

# A Case-Control Study of Mercury Burden in Children with Autistic Spectrum Disorders

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## ABSTRACT

Large autism epidemics have recently been reported in the United States and the United Kingdom. Emerging epidemiologic evidence and biologic plausibility suggest an association between autistic spectrum disorders and mercury exposure.

This study compares mercury excretion after a three-day treatment with an oral chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders and a matched control population. Overall, urinary mercury concentrations were significantly higher in 221 children with autistic spectrum disorders than in 18 normal controls (Relative Increase (RI)=3.15;  $P < 0.0002$ ). Additionally, vaccinated cases showed a significantly higher urinary mercury concentration than did vaccinated controls (RI=5.94;  $P < 0.005$ ). Similar urinary mercury concentrations were observed among matched vaccinated and unvaccinated controls, and no association was found between urinary cadmium or lead concentrations and autistic spectrum disorders.

The observed urinary concentrations of mercury could plausibly have resulted from thimerosal in childhood vaccines, although other environmental sources and thimerosal in Rh<sub>0</sub>(D) immune globulin administered to mothers may be contributory.

Regardless of the mechanism by which children with autistic spectrum disorders have high urinary mercury concentrations, the DMSA treatment described in this study might be useful to diagnose their present burden of mercury.

**KEY WORDS:** autism, autistic spectrum disorders, chelation, DMSA, mercury, thimerosal

## Background

Recent studies have analyzed the prevalence of autism from the mid-1980s through 2002 in the United States and the United Kingdom.<sup>1-5</sup> The prevalence of autism is estimated to have risen from one in about 2,500 children in the mid-1980s to as common as one in 150 by 2002. Further, since all of these studies find the prevalence of autism in males to be four times that of females, the male prevalence of this disorder exceeds one in 100. These studies show that the rise in the prevalence in autism is genuine and not the result of population migration, differences in diagnostic criteria, or other potential confounders.

In 2001, the Institute of Medicine (IOM) of the United States National Academy of Sciences<sup>6</sup> determined that a link between mercury from thimerosal contained in childhood vaccines and the recent dramatic increase in neurodevelopment disorders is biologically plausible. Recent studies demonstrate a strong epidemiologic

link between exposure to mercury from thimerosal contained in childhood vaccines and neurodevelopment disorders.<sup>7-9</sup>

The purpose of this study was to evaluate the concentration of mercury in the urine following a three-day treatment with an oral chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders in comparison to a control population. Forman et al.<sup>10</sup> have reported on the use of oral treatment with DMSA in children exposed to metallic mercury. The authors found that oral chelation with DMSA produced a significant mercury diuresis in these children. They observed no adverse side effects of treatment. The authors concluded that DMSA appears to be an effective and safe chelating agent for treatment of pediatric overexposure to metallic mercury. In addition, extensive literature supports its safety in the chelation of lead from exposed children.

## Methods

This study is a retrospective analysis of 221 consecutive children with previously established autism spectrum disorders referred and admitted to the International Child Development Resource Center (ICDRC). Each child had been diagnosed with autism (DSM-IV 299.00) or pervasive developmental disorder (DSM-IV 299.80) by outside physicians. A control population of 18 children was also identified without autism spectrum disorders in themselves or among their siblings or their first-degree family members. These healthy children presented to the ICDRC for elective determination of their levels of environmental mercury exposure at the request of their families, and are included here for case comparison. The Arizona State University Institutional Review Board approved our retrospective examination of cases and controls in this study.

All children were examined to exclude those who had dental amalgams. Among the 221 cases, all had received their full scheduled childhood immunizations appropriate for their respective ages. Among the 18 controls, 10 children had received their full childhood immunization schedules, and 8 children had received no childhood immunizations because of religious objections.

Informed consent was obtained from both cases and controls for DMSA chelation treatment. Controls and cases were both challenged with a three-day oral treatment of DMSA (10 mg/kg per dose given three times daily). After the ninth dose, the first voided morning urine was collected (when possible), or an overnight urine collection bag was worn. All laboratory analyses were performed by the Doctors' Data, Inc., in Chicago, Ill. The response to DMSA was measured as micrograms of mercury per gram of creatinine using inductively coupled mass spectrometry, and creatinine was measured using the Jaffe method. The laboratory was not informed whether the specimens were from cases or controls.

In addition to the overall excretion data, several epidemiologic case-control studies were conducted using the available populations. First, it was possible to match 88 cases against 16 controls for age (within one year) and sex, and overall post-DMSA urinary

mercury concentrations were determined. Second, it was possible to match 55 cases against 8 vaccinated controls for age, sex, and vaccine status, and overall post-DMSA urinary mercury, cadmium, and lead concentrations were determined. Finally, as epidemiologic controls, it was possible to match 5 each of vaccinated and unvaccinated controls for age and sex, and overall post-DMSA urinary mercury, cadmium, and lead concentrations were determined.

The statistical package contained in Excel® and SISA® was employed in this study. We determined means, relative increase (RI) in mean heavy metal excretion in cases compared with controls ( $\text{mean}_{\text{cases}}/\text{mean}_{\text{controls}}$ ), standard deviation, and statistical significance using a t-test. Our null hypothesis was that the populations under study should have similar distributions of excreted heavy metals, and we accepted a double-sided P-value of <0.05 as statistically significant.

Population Type	Number of Boys	Number of Girls	Mean Age in Years (Range)	Mean Urinary Mercury (mcg/g creatinine) (Range)
Cases	183	38	6.25 (3 to 15)	4.06 ± 8.59 (0 to 58.65)
Controls	14	4	8.85 (3 to 16)	1.29 ± 1.54 (0 to 6.2)

**Table 1.** Summary of 221 Cases and 18 Controls

## Results

Table 1 summarizes the number of males and females, mean age in years, and average mcg Hg/g creatinine after DMSA treatment among our 221 cases and 18 controls. Among our 221 cases the boy:girl ratio was 4.88:1, and among our 18 controls the boy:girl ratio was 4:1. Urinary mercury concentrations were significantly higher in cases than in controls (RI=3.15; P < 0.0002; 95% CI: 1.43 to 4.11).

In the first part of our case-control analysis, we determined the mean and standard deviation of the concentrations of urinary mercury in the 88 cases (5.45 ± 10.9 mcg Hg/g creatinine) and 16 age and sex-matched controls (1.45 ± 1.57 mcg Hg/g creatinine) after DMSA treatment. The urinary mercury concentrations were significantly higher in cases than in controls (RI=3.76; P < 0.002; 95% CI: 1.60 to 6.41).

The results of the second part of our case-control analyses are summarized in Table 2. We determined the mean and standard deviation of the urinary mercury concentrations in the 55 cases (6.42 ± 12.69 mcg Hg/g creatinine) and 8 age, sex, and vaccine-

	Heavy Metal Examined	Population Examined	Heavy Metal Level (mcg/g creatinine)
	Mercury	55 Cases	6.42 ± 12.69
	Mercury	8 Controls	1.08 ± 1.12
Statistical Assessment			Relative Increase = 5.9 P < 0.005 95% CI: 1.90 to 8.79
	Cadmium	55 Cases	0.48 ± 0.42
	Cadmium	8 Controls	0.36 ± 0.22
Statistical Assessment			Relative Increase = 1.3 P = 0.35 Not Significant
	Lead	55 Cases	18.2 ± 43.3
	Lead	8 Controls	11.8 ± 8.6
Statistical Assessment			Relative Increase = 1.5 P = 0.34 Not Significant

**Table 2.** Matched Cases and Controls for Heavy Metal Levels Following a 3-Day DMSA Treatment

status-matched controls (1.08 ± 1.12 mcg Hg/g creatinine). We determined that cases had a significantly higher urinary concentrations of mercury after DMSA treatment than did controls (RI=5.94; P < 0.005; 95% CI: 1.90 to 8.79). As shown in Table 2, both groups had similar urinary concentrations of cadmium and lead after DMSA treatment. Among our age and sex-matched healthy children, we determined that 5 vaccinated controls had similar urinary concentrations of mercury, cadmium, and lead after DMSA treatment compared with 5 unvaccinated controls, as is summarized in Table 3.

	Heavy Metal Examined	Population Examined	Heavy Metal Level (mcg/g creatinine)
	Mercury	5 Vaccinated Controls	0.70 ± 0.71
	Mercury	5 Unvaccinated Controls	1.98 ± 2.40
Statistical Assessment			P = 0.35 Not Significant
	Cadmium	5 Vaccinated Controls	0.42 ± 0.27
	Cadmium	5 Unvaccinated Controls	0.50 ± 0.27
Statistical Assessment			P = 0.65 Not Significant
	Lead	5 Vaccinated Controls	14.0 ± 10.1
	Lead	5 Unvaccinated Controls	16.1 ± 8.5
Statistical Assessment			P = 0.73 Not Significant

**Table 3.** A summary of a comparison of matched vaccinated and unvaccinated controls for heavy metal levels following a three-day DMSA treatment

## Discussion

This study shows a strong association between increased urinary mercury concentrations following three days of treatment with DMSA and the presence of an autistic spectrum disorder. The statistically significant association persists when vaccinated cases are compared with matched vaccinated controls. No association was found between post-DMSA urinary cadmium or lead concentrations and autistic spectrum disorders. Lastly, although the study populations were small, the heavy-metal concentrations measured in matched vaccinated and unvaccinated control children were small and showed no statistically significant differences in urinary mercury, cadmium, and lead concentrations following a three-day treatment with DMSA.

Previously, Stajich et al.<sup>11</sup> showed that newborn infants had significant (P < 0.01) several-fold increases in the blood concentrations of mercury during the 48 to 72-hour period following immunization with thimerosal-containing childhood vaccines, compared with pre-vaccination levels.

Pichichero et al.<sup>12</sup> examined the concentrations of mercury in the blood, urine, and stool 3 to 28 days following thimerosal-containing vaccines in 40 full-term infants of age 6 months and younger in comparison to 21 control infants receiving thimerosal-free vaccines. The mean mercury doses received by thimerosal-exposed subjects were 45.6 mcg (range 37.5-62.5) for 2-month-old infants and 111.3 mcg (range 87.5-175.0) for 6-month-old infants. Blood mercury concentrations in thimerosal-exposed 2-month-old infants ranged from less than 3.75 to 20.55 nmol/L; in 6-month-old infants, all values were lower than 7.50 nmol/L. Only 15 blood samples from controls contained quantifiable mercury.

Concentrations of mercury were low in the urine after vaccination but were high in the stools of thimerosal-exposed 2-month-old infants (mean 82 ng/g dry weight) and 6-month-old infants (mean 58 ng/g dry weight). The authors estimated that the blood half-life of ethylmercury was 7 days (95% CI 4-10 days). The study was unable to determine the ultimate disposition of most of the mercury with which infants were injected.

Our analysis shows that children who developed autistic spectrum disorders had significantly greater accumulated mercury than controls. Our results are similar to those of the retrospective study by Holmes et al.<sup>13</sup> They observed that there was a significant relationship between increasingly severe autism and decreasing mercury levels in first baby haircuts in comparison to normal controls. Our results and those of Holmes et al. probably result from a decreased ability of children with autistic spectrum disorders to excrete mercury, resulting in the retention of potentially toxic mercury levels.

Impaired sulphation is observed in autistic spectrum disorders, and this biochemical deficit, possibly a pre-existing genetic condition, may contribute to the observed mercury accumulation, since the normal mechanism of clearing mercury from the body is thought to involve the binding of mercury compounds to sulfhydryl groups.<sup>14</sup>

Mercury concentrations in the human brain are six times greater than the blood.<sup>15</sup> This stems from the fact that thimerosal contains the ethylmercury radical attached to the sulfur atom of the thiol group of salicylic acid. Generally, mercuric ions bind tightly but reversibly to thiol ligands.<sup>16</sup> It is likely, therefore, that the ethylmercury cation of thimerosal dissociates from the thiosalicylic acid moiety immediately after injection to bind to the surrounding thiol ligands present in great excess in tissue proteins.<sup>17</sup>

The buildup of mercury in the tissues of children is particularly alarming in light of a recent article by Baskin et al.<sup>18</sup> They have examined the toxic effects of micromolar concentrations of thimerosal in cultured human cerebral cortical neurons and in normal human fibroblasts. The results demonstrated that thimerosal in micromolar concentrations induced membrane and DNA damage, and initiated caspase-3 dependent apoptosis in human neurons and fibroblasts. In addition, the authors report that thimerosal toxicity may occur at even lower doses than those utilized in their experiments with longer times of exposure. Another recent study by Makani et al.<sup>19</sup> has also demonstrated high cellular toxicity of thimerosal in low micromolar concentrations in T-cells incubated with thimerosal for 24 hours.

A recent article by Nelson and Bauman<sup>20</sup> stated that the overall clinical picture of mercurism—from any known form, dose, duration, or age of exposure—does not mimic that of autism and that no evidence has yet been brought forward to indicate that children exposed to vaccines containing mercurials have more autism than children with less or no such exposure. However, the National Toxicology Program (NTP) within the U.S. Department of Health and Human Services, an interagency program headquartered at the National Institutes of Health's National Institute of Environmental Health Sciences (NIEHS), reports that clumsiness, speech impairment, and emotional disturbances are commonly observed with both acute and chronic thimerosal exposure. These mercurial symptoms are core to the observed abnormalities in autistic spectrum disorders. This observation is supported by Green et al.,<sup>21</sup> who recently reported that clumsiness is a com-

monly observed comorbidity in Asperger's Syndrome, an autistic spectrum disorder.

The results of our present study, combined with the published observations included above, disagree with the views expressed by Nelson and Bauman and support the hypothesis of Bernard et al.,<sup>22</sup> who have compared the similar biological abnormalities commonly found in autism and the corresponding pathologies arising from mercury exposure. Distinct similarities were found between autism and mercury exposure in their effects upon biochemistry, the immune system, the central nervous system structure, neurochemistry, and neurophysiology.

Another study by Bernard et al.<sup>23</sup> has further examined the relationship between thimerosal and autism. They determined that thimerosal was first added to childhood vaccines in the 1930s, and autism was first described in 1943 among children born in the 1930s, suggesting that autism may indeed be an iatrogenic effect of thimerosal.

In addition, Redwood et al.<sup>24</sup> have reported that mercury exposure from childhood immunization is a cause for concern because exposure to low levels of mercury during critical stages of development has been associated with neurological disorders in children, including attention deficit disorder (ADD), learning difficulties, and speech delays.

Moreover, our findings appear to confirm previously published epidemiologic evidence showing a direct association between increasing mercury from thimerosal-containing childhood vaccines and neurodevelopment disorders in children.<sup>7-9</sup> These studies showed that there was a two to sixfold, statistically significant increased incidence of neurodevelopment disorders following an additional 75-100 mcg dosage of mercury from thimerosal-containing childhood vaccines in comparison to thimerosal-free childhood vaccines. These studies showed dose-response curves demonstrating a close, statistically significant correlation between increasing mercury doses from childhood vaccines and childhood neurodevelopment disorders.

The results of our analyses suggest that mercury should be removed immediately from all biologic products, and others have reached a similar conclusion. Kravchenko et al.<sup>25</sup> stated, "Thus thimerosal, commonly used as a preservative, has been found not only to render its primary toxic effect, but also [to be] capable of changing the properties of cells. This fact suggests that the use of thimerosal for the preservation of medical biological preparations, especially those intended for children, is inadmissible." Cox and Forsyth<sup>26</sup> reported, "However, individual cases of severe reactions to thimerosal demonstrate a need for vaccines with an alternative preservative." Similarly, "...reactions can be expected in such a high percentage of merthiolate-sensitive persons that merthiolate in vaccines should be replaced by another antibacterial agent."<sup>27</sup> Rohyans et al.<sup>28</sup> revealed in 1984, "Although aqueous merthiolate has been used for years as a topical antiseptic, a recent review of its use by the Food and Drug Administration resulted in its classification as 'less than effective.' Furthermore, two of the ingredients (thimerosal and borate) in merthiolate are toxic if absorbed or injected." In addition, Seal et al.<sup>29</sup> reported in *The Lancet*, "Thimerosal is a weak antibacterial agent that is rapidly broken down to products, including ethylmercury residues, which are neurotoxic. Its role as a preservative in vaccines has been questioned, and the pharmaceutical industry itself considers its use as historical."

## Conclusion

Analysis of post-DMSA urinary mercury excretion found a strong, statistically significant association between greatly increased urinary mercury concentrations and the presence of autistic spectrum disorders in vaccinated children.

The mercury levels measured in this study could plausibly have resulted from exposure to mercury in routine childhood vaccines in the United States, while thimerosal in Rh<sub>0</sub>(D) immune globulin and other potential environmental sources of mercury may be contributory.

Our study is unable to determine whether the statistically significantly higher urinary concentrations of mercury measured in cases in comparison to controls is caused by higher exposure to mercury, reduced ability to excrete mercury, or a combination of these explanations. Regardless of the mechanism by which children with autistic spectrum disorders accumulate high mercury levels, the DMSA treatment course described in this study appears useful and important in determining mercury body burden.

The data from this study, along with emerging epidemiologic data showing a link between increasing mercury doses from childhood vaccines and childhood neurodevelopment disorders, increases the likelihood that mercury is one of the main factors leading to the large increase in the rate of autism and other neurodevelopment disorders. It is to be hoped that removing thimerosal from all childhood vaccines will contribute to a decline in the numbers of new cases of autistic spectrum disorders.

Unfortunately, as discussed in a recent publication,<sup>30</sup> many of the vaccines recommended for the childhood immunization schedule contained the full doses of thimerosal through 2002 (FDA, personal communication), and in addition, pediatric vaccines such as influenza, diphtheria-tetanus (DT), and possibly others, still contain the full amounts of thimerosal in 2003. Therefore, it may be quite some time before a decrease in the prevalence of neurodevelopment disorders is seen.

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Potential conflict of interest: Dr. Mark Geier has been an expert witness and a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation. David Geier has been a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation.

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