AN INVESTIGATION INTO THE SUPPOSED LINK BETWEEN THIMEROSAL-CONTAINING VACCINES AND AUTISM

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Abstract

Thimerosal, a mercury-containing preservative used in vaccines, was implicated in 1997 to be linked to autism. Recent in vitro studies suggest that the concentration of thimerosal, and subsequently ethyl mercury, present in vaccines is not cytotoxic. A paucity of studies done specifically with thimerosal has led lawmakers to base regulations on the toxicological profile of methyl mercury. However, recent in vivo studies show a marked difference in the toxicological profiles of ethyl mercury, a metabolite of thimerosal, and methyl mercury. Neither in vitro nor in vivo studies, then, implicate thimerosal as a toxic agent at the concentrations present in vaccines. Epidemiological studies have been conducted in various countries assessing if a causal relationship exists between autism and thimerosal-containing vaccines. Though there is a concern that the ethyl mercury in thimerosal may cause neurotoxicity, which may lead to autism, large scale epidemiological studies have not been able to support this claim. In 2004, after critical review of major epidemiological studies, the Institute of Medicine's Immunization Safety Committee released a report stating that a relationship between autism and mercury was not found. The media, however, (mainly small, local sources) have disproportionately covered stories supporting a link, taking advantage of the desperation felt by those personally affected by autism in order to increase sales. This, in turn, has caused many parents, especially parents of autistic children, to be hesitant to vaccinate their children. In order to address this, thimerosal was removed from all vaccines in the US market. While not justified in terms of reducing the risk of autism, the removal of thimerosal was justified in that it ensures that mislead parents will continue to vaccinate their children.

Autism is a neurological disorder that is characterized by deficits in verbal and nonverbal communication, social interaction, and repetitive behaviors or interests. Autism technically consists of five disorders with varying severity—termed autism spectrum disorders—and is now estimated to affect as many as one in every 166 children. While research continues to investigate this disorder, which was first identified in 1943, as of now there are no known causes of autism and no consistent finding as to the changes that occur in the brains of autistic patients. Because of this, diagnosing and treating autism are challenging endeavors.[1]

The Food and Drug Administration (FDA) Modernization Act of 1997 prompted a review of mercury-containing drugs by the FDA. This review lead to a concern for the usage of thimerosal-containing vaccines, revealing that certain vaccines used since 1991 contained ethyl mercury levels that, for a six-month-old infant, were higher than the Environmental Protection Agency (EPA) standard set in 1995. Due to the neurotoxicity known to be caused by methyl mercury,[2] a concern arose for the possibility of a causal relationship between ethyl mercury poisoning in children under two years of age who were receiving thimerosal-containing vaccines.[3] This has raised concern among the general population for the potential of thimerosal-containing vaccines as being instrumental in producing the developmental defects observed in autistic children. However, given the low doses of mercury present in vaccines, subsequent in vitro, in vivo, and epidemiological studies do not corroborate the claim.

History of Thimerosal and Its Toxicological Mechanism

The mercurial compound added to vaccines is sodium ethylmercurithiosalicylate, also known as thimerosal. The structure of thimerosal is shown in Figure 1. Thimerosal is first metabolized to

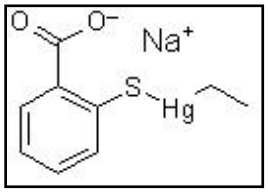

Figure 1: Thimerosal[4]

ethyl mercury and then inorganic mercury. The inorganic mercury is found to preferentially build up in the brain and kidneys of animals and humans at high doses. When added to vaccines, thimerosal is found to challenge the growth of bacteria and fungi.

A review article written by Leslie Ball nicely outlines the history of thimerosal use in childhood vaccines.[5] Ball claims public health disasters in the early 1900s confirmed the need for preservatives in vaccines. In 1916 the administration of Typhoid vaccine, contaminated with the bacteria *Staphylococcus Aureus*, led to four fatalities and 60 cases of morbidity in South Carolina.[6] In 1928, vaccinations with diphtheria toxin-antitoxin vaccine, contaminated with *Staphylococcal*, resulted in 12 of 21 children given the vaccine from the same vial dying. In light of these and other events, the United States introduced thimerosal as a preservative in multi-dose vials of vaccine in the 1930s. Few high-dose toxicity studies were done before the mass distribution of thimerosal-infused vaccines. The studies were conducted in rabbits, rats, mice, dogs, and guinea pigs.[7] The animals were given a 1% intravenous dose of thimerosal and observed for seven days. The maximum tolerated doses were found to be 20 mg/kg in rabbits and 45 mg/kg in rats. Autopsies of the dogs revealed no major tissue changes. The guinea pigs underwent severe pain after administration of a 0.1% dose, but no pain was seen with 1/4000 and 1/8000 dose dilutions. No control animals were listed in the study. The subject was revisited in 1971, when Fisher rats were given doses of thimerosal between 30 and 1000 µg/Kg for one year. The dosed rats were found to exhibit a dose-dependent incidence of bronchopneumonia. However, quantitative data were only collected in the high-dose range.

Studies attempting to define the toxicity of thimerosal at low doses are a recent phenomenon, and therefore data is still sparse. Current health regulations are based on the toxic

effects of methyl mercury, a related compound with a supposedly similar toxicological profile as ethyl mercury.

The differences between methyl and ethyl mercury uptake and metabolism highlight the need for more research to be done on ethyl mercury so that thimerosal can be regulated more effectively. A study done in 1985 on the comparative toxicology of methyl and ethyl mercury in animals demonstrates that the metabolism of ethyl mercury, a metabolite of thimerosal, leads to higher concentrations of inorganic mercury in the blood, brain, and kidneys of rats than does an equivalent dose of methyl mercury.[8] Weight loss and nephrotoxicity (renal damage) were greater in rats dosed with ethyl mercury. However, the neurotoxic effects of ethyl and methyl mercury were similar at high-doses around 10mg/kg. The study also showed that clearance rates of ethyl mercury were faster than methyl mercury; thus, the total time cells were exposed to mercury was less in the case of ethyl mercury.

A newer study, done in 2004 at the National Institutes of Health, suggests a significant difference between the pharmacokinetics of methyl and ethyl mercury and different routes of administration.[9] Less of the dose containing ethyl mercury or thimerosal reached the brain than did the dose of containing methyl mercury. Furthermore, "intramuscular injections resulted in lower total mercury concentration in the blood, brain, and kidney as compared to oral administration…The percent of mercury that reached the brain was significantly less in the mature animal as compared to the young." Comparing oral methyl mercury exposures to intramuscular ethyl mercury exposures, then, may not be permissible.

An in vitro study exposing human neuroblastoma and glioblastoma cells to 2.5 – 20 μM/L of thimerosal produced results similar to a study exposing neural cells to methyl mercury, suggesting a similarity in mechanism at the cellular level.[10] The study, completed at the University of Arkansas, proposes that the intracellular defense against inorganic mercury is

glutathione (GSH), a molecule made of cysteine, glycine, and glutamic acid. The sulfhydryl group on cysteine binds the inorganic mercury and prevents it from binding to sulfhydryl groups on cellular proteins. It is thought that cytotoxicity results from inorganic mercury binding to proteins and decreasing their stability. Neural cells do not synthesize cysteine because they do not have the key enzyme cystathionine gamma lyase, which is instrumental in the production of glutathione. Thus, neural cells depend on the liver for producing cysteine. If levels of GSH are low, the unbound inorganic mercury is free to engage with proteins. Incubation of glioblastoma and neuroblastoma cells with thimerosal show a decrease in cell viability with an increase in dose, from 2.5 to 20 μM. A dose of 10 μM thimerosal resulted in 50% cell viability in comparison to control cells. Neuroblastoma cells were found to be more sensitive to thimerosal than glioblastoma cells. Overall, this study confirmed the similarity between methyl mercury and ethyl mercury in neural cells at the cellular level. The concentrations of thimerosal used in the study were significantly higher than those used in vaccines, therefore the study did not investigate the effect of low-doses of thimerosal on neural cells.

A study done at Baylor College of Medicine shows the effect of nanomolar concentrations of thimerosal on human cortical neuronal cells and human fibroblasts.[11] After six hours of incubation with 2 μM of thimerosal, 11% of the cortical neurons had compromised cellular membranes, and after six hours of incubation with 250 μM of thimerosal, 100% of the cortical neurons had compromised cellular membranes. Cells with compromised cell membranes were also found to have DNA damage (measured using a TUNEL assay). Incubation time was increased from six hours to 24 hours for concentrations below 2μM. No DNA damage was seen at concentrations of thimerosal below 1μM (125, 250, and 500 nM) after 24 hours of incubation. Apoptotic morphology, assessed under a fluorescent microscope qualitatively, was seen after six hours of incubation in 2μM of thimerosal and after 24 hours of incubation in 1μM of thimerosal.

Caspase-3, a marker of apoptosis, was also present in 20% of the cells incubated in 2 μM and 97% of the neurons incubated in 250 μM of thimerosal. Overall, this study implicates concentrations of 1 – 250μM of thimerosal in neural toxicity. However, the study does not extend incubation time beyond 24 hours. Just as the toxic effects that were seen at 2μM of thimerosal after six hours were not seen at the two-hour mark, it is possible that neurons incubated with concentrations of thimerosal below 1 μM may need to be incubated for more than 24 hours to observe cytotoxicity.

In vivo studies show that ethyl mercury is able to cross cell membranes and get converted to inorganic mercury intracellularly.[12] The buildup of inorganic mercury occurs preferentially in the brain and kidneys. A study done at the NIH shows the differential uptake in methyl-, ethylmercury, and thimerosal in postnatal mice (see Table below).[13]

Table 1
Mercury distribution in PND 16 mice

	Blood	Brain			Kidney			Muscle		
	μgHg/g tissue	μgHg/g tissue	% Dose	Tissue/ Blood	μgHg/g tissue	% Dose	Tissue/Blood	μgHg/g tissue	% Dose	Tissue/ Blood
Methylmercury (MeHg)										
[0,1-11](17.4 μg mercury oral)										
24 h	17.9 ± 1.0	16.1 ± 1.2	1.5 ± 0.1	0.9 ± 0.1	64.9 ± 6.3	2.7 ± 0.3	2.7 ± 0.3			
7 days	7.8 ± 2.1[a]	14.4 ± 0.9	1.5 ± 0.1	2.2 ± 0.4	57.3 ± 0.9	3.7 ± 0.4[a]	3.7 ± 0.4[a]			
[0,1-11](17.4 μg mercury IM)										
24 h	2.5 ± 0.3[a]	5.6 ± 1.3[b]	0.6 ± 0.1[b]	1.0 ± 0.2	25.2 ± 5.6[b]	1.1 ± 0.3[b]	5.2 ± 2.1[b]	9 ± 1.4	0.7 ± 0.1	1.6 ± 0.3
7 days	2.1 ± 0.3[a,b]	4.2 ± 0.5[a]	0.5 ± 0.1[b]	2.2 ± 0.5[a]	27.2 ± 3.3[b]	2.2 ± 0.3[a,b]	13.4 ± 2.0[a,b]	2.4 ± 0.2[a]	0.4 ± 0.03[a]	1.30 ± 0.3
Ethylmercury (EtMg)										
[0,1-11](6 μg mercury IM)										
24 h	2.5 ± 0.5	0.9 ± 0.2	0.4 ± 0.06[c]	0.4 ± 0.1	18.8 ± 3.0	3.5 ± 0.6[c]	9.4 ± 2.7[c]	3.2 ± 0.6	1.4 ± 0.3[c]	1.7 ± 0.6
7 days	0.5 ± 0.1[a]	0.70 ± 0.2	0.2 ± 0.05[c]	1.7 ± 0.4[a]	8.7 ± 1.6[a]	1.6 ± 0.3[a,c]	28.3 ± 12.2[a,c]	0.5 ± 0.1[a]	0.2 ± 0.02[a,c]	1.3 ± 0.4
Thimerosal										
[0,1-11](15.4 μg mercury IM)										
24 h	4.2 ± 0.3	1.7 ± 0.2	0.22 ± 0.04[c]	0.4 ± 0.06	28.9 ± 4.2	1.7 ± 0.3	6.8 ± 0.9	5.4 ± 0.8	0.7 ± 0.2[c]	1.30 ± 0.2
7 days	1.5 ± 0.1	1.8 ± 0.3	0.17 ± 0.03[c]	1.2 ± 0.2[a]	17.8 ± 2.2	1.2 ± 0.1[c]	11.8 ± 1.5[c]	1.1 ± 0.1[a]	0.15 ± 0.02[a,c]	0.7[a] ± 0.1

Values represent mean ± S.D. ($n = 6$). Values were <0.002 μgHg/g brain and <0.01 μgHg/g kidney. Calculated on the basis of total mercury in tissue/total mercury administered × 100. Actual amount of mercury per 2 μl injection volume was used for calculations.
[a] Indicates statistical significance ($p < 0.05$) relative to 24 h within each dose group.
[b] Indicates statistical significance ($p < 0.05$) relative to oral MeHg.
[c] Indicates statistical significance ($p < 0.05$) relative to IM MeHg as determined by Fisher's LSD following significant overall ANOVA. Limited to % dose only.

This dataset has several implications. First, it shows how the distribution of inorganic mercury in the postnatal mouse varies. Second, it demonstrates that oral administration of methyl mercury results in much greater accumulations of mercury than does the intramuscular administration. Additionally, it is evident that once mercury reaches the tissues, it is cleared at a

very slow rate. Finally, the data confirm previous findings that mercury situates itself mainly in the kidneys and brain. This dataset also varies from that of adult mice, suggesting that the uptake and metabolism of mercury differs in adult and postnatal mice. These results have strong implications for the regulation of thimerosal in vaccines. This study focuses on mercury distribution, and does not examine the effect of low doses of thimerosal on developing mice.

No extensive toxicological studies have been done using humans. Cases of accidental methyl mercury poisoning in Iraq, Japan, and the Ferroe Islands suggest that high doses of mercury are toxic to humans. "Maternal methyl mercury exposure in these epidemics was associated with neurological abnormalities, such as delays in motor function, among children exposed in utero."[14] However, the differences between methyl mercury and thimerosal and the differences between oral and intramuscular doses make it difficult to extrapolate from such case-studies to the effect of vaccines containing thimerosal on young children.

Overall, the toxicological data from in vitro and in vivo studies suggest that thimerosal and ethyl mercury, at the concentrations found in vaccines, are not toxic. The in vitro study done at Baylor College of Medicine implicated 1 µM (405 µg/l) to 250 µM (101mg/l) of thimerosal as inducing toxic effects, such as compromised cell membranes and apoptotic morphology.[15] However, during vaccination, children receive at most 403 µg of thimerosal, which is equivalent to 200 µg of mercury (thimerosal is roughly 49% ethyl mercury). At the 5^{th}, 50^{th}, and 95^{th}% of female weight at birth, the Baylor group contends that 200/3.81 = 52 µg/kg, 200/5.22 = 38 µg/kg, and 200/6.27 = 32 µg/kg dose of mercury is administered, respectively. The 1 µM dose is claimed to be "less than four times higher than some of these estimated concentrations." Few other studies have sought to determine the effect of thimerosal at low doses because most cellular changes were only seen at higher doses. Thus, the studies focused more on mechanisms than determining a safe threshold.

The in vivo studies show the distribution of mercury in animal bodies. The accumulation of inorganic mercury in the brain and kidneys has been confirmed by several in vivo studies. Similar to the in vitro studies, few in vivo studies have been done to study the safe threshold level of thimerosal in animals. Human cases of methyl mercury poisoning show that toxic effects occur at 3mg/kg. However, given the differences in methyl mercury and thimerosal metabolism, this number has little significance to the issue of childhood vaccinations. Although toxicological data have not yet been able to determine a concrete threshold level, below which no toxic effects occur, it is most likely true that the concentration of thimerosal found in vaccines is not toxic according to data presented thus far. Epidemiological data also suggest that the levels of thimerosal found in vaccines do not contribute to toxicity.

Epidemiological Studies

Following the discovery that the ethyl mercury dose in certain thimerosal containing vaccines administered to infants exceeded the 1995 EPA standard, the scientific community followed suit by performing various epidemiological studies. The uproar in the concern for thimerosal-containing vaccines and their possible association with autism was due to a link made with the neurotoxic effects of another mercurial compound, methyl mercury. However, major studies with varying population sizes, designs, controls, and locations have not been able to show that a causal relationship exists.

In 2003, an ecological study was released by Madsen et al. evaluating the incidence of autism from 1971 to 2000 from Danish population based data, where vaccine coverage has been greater than 90% since 1979. Thimerosal-containing vaccines administered to children were

Figure 2. Results of Madsen et al. study

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Figure 1 from Madsen et al.

used in Denmark from the 1950s until 1992. From 1961 to 1970 the thimerosal-containing diphtheria-tetanus-pertussis vaccine was administered in four doses at ages 5, 6, 7 and 15-months; this means that each child who was administered all four doses received 400 μg of thimerosal (200 μg of ethyl mercury). From 1970 to 1992 the thimerosal-containing whole-pertussis vaccine was administered in three doses at ages 5-weeks, 9-weeks, and 10-months; this means that each child who was administered all three doses received 250 μg of thimerosal (125 μg ethyl mercury).

The hypothesis of the Madsen et al. study is that if thimerosal-containing vaccines and autism are correlated, then the incidence of autism among the population of interest would decrease following the removal of thimerosal-containing vaccines. The cases observed (958) are obtained through the Danish Pediatric Central Research Register and consist of children between the ages of 2 and 10-years-old diagnosed with autism.[16]

The study measures the annual incidence of autism by differentiating by age and gender. The first day that symptoms of autism were recorded is referred to as the point time for each autistic case. Each calculation of incidence was done by taking the number of people with their first diagnosis of autism in each age band for each year (see Figure 2) and divided by the total

number of people in Denmark within that age band and year. A time series analysis of incidence of autism per 10,000 people of the population at risk was generated and compared with the key dates concerning thimerosal usage.[17]

The results of the study (see Figure 2) revealed interesting findings. During the period of 1970 to around 1990, when thimerosal vaccines were administered throughout the country, the incidence of autism remained relatively constant. The incidence began to rise around 1991 and continued to do so even after the gradual removal of thimerosal-containing vaccines after 1992. As Madsen et al. discuss, the increase in incidence may be attributable to a shift in the criteria requirements for diagnosis of autism, from the International Classification of Diseases (ICD)-8 to the more recent ICD10 as well as a growing awareness for autism. Moreover, Madsen et al. recognize that incidence rates may have shown an increase after 1995 because the methodology of the ecological study does not account for the inclusion of outpatients (prior to 1995, only inpatients were included in the registry) who were labeled as "first diagnosed" for a certain year but actually were probably diagnosed outside of the registry earlier than that.[18]

A prospective study was released in 2003 by Hviid et al. that included the entire population of Denmark born from January 1, 1990 until December 31, 1996 in its study attempting to determine if a causal relationship exists between thimerosal-containing vaccines and autism. The study consists of a total population size of 467,450 people. The aim of this particular study was to determine the rate ratio of autism cases among two cohorts: one

Table 1. Results of Hviid et al. study

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See Hviid et al.

consisting of children who received the thimerosal-containing whole-cell pertussis vaccine and the other consisting of children who received the same vaccine but without thimerosal. This particular type of study is possible because of the whole-cell pertussis vaccination history of Denmark. Until late March 1992, all three doses of the whole-cell pertussis vaccine combined contained 250 μg of thimerosal (125 μg of ethyl mercury), and after this the thimerosal-free version of the vaccine was used until January 1, 1997. Hviid et al. also attempts a dose-response analysis in children with respect to thimerosal by comparing cohorts differentiated by the number of doses of whole-cell pertussis vaccine actually received by the children.[19]

The Hviid et al. study consists of a total sample size of 467,450 people. Information on the autistic spectrum disorder diagnoses was obtained from the Danish Psychiatric Central Register. The researchers breakdown of the population according to who received the whole-cell pertussis vaccine and which type, and the number of thimerosal-containing vaccinations received can be viewed in Table 1. The results of the study did not reveal a significant difference in the rate of autism among a population of children who received the thimerosal-containing whole-cell pertussis vaccine and those who did not. The rate ratio fully adjusted for age, calendar period, child's sex, location of birth, birth weight, etc (see Table 1) was 0.85 (95% CI: 0.60-1.20) and 1.12 (95%CI: 0.88-1.43) for autism and other autistic spectrum disorders, respectively. In addition, a trend was not observed that revealed a dose-response relationship between autism and thimerosal (which was calculated as the increase in the rate ratio per 25 μg increase in ethyl mercury). Hviid et al. also found a statistically significant increase in the incidence of autism and other autistic spectrum disorders from 1990 to 1996, with rate ratios of 1.24 (95% CI: 1.17-1.31) for autism and 1.21 (95% CI: 1.16-1.27) for other autistic spectrum disorders.[20]

The study evaluated various biases that may have affected the outcome of the study. After readjusting the period for which the whole-cell pertussis vaccine was considered to be free

of thimerosal to correct for any stray thimerosal-containing vaccines that may have been administered after thimerosal was "officially" removed from vaccines, the rate ratios for both autism and other autistic spectrum disorders did not change much; 0.87(95% CI: 0.61-1.23) and 1.15 (95% CI: 0.90-1.47), respectively. A dose response was again not observed. Furthermore, the study attempted to increase its robustness by using a more homogenous sample cohort by limiting the study to children born between 1991 to 1993. Again, similar rate ratios resulted, and no dose-response relationship was observed.[21]

There are a few notable factors that may have affected the outcome of this study. It is important to note, as with the Madsen et al. study, the different criteria used to classify autism throughout the study period. From 1991-1993, the IOD8 was used, and from 1994 to 2000, the IOD10 was used by the child psychiatrists who made the diagnosis of each autism case. In addition, the date of diagnosis of autism for each case may vary significantly from the actually onset of symptoms because of the lengthy process involved in diagnosing autism.

The Wakefield et al. study of 1998 brought much attention to autism among both the academic and general population with its suggestion of an association between "autistic enterocolitis" and the MMR vaccine. Though the MMR vaccine never contained thimerosal, the study of its relationship with autism is very similar to that of thimerosal-containing vaccines and is important in trying to determine if causality exists between vaccines and autism in general, since epidemiological studies for thimerosal and autism have not shown causality. Wakefield et al. proposed causation of autism by the administration of live measles virus. Though conclusions made by the Wakefield et al. study received much attention from concerned parents (similar to that of the thimerosal and autism situation), the study has not been supported by the majority of epidemiological studies that followed by it. In addition, though the Wakefield et al. study discusses much about possible links between autism and gastrointestinal diseases, it did not

mention explicitly where or how MMR vaccines factor into this, only that "the onset of behavioural symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children."[22] An important note to make is that 10 of the 12 original authors of the Wakefield et al. publication retracted their claims in the article:

> We wish to make it clear that in this paper no causal link was established between MMR vaccine and autism as the data were insufficient. However, the possibility of such a link was raised and consequent events have had major implications for public health. In view of this, we consider now is the appropriate time that we should together formally retract the interpretation placed upon these findings in the paper, according to precedent.[23]

Studies following the publication of the Wakefield et al. study suggest that the timing of the MMR vaccination and the onset of autism are merely a coincidence, and the relationship suggested in the Wakefield et al. study has not been supported.[24]

In June of 1999 *The Lancet* published an epidemiological study by Taylor et al. The main goal of the study was to analyze the trends in the incidence of autism before and after the introduction of the MMR vaccine in October of 1988. The results of the study suggest that a "step-up" in incidence of autism did not occur after the introduction of the MMR. The study consisted of the evaluation of 498 cases of autism, and the experimental subjects were children with autism born since 1979, from eight health districts of North Thames, UK. The 498 cases were identified as core autism (261), atypical autism (166), or Asperger's syndrome (71). The autistic diagnosis of each case was checked against the criteria set by the ICD10 for the ages between 18-months and 3-years.[25]

The evaluation of the variables in the study consisted of a Poisson regression of autism cases from 1979 to 1992 to analyze trends in the time series of cases. The second analysis consisted of comparing the age of diagnosis for autistic children who did receive the MMR vaccine and those who did not. This was done by splitting the cases up into three categories

(with the effect of each birth cohort controlled for): children who received the MMR vaccine before the age of 18 months, children who did not receive the vaccine, and children who received the vaccine after 18 months of age. As a correction factor for this analysis, the MMR vaccine coverage was shown to not differ significantly between the cases evaluated and the entire birth cohort of the North East Thames region. The third analysis consisted of a case-series method, which does not involve a control group. Three variables were analyzed in order to assess a possible temporal association between autism and the MMR vaccination: the age of diagnosis of autism, the recorded age of parental concern, and the onset age at regression (the point at which behavioral retardation begins).

The time series analysis revealed a statistically significant upward trend in core autism and atypical autism and a "nearly significant" trend for Asperger's syndrome. A "step-up" in autism cases was not evident in or after 1987. The study also did not find a significant difference of age at which diagnosis occurred upon evaluation of autistic children who were vaccinated before 18 months (233/346 cases), who were never vaccinated (64/356 cases) and who were vaccinated after 18months of age (59/356). Concerning the onset of autism within 1 or 2 years after vaccination of MMR, there was no temporal association detected, with the relative incidence being 1.09 (0.79-1.52) and the control being 0.94 (95% C.I: 0.60-1.47)

There were two major limitations to the study. The first was that there was wide variation in the quality of the clinical notes involved with each diagnosis of autism, which limits the accuracy of the study. Taylor et al. used these clinical notes to evaluate the point reference signifying the time of diagnosis. Another limitation of the study is that it does not determine whether prevalence of autism increased over the period of study, though it does suggest an increase in incidence.[26]

The epidemiological studies conducted following the FDA review of thimerosal-containing vaccines have not shown that a causal relationship exists with autism. A critical analysis of autism and thimerosal studies by the Institute of Medicine (IOM) resulted in the release of the eighth and final report in 2004 stating that no causal relationship exists between thimerosal-containing vaccines and autism. In addition, a causal relationship between the childhood MMR vaccine and autism has not been supported by epidemiological data as well, supporting further that some environmental agent outside of vaccines is perhaps the cause for the notable increase in incidence of autism over the years. However, it is important to note that incidence measurements of autism may be on the rise due to better awareness of autism and therefore higher rates of diagnosis over time. Though these epidemiological studies have thus far strongly favored that a causal relationship does not exist between thimerosal-containing vaccines and autism, the concern of parents of autistic children on this issue has not waned over the years, largely due to influences of the media and the lack of knowledge of the actual causes of autism.

Media Coverage and Public Opinion

In an online survey conducted in February of 2005 by MSNBC, over 88,000 people voted for what they considered to be responsible for the recent rise in autism cases. From the choices, which included, among others, "more awareness," "better diagnosis," and "genetics," 22% of the respondents selected "childhood exposure to mercury or other toxins," making it the second most popular selection behind only "a combination of factors."[27] Despite the epidemiological evidence just discussed that suggests otherwise, a large portion of the population (or at least a large portion of the population that would be looking at MSNBC's online autism coverage—arguably parents and other persons affected by autism) believes that mercury exposure is

responsible for the rise in autism cases. If the evidence suggests that there is no connection between mercury and autism, how and why are the people most affected by autism believing otherwise?

To answer this question one must look at the media coverage of the autism epidemic. There are two purposes a medium can choose to fulfill: informing its audience or making as large a profit as possible. Recognized national media sources tend, more times than not, to provide objective views of issues by presenting all the facts. These sources, however, have such strong reputations that perhaps they do not need to concern themselves as much with profits. Smaller sources, on the other hand, such as local newspapers, obviously have to focus more on making profits, and their purpose, therefore, may not always be to provide the most informative stories but rather to provide the stories that will sell best.

With this said, both of these types of media have covered the issue of autism and its possible link to mercury; with the number of autism cases soaring within the past couple of decades, audiences have been intrigued by this mysterious syndrome and have demanded media coverage. The contrasting ways by which the two types of media sources have covered the issue are striking.

Recognized national media sources such as CNN and MSNBC have covered stories of autism in a relatively objective manner. MSNBC's recently aired special on autism featured a specific segment on *Nightly News with Brian Williams* addressing mercury's potential cause-and-effect relationship with autism. The segment featured two scientists who held contrasting views—Dr. Marie McCormick of Harvard University, whose research strongly suggests that there is no link, and Dr. Richard Deth of Northeastern University, who believes that a link does indeed exist. Both sides of the argument were presented. Since the scientific community, however, is not split 50-50 on this issue, the segment also emphasized that scientific evidence

strongly points to there not being a connection between mercury and autism.[28] The purpose of this media coverage was clearly to inform.

Smaller media sources, on the contrary, have disproportionately covered stories suggesting a link between mercury and autism despite the overall belief of the scientific and medical communities. This is because stories against connections between illnesses and supposed causes are not nearly as interesting to the general public as stories supporting connections. While there are an infinite number of things that *do not* cause a particular illness, something suggesting that a specific agent causes an illness is rare and therefore captivating.

Moreover, the main market in terms of autism coverage is the parents and other relatives of autistic children. This audience is not only interested in there being a connection between autism and a specific agent, they are in some ways desperate for a connection. As of now there are still no known causes of autism; in fact, the scientific and medical communities still lack a basic understanding of the syndrome itself, let alone its cause or causes. This almost complete lack of knowledge has left parents with many questions for which there just are not any answers at this time. Many, therefore, are practically begging for stories that claim to provide an answer…any answer.

This is where profit-driven media come in. The media know that people whose lives have been affected by autism are tired of being led to dead ends and are instead anxious to hear possible answers. This is why mainly smaller media sources often cover stories supporting a link between mercury and autism. For example, the *Joplin Globe*, a local newspaper of Joplin, Missouri, featured an article on the front page of its Sunday edition on October 24, 2004 telling the story of Alan and Lujene Clark, who firmly believe that mercury in a flu vaccine triggered their son Devon's autism. The Clarks, according to the article, are now devoted to spreading awareness of mercury's cause-and-effect relationship with autism.[29] Stories like these—stories

that offer answers—are the ones that people are eager to hear about and are therefore the ones that will draw the largest profits (hence its placement on the front page of the Sunday edition).

Woo et al. addressed the influence media coverage has on the public perception of autism's causes, particularly the perception of parents with autistic children. Woo compared questionnaire responses by parents participating in the Vaccine Adverse Event Reporting System (VAERS) with responses by parents in the general population. VAERS, which was established in 1990 and is jointly managed by the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC), receives reports from a variety of sources concerning the effects of vaccines. Woo and her colleagues searched VAERS and identified adverse event descriptions that suggested autism. Utilizing a survey designed by Gellin et al.,[30] Woo questioned VAERS reporters about their impressions of vaccines.[31]

The study's results strongly suggest that parents of autistic children have less trust of vaccines than the general population. Only 38% of VAERS respondents agreed that they are "more likely to trust vaccines that have been around for a while", compared to 88% of the general population. In addition, only 7% of VAERS respondents agreed that "vaccines are always proven to be very safe before they are approved for use", compared to 71% of the general population. The results also suggest that the media more easily influence persons personally affected by autism. In response to the open-ended question, "What made you think that ___'s symptoms might be related to a vaccination?" 24.2% of VAERS respondents said magazine or newspaper and 19.4% said web or internet, making these the second and fourth most common reasons, respectively. Woo and colleagues discussed the implications of their findings:

> Faced with such a serious diagnosis as autism, parents naturally look for explanations in events, such as vaccinations, occurring just before the onset of autistic signs and symptoms…These findings illustrate that the media and the internet influence parental perceptions of vaccine safety and reinforce the need to develop fair and effective ways to communicate with the public about the benefits and risks of vaccines.[32]

Parents' desperation leaves them not only susceptible to unproven explanations but also susceptible to unproven treatments. As a side effect of the media's often unbalanced coverage of the autism epidemic and the potential link between autism and mercury, a market has been created for "detoxification treatment". Mary Ann Block, an osteopath in Hurst, Texas, and Dr. Kenneth Bock of the Rhinebeck Health Center in Rhinebeck, New York, for example, are treating autistic patients with chelation therapy—this involves injections of different chemicals that bind to metals and allow them to be removed from the body through the urine.[33] While the treatment is FDA approved for lead poisoning, no published clinical studies show that chelation is effective at treating autism. Bock, however, asserts that "there may be a subset of children that are more susceptible to mercury and therefore react this way in terms of the autism spectrum," and these children, he claims, will respond to chelation.[34] With no scientific evidence supporting the supposed effectiveness of the treatment, what *is* known is that chelation has serious side effects, including liver and kidney problems. Moreover, chelation is not cheap: initial assessments alone cost thousands of dollars (Block charges $2500), and then treatments and supplements end up costing families even more. Additional methods of detoxification include saunas, which some parents even have installed in their homes (such is the case of the Cravens, who spent $3,500 to install an in-home sauna to help remove metals from their two autistic sons).[35]

But parents are willing to ignore scientific data (or the lack thereof) if they are led to believe by convincing enough sources that what they are doing may help their sick children. Kacey Dolce, whose autistic son Hank is one of Block's chelation patients, said, "We don't know enough yet to say no…I'll do anything to help our child."[36] Clearly, some parents require sufficient data disproving a treatment before they will *stop* considering it, as opposed to

requiring sufficient data supporting a treatment before they will *start* considering it. This allows many people to capitalize on media coverage and profit from parents' desperation.

So long as the causes of autism remain a mystery, parents' desire for answers will continue to leave them easily impressionable. Capitalizing on heresy, misleading media sources will inevitably continue to profit from parents' susceptibility and by doing so will continue to shape public opinion.

Government Regulations

Although ethyl mercury is the form contained in thimerosal, no guidelines exist specifically for ethyl mercury exposure. Rather, guidelines for methyl mercury, which has different toxicological mechanisms, as was previously discussed, are used for ethyl mercury as well. The EPA has set a limit of 2 parts of methyl mercury per billion parts of drinking water (2 ppb); the FDA has set a maximum permissible level of 1 part of methyl mercury per million parts of food (1 ppm); and the Occupational Safety and Health Administration (OSHA) has set limits of 0.1 milligrams of organic mercury per cubic meter of workplace air (0.1 mg/m^3) and 0.05 mg/m^3 of metallic mercury vapor for 8-hour shifts and 40-hour work weeks.[37] The EPA's guideline, which is the most conservative, is designed to serve as a warning to trigger additional investigation, while the FDA guideline is designed as a safe limit for long-term consumption of food contaminated with mercury (particularly fish).[38] Each of these regulations was in place prior to when the supposed association between mercury in vaccines and autism was discovered, and none address the issue of intramuscular exposure.

When the FDA's Center for Biologics Evaluation and Research (CBER) began reassessing the safety of mercury in vaccines in April 1998, they determined that thimerosal was

present in over 30 licensed vaccines in the US. Looking at cumulative exposure over the first six months of life, an infant who received all recommended vaccines on schedule could be exposed to up to 187.5 μg of mercury. Adjusting for average weight at various percentiles in infants between birth and six-months of age, this exposure exceeded the EPA guideline for methyl mercury.[39] The EPA guideline, however, is based on oral exposure, which has different toxicological effects than exposure through vaccination, as was discussed. Nonetheless, this prompted more research, which eventually led to the release of a joint American Academy of Pediatrics/US Public Health Service statement on thimerosal, in which it was recommended—as a precautionary measure—that thimerosal be removed from vaccines. The statement mentions, however, that the risk associated with the diseases that vaccines are designed to prevent is much higher than the risk associated with thimerosal, so until thimerosal is removed from all vaccines parents should still vaccinate their children.[40] At this time, with the exception of influenza vaccines (thimerosal-free influenza vaccines are approved for use in children 6 to 35 months of age),[41] all routinely recommended pediatric vaccines that are manufactured for the US market contain no thimerosal or contain significantly reduced amounts (e.g., trace amounts) of thimerosal.[42] In order to remove thimerosal, single-dose vials, as opposed to the previously used multi-dose vials, are now used to store vaccines, greatly reducing bacterial contamination.[43]

The process of removing thimerosal from vaccines and the production and storage of single-dose vials are costly alternatives to leaving the preservative in vaccines. Because the toxicological and epidemiological data strongly suggest that there is no link between thimerosal in vaccines and autism, the authors of this paper feel that the precautionary measure of removing thimerosal was unnecessary. Taking into account, however, the effects that media coverage have on the public, particularly parents of autistic children, not removing thimerosal may have resulted in parents refusing to vaccinate their children, as was suggested by the responses in the

Woo et al. study. Since it was plausible for thimerosal to be removed from vaccines manufactured for the US market, the authors agree that the measure was justified in that it ensures that parents will continue to vaccine their children.

For countries in which the removal of thimerosal would be too great a financial burden, the authors feel that the removal is not justified. Persons living in countries that cannot afford alternatives to thimerosal are most likely not as frequently exposed to the media as citizens of the US. This means that they are less likely to not vaccinate their children because of misleading media coverage, which is the only reason the authors feel the measure was justified in the US.

Conclusion

Upon review of mercurial-containing drugs by the FDA in 1997, the discovery that certain thimerosal-containing vaccines administered to infants contained more ethyl mercury than the 1995 EPA standard was made. This became an issue of concern and interest to the scientific and medical communities because of the studies that have linked methyl mercury to neurotoxicity and its possible association with autism. The scientific and medical communities quickly responded by conducting a number of toxicological and epidemiological studies to assess the possible causal relationship between thimerosal-containing vaccines and autism. All these studies suggest that the low doses of ethyl mercury present in the thimerosal of vaccines are not toxic and that vaccinations containing thimerosal do not correspond with increases in autism. Nonetheless, parents and other relatives of autistic children, all desperate for answers, have made media coverage supporting a causal link marketable. This media coverage, in turn, has influenced public opinion, leaving many parents weary of vaccinations. Because of this, the measure of removing thimerosal from vaccines in the US was justified. The removal, however,

was not necessary for the prevention of autism, so nations that cannot afford to remove thimerosal from their vaccines should not make this process a priority.

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