Exposure to Heavy Metals, Physical Symptoms, and Developmental Milestones in Children with Autism

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Abstract:
A questionnaire was used to assess heavy metal exposure in the mothers of children with autistic spectrum disorders (ASD) vs. controls. Four exposures were found to be statistically significant, including maternal seafood consumption, oral antibiotic use (which greatly decreases excretion of mercury), adverse reactions to vaccinations (many of which contain thimerosal), and pica (which leads to increased consumption of heavy metals).

Several major physical problems were much more common and severe in the ASD population, including chronic gastrointestinal problems, sleep problems, excessive ear infections, low muscle tone, and excessive salivation/drooling.

Finally, 62% of the children with ASD were reported to have developed normally, with normal developmental milestones, and then had a major regression at an average age of 18 months.

Keywords: autism; mercury; thimerosal; regression

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Introduction

The causes of autism are unknown, but it has been suggested that one possible cause is mercury poisoning. Bernard et. al. [1] pointed out that all of the major symptoms of autism are also observed in infantile mercury poisoning, and there are many similarities of autism to acrodynia (pink disease), which was traced to the use of mercury-based teething powders. Also, a recent study by Geier and Geier [2] found that injection with thimerosal (a mercury-based preservative) in vaccines led to a much greater risk of a child developing autism, speech disorders, or heart arrest. Most importantly, a recent study by Holmes et al. [3] found that infants who became autistic had 1/8 of the normal amount of mercury in their baby hair compared to controls, which suggests an inability to excrete mercury. They also found that the severity of autism had a strong inverse relationship with the level of mercury, with the most severe group having the lowest levels of mercury in their hair. This is consistent with the hypothesis that the group with the most inhibition of mercury excretion would be the most severely affected. They also found that maternal seafood consumption, maternal dental fillings, and infant exposure to thimerosal in vaccines correlated with the amount of mercury in the baby hair of the typical infants, demonstrating that all are significant sources of mercury exposure in children.

There are many possible sources of exposure to mercury. Since thimerosal is a risk factor, then it is likely that other sources of mercury are also risk factors, and would act to increase the body burden of mercury. The major sources of mercury exposure include:

1. Seafood: Certain types of seafood, especially predators high on the food chain such as shark and swordfish, are known to contain high levels of methyl mercury. The FDA has advised pregnant women to completely avoid the fish highest in mercury (such as shark and swordfish), and limit their consumption of other mercury-containing fish [4]. One of the major studies of mercury poisoning involved people on the Faroe Islands[5]. It was found that their high consumption of fish resulted in an increased incidence of neurological damage, and in fact those studies were used by the EPA to set safe limits on mercury exposure.
2. Vaccines: Thimerosal is used as a preservative in some vaccines, at a concentration of typically 25-50 mcg/dose. Childhood vaccines which may contain thimerosal include hepatitis B, haemophilus influenza B (Hib), and diptheria-pertussis-tetanus (DPT). In 1999 the American Association of Pediatrics (AAP) recommended thimerosal be removed from childhood vaccines as a precaution [6], and the FDA has requested vaccine manufacturers to reduce their use of thimerosal. However, as of 2003, several vaccines still contain thimerosal. In addition, thimerosal was also used in other medications, such as the Rho-D immunoglobulin injections given to pregnant mothers who are Rh-negative blood type.
3. Dental Amalgams: Approximately 80% of the dental fillings used in the US are a silver-mercury alloy, and they are approximately 50% mercury by weight. Studies of dental fillings in sheep [7] and in monkeys [8] using radioactive tracers have shown that mercury slowly leaches out of the fillings and enters many major organs of the body including the brain. Studies of pregnant ewes [9] showed that mercury accumulates in the placenta, and is passed on to the fetus and later to the infant through breast milk. The amount of mercury that leaches out of fillings varies according to several factors, including the acidity of the mouth, the presence of other metals (stainless steel crowns and gold caps can cause galvanic corrosion of silver-mercury amalgams), and gum chewing. According to a 1996 EPA report to Congress [10], typical levels of mercury release have been reported at 2-20 mcg per day, with the data at the lower end being more reliable. On the one hand this is a small enough level that dental amalgams could last for decades, but it is high enough to be a significant source of mercury
4. Coal: In 1996, the EPA estimated that coal-burning power plants in the US emitted 52 tons of mercury into the air. That mercury can remain airborne for a year or more, and is one of the major sources of mercury in air, water, and fish.
5. Fungicides: Some fungicides and pesticides contain mercury. Methylmercury poisoning occurred twice in Iraq following consumption of seed grain that had been treated with a fungicide containing methylmercury[11]. The number of people admitted to the hospital with symptoms of poisoning has been estimated to be approximately 6,500, with 459 fatalities reported. The Iraqi babies who were exposed prenatally either failed to develop language or presented with severe language deficits in childhood.
6. Water: The EPA regulates the allowable level of mercury in water, and requires that it be below 2 ppb. There are several possible sources of groundwater contamination, including emissions from coal power

plants, mining operations that use mercury to extract metals from their ore, dental offices, and a variety of natural sources.

Overall, dental amalgams and fish are the major source of mercury in the general population, and thimerosal-containing vaccines are also a major source in some people, including young children and the armed forces.

Recently, as part of the ongoing National Health and Nutrition Examination Survey (NHANES), CDC researchers measured levels of mercury in blood and hair samples from 700 women and 300 children[12]. They found that nearly 1 in 10 US women have levels of mercury in their blood that are close to hazardous. The levels were lower in the children, presumably because they eat less fish. Thus, those women who have high levels of mercury and other metals will pass some of those metals on to their fetuses and nursing infants. Since there is a wide variation in the susceptibility/sensitivity to mercury, it seems possible that some infants may suffer neurological damage.

The 1999 report by the Agency for Toxic Substances and Disease Registry (ATSDR) report on mercury toxicity has a 2-page summary of the effects of prenatal exposure which includes the following quotes[13]

- "Mercury is considered to be a developmental toxicant. … The symptoms observed in offspring of exposed mothers are primarily neurological in origin and have ranged from delays in motor and verbal development to severe brain damage."
- "The infant may be born apparently normal, but later show effects that may range from the infant being slower to reach developmental milestones, such as the age of first walking and talking, to more severe effects including brain damage with mental retardation, incoordination, and inability to move."
- "Other severe effects observed in children whose mothers were exposed to very toxic levels of mercury during pregnancy include eventual blindness, involuntary muscle contractions and seizures, muscle weakness, and inability to speak."
- "It is important to remember, however, that the severity of these effects depends upon the level of mercury exposure and the time of dose."

Most of the symptoms described above are commonly observed in autism.

There are several factors that are known to significantly increase the toxicity of mercury. One important factor is synergistic toxicity with other metals. In a study of rats,[14] it was found that a combination of the LD1 of lead (the amount of lead required to kill 1% of the rats) with an LD1 of mercury resulted in an LD100 (all of the rats died). Similar effects were observed for mercury with cadmium. It is likely that exposure to other heavy metals has a similar synergistic effect, because they have a similar excretion mechanism, so that exposure to multiple heavy metals will competitively slow excretion of any one metal.

Another important factor in the toxicity of mercury is the use of oral antibiotics, because they dramatically slow the excretion of mercury. In a study of rats,[15] it was found that the use of oral antibiotics led to an increase in the half-life for excretion of mercury from 10 days to 100 days. If the rats were also placed on an all-milk diet (which is very relevant to nursing human infants), then the half-life increased to over 300 days.

Objectives:
The purpose of this study is to examine possible sources of exposure to mercury and other heavy metals in children with autism vs. controls, to determine if any are possible risk factors for autism. As part of this study, we will also investigate the physical symptoms and developmental milestones of the children with autism.

Participant Selection:
The participants were invited to participate by a mass mailing sent to approximately 1000 families of people with autism in the state of Arizona, using the mailing lists of the Greater Phoenix Chapter and the Pima County Chapter of the Autism Society of America. They were informed that the study involved

investigation of exposure to heavy metals in general, including mercury. Parents of the participants with ASD asked friends and neighbors to act as controls from the study, and a few participants were also solicited from two elementary schools in Tempe, Arizona. The inclusion criteria for the study were:

1) Age of 3-15 years
2) For ASD children, a psychiatrist or developmental pediatrician diagnosed them with autism, PDD/NOS, or Asperger's.
3) For the control children, they must be: a) mentally and physically healthy individuals without any developmental delays, illness, or other medical conditions, and b) unrelated to a person with ASD.

There were 53 children with ASD and 48 control children enrolled in the study. The children with ASD included 49 with autism, 2 with PDD/NOS, and 2 with Asperger's. (Some of the children had received multiple diagnoses, in which case they were counted in the most severe category). However, in Arizona, only children diagnosed with autism receive significant services from the state (respite, behavioral therapy, speech therapy, occupational therapy, physical therapy, music therapy); children with diagnoses of Asperger's or PDD/NOS do not receive such services. Therefore, even though qualified psychologists, psychiatrists, or developmental pediatricians gave the diagnoses, there is some question as to their validity. That is a limitation of this initial study, and future studies can include a more rigorous assessment of the child's diagnosis.

There were 12 girls and 11 girls in the ASD and control groups, respectively, comprising 23% of each of their groups. There were two sets of twins in the ASD group, and one set of twins and one pair of siblings in the control group. Ages ranged from three to 15 years of age, with a mean age of 7.1 and 7.5 for the children with ASD and the controls, respectively, with standard deviations of 3.0 years for each. Thus, there was overall a good match between the groups in terms of gender and age.

A subset of this group and their mothers submitted current hair samples for elemental analysis, and those results are reported on in another paper.[16]

Methodology:

For this study we created a heavy metal exposure and child development questionnaire (see Appendix 1). It is divided into four areas: Prenatal Exposure, Infantile Exposure, Physical Symptoms, and Developmental Milestones. We also created a mother's health questionnaire (see Appendix 2).

The mothers of the children were all interviewed by the same researcher, to determine their response to the questionnaire. It was not possible to blind the interviewer as to whether or not the child had autism, due to the nature of the questions. The results of the questionnaires are listed in Tables 1, 2, 3, and 4.

In addition, all of the children and their mothers were examined by a board-certified dentist with over 20 years of clinical experience, to determine their dental status. The results of the examination are listed in Table 5.

Statistical analysis of the data was carried out with an unmatched t-test, assuming 2-sided distributions of unequal variance.

Results:

I. Heavy Metal Exposure and Child Development Questionnaire

A. Prenatal Exposure:

The two groups of mothers did not have a significant difference ($p<0.05$) in most of the investigated risk factors, including vaccinations during pregnancy, exposure to paint, smoking or second-hand smoke exposure, thimerosal in contact lens solution, tattoos, or pesticide use in the home. They were equally likely to take prenatal supplements, which can help prevent absorption of heavy metals. The average age of the mothers at the birth of their child in the study was 32.0 years for the mothers of children with ASD, vs. 30.1 years for the mothers of the typical children. The difference was not statistically significant, but warrants consideration in future studies.

The autism mothers reported slightly less injections with Rhogam, which is in marked contrast to the study by Holmes et al. that reported on a high use of Rhogam in mothers of children with ASD.[3] It is possible that the difference is partially due to a sampling bias, since the Holmes' study involved her patients, some of whom knew of her interest in Rhogam before becoming her patients. Regardless, the Rhogam issue needs further investigation.

Regarding maternal seafood consumption during pregnancy, it was found that 58% of the mothers of children with ASD reported consuming more than 2 servings of seafood per month, vs. only 33% of the mothers of typical children. This is consistent with our finding of 57% more mercury in the hair of a subset of the ASD group vs the control group [16]. This difference was not statistically significant when analyzed with a simple ttest, but a logistic regression model yielded a relative risk of 2.7, with a confidence interval of 1.1-6.2, p=0.02. This relative risk is presumably due to the level of mercury in the fish.

B. Infantile Exposure:

Mothers of children with ASD nursed their children for approximately the same length of time as did the mothers of typical children. Both groups of children ate similar amounts of seafood. The children with ASD were slightly more likely to eat/lick paint, but it was rare (3 severe cases, 1 moderate case, 2 mild cases, vs 1 moderate case in the controls), and the difference was marginally statistically significant (p=0.05).

Pica: A large fraction of the children with ASD (16 of 53, or 30%) were reported by their parents to have moderate to severe pica, whereas only one of the typical children was reported to have moderate pica. This result was extremely statistically significant (p=0.00002). The pica generally involved a large number of types of inedible objects, typically including sand, dirt, paper, and other objects. This is likely to result in a significantly higher intake of toxic metals, and indeed higher levels of strontium, aluminum, uranium, and barium were observed in the hair of these children.[16]

Vaccinations: As shown in Table 2, mothers of children with ASD reported significantly more adverse reactions, and of a more serious nature, than did the mothers of the typical children. The difference was highly statistically significant (p=0.001). Note that the criteria for these reactions was that it occurred within a few days of the vaccination, so that there was a close temporal association. A description of individual case studies that were moderate or severe is given in Table 2. This data strongly suggests that further investigation into the adverse vaccine reactions in autism is warranted.

C. Physical Symptoms

All of the physical symptoms investigated except for thrush were found to be much more common in ASD, with a very high statistical significance.

Gastrointestinal Problems: 33 of the 53 children with ASD (62%) were reported to have moderate to severe gastrointestinal problems (chronic diarrhea and/or constipation) versus one of the typical children (2%) reported to have moderate gastrointestinal problems. This result is extremely statistically significant ($p<10^{-13}$).

Sleep Problems: Similarly, 32 of the 53 children with ASD (60%) were reported to have moderate to severe sleep problems (falling asleep and/or night wakening), versus one of the typical children (2%) reporting moderate sleep problems. This result was highly statistically significant ($p<10^{-11}$).

Also, it should be noted that there was a significant correlation between sleep problems and gastrointestinal problems (correlation coefficient = 0.31). In a previous small pilot study of a vitamin/mineral supplement,[17] we found that the supplement resulted in statistically significant improvements in gastrointestinal and sleep problems. Thus, it could be that some sleep problems are due to gastrointestinal problems, and that treatment of the gastrointestinal problems may reduce some sleep problems.

Low Muscle Tone: 16 of the children with ASD (30%) were reported to have moderate to severe loss of muscle tone, compared to one of the typical children (2%) reported to have moderate loss of muscle tone. This result was highly statistically significant, with p=0.000000002.

Excessive Salivation/Drooling: The mothers reported that 3 children had severe salivation/drooling problems, 5 had moderate problems, and 9 had mild problems, compared to 2 of the typical children reporting mild problems. The difference was highly statistically significant, with p=0.0003. The salivation/drooling problem partially correlated with muscle tone problems (correlation coefficient=0.47), and parents often reported that they thought the drooling was due to poor muscle tone.

Ear Infections: Children with ASD had an average of 10.9 ear infections during their first three years of life, versus 4.3 for the typical children, with medians of 10.0 and 2.5, respectively. (Ear infections lasting longer than one month were counted separately for each month they lasted – some children had continuous ear infections for many months in a row). This result is highly statistically significant, p=0.00006. In the ASD group, 53% had 9 or more ear infections, vs. 12% for the control group. In contrast, only 17% of the ASD group had zero or one ear infections, vs. 35% of the control group. According to the parents, virtually all of the ear infections were treated with oral antibiotics, often for months at a time. The predominant use of oral antibiotics in children is for ear infections, so that the increase in ear infections corresponds to a much higher overall usage of oral antibiotics as well. This extended use of oral antibiotics is likely to have had two major effects:

1) Destroy most of the normal beneficial bacteria in the gastrointestinal tract, possibly resulting in overgrowth of yeast and possibly pathogenic bacteria. This may explain some of the gastrointestinal problems observed in ASD, since the presence of the normal flora promotes gut motility. The recent study of the temporary effectiveness of oral vancomycin in reducing autistic symptoms supports the idea that pathogenic bacteria are important in the etiology of ASD.[18] [19]
2) Inhibit excretion of mercury, based on the study of rats which found that oral antibiotics increase the half-life for excretion of mercury tenfold or more.

Future studies should count the total usage of oral antibiotics from all sources, and break it down into several time intervals during pregnancy and the first three years of life.

D. Regression and Developmental Milestones

62% of the children with ASD were reported by their mothers to have had a period of normal development, followed by a major regression at age 12-30 months (18 months on average, standard deviation of 4 months). Two other children possibly had symptoms of regression, whereas the remaining 34% of the children seemed to have had developmental delays from birth. Although it is possible that the mothers missed some early signs of ASD, these mothers all reported a major decline in their children's language, social interactions, and/or behavior. Moreover, table 2 shows that the children with regressive ASD reached their developmental milestones in sitting, crawling, walking and talking at almost exactly the same age as the typical children. In contrast, the children with non-regressive ASD were on average 2 months late in learning to sit up and crawl, 4 months late learning to walk, and 17 months late learning to talk (not counting 4 children aged 5-6 years who had not learned to talk). All of those delays are statistically significant, and most are highly statistically significant.

Mother's Health:

Since the child's mother is their major source of environmental exposure and source of nutrients during pregnancy and breastfeeding, they were surveyed as to their current health status. Each symptom was reported on a 4-point scale: none/mild/moderate/severe. Table 4 lists the survey results. There was also an "other symptom" category, but it was usually blank, and there was no pattern in the few that were listed. The mothers of children with ASD tended to have a higher incidence of certain physical symptoms (chronic fatigue, arthritis, muscle/joint pain, asthma, night vision problems, hair loss) and mental symptoms (anxiety, depression, memory problems, insomnia). Some of those symptoms could have been due to dealing with a child with ASD. However, the overall incidence of any one condition was low, and there was no statistically significant difference between the two groups for any of those categories.

II. Dental Data and Discussion

The results of the dental examinations are listed in Table 5. The mothers of children with ASD has slightly more mercury amalgam surfaces (10.0 vs. 8.3), but the difference was not statistically significant. There was a trend that the mothers of children with ASD were more likely to have gold fillings (p=0.10), porcelain bonded metal crowns (p=0.06), and composite fillings (p=0.09).

There was a trend that mothers of children with ASD were more likely to have had a mercury amalgam filling placed or removed during pregnancy (5 cases of placement and 2 of removal, vs. 1 placement and 0 removals for the controls, p=0.08). Most of the cases involved a single filling, but one of the autism cases involved 5 fillings being placed. We believe that the placement of a mercury filling during pregnancy could be a major risk factor for mercury exposure because our recent research [20] has found that a new mercury amalgam releases approximately 500 mcg/day of mercury the first day it is made, and that it takes several months to decrease to the low levels of approximately 1 mcg/day for older fillings. This exposure level is far higher than for a typical vaccination (25-50 mcg of thimerosal, or 12.5-25 mcg of mercury). Similarly, the removal of a filling is expected to result in a high exposure to mercury unless special precautions are taken, because the removal usually involves drilling out the old filling, which creates a high amount of mercury vapor and mercury particles.

For the children, the only statistically significant difference (p=0.01) was that the typical children had roughly three times as many sealants/composite fillings: 1.6 vs 0.5 on average. In contrast, the children with ASD were more likely to have mercury amalgam fillings (0.98 vs 0.43), but the difference was not statistically significant. One likely explanation of this finding is that the sealants were more likely to be used in the typical children due to compliance issues, and that they reduced the likelihood of developing cavities that required a filling. None of the children had root canals, gold fillings, porcelain-bonded metal crowns, or porcelain crowns. A few children had stainless steel crowns, but the difference between the groups was not statistically significant.

Limitations of this study:

This study has several limitations, including:

1) Sample bias: The participants knew that this study involved an investigation of heavy metal toxicity, so their choice to participate may have biased the results. This is probably most relevant to the issue of maternal seafood consumption and number of mercury dental fillings, which some of the mothers had some knowledge of. However, their responses to other questions are probably not significantly biased.
2) Sample Size: A much larger study, involving multiple sites, is needed to verify or refute the findings of this study.

Summary:

In summary, many different possible sources of exposure to heavy metals were considered. The ones that were found to be statistically significant included:

1) Seafood: Maternal consumption of more than 2 servings per month was found to be a risk factor for ASD. This was true of 57% of the mothers of children with ASD, vs. 33% of the controls.
2) Oral Antibiotics: Due to a high incidence of ear infections, the children with ASD received much higher levels of oral antibiotics. This is important because:
    a. Oral antibiotics destroy normal beneficial flora in the gastrointestinal tract, and hence can lead to overgrowths of abnormal bacteria and yeast
    b. Oral antibiotics greatly decrease the excretion of mercury, causing it to build up to higher levels and greatly increasing its toxicity.
3) Vaccines: Children with ASD were more likely to have an adverse reaction to vaccines, and those adverse reactions tended to be more severe. This could be due to the thimerosal in the vaccines.
4) Pica: The children with ASD were reported to be much more likely to have moderate to severe pica (30%) than the typical children (2%). Using a severity scale of 0-3, the difference was highly significant (0.9 vs 0.04, p=0.00001). This consumption of non-food items, including sand, dirt,

paper, and other objects, probably resulted in a significant increase in their exposure to toxic metals.

5) Dental Fillings: There was a trend ($p=0.08$) that the mothers of children with ASD were more likely to have had a mercury filling placed or removed during their pregnancy (7 cases vs. 1 case for the controls). This is relevant because our recent study has found that fillings release much more mercury when initially placed.

Several physical symptoms were found to be very common in ASD, with an extremely high statistical significance:

1) Gastrointestinal: 62% of the children with ASD had moderate or severe problems with chronic constipation and/or diarrhea, far more than the typical children (2%). Using a severity scale of 0-3, the difference was highly statistically significant, 1.9 vs 0.1, $p=1 \times 10^{-12}$. This may be partly due to the high usage of oral antibiotics, which can disrupt normal GI flora, which are important in promoting normal motility.
2) Sleep: 60% of the children with ASD were reported to have moderate or severe sleep problems, far more than the typical children (2%). Using a severity scale of 0-3, the difference was highly statistically significant, 1.8 vs 0.2, $p=1 \times 10^{-13}$).
3) Sleep and gastrointestinal problems were moderately correlated, with a correlation coefficient of 0.31.
4) Muscle Tone: 30% of the children with ASD had moderate or severe problems with low muscle tone, far more than the typical children (on a scale of 0-3, 1.0 vs 0.06, $p=0.000000002$).
5) Salivation/Drooling: 15% of the children with ASD had problems with salivation/drooling, much more than the typical children ($p=0.0003$). Salivation/drooling problems were moderately correlated with muscle tone problems (correlation coefficient = 0.47).
6) Ear infections: Children with ASD were reported to have had many more ear infections than typical children during their first three years of life (10.9 vs 4.3, $p=0.00006$). Since almost all ear infections were treated with oral antibiotics, this resulted in much higher oral antibiotic use in children with ASD.

Regressive ASD was found to occur in 62% of the children with ASD, at an average age of 18 months. Those children met their developmental milestones (age of crawling, sitting, walking, and talking) at the normal time. In contrast, the children with non-regressive ASD were two months late in crawling and sitting, four months late in walking, and 17 months late in talking.

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Table 1: Results of Questionnaire on Heavy Metal Exposure during Pregnancy and Infancy. For the ttest, n.s. means "not significant."

	Mothers of children With ASD	Mothers of control Children	TTest
Maternal age at childbirth	32.0 years	30.1 years	n.s.
Seafood consumption	4.8 servings/month	3.6 servings/month	See text
Rhogam Shot	13%	21%	n.s.
Vaccinations during preganancy	14%	8%	n.s.
Paint exposure	0.5*	0.3*	n.s.
Smoke cigarettes?	6%	6%	n.s.
Secondhand Smoke	0.48*	0.37*	n.s.
Wear contact lenses?	47%	38%	n.s.
Take prenatal supplement	92%	95%	n.s.
Tatoos	2%	4%	n.s.
Pesticide Use in Home	1.1*	0.9*	n.s.
Months of Breastfeeding	7.9 months	7.9 months	n.s.
Child's seafood consumption	2.1 servings/month	1.8 servings/month	n.s.
Eat/Lick Paint	0.26*	0.04*	p=0.05
Eat Non-Food Items (Pica)	0.9*	0.0*	p=0.00002

* Severity scale of 0-3: none=0, mild=1, moderate=2, severe=3.

Table 2a: Immediate Adverse Reactions to Vaccinations

	No reaction	Mild	Moderate	Severe
ASD	48%	23%	11%	18%
Controls	68%	26%	4%	2%

Table 2b: Case Reports of Adverse Reactions to Vaccinations: Children with ASD and Controls.

Reaction Severity	**Vaccination**	**Age-** months	**Reaction**
ASD Group			
Severe	MMR	15	high fever, very listless/passive for 5 days with no eating or drinking, then began regression into autistic behavior
	HIB/HepB DTaP, IPV, MMR, HIB, Varicella	8 14	• high fever, crying, diarrhea for 3-4 days • began wheezing within a few days, developed asthma within 2 weeks,
	Petusssis	8	severe diarrhea for 3 months, continued to some extent for 27 months
	DTaP MMR	2 15	103 fever for 1.5 days high fever for one week; didn't eat for 3 months, only drank milk; regression began
	MMR, DTaP, varicella	12	respiratory arrest led to hospitalization for 5 days, and then autistic symptoms began
	DTaP	6	screamed loudly for six hours, and then began long-term regression resulting in autism
	DTaP	18	high fever the next day, which lasted for 10 days; hospitalized on day 6 for 3 days; very lethargic; major regression started 4 months later
	DTP, OPV, HIB	3	high fever, very sick for 2 weeks, then prolonged ear infection and little sleep; slow development before, and slower afterwards – possible regression
	MMR	13	within 1 week started seizures (none prior) each round of vaccinations usually caused mild fevers (101-102) for several days
Moderate/ Severe Reactions	DTaP, OPV, HIB HepB	4 21	• fever for 2 days • fever, cough; after several weeks developed ITP (severe blood disorder) and was bruised head to toe for 9 months;
Moderate	HepB	21	high fever for 2 days
	DTaP	2	greatly swollen thigh, crying for 1 day
	DTP/MMR DTP	6 30	very high fever, screaming, hives for 2 days, then recovered; autism became more noticeable
	Misc.	N/A	Most vaccinations caused high fever for 1-2 days
Mild/ Moderate	Misc.	N/A	every vaccination resulted in 101-102 fever for about 1 day
	Tetanus		1 inch diameter lump in leg for 6-8 months

CONTROLS			
Moderate/ Severe	Chicken pox	12	lethargic for 2 weeks, rested in bed
	MMR	12	four hours after vaccination developed 104 fever, which lasted 2 days; several seizures lasting 1-2 minutes for 2 days after vaccination; then okay
Moderate	MMR/DTP/ Polio	18	103 fever, lasting 3 days; rash; lethargic

Table 3: Developmental Milestones for Children: in months

Developmental Milestone	Non-regressive ASD	Regressive ASD	Controls	Ttest: non-regressive ASD vs. controls
Crawling	9.5	7.5	7.4	p=0.02
Sitting	7.7	5.8	5.5	p=0.007
Walking	16.0	11.9	11.4	p=0.0007
Talking	28.0*	11.2	11.2	p=0.0002

* does not include 4 children who had not learned to talk by age 5-6 years

Table 4: Health problems of mothers of children with ASD (first row) vs. mothers of typical children (second row), reported as a percentage. There were no statistically significant differences reported. Sorted by frequency of occurrence.

	None	Mild	Moderate	Severe
Mental Problems				
Depression	77	6	10	7
	83	10	7	0
Memory Problems	78	7	11	4
	83	11	3	3
Difficulty Concentrating	81	7	10	2
	83	9	6	2
Anxiety	81	8	9	2
	85	12	1	2
Insomnia	82	6	6	6
	82	18	0	0
Hyperactivity	94	0	6	0
	96	2	2	0
Physical Problems				
Night vision	67	21	8	4
	79	17	4	0
Muscle/Joint pain	73	16	7	4
	85	11	4	0
Chronic fatigue	83	8	9	0
	85	13	2	0
Hair loss	84	8	6	2
	92	6	2	0
Gastrointestinal problems	85	9	4	2
	87	9	2	2
Vaginal yeast	82	10	8	0
	79	10	11	0
Asthma	85	9	4	2
	91	7	2	0
Arthritis	88	8	2	2
	96	4	0	0
Thrush	98	0	2	0
	98	2	0	0
Anemia	96	4	0	0
	91	9	0	0
High blood pressure	96	2	2	0
	96	4	0	0
Diabetes	96	4	0	0
	96	4	0	0
Heart disease	98	2	0	0
	98	2	0	0
Fibromyalgia	100	0	0	0
	96	2	2	0
Excessive salivation	98	2	0	0
	100	0	0	0
Lupus	100	0	0	0
	100	0	0	0
Multiple sclerosis	100	0	0	0
	100	0	0	0

Table 5: Dental status of children with ASD and their mothers compared to controls.

	Hg surfaces	Root Canals	Pulpotomies	Gold fillings	Porcelain-bonded metal crowns	Porcelain crowns	Stainless Steel Crowns	Composites/ sealants
ASD	0.98	0	.10	0	0	0	0.53	0.47
controls	0.43	0	0.02	0	0	0	0.18	1.61
ttest	.24	.30	.27				0.07	0.012
ASD mothers	10.0	0.25	0	0.27	1.83	0.10	0.02	2.65
Control mothers	8.31	0.30	0	0.08	0.74	0.04	0	1.30
ttest	0.22	0.73		0.10	0.06	0.42	0.34	0.09

Appendix 1: Questionnaire on Exposure to Heavy Metals, Physical Symptoms, Child Development:

Child's Date of Birth
Mother's Date of Birth

A. Exposure to Heavy Metals during Pregnancy:

1) How often did you eat seafood: _____ servings/month

2) Did you have a Rhogam shot (due to an Rh difference between mother and child)?
____ Yes ___No

3) Did you have any vaccinations during your pregnancy/breastfeeding?
If yes, what and when? _____

4) Did you do any painting or reside in a home that had been recently painted either during your pregnancy or prior to it? ___Yes ___No If yes, please explain:

5) Did you smoke while pregnant or while breast feeding? ___Yes ___No
If yes, please estimate the number of cigarettes/day:

6) Were you frequently exposed to second-hand smoke due to a spouse, officemates, etc?
___Yes ___No
If yes, please estimate the extent:
___mild/infrequent ____moderate _____severe

7) Did you wear contact lenses while pregnant or while breast feeding? ___Yes ___No

8) Did you take a prenatal supplement? ___Yes ___No

9) Did you have any tattoos present during your pregnancy? ____ Yes ___No
If yes, approximate area (___ inches by ___ inches)

10) Did you use any pesticides in your home during your pregnancy? ___Yes ___No
If yes, list number of times/year: _____

B. Exposure to Heavy Metals during Infancy

1) If you breastfed, how long was it for? _____ months

2) How often does he/she eat seafood: ____ servings/month

3) Did he/she seem to have any negative reactions to any vaccination?
___Yes ___No
If yes, please rank the severity on a scale of:
1=mild fever for 1 day;
2= high fever, up to 2 days;
3=up to 1 week, major reaction, long term effects
Please explain the reaction:

4) Did he/she frequently eat or lick paint?
____never/rarely ___mild ____moderate _____ severe

5) Did he/she frequently eat any non-food items?
____never/rarely ___mild ____moderate _____ severe
If yes, what did he/she primarily eat?

C. Physical Symptoms:

Please rank the following symptoms on a scale of 0-3, with 0= none, 1=mild, 2=moderate, and 3=severe

___ Gastrointestinal Problems: chronic diarrhea/constipation after 1 year of age

___ Sleep Problems: problems falling asleep and/or waking during the night

___ Low Muscle Tone: general muscle weakness

___ Excessive Salivation/Drooling

___ Thrush: white yeast infection in the mouth

Ear Infections: Please estimate the total number of ear infections during the first 3 years of life. If an ear infection lasted longer than one month, count each month as a separate infection: _____ ear infections

D. Developmental Landmarks (please give age at which these milestones occurred):

Crawling _____ months
Sitting _____ months
Walking _____ months
Talking _____ months (using meaningful words like “ma-ma” or “da-da”)

Did you child experience a period of major regression, during which they lost important skills?____Yes ____ No If yes, what age did this occur at?_____

Appendix 2: Questionnaire on Health Status of Mothers:

Do you have any of the following problems?
If yes, please indicate severity (1=mild, 2=moderate, 3=severe)

__ Chronic Fatigue
__ Fibromyalgia
__ Lupus
__ Diabetes
__ Multiple Sclerosis
__ Arthritis
__Muscle/Joint Pain
__Asthma
__ Anemia
__ Heart Disease
__High Blood Pressure
__Anxiety
__Depression
__Memory Problems
__Difficulty Concentrating/Thinking
__Hyperactivity
__Insomnia
__Excessive Salivation
__Vaginal Yeast Infections
__Thrush
__Gastrointestinal Problems (diarrhea/constipation)
__Night Vision Problems (sensitive to bright lights)
__Hair loss
__Other (please explain)

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