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# The Vaccine Debate:

Safe and effective or toxic and defective?

You know we're in trouble…when a real estate agent wins a science debate against the CDC.

**Conclusion:  Vaccine Science = Tobacco Science**

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## Introduction:

This discussion is not only scientific, but on many levels, it involves religion. For the most part I'm going to focus on the science, but I will occasionally throw in a religious citation:

"*The heavens declare the glory of G-d, and the expanse of the sky tells of His handiwork." [Psalm 19]* Nature and science communicate with us, proving to us that G-d exists and that He created the world. Therefore, anyone who distorts science or degrades nature, is impairing our ability to perceive and understand G-d's existence in the world. There are physicians and scientists on both sides of the debate. Some will say vaccines are safe and effective, while others will say they are toxic and defective. Are they the greatest invention since sliced bread, or are they the biggest mistake in the history of medicine? Do vaccines enhance our immune system, or do they ruin it? The goal of this discussion is to determine which scientists are telling us the truth.

I would also say that the purpose of this paper is to prove that there is in fact a debate. Many people are under the delusion that "the science is settled" and there's nothing to talk about. As you will soon find out, the science is *not* settled by any stretch of the imagination. Charles H. Duell, Commissioner of the US Patent Office, made the same mistake in 1899 when he purportedly said: "everything that can be invented has been invented." We are constantly discovering new aspects of our remarkable immune system. As our understanding of the immune system develops, the way in which we would like to manipulate that system should also develop. Every time a new discovery is made, we should reevaluate every assumption we have made and ask: are vaccines the best way to go, or should we try an entirely different approach? That's how science usually works. Unfortunately, this question has never been asked in nearly 200 years and counting.

At a minimum, we should always be asking: how can we improve vaccines? But even this basic request is like pulling teeth. After decades of scientific literature on the hazards of mercury, the industry finally agreed to begin a phase out process. However, they will never admit there was anything wrong with it. They only removed it due to the "concern" that people had, but of course mercury is completely harmless. As I point out later, they even regretted the decision of removing mercury from vaccines, because parents and physicians might G-d forbid think that there used to be a slight glitch in the infallible vaccine program.

How did I get involved in a vaccine debate with the CDC without having any background in medicine or science? Here's how it started: I live in a Jewish community in Maryland, together with my wife and six children. Maryland is one of 47 states which has a religious exemption to vaccines. For all practical purposes, public school isn't an option for us because we send our children to private school. The problem is, around 3 years ago, all the private Jewish schools in our community got together and decided that they would not accept a religious exemption. Now we find ourselves in the same predicament as CA, MS, and WV.

My wife and I were always hesitant about vaccines, and we delayed the schedule as much as possible. Around 2 years ago, I started investigating the matter a little deeper. There seemed to be valid room for concern, but not that many answers were available. At that point, we put all vaccines on hold until I could get to the bottom of it. My older three children were in school because they had received all the required vaccines. My 5-year-old, our 4th child, wanted to

attend pre-school so we decided to check his antibody titers, being that he got at least the first dose of all the vaccines. Surprisingly, he passed every one of them. We were thrilled that we wouldn't have to complete the series of any of the vaccines. However, at the last minute, the school administration discovered that although Maryland state law allows titers in lieu of vaccination, they do not accept titer levels for DTaP. Of course, the big question was, WHY? I was determined to find the answer. This seemed like a very random rule, and I figured if I could determine that there wasn't a very compelling reason behind it, they might let it go.

Also, if I could determine that there is no compelling medical reason behind it, the school administration should agree that a religious exemption is warranted. The entire goal of vaccination is to achieve a certain level of antibodies, (which proves how archaic vaccine science is, but that's a different discussion). If we know his titer level is high enough, why is it necessary to give an additional vaccine? Rabbi Moshe Feinstein, of blessed memory, who I am quoting in one of my first emails, says that it might be a violation of our religion to visit a doctor without a valid medical reason. Just to clarify, not only are we permitted to see a doctor, our religion *obligates* us to visit a medical professional in any situation where someone is in any need of medical help. But in the Responsa which I am quoting, written approximately 50 years ago, Rabbi Moshe Feinstein is referring to someone who would like to take some sort of injection in order to help him fast on the High Holiday of Yom Kippur. Apparently, he was too sick to be able to safely get through the entire day without eating or drinking. The injection would give him the strength to be able to fast, and he was asking Rabbi Feinstein if Jewish law would permit, or even obligate him to take the injection, allowing him to participate in the fast. His response was that he is *not* permitted to take the injection. One reason he gives is what I just explained. He has no medical need for the procedure. He only wants it in order to give him the ability to fast. Therefore, he would not be permitted to visit a doctor in that type of situation.

Interestingly, he offers another reason. He says that every medical injection runs the risk of injury, even if doctors are not aware of any risk. Putting ourselves at risk, unless medically required, is a violation of our religion. To clarify, just about every single drug comes with an insert describing all the known side effects and risks. Rabbi Feinstein wasn't talking about those. He was talking about something which has no risks at all. He's saying that although doctors are not aware of it, there still is a risk of injury. I think a perfect example is the saline IV bag, which is exactly what I would like to be hooked up to during the next fast day. Receiving hydration through IV is the most basic, harmless medical procedure known to mankind. According to every doctor in the world, it is 100% safe. And yet, if I were to ask Rabbi Feinstein, he would tell me not to use it unless absolutely necessary because of the risks that are involved. So, I ask you, who do you think is right: Rabbi Feinstein, or every doctor on the planet? As it turns out, the Rabbi was right. We now know that the "harmless" saline IV bag is responsible for 50 to 70 thousand hospital deaths and 100,000 kidney failures *annually*. Go figure!

https://nypost.com/2018/02/27/the-saline-used-in-iv-bags-could-be-killing-you/

Getting back to my question, I contacted the Maryland Health Department to find out why they don't let us use titer levels for DTaP. They made two failed attempts in trying to explain the reason - See the email conversation I attached in Appendix A. The 2nd attempt, which they emailed me on 11/15/17, was quite amusing. She gave an explanation based on waning immunity of pertussis, basically showing that the vaccine doesn't work that well because the immunity only lasts a few years, at best. And that's why we require so many doses of the vaccine, even if the titer levels are good. But as I pointed out to her, that makes no sense. She

later admitted to me, on 11/20/17, that the waning immunity approach did not answer my question, claiming that the previous email was "not intended to explain why Maryland will not accept a titer in lieu of vaccination", rather it was only intended to help me "gain some understanding for the immunization requirements in the state of Maryland". If that's true, why was the subject of her email: "Why vaccination is required for DTaP immunity in lieu of blood testing"? And the best part is what the school said: After I forwarded them the 11/15 email about waning immunity from the Department of Health, I explained to them the same thing which I responded to the State, that it makes no sense. The school responded to me the next day, on 11/16/17, saying that "the Maryland department of health has answered your questions and their answers are medically sound". Seriously? 4 days later, the state admitted to me that she did *not* answer my questions because as I explained to her, it made no sense. I later informed the school administration of their error, and to date I have not received a response from them.

It seemed like a hopeless case. But I figured I would make one more phone call to see if I could get to the bottom of it, and that's when I called the CDC. I spoke to the operator who typed up my question and sent it off to the appropriate department. Within 2 days they emailed me with a response. They were asking me to clarify a few points so they could better understand what I wanted. At the beginning of our conversation, my intention was only to ask that one specific question. But as things progressed, I decided to take the opportunity and ask more questions. I kept asking questions for 7 months, until they told me that they have no more answers to give me.

We all want to do what's best for our children. I'm sharing this with the hope that it will assist other parents in making an educated and responsible decision. After you read the introduction, I recommend skipping to page 38 to my 6/18 email, where I wrote up a summary of the entire discussion with CDC. Then you can go back and read through all the emails with a better understanding of the main points which I'm trying to prove. At the end of the paper, I include an appendix with all the attachments that were included in the emails for your reference. To all parents out there: I'm not telling you what to decide, but I will tell you to keep asking questions. Don't ever assume that "someone" out there must have an answer. I have 27 emails proving that *nobody* out there has *any* good answers. If you have any questions or comments, please feel free to shoot me an email. Or, if you think you're smarter than CDC and FDA and you would like to continue the debate, by all means. You can reach me at rszendro@gmail.com

There are a few people who I have to thank. I can't mention everyone, because the list is too long, but there are just a few people that I feel like I have to mention: Dr. Yoni and Rena Baron, thank you for all your help, and for introducing me to "Dissolving Illusions", by Suzanne Humphries MD and Roman Bystrianyk. I highly recommend that book to everyone. If your jaw doesn't hit the ground after reading it, you should probably see a doctor to make sure you have a pulse. Roman Bystrianyk, who I spoke to several times, thank you for all your help and for introducing me to Dr. Toni Bark. Toni, thank you for always being there, helping out in many different ways throughout this very interesting ordeal. And of course, to my wife, thank you for putting up with all my crazy projects, and allowing me to spend countless hours working on my computer, day and night.

Signing off,

Raphael Szendro, Team Leader, R&R Executive Team of ExecuHome Realty, Pikesville, MD

**11/20/17**

General Public:

Caller's child is 5 years old, and recently had his titers checked for school. Caller wants to know if there is any reason that they shouldn't rely on the tests for polio, DTaP, and varicella.

**11/21/17**

Thank you for your inquiry to CDC-INFO.

So CDC can verify the most current information and best respond to your inquiry, would you please elaborate further on your question? Are you looking for more information on what is required by your state for your son to attend school, or was there another concern you had regarding his vaccination status? Has your child completed the vaccine series for the diseases that you have listed? Have you spoken to your child's doctor about the titers that were taken? This information will help us respond to your inquiry.

**11/22/17**

I'm not asking about state law. I just want to know from a medical perspective, are there any scientific studies or medical literature available that would explain why I should have any concern regarding the immunity levels for my son if his titer levels were tested to be adequately high enough.

He has had 3 doses of DTaP, 2 polio, 1 MMR, and 1 varicella. I attached his records and blood work and a letter written by his pediatrician. The main question is really regarding DTaP. The school is telling me that the state won't allow titer levels for that vaccine, but they will allow it for the other vaccines. For the other vaccines, they will require him to recheck his titers every year. I wanted to know if you would consider it to be medically necessary to give him another dose of DTaP at this point in time. His pediatrician said she's not aware of any reason. If it is medically necessary, I would let him have it. If not, I wouldn't be able to give it to him for religious reasons, and I would have to sign a religious exemption. In my religion, medical interventions and procedures can only be done if it's medically necessary.[1]

Thank you

**11/24/17**

Good morning Mr. Szendro,

For some vaccine preventable diseases, research has demonstrated the titer levels needed for protection against the disease. This is considered a serologic correlate of protection for that disease. MMR and varicella, for example, have well known correlates of protection and thus when a specific titer level is present, your child is considered protected.

[1] אגרות משה או"ח ח"ג ס' צ

However, there are no well-defined serologic correlates of protection for pertussis. In other words, titers are not a reliable measurement for protection against pertussis disease. Thus, even if your son has positive pertussis titers, he may not be fully protected against pertussis disease. Therefore, your son would be recommended to receive a dose of DTaP between 4-6 years of age in accordance with the recommended schedule. This dose is to provide protection against pertussis during his early school years.

Kind regards,

Candice Robinson, MD, MPH
Immunization Services Division
National Center for Immunization and Respiratory Diseases
Centers for Disease Control and Prevention

**11/24/17**

Good morning. Thank you for getting back to me.

It sounds like you're saying that although the research has demonstrated reliable titer levels for other diseases, they haven't gotten around to doing any research for pertussis to determine what the reliable titer level would be. So we just don't know whether or not positive titer levels are reliable for pertussis.

Or are you saying that research was done for pertussis, and the conclusion of the research was that positive titer levels are unreliable.

If what you're saying is the 2nd option, can you please send me a copy of the research that was done?

Thank you

**11/24/17**

Good afternoon Mr. Szendro,

We have consulted with our pertussis subject matter experts who have provided the following response and reference:

"While multiple studies have evaluated potential correlates of protection for pertussis, no consistent, reliable correlate has yet been identified. Thus, titers cannot be used to determine whether someone is protected against pertussis. Receipt of DTaP at age 4-6 years is the best way to ensure protection against protection. Here is a review article that summarizes data on correlates of protection for various vaccine-preventable diseases, which includes references for pertussis: Plotkin SA. Correlates of protection induced by vaccination. Clinical and vaccine immunology. July 2010. 17(7):1055-1065."

Kind regards,

Candice Robinson, MD, MPH
Immunization Services Division
National Center for Immunization and Respiratory Diseases
Centers for Disease Control and Prevention

**11/28/17**

Thank you for the information. I went through some of the studies which you referenced. The article states regarding pertussis: The exact level of each of these antibodies that is protective is controversial...
Other studies that are referenced in that article state that:
1. Anti-pertactin, anti-fimbriae 2/3, and anti-PT may be used as surrogate markers of protection for multicomponent acellular and whole-cell vaccines against pertussis.
PMID: 9796042
2. High susceptibility to symptomatic pertussis was found among persons with low initial IgG antibody concentrations against pertussis toxin, especially those without previous history of pertussis vaccination or disease.
PMID: 12922081
3. Thus, there is a highly significant correlation between the level of vaccine-induced serum PT IgG and protection against pertussis.
PMID: 10720524

I suspect that the reason why titers aren't showing a clear level of protection is because the vaccine itself is flawed, and doesn't protect the individual the way we thought it did. (The 3rd source seems to be saying that antibody titers *are* a good proof of immunity.)

The basic science behind vaccines is over 200 years old. It always bothered me, how can we expect to know what we're doing in manipulating the immune system when we know very little about the way the system works. We certainly knew much less 200 years ago. After doing more research, I found that my suspicions were correct. Recent studies are showing that there are fundamental flaws in the pertussis vaccine.

1. PMCID: PMC4038146:
Current acellular vaccines against *Bordetella pertussis* are effective in preventing severe disease but have little effect on less severe coughing illness that can mediate transmission.
the incidence of whooping cough has increased to levels 50-fold higher than the all-time low in the United States, in 1976
there is debate about whether acellular vaccines induce the most effective type of immune response to protect vaccinated individuals and prevent the spread of disease
Ultimately, control of whooping cough is dependent on understanding the transmission mechanisms of *Bordetella* in naive and immune populations. . . Understanding these mechanisms is necessary before there can be rational design of improved vaccines and therapeutic interventions to block transmission.
current vaccines do not effectively prevent transmission of *Bordetella*and thus fail to confer the full benefits of herd immunity in reducing clinical cases.
It is possible that the effects of acellular vaccination could mask symptoms to allow infected individuals to act as unsuspecting reservoirs for potential spread to more-susceptible individuals.
Importantly, full implementation of acellular-only vaccination began relatively recently, in the

1990s. Therefore, the proportion of the population that has only received the acellular vaccine will continue to rise for several years to come, raising the possibility that we may observe further increases in whooping cough cases.

2. PMID: 22718262
New generation aP that induce Th1 rather than Th2 responses are required to improve vaccine efficacy and prevent further spread of B. pertussis.

3. PMID: 24277828
Pertussis rates in the United States have been rising and reached a 50-y high of 42,000 cases in 2012. Although pertussis resurgence is not completely understood, we hypothesize that current acellular pertussis (aP) vaccines fail to prevent colonization and transmission. . . optimal control of pertussis will require the development of improved vaccines.
key deficiencies remain in our understanding of pertussis-induced helper T-cell immune responses in humans and primates.

4. PMID: 28289064
the increase in pertussis appears to be the result of waning immunity. . .aP is less able to prevent nasopharyngeal colonization of *Bordetella pertussis* than wP or natural infection.

5. PMID: 24216286
Despite high levels of vaccination coverage, pertussis circulation cannot be controlled at all. The results question the efficacy of the present immunization programmes.

> **Here's a recent article from Boston University:**
> https://www.bu.edu/sph/2017/09/21/resurgence-of-whooping-cough-may-owe-to-vaccines-inability-to-prevent-infections/
>
> The startling global resurgence of pertussis, or whooping cough, in recent years can largely be attributed to the immunological failures of acellular vaccines,
>
> "This disease is back because we didn't really understand how our immune defenses against whooping cough worked, and did not understand how the vaccines needed to work to prevent it," said Christopher J. Gill, associate professor of global health and lead author of the article. "Instead we layered assumptions upon assumptions, and now find ourselves in the uncomfortable position of admitting that we may made some crucial errors.

**Conclusion:** I'm still searching for a compelling reason to give my son another dose of DTaP. The current science seems to be saying that we don't understand the immune system, and the current vaccine for pertussis doesn't work. Not only does the vaccine not protect the individual, it could "mask symptoms to allow infected individuals to act as unsuspecting reservoirs for potential spread to more-susceptible individuals".

**1/1/18**

Happy new year!

I hope you had a chance to review the medical literature that I referenced in my last email on Nov 28th.

Based on recent studies, does the CDC still recommend that I vaccinate my child with DTaP?

Please let me know what you recommend, and show how your recommendation is consistent with *all* available medical literature. On your website you made a "pledge to the American People": #3 - "Base all public health decisions on the highest quality scientific data that is derived openly and objectively"

Let's make 2018 an amazing and healthy year for everyone!

**1/8/18**

Thank you for contacting CDC-INFO. We hope you find the following information about titer results helpful.

Unfortunately, CDC cannot provide individualized medical consultations. Interpretation of clinical test results requires discussion between the ordering physician and the lab's medical director. The physician then discusses the result with the patient. CDC recommends that you speak with your child's doctor for a better understanding of what these results mean for your child, specifically. If your child's healthcare provider is unsure of how to interpret your results, he/she can contact CDC or a local infectious disease specialist for assistance.

Unfortunately, CDC is not able to provide medical advice. CDC does not have public hospitals or doctors' offices, and is unable to do the following:

• See patients,
• Diagnose illnesses,
• Give specific opinions or advice about symptoms,
• Interpret laboratory results,
• Provide medical or veterinary treatment, or
• Prescribe medicine.

For more information about vaccination, please visit the CDC website:

Vaccines & Immunizations
https://www.cdc.gov/vaccines/index.html

Diphtheria, Tetanus, and Pertussis Vaccine Information Statement (VIS)
https://www.cdc.gov/vaccines/hcp/vis/vis-statements/dtap.html

Diphtheria, Tetanus, and Pertussis Vaccine Safety
https://www.cdc.gov/vaccinesafety/vaccines/dtap-tdap-vaccine.html

**1/8/18**

Thank you for getting back to me. I already resolved the specific question that I had regarding my son. I gave him one more dose of DTaP. I couldn't get him into school without it, so I had no choice.

However, after reading through many of the relevant studies, I still have a few general questions for you, one of which I explained in my 1/1/18 email.

https://www.cdc.gov/about/organization/mission.htm

You stated there:

## Pledge to the American People

1. Be a diligent steward of the funds entrusted to our agency
2. Provide an environment for intellectual and personal growth and integrity

3. Base all public health decisions on the highest quality scientific data that is derived openly and objectively
4. Place the benefits to society above the benefits to our institution
5. Treat all persons with dignity, honesty, and respect

My question is regarding #3. Based on what I emailed you on 11/28/17, it looks like the most current research is showing that the pertussis vaccine doesn't work that well in protecting the individual, nor does it help in creating herd immunity. It actually creates an *opposite* effect of herd immunity, "to allow infected individuals to act as unsuspecting reservoirs for potential spread to more-susceptible individuals".

1. Do you still recommend that Americans vaccinate their children for pertussis?

2. You stated in one of the links that you sent me: *PERTUSSIS (Whooping Cough) causes coughing spells so bad that it is hard for infants to eat, drink, or breathe. These spells can last for weeks. It can lead to pneumonia, seizures (jerking and staring spells), brain damage, and death.* Can you please tell me what are the chances that whooping cough can cause brain damage or death? Please show me where you got the data that show the risks.

3. You also stated on your website: *Getting diphtheria, tetanus, or pertussis disease is much riskier than getting DTaP vaccine*. Can you please tell me what are the chances that the DTaP vaccine can cause brain damage or death? Please show me where you got the data that show the risks.

4. You also stated on your website: *Most children who are vaccinated with DTaP will be protected throughout childhood*. The last dose that you recommend is 4-6 years. What exactly did you mean that most children will be protected "throughout childhood"? Did you mean until age 18? Does that mean most children will be immune from pertussis for 12 years?

Thank you

**1/10/18**

Mr. Szendro:

Your message was referred to me for reply. My answers are prefaced by A:

You asked:

> 1. Do you still recommend that Americans vaccinate their children for pertussis?

A: Yes; although pertussis cases have increased from their low point in the 1980's to the present, the number of cases is still far less than reported prior to use of any vaccine; see https://www.cdc.gov/pertussis/surv-reporting.html

> 2. You stated in one of the links that you sent me: *PERTUSSIS (Whooping Cough) causes coughing spells so bad that it is hard for infants to eat, drink, or breathe. These spells can last for weeks. It can lead to pneumonia, seizures (jerking and staring spells), brain damage, and death.* Can you please tell me what are the chances that whooping cough can cause brain damage or death? Please show me where you got the data that show the risks.

A: The pertussis chapter in the textbook Vaccines by Plotkin, Orenstein, and Offit, which you should find in medical libraries, indicates a range of pneumonia incidence of up to 23.8% in infants <6 months of age, and 5.8% in children and infants overall. The rat of encephalopathy is quoted from various studies as between 8 and 80 per 100,000 cases. CDC cites other studies at https://www.cdc.gov/pertussis/clinical/complications.html, with a death rate in infants <12 months of age, of approximately 0.5% (1% of hospitalized infants, and about ½ of all infants with pertussis are hospitalized).

3. You also stated on your website: *Getting diphtheria, tetanus, or pertussis disease is much riskier than getting DTaP vaccine*. Can you please tell me what are the chances that the DTaP vaccine can cause brain damage or death? Please show me where you got the data that show the risks.

A: The most recent summary of evidence on association of encephalopathy and /or encephalitis from 2011 by the Institute of Medicine said the evidence was inconclusive about a causal relationship between acellular pertussis vaccines and these conditions. You can read the report on pp. 534-8 in the attached document.[2]

4. You also stated on your website: *Most children who are vaccinated with DTaP will be protected throughout childhood*. The last dose that you recommend is 4-6 years. What exactly did you mean that most children will be protected "throughout childhood"? Did you mean until age 18? Does that mean most children will be immune from pertussis for 12 years?

A: We believe protection lasts at least 5 years after the last DTaP dose, with an estimated 71% of children still protected after 5 years, hence the recommendation to give Tdap at age 11-12 years. More information is at https://www.cdc.gov/vaccines/vpd/dtap-tdap-td/hcp/about-vaccine.html.

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[2] The full report is 895 pages long. You can download it here: https://www.nap.edu/catalog/13164/adverse-effects-of-vaccines-evidence-and-causality

**2/22/18**

Thank you for responding to my questions. It took me a while to go through the information since I recently had a baby boy on 1/15. We have our hands full, to say the least.

If you're going to recommend a vaccine, I think you would agree that it's important to look at the full picture when evaluating the risk-to-benefit ratio. There were several points that I made which prompted me to ask the question: "do you still recommend that Americans vaccinate their children for pertussis?" You focused on one specific point, that cases of pertussis today are fewer than the number of cases prior to the use of a vaccine. On the surface, that seems to be true. But when you look at the big picture, not only does that fact become irrelevant, it might not even be accurate.

What we see from the peer reviewed studies which I referenced in my Nov 28th email, is that the pertussis vaccine only prevents or minimizes the severity of the symptoms. It does NOT prevent infection of the disease, or the spread of the disease. Fundamental flaws were made, incorrect assumptions were made, and the number of cases of whooping cough are likely to increase over the coming years.

What do we gain by vaccinating for pertussis? It would appear that for now, fewer people will show severe symptoms of the disease. That sounds like a good thing. Now let's look at the bigger picture: There's a child at my son's school who is immunosuppressed. He's at risk of dying if he gets sick. If my child gets whooping cough, he's going to stay home with me for a week or 2, and then return to school after fully recovering, completely disease free. But if my son is vaccinated for pertussis, he might be unknowingly carrying and spreading the disease to others, including the boy at school who is at risk of death. It would seem to be very selfish of me to vaccinate my son in order to prevent one or two weeks loss of work, at the expense of another child's life. The same thing is true with a newborn infant. If a family member has a terrible cough, they're not going anywhere near that baby. But if everyone in the family including grandparents are immunized, they might be bringing the pertussis bacteria directly to my infant. This is exactly what I quoted in my previous email from recent studies: "There is debate about whether acellular vaccines induce the most effective type of immune response to protect vaccinated individuals and prevent the spread of disease . . . It is possible that the effects of acellular vaccination could mask symptoms to allow infected individuals <u>to act as unsuspecting reservoirs for potential spread to more-susceptible individuals</u> . . . Despite high levels of vaccination coverage, pertussis circulation cannot be controlled at all." The benefit of the vaccine doesn't turn out to be that much of a benefit when you look at the big picture.

Now let's look at the risks of the vaccine: First we'll talk about what you said regarding the risk of encephalopathy. You were responding to my question which was: "You also stated on your website: *Getting diphtheria, tetanus, or pertussis disease is much riskier than getting DTaP vaccine*. Can you please tell me what are the chances that the DTaP vaccine can cause brain damage or death? Please show me where you got the data that show the risks." To which you responded: "The most recent summary of evidence on association of encephalopathy and /or encephalitis from 2011 by the Institute of Medicine said the evidence was inconclusive about a causal relationship between acellular pertussis vaccines and these conditions." Correct me if I'm wrong, but what it sounds like you're trying to tell me is that the risk of encephalopathy, which is one specific type of brain damage, is small to none. And how do we know that the risk is small to none? Because it says so in the IOM report from 2011. My friend, you have to read the instructions. In the very same report which you quoted, the authors wrote in the Preface to the report, found on page 12 of the PDF: "The committee particularly counsels readers not to interpret a conclusion of inadequate data to accept or reject causation as evidence either that causation is either present or absent. Inadequate data to accept or reject causation

means just that—inadequate." When you say on your website that A is much riskier than B, that means you know the level of risk of both A and B. You already showed me where you got the data for the level of risks of the disease. But you still haven't shown me where you got the data for the risks of DTaP.

I have the exact same problem with another page on the CDC website which states: "Vaccines Do Not Cause Autism… studies have shown that there is no link between receiving vaccines and developing ASD." The CDC then quotes the exact same IOM report from 2011. Let me quote you the exact words found in the report on page 546: "**Conclusion 10.6**: The evidence is inadequate to accept or reject a causal relationship between diphtheria toxoid–, tetanus toxoid–, or acellular pertussis–containing vaccine and autism." How can you say that this study is showing us that there is no link? The authors specifically instructed us not to interpret a conclusion of inadequate data as evidence that causation is absent.

Please show me where you got the data proving that DTaP does not cause autism, as it is claimed on the CDC website: "Vaccines Do Not Cause Autism", which includes DTaP.

Here's some information that I found regarding the risks of DTaP, showing that it *does* in fact cause autism, and several other types of neurological and autoimmune disorders. Most of these studies focus on the risk of injecting aluminum, which is one of the ingredients used in DTaP.
PMID: 22099159
PMID: 23609067

PMID: 21568886

PMID: 22235057

PMID: 26948677

PMID: 23932735

PMID: 25506338

PMID: 20882368

PMID: 11335699

PMID: 23557144

When you finish reading these, I can send you a few dozen more. I would like to know if you have any studies that you can show me that prove the safety of injecting aluminum into animals and/or humans?

Here's an interesting study from 2010 showing that: "Boys vaccinated as neonates had threefold greater odds for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life."
PMID: 21058170

When we were in the hospital on Jan 15th with our newborn son, my wife told me that the pediatrician on staff came in and recommended the Hepatitis B vaccine, explaining the urgency of getting it done in the hospital and not waiting until our next visit to our pediatrician. My wife wisely declined. Personally, I don't see the great need to inject an STD into any newborn child on his first day of life, especially considering all the current studies I have read.

I believe we still have a few unanswered questions. I would greatly appreciate a response.

Thank you

**2/22/2018**

Some answers to your additional questions:

1. Despite the imperfect, limited duration of immunity from the current pertussis vaccines, the number of pertussis cases in the U.S. is still far less than the time before vaccines, hence they offer real benefit; see https://www.cdc.gov/pertussis/surv-reporting.html. We do need better pertussis vaccines that can interrupt transmission as you point out.

2. Studies finding no association between DTaP vaccine and encephalopathy are listed here: https://www.cdc.gov/vaccinesafety/vaccines/dtap-tdap-vaccine.html.

3. Regarding vaccines and autism, this earlier compendium of studies rejected an association of vaccines, particularly MMR vaccine, and thimerosal, as related to, or causing autism, which is attached, and here: http://nationalacademies.org/hmd/reports/2004/immunization-safety-review-vaccines-and-autism.aspx. The CDC data and related information about autism and vaccines are at https://www.cdc.gov/vaccinesafety/concerns/autism.html.

4. We, as adults and children, ingest more aluminum from our food than received in vaccines. The attached article discusses this issue, and does not identify a measurable risk from aluminum in vaccines.[3]

5. The authors of the article about hepatitis B vaccine and autism focus on the thimerosal association, which we believe is not valid, and even if one is concerned about thimerosal in vaccines, there is little or no thimerosal in any vaccines given to children now, other than some influenza vaccines;
see https://www.fda.gov/BiologicsBloodVaccines/Vaccines/QuestionsaboutVaccines/ucm070430.htm.

I wish you and your family good health.

Raymond A. Strikas, MD, MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

[3] Appendix B

Immunization Services Division

National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention

Ras8@cdc.gov

Tel. 404 639 6465

**3/6/2018**

Good evening,

Thank you for taking the time to answer my questions. I have a few things to say regarding your last email:

**1.** A. I agree that you can view this as a benefit to a certain degree, but it's very superficial when you keep in mind that "less cases of pertussis" just means less severe reactions to the infection. It also means putting vulnerable individuals at higher risk. I would say that due to the deficiencies of the vaccine, we should stop using it until a safer and more effective one is developed. But it doesn't seem like you agree with me on that.

B. What we *do* agree on is that the current pertussis vaccine does not provide herd immunity. As you stated: "We do need better pertussis vaccines that can interrupt transmission".

**2.** My original question was, what are the risks of brain damage and death? You gave me information on one specific type of brain damage, which is helpful, but it doesn't answer my main question. I want to know about the risks of the vaccine in general, which is very important if you want to assess the risk to benefit ratio. Let me put the question a little differently: Can you tell me about the safety studies that were done on DTaP before and/or after being put on the childhood schedule. I recently read something that was very puzzling: The control group for the safety trial doesn't get a saline placebo. The control group is given all the ingredients in the vaccine except for the virus or bacteria. Or they might get a different vaccine all together.

A. Is that true?

B. If it's true, why would they do that? Every other drug in the world that gets tested for safety will have one group who receives the drug, and a control group which receives an inert placebo. Why would they do it any differently with vaccines? You're practically testing it against itself. How does that prove any level of safety?

**3.** I haven't had a chance to read all of the studies that you referenced, but what it sounds like you're saying is that MMR was studied to see whether or not it causes autism, as well as thimerosal. DTaP does not contain thimerosal. That means DTaP was never studied to determine whether or not it causes autism. The question from my last email still remains: How can the CDC say: "Vaccines Do Not Cause Autism" – including DTaP and other vaccines which

were never studied? Also, the IOM report which they quote as a source is completely erroneous.

**4.** A. You said: "We, as adults and children, ingest more aluminum from our food than received in vaccines." In the document that you attached (PMID: 22001122), it says: "The body burden of aluminum from vaccines is not more than 2-fold higher than that received in the diet." The study which is quoted there (PMID: 12184359) says: "The calculated body burden of aluminum from vaccinations exceeds that from dietary sources". It sounds like the amount of aluminum in vaccines is greater than the amount ingested. Furthermore, I'm a real estate agent, not a scientist. But I know that you can't compare apples to oranges. Wouldn't you agree that there's a big difference between eating and injecting? I can drink a half gallon of Kool-Aid on a hot summer day. It's not the healthiest choice, but I'll be in pretty good shape when I'm done with it. What do you think would happen to me if I injected a half gallon of Kool-Aid through IV? I wouldn't want to find out, but probably not a good situation. What if you took your recommended list of vaccines, and instead of injecting them, you shot the contents into a cup and drank it? What if a pediatrician tried that technique on a child? It would probably result in a lawsuit and possible jail time. Obviously, there's a clear difference between ingesting and injecting. That's why I asked in the last email if you "have any studies that you can show me that prove the safety of *injecting* aluminum into animals and/or humans".

B. Please look up a recent study (PMID: 28576261) published less than a year ago. The title is: "Critical analysis of reference studies on aluminium-based adjuvants toxicokinetics".[4] It explains numerous flaws with the study which you referenced. Do you agree that those flaws exist? If not, please explain why?

C. Please see the attached report published Aug 2017 titled: "AUTISM & ALUMINUM ADJUVANTS IN VACCINES".[5] It's a 23 page report showing scientific evidence that aluminum adjuvants can cause autism and other brain injuries, with roughly 75 references to other published studies. It also describes other flaws with the study that you cited. We also had the 10 studies which I listed in my last email showing the hazards of injecting aluminum into animals and humans. And as I said before, there are dozens more. I'm trying to look at the full picture, and what I see in front of me is well over 100 studies showing that injecting aluminum is extremely dangerous and toxic. Then you have the one study which you sent me, together with another one or two, suggesting that aluminum is safe. Not only are those 2 or 3 studies greatly outnumbered, there's a whole laundry list of reasons why those studies are flawed. Please explain to me why you feel it makes sense as a scientist to rely on the study which you referenced. Or, maybe some of this information is new to you, and perhaps you will change your opinion regarding the safety of aluminum in vaccines.

[4] Appendix C
[5] Appendix D

**3/8/2018**

Mr. Szendro:

I offer some more information below. It appears you are convinced of your points of view, and I of CDC's. You may choose to vaccinate your children or not, as per local and state laws requirements, of which you seem to be well aware. If you have additional questions about the contents of vaccines, given CDC does not approve or license vaccines, it may be more beneficial to ask your questions of the Food and Drug Administration at

(800) 835-4709

(240) 402-8010

ocod@fda.hhs.gov

**Consumer Affairs Branch (CBER)**

Division of Communication and Consumer Affairs

Office of Communication, Outreach and Development

Food and Drug Administration

10903 New Hampshire Avenue

Building 71 Room 3103

Silver Spring, MD 20993-0002

Item 1 does not require a response.

2. : Can you tell me about the safety studies that were done on DTaP before and/or after being put on the childhood schedule?

The largest group of safety studies occurred in Europe prior to licensure, and these are described here with references listed: https://www.cdc.gov/mmwr/PDF/rr/rr4607.pdf. Per this reference, and the chapter on pertussis vaccines in the textbook Vaccines by Plotkin, Orenstein, Offit, and Edwards (which you can find in most medical libraries), the comparative arms of the DTaP studies were usually either DTP vaccine, and/or DT. Only one study used diluent as a placebo. The rationale appears to be withholding vaccine could have been considered unethical, given DTP vaccine was the standard of care. The rates of serious adverse events, including temporary encephalopathy in these studies were much lower than with DTP

vaccines. The second article abstract below cites the Japanese experience, with very little reported encephalopathy since DTaP vaccine was adopted there.

Also, many cases of encephalopathy attributed to DTaP vaccination have now been found to have occurred in children with Dravet's syndrome; see

Lancet Neurol. 2010 Jun;9(6):592-8. doi: 10.1016/S1474-4422(10)70107-1. Epub 2010 May 4.

# Effects of vaccination on onset and outcome of Dravet syndrome: a retrospective study.

McIntosh AM[1], McMahon J, Dibbens LM, Iona X, Mulley JC, Scheffer IE, Berkovic SF.

## Author information

1

Epilepsy Research Centre and Department of Medicine (Neurology), University of Melbourne, Victoria, Australia.

### Abstract

**BACKGROUND:**

Pertussis vaccination has been alleged to cause an encephalopathy that involves seizures and subsequent intellectual disability. In a previous retrospective study, 11 of 14 patients with so-called vaccine encephalopathy had Dravet syndrome that was associated with de-novo mutations of the sodium channel gene SCN1A. In this study, we aimed to establish whether the apparent association of Dravet syndrome with vaccination was caused by recall bias and, if not, whether vaccination affected the onset or outcome of the disorder.

**METHODS:**

We retrospectively studied patients with Dravet syndrome who had mutations in SCN1A, whose first seizure was a convulsion, and for whom validated source data were available. We analysed medical and vaccination records to investigate whether there was an association between vaccination and onset of seizures in these patients. Patients were separated into two groups according to whether seizure onset occurred shortly after vaccination (vaccination-proximate group) or not (vaccination-distant group). We compared clinical features, intellectual outcome, and type of SCN1A mutation between the groups.

**FINDINGS:**

Dates of vaccination and seizure onset were available from source records for 40 patients. We identified a peak in the number of patients who had seizure onset within 2 days after vaccination. Thus, patients who had seizure onset on the day of or the day after vaccination (n=12) were included in the vaccination-proximate group and those who had seizure onset 2 days or more aftervaccination (n=25) or before vaccination (n=3) were included in the vaccination-distant group. Mean age at seizure onset was 18.4 weeks (SD 5.9) in the vaccination-proximate group and 26.2 weeks (8.1) in the vaccination-distant group (difference 7.8 weeks, 95% CI 2.6-13.1; p=0.004). There were no differences in intellectual outcome, subsequent seizure type, or mutation type between the two groups (all p values >0.3). Furthermore, in a post-hoc analysis, intellectual outcome did not differ between patients who received vaccinations after seizure onset and those who did not.

**INTERPRETATION:**

Vaccination might trigger earlier onset of Dravet syndrome in children who, because of an SCN1A mutation, are destined to develop the disease. However, vaccination should not be withheld from children with SCN1A mutations because we found no evidence that vaccinations before or after disease onset affect outcome.



The article below is one example, where most recently, with DTaP vaccine, Japan reported encephalopathy at the very low rate of 1 to 1.3 per 10 million children following DTaP vaccine.

Pediatr Int. 2004 Dec;46(6):650-5.

# Safety and efficacy of acellular pertussis vaccine in Japan, evaluated by 23 years of its use for routine immunization.

Kuno-Sakai H[1], Kimura M.

**Author information**

1

Department of Public Health and Social Medicine, Tokai University, Isehara City, Japan. harumi@is.icc.u-tokyo.ac.jp

## Abstract

**BACKGROUND:**

Real evaluation of any vaccine can only be done after the vaccine has been in routine use for a substantially long period of time. In Japan, acellular pertussis vaccine was introduced and totally replaced whole cell pertussis vaccine in 1981. From 1982 to 1988 40.3 million doses of acellular pertussis vaccine were given to 2-year-olds and from 1989 to 2001 59.3 million doses of acellular pertussis vaccine were given to 3-month-olds. It is now time to evaluate the efficacy and safety of acellular pertussis vaccine by the use of national data officially supplied by the Government.

**METHODS:**

Government national surveillance of pertussis, which began in 1981, was used to analyze epidemiology of pertussis. Official Government reports on acceptance rates of pertussis were analyzed. A peer review has been made on all severe neurological illnesses/death occurring after pertussis immunization which have been applied for through the Vaccine Injury Compensation System, Ministry of Health Labor and Welfare, Japan.

**RESULTS:**

High acceptance rates of acellular pertussis vaccine combined with diphtheria and tetanus toxoids (DTaP) has been maintained and a dramatic decrease in pertussis was noted over the past 23 years. Neurological illnesses temporally associated both with whole cell and with acellular pertussis vaccination has been a rare phenomenon. However, incidences ofencephalopathy/encephalitis and status epileptics/frequent convulsions, febrile seizures/provocation of convulsions, and sudden deaths were significantly lower with acellular pertussisvaccination than with whole cell pertussis vaccination.

**CONCLUSION:**

With the use of acellular pertussis vaccine which has been accepted by the public, pertussis has been well controlled in Japan.

3. You asked: "How can the CDC say: "Vaccines Do Not Cause Autism" – including DTaP and other vaccines which were never studied?"

Please see the Autism Speaks website for their assessment of DTaP vaccines and incidence of autism: https://www.autismspeaks.org/node/20441. You did not state you had reviewed the studies at this page: Then you can send information demonstrating these are flawed or mistaken. At present, there are no convincing data vaccines cause autism.

4. Response to question #4:
In 2000, the National Vaccine Program Office sponsored a workshop on aluminum in vaccines. A summary of that workshop is available in the journal Vaccine 20 (2002) S1-S4. It contains a detailed summary of what was known and unknown about aluminum containing adjuvants at that time. It states: " … Minor reactions have occurred but there have been few serious reactions." Available athttp://www.infovaccini.it/lib/exe/fetch.php?media=aluminum_nvpo_usa.pdf

After this workshop, the Mitkus paper that you have referenced was done, as well as the Glanz et al 2015 study (available at https://www.ncbi.nlm.nih.gov/pubmed/26518400) which concluded that "The safety of vaccine aluminum exposure can be feasibly studied in the Vaccine Safety Datalink (VSD). However, possible biological mechanisms and confounding variables would need to be considered before conducting any studies."

A summary on this topic is in the paper by Offit and Jew which states: "The safety of aluminum has been established by experience during the past 70 years, with hundreds of millions of people inoculated with aluminum containing vaccines. Adverse reactions including erythema, subcutaneous nodules, contact hypersensitivity, and granulomatous inflammation have been observed rarely." These authors also concluded that "the burden of aluminum to which infants are exposed in food and vaccines is clearly less than the guideline established by the ATSDR (minimum risk level for exposure to aluminum was 2

mg/kg/day) and far less than that found to be safe in experimental animals." Available athttp://pediatrics.aappublications.org/conten t/112/6/1394

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**From:** Raphael Szendro [mailto:rszendro@gmail.com]
**Sent:** Tuesday, March 6, 2018 9:20 PM
**To:** Strikas, Raymond A. (Ray) (CDC/OID/NCIRD) <ras8@cdc.gov>
**Cc:** NIPINFO (CDC) <NIPINFO@cdc.gov>; RDB Inquiries (CDC) <RDB@cdc.gov>; MVPDB Inquiries (CDC) <MVPDB@cdc.gov>
**Subject:** Re: multiple safety gp 2

**3/21/2018**

Good afternoon,

We're having fun with the kids today on our day off thanks to the snow storm. I hope you're enjoying better weather down south. I just finished writing up my response to your last email. It's a little bit long-winded, but I hope you have some time to read through it and let me know what you think.

Thank you

"CDC does not approve or license vaccines"

CDC does, however, recommend vaccines. The Advisory Committee on Immunization Practices, which I'm assuming is part of CDC, "develop recommendations on the use of vaccines in the civilian population of the United States. The ACIP holds three meetings each year at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia to review scientific data and vote on vaccine recommendations." As I mentioned in a previous email, CDC made a pledge to the American people to "base all public health decisions on the highest quality scientific data that is derived openly and objectively". I'm asking you to show me the "highest quality scientific data" which was used when recommending the current vaccine schedule. I think we're almost ready to resolve that question. I would rather not start an entirely new conversation with the FDA.

"It appears you are convinced of your points of view, and I of CDC's."

I wouldn't say I'm convinced one way or the other. I used to vaccinate my children. My oldest 3 are fully up to date. I only stopped for the time being because I had so many unanswered questions. I'm glad I finally found someone who is willing to talk to me. Science is the only thing that will convince me of anything. The question is, which science is accurate, and which one isn't? For example, injecting aluminum into humans: is it perfectly harmless, or is it extremely toxic? Which science is correct? They can't both be right. We'll get back to that shortly. So far, I would say that I'm leaning towards not continuing to vaccinate my children. As far as I can tell, the vast majority of scientific data shows that the risks of vaccines outweigh any possible benefit, and the logic and evidence of these studies is much more compelling compared to the studies which are in favor of vaccination. If you can show me why I'm making a mistake, I would be more than happy to get all my children back on schedule with their vaccines.

"Item 1 does not require a response."

I'm glad we agree on that point.

"The largest group of safety studies occurred in Europe prior to licensure… the comparative arms of the DTaP studies were usually either DTP vaccine, and/or DT."

Check out this study: "The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natural Experiment." PMID: 28188123[6]

The study shows that infants who received DTP were 5 times more likely to die compared to those who were not vaccinated. It concludes: "All currently available evidence suggests that DTP vaccine may kill more children from other causes than it saves from diphtheria, tetanus or pertussis." What you're telling me is that DTaP is just as safe as DTP. Not very reassuring.

"The rationale appears to be withholding vaccine could have been considered unethical, given DTP vaccine was the standard of care."

So you agree with me that for the most part, an inert placebo is not used when testing the safety of vaccines, because that would be unethical. It's unethical to make sure a drug is safe before approving it for public use? I find that remarkably paradoxical. Furthermore, there is almost always a small percentage of the population who does not vaccinate their children. Was any attempt made to locate or invite those children and include them in the studies that you referenced?

"Also, many cases of encephalopathy attributed to DTaP vaccination have now been found to have occurred in children with Dravet's syndrome; see Lancet Neurol. 2010 Jun . . ."

"The article below is one example, where most recently, with DTaP vaccine, Japan reported encephalopathy at the very low rate of 1 to 1.3 per 10 million children following DTaP vaccine. Pediatr Int. 2004 Dec;46(6):650-5. . ."

Are you trying to prove that vaccines do not cause encephalitis or encephalopathy? If that's the case, I'm very confused. The first study you quoted is from 2010. The second study is from 2004. And yet, the 2012 IOM report which we discussed earlier states as follows:

[6] Appendix E

**Conclusion 10.1**: The evidence is inadequate to accept or reject a causal relationship between diphtheria toxoid–, tetanus toxoid–, or acellular pertussis–containing vaccine and encephalitis.

**Conclusion 10.2**: The evidence is inadequate to accept or reject a causal relationship between diphtheria toxoid–, tetanus toxoid–, or acellular pertussis–containing vaccine and encephalopathy.

Are you saying that you disagree with the Institute of Medicine which concluded that, as of 2012, there was no available data to determine whether or not DTaP causes encephalitis or encephalopathy? Or are you saying that the Institute of Medicine was unaware of the studies that you referenced?

"Please see the Autism Speaks website for their assessment of DTaP vaccines and incidence of autism: https://www.autismspeaks.org/node/20441. You did not state you had reviewed the studies at this page:"

There are no studies at this page. All they do is quote the IOM report, which says that MMR doesn't cause autism, and DTaP has inadequate data to determine whether or not it causes autism. Therefore, my question remains: How can the CDC say: "Vaccines Do Not Cause Autism" – including DTaP and other vaccines which were never studied?

"At present, there are no convincing data vaccines cause autism."

Really? First let's look at mercury. Here are a few quotes from the FDA website:

"Since 2001, all vaccines manufactured for the U.S. market and routinely recommended for children ≤ 6 years of age have contained no thimerosal or only trace amounts (≤ 1 microgram of mercury per dose remaining from the manufacturing process), with the exception of inactivated influenza vaccine. . . Currently, all hepatitis vaccines manufactured for the U.S. market contain either no thimerosal or only trace amounts. Also, DT, Td, and Tetanus Toxoid vaccines are now available in formulations that contain no thimerosal or only trace amounts . . . Thimerosal may be added at the end of the manufacturing process to act as a preservative to prevent bacterial or fungal growth in the event that the vaccine is accidentally contaminated, as might occur with repeated puncture of multi-dose vials. When thimerosal is used as preservative in vaccines, it is present in concentrations up to 0.01% (50 micrograms thimerosal per 0.5 mL dose or 25 micrograms mercury per 0.5 mL dose). In some cases, thimerosal is used during the manufacturing process and is present in small amounts in the final vaccine (1 micrograms mercury or less per dose)."

In conclusion, inactivated influenza vaccines still contain mercury, and other vaccines may contain trace amounts of mercury. The flu vaccine is recommended to pregnant women during all stages of pregnancy. Please read the following article: "Methodological Issues and Evidence of Malfeasance in Research Purporting to Show Thimerosal in Vaccines Is Safe".[7]

https://www.hindawi.com/journals/bmri/2014/247218/

Towards the beginning of the article, the authors include a link giving you access to a spreadsheet that contains a list of 168 scientific studies (attached)[8] showing numerous injuries caused by mercury, including autism. Then they list 6 studies which are commonly used to support the belief that mercury is safe. The obvious question is, which one is correct? Is it the 168 studies that show several injuries triggered by mercury, or is it the 6 studies showing that mercury is harmless? They can't both be

[7] Appendix F
[8] Appendix G

right. Logically, the authors side with the majority opinion, giving a detailed explanation for each of the 6 studies showing why their data is not a contradiction to the obvious fact that mercury is highly toxic.

You stated that you are convinced of CDC's point of view. What was it about those 6 studies that convinced you that they were correct, and why would you disagree with the authors' explanations on each of the 6 studies? Also, what was it about the 168 studies that you found unconvincing?

You also have to read the following article: "New CDC Research Debunks Agency's Assertion That Mercury in Vaccines Is Safe", by Robert F. Kennedy, Jr. and Lyn Redwood, RN, MSN, published February 2017.

https://www.ecowatch.com/cdc-mercury-vaccines-kennedy-2226257805.html

They start off the article with a very interesting question: "The U.S. Environmental Protection Agency (EPA) and U.S. Food and Drug Administration (FDA) once again advised pregnant women to curb consumption of fish in order to limit fetal exposures to neurotoxic mercury. This warning raises the baffling query: How can the Centers for Disease Control and Prevention (CDC) justify its recommendations that pregnant women get flu shots which are laden with far more mercury than what's found in a can of tuna?"

You have to read the entire article, but here are a few other quotes that I found interesting:

A single Thimerosal-preserved flu vaccine contains 25 micrograms of ethylmercury. If the EPA RfD for ingested methylmercury is applied to this injected ethylmercury figure, an individual would have to weigh more than 250 kilograms (551 pounds) for the 25 microgram exposure to be considered safe. Back in the 1990s, a two-month-old child could have received 62.5 micrograms from three vaccines in a single doctor's visit. Assuming the child weighed about 5 kilograms (11 pounds), he or she would have received 125 times the EPA RfD for methylmercury.

The WHO's conclusion that ethylmercury is safer because of its "short" half-life may be based on observations that ethylmercury disappears from blood samples quicker than methylmercury. However, this tendency may be evidence not of ethylmercury's comparative safety, but of its greater danger if, as science has suggested, ethylmercury is not leaving the body but simply migrating more rapidly to the organs, including the brain. Indeed, studies have shown that an ethylmercury compound's short residence in the blood stems from its ability to more easily pass into the organs, where it can remain for long periods and possibly cause injury.

Beyond a possibly greater capacity to have inorganic mercury accumulate in organs, Thimerosal also passes more easily from a mother's bloodstream through the placenta into a developing baby than does methylmercury. That was the evaluation made in a 1983 review study by A. Leonard. In addition, a 1995 study demonstrated that both ethylmercury and methylmercury cause mutagenic changes at similar concentrations in bacterial cells.

Other research had clarified that, while ethylmercury disperses quickly from the bloodstream, this is not evidence of safety. For example, a 2004 study by G. Jean Harry of the National Institute of Environmental Health Sciences noted that mice injected with Thimerosal accumulated mercury in both the brain and kidneys. "By seven days" post-treatment, the study authors wrote, "mercury levels decreased in the blood but were unchanged in the brain" compared to levels measured just 24 hours after treatment, indicating slow clearance.

The authors conclude: "Overwhelmingly, the literature presents clear evidence that ethylmercury is invasive and persistent in the brain. Emerging evidence suggests that ethylmercury is more toxic than methylmercury, in direct contrast with the CDC's historic position."

I found the article fascinating and highly informative. Please explain to me why you are convinced that the authors are incorrect in their evaluation and understanding of the current medical literature.

Now let's get back to aluminum:

"In 2000, the National Vaccine Program Office sponsored a workshop on aluminum in vaccines. A summary of that workshop is available in the journal Vaccine…"

I read the 4 page workshop summary, which states:

There seems to be abundant data concerning risk levels for ingested aluminum, but scant data about risk levels for injected aluminum. The oral minimum risk level, for example, appears to be in the range of 2–60 mg/kg of aluminum per day but there are no comparable data for injected aluminum. The uncertainties notwithstanding, there appeared to be a large margin of safety for aluminum adjuvants.

I'm glad to see they acknowledged the obvious fact which I pointed out in my last email, that there's a difference between ingested aluminum and injected aluminum. Apparently back then there wasn't much research on injected aluminum, which didn't seem to bother them. A lot has happened since 2000. I attached letters from three independent aluminum adjuvant experts, stating that injecting aluminum may cause various neurological disorders including autism.[9] Between the three of them, they have published over 200 peer reviewed papers on the subject. I asked you this question twice before so I'm assuming the answer is no, but I'll ask you again just to make sure: Do you have any studies that you can show me proving the safety of injecting aluminum into animals and/or humans?

"After this workshop, the Mitkus paper that you have referenced was done…"

Actually, you were the one who referenced the Mitkus paper. You attached it in your 2/22 email. I responded by showing you a study entitled "Critical analysis of reference studies on aluminium-based adjuvants toxicokinetics", where the authors discuss the three toxicokinetic studies commonly used to suggest innocuity of aluminum, including Mitkus. They describe various flaws in each of the three studies. I also showed you a paper entitled "AUTISM & ALUMINUM ADJUVANTS IN VACCINES". Towards the end of the paper, they discuss three critical flaws specifically regarding Mitkus.

I'll quote one of the more obvious problems discussed in the paper:

"Mitkus does not cite any toxicity data for injected Al adjuvants. Mitkus instead uses toxicity data for ingested, non-particulate, water-soluble Al (Golub 2001, which used Al lactate) to derive the MRL. This data comes from a single study (Golub 2001). So, remarkably, Mitkus claims a safe level of injected Al adjuvant exposure, without citing any Al adjuvant toxicity data. The error is unnecessary and neglectful because at least two animal studies of injected Al adjuvant toxicity were available prior to the Mitkus publication in 2011 (Petrik 2007, Shaw 2009). These papers were not cited or mentioned by Mitkus 2011."

[9] Appendix H

Please explain to me why you are convinced that the Mitkus paper is correct in its conclusion, despite the flaws described in the two papers quoted above. Also, as I said before, over 200 papers have been published on the toxicity of injected aluminum, showing that it can cause neurological disorders including autism. And yet, you maintain that the CDC is accurate in its statement: "Vaccines Do Not Cause Autism". Meaning, there isn't even a possibility that these 200 papers might have some truth to them. Please explain to me what you saw in those papers that convinced you that they were absolutely and categorically wrong in their conclusion.

"The safety of vaccine aluminum exposure can be feasibly studied in the Vaccine Safety Datalink (VSD). However, possible biological mechanisms and confounding variables would need to be considered before conducting any studies."

Does CDC ever intend on doing such a study? I think it would be very beneficial. According to the Institute of Medicine in 2012, there are 135 possible vaccine related injuries that have never been studied. Conducting such a study might provide data for many of those injuries. If they don't intend on conducting the study, why not?

"A summary on this topic is in the paper by Offit and Jew…"

You are referring to: "Addressing Parents' Concerns: Do Vaccines Contain Harmful Preservatives, Adjuvants, Additives, or Residuals?", by Paul A. Offit and Rita K. Jew. I have a few comments to make regarding this article:

"However, the pharmacokinetics of ethylmercury and methylmercury are not the same. Methylmercury has a biological half-life in blood of approximately 50 days compared with that of approximately 7 days for ethylmercury. Because ethylmercury is excreted from the body far more quickly than methylmercury..."

The authors start by comparing the two types of mercury half-life "in blood", and they show that ethylmercury isn't found in the blood after 7 days. Ok, so it left the blood. Where did it go? The authors make the assumption that since it's no longer found in the blood, it must have been excreted from the body. If you read the article that I quoted above by Robert F. Kennedy, Jr. and Lyn Redwood, RN, MSN, you would see several studies proving that the mercury is *not* excreted from the body after leaving the blood, instead, it is lodged in the brain.

"...the removal of thimerosal from vaccines caused some parents and physicians to believe that vaccines that contain thimerosal were harmful…"

Please read the 186 published and peer reviewed studies I quoted above and you will find that vaccines that contain thimerosal are indeed quite harmful. It's unbelievable that they actually regret making an improvement in safety due to fear of losing public allegiance to the vaccine agenda. If anything, it's the refusal to make improvements that will affect public trust.

"The safety of aluminum has been established by experience during the past 70 years, with hundreds of millions of people inoculated with aluminum-containing vaccines."

The safety has been established by experience? You can't prove the safety of something just by doing it over and over for 70 years. You have to go back and compare your results with a control group who did not receive aluminum-containing vaccines, like the VSD study described by Glanz et al 2015 that you

quoted. To my knowledge, this study has never been done. Until such a study takes place, I can just as easily make the following statement: The dangers of aluminum have been established by experience during the past 70 years, with hundreds of millions of people inoculated with aluminum-containing vaccines, as we witness the rates of autism to skyrocket, as well as the rates of ADHD, other neurological disorders, autoimmune disorders in children and childhood cancer.

"For determining the quantity of aluminum below which safety is likely, data were generated in mice that were inoculated orally with various quantities of aluminum lactate. No adverse reactions were observed when mice were fed quantities of aluminum as high as 62 mg/kg/day."

How dumb do they think we are? We're not feeding vaccines to our children. We're injecting them. Stop feeding the mice aluminum, you're wasting your time. Take a look at any of the numerous studies that have been done by INJECTING aluminum into animals, and then tell me how safe you think it is to do the same thing to children.

I can go on, but I'm starting to lose patience, and you probably are as well.

I just have one last question about something you said in your 2/22 email:

"The authors of the article about hepatitis B vaccine and autism focus on the thimerosal association, which we believe is not valid, and even if one is concerned about thimerosal in vaccines, there is little or no thimerosal in any vaccines given to children now, other than some influenza vaccines…"

Can you please give me more information regarding the attached paper?[10] It's a study that was apparently conducted by CDC in 2001 but was never published. It compared children receiving the thimerosal containing Hepatitis B vaccine during the first month of life versus those who did not. It showed that the children who received the vaccine had an increased risk of 829% for ADHD, 762% for autism, 638% for ADD, 565% for tics, 498% for sleep disorders, and 206% for speech delays.

**3/21/2018**

Thank you for your information. I will review it.

Raymond A. Strikas, MD , MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

Immunization Services Division

National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention

[10] Appendix I

Ras8@cdc.gov

Tel. 404 639 6465

**From:** Raphael Szendro [mailto:rszendro@gmail.com]
**Sent:** Wednesday, March 21, 2018 1:34 PM
**To:** Strikas, Raymond A. (Ray) (CDC/OID/NCIRD) <ras8@cdc.gov>
**Cc:** NIPINFO (CDC) <NIPINFO@cdc.gov>; RDB Inquiries (CDC) <RDB@cdc.gov>; MVPDB Inquiries (CDC) <MVPDB@cdc.gov>
**Subject:** Re: FW: multiple safety gp 2

**4/5/2018**

Mr. Szendro:

I have some responses to your comments:

DTP and DTaP:

See this summary by the Strategic Group of Experts at WHO: http://www.who.int/immunization/sage/meetings/2014/april/1_NSE_Backgroundpaper_final.pdf?ua=1, which casts significant doubt on the Guinea Bissau study; it is most curious why they did not publish these results until 30 years after the study was conducted.

As I noted earlier it is unethical to withhold a standard of care, such as vaccination if one is testing a new vaccine against an older, similar one (DTaP compare to DTP in this instance). Looking at children who have received no doses of the vaccine in question would be very difficult, because there are relatively few of them, and they usually differ in various ways from the vaccinated population, e.g., socioeconomic status, racial/ethnicity makeup, which can make comparative analyses very difficult if not impossible.

Thimerosal and Vaccines:

If thimerosal had the toxic effects you cite from various sources, inducing neurodevelopmental disorders in children, including autism spectrum disorders (ASD), why is it that since 2000, when thimerosal was removed from childhood vaccines, except for some influenza vaccines, the rate of autism diagnosis has

steadily increased (see prevalence data at https://www.cdc.gov/ncbddd/autism/data.html? Why is autism 4.5 more common in boys than girls, when there is no differential rate of vaccination by gender? I don't know, but vaccines would seem unlikely to have a role in the increases in ASD. Vaccination rates have remained stable for the vaccines used in 2000 and still used in 2016; see https://www.cdc.gov/mmwr/volumes/66/wr/pdfs/mm6643a3-H.pdf, andhttps://www.cdc.gov/mmwr/preview/mmwrhtml/mm5030a1.htm, but ASD has not diminished, and may well have increased, or at least its recognition has increased.

Thimerosal is present only in some influenza vaccines, and according to reports to CDC by vaccine manufacturers, is included only in between 14% to 22% of influenza vaccine doses for the 2017-18 influenza season. So its use is diminishing, while, as noted autism rates are increasing.

Aluminum and vaccines:

We agree studies need to be done to understand the role of injected aluminum better in humans, but it seems difficult to ascribe a host of neurodevelopmental disorders such as ASD, particularly in light of those disorders' increasing prevalence, but only two aluminum containing vaccines have been added to the childhood vaccination schedule since 2000 (hepatitis A and pneumococcal conjugate vaccines).

Colleagues from the Agency for Toxic Substances and Disease Registry offer this additional information:

"As you pointed out, Mitkus et al. (2011) addressed aluminum by ingestion of a soluble compound. Those researchers estimated the systemic uptake of that aluminum, and used relevant parameters to place on a common scale the systemic burden of aluminum from uptake at the MRL level and that from vaccinations. It appears that their approach was appropriate, did not suffer from a lack of injection study data, and produced reasonable results. You mentioned two injection study articles which addressed neurological effects of injected aluminum hydroxide as being relevant. Those were Shaw and Petrik (2009) and Petrik, Wong, Tabata, Garry, and Shaw (2007). However, Shaw's aluminum hydroxide injection work has been challenged scientifically. According to the National Institutes of Health, that same group faces a second retraction of their study of injected aluminum hydroxide on the brain (Li et al. 2017) "due to evidence of incorrect data" and the conclusion that "the data and results presented in this paper are clearly not reliable." (The retraction and removal notices are published at https://www.sciencedirect.com/science/article/pii/S0162013417300417?via%3Dihub, https://www.ncbi.nlm.nih.gov/pubmed/28923356, and https://www.ncbi.nlm.nih.gov/pubmed/29269133.) Another of their articles (Inbar et al. 2016) was withdrawn "due to serious concerns regarding the scientific soundness of the article, … the methodology is seriously flawed, and the claims that the article makes are unjustified…." (See the withdrawal notice and an additional relevant comment at https://www.ncbi.nlm.nih.gov/pubmed/26778424 and https://www.ncbi.nlm.nih.gov/pubmed/29061567.) These are serious concerns which significantly reduce confidence in the research conducted by this group despite their number of publications. Thus, we consider it prudent to rely on the Mitkus et al.

(2011) study, which we consider provides a relevant and reliable evaluation of the safety of aluminum in vaccines."

Verstraeten article:

The study related to the abstract was published, abstract of the paper below, and a later commentary by Dr. Verstraeten written after he left CDC is at the lower link, where he discusses the analyses and findings of no clear association with neurodevelopmental disorders:

Pediatrics. 2003 Nov;112(5):1039-48.

# Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases.

Verstraeten T[1], Davis RL, DeStefano F, Lieu TA, Rhodes PH, Black SB, Shinefield H, Chen RT; Vaccine Safety Datalink Team.

## Author information

1

Epidemic Intelligence Service Program, Epidemiology Program Office, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.

### Erratum in

- Pediatrics. 2004 Jan;113(1):184.

### Abstract

**OBJECTIVE:**

To assess the possible toxicity of thimerosal-containing vaccines (TCVs) among infants.

**METHODS:**

A 2-phased retrospective cohort study was conducted using computerized health maintenance organization (HMO) databases. Phase I screened for associations between neurodevelopmental disorders and thimerosal exposure among 124 170 infants who were born during 1992 to 1999 at 2 HMOs (A and B). In phase II, the most common disorders associated with exposure in phase I were reevaluated among 16 717 children who were born during 1991 to 1997 in another HMO (C). Relative risks for neurodevelopmental disorders were calculated per increase of 12.5 micro g of estimated cumulative mercury exposure from TCVs in the first, third, and seventh months of life.

**RESULTS:**

In phase I at HMO A, cumulative exposure at 3 months resulted in a significant positive association with tics (relative risk [RR]: 1.89; 95% confidence interval [CI]: 1.05-3.38). At HMO B, increased risks of language delay were found for cumulative exposure at 3 months (RR: 1.13; 95% CI: 1.01-1.27) and 7 months (RR: 1.07; 95% CI: 1.01-1.13). In phase II at HMO C, no significant associations were found. In no analyses were significant increased risks found for autism or attention-deficit disorder.

**CONCLUSIONS:**

No consistent significant associations were found between TCVs and neurodevelopmental outcomes. Conflicting results were found at different HMOs for certain outcomes. For resolving the conflicting

findings, studies with uniform neurodevelopmental assessments of children with a range of cumulative thimerosal exposures are needed.

Further explanation[11] at http://pediatrics.aappublications.org/content/113/4/932.long

END

Raymond A. Strikas, MD, MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

Immunization Services Division

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**Subject:** Re: FW: multiple safety gp 2

**5/9/2018**

Good morning,

You began your last email by referencing the Strategic Group of Experts at World Health Organization, stating that they "cast significant doubt on the Guinea Bissau study" regarding the safety outcomes of DTP. I wouldn't lose too much sleep over their "doubts", my reasoning being as follows: World Health Organization has a history of wrongfully "casting doubt" on any study containing even the slightest hint

[11] Also see Appendix F section 6. https://www.hindawi.com/journals/bmri/2014/247218/#B21

of incriminating evidence against the safety or efficacy of vaccines. Case in point, the 1998 Lancet paper by Dr. Andrew Wakefield and 12 other world-renowned scientists, including the world's leading pediatric gastroenterologist, Dr. John Walker-Smith. From what I understand, the primary findings of the 1998 Lancet paper was the discovery of a link between bowel disease and autism. Today, those findings have been confirmed with numerous studies from across the world, and the link between autism and inflammatory bowel disease is widely accepted. Here is a list of 28 studies which replicated the results of the Lancet study.
http://currenthealthscenario.blogspot.com/2012/06/28-studies-that-support-dr-andrew.html
Here is a paper that came out very recently also showing the same results:
http://www.ucdmc.ucdavis.edu/publish/news/newsroom/12807
Additionally, the researchers reported that the parents of 8 out of the 12 children in the case series indicated that regression began immediately following the MMR vaccine. This observation was included in the report. The report did not say that MMR causes bowel disease or autism. Rather, they simply stated what they observed, and recommended that more research be done to determine if any link exists. Instead of taking the advice of leading scientists, the U.K. General Medical Council attacked the authors and accused them of serious medical misconduct, among other fabricated accusations. In fear of losing their licenses, most of the authors retracted from the study. Dr. Andrew Wakefield and Dr. John Walker-Smith did not retract, and their licenses were revoked. Dr. John Walker-Smith sued the GMC, and several years later was exonerated and his license was reinstated. That last little detail usually gets left out of the story for some reason. Here is a link to the full court ruling:
http://www.bailii.org/ew/cases/EWHC/Admin/2012/503.html
Not only was the GMC unable to prove any trace of medical misconduct during the course of five hearings, the judge reprimanded them for revoking Dr. John Walker-Smith's license stating they had absolutely no basis for doing so. Dr. Wakefield did not pursue legal action, but had he done so, he most definitely would have been proven innocent of any misconduct.

Then you have the World Health Organization, stating in a bulletin from 2017:
http://www.who.int/bulletin/volumes/95/10/17-021017/en/
"Last year, the Italian government intervened to stop *Vaxxed*, an anti-vaccination film by Andrew Wakefield, author of a fraudulent 1998 paper falsely linking the measles, mumps and rubella (MMR) vaccine to autism, from being shown in Italy."

There are several problems with this statement: 1. *Vaxxed* is not an anti-vaccination film. They obviously never watched the film. 2. The 1998 Lancet paper does not link MMR to autism. They obviously never read the paper. 3. Fraudulent? They obviously never read the court ruling. The judgement thoroughly proves that the paper was not fraudulent in any way. If any fraud was present, the judge would not have ordered GMC to reinstate his license.

Therefore, from the fact that the World Health Organization can misquote a paper and call it fraudulent when they clearly haven't read the paper, proves to me that they have an extreme bias when it comes to vaccines. Let them cast their doubts. In my opinion, WHO has zero credibility.

"As I noted earlier it is unethical to withhold a standard of care, such as vaccination if one is testing a new vaccine against an older, similar one (DTaP compare to DTP in this instance)."

What is your source for this? It's true, that in certain instances, it may be considered unethical to conduct a placebo-controlled trial. For example, you want to test a new cancer treatment. You take 1,000 patients who have cancer, and half of the patients will end up receiving a placebo. It would be

unethical to withhold treatment if there is an effective treatment available that they could have received during the trial period. Cases like this are discussed at length in medical literature. Several factors are taken into account to determine if a placebo trial would be appropriate. Here's an example from an article I found: "Placebo-controlled trials - good science or medical neglect?" (PMCID: PMC1070847). The authors conclude with the following questions that should be considered when determining if the use of a placebo would be appropriate:

1. Do participants have a disease or condition for which treatment is available, normally prescribed, and of known efficacy?
2. Will lack of treatment likely result in progression of the disease or condition or the infliction of pain or suffering during the trial?
3. If the disease or condition progresses, is this likely to be reversible?
4. If the disease process is irreversible, how great is the burden of this progression, and how likely is existing treatment to resolve or reduce this burden?
5. Is there substantial evidence that the experimental treatment is of therapeutic benefit?

Let's answer these questions as they relate to vaccine trials.
1. No, the participants do not have a disease or condition. We're testing vaccines which are designed to prevent a possible infection in the future. At the start of the trial period, each individual is perfectly healthy.
2. No, it will not likely result in progression of the disease or condition, because there is no disease or condition to begin with. However, you might want to ask a related question: Will lack of treatment likely result in becoming infected with the disease? The key word here is "likely". It might be true that if you remove the measles vaccine from the entire population of the country or the world, eventually the disease will slowly come back and begin to spread over time. But if you're just taking a relatively small group of children, let's say 1,000, and withholding the measles vaccine for one year while you conduct a clinical trial, it is not likely whatsoever that any of the children in the control group will contract measles during the trial period. Especially when everyone else around them is vaccinated and you have herd immunity. An even better example is hepatitis B. Why have you not done a placebo study on the HepB vaccine? Are you worried that the infants are going to share heroin needles with their friends in the NICU? How can you call it unethical to temporarily withhold the HepB vaccine from an infant? And if you want to be paranoid about it, we can even test the parents and siblings before hand to make sure they aren't infected, just in case the sibling decides to bite his newborn baby as soon as she gets back from the hospital.
3. Again, there is no disease or condition. And even if you want to talk about the extremely rare possibility of contracting the disease during the trial period, the answer is yes, it is very likely to be reversible. Chicken pox is reversible. The child will heal on his/her own within 1-2 weeks. The same thing is true with whooping cough, measles and mumps, all of which are typically mild childhood diseases. Even polio is relatively mild. Almost 75% of anyone who becomes infected with polio, according to CDC, "will not have any visible symptoms. About 1 out of 4 people with poliovirus infection will have flu-like symptoms. These symptoms usually last 2 to 5 days then go away on their own." Paralysis can occur "in about 1 out of 200 people with poliovirus infection". Which means, on the very rare chance that a child in the control group will contract polio during the trial period, there would be a 0.5% chance of paralysis. And, the majority of polio related paralysis is relatively mild, causing "weakness in the arms, legs, or both", which in most cases is reversible with a full recovery. https://www.cdc.gov/polio/about/
4. N/A
5. It depends who you ask.

Based on the above analysis, it would be unquestionably beneficial and appropriate to perform placebo-controlled trials when testing the safety and efficacy of new vaccines. I would like to know what your source is that led you to believe it would be unethical.

Here's another major flaw in what you're saying. Even if we were to determine that it is unethical to perform placebo-controlled trials for new vaccines, and we will therefore give the control group a different vaccine instead of a placebo, you can no longer call it a safety study. At best, you're showing that the new vaccine isn't more dangerous and more hazardous than the old vaccine. But you're not proving that it's safe. The old vaccine was never proven to be safe, because it was also tested against another vaccine. Essentially, the only thing you might be testing for is efficacy. No safety testing is being done. Which means, you are recommending that my children should participate in a human experiment. It's even worse than a human experiment. In most experiments, the results are carefully tracked. But with vaccines, the only post marketing tracking system is an unreliable, inadequate, voluntary reporting system called VAERS. It is estimated that only 1% of all adverse events are reported to VAERS. There are no safety tests being done on vaccines prior to FDA approval, and there is practically no system in place to track the post market effects of vaccines. Doesn't sound very ethical to me.

Speaking about human experimentation, here's a conversation that took place at a recent ACIP meeting in Feb 2018. The issue at hand was whether or not to approve a new vaccine which contained an adjuvant that has never been used before: "HEPLISAV-B is a Hepatitis B vaccine that may be used to vaccinate persons aged 18 years and older against infection caused by all known subtypes of HBV."
https://www.youtube.com/watch?v=rpB4w9USEbc
The sections that I'm quoting from start at about 46 minutes into the video:

*-. . .my question though, is related to the 1018 adjuvant and I'm wondering, is this adjuvant used in other licensed products either in the US or elsewhere, or is it a new adjuvant?*
*-This is the first vaccine for humans in which this adjuvant is being used.*
*-With the number of adjuvanted vaccines now available including flu vaccine and Shingrix, is there any comment on using this vaccine the same time as other adjuvanted vaccines?*
*-We have no data to make a recommendation one way or the other.*
*-So, um, just to put this in context with other vaccines, um, whilst preclinical studies were not done using these vaccines simultaneously, our general approach to immunizations is that, um, they should be given- they can be given the same time in different, um, limbs.*
*-Are multiple adjuvanted vaccines used in Europe or other markets?*
*-Not to my knowledge.*
-Public comments
-Vote
*-Thank you very much. So the voting is completed, um, and it is unanimous to support this recommendation. Thank you all.*
*-Does anyone around the table, we don't need to go around and verify *** but does anyone have any comments they wish to make about their vote?*
*-So, just a slight reservation. I think this is a huge advance and a step forward. I am concerned about that signal, that myocardial infarction signal. I am concerned about the use of this new adjuvant, and certainly urge us to continue to look at the post marketing data carefully.*
*-Dr. Hunter?*

*-Just a question about that: would we, how soon would we be getting that post marketing data update here?*
*-Dr. Ward?*
*-There's two kinds of data. Um, the vaccine safety data link data will require people to be using the vaccine to develop substantive database. And Dr. Sun, do you want to comment on the post marketing data that FDA is requiring?*
*-In our approval letter which I'm looking at it makes references to the specific dates. Um, I think the myocardial infarction study we're, um, seeing that the date, for uh, likely for May 31, 2020.*

To summarize, they were voting on a vaccine which contained a brand new adjuvant, never used before anywhere in the world. One of the members of the committee asked a good question: has this new adjuvant ever been tested to see what happens when you use it together with other vaccines and other adjuvants? A: No data. And by the way, we intend on using it together with other vaccines. Just make sure you inject them in different limbs. Everyone then unanimously voted to approve the vaccine. After the vote was complete, one of the members made a comment that he's slightly concerned about the myocardial infarction signal (it caused heart attacks during the clinical trial), and the fact that it's a completely new adjuvant. He recommended that we keep an eye on that annoying heart attack issue, and it would be a good idea to check out the post marketing data. Q: How soon will we have that data? A: Hopefully in 2 and a half years. No data, no testing, no matter, just vote yes. The human test subjects will let us know how things are going in 2 years.

"Looking at children who have received no doses of the vaccine in question would be very difficult, because there are relatively few of them…"

I'm not sure what you mean by "relatively few". How many do you need? According to CDC, in the 2016-17 school year, "the median percentage of kindergartners with an exemption from at least one vaccine was 2.0%, similar to 2015–16 (1.9%)". This doesn't include home school children. It is estimated that nearly 4 million babies are born each year in the United States. If you do the math, you should end up with at least 80,000 unvaccinated and partially vaccinated children every year. My 4-month-old baby hasn't had any vaccines yet. I give full permission to include him in the control group for any vaccine trial that you want. Just show me where to sign up.

"…and they usually differ in various ways from the vaccinated population, e.g., socioeconomic status, racial/ethnicity makeup, which can make comparative analyses very difficult if not impossible."

What is your source? I found an article in Pediatrics which states as follows: "Undervaccinated children tended to be black, to have a younger mother who was not married and did not have a college degree, to live in a household near the poverty level, and to live in a central city. Unvaccinated children tended to be white, to have a mother who was married and had a college degree, to live in a household with an annual income exceeding $75,000…"
http://pediatrics.aappublications.org/content/114/1/187
Where did you get the idea that fully vaccinated children are so much different than everyone else? How can they possibly be so much different from the two groups described above? Do their parents tend to be gay, Hispanic/Asian, have a PHD, and have an annual income less than $75k but above the poverty level?

Furthermore, in your 3/8/18 email, you quoted "the Glanz et al 2015 study which concluded that "The safety of vaccine aluminum exposure can be feasibly studied in the Vaccine Safety Datalink (VSD)…",

referring to a database comparing vaccinated vs unvaccinated children. Glanz, who you quoted, is saying that it would be feasible to do a study comparing the two groups. Where did you get the idea that it would be "very difficult if not impossible"?

"…why is it that since 2000, when thimerosal was removed from childhood vaccines, except for some influenza vaccines, the rate of autism diagnosis has steadily increased… Thimerosal is present only in some influenza vaccines, and according to reports to CDC by vaccine manufacturers, is included only in between 14% to 22% of influenza vaccine doses for the 2017-18 influenza season. So its use is diminishing, while, as noted autism rates are increasing."

I received the following information from an acquaintance of mine:

The rate of autism steadily increased since 2000 because the 2000 U.S. autism rate, as announced by the CDC, is based on data on TWELVE-YEAR-OLDS, who were born in 1988. The 2014 autism rate is based on data on children born in 2002.

That's how the CDC collects and analyzes the data in order to come up with that rate. Data is collected on 8-year-olds, and 4 years later, the CDC finishes analyzing it and announces the official autism rate, NOT for the year in which the children were born, but for the year the announcement is made.

After the request was made to vaccine manufacturers to make thimerosal-free pediatric vaccines, thimerosal-preserved pediatric vaccines still continued to be distributed and administered in the US for as long as their shelf life was good. Most sources agree that this was until 2004.

So 2016 was when we saw the rate hold steady at 1 in 168[12] as SOME children were given thimerosal-free vaccines while others were given the old, thimerosal-preserved vaccines.

The reason we didn't see the autism rate DROP in 2016 is because, as thimerosal was phased out of the pediatric vaccines, flu shots — the vast majority of which came in thimerosal-preserved multi-use vials before just the last few years — were added to the pediatric schedule, AND administered to pregnant women.

In addition, the number of aluminum-adjuvanted vaccines on the pediatric schedule skyrocketed, with 4 doses of pneumococcal conjugate (Prevnar) added to the pediatric schedule in 2001, given at 2, 4 6, and 12 months, adding 125 micrograms of aluminum per dose, AND they were injected simultaneously with other vaccines (including some in 2001/2002, and possibly later that were thimerosal-preserved, if the pediatrician was using up already-purchased vaccines).

For reference, the "safe limit" recognized by the FDA for aluminum in IV's is 5 mg per kg of body weight. They note here that higher amounts could result in central nervous system and bone toxicity: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=201.323

For an average 2-month-old, that would be about 17.5 micrograms.

In 1953 , aluminum was injected to induce seizures in monkeys for study purposes: "Experimental Epilepsy in the Monkey Following Multiple Intracerebral Injections of Alumina Cream" https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1877387/?page=1

[12] This was a typo. It should say "1 in 68", not "1 in 168". See https://www.cdc.gov/ncbddd/autism/data.html

So if we know that aluminum can induce seizures in primates, and we also know that mercury can cause neurotoxic effects, what happens when both aluminum adjuvants *and* mercury-based preservatives are *simultaneously* injected into an infant who, for whatever reason, is unable to excrete both of them?

"Why is autism 4.5 more common in boys than girls, when there is no differential rate of vaccination by gender?"

Here are two possible explanations how vaccines can cause autism at a significantly different rate between boys and girls.

1. I found the following study from the Journal of Toxicology and Environmental Health: https://www.tandfonline.com/doi/abs/10.1080/15287394.2011.573736 which states as follows: "Although individuals probably have a genetic predisposition to develop autism, researchers suspect that one or more environmental triggers are also needed. One of those triggers might be the battery of vaccinations that young children receive." Although it may not be politically correct to say this, boys and girls are different. Therefore, their genetic predisposition to develop autism might be different. That's why the same environmental triggers, like vaccines, might affect them differently.

2. This is an article that I found which directly addresses your question: It's called "Why Autism Affects Boys More Than Girls". http://time.com/4663196/autism-spectrum-disorder-gender/ They quote a study published in JAMA Psychiatry. Here's what they said: "Brain scientists know that some structures in the brain differ between the sexes. One is the thickness of the cortex, the brain's outer layer that is embedded with nerves involved in memory, thinking, language and other higher cognitive functions. Men tend to have thinner cortex measurements, while women tend to have thicker ones, and this difference is a pretty reliable way to distinguish males from females. . . The thinner the cortex, regardless of gender, the more likely the person was to have ASD. Even for the women with thinner, more male-like cortical thickness readings, the risk of ASD was three times higher than for women with thicker measurements more in line with unaffected women." The authors don't give any explanation as to how this physical difference has any causal affect on the prevalence of autism. However, we do know that injected mercury, as well as injected aluminum, directly affect the brain. Therefore, as a possible answer to your question, we could suggest that for some reason, a thinner cortex is more vulnerable to mercury and aluminum toxicity compared to a thicker cortex.

"We agree studies need to be done to understand the role of injected aluminum better in humans, but it seems difficult to ascribe a host of neurodevelopmental disorders such as ASD, particularly in light of those disorders' increasing prevalence, but only two aluminum containing vaccines have been added to the childhood vaccination schedule since 2000 (hepatitis A and pneumococcal conjugate vaccines)."

As stated above, the 4 doses of the pneumococcal conjugate vaccines added 125 micrograms of aluminum per dose to the schedule, when the safe limit recognized by the FDA for aluminum in IV's for an average 2-month-old would have been around 17.5 micrograms. For a much more detailed report, please see this recently published paper[13] (attached): https://www.sciencedirect.com/science/article/pii/S0946672X17300950

Also see this one[14]: http://www.jpands.org/vol21no4/miller.pdf Which states: "Vaccines containing aluminum were added to the childhood immunization schedule when some vaccines containing mercury

[13] Appendix J
[14] Appendix K

were removed. Prior to the mercury phase-out (pre-2000), babies received 3,925 mcg of aluminum by 18 months of age. After pneumococcal and hepatitis A vaccines were added to the schedule, babies began receiving 4,925 mcg of aluminum during the same age period—a 25% increase." An increase of 25% would seem to be a significant amount that could very likely increase the prevalence of neurodevelopmental disorders.

Furthermore, think about what you're saying: We agree that we haven't done a single study to directly test the toxicity of injected aluminum. But we still recommend that you inject it into your children. After all, how bad could it be? And don't worry about the growing epidemic of brain injured children in the United States. We only added 2 more aluminum containing vaccines since 2000, so it's probably not because of that.

"As you pointed out, Mitkus et al. (2011) addressed aluminum by ingestion of a soluble compound. . . It appears that their approach was appropriate, did not suffer from a lack of injection study data, and produced reasonable results. . . However, Shaw's aluminum hydroxide injection work has been challenged scientifically. . . These are serious concerns which significantly reduce confidence in the research conducted by this group despite their number of publications. Thus, we consider it prudent to rely on the Mitkus et al. (2011) study, which we consider provides a relevant and reliable evaluation of the safety of aluminum in vaccines."

"It appears that their approach was appropriate…". Comparing apples to oranges does not appear to be appropriate, at least not according to my 8th grade science teacher. "…did not suffer from a lack of injection study data…". There was a total of zero injection study data. How do you call that *not* suffering from a lack of injection study data?

As I have mentioned to you in previous emails (2/22/18 and 3/6/18), it's always important to look at the full picture before we can make an honest, responsible conclusion:

Aluminum has been used in vaccines, according to CDC, since the 1930s. And yet, not a single safety study has ever been conducted. All the CDC has to say about aluminum safety is: "The amount of aluminum present in vaccines is low and is regulated by the U.S. Food and Drug Administration (FDA)." And, "Aluminum salts, such as aluminum hydroxide. . . have been used safely in vaccines for more than 70 years." https://www.cdc.gov/vaccinesafety/concerns/adjuvants.html I kept the hyperlink attached to "aluminum present in vaccines is low" so you can see what happens when you click on it. Nothing. It says "page not found" or "unable to open…". There is no source because it's a false statement. The next part of the statement, that it is regulated by FDA, is also a false statement, to which CDC also provides no source or link. Based on the numbers I showed you previously, the amount of aluminum we are giving children in vaccines, according to FDA regulations, is hundreds of times higher than what is considered to be a safe level. The final statement, that aluminum has been "used safely in vaccines for more than 70 years", again with no source sited, is groundless. You quoted a similar statement from Offit and Jew in your 3/8/18 email, which is likewise baseless, as I explained in my 3/21/18 email. This is probably what doctors and health agencies said for decades regarding smoking up until 1964: Tobacco is good for you, we have been safely smoking for hundreds of years. And this is what every doctor in the world said in the 1840s when countless mothers were dying during child birth, and Dr. Semmelweis demonstrated that washing his hands before delivery drastically increased the mother's chance of survival. We insisted that he was wrong, and instead of giving it a try to see if it in fact is a life saver, we had him institutionalized. After all, we have been safely delivering babies for centuries without washing our hands. Dr. Semmelweis is obviously crazy.

If there were no safety studies since 1930, why did Dr. Mitkus all of a sudden feel the need to write a paper on the safety of alulminum in 2011? He answers this question when he writes: "Because concerns have been expressed by the public that aluminum in vaccines may pose a risk to infants, we developed an up-to-date analysis of the safety of aluminum adjuvants." Which I interpret to mean that the FDA, who Dr. Mitkus works for, hired him to take care of these concerns. One of the public concerns that he is referring to is a 2007 study by Dr. Chris Shaw, as well as another study from 2009, where they injected aluminum hydroxide into baby mice. According to Dr. Shaw, FDA ignored his study, stating it "does not believe that this particular paper brings to light the need for additional research that is not already underway".

https://www.youtube.com/watch?time_continue=108&v=HK-93SHnTFk

Why did FDA and Dr. Mitkus ignore the studies from Dr. Shaw? And these weren't the only two that were ignored. Here's two more from other scientists: "A role for the body burden of aluminium in vaccine-associated macrophagic myofasciitis and chronic fatigue syndrome" (2009), and "Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle" (2001). Did he ignore these studies when he wrote his paper in 2011 because he knew that 6 years later, one of the authors would publish a study that would later be retracted because of some sort of technical error in the way the data was reported? Or, did Dr. Mitkus have an agenda? I would put money on the latter.

The most important question that we have to address is today, based on all of the current literature that we have available, which science is correct? Is aluminum in vaccines perfectly safe, or is it extremely toxic? They can't both be right. Should we go with hundreds of studies showing numerous injuries that occur after injecting baby mice with aluminum hydroxide, the exact same type of aluminum used in vaccines? Or should we go with one single study which analyzes data from water-soluble aluminum, an entirely different type of aluminum NOT used in vaccines, after being fed to adult mice?

We should also look at the number of scientists on either side of the debate. On the one hand, we have one FDA scientist, Dr. Mitkus, who didn't do any biological experiments on his own. All he did was analyze data from ingested aluminum, and for some reason found it suitable to compare apples to oranges. On the other hand, we have at least 6 world renowned aluminum experts, all of which conducted their own biological experiments and studies, and they all came to the exact same conclusion: Dr. Chris Exley, Dr. Romain Gherardi, Dr. Guillemette Crepea, Dr. Christopher Shaw, Dr. Lucija Tomljenovic, and Dr. Yehuda Shoenfeld. I strongly urge you to look up the work that each of these scientists have published. There's one in particular that I think is very important to look at: "Aluminium in brain tissue in autism" (2018). See attached.[15] Dr. Chris Exley and his team have dissected over 100 brains. They recently dissected 5 brains from people with autism and found that the levels of aluminum in all 5 brains were extremely high.

Let's pretend that the two retracted articles that you quoted were the only ones ever written on the topic of aluminum toxicity. I would still want to investigate the matter further, and make sure that the errors were significant, and the authors didn't just forget to dot an "i". If we were in court, and you pulled out a document proving that I owe you $1 million, I would look at every single detail in that document to make sure it's valid. If I could find even the slightest reason to invalidate the document, I

[15] Appendix L

would, and the court wouldn't award you a dime. But that only works for monetary issues, not when we're talking about the safety of our children. We don't look for loopholes when it comes to safety.[16] Dr. Shaw is an expert witness who is telling me to stay away from aluminum-containing vaccines. You're telling me that you found a loophole, and *maybe* his conclusion is wrong, to which I respond, *maybe* it's not. It would be irresponsible to ignore him unless you can *prove* that his conclusions are wrong, or at a minimum, prove that the errors are significant errors. But those weren't the only two papers that have ever been written on aluminum toxicity. Dr. Shaw himself wrote at least 14 others, and there are at least 5 other scientists from around the world who came to the exact same conclusion as Dr. Shaw, with hundreds of published and peer reviewed studies to prove it. So even if the errors were significant, that doesn't prove that the conclusion was wrong. It just means that there was human error found in 2 studies, a tiny fraction of the extensive body of published literature, which is perfectly acceptable. But you're suggesting that we reject everything Dr. Shaw has ever published, along with everything that all the other scientists have published. And to top it off, you have not shown me a single study that directly contradicts what all of these scientists have proven. Rather, you find it prudent to compare apples to oranges. With all due respect, this isn't just irresponsible, it's insane.

**5/9/2018**

Thank you for your comments.

Raymond A. Strikas, MD, MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

Immunization Services Division

National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention

Ras8@cdc.gov

Tel. 404 639 6465

**From:** Raphael Szendro <rszendro@gmail.com>
**Sent:** Wednesday, May 9, 2018 6:16 AM

[16] עבודה זרה דף ל. פירוקא לסכנתא, וע' תרומת הדשן ס' ריא

**5/11/2018**

One more thing I would like to add. If you want more information about Dr. Andrew Wakefield, he was interviewed just yesterday and he discussed many aspects of the story. You can see the video here:

https://www.youtube.com/watch?v=Sh8yjUqzhNs

Thank you,

Have a great weekend.

**6/11/2018**

Good morning,

I haven't heard back from you in a while. I don't mean to rush you. I just wanted to know if you were planning on getting back to me with any additional information.

Thank you

**6/11/2018**

I have no additional information at this time.

Raymond A. Strikas, MD, MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

Immunization Services Division

National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention

Ras8@cdc.gov

Tel. 404 639 6465

**From:** Raphael Szendro <rszendro@gmail.com>
**Sent:** Monday, June 11, 2018 9:37 AM

**To:** Strikas, Raymond A. (Ray) (CDC/OID/NCIRD) <ras8@cdc.gov>
**Cc:** NIPINFO (CDC) <NIPINFO@cdc.gov>; RDB Inquiries (CDC) <RDB@cdc.gov>; MVPDB Inquiries (CDC) <MVPDB@cdc.gov>
**Subject:** Re: FW: multiple safety gp 2

**6/18/2018**

I wanted to thank you for taking the time to address my questions and concerns over the last few months. I think we had a very productive conversation. Here is a summary of some of the things I have learned throughout the course of our conversation:

- We agree that the pertussis vaccine is lacking and requires improvements. You stated that the current pertussis vaccine is "imperfect", it provides a "limited duration of immunity" [2/22/18], and "pertussis cases have increased from their low point in the 1980's to the present" [1/10/18].

- We agree that the current pertussis vaccine does not create herd immunity. You stated: "We do need better pertussis vaccines that can interrupt transmission as you point out" [2/22/18]. Meaning, the *current* pertussis vaccine does *not* interrupt transmission. When you said: "as you point out", you were referring to the numerous published articles which I quoted [11/28/17], for example: "current acellular pertussis (aP) vaccines fail to prevent colonization and transmission", and "Despite high levels of vaccination coverage, pertussis circulation cannot be controlled at all."

- Not only does DTaP fail to protect the "herd" from colonization and transmission, it puts vulnerable individuals, like infants and immunosuppressed at a higher risk of infection and death [11/28/17 and 1/8/18]. The only thing that we disagreed on was how to deal with the problematic situation. I suggested that we stop using DTaP until a better quality product is produced, while you recommended that we continue using it [1/10/18, 2/22/18 and 3/6/18]. But the fact that the use of DTaP puts certain individuals at a higher risk of death, remains undisputed. In other words, we both agree that if everyone in the country stopped using DTaP, there would likely be fewer infant deaths from pertussis.

- It was unclear to me why titers cannot be used to determine whether someone is protected against pertussis. But as I said [11/28/17], I suspect it has something to do with the fact that the pertussis vaccine is fundamentally flawed and doesn't work the way it was intended. Cases of pertussis have been increasing over the past several years, despite high levels of vaccination coverage. For some reason, we think that if we give everyone as many doses of the defective vaccine as we possibly can, even if they already show sufficient titer levels, it will somehow correct the problem. Sounds to me like a textbook case of insanity.

- I quoted the following statement from the CDC website: *Getting diphtheria, tetanus, or pertussis disease is much riskier than getting DTaP vaccine* [1/8/18]. To date, this statement has never been scientifically verified. In order to make the above statement, you would have to know the risks of getting the diseases, AND you would have to know the risks of getting DTaP vaccine. According to the Institute of Medicine, as of 2011, we have no idea whether or not DTaP causes encephalopathy and/or

encephalitis [2/22/18]. You have not shown me any data since 2011 showing whether or not DTaP causes encephalopathy and/or encephalitis. Therefore, the above statement from the CDC website remains unjustified. In fact, according to the same report from the Institute of Medicine, we also have no idea if DTaP causes autism. Maybe it does, and maybe it doesn't.

- **Thimerosal (mercury) in vaccines:** We agree that although the amounts have been minimized in recent years, mercury can still be found in many vaccines [3/21/18]. I expressed concern that mercury in vaccines may cause various neurodevelopmental disorders including autism. You responded by saying: "At present, there are no convincing data vaccines cause autism" [3/8/18]. I then showed you a list of 168 published studies which prove quite convincingly that mercury causes autism [3/21/18]. I asked you what you felt was unconvincing about those 168 studies, but you offered no response. I then referenced an article which lists 6 studies commonly used by CDC and others, to support the belief that mercury is safe. The authors of the article explain: "The purpose of this review is to examine these six publications which were "overseen" by the CDC and which claim that prenatal and early childhood vaccine-derived Thimerosal exposures are not related to the risk of a subsequent diagnosis of autism or autism spectrum disorder (ASD). This review analyzes possible reasons why their published outcomes are so different from the results of investigations by multiple independent research groups over the past 75+ years." After reading this article, we are left with the conclusion that mercury is unquestionably toxic. A. The overwhelming majority of literature supports this conclusion. B. The small handful of literature claiming that mercury is safe has been reconciled, and therefore does not contradict the clear and obvious conclusion that mercury is toxic and dangerous. I asked why you would disagree with the logical explanations stated in the article, but you never responded.

- **Aluminum in vaccines:** I gave you the names of six aluminum adjuvant experts from around the world [3/21/18 and 5/9/18], all of whom have done extensive research, and they all came to the same conclusion: aluminum in vaccines is extremely toxic. Baby mice were injected with the same type of aluminum used in vaccines, at the exact proportion that children receive in vaccines. The results were devastating. Five human brains were dissected from individuals with autism, and the levels of aluminum found in all five brains were the highest they have ever seen. Between all the scientists from around the world, over 200 peer reviewed articles have been published on the subject. You agreed with me that to date, not a single animal experiment has ever been done showing that injected aluminum is safe. And yet, you insist that we should continue injecting aluminum into our children based on one scientist who was hired by the FDA to do something about the growing concern of scientists and parents around the world regarding the safety of aluminum in vaccines. Surprisingly, he concluded that aluminum in vaccines is perfectly safe. He looked at levels of ingested aluminum in adult mice, and tried to draw a comparison to injected aluminum in infants. I presented at least three articles which showed numerous flaws with that study, to which you never responded with any explanations or answers. You did respond to one of the flaws, however, which you quoted from the Agency for Toxic Substances and Disease Registry [4/5/18]. But as I explained [5/9/18], I don't see how their response resolves the issue. The other flaws which I referenced were never addressed at all. Out of the 200+ studies supporting the fact that aluminum is harmful to children, you showed me one or two of them that were retracted for some sort of technical reason. Based on that, you were willing to reject every scientist from around the world who has been studying aluminum adjuvants for decades, and have all come to the exact same conclusion. The question is: if you really don't like the way scientists have been studying aluminum adjuvants, why have you not conducted an experiment of your own to prove them wrong?

- Other than one or two aluminum studies which were noted, you never found any factual or scientific flaws in the hundreds of published articles that I referenced which showed the toxic effects of mercury and aluminum. You did, however, raise two concerns [4/5/18] in an attempt to disprove all 168

mercury studies and 200+ aluminum studies. You asked, how is it possible that mercury, which has been steadily decreasing in recent years, causes autism which has been steadily rising? And we only added 2 aluminum containing vaccines to the schedule since 2000. You also asked, "Why is autism 4.5 more common in boys than girls, when there is no differential rate of vaccination by gender?" I sufficiently answered both of those questions [5/9/18], showing that the rise in autism each year lines up perfectly with the usage levels of mercury and aluminum in vaccines. I also gave a few possible explanations for the different autism rates that we find between boys and girls. You did not challenge any of the answers which I offered. Therefore, the published science which I referenced remains valid and undisputed.

- Besides ignoring the vast majority of current scientific literature, vaccine science is also based on guess work, false assumptions, misquoted data, and fabricated claims: **1.** Boston University [11/28/17]: "This disease is back because we didn't really understand how our immune defenses against whooping cough worked, and did not understand how the vaccines needed to work to prevent it… Instead we layered assumptions upon assumptions, and now find ourselves in the uncomfortable position of admitting that we may [have] made some crucial errors." **2.** Kennedy and Redwood [3/21/18]: In this article, the authors explained that scientists have observed mercury rapidly leaving the bloodstream after vaccination. They assume, therefore, that mercury from the vaccine is rapidly excreted from the body. The authors prove from numerous studies that this assumption is wrong. Although it leaves the bloodstream, it is NOT excreted from the body. Rather, the mercury is lodged in various organs, including the brain. You never questioned or challenged anything that was stated in that article. **3.** [1/10/18] You misquoted the 2011 IOM report, stating that "the evidence was inconclusive about a causal relationship between acellular pertussis vaccines and these conditions", implying that there is little to no risk of encephalopathy and/or encephalitiswhen using the DTaP vaccine. **4.** [2/22/18] The CDC misquotes the same report when it says on its website: "Vaccines do not cause autism…studies have shown that there is no link between receiving vaccines and developing ASD. In 2011, an Institute of Medicine (IOM) report on eight vaccines given to children and adults found that with rare exceptions, these vaccines are very safe." The report, which is 895 pages long, makes no such claims. CDC must have been relying on the assumption that nobody was going to take the time to read the report. **5.** [5/9/18] World Health Organization misquoted the 1998 Lancet study stating that the authors linked the MMR vaccine to autism. They also claimed that the paper was fraudulent, after it was proven in court that no fraud existed. **6.** [2/22/18] You stated: "We, as adults and children, ingest more aluminum from our food than received in vaccines." The study which you attached in your email says the exact opposite. **7.** [5/9/18] The CDC website states: "The amount of aluminum present in vaccines is low and is regulated by the U.S. Food and Drug Administration (FDA)." These claims clearly were fabricated, being that the amount of aluminum in vaccines is much higher than the safe limit recognized by the FDA for aluminum in IV's. **8.** You mentioned that it would be unethical to conduct placebo-controlled trials for vaccines. I asked if you had a source for this [5/9/18], but you never offered one. I even showed you, based on published literature, that it would in fact be perfectly appropriate and ethical to conduct placebo-controlled trials for vaccines. **9.** You stated [4/5/18]: "Looking at children who have received no doses of the vaccine in question would be very difficult, because there are relatively few of them". I asked for a source, but never received one. I showed, based on statistics, that there are more than enough unvaccinated children who can take part in a study. **10.** You also stated [4/5/18] that unvaccinated children usually differ in various ways from the vaccinated population. I couldn't figure out what you meant by that, and you didn't provide any source.

- The concept of "safety" is entirely nonexistent when it comes to vaccine science. **1.** In over 70 years of using aluminum in vaccines, the industry has never conducted a single animal experiment to determine whether injected aluminum was safe for humans. **2.** I showed you that based on the "safe

limit" recognized by the FDA for aluminum in IV's, we are injecting 100s of times that amount into our children [5/9/18]. You did not dispute this fact. **3.** At a recent ACIP meeting, it was admitted that there was no data available to determine if the new adjuvant in question was safe to use together with other vaccines and other adjuvants. Still, everyone voted unanimously to approve it, relying on human test subjects to let us know in a few years if there are any serious problems, assuming we're actually going to follow up with them [5/9/18]. **4.** We agreed that an inert placebo is not used when testing vaccines, which means that the control group is receiving practically the exact same hazardous materials as the test group. Therefore, the only thing they might be testing for is efficacy. Safety trials have not been performed on any vaccine that has ever been put on the market.

The vaccine agenda has consistently demonstrated over the years to have a complete disregard for vaccine safety. DHHS Federal Register, Vol 49 No 107 from June 1, 1984: "...any possible doubts, whether or not well founded, about the safety of the vaccine cannot be allowed to exist in view of the need to assure the vaccine will continue to be used to the maximum extent consistent with the nation's public health objectives."

This discussion has helped me gain a much better understanding of what vaccine science is all about. I was able to reduce all the information from our conversation into one simple formula:

Vaccine Science = Tobacco Science

Thank you again for all your help. I feel like I'm now in a much better position to be able to make a responsible, educated decision for my children.

**6/18/2018**

Thank you. I have nothing further to offer at this time.

Raymond A. Strikas, MD, MPH, FACP, FIDSA

Lead, Education Team

Communication and Education Branch

Immunization Services Division

National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention

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Tel. 404 639 6465

**From:** Raphael Szendro <rszendro@gmail.com>
**Sent:** Monday, June 18, 2018 11:59 AM

## Email to FDA:

### 6/18/2018

Good afternoon,

Attached is a copy of the correspondence between myself and the CDC. I have been in contact with them since Nov 2017. I had several questions and concerns regarding vaccines, and in particular, the DTaP vaccine. At one point during the conversation, in an email from CDC on 3/8/18, it was recommended to me that I contact FDA with my questions. Please review our correspondence and let me know if you have anything to add to our conversation.

Thank you

--

**Raphael Szendro, Team Leader**
**The R&R Executive Team**
**of ExecuHome Realty**

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## Response from FDA:

### 6/19/2018

Dear Mr. Szendro:

Thank you for writing to the Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER). One of seven centers within FDA, CBER is responsible for the regulation of many biologically-derived products, including blood intended for transfusion, blood components and derivatives, vaccines, allergenic extracts, tissues, and cell and gene therapy products.

We appreciate your interest in this matter. Please know that we have no additional information to add in response to your inquiry.

You may access our vaccine information and related links, including the list of Vaccines Licensed for Distribution in the U.S. with Supporting Documents (e.g. package insert), at: https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm

We hope this information is helpful.

Sincerely,
**Amy Temple**
*Consumer Safety Officer*

**Center for Biologics Evaluation and Research**
**Office of Communications, Outreach and Development**
**U.S. Food and Drug Administration**
Tel: 800-835-4709
OCOD@fda.hhs.gov

# Appendix A

Emails with Maryland Department of Health

## MD Dept of Health: 11/13/17

Hello Mr. Raphael,

Studies done (example here) have shown that titers have been low for a large proportion of children, which is why the state will not accept titers and recommends the 4-6 year-old booster instead.

You can see this webpage for the state regulations:
http://www.dsd.state.md.us/comar/comarhtml/10/10.06.04.03.htm

Please let me know if you have further questions.

Thank you,

Patricia Swartz, MS, MPH
ImmuNet Coordinator
Maryland Department of Health
Prevention and Health Promotion Administration
Center for Immunization
201 West Preston Street Suite 319
Baltimore, MD 21201
office: 410-767-3029
email: patricia.swartz@maryland.gov

## Me: 11/13/17

Thank you for giving me that information. I looked at the study that you were referring to (PMID: 11332669).
It looks like they are recommending a 5th dose of DTaP since the titers are often too low after 4 doses. But what if a child has proven to have enough titers after 3 or 4 doses? Where does it say in that study that we shouldn't rely on the blood work? All the study is showing is that the DTaP doesn't work that well, and in most cases it won't achieve the desired titers until the child has had at least 5 doses. But in some cases, like my son, if the blood work proves that there are enough titers after 3 doses, why shouldn't that be sufficient?

## MD Dept of Health: 11/15/17

Good afternoon Mr. Szendro

Here are several articles on waning immunity for pertussis. Per COMAR, students are required to receive the appropriate number of diphtheria, tetanus, and pertussis containing vaccines based on age (4 for students under age 7 and 3 for students 7 years old and older). This is because immunity to pertussis will

wane in all populations over time. Blood testing is only a snap shot of immunity for the moment that the blood was drawn and is not predictive of ongoing immunity. Based on information from the US Department of Health and Human Services and the Centers for Disease Control and Prevention the best way to determine ongoing immunity is to make sure students are vaccinated with the appropriate number of diphtheria, tetanus, and pertussis containing vaccines based on age.

Please see the links below for CDC's explanation on why they recommend 4 DTaP vaccines and a Booster dose.

Please feel free to contact me with any other questions. I hope this helps.

https://www.cdc.gov/pertussis/about/faqs.html

https://www.cdc.gov/media/matte/2011/10_whooping_cough.pdf

https://www.cdc.gov/vaccines/parents/downloads/parent-ver-sch-0-6yrs.pdf

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***Elease Booker- Ragin, M.S., CHES***

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Maryland Department of Health

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## Me: 11/17/17

Elease, thank you for getting back to me with the additional information.

I'm trying to get a little more clarification on this. I showed your email to two physicians, and both of them had trouble understanding the logic behind the state regulation. One of those physicians is Dr. Toni Bark. Here is what she wrote in a previous email with Patricia Swartz:

"The state won't accept titer levels because so many are low from vaccination due to poor stimulation. The act of vaccination is not magic. If the titers remain low who cares how many you relieve. It's a ridiculous argument
If the industry uses titers as a surrogate for immunity (which it actually isn't in reality) then they must take titer levels which appear to be in their protective range as proof of immunity. This is standard and allowable in all states but crazy California.
To imply the child is protected even with low titers just because they received a vaccination, is magical thinking."

When I spoke to Patricia on the phone, she seemed to agree with what Dr. Bark was saying.

The CDC never directly addresses my question: They admit that even after all 5 doses, it's only 80% to 90% effective. My child didn't have all of the recommended doses in the series, but he did have his blood tested. Right now, my child is 100% guaranteed immune from pertussis, while every other kid his age is only 80-90% protected. Where does Maryland State see from CDC that they don't recommend using titers as acceptable proof of immunity? Why wouldn't they? It's much more reliable. You keep saying that we often find problems with "waning immunity". But that's true with all vaccines, immunity doesn't last forever. That's why my son will have to get his titers checked again next year, and again the year after that. As long as he keeps passing the test like he did this year, that will prove that in this particular case, his immunity did NOT fade over time. He will be 100% guaranteed safe and protected, unlike everyone else. If he ever fails the test, he will be required to finish off the series of vaccines, at which point he will be equally protected at 80-90%.

I didn't see anywhere in the 3 links that you sent me any mention of whether or not titers should be an acceptable form of proof of immunity. Please help me understand where the State got that from.

Thank you

## MD Dept of Health: 11/20/17

Mr. Szendro

I'm sorry for the delay in responding to your email. My previous email was to help you gain some understanding for the immunization requirements in the state of Maryland. This email was not intended to explain why Maryland will not accept a titer in lieu of vaccination, however I do hope that you found some of the information about waning immunization protection in pertussis vaccines helpful.

# Appendix B

Mitkus et al, 2011

Vaccine 29 (2011) 9538–9543

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Updated aluminum pharmacokinetics following infant exposures through diet and vaccination

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Modeling

ABSTRACT

Aluminum is a ubiquitous element that is released naturally into the environment via volcanic activity and the breakdown of rocks on the earth's surface. Exposure of the general population to aluminum occurs primarily through the consumption of food, antacids, and buffered analgesics. Exposure to aluminum in the general population can also occur through vaccination, since vaccines often contain aluminum salts (frequently aluminum hydroxide or aluminum phosphate) as adjuvants. Because concerns have been expressed by the public that aluminum in vaccines may pose a risk to infants, we developed an up-to-date analysis of the safety of aluminum adjuvants. Keith et al. [1] previously analyzed the pharmacokinetics of aluminum for infant dietary and vaccine exposures and compared the resulting body burdens to those based on the minimal risk levels (MRLs) established by the Agency for Toxic Substances and Disease Registry. We updated the analysis of Keith et al. [1] with a current pediatric vaccination schedule [2]; baseline aluminum levels at birth; an aluminum retention function that reflects changing glomerular filtration rates in infants; an adjustment for the kinetics of aluminum efflux at the site of injection; contemporaneous MRLs; and the most recent infant body weight data for children 0–60 months of age [3]. Using these updated parameters we found that the body burden of aluminum from vaccines and diet throughout an infant's first year of life is significantly less than the corresponding safe body burden of aluminum modeled using the regulatory MRL. We conclude that episodic exposures to vaccines that contain aluminum adjuvant continue to be extremely low risk to infants and that the benefits of using vaccines containing aluminum adjuvant outweigh any theoretical concerns.

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## 1. Introduction

In the first year of life, infants receive vaccinations according to a schedule recommended by the Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention [2]. Some of these vaccines utilize aluminum salts as adjuvants (for example, aluminum hydroxide, $Al(OH)_3$, or aluminum phosphate, $AlPO_4$). The particular vaccines (and therefore aluminum exposures) that an infant may receive at any point in the immunization schedule may vary depending on the vaccine chosen by the health care provider, parents, and caregivers from the available FDA-licensed vaccines. Potential aluminum exposures associated with vaccine administration, however, are different from dietary exposures to aluminum, since aluminum in vaccines does not have to pass through the walls of the gastrointestinal tract, which is a significant barrier to systemic aluminum absorption. Rather, it is expected that the whole amount of aluminum in the adjuvant will be absorbed from muscle into the blood following vaccination, albeit at some rate over time.

In an effort to evaluate the relative contribution to aluminum levels in infants from vaccines and from diet, Keith et al. [1] analyzed the pharmacokinetics of aluminum for infant dietary and vaccine exposures and compared these exposures to the level set by the Agency for Toxic Substances and Disease Registry, which is called the minimal risk level or MRL (ATSDR [29]). Exposures below this level are considered to be safe, but levels of exposure at or slightly above the MRL may also be safe due to safety factors that are built into the process of calculating the MRL. Keith et al. [1] concluded that the calculated body burden from aluminum exposures in infants from vaccines is below the MRL equivalent curve for all but a few brief periods during the first year of life. We updated the analysis of Keith et al. [1] with a current vaccination schedule, a more recent aluminum retention function from human volunteers, incorporation of infant glomerular filtration rates, an adjustment for the kinetics of aluminum efflux from the site of injection, contemporaneous MRLs, and the most recent infant body weight data for children 0–60 months of age [3].

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### 1.1. Exposure to aluminum

Aluminum is a ubiquitous environmental metal with no known nutritional role in humans. Because of aluminum's abundance in the environment, it is frequently consumed as an incidental component of water or food, including infant formula [4]. Aluminum is also intentionally added to food as a caking or emulsifying agent. As a result, bread made with aluminum-based baking powder can contain up to 15 mg aluminum per slice, and processed American cheese can contain as much as 50 mg aluminum per slice [5]. Another potential means of exposure to aluminum in humans can occur through vaccination. Certain vaccines may contain specific aluminum salts (primarily aluminum hydroxide and aluminum phosphate) as an adjuvant. Aluminum adjuvants are important components of vaccines, since they stimulate the immune system to respond more effectively to protein or polysaccharide antigens that have been adsorbed to the surface of insoluble aluminum particles. Specifically, these coated particles are phagocytized by cells of the innate immune system (e.g., macrophages) and activate intracytoplasmic sensors of pathogen-associated molecular patterns located within the cells, such as the nucleotide-binding domain leucine-rich repeat-containing family of sensors ([6]; Schroder and Tschopp [30]). The functional consequence of activation of this intracellular system is the activation of certain enzymatic caspases that cleave pro-interleukin (IL)-1β to interleukin (IL)-1β. The secretion of the mature cytokine, IL-1β, leads to an inflammatory reaction and a downstream Th2-dependent antibody response [7], which amplify the immune response to the antigen. Adjuvanted aluminum, therefore, plays a vital role in facilitating the response that underlies the immunoprotection afforded by vaccines.

### 1.2. Aluminum disposition and toxicity

Dietary exposure to aluminum (usually as the citrate) results in small amounts of aluminum being absorbed from the gut (<1%) and reaching the bloodstream [4]. Following enteral absorption, aluminum is transported mainly in the plasma in association with the iron-binding protein transferrin [8]. Aluminum is distributed well throughout the body with the skeleton and lungs (due to inhalation exposures) containing the highest mass of aluminum (approximately 50% and 25% of the body burden, respectively). As for many divalent and polyvalent metals, the skeleton can be a long-term storage depot for aluminum, with the half-life of aluminum in bone being on the order of years [5]. It is anticipated that bone will serve as a stable depot for aluminum in infants, as well as adults, due to the increase in bone mass and volume that takes place during an infant's rapid growth and development. With regard to the non-skeletal compartment, the half-life of aluminum in soft tissues such as the liver is short (<2 days), which indicates very little accumulation in these organs. The majority of bioavailable aluminum is excreted shortly after exposure, primarily in the urine [5], and there appears to be little difference in the renal clearance of aluminum in infants and adults at low exposures [9]. Although aluminum accumulates in the brain as well as bone over time, the concentration of aluminum in brain is lower than that in many other tissues of the body (e.g., liver, spleen), and only 1% of whole-body aluminum is present in the brain or central nervous system at any given time [8,5].

The toxicity of aluminum depends largely on the route and length of exposure. Following single injections, occasional irritation (dermal) at the site of injection is the only adverse effect that has been reported in the published literature. Neurotoxicity in rats has been demonstrated following long-term injections of aluminum leading to aluminum overload or aluminum toxicosis [10,11]. However, the doses tested in these studies were much higher than the maximal exposures that infants might be exposed to from vaccines, and the dosing schedules, the species of aluminum (soluble), and the routes of exposure (intraperitoneal) tested were not relevant to how infants might be exposed to aluminum through vaccination. There is no evidence for neurotoxic effects in humans who may be exposed to aluminum following single, episodic injections [12]. In addition, while aluminum hydroxide has been detected in biopsy samples of muscle obtained from some children with macrophagic myofasciitis (MMF), a rare inflammatory myopathy characterized by clinical symptoms of myalgia or arthralgia and an inflammatory infiltrate at muscle biopsy, this condition has not been shown to be caused by aluminum in vaccines [13]. The clinical symptoms that have been observed in the limited number of patients that have been diagnosed with this rare condition are considered to be due to separate, coincidental immune or neurological disorders that are unrelated to the presence of aluminum in vaccines [14,15].

## 2. Materials and methods

### 2.1. Baseline aluminum levels at birth

Rather than starting from a zero amount of aluminum in the body, we assumed a baseline level of aluminum in an infant at birth. Although whole-body aluminum levels have not been measured in human fetuses, they were measured in only one published animal study, i.e. Cranmer et al. [16], who measured "total" aluminum in fetal mice following maternal exposure to aluminum chloride or saline (control). In this study, saline-treated fetuses contained approximately 592 ppb aluminum. However, since the aluminum content of the saline was unreported and since we consider results in humans to be more relevant to human exposures, we estimated aluminum levels in newborns using the results of Moreno et al. [17], who measured background levels of aluminum in the *serum* of children at birth to be $0.16 \pm 0.05$ μmol/l, which is equivalent to a mean value of 4.32 ppb (MW, Al = 27 g). Next, we estimated levels of aluminum in *whole blood* to be 0.18 μmol/l (4.8 ppb), by taking into consideration published results indicating that approximately 90% of aluminum in blood resides in serum or plasma, with 10% of blood Al located in erythrocytes [5]. This value is in excellent agreement with a background blood concentration in newborns of $0.19 \pm 0.11$ μmol/l reported earlier by Sedman et al. [9]. Since aluminum in blood accounts for approximately 4% of total aluminum in the body at any given time ([5] based on [18]), a blood concentration of 4.8 ppb yields a total background concentration of aluminum in newborns of 120 ppb. Because a newborn infant weighs approximately 3.2 kg (50th percentile for girls; [19]), this concentration corresponds to an estimated body burden of 384 μg, or about 0.4 mg Al, at birth. This natal body burden of aluminum is considered to be low due to the fact that the placenta partially protects the developing fetus from exposures from the mother during pregnancy [20,16,28].

### 2.2. Schedule of vaccination

Using the most recent recommended immunization schedule for persons aged 0–6 years [2], potential combinations of FDA-licensed routine childhood vaccines were compiled and analyzed to determine the maximum doses ($d$) of aluminum that a child might receive over the course of a year. This information was derived from FDA-approved vaccine prescribing information, and the sequence of maximum exposures was determined to be as follows: 0.25 mg at birth, 0 mg at 30 days, 1.2 mg at 60 days, 1.2 mg at 120 days, 0.975 mg at 180 days, and 0.6 mg at 365 days of age. These amounts are summarized by vaccine in Table 1. By way of comparison, Keith et al. [1] calculated aluminum exposures as 0.25 mg at birth, 1.1 mg at 60 days, 0.85 mg at 120 days, 1.1 mg at 180 days, and 0.85 at 365

**Table 1**
Sequence of vaccine administrations leading to maximal aluminum exposures in infants over the first year of life. Based on 2011 ACIP vaccination schedule.

| Vaccine | Postnatal day of administration | Aluminum content (mg) |
|---|---|---|
| Hep B | 0 | 0.25 |
| DTaP + HepB + IPV + Hib + PCV | 60 | 1.2 |
| DTaP + HepB + IPV + Hib + PCV | 120 | 1.2 |
| DTaP + HepB + IPV + PCV | 180 | 0.975 |
| Hib + PCV + HepA | 365 | 0.6 |

days of age using an immunization schedule which is no longer current.

### 2.3. Aluminum retention in children

Aluminum retention in humans has been measured in adult volunteer studies using radioactive aluminum tracers following intravenous administration [21]. Priest [5] re-analyzed the retention of aluminum in the body using longer timecourse data and reported a retention function for adults that is a three-component exponential function of time:

$$R = 29.3 \times e^{-0.595 \times t} + 11 \times e^{-0.172 \times t} + 6.5 \times e^{-0.000401 \times t} \quad (1)$$

where "$R$" refers to the percentage of administered aluminum in the body at time, $t$, beginning approximately one day after injection. This retention function reflects three whole-body half-lives for aluminum of 1.4, 40, and 1727 days, respectively, which mirrors aluminum residence in three compartments in the body, with long-term storage in bone most likely responsible for the longest half-life [5]. The relevant adult rate constants in this 3-compartment model were determined from Eq. (1) using the method of Gibaldi and Perrier [22] and are presented in Fig. 1.

Because glomerular filtration, the primary pathway of excretion of aluminum from the body as well as the main process of renal elimination for xenobiotics in newborns, is not fully developed at birth [23,24], it is expected that aluminum is not cleared from the blood of infants as quickly as that of adults. As a result, the elimination rate constant, $k_{10}$ (Fig. 1), would be expected to be lower in children than adults, but would also increase over time as renal function developed throughout childhood. We therefore modeled glomerular filtration in childhood based on aggregate mean creatinine clearance rates ($C_{Cr}$) measured in 122 children over the first thirteen years of life [25]. Since the data for $C_{Cr}$ seemed to start out small and rise quickly and asymptotically approach a maximum between ages 5 and 13, we utilized a Michaelis–Menten function to describe the rapid increase in renal function. The functional form for $C_{Cr}$ was estimated as follows:

$$C_{Cr}(t) = \hat{a} + \hat{b}\left(\frac{t}{t+\hat{c}}\right) = 50.871 + 90.044\left(\frac{t}{t+231.462}\right) \quad (2)$$

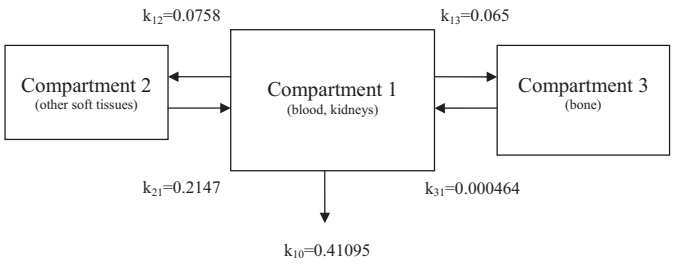


**Fig. 1.** Three-compartment model of aluminum disposition in adults. Rate constants were derived from the retention equation of Priest [5].

Since the horizontal asymptote of the creatinine clearance represents the adult rate of clearance, the function:

$$f(t) = \frac{\hat{a}}{\hat{a}+\hat{b}} + \frac{\hat{b}}{\hat{a}+\hat{b}}\left(\frac{t}{t+\hat{c}}\right) = 0.361 + 0.639\left(\frac{t}{t+231.462}\right) \quad (3)$$

which has a horizontal asymptote of unity, should roughly represent the filtration efficiency of the renal system relative to the adult efficiency. Since the primary means of aluminum removal is through the kidney, it follows that the rate of aluminum removal in children should be:

$$k_{10}(t) = \hat{k}_{10} \times f(t) = 0.41095\left(0.361 + 0.639\left(\frac{t}{t+231.462}\right)\right) \quad (4)$$

where $\hat{k}_{10}$ is the estimated elimination rate constant in adults based upon the equation from Priest [5] and $f(t)$ represents the fraction of adult aluminum removal for children at age $t$. Upon substitution of the function from Eq. (4) into the ordinary differential equations that describe the 3-compartment model for aluminum it follows that:

$$\frac{dX_1}{dt} = -k_{10}(t)X_1 + k_{21}X_2 + k_{31}X_3 - k_{12}X_1 - k_{13}X_1 \quad (5)$$

$$\frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2 \quad (6)$$

$$\frac{dX_3}{dt} = k_{13}X_1 - k_{31}X_3 \quad (7)$$

Because this set of differential equations includes a non-constant coefficient, $k_{10}(t)$, the exact solution is non-tractable. Therefore, we utilized numeric Runge–Kutta type methods to solve the set of differential equations numerically using the statistical program R (R Foundation for Statistical Computing [31]).

### 2.4. Infant body weight

Because the safe, oral daily dose of aluminum (i.e., MRL = 1 mg/kg bw/day) is expressed by ATSDR [4] as normalized to body weight, it was necessary to multiply this MRL value by infant body weight to obtain safe doses ($d$) of aluminum over the first year of life. Because infant body weight is not constant and increases rapidly after birth, it was necessary to determine the relevant mathematical functions that describe infant body weight during this time. We, therefore, modeled the most recent infant body weight data for US children 0–60 months of age [3]. We estimated the 5th and 50th percentiles of infant body weight (kg) for age (months) for males and females combined using quantile regression. The model describing the relationship between weight and age was estimated using the best-fitting polynomial functions of age, since the data indicate that this relationship is non-linear. The degree of the polynomial was determined by minimizing a cross-validation criterion, and the following functions were calculated from the NHANES [3] data:

$$BW_{5th} = 2.65899 - \left(\frac{1.86774}{(1+age)^{0.5}}\right) + 1.59926(1+age)^{0.5} \quad (R^2 > 0.99) \quad (8)$$

$$BW_{50th} = 3.35319 + (1.74026(1+age)^{0.5}) + 0.618471(nl(0.1 + 0.1age)) \quad (R^2 > 0.99) \quad (9)$$

### 2.5. Calculations of aluminum body burdens

The ATSDR MRL of 1 mg/kg bw/day was multiplied by the relevant functions for infant body weight [Eqs. (8) and (9)] and corrected for the low absorption of aluminum from the gastrointestinal tract (0.78%; [26]), to estimate correspondingly safe oral doses ($d$) of

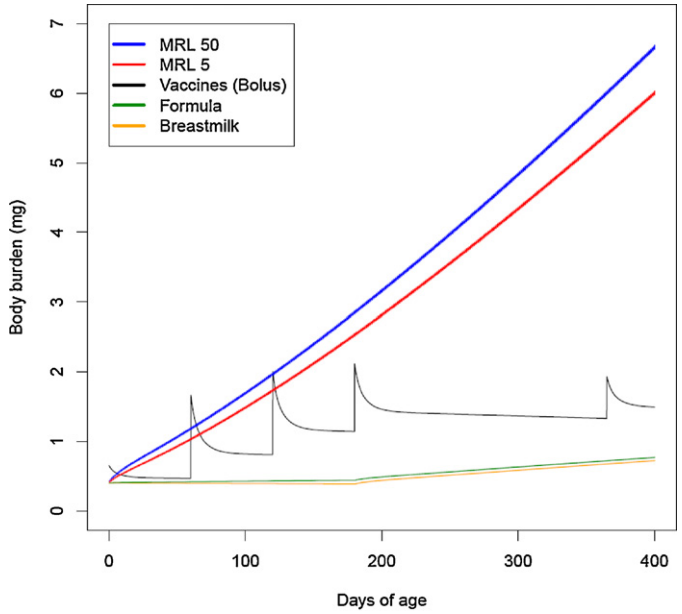


**Fig. 2.** Aluminum body burden contributions from diet and vaccines (100%, instantaneous absorption assumed) relative to current MRL level intake in infants. *Note*: the body burden of aluminum is greater than zero at birth, since infants are exposed to aluminum from their mothers *in utero*.

aluminum. The following dietary exposures of infants to aluminum, published previously by Keith et al. [1] and adjusted for 0.78% oral absorption, were utilized in our model: (1) age 0–6 months: 0.03 mg (breast milk) and 0.15 mg (formula); (2) age >6 months: 0.7 mg (breast milk or formula). Retention of aluminum following infant dietary exposures, exposures from vaccines according to the 2011 ACIP schedule, and safe doses of aluminum were then estimated over the first 400 days of life using Eqs. (1)–(7). Retention curves were generated using the publicly available statistical modeling software, R (R Foundation for Statistical Computing [31]).

## 3. Results and discussion

Fig. 2 shows the amount of aluminum that is retained by an infant following exposure from vaccines (assuming complete and instantaneous absorption) or the diet (formula or breast milk) throughout the first 400 days of life. The two upper curves show the amount of aluminum retained by infants of median or low birth weight, if the infant consumed the MRL of aluminum (1 mg/kg bw/day) every day over the first year of his/her life. The MRL is based on the infant's weight, so the upper curve shows the body burden of aluminum associated with infants at the median or 50th percentile weight, and the lower curve shows the level associated with infants at the 5th percentile of weight. Both curves assume intestinal absorption of 0.78% [26] and retention according to Eq. (1) that was modified to reflect glomerular filtration rates in infants. Fig. 2, as well as the equivalent curve published previously by Keith et al. [1], demonstrate that there are brief "excursions" of bodily aluminum levels above the MRL following vaccination, when complete and instantaneous absorption of aluminum from the site of injection is assumed; however, due to the rapid elimination of aluminum, the levels quickly fall back below the MRL. The curves for aluminum retention associated with formula and breastmilk show a slight change at six months that is due to the assumption that infants switch from breastmilk or formula to solid food on this date and therefore begin to receive a higher aluminum dose from baby food.

The determinations of the kinetics of aluminum retention by Priest [21,5] were based on experiments where human volunteers were given an intravenous injection of aluminum citrate. For vaccines, the injection is intramuscular, the aluminum is in an insoluble form (e.g., as the phosphate or hydroxide of aluminum), and muscle at the site of injection is considered to be a storage depot for aluminum. Over time the insoluble aluminum hydroxide or aluminum phosphate particles are solubilized by citrate ions in the interstitial fluids of muscle. After solubilization, the uptake and distribution kinetics of aluminum will likely be similar to the kinetics determined by the human volunteer studies. However, it is unlikely that the process of absorption from the site of intramuscular injection into the blood is instantaneous, as is assumed for intravenous exposures and as presumed by the retention functions used to generate Fig. 2 and by Keith et al. [1].

Flarend et al. [27] investigated the absorption into the blood of aluminum hydroxide and aluminum phosphate following intramuscular injection into New Zealand White rabbits. Two important observations were made in their experiments: (1) only a fraction of the injected aluminum was taken up from the site of injection into blood over the 28-day experimental period, and (2) absorption of neither adjuvant was instantaneous. Specifically, blood concentrations of aluminum hydroxide decreased to a minimum by the end of the experiment (reached a terminal phase), where as aluminum phosphate blood concentrations were relatively constant over the 28-day period and did not reach a terminal phase. These results likely reflected differences in the rate of absorption of each adjuvant from the site of injection and not differences in excretion, since all other experimental conditions were equivalent in each group. By comparing with the area under the curve of the blood concentration–time curve for an intravenous administration of 0.85 mg aluminum citrate, the authors determined that only 17% and 51% of injected aluminum hydroxide and aluminum phosphate, respectively, was absorbed into the blood over 28 days. If the results of the rabbit studies by Flarend et al. [27] are reflected in similar kinetics in humans, then the dose of aluminum that enters into the bloodstream after intramuscular injection of vaccines in infants is at best only one half of what has been modeled in Fig. 2.

Therefore, based on the results of [27], we assumed: (1) that only 51% (for aluminum phosphate, AP) or 17% (for aluminum hydroxide, AH) of injected aluminum would be absorbed into the blood following a single intramuscular vaccine injection over the first 28 days after exposure, and (2) that absorption of the remaining adjuvant at the site of injection would take place at a constant rate over the next 28 days for AP and 137 days for AH, rather than instantaneously, as modeled in Fig. 2. In order to make this calculation, we assumed that the rate of absorption after 28 days was the same as that during the 28-day experimental period in [27], i.e., 0.51/28 $day^{-1}$ for AP and 0.17/28 $day^{-1}$ for AH. These rates are considered to be highly conservative, since blood concentrations of AH approached zero by the end of the experiment, thereby implying a very low rate of uptake into blood, and the blood concentration–time curve for AP appeared to be entering a terminal phase 28 days post-injection. Using these assumptions for the absorption of aluminum from intramuscular injection of vaccines, we repeated our analysis.

Figs. 3 and 4 demonstrate that modeling the slower release of aluminum from the injection site eliminates the "excursions" of whole-body aluminum above MRL levels shown in Fig. 2. The body burden of aluminum is less than 50% of the oral safe level for either AP (Fig. 3) or AH (Fig. 4) at all times during the first year or so of life. Using the assumptions of slower release of aluminum adjuvant from the site of injection, the estimated level of aluminum in infants exceeds the MRL (safe) body burden at no time, and the margin of exposure between aluminum body burden from vaccine and the

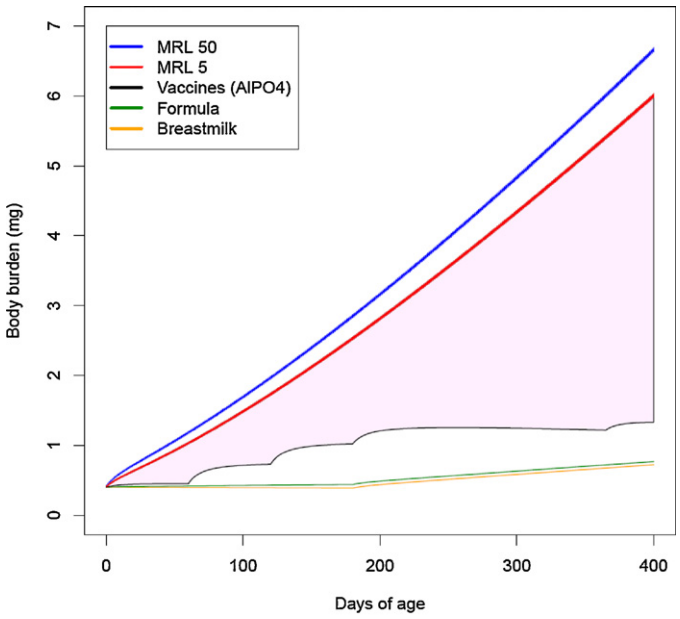


**Fig. 3.** Body burden contributions of aluminum from diet and vaccines (constant absorption of aluminum phosphate over 56 days based on results of Flarend et al. [27]) relative to current MRL level intake in new born infants. Margin of exposure in pink. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MRL increases with age. It was also observed that the body burden of aluminum following injection of AH increased more gradually than that for AP. This was due to the slower rate of efflux of AH from the site of injection reported in rabbits.

Although based on the most recent data available, there are several uncertainties in our analysis. First, the published retention function for aluminum (Eq. (1)) is based on results for only one person, albeit data have been acquired from this adult for twelve years [5]. Ideally, the retention function would have been derived from pharmacokinetic data in infants or in more than one adult; however, an expansion of this analysis is unlikely. An infant monkey study could provide these data, however, given the present lack of evidence of harm due to the current aluminum levels, such studies may be a low priority. Second, the results of Flarend et al. [27], from which we obtained our estimate of the rate and extent of absorption of aluminum hydroxide and phosphate following intramuscular injection, are based on data from only two rabbits per adjuvant tested. The low number of animals in that study is not surprising, given that it was primarily an exploratory investigation into the disposition of injected aluminum, utilizing, at that time, a new method for detecting radioactive Al in the body (accelerator mass spectrometry). Ideally, the results of that study should be confirmed using a larger number of animals, in order to increase our confidence in the results. Nevertheless, the study clearly showed that the absorption of aluminum, at least in rabbits, is neither instantaneous nor complete up to one month following intramuscular injection [27]. We consider this behavior to be more biologically plausible than complete and instantaneous absorption from the site of injection, and more consistent with the view of muscle tissue being a storage depot for aluminum adjuvant following intramuscular vaccination. A third uncertainty in the analysis is the extent to which the use of maximum aluminum exposures (modeled here) is relevant to aluminum body burdens estimated following more typical exposures to aluminum adjuvant, which are considered to be lower. Ideally, one would like to model aluminum exposures to reflect typical exposures in the population. However, modeling body burdens following maximum exposures to aluminum provides a "worst case" scenario, since more typical exposures to aluminum will obviously lead to a lower body burden and therefore a greater margin of exposure (safety), the distance between safe and expected body burdens of aluminum. Our results indicate that body burdens following maximal exposure to aluminum adjuvant do not exceed those based on an accepted regulatory standard of safe aluminum levels, i.e., the MRL established by ATSDR.

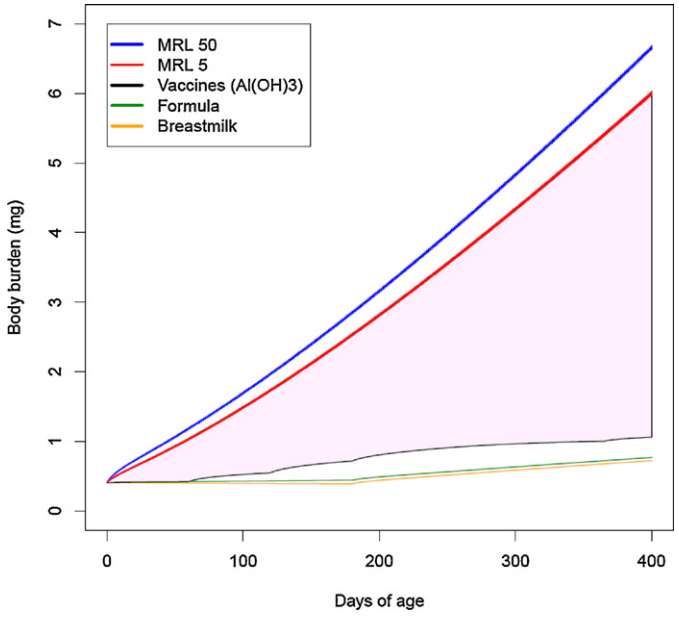


**Fig. 4.** Body burden contributions of aluminum from diet and vaccines (constant absorption of aluminum hydroxide over 165 days based on results of Flarend et al. [27]) relative to current MRL level intake in new born infants. Margin of exposure in pink. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## 4. Conclusions

Using the previous work of Keith et al. [1] as our starting point, we re-evaluated aluminum levels in infants using a number of updated parameters, including a current pediatric vaccination schedule, baseline aluminum levels at birth, a recent aluminum retention function from human volunteers that incorporates glomerular filtration rates in infants, an adjustment for the kinetics of aluminum efflux at the site of injection, the most recent MRL for aluminum, and up-to-date infant body weight data for children 0–60 months of age. Assuming slow release of aluminum adjuvant from the site of injection into the systemic circulation, we have demonstrated that aluminum levels in infants are well below the minimal risk level curves for either median or low-birth weight babies. We also compared the body burden of aluminum contributed by vaccines with that contributed by diet. The body burden of aluminum from vaccines is not more than 2-fold higher than that received in the diet. While the contribution of vaccines to an infant's aluminum body burden can be slightly higher than that of the dietary contribution in our model, the fact that the primary pool where the aluminum is residing, as a long-term storage depot, is likely to be skeletal and not a more sensitive soft organ system is reassuring [5]. Although aluminum toxicosis is known to occur in humans, it is found exclusively in individuals suffering from kidney disease or in those exposed to high levels of aluminum via occupational inhalation. However, for infants, our study demonstrates that there is little risk for aluminum toxicity following immunizations administered according to ACIP recommendations even

with maximal exposures to aluminum adjuvant. For the general population of infants, who receive less than the maximal dose, the risk is even lower.

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# Appendix C

Critical analysis of reference studies on aluminium-based adjuvants toxicokinetics

**Format**: Abstract



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# [Critical analysis of reference studies on aluminium-based adjuvants toxicokinetics].

[Article in French]

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## Author information

## Abstract

We reviewed the three reference toxicokinetic studies commonly used to suggest innocuity of aluminum (Al)-based adjuvants. A single experimental study was carried out using isotopic $^{26}$Al (Flarend et al., 1997). This study ignored adjuvant cell capture. It was conducted over a short period of time (28 days) and used only two rabbits per adjuvant. At the endpoint, Al retention was 78% for aluminum phosphate and 94% for aluminum hydroxide, both results being incompatible with quick elimination of vaccine-derived Al in urines. Tissue distribution analysis omitted three important retention sites:

the injected muscle, the draining lymph node and bone. Two theoretical studies have evaluated the potential risk of vaccine Al in infants, by reference to the oral Minimal Risk Level (MRL) extrapolated from animal studies. Keith et al., 2002 used a too high MRL (2mg/kg/d), an erroneous model of 100% immediate absorption of vaccine Al, and did not consider renal and blood-brain barrier immaturity. Mitkus et al. (2011) only considered absorbed Al, with erroneous calculations of absorption duration. They ignored particulate Al captured by immune cells, which play a role in systemic diffusion and the neuro-inflammatory potential of the adjuvant. MRL they used was both inappropriate (oral Al vs injected adjuvant) and far too high (1mg/kg/d) with regard to experimental studies of Al-induced memory and behavioral changes. Both paucity and serious weaknesses of these studies strongly suggest that novel experimental studies of Al adjuvants toxicokinetics should be performed on the long-term, including post-natal and adult exposures, to ensure innocuity and restore population confidence in Al-containing vaccines.

**KEYWORDS:** Adjuvants vaccinaux; Aluminium; Sécurité vaccinale; Toxicocinétique; Toxicokinetics Vaccine safety; Vaccine adjuvant

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# Publication types

# LinkOut - more resources

# Appendix D

## AUTISM & ALUMINUM ADJUVANTS IN VACCINES

How Aluminum Adjuvants in Vaccines Can Cause Autism

Informed Consent Action Network, 2017

# AUTISM & ALUMINUM ADJUVANTS IN VACCINES

## How Aluminum Adjuvants in Vaccines Can Cause Autism

ICAN
Informed Consent Action Network

Published: August 18, 2017 (Version 1.0)

The Centers for Disease Control (CDC) asserts that vaccines and vaccine ingredients have been disproven as potential causes of autism. Statements by the CDC are generic and encompass all vaccines and vaccine ingredients. For example, the CDC states:

> *"Vaccines Do Not Cause Autism" "There is no link between vaccines and autism." "…no links have been found between any vaccine ingredients and autism spectrum disorder." (CDC website, August 2017)*

These statements are not supported by available science. The CDC's evidence supporting these statements is limited to the MMR vaccine (Taylor 2014), thimerosal preservative (Taylor 2014) and vaccine antigen exposure (DeStefano 2013).

Dr Frank DeStefano of the CDC's Immunization Safety Office is co-author of a paper (Glanz 2015) which states:

> *"To date, there have been no population-based studies specifically designed to evaluate associations between clinically meaningful outcomes and non-antigen ingredients, other than thimerosal."*

This statement applies to, among other vaccine ingredients, aluminum adjuvant. Studies of MMR vaccine cannot be used as evidence of safety for other vaccines, for example vaccines that contain aluminum adjuvant. The overly-broad, generic assertions that no vaccines and no ingredients cause autism are thus not supported by scientific evidence. In fact, the CDC statements are contradicted by a large, consistent and growing body of scientific evidence, including:

1) studies showing neurotoxic and neuroinflammatory effects (e.g. microglial activation) from dosages of aluminum adjuvants lower than or approximately equal to dosages received by infants according to the CDC vaccine schedule (Crepeaux 2017, Petrik 2007, Shaw 2013, Shaw 2009);

2) studies linking vaccines to immune activation brain injury (Zerbo 2016, Li 2015);

3) studies showing that early-life immune activation is a causal factor in autism and other neurodevelopmental disorders and mental illnesses (e.g. schizophrenia) (Meyer 2009, Deverman 2009, Estes 2016, Kneusel 2014, Careaga 2017, Meyer 2014).

The accumulating evidence indicates that vaccine-induced immune activation, and aluminum adjuvants in particular, may cause mental illnesses and neurodevelopmental disorders, including autism.

In this paper, we present scientific evidence that aluminum adjuvants can cause autism and other brain injuries. Also, we explain why the studies allegedly supporting the safety of aluminum adjuvants do not show safety for adverse neurological outcomes.

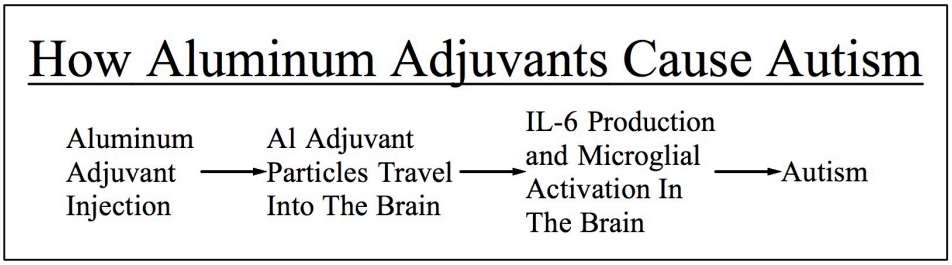


**Fig 1: Proposed mechanism for how aluminum adjuvants cause autism. Each step is supported by replicated scientific studies.**

## Immune Activation: A Cause of Autism and Mental Illness

The term "immune activation" describes the activation of the cellular components of the immune system. The developing brain can be injured by immune activation, with life-long consequences (Meyer 2009, Deverman 2009, Estes 2016, Kneusel 2014, Careaga 2017, Meyer 2014). Immune activation injury is linked to autism, schizophrenia, depression and other mental illnesses or neurodevelopmental disorders. Immune activation effects on the brain are mediated by immune system signaling molecules, especially cytokines (Estes 2016, Meyer 2014, Smith 2007, Choi 2016, Pineda 2013).

It is generally accepted that immune activation (e.g., from infection) during pregnancy is a risk factor for autism and schizophrenia in the offspring (Ciaranello 1995, Atladottir 2010, Brown 2012). The intensity and duration of immune activation and cytokine expression appear to be important factors influencing autism risk (Meyer 2014). Intense immune activation is associated with greater risk of autism (Careaga 2017, Atladottir 2010). Chronic inflammation is associated with greater risk of autism (Jones 2016, Zerbo 2014). However, there is no evidence that short-duration, low-intensity immune activation resulting from common childhood illnesses increase autism risk. Timing of immune activation in relation to stages of brain development is also an important factor (Meyer 2006, Meyer 2009).

Animal experiments have tested the effects of immune activation during pregnancy and postnatally on the development of offspring (Meyer 2009, Deverman 2009, Estes 2016, Kneusel 2014, Careaga 2017, Meyer 2014). In these experiments, pregnant animals (mice, rats and monkeys) or neonates are injected with a non-infectious immune activating substance such as "poly-IC" (which mimics a viral infection) or lipopolysaccharide (LPS, which mimics a bacterial infection). These substances cause immune system activation without infection. They induce fever and cytokine production and can have substantial effects on brain development if activation is sufficiently intense or prolonged and if exposure occurs during vulnerable developmental stages.

Immune activation has been demonstrated in mice to cause the three core behavioral symptoms of autism: decreased socialization and communication, and increased repetitive behaviors (Malkova 2012). Immune activation has also been shown to cause neuropathology (Weir 2015) and behavioral abnormalities in monkeys that resemble behaviors in human schizophrenia and autism (Bauman 2014, Machado 2015). See Fig. 2.

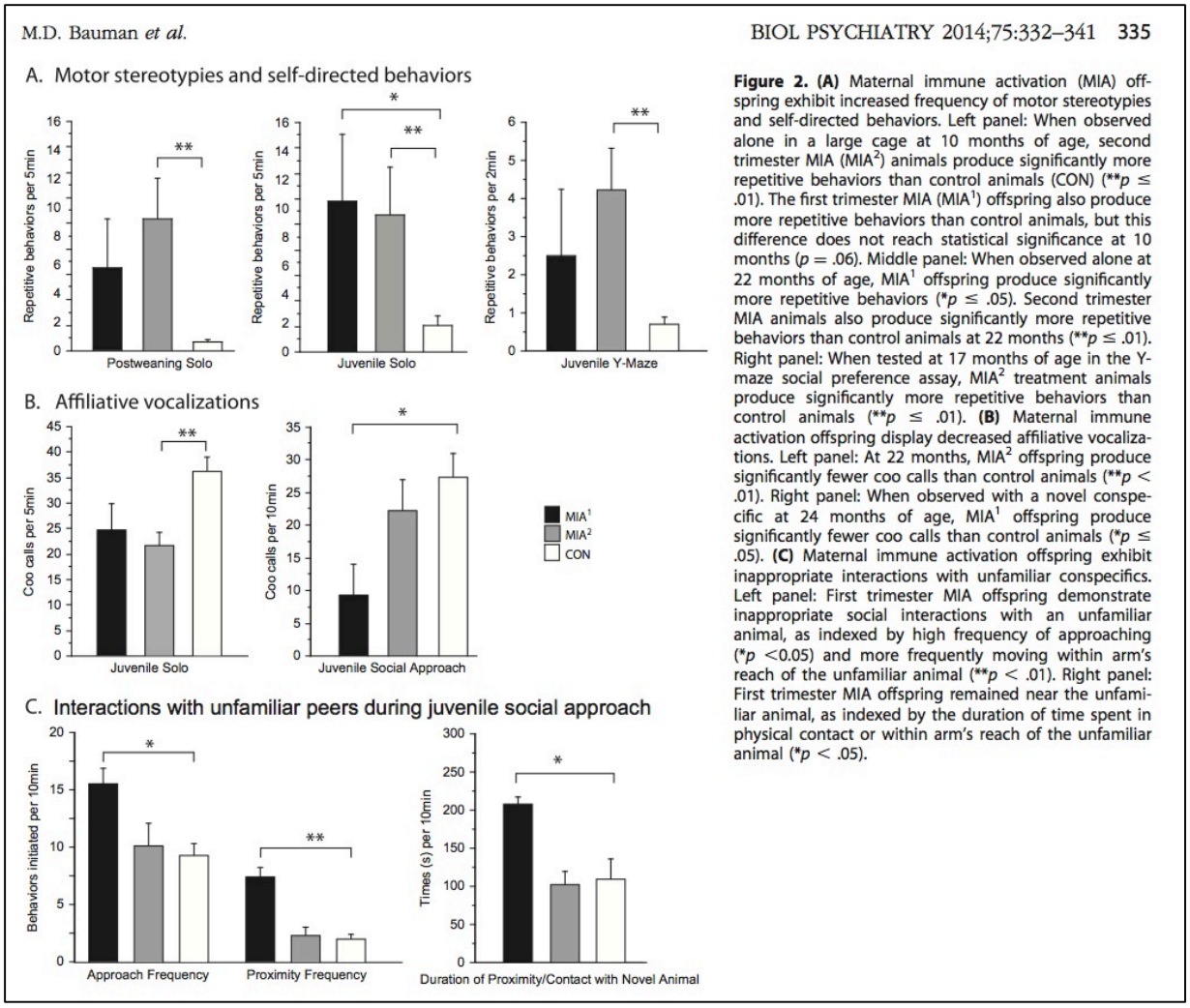


**Figure 2. (A)** Maternal immune activation (MIA) offspring exhibit increased frequency of motor stereotypies and self-directed behaviors. Left panel: When observed alone in a large cage at 10 months of age, second trimester MIA ($MIA^2$) animals produce significantly more repetitive behaviors than control animals (CON) (**$p \leq .01$). The first trimester MIA ($MIA^1$) offspring also produce more repetitive behaviors than control animals, but this difference does not reach statistical significance at 10 months ($p = .06$). Middle panel: When observed alone at 22 months of age, $MIA^1$ offspring produce significantly more repetitive behaviors (*$p \leq .05$). Second trimester MIA animals also produce significantly more repetitive behaviors than control animals at 22 months (**$p \leq .01$). Right panel: When tested at 17 months of age in the Y-maze social preference assay, $MIA^2$ treatment animals produce significantly more repetitive behaviors than control animals (**$p \leq .01$). **(B)** Maternal immune activation offspring display decreased affiliative vocalizations. Left panel: At 22 months, $MIA^2$ offspring produce significantly fewer coo calls than control animals (**$p < .01$). Right panel: When observed with a novel conspecific at 24 months of age, $MIA^1$ offspring produce significantly fewer coo calls than control animals (*$p \leq .05$). **(C)** Maternal immune activation offspring exhibit inappropriate interactions with unfamiliar conspecifics. Left panel: First trimester MIA offspring demonstrate inappropriate social interactions with an unfamiliar animal, as indexed by high frequency of approaching (*$p < 0.05$) and more frequently moving within arm's reach of the unfamiliar animal (**$p < .01$). Right panel: First trimester MIA offspring remained near the unfamiliar animal, as indexed by the duration of time spent in physical contact or within arm's reach of the unfamiliar animal (*$p < .05$).

**Fig 2: Maternal immune activation in monkeys caused behavioral abnormalities in juvenile offspring resembling behaviors in both autism and schizophrenia. MIA1 (Black)= first trimester immune activation; MIA2 (grey) 2nd trimester immune activation; CON (white) saline control. From Bauman et al. 2014**

Immune activation also causes non-behavioral effects associated with human autism (citations here link immune activation with these effects):

1) reduction in Purkinje cells (Shi 2009);
2) mitochondrial dysfunction (Giulivi 2013);
3) increase in brain volume (from IL-6 exposure, Wei 2012(b)) and neuron density in the brain (Smith 2012);
4) long term chronic brain inflammation (Garay 2012); and
5) microbiome disruption (dysbiosis) (Hsiao 2013).

These non-behavioral similarities further support the relevance of the immune activation models to human autism. The non-behavioral (e.g., physiological) effects of immune activation have been reviewed (Labouesse 2015).

The cytokines interleukin-6 (IL-6) and interleukin-17a (IL-17) have been identified as mediating the behavioral effects of immune activation (Smith 2007, Malkova 2012, Choi 2016, Pineda 2013, Wei 2012(a), Wei 2013, Parker-Athill 2010, Wei 2016). The IL-6 findings have been replicated by different researchers using a variety of experimental methods. For example, in an experiment with poly-IC, abnormal behavior is almost completely prevented by simultaneous administration of IL-6-blocking antibody (Smith 2007, Pineda 2013). Injection of IL-6 by itself causes abnormal behavior that closely matches

behavior resulting from poly-IC immune activation (Smith 2007). Inhibition of IL-6 signaling in a genetic autism model (BTBR mice) normalized social and repetitive behavior (Wei 2016). These results demonstrate that IL-6 is responsible for causing abnormal autism-like behavior.

The Patterson laboratory at CalTech was the first to report that IL-6 is responsible for causing the autism-like behavioral effects of immune activation (Smith 2007). Two papers from this research group state:

> *"IL-6 is central to the process by which maternal immune activation causes long-term behavioral alterations in the offspring." (Smith 2007)*

> *"…blocking IL-6 prevents >90% of the changes seen in offspring of poly(I:C)-injected females, showing that gene expression changes, as well as behavioral changes, are normalized by eliminating IL-6 from the maternal immune response." (Smith 2007)*

> *"IL-6 is necessary and sufficient to mediate these effects since the effects…are prevented by injection of pregnant mice with poly-IC combined with an anti-IL-6 antibody, and are mimicked by a single maternal injection of IL-6." (Garay 2013)*

Brain exposure to elevated IL-6 by engineered virus showed that IL-6 exposure, initiated after birth, caused autism-like behaviors (Wei 2012(a)). The Wei 2012(a) paper states:

> *"We demonstrated that IL-6 is an important mediator of autism-like behaviors. Mice with an elevated IL-6 in brain developed autism-like behaviors, including impaired cognition ability, deficits in learning, abnormal anxiety-like trait and habituation, as well as a decreased social interaction initiated at later stages. These findings suggest that an IL-6 elevation in the brain could modulate certain pathological alterations and contribute to the development of autism." (Wei 2012(a))*

More recent evidence shows that IL-17 acts downstream of IL-6 to cause autism-like behavioral abnormalities and atypical cortical development in mice (Choi 2016). Blocking either IL-6 or IL-17 prevents the autism-like behavior; an injection of IL-17 by itself causes the autism-like behavior (Choi 2016). IL-6 is known to induce IL-17 by promoting the development of Th17 cells which produce IL-17.

Immune activation animal models appear to be valid models for human neurological/psychiatric disorders, including autism (Estes 2016, Careaga 2017, Meyer 2014). The Estes 2016 review argues for the validity of the immune activation models to humans:

> *"These MIA (maternal immune activation) animal models meet all of the criteria required for validity for a disease model: They mimic a known disease-related risk factor (construct validity), they exhibit a wide range of disease-related symptoms (face validity), and they can be used to predict the efficacy of treatments (predictive validity)." (Estes 2016)*

Evidence suggests a mediating role for IL-6 and IL-17 in human autism. For example, IL-6 is significantly elevated in the cerebellum in human autism (Wei 2011) and is highly elevated in some brain regions of some autistic individuals (Vargas 2005). Treatment of human autistics with the anti-inflammatory flavonoid luteolin improves autistic behaviors in the individuals that also experience a decline in IL-6 blood levels (Tsilioni 2015). This result is consistent with a causal role for IL-6 in human autism. Also, IL-17 is elevated in human autism (Akintunde 2015, Al-Ayadhi 2012, Suzuki 2011). Vitamin D reduces IL-17 production (Bruce 2011, Wobke 2014, Drozdenko 2014) and improves autistic behaviors in humans (Saad 2016, Jia 2015). The vitamin D findings are consistent with a causal role for IL-17 in human autism.

IL-6 functioning appears to be similar or identical in mice and humans. No mouse-human differences in IL-6 functioning are described in a 2004 review (Mestas 2004). IL-6 functioning is quite conserved across species (Brown 2014). Central nervous system development in rodents

and humans is governed by the same principles (Brown 2014). Hence, the fact that IL-6 causes autism-like behavioral abnormalities in animal models deserves a presumption of validity to humans.

Immune activation is a risk factor for autism, schizophrenia and other neurological/psychiatric disorders. The cytokines IL-6 and IL-17 are responsible for mediating the autism-like behavioral effects of immune activation in the animal models. The available evidence supports a causal role for IL-6 and IL-17 in human autism.

## Maternal vs. Postnatal Immune Activation

The timing of immune activation is an important factor influencing effects on the brain. The developing brain is vulnerable to immune activation injury; the mature, adult brain is apparently not nearly as vulnerable. Sensitivity to immune activation likely declines as the brain matures (Meyer 2014, Meyer 2007).

In most immune activation experiments, the offspring are exposed to immune activation during gestation (by stimulating the maternal immune system). In contrast, most vaccines are administered postnatally. This raises the question of whether postnatal immune activation can have similar effects on the brain as maternal immune activation. Diverse evidence indicates that the brain can be adversely affected by postnatal immune activation. Postnatal immune activation experiments, human case reports, and consideration of brain development timelines suggest that the human brain is vulnerable to immune activation injury for years after birth.

In the maternal immune activation experiments, inflammatory signaling and some cytokines (e.g. IL-6) traverse the placenta into the fetus. Consequently, immune activation in the mother causes immune activation and elevated cytokines in the fetus, and in the fetal brain (Oskvig 2012, Ghiani 2011).

Postnatal immune activation can have adverse neurological effects, including increased seizure susceptibility (Chen 2013, Galic 2008), learning and memory deficits (Harre 2008), and an increase in excitatory synapse formation (Shen 2016). Seizure disorders, learning and memory dysfunction, and elevated excitatory signaling are associated with autism.

Elevated IL-6 in the brain in the postnatal period causes neuronal circuitry imbalance and mediates autism-like behaviors in mice (Wei 2012(a)). The circuitry imbalance observed in Wei 2012(a) was an excess of excitatory synapses and a deficit of inhibitory synapses. See Fig. 3. Excessive excitatory signaling is observed in human autism (Robertson 2016, Freyberg 2015). In fact, an imbalance between excitatory and inhibitory signaling (towards excess excitation) has been posited as a central characteristic of autism (Robertson 2016, Freyberg 2015).

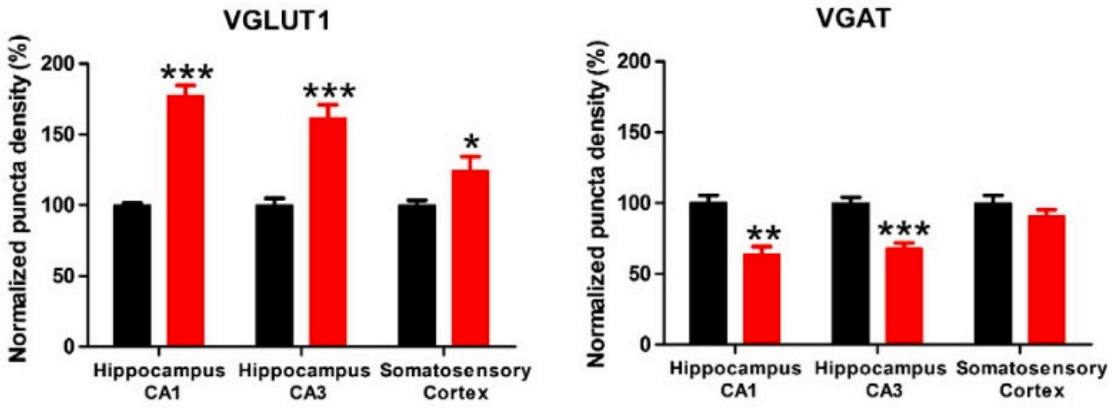


**Fig 3: Elevation of IL-6 in the brains of mice (initiated shortly after birth) caused an increase in excitatory synapses (VGLUT1) and a decrease in inhibitory synapses (VGAT). Excessive excitatory signaling is observed in human autism. Red=Elevated IL-6; Black=Control. VGLUT1=excitatory synapses; VGAT=inhibitory synapses. *P<0.05, **P<0.01 and ***P <0.001. Adapted from Wei et al 2012(a).**

In a maternal immune activation experiment with mice (Coiro 2015), autism-relevant behavior and dendritic spine abnormalities (relevant to autism and schizophrenia) were ameliorated by administering an anti-inflammatory drug postnatally. The drug was started at birth and continued for 2 weeks, which roughly corresponds to age 2 in humans (Semple 2013). This result indicates that brain development is affected by postnatal inflammation, at times corresponding to when vaccines are given to humans.

Several case reports describe previously-healthy children that displayed sudden-onset autistic behavior during or subsequent to infection in the brain. All the cases had signs of intense brain inflammation. Here are brief descriptions:

*Delong 1981*: describes 3 children, ages 5, 7 and 11 with full-blown autistic behavior associated with brain inflammation. Brain inflammation was presumed in two cases and confirmed in one. The 5 and 7 year olds recovered completely, and the 11-year recovered partially.

*Marques 2014*: describes a previously healthy 32-month-old girl that suffered autistic regression from a viral central nervous system infection with associated brain inflammation.

*Ghaziuddin 2002*: describes a previously healthy 11-year-old boy that suffered permanent autistic regression after sudden onset herpes brain infection with associated brain inflammation.

*Gillberg 1986*: describes a previously healthy 14-year-old girl with permanent autistic regression from herpes brain infection with associated brain inflammation.

The most parsimonious explanation for these cases is that autistic behavior resulted from intense inflammation and cytokine production in the brain. Accordingly, these cases indicate that the human brain remains vulnerable to immune activation injury well into childhood, though the vulnerability almost certainly decreases with maturation. The susceptibility of older children to inflammation-induced autistic behavior strongly suggests that younger infants, of 0-2 years of age, are also vulnerable. It is not reasonable to claim, and there is no evidence to suggest, that the age range of 0-2 years (when most vaccines are given) is uniquely resistant to immune activation injury. All the available evidence indicates the opposite.

The immune activation experiments and case reports are consistent and indicate that

immune activation and elevated cytokines in the postnatal period can cause brain injury.

The next critical question to consider is whether vaccines can cause immune activation and elevated cytokines in the brain.

## Postnatal Vaccination Affects Brain Development in Animal Model

The first study to test the effect of postnatal vaccination on brain development was published in 2015 (Li 2015). In this experiment, neonatal rats were administered bacillus calmette-guerin (BCG) vaccine, hepatitis B (HBV) vaccine or a combination (BCG+HBV) timed to imitate human infant vaccination schedules. BCG and HBV vaccines produced opposite effects on the brain. Specifically, BCG enhanced synaptic plasticity and long-term potentiation (LTP, the basis for learning and memory); HBV inhibited synaptic plasticity and LTP. BCG and HBV vaccines also caused opposite changes in some synapse protein levels.

HBV vaccine (but not BCG vaccine) increased IL-6 gene expression in the brain; increased gene expression likely indicates an elevation in brain IL-6. The HBV vaccine contains aluminum adjuvant, and the BCG does not contain aluminum adjuvant. Hence, the aluminum adjuvant may be the ingredient responsible for the elevated IL-6 gene expression. See Fig. 4.

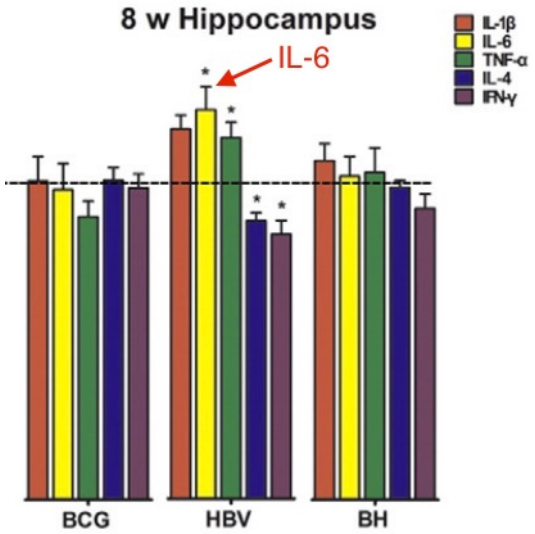


**Fig. 4: Hepatitis B vaccine, but not BCG vaccine, increased IL-6 gene expression in the brain at 8 weeks after neonatal vaccination. Hepatitis B vaccine contains aluminum adjuvant; BCG vaccine does not. Elevated IL-6 causes autism-like behaviors in animal models. *P<0.05 Adapted from Li et al 2015.**

The Li et al study showed that the vaccines caused other changes in the brain, including 1) changes in long-term potentiation (LTP) (Hep B decreased LTP), 2) changes in dendritic spines, and 3) changes in synapse protein expression. Changes in synapse proteins and dendritic spines have been observed in human brain disorders.

Li et al. attribute the brain effects to changes in cytokine levels and immune polarization (Th1/Th2 polarization) induced by the vaccines. Aluminum adjuvants cause Th2 polarization. Li et al. state that the results suggest vaccines can interact by way of immune activation effects:

> *"…our data suggested that combinations of different vaccines can mutually interact (enhance or counteract). The mechanism of synaptic plasticity modulation through neonatal BCG/HBV vaccination may be via*

*systemic Th1/Th2 bias accompanied by a specific profile of cytokines and neurotrophins in the brain." (Li 2015)*

Li 2015 demonstrates that vaccines affect brain development by an immune activation mechanism. Further, since aluminum adjuvants induce Th2 activation and long term Th2 polarization, the Li 2015 results suggest that all aluminum-adjuvanted vaccines may cause adverse effects similar to the HBV vaccine. Accordingly, the Li 2015 results suggest that studies showing that immune activation causes neurological/psychiatric disorders are relevant to vaccine adverse effects.

## Vaccines Are Given During Synaptogenesis

Another way to answer the question of brain vulnerability to immune activation is to consider the types of brain development processes occurring when vaccines are administered. Vaccines are given primarily in the first 18 months after birth. The human brain undergoes intense and rapid development during this period. Synaptogenesis (formation of synapse connections between neurons) is especially intense in this period.

The vulnerability of the developing brain to immune activation is apparently related to the specific types of brain development processes occurring (Tau 2010, Meyer 2006, Meyer 2007). Such processes include migration (movement of neurons to final locations in the brain), adhesion (formation of chemical-mechanical attachments between brain cells), and synaptogenesis (formation of synapse connections between neurons), among others (neurogenesis, gliogenesis, myelination etc).

Cytokines affect brain development processes. For example, elevated IL-6 affects migration, adhesion and synaptogenesis (Wei 2011). Elevated IL-6 in the postnatal period promotes an excess of excitatory synapses and a deficit of inhibitory synapses, and mediates autism-like behaviors (Wei 2012(a)).

In humans, a dramatic increase in synaptogenesis begins around the time of birth, and continues until about age 3 (Huttenlocher 1997, Tau 2010, Stiles 2010, Semple 2013). Vaccines are administered during this intense synaptogenesis. See Figs. 5-6. Elevated brain IL-6 induced by vaccination during synaptogenesis may cause an excitatory-inhibitory imbalance, towards excitation. An excitatory imbalance has been observed in human autism (Robertson 2016, Freyberg 2015).

Synaptogenesis tapers off through childhood and adolescence. This fact may explain why some older children and teens can suffer autistic regression after intense brain inflammation, but apparently become less vulnerable to immune activation brain injury with age.

Intense synaptogenesis occurs at ages 0-18 months, when many vaccines are administered. Consequently, vaccines may adversely impact synaptogenesis if they induce inflammation or IL-6 in the brain.

The timing of brain development processes in humans supports the idea that the human brain is vulnerable to immune activation and cytokines in the first few years after birth, when vaccines are administered. Disruption of synaptogenesis by vaccine-induced immune activation is a particular concern.

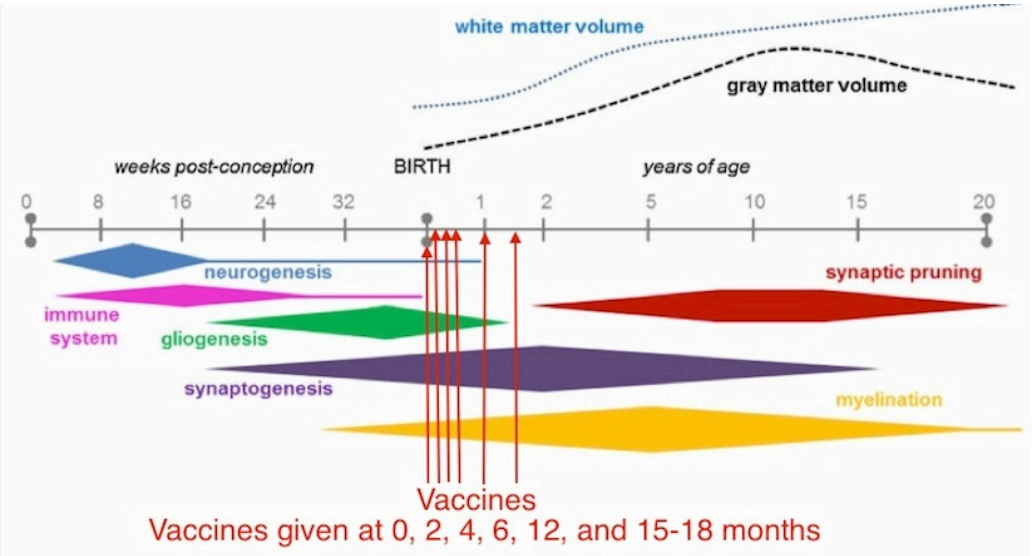


**Fig. 5: Timeline of specific brain developmental processes in humans. Synaptogenesis is most intense during the first couple years of life, when vaccines are administered. Timing of vaccination according to the CDC vaccine schedule is shown. Elevated IL-6 during synaptogenesis may cause an excitatory-inhibitory synapse imbalance, towards excitation. Adapted from Semple 2013.**

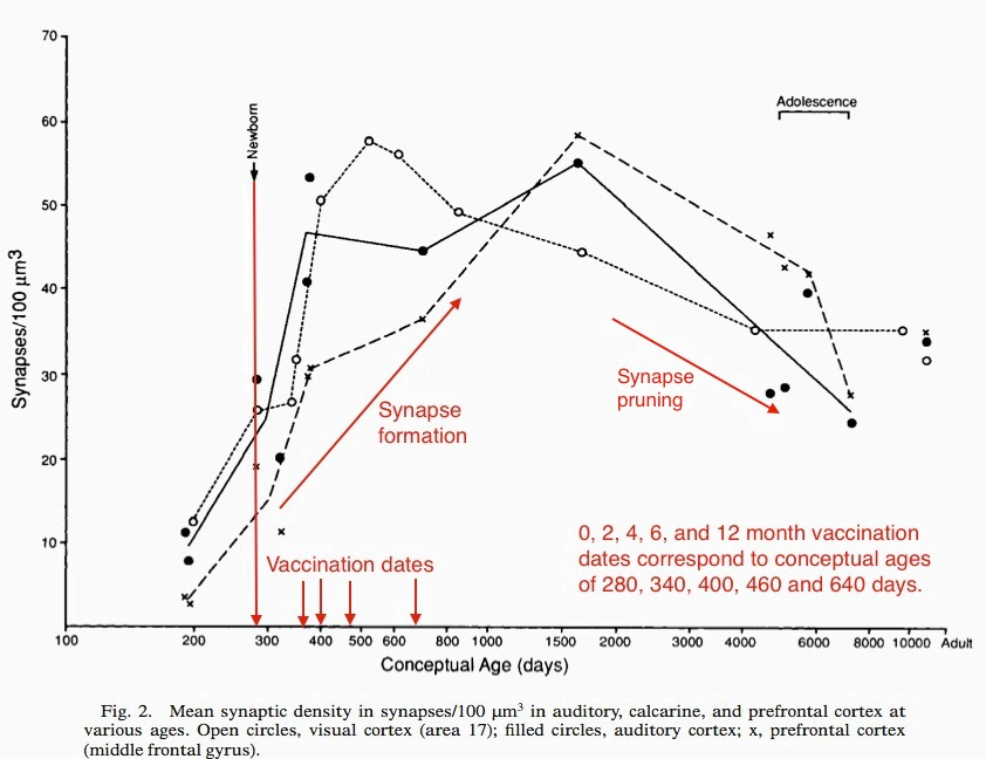


Fig. 2. Mean synaptic density in synapses/100 µm$^3$ in auditory, calcarine, and prefrontal cortex at various ages. Open circles, visual cortex (area 17); filled circles, auditory cortex; x, prefrontal cortex (middle frontal gyrus).

**Fig. 6: Measurements of synapse density in human cadavers of various ages indicate a dramatic increase in synapses in the first few years of life. Vaccines are administered during intense synapse formation. Elevated IL-6 during synaptogenesis may cause an excitatory-inhibitory synapse imbalance, towards excitation. Image adapted from Huttenlocher and Dabholkar 1997.**

# Aluminum Adjuvants: Neurotoxic At Vaccine Dosages

Aluminum (Al) adjuvants have an essential role in many vaccines: to stimulate immune activation. Without Al adjuvants, these vaccines would have greatly reduced efficacy.

Aluminum adjuvants comprise sub-micron particles (primary particles) of aluminum compounds, typically AlOH, AlPO4, AlSO4 or mixtures. The primary particles are typically agglomerated into larger particles with sizes of about 2-20 microns (Harris 2012). The Al adjuvant materials have low solubility in water and body fluids. Al adjuvant particles are biopersistent and can remain in the body for months or years (Flarend 1997, Khan 2013, Gherardi 2001).

Aluminum ingested in the diet has low oral absorption (about 0.3%), is rapidly excreted by the kidneys, is (mostly) excluded from the brain by the blood-brain barrier, and is in a solubilized, Al3+ ionic form (not particulate) These defenses are adequate for protecting the brain from natural levels of aluminum exposure. These protective mechanisms are unable to protect the brain from injected aluminum adjuvant particles. Al adjuvant particles are too large to be removed by the kidneys, and are carried across the blood-brain barrier by macrophages.

Dosages of aluminum adjuvants received by infants according to the CDC vaccination schedule are:

**Birth (Hep B):**
74 mcg/kg (250 mcg for 3.4 kg infant)

**2 month:**
245 mcg/kg (1225 mcg for 5 kg infant)

**4 month:**
150 mcg/kg (975 mcg for 6.5 kg infant)

**6 month:**
153 mcg/kg (1225 mcg for 8 kg infant)

These are maximum-possible dosages (because different vaccine products have different amounts) for average-weight infants.

Accumulating evidence shows that aluminum adjuvants have adverse neurological effects at dosages lower than or approximately equal to dosages infants receive from vaccines. These effects appear to depend on the particulate nature and biopersistence of the aluminum adjuvant. Injected Al adjuvant has adverse effects that are apparently mediated by the particles and independent of solubilized Al3+ ions released by the slowly dissolving particles (Crepeaux 2017).

Al adjuvant injections in mice cause adverse effects at vaccine-relevant dosages of 100, 200, 300 and 550 mcg/Kg body weight (Crepeaux 2017, Shaw 2009, Petrik 2007, Shaw 2013). These include deficits in learning and memory (Shaw 2009), deficits in neuromuscular strength/function (Petrik 2007), and changes in locomotor activity and/or gait (Shaw 2009, Shaw 2013). Autism is associated with gait and movement abnormalities (Kindregan 2015) and memory dysfunction (Williams 2006).

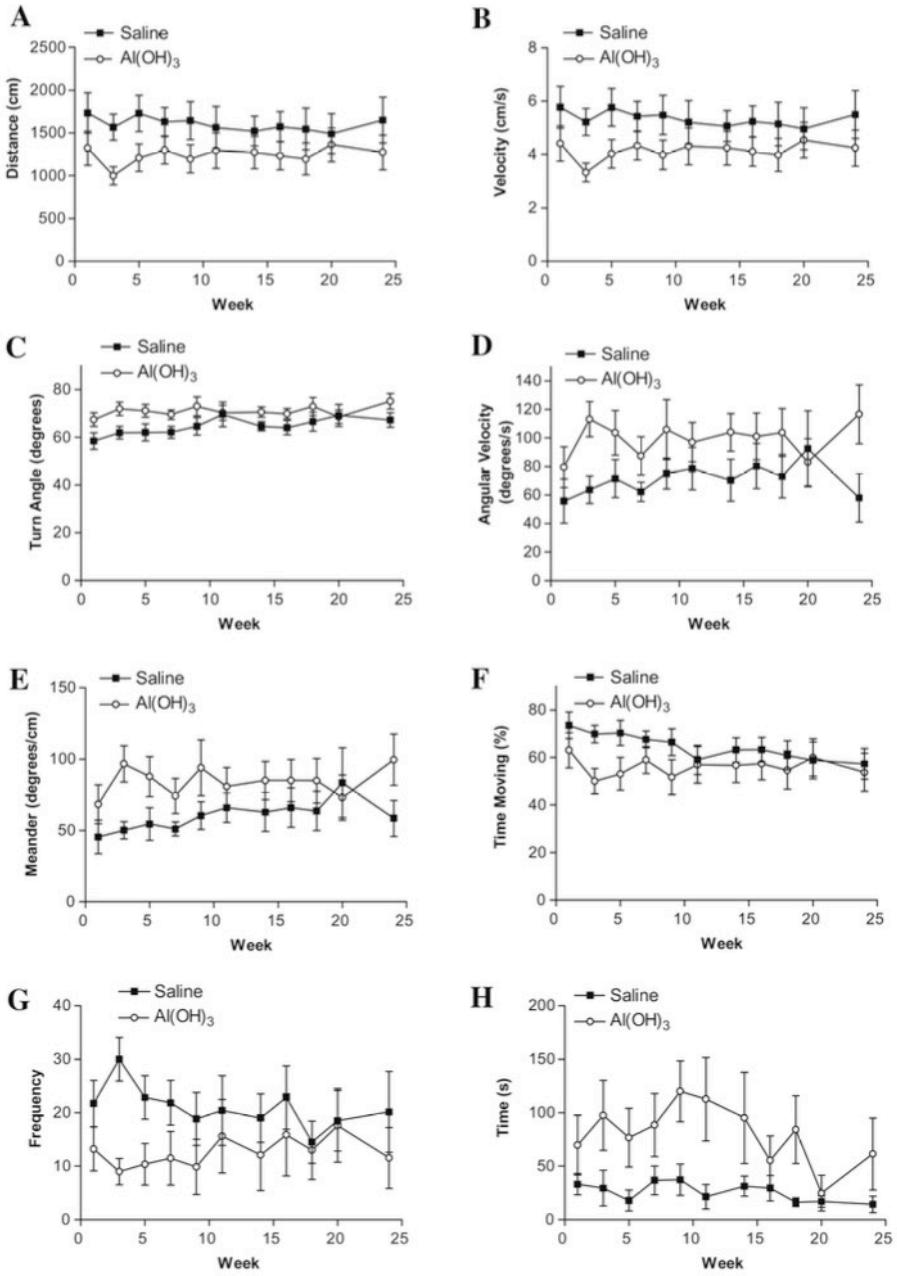


**Fig. 4.**
Open field movement analysis as an assessment of spontaneous activity and anxiety in control mice vs. mice injected six times with aluminum hydroxide. Aluminum hydroxide injected mice showed the following behavioural changes: (A) Shorter distances moved (***$p < 0.0001$). (B) Slower movement (***$p < 0.0001$). (C) Greater mean turn angle (***$p < 0.0001$). (D) More rapid turning (***$p < 0.0001$). (E) Greater meander (***$p < 0.0001$). (F) Smaller percentage of time in overall movement (**$p = 0.0030$). (G) Fewer entries into the centre of the open field (***$p < 0.001$). Late entry into centre (***$p < 0.0001$). (All measures, two-way ANOVA).

**Fig. 7: Dosage of 300mcg/Kg AlOH adjuvant caused large and persistent changes in exploratory behavior and movement in open field tests. This is an indicator of neurotoxicity. Human autistics also display abnormal movement and exploratory behavior. Adapted from Shaw and Petrik 2009.**

Al adjuvant dosages of 200mcg/Kg (as 3 x 66mcg/Kg) (Crepeaux 2017) and 300mcg/Kg (as 6 x 50mcg/Kg) (Shaw 2009) increased microglial activation in the ventral forebrain and lumbar spinal cord, respectively. The elevated microglial activation was measured about 6 months after Al adjuvant injection, which suggests that the microglial activation is chronic. Activated microglia indicate an ongoing inflammatory process and suggest the presence of elevated cytokines. Human autistics have activated microglia and elevated cytokines throughout the brain (Vargas 2005, Suzuki 2013, Li 2009).

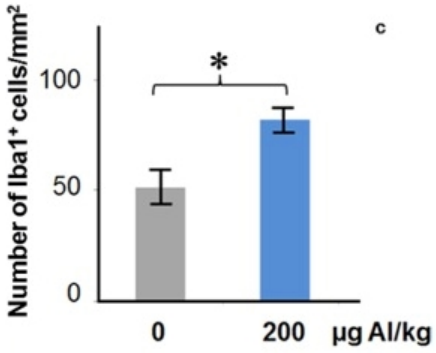


**Fig. 8: Al adjuvant (200mcg/Kg) caused an increase in microglial activation in the brain of mice. The protein iba1 indicates activated microglia. Measurements were performed 6 months after Al adjuvant injection, indicating that the microglial activation is a chronic condition. * P<0.05. From Crepeaux et al., 2017.**

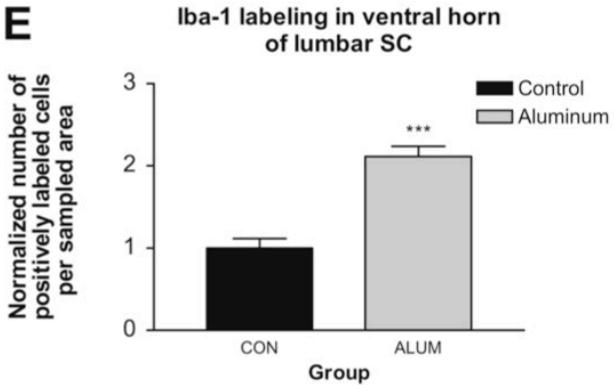


**Fig. 9: Al adjuvant (300mcg/Kg) caused an increase in microglial activation in the lumbar spinal cord of mice. The protein iba1 indicates activated microglia. Measurements were performed 6 months after Al adjuvant injection, indicating that the microglial activation is a chronic condition. ***$p < 0.001$, one-way ANOVA. From Shaw and Petrik 2009.**

Activated microglia are implicated as a causal factor in autism, because microglia mediate inflammation in the brain. Microglia can produce IL-6 when in an activated state. A recent review on microglia and autism (Takano 2015) states:

> *"…any factors that alter the number or activation state of microglia either in utero or during the early postnatal period can profoundly affect neural development, thus resulting in neurodevelopmental disorders, including autism." (Takano 2015)*

Microglia appear to play an important role in the causation of autism (Takano 2015, Kneusel 2014). Hence, the microglial activation caused by aluminum adjuvants suggests a role in autism.

Several studies show that Al adjuvants increase brain aluminum content (Crepeaux 2017, Flarend 1997, Shaw 2009, Khan 2013, Crepeaux 2015). A dosage of 200 mcg/Kg Al adjuvant caused a 50-fold increase in brain aluminum content in mice, from 0.02 ug/g to 1.0 ug/g dry weight of brain (Crepeaux 2017). These measurements were performed 6 months after the final injection, indicating that the Al persists in the brain long-term (Crepeaux 2017). See Fig. 10. Al adjuvants have been found to accumulate in the brain of mice up to one year after injection (Khan 2013). Crepeaux 2015 demonstrated persistence and increasing accumulation of Al adjuvant particles up to 270 days in spleen and lymph nodes of mice. Increasing accumulation of Al in distant organs over time suggests that toxic effects may increase with time, and may be delayed by months or years after exposure.

The 400 and 800 mcg/Kg doses used in the Crepeaux 2017 study did not cause adverse effects or elevated brain aluminum. The authors attribute this surprising inverted dose-response relationship to granulomas induced by the higher dosages. Granulomas trap the Al adjuvant at the injection site, thereby preventing its transport into the brain and other sensitive tissues. Granulomas occur after about 1% of vaccinations (Bergfors 2014). This is cause for concern because it indicates that, for 99% of vaccinations, the Al adjuvant can be transported around the body. It is not confined to a granuloma. See Fig. 11.

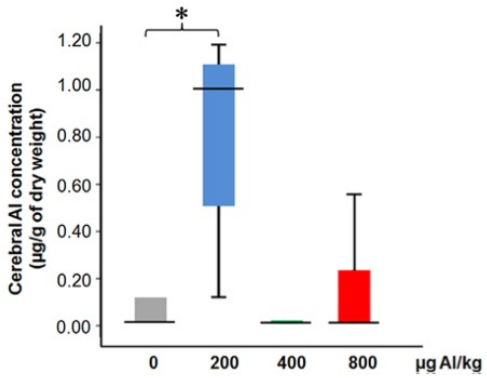


**Fig. 10: Dosage of 200 mcg/Kg Al adjuvant caused a 50-fold increase in brain aluminum content, from 0.02 to 1.00 ug/g dry weight, in mice. Higher dosages (400 and 800 mcg/Kg) did not increase brain Al content, presumably because the higher dosages caused a granuloma at the injection site. A granuloma traps the Al adjuvant at the injection site, thereby preventing systemic dispersal and transport into the brain. These measurements were performed 6 months after the final injection, indicating that the Al persists in the brain long-term. *P<0.05. From Crepeaux et al., 2017.**

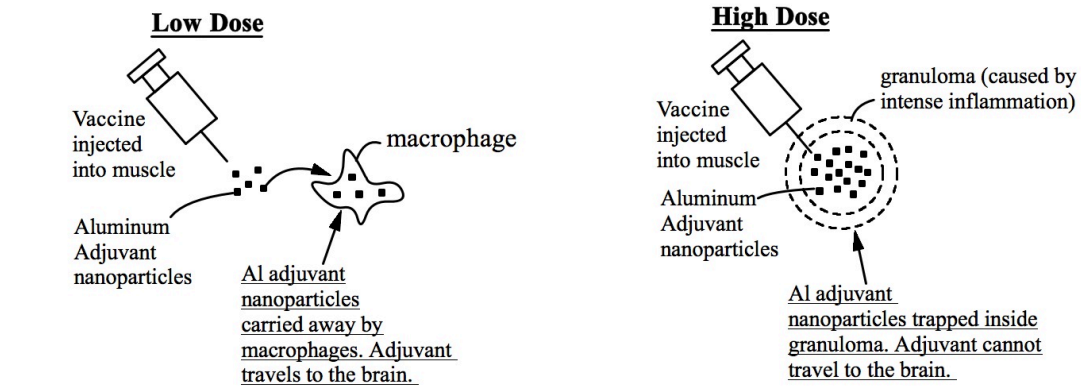


**Fig. 11: High dose Al adjuvant injection into the muscle causes a granuloma, which traps the Al adjuvant and prevents it from traveling into the brain. Low dose does not form a granuloma. Hence, the lower dose is free to travel to the brain. Consequently, the lower dose is more toxic than the higher dose. This mechanism explains the surprising inverted dose-toxicity results of Crepeaux et al. 2017.**

## Particle Transport and Macrophage Chemotactic Protein (MCP-1)

Aluminum adjuvants travel into the brain (Khan 2013, Crepeaux 2015, Crepeaux 2017, Shaw 2009, Flarend 1997). Al adjuvant particles are carried through the blood-brain barrier and into the brain by macrophages (Khan 2013). Transport is promoted by macrophage chemotactic protein-1 (MCP-1) (Khan 2013). MCP-1 causes macrophages to travel around the body and into the brain. Particle transport into the brain by macrophages is well-established and has been investigated for therapeutic applications (Choi 2012, Pang 2016).

MCP-1 is elevated in the brains of human autistics (Vargas 2005) and is elevated in the blood of neonates later diagnosed with autism (Zerbo 2014). This suggests that neonates with high MCP-1 will experience elevated Al adjuvant transport into the brain when injected with Al adjuvanted vaccines. This is consistent with Al adjuvants causing autism by inducing immune activation and elevated cytokines in the brain.

## Aluminum Induces IL-6 Expression In The Brain

Water-soluble aluminum salts (e.g. AlCl3, Al lactate) induce elevated IL-6 in the brain and other tissues. In fact, aluminum appears to selectively induce IL-6 (Viezeliene 2013). Studies of aluminum exposure and IL-6 expression in the brain include:

Cao 2016: Ingestion of 30 or 90 mg/kg/day aluminum (as AlCl3) for 90 days significantly increased gene expression of IL-6 and other cytokines in the brain (hippocampus).

Alawdi 2016: Ingestion of 3.4 mg/kg/day aluminum (as AlCl3) for 6 weeks caused a 4-fold increase in IL-6 in the brain (hippocampus). This dosage is far lower than the outdated "no observed adverse effects level" (NOAEL) oral dosages (26 and 62 mg/kg/day) used as benchmarks for toxicity threshold (Mitkus 2011, Offit 2003).

In fact, other experiments show that oral dosages of 3.4, 4, 5.6, 6, and 20.2 mg/Kg/day aluminum cause numerous adverse effects in mice or rats and hence the NOAEL for orally ingested

Al is currently unknown (Alawdi 2016, Dera 2016, Sethi 2008, Sethi 2009, Bilkei-Gorzo 1993).

The induction of IL-6 may occur because aluminum strongly induces oxidative stress (Exley 2003). Oxidative stress induces IL-6 expression (Viezeliene 2013).

## CDC Website Cites Fatally Flawed Study Of Al Adjuvants (Mitkus 2011)

Dosages of Al adjuvants received by infants increased dramatically as the vaccine schedule was expanded in the 1980s and 1990s. However, as the vaccine schedule expanded, the increasing dosages of Al adjuvants were not tested for safety. Government agencies (HHS, NIH, CDC, FDA) have not pursued any new experimental work on Al adjuvant toxicity.

To support the safety of Al adjuvants at today's higher dosages, the CDC cites a 2011 FDA study of aluminum exposure from vaccines (Mitkus 2011). This study is the only scientific evidence cited by the CDC and FDA websites to support the safety of Al adjuvants.

The Mitkus 2011 study is a theoretical modeling study of Al adjuvant kinetics; it contains no new data concerning Al adjuvant toxicity (from animal models or epidemiology). Mitkus 2011 calculates a body burden of aluminum resulting from the slow dissolution of Al adjuvant particles, and compares the dissolved-aluminum body burden to a "minimal risk level" (MRL). The MRL is derived from a study of ingested Al toxicity in mice (Golub 2001). The Golub 2001 study provides the NOAEL (26 mg/kg/day ingested), which is converted into the MRL for human infants (based on 1mg/kg/day ingested) by using a safety factor of about 30.

The Mitkus study is fatally flawed for these reasons:

### 1) MITKUS ASSUMES AL ADJUVANT PARTICLES ARE HARMLESS

Mitkus makes an unstated assumption that Al adjuvants have zero toxicity while in particulate form. Mitkus only considers the potential toxicity of aluminum ions (Al3+) released by the slowly-dissolving Al adjuvant particles.

Al adjuvants comprise low-solubility and biologically-persistent microscopic particles. The Mitkus analysis assumes that the particles are absolutely nontoxic and perfectly harmless, even when present in the brain and other organs. Mitkus provides no justification for this unstated assumption. Further, the assumption is contradicted by recent findings on Al adjuvant toxicity (Crepeaux 2017) and particulate toxicity generally. Particles can have toxic effects mediated by surface chemistry (e.g. surface charge and surface catalytic activity) and particle shape, among other characteristics of solid particles (Sharifi 2012, Podila 2013).

Several studies show injected Al adjuvants cause behavioral abnormalities, abnormal weight gain, learning and memory impairment, motor neuron death/apoptosis, neuromuscular strength deficits, chronic microglial activation/brain inflammation, and large (e.g. 50X) increases in brain and spinal cord aluminum content (Petrik 2007, Shaw 2009, Shaw 2013, Crepeaux 2017). These adverse effects occur at dosages less than or approximately equal to dosages received by infants according to the CDC vaccine schedule.

### 2) NEW RESEARCH SHOWS INGESTED AL HARMFUL AT DOSAGES LOWER THAN 26 MG/KG/DAY

Mitkus assumes that Al adjuvant toxicity is mediated exclusively by solubilized Al (Al3+ ions) released by the slowly-dissolving Al adjuvant particles. To establish a threshold toxicity level from the solubilized Al, Mitkus relies on a mouse feeding study (Golub 2001) reporting a "no-observed adverse effects level" (NOAEL) oral dosage of 26 mg/Kg/day ingested aluminum. Mitkus used a 30X safety factor for applying this dosage to humans, which is reasonable.

However, other experiments show that much lower oral dosages of 3.4, 4, 5.6, 6, and 20.2 mg/Kg/day aluminum cause adverse effects in mice or rats (Alawdi 2016, Dera 2016, Sethi 2008, Sethi 2009, Bilkei-Gorzo 1993). The adverse effects include chronic brain inflammation, learning and memory impairment, and kidney inflammation. So, the Mitkus analysis is wrong because 26 mg/kg/day is not a NOAEL. The "minimal risk level" (MRL) determined by Mitkus is too high by a factor of at least 26/3.4 = 7.6. Using a corrected NOAEL of 3.4 mg/Kg/day (based on Alawdi 2016) results in vaccine aluminum exposure exceeding the MRL for AlPO4 adjuvant, and approximately matching the MRL for AlOH adjuvant. The new, corrected MRL lines indicate that Al phosphate adjuvant (Fig. 12) and Al hydroxide adjuvant (Fig. 13) from the CDC vaccine schedule may cause toxicity from the solubilized Al per se.

Since 3.4mg/Kg/day is not a NOAEL (adverse effects were observed at this dosage) the true NOAEL is less than 3.4/mg/Kg/day. See Figs. 12-13.

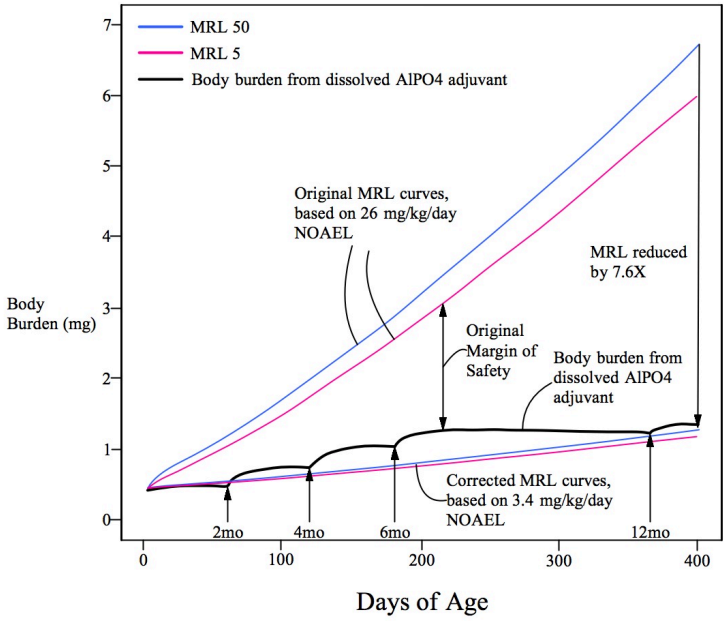


**Fig. 12: Body burden vs. MRL comparison chart for Al <u>phosphate</u> adjuvant (AlPO4) corrected in accordance with the new discovery (Alawdi 2016) that ingestion of 3.4 mg/kg/day Al causes adverse effects. The body burden exceeds the corrected MRL curve for almost the entire first year of life, indicating toxicity. The toxicity of Al adjuvant <u>particles</u> is a separate, additional issue. MRL 50 and MRL 5 refer to two different infant growth rates. Adapted from Mitkus et al., 2011.**

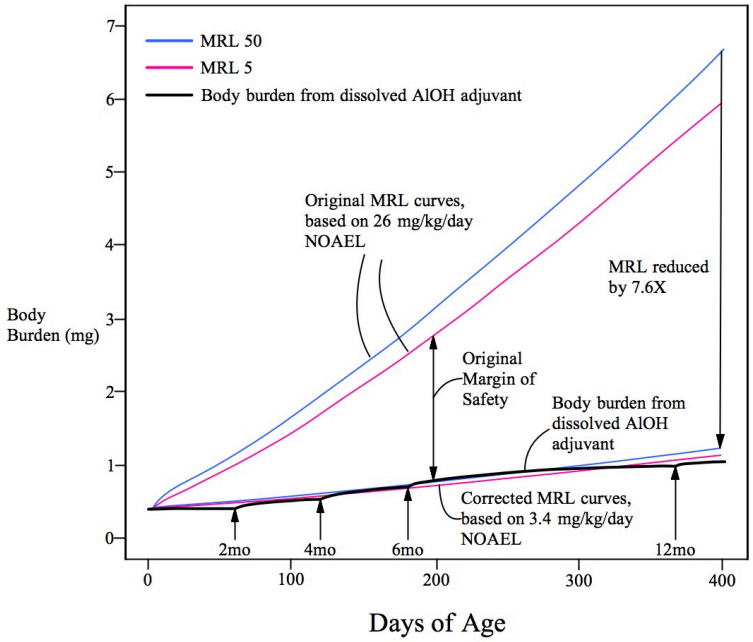


**Fig. 13: Body burden vs. MRL comparison chart for Al hydroxide adjuvant (AlOH), corrected in accordance with the new discovery (Alawdi 2016) that ingestion of 3.4 mg/kg/day Al causes adverse effects. The body burden overlaps the new, corrected MRL, indicating borderline toxicity. The margin of safety is gone. MRL 50 and MRL 5 refer to two different infant growth rates. The toxicity of Al adjuvant particles is a separate, additional issue. Adapted from Mitkus et al., 2011.**

### 3) NO AL ADJUVANT TOXICITY DATA CITED, DESPITE AVAILABILITY

Mitkus does not cite any toxicity data for injected Al adjuvants. Mitkus instead uses toxicity data for ingested, non-particulate, water-soluble Al (Golub 2001, which used Al lactate) to derive the MRL. This data comes from a single study (Golub 2001).

So, remarkably, Mitkus claims a safe level of injected Al adjuvant exposure, without citing any Al adjuvant toxicity data. The error is unnecessary and neglectful because at least two animal studies of injected Al adjuvant toxicity were available prior to the Mitkus publication in 2011 (Petrik 2007, Shaw 2009). These papers were not cited or mentioned by Mitkus 2011.

Each of these three flaws is fatal for the validity of the Mitkus study in establishing the safety of aluminum adjuvants. Hence, the CDC is completely lacking valid evidence for the safety of Al adjuvants. This is especially true for safety regarding neurological and long-term outcomes, because other available studies of Al adjuvant safety (e.g., Jefferson 2004) do not consider (or are incapable of detecting) these outcomes.

## CDC Fails To Investigate Toxicity of Al Adjuvants

The CDC has conducted no epidemiological studies on long term safety (e.g. considering neurological outcomes) of Al adjuvants. There is one ecological study of country-level data, which reported an association between Al adjuvant exposure and autism (Tomljenovic 2011). However, being an ecological study, it is highly susceptible to confounding and biases.

Dr Frank DeStefano of the CDC's Immunization Safety Office is co-author of a feasibility study (Glanz 2015) on using the Vaccine

Safety Datalink (VSD) to investigate the safety of individual vaccine ingredients. The paper focuses on Al adjuvants. It acknowledges that thimerosal is the only vaccine ingredient studied for autism or neurological safety, and that a possible association between Al adjuvants and autism has not been explored in epidemiological studies. Glanz 2015 states:

> *"To date, there have been no population-based studies specifically designed to evaluate associations between clinically meaningful outcomes and non-antigen ingredients, other than thimerosal."*

The CDC has not investigated Al adjuvant safety concerns, despite the accumulating scientific evidence of harm and evidence linking Al adjuvants to immune activation mechanisms of brain injury.[1]

# Conclusion

The science reviewed here tells a consistent and compelling story: that vaccines may cause autism by stimulating immune activation and elevated cytokines in the brain. Al adjuvants are implicated as a cause of autism because they can be transported into the brain, because they cause microglial activation at vaccine-relevant dosages, and because aluminum induces IL-6 in the brain.

In statements asserting no vaccine-autism link, the CDC cites scientific evidence that is not relevant to Al adjuvant safety or is incapable of disproving an Al adjuvant-autism link (Taylor 2014, DeStefano 2013, Mitkus 2011). In support of claims for Al adjuvant safety, the CDC relies on a profoundly flawed theoretical modelling study (Mitkus 2011). There is little scientific evidence supporting the safety of Al adjuvants, especially in relation to autism and other long term neurological outcomes.

[1] However, the Glanz paper notes that studies of aluminum adjuvants are problematic because of expected small differences in exposures in the low and high exposure groups. Glanz 2015 concludes: "*…children below the 10th percentile would be exposed to between 0 mg and 3.1mg, while children above the 90th percentile would be exposed to between 4.8 mg and 5.3 mg of aluminum from vaccines. It is unclear if such differences in aluminum exposure would be biologically meaningful.*" (Glanz 2015). So, epidemiological studies may not provide reliable evidence for safety or harm. Controlled, prospective human trials of aluminum adjuvant exposure from vaccines will likely be prohibited for ethical reasons. Also, Al adjuvants are essential ingredients for Al adjuvanted vaccines. Consequently, it will be challenging to design studies of long term adverse effects of Al adjuvants in humans. Experiments in animal models can provide valuable information. Al adjuvants should be tested for effects on: 1) excitatory/inhibitory imbalance; 2) core symptoms of autism (social, communicative and repetitive/stereotyped behaviors); 3) IL-6, IL-17, and other cytokine levels in the brain; 4) other physiological abnormalities associated with autism (e.g. mitochondrial dysfunction, microbiome dysbiosis, Purkinje cell loss, cerebellum abnormalities etc); and 5) microglial activation and immune activity in the brain. Investigating these outcomes can provide valuable information concerning the safety of Al adjuvants.

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# Appendix E

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# The Introduction of Diphtheria-Tetanus-Pertussis and Oral Polio Vaccine Among Young Infants in an Urban African Community: A Natural Experiment

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ABSTRACT

*Background:* We examined the introduction of diphtheria-tetanus-pertussis (DTP) and oral polio vaccine (OPV) in an urban community in Guinea-Bissau in the early 1980s.
*Methods:* The child population had been followed with 3-monthly nutritional weighing sessions since 1978. From June 1981 DTP and OPV were offered from 3 months of age at these sessions. Due to the 3-monthly intervals between sessions, the children were allocated by birthday in a 'natural experiment' to receive vaccinations early or late between 3 and 5 months of age. We included children who were <6 months of age when vaccinations started and children born until the end of December 1983. We compared mortality between 3 and 5 months of age of DTP-vaccinated and not-yet-DTP-vaccinated children in Cox proportional hazard models.
*Results:* Among 3–5-month-old children, having received DTP (±OPV) was associated with a mortality hazard ratio (HR) of 5.00 (95% CI 1.53–16.3) compared with not-yet-DTP-vaccinated children. Differences in background factors did not explain the effect. The negative effect was particularly strong for children who had received DTP-only and no OPV (HR = 10.0 (2.61–38.6)). All-cause infant mortality after 3 months of age increased after the introduction of these vaccines (HR = 2.12 (1.07–4.19)).
*Conclusion:* DTP was associated with increased mortality; OPV may modify the effect of DTP.


## 1. Introduction

Individually randomized studies to measure impact on child survival of different vaccines were not conducted when the Expanded Program on Immunization (EPI) was introduced in low-income countries in the 1970s. The disease-protective effects were well documented, so the main issue was at which age to introduce the vaccine most effectively (The Expanded Programme on Immunization, 1982). Except for measles vaccine (MV), surprisingly few studies examined the introduction of vaccines and their impact on child survival (Aaby et al., 1983, 2003a; Holt et al., 1990; The Kasongo Project Team, 1981). One trial of measles-vaccinated and measles-unvaccinated communities in Congo showed a larger than expected reduction in child mortality (Aaby et al., 1981); this observation was subsequently corroborated by community "trials" and before-after studies in several countries (Aaby et al. 1984, 1993, 2003a; Holt et al., 1990; Kapoor and Reddaiah, 1991). Hence, a vaccine may have non-specific effects (NSEs) on susceptibility to other infections (Aaby et al., 1995). WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recently reviewed the potential NSEs of BCG, diphtheria-tetanus-pertussis (DTP) and MV and recommended further research (Higgins et al., 2014; Strategic Advisory Group of experts on Immunization, 2014).

Though protective against the target diseases, DTP may increase susceptibility to unrelated infections (Aaby et al., 2003b, 2004a, 2012) (Appendix A). The SAGE review noticed that the majority of studies found a detrimental effect of DTP (Higgins et al., 2014). However, SAGE considered the evidence inconsistent because two studies reported beneficial effects (Higgins et al., 2014) and that most studies underestimated the benefit of DTP because studies were conducted in situations with herd immunity. Furthermore, all studies gave DTP and OPV together, making it impossible to separate effects of DTP and OPV (SAGE non-specific effects of vaccines Working Group, 2014).

On the other hand, the "unvaccinated" children in these studies have usually been frail children too sick or malnourish to get vaccinated, and the studies may therefore have underestimated the negative effect of DTP. We therefore examined what happened when DTP and OPV were first introduced, but not always given together, in 1981–1983 in the capital of Guinea-Bissau. In this situation the children were allocated by birthday to receive vaccines early or late and the "unvaccinated" were therefore not frail children.

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## 2. Methods

### 2.1. Background

Bandim Health Project (BHP) has followed an urban community with a demographic surveillance system since December 1978, and took part in the introduction of vaccines well before a full-fledged national program was implemented with UNICEF support in 1986 (Aaby et al., 1984, 2004a).

### 2.2. Demographic Surveillance

In 1978–1979, under-five mortality was nearly 500/1000. Since malnutrition was assumed to be the main cause, a study was initiated to determine why children were malnourished (Aaby et al., 1983). However, severe malnutrition was not evident, and to understand the high mortality we started a health and demographic surveillance system (HDSS). The area was mapped and a census conducted. Four health workers were employed to identify pregnant women, encourage women to attend ante-natal clinics, and to follow children with anthropometric measurements to assess growth patterns and detect malnourished children. Each health worker followed a population of 1500–2000 individuals. The health workers were supervised by an expatriate nurse.

For each sub-district in Bandim, the responsible health worker kept a list of children under three years of age. BHP had no computerized surveillance system until 1990 but kept an A5 card ("BHP card") for each child, where weights and vaccination dates were noted. The child's growth card was kept by the mother.

The Bandim population was very mobile. It was important to maintain contact with the natal village for ceremonial purposes and to secure rice. Furthermore, mothers were not supposed to have sexual relations during breastfeeding (Jakobsen et al., 2004). Breastfeeding was prolonged in Guinea-Bissau. Thus, many women stayed in the rural areas with their natal family while breastfeeding. These cultural traditions introduced variability in the participation in weighing and vaccination sessions.

### 2.3. Anthropometry

We arranged quarterly weighing sessions in each sub-district. The responsible health worker advised mothers the day before a community weighing. The following morning, the weight was measured and noted on the child's growth card and the BHP card. When the World Food Program provided supplementary feeding this was given to families with malnourished children.

### 2.4. Vaccinations

There was no community vaccination program in 1981 except that we had organized a few measles vaccination campaigns (Aaby et al., 1984). Mothers could take their children to the Mother and Child Health Program in town. However, this clinic was mainly attended by the urban elite. Few children were vaccinated before BHP organized vaccination sessions (Table 1).

In June 1981, BHP started to provide vaccinations at the quarterly weighing sessions. A health center nurse accompanied the weighing team and vaccinated eligible children. DTP and OPV were provided from 3 months and MV from 9 months of age. OPV-at-birth was not given then. The three DTP and OPV doses could be given with an interval of one month but since we only arranged weighing every three months, most children had longer intervals between doses. DTP was administered intramuscularly and OPV as an oral drop. When both vaccines were administered at the same session OPV was usually given first and then DTP; the children would usually start crying after DTP due to the pain of the injection and it would therefore have complicated the administration of OPV to give DTP first. There were several periods where either OPV or DTP was missing (Fig. 1). BCG was rarely provided at the weighing sessions since most nurses were not trained to administer intra-dermal vaccination. A total of 269 children may have been BCG vaccinated as they had a vaccination date on their card (N = 192) or were noted to have received BCG but no date given (N = 77).

The expatriate nurse sometimes organized additional vaccination sessions in which the children were not weighed. During these sessions,

**Table 1**
Median age of vaccination and coverage for BCG, DTP and OPV of study cohort.

| | 1980 | 1981 | 1982 | 1983 | 1981–1983 |
|---|---|---|---|---|---|
| Median age in days (N vaccines) | | | | | |
| BCG | 9 (4) | 48.5 (50) | 34 (46) | 25 (68) | 33 (164) |
| DTP1 | 97 (12) | 127 (147) | 121 (164) | 117 (278) | 121 (589) |
| OPV1 | 98 (12) | 118 (185) | 121.5 (170) | 117 (225) | 118 (580) |
| MV | 181 (5) | 141 (53) | 157 (2) | 110 (1) | 141.5 (56) |
| Coverage at 6 months of age | | | | | |
| BCG | 1.7% (5/289) | 3.5% (12/342) | 23.7% (72/304) | 17.4% (57/327) | 14.5% (141/973) |
| DTP1 | 4.2% (12/289) | 31.3% (107/342) | 61.2% (186/304) | 73.1% (239/327) | 54.7% (532/973) |
| DTP3 | 2.4% (7/289) | 0.9% (3/342) | 4.3% (13/304) | 4.0% (13/327) | 3.0% (29/973) |
| OPV1 | 4.2% (12/289) | 43.0% (147/342) | 62.5% (190/304) | 69.7% (228/327) | 58.1% (565/973) |
| OPV3 | 2.4% (7/289) | 2.0% (7/342) | 4.3% (13/304) | 4.0% (13/327) | 3.4% (33/973) |
| MV | 2.8% (8/289) | 15.2% (52/342) | 0.7% (2/304) | 0% (0/327) | 5.5% (54/973) |
| Coverage at one year of age | | | | | |
| BCG | 2.6% (3/116) | 2.4% (7/294) | 15.4% (51/332) | 17.4% (46/264) | 11.7% (104/890) |
| DTP1 | 2.6% (3/116) | 32.7% (96/294) | 71.1% (236/332) | 83.0% (219/264) | 61.9% (551/890) |
| DTP3 | 2.6% (3/116) | 4.4% (13/294) | 18.4% (61/332) | 43.2% (114/264) | 21.1% (188/890) |
| OPV1 | 2.6% (3/116) | 37.4% (110/294) | 77.4% (257/332) | 84.8% (224/264) | 66.4% (591/890) |
| OPV3 | 2.6% (3/116) | 12.2% (36/294) | 32.5% (108/332) | 44.3% (117/264) | 29.3% (261/890) |
| MV | 15.5% (18/116) | 68.0% (200/294) | 34.0% (113/332) | 51.1% (135/264) | 50.3% (448/890) |

Notes: The inclusion criteria for the cohort in Table 1 are the same as for our study cohort: weight examination after 15 days of age and contribute time between 91 and 183 days of age. Median age: 'year' means the year the vaccination was given, and median age is the median age at time of vaccination with a given vaccine among children vaccinated before turning 6 months. E.g. the 4 BCG vaccines in the 1980 column were given in 1980 to children with a median age of 9 days.
Coverage: 'year' means the year when the child turned exactly 1 year (or 6 months) old and coverage was assessed. Only children surviving to 1 year (or 6 months) of age were assessed for coverage. Children turning 1 year in 1984 were thus not presented in the table.

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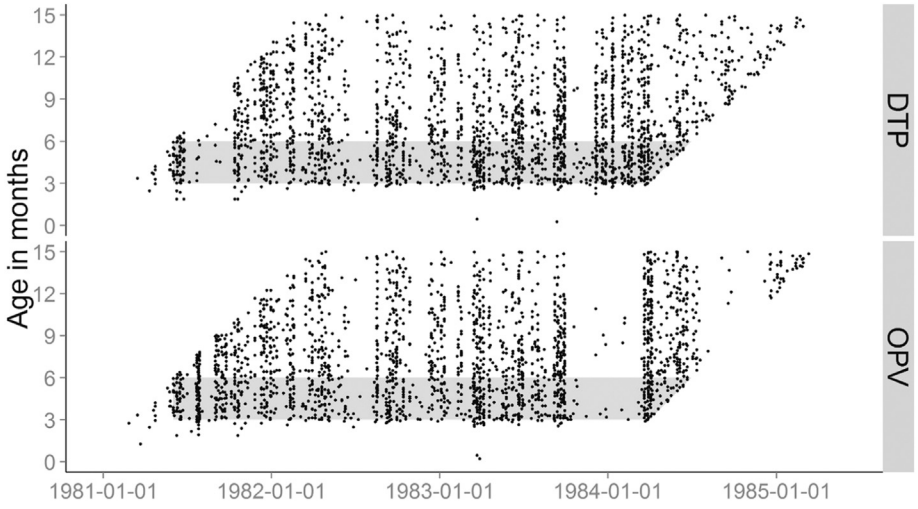


**Fig. 1.** Each vaccination of the specified type is plotted according age of the recipient and date of vaccination.

vaccinations were noted on the BHP cards. Both nurses and mothers thought that sick children should not be vaccinated; the BHP card often indicated that the child was 'sick', 'malnourished' or 'orphan' as an explanation of why an age-eligible child had not been vaccinated.

### 2.5. Data Control

When a computerized system became available in 1990–1991, weights and vaccinations from the BHP cards were entered. For the present analysis, all information on dates of visit, weights and vaccination dates was checked against the original cards. A few cards were not available or could no longer be found (Fig. 2).

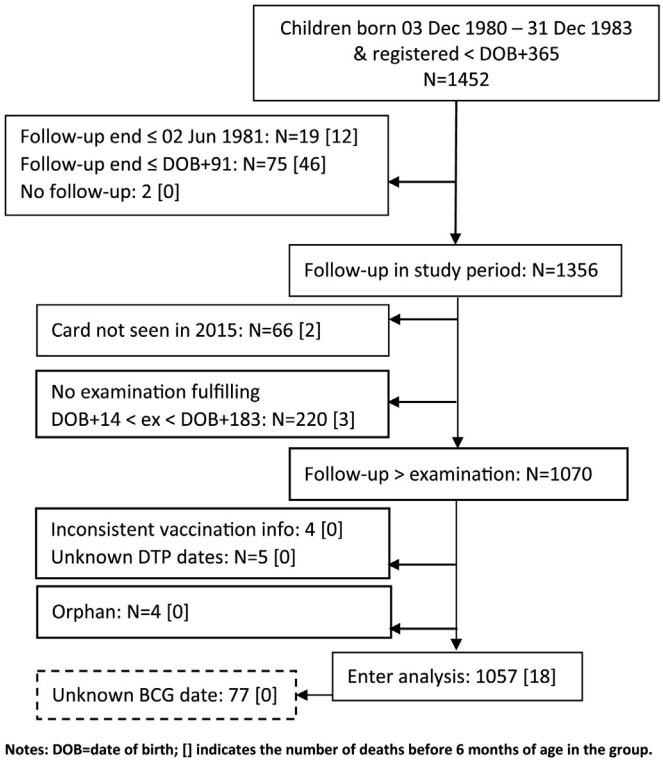


**Fig. 2.** Flowchart of study population and children included in the analyses. Notes: DOB = date of birth; [] indicates the number of deaths before 6 months of age in the group.

### 2.6. The Study Cohort

We included children born from December 3, 1980 as they would become eligible for vaccination before 6 months of age (Fig. 2). Few children were vaccinated with BCG (Table 1). Children who travelled and never attended any session were not included in the 'unvaccinated' group. Children weighed within a fortnight of their birth to obtain a birth weight were only included if they took part in a subsequent community weighing session. Furthermore, we excluded orphans since they were not breastfed and were likely to have different care. The cohort is depicted in Supplementary Fig. 1.

### 2.7. Natural Experiment for 3–5-month-old Children

Though not individually randomized, the present study is a natural experiment with limited bias in group allocation: With 3-monthly intervals between weighing sessions, children were allocated by their birthday to receive their first vaccinations early or late between 3 and 5 months of age (Fig. 3). We therefore compared 3–5-month-old children who had received DTP (±OPV) vaccinations early with children who had not yet received these vaccinations. Since there were no healthy "unvaccinated" children after 6 months of age unless they had travelled, we censored follow-up of all children at 6 months of age (Fig. 3).

Sick children were not vaccinated, in the main analysis we therefore censored 'unvaccinated' children who attended a weighing session but did not receive a vaccination (Fig. 3). Since the censoring of sick children could have introduced a bias, we also conducted an intention-to-treat analysis in which the censored children were transferred to the DTP group. Hence, in this analysis we compared the mortality of the intended-DTP-vaccinated group and the not yet DTP-vaccinated group.

Children were included from 91 days of age if they had been examined in a weighing session before 91 days; if they were only seen in a weighing session after 3 months of age they were only included from the day seen. DTP was not administered elsewhere and the follow-up time of children was therefore counted as DTP-unvaccinated time in the survival analysis until BHP provided the vaccine. Time as DTP-unvaccinated also came from children who did not turn up at the weighing sessions between 3 and 5 months of age but had been seen before 3 months of age and therefore were part of the community cohort (Fig. 3). Hence, the DTP-vaccinated and DTP-unvaccinated children were all children from the same cohort of children born in Bandim and their allocation depended on the timing of their birth date, the timing of the weighing sessions and their travelling pattern. We

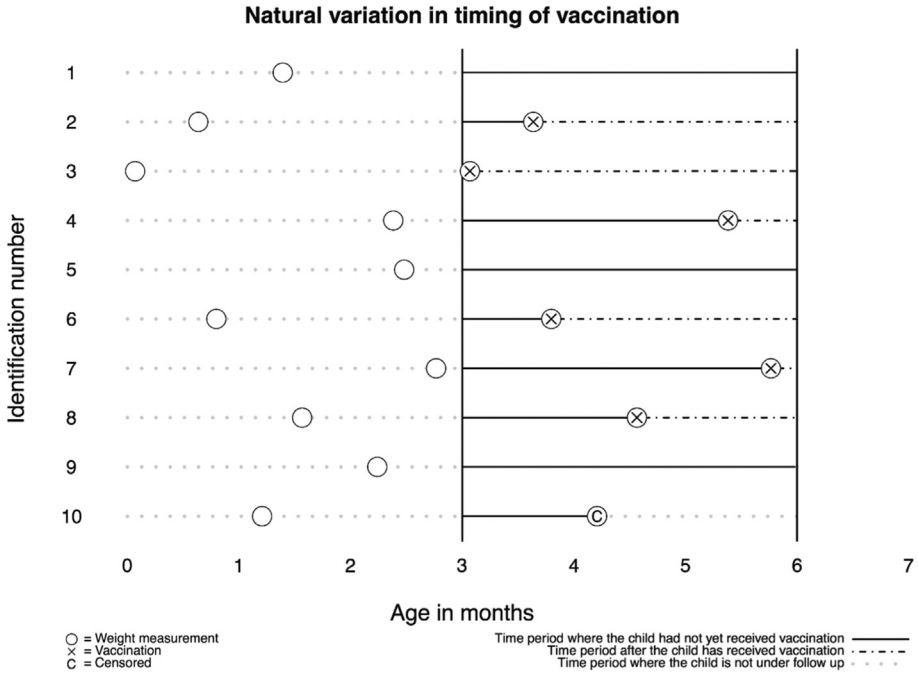


**Fig. 3.** Natural experiment study design. Note: Children were weighed every third month. After 3 months of age they received DTP and OPV on weighing days if they were healthy. Children who attended but were not vaccinated at a weighing session after 3 months of age were censored in the survival analysis comparing DTP-vaccinated and unvaccinated children.

compared the background factors for the children who were DTP vaccinated, attended a weighing session between 3 and 5 months but were not vaccinated and those who did not attend a weighing session (Table 2).

We also examined the mortality of children who due to logistic reasons had received DTP-only. Absences and travelling patterns are unlikely to differ between children who at their first vaccination had received DTP1 + OPV versus DTP1-only; these two groups were equally likely to receive subsequent vaccinations both with respect to timing of subsequent vaccinations and coverage (data available on request).

### 2.8. Statistical Methods

First possible enrolment date was June 2, 1981, when DTP and OPV vaccinations were introduced. Different vaccination groups were compared using a Cox proportional hazard model with age as underlying time.

Children were classified according to their most recent vaccination (Supplementary Table 1). We ignored BCG vaccinations in the main analysis because we gave few BCG vaccinations (Table 1) and some children had received BCG at the maternity ward without proper documentation as some children had a BCG scar but no vaccination card. To avoid survival bias, we used a landmark approach (Jensen et al., 2007); hence, a child's vaccination status was only updated from the day the information was collected. Due to the additional vaccination sessions organized by the expatriate nurse some "unvaccinated" children received a vaccine before the weighing session where they changed status to "vaccinated"; it is noted in the footnote to Table 3 how many had received such vaccinations. As a sensitivity analysis we also did an analysis including the additional vaccination sessions as landmarks. For the remainder of this paper, we will refer to these landmarks as vaccination-days-without-weighing.

The WHO z-score for weight-for-age was used to assess nutritional status. Control for sub-district, ethnic group and twinning did not change the results (data not shown). There was no obvious clustering

**Table 2**
Background factors children in the main analysis of vaccination and mortality between 3 and 5 months of age.

| | | DTP-vaccinated at 3–5 months | Attended weighing session at 3–5 months, not vaccinated | Did not attend weighing session at 3–5 months |
|---|---|---|---|---|
| Number | | 662 | 186 | 209 |
| Male sex | | 52.1% | 53.2% | 54.1% |
| Twin | | 2.7% | 2.2% | 2.9% |
| Birth weight (SD) | | 3.23 (0.025) | 3.28 (0.061) | 3.22 (0.051) |
| Ethnic group | | | | |
| • Pepel | | 46.8% | 54.8% | 45.0% |
| • Balanta | | 11.8% | 13.4% | 16.3% |
| • Other ethnic groups | | 41.4% | 31.7% | 38.8% |
| Mean weight-for-age z-score (SD) at examination before 3 months of age | | −0.30 (0.037) | −0.34 (0.084) | −0.43 (0.066) |
| Follow-up time (person-years) between 3 and 5 months; | All time | 135.5 [92] | 36.8 [86] | 47.4 [92] |
| [Median number of days of follow] | As DTP vaccinated | 73.3 | 1.8 | 2.0 |
| | As unvaccinated | 62.2 | 35.1 | 45.4 |
| Mean number (SD) of weighing sessions per year between 6 and 11 months of age | | 2.7 (0.03) | 2.2 (0.07) | 1.6 (0.08) |

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**Table 3**
Mortality rate and hazard rate (HR) for children from 3 months of age until first examination without vaccination or 6 months of age. Natural experiment.

| Age group 3–5 months | Mortality rate (deaths/person-years) | | | HR (95% CI) for DTP vs unvaccinated |
|---|---|---|---|---|
| All | | | | |
| Unvaccinated (N = 651) | 4.5 (5/111.4) | DTP (±OPV) (N = 462) | 17.4 (11/63.1) | 5.00 (1.53–16.3) |
| | | DTP only (N = 101) | 35.2 (5/14.2) | 10.0 (2.61–38.6) |
| | | DTP + OPV (N = 361) | 12.3 (6/48.9) | 3.52 (0.96–12.9) |
| Girls | | | | |
| Unvaccinated (N = 313) | 1.9 (1/51.9) | DTP (±OPV) (N = 222) | 13.3 (4/30.1) | 9.98 (0.81–123.0) |
| | | DTP only (N = 44) | 16.2 (1/6.2) | 12.0 (0.56–257.2) |
| | | DTP + OPV (N = 178) | 12.5 (3/23.9) | 9.50 (0.73–124.0) |
| Boys | | | | |
| Unvaccinated (N = 338) | 6.7 (4/59.5) | DTP (±OPV) (N = 240) | 21.2 (7/33.0) | 3.93 (1.01–15.3) |
| | | DTP only (N = 57) | 49.8 (4/8.0) | 8.93 (2.01–39.7) |
| | | DTP + OPV (N = 183) | 12.0 (3/24.9) | 2.21 (0.44–11.0) |

Notes: There were no deaths due accidents in this age group. BCG is disregarded in the analysis. Hence, the unvaccinated children have not received DTP, OPV or MV but may have received BCG. Of the 651 unvaccinated children, 219 received DTP and/or OPV before their first weighing examination. These children counted as 'unvaccinated' until their first weighing examination. Of the 462 children who received DTP (±OPV), 177 received an additional DTP or OPV before 6 months of age. The OPV-only is not presented in the table because there were no deaths and very little follow-up time in this age group.

of deaths and control for season and calendar time did not change estimates (data not shown).

There were 18 deaths between 3 and 5 months of age: 3 had cough and respiratory infections as the main symptom, 3 had fever (presumed malaria), 2 were due to diarrhea, 5 had diarrhea and vomiting, 1 was a sudden death, and 4 had no information on cause.

### 2.9. Ethics

The study of nutritional status was planned by SAREC (Swedish Agency for Research Collaboration with Developing Countries) and the Ministry of Health in Guinea-Bissau.

## 3. Results

Of 1356 children registered in Bandim and followed to 3 months of age (Fig. 2), 286 were never weighed, had no card or their card was lost. An additional 13 children had inconsistent information, vaccinations marked with a cross but without dates or were orphans. Hence, 1057 children were included in the study cohort. The median ages for DTP1 and OPV1 were 121 and 118 days, respectively (Table 1). The vaccination coverage at 6 months of age was 55% for DTP1; 3% got DTP3 (Table 1). Coverage for MV was only 6%. Of the DTP1, OPV1 and MV vaccinations noted on the BHP card 90–95% had been administered by the BHP.

For children examined after 91 days, a one-unit increase in w/a z-score was associated with an odds ratio of 1.07 (0.93–1.24) for receiving a vaccination at that weighing session.

### 3.1. Natural Experiment with 3–5-month-old Children

There were no marked differences in background factors for the three groups of children who were DTP vaccinated at 3–5 months of age, those who attended a weighing session but were not vaccinated, and those who did not attend a weighing session at 3–5 months of age (Table 2). Birth weight was similar in the three groups. Weight-for-age z-score before 3 months of age did not differ for the three groups (Table 2). Those who did not attend a weighing session at 3–5 months of age were significantly less likely to attend later weighing sessions during infancy, the mean number of visits being lower for those not attending than for those being DTP-vaccinated ($p < 0.001$) (Table 2); hence, they are likely to have travelled more than those who were DTP-vaccinated.

In the main experiment depicted in Fig. 3, DTP vaccination (±OPV) compared with 'DTP-unvaccinated' was associated with a HR of 5.00 (1.53–16.3) (Table 3); the HR was 9.98 (0.81–123) for girls and 3.93 (1.01–15.3) for boys. If we also included vaccinations given on vaccinations-days-without-weighing in the landmark analysis, DTP (±OPV) compared with unvaccinated was associated with a HR of 3.90 (1.20–12.3). When DTP had been given alone without OPV the HR was 10.0 (2.61–38.6) (Table 3). The difference between DTP-only children and DTP-plus-OPV does not reflect differences in follow-up and other vaccinations since the time to DTP2 and prevalence of DTP2 was the same for DTP-only and DTP-plus-OPV vaccinated children (data not shown). If we excluded the 269 children who may have been BCG vaccinated results were similar (Supplementary Table 2).

If the analysis was conducted as an intention-to-treat analysis in which the children weighed but not vaccinated were not censored but transferred to the DTP group, the intended-DTP-vaccinated group had a HR of 3.92 (1.20–12.8) compared with the not-yet vaccinated group (Supplementary Table 3).

### 3.2. Secondary Analyses

Since the introduction of DTP and OPV apparently was associated with increased mortality, we examined what happened to infant mortality from 3 to 12 months of age after the introduction of these vaccines. The mortality rate for all 3–11 months old children increased 2-fold (HR = 2.12 (1.07–4.19)) from 1980, before vaccinations, to 1982–1983, after the introduction of DTP and OPV (Table 4).

## 4. Discussion

### 4.1. Main Observations

DTP vaccinations were associated with increased infant mortality even though there was no vaccine-induced herd immunity. When unvaccinated controls were normal children who had not yet been eligible for vaccination, mortality was 5 times higher for DTP-vaccinated children. Co-administration of OPV with DTP may have reduced the negative effects of DTP.

### 4.2. Strength and Weaknesses

The present analysis assessed DTP and child survival in a "natural experiment" in which the children were allocated by the timing of their birth and community weighing sessions and the group allocation was therefore not influenced by the usual selection biases to the same extent as most other studies of DTP (Aaby et al., 2016). To assure that the censoring from the main analysis of children who were not vaccinated had not produced the unexpected strong result we made an intention-to-treat analysis but this did not change the result. If anything the unvaccinated children had slightly worse nutritional status before 3 months of age than the children who were subsequently DTP vaccinated ($p = 0.09$) (Table 2); the unvaccinated children travelled more than the DTP vaccinated children. These biases would tend to favor rather than increase mortality in the DTP group and the

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**Table 4**
Mortality rates (deaths/100 person-years) between 3 and 11 months of age by study year.

| Mortality rate | 1980 | 1981 | 1982 | 1983 | HR (95% CI) for 1982–1983 versus 1980 |
|---|---|---|---|---|---|
| Children aged 3–11 months | 4.7 (10/211.8) (N = 547) | 7.2 (18/250.8) (N = 678) | 8.0 (19/237.1) (N = 632) | 12.1 (30/247.5) (N = 638) | 2.12 (1.07–4.19) |

Notes: Event recorded as accidents were not removed from this analysis.

estimates from the natural experiment may therefore still be conservative.

The estimated effects of DTP and OPV are unlikely to have been influenced by other vaccinations since very few had received other vaccines; if the children who may have received BCG were censored in the analysis the result was essentially the same (Supplementary Table 2).

The 3-monthly community examinations assured that we had follow-up information for all children and relatively accurate information on the time of death. Some children were excluded because a BHP card could not be found and we did not know whether they had been vaccinated or were travelling. Most likely, BHP cards may never have been made because the child was not coming for examination, or the card may have disappeared at community examinations, at the later handling of BHP cards by field workers or data entry clerks, or due to mice. However, the few missing cards are unlikely to have affected the main analysis as the mortality rate in this group was similar to the general mortality rate (Fig. 2).

To assure comparability of vaccinated and unvaccinated groups, also with respect to travelling, we included only children who had been weighed in Bandim in connection with the 3-monthly community examinations. This meant that children who mostly stayed outside the area were not included in the analysis; these children had no access to community vaccinations and they lived elsewhere where the mortality risk might have been quite different, e.g. due to a higher risk of malaria infection.

The present study was not a planned trial. The study would have been a cleaner natural experiment if vaccinations had only been administered at the weighing sessions. However, the expatriate nurse did organize additional vaccinations and some 'unvaccinated' children had therefore already received a vaccination before coming for a weighing session. These 'misclassifications' do not explain the increased mortality in the DTP group. The estimate for DTP-vaccinated (±OPV) compared with DTP-unvaccinated children was 4-fold higher mortality when we included these additional landmarks in the analysis.

### *4.3. Comparison with Previous Studies of DTP and OPV*

There is only one other study of the introduction of DTP. In rural Guinea-Bissau, DTP (±OPV) was associated with 2-fold higher mortality (Aaby et al., 2004a). All studies that documented vaccination status and followed children prospectively indicate that DTP has negative effects; a meta-analysis of the eight studies found 2-fold higher mortality for DTP-vaccinated compared with DTP-unvaccinated, mostly BCG-vaccinated controls (Aaby et al., 2016) (Appendix A).

The negative effect of DTP was much worse in this natural experiment than has been reported in previous studies of DTP. This is presumably due to the "unvaccinated" control children in previous studies having been a frail subgroup too frail to get vaccinated. Previous studies have not been able to compare DTP-vaccinated children with "normal" controls. Hence, most previous studies have probably underestimated the negative effect of DTP.

The potentially differential effects of DTP and OPV have only been examined in few studies. However, we have recently been able to document marked beneficial NSEs of OPV. In an RCT, OPV at birth (OPV0) reduced infant mortality by 32% (0–57%) before the children received campaign-OPV (Lund et al., 2015). In Bissau campaign-OPV reduced the mortality rate by 19% (5–32%) (submitted). When DTP was missing for several months in Bissau, we showed that the all-cause case-fatality at the pediatric ward was 3-fold lower if the children had OPV-only as their most recent vaccination rather than the recommended combination of DTP and OPV (Aaby et al., 2004b). Thus, OPV may have modified the negative effect of DTP.

This pattern was also seen when DTP was first introduced in the rural areas of Guinea-Bissau in 1984 (Aaby et al., 2004a). OPV was not used the first year and the HR for DTP versus unvaccinated was 5.00 (0.63–39.7). In the period from 1985 to 1987, when DTP and OPV were nearly always administered together, the MRR was 1.90 (0.91–3.97). In the present study, the hazard ratio was 10.0 (2.61–38.6) for DTP-only but 3.52 (0.96–12.9) for children who received DTP and OPV simultaneously (Table 3). Based on these two studies of the introduction of DTP, the HR compared with DTP-unvaccinated children was significantly different for children who had received DTP-only (HR = 8.14 (2.63–15.2)) and for children who received both DTP and OPV (HR = 2.21 (1.16–4.19)) (test of interaction, p = 0.049). Hence, simultaneous administration of DTP and OPV may have alleviated the negative non-specific effect of DTP.

## 5. Conclusions

DTP was associated with 5-fold higher mortality than being unvaccinated. No prospective study has shown beneficial survival effects of DTP. Unfortunately, DTP is the most widely used vaccine, and the proportion who receives DTP3 is used globally as an indicator of the performance of national vaccination programs.

It should be of concern that the effect of routine vaccinations on all-cause mortality was not tested in randomized trials. All currently available evidence suggests that DTP vaccine may kill more children from other causes than it saves from diphtheria, tetanus or pertussis. Though a vaccine protects children against the target disease it may simultaneously increase susceptibility to unrelated infections.

The recently published SAGE review called for randomized trials of DTP (Higgins et al., 2014). However, at the same time the IVIR-AC committee to which SAGE delegated the follow-up studies of the NSEs of vaccines has indicated that it will not be possible to examine the effect of DTP in an unbiased way. If that decision by IVIR-AC remains unchallenged, the present study may remain the closest we will ever come to a RCT of the NSEs of DTP.

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## Conflict of Interest

Nothing to declare

## Contributions

CSB and PA proposed the study. PA collected the original data. AR is responsible for the demographic surveillance system. SWM and PA cleaned the data. SWM and AA conducted the statistical analyses. The first draft was written by PA; all authors contributed to the final version of the paper. PA and SWM will act as guarantors of the study.

## Independence

The funding agencies had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report.

## Data Sharing

Through request to the authors

## Appendix A. The DTP Controversy

The issue of DTP vaccination and child mortality in high mortality areas was raised 15 years ago when a study from rural Guinea-Bissau showed 1.84-fold higher mortality for children who had received DTP1 vaccination (Aaby et al., 2016; Kristensen et al., 2000). All subsequent prospective studies have supported a negative effect (Aaby et al., 2016). Furthermore, DTP may have a negative effect when given simultaneously with or after MV (Aaby at el., 2003b, 2012). For example, the negative effect of high-titer measles vaccination (HTMV) in girls, which led to the global withdrawal of HTMV, was due to DTP being administered after MV because HTMV had been given early at 4–5 months of age (Aaby et al., 2003b).

DTP has not been shown to have beneficial effects in RCTs or natural experiments. The current policy for DTP has only been examined by reanalyses of existing data sets collected for other purposes. All such studies have had methodological problems related to different forms of frailty and survival bias (Aaby et al., 2012). These studies have updated follow-up time for DTP-vaccinated children who survived but children who died without their vaccination status being documented were classified as “unvaccinated”. Such procedures give a misleading high mortality rate in the unvaccinated group, and the comparison of DTP-vaccinated survivors and “unvaccinated” children will therefore give a beneficial estimate for DTP (Aaby et al., 2016). If the mortality rate of unvaccinated children is unnaturally increased, the HR of unvaccinated children versus children who have received at least one vaccine may indicate how much bias there might be in the study, and we have called this HR the “bias-index”. All studies with prospective follow-up have had a bias index below 2.0 (Aaby et al., 2016); in the present study the bias index was 0.41 (0.15–1.15) in the 3–5 months age group (Supplementary Table 2). In studies with survival bias and unnaturally high mortality in the unvaccinated group, the bias index has been 3–8 times higher (Aaby et al., 2016).

SAGE recently reviewed the potential NSEs of BCG, MV and DTP (Higgins et al., 2014; Strategic Advisory Group of experts on Immunization, 2014). The reviewers indicated that the majority of studies showed a deleterious effect of DTP but they concluded that the results were inconsistent because two studies showed a beneficial effect. The beneficial effect in these studies was not surprising because the mortality rate in the unvaccinated group was unnaturally high, and the bias index was 3.40 (2.93–3.95) and 7.52 (5.15–10.97), respectively (Aaby et al., 2016).

SAGE's working group on non-specific effects of vaccines further emphasized that the overall effect remains unclear because DTP has been given in combination with other vaccines and under circumstances where the burden of the target diseases has been reduced to a very low level. However, several previous studies have shown that the negative effect of DTP-plus-OPV was not due to OPV (Aaby et al., 2004a,b, 2012). OPV has probably reduced the overall negative effect of DTP. Previous studies have indicated that DTP (±OPV) was associated with a 2-fold higher mortality than DTP-unvaccinated children (Aaby et al., 2016). Since pertussis did not account for >5–6% of infant deaths in the only existing African study of the impact of pertussis on child mortality (Mahieu et al., 1978), it is not surprising that DTP is also associated with a strong negative effect prior to vaccine-induced herd immunity (Aaby et al., 2012).

## Appendix B. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2017.01.041.

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# Appendix F

Methodological Issues and Evidence of Malfeasance in Research Purporting to Show Thimerosal in Vaccines Is Safe

Dr. Brian Hooker, 2014

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*Review Article*

# Methodological Issues and Evidence of Malfeasance in Research Purporting to Show Thimerosal in Vaccines Is Safe

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There are over 165 studies that have focused on Thimerosal, an organic-mercury (Hg) based compound, used as a preservative in many childhood vaccines, and found it to be harmful. Of these, 16 were conducted to specifically examine the effects of Thimerosal on human infants or children with reported outcomes of death; acrodynia; poisoning; allergic reaction; malformations; autoimmune reaction; Well's syndrome; developmental delay; and neurodevelopmental disorders, including tics, speech delay, language delay, attention deficit disorder, and autism. In contrast, the United States Centers for Disease Control and Prevention states that Thimerosal is safe and there is "no relationship between [T]himerosal[-]containing vaccines and autism rates in children." This is puzzling because, in a study conducted directly by CDC epidemiologists, a 7.6-fold increased risk of autism from exposure to Thimerosal during infancy was found. The CDC's current stance that Thimerosal is safe and that there is no relationship between Thimerosal and autism is based on six specific published epidemiological studies coauthored and sponsored by the CDC. The purpose of this review is to examine these six publications and analyze possible reasons why their published outcomes are so different from the results of investigations by multiple independent research groups over the past 75+ years.

## 1. Introduction

Thimerosal is an organic-mercury (Hg) based compound, used as a preservative in many childhood vaccines, in the past and present. To date, there have been over 165 studies that focused on Thimerosal and found it to be harmful [1, 2]. (A comprehensive list of these studies is shown at http://mercury-freedrugs.org/docs/20140329_Kern_JK_ExcelFile_TM_sHarm_ReferenceList_v33.xlsx.) Of these studies, 16 were conducted to specifically examine the effects of Thimerosal on human infants and/or children [3–18]. Within these studies, which focused on human infants and/or children, the reported outcomes following Thimerosal exposure were (1) death [3]; (2) acrodynia [4]; (3) poisoning [5]; (4) allergic reaction [6]; (5) malformations [7]; (6) autoimmune reaction [8]; (7) Well's syndrome [9]; (8) developmental delay [10–13]; and (9) neurodevelopmental disorders, including tics, speech delay, language delay, attention deficit disorder, and autism [10, 11, 14–18].

However, the United States (US) Centers for Disease Control and Prevention (CDC) still insists that there is "no relationship between [T]himerosal[-]containing vaccines and autism rates in children" [19]. This is a puzzling conclusion because, in a study conducted directly by the CDC, epidemiologists assessed the risk for neurologic and renal impairment associated with past exposure to Thimerosal-containing vaccine (TCV) using automated data from the Vaccine Safety Datalink (VSD) and found a 7.6-fold increased risk of autism from exposure to Thimerosal during infancy [20]. The database for that study was "from four health

maintenance organizations [HMOs] in Washington, Oregon, and California, containing immunization, medical visit, and demographic data on over 400,000 infants born between 1991 and 1997." In that initial study, Verstraeten et al. [20] "categorized the cumulative ethyl-Hg exposure from [T]himerosal[-]containing vaccines after one month of life and assessed the subsequent risk of degenerative and developmental neurologic disorders and renal disorders before the age of six." They "applied proportional hazard models adjusting for HMO, year of birth, and gender, and excluded premature babies." The reported results showed that "the relative risk (RR) of developing a neurologic development disorder was 1.8 (95% confidence intervals [CI] 1.1–2.8) when comparing the highest exposure group at 1 month of age (cumulative dose > 25 $\mu$g) to the unexposed group." Similarly, they "also found an elevated risk for the following disorders: autism (RR 7.6, 95% CI = 1.8–31.5), nonorganic sleep disorders (RR 5.0, 95% CI = 1.6–15.9), and speech disorders (RR 2.1, 95% CI = 1.1–4.0)" in the highest exposure group.

Considering the many peer-reviewed published research studies that have shown harm from Thimerosal, including studies in which Thimerosal exposure is associated with the subsequent diagnosis of neurodevelopmental disorders (16 studies) such as autism, and the just-described evidence from the CDCs own research, which found evidence of a relationship between the level of Thimerosal exposure and the risk of a subsequent autism diagnosis, how does the CDC conclude that there is no evidence of that relationship? The foundation for the CDC's current stance apparently is based primarily on six specific published epidemiological studies that the CDC has completed, funded, and/or cosponsored, starting in the late 1990s. These studies include (1) the Madsen et al. [21] ecological study of autism incidence versus Thimerosal exposure in Denmark, (2) the Stehr-Green et al. [22] ecological study of autism incidence versus Thimerosal exposure in Denmark, Sweden, and California, (3) the Hviid et al. [23] study of autism incidence versus Thimerosal exposure in Denmark (also ecological), (4) the Andrews et al. [24] cohort study of autism incidence and Thimerosal exposure in the United Kingdom, (5) the published Verstraeten et al. [25] CDC cohort study of autism incidence and Thimerosal exposure in the United States, and (6) the more recent Price et al. [26] case-control study of autism incidence and Thimerosal exposure in the United States. Although the CDC cites several other publications to purport the safety of Thimerosal, only these six specifically consider its putative relationship to autism.

The purpose of this review is to examine these six publications [21–26] which were "overseen" by the CDC and which claim that prenatal and early childhood vaccine-derived Thimerosal exposures are not related to the risk of a subsequent diagnosis of autism or autism spectrum disorder (ASD). This review analyzes possible reasons why their published outcomes are so different from the results of investigations by multiple independent research groups over the past 75+ years. The review begins with an examination of the Madsen et al. [21] study.

## 2. The Madsen et al. 2003 Study

The CDC-sponsored Madsen et al. [21] study examined whether discontinuing the use of TCVs in Denmark led to a decrease in the incidence of autism. Data were obtained from the Danish Psychiatric Central Research Register, which contains all psychiatric admissions since 1971 and all outpatient contacts in psychiatric departments in Denmark since 1995. The study authors examined the data from 1971 to 2000 and reported that rate of autism increased with the removal of Thimerosal from vaccines (starting in 1992, the year that Thimerosal-containing early childhood vaccines were phased out).

Although there are several concerns about the methodology used, the most serious concern involves diagnosis. As described in the paper, estimates of total autism cases in Denmark were only based on diagnoses occurring during inpatient visits from 1971 to 1994 and then during both inpatient and outpatient visits from 1995 to the last year of the study in 2000. Thus, the inclusion criteria are greatly expanded two years after the phaseout of Thimerosal from infant vaccines in Denmark, creating an "artificial increase" in autism prevalence. The authors conceded that "the proportion of outpatient to inpatient activities was about 4 to 6 times as many outpatients as inpatients with variations across time and age bands." However, in an earlier publication by Madsen et al. [27], the same authors had stated regarding this same data, "in our cohort, 93.1% of the children were treated only as outpatients. . ." Unlike the statement in the Madsen et al. [21] study, the 2002 paper indicates that the ratio between outpatients and inpatients in the 1971–2000 dataset was 13.5 : 1, which would account for an even greater increase in cases diagnosed starting in 1995 (i.e., after the probable completion of the phaseout of TCVs that started in 1992).

In addition, the authors stated that the Danish registry which was used to count cases did not include a large Copenhagen clinic before 1993. This clinic accounted for as many as 20% of the autism cases nationwide, which would again artificially inflate the autism incidence observed in Denmark after the phaseout of TCVs was initiated in 1992. The authors do not mention this change in inclusion criteria (i.e., the addition of a new clinic in the registry) neither do they attempt to adjust their analysis in accordance with the anomaly. It was revealed, instead, in a similar paper by Stehr-Green et al. [22] where the authors state regarding the Denmark registry of autistic patients, "Prior to 1992, the data in the national register did not include cases diagnosed in one large clinic in Copenhagen (which accounts for approximately 20% of cases occurring nationwide)."

Also, the diagnosis criteria for "autism" changed within the course of the study. From 1971 to 1993, the ICD-8 standards for diagnosis (psychosis protoinfantilis 299.00 or psychosis infantilis 299.01) were used to measure autism incidence. However, from 1994 to 2000, the ICD-10 standard (infantile autism, F84.1) was used. Although the authors did not address the impact of the change in diagnostic criteria, this could result in as much as a 25-fold increase in cases as the instantaneous change in autism prevalence in Denmark,

due to this change, went from a low of 1.2/10,000 to a high of 30.8/10,000 [28].

Another disconcerting methodological issue was that the 2001 data, which showed a strong downward trend in autism rates in at least two of the three age groups (continuing from 1999 through 2001), was not included in the final publication. This was apparent because when the paper was initially submitted for publication, it included the 2001 data. After the paper was rejected for publication by the Journal of the American Medical Association (JAMA) and the Lancet, it was submitted to the journal Pediatrics again including the 2001 data. As stated by one of the peer-reviewers of the Pediatrics submission, "The drop of incidence shown for the most recent years is perhaps the most dramatic feature of the figure, and is seen in the oldest age group as well as the youngest. The authors do not discuss whether incomplete ascertainment in the youngest children or delay in recording of data in the most recent years might play a role in this decline, or the possibility that this decrease might have come about through elimination of [T]himerosal" (January 23, 2003, communication between Dr. Poul Thorsen, Aarhus University, and Dr. Coleen Boyle, CDC scientist). In response to this criticism, the authors removed the 2001 incidence numbers. The authors' decision to withhold these data resembles scientific malfeasance, especially when coupled with the previously discussed problematic methods for counting autism cases. If the scientists believed that downward trend between 1999 and 2001 was caused by some phenomenon unrelated to the phaseout of the TCVs, these scientists should have included those data and then explained the trend within the discussion of the data.

If the 2001 data had been included in the final publication, the results would have been consistent with a more recent CDC study [29] where a decreasing trend of autism prevalence in Denmark after the removal of Thimerosal in 1992 was reported. Instead of large increases in autism prevalence after 1992, the recent Danish study revealed that the autism spectrum disorder prevalence in Denmark fell steadily from a high of 1.5% in 1994-95 (when children receiving Thimerosal-free formulations were too young to receive an autism diagnosis and, because of the known offset in diagnosis, most of those being diagnosed had been born 4 to 8 years earlier [from 1985 to 1990]) to a low of 1.0% in 2002–2004 (more than 10 years after the phasein of the use of Thimerosal-free vaccine formulations was started in 1992).

## 3. The Stehr-Green et al. 2003 Study

The CDC's Stehr-Green et al. [22] study compared the prevalence/incidence of autism in California, Sweden, and Denmark with average exposures to TCVs. Graph-based ecologic analyses were used to examine population data from the state of California (national immunization coverage surveys and counts of children diagnosed with autism-like disorders seeking special education services in California); Sweden (national inpatient data on autism cases, national vaccination coverage levels, and information on use of all vaccines and vaccine-specific amounts of Thimerosal); and Denmark (national registry of inpatient/outpatient-diagnosed autism cases, national vaccination coverage levels, and information on use of all vaccines and vaccine-specific amounts of Thimerosal).

The study followed and appeared to be conducted in response to California study data [30], which was presented to the Institute of Medicine's Immunization Safety Review Committee. The California data showed that increased uptake of Thimerosal-containing vaccines in California during the 1990s correlated with a corresponding increase in autism diagnoses. In the Stehr-Green et al. [22] study, the researchers stated that the reliability of the autism prevalence data, citing that the California data included autism spectrum disorder diagnoses such as pervasive development disorder (PDD), could account for the increase. However, in a published response to this paper, Blaxill and Stehr-Green [31] stated that the California prevalence rates were reported based solely on autism cases.

In the Stehr-Green paper, the Sweden autism prevalence data showed an increase in autism rates from 5- 6 cases per 100,000 in 1980–82 to a peak of 9.2 cases per 100,000 in 1993. In Sweden, TCVs were phased out starting in 1987. Denmark's autism prevalence data was identical to that reported in the Madsen et al. [21] study critiqued previously. For Denmark, the authors reported an astounding 20-fold increase in autism prevalence between 1990 and 1999, despite the phaseout of TCVs that started in 1992.

In addition, the data from Sweden were based on inpatient (hospital) visits only. This limitation (counting a small fraction of the total number of cases) likely accounted for the erratic swings in the annual numbers of autism cases reported in that country. Also, the Thimerosal exposure level based on the Swedish vaccination schedule during this time period was much less (a nominal maximum of 75 $\mu$g of Hg by two years of age) than that possible in California (and the United States as a whole) where developing children nominally received up to 237.5 $\mu$g of Hg by 18 months of age through the standard immunization schedule. In conclusion, the Stehr-Green et al. study was problematic in its attempt to combine ecological data from three different countries that, relative to each other, demonstrated different vaccination policies and widely different Thimerosal exposure levels.

## 4. The Hviid et al. (2003) Study

The Hviid et al. [23] population-based cohort study, widely cited by the CDC, compared rates of autism prevalence among individuals who received Thimerosal-free vaccines to those receiving TCVs. The authors report that there was no evidence of increased autism prevalence with Thimerosal exposure.

The study authors stated that the mean age of autism diagnosis within their population was 4.7 years with a standard deviation of 1.7 years. However, cases and controls as young as 1 year of age were included within the analysis. Accordingly, controls that were less than the mean age of diagnosis minus two standard deviations (1.3 years) from that age had a 97.5% probability of actually being individuals who will later develop autism and are therefore possibly misclassified. Similarly, in this study, the mean age for an

ASD diagnosis was 6.0 years with a standard deviation of 1.9 years. Thus, the study methodology is questionable because it appears to have underascertained the number of cases diagnosed with autism and ASD.

In addition, rather than counting persons within the cohort, the authors counted "person-years of follow up." With this technique, each age group (one-year-olds, two-year-olds, etc.) was considered equally, despite the fact that younger age groups were much less likely to receive an autism diagnosis. This again contributed to the undercounting of the cases with a diagnosis of autism and ASD and biased the study towards the null hypothesis (that there is no statistically significant Thimerosal exposure effect on the outcomes observed).

## 5. The Andrews et al. (2004) Study

The Andrews et al. [24] study was a retrospective cohort study completed using records from a database in the United Kingdom, where autism prevalence rates were compared for children receiving Thimerosal-containing DTaP and DT vaccines. In the Andrews et al. [24] study, Cox's proportional-hazards ratios were used to evaluate periods of followup in the cohort examined by the investigators using the records in the general practitioner research database (GPRD), a database that was known to have a significant level of errors. These investigators reported that increased organic-Hg exposure from TCVs was associated with a significantly reduced risk for diagnosed general developmental disorders and for unspecified developmental delay (although there was a significantly higher risk for diagnosed tics).

Considering that there are several studies conducted by independent investigators that have found that exposure to Thimerosal is a risk factor for neurodevelopmental delay and disorders [10, 11, 16], the reduced rate of neurodevelopmental delay and disorders with Thimerosal exposure found in the Andrews et al. [24] study suggests possible methodological issues.

This result may have occurred, in part, because other studies examined cohorts with significantly different childhood vaccine schedules and with different diagnostic criteria for outcomes. This difference may also exist because these other studies that found Thimerosal to be a risk factor for neurodevelopmental delay and disorders employed different epidemiological methods, especially with respect to the issue of follow-up period for individuals in the cohorts examined. The method used to measure follow-up period for individuals is a critical issue in all studies examining the relationship between exposures and the subsequent risk of a neurodevelopmental disorder diagnosis, especially in those instances where the postexposure periods for all of the participants in the study are essentially the same. This is because the risk of an individual being diagnosed with a neurodevelopmental disorder is not uniform throughout his/her lifetime. As observed in the present study, the initial mean age for any neurodevelopmental disorder diagnosis was 2.62 years old, and the standard deviation of mean age of the initial diagnosis of neurodevelopmental disorder was 1.58 years old. These findings are highly problematic because (1) any follow-up method that fails to consider the lag time between birth and age of initial neurodevelopmental disorder diagnosis will likely not be able to observe the true relationship between exposure and the subsequent risk of a neurodevelopmental disorder diagnosis and (2) statistically, the mean and standard deviation age of diagnosis as reported lead to the nonsensical result that a significant portion (2.5%) of the children in this study were diagosed with a neurodevelopmental disorder more than six months before they were born (i.e., the mean age minus two standard deviations, $2.62 - [2 \times 1.58] = -0.54$ years of age).

Another issue with this study is that the authors used a nontransparent, multivariate regression technique to analyze vaccine uptake and autism prevalence data. The study included one dependent variable (autism) and multiple independent variables, including two independent variables (Thimerosal exposure levels and year of birth) that were "correlated" with each other, since Thimerosal exposures increased with time. Thus, the researchers did not report a statistical analysis of the effect of Thimerosal exposure on autism incidence, despite the fact that the authors stated that no such effect was observed. Moreover, the methods used in this study can create a problem in regression known as "multicolinearity." In this case, since the time variable and the vaccine exposure variable are correlated, they actually compete to explain the outcome effect. Inclusion of the time variable reduces the significance of the exposure variable. Yet, the authors did not explain why they included a time variable that competes with the exposure variable. Unfortunately, the authors of this study never released the raw data so that a valid single-variable analysis could be conducted to ascertain the probability of an association between Thimerosal exposure and the risk of autism.

It is also important to note that the UK Thimerosal exposure (a maximum of 75 $\mu$g of Hg by 4 months of age) was not comparable to that in the United States (a maximum of 75 $\mu$g of Hg by 2 months of age and 187.5 $\mu$g of Hg by 6 months of age). Thus, this study should not be extrapolated to the probability of an autism-Thimerosal association based on the US vaccination schedule.

## 6. The Verstraeten et al. (2003) Study

The CDC's published Verstraeten et al. [25] study consists of a cohort analysis of a subset of records from the medical records databases for several of the HMOs whose records were maintained in a central data repository, the Vaccine Safety Datalink (VSD). This study was conducted in at least five separate phases. In the final phase (i.e., the results reported in the publication), the authors stated that there was no relationship between Thimerosal exposure in vaccines and autism incidence. However, no data are reported in the published study to support this conclusion.

Results from the first phase of the study released in an internal presentation abstract by Verstraeten et al. [20] (mentioned earlier) using records from four (4) HMOs showed that infants who were exposed to greater than 25 $\mu$g of Hg in vaccines and immunoglobulins at the age of one month were 7.6 times more likely to have an autism diagnosis than those not exposed to any vaccine-derived organic Hg.

Verstraeten, Thomas

From: Verstraeten, Thomas
Sent: Friday, July 14, 2000 10:42 AM
To: "Philippe Grandjean"; Verstraeten, Thomas
Cc: Chen, Robert (Bob) (NIP); Destefano, Frank; Pless, Robert; Bernier, Roger; Tom Clarkson; Pal Weihe
Subject: RE: Thimerosal and neurologic outcomes

Dear Dr. Grandjean,
Thank you for a very rapid response!
I apologize for dragging you into this nitty gritty discussion, which in Flemish we would call "muggeziften". I know much of this is very hypothetical and personally I would rather not drag the Faroe and Seychelles studies in this entire thimerosal debate, as I think they are as comparable to our issue as apples and pears at the best. Unfortunately I have witnessed how many experts, looking at this thimerosal issue, do not seem bothered to compare apples to pears and insist that if nothing is happening in these-studies then nothing should be feared of thimerosal. I do not wish to be the advocate of the anti-vaccine lobby and sound like being convinced that thimerosal is or was harmful, but at least I feel we should use sound scientific argumentation and not let our standards be dictated by our desire to disprove an unpleasant theory.
Sincerely,
Tom Verstraeten.

Figure 1: July 14, 2000, email from Verstraeten to Philippe Grandjean regarding the risk of harm due to Thimerosal (obtained by the authors via the US Freedom of Information Act of 1950 as amended).

Within the same abstract, Verstraeten reports that the risk for any neurodevelopmental disorder was 1.8, the risk for speech disorder was 2.1, and the risk for nonorganic sleep disorder was 5.0. All relative risks were statistically significant.

In the second phase of the study, a different approach was taken: exposure was compared at 3 months of age, rather than one month. Results of this phase showed that children exposed to the maximum amount of organic Hg in infant vaccines (62.5 $\mu$g) were 2.48 times more likely to have autism diagnosis compared to those exposed to less than 37.5 $\mu$g of Hg in vaccines. These results were also statistically significant. No assessment against a "no exposure" control was apparently completed in this study phase.

In the third phase of the study, in which more data stratification methods and different inclusion/exclusion criteria were applied to the analysis, the relative risk of autism for children at three months of Thimerosal exposure dropped to 1.69. At this point, evidence in an email from Verstraeten, the lead investigator, written to a colleague outside of the CDC (obtained by the authors via the US Freedom of Information Act of 1950 as amended), suggests that Verstraeten could have been receiving pressure within the CDC to apply unsound statistical methods to deny a causal relationship between Thimerosal and autism. In this email, Verstraeten states (Figure 1), "I do not wish to be the advocate of the anti-vaccine lobby and sound like being convinced that thimerosal is or was harmful, but at least I feel we should use sound scientific argumentation and not let our standards be dictated by our desire to disprove an unpleasant theory."

The fourth and fifth phase of the study used records from only two of the original HMOs and incorporated a third HMO, Harvard Pilgrim, into the analysis. Some critics of the study questioned the use of Harvard Pilgrim, as this HMO appeared to be riddled with uncertain record keeping practices, and the state of Massachusetts had been forced to take it over after it declared bankruptcy. In addition, the HMO used different diagnostic codes than the other two HMOs used in phases 2 and 3. Other criticisms include that the study used younger children, from 0 to 3 years of age, even though the average age for an autism diagnosis at the time was 4.4 years. Since half of the children receiving an autism diagnosis would be over 4.4 years of age, far greater than the maximum age in the study at 3 years, this analysis excluded more than 50% of all autism cases from this HMO. Also, the cohort from this HMO contained 7 times fewer individuals than the main cohort from the previous study (i.e., HMO B), and there was no apparent attempt to assess the power of this HMO to show any statistically significant effect.

Also of note is the lack of variability within strata among the different HMOs in the Verstraeten et al. [25] study. By design, a cohort study seeking to assess the effect of some treatment on a subsequent outcome should be designed to maximize the range of the independent "treatment" variable (Thimerosal exposure in this instance) in order to determine if there is indeed an "effect" in the dependent postexposure outcome variable (neurological disorders in this study). However, the authors knowingly stratified the analysis based on the participants' gender, year of birth, month of birth, and clinic most often visited. This effectively reduced the variability of Thimerosal exposure within the strata to the point that it reduced the capability of the final analysis to find any but the "strongest" Thimerosal exposure-related outcome effects. The problems with such "overmatching" practices have been discussed in detail in peer-reviewed scientific literature and will be treated in greater detail in the forthcoming review of the CDC's Price et al. [26] paper.

Another methodological concern about the Verstraeten et al. [25] study is related to the issue of the minimum follow-up period required for individuals in the cohorts examined to ensure that all the cases in the cohort will have been identified with a high degree of certainty. This issue has been mentioned as a problem in the previous studies. As mentioned earlier, the method used to determine the minimum follow-up period for individuals is a critical issue

in all studies examining the relationship between exposures and the subsequent risk of a neurodevelopmental disorder diagnosis, especially in those instances where the exposures to all participants in the study are the same or essentially the same. This is the case because the risk of an individual being diagnosed with a neurodevelopmental disorder is not uniform throughout his/her lifetime. Any follow-up method that fails to consider the lag time between birth and age of initial neurodevelopmental disorder diagnosis will likely not be able to observe the true relationship between exposure and the subsequent risk of a neurodevelopmental disorder diagnosis. Verstraeten et al. [25] included children in the control group who were too young (down to "0" years of age) to receive a neurodevelopmental disorder diagnosis.

Within this study, Verstraeten et al. [25] still found significantly increased risk ratios for tics and language delay. However, the authors stated that, because these results were not consistent between the HMOs tested, these significantly increased risk ratios could not be used to make a determination of the potential adverse consequences of organic-Hg exposure from TCVs.

## 7. The Price et al. 2010 Study

In 2010, the CDC published another epidemiology study on Thimerosal and autism [26]. This case-control study was conducted using the records from three managed care organizations (MCOs) consisting of 256 children with an ASD diagnosis and 752 controls that were matched by birth year, gender, and MCO to the children with an ASD diagnosis. Exposure to Thimerosal in vaccines and immunoglobulin preparations was determined from electronic immunization registries, medical charts, and parent interviews. Conditional logistic regression was used to assess associations between ASD, autistic disorder (AD), and ASD with regression and exposure to ethyl-Hg during prenatal, birth-to-1-month, birth-to-7-month, and birth-to-20-month periods. Their published finding was that prenatal and infant Thimerosal exposure from TCVs and Thimerosal-containing immunoglobulin posed no statistically significant risk of autism.

As mentioned earlier, in case-control studies, the main methodological concern is the phenomenon called "overmatching." This concern for overmatching in the Price et al. [26] study was voiced previously by DeSoto and Hitlan [32]. In their comprehensive analysis of overmatching errors specific to the Price paper, DeSoto and Hitlan [32] stated that "Matching cannot—or should not—be done in a way that artificially increases the chance that within[-] strata exposure is the same; this happens when a matching variable is a significant predictor of exposure and is called overmatching."

Cases were matched with controls of the same age and sex, within the same HMO and essentially the same vaccination schedule, using the same vaccine manufacturers. DeSoto and Hitlan then state further, regarding the lack of variability of Thimerosal exposure in the Price study, "Across the different years, the average cumulative exposure varies from 42.3 $\mu$g to 125.46 $\mu$g; while within the birth year stratas (sic), the mean exposures do not vary by more than 15 micrograms." In other words, the maximum level of variation in Thimerosal exposure in the cases and controls being compared was 15 $\mu$g of Hg, as compared to the "83" $\mu$g of Hg range for the average cumulative exposures in the cohort studies. Moreover, this range is much less than the range of Thimerosal exposures that could have been used to determine risk including (a) 0 to 50 $\mu$g of Hg for one-month exposures, (b) 0 to 190 $\mu$g of Hg for seven-month exposures, and (c) 0 to 300 $\mu$g of Hg for 20-month exposures. Finally, regarding the Price study, DeSoto and Hitlan [32] concluded, "this paper is flawed. Unfortunately, there is not an analytic fix for overmatching: it is [a] design flaw."

Prenatal Thimerosal exposure for the children within the study arose from the Thimerosal-preserved inactivated-influenza vaccine given during pregnancy and the Rho immunoglobulin administered to pregnant women to prevent Rh-factor incompatibility injury to the developing child. Unlike postnatal exposure from TCVs in the recommended childhood vaccination schedule, prenatal exposures would not be overmatched in a study design that stratified the participants based on their birth year or HMO. Evidence from the background CDC report regarding the Price study showed a significant risk of regressive autism due to prenatal Thimerosal exposure levels, at exposure levels as low as 16 $\mu$g of Hg [33]. However, the risk of regressive autism due to prenatal Thimerosal exposure reported in that paper was 1.86 and yielded a $P$ value of 0.072 which was deemed as insignificant based on the authors' "cut-off" value of $P < 0.05$. However, $P$ values between 0.05 and 0.10 are "marginally significant" and should merit further study. In addition, upon further analysis, it was found that the 2009 background report [33] to the Price et al. [26] study showed that the prenatal Thimerosal exposure model was run in six different ways and that the most reliable methods (those that factored out the postnatal Thimerosal exposure effects) found highly statistically significant relative risks of up to 8.73 ($P = 0.009$) for regressive ASD due to prenatal Thimerosal exposures from Thimerosal-containing influenza vaccines and Rho immunoglobulin products relative to no such prenatal Thimerosal exposures. Curiously, these more compelling results were not reported in the paper. Withholding these data from the publication and, instead, reporting a significantly lower value could appear to constitute scientific malfeasance on the part of the authors of this study.

## 8. Conclusion

As seen in this review, the studies upon which the CDC relies and over which it exerted some level of control report that there is no increased risk of autism from exposure to organic Hg in vaccines, and some of these studies even reported that exposure to Thimerosal appeared to decrease the risk of autism. These six studies are in sharp contrast to research conducted by independent researchers over the past 75+ years that have consistently found Thimerosal to be harmful. As mentioned in the Introduction section, many studies conducted by independent investigators have found Thimerosal to be associated with neurodevelopmental disorders. Several studies, for example, including three of the six studies covered in this review, have found Thimerosal to

Table 1: Methodological issues most common in each of the six reviewed studies.

| Study reviewed | Methodological issues |
|---|---|
| Madsen et al. [21] | (i) Changing entrance criteria in ecological studies.<br>(ii) Withholding important results from the final publication.<br>(iii) Conclusions not generalizable to the US vaccination schedule due to widely different vaccination schedules and different levels of Thimerosal dosing in other countries. |
| Stehr-Green et al. [22] | (i) Changing entrance criteria in ecological studies.<br>(ii) Withholding important results from the final publication.<br>(iii) Conclusions not generalizable to the US vaccination schedule due to widely different vaccination schedules and different levels of Thimerosal dosing in other countries. |
| Hviid et al. [23] | (i) Accounting for "person-years" regarding exposure rather than actual exposure levels.<br>(ii) Conclusions not generalizable to the US vaccination schedule due to widely different vaccination schedules and different levels of Thimerosal dosing in other countries. |
| Andrews et al. [24] | (i) Accounting for "person-years" regarding exposure rather than actual exposure levels.<br>(ii) Conclusions not generalizable to the US vaccination schedule due to widely different vaccination schedules and different levels of Thimerosal dosing in other countries. |
| Verstraeten et al. [25] | (i) Cohort of children too young for followup for an autism diagnosis.<br>(ii) "Overmatching" phenomena due to too closely matched cases and controls.<br>(iii) Withholding important results from the final publication. |
| Price et al. [26] | (i) "Overmatching" phenomena due to too closely matched cases and controls.<br>(ii) Withholding important results from the final publication. |

be a risk factor for tics [10, 17, 24, 25, 34, 35]. In addition, Thimerosal has been found to be a risk factor in speech delay, language delay, attention deficit disorder, and autism [10, 11, 15–17, 24, 25, 34].

Considering that there are many studies conducted by independent researchers which show a relationship between Thimerosal and neurodevelopmental disorders, the results of the six studies examined in this review, particularly those showing the protective effects of Thimerosal, should bring into question the validity of the methodology used in the studies. A list of the most common methodological issues with these six studies is shown in Table 1. Importantly, other than the Hviid et al. [23] study, five of the publications examined in this review were directly commissioned by the CDC, raising the possible issue of conflict of interests or research bias, since vaccine promotion is a central mission of the CDC. Conceivably, if serious neurological disorders are found to be related to Thimerosal in vaccines, such findings could possibly be viewed as damaging to the vaccine program.

## Conflict of Interests

All of the investigators on the present study have been involved in vaccine/biologic litigation.

## Acknowledgment

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## References

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# Appendix G

168 studies showing that mercury in vaccines is unsafe

| Authors | Year | Journal |
|---|---|---|
| Li et al. | 2014 | ToxSci Advance Access |
| Trumpler et al. | 2014 | Trace Elements |
| Dorea et al. | 2014 | Journal of Toxicology & Environmental Health |
| Pieper et al. | 2014 | Metallomics. |
| Zhang et al. | 2014 | Graefes Arch Clin Exp Ophthalmol. |
| Staab et al. | 2014 | PLoS Genet. |
| Wehe et al. | 2014 | Anal Bioanal Chem. |
| Marques et al. | 2014 | Environ Pollut. |
| Geier et al. | 2013 | Transl Neurodegener |
| Grønborg et al. | 2013 | JAMA Pediatr. |
| Chen et al. | 2013 | World J Pediatr. |
| Zimmermann et al. | 2013 | Neurotoxicology. |
| Goldman | 2013 | Hum Exp Toxicol. |
| Sharpe et al. | 2013 | J Toxicol. |
| Abdel-Rahman | 2013 | J Applied Pharmaceutical Science |
| Guzzi et al. | 2012 | Interdiscip Toxicol. |
| Zimmer et al. | 2012 | Environ Health Perspect. |
| li et al. | 2012 | PLoS One. |
| Mrozek-Budzyn et al. | 2012 | Neurotoxicol Teratol. |
| Blanuša et al. | 2012 | J Biomed Biotechnol. |
| Sharpe et al. | 2012 | J Toxicol. |
| Khan et al. | 2012 | J Physiol Pharmacol. |
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| Hunter et al. | 2010 | Dis Model Mech. |
| Olczak et al.a | 2010 | Folia Neuropathol. |
| Wyrembek et al. | 2010 | J Physiol Pharmacol. |
| Gallagher & Goodman | 2010 | J Toxicol Environ Health A. |
| Olczak et al.b | 2010 | Neurochem Res. |
| Hewitson et al. a | 2010 | J Toxicol Environ Health A. |
| Zieminska et al. | 2010 | Toxicology. |
| Rodrigues et al. | 2010 | Arch Toxicol. |
| Hewitson et al. b | 2010 | Acta Neurobiol Exp (Wars). |
| Paradis et al. | 2010 | Northeast Bioengineering Conference |
| Gardner et al. | 2010 | Toxicol Lett. |
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| Migdal et al. | 2010 | Toxicol Appl Pharmacol. |
| Geier et al. | 2010 | Med Sci Monit. |
| Marques et al. | 2010 | Acta Paediatr. |
| Minami et al. | 2010 | Cell Biol Toxicol. |
| Geier et al. | 2009 | Toxicol Environ Chem |
| Olczak et al. | 2009 | Brain Res. |
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| Young et al. | 2008 | J Neurol Sci. |
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| Laurente et al. | 2007 | Anales de la Facultad de Medicina |
| Park et al. | 2007 | J Toxicol Environ Health A. |
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| Geier & Geier | 2007 | J Matern Fetal Neonatal Med. |
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| Lawton et al. | 2007 | Toxicol In Vitro. |
| Ribeiro et al. | 2007 | Neurosci Res. |
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| Hagele et al. | 2007 | Int J Toxicol. |
| Yole et al. | 2007 | Toxicology. |
| Karsen et al. | 2007 | J Infect Dev Ctries. |
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| Zheng & Dreskin | 2007 | Ann Allergy Asthma Immunol. |
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| Orct et al. | 2006 | J Appl Toxicol. |
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| Pérez et al. | 2006 | J Lipid Res. |
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| Havarinasab et al. | 2005 | Toxicol Appl Pharmacol. |
| Chang et al. | 2005 | Pharmacol Res. |
| Parran et al. | 2005 | Toxicol Sci. |
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| James et al. | 2005 | Neurotoxicology. |
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| Nakao et al. ordered, but not able to retrieve | 2003 | Natural Science Research |
| Qvarnström et al. | 2003 | Anal Chem. |
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| Makani et al. | 2002 | Genes Immun. |
| Kerst et al. | 2002 | J Membr Biol. |
| Müller et al. | 2001 | Int J Hyg Environ Health. |
| Montero et al. | 2001 | Cell Calcium. |
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| Westphal et al. | 2000 | Int Arch Occup Environ Health. |
| Song et al. | 2000 | Brain Res. |
| Donoso et al. | 2000 | Biophys J. |
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| Mihai et al. | 1999 | Cell Calcium. |
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| Karhapää et al. abstract | 1996 | Cell Calcium. |
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| Wiktorek et al. abstract | 1996 | Biochem Biophys Res Commun. |

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| Brunner et al. abstract | 1991 | Mutagenesis. |
| Withrow et al. abstract | 1989 | Photochem Photobiol. |
| Chanez et al. | 1989 | Neurotoxicology. |
| Chervonskaia et al. | 1988 | Zh Mikrobiol Epidemiol Immunobiol. |
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| Fagan et al. | 1977 | Arch Dis Child. |
| Parry abstract | 1977 | Mutat Res. |
| Heinonen et al. | 1977 | Publishing Sciences Group (book) |
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| Braaten et al. | 1975 | Tissue Cell. |
| Gasset et al. abstract | 1975 | Arch Ophthamol. |
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| Nelson & Gottshall | 1967 | Appl Microbiol. |
| Ellis | 1943 | Arch Ophthamol. |
| Kinsella | 1941 | Ann Intern Med. |
| Welch & Hunter | 1940 | Am J Public Health. |
| Welch | 1939 | J. Immunol. |
| Cummins | 1937 | Lancet |
| Salle & Lazarus | 1935 | Proc Soc Exp Biol Med. |
| Powell & Jamieson | 1931 | Am J Hyg. |
| Smithburn et al. | 1930 | J. Am. Med. Assoc. |

# Appendix H

a place of mind
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June 24, 2017

United States Department of Health & Human Services
National Institutes of Health
Food & Drug Administration
Centers for Disease Control & Prevention
200 Independence Avenue, S.W.
Washington, D.C. 20201

Re: *Aluminum Adjuvants*

Dear Directors:

I am writing to you in regard to aluminum adjuvants in vaccines. This subject is one my laboratory works on intensively and therefore one where I feel that I have some expertise. In particular, we have studied the impact of aluminum adjuvants in animal models of neurological disease, including autism spectrum disorder (ASD). Our relevant studies on the general topic of aluminum neurotoxicity in general and specifically in regard to adjuvants are cited below.

These studies and the broader existing literature regarding aluminum toxicity, lead almost invariably to the conclusion that aluminum in any chemical form is always neurotoxic when administered to humans. Further, I am convinced that aluminum adjuvants in vaccines may contribute to neurological disorders across the lifespan. In adults, such adjuvant may induce macrophagic myofasciitis, a disease with neuropathological aspects. In children, there is growing evidence that aluminum adjuvants may disrupt developmental processes in the central nervous system and therefore contribute to ASD in susceptible children.

Despite the foregoing, the safety of aluminum adjuvants in vaccines has not been properly studied in humans even though, pursuant to the recommended vaccine schedule published by the Centers for Disease Control (CDC), a baby may be injected with up to 3,675 micrograms of aluminum adjuvant by six months of age.

In regard to the above, it is my belief that the CDC's claim on its website that "Vaccines Do Not Cause Autism" is wholly unsupported. Given this, I remain convinced that much more research on the role of aluminum adjuvant in vaccines and neurological disorders, including ASD, is warranted and should be a research priority for the NIH and other funding bodies.

Yours sincerely,

Christopher A. Shaw, Ph.D
Professor
Dept. of Ophthalmology and Visual Sciences
University of British Columbia
828 W. 10th Ave.
Vancouver, British Columbia
Canada, V5Z1M9
Tel: 604-875-4111 (ext. 68373)
Email: cashawlab@gmail.com

Relevant Publications (Shaw Laboratory)


1. Crepeaux G, Eidi H, David MO, Baba-Amer Y, Tzavara E, giros B, authier FJ, Exley C, Shaw CA, Cadusseau J, Gherardi RK. Non-linear dose-response of aluminium hydroxide adjuvant particles: Selective dose neurotoxicity. *Toxicology*. 375:48-57. (2016).

2. Crepeaux G, Eidi H, David M-O, Tzavara E, Giros B, Exley C, Curmi PA, Shaw CA, Gherardi RK, Cadusseau J. Highly delayed systemic translocation of aluminium-based adjuvant in CD1 mice following intramuscular injections. *J. Inorg. Biochem*. 152:199-205. (2015).

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8. Shaw CA, Li Y, Tomljenovic L. Administration of aluminum to neonatal mice in vaccine in vaccine-relevant amounts is associated with adverse long term neurological outcomes. *J Inorg Chem*. (2013).

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14. Petrik MS, Wong MC, Tabata RC, Garry RF, and Shaw CA. Aluminum adjuvant linked to Gulf War illness induces motor neuron death in mice. *J Neuromolecular Medicine*. 9: 83-100. (2007).

institutmondor
de recherche biomédicale

UNIVERSITÉ
PARIS-EST

June 15, 2017

United States Department of Health & Human Services
National Institutes of Health
Food & Drug Administration
Centers for Disease Control & Prevention
200 Independence Avenue, S.W.
Washington, D.C. 20201

Re: *Aluminum Adjuvants*

Dear Directors:

I am an expert in the field of aluminum adjuvants toxicity in humans and animal models. I have been working in this field since the initial description of the Al vaccine-induced macrophagic myofasciitis in 1998. Since that time I have written 40 peer-reviewed scientific publications and one book on this subject.

I strongly support the contention that aluminum adjuvants in vaccines may have a role in the etiology of autism spectrum disorder (ASD). My view is founded on a significant and burgeoning body of peer-reviewed scientific evidence which makes the link between ASD and exposure to aluminum through vaccinations and other sources. Examples of this literature from my own group are detailed below and I urge the HHS to take them into consideration in forming any future opinion on the safety of aluminum adjuvants in vaccines.

The Center for Disease Control's claim on its website that "Vaccines Do Not Cause Autism" is unsupported with respect to aluminum adjuvants and this claim stifles the important research to determine the safety of aluminum adjuvants used in vaccines. As an expert in the field of aluminum adjuvants and aluminum toxicity I solemnly declare that more research on the role of aluminum adjuvant in vaccines and neurological disorders, including ASD, is essential and urgently required.

Yours very sincerely

Romain K. Gherardi
Professor, Neuromuscular Pathology Expert Centre
University Paris-Est, INSERM U955-E10,
Henri Mondor hospital, Créteil France
Contact at the hospital
Tel 00 (33) 1 49812746
romain.gherardi@hmn.aphp.fr

**UMR U955 INSERM / UPEC**

**Team 10**
« Biology of the neuromuscular system»

Fred Relaix, director
FrançoisJérome Authier, co-director

Romain Gherardi, former director
Tél. +33 (0)1 49 81 27 42
Fax. +33 (0)1 49 81 27 33
romain .gherardi@inserm.fr

## ***Selection of significant publications from our group in the field***

Gherardi R. Toxic Story: deux ou trois vérités embarrassantes sur les adjuvants des vaccins. **Actes Sud** (publisher), Paris, 2016, 250 pages

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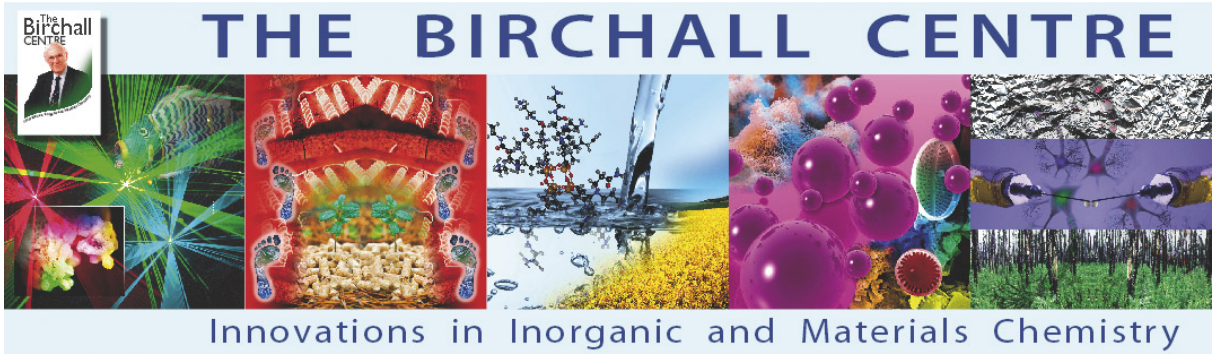


**Tel: 01782 734080**
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**e-mail: c.exley@keele.ac.uk**
**http://www.keele.ac.uk/aluminium**

June 15, 2017

United States Department of Health & Human Services
National Institutes of Health
Food & Drug Administration
Centers for Disease Control & Prevention
200 Independence Avenue, S.W.
Washington, D.C. 20201

Re: *Aluminum Adjuvants*

Dear Directors:

I am an expert in the field of aluminum adjuvants and aluminum toxicity. I have been working in this field for more than 30 years during which time I have written in excess of 150 peer-reviewed scientific publications on this subject.

I strongly support the contention that aluminum adjuvants in vaccines may have a role in the etiology of autism spectrum disorder (ASD). My view is founded on a significant and burgeoning body of peer-reviewed scientific evidence which makes the link between ASD and exposure to aluminum through vaccinations and other sources. Examples of this literature from my own group are detailed below and I urge the HHS to take them into consideration in forming any future opinion on the safety of aluminum adjuvants in vaccines.

The Center for Disease Control's claim on its website that "Vaccines Do Not Cause Autism" is unsupported with respect to aluminum adjuvants and this claim stifles the important research to determine the safety of aluminum adjuvants used in vaccines. As an expert in the field of aluminum adjuvants and aluminum toxicity I solemnly declare that more research on the role of aluminum adjuvant in vaccines and neurological disorders, including ASD, is essential and urgently required.

Yours faithfully

Christopher Exley PhD
Professor in Bioinorganic Chemistry

Honorary Professor, University of the Highlands and Islands

**List of Recent, Relevant and Significant Publications From Our Group**

Exley C, Siesjö P & Eriksson H (2010) The immunobiology of aluminium adjuvants: how do they really work? Trends in Immunology 31, 103-109.

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Exley C (2017) Aluminium should now be considered a primary aetiological factor in Alzheimer's disease. Journal of Alzheimer's Disease Reports 1, 23-25.

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# Appendix I

Verstraeten, Thomas M., MD, NIP, Division of Epidemiology and Surveillance, Vaccine Safety and Development Branch, Mailstop E-61, 770-639-8327.
EIS Class Year of Entry: 1999
No previous EIS Conference presentations
Mackel Award consideration: No
Number of abstracts submitted: 2, priority this abstract: 1
Strong preference for poster presentation: No

Thomas M. Verstraeten, R. Davies, D. Gu, F DeStefano

**Increased risk of developmental neurologic impairment after high exposure to thimerosal-containing vaccine in first month of life.**

**Background**: Concern has risen on the presence of the ethylmercury containing preservative thimerosal in vaccines. We assessed the risk for neurologic and renal impairment associated with past exposure to thimerosal-containing vaccine using automated data from the Vaccine Safety Datalink (VSD). VSD is a large linked database from four health maintenance organizations in Washington, Oregon and California, containing immunization, medical visit and demographic data on over 400,000 infants born between '91 and '97.

**Methods**: We categorized the cumulative ethylmercury exposure from thimerosal containing vaccines after one month of life and assessed the subsequent risk of degenerative and developmental neurologic disorders and renal disorders before the age of six. We applied proportional hazard models adjusting for HMO, year of birth, and gender, excluding premature babies.

**Results**: We identified 286 children with degenerative and 3702 with developmental neurologic disorders, and 310 with renal disorders. The relative risk (RR) of developing a neurologic development disorder was 1.8 ( 95% confidence intervals [CI] = 1.1-2.8) when comparing the highest exposure group at 1 month of age (cumulative dose > 25 ug) to the unexposed group. Within this group we also found an elevated risk for the following disorders: autism (RR 7.6, 95% CI = 1.8-31.5), nonorganic sleep disorders (RR 5.0, 95% CI = 1.6-15.9), and speech disorders (RR 2.1, 95% CI=1.1-4.0) . For the neurologic degenerative and renal disorders group we found no significantly increased risk or a decreased risk.

**Conclusion**: This analysis suggests that high exposure to ethylmercury from thimerosal-containing vaccines in the first month of life increases the risk of subsequent development of neurologic development impairment, but not of neurologic degenerative or renal impairment. Further confirmatory studies are needed.

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# Appendix J

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Toxicology

# Reconsideration of the immunotherapeutic pediatric safe dose levels of aluminum

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ABSTRACT

FDA regulations require safety testing of constituent ingredients in drugs (21 CFR 610.15). With the exception of extraneous proteins, no component safety testing is required for vaccines or vaccine schedules. The dosing of aluminum in vaccines is based on the production of antibody titers, not safety science. Here we estimate a Pediatric Dose Limit that considers body weight. We identify several serious historical missteps in past analyses of provisional safe levels of aluminum in vaccines, and provide updates relevant to infant aluminum exposure in the pediatric schedule considering pediatric body weight. When aluminum doses are estimated from Federal Regulatory Code given body weight, exposure from the current vaccine schedule are found to exceed our estimate of a weight-corrected Pediatric Dose Limit. Our calculations show that the levels of aluminum suggested by the currently used limits place infants at risk of acute, repeated, and possibly chronic exposures of toxic levels of aluminum in modern vaccine schedules. Individual adult exposures are on par with Provisional Tolerable Weekly Intake "limits", but some individuals may be aluminum intolerant due to genetics or previous exposures. Vaccination in neonates and low birth-weight infants must be re-assessed; other implications for the use of aluminum-containing vaccines, and additional limitations in our understanding of neurotoxicity and safety levels of aluminum in biologics are discussed.

## 1. Introduction

Aluminum is used as an adjuvant in vaccines licensed by the US Food and Drug Administration [1–7] to enhance the immunogenicity of the vaccine in various forms (e.g., aluminum oxyhydroxide and aluminum hydroxyphosphate) [9,10] (Fig. 1). The Center for Biologics Evaluation and Research (CBER) sets the amount of aluminum per dose in biological products, including vaccines, to 850 μg aluminum if measured by assay. Two additional levels are specified by the regulations (1140 and 1250 μg respectively), depending on how the level is measured [8].

The 850 μg of aluminum per vaccine FDA amount was derived from data that demonstrated that this amount of aluminum per dose enhanced the antigenicity and effectiveness of the vaccine [9,10], but does not include safety considerations. Current amounts of aluminum are not adjusted to body weight of an infant. To avoid toxicity associated with variation in body weight between adults and children related to aluminum in vaccines, standard of care dose levels convert mg to mg/kg for the weight range being considered [28,39]. At the current time, there are no known or published studies specifically defining levels of Al in any vaccine product based on safety studies of Al.

Safety for aluminum from all sources is based on the No Observed Adverse Effect Level (NOAEL), Minimal Risk Level (MRL), and the Lowest Observed Affect Level (LOAEL) [15–20]. The Joint Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) for aluminum to be 7000 μg/kg body weight per week in 1989, which applies to all aluminum compounds in food, including additives. That level remained in effect until 2011 when the PTWI was revised to 2000 μg Al/kg per week [12,13]. The Agency for Toxic Substances and Disease Registry (ATSDR) had used an MRL of 1000 μg Al/kg per day (7000 μg/kg per week) [24–27].

We found two important errors in the provenance and derivation of provisional aluminum intake levels from World Health Organization (WHO; Supplementary Material) which, unfortunately, led to overestimation of safe exposure levels.

Here we consider adjusted child equivalent aluminum doses (CED) in vaccines by body weight, to determine putative pediatric dose limits

*Abbreviations:* NOAEL, no observed adverse effect level; LOAEL, lowest observed adverse effect level; MRL, minimal risk level; JECFA, joint expert committee on food additives; ATSDR, agency for toxic substances and disease registry; PTWI, provisional tolerable weekly intake; PDL, pediatric dose limit; CED, child equivalent dose; HED, Human Equivalent Dose

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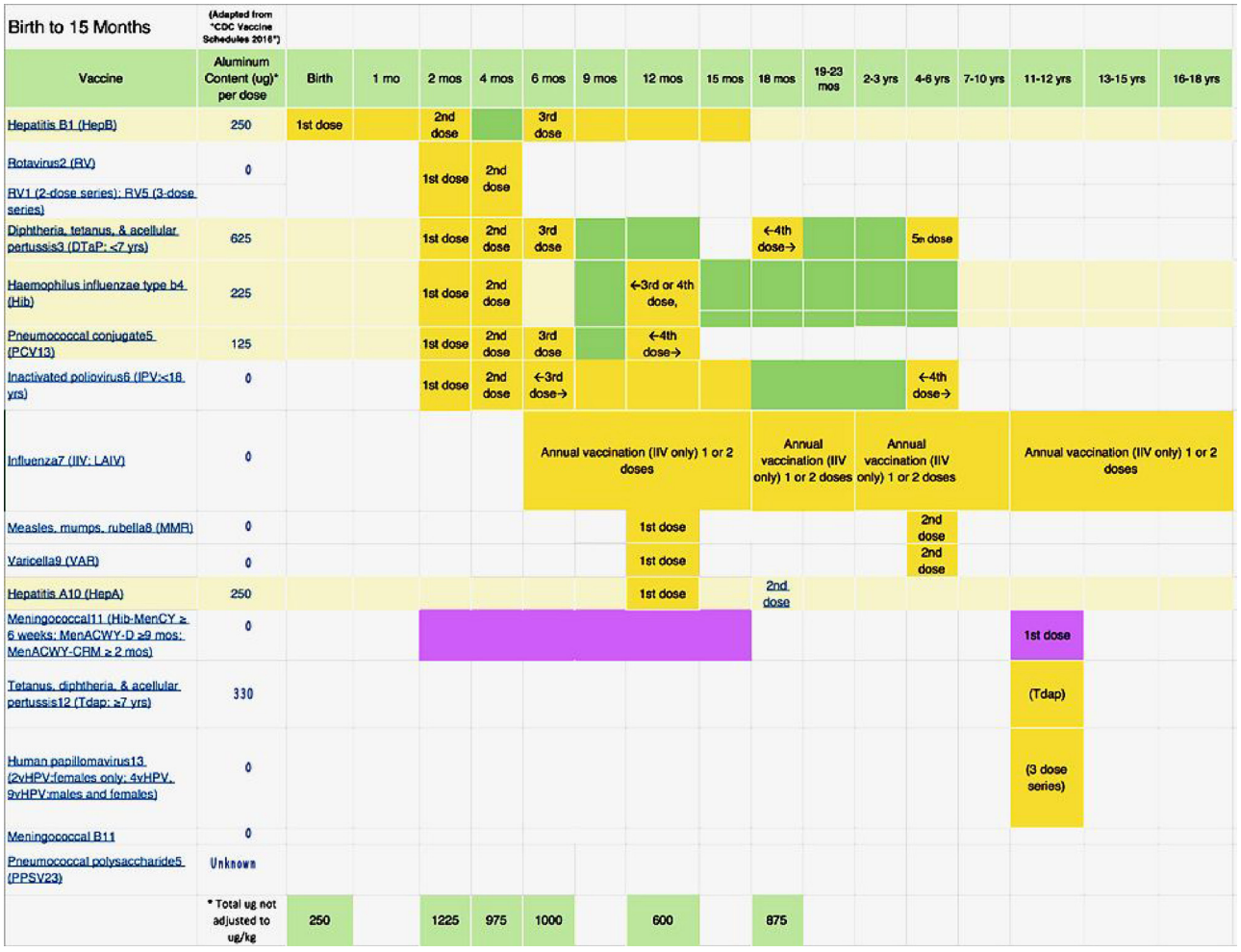


**Fig. 1.** Pediatric Vaccine Schedule 2016–2017.
The CDC schedule reflects the expected timing of administration of vaccines containing aluminum (shaded light yellow) as adjuvant at birth, 2, 4, 6, 12, and 18 months. The total amount of aluminum per vaccine visit (green shaded box below each scheduled interval) is reported from birth through 24 months.

(PDLs) of aluminum estimated by Clark's Rule for the pediatric population, to investigate further the effect those discrepancies that exist between the JECFA and ATSDR may have regarding the MRL of aluminum in biologics, and to compare relative dosing from dietary and injected sources in the pediatric population.

## 2. Materials and methods

### 2.1. FDA dose amounts of aluminum adjusted by body weight in infants and adults

FDA regulations require that proteins in vaccines be tested for safety. Aluminum is a known neurotoxin and it is unfortunate that additives in vaccines are not required to be subjected to animal safety studies prior to use on human subjects. Several known methods exist for pediatric dosing by weight. In Clark's Rule [28–39] of pediatric dose calculations, for example, the adult body weight reference is usually (as published) considered to be 150 bs. (68 kg) with the calculated dose being converted to mg/kg.

Aluminum toxicity studies use 60 kg as the reference adult body weight to calculate the MRL and LOAEL [16–18]. For that reason, we used 60 kg as the adult body weight reference rather than the more commonly used 68 kg adult body weight reference in Clark's Rule of pediatric calculations. Our calculations are thus consistent with past aluminum toxicity studies [16–18], and more comparable to the toxicities at the No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effects Level (LOAEL).

Each of the established FDA-approved doses of 850 μg, 1140 μg, and 1250 μg were converted to the equivalent dose expressed in mg/kg using Clark's Rule [28,39]:

$$Child's\ Dose\ (\mathrm{mg}) = Adult\ Dose\ (\mathrm{mg}) \times \frac{BW\,(Child)lbs}{BW\,(Adult)lbs}$$

The body weights for infants from birth through 24 months used in the Clark's Rule calculation were obtained using calculated monthly growth velocities obtained from Weight for Age standards in males and females from the 5th to the 95th percentile [40,41]. The resulting pediatric doses were compared to the same doses in an adult also adjusted by the body weight of 60 kg.

### 2.2. Minimal risk level of aluminum in children

Minimal Risk Levels (MRLs) are usually derived for hazardous substances using the NOAEL/uncertainty factor approach [16,17] to avoid toxicities [21]. The resulting exposures using the adjusted body weight calculations are presented by plotting the calculated MRL in children against the FDA doses of 850 μg adjusted by body weight at the 50th percentile in children birth through 24 months.

We estimated the human equivalent dose (HED) [11,20,21] in a child first obtaining the adult HED using the equation

HED = Animal dose NOAEL (mg/kg) × [Animal weight (kg)/Human

weight (kg)]$^{(1 - \text{BSA exponent } 0.67)}$

The HED of the NOAEL/MRL may be calculated using a $K_m$ ratio or Rule of Exponents equation [21] with a provisional additional safety factor of 10 applied. The results of these two calculations differ significantly. The anatomic compartment from which exogenous aluminum is absorbed also needs to be taken into consideration (intestinal vs. intramuscular).

The animal dose reference used by the ATSDR is 260 μg/kg and the reference animal weight of the mouse is 0.02 kg [15]. The adult human body weight reference used was 60 kg to be consistent with the previous ATSDR calculations of MRL [16,17]. A safety factor of 10 is applied to the final calculation of the adult HED to obtain the Minimal Risk Level (MRL) for an adult human [21].

To obtain the Child Equivalent Dose (CED) of the adult MRL, we multiplied the $MRL_{(adult)}$ by the body weight ratio between child and adult:

$$CED\ (mg/kg) = HED_{(adult)}\ mg/kg \times BW_{(child)}\ (kg)/BW_{(adult)}\ (kg)$$

Additionally, we calculated the pediatric equivalent of the daily provisional tolerable intake using the JECFA adult reference of 286 μg (2 μg/kg per week JECFA provisional tolerable weekly intake divided by 7 days converted to micrograms) to establish a revised and corrected provisional tolerable daily intake from the weekly intake adjusted by the BW of the child at the 5th through the 95th percentile from birth to 24 months. It should be recalled that animal levels (ATSDR and JECFA MRL) contributing to these revised estimated levels were based on enteral (dietary) exposures, and in adult animals.

The only available safety dosing reference point for for large—and small—volume parenteral exposures of aluminum is from CFR/FDA 21CFR201.323 from intravenous exposure. That safety limit is placed at 4–5 μg/kg/day, without reference to duration of treatment and applies to individuals with renal dysfunction, a condition that is very common among premature infants.

## 3. Results

### 3.1. FDA doses adjusted by body weight in infants and adults

Each of the FDA doses for aluminum (850 μg, 1100 μg, and 1250 μg) were divided by the daily body weights per percentile weight class by age from birth to 2 years and expressed as μg/kg. Similarly, these same dose limits were divided by the adult body weights of 60 kg for comparison (μg/kg).

### 3.2. FDA 850 μg dose adjusted by body weight in infants and adults

If infants were given 850 μg of aluminum (injected), the exposure would vastly exceed the only available CFR/FDA 4–5 μg/kg/day safety limit (Fig. 2). Compared to an adult whose body weight is 60 kg, a male child at birth receives 254 μg/kg, 152.7 μg/kg at 2 months, 121.4 μg/kg at 4 months, 107.1 μg/kg at 6 months, 92.8 μg/kg at 1 year, and 69.9 μg/kg at 2 years as compared to 12.5-14.2 μg/kg in an adult. A female child whose body weight is generally less than the male receives a slightly higher burden of aluminum comparatively. At the 50th percentile body weight, a male child at birth receives 1800% more aluminum per body weight as compared to a 60-kg adult male, 1074.6% at 2 months, 954.9% at 4 months, 754.2% at 6 months, 876% at 1 year, and 493% at 2 years of age more aluminum per body weight as compared to a 60-kg adult (Table 1, Fig. 2).

### 3.3. FDA 1140 μg dose adjusted by body weight in infants and adults

Compared to an adult whose body weight is 60 kg, a male child at birth receives 340.7 μg/kg, 204.8 μg/kg at 2 months, 162.8 μg/kg at 4 months, 143.7 μg/kg at 6 months, 124.4 μg/kg at 1 year, and 93.8 μg/kg at 2 years as compared to 16.8-19.0 μg/kg in an adult. Similarly, a female child whose body weight is generally less than the male receives a slightly higher burden of aluminum comparatively.

### 3.4. FDA 1250 μg dose adjusted by body weight in infants and adults

Compared to an adult whose body weight is 60 kg, a male child at birth receives 373.5 μg/kg, 224.5 μg/kg at 2 months, 178.5 μg/kg at 4 months, 157.5 μg/kg at 6 months, 136.4 μg/kg at 1 year, and 102.9 μg/kg at 2 years as compared to 18.4-20.8 μg/kg in an adult. Similarly, a female child whose body weight is generally less than the male receives a slightly higher burden of aluminum comparatively.

### 3.5. Comparison of FDA dose adjusted by body weight between infants and adults

To define an appropriate modification in the amount of aluminum per dose in a pediatric vaccine, and separate from the previous HED based upon the MRL, we applied Clark's Rule at both 68 kg and 60 kg to the 850 μg FDA dose by assay (0.85 mg per dose by assay). The calculated 850 μg per dose at the 50th percentile is lower when converting the adult body weight reference to 60 kg, the adult body weight typically used in toxicity studies (Fig. 3).

At birth, and in consideration of Clark's Rule in pediatric dosing (Adult BW = 68 kg), these calculations, based on assumptions, suggest that a child at the 50th percentile BW should receive no more than 44 μg/kg. That modification in the actual amount of aluminum per dose of a pediatric vaccine (or vaccines per day) should be at or below the current adult-based, diet-based MRL. Unfortunately, that would exceed the calculated MRL of 10.31 μg/kg at birth, and 37.48 μg/kg at 2 years of age.

### 3.6. Aluminum daily minimal risk level (MRL) in children, all sources with applied safety factor

In an adult weighing 60 kg whereby the human $K_m$ is 37 and mouse $K_m$ is 3 ($K_m$ ratio = 0.081), the Minimal Risk Level (MRL) of 26 mg Al/kg mouse dose (26) would be 26 × 0.081 = 2.11 mg/kg/day. Applying the safety factor of 10 would correct the MRL to 0.21 mg/kg/day, not 1 mg/kg/day. The application of an additional safety factor of 10 is the accepted final step prior to establishing first dose during trial dosing 12,13,15-20].

The $K_m$ in an 8-year-old child weighing 20 kg is 25 [15]. The calculated pediatric HED of the Minimal Risk Level (MRL)/NOAEL using the $K_m$ ratio formula would be 26 mg/kg times 0.12 ($K_m$ ratio = 3/25) divided by the safety factor of 10 would result in an HED of 3.12 mg Al/kg before a safety factor of 10 is applied using the $K_m$ ratio. With the safety factor of 10, the estimated MRL would be 312 μg Al/kg/day in an 8-year-old child weighing 20 kg. That would effectively lower the ATSDR MRL estimate from 1000 μg Al/kg day to 312 μg Al/kg/day by a factor of 3.2 with the safety factor applied.

Without a provisional safety factor, the MRL would be greater than the ATSDR provisional tolerable daily intake of 1 mg Al/kg per day, but less than the JECFA provisional tolerable daily intake of 290 μg Al/kg which is a concern. With the safety factor of 10, the estimated MRL in the pediatric population (< 8 years of age) is less than the 500 μg/kg body weight from all sources including additives range 100–350 μg/day identified in the JECFA report regarding children 2 years of age.

### 3.7. MRL based upon rule of Exponents/Safety factor of 10

In consideration that the HED calculated by the $K_m$ ratio may not be appropriate for use in intramuscular exposures [22,23], we used the Rule of Exponents equation [21]

HED = Animal dose NOAEL (mg/kg) × [Animal weight (kg)/Human

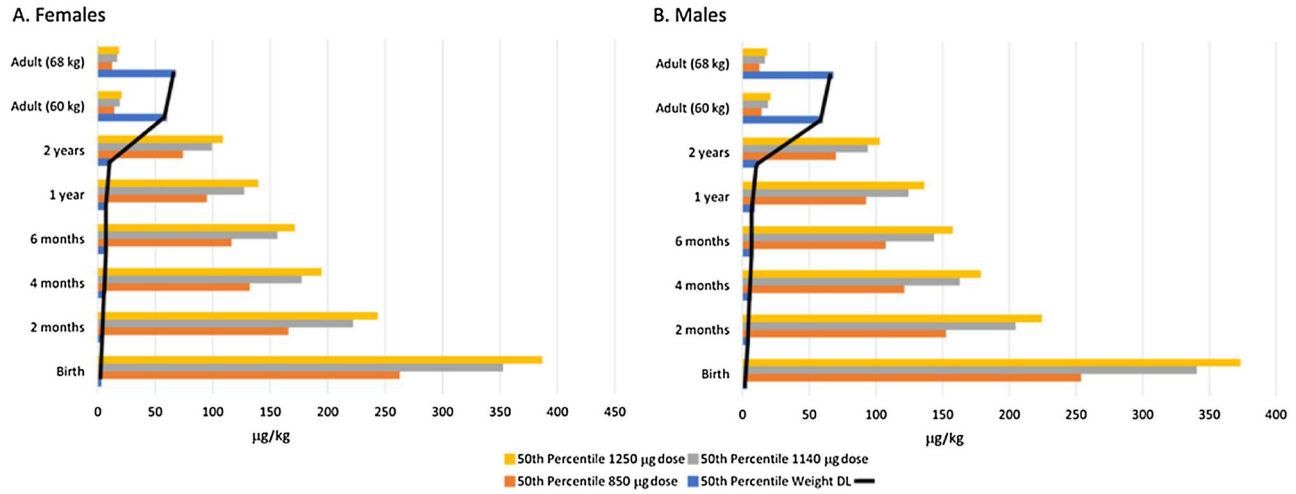


**Fig. 2.** FDA Doses and exposures adjusted by body weight: Comparison between Infants and an Adult.
In a male child from birth through 36 months at the 50th percentile body weight, the FDA dose of 850 µg adjusted by body weight demonstrates that an adult weighing 60 kg receives significantly less aluminum per injection per kg compared to a child, particularly those children with lower body weights.

**Table 1**
FDA Dose Adjusted by Body Weight (µg/kg), Birth through Adulthood, US Population.

| MALES (50th Percentile Body Weight) | | | | |
|---|---|---|---|---|
| Age | Body Weight (kg) | 850 µg dose (µg/kg) | 1140 µg dose (µg/kg) | 1250 µg dose (µg/kg) |
| Birth | 3.35 | 254.00 | 340.66 | 373.54 |
| 2 months | 5.57 | 152.67 | 204.76 | 224.52 |
| 4 months | 7.00 | 121.39 | 162.80 | 178.51 |
| 6 months | 7.93 | 107.13 | 143.67 | 157.55 |
| 1 year | 9.17 | 88.10 | 118.16 | 129.56 |
| 2 years | 12.15 | 69.95 | 93.82 | 102.87 |
| Adult Reference (60 kg) | 60 | 14.17 | 19.00 | 20.83 |
| Adult Reference (68 kg) | 68 | 12.5 | 16.76 | 18.38 |
| FEMALES (50th Percentile Body Weight) | | | | |
| Age | Body Weight (kg) | 850 µg dose (µg/kg) | 1140 µg dose (µg/kg) | 1250 µg dose (µg/kg) |
| Birth | 3.23 | 262.98 | 352.70 | 386.73 |
| 2 months | 5.13 | 165.75 | 222.30 | 243.75 |
| 4 months | 6.42 | 132.32 | 177.47 | 194.59 |
| 6 months | 7.29 | 116.49 | 156.23 | 171.30 |
| 1 year | 8.95 | 94.99 | 127.40 | 139.69 |
| 2 years | 11.48 | 74.06 | 99.92 | 108.91 |
| Adult Reference (60 kg) | 60 | 14.17 | 19.00 | 20.83 |
| Adult Reference (68 kg) | 68 | 12.5 | 16.76 | 18.38 |

weight (kg)]$^{(1 - \text{BSA exponent } 0.67)}$

In an adult weighing 60 kg, the calculated HED using the above equation would be 1850 µg/kg without a safety factor of 10. Applying the safety factor of 10 would result in the HED (MRL) of 185 µg/kg, significantly lower than the current ATSDR MRL/NOAEL of 1000 µg Al/kg/day. This approximates the corrected 2007 JECFA calculation of 140 µg/kg/day (PTWI of 1000 µg Al/kg per week).

We do not mean to imply this level exposure is safe for pediatric injection. The corresponding HED in a child should take into consideration the ratio of the $BW_{(child)}/BW_{(adult)}$, such that $MRL_{(child)}$ = Adult MRL(mg/kg) X $BW_{(child)}/BW_{(adult)}$.

At birth, for 50th percentile body weight males the daily MRL would be 16.01 µg/kg/day (0.01601 mg/kg/day) and 58.12 µg/kg/day at 2 years (See Supplemental Files). As expected, a female child would have a corrected value of 15.46 µg/kg/day at birth and 54.9 µg/kg/day at 24 months. In a child, that recalculated MRL would be less than the 1989 JECFA provisional tolerable daily intake from dietary and additive exposures of 140 µg/kg/day and current provisional tolerable daily intake of 290 µg/kg/day per day both before and after the safety factor of 10 is applied (Fig. 3).

As an example, using a specific vaccine, the weight-adjusted MRLs and aluminum exposures from DTaP vaccine (with 625 µg aluminum per dose) show exposures in children at 2 months that vastly exceed the dietary adult mouse-derived MRL considering body weight (Fig. 4).

## 4. Discussion

Aluminum in various forms is commonly used as an adjuvant in many vaccines licensed by the US Food and Drug Administration [1–7]. In 2002, the scheduled childhood vaccines that included aluminum as an adjuvant were limited to Diphtheria-Pertussis-Tetanus (DPT) and Hepatitis B (HepB). The amount of aluminum per vaccine dose ranged from 250 µg/dose (HepB) to 625 µg/dose (DPT). In 2016, however, the number of individual pediatric vaccines containing aluminum as adjuvant from birth to 36 months has increased significantly and ranges from 250 to 625 µg/dose [3–6] (Table 1; Fig. 1). Those vaccines, which contain aluminum as an adjuvant to increase antigenicity [33,34] include Hepatitis B (HepB)[2], Diphtheria, Tetanus, acellular Pertussis (DTaP) [3], Haemophilus influenza B (HiB) [4], Hepatitis A (HepA) [5], and pneumococcal conjugate (PCV13) [6]. Here, we further discuss the background and provenance of the derivation of aluminum doses, issues that may be currently causing unanticipated dose-related toxicity.

The FDA referenced doses of 850 µg, 1140 µg, and 1250 µg have not been estimated considering body weight of the pediatric population, nor do they necessarily directly reflect established non-toxic doses in that population prior to this report. The current aluminum amounts in vaccines are not sufficiently characterized: the doses of aluminum used in vaccines, and the per day exposure that results from the CDC vaccine schedule, are not determined based on animal dose escalation safety (NOAEL) studies. They are also determined considering neither injected dose-related toxicity, nor mass differences between adults and infants. This issue must be addressed.

Our results demonstrate that the aluminum exposure from vaccines would exceed the calculated Pediatric Dose Limit, or PDL 850 µg aluminum/dose by assay, when corrected to 44 µg by Clark's Rule estimated from the FDA adult dose of 850 µg/dose (850 µg $x\ BW_{(child)}$ 3.35/$BW_{(Adult)}$ 68 $kg$) at birth, 2.5, 4.5, and 6.5 months. It must be emphasized that at birth, only the aluminum content in the HepB vaccine is under consideration. Only at 6.5 months does the combined aluminum

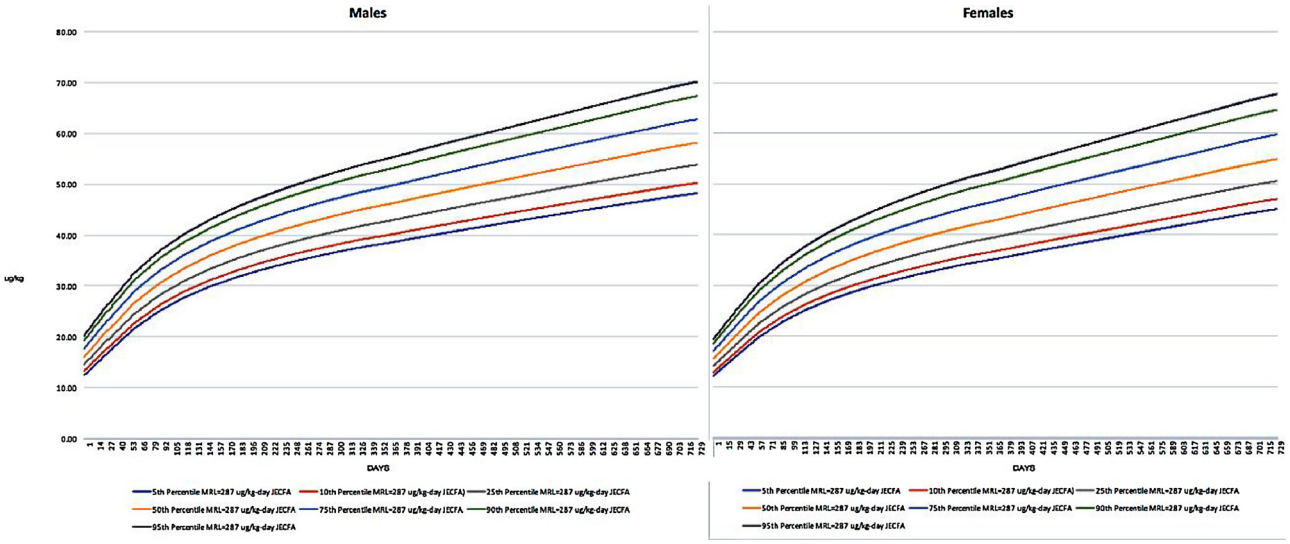


**Fig. 3.** MRL Males and Females, Birth-24 years (5th-95th Percentile Body Weight).
The most recent daily JECFA MRL of 287 μg/kg/day in a child from birth through 24 months (729 days) was calculated by multiplying the daily Child BW/Adult BW ratio using a referenced adult body weight of 60 kg and the daily child body weight at the 5th-95th percentile. The final calculation is expressed in μg/kg/day (Y-axis). The 50th percentile body weight daily calculation is demonstrated with the orange line (see Legend at bottom of graph).

level fall below the 1140 and 1250 μg/kg calculated dose level at the 50th percentile body weight. This is a clearly significant concern for the current vaccine schedule, especially in the context of the recommended (and increasingly strongly enforced) time intervals at birth, 2, 4, and 6 months.

All individual doses are at or below the FDA dose of 850 μg/dose by assay. However, when administered simultaneously at the recommended CDC schedule, the "limit" is significantly exceeded if not modified in accordance with standard pediatric dose calculations. These doses are given regardless of body weight. The product data sheet for DTaP states, for example:

> "Each 0.5-mL dose contains aluminum salts as adjuvant not more than 0.85 mg (850 μg) aluminum by assay"

When adjusted to body weight (μg/kg) and compared to a 60–68 kg adult, the aluminum load is significantly higher in the birth through 24-month age cohort.

The scheduled pediatric vaccinations in 2016 have significantly expanded since 2002, and the amount of aluminum per vaccine dose, particularly the use of TDaP, has changed. The combined doses of aluminum at 2, 4, and 6 months are 1225 μg, 975 μg, and 1225 μg respectively (Table 2), and are not determined considering infant and

**Table 2**
ATSDR References for NOAEL and LOAEL.

| Population | Year Published | Route of Exposure | NOAEL | LOAEL | Reference |
|---|---|---|---|---|---|
| Mice | 1989 | Dietary | 62 mg Al/kg | 130 mg Al/kg | Golub et al. [24] |
| Mice | 2001 | Dietary | 26 mg Al/kg | 130 mg Al/kg | Golub et al. [25] |
| Mice | 2005 | Dietary | 53 mg Al/kg | 103 mg Al/kg | Colomina et al. [26] |
| Mice | 2000 | Dietary | – | 100 mg Al/kg | Golub et al. [27] |

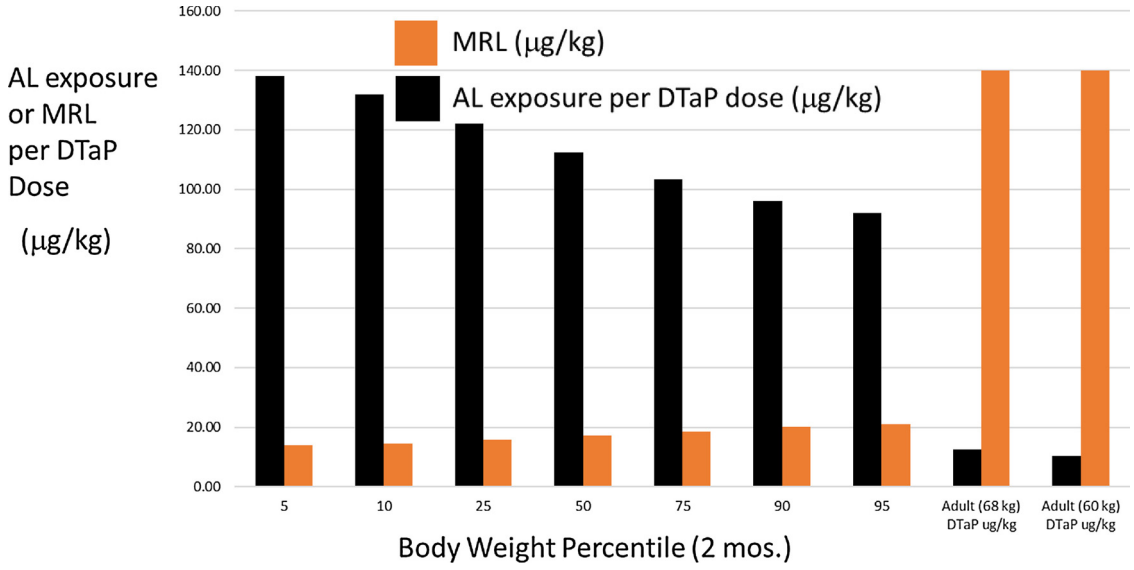


**Fig. 4.** Comparison of the Calculated Pediatric MRL and the AL Exposures from DTaP Vaccine for Children (and Adults) using Clark's Rule to Accommodate Pediatric Body Weights (μg/kg, per day, at 2 months and for Adult).

child body weight.

When expressed considering infant and child body weight ($BW_{(child)}$) obtained from the CDC growth data sheets, the individual aluminum levels (μg/kg) in the HepB, DTaP, Hib, and PCV vaccines remain below the limits of 850 μg/kg, 1440 μg/kg, and 1225 μg/kg at birth. However, at 2.5 months, 4.5 months, and 6.5 months, the combined aluminum levels (μg/kg) in the scheduled DTaP, HiB, and PCV vaccines exceed the FDA 850 μg limit by a factor of 1.15.

### 4.1. The PTWI propagated error

In our review of the provenance of information on Al limits, we discovered an unfortunate but serious error in the calculation in the MRL. The JECFA established a PTWI for aluminum to be 7000 μg/kg body weight per week in 1989. The PTWI applied to all aluminum compounds in food, including additives which remained in effect until 2011. The provisional tolerable daily intake (all sources) would therefore have been around 1000 μg/kg/day.

From 1989 to 2011, the MRL was reported to be 1000 μg/kg/day from all sources. That number was withdrawn in 2011, therefore the total provisional tolerable daily intake should be currently 0.29 mg Al/kg per day, based upon the provisional tolerable weekly intake (PTWI) of 2 mg Al/kg week as expressed by the Joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) in 2011.

In 1996, the Committee on Nutrition in their article on aluminum neurotoxicity in children reported the 1989 JECFA provisional tolerable weekly intake of 1000 μg/kg [12] as a provisional daily intake [14]. Unfortunately, that error overestimates the provisional tolerable daily intake of aluminum from all sources in adults by a factor of at least 2. As the 1000 μg/kg/week PTWI was in fact replaced with a PTWI of 2000 μg/kg/week in 2011, the daily provisional tolerable intake should be around 286 μg/kg per day, and in consideration that the highest mean intake of a child at 2 years is 500 μg/kg per day [15]. The value 1000 μ/kg/day would seem to bring the 850 μg per dose into range, but it is off by a factor of at least 2 and perhaps seven. The role of the reliance on the incorrect PTWI on public health may be significant, especially for infants, especially for low-birthweight infants and those born prematurely.

By our calculations, and in consideration of the route of exposure using the Rule of Exponents to calculate the HED, the correct daily (all sources, all doses) MRL in the pediatric population should have been determined to be no more than 10.31-16.01 μg/kg per day at birth to 58.12 μg/kg per day at 2 years of age. Current exposures from pediatric vaccines exceed these levels; for a median weight (US) 3.3 kg male, HepB vaccine with 250 μg leads to 75.75 μg/kg/day. The two-month vaccination visit repeats the excess. Excess exposures in low birthweight and neonatal infants is obviously even more problematic. The use of HepB vaccine in a 2-kg infant (FDA's unofficial cut-off for vaccination in the Neonatal Intensive Care Unit, NICU) leads to 150 μg/kg/day. Vaccination practices in the NICU must be revisited.

Our results demonstrate that the aluminum load from vaccines would exceed the estimated PDL 850 μg aluminum/dose by assay, when corrected to 47.4 μg by Clark's Rule estimated from the Federal adult dose limit of 850 μg/dose (850 μg $x$ $BW_{(child)}3.35$ kg/$BW_{(Adult)}$ 68 kg) at birth to 24 months. The adjusted dose limits would still be higher than the calculated MRL per day.

The NOAEL and LOAEL have been established to reduce the incidence of known harmful neurotoxic effects and are based on studies of adult mice using poorly-absorbed, ingested aluminum not highly-absorbed injected aluminum. The entire paradigm to aluminum dosing in vaccines has not been determined considering body weight, based on NOAEL (not the LOAEL), which is more in line with the universal standard medical practices during pediatric dosing [28,39]. These should be calculated per child given their body weight prior to vaccination, and daily limits placed on total aluminum injected considering all doses and all sources. However, even when the appropriate and necessary adjustments are made, our results predict an increased risk of neurotoxicity from birth through 36 months particularly when the accumulating body burden is taken into consideration at every scheduled vaccine interval. Although not considered in this current analysis, we are aware that the accumulated aluminum body burden at each vaccination interval will be higher than an individual aluminum level in a single vaccine. This is because there will be a retained body burden fraction of aluminum resulting from the previous dosing intervals considering body weight (in progress) that needs to be considered, particularly in consideration of potential toxicities.

Mitkus et al. [32]'s calculations were based on the day/week propagated error. Mitkus et al. [32] published their study in 2011 when the PTWI was still at 1 mg/kg, further propagating the day/week error. Thus, current assessments of aluminum accumulation from vaccination and dietary exposures are not correct. Some dietary sources contain unacceptably high levels of aluminum, such as certain brands of antacids. Concerned individuals can exercise consumer choice and avoid food products that include aluminum.

While the effect of our proposed reduction on the final antigenicity of the vaccine is unknown, the full effects of the high injected doses of aluminum on the developing brain are also unknown. Indications of accumulation of aluminum associated with autism were recently published [42] in which the majority of tissue samples from post-mortem brains of patients diagnosed with autism spectrum disorder (ASD) were found to contain high concentrations of aluminum. Many samples contained extremely high concentrations, and the study also localized aluminum in glial cells in the brain, consistent with aluminum-induced gliosis models of neurodevelopmental disorders.

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## Conflict of interest

RR has no real or potential conflict of interest. JLW has a potential conflict of interest as he has consulted on two vaccine injury cases on behalf of complainants.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2018.02.025.

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# Appendix K

Neil Z. Miller, 2016

# Aluminum in Childhood Vaccines Is Unsafe

Neil Z. Miller

**ABSTRACT**

Aluminum is a neurotoxin, yet infants and young children are repeatedly injected with aluminum adjuvants from multiple vaccines during critical periods of brain development. Numerous studies provide credible evidence that aluminum adversely affects important biological functions and may contribute to neurodegenerative and autoimmune disorders. It is impossible to predetermine which vaccinated babies will succumb to aluminum poisoning. Aluminum-free health options are needed.

## Introduction

From 1999 through 2002, several vaccines containing mercury were phased out of the childhood immunization schedule. Manufacturing of childhood vaccines with thimerosal ceased in 2001, but those that were not past their expiration date remained on the market for sale until January 2003.[1] They were replaced with low-mercury or "thimerosal-free" vaccines. In the years that followed, autism rates continued to rise, prompting health authorities to assert that autism is not linked to mercury in vaccines and that vaccination policies are safe and appropriate.[2-4] (If mercury in vaccines contributed to autism, then rates should have dropped after mercury was removed.) However, in 2002, during this so-called phase-out period, the Centers for Disease Control and Prevention (CDC) actually added two doses of mercury-containing influenza vaccines to the list of inoculations urged for all babies 6 to 23 months of age.[5] Two years later, the CDC also added *pregnant women in their first trimester* to the list of people officially recommended and actively encouraged to receive influenza vaccines, even though a majority of available doses contained mercury.[6]

In addition to these questionable actions during this highly publicized "phase-out" of mercury, four doses of a new vaccine with high *aluminum* content were added to the childhood immunization schedule in February 2000 (for pneumococcus) and two doses of another aluminum-containing vaccine (for hepatitis A) were added in 2005.[7,8] These changes to the vaccine schedule resulted in a substantial increase of aluminum-containing vaccine doses—from 10 to 16 injections—that babies are still mandated to receive by 18 months of age.

Prior to the mercury phase-out (pre-2000), babies received 3,925 micrograms (mcg) of aluminum in their first year-and-a-half of life. After pneumococcal and hepatitis A vaccines were added to the immunization schedule, babies began receiving 4,925 mcg of aluminum during the same age period—a 25% increase (Figure 1).[9,10] In 2011, CDC recommended that pregnant women receive a pertussis vaccine (Tdap), which also contains aluminum.[11] Studies show that aluminum crosses the placenta and accumulates in fetal tissue.[12] Thus, millions of babies in utero, infants, and young children were injected with, and continue to receive, unnaturally high doses of neurotoxic substances—mercury and aluminum—long after unsuspecting parents were led to believe that vaccines were purified and made safe.

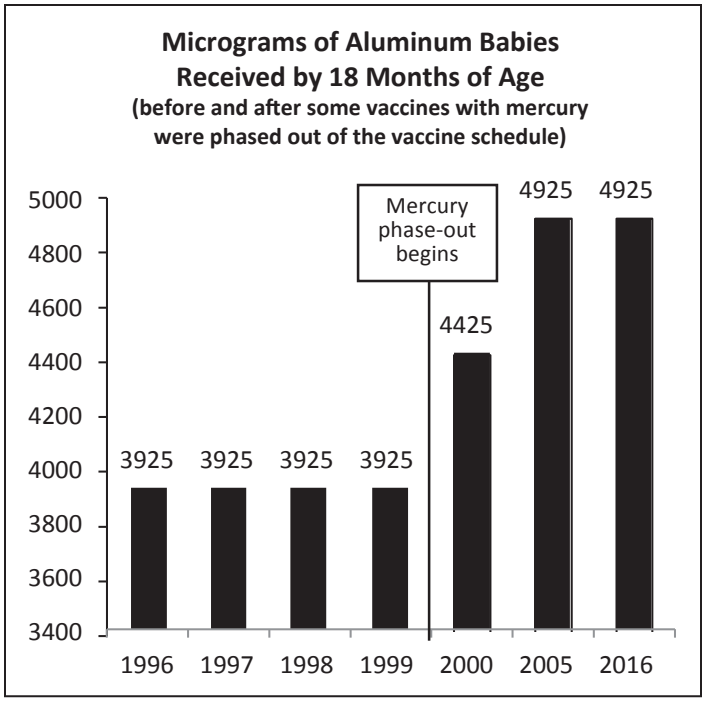


**Figure 1.** Aluminum Content from Childhood Vaccines

Vaccines containing aluminum were added to the childhood immunization schedule when some vaccines containing mercury were removed. Prior to the mercury phase-out (pre-2000), babies received 3,925 mcg of aluminum by 18 months of age. After pneumococcal and hepatitis A vaccines were added to the schedule, babies began receiving 4,925 mcg of aluminum during the same age period—a 25% increase.

Source: The vaccine manufacturers' product inserts and the CDC's annual childhood vaccination schedules.

## Aluminum

Aluminum adjuvants are added to several vaccines to elicit a more robust immune response and increase vaccine efficacy. In the United States, Canada, Europe, Australia, and many other parts of the world, infants and young children receive high quantities of aluminum from multiple inoculations. For example, in the U.S. the hepatitis B, DTaP (for diphtheria, tetanus and pertussis), pneumococcal (PCV), *Haemophilus influenzae* type b (Hib), and hepatitis A vaccines are all administered during early childhood. Each of these

vaccines contains aluminum, and multiple doses (booster shots) are required (Table 1). Babies are injected with 1,225 mcg of aluminum instantaneously at age 2 months, and 4,925 mcg of accumulated aluminum by age 18 months (Figure 2).[9,10]

**Table 1.** Aluminum Exposures in Early Childhood from Recommended Vaccines

| Vaccine | Aluminum Content | Vaccine Schedule |
|---|---|---|
| Hep B | 250 mcg x 3 doses | Birth, 2, 6 months |
| DTaP | 625 mcg x 4 doses | 2, 4, 6, 15 months |
| PCV | 125 mcg x 4 doses | 2, 4, 6, 12 months |
| Hib | 225 mcg x 3 doses | 2, 4, 12 months |
| Hep A | 250 mcg x 2 doses | 12, 18 months |

Source: The vaccine manufacturers' product inserts and the CDC's 2016 childhood vaccination schedule.

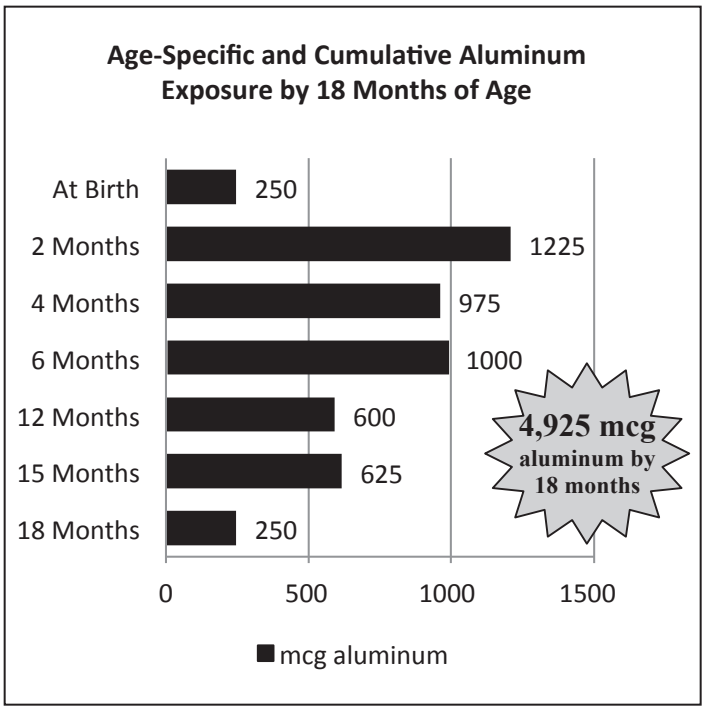


**Figure 2.** Cumulative Aluminum Exposure from Recommended Childhood Vaccines

Source: The vaccine manufacturers' product inserts and the CDC's 2016 childhood vaccination schedule.

Babies are not the only age group exposed to high quantities of aluminum from vaccines. The HPV vaccine (indicated for the prevention of cervical cancer and genital warts associated with some strains of human papillomavirus) is marketed to pre-teens and adolescents. Each dose in the three-dose series contains 500 mcg of aluminum. The Tdap vaccine (for tetanus, diphtheria, and pertussis) is given to pre-teens as well, and contains 390 mcg of aluminum.[13] Several adult vaccines also contain aluminum.

Aluminum is neurotoxic and has a long history of well-documented hazards.[14] For example, as early as 1921 The *Lancet* described a 46-year-old metal worker in whom "aluminium produced a rather slow intoxication. In this case it caused memory loss, tremor, jerky movements and incontinence of urine."[15] In 1927, Dr. Victor Vaughn, a toxicologist with the University of Michigan, testified before the Federal Trade Commission that "all salts of aluminum are poisonous when injected subcutaneously or intravenously."[16] By 1951, Chusid et al. showed that chronic epilepsy could be induced in monkeys through intra-cerebral administration of aluminum hydroxide cream.[17] In 1968, Driver et al. performed a similar experiment by placing aluminum hydroxide cream unilaterally on the posterior parietal cortex of six monkeys.[18] From 3 to 8 weeks after surgery, electrical abnormalities could be seen on an electroencephalogram and the monkeys exhibited "episodic twitching of the limbs and face." The animals were also impaired at learning new tasks and at re-learning tasks first learned prior to the intervention.

According to the American Academy of Pediatrics (AAP), "Aluminum is now being implicated as interfering with a variety of cellular and metabolic processes in the nervous system and in other tissues."[19] Bishop et al. published data showing that "aluminum accumulates in the body when protective gastrointestinal mechanisms are bypassed, renal function is impaired, and exposure is high."[20] For example, in premature infants, "prolonged intravenous feeding with solutions containing aluminum is associated with impaired neurologic development" by 18 months of age. More recently, Kawahara et al. published research confirming that "aluminum can cause severe health problems in particular populations, including infants."[21] The authors of this paper also declared that "whilst being environmentally abundant, aluminum is not essential for life. On the contrary, aluminum is a widely recognized neurotoxin that inhibits more than 200 biologically important functions and causes various adverse effects in plants, animals, and humans."

## Neurologic and Autoimmune Disorders

Numerous studies provide compelling evidence that injected aluminum is detrimental to health. For example, a recent paper by Tomljenovic and Shaw affirmed that aluminum is a neurotoxin and may be a co-factor in several neurodegenerative disorders and diseases, including Alzheimer's, Parkinson's, multiple sclerosis, amyotrophic lateral sclerosis (ALS), autism, and epilepsy.[22] According to the authors, "The continued use of aluminum adjuvants in various vaccines for children as well as the general public may be of significant concern. In particular, aluminum presented in this form carries a risk for autoimmunity, long-term brain inflammation and associated neurological complications and may thus have profound and widespread adverse health consequences."

Recent data by Perricone et al. showed that aluminum adjuvants in vaccines have been linked to multiple sclerosis, systemic lupus erythematosus, chronic fatigue syndrome, Gulf War syndrome, macrophagic myofasciitis, arthritis, and autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome), an autoimmune disease with neurological and cognitive manifestations.[23] Clinical symptoms associated with vaccine-induced autoimmunity can take months or years to manifest, much longer than the time intervals utilized in most vaccine safety studies.

Although aluminum is a neurotoxin, pre-school children are repeatedly injected with aluminum adjuvants from multiple vaccines during critical periods of brain development. A recent paper published in the journal *Lupus* found that this may lead to neuro-developmental and autoimmune disorders.[24] During early development, the child's blood-brain barrier is more permeable to toxins, and the kidneys are less able to eliminate them. Thus, children have a greater risk than adults of adverse reactions to aluminum adjuvants in vaccines. The authors of this paper issued the following warning: "Because children may be most at risk of vaccine-induced complications, a rigorous evaluation of the vaccine-related adverse health impacts in the pediatric population is urgently needed."

**Macrophagic Myofasciitis (MMF)**

Some people develop macrophagic myofasciitis (MMF) after receiving an aluminum-containing vaccine.[25-39] MMF is characterized by an aluminum-filled lesion (wound) at the site of an earlier vaccination. MMF lesions occur when the aluminum adjuvant from a vaccine remains embedded in the muscle tissue and causes a continuous immune reaction. The lesions are persistent, long-term granulomas (or inflammatory tumors) found in the quadriceps in children and deltoid muscles of adults, common vaccination sites. Several vaccines contain aluminum hydroxide, which has been identified as the causal factor of MMF lesions.[25]

Although MMF is associated with a macrophagic lesion at the site of vaccination, it is a systemic ailment. Symptoms include chronic fatigue, chronic diffuse myalgia (muscle weakness), arthralgia (joint pain), and disabling headaches. Aluminum's toxic effects can also manifest as impaired psychomotor control, repetitive behavior, speech disorders, sleep disturbances, seizures, confusion, and anxiety, as well as deficits of concentration, learning, and memory. Nearly 20% of patients with MMF develop an autoimmune disease, including neuromuscular and multiple sclerosis-like demyelinating disorders.[26-28]

Several descriptive studies document MMF in pediatric populations. For example, Spanish scientists presented data on seven children younger than 3 years of age with lesions of macrophages on muscle biopsies at the site of vaccination.[29] In three of four cases tested, elevated levels of aluminum in muscle were detected (indicative of a reaction to aluminum adjuvants in vaccines). All of the children developed hypotonia (a lack of normal muscle tone) and motor or psychomotor delay. Six of the children also had abnormal neuro-imaging, associated with neurological anomalies, including atrophy and abnormal myelination.

In the U.S., Gruis et al. evaluated four cases of MMF in young children with hypotonia, motor delay and failure to thrive, likely due to intramuscular injections of aluminum-containing vaccines.[30] Another team of American physicians evaluated MMF in two fully vaccinated children. Both showed typical aluminum-filled macrophages at muscle biopsies.[31] One child had abnormal pupillary reflexes and urinary retention suggesting dysautonomia while the other child had developmental delay and hypotonia.

Israeli researchers documented MMF in six Arab children.[32] Reactions included hypotonia, seizures, motor delay, and developmental delay. The authors of this paper believe that genetic predisposition is a factor in determining the prevalence of MMF in different populations.

German researchers documented MMF in a 3-month-old East Indian child following his hepatitis B vaccine at birth, "after which he developed generalized hypotonia, and central nervous system and peripheral nervous system manifestations at one month of age."[33] The child also had respiratory failure, decreased spontaneous movements, apnea spells, and generalized seizures. Aluminum was detected in the muscle biopsy macrophages. The authors recommend that "after vaccination, children should be closely followed to detect these complications at early stages."

Italian researchers believe that MMF in children "is probably more common than reported. Diagnosis requires a high index of suspicion and can be missed if biopsy is performed outside the vaccination site."[34] According to Canadian MMF researchers, "aluminum has been demonstrated to impact the central nervous system at every level, including by changing gene expression. These outcomes should raise concerns about the increasing use of aluminum salts as vaccine adjuvants." Moreover, "based on the current and emerging literature, it seems unlikely that in the future aluminum will be considered safe for human use in any of the current medicinal applications."[28]

**Animal Studies**

A recent paper by Luján et al. found that sheep developed a new type of autoimmune and inflammatory disorder—ovine autoimmune/inflammatory syndrome induced by adjuvants (ASIA)—after receiving vaccines containing aluminum adjuvants.[40] The condition appears in some sheep two to six days after they are vaccinated. Symptoms of the acute phase include poor response to external stimuli and acute meningoencephalitis. The chronic phase causes muscular atrophy, neurodegeneration of the gray matter of the spinal cord, and death.

Khan et al. conducted several mouse experiments to determine the long-term biological distribution of vaccine-related aluminum nanoparticles.[41] They discovered that aluminum travels from the injection site to distant organs such as the spleen and brain, where aluminum deposits could still be detected one year later. Aluminum remains in monocyte-lineage cells long after vaccination and may cause neurologic and autoimmune disorders. According to these scientists, "Alum has high neurotoxic potential, and administration of continuously escalating doses of this poorly biodegradable adjuvant in the population should be carefully evaluated by regulatory agencies since the compound may be insidiously unsafe."

Scientists also looked at whether Gulf War Syndrome, which afflicted many veterans of Western militaries with cognitive and behavioral deficits similar to ALS (a progressive neurodegenerative disease that destroys nerve cells), could be related to the aluminum-containing anthrax vaccines they received. In a series of studies, mice were injected with adjuvants at doses equivalent to those given to vaccinated U.S. Gulf War veterans.[42,43] The aluminum-injected mice exhibited significant deficits in memory and motor functions. Testing showed motor neuron loss and progressive deficiencies in strength. The mice also had pathological abnormalities that are characteristic of neurological diseases such as Alzheimer's and dementia. According to the authors of these studies, "The demonstrated neurotoxicity of aluminum hydroxide and its relative ubiquity as an adjuvant suggest that greater scrutiny by the scientific community is warranted."[43]

Israeli scientists recently evaluated an aluminum adjuvant and the HPV vaccine Gardasil to determine behavioral and inflammatory effects.[44] Female mice were injected with either aluminum or Gardasil in amounts equivalent to human exposure, or they received a true placebo. (Vaccine safety trials for the HPV vaccine did not provide the control group with an inert substance or true placebo; the "control" group was injected with aluminum.) The Gardasil and aluminum-injected mice spent significantly more time exhibiting depressive behavior when compared to the placebo-injected mice. In addition, anti-HPV antibodies from the sera of Gardasil-injected mice showed cross-reactivity with the mouse brain protein extract. Analysis revealed microglial activation in the hippocampi of Gardasil-injected mice. According to the authors, "It appears that Gardasil via its aluminum adjuvant and HPV antigens has the ability to trigger neuroinflammation and autoimmune reactions, further leading to behavioral changes."

## Autism

There is evidence that aluminum in vaccines may be linked to autism. For example, the *Journal of Inorganic Biochemistry* published data showing a highly significant positive linear correlation between the amount of aluminum infants receive from their vaccines and the rates of autism in several developed nations (Pearson r = 0.89-0.94).[45] The authors of this ecological study commented on their findings: "Our results...suggest that a causal relationship may exist between the amount of aluminum administered to preschool children at various ages through vaccination and the rising prevalence of autism spectrum disorders."

In another recently published paper, Shaw et al. found that genetic predispositions may sensitize some children to central nervous system damage induced by aluminum-containing pediatric vaccines.[46] Moreover, vaccines with aluminum adjuvants are *injected* into the body, bypassing protective barriers of the gastrointestinal tract and skin. Absorption of aluminum by this mode is more efficient than through ingestion, increasing the likelihood of a toxic outcome. The authors summarized their findings: "Evidence has now emerged showing that autism may in part result from early-life immune insults induced by environmental xenobiotics. One of the most common xenobiotic with immuno-stimulating as well as neurotoxic properties to which infants under two years of age are routinely exposed worldwide is the aluminum vaccine adjuvant."

Recent research published in the *Journal of Toxicology* found that aluminum exposure produces adverse effects in living organisms and is especially damaging to the central nervous system.[47] Aluminum from vaccine adjuvants crosses the blood-brain and blood-cerebrospinal fluid barriers, provoking harmful immuno-inflammatory responses in neural tissues. Yet, clinical studies on vaccine safety often give aluminum-containing injections to a "control" group as a harmless "placebo" despite evidence that aluminum is toxic to humans and animals. The use of aluminum as a placebo cannot be justified. According to the authors of this paper, "Studies on animal models and humans have shown that aluminum adjuvants by themselves cause autoimmune and inflammatory conditions. These findings plausibly implicate aluminum adjuvants in pediatric vaccines as causal factors contributing to increased rates of autism spectrum disorders in countries where multiple doses are almost universally administered."

In another recent animal study, young mice were injected with either high or low levels of aluminum adjuvants (designed to correlate with U.S. or Scandinavian childhood vaccine schedules).[48] Significant changes in the mice were observed, affirming the role of aluminum adjuvants in adversely altering the central nervous system. The authors commented on their findings: "These current data implicate aluminum injected in early postnatal life in some central nervous system alterations that may be relevant for a better understanding of the etiology of autism spectrum disorders."

## Vaccine Industry Conferences and Concerns

In May 2000—3 months *after* the CDC added the aluminum-containing pneumococcal vaccine to the recommended immunization schedule for children—the U.S.

Department of Health and Human Services (HHS) sponsored a Workshop on Aluminum in Vaccines.[49,50] The workshop, given in San Juan, Puerto Rico, was attended by members of the vaccine industry, including government officials, immunologists, pathologists, vaccine manufacturers, metal ion specialists, and other interested people. It was organized to increase knowledge about aluminum as an adjuvant in vaccines, investigate potential adverse reactions associated with aluminum in vaccines, and develop a research agenda on the effect of aluminum in the human body. Experts from around the world were invited to give their presentations on vaccines and aluminum.

Dr. Romain Gherardi, a specialist in neuromuscular disease and professor at the Mondor Institute of Biomedical Research, showed that MMF without vaccination does not occur. In fact, it often begins after receiving a hepatitis B vaccine. Myalgia was present in 94% of patients with MMF, and 85% of these people were disabled. Although 30% of patients had their first myalgias within 3 months after their last vaccination, 20% of patients' symptoms took longer than 2 years to manifest. These myalgias begin in the calves and legs, then progress to diffuse myalgia. Fatigue was present in 93% of patients with MMF, and 87% of these people were disabled. In addition, 34% of MMF patients had autoimmune disease, including multiple sclerosis and arthritis.[50, pp 48-74]

In June 2000, the CDC sponsored a conference on thimerosal (mercury) in vaccines, although aluminum was discussed as well.[51] CDC scientists analyzed the agency's Vaccine Safety Datalink (VSD) database containing thousands of medical records of vaccinated children and found statistically significant relationships between mercury in vaccines and developmental delay, tics, and attention deficit disorder.[51, pp 40-41] However, Dr. Tom Verstraeten, CDC epidemiologist, analyzed the data and determined that the injuries could have been caused by aluminum in the vaccines.[51, p 77] It is also possible that the neurological damage was due to the synergistic effects of both aluminum and mercury in the vaccines given to the affected children.

Although millions of children every year are required to receive vaccines containing aluminum and mercury, evidence supporting the safety of this practice is lacking. For example, according to Dr. Richard Johnston, immunologist and professor of pediatrics at the University of Colorado School of Medicine, "Aluminum and mercury are often simultaneously administered to infants, both at the same site and at different sites. However...there is absolutely no data, including animal data, about the potential for synergy, additivity or antagonism, all of which can occur in binary metal mixtures."[51, p 20] Dr. Alison Maule, who attended the Workshop on Aluminum in Vaccines, voiced similar concerns: "We need to bear in mind that we are not only putting aluminum in here, we are putting in mercury.... Often these effects are additive but there is always the possibility of synergy. We know nothing about that."[50, p 106] Dr. Vito Caserta, chief medical officer for the Vaccine Injury Compensation Program, had this to say: "One of the things I learned at the aluminum conference in Puerto Rico…that I never really understood before, is the interactive effect of different metals when they are together in the same organism. It is not the same as when they are alone, and I think it would be foolish for us not to include aluminum as part of our thinking with this."[51, p 234] Dr. William Weil, pediatrician, former member of the National Institutes of Health, and representative for the AAP Committee on Environmental Health, was also present at the CDC conference and made his concerns known: "In relationship to aluminum, being a nephrologist for a long time, the potential for aluminum and central nervous system toxicity was well established by dialysis data. To think there isn't some possible problem here is unreal."[51, pp 24-25]

Some health authorities who oversee federal vaccine initiatives candidly acknowledge their limited understanding of metals—aluminum and mercury—that are added to several vaccines. For example, Dr. Martin Myers, director of the National Vaccine Program Office and host of the HHS-sponsored Workshop on Aluminum in Vaccines, made a frank admission: "Perhaps the most important thing that I took away from the last meeting was that those of us who deal with vaccines have really very little applicable background with metals and toxicological research."[49, pp 1-2] Dr. Neal Halsey, director of the Institute for Vaccine Safety, Johns Hopkins Bloomberg School of Public Health, and former member of the CDC's Advisory Committee on Immunization Practices (ACIP), was also present at the workshop on aluminum. He had concerns regarding missing data: "We do not seem to have information on the age-related toxicity of aluminum, especially when we are dealing with very young infants…. We do not know whether or not there is a difference in susceptibility by age, as there [is] with other metals."[50, pp 83-84]

Some health authorities seemed to admit that even if aluminum is dangerous, it would be burdensome to remove it. For example, according to Dr. John Clements with the World Health Organization's Expanded Programme on Immunization, "There are not easy and obvious substitutes to aluminum adjuvants…. The existing vaccines, if they change the adjuvant for any reason, would need to be resubmitted for clinical trials for safety and efficacy and it would take a great deal of time to do that."[50, p 75] Furthermore, "Aluminum is not perceived, I believe, by the public as a dangerous metal. Therefore, we are in a much more comfortable wicket in terms of defending its presence in vaccines."[49, p 64]

Note: In 2005, 5 years after conference attendees spoke out about a lack of data on the effects of mixing different metals in childhood vaccines, Dr. Boyd Haley, former professor of medicinal chemistry and chairman of the chemistry department at the University of Kentucky, published a study in which he investigated the effect of combining aluminum hydroxide with thimerosal.[52] In this study, cultured neurons showed no significant cell death six hours after they were exposed to just aluminum; more than 90% survived. Thimerosal alone also caused few neurons

to die after six hours of exposure. Again, more than 90% survived. However, when cultured neurons were exposed to aluminum and thimerosal, only about 40% survived after six hours, clearly demonstrating synergistic toxicity (Figure 3).

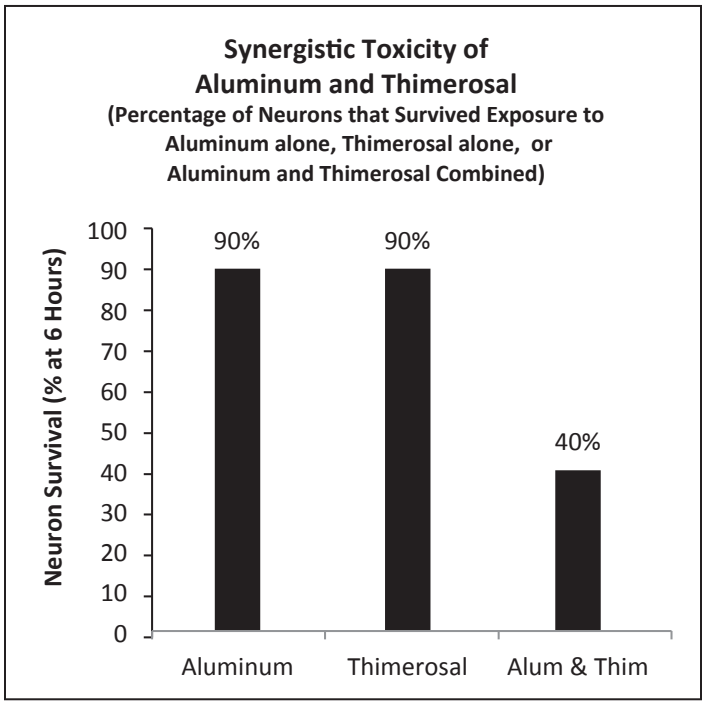


**Figure 3.** Survival of Neurons Exposed to Aluminum, Thimerosal, or Both

### Unconvincing Evidence of Adjuvant Safety

Although several high-level representatives of the CDC, World Health Organization (WHO), American Academy of Pediatrics, Institute for Vaccine Safety, National Vaccine Program Office, and Vaccine Injury Compensation Program who attended the conferences on aluminum and thimerosal had serious concerns about the potential hazards associated with aluminum in vaccines, a conference report and workshop summary published in the journal *Vaccine* 2 years later declared that "the message from this conference for the global public should stress the safety of both these adjuvants and these vaccines," despite acknowledging that "we don't know" how aluminum adjuvants interact with the immune system and how it is processed by infants and children.[53] The conference report minimized risks by claiming that aluminum has been used as a vaccine adjuvant for more than 70 years and "has an established safety record with low incidence of reported adverse events." However, no one is warning vaccine recipients to consider the possibility that their adverse event could be related to aluminum in their vaccines nor encouraging them to report it to health authorities. Furthermore, research indicates that many people who have adverse reactions to aluminum-containing vaccines won't exhibit symptoms for several weeks, months, or years, so it's very difficult for vaccine recipients to recognize that the vaccines they received some time ago may be related to their current disabling autoimmune ailments.

A few years later, the FDA published a study, Mitkus et al., in which the authors concluded that "the benefits of using vaccines containing aluminum adjuvant outweigh any theoretical concerns."[54] This study is often cited as a confirmation that injecting babies with multiple doses of aluminum-containing vaccines is safe. However, there are major flaws in the FDA's analysis:

1. To determine an aluminum intake "minimal risk level" (MRL) for humans, a single animal study was used.[55] This study found that mice could receive up to 26 milligrams of aluminum per kilogram of body weight per day (26 mg/kg/day) with no adverse effects. After considering differences between mice and humans (and other factors), this number was reduced to create a margin of safety, and an MRL of 1 mg/kg/day was established for humans, including infants.[56] But there is a problem: 26 mg/kg/day is not a safe amount of aluminum for animals. Several studies confirm that animals are harmed by much lower quantities of aluminum—3.4 to 6.1 mg/kg/day—and at least three of these studies were published before the FDA paper in 2011, so the FDA study was fallacious at its inception.[57-60] Rats that were given just 6.1 mg/kg/day aluminum (30 mg/kg/day $AlCl_3$) needed significantly more repetitions to learn a maze when compared to a control group.[57] Rats that were given just 5.6 mg/kg/day aluminum (50 mg/kg/day $AlCl_3$-$6H_2O$) had significantly impaired spatial learning and memory abilities when compared to a control group. They also had cellular shrinking, plus behavioral, biochemical, and histological alterations.[58] Rats that were given just 3.4 mg/kg/day aluminum (17 mg/kg/day $AlCl_3$) "showed behavioral, biochemical, and histological changes similar to those associated with Alzheimer's disease."[60]

2. The MRL for humans is derived from dietary aluminum *fed* to mice. But infants are *injected* with aluminum. Injected aluminum bypasses the gastrointestinal tract and has unique toxic properties compared to aluminum that is ingested. To determine the safety of injected aluminum, scientists must conduct experiments with injected—not ingested—aluminum.

3. After vaccines containing aluminum adjuvants are injected into the body, aluminum nanoparticles can be transported by monocyte-lineage cells to draining lymph nodes, blood and spleen—and may also penetrate the brain.[41] Aluminum is unsafe even in trace quantities. For example, just 50 nanomolars of aluminum are sufficient to generate reactive oxygen species (ROS), or oxidative stress, in human primary neuronal-glial cell cultures and induce inflammatory gene expression.[61] In another study, just 10 nanomolars of aluminum increased C-reactive protein (CRP) levels four-fold, causing inflammation in human brain microvessel endothelial cells.[62] But the FDA assumes, without evidence, that these poorly biodegradable aluminum nanoparticles,

which have been detected in body organs up to a year after vaccination, are harmless, and they are not calculated by the FDA as part of the aluminum "body burden" until they dissolve.

4. The "retention function for aluminum," a mathematical equation that the FDA used to help estimate levels of aluminum in infants, was derived from data on only one person, an adult (rather than from numerous infants), and an estimate on the rate of absorption of aluminum hydroxide following injection was based on data from just two rabbits.

The FDA paper also falsely claimed that "occasional irritation (dermal) at the site of injection is the only adverse effect that has been reported in the published literature" following injections of aluminum-containing vaccines. And the clinical symptoms in patients diagnosed with MMF "are considered to be due to separate, coincidental immune or neurological disorders that are unrelated to the presence of aluminum in vaccines."[54] The Global Advisory Committee on Vaccine Safety, established by WHO, welcomed the FDA's analysis endorsing the safety of aluminum in vaccines.[63] The CDC vigorously defends the presence of aluminum in vaccines as well.[64] Clearly, FDA, CDC, and WHO agree on continuing indefinitely with their current policies of injecting babies with multiple doses of aluminum-containing vaccines.

**Aluminum Toxicity Acknowledged for Parenteral Nutrition**

Although the FDA's recent paper advocates the continued use of aluminum in childhood vaccines, FDA has known for many years that aluminum can be dangerous. For example, some infants require parenteral nourishment (administered by intravenous injection). All parenteral nutritional formulas contain aluminum. According to the FDA, "when medication and nutrition are administered orally, the gastrointestinal tract acts as an efficient barrier to the absorption of aluminum, and relatively little ingested aluminum actually reaches body tissues. However, parenterally administered drug products containing aluminum bypass the protective mechanism of the gastrointestinal tract and aluminum circulates and is deposited in human tissues."[65]

In a 1997 study published in the *New England Journal of Medicine*, scientists assessed 182 infants who received intravenous injections of nutritional formula that contained differing quantities of aluminum.[20] They calculated that infants who received aluminum at greater than 4 to 5 mcg/kg/day would lose 1 point per day on the Bayley Mental Development Index ($p = 0.03$). Babies who score low on this test are at risk for subsequent developmental and educational problems. This study contributed to FDA's decision to set limits on aluminum content in parenteral drug products and require warning labels on the package inserts—safety measures that were never required with aluminum-containing vaccines. In the Code of Federal Regulations, Title 21, published in the Federal Register, aluminum toxicity levels are revealed:

> WARNING: This product contains aluminum that may be toxic.... Research indicates that patients with impaired kidney function, including premature neonates, who receive [injections] of aluminum at greater than 4 to 5 mcg per kilogram of body weight per day, accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates.[66]

This means that for a 6-pound baby with impaired kidney function, 11-14 mcg of injected aluminum would be toxic. The hepatitis B vaccine given at birth contains 250 mcg of aluminum—20 times higher than safety levels indicated for preemies. Babies weigh about 12 pounds at two months of age when they are injected with 1,225 mcg of aluminum from their CDC-recommended vaccines—50 times higher than safety levels for preemies.

Healthy babies may be able to handle quantities of aluminum above FDA toxicity levels indicated for patients with impaired kidney function. However, no one knows how much more aluminum is safe because adequate studies were never conducted. In addition, babies are not screened for renal function prior to vaccination. Therefore, it is impossible to know ahead of time which babies will succumb to aluminum poisoning. Instead, parents are expected to play Russian roulette with their children.

**Summary**

Aluminum adjuvants are added to several vaccines to elicit a more robust immune response and increase vaccine efficacy. Infants and young children throughout the world receive high quantities of aluminum from multiple inoculations. Incremental changes to the vaccination schedule during the past several years significantly increased the quantity of aluminum in childhood shots. Numerous studies provide compelling evidence that injected aluminum can be detrimental to health. Aluminum is capable of remaining in cells long after vaccination and may cause neurologic and autoimmune disorders. During early development, the child's brain is more susceptible to toxins and the kidneys are less able to eliminate them. Thus, children have a greater risk than adults of adverse reactions to aluminum in vaccines.

Millions of children every year are injected with vaccines containing mercury and aluminum despite well-established experimental evidence of the potential for additive or synergistic toxicity when an organism is exposed to two or more toxic metals. Dr. Haley's study in which cultured neurons died at an accelerated rate following concurrent exposure to aluminum and thimerosal provides evidence of an enhanced detrimental effect. In addition, aluminum toxicity levels published by FDA indicate that two-month-old babies who are vaccinated according to CDC guidelines may

be receiving quantities of aluminum that are significantly higher than safety levels.

## Conclusion

Toxic metals such as aluminum do not belong in prophylactic medications administered to children, teenagers, or adults. Vaccines are normally recommended for healthy people, so safety (and efficacy) standards must be impeccable. Parents, especially, should not be compelled to permit their loved ones to receive multiple injections of toxic metals that could increase their risk of neurodevelopmental and autoimmune ailments. Safe alternatives to current disease prevention technologies are urgently needed.

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# Appendix L

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# Aluminium in brain tissue in autism

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ABSTRACT

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.

## 1. Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions of unknown cause. It is highly likely that both genetic [1] and environmental [2] factors are associated with the onset and progress of ASD while the mechanisms underlying its aetiology are expected to be multifactorial [3–6]. Human exposure to aluminium has been implicated in ASD with conclusions being equivocal [7–10]. To-date the majority of studies have used hair as their indicator of human exposure to aluminium while aluminium in blood and urine have also been used to a much more limited extent. Paediatric vaccines that include an aluminium adjuvant are an indirect measure of infant exposure to aluminium and their burgeoning use has been directly correlated with increasing prevalence of ASD [11]. Animal models of ASD continue to support a connection with aluminium and to aluminium adjuvants used in human vaccinations in particular [12]. Hitherto there are no previous reports of aluminium in brain tissue from donors who died with a diagnosis of ASD. We have measured aluminium in brain tissue in autism and identified the location of aluminium in these tissues.

## 2. Materials and methods

### 2.1. Measurement of aluminium in brain tissues

Ethical approval was obtained along with tissues from the Oxford Brain Bank (15/SC/0639). Samples of cortex of approximately 1 g frozen weight from temporal, frontal, parietal and occipital lobes and hippocampus (0.3 g only) were obtained from 5 individuals with ADI-R-confirmed (Autism Diagnostic Interview-Revised) ASD, 4 males and 1 female, aged 15–50 years old (Table 1).

The aluminium content of these tissues was measured by an established and fully validated method [13] that herein is described only briefly. Thawed tissues were cut using a stainless steel blade to give individual samples of *ca* 0.3 g (3 sample replicates for each lobe except for hippocampus where the tissue was used as supplied) wet weight and dried to a constant weight at 37 °C. Dried and weighed tissues were digested in a microwave (MARS Xpress CEM Microwave Technology Ltd.) in a mixture of 1 mL 15.8 M $HNO_3$ (Fisher Analytical Grade) and 1 mL 30% *w/v* $H_2O_2$ (BDH Aristar). Digests were clear with no fatty residues and, upon cooling, were made up to 5 mL volume using

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**Table 1**
Aluminium content of occipital (O), frontal (F), temporal (T) and parietal (P) lobes and hippocampus (H) of brain tissue from 5 donors with a diagnosis of autism spectrum disorder.

| Donor ID | Gender | Age | Lobe | Replicate | [Al] μg/g |
|---|---|---|---|---|---|
| A1 | F | 44 | O | 1 | 0.49 |
| | | | | 2 | 4.26 |
| | | | | 3 | 0.33 |
| | | | | Mean (SD) | 1.69 (2.22) |
| | | | F | 1 | 0.98 |
| | | | | 2 | 1.10 |
| | | | | 3 | 0.95 |
| | | | | Mean (SD) | 1.01 (0.08) |
| | | | T | 1 | 1.13 |
| | | | | 2 | 1.16 |
| | | | | 3 | 1.12 |
| | | | | Mean (SD) | 1.14 (0.02) |
| | | | P | 1 | 0.54 |
| | | | | 2 | 1.18 |
| | | | | 3 | NA |
| | | | | Mean (SD) | 0.86 (0.45) |
| | | | All | Mean (SD) | 1.20 (1.06) |
| A2 | M | 50 | O | 1 | 3.73 |
| | | | | 2 | 7.87 |
| | | | | 3 | 3.49 |
| | | | | Mean (SD) | 5.03 (2.46) |
| | | | F | 1 | 0.86 |
| | | | | 2 | 0.88 |
| | | | | 3 | 1.65 |
| | | | | Mean (SD) | 1.13 (0.45) |
| | | | T | 1 | 1.31 |
| | | | | 2 | 1.02 |
| | | | | 3 | 2.73 |
| | | | | Mean (SD) | 1.69 (0.92) |
| | | | P | 1 | 18.57 |
| | | | | 2 | 0.01 |
| | | | | 3 | 0.64 |
| | | | | Mean (SD) | 6.41 (10.54) |
| | | | Hip. | 1 | 1.42 |
| | | | All | Mean (SD) | 3.40 (5.00) |
| A3 | M | 22 | O | 1 | 0.64 |
| | | | | 2 | 2.01 |
| | | | | 3 | 0.66 |
| | | | | Mean (SD) | 1.10 (0.79) |
| | | | F | 1 | 1.72 |
| | | | | 2 | 4.14 |
| | | | | 3 | 2.73 |
| | | | | Mean (SD) | 2.86 (1.22) |
| | | | T | 1 | 1.62 |
| | | | | 2 | 4.25 |
| | | | | 3 | 2.57 |
| | | | | Mean (SD) | 2.81 (1.33) |
| | | | P | 1 | 0.13 |
| | | | | 2 | 3.12 |
| | | | | 3 | 5.18 |
| | | | | Mean (SD) | 2.82 (1.81) |
| | | | All | Mean (SD) | 2.40 (1.58) |
| A4 | M | 15 | O | 1 | 2.44 |
| | | | | 2 | 1.66 |
| | | | | 3 | 22.11 |
| | | | | Mean (SD) | 8.74 (11.59) |
| | | | F | 1 | 1.11 |
| | | | | 2 | 3.23 |
| | | | | 3 | 1.66 |
| | | | | Mean (SD) | 2.00 (1.10) |
| | | | T | 1 | 1.10 |
| | | | | 2 | 1.83 |
| | | | | 3 | 1.54 |
| | | | | Mean (SD) | 1.49 (0.37) |
| | | | P | 1 | 1.38 |
| | | | | 2 | 6.71 |
| | | | | 3 | NA |
| | | | | Mean (SD) | 4.05 (3.77) |
| | | | Hip. | 1 | 0.02 |
| | | | All | Mean (SD) | 3.73 (6.02) |
| A5 | M | 33 | O | 1 | 3.13 |
| | | | | 2 | 2.78 |
| | | | | 3 | 1.71 |
| | | | | Mean (SD) | 2.54 (0.74) |
| | | | F | 1 | 2.97 |
| | | | | 2 | 8.27 |
| | | | | 3 | NA |
| | | | | Mean (SD) | 5.62 (3.75) |
| | | | T | 1 | 1.71 |
| | | | | 2 | 1.64 |
| | | | | 3 | 17.10 |
| | | | | Mean (SD) | 6.82 (8.91) |
| | | | P | 1 | 5.53 |
| | | | | 2 | 2.89 |
| | | | | 3 | NA |
| | | | | Mean (SD) | 4.21 (1.87) |
| | | | All | Mean (SD) | 4.77 (4.79) |

ultrapure water (cond. < 0.067 μS/cm). Total aluminium was measured in each sample by transversely heated graphite furnace atomic absorption spectrometry (TH GFAAS) using matrix-matched standards and an established analytical programme alongside previously validated quality assurance data [13].

### 2.2. Fluorescence microscopy

All chemicals were from Sigma Aldrich (UK) unless otherwise stated. Where available frontal, parietal, occipital, temporal and hippocampal tissue from 10 donors (3 females and 7 males) with a diagnosis of ASD was supplied by the Oxford Brain Bank as three 5 μm thick serial paraffin-embedded brain tissue sections per lobe for each donor (Table S1). Tissue sections mounted on glass slides were placed in a slide rack and de-waxed and rehydrated via transfer through 250 mL of the following reagents: 3 min in Histo-Clear (National Diagnostics, US), 1 min in fresh Histo-Clear, 2 min in 100% *v/v* ethanol (HPLC grade) and 1 min in 95, 70, 50 & 30% *v/v* ethanol followed by rehydration in ultrapure water (cond. < 0.067 μS/cm) for 35 s. Slides were agitated every 20 s in each solvent and blotted on tissue paper between transfers to minimise solvent carry-over. Rehydrated brain tissue sections were carefully outlined with a PAP pen for staining, in order to form a hydrophobic barrier around the periphery of tissue sections. In between staining, tissue sections were kept hydrated with ultrapure water and stored in moisture chambers, to prevent sections from drying out. Staining was staggered to allow for accurate incubation times of brain tissue sections. We have developed and optimised the fluor lumogallion as a selective stain for aluminium in cells [14] and human tissues [15]. Lumogallion (4-chloro-3-(2,4-dihydroxyphenylazo)-2-hydroxybenzene-1-sulphonic acid, TCI Europe N.V. Belgium) was prepared at *ca* 1 mM via dilution in a 50 mM PIPES (1,4-Piperazinediethanesulphonic acid) buffer, adjusted to pH 7.4 with NaOH. Lumogallion staining was performed via the addition of 200 μL of the staining solution to rehydrated brain tissue sections that were subsequently incubated at ambient temperature away from light for 45 min. Sections for autofluorescence analyses were incubated for 45 min in 200 μL 50 mM PIPES buffer only, pH 7.4. Following staining, glass slides containing tissue sections were washed six times with 200 μL aliquots of 50 mM PIPES buffer, pH 7.4, prior to rinsing for 30 s in ultrapure water. Serial sections numbered 1 and 2 for each lobe were incubated in 50 mM PIPES buffer, pH 7.4 or stained with 1 mM lumogallion in the same buffer, respectively, to ensure consistency across donor tissues. All tissue sections were subsequently mounted under glass coverslips using the aqueous mounting medium, Fluoromount™. Slides were stored horizontally for 24 h at 4 °C away from light, prior to analysis via fluorescence microscopy.

Stained and mounted human brain tissue sections were analysed via

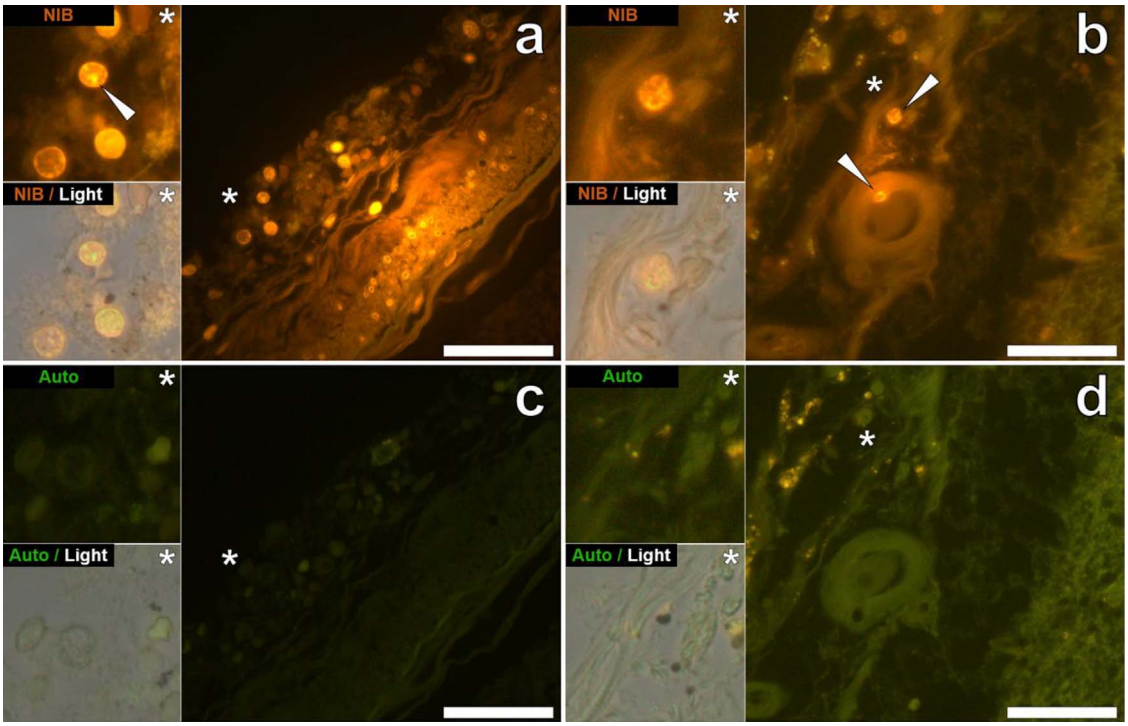


**Fig. 1.** Mononuclear inflammatory cells (probably lymphocytes) in leptomeningeal membranes in the hippocampus and frontal lobe of a 50-year-old male donor (A2), diagnosed with autism. Intracellular lumogallion-reactive aluminium was noted via punctate orange fluorescence emission (white arrows) in the hippocampus **(a)** and frontal lobe **(b)**. A green autofluorescence emission was detected in the adjacent non-stained (5 μm) serial section **(c & d)**. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the use of an Olympus BX50 fluorescence microscope, equipped with a vertical illuminator and BX-FLA reflected light fluorescence attachment (mercury source). Micrographs were obtained at X 400 magnification by use of a X 40 Plan-Fluorite objective (Olympus, UK). Lumogallion-reactive aluminium and related autofluorescence micrographs were obtained via use of a U-MNIB3 fluorescence filter cube (excitation: 470–495 nm, dichromatic mirror: 505 nm, longpass emission: 510 nm, Olympus, UK). Light exposure and transmission values were fixed across respective staining treatment conditions and images were obtained using the CellD software suite (Olympus, Soft Imaging Solutions, SiS, GmbH). Lumogallion-reactive regions identified through sequential screening of stained human brain tissue sections were additionally imaged on autofluorescence serial sections, to assess the contribution of the fluorophore. The subsequent merging of fluorescence and bright-field channels was achieved using Photoshop (Adobe Systems Inc. US). When determining intracellular staining the type of cells stained were estimated by their size and shape in the context of the brain area sampled and their surrounding cellular environment.

## 3. Results

### 3.1. Aluminium content of brain tissues

The aluminium content of all tissues ranged from 0.01 (the limit of quantitation) to 22.11 μg/g dry wt. (Table 1). The aluminium content for whole brains (n = 4 or 5 depending upon the availability of hippocampus tissue) ranged from 1.20 (1.06) μg/g dry wt. for the 44 year old female donor (A1) to 4.77 (4.79) μg/g dry wt. for a 33 year old male donor (A5). Previous measurements of brain aluminium, including our 60 brain study [13], have allowed us to define loose categories of brain aluminium content beginning with ≤1.00 μg/g dry wt. as pathologically benign (as opposed to 'normal'). Approximately 40% of tissues (24/59) had an aluminium content considered as pathologically-concerning (≥2.00 μg/g dry wt.) while approximately 67% of these tissues had an aluminium content considered as pathologically-significant (≥3.00 μg/g dry wt.). The brains of all 5 individuals had at least one tissue with a pathologically-significant content of aluminium. The brains of 4 individuals had at least one tissue with an aluminium content ≥5.00 μg/g dry wt. while 3 of these had at least one tissue with an aluminium content ≥10.00 μg/g dry wt. (Table 1). The mean (SD) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. There were no statistically significant differences in aluminium content between any of the 4 lobes.

### 3.2. Aluminium fluorescence in brain tissues

We examined serial brain sections from 10 individuals (3 females and 7 males) who died with a diagnosis of ASD and recorded the presence of aluminium in these tissues (Table S1). Excitation of the complex of aluminium and lumogallion emits characteristic orange fluorescence that appears increasingly bright yellow at higher fluorescence intensities. Aluminium, identified as lumogallion-reactive deposits, was recorded in at least one tissue in all 10 individuals. Autofluorescence of immediately adjacent serial sections confirmed lumogallion fluorescence as indicative of aluminium. Deposits of aluminium were significantly more prevalent in males (129 in 7 individuals) than females (21 in 3 individuals). Aluminium was found in both white (62 deposits) and grey (88 deposits) matter. In females the majority of aluminium deposits were identified as extracellular (15/21) whereas in males the opposite was the case with 80 out of 129 deposits being intracellular. We were only supplied with 3 serial sections of each tissue and so we were not able to do any staining for general morphology which meant that it was not always possible to determine which subtype of cell was showing aluminium fluorescence.

Aluminium-loaded mononuclear white blood cells, probably lymphocytes, were identified in the meninges and possibly in the process of

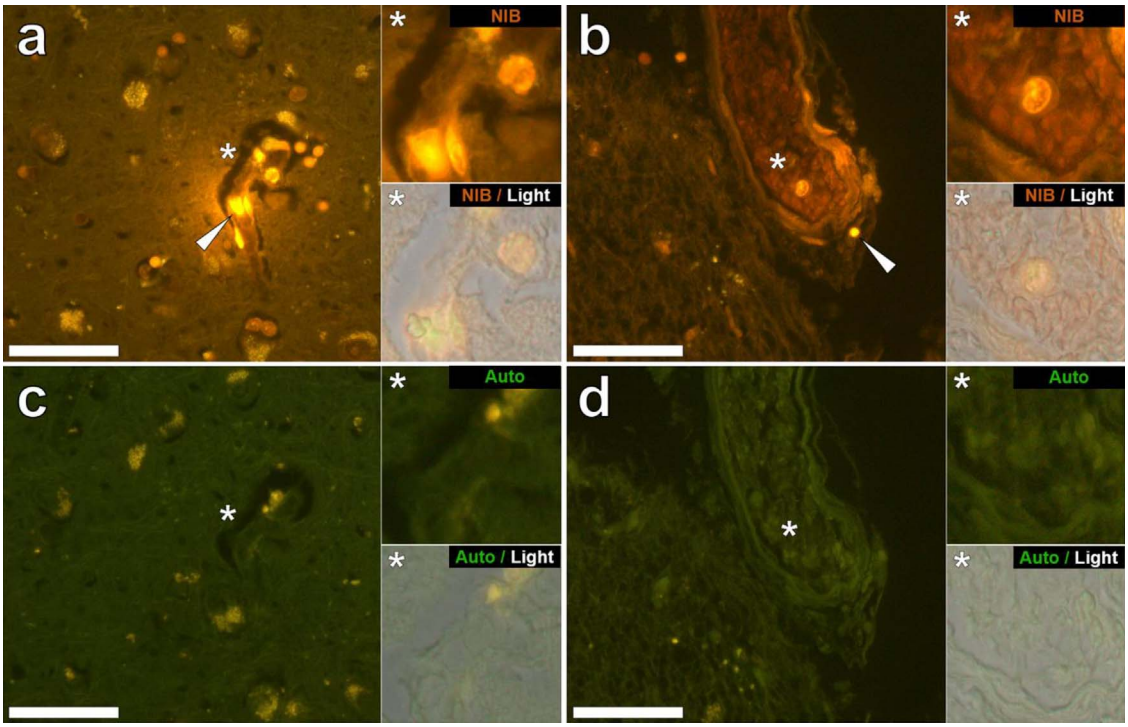


**Fig. 2.** Intracellular lumogallion-reactive aluminium in the vasculature of the hippocampus of a 50-year-old male donor (A2), diagnosed with autism. Aluminium-loaded inflammatory cells noted in the hippocampus in the vessel wall (white arrow) **(a)** and depicting punctate orange fluorescence in the lumen **(b)** are highlighted. An inflammatory cell in the vessel adventitia was also noted (white arrow) **(b)**. Lumogallion-reactive aluminium was identified via an orange fluorescence emission **(a & b)** versus a green autofluorescence emission **(c & d)** of the adjacent non-stained (5 μm) serial section. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

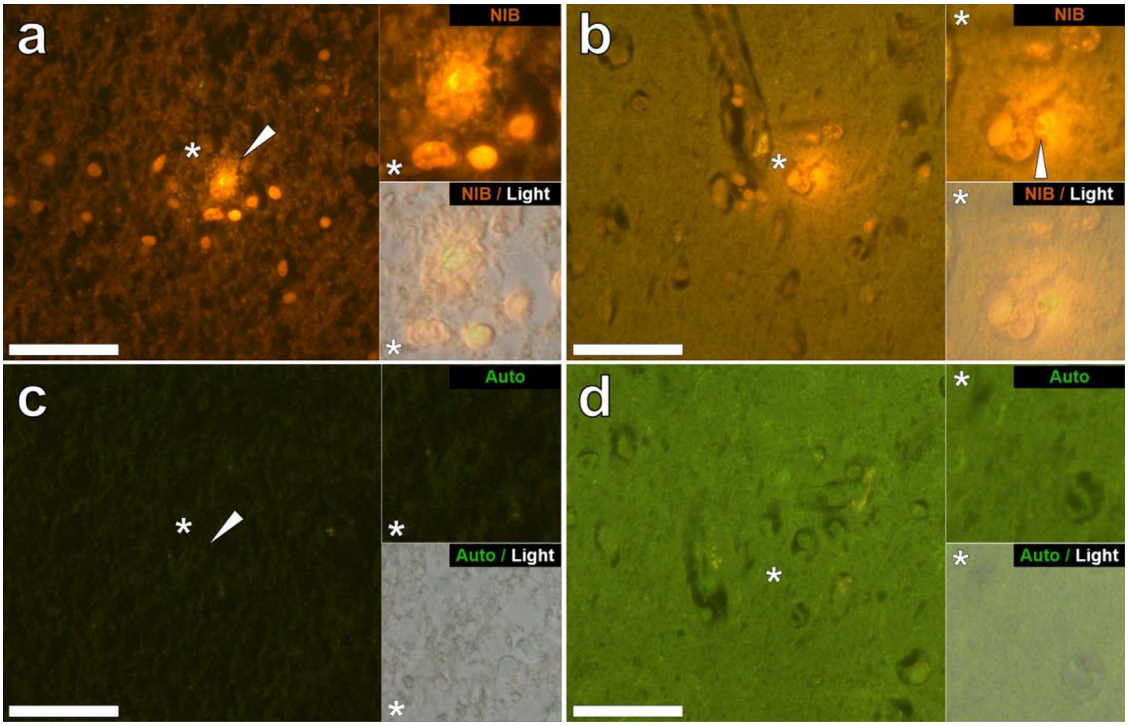


**Fig. 3.** Intracellular aluminium in cells morphologically compatible with glia and neurones in the hippocampus of a 15-year-old male donor (A4), diagnosed with autism. Lumogallion reactive cellular aluminium identified within glial-like cells in the hippocampus **(a)** and producing a punctate orange fluorescence in glia surrounding a likely neuronal cell within the parietal lobe **(b)** are highlighted (white arrows). Lumogallion-reactive aluminium was identified via an orange fluorescence emission **(a & b)** versus a green autofluorescence emission **(c & d)** of the subsequent non-stained (5 μm) serial section (white arrow/asterisk). Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

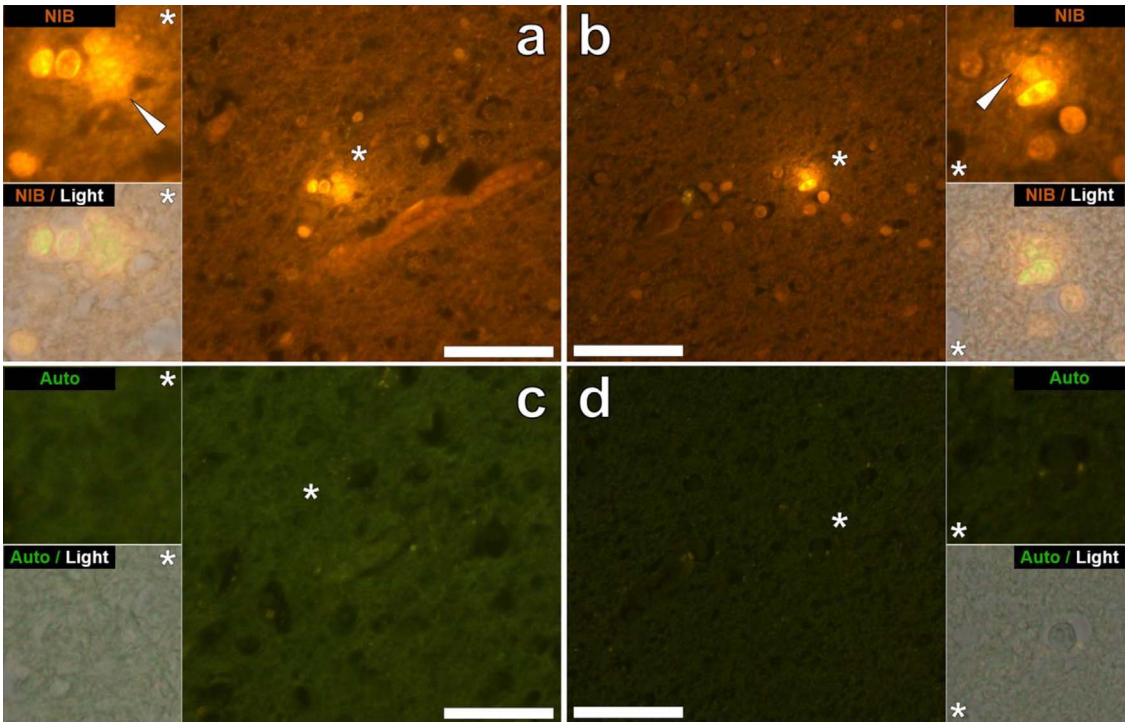


**Fig. 4.** Intracellular aluminium in cells morphologically compatible with microglia within the parietal and temporal lobes of 29-year-old (A8) and 15-year-old (A4) male donors, diagnosed with autism. Lumogallion-reactive extracellular aluminium (white arrows) producing an orange fluorescence emission was noted around likely microglial cells in the parietal **(a)** and temporal lobes **(b)** of donors A8 and A4 respectively. Non-stained adjacent (5 μm) serial sections, produced a weak green autofluorescence emission of the identical area imaged in white **(c)** and grey matter **(d)** of the respective lobes. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

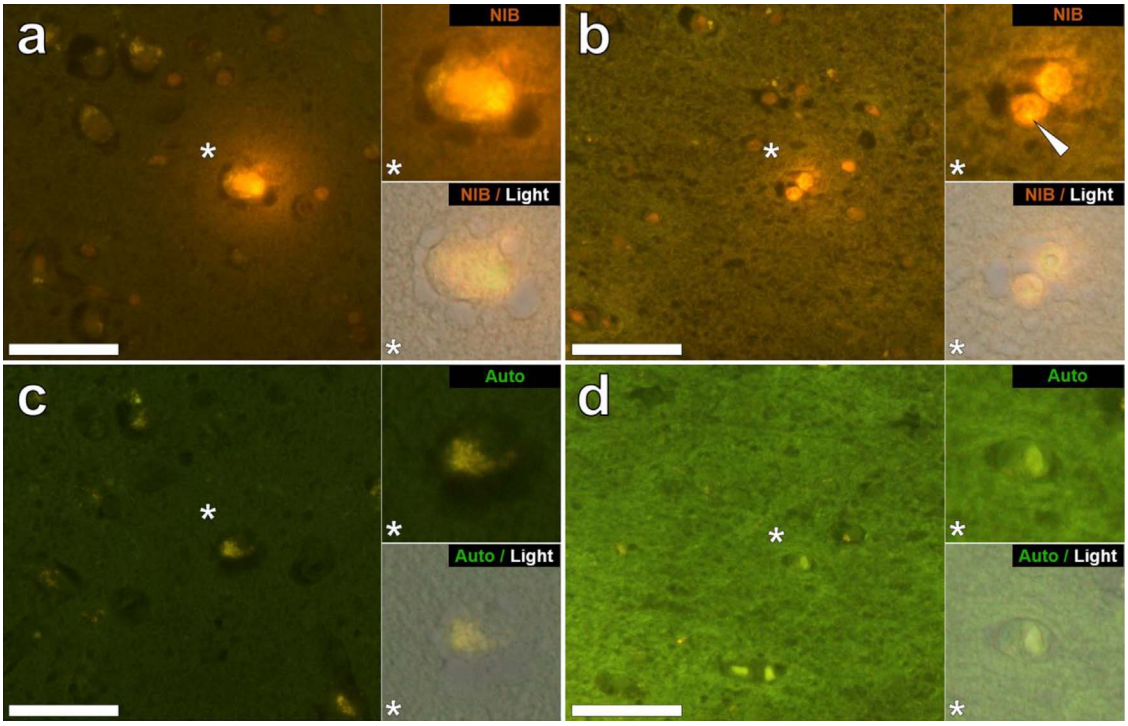


**Fig. 5.** Lumogallion-reactive aluminium in likely neuronal and glial cells in the temporal lobe and hippocampus of a 14-year-old male donor (A10), diagnosed with autism. Intraneuronal aluminium in the temporal lobe **(a)** was identified via an orange fluorescence emission, co-deposited with lipofuscin as revealed by a yellow fluorescence in the non-stained auto-fluorescence serial (5 μm) section **(c)**. Intracellular punctate orange fluorescence (white arrow) was observed in glia in the hippocampus **(b)** producing a green autofluorescence emission on the non-stained section **(d)**. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

entering brain tissue from the lymphatic system (Fig. 1). Aluminium could be clearly seen inside cells as either discrete punctate deposits or as bright yellow fluorescence. Aluminium was located in inflammatory cells associated with the vasculature (Fig. 2). In one case what looks like an aluminium-loaded lymphocyte or monocyte was noted within a blood vessel lumen surrounded by red blood cells while another probable lymphocyte showing intense yellow fluorescence was noted in the adventitia (Fig. 2b). Glial cells including microglia-like cells that showed positive aluminium fluorescence were often observed in brain tissue in the vicinity of aluminium-stained extracellular deposits (Figs. 3 and 4). Discrete deposits of aluminium approximately 1 μm in diameter were clearly visible in both round and amoeboid glial cell bodies (e.g. Fig. 3b). Intracellular aluminium was identified in likely neurones and glia-like cells and often in the vicinity of or co-localised with lipofuscin (Fig. 5). Aluminium-selective fluorescence microscopy was successful in identifying aluminium in extracellular and intracellular locations in neurones and non-neuronal cells and across all brain tissues studied (Figs. 1–5). The method only identifies aluminium as evidenced by large areas of brain tissue without any characteristic aluminium-positive fluorescence (Fig. S1).

## 4. Discussion

The aluminium content of brain tissues from donors with a diagnosis of ASD was extremely high (Table 1). While there was significant inter-tissue, inter-lobe and inter-subject variability the mean aluminium content for each lobe across all 5 individuals was towards the higher end of all previous (historical) measurements of brain aluminium content, including iatrogenic disorders such as dialysis encephalopathy [13,15,16–19]. All 4 male donors had significantly higher concentrations of brain aluminium than the single female donor. We recorded some of the highest values for brain aluminium content ever measured in healthy or diseased tissues in these male ASD donors including values of 17.10, 18.57 and 22.11 μg/g dry wt. (Table 1). What discriminates these data from other analyses of brain aluminium in other diseases is the age of the ASD donors. Why, for example would a 15 year old boy have such a high content of aluminium in their brain tissues? There are no comparative data in the scientific literature, the closest being similarly high data for a 42 year old male with familial Alzheimer's disease (fAD) [19].

Aluminium-selective fluorescence microscopy has provided indications as to the location of aluminium in these ASD brain tissues (Figs. 1–5). Aluminium was found in both white and grey matter and in both extra- and intracellular locations. The latter were particularly preeminent in these ASD tissues. Cells that morphologically appeared non-neuronal and heavily loaded with aluminium were identified associated with the meninges (Fig. 1), the vasculature (Fig. 2) and within grey and white matter (Figs. 3–5). Some of these cells appeared to be glial (probably astrocytic) whilst others had elongated nuclei giving the appearance of microglia [5]. The latter were sometimes seen in the environment of extracellular aluminium deposition. This implies that aluminium somehow had crossed the blood-brain barrier and was taken up by a native cell namely the microglial cell. Interestingly, the presence of occasional aluminium-laden inflammatory cells in the vasculature and the leptomeninges opens the possibility of a separate mode of entry of aluminium into the brain i.e. intracellularly. However, to allow this second scenario to be of significance one would expect some type of intracerebral insult to occur to allow egress of lymphocytes and monocytes from the vasculature [20]. The identification herein of non-neuronal cells including inflammatory cells, glial cells and microglia loaded with aluminium is a standout observation for ASD. For example, the majority of aluminium deposits identified in brain tissue in fAD were extracellular and nearly always associated with grey matter [19]. Aluminium is cytotoxic [21] and its association herein with inflammatory cells in the vasculature, meninges and central nervous system is unlikely to be benign. Microglia heavily loaded with aluminium while potentially remaining viable, at least for some time, will inevitably be compromised and dysfunctional microglia are thought to be involved in the aetiology of ASD [22], for example in disrupting synaptic pruning [23]. In addition the suggestion from the data herein that aluminium entry into the brain via immune cells circulating in the blood and lymph is expedited in ASD might begin to explain the earlier posed question of why there was so much aluminium in the brain of a 15 year old boy with an ASD.

A limitation of our study is the small number of cases that were available to study and the limited availability of tissue. Regarding the latter, having access to only 1 g of frozen tissue and just 3 serial sections of fixed tissue per lobe would normally be perceived as a significant limitation. Certainly if we had not identified any significant deposits of aluminium in such a small (the average brain weighs between 1500 and 2000 g) sample of brain tissue then such a finding would be equivocal. However, the fact that we found aluminium in every sample of brain tissue, frozen or fixed, does suggest very strongly that individuals with a diagnosis of ASD have extraordinarily high levels of aluminium in their brain tissue and that this aluminium is pre-eminently associated with non-neuronal cells including microglia and other inflammatory monocytes.

## 5. Conclusions

We have made the first measurements of aluminium in brain tissue in ASD and we have shown that the brain aluminium content is extraordinarily high. We have identified aluminium in brain tissue as both extracellular and intracellular with the latter involving both neurones and non-neuronal cells. The presence of aluminium in inflammatory cells in the meninges, vasculature, grey and white matter is a standout observation and could implicate aluminium in the aetiology of ASD.

## Competing interests

The authors declare that they have no competing interests.

## Author contributions

CE designed the study, carried out tissue digests and TH GFAAS. DU carried out tissue digests and TH GFAAS. AK carried out brain neuropathology on sections prepared by MM. MM carried out all microscopy and with CE wrote the manuscript. All authors read and approved the manuscript.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2017.11.012.

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