, consensus exists on the part of the above cited public health organizations that adverse health consequences can occur. ⁽⁶³⁾

To clarify the discrepancies between the different exposure guides, the U.S. Congress commissioned the National Academy of Sciences of that country to carry out a study on the toxicological effects of methylmercury, with the object of offering recommendations in establishing the reference dose scientifically most appropriate. ⁽⁶⁴⁾ This report concluded that the current reference dose of the EPA was the scientifically justified level most adequate for the protection of human health, that is, 0.1 µg/kg/day. Because of the fact that, despite more than 70 years of vaccinations with thimerosal, it does not currently take into account the definitive data that compare the toxicity of ethylmercury versus that of methylmercury; the U.S. FDA considers both as equivalent in the evaluation of risk. ⁽²⁸⁾

Vaccines for human beings contain concentrations of thimerosal from 0.001% (1 part in 100,000) up to 0.01% (1 part in 10,000). Thimerosal is a compound made up of practically 50% ethylmercury; therefore, that vaccine contains 0.01% of thimerosal as preservatives, has 50 µg of thimerosal per dose of 0.5 millilitres (mL) or approximately 25 µg of ethylmercury per dose of 0.5 mL. ⁽²⁸⁾ The content of organic Hg, under the form of ethylmercury, on vaccines currently available in Peru is shown in Table 2.

The great majority of the pediatric vaccines employed in our country contains the highest doses permitted of ethylmercury (25 µg in each of them). These immunizations are: the vaccine against diphtheria-pertussis-tetanus (DPT), Haemophilus influenzae type B (Hib), viral hepatitis B (HvB), influenza, and the vaccine against meningococcal meningitis.

The Table 3 shows the current vaccination plan established by the Ministry of Health of Peru (MINSA), the average weight of a Peruvian child at different ages in which he is immunized, the maximum levels of exposure to mercury permitted according to the EPA and the WHO in those vaccinated, and the levels of overexposure corresponding to those subjected employing vaccines that contain 25 µg of ethylmercury. Note that the immunizations administered during pregnancy, birth and at three months of age largely exceed all of the established levels of maximum exposure, while those at 2 and 4 months of age surpass the reference levels of the EPA.

It is notable that MINSA includes only acellular Hib in its vaccination plans, the same that lacks Hg. Recently, it removed the two doses of DPT, before it given at 2 and 4 months of age, by a pentavalent combination vaccine (DPT + Hib + HvB), which was prepared starting with the combination of two vaccines: Tritanrix HB (DPT + HvB) that contains only what appears to be Hg (approximately 3 µg, in accordance with the producer) and Hiberix (acellular Hib), that does not contain Hg. Thanks to these changes, the total dose of ethylmercury received in the first four months of life of a Peruvian child has been reduced to 56 µg, in accordance with the current vaccination schedule of MINSA.

However, in private vaccination centers multiple immunizations have continued to be employed, with vaccines that contain high doses of Hg. For example, if simultaneous vaccines of DPT, Hib and HVB were given the same day, they would add up to a total exposure of 75 µg of Hg; therefore, according to the EPA the child would have to weigh 750 kg and in accordance with the WHO, the child would have to weigh 159 kg, in order not to exceed the widest corresponding permissible margins.

In addition, two types of vaccines are given to pregnant Peruvian women, containing large quantities of ethylmercury. Eventually, using human anti-D immunoglobulins is required, to prevent hemolysis from blood incompatibility in the newborn infants of mothers carrying the negative Rh blood factor. However, routinely, the antitetanus vaccine (absorbed tetanus toxoid) is administered in two doses, beginning from the fourth month of pregnancy; each dose contains 25 µg of added ethylmercury, the same that are injected in a very susceptible period to fetal life. Given that, the mercurial concentrations in the fetal blood become 4,3 times higher than that found in maternal blood ⁽⁶⁵⁾, fetal mercury exposure from these vaccines exceeds 200 and 42 times the maximum permitted references, in accordance with the EPA and WHO, respectively (Table 3). It's possible that, these immunizations are applied throughout to an urban population, whose childbirths occur in medical centers that fulfill optimal conditions of sterility, which limits significantly the risk of neonatal tetanus to levels that are practically nonexistent.

Table 2: List of registered vaccines in Peru, that contain thimerosal as preservatives.*

DESCRIPTION	THIMEROSAL RALLY	EQUIVALENCE gr%	VACCINE
Antiflu	0,05 mg/0,5ml	0,01	Influenza
Anatoxal Di Te Berna adults	0,05 mg/0,5ml	0,01	Diphtheria
Anti-D 250 µg/2ml	200µg/2ml	0,01	Ig anti-D
Anti-D 250 UI/2ml	200µg/2ml	0,01	Ig anti-D
Anti-D 250 UI/2,5ml	250µg/2ml	0,01	Ig anti-D
Biovac-B 200µg/10ml	0,50mg/10ml	0,005	HvB
Euvax-B pediatric 10µg/0,5ml	0,01% m/v	0,01	HvB
Euvax-B adult 20 µg/0,5ml	0,01%	0,01	HvB
Fluarix	0,0025mg/0,6ml	0,0005	Influenza
Gene Vac-B 10ug/0,5ml	0,025mg/0,5ml	0,005	HvB
Gene Vac-B 20ug/ml	0,05mg/ml	0,005	HvB
Heberbiovac Hib 0,05 mg/ml	0,05mg/ml	0,005	HvB
Heberbiovac Hib 10 µg/0,5ml	0,025mg/0,5ml	0,005	HvB
Hepavax-Gene 10 µg/0,5ml	0,01% p/v	0,01	HvB
Hepavax-Gene 20 µg/0,5ml	0,01% p/v	0,01	HvB
Imovax DT adult	0,05mg/0,5ml	0,01	DT
Inflexal Berna V	50µg/0,5ml	0,01	Influenza
Revac-B 10 µg/0,5ml	0,025mg/0,5ml	0,005	HvB
Revac-B 20 µg/0,5ml	0,05mg/ml	0,005	HvB
Resuman Berna 300µg	0,1mg/ml	0,01	Ig anti-D
Tritanrix HB	6µg/0,5ml	0,0012	Hib
Absorbed tetanus toxoid	0,1mg/ml	0,01	T
Va-Meningoc-BC	0,05mg/0,5ml	0,01	Meningococcal
Absorbed diphtheria, tetanus and pertussis vaccine	0,01%	0,01	DPT
Absorbed diphtheria and tetanus vaccine/Adults and teenagers	0,01%	0,01	DT
Recombinant antihepatitis B vaccine 20µg/ml	0,1mg/ml	0,01	HvB
Vaxigrip	22µg/1000ml	0,0000022	Influenza

* *Peruvian Medical College (update November, 2005).*

HvB (viral hepatitis B), Hib (Haemophilus influenzae type B), DPT (diphtheria, pertussis and tetanus), DT (diphtheria and tetanus), T (tetanus), Ig anti-D (human anti-D immunoglobulin).

Table 3. MINSA vaccination plan 2005-2006, containing ethylmercury in vaccines and levels of mercurial overexposure.

DATE OF APPLICATION	AVERAGE WEIGHT AT VACCINATION (kg)	DOSE OF MAXIMUM EXPOSURE (µg)		VACCINE ADMINISTERED	ETHYL MERCURY CONTENT (µg)	OVER EXPOSURE	
		Per EPA ⁽¹⁾	Per OMS ⁽²⁾			Per EPA	Per OMS
Pregnancy	1.00	0.100	0.470	Tetanus	25	200 times ⁽³⁾	42.4 times ⁽³⁾
Birth	3.00	0.300	1.410	BCG	0	0	0
				HvB	25	83 times	18 times
2 months	4.35	0.435	2.045	Pentavalent	3	6.9 times	1.5 times
				Oral Polio	0	0	0
3 months	5.15	0.515	2.421	DPT	25	49 times	10.3 times
				Acellular Hib	0	0	0
				Oral Polio	0	0	0
4 months	5.95	0.595	2.797	Pentavalent	3	5 times	1.1 times
				Oral Polio	0	0	0
12 months	10.0	1.000	4.700	MMR	0	0	0
				Yellow fever	0	0	0

⁽¹⁾ Based on the reference by EPA of 0,1 µg/kg/day.

⁽²⁾ Based on the reference by WHO of 0,47 µg/kg/day.

⁽³⁾ Based on the preferential accumulation 4:1 of Hg in the blood of the umbilical cord. U.S. Environmental Protection Agency, *Integrated Risk Information System*, April, 2006.

BCG (tuberculosis), HvB (viral hepatitis B), DPT (diphtheria, pertussis and tetanus), Hib (Haemophilus influenzae type B), Pentavalent (DPT + Hib + HvB), MMR (measles, mumps, rubella).

Despite the striking levels of observed overexposures, and as a major concern, it is necessary additionally to consider the following points:

1. The levels of exposure previously mentioned take into account uniquely the content of Hg in the vaccines and does not consider other sources of mercurial exposure, such as foods (especially fish), mother's milk, water and other sources of environmental pollution. The European Medical Evaluation Agency (EMEA) has estimated that such sources, outside of vaccines, represent between 80 and 100 additional µg of Hg per year. ⁽⁶⁶⁾ In addition, studies done in Canada have estimated

that vaccines containing thimerosal represent only 50% of the mercurial exposure to which children are exposed during only their first year of life. ⁽⁶⁷⁾

2. The recommended maximum exposure levels were established by the oral exposure to organic mercurials; it is expected that, if these are administered parenterally (as in the case of vaccines), the exposure may be even higher, since the peak blood levels appear much more rapidly.

3. These ranges of maximum permitted exposure have been established for adults with an average weight of 70 kg; it is known that fetuses in development and small children are much more susceptible to mercurial toxicity in respect to adults, in proportion to weight and body surface area. ⁽⁶⁸⁾

4. Recently, data have become available that show that ethylmercury from thimerosal has less lifetime in the blood, since its higher liposolubility gives it greater capacity to cross the blood-brain barrier in relation to methylmercury, depositing itself seven times more in the central nervous system, as inorganic Hg (considered the most toxic of all mercury forms). ⁽⁶⁹⁾

EVIDENCE

1. Epidemiological studies.

In the U.S., six large retrospective population based epidemiological studies have been done, that evaluated the association between thimerosal contained in pediatric vaccines and ASD. Of those, five investigations found that there was a causal relationship between exposure to thimerosal and child neurodevelopment disorders ^(25-27, 70, 71), while one eventually concluded that it could neither accept nor reject such a hypothesis. ⁽⁷²⁾

Other epidemiological studies ⁽⁷³⁻⁷⁷⁾ conducted outside the U.S. have not shown an apparent association. However, all of those, small quantities of exposure to mercury in vaccines were evaluated, these being the third part of the quantities administered in the U.S. In effect, the total doses of exposure of ethylmercury in pediatric vaccines in the United Kingdom, Sweden and Denmark of 75 µg, 75 µg, and 125 µg, respectively, are far enough from the 237,5 µg of potential maximum exposure, in accordance with the North American schedule of immunizations. Precisely, this fact was pointed out as one of the studies' principal limitations. ⁽⁷⁸⁾ In addition, given it would seem that it is very important to consider the exact moment of occurrence of the exposure in relation to cerebral maturation, the experts also have noted that, in countries where these studies have been done, the vaccination plan differ largely from the immunization schedules established in the U.S., such that their conclusions cannot be extrapolated to other countries. ⁽⁷¹⁾ Finally, it has been noted that the statistical power of all of them has not been adequately validated, so that its capacity to detect uncommon conditions, like the studies in these large population cohorts, is fairly limited. None of the referenced studies has estimated such statistical power, nor did they define the form in which they established the sample sizes. ⁽⁷⁸⁻⁸¹⁾

The ASD cases in the U.S., with 60 of 10 000 children (1 in every 166) currently affected, are much more prevalent than in the countries in northern Europe, such as Denmark, one of the countries where the U.S. CDC decided to conduct several of its epidemiological studies ^(74, 75) that have not shown an association. In that country,

thimerosal was removed from vaccines in 1992, and nowadays, it reports 7,7 cases of autism per 10 000 children (1 in every 1 300). Despite that, the authors have not known how to justify such important epidemiological differences. Other investigators have also observed that, given the great differences between the Danish and North American vaccination plans, the conclusion of such investigations, even if they were relevant to Denmark, certainly are not applicable to the United States.⁽⁸²⁾

The first (and unfortunately only) large official study conducted by the U.S. CDC in more than 70 years of the history of vaccinations, was conducted by T. Verstraeten and colleagues, using the Vaccine Safety Datalink (VSD), the largest registry of data related to the health and immunizations in the U.S.⁽⁷²⁾ The investigation was begun in 1997, with its initial results presented 3 years later in a confidential form, in June of 2000, in Simpsonwood, Georgia. In accordance with the transcripts obtained thanks to an application requested under the U.S. Freedom of Information Act,⁽⁸³⁾ only 52 people attended that conference: authorities from the WHO, the U.S. CDC and the U.S. FDA, experts in vaccination at the time, and representatives of the four companies that manufactured vaccines in the U.S. (GlaxoSmithKline, Merck, Wyeth and Aventis Pasteur). Those results were never officially published. Nevertheless, according to the transcripts of the conference, the authors found a statistically significant causal relationship between mercury exposures contained in vaccines at three months of age, as much with autism as with attention deficit syndrome, among other child development disorders. The CDC decided to keep this information confidential and ordered the authors to continue working with the data from the VSD. After three more years and after multiple changes in the design and methodology of the study, the results were eventually published in the journal *Pediatrics*, official organ of the AAP, changing its initial conclusions and rejecting a causal relationship.

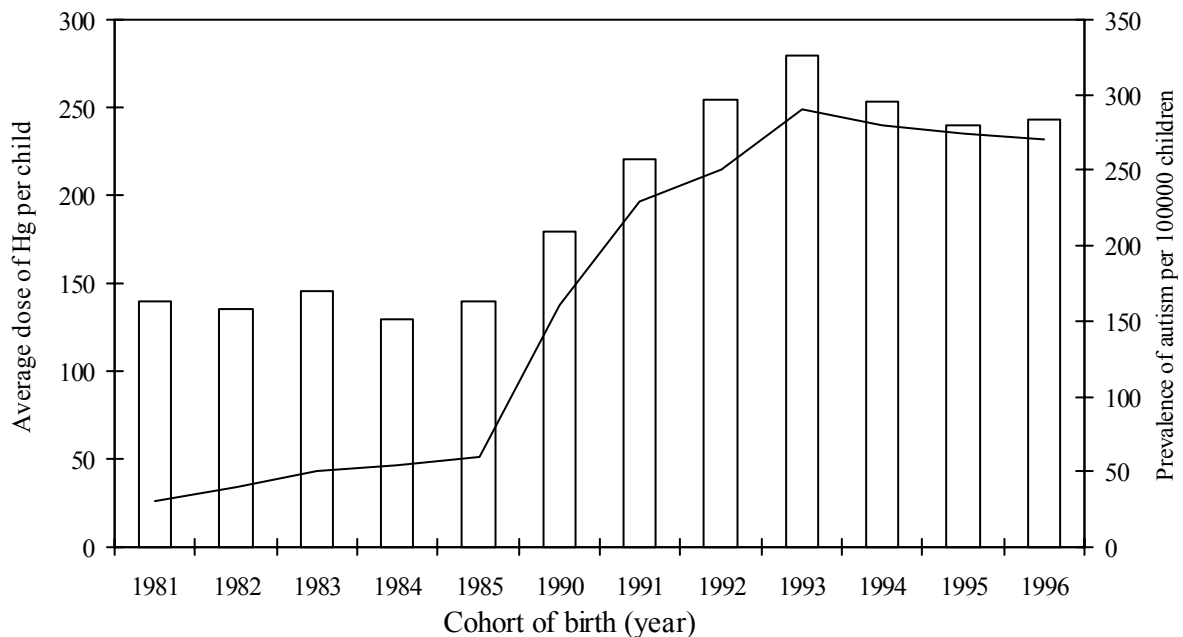
The publication of this study has received the most number of criticisms and suspicions of lack of independence by different American organizations involved in the fight against autism, and the autism community in general, in the U.S. Curiously, the principal author of the investigation (Verstraeten) was hired by one of the above-cited North American pharmaceutical companies, well before the final publication of his investigation, and was taken outside of the U.S., to Belgium. In addition, in a letter to the editor, which appeared in *Pediatrics* one year after the publication of his study⁽⁸⁴⁾, Verstraeten contradicted the conclusions of his own study; affirming, in reality, his study eventually concluded in a neutral result, that is, that he could not accept but neither could he reject the causal association.

Since 2000, the U.S. CDC had encrypted the data from the VSD, restricting its analysis by independent investigators. It required the intervention of a committee of the U.S. House of Representatives⁽¹⁶⁾ to permit for the first time access to that database to investigators who had no conflicting relationship with any North American federal office or with pharmaceutical companies. That occurred in a limited form in 2004 and a few months later, the resulting study was published. The investigation concludes that exposure to Hg, through vaccines containing thimerosal administered in the U.S., was a significant risk factor for the appearance of neurodevelopmental disorders.⁽⁷¹⁾

2. Ecological Studies

A recent study, which evaluated the relationship between the average dose of Hg received through vaccines containing thimerosal and the prevalence of autism in the U.S., found that the cohorts of children born since the mid-part of the 1980s until the end of the 1990s showed a positive ascending line of correlation, of statistical significance, between mercury exposure from vaccines and the prevalence of illness.⁽⁸⁵⁾ In this work, the authors also found that, upon reducing the dose of exposure to Hg, a corresponding decrease was observed in the prevalence of the disorder (Figure 2).

Figure 2: Prevalence of autism in the U.S. in comparison to the average dose of mercury administered in vaccines.*



* Geier D, Geier M. *Med Sci Monit.* 2004.

The bars show the average dose of Hg (µg) administered per child in the infant vaccines. The continuing line shows the prevalence of autism per 100,000 children in the U.S.

In addition, a recent investigation conducted in the U.S., using two different sources of data, the Vaccine Adverse Event Reports (VAERS) and the California Department of Development Services (CDDS), indicates that both registries have coincided in showing significant growths in the reporting curves of new cases of autism and language disorders, from the year 1994 until 2002, observing a direct correlation with the content of thimerosal in children's vaccines. Nevertheless, and after the progressive removal of ethylmercury from the pediatric immunizations in the U.S. beginning in 1999, both registries also show a significant decline in the same curves, from the middle of 2002 until the year 2005. The authors conclude that such results clearly indicate that the reporting curves of new cases of both illnesses are directly related, first to the increase and subsequently to the decrease, in the cumulative dose of Hg in children exposed to vaccines containing thimerosal, in the North American immunization programs.⁽⁸⁶⁾

Furthermore, the provisional data from the U.S. Department of Education show a recent decrease in the number of new cases of autism diagnosed among children from 3 to 5 years of age, the same that had been immunized with vaccines free of thimerosal. After an annual sustained growth, observed since the beginning of the past decade, 1 451 new cases were reported in the period 2001-2002; 1 981 in the period 2002-2003; 3 707 between 2003-2004; and 3 178 in the period 2004-2005, which represents 529 fewer cases.⁽⁸⁷⁾

Another very recent study also offers epidemiological evidence that demonstrates that, since the removal of thimerosal from pediatric vaccines, the number of new cases of child neurodevelopmental disorders has decreased significantly in the U.S.⁽⁸⁸⁾ In that investigation, Geier and colleagues carried out an ecological study, monitoring the VAERS from the year 1991 until 2004, evaluating both the date of receipt of reports of harm attributed to vaccines, as well as the date of administration of the immunizations. The diseases studied were autism, mental retardation and language disorders. They found that, the peak of reports received in the VAERS occurred in the period 2001-2002, corresponding to the administration of vaccines with thimerosal from the year 1998. However, they also observed a significant reduction of the reporting of new cases of these illnesses from when the preservative began to be removed from the pediatric vaccines in the U.S., in the year 1999 and forward.

In addition, two recent studies have documented how environmental pollution, particularly exposure to heavy metals (especially Hg) has plays a role as a risk factor in the development of ASD. Palmer and colleagues⁽⁸⁹⁾ studied the association between the prevalence of autism and environmental pollution by Hg and other toxins in Texas, one of the states of the American Union with the largest petroliferous resources. The investigators compared the growth of cases of autism, from 1990 until the year 2000, in 31 districts of Texas, with the rate of environmental release of Hg. They found that, on average, for each 1000 pounds of Hg released to the environment, a 43% increased was observed in the need for special education services and a 61% growth in the prevalence of autism. Also, in one study that has just been published, conducted by Windham and colleagues, of the National Institute of Environmental Health Sciences⁽⁹⁰⁾, the authors compared the environmental exposure to 19 chemical substances identified as potential neurotoxins, developmental toxins and/or disruptors of the endocrine system, in 284 children with ASD and 657 healthy children as controls, in San Francisco Bay, U.S. After the corresponding statistical adjustments, the authors found an association between the risk of developing autism and the exposure in the ambient air surrounding the place of residence to heavy metals (Hg, cadmium and nickel), trichloroethylene and vinyl chloride, as much in the prenatal stage as in the first years of life in children with ASD.

3. Biomolecular and Toxicology Studies

We must point out that, science in general accepts that the epidemiological type studies can contribute to just one part of the scientific evidence, indicating that an area of investigation requires larger clinical and molecular studies, in order clarify a certain phenomenon. In the case of thimerosal, despite the ethical and medical-legal problems that may be involved to complete laboratory studies on ethylmercury in children, recently, extensive clinical investigations have been published, both in human beings as in laboratory animals, *in vivo* and *in vitro*, as well as numerous biomolecular studies,

which describe the capacity of thimerosal to cause neurological disorders and that link it as the causal agent to several disorders of child neurodevelopment.⁽⁹¹⁾

The following evidence has fully established that, all forms of Hg (including ethylmercury) are neurotoxic, especially during the early phases of cerebral development.

3.1 Studies on children with ASD

At this date, there are investigative works available, involving children with ASD, in which they have shown a small capacity to excrete Hg and other heavy metals in the urine and hair⁽⁹²⁻⁹⁶⁾ and how this capacity increases significantly after they have been administered chelants (substances capable of eliminating heavy metals from the body). These works conclude that, children affected by ASD have a significantly higher quantity of Hg accumulated in their bodies, in comparison with healthy children in controls, as a result of diminished capacity to excrete Hg.

The excess of urinary excretion of porphyrins (porphyrinuria) is a discovery described in several fields (genetic deficiencies, hematological diseases, hepatologies, nephropathologies, etc.); but, it has also been described as a biological marker for chronic poisoning from heavy metals, both in animals as well as human beings.⁽⁹⁷⁻¹⁰⁰⁾ Furthermore, a singular altered pattern of porphyrins in urine, with elevations of precoproporphyrin (keto-isocoproporphyrin) and of pentacarboxyporphyrin, has been characterized as specific to poisoning by Hg.⁽¹⁰¹⁻¹⁰⁵⁾ Nataf and colleagues, in an innovative investigation about to be published in the journal *Toxicol Appl Pharmacol*, has studied for the first time the urine levels of porphyrins in 269 children with ASD, in a clinic in Paris, comparing them with values in healthy children controls. The authors found values of porphyrins 2,6 times higher in children with ASD, especially in the autistic group. The determinations of precoproporphyrin and of pentacarboxyporphyrin also turned out to be significantly raised. In addition, a subgroup of autistic children who had shown abnormal values of porphyrinuria were treated with dimercaptosuccinic acid (DMSA), a chelant of heavy metals; they were observed to have normalization of urinary excretion of porphyrins. The investigators conclude that these discoveries suggest that heavy metals, particularly Hg, are implicated in the causality of ASD. They also point out that porphyrinuria, besides being a biomarker of toxicity, could also have a role in behavioural manifestations of autism, given that these abnormal metabolites bind to benzodiazepine receptors⁽¹⁰⁶⁻¹⁰⁸⁾ and have been associated with neurological disturbances, epilepsy and autism.⁽¹⁰⁹⁻¹¹³⁾

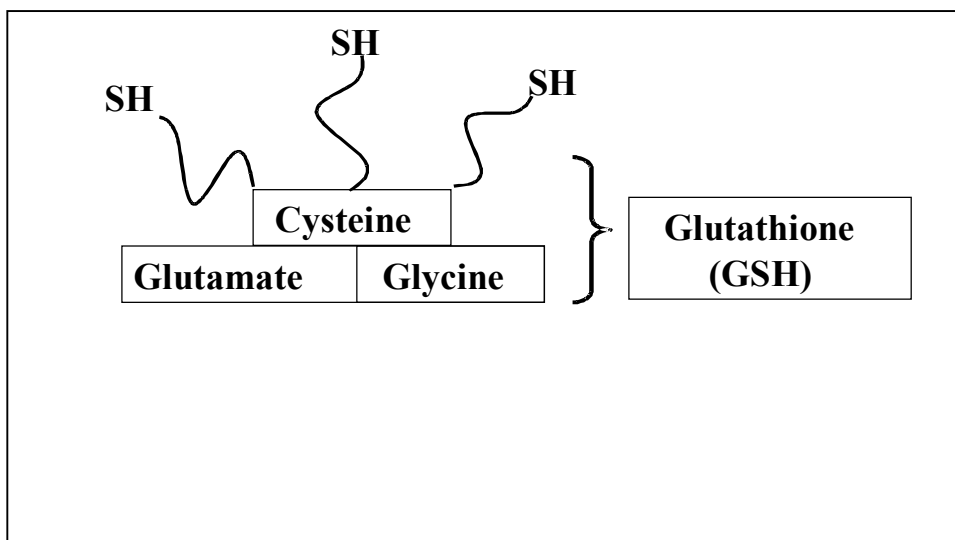
The ARI has been using in a protocolizing manner, several chelants, in the treatment for detoxification of child developmental disorders, since for several years, they have been finding several significant improvements (and in numerous cases the loss of diagnosis of ASD), in children affected by this group of disorders.⁽¹¹⁴⁻¹¹⁵⁾

A lesser ability to desulfate has also been described in children with ASD. This biochemical deficit seems to contribute to the accumulation of Hg observed in these patients, given that the physiological process of detoxification of Hg in the human body includes the binding of mercurial compounds to sulfhydryls (thiols), to permit its subsequent excretion.⁽¹¹⁶⁾ In addition, it has been found that children affected by ASD have plasmatic levels significantly lower of cysteine (19% reduction) and of glutathione

(46% reduction) in comparison to healthy children; both substances are crucial for the excretion of Hg. ⁽¹¹⁷⁾ People with genetic deficiencies in the synthesis of glutathione will be less capable of excreting Hg and, therefore, more sensitive to its adverse effects. ⁽¹¹⁸⁻¹¹⁹⁾

Glutathione (GSH) is a tripeptide produced at the liver, beginning with the amino acids glycine, glutamate and cysteine (Figure 3). It acts to protect the human body from toxic agents, such as heavy metals, by means of binding these to its sulfhydryl groups. It is also a powerful antioxidant, which prevents the formulation of free radicals and inhibits cellular damage. Significantly lower levels of GSH has been found in children with ASD, thereby increasing the susceptibility of these patients to damage by heavy metals, in particular Hg.

Figure 3. Structure of glutathione



Precisely, genomic polymorphisms have been found, for specific enzymes in the cycle of methionine and transulfatation, which explains the genetic susceptibility increased by poisoning from heavy metals in the autistic population, since these metabolic routes are precisely those responsible for the elimination of Hg and other heavy metals. ⁽¹¹⁷⁻¹²⁰⁾ Additionally, it is known that the developing brain is unique in its susceptibility of neurotoxicity induced by mercurial agents; factors such as cerebral maturation, metabolism, nutritional state, sex and immunogenicity influence, as well as genetic vulnerability, the prognosis of cerebral damage. ⁽⁶⁸⁾ Table 4 lists the genetic factors associated with increased susceptibility to developing ASD, according to the ARI.

Redwood and colleagues ⁽¹²¹⁾ have stated that the exposure to Hg through immunizations must be grounds for concern, due to the small quantities of Hg administered during critical periods of development; they have been associated with various child neurological disorders, which include attention deficit syndrome, difficulty with speech and language disorders.

Table 4. Genetic factors that predispose to ASD*

<ul style="list-style-type: none">- Male gender.- HLA C48 (false allele).- <u>Family history of autoimmunity</u>: allergies, bronchial asthma, diabetes, rheumatoid arthritis, intestinal inflammatory disease, celiac disease, autoimmune thyroiditis.- <u>Specific nucleotide polymorphisms</u>:<ul style="list-style-type: none">➤ MTHFr: Tetrahydrofolate methylene reductase.➤ COMT: Catecholamine O-methyltransferase.➤ MTRR/MTR: Methionine synthase and methionine synthase reductase.➤ TCII: Transcobalamine.➤ GABRB3: GABA receptor.➤ ADA: Adenosine deaminase.➤ Mutant UBE3A (ubiquitin ligase).➤ CBS: Cystathione Beta-synthase.
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* *Usman A. DAN!TM Conference Proceedings – Spring 2006, April 7-10, 2006. Autism Research Institute. Washington, D.C.*

3.2 In vitro studies on human cells

Thimerosal has been irrefutably proven to be toxic, even in micromolecular concentrations, to nerve cells, fibroblasts and human T-lymphocytes, damaging their mitochondrial mechanisms and inducing oxidative stress and the depletion of glutathione, the principle physiological detoxifying agent for heavy metals.

The studies recently conducted in the Department of Biochemistry at the University of Kentucky by Professor Boyd Haley are revealing. ⁽¹²²⁾ The investigators have found thimerosal to be even more neurotoxic than the ionic form of mercury (Hg^{2+}), due to the enormous power of penetration it possesses in fatty tissue, such as the central nervous system (greater liposolubility). In addition, they have observed that the toxicology of ethylmercury does depends not only on blood levels, but also on other numerous associated factors, such as: concomitant illness, simultaneous exposure both to other heavy metals (ionic zinc, cadmium, lead, aluminium) as well as antibiotics (tetracyclines, ampicillin, neomycin), genetic susceptibility, levels of testosterone, the beginning/end of life, oxidative stress, etc., all of which increase the damage in relation to a healthy individual. Their studies on human nerve cells exposed to extremely small quantities of thimerosal (even at concentrations of 1:10 000, as in vaccines) show how this toxin destroys neurons and/or severely affects their growth. They have observed that a solution of 50 nanomolars of thimerosal causes the death of 43% of exposed neurons, in the first 24 hours, while a solution with double the concentration causes close to 83% of cellular death in the same time period, concluding that its medical use is unacceptable, especially in the pediatric field. The same authors have demonstrated that, the toxicity of thimerosal is exacerbated when the preservative is used simultaneously with other heavy metals, such as ionic zinc, cadmium, lead, or aluminium. As a result, their investigations have shown that the same levels of thimerosal cause 50% of

neuronal death, produce death of approximately 90% of human nerve cells in the presence of non-toxic levels of aluminium chloride. ⁽¹²²⁾

This power of synergistic toxicity is of great concern, since many of the vaccines with thimerosal also contain aluminium; no study has been done on the biosafety at this point. We must point out that aluminium is also a known neurotoxin that shares many common mechanisms with Hg. For example, both are toxic to neurotubules; they interfere with antioxidant enzymes, harm enzymes that repair DNA, interfere with the mitochondrial production of energy, block protein receptors of cerebral glutamates (GLT1 and GLAST), they bind to DNA and disturb the functions of neuronal membranes. ⁽¹²³⁻¹²⁸⁾ Aluminium, used as a preservative in vaccines, has had a causal relationship with macrophagic myofasciitis, a condition that produces severe muscular weakness and multiple neurological syndromes. Gherardi and colleagues have emphasized that, even in the absence of an obvious systemic autoimmune disease, in the majority of cases diagnosed with macrophagic myofasciitis, there has been evidence of a chronic immunologic overstimulation caused by the injection of aluminium. ⁽¹²⁷⁾ This phenomenon is very important, since recently, this pathological mechanism has been pointed out as one of the principal causes of damage in numerous neurodegenerative illnesses (multiple sclerosis, Alzheimer's disease, Parkinson's disease), ASD and attention deficit syndrome. ⁽¹²⁹⁻¹³¹⁾ In addition, it must be emphasized that for decades, studies have existed showing that the toxicity of both metals is highest when they are used in combined form. ⁽¹³²⁾

Gaskin and colleagues ⁽¹³³⁾ have estimated that the quantities of thimerosal contained in the pediatric vaccination programs reach values 4 times higher than the lowest concentrations, at which, in their study, these authors found neurological toxicity (201 ug/L). They conclude that, the rapid appearance of damage caused by thimerosal in low micromolar concentrations, observed in short periods of time, is extremely worrying, since they estimate that prolonged exposure to thimerosal in small quantities (as happens during vaccinations) could produce neurological damage at even lower doses.

Leong and colleagues examined neuronal growth *in vitro* after exposure of human nerve cells to similar concentrations of Hg, aluminium, lead, cadmium and manganese. They showed that nanomolar concentrations of Hg markedly altered the structures of the membranes and growth lines of 77% of exposed neurons, while with the other heavy metals, such damage was not observed. ⁽¹³⁴⁾ Similar results have been described in numerous additional studies using thimerosal. ⁽¹³⁵⁻¹³⁷⁾

James and colleagues ⁽¹¹⁹⁾ have demonstrated that thimerosal induces oxidative stress and apoptosis (self-programmed cell death) of neurons, astrocytes, and human T cells, activating mitochondrial metabolic pathways of cellular death. They observed that neuronal death would occur just three hours after being exposed to thimerosal, while the cells of the glia (astrocytes) exhibited the same cytotoxic effects at 48 hours after exposure to the same concentrations of the preservative. It should be emphasized that known toxins, such as antineoplastic agents used in chemotherapy, ultraviolet radiation and stress molecules (reactive oxygen and species of reactive nitrogen), also act at the same cellular level. ⁽¹³⁸⁾ In addition, their studies have shown how this mercurial toxicity is correlated with intracellular levels of glutathione, and the way this last substance could be of therapeutic use in children, elderly and pregnant women who

receive vaccinations or immunoglobulins with thimerosal and in individuals who regularly consume fish containing appreciable quantities of Hg. ⁽¹¹⁹⁾

Recently, it has also been reported that methylation plays a critical role in the ability of tissue growth factors to promote normal development. In this important metabolic route, the liver is capable of completing the pathway of synthesis of homocysteine to methionine and of cysteine to glutathione (Figure 4). The astrocytes and neurons do not possess the enzyme cystathionine lyase. Therefore, they are incapable of synthesizing cysteine. As a result, these cells are dependent on the levels of plasmatic cysteine derived primarily from the hepatic synthesis of glutathione. It has been reported that thimerosal is also a powerful antagonist of the enzyme methionine synthase, impeding the formation of methionine, which is essential for the methylation of several structures, such as DNA, RNA, various proteins, phospholipids, histones, and neurotransmitters, all of which have been found to be altered in children with ASD. In addition, thimerosal inhibits the synthesis of glutathione, the principal physiological mechanism for detoxification of heavy metals. This way, it has been shown to be a potent inhibitor of this function, allowing a molecular explanation of how vaccines containing thimerosal can increase the incidence of neurodevelopment disorders. ^(117-119, 134)

Waly and colleagues, in a fascinating study ⁽¹³⁹⁾, has shown how the exposure to heavy metals (including lead, Hg and aluminium) can contribute to neurodevelopmental disorders, through the inhibition of metabolic pathways of methylation – especially the methylation of DNA – and the decrease in several factors of development (neurotropic growth factor, cerebral neurotropic factor and growth factor similar to insulin type 1), all of which are indispensable for the promotion of neurological development and support the function and survival of the nervous system. ^(140, 141)

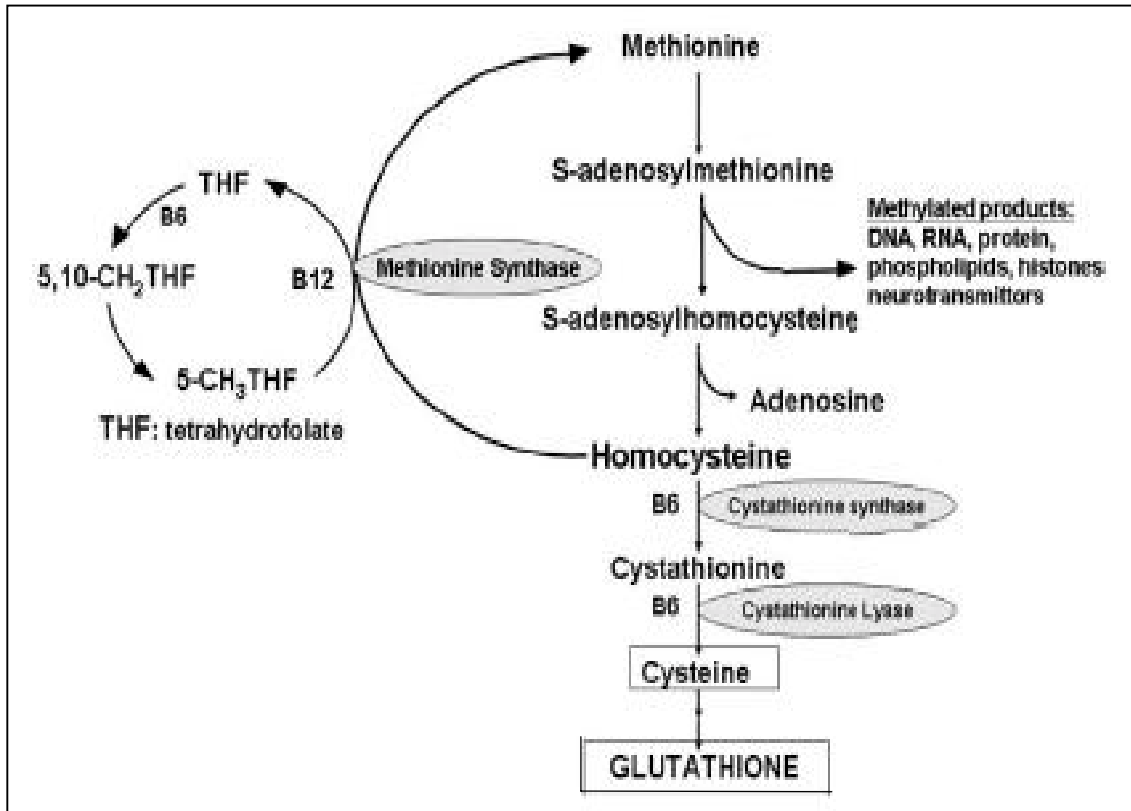
Numerous other *in vitro* studies on human neurons have demonstrated that nanomolar and micromolar concentrations of thimerosal are capable of inducing neuronal death, neurodegeneration, damage to the cellular membranes and alterations of DNA, within a few hours of exposure. ⁽¹⁴²⁻¹⁴⁴⁾ Recently, it has also been shown that extremely small quantities of thimerosal are equally capable of critically interrupting the interneuronal pathways of communication and the biochemical events necessary for the adequate neurological development in human beings. ^(145, 146)

3.3 Studies in animal models

Autism has been reproduced in laboratory animals, satisfying one of the earlier etiological principles of Pasteur. In fact, Hornig and colleagues ⁽¹⁴⁷⁾, utilizing selected stocks of rats whose brains were in development and exposing them to vaccines with thimerosal (at doses equivalent to the weight and age of the human immunization schedules), have been able to replicate several similar characteristics to those of the disease, such as retardation in growth, decrease in locomotion, inappropriate responses to novelty, increase in cerebral size, significant abnormalities in the architecture of areas of the brain related to emotion and cognition, and disorders in the cells of the hippocampus, among others. Using the same model of investigation, damaging interaction between thimerosal and testosterone ^(122, 148) was also observed, confirming the major susceptibility to mercurial damage among animals of masculine gender. It is notable that child neurodevelopment disorders are four times more frequent among boys

than girls. ^(5, 8, 15) In addition, in human fetuses and small children of male sex, greater sensitivity to neurological damage has been observed after the exposure to organic mercurials. ⁽¹⁴⁹⁾

Figure 4. Pathway for the synthesis of glutathione and methylation.



Studies conducted in animal models have documented substantial quantities of thimerosal in blood and in different tissues (especially the brain), showing its passage through the blood-brain and placental barrier, determining that the time of half-life of Hg in the animal brain is from 28 days after being administered as thimerosal. ⁽¹⁵⁰⁻¹⁵¹⁾

For several years, *in vivo* studies done in rodents had shown that ethylmercury was capable of crossing cellular membranes and later being converted intracellularly to inorganic Hg, the same that accumulates preferentially in the brain and the kidneys. This intracellular accumulation of inorganic Hg proved to be higher for ethylmercury (thimerosal) in relation to methylmercury, using equimolar exposures, in spite of the fact that the speed of blood purification of ethylmercury is definitely more rapid than that of methylmercury. ⁽¹⁵²⁾

Nevertheless, it has been determined that there are no major differences between the toxicity of methylmercury and thimerosal. Small quantities of thimerosal, in the order of micrograms in the brain of laboratory animals, were capable of producing similar neurological damage as the first, indicating that the presence of Hg in appreciable quantities was not necessary for such neurotoxicity to occur. ⁽¹⁵²⁾

A recent study confirms these same observations: the blood levels of thimerosal are significantly shorter than those shown by methylmercury, 8,6 days vs. 21,5 days, respectively. However, the first crosses to a large degree the blood-brain barrier to deposit itself in the brain, transforming itself later into inorganic Hg (considered the most toxic of all mercury forms), seven times more (71% vs. 10%), in comparison to methylmercury. It is also known that, the time of half-life of intracerebral inorganic Hg varies between 227 and 540 days.⁽⁶⁹⁾

Multiple immunological disruptions have been documented in patients with ASD. Recently, it has been found that thimerosal, at nanomolar concentrations, dramatically alters the dendrite cells of rats *in vitro*. These cells play a central role in the immunological response, since they act as hosts to antigens and stimulate the activation of T lymphocytes. Goth and colleagues⁽¹⁵³⁾ have reported that thimerosal alters the properties of dendrite cells, suppressing their internal signals; at small doses, the preservative induced the release of abnormal quantities of interleukins, while at higher doses it caused death. Such effects were observed even at very small concentrations and within a few minutes of exposure, also showing that the most immature cells were particularly sensitive to thimerosal. The authors conclude that their discoveries have implications in the commercial use of thimerosal in vaccines. The fact that dendrite cells are so sensitive to damage induced by the mercurial agent, and knowing the important role that they play in cellular immunity and immunologic tolerance, constitutes an explanation of the possible contribution to damage to the function of the immune system which the preservative causes.

4. Studies of biosafety

Although it may be difficult to believe, according to the investigation of the North American Congress, no large study of biosafety of thimerosal has been conducted by the pharmaceutical companies, nor has it been required by the U.S. FDA or the U.S. CDC in more than 70 years of history of human immunizations.⁽¹⁶⁾ On the other hand, multiple investigations have long demonstrated its toxicity.

Kravchenko and colleagues⁽¹⁵⁴⁾, working with cultures of human cells, showed that thimerosal not only was damaging in its primary toxic effects, but they also observed that it was capable of changing the properties of cells. These authors concluded that the use of thimerosal in biologic medical preparations, especially directed toward children, was unacceptable. After this publication, the Soviet countries retired thimerosal from its child vaccines, at the beginning of the decade of the 1980s. Many other studies arrived at similar conclusions, pointing out the unsuitability of thimerosal in the vaccines, since it is known for its capacity to induce allergic responses^(155, 156), its poor antiseptic effectiveness⁽¹⁵⁷⁾ and/or its degradation into neurotoxic substances.⁽¹⁵⁸⁾

A descriptive study published in 2002 showed, apparently, good news: thimerosal, after inoculation by vaccines in newborn children, resulted in blood levels significantly less and shorter than methylmercury, being excreted in appreciable quantities in the feces.⁽¹⁵⁹⁾ However, this work did not study other body parts (such as the nervous system); nor did it measure the peak serum levels of ethylmercury after the first hours of inoculation, despite the fact that other investigations have documented substantial elevations of their blood concentrations in the first 24 to 72 hours after being

administered as thimerosal from pediatric vaccines. ⁽¹⁶⁰⁾ In addition, the study was not designed to measure the biological effect of the preservative (it just measured pharmacokinetic variables) and was conducted in an extremely small population (33 children). It should be emphasized that, it has been fully determined that the nervous system preferentially takes Hg originating from thimerosal; intracerebral concentrations were observed to be between 5 to 7 times higher than levels measured in the blood. ⁽¹⁶¹⁻¹⁶⁴⁾

In the Material Safety Data Sheet of thimerosal, dated June of 1991, written by the pharmaceutical company that licensed the product ⁽¹⁶⁵⁾, confirms that, “...*the mercurial component has caused systemic effects in laboratory animals, including mild to serious mental retardation and compromises motor coordination.*” For those reasons, thimerosal was considered toxic and removed from vaccines for animals, in the year 1992, in the U.S. But what was never explained is that, in the same document, in which it refers to human exposure, it states: “...*the exposure to Hg in utero and in children can cause mild to severe mental retardation and cause mild to severe compromise to motor coordination.*” In addition, the Information Safety Report from Merck laboratories in Europe ⁽¹⁶⁶⁾ also describes thimerosal as a product extremely toxic, dangerous to the environment and damaging in its cumulative effects.

The U.S. National Toxicology Program declares thimerosal as “...*a poison to the oral, subcutaneous, intravenous tracks and possibly to other routes;*” it classifies thimerosal as a laboratory carcinogen and teratogen, concluding that child exposure can result in “...*mental retardation, loss of coordination in walking, speech and writing; stupor, irritability and severe disturbances of personality and behaviour.*” ⁽¹⁶⁷⁾ For several years, the AAP knew about the harmfulness of mercurial exposure. In a technical report from 2001 ⁽⁶⁸⁾, they conclude that: “*the developing fetus and small children are disproportionately affected by the exposure to Hg, because of the fact that many aspects of development, particularly cerebral maturation, can be affected by the presence of Hg. To minimize the exposure to Hg is, therefore, essential to optimize the health of children.*” For these reasons, the AAP, since 1999, demands urgently for the reduction or elimination of vaccines that contain thimerosal as a preservative. ⁽¹⁶⁸⁾ Two years later and to date, all of the vaccines included in the pediatric vaccination calendar stipulated by the AAP do not contain thimerosal ⁽²⁸⁾, there is only the flu vaccine, the only child immunization used in the U.S. that still possesses high elevated quantities of Hg.

A recent report of the Environmental Working Group, after an extensive investigation, has developed multiple connections between mercurial exposure – especially coming from child vaccines – and ASD, confirming the possibility that Hg is the factor that causes or contributes to these disorders. ⁽¹⁶⁹⁾

5. Studies on fetal toxicology and reproductive health

Thimerosal has been recognized recently, by the California EPA, Office of Environmental Health Assessment, as a developmental toxin, which means that it can cause congenital defects, low birth weight, biological and psychological dysfunctions, or behavioural disturbances, that can manifest in the early childhood; in addition, that maternal exposure during pregnancy can compromise the development or even cause fetal death. ⁽¹⁶⁷⁾ A report conducted by that organization, in response to a petition by a

pharmaceutical company to declare thimerosal as harmless, concluded: “...*The scientific evidence that demonstrates that thimerosal causes reproductive toxicity is clear and voluminous. Thimerosal breaks down in the body into ethylmercury. The evidence of its reproductive toxicity includes severe mental retardation and malformations in children that were exposed when their mothers received ethylmercury or thimerosal while they were in gestation. The studies in animals demonstrate toxicity in development after exposure to ethylmercury or thimerosal, and the data show the conversion into other forms of Hg that also clearly produce reproductive toxicity*”.

Several laboratory studies, both in animals and in humans, have demonstrated that the exposure to thimerosal in specific stages of prenatal life carry as a consequence the passage of appreciable quantities of Hg across the placental barrier, resulting in significant fetal lethality and teratogenicity.⁽¹⁷¹⁾

Vaccines for tetanus prevention, used in our midst contain 25 µg of ethylmercury per dose; a fetus in development can, therefore, potentially receive extremely high doses of Hg that dramatically exceed the limits established by the EPA or the WHO (Table 3). It is also known that, the highest proportion of injected ethylmercury accumulates in fetal tissues in relation to the maternal organs, especially in the central nervous system.⁽¹⁷²⁾ All of these observations should cause even more concern, since although in our country the average concentration of Hg in the blood of the umbilical cord of newborns has not been estimated, in the U.S. it has been reported that 7,8 to 15,7% of the cases studied showed Hg values associated with reduction of intellectual coefficients.⁽¹⁷³⁾

Holmes and colleagues⁽⁹³⁾ determined that the mothers of autistic children had received approximately six times higher quantities of thimerosal during pregnancy, due to the exposure contained in human anti-D immunoglobulins, in relation to mothers whose children had a normal neurodevelopment; these authors suggest an important role of prenatal exposure to Hg in the later appearance of ASD.

The studies in animals do not show different results. Gasett and colleagues⁽¹⁷⁴⁾ observed significantly more fetal deaths after the maternal exposure to thimerosal compared to control animals, indicating that, even topically, the preservative showed abortive properties. These discoveries were replicated by Itoi and colleagues⁽¹⁷⁵⁾, who demonstrated a range five times greater of fetal death when a topical solution of thimerosal was applied to the connective tissue of pregnant rabbits. In this same study, the congenital malformations occurred only in the group of animals exposed to the preservative (9,1% vs. 0,0%). Digar and colleagues⁽¹⁷⁶⁾ found four times greater mortality when 0,1 mg of thimerosal was injected in the vitalen sack of chicken eggs. They also observed serious malformations in 36% of the exposed embryos, but in none of the controls; these animal malformations included syndactilia, visceral ptosis, thinning of the abdominal wall and abnormalities in the growth of the wings and the body.

Thimerosal also has the potential to decrease fertility. Batts and colleagues⁽¹⁷⁷⁾ documented that this substance was toxic to ciliary function, when it was topically applied over the trachea of sheep, indicating a potential pathological mechanism to compromise the reproductive capacity in women (fallopian tubes) and males (motility of sperm). In fact, this hypothesis has been proposed to justify the high incidence of

infertility among adults who in their infancy suffered acrodynia, another entity caused by exposure to mercury. ⁽¹⁷⁸⁾ Goncharuk ⁽¹⁷⁹⁾ described a dose dependent rate of lethality in rats exposed to inhaled compounds of ethylmercury. In addition, the investigator reported that, when this form of organic Hg was administered to male rates before being cross-bred, a decrease in fertility was observed, not only in those who received it, but also in the two following generations.

Inorganic Hg, another one of the metabolites of thimerosal, also has been shown to be genotoxic and to diminish reproductive capacity in several laboratory animals in vitro. ⁽¹⁸⁰⁾ Kahn and colleagues ⁽¹⁸¹⁾ observed that fertility and survival were reduced in rats exposed to Hg chloride. These effects, including ovarian atrophy, were seen in the absence of systemic manifest toxicity, underscoring the necessity to conduct clinical studies to evaluate the prenatal risk of exposure to thimerosal.

Also, studies have been done on the toxicity of thimerosal on human reproductive health. Heinonen and colleagues ⁽¹⁸²⁾ demonstrated that topical exposure to thimerosal during pregnancy significantly increased the risk of congenital defects.

Reproductive toxicity and the damage to human fetuses from methylmercury have been widely studied and accepted. Some health authorities proclaim that methylmercury is more toxic than ethylmercury in thimerosal; but these conclusions are not based on scientific literature. ⁽¹⁷¹⁾ A laboratory study done on pigs found thimerosal significantly more toxic than methylmercury. ⁽¹⁸³⁾ In addition, Leonard and colleagues ⁽¹⁸⁴⁾ found that ethylmercury crosses the placenta more easily than methylmercury and was capable of causing mutagenic changes in the cells studied. Despite these multiple investigations, the U.S. FDA has never given preference to vaccines free of thimerosal over those that still contain it. In fact, even though since 1999, all of the medical and scientific organizations and government health authorities in the U.S. decided on the prompt and urgent removal of this preservative in their vaccines ^(185, 186), such a process never carried out in an adequate manner; there was no ending of the consumption and/or withdrawal of the vaccines containing thimerosal until the year 2003, which has been sharply criticized on the part of the U.S. Congress. ⁽¹⁶⁾

On this view, it has been commented that the history of acquired autism may perhaps end like the case of acrodynia, where the withdrawal of the causal substance (*i.e.*, Hg contained in toothpastes) led to the disappearance of the disease and the subsequent identification of the etiologic cause. ⁽¹²²⁾ It should be mentioned that it is estimated that only 1 of every 500 children exposed developed the disease, which reaffirms the concept that, besides the causal exposure, specific factors for vulnerability are required among children susceptible to the damage caused by Hg. More than 10 years after the suspension of this form of mercury exposure was needed so that medicine formally would recognize that this had been the agent responsible for the disorder. It is advisable to point out that much of the symptoms described in acrodynia are similar to those observed recently in children identified as autistic or with attention deficit syndrome.

CONCLUSIONS AND RECOMMENDATIONS

1. Mercury (Hg) has been considered the third most toxic element on earth, exceeded only by plutonium and uranium. Although, paradoxically, it has been used for medical purposes for a long time, the documentation of its toxicity has led to its gradual withdrawal from practically all medications and biological agents used nowadays.

2. For some years, its large negative impact on human health has been demonstrated, especially in the most susceptible groups, such as fetuses and children. This is the reason why different experts and international health agencies have pronounced in agreement that the reduction or elimination of exposure to Hg, whether it comes from thimerosal in immunization or whatever other source, constitute measures of capital importance for the public health.

3. For the past several years, the pharmaceutical company manufacturers of vaccines have developed alternative preservatives to thimerosal, which have shown efficacy and safety.

4. Gradually, all of the developed countries (United States, European Community, Eastern Europe countries) have withdrawn thimerosal from their vaccines for human beings. Those that have done so previously show incidence and prevalence rates of child neurodevelopmental disorders that are significantly lower in relation to those that have recently prohibited it and also, evidently, in relation to the countries like ours, in which this toxin has not yet been abolished.

5. Recently, numerous scientific investigations have been conducted that have contributed multiple epidemiological, genetic, metabolic, biochemical, clinical and therapeutic evidence, both in human beings and in animal models, that implicate thimerosal and living measles, rubella and mumps contained in pediatric vaccines, as causal agents, triggering or aggravating child neurodevelopment disorders.

6. Recently, it has been reported, through the use of diverse data sources, that the withdrawal and/or reduction of thimerosal in pediatric vaccines has been followed by a significant decline in the incidence of new cases of child neurodevelopmental disorders, including autism and language disorders.

7. The quantities of organic Hg, still contained in some of the vaccines that are used in pediatric immunization programs in Peru, widely and significantly exceed the margins for maximum permitted exposure to organic mercurials from all of the public health agencies in the world, including the World Health Organization itself.

8. Having presented the evidence in relationship to thimerosal and child neurodevelopmental disorders, we request to establish the legal framework and all of the financial and health conditions in order to withdraw thimerosal contained in some of the vaccines for human beings in Peru, in the most expedient and pressing way possible, and having as well making every necessary effort to minimize the environmental exposure to other mercurial sources and toxic chemical agents, given the profound significance to the health of our children.

9. We request the urgent announcement for a meeting of experts, presided over by MINSA, in order to reassess the Expanded Program for Vaccinations of Peru, in which, in accordance with our current epidemiologic necessities, the policies for the most effective vaccination are discussed, but at the same time safer, in which we consider separately the immunization against measles, rubella and mumps, or in any case, the use of acellular vaccines for these illnesses.

10. Children and pregnant mothers constitute the most exposed segments to these illnesses. The State must assume its responsibility in the duty toward the care of their lives and health, since they represent our most precious human resource, on which the future of our nation depends.

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Correspondence:

Luis Alberto Maya Pérez.
Av. Javier Prado Este 3801, Santiago de Surco.
Lima 33, Perú.
E-mail: mayapuy@qnet.com.pe