Testimony of Dr. James Lyons-Weiler (PhD) Re: Dr. Kelly Sutton's Case

Relevant Experience: Dr. Lyons-Weiler has 20+ years research in biomedical sciences (basic, translational, clinical). He has been a teacher of research study design courses to graduate students and medical residents. He led the design and analysis on over 100 basic, translation and clinical research studies at The University of Pittsburgh. He taught the design and analysis of health outcome-related designs of studies to residents and physicians at the University of Pittsburgh Cancer Institute; He is a world-renowned expert in molecular diagnostics and treatment prognostics using mixed data (biomarkers, clinical & demographic data) and has an extensive recent record of research in vaccine safety science, including studies on the toxicity of aluminum dosing in pediatric vaccines, comparisons of alternative vaccine schedules, and a recent vaccinated vs. unvaccinated study. He has published a peer-reviewed study on the likelihood of disease caused by proteins included in COVID19 vaccines, and he has provided peer-review for studies on the development of COVID19 vaccines.

Re: The Case of Dr. Kelly Sutton

When physicians are confronted with differences among patients in their ability to tolerate medicine in cancer treatments, it is widely understood and appreciated that underlying, unseen biological differences may exist among patients with respect to their ability to tolerate and indeed survive specific treatment regimens. No one expects that all patients will respond equally well (or poorly) to all prescription drugs; physicians often attempt a particular route of treatment, only to discover it is either ineffective or that the patient is intolerant of the initial treatment, and alternative therapies are sought.

With vaccines, the presumption of the population-wide approach to vaccination is that the vaccines are sufficiently safe (i.e., tolerable by a sufficient percentage of patients) such that the specific approach for any given vaccine should be to vaccinate as many people as possible, with as few exceptions as possible. I wish I could say that the science on vaccine safety supported the notion that vaccine adverse events are rare. Instead, I am sorry to have to report that the field of vaccine safety science is rife with studies that smack of tobacco science, including all of the following flaws in the design of the study, or its analysis:

- Over-reliance on observational studies, which fall short of providing sufficient evidence for, or against, causality assessment adverse events;
- Insufficient statistical power to detect serious adverse events (small sample numbers);
- Non-representativeness of the population studied;
- Testing irrelevant and unrelated hypotheses;
- Adjustment of the study design after peeking at the results;

- Repeated rounds of data analysis in search of a result that fits a pre-supposed outcome favoring vaccine safety;
- Non-reporting of clear evidence of association of adverse events with exposure to vaccines;
- Manipulation of study group composition to obtain the desired analysis outcome
- Reliance on causal non-determination inferences by physicians of new conditions that present during the course of clinical trials for new vaccines in search of adverse events that might be caused by the vaccine;
- Failure to use an inert placebo for the control group exposure, favoring instead either an active adjuvant (like aluminum oxyhydroxide) or another vaccine
- Direct influence of funding source personnel on the design of studies or reviews, representing a direct and overt serious conflict of interest (Delong, 2012)

These and other flaws have been reviewed by myself carefully (see IPAK Report (Lyons-Weiler, 2018)), and by others (e.g., Hooker et al., 2014).

Physicians like Dr. Sutton are confronted with patients presenting with symptoms or sets of symptoms following vaccination for which the consensus holds that the vaccines did not cause the adverse health outcomes, they are faced with either accepting the consensus opinion (which emits primarily from the Advisory Committee on Immunization Practices, or ACIP), or from directives from the US Centers for Disease Control and Prevention. This has motivated scientific research in my research program.

Both ACIP and the CDC fall under the administration of the US Department of Health and Human Services (US HHS). US HSS is the defendant in the National Vaccine Injury Compensation Program (NVICP), which it also administers, a clear breach of the separation of powers clause.

My research on vaccine safety science began in an innocuous manner. I was writing a book, and had decided to add a chapter on vaccines to celebrate their successes. Given my expertise and experience in the design of clinical research - and in the development of algorithm to predict patient health outcomes using biomarkers in cancer and other diseases - I had expected that vaccine safety science would be top-notch, with gold-standard, double-blinded, inert placebo studies being used to determine the causality of any adverse health outcomes. The general concept at the time (2015) was that vaccines were effective, had saved millions of lives, and were, for the most part, safe, save the rare bad luck of a “hot lot” of vaccines. We have since learned that vaccine lots are distributed geographically at random to prevent communities from learning about hot lots. By that time, mercury (ethylmercury, specifically) had been removed from pediatric vaccines out of an abundance of caution, and it had been determined (so we were told) that vaccines do not cause autism. In my personal experience with vaccines, I had decided

to space my own children's' vaccines out, in an abundance of caution. Importantly, no pediatrician ever expressed any concern over the decision to space out vaccines for my two sons.

When I tried to write the chapter on vaccines, I found that I could not in good faith report that vaccine safety science was rigorous and robust, and therefore I could not report that vaccines were safe & effective. What I reported instead were some of that mentioned in the list above on the flaws and shortcomings of vaccine safety science. When the chapter was complete, I knew that the chapter itself would kill the popularity of my book, but I felt obligated to report what I had found.

The most damning evidence of scientific wrongdoing came in the form of the interviews between Dr. Brian Hooker and Dr. William Thompson of the CDC. Dr. Thompson had been put on administrative leave for bringing concerns over his co-authors' plan to exclude certain results from a report of the Institutes of Medicine (IOM), an arm of the National Academy of Sciences that was changed by Congress to conduct regular reviews of the safety of pediatric vaccines. Time and again the IOM had determined that there was insufficient evidence to rule in or rule vaccines as a contributing cause of autism (See NAS, 2012). Dr. Thompson provided Dr. Hooker, as well as Congressman Dan Burton (FL) with evidence that the CDC willfully altered the study design of a study of on-time vs. delayed Measles, Mumps & Rubella (MMR) to bring about a different result than had been found via the initial analysis. The hacked study was eventually published infamously as Destefano et al., (2014). I immediately recognized this as a flaw known as "analysis-to-result" (aka "p-hacking"), in which a scientific investigator might wish to find a particular relationship or result in a collection of data that favored their pet hypothesis, but, upon conducting their experiment or study, found the "wrong" result. After looking at the initial result, either by changing the study design or the design of analysis, investigators can "find" the result that fits their pet hypothesis, assuring publication and grants. This type of activity is universally recognized as posing a serious threat to the validity of any scientific study (e.g., Head et al., 2015; Bruns and Ioannidis, 2016; Bin Abd Razak et al., 2016; Raj, 2018; Pedroni et al., 2019).

Dr. Thompson's revelations to Dr. Hooker were a stunning indictment not only of the scientific validity of that single study (which was eventually published as Destefano et al., 2008), but also of the way CDC handled the actual doing of science, and also of the integrity of the investigators and CDC leadership itself. Instead of seeking to resolve the issue raised by Dr. Thompson's appropriate, earnest and forthright expression of concern, Dr. Julie Gerberding saw fit to put Dr. Thompson (through an intermediary) on leave so Dr. Frank Destefano could present the results instead. Dr. Frank Destefano, the lead author, admitted to news journalist Sharyl Attkisson that their decisions to change the study design were arbitrary and unable to rule out a causal link between vaccines and autism (Appendix 2; Transcript of the Interview between Dr. Frank Destefano, CDC and Sharyl Attkisson, Journalist). As egregious and disturbing as these

revelations were, the most disturbing revelation to me was the reveal from Dr. Thompson that CDC had a “sanitation” committee to which all manuscripts for all vaccine-related studies had to be submitted for review prior to submission to a research journal so any negative inferences about vaccines could be modulated (minimized) via language change.

Upon listening to those recordings, reading the transcripts, and eventually reading Dr. Thompsons’ admission issued via his lawyer, I decided to embark on a path in my career from which I knew there could be no reward - and from which there could be no return. I read and evaluated all of the studies that CDC had cited that claimed to show that vaccines were not related to autism (see IPAK report (Lyons-Weiler, 2018). I found them deeply disturbing. I found, first of all, that not all vaccines had even been studied for association with autism, and that some studies exist that have, in fact, reported association (Fig. 1). This was utterly unexpected, because the CDC website proffered the knowledge claim that “Vaccines Do Not Cause Autism” (CDC, 2021). In my extensive literature review, I found that most of the studies that failed to find any association were insufficiently powered (too small) to be able to have found an association of the diagnosis of autism with vaccination. I found that I agreed with the Institutes of Medicine that reported that there was insufficient evidence for or against association of the vaccines there were studied, and autism. I wrote two reports on this, which are currently still under peer-review; they are provided as one Exhibit (see Lyons-Weiler, 2018. Systematic Review of Historical Epidemiologic Studies Influencing Public Health Policies on Vaccination IPAK REPORT 2018-1.)

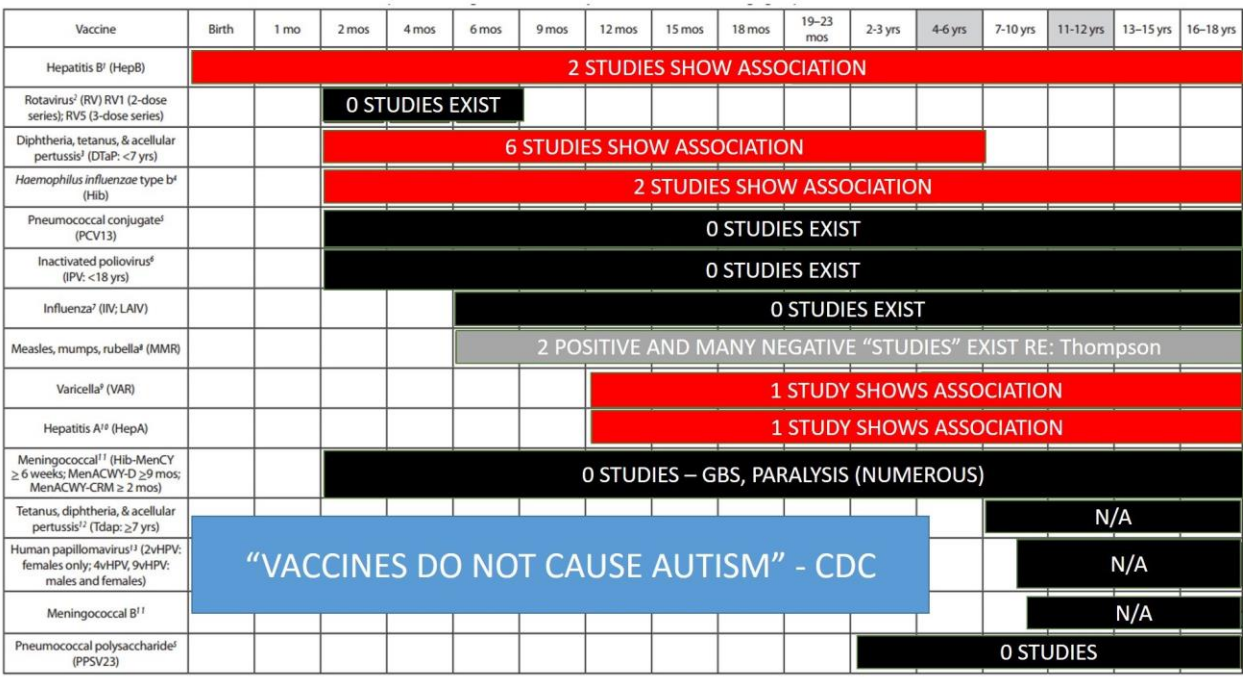


Fig. 1. CDC Schedule with available peer-reviewed literature on whether vaccines contribute to risk of autism. Black applies to vaccines that have not been studied; red includes vaccines for which positive associations have, in fact, been reported.

Fig. 2. Review of the sample sizes of the studies usually cited to conclude that vaccines do not cause autism. I have determined via power analysis that most of these studies could not have detected an odds ratio of >1.1 if an association had, in fact, existed (See IPAK REPORT 2018-1 for a full description)

			Sample Size			Power > x?
Author	**Year**	**Study Type**	**Group 1**	**Group 2**	**Total N**	**0.8**
Madsen	2002	Tx/Control Cohort	440655	96648	537303	N
Mrozek-Budzyn	2010	Case/Control	96	192	288	N
Smeeth et al.	2004	Case/Control	1294	4467	5761	N
Taylor et al.	1999	Case series	233	64	297	Y
Farrington et al.	2001	Case series	233	64	297	Y

The rise in autism cannot be explained by improved diagnosis (aka “diagnostic substitution), and my research into the literature has revealed that the largest genetic studies leave room for, on the average, 50% liability for unspecified environmental factors (e.g., Hallmayer et al., 2011; Klei et al., 2012; Sandin et al., 2014). The correct studies that include both vaccines and genetics to assess risk have never been conducted (Lyons-Weiler, 2018b).

On the question of aluminum safety in vaccines, I set about (with colleagues and co-authors) to determine how the US FDA came to conclude that a dose of 850 micrograms (mcg) of aluminum were safe in pediatric vaccines. The answer is that in fact neither FDA nor CDC ever conducted any science to determine the safety of aluminum adjuvants in vaccines; instead, they cited (out of many available studies) a single study that alleged showed no adverse health associated with a particular low dose of ingested aluminum (not injected, ingested). We published these findings in 2019 (Lyons-Weiler and Ricketson, 2018). Instead of using animal studies to assess the dose toxicity of aluminum in vaccines, as is currently conducted for other adjuvants, FDA used a mathematical exercise to assess the safety of injecting human infants with vaccine forms of aluminum via assumption-laden mathematical transformations from inappropriately interpreted safety assessment from oral forms of aluminum fed to adult mice (Mitkus et al., 2011). Most

remarkably, we could find no empirical data that showed that injected single dose amounts of aluminum oxyhydroxide were safe and did not lead to short-term or long-term chronic illness. Worse, there were no data on the safety of multiple doses, and yet numerous vaccines can be administered on the same day on the CDC schedule or make-up schedule.

In search for the mechanisms of action for drugs, and for dosage safety testing in drugs, mice are often used to determine the dose at which half of the mice in a study die. This toxicity level is called the LD50. We could not find any study that reported an LD50 of injected aluminum hydroxide, and yet it was being injected into hundreds of millions of children in the US every year. We could not find any pediatric dose limit (PDL) for aluminum for injected forms of aluminum. We did find one guidance from FDA that limited parenteral (non-oral) exposure to aluminum to 5 mcg/kg/day for persons with renal (kidney) disease (21CFR201.323), and yet the doses in vaccines are modulated by body weight.

We realized that from the adult dose of 850 mcg per vaccine, we could estimate a PDL using Clark's rule, which led to our first study on aluminum toxicity in vaccines (Lyons-Weiler and Ricketson, 2018). From this, we could then model the injection and clearance of any schedule, including the CDC's recommended schedule (McFarland Lyons-Weiler et al, 2019). We found that the CDC's schedule leads to infants being in aluminum toxicity (beyond the estimated PDL) 70% of their days in the first seven months of life. A later analysis, which adjusted for kidney development in infants, led up to conclude that the CDC schedule leads to infants being in aluminum toxicity 100% of their days in the first year of life. We have subsequently published a third analysis of the US CDC's vaccine catch-up schedule (Lyons-Weiler et al, 2020), and our research has impacted international medical practice (Yasuda et al., 2020).

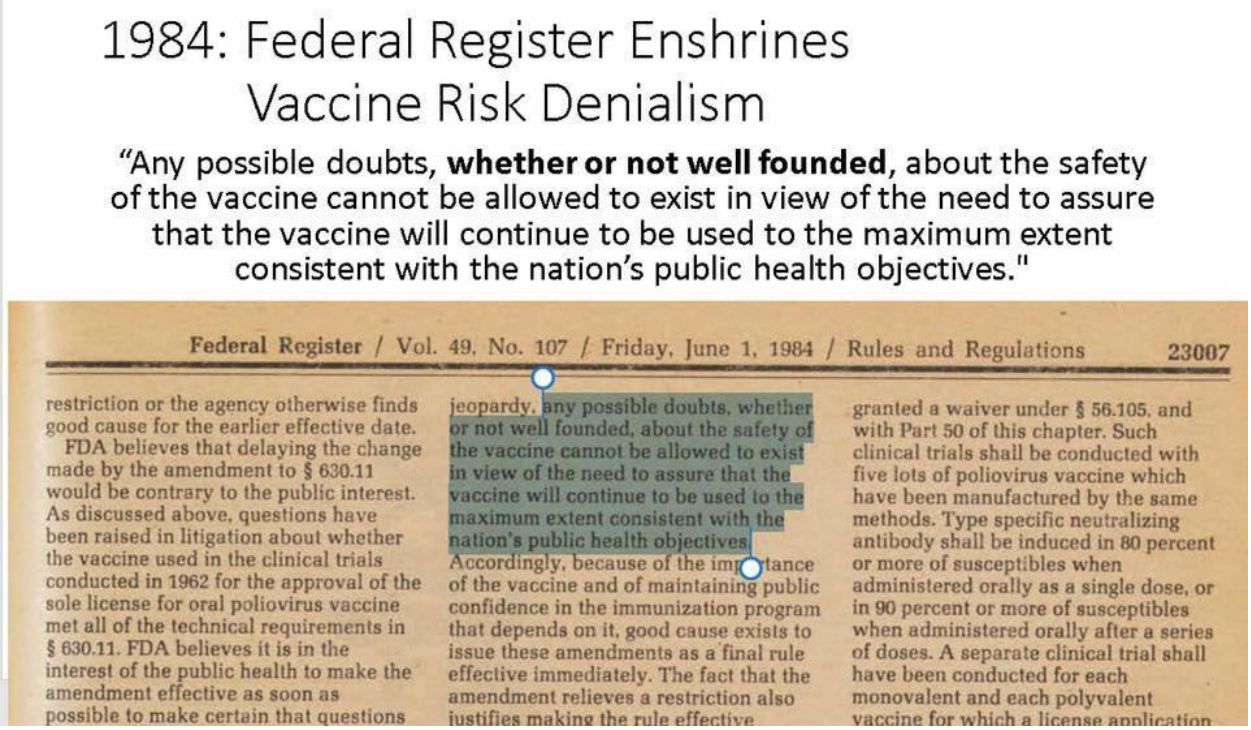
The health effects of aluminum depend on the species of aluminum, but I can assure the court that aluminum oxyhydroxide - the same form used in pediatric vaccines - is used to routinely and reliably induce autoimmunity in mice and rats. I have published a report on that as well (Lyons-Weiler, 2019); I show that the doses given in some of the animal studies overlap doses (per body weight) experienced by children in the CDC's vaccination schedule.

Per the US Congress, it is the responsibility of the CDC to issue, every two years, a report on the safety of pediatric vaccines (per the National Vaccine Childhood Injury Act). Given this fact, it will come as a surprise that US HSS stipulated in a major concussion to The ICAN Decide Network (ICAN) that they have not been conducting the studies, nor commissioning them, nor filing the reports as required by the NVICP. This abrogation of duty has left a knowledge vacuum under which recommendations by ACIP to add more and more vaccines to the schedule are increasingly based on less and less science, and more and more on mere opinion by those holding seats at ACIP. Two years ago, I led a committee to explore the conflicts of interest of ACIP committee members for Robert F. Kennedy, Jrs' organization, Children's Health Defense.

We found that every member of ACIP, with the exception of a representative of the US Department of Defense, had direct financial relationships to vaccine manufacturing pharmaceutical companies, or had other non-material conflicts of interest.

The safety of the entire CDC vaccination schedule has not been adequately studied. Since CDC is not keeping up with vaccine safety science, and are basing their continued recommendations on less and less science. Prior to 2020, no comparisons of the medical records of completely unvaccinated and vaccinated pediatric patients had been conducted. A survey study had found increased association of numerous health outcomes with exposure to pediatric vaccines (Mawson et al.); their findings, which included increased rates of chronic illness (2.4x), eczema (2.9x), neurodevelopmental disorder (3.7x), autism (4.2x), ADHD (4.2x), learning disabilities 5.2x) and allergic rhinitis (30.1x), helped motivate our own study of the effects of vaccination on overall childhood health comparing the vaccinated and unvaccinated in a practice in Oregon (Lyons-Weiler and Thomas, 2020).

All physicians, including Dr. Kelly Sutton, have a duty, and obligation to their patients to keep up with published studies on the medicines they prescribe (or do not prescribe), the decision on the part of parents to refuse vaccines must seem realistic and well-reasoned. This obligation exists in spite of the record in the Federal Register insisting that the American public never be allowed to become aware of risks associated with vaccination:



Like all human endeavors, medicine must be allowed to advance via trial and error. Denial of the errors leads to stagnation and places limits on knowledge. Any reasonable definition of standard-of-care (SOC) must be comprehensive and must include the professional responsibility of physicians to stay informed. Physicians who remain updated in their practice must consult a diverse amount of information from various sources, including their peers, peer-reviewed studies, professional journals and societies, and recommendations and guidances from FDA and CDC. Unfortunately, the systems put into place on vaccine safety are woefully insufficient to capture accurate data. The CDC's own Vaccine Adverse Events Reporting System (VAERS), admonishes users that the data cannot be used to assess causality; users must click a button acknowledging this information prior to accessing and using VAERS data (Fig 3 and Fig 4; CDC 2021). This particular system was found in a study conducted by Harvard-Pilgrim to miss 99 out of 100 vaccine adverse events (Lazarus, 2011). According to the Harvard-Pilgrim report, when CDC was informed of this fact, they stopped returning phone calls to the principal investigator on the project (Lazarus, 2011).

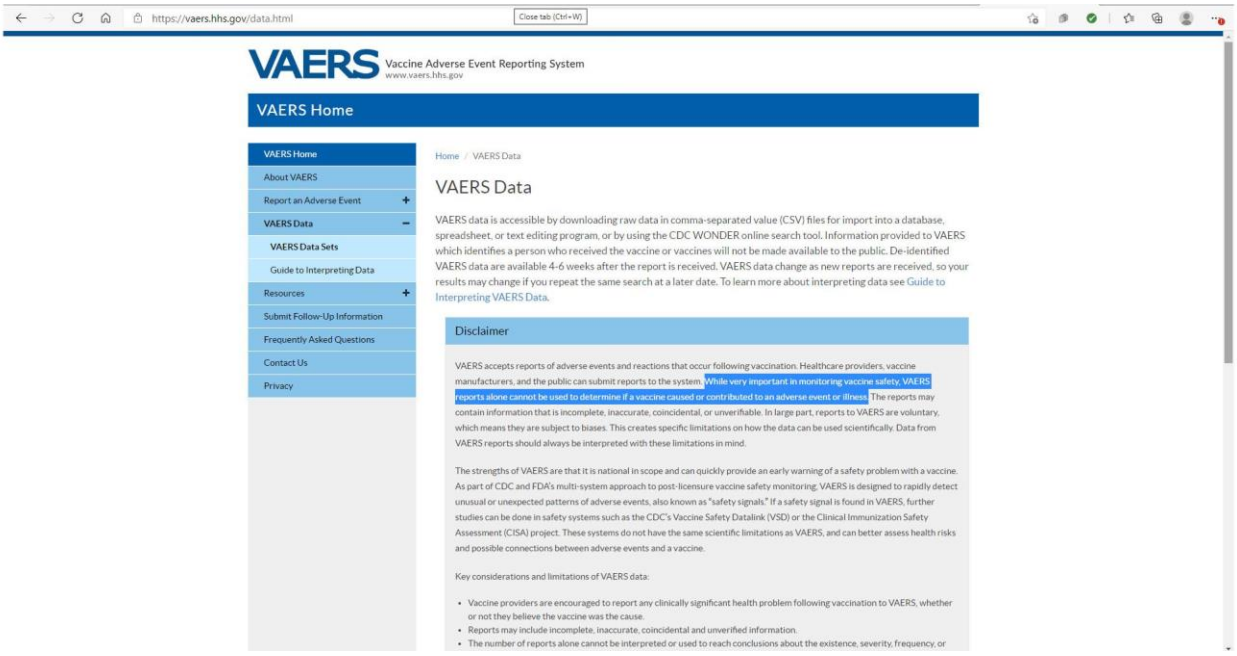


Fig. 3. The CDC's warning that VAERS data are not able to be used to determine causality of vaccine adverse events.

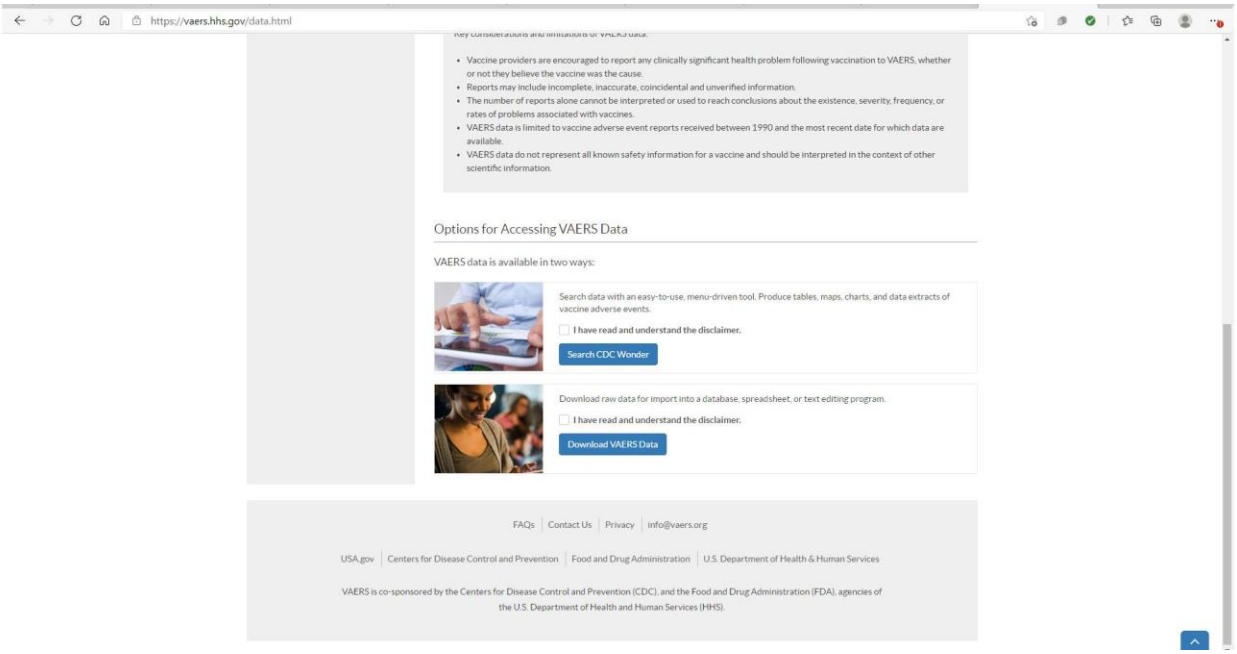


Fig. 4. Users seeking to access vaccine adverse events data are required to acknowledge the limitations of the CDC's vaccine adverse event tracking system for determination of causality.

In failing to report to Congress every two years as required, the CDC and the HHS at large have left a massive knowledge vacuum. This knowledge vacuum is massive, and my own, and others' research has only partly filled in the gaps. Compelling and credible studies have, in fact, found

associations between vaccines and autism (Gallagher and Goodman, 2010), vaccine and increased risk of death (Aaby et al., 1993; 2018; Benn et al., 2008; Mogensen et al., 2017); vaccines and asthma (McDonald et al., 2008); vaccines and thrombocytopenic purpura (Rinaldi et al. 2014). Mogensen et al., reported an 10-fold increased risk of death from all causes in children who received the DPT vaccine compared to the unvaccinated, and a 5-fold increase in risk of death in those who received both the DPT and oral polio vaccine.

Numerous studies exist showing autism occurring following acetaminophen exposure following MMR-vaccine induced fever (Schultz et al., 2008; Bauer et al., 2013; Claudia et al., 2016; Shoffner et al. (2010)). In 2010, Shoffner et al., (2010) found that 71% of kids with regressive autism had an episode of fever > 101°F In 33% of these cases, the fever occurred right after vaccination – and none showed regression unless fever had occurred.

It has been established that contaminants in vaccines include other metals - including copper - which binds well w/aluminum with non-specific proteins and create aggregates - this weakens the ability of aluminum to act as an adjuvant (Schlegl et al., 2015). An evidence-based review of the published literature just published (Boretti, 2021) has concluded that sufficient evidence exists to take seriously the hypothesis that exposure to aluminum from vaccines may be a causal factor in autism spectrum disorder. After reviewing the literature, a leading research team on autism (Morris et al., 2017) has called for end to the use of aluminum adjuvants in vaccines.

None of these studies purport to show that every vaccination harms every patient, nor even that vaccines predominantly cause harm. But they do lend credible support to the claims of specific harm by vaccines to specific individuals - and families, if a genetic risk of vaccine intolerance exists. Every physician who administers vaccines should be aware of the balance of the studies, not just those promoted by ACIP and the CDC. It is neither the parents', nor Dr. Sutton's responsibility to ensure that vaccine safety studies are conducted in an objective manner, free from conflicts of interest, or that knowledge claims made by vaccine manufacturers and regulatory agencies are based on solid studies. But it is their duty to abide by their patients' rights to informed consent. And it is the duty of all medical boards and societies to give the minority view due attention, for new information contrary to the consensus will always start in a minority of professionals. Instead, physicians who operate under the professional definition of Standard of Care are persecuted and their careers are being systematically destroyed, as if the destruction of their careers will in any stop the spread of vaccine risk awareness that is occurring as a result of the abrogation of the CDC to do its duty under the NVICP, and as a result of the regulatory capture that allows pharmaceutical companies to open up new vaccine markets by approving their "competitors" vaccines, for which they have their own vaccine waiting in the wings. In 2018, ACIP held a meeting to approve or not approve a new vaccine with a new, untested adjuvant and failed to have any discussion on safety until after the vote (see transcript,

Appendix 1)

It must be understood that argument from authority is acknowledged to be a logical fallacy (appeal to authority); the entirety of the rule by consensus in vaccines is resting squarely on this logical fallacy, which is especially flawed given that the authority is limited in the scope of the evidence it is willing to consider, biased in their views on vaccine safety in particular, and conflicted by perverse incentives resulting from profits stemming from vaccine administration in their practices (See Lyons-Weiler and Thomas, 2021). The ACIP committee is a particularly robust example of regulatory capture. In one meeting in 2019, members voted to recommend a flu vaccine that used a new adjuvant without holding any discussion on safety; directly after the vote, a member raised the question of evidence of cardiac events that had been reporting following the vaccine (see Transcript, Appendix 1).

May 5, 2021

James Lyons-Weiler PhD

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Appendix 1

Transcript of Proceedings of Advisory Committee on Immunization Practices (Source: (Source: ACIP Voting on a new vaccine, YouTube, Published March 15, 2018, https://www.youtube.com/watch?v=7UzQqan3uF8a)

00:00

um is there any comment on using this

00:02

vaccine at the same time with other edge

00:04

of any vaccines we have no data to make

00:10

a recommendation one way or the other so

00:16

um just so you just to sort of put this

00:19

in context of other vaccines while

00:21

preclinical studies were not done using

00:23

these vaccines simultaneously our

00:25

general approach to immunizations is

00:27

that they should be given they can be

00:30

given at the same time in different

00:32

limbs dr. hunter are adjuvanted multiple

00:38

adjuvanted vaccines used in Europe or

00:42

other markets dr. Ward do you want to

00:49

comment that's not to my knowledge

00:53

okay um I think unless there's any

00:56

further discussion we will take a vote

00:58

on this recommendation I want to remind

01:02

everyone to please um check your voting

01:05

whatever machine thing and voting is

01:10

open thank you very much

01:12

so the voting is completed and it is

01:15

unanimous to support this recommendation

01:18

thank you all and does anybody around

01:23

the table we don't need to go around and

01:24

verify your votes but does anybody have

01:26

any comments they wish to make about

01:28

their vote dr. great so just a slight

01:34

reservation I think this is a huge

01:36

advance and the step forward I am

01:38

concerned about that signal that

01:40

myocardial infarctions signal I am

01:41

concerned about the use of this new age

01:44

of Emma and certainly urge us to

01:46

continue to look at the post-marketing

01:49

data carefully dr. hunter just a

01:52

question about that would we how soon

01:54

would we be getting that post-marketing

01:57

data update here there's two kinds of

02:00

data

02:00

um the vaccine Safety Data Link data

02:03

will require people to be using the

02:05

vaccine to develop substantive database

02:08

and dr. son do you want to comment on

02:12

the post marketing

02:14

data that FDA is requiring I think for

02:18

the myocardial infarctions study we're

02:23

seeing that the date for likely for May

02:27

28 31 2020 it will also be studies

02:32

looking at autoimmune diseases as well

02:35

as disaster and you know there will be a

02:38

pregnancy registry as well so that sure

02:41

that's all included in the

02:42

post-marketing surveillance thank you

Appendix 2

Transcript of Interview Between Dr. Frank Destefano, CDC, and Sharyl Attkisson, Journalist.

Source: CDC: "Possibility" that vaccines rarely trigger autism (AUDIO) | Sharyl Attkisson https://sharylattkisson.com/2018/12/cdc-possibility-that-vaccines-rarely-trigger-autism/ Dec 19, 2018, Accessed 5/4/2021

Attkisson: And is, is the pos—the current position that any potential link between vaccines and autism, secondary, any kind at all, has been entirely ruled out 100%?

DeStefano: I re, you know, I re—uh, I think every hypothesis that's been looked at has been, uh, ruled out.

Attkisson: But, I mean, are you, are you, can I say the CDC's position is that if anybody thinks there's anything anymore, it's a myth? It's all been disproven?

DeStefano: Wouldn't say it's a myth, I'd say, you know, all the evidence, thus far, points to that there's not a causal association between vaccines and autism.

Attkisson: What about secondary?

DeStefano: Sec—I don't understand what do you mean "secondary"?

Attkisson: What about not "causal," but "as a result of" vaccines, as in the Poling case? The medical expert found, you know, as a result of the damages she had from the vaccines, she ended up with autism. And the distinction was made in the medical expert, 'well, that's not 'causal', it's sort of a 'but for' but it's not a 'causal.'

DeStefano: Yeah, I mean, I mean in that case, you know, she had a, I mean, you know, she had an underlying uh biological illness that uh either vaccination, or it could've been an infection that that would trigger some physiological stress in her, uh, seems to have, you know, could've, could've caused uh, um, manifestations that, characteristics of autism which, you, you know, appears to be what happened in her case.

Attkisson: But I mean doesn't that, is—isn't that a "link"? It's not a "causal" link, but isn't that a potential link between vaccination and autism if certain children with a "underyling (SIC) biological illness" can have a "trigger" through vaccination?

DeStefano: [Unintell] as you call it, a secondary link if you wanna call it that way, w-- in certain children, I mean ri—I mean, I, maybe that, but, you know, then I guess, that, that is a possibility.

Attkisson: Do you think that's an important area of study so we could figure out which kids might have that predisposition?

DeStefano: uh, [phone noise] Yeah, I mean, I think um…You know, I think it's something that, uh, well I mean, you know, in terms of uh… I mean, It's hard, it's hard to say, you know, I mean it's like, um…I mean how how important that is. I mean, it's a theoretical possibility, I guess the, the Poling case maybe suggested it could happen. Uh, but [unintell] cause it's hard to predict who those children might be, but certainly, um individual cases, uh, can be studied to try to, uh, to look at those, uh, those possibilities.

Attkisson: Well I would just think—and then, then I'll let you go in a few minutes unless you have more time—but as a parent, if my kid had whatever Poling had and we could figure that out, that would be one kid you would cull out [from vaccination] versus not worry about other kids if they don't have that predisposition. But maybe you could identify the ones that would be vulnerable. But I haven't seen that there's any—is there an area of study trying to do such a thing within CDC or funded by CDC? Or NIH?

DeStefano: Well, in terms of like, you know, the area at CDC that's that's studying autism and possible causal relationships of autism, uh, you know, whatever they may be, uh, is in the Center the National Center for Birth Defects and Developmental Disability, and they, they do monitoring for autism prevalence and they do have, uh, studies trying to go on, you know, going on to, to look at, uh, a number of factors that could be, uh, related to, uh, increasing the risk of autism or causing autism.

Attkisson: I mean I think to sum up, you're you're saying what I, what I think is also the case just based on my own research: that while the government has ruled out any known "causal" link between autism and vaccines, it hasn't ruled out the possibility, and in fact there seems to be at least one case where it's acknowledged what I called a "secondary" link, meaning not "causal" but uh "triggered." And the result for the parent, you know, may--to them it may be one and the same. And they may be trying to figure out which kids, you know, might have that predisposition.

DeStefano: Yeah, but you know, that's very difficult to do. That's almost circular reasoning, say, you know, kind of, you can't, I mean, you know, the, the useful thing for parents who are clinically would be able to identify the kids who are gonna have, I mean, this way we're identifying one certain child after the fact and say, you know, maybe in that one child, it was this or that that happened to him. But uh, it's very difficult to make a causal link in in just one case.

Attkisson: Well, but isn't that what you guys are supposed to do, figure it out? That's a, as you know, autism is such a huge problem, even if a teeny percentage is perhaps triggered by vaccination, I would think that'd be very, very important to, to learn and try to figure out. You guys are the best at it, I'm sure somebody there can do it over time.

DeStefano: Yeah…[unintell] I think…[unintell] have a better understanding of uh of the pathogenesis of autism and the genetics and the biology and then, I think, I mean, and then, and then, with these individual cases, it'd be, you know, more feasible to try to establish if, uh, if, if vaccines in an individual case, say a person with a certain, certain set of genes or something, you know, if we ever get to that point, then that kind of research, uh, might be fruitful, you know.

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Review

Reviewing the association between aluminum adjuvants in the vaccines and autism spectrum disorder

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ABSTRACT

The manuscript reviews the association between aluminum adjuvants (AlAd) in vaccines and autism spectrum disorder (ASD). Aluminum (Al) is neurotoxic. Infants who have received AlAd in vaccines show a higher rate of ASD. The behavior of mice changes with Al injection. Patients suffering from ASD have higher concentrations of Al in their brains. Thus, AlAd is an etiologic factor in ASD. Immune efficacy led to the use of the AlAd in vaccines; however, the safety of those who are vaccinated with such vaccines has not been considered. The mechanisms of action of AlAd and the pharmacodynamics of injected AlAd used in vaccines are not well-characterized. The association between aluminum adjuvants in the vaccines and autism spectrum disorder is suggested by multiple lines of evidence.

1. Introduction

Some vaccines contain different types of aluminum-based adjuvants [1], including amorphous aluminum hydroxyphosphate sulfate, aluminum hydroxide, aluminum phosphate, and potassium aluminum sulfate [2]. Their function is to help boost the body's response to the vaccine. The dramatic increase over the last 40 years in the number of vaccines, administered especially to infants has brought attention to the safety of these adjuvants. Apart from preliminary safety studies, public health surveillance is needed to identify the vaccine adverse events, due to the possibility of accumulation of aluminum from multiple vaccine administrations over the lifetime. Autism spectrum disorder (ASD) is a complex developmental condition that involving challenges in social interaction [3,4]. While ASD is usually diagnosed in childhood, but diagnosis may also be given as an adolescent or adult. ASD is a lifelong condition. The management of children with ASD has become more and more challenging during Covid19 restrictions [5–8].

For those born in the 1950s and 1960s, when the number of vaccines administered to infants was minimal, autism spectrum disorder (ASD) was a rare issue during their childhood. The increase in the number of vaccines administered to infants was followed by an increase in the prevalence of ASD. This simple association and the fact that aluminum (Al) is a neurotoxic substance [9] is not proof that aluminum adjuvants (AlAd) are the cause of ASD. However, associations are a first requirement in the line of evidence in the investigation. Diagnosis of ASD is challenging, and based on the child's developmental history and behavior [10,11].

Figs. 1.a, b present the 2017 incidence of autistic spectrum disorders, and the male vs. female prevalence. Autistic spectrum disorder includes autism and Asperger Syndrome or other autistic spectrum disorders. Figs. 1.b, c present the 2016 incidence of Autism, as well as the male vs. female prevalence of 2016. Autism is considered a sub-category of autistic spectrum disorders. Figs. 1.d, e present the 2017 attention-deficit/hyperactivity disorder (ADHD) incidence and male vs. female prevalence [12]. ASD is more prevalent in countries, such as United Kingdom (UK), Australia, Canada, New Zealand, and Japan. These are the leading countries in the introduction of vaccines over the years, especially in the number of those administered to infants under 12 months of age. The United States (US) follows this group closely. Canada has more than 1.5 % of male citizens affected by ASD. Australia has almost 0.7 % of male citizens affected by autism, and more than 3.5 % of male citizens are affected by ADHD.

The vaccines' history completes the picture. If we look for example at [13], detailing the history of the vaccines in the US, in late 1940 there were 4 recommended vaccines. There were five in the late 1950s and eight in the late 1960s. In the 1970s, one vaccine was eliminated. From 1985 to 1994, there were eight recommended vaccines, which become nine from 1994 to 1995. The recommended vaccines of 2000 were eleven, and the recommended vaccines of 2005 were thirteen. The recommended vaccines of 2010 were fourteen. The 2019 recommended Vaccines list is also made up of fourteen entries.

While it may be argued that the results of Fig. 1 may suffer from the different methods adopted in different countries to detect ASD, this is

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Nomenclature

ADHD	Attention-deficit/hyperactivity disorder
Al	Aluminium
AlAd	Aluminum adjuvants
ASD	Autism spectrum disorders
CAA	Cerebral amyloid angiopathy
CDC	Centers for disease control and prevention
fAD	Familial Alzheimer's disease
MMR	Measles, Mumps, Rubella
NNV	Number needed to vaccinate
VAERS	Vaccine adverse events reporting system

unlikely the general case. While some commentaries have the premise that all countries have the same numbers no matter their policies, this conflicts with the huge differences between countries of similar socio-economic characteristics.

Almost all the nations are converging towards an increased number of recommended immunizations for infants. These recommended immunizations are in many cases compulsory, as children are not accepted without their up-to-date in childcare centers and schools. For example, under the Australian "*No Jab, No Play*" laws, evidence of immunization is necessary for enrolment [14,15].

If AlAd is responsible for ASD, considering the lag time, the present geographical distribution of ASD prevalence rate is correlated to the geographical distribution of the number of vaccines administered in the past, as it seems the case. For example, Italy or Russia have a present reduced prevalence of ASD vs. the UK and reduced past immunization schedules.

The US Centers for Disease Control and Prevention (CDC) admits that ASD affected 1 in 59 children aged 8 years in 2014 (2018 report). This incidence is 15 % higher than the prior report of two years earlier [16]. Since the year 2000, when the CDC began tracking ASD, the incidence has increased dramatically. The 2007 report, stated that ASD was detected in 1 in 150 children. The 2009 report, stated that ASD was detected in 1 in 110 children. The 2012 report, stated that ASD was

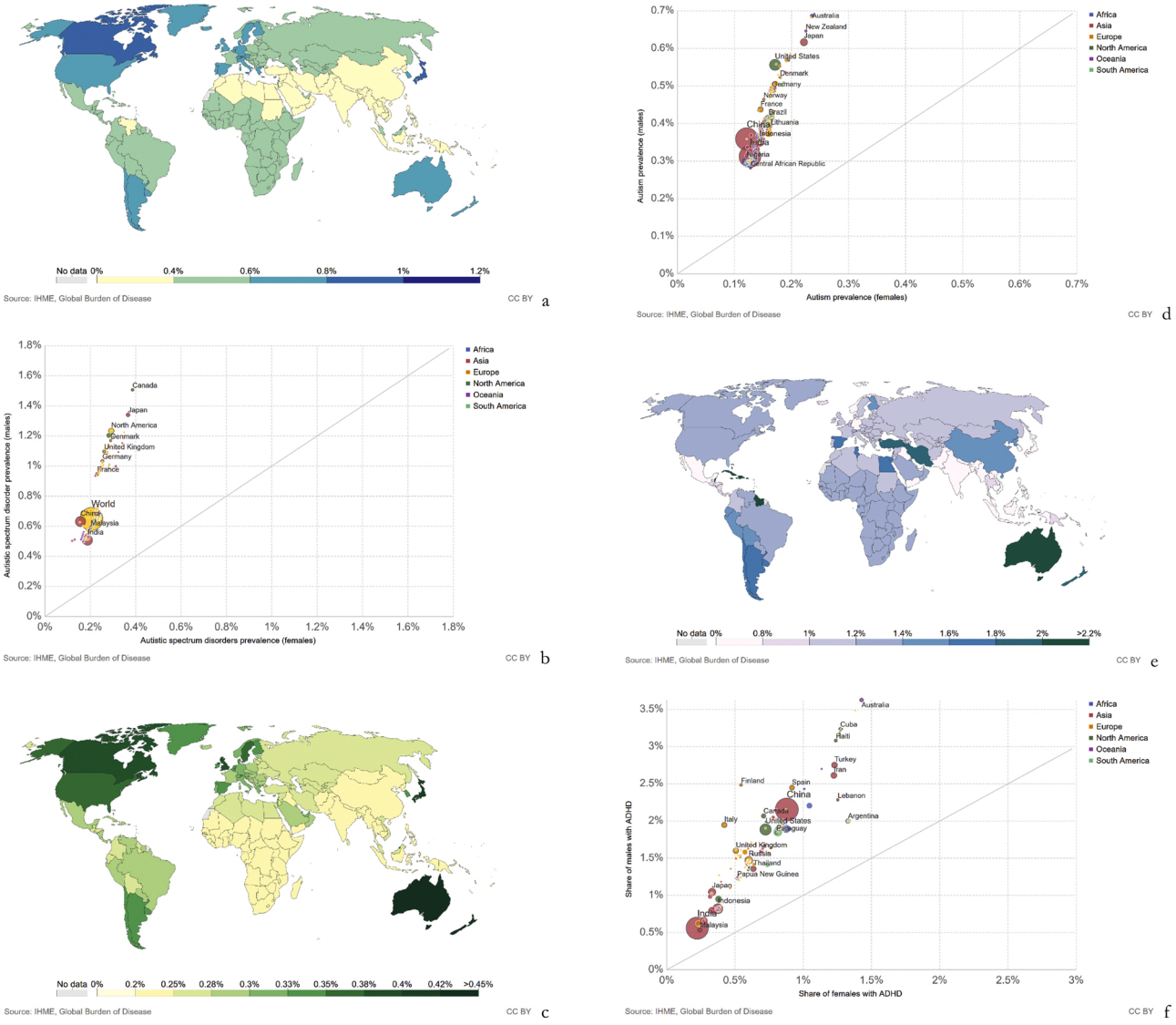

Fig. 1. (a) Share of the population with autistic spectrum disorder for the year 2017. Autistic spectrum disorder includes autism and Asperger Syndrome. (b) Share of males vs. females with autistic spectrum disorder for the year 2017. (c) Share of the population with autism for the year 2016. (d) Share of males vs. females with autism for the year 2016. (e) Share of the population with attention-deficit/hyperactivity disorder (ADHD) for the year 2017. (f) Share of males vs. females with attention-deficit/hyperactivity disorder (ADHD) for the year 2017. Images from [12]. Data from [17]. CC BY.

detected in 1 in 68 children, the same as the 2016 report. The 2018 report, stated that ASD was detected in 1 in 59 children. ASD is four times more likely in boys (1 in 38) than girls (1 in 152). There is a correlation between the prevalence of ASD and the increased number of vaccines. However, there are to mention other factors such as the increased exposure of infants to electromagnetic fields, ultrasounds, and toxins in the foods that also correlate to the increased prevalence of ASD. These correlations do not rule out vaccines, however, as different environmental factors may interact.

While correlation is not proof for causation, this information provides more evidence in support for the hypothesis that the increase in aluminum exposure via vaccines may have a causal role in the increases in ASD prevalence. The following section substantiates the growing body of evidence in the scientific literature supporting this association.

A significant body of literature supports the notion that aluminum is a serious problem. In addition to those references used to support the three lines of evidence, additional lines of evidence on the mechanism are worth considering [18–25]. Al is a neurotoxin. These additional studies offer plausible substantiation that Al may unfavorably influence biological functions and contribute to neurodegenerative and autoimmune disorders.

This review outlines the evidence necessary to conclude that we should raise an alarm on aluminum in vaccines, that current formulations of aluminum-containing vaccines need revision, as AlAd is more likely harmful than safe. AlAd in vaccines may explain the increasing prevalence of ASD. The "Results" section will propose multiple other lines of evidence emerging in the literature that supports the hypothesis that AlAd is likely responsible for the increases in ASD. These lines of evidence include ecological studies, animal models, and measurements of Aluminum (Al) in brain tissues of subjects with ASD. Mechanisms of action are also proposed in this section.

2. Materials and methods

A literature review is performed for the possible link between AlAd in vaccines and ASD. The flow chart of arguments - diagram of evidence supporting the correlation between AlAd and ASD is proposed in Fig. 2.

3. Results

Much published research has concluded that AlAd may be unsafe, with some of them suggesting a clear link between AlAd and ASD. As shown in Fig. 2, in between the works claiming a link between ASD and AlAd, there are three lines of scientific evidence suggesting correlation [26], ecological comparisons associating immunization with AlAd and ASD, experiments in mice connecting AlAd and behavioral disorders, and measurements of much higher concentration of Al in brain cells of subjects with ASD.

Aluminum is used in many pediatric vaccines as an adjuvant [27–29] despite its neurotoxicity. There is concern about the role of AlAd in the rising number of vaccines administered and the rising number of ASD

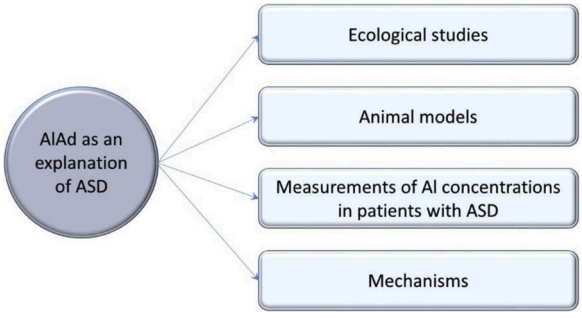

Fig. 2. Flow chart of arguments - diagram of evidence supporting the correlation between AlAd and ASD.

cases. As Al is a neurotoxin and immune stimulator it may induce neuroimmune disorders such as dysfunctional immunity and impaired brain function. AlAd in vaccines are based on immune efficacy but ignore bodyweight for safety [30]. The safety inferences on doses of AlAd in vaccines are theoretical and are derived from dietary studies of different forms of aluminum in adult mice [30]. Doses adjusted per body weight would limit aluminum doses to 15–17 times compared to adults [30,31]. The mechanisms of action of AlAd and the pharmacodynamics of injected AlAd used in vaccines are not well-characterized [31]. Aluminum salts are not solvable in plasma, and therefore serum/plasma level clearance rates are not good measures of whole-body toxicity. How differences in schedules impact accumulation and the influence of genetic and environmental factors on AlAd detoxification are unknown.

As shown in [32], the French vaccination requirements and recommendations of 2018 required the injection of 2545 and 7735 μg of Al3+, with at least 50 % before 12 months of age. The vaccines with higher doses of aluminum are mainly injected at the beginning of life. The ecological study [33] suggested a correlation between the rising ASD and the increased AlAd in vaccines administered during early postnatal life. They found that children from Western countries with the highest ASD frequency have the highest exposure to AlAd in vaccines. The increase in exposure to AlAd correlates well with the increase in ASD especially in the US during the two decades before the study. The amounts of AlAd administered at 3–4 months of age also correlate well with the increase in ASD in seven Western countries. According to [34], AlAd in vaccines carries a risk for autoimmunity, long-term brain inflammation, and associated neurological complications. The risk of potential adverse effects of AlAd is being underestimated. The ecological study [35] suggests a link between AlAd in vaccines and ASD by examining the word frequency patterns in the US CDC Vaccine adverse events reporting system (VAERS) database. ASD in VAERS increased steadily in the late 1990s when mercury was phased out, and the load of AlAd was increased. Signs and symptoms most frequently reported after the start of this century include cellulitis, seizure, depression, fatigue, pain, and death. The authors propose that children with ASD may be vulnerable to Al due to insufficient serum sulfate and glutathione. They also explain the link between ASD and the MMR (Measles, Mumps, Rubella) vaccine via the increased sensitivity to acetaminophen administered to control fever [36].

The evidence for concern over Al and ASD is also proposed in [37, 38]. AlAd injected in the early period of postnatal development, may affect the later social behavior of humans. The persistent claims that vaccines do not contain toxic substances or that they contain them in physiologically negligible amounts are questionable. Al is neurotoxic [39–41]. Transport, distribution, accumulation, and excretion rates of administered aluminum adjuvants are covered in [42–44,34], making plausible the accumulation of Al in brain tissues of patients subjected to multiple vaccinations when infants and young child.

While synergistic toxicity of Al and mercury is known, studies of adverse neurological and immunologic outcomes in children receiving both thimerosal-containing influenza vaccines and Al in the same visit, or the same month, have not been conducted [45]. Countries with higher AlAd exposure – for example, the US – have a much larger ASD than a country with much smaller AlAd exposure – for example, Serbia [46]. According to [46] the 1.09×10^{20} Al ions administered to US children are not a physiologically negligible quantity of toxic Al and they can endanger the health of children.

An animal study [47] found behavioral anomalies in mice injected with AlAd. They injected Al-hydroxide in early post-natal CD-1 mice, both male and female. "High" and "low" AlAd levels correlated to the US or Scandinavian pediatric vaccine schedules. Male and female mice in the "high" AlAd group showed significant weight gains. Male mice in the "high" AlAd group had changes in light-dark box tests and alterations in open-field behavior. "High" and "low" female mice had changes in the light-dark box, but no alterations in open-field behavior. The animal study [48] also investigated the effect of AlAd on the social behavior of

mice. Postnatal exposure to AlAd was associated with behavioral abnormalities. Exposure to AlAd was associated with diminished social interest. Cognitive and behavioral changes were observed in sheep subjected to repetitive inoculation with Al-containing products [49]. Animals in both the "Vaccine" and "Adjuvant-only" groups, both subjected to multiple inoculations with AlAd, exhibited individual and social behavioral changes vs. the "Control" group. Aluminum was detected in the lumbar spinal cord of sheep subcutaneously inoculated with aluminum-hydroxide-containing products [50].

The theoretical study that claimed that doses of Al in pediatric doses were safe [51] was seriously flawed [30]. The presumption of toxicity was derived from different studies by the same research team [52–54]. To be noted, Golub, Donald, Gershwin, and Keen [52] considered both repeated acute and long-term (chronic) toxicity to be an issue; repeated instances of microglia activation can lead to chronic microglial activation, which is seen in children and adults with ASD [55]. Ingested, and not injected, forms of Al, were used in adult, and not infant mice. Ingested and injected forms of Al may have dramatically different consequences [56]. Al injected in infant mice may have dramatically different consequences than Al ingested in adult mice.

If the statistical samples of ecological studies supporting a correlation between AlAd and ASD are often considered small, even smaller statistical samples have been used to support the opposite findings. The study of Al whole-body clearance rates [57] was practically based on repeated measurements of retained aluminum over 12 years for a single adult volunteer after a single injection with citrate solution containing ^{26}Al. A proper safety statistic is certainly missing not only to oppose but also to support the use of AlAd in vaccines, and the issue may only be solved by further unbiased research conducted by applying the scientific method. An in vitro THP-1 cell model was used in [58] to study the cellular uptake of AlAd in vaccines. It is shown that not all AlAds are equal for physical properties, biological reactivity, and potential toxicities. It is argued that high loading of Al oxyhydroxide in the cytoplasm of THP-1 cells may be subjected to subsequent transport to the brain.

Mold, Umar, King, and Exley [59] measured the Al content in the brain tissues of donors with ASD. They used transversely heated graphite furnace atomic absorption spectrometry and an Al-selective fluor to permit fluorescence microscopy. Al content in brain tissue was higher with ASD. Al associated with neuronal cells was present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey, and white matter. Intracellular Al associated with non-neuronal cells was observed in ASD brain tissues.

The above 3 lines of evidence, ecological comparisons correlating immunization with vaccines having AlAd and ASD, an animal study of Al-exposure in mice and behavioral changes, and measurements of Al in ASD brain tissues, make a strong case for AlAd being responsible for ASD. The evidence supporting the claim that AlAd in vaccines causes ASD is incomplete, as a causative link is missing. However, the evidence that AlAd in vaccines does not cause ASD is certainly much less than for the opposite claim.

An explanation of the AlAd neurotoxicity is proposed in [46]. Metabolic cell functions are related to enzymes. Most enzymes are metalloenzymes (biometal incorporated in the enzyme). After a time of active work, an enzyme is degraded, metabolized, and synthesized again. During the resynthesis of an enzyme, if a not-biometal (such as Al) is used instead of a particular biometal, an afunctional enzyme is formed. The afunctional enzyme will then cause a failure of particular metabolic functions.

Ivanovski, Fletcher, Ivanovski, Garavelli, Nikolić, and Ivanovski [46] highlights the similarity between the AlAd neurotoxicity mechanism and the mechanism of the generation of microcytic anemia in chronic lead poisoning [60]. The amount of AlAd in vaccines is not minuscule [61], and it may have a role in ASD. According to [62,63] scientific evidence confirms the presence of Al brain tissue from donors with diagnoses of familial Alzheimer's disease, autism spectrum disorder, multiple sclerosis, and epilepsy. Finally, McFarland, La Joie, Thomas, and Lyons-Weiler [31] highlights how mechanisms of action and pharmacodynamics of injected AlAd in vaccines are not well-characterized, particularly concerning accumulation and clearance.

The possible role of AlAd in ASD following various mechanisms is further stressed in many other recent works. Bjørklund et al. [64] discuss toxic metal pollutants. Arumugham, and Trushin [65] and Swierczynski [66] examine ASD pathogenesis. Bjørklund et al. [67] discusses oxidative stress and ASD. The association of trace elements and minerals and ASD is discussed in [68]. McFarland, La Joie, Thomas, and Lyons-Weiler [31] argue about acute exposure and chronic retention of Al in different vaccines. Dórea [69] discusses the role of multiple low-level exposures in early life. Grochowski et al. [70] discusses methods to detect trace elements in the human brain.

Other pathologies also being related to Al in the brain are stressed in other works such as multiple sclerosis [71], epilepsy [72], cerebral amyloid angiopathy [73], Alzheimer′ s disease [74], or cerebral palsy [75]. Gherardi, Crépeaux, and Authier [76] discuss myalgia and chronic fatigue syndrome following immunization.

Fig. 3 (from [62]) presents the result of measurements of Aluminum in brain tissue from donors diagnosed with (a–c) ASD, (d) cerebral amyloid angiopathy (CAA), (e) epilepsy, and (f) familial Alzheimer's disease (fAD). In healthy subjects, the concentration of Aluminum is practically undetectable. Accumulation of injected aluminum is one explanation. Alternative explanations have not been proposed so far.

Many more studies have demonstrated the presence of Al in brain tissue in subjects with a neurodegenerative and neurodevelopmental disease, for example [77]. This work used microwave-assisted acid digestion and transversely heated graphite furnace atomic absorption spectrometry to measure Al in brain tissues from donors without and with perceptible neurodegenerative disease. About 80 % of tissue samples had Al content below 1.0 μg g of the dry weight of tissue. In the case of sporadic or familial Alzheimer's disease [78,79], ASD [62], and multiple sclerosis [71], Al was significantly increased in the disease groups compared to control [77].

A causative explanation on a molecular level of the role of Al in neurological disorders is given in [80] or [81]. Al negatively impacts the central nervous system in all species that have been studied, including humans.

4. Discussion

The adjuvants are explicitly intended to multiply the immunogenicity of the antigens but they also multiply the incidence of adverse reactions that are associated with the antigen. While adjuvants are essential to vaccines, as they multiply the reactogenicity, they also multiply the toxicity of vaccines: Pre-licensure clinical trials are not powered enough to be significant and are not of long enough duration to detect long-term effects. Studies on the potential synergistic damage from the administration of more than two vaccines when more than two vaccines are injected at the same time are missing [18]. Clinical trials are not designed to determine long-term implications. During Phases I to III, a vaccine is given to thousands of people to test for efficacy and safety [82]. While some vaccines undergo a Phase IV study after the vaccine is approved and licensed, still these studies are not designed to detect neurologic disorders that may develop also many decades after administration of many different vaccines. A better understanding of the work of adjuvants in adverse events is necessary.

What is under discussion is not the general adult body's handling of Al. Studies of adults receiving intravenous nutrition, or total parenteral nutrition, where certainly much larger doses of Al were administered directly into the circulation or in renal disease via dialysis water, do not address the issues of AlAd in vaccines administered to infants. In these cases of adults receiving intravenous nutrition, or total parenteral nutrition the Al load was considerable, but the only patients to develop neurologic findings were those who were uremic. The point in question is the injection in infants of AlAd through their particularly intense

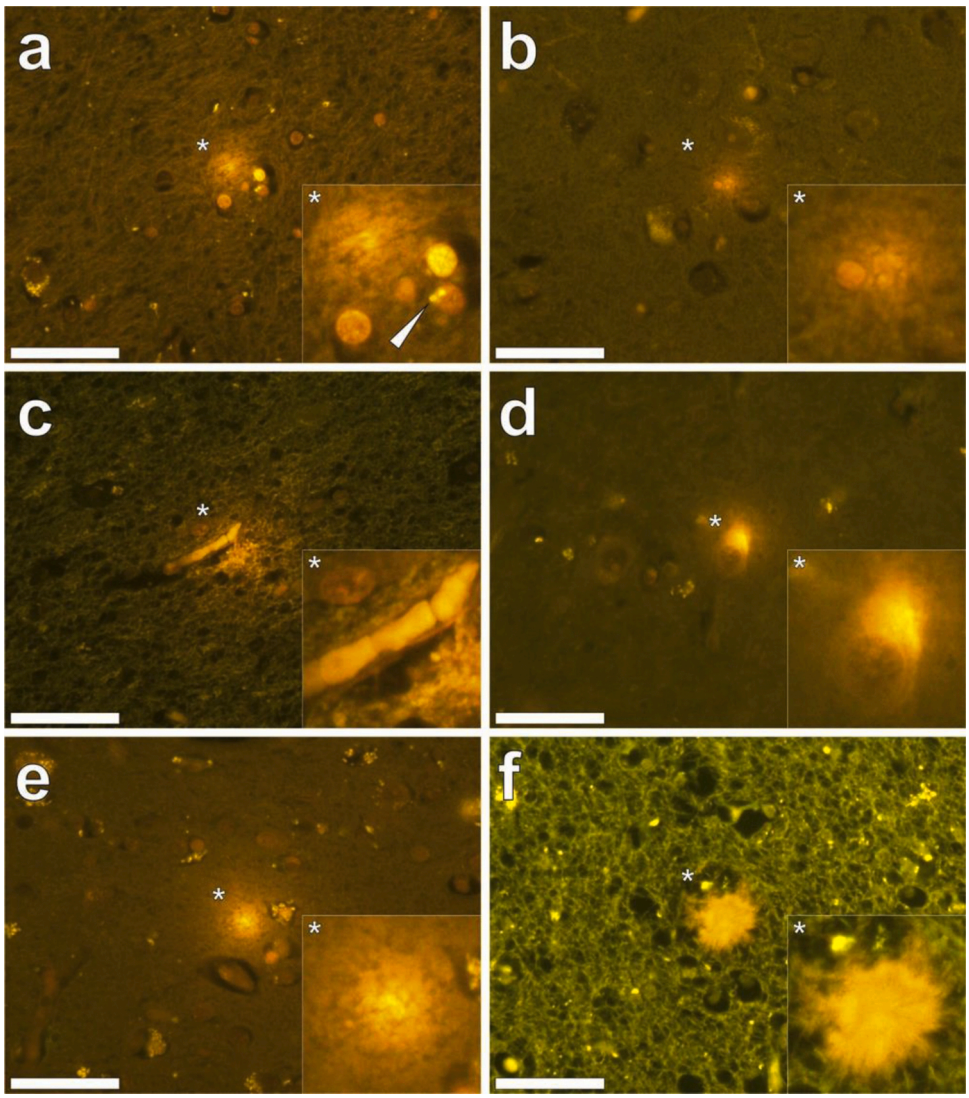

Fig. 3. Measurements of Aluminum in brain tissue from donors diagnosed with (a–c) ASD, (d) cerebral amyloid angiopathy (CAA), (e) epilepsy, and (f) familial Alzheimer's disease (fAD). Image reproduced from [62]. Open-access article distributed under the terms of the Creative Commons CC BY license.

immunization schedules. Not one isolated, but repeated intramuscular injections of AlAd in infants may dramatically contribute to a neurologic disease.

The number needed to vaccinate (NNV), i.e. the number of patients that need to be vaccinated for one patient to benefit [83], is sometimes very large. The larger the number, the worse is the efficacy of the vaccine. According to [84], 1, 852 children have to be vaccinated against influenza to avoid 1 hospitalization [85]. also states that for 1 hospital admission for influenza prevented, vaccination with Fluvax or Fluvax Junior may have caused 2–3 hospital admissions due to febrile convulsions. According to [86] 4, 255 to 6, 897 children 24–59 months of age have to be vaccinated for influenza to prevent 1 hospitalization. Hence, the number of vaccines being administered should be carefully considered.

The safety vs. efficacy of current formulation of vaccines adopting AlAd needs revision. Vaccines that include AlAds that show properties of long-term toxicity should be replaced with alternative formulations [85, 87], or their impact should be otherwise mitigated. Many of the vaccines currently administered may not be essential.

Conflicts of interest prevent a healthy scientific debate on the effect of aluminum adjuvants, [88–92]. The manufacture of consent through mass corporate media [93] defined as "*effective and powerful ideological institutions that carry out a system-supportive propaganda function*" biases a proper debate. The bias of science by mainstream media is a growing concern. As outlined in The Lancet article [94] "Corruption in global health: the open secret" every health professional knows this, but no one addresses it.

5. Conclusion

This work has summarized the three lines of evidence: ecological studies, animal studies, and measurements of Al in brain tissues of subjects with ASD, that together suggest a possible causal relationship between AlAd and the increasing prevalence of ASD. Mechanisms are explaining the Al neurotoxic effects. There is a causative explanation on a molecular level of the role of Al in neurological disorders.

The work has evidenced likely long-term neurotoxicity of AlAd in vaccines. This suggests a reduction of non-essential uses of AlAd: by reducing the number of vaccines being administered to those essential; reducing the amount of adjuvant based on weight; changing the formulation of the adjuvant, or avoiding multiple vaccinations during a single session in infants.

This review of lines of evidence in support of the hypothesis that aluminum adjuvants in vaccines might be a causal factor in neurodevelopmental disorders concludes that this correlation is clear.

Data availability statement

As a review of published information, supporting data when available may be found in the cited references.

Declaration of Competing Interest

The authors report no declarations of interest.

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Autism and Vaccines

Questions and Concerns

Autism spectrum disorder (ASD)is a developmental disability that can cause significant social, communication, and behavioral challenges. Recent estimates from CDC's Autism and Developmental Disabilities Monitoring Network found that about 1 in 54 children have been identified with ASD in communities across the United States. CDC is committed to providing essential data on ASD, searching for causes of and factors that increase the risk for ASD, and developing resources that help identify children with ASD as early as possible.

Vaccines do not cause autism.

Some people have had concerns that ASD might be linked to the vaccines children receive, but 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.

A 2013 CDC study [PDF – 7 pages] added to the research showing that vaccines do not cause ASD. The study looked at the number of antigens (substances in vaccines that cause the body's immune system to produce disease-fighting antibodies) from vaccines during the first two years of life. The results showed that the total amount of antigen from vaccines received was the same between children with ASD and those that did not have ASD.

Vaccine ingredients do not cause autism.

One vaccine ingredient that has been studied specifically is thimerosal, a mercury-based preservative used to prevent contamination of multidose vials of vaccines. Research shows that thimerosal does not cause ASD. In fact, a 2004 scientific review by the IOM concluded that "the evidence favors rejection of a causal relationship between thimerosal–containing vaccines and autism." Since 2003, there have been nine CDC-funded or conducted studies [PDF – 2 pages] that have found no link between thimerosal-containing vaccines and ASD, as well as no link between the measles, mumps, and rubella (MMR) vaccine and ASD in children.

Between 1999 and 2001, thimerosal was removed or reduced to trace amounts in all childhood vaccines except for some flu vaccines. This was done as part of a broader national effort to reduce all types of mercury exposure in children before studies were conducted that determined that thimerosal was not harmful. It was done as a precaution. Currently, the only childhood vaccines that contain thimerosal are flu vaccines packaged in multidose vials. Thimerosal-free alternatives are also available for flu vaccine. For more information, see the Timeline for Thimerosal in Vaccines.

Besides thimerosal, some people have had concerns about other vaccine ingredients in relation to ASD as well. However, no links have been found between any vaccine ingredients and ASD.

More Information

- Facts About Autism Spectrum Disorders
- Fact Sheet: Understanding Thimerosal, Mercury, and Vaccine Safety [PDF – 2 pages]
- IOM Report: Adverse Effects of Vaccines: Evidence and Causality, 2011
- Timeline: Thimerosal in Vaccines (1999-2010)
- Frequently Asked Questions about Thimerosal

Related Scientific Articles

Taylor LE, Swerdfeger AL, Eslick GD. Vaccines are not associated with autism: An evidence-based meta-analysis of case-control and cohort studies . *Vaccine*. 2014 June;32(29):3623–3629.

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Conflicts of Interest in Vaccine Safety Research

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Conflicts of interest (COIs) cloud vaccine safety research. Sponsors of research have competing interests that may impede the objective study of vaccine side effects. Vaccine manufacturers, health officials, and medical journals may have financial and bureaucratic reasons for not wanting to acknowledge the risks of vaccines. Conversely, some advocacy groups may have legislative and financial reasons to sponsor research that finds risks in vaccines. Using the vaccine-autism debate as an illustration, this article details the conflicts of interest each of these groups faces, outlines the current state of vaccine safety research, and suggests remedies to address COIs. Minimizing COIs in vaccine safety research could reduce research bias and restore greater trust in the vaccine program.

Keywords: adverse effects of vaccines, autism, conflicts of interest, vaccine research, vaccine safety

INTRODUCTION

How safe are vaccines? Health officials caution that no vaccine is 100% safe, but they sponsor studies that conclude the benefits of vaccines far outweigh the risks. Yet conflicts of interest (COIs) cloud the study of adverse effects of vaccines, and public skepticism about vaccine safety information is widespread (ASTHO, 2010). Investigation into the possible link between childhood vaccines and autism provides an illustration of the competing interests that sponsors of vaccine safety research face that could affect their objectivity in choosing which studies to support. Much research is sponsored by vaccine manufacturers and public health bodies, who have financial and bureaucratic interests that could impede the objective study of vaccine safety. These companies and agencies adamantly deny a link between vaccines and autism, and argue that vaccines are one of the most important innovations in disease reduction in the 20th Century (CDC, 1999). They cite several studies that conclude a

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link between vaccines and neurological disorders cannot be established (Offit, 2008). Such research is often disseminated by medical journals that have financial reasons to promote the views of the research sponsors. Conversely, research promoted by some autism advocacy groups presents several overlapping and interwoven theories that link vaccines to autism. Researchers suggest that live viruses and the neurotoxins mercury and aluminum in some vaccines may be associated with neurological disorders (Jepson and Johnson, 2007).

This article examines COIs among people who conduct vaccine safety research as well as institutions that support the research. Using the investigation into the possible link between childhood vaccines and autism as an illustration, this article discusses the current state of vaccine safety research. Gaps in current research are discussed as well as the low level of public trust in the research. To address COIs, Resnik's (2004) framework is used to determine which conflicts to prohibit, which to manage, and which merely to disclose. The existence of COIs does not necessarily mean that the research is fraudulent or that the system that sponsors the research is wholly corrupt. To be sure, many honest and unbiased researchers are examining vaccine safety. However, COIs are widespread, and research consumers cannot know the extent of the problem. Thus, the reliability of any of the information generated is uncertain. If an unbiased researcher bases his or her work on biased research, the result could be an unintentional perpetuation of the bias. Acknowledging and ameliorating the COIs could lead to better and more trusted vaccine safety research.

COIS AMONG SPONSORS OF VACCINE SAFETY RESEARCH

Funding for research on vaccines and vaccine safety comes from several sources. Vaccine developers must provide regulators with studies showing the safety of their products, and they sometimes sponsor similar studies for the medical community at large. Public health agencies, such as the Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) in the United States, sponsor studies that promote public health. Other sponsors of research include Congressional committees, special medical panels, advocacy groups, and indirectly, medical journals. All of these sponsors face competing interests that could affect their objectivity in determining which research to promote.

Vaccine Manufacturers

Vaccine manufacturers have a COI related to the tension between making profits and studying the negative side effects of their products. Vaccines are a big and growing business: Worldwide sales of pediatric vaccines in 2009 were about $11.5 billion, and sales are expected to reach close to $20 billion by 2014 (Sahoo, 2010). Once manufacturers have met the expensive regulatory hurdles

of vaccine approval, they have little incentive to research the safety of their products. Although postlicensure analyses are typically undertaken to ensure the safety of the products, such analyses in the United States, for example, are performed by the same regulatory agencies that initially approved the vaccines (Salmon et al., 2004). Moreover, vaccine manufacturers do not face the threat of lawsuits that might motivate other industries to seek to improve safety. The National Childhood Vaccine Injury Act of 1986 protects vaccine companies in the United States from being sued. The protection was deemed necessary, because vaccine manufacturers were facing increasing tort litigation and an adequate supply of vaccines at stable costs was considered essential for public health (Supreme Court, 2011). One implication of the legislation was to provide incentives for the development of new vaccines, which typically earned smaller profit margins per dose than other drugs. Citizens in the United Kingdom may sue vaccine manufacturers, but no plaintiff has ever been successful (Hanson, 2007).

Concern about adverse effects of vaccines on sales is evident in intra-office correspondence at Merck, a vaccine manufacturer. In a 1991 internal memo to executives at Merck, Maurice Hilleman, a vaccine researcher, reported that some countries were considering banning thimerosal, the mercury-containing preservative. He admitted he did not know whether thimerosal was dangerous, but he warned that sales could be affected by public perceptions. He suggested reducing the thimerosal content of vaccines being exported. In the memo, Dr. Hilleman gave no indication that he would investigate whether the thimerosal in vaccines could harm infants and young children despite his stated concern (Hilleman, 1991).

Compounding the COIs inherent in the business of manufacturing vaccines is the fact that vaccine manufacturers sponsor research. The influence of industry is wide-spread: It affects individuals as well as institutions and study outcomes as well as research initiatives. In a survey of faculty at top U.S. medical research institutions, Tereskerz et al. (2009) found over two-thirds of researchers (338 out of 506) received some support from industry. Studies show that the financial interests of researchers are positively associated with outcomes favorable to the sponsor in medical studies (Friedman and Richter, 2004; Jefferson et al., 2009; Yank et al., 2007). Not only individual researchers, but also research institutions can be influenced by industry sponsorships such as grants, endowed chairs, and other gifts (Tereskerz, 2003). Industry sponsorship can influence not only outcome, but research initiatives as well: The Tereskerz et al. (2009) survey mentioned above also found 35% of respondents knew of industry-sponsored researchers who compromised their research agenda because the researchers were sponsored by industry. Where industry support was important to the research unit, over half of respondents knew of researchers who compromised their research initiatives. The same study noted that industry support tended to go to senior or well-established researchers,

so industry influence on research agenda could reach younger researchers who work with or for their more established mentors.

Although authors of research articles are supposed to declare COIs, authors do not always fully disclose important information. For example, the tobacco industry was adept at recruiting medical researchers to refute any link between smoking and cancer without having the researchers reveal their sources of the funding (Drope and Chapman, 2001). Few consequences seem to be in place for authors who do not declare COIs, and at least one major medical journal, *Journal of the American Medical Association (JAMA)*, has modified its policy to make the investigation of COIs less transparent (DeAngelis and Fontanarosa, 2009). Besides receiving research funds from industry, researchers are sometimes paid to put their names on articles they did not write. The true industry-sponsored author is not revealed, so the reader is often not aware of the industry influence on these ghost-written articles (Ngai et al., 2005).

U.S. FDA

The U.S. FDA faces at least three COIs when it considers sponsoring research into the possible link between vaccines and autism. The first is the mission of the FDA, which is to protect "the public health by assuring the safety, efficacy, and security of human and veterinary drugs. . . . The FDA also helps the public get the accurate, science-based information they need to use medicines and foods to improve their health" (FDA, 2009). The FDA evaluates and approves vaccines for safety and efficacy. Sponsoring research that finds a link between autism and vaccines that the FDA has approved could greatly damage the Administration's reputation and reduce public trust in the FDA.

A second major COI in the FDA lies in the way the Administration is funded. In 1992, the Prescription Drug User Fee Act was adopted whereby pharmaceutical companies paid fees to have their drugs evaluated. The intent of the legislation was to enhance the resources of the FDA and thereby speed up evaluations. However, industry funding could result in industry influence (Angell, 2004). While the Act refers only to prescription drugs and not vaccines, many vaccine manufacturers also produce prescription drugs. The user fees paid by drug manufacturers provide incentives for the FDA to be more friendly to the industry since it is dependent upon industry funding.

A third conflict involves the National Vaccine Injury Compensation Program. Parents who believe their child may have been injured by a vaccine can file a claim in the Division of Vaccine Injury Compensation (DVIC). Both DVIC and the FDA are divisions of the U.S. Department of Health and Human Services (DHHS). If the information that the FDA is mandated to provide the public includes studies that show vaccines could be related to autism, it would be providing evidence for claims being filed within its own agency.

As of December 2011, over 5,600 autism claims have been filed in DVIC. The average payout for vaccine-related injuries is close to $825,000 (DHHS, 2011), so the autism claims could cost the Program over $4.6 billion. Additionally, more parents would seek compensation if DVIC recognized autism as a vaccine injury.

U.S. Centers for Disease Control and Prevention (CDC)

After a vaccine receives approval from the U.S. FDA, the U.S. Centers for Disease Control and Prevention (CDC) decides whether to add a vaccine to its recommended schedule for the U.S. civilian population. The CDC also sponsors research on vaccine safety. It has at least three major COIs that could hamper its ability to provide objective research about vaccines. The first is the nature of the CDC's mandate, which is to prevent and control disease, injury, and disability (CDC, 2012). Thus, the CDC is obligated to prevent disease, which it does largely by promoting vaccination. It is also charged with controlling disabilities. If the research it sponsors were to identify vaccines as being hazardous and if the vaccination schedule it recommends is associated with autism, it would be forced to concede that its policies did not support its goals and actually promoted disabilities. Since the CDC is charged with promoting vaccination programs as well as assessing vaccine risks, it might be reluctant to sponsor research that uncovers risks it may have created.

An example of the CDC being concerned about research into a problem it may have created occurred in 2000, when the CDC commissioned the Institute of Medicine (IOM) to evaluate vaccine safety, particularly the possible links between the mumps-measles-rubella vaccine and the mercury-containing preservative thimerosal with autism. In a discussion concerning the proposed study (IOM, 2001), Dr. Marie McCormick, then Chair of the Immunization Safety Review Committee of the IOM, said (p. 33), "[The CDC] wants us to declare, well, these things [vaccines] are pretty safe on a population basis." Later in this planning discussion, Dr. McCormick decided (p. 97), "[W]e are not ever going to come down that [autism] is a true side effect [of vaccines] . . . ", thereby declaring a conclusion before the study was undertaken. In its final report, the IOM stated that although a link between vaccines and autism was possible theoretically, epidemiological studies favored no causal link and suggested funds be channeled to more promising areas of research (IOM, 2004). Other researchers who receive grants from the CDC may also be leery of investigating problems their benefactor may have created.

A second conflict involves the National Vaccine Injury Compensation Program. Parents who believe their child may be have been injured by a vaccine can file a claim in the DVIC, which, along with the CDC, is part of DHHS. Thus, if the CDC sponsored research that found vaccines had side effects such as autism, it would be providing evidence for claims filed against its own agency.

Finally, officials at the CDC may see working for the government as a stepping stone to employment at a vaccine manufacturer. A year after leaving as director of CDC in 2009, Dr. Julie Gerberding took a position as president of Merck Vaccines. During her tenure as CDC director from 2002 to 2008, Dr. Gerberding supported the above-mentioned IOM study as well as other studies that concluded no link between vaccines and neurological disorders could be found (see CDC, 2010, for an overview of the studies). Another former CDC employee, Dr. Thomas Verstraeten, began working for GlaxoSmithKline when he was in the process of completing a major study on the potential negative side effects of thimerosal at the CDC (Verstraeten, 2004); the study found no consistent significant associations between thimerosal and negative neurological outcomes (Verstraeten et al., 2003). While the studies may have been good analyses, the COI regarding research emphasis or conclusion is unavoidable when a public official takes a lucrative position in the industry that s/he previously regulated.

U.S. Congress

While U.S. Congressional committees have undertaken a few investigations into the possible link between vaccines and autism (US HR, 2000a,b, 2003), they have not actively pursued the issue. Members of Congress may be reluctant to sponsor research into vaccine safety for at least two reasons: contributions and prospects of future employment. According to the Center for Responsive Politics, the pharmaceutical/health products industry spent over $2.3 billion between 1998 and 2011 to lobby elected officials and candidates, more than any other industry (CRP, 2011). CRP also reports that the number of lobbyists increased steadily from 729 in 1998 to a peak of 1,803 in 2008, declining to 1,612 in 2010 (CRP, 2010). Since 2005, the industry employed at least three lobbyists for every member of Congress. Additionally, a revolving door exists between Congress and the pharmaceutical industry. Over half of the lobbyists employed by the pharmaceutical industry in 2008 had worked in Congress or another branch of the federal government, and 35 had been former members of Congress (Beckel, 2009). Mandating a study that could hurt major contributors or future employers could result in fewer contributions or no offers of employment or both.

Special Medical Panels

Special panels in the medical community can sponsor vaccine safety studies. One special panel that could provide grants for vaccine safety studies is the U.S. Interagency Autism Coordinating Committee (IACC), which coordinates the various agencies within the DHHS that explore autism. Part of IACC's mandate is to fund research into possible causes of autism. In January

2009, Dr. Thomas Insel, chair of the Committee, called a surprise re-vote on whether to support the funding of two studies that were to have investigated the possible link between vaccines and autism. Although the committee had voted in December 2008 to support the studies (IACC, 2008), the committee decided against conducting the studies in the re-vote. Dr. Insel said DHHS' Health Resources and Services Administration (HRSA), which administers both the grants for IACC as well as the vaccine-injury compensation funds, faced at least the appearance of a COI:

> So the optics of having HRSA vote on issues related to autism and vaccines, when they have a large court case, the optics of having people who could be perceived to have or to represent those who have a financial investment in this issue. It takes it out of the realm of a scientific question, a research question, and it raises the possibility that some could see whatever comments we make as being biased by non-scientific issues. . . . If we say, yes, we think it's important to look at this and to provide additional information, it implies that we believe that there's a relationship between autism and vaccines . . . If we say we don't think that this needs to be pursued, it opens us up to the possibility, at least the optics, that we were trying to keep HRSA from having to go down this road legally. (IACC, 2009)

Advocacy Groups

Some independent advocacy groups are skeptical of vaccines and are interested in exposing the dangers of vaccines. These non-profit organizations sponsor research into the possible association between vaccines and autism. Groups such as the Autism Research Institute (ARI), the National Vaccine Information Center (NVIC), and Sensible Action for Ending Mercury-Induced Neurological Disorders (SafeMinds) provide limited grants for the study of vaccine safety. These groups consider that vaccines or vaccine ingredients may be associated with autism and have a reputational interest in the outcome of the research. Some members of these organizations also have a legislative agenda that includes enacting laws to allow vaccination choice and allocating more resources to the study of vaccine side effects (Habakus and Holland, 2011). Parents of children with autism or other neurological disorders founded many of these groups; some of the parents have filed claims under the U.S. Vaccine Injury Compensation Program. Therefore, some individuals associated with these groups have a financial interest in seeing research that establishes a link between vaccines and autism. These organizations sponsor relatively small projects: ARI grants average about $20,000 (ARI, 2012), and SafeMinds grants range from $5,000 to $75,000 per year (SafeMinds, 2012); the entire research budget for NVIC is roughly $100,000 (NVIC, 2012). While these organizations are not as well-staffed or well-funded as government agencies or vaccine manufacturers, their main task is to generate information to refute agency

or industry claims. In so doing, they are known to fund research to help bolster their position. Although there is limited oversight concerning the general information these groups disseminate, the research they sponsor goes through the same vetting process as any other research that appears in peer-reviewed journals.

Medical Journals

While medical journals do not sponsor vaccine safety research directly, they disseminate research and thereby influence the type of research that is sponsored. If an area of research is sponsored, but not published, sponsors will not continue to fund the area.

Medical journals should be the repository of objective, unbiased research. However, some authors of articles as well as publishers of journals have COIs concerning the dissemination of research on vaccine safety. An author who is a paid consultant for or receives grant money from a vaccine manufacturer has a COI when publishing a paper analyzing the safety of vaccines. This COI does not mean that the analysis is incorrect, but the conflict could influence the analysis. An editorial in the *New England Journal of Medicine (NEJM)* noted generally:

> What is at issue is not whether researchers can be 'bought' in the sense of a quid pro quo, it is that close and remunerative collaboration with a company naturally creates goodwill on the part of researchers and the hope that the largesse will continue. This attitude can subtly influence scientific judgment. (Angell, 2000)

Medical journal authors' ties to vaccine manufacturers are pervasive, as revealed in a review of authors of vaccine safety articles published in top journals. Table 1 reports the number of articles found by searching in EBSCO*host* for the terms 'vaccine' and 'safety' in the abstracts of original research articles of selected journals from 2006 to 2010. Lundh et al. (2010) have identified the following as major medical journals based on their impact factors: *Archives of Internal Medicine (Archives), Annals of Internal Medicine, British Medical Journal (BMJ), JAMA,* the *Lancet,* and the *NEJM*. All these journals were searched as well as *Pediatrics,* because of its emphasis on children's health. *Archives* had no studies meeting the search criteria, but the remaining journals contained a total of 39 studies that did meet the criteria. Thirty-one studies, or 79.5%, included at least one author who declared a COI with a vaccine manufacturer, and 24 studies, or 61.5%, included at least three authors with COIs.

Not only authors, but also journals themselves can be conflicted. Washington (2011) details the reliance of medical journals on advertising from pharmaceutical companies, which can account for up to 99% of a journal's advertising revenue. Fugh-Berman et al. (2006) point out that some journals

Table 1: Original research articles with "vaccine safety" in abstract, 2006–2010.

Journal	Total number	At least 1 author discloses COI	At least 3 authors disclose COI
Annals of Internal Medicine	1	1	0
BMJ (international edition)	1	1	1
JAMA	3	3	2
Lancet	9	9	7
New England Journal of Medicine	11	9	7
Pediatrics	14	8	7
Total	39	31	24

accept advertising revenue only from companies that sell products relevant to medicine, thereby increasing the reliance of the journal on drug companies. In testimony before the U.K. House of Commons Science and Technology Committee, Dr. Fiona Godlee, editor-in-chief of the *BMJ*, further characterized the relationship between journals and drug companies as follows:

> Even on the peer-reviewed side of things, it has been said that the journals are the marketing arm of the pharmaceutical industry. That is not untrue. To a large extent, that is true. (UK HC Science and Technology Committee, 2011)

Additionally, pharmaceutical companies provide funds to medical journals by purchasing article reprints and subscriptions that the companies distribute to physicians. Although information on the amount of revenue generated through reprints is not publically available, Lundh et al. (2010) queried six major medical journals to determine the influence of reprints on their total revenue in 2005–2006. The two journals that responded were *BMJ*, which reported that the selling of close to one million reprints represented 3% of its income, and the *Lancet* for which the selling of over 11 million reprints represented 41% of its income. Lundh et al. determined from public sources that the American Medical Association, which publishes *Archives* and *JAMA*, earned approximately 12% of its revenue from reprints. Testimony by Dr. Richard Horton, editor of the *Lancet*, to the U.K. House of Commons Health Committee provides further evidence of the importance of reprint income. Horton discussed his experience with calls from investigators about their research. If Horton expressed interest in the work, the investigator might indicate the article could generate reprint revenue. Horton explained:

> Then the conversation might go: 'It is likely that the company will want to buy several hundred thousand reprints' and of course several hundred thousand reprints might translate into half a million pounds, a million pounds revenue to the journal. There is an implicit connection between the submission of a paper and the revenue that comes into a journal. (UK HC Health Committee, 2005)

Horton further testified that if a reviewer were too critical of an article, the research sponsor might call Horton and demand that the journal be less critical. The company representative might threaten to pull the paper and point out that if the paper were pulled, there would be no reprint income for the journal.

VACCINE SAFETY RESEARCH TODAY

Resnik (2004) points out that COIs can lead to biased research, injuries and low trust. While we cannot know with certainty whether the COIs discussed above have influenced the current state of vaccine safety research, we do know that gaps exist in research, reports of vaccine injuries are not studied, and public trust is low. Effectively addressing these issues involves minimizing the COIs that vaccine safety researchers face. However, unlike other medical researchers, almost all vaccine safety researchers face some kind of COI.

Most Researchers Face COIs

Typically, COIs in medical research are confined to industry influence. For example, industry-sponsored research showed smoking was safe. Researchers who wrote on the dangers of smoking wanted to refute the industry safety studies, but, until the desire to enact laws to ban smoking arose, the researchers did not have a political agenda. However, some vaccine skeptics have formed organizations (see *Advocacy Groups* above) and have a legal agenda: They want vaccination choice and they want compensation for alleged victims (Habakus and Holland, 2011).

The fact that COIs exist for sponsors of research that promote vaccines as well as those who are skeptical of vaccines could contribute to several trends in vaccine research today. One trend is the increased interest in research concerned with vaccine safety since the 1990s, when a link between vaccines and autism was first hypothesized. Table 2 shows the steady growth in the proportion of vaccine articles indexed in PubMed that include safety. Of the articles indexed in PubMed that contained the term "vaccine" in the abstract, a little over 2% also contained the term "safety" in 1980. This percentage grew to 5% in 1990 and 7% in 2000. By 2010, the percentage was close to 10%.

Another trend is the increase in contradictory research. Some studies show that a link between autism and vaccines cannot be established, while others conclude the question is open and more study is needed. Parents, typically the ultimate decision makers concerning vaccines, are confused, yet they can find more information to assist in answering their questions. Moreover, the search for information is an on-going process. Even if parents decide to fully vaccinate their infants, they may decide later to delay or refuse shots, especially if their

Table 2: Articles indexed in PubMed concerning vaccine safety.

Year	"Vaccine" in abstract (#)	"Vaccine" and "safety" in abstract (#)	"Vaccine" and "safety" in abstract (%)
1980	1,131	24	2.1%
1990	2,118	105	5.0%
2000	3,988	281	7.0%
2010	8,288	792	9.6%

child shows signs of neurological impairments. This course of action is easier to pursue since research exists on both sides of the vaccine safety question.

Consumers of medical research must in all likelihood accept that almost all researchers of vaccine safety face COIs, and discount accordingly. They already know how to discount industry-sponsored research, but they must learn how to discount research sponsored by groups that are skeptical of vaccines. Regardless of the research sponsor, consumers must be exceptionally vigilant in assessing the research questions being asked, the manner in which the study is designed, which data are collected and how, and whether the conclusion follows from the analysis. For example, epidemiological studies conclude that a link between vaccines and autism cannot be established (Hviid et al., 2003; Madsen et al., 2003; Smith and Woods, 2010), yet such studies are designed to create hypotheses, not determine causation or lack thereof (Washio et al., 2008).

Some vaccine safety researchers appear to have few, if any, connections with vaccine manufacturers, North American or Western European regulators or groups that question vaccine safety. Research is emerging from outside North America and Western Europe and appears to be written by people with few or no conflicts. Researchers such as Wu et al. (2010) from China, Dorea and Marques (2010) from Brazil, and Duszczyk-Budharthoki et al. (2011) from Poland declare in their publications that they have no COIs. The results of these vaccine safety studies are mixed: Wu et al. conclude the H1N1 vaccine is safe, while Dorea and Marques and Duszczyk-Budharthoki et al. conclude mercury and aluminum in vaccines can be harmful.

Gaps in Vaccine Safety Research Exist

While the safety of an individual vaccine is considered in the regulatory approval process, studies tend to observe the effects of a vaccine for only a few weeks after the administration of the shot, so long-term effects are unknown. Manufacturers and regulators are to perform postlicensure studies, but resources for such studies are limited: Cooper et al. (2008) report that the U.S. Immunization Safety Office has a budget of only $20 million, which is a fraction of the close to $3 billion allocated to the U.S. National Center

for Immunization and Respiratory Diseases, which distributes vaccines and monitors vaccine-preventable diseases. Moreover, no study of the safety of the entire U.S. vaccine schedule has ever been undertaken. That is, the safety of the combination of vaccines is unknown.

Additionally, questions about vaccine safety are not addressed. For example, questions surrounding the safety of thimerosal, which is half ethylmercury, persist. The typical influenza vaccine contains 50 micrograms of thimerosal, and the U.S. state of California classifies thimerosal as a mercury compound, which can cause developmental toxicity (CA EPA, 2004). However, no study offers guidelines for safe levels of injecting ethylmercury (FDA, 2011). Aluminum is another ingredient found in vaccines, yet the risks are not well understood (Dorea and Marques, 2010; Tomljenovic and Shaw, 2011a,b).

At least one public health official has raised concerns about the gaps in vaccine safety research. Dr. Bernadine Healy, former director of the U.S. National Institutes of Health, commented that public health officials were not pursuing a possible link between vaccines and autism out of fear for what they might find and the effects on the vaccination program:

> There is a completely expressed concern that they don't want to pursue a hypothesis because that hypothesis could be damaging to the public health community at large by scaring people . . . I think the public's smarter than that. The public values vaccines. But more importantly, I don't think you should ever turn your back on any scientific hypothesis because you're afraid of what it might show. (Attkisson, 2009)

One doctor who explored the possible hazards of vaccines became the center of a storm of controversy. In a series of case studies, Dr. Andrew Wakefield and colleagues suggested a possible association between gastrointestinal issues—perhaps precipitated by the measles-mumps-rubella vaccine—and autism:

> We have identified a chronic enterocolitis in children that may be related to neuropsychiatric dysfunction. In most cases, onset of symptoms was after measles, mumps, and rubella immunisation. Further investigations are needed to examine this syndrome and its possible relation to this vaccine. (Wakefield et al., 1998)

The U.K. General Medical Council, a professional self-governing body that licenses doctors, created a Fitness to Practice (FTP) hearing panel that found Wakefield guilty of serious professional misconduct (GMC, 2010) and revoked his license to practice medicine in the United Kingdom. Although some say the research is fraudulent (Deer, 2011), others point to research that substantiates Wakefield et al.'s conclusions (PR Newswire, 2011). Putting aside the hotly debated question of Wakefield's guilt or innocence, Wakefield's experience could have a chilling effect on any researcher considering the study of vaccine risks.

Reports of Vaccine Injuries Are Not Investigated

Although many parents report that vaccines have caused or are associated with autism, no research sponsor has launched a major investigation of the children who are alleged to have developed autism from vaccines. One study found at least 83 vaccine-injured people who received compensation from the U.S. Vaccine Injury Compensation Program (VICP) had autism along with other disabilities (Holland et al., 2011). Besides those compensated, more than 5,600 people have filed claims in VICP stating that vaccines triggered their child's autism (DHHS, 2011) and over 2,000 reports of autism or autism spectrum disorder as a vaccine reaction have been reported to the Vaccine Adverse Event Reporting System (VAERS), which collects information from people who believe they or their child have been injured by a vaccine. The system is an unreliable measure of vaccine reactions: Scott et al. (1990) note that such a passive system vastly underreports the true number of adverse events, whereas Ellenberg and Chen (1997) point out that reports could be mere coincidences so that overreporting could be a problem. Regardless of the accuracy of the claims or of the reporting system, many parents suspect vaccines caused their child's autism. One study found almost half of all parents of children with autism believe that vaccines triggered their children's disorder (Law et al., 2010). These parents could be wrong, but they have not yet been convinced by current research that vaccines are safe. Despite these reports and parental suspicions, no research sponsor has supported a large-scale study of the prevalence of antism among vaccinated versus unvaccinated children, nor are vaccination records included in prospective studies. For example, the Columbia Center for Children's Environmental Health sponsors studies that track pre- and post-natal exposures to a variety of environmental pollutants to determine possible adverse health outcomes, yet exposures to vaccines are not included (CCCEH, 2011). Nor are vaccinations included in the National Child Study, which also looks prospectively at the influence of other environmental factors on children's health (NCS, 2012).

Trust in Vaccine Safety Research Is Low

Some industry analysts have characterized public confidence in vaccines as a crisis (Black and Rappuoli, 2010). Kennedy et al. (2011) report that 77% of U.S. parents surveyed have at least one concern about vaccine safety. According to a CDC report, 39% of parents surveyed in the United States said they either delayed or refused vaccinations for their children (DeNoon, 2010). In a survey by WebMD, almost 70% of U.S. parents wanted information about vaccine risks, and 66% said they either questioned or refused a vaccination for their child (DeNoon, 2011). Almost half of all parents surveyed in the United States question the validity of vaccine safety data because of the influence

of pharmaceutical companies, and over 40% believe the government is covering up information about vaccine safety (ASTHO, 2010). In one study, parents reported being most concerned about the MMR, HPV, and influenza shots; parents' most common fears were autism, too many shots, and serious side effects (Tryon et al., 2011).

Not only parents, but health care workers including new doctors are also raising questions about vaccine safety. One study revealed that only 40% of health care workers received an influenza shot (King et al., 2006). In another study, reasons for refusal by health care workers included concern over adverse reactions (Clark et al., 2009). When the state of New York mandated the influenza and H1N1 shots for medical professionals, health care workers protested, citing safety concerns about the shots (Matthews, 2009). The mandate was withdrawn within two months of being issued and before it ever took effect (Chan and Hartocollis, 2009). In another study, new doctors were found to be more skeptical about vaccine safety than their older peers. They particularly questioned the safety of the polio, MMR, and varicella vaccines (Mergler and Omer, 2011).

DISCUSSION

Addressing the COIs among the people who conduct research and institutions that study vaccine safety could reduce bias while restoring public trust. Research suggests egregious COIs should be prohibited, while some COIs can be managed and others need only to be disclosed (Resnik, 2004). Such a framework is useful in determining how to address COIs in vaccine safety research.

Prohibit Agencies that Promote Vaccines from Overseeing Vaccine Safety

The U.S. airline industry offers a model for addressing the conflict of the CDC both promoting vaccines and overseeing vaccine safety (Salmon et al., 2004). Similar to the U.S. National Transportation Safety Board (NTSB), which oversees transportation safety issues of national importance, an independent agency to oversee vaccine safety could be established. The NTSB is an independent agency, which receives no funding or administrative support from the U.S. Department of Transportation. Likewise, separating a National Vaccine Safety Board from the DHHS would be important, based on the experience of the NTSB. Although the NTSB had been part of the Transportation Department, officials decided to separate the two entities for proper oversight, declaring ". . . No federal agency can properly perform such (investigatory) functions unless it is totally separate and independent from any other . . . agency of the United States" (NTSB, 2011).

However, creating a credible independent vaccine safety agency would be difficult since airline accidents differ from vaccine injuries in at least two crucial ways. First, an airline accident is obvious. The airline industry cannot sponsor studies that claim that the accident did not occur, nor can it suppress media reports of the accident. Vaccine injury, however, can be explained away as coincidence, especially if the injury does not manifest itself until several years after the administration of the shot. Since vaccine injury can be difficult to determine, it is all the more important that the agency that oversees vaccine risks should be separate from the agency that promotes vaccines. If a parent reports an injury to an agency that is specifically charged with vaccine risk, the agency has an incentive to investigate.

A second major difference stems from the first: Since accidents are so apparent in the airline industry, the industry is forced to promote safety. An airline with a bad safety record would lose customers and face ruinous lawsuits. The vaccine industry has no such constraints; the state mandates that children receive the industry's products and the only legal avenue of redress is a vaccine injury panel, not a court of law. Indeed, once a vaccine has gone through the expensive approval process, a vaccine manufacturer has a disincentive to study negative side effects. Admitting the existence of such side effects might compel the manufacturer to withdraw the vaccine, make improvements that reduce the side effect, and then seek regulatory approval once again, a very expensive process. A credible vaccine safety board could address some of these issues. Any report by a parent or doctor of an adverse reaction to a vaccine would be investigated. Moreover, this agency could compare the long-term health outcomes of children who were vaccinated versus those who were not vaccinated: No such study has yet been performed. If certain vaccines were found to be associated with autism, bad publicity would force vaccine manufacturers to be concerned about the risks of those vaccines.

Despite the difficulties, creating an independent vaccine safety agency would assist in restoring public confidence in the vaccine program. As Salmon et al. (2004) noted: "The public must know that vaccine safety concerns are taken seriously and investigated by independent professionals whose primary responsibility is safety, not financial gain, public image, or program goals."

Prohibit Government Officials from Working for Vaccine Manufacturers

Closing the revolving door between public office and vaccine manufacturers could help to restore confidence in the vaccine program. Parents would trust public health officials in the government if parents knew the officials could not use public service as a stepping stone to a lucrative position in private industry. Specifically, any person in a vaccine policymaking position or any member of a vaccine advisory committee could have no past funding or salary from a vaccine

manufacturer. Nor should they own stock in a vaccine company. Moreover, the vaccine policymakers and vaccine advisors would not be allowed to receive grants or salary from or purchase stock in vaccine manufacturers after leaving their posts. The same policy could be in effect for special masters who decide vaccine injury cases as well as legislators who enact laws that affect vaccine regulations.

Since finding qualified vaccine experts who have no past ties to pharmaceutical companies is very difficult (Drazen and Curfman, 2002), the implementation of this policy would be step-wise and long-term. The first step would be to prohibit vaccine policymakers from receiving funds after leaving their positions. Currently, in the United States, policymakers must wait one year before accepting a position in an industry they regulated. This waiting period should be extended to at least five years, or, ideally, ten years. The government officials could work in other industries or academia, but not in the industry they regulated or in an academic capacity where they would receive funds from industry. The prohibition of past funding could be phased in by setting caps on the amount a policymaker received in the past. The caps could be lowered over time, and ultimately reach zero. Phasing in this program might not take as long as one might expect: The American Medical Student Association launched a PharmFree Campaign in 2002 whereby medical students can pledge not to accept funds from pharmaceutical companies either as students or as doctors (AMSA, 2011).

Manage the Influence of Vaccine Manufacturers on Medical Journals

To ensure the objectivity of published medical research on vaccine safety, vaccine manufacturers should not influence medical journals. One way to improve the integrity of medical publications is for central repositories of research to require disclosure of ties between journals and pharmaceutical companies. PubMed, an on-line index of biomedical literature maintained by the U.S. National Institutes of Health, is such a repository. PubMed could require journals that it cites to disclose any COIs, including the amount of revenue the journal receives from pharmaceutical companies—including advertising and reprints—as well as whether owners or editors of the journal are stockholders or board members of pharmaceutical companies.

The benefits of information must be balanced with the costs of obtaining the information. Collecting and reporting information costs both time and effort. Institutions that request information should attempt to obtain information that is already being collected by the journals. Such institutions could work with the journals' accountants to determine which information is readily available and to create a standardized form—similar to the form that the International Committee of Medical Journal Editors (ICMJE) suggests its

member journals require of authors in the journals (ICMJE, 2011). The benefit of such an undertaking is similar to the benefit of requiring individual authors to disclose COIs: Knowing the extent of private industry involvement would go far in assuring an increasingly wary research consumer that major medical journals are not unduly influenced by pharmaceutical companies.

Disclose All Financial Payments to Doctors

One step to address COIs in the vaccine safety research in the United States is the Physician Payment Sunshine Act (PPSA). Beginning in 2012, drug manufacturers must report to the DHHS all payments they make to doctors or teaching hospitals. DHHS is then to make the data easily available to the public, including the names and addresses of doctors or hospitals as well as the types and amounts of payments. Such disclosures are particularly important for doctors who are researching vaccine safety or sponsoring such research.

Besides reports from drug companies, medical researchers themselves as well as research hospitals should disclose the financial ties they have with third parties. Disclosures should be easily available to the public and, to the extent possible, streamlined. Authors of articles published in journals that belong to the ICMJE must already report their funding sources and their financial ties with third parties; researchers and research institutions could post the same form on a central website. The form is electronic and can be easily up-dated. It should include not only that a money transfer to a doctor or institution has been made, but also the amounts that the researchers receive, since even small transfers can influence behavior (Katz et al., 2003). To present a complete profile of financial ties, medical researchers should also disclose stock holdings and stock options. Not only researchers, but also people who serve in national health offices such as DHSS or on special health panels such as IACC should openly disclose all financial ties to vaccine manufacturers.

Some may argue that having both companies and individual doctors or institutions report financial ties would be redundant. However, such a system is currently in place in the United States regarding income tax. Companies report how much they pay employees, and individuals report how much they earn as well as their income from sources other than working. Likewise, individual doctors may own stock in vaccine manufacturers or receive funds from non-U.S. manufacturers that are not covered under the PPSA. Therefore, both sources of information are needed.

Enforcing the disclosure requirements would be a challenge. Since vaccine manufacturers and public health regulators face COIs, the enforcement agency should be independent of industry and current regulators. In the United States, the Internal Revenue Service has experience auditing financial claims and could extend its work to ensure vaccine manufacturers, doctors, and medical institutions accurately disclose financial ties. Fines could be exacted for

non-compliance as well as banning researchers from publishing in journals for a period of time or receiving government grants for research or both.

Disclose All Data in Vaccine Safety Studies

Since the consumers of vaccine safety research must be exceptionally vigilant in understanding the studies (see section *Most Researchers Face COIs*), the readers should have easy access to the data used in the studies. The U.S. National Academies recognized the importance of sharing data when they created the Committee on Responsibilities of Authorship in the Biological Sciences. The Committee created the "uniform principle for sharing integral data and materials expeditiously (UPSIDE), which includes the following tenet:

> An author's obligation is not only to release data and materials to enable others to verify or replicate published finds (as journals already implicitly or explicitly require) but also to provide them in a form on which other scientists can build with further research. (NRC, 2003)

Data could be posted with the electronic version of studies or deposited with the journal as a requirement for being published.

Implementing this recommendation could be difficult. Although Public Library of Science (PLoS) journals require all authors to honor requests for data from independent researchers, Savage and Vickers (2009) found that most researchers did not comply. Only one of the ten authors Savage and Vickers contacted provided them with the raw data they requested. Some of the authors were not permitted to provide data, as they had changed institutions; other authors said providing annotated data would take too much time to prepare. These comments led Savage and Vickers to recommend that data be deposited at the time of publication. Their findings also suggest that the impetus for data sharing must come from the journals themselves.

SUMMARY

COIs can influence the objectivity of vaccine safety researchers. Using the vaccine-autism debate as an illustration, this article describes the COIs faced by various research sponsors. Vaccine manufacturers have financial motives and public health officials have bureaucratic reasons that might lead them to sponsor research that concludes vaccines are safe. Advocacy groups have members with legal and financial reasons to support studies that find adverse effects in vaccines. These conflicts do not mean the research is incorrect, but the research could be selective and biased. Currently, most vaccine safety researchers face conflicts, which contribute to consumer confusion as well as more studies concerned with vaccine safety. Reported injuries from vaccines

are not investigated and both the public as well as some health workers question vaccine safety research. Ameliorating the COIs--through bureaucratic restructuring and enforced transparency—could lead to less bias, more investigation into reported injuries and increased trust in vaccine safety research.

DISCLOSURES

The author has two daughters with pervasive development disorder, not otherwise specified. She has filed a petition in the U.S. Court of Federal Claims under the National Vaccine Injury Compensation Program for one of her daughters. The author is a former member of the board of directors of Sensible Action for Ending Mercury-Induced Neurological Disorders (SafeMinds).

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TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
SUBCHAPTER C - DRUGS: GENERAL

PART 201 -- LABELING

Subpart G - Specific Labeling Requirements for Specific Drug Products

Sec. 201.323 Aluminum in large and small volume parenterals used in total parenteral nutrition.

(a) The aluminum content of large volume parenteral (LVP) drug products used in total parenteral nutrition (TPN) therapy must not exceed 25 micrograms per liter ([micro]g/L).

(b) The package insert of LVP's used in TPN therapy must state that the drug product contains no more than 25 [micro]g/L of aluminum. This information must be contained in the "Precautions" section of the labeling of all large volume parenterals used in TPN therapy.

(c) Except as provided in paragraph (d) of this section, the maximum level of aluminum present at expiry must be stated on the immediate container label of all small volume parenteral (SVP) drug products and pharmacy bulk packages (PBPs) used in the preparation of TPN solutions. The aluminum content must be stated as follows: "Contains no more than __ [micro]g/L of aluminum." The immediate container label of all SVP's and PBP's that are lyophilized powders used in the preparation of TPN solutions must contain the following statement: "When reconstituted in accordance with the package insert instructions, the concentration of aluminum will be no more than __ [micro]g/L." This maximum level of aluminum must be stated as the highest of:

(1) The highest level for the batches produced during the last 3 years;

(2) The highest level for the latest five batches, or

(3) The maximum historical level, but only until completion of production of the first five batches after July 26, 2004.

(d) If the maximum level of aluminum is 25 [micro]g/L or less, instead of stating the exact amount of aluminum as required in paragraph (c) of this section, the immediate container label may state: "Contains no more than 25 [micro]g/L of aluminum." If the SVP or PBP is a lyophilized powder, the immediate container label may state: "When reconstituted in accordance with the package insert instructions, the concentration of aluminum will be no more than 25 [micro]g/L".

(e) The package insert for all LVP's, all SVP's, and PBP's used in TPN must contain a warning statement. This warning must be contained in the "Warnings" section of the labeling. The warning must state:

WARNING: This product contains aluminum that may be toxic. Aluminum may reach toxic levels with prolonged parenteral administration if kidney function is impaired. Premature neonates are particularly at risk because their kidneys are immature, and they require large amounts of calcium and phosphate solutions, which contain aluminum.

Research indicates that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 4 to 5 [micro]g/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration.

(f) Applicants and manufacturers must use validated assay methods to determine the aluminum content in parenteral drug products. The assay methods must comply with current good manufacturing practice requirements. Applicants must submit to the Food and Drug Administration validation of the method used and release data for several batches. Manufacturers of parenteral drug products not subject to an approved application must make assay methodology available to FDA during inspections. Holders of pending applications must submit an amendment under § 314.60 or § 314.96 of this chapter.

[65 FR 4110, Jan. 26, 2000, as amended at 67 FR 70691, Nov. 26, 2002; 68 FR 32981, June 3, 2003]

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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 μg) 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 μg 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 μg 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 μg of Hg by 4 months of age) was not comparable to that in the United States (a maximum of 75 μg of Hg by 2 months of age and 187.5 μg 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 μg 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 μg) were 2.48 times more likely to have autism diagnosis compared to those exposed to less than 37.5 μg 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 μg to 125.46 μg; 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 μg of Hg, as compared to the "83" μg 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 μg of Hg for one-month exposures, (b) 0 to 190 μg of Hg for seven-month exposures, and (c) 0 to 300 μg 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 μg 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. (ii) Withholding important results from the final publication. (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. (ii) Withholding important results from the final publication. (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. (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. (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. (ii) "Overmatching" phenomena due to too closely matched cases and controls. (iii) Withholding important results from the final publication.
Price et al. [26]	(i) "Overmatching" phenomena due to too closely matched cases and controls. (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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Endoplasmic Reticulum Hyperstress from Aluminum and Mercury Exposures Drives Autoimmune Diseases of Unknown Origin with a Genetic Risk and Food Allergies

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Abstract

Aluminum and mercury both cause Endolasmic Reticulum Stress (ER Stress). When combined with genetic variations that lead to protein folding issues in the ER, these metals contribute to ER Hyperstress, including ER Overload and apoptosis. The basic mechanistic exposure of otherwise cryptic potential self-antigens via ER Hyperstress induced cell death leads to immunologic exposure to unusual post-translationally modified proteins. Interrupted protein production means partial (incomplete) acetylation, lipidation, citrullination, and glycosylation. This mode of production of near-self antigen sources is now recognized as crucial for specific autoantibody recognition in autoimmune diseases. Prototypical autoimmune disorders (ADs) like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) have a genetic risk, exhibit female predominance, presence of autoantibodies and response to T-cell or B-cell-targeted therapies. Mechanistic evidence exists that shows that they also, without fail, involve pathophysiological outcomes of the Unfolded Protein Response (UPR). Animal models routinely employ injections of aluminum hydroxide as the environmental causal factor to reproduce the symptoms of a surprising number of ADs seen in humans. Human studies have linked aluminum exposures to the onset of ADs, and ER Stress and the Unfolded Protein Response (UPR) both are observed across many ADs in humans. The assumption that low doses of aluminum are not toxic now appears to be incorrect. The literature of autoimmune disorder pathophysiology strongly supports the ER Hyperstress model of autoimmunity, in which generic, otherwise harmless mutations that evoke the UPR, usually to a non-pathophysiological conclusion, contribute to ER Stress that is severely compounded by exposures to metals, including aluminum and mercury. Food allergies also involve ER Hyperstress and are inducible in animals with aluminum hydroxide injections. In a comparison of the protein intrinsic disorder (PID) of proteins known to carry mutations that confer genetic risk of autoimmune disorders to general proteins in the human proteome, the PID of autoimmune-related proteins was significantly lower than that of general proteins (average PID AID vs. PID null 8.58 vs. 16.165, $p=0.0003$). This result suggests that proteins involved in ADs cannot tolerate non-synonymous substitutions that change folding energies – and it suggests that genetic risk of autoimmunity aided by immune activation will have a cryptic component in which proteins unrelated to the functional aspects of proteins elicit an autoimmune attack. It is predicted that, due to ER Hyperstress and the direct cellular toxicity of metals, autoimmune disorders will also have a component of acquired cellular detoxification deficiency disorder (ACDD), consistent with the oft-reported comorbidity between autoimmune/autoinflammatory disorders and multiple chemical sensitivity. ACDD is a disorder that makes causality assessment challenging but that can be alleviated by aluminum chelation, pointing to ER stress as a shared root cause leading to toxin accumulation. Direct genetic risk exists due to variation in genes involved in detoxification (e.g., HLA and CYP genes), but more generic risk likely exists due to conformational differences in proteins that challenge the unfolded protein response. These realizations point to the importance of mutations in proteins associated with autoimmune disorders cognizant of (upweighting) low protein intrinsic disorder as potential biomarkers of the likely occurrence of autoimmunity following environmental exposures that can trigger ER stress and the UPR. If done well, such mutations may be useful in predicting the specific type of autoimmunity that could occur if exposed to aluminum and mercury containing vaccines and other compounds known to elicit ER stress, such as glyphosate. Overwhelming, convincing evidence supports a genetic susceptibility to aluminum and mercury exposures exist in humans. It must be accepted that some percentage of the population cannot tolerate aluminum exposures as well as others and exposure to aluminum and mercury will caused them to fall into a

cascade of events caused by ER Hyperstress. Medicine must shift away from a culture that turns a blind eye to the evidence in support of the fact that AD, NDs and food allergies are caused, in part, by aluminum exposures. Phased biomarker development studies will help Identify the genetic minorities at increased risk of autoimmunity from aluminum and mercury exposure that lead to ER Hyperstress. All future studies of the association of vaccines with autoimmune disorders should focus on the prevalence of autoimmune disorders in vaccinated individuals with known high-risk genotypes compared to individuals without high-risk genotypes, no evidence of familial risk of autoimmunity, and no mutations in the AD-related genes. The entirety of the evidence in the literature supports the clinical existence of ASIA, and ER Hyperstress is a plausible biological mechanism by which genetic variation and injected forms of metals interact to cause autoimmune/autoinflammatory condition in some people.

Introduction

The primary shared characteristics of autoimmune disorders are infiltration of eosinophils, neutrophils, macrophages, activated mast cells, and T helper cell type 1 (Th1) cells, Th17 increase, bias in and release of a diversity of cytokines, intracellular protein aggregates and inclusion bodies, autophagy, and poor understanding of pathogenic mechanisms of the disease. Autoimmune disorders are variably IgE-mediated or IgG and complement-mediated, and delayed-onset T cell-mediated systemic reaction are seen, with differences both within and among autoimmune conditions. Alterations in Th1/Th2 cytokine levels accompany serious adverse events from vaccines[1]. Th17 cells are pro-inflammatory subset whereas Treg cells reflect reductions in inflammation[2]. The Th17/Treg balance has importance in the control of immunity mediated by Th17 cells. Autoimmunity also involves shifts in the T17/Treg balance[2]. A study of pro-inflammatory Th17 cells found that the cytokine IL10 is reduced in multiple sclerosis[3]. Hashimoto (2017)[4] reported that in rheumatoid arthritis, inflammation prevents T cells from regulating T17 cells. T-cell imbalances favoring Th1, Th2 and Th17 cells was found in Hashimoto's Thyroiditis [5]. Adjuvant-induced arthritis in mice is attenuated by Ras signaling inhibitors [6] including farnesylthiosalicylic acid [7]. A role of aluminum adjuvants in impairing regulatory T cell function is suspected, and genetic mechanisms have been explored [8].

Vaccines sometimes contain, as an adjuvant, aluminum in one of two forms - aluminum hydroxide, or, more rarely, amorphous aluminum hydroxysulfate (e.g., the anti-human papillomavirus vaccine, Gardasil). The specific mechanisms of action of aluminum presence of aluminum salts as adjuvants are not well characterized, but likely involve multiple effects including (a) increasing the duration of availability of antigen for immune surveillance, (b) aluminum-induced cell death leading to cytokine release, triggering the release of pro-inflammatory cytokines [9]. It is known that mice deficient in Nalp3, ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) or caspase-1 failed to mount a significant antibody response to an antigen administered with aluminum adjuvants, further pointing to apoptosis-mediated immune activation [10]; the Nalp3 inflammasome is a critical factor in the effects of aluminum adjuvants. In fact, the innate inflammasome pathway directs a humoral adaptive immune response [10].

Adjuvants are used to strengthen the immune response to pathogen antigens and reduce both the dose of antigen and frequency of vaccination injections. The effectiveness of aluminum as an adjuvant is exemplified by the finding that that AAHS was sufficiently strong to overcome genetic immunoresistance in mice[11]; however, given adverse events and toxicity of aluminum adjuvants, new adjuvants are being sought [12]. It is generally hoped that the immune response developed following injection of vaccines with adjuvants is specific, but this is not always the case. Animal studies have now routinely demonstrated that concurrent exposure to other antigen sources such as foods, infections can induce allergic sensitivity detected by secondary exposures. Similarly, exposure to self-antigens can occur leading to non-specific immune activation, potentially leading to autoimmunity. Ironically, treatment of autoimmune disorders involves further allergen and aluminum vaccination using aluminum adjuvants to induce TH1 helper T cells to compete with TH2 associated inflammation, usually in more complex staged protocol[12]. The effect of immunomodulatory treatments varies with each patient’s immunogenic profile, and multiple contributors to the onset and exacerbation of autoimmunity are known.

Various specific mechanisms of autoimmunity are now widely recognized, and can involve pathogen proteins either during infection or from vaccination:

Molecular Mimicry. A patient is exposed to an antigen from a pathogen or nonpathogen (such as the yeast in come vaccines) which carries elements that are similar enough in amino acid sequence or structure to self-antigen that the alloantigen acts as a self-'mimic'. In molecular mimicry, T or B cells activated in response to the pathogen also happen to be cross-reactive to self proteins. This can lead to direct autoimmune damage and false-positive "friendly-fire" activation of the immune system (via, for example, cytokine signaling due to the release of cytokines as result of autoimmune attacks on self proteins, cells, and tissues).

Epitope Spreading. Antigens from pathogens can also cause autoimmune disease via epitope spreading. In epitope spreading, damage to self-tissue occurs due either to the immune response to tissue infected by a persisting pathogen, or direct lysis of healthy cells by the pathogen. Antigen-presenting cells (APCs) take up antigens released from damaged tissue, initiating a self-specific immune response.

Bystander Activation. In this model, an indirect or non-specific activation of autoimmune cells is caused by the generally inflammatory environment that results from infection. The non-specific activation of one part of the immune system leads to the activation of other parts.

Cryptic Antigens. Foreign antigens can lead to autoimmunity via the activation of immunity to antigens that are not usually dominant – they are instead normally invisible to the immune system. It is generally described as an increase in "subdominant" antigens, and is usually attributed, like bystander activation, to the inflammatory environment that arises after infection. The involvement of cryptic antigens is considered likely when one observes increased protease production, and differential processing of released self-epitopes by APCs.

Of these mechanisms, the most discussed is molecular mimicry. Cross-reactive antibodies between foreign (e.g., viral, bacterial) epitopes and human proteins can occur either via infection (e.g., Guillan Barre Syndrome, or GBS from *C. jejuni* infection;[13]), or artificial immunization (e.g., narcolepsy due to similarity between human orexin protein and epitopes in the H1N1 swine flu vaccine; [14]). The evidence of autoimmunity due to vaccination is quickly gaining increasing support. In 2017, The National Vaccine Compensation Program added GBS to the list of injuries recognized by the National Vaccine Compensation Program [15]. The syndrome was first noted after the universal vaccination program against swine flu in 1976 [16].

A general syndrome called Autoimmune/Autoinflammatory Syndrome Induced by Adjuvants (ASIA [17]) now has mounting evidence as a contributor to autoimmunity/autoinflammatory conditions in some patients, and has been described as encompassing numerous autoimmune disorders of otherwise mysterious origin, including systemic lupus erythematosus [18]; undifferentiated connective tissue disease[19], Hashimoto's thyroiditis and/or subacute thyroiditis[20]; antiphospholipid syndrome (APS)[20]; Sjögren's Syndrome [21,22]; Postural Orthostatic Tachycardia with chronic fatigue [23], and primary ovarian failure following HPV vaccination [24]. Within the more specific diagnosed autoimmune conditions, general shared symptoms of arthralgia, myalgia, and chronic fatigue symptoms predominate [25].

The scientific and medical (clinical) recognition of ASIA is expanding [26-29]. There is overwhelming evidence from animal and human studies that - for some individuals - vaccine-level exposures to injected forms of aluminum is unsafe (see [30] for a recent review). Understanding autoimmunity in humans from adjuvants is impossible without due consideration of the role of genetic susceptibility, because not all humans develop autoimmune disorders and many who are vaccinated do not.

It was recently shown that the amount of aluminum in vaccines is not based on relevant safety data from dose escalation studies of vaccine-type aluminum into rat or mice pups, and that the doses used in vaccines are well

beyond acute toxicity levels at certain points in the CDC's recommended pediatric schedule [31-33]. Infants acutely receive more biologically available aluminum from vaccines during the schedule than from dietary sources, and the basis of our understanding of tissue fates, clearance, and accumulation is fundamentally flawed, relying on dietary types of aluminum given orally to adult mice to infer safety of injected forms of aluminum given to human infants [31]. Numerous calls have been made for consideration of alternative adjuvants [28,31-32,40] and for the cessation or reduction of the use of aluminum salts in vaccines (Morris et al., 2017[28]).

While the general idea that aluminum adjuvants, or other adjuvants may induce autoimmunity conditions, the mechanism of autoimmune action have been broadly elucidated, a precise description of why risk is higher in some individual than others – that is, the manifestation of a genetic x environment interaction – has not been provided. Here, I review the evidence that aluminum hydroxide from vaccines (and thimerosal in some flu vaccines), and, by implication from published evidence aluminum from other sources as well - may be root causes contributing to the epidemic of diseases of mysterious origin in humans. As in autism spectrum disorders (ASD) [14], the literature very strongly supports ER Hyperstress as an important direct root causal mechanism for autoimmunity syndrome induced by adjuvants leading to other manifestations of autoimmunity. The main conclusion of this review is that the evidence of neuroimmune toxicity of aluminum-types found in vaccines is overwhelming, and that the specific genetic x environmental interaction needed to explain why only some individuals appear hypersensitive to aluminum – and other toxins – is found in the ER Hyperstress model[34], in which genetic ER stress interacting with environmental ER stress. The ER Hyperstress model explains why some, but not all, individuals have increased susceptibility to serious vaccine injury, and why a confusing massive list of genes can be found associated with autism and autoimmunity.

Aluminum Adjuvanticity and Toxicity

The cellular effects of aluminum are myriad and diverse. At high doses, aluminum can inhibit the formation of α-ketoglutarate and can cause toxic levels of ammonia in tissues. Aluminum can also bond to phosphorylated bases on DNA, disrupting protein synthesis and catabolism. It has long been known that brain cells, including neurons and glial cells, are susceptible to long-term accumulation of aluminum. When aluminum bonds to phosphate, it can inhibit normal catabolism of neuronal filaments in the central nervous system [35]. Among the most important of these effects include disturbance of normal protein folding, and initiation of the Unfolded Protein Response due to aluminum-induced ER stress [36-38].

Aluminum adjuvants induce immune responses that vary with type, and location of administration. When aluminum overstresses the ER, cell contents are released from dying cells, including dsDNAs, partially folded proteins, cytokines, and in tissues of the central nervous system, excitotoxins such as glutamate. The cell contents usually initiate a T helper type 2 (Th2) responses, IgE isotype switching and peripheral effector responses, through Irf3-dependent mechanisms [39]. Exposure locations, dosage amounts, and specific forms injected determine the degree of localized vs. systemic short-term exposure. Intramuscular injections lead to the formation of granulomas, with slow the release of aluminum over time. Surprisingly, chronic exposures to low doses of aluminum appear to induce higher short-term toxicity by escaping tissue sequestration responses [40].

A systematic review of the dosing of aluminum hydroxide in animal studies that routinely and reliably induce the symptoms of autoimmune conditions reveals that the dosing used ranges from 5 to 10,000 times that typically used in vaccines (Table 1). Many of the studies used protocols with repeated injections over a period of weeks. Not all studies reported sufficient detail to be included. Notable, studies that employed mouse models with genetic risk used the lowest amount of aluminum (Table 1). For reference, in the CDC schedule, infants receive seven doses of aluminum-containing vaccines by the second month, totaling 1445 mcg aluminum injected, and sixteen doses for a total of 4925 mcg aluminum injected by 18 months of age.

Table 1. Dosing of aluminum hydroxide used to induce autoimmune symptoms in animal models for pharmacological treatment studies relative to human dosing in vaccines (last column)

Citation (et al.)	Condition	Animal	bw (g)*	bw (kg)	AL dose (mcg)	AL dose (mg)	mcg/kg	mg/kg	human max exposure** (mcg/kg)	x human max exposure
Zhu[41]	Atherosclerosis	apoE null C57BL/6 mice	20	0.02	25	0.025	1250	1.25	230	5
Zhu[41]	Atherosclerosis	LDLR null C57BL/6 mice	20	0.02	25	0.025	1250	1.25	230	5
Kelly-Scumpia [42]	Lupus	C57bl/6 mice	20	0.02	50	0.05	2500	2.50	230	11
Yasar[43]	Allergic rhinitis	Rats	250	0.25	1000	1	4000	4.00	230	17
Elsakkar[44]	Asthma	CD1 mice (male)	25	0.025	292	0.292	11680	11.68	230	51
Elsakkar[44]	Asthma	CD1 mice (male)	20	0.02	292	0.292	14600	14.60	230	63
Qi[45]	CP/CPPS	Wistar rats	250	0.25	6250	1.25	25000	5.00	230	109
Brandt[46]	GI allergy, asthma	BALB/c	20	0.02	1000	1	50000	50.00	230	217
Qi[45]	CP/CPPS	Wistar rats	250	0.25	12500	2.5	50000	10.00	230	217
Agmon-Levin[47]	Lupus	NZBWF1 mice (female)	38	0.038	2000	40	52631	1052.63	230	229
Yang[48]	Rhinitis	SD rats	400	0.4	30000	30	75000	75.00	230	326
Xi[49]	Rhinitis	BALB/c mice (female)	24	0.024	5000	5	208333	208.33	230	906
Xi[49]	Rhinitis	BALB/c mice (female)	17	0.017	5000	5	294117	294.12	230	1279
Sagawa[50]	Arthritis	BALB/c mice (female)	20	0.02	40000	40	2000000	2000.00	230	8696
Sagawa[50]	Arthritis	DBA/1 mice (male)	18	0.018	40000	40	2222222	2222.22	230	9662

** estimated at 1225 mcg AL/5.326 kg (median body weight @ 2 mos). Meant to be typical.

ER Hyperstress: How Metals and Genetic Variations Collide

Unfortunately, the safety of pediatric dosing of aluminum in vaccines and in the CDC pediatric vaccine schedule is not well-founded [31-32]. Aluminum causes ER stress [36-38], which introduces problems with resolving challenges in protein folding protein and maintaining cellular homeostasis, and it also directly causes mitopathies [51]. The combined effects make cellular detoxification challenging. This likely explains why some individuals tend to accumulate toxins, including aluminum, faster than others, and may explain genetic susceptibility to aluminum toxicity in specific tissues. Part of this susceptibility is the role of non-synonymous substitutions that cause problems with ER-mediated protein folding, leading to genetically induced UPR, which generally resolves the issue without incident. When the ER stress caused by these proteins is compounded by metal-induced ER stress, this is called ER Hyperstress [34], and it appears to represent an exact genetic X environment interaction point that explains both why some individuals are more susceptible to serious adverse events from vaccines, and why much of the genetic variation related to that risk may not appear to be functionally related to the specific condition thought to be caused by vaccination, as in ASD.

The Unfolded Protein Response

The unfolded protein response (UPR) is a highly conserved, adaptive signaling pathway activated by accumulation of misfolded proteins within the endoplasmic reticulum (ER). The ER is mainly responsible for the translational biosynthesis, cellular trafficking, and posttranslational modification of proteins. These processes in the ER ensure timely and proper delivery and release of folded proteins via secretory pathways. Improperly or folded or modified proteins are degraded via ER-associated degradation (ERAD), or autophagy. Three transmembrane ER stress sensor proteins monitor and signal upon ER stress: inositol-requiring protein 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6α (ATF6α). When unfolded proteins build

up due to ER stress, the UPR either resolves the issue via a slowdown in transcription via ATF6α expression, or translational control via PERK and IRE1 α. When these fail, cellular apoptosis is initiated, and cellular contents are dumped into the extracellular matrix, initiating the cascade of signals due to cellular injury [52].

Proteins with high intrinsic disorder require assistance in folding within the lumen of the ER [53]. When mutations lead to abnormally folded proteins, they can accumulate in the ER, resulting in the induction of the unfolded protein response (UPR). The issue can be resolved via a rate reduction in protein production. For serious problems, proper degradation by the proteasome, or chaperoning out of the cell is usually possible. However, the exposure of cells to environmental toxins that induce ER stress can combine with the ER stress induced by unfolded proteins, leading to severe endoplasmic reticulum stress, and to cell death by apoptosis. These factors and ER Hyperstress have been implicated in ASD, specifically evidenced by the role of mutation that lead to generic non-synonymous substitutions contributing to ER Stress, compounded by ER Stress from environmental toxins. Injected and ingested doses of aluminum and synergistic toxicity with mercury from thimerosal are likely root-cause culprits for susceptible individuals [54].

Eupedia[55] is a searchable online resource that stores information on genes and mutations associated with autoimmune diseases, and it includes entries for various autoimmune disorders, including allergies, Ankylosing Spondylitis, Celiac disease, Crohn's disease, Grave's disease, Intestinal Bowel Disorder, Psoriasis, Rheumatoid Arthritis, Sjögren's syndrome, SLE, Type I Diabetes, Type II Diabetes, and Ulcerative colitis (https://www.eupedia.com/genetics/autoimmune_diseases_snp.shtml). As a test of the potential contribution of diffuse genetic risk due to non-synonymous changes in proteins that are already intrinsically disordered, the protein intrinsic disorder (PID) of proteins encoded by genes known to carry mutations that confer genetic risk of autoimmune disorders to randomly selected general proteins in the human proteome was compared to the PID of general proteins (N=200). PID values were retrieved from Mobidb (http://mobidb.bio.unipd.it/search). The average PID of AID-associated proteins vs. PID null was 8.58 vs. 16.165, p=0.0003).

This opportunistic result suggests that proteins involved in autoimmune disorders cannot tolerate non-synonymous substitutions that change folding entropies. It also suggests that family-specific genetic risk of autoimmunity aided or exacerbated by immune activation from infections or vaccination will have a cryptic component in which proteins unrelated to the functional aspects of proteins can elicit an autoimmune attack.

When these toxins are metals like aluminum and mercury, the metals that are released upon cell death can be taken up by other, nearby cells. Double-stranded DNA (dsDNA) is also released upon apoptosis, activating cyclic GMP-AMP synthase, contributing to lethal autoimmunity in a SLE mouse model [56]. In conditions in which ER Hyperstress is sublethal to the cell, environmental toxins such as aluminum and mercury (and others) can also contribute to direct damage to mitochondria and the Golgi system, impairment of cellular detoxification, and a run-away accumulation of additional neotoxins in a vicious cycle of causality. By directly interfering with phosphate and ATP metabolism, aluminum can further impair cellular energy transfer processes.

The UPR and ER Stress are now widely known to be involved in the pathophysiology of numerous autoimmune diseases "of unknown origin". The UPR is now recognized as a central process in neurodegenerative diseases including Alzheimer's disease [53] and Parkinson's disease {58], metabolic diseases (e.g., type II diabetes [59], inflammatory diseases (type I diabetes [60] and inflammatory bowel disease), and autoimmune diseases (e.g., Systemic Lupus Erythematosus [61]).

Here I systematically compile the evidence that symptoms consistent with those of autoimmune disorders in humans are reliably and consistently reproduced in animal models; studies of autoimmune disorders following vaccination in humans. I also review examples of studies that demonstrate the central causal role of ER Stress and the UPR in human studies of autoimmune disorders. I review the literature of the role of metals in vaccines in the UPR and ER Stress in autoimmune disorders focused on the ER Hyperstress model, and then point to evidence that, as in ASD, mutations that lead to ER protein folding problems interact with the ER Stress caused by vaccine doses

of aluminum and mercury. An underappreciated result of the fatal outcome of UPR for cells is the sudden release of strangely folded proteins, and autoinflammation and autoimmunity resulting from this release of improperly processed, aberrantly folded proteins, which are a rich source of neoantigens for transient and persistent autoimmunity. Ethyl mercury induces mutations in mitochondria in astrocytes, and aluminum impairs astrocytic cytoskeletal dynamics [62].

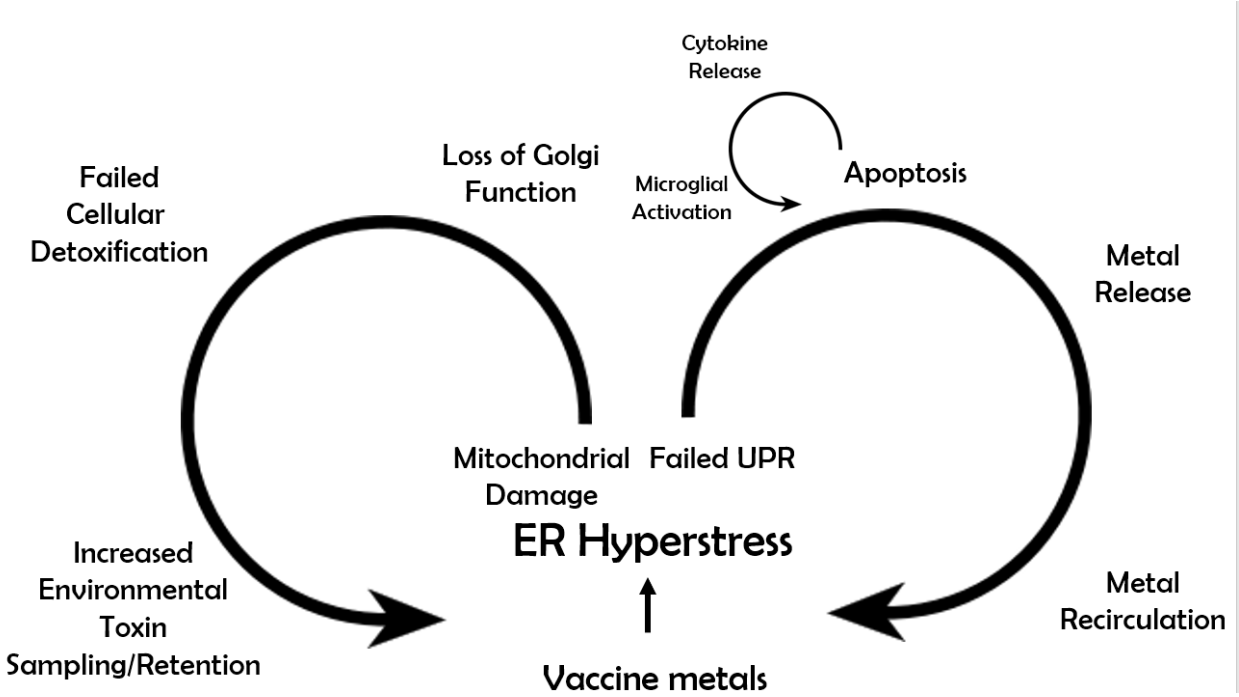

Figure 1. The ER Hyperstress model focuses on the interaction and combined effects of early-life exposure to aluminum and ethyl mercury and specific mutations that also cause ER stress. Along with metal release, spillage of oddly folded proteins (necrosis) and the consequent local immunological reactions likely contribute to autoimmunity risk. The combined effects of genetic and environmental ER stresses are called ER Hyperstress[30] and provide a specific biological mechanism for ASIA.

ER Hyperstress Model of Neuroimmune Dysfunction

Additional aspects of ER Hyperstress include acquired cellular detoxification deficiency via mitochondrial and Golgi damage. When these cellular systems are impaired, individuals begin to take up parochial industrial and agricultural neotoxins from their environment. Thus, individuals with ER Hyperstress induced AD's, DD's and ND's likely will be found to have a constellation of toxins, including organic pollutants and other metals, making causality assessment within individuals and at the population level difficult. The rates of food allergy have skyrocketed since the expansion of the use of aluminum adjuvants in vaccines [63,64], and a pilot study [65] found higher rates of allergies in children compared to unvaccinated children.

Asthma/Allergic Rhinitis

Individuals with a diagnosis of asthma usually have concurrent or prior atopy [66], or a family history of atopy. It has long been known that familial history of atopy reduces the capacity of infants to produce the TH1 cytokine interferon (IFN)-γ compared with infants from nonatopic families [67]. A large proportion of patients with asthma have increased CD4+ T cells, weak Th1 responses, strong Th2 immune responses, with Th2-based cytokine profiles including interleukin (IL)-4, IL-5, IL-9, and IL-13, which promote eosinophilic inflammation and immunoglobulin E

(IgE) production in B cells. IL-5 drives eosinophil differentiation in the bone marrow, and IL-9 causes the differentiation of mast cells.

Children are exposed to large numbers of allergens throughout childhood, and simultaneous exposure to Th2- and IgE-enhancing adjuvants presents the opportunity to develop numerous triggers. The occurrence of anaphylaxis after immunization points to a pathogen's antigens as potential allergens. The role of airborne environmental triggers of asthma are well known [68], but it is important to distinguish between antigen/allergen source and root cause of autoimmunity. McDonald et al. 2008)[69] found that delay in the DTP vaccine was associated with a significant reduction in the risk of asthma.

Some animal studies have shown it is possible to develop allergic rhinitis and asthma without special exposure to airborne antigen sources. The drug development literature is replete with animal studies that routinely induce allergic rhinitis using aluminum hydroxide and ovalbumin, mainly for the purpose of testing drugs and other treatments for allergies. For example, Xi et al. 2014[49] found that dosage of aluminum hydroxide determined the strength of the autoimmune reaction in a BALB/c mice model of allergic rhinitis. They found eosinophils in the nasal mucosa of mice who were injected with the aluminum hydroxide powder in solvent, but none resulting from the injection of the hydrogel form. No behavioral or neurological outcomes were studied. They used common "doses" of 5 mg per type of aluminum mixed with ovalbumin and provide a good example of the difficulties of comparing the biological equivalence of the same dose levels of different forms of aluminum. They found that high doses were immunosuppressive and lead to granulomas that could potentially lead to ascites (blockages) in organs.

Li and Geng (2015) [70] studied the effects of budesonide on the symptoms of allergic rhinitis induced by aluminum hydroxide and ovalbumin. They used 0.5 mL aluminum hydroxide gel in their induction model. Brandt et al., (2006) [46] developed a model of gastrointestinal allergy following asthma with Th2-associated humoral and cellular responses involving intestinal eosinophilia, mastocytosis and diarrhea by injecting 50 μg of ovalbulmin in the presence of 1 mg of the aluminum potassium sulfate adjuvant (alum).

Many other similar studies with similar findings exist [e.g., 43,48,71). Each of these conducted similar studies of treatments of allergic rhinitis caused by the administration of aluminum hydroxide and ovalbumin. Similarly, aluminum hydroxide is routinely used to create bronchial asthma in animals with high reliability. To study the efficacy of fruits of the *Vitis vinifera* plant, Arora et al. (2016) [72] sensitized rats to ovalbumin with 2 mg of aluminum hydroxide. Arora et al. (2017) [73] later used the same method to study the efficacy of Kanakasava in alleviating bronchial asthma. Zeng et al., (2014) [74] induced bronchial asthma using ovalbumin and aluminum hydroxide in Sprague-Dawley rats.

In humans, both Th1/Th2 and Th17/Treg imbalances can be found in patients with asthma [75]. This effect was produced using aluminum hydroxide and ovalbumin in mice; Liu et al., 2015 [76] found that pingchuan formula, a traditional Chinese treatment, could restore Th17/Treg balance. T-regulatory cells modulate both Th1 and Th2 type responses [77]. Workers in aluminum production plants have increased risk of asthma [78] involving granulomatous bodies in the lungs. The presence of aluminum within the granulomas differentiates "aluminum lung" from sarcoidosis and beryllium disease. Pulmonary fibrosis and pulmonary alveolar proteinosis and desquamative interstitial pneumonia, which involves the accumulation of macrophages in response to aluminum in the alveola all represent interstitial lung diseases that can result from chronic exposures to aluminum by workers in aluminum production [78].

The improvement of aluminum-hydroxide induced asthma symptoms observed by Bibi et al. [79] after chelation aimed at sequestration of aluminum is evidence of reversibility, a key component of causal inference. The fact that volatile organic compounds can trigger asthmatic episodes, and individuals with asthma can accumulate

persistent organic pollutants [80], point to cellular detoxification deficiency. A study of the cord blood of 2,050 infants found levels of pesticides in blood were associated with allergies and eczema [81]; children with increased levels of pesticides in their blood were at increased risk of asthma [82]. Both pesticides and IgE levels (marker for allergic response); pesticides were associated with higher IgE levels in the infants whose mothers had used pesticides during pregnancy [83]. The accumulation of pesticides was correlated with familial risk as reflected in the presence of IgE in the cord blood of mothers with prior atopy only. This provides evidence of environmental toxin sampling and cellular detoxification deficiency. Known and suspected causal agents of asthma such as exposure to aluminum-or mercury containing vaccines were not included as factors in the study.

While the doses used in animal studies tends to be higher (per body weight) than used in vaccines, there are three good reasons to suspect that they are indeed highly relevant to the development of autoimmune disorders in humans. First, aluminum used in vaccines has a non-linear selective toxicity, and small doses may fly "under the radar" and fail to elicit tissue protective granuloma responses [40]. Second, many autoimmune disorders involve genetic predisposition, a component not necessary for effecting autoimmunity in animal models.

Antiphospholipid syndrome (APS)/Immune System Activation of Coagulation (ISAC)
Antiphospholipid syndrome is an autoimmune condition in which the immune system mistakenly attacks phospholipids in cell membranes, causing vascular leakage and hypercoagulation. APS is diagnosed by the occurrence of antiphospholipid antibodies (aPL) and can provoke blood clots (thrombosis) arteries and veins, and can cause pregnancy-related complications such as stillbirth, miscarriage, short gestation, and severe preeclampsia. A moderate thrombocytopenia is usually involved. APS has been induced in mice using alhydrogel [84] and aluminum hydroxide combined with tetanus toxoid resulting in hyperimmunization [85]. In one of the earliest studies, Pierangeli and Harris (1993) [85] successfully induced antiphospholipid antibodies (APAs) using aluminum hydroxide and either human beta 2 glycoprotein 1 or anticardiolipin antibodies.

Cross-reactive antibodies have been found following Hepatitis B vaccination in patients who developed thrombocytopenia. Cross-reactivity to platelet antigens has been detected in about 80% of cases. In thrombocytopenia occurring post- measles-mumps-rubella (MMR) vaccination, the presence of anti-rubella and anti- measles IgG antibodies that cross-react with platelet antigens has been consistently detected. D'alò et al. (2017) [86] reviewed the evidence determined that the role of vaccines as a pathophysiological mechanism in thrombocytopenia is "certain".

Given that antiphospholipid antibodies have been found in patients with persistent macrophagic myofasciitis [87], and APS patients often experience a decline in kidney function [88], doses of aluminum in APS patients should be kept below the 4-5 mcg/kg/day limit required by the CFR/FDA (FDA/CFR 21CFR201.323, 2017) [89]. Surprisingly, no specific limit is provided by FDA for injected aluminum exposures from vaccines for the general population. To date, no studies of the efficacy of aluminum-focused chelation therapy have been conducted on patients with APS.

Arthritis
Injections of either ovalbumin or collagen in the presence of aluminum hydroxide produced lymphocyte proliferation and interferon gamma production in mice [50]. The study found that arthritis in mice induced by aluminum hydroxide and these allergens could be alleviated by angiotension II receptor blockers. Xiao et al. (2008) [91] used aluminum potassium sulfate and ovalbumin to induce collagen-induced arthritis. Vaccination with OSPA and aluminum hydroxide caused severe destructive Lyme arthritis in hamsters upon infection with *Borrelia burgdorferi* [92]. Lyme arthritis sufferers often have lower frequencies of Treg cells, and higher expression of activation coreceptors that augment Teff cell function [93].

Chelation with EDTA has been effective in reducing the severity of rheumatoid arthritis in a patient with aluminum, cadmium and lead intoxication [94]. Like other types of autoimmunity, co-morbidity of various types of arthritis

with multiple chemical sensitivity [95], points to involvement of acquired cellular detoxification deficiency in arthritis. Evidence that RA pathogenesis involves the ER chaperone GRP78 is reviewed by Park et al. (2014[96]), including the release of pro-inflammatory cytokines.

One of the two studies conducted by Ray et al., 2011 [97] to test the hypothesis of association of RA with vaccines found a significant association – yet the authors favored the interpretation of no association. The HLA-DR class II genetic component of RA risk in some families is well known; studies such as Mitchell et al. (1998) [98] that are more appropriately designed (via genetic stratification) report much higher odds ratios of association with vaccines. It is accepted that the MMR vaccine can cause arthralgia and arthritis in a significant number of women with no preexisting MMR vaccine exposure (10-25%). The contribution of vaccines to chronic arthralgia or arthritis has not been fully studied. This conclusion is based on an assessment in 2012 by the Institute of Medicine [99], who cited numerous studies reporting transient arthritis after vaccination. The IOM concluded they were unable to find sufficient evidence to conclude that vaccines do, or do not cause chronic arthralgia or arthritis; however, they did reference a variety of poorly conducted studies that failed to detect positive associations. For example, the study cited that reported no association between a recombinant DNA Hepatitis B vaccine and arthritis only had 44 patients with RA, and a control group of only 22 patients. The lack of studies of sufficient quality stymied the IOM's ability to determine (either way) whether vaccines contribute to chronic arthritis. Some studies did report a statistically significant association between influenza and HPV vaccines and arthralgia, but one cannot determine whether the negative results were due to low power or other problems with design of analysis, which is common in retrospective ecological correlation studies. One study of a vero-cell culture-derived trivalent influenza vaccine found a relative risk of arthralgia of 2.0 (95% CI: 1.6-2.5), and another study of an AS04-adjuvanted HPV-16/18 vaccine (Cervarix) among women in Korea (Kim et al., 2011[100]) found an odds ratio of grade 3 arthralgias of 2.68 (95% CI: 1.29-5.59) with vaccination use.

The reliance on weak retrospective studies, often underpowered, hinders causal inferences based on association only because correlation studies fall short of sufficiently critical test of causality [34]. They are almost always not designed to test the hypothesis of association in a genetically susceptible subgroup, which contributes to the inability to detect associations. Even in randomized clinical trials, inclusion criteria often exclude persons with known comorbid conditions – potentially excluding people from the study who carry the genetic risk of the autoimmune disorder(s) of concern. The study of Jackson et al. (2010) [101] is such an example, where patients with new medical conditions are excluded – as were patients with kidney issues. Such exclusions may remove those who are genetically susceptible – and the exclusions are not carried over into clinical practice as contraindications, and thus true associations are weakened. Thus, negative results should be reported as "failing to detect an association (or effect)" instead of "finding no evidence". Further, because correlations are not sufficiently critical tests of causality, finding of "no evidence" in epidemiological correlation studies should never be confused with proof of no causal relationship. Inconsistencies between strong evidence of vaccine-induced autoimmunity at the individual level and negative results at the population level are likely explicable due to the application of inappropriate epidemiological study designs that do not consider genetic and familial risk. Failure to exclude patients from exposure in the clinic when they have clearly been excluded during safety and efficacy trials is "translational failure" and must be eschewed.

Atherosclerosis

ER stress plays an important role in atherosclerosis and other macrovascular complications [102-103]. Numerous types of ER stress contributors have been identified, but many of these are manifestations of ongoing processes, not root causes. These include insulin resistance, hyperhomocysteinuria, oxidation and stress [104]. Once ER stress is manifest, multiple additional processes lead to the recruitment of macrophages to the site of lesions where "foam cells" provide structure within which pro-inflammatory cytokines recruit localized immune responses. While experimental animal models such as APOE deficient mice fed a special diet can induce sufficient ER stress to cause atherosclerosis, Nishizono et al. [105] were able to induce atherosclerosis via injection of

ovalbumin combined with aluminum hydroxide. It is interesting to note that inclusion of AGE-LDL in the injection led to reduction in atherosclerosis compared to aluminum hydroxide alone in diabetic ApoE and LDLR null mice [41]. Incidental data suggest that heavy metal chelation, including types aimed at aluminum, seem to improve outcomes of people with atherosclerosis-induced myocardial infarction [106]. Patients with end-stage renal disease also show signs of calcifications in blood vessels and atherosclerosis [107-108], correlated with serum levels of phosphate-binding medicines in the serum. Since aluminum-containing drugs are dangerous to neonates, and the toxicity of aluminum is dosage and body-weight dependent [31], it is prudent to avoid injections of aluminum in the NICU to avoid adding to renal and cardiovascular toxicity.

Oddly, while aluminum tends to increase Th2 immunity, atherosclerosis typically involves Th1 cytokines, and aluminum (alone) could be protective against atherosclerosis in such cases (see [109] for a review). In the presence of unsafe foreign epitopes (i.e., epitopes that are immunogenic but that are similar to human proteins), however, the shifting in the immunity profile could have multiple effects that vary over time, or with genetic background. The UPR is involved in AS lesion development at every stage in APOE deficient mice [110].

Systemic Lupus Erythematosus

Katz-Agranov and Zandman-Goddard (1985)[111] reported that SLE has been observed after "HBV vaccines, human papillomavirus (HPV) vaccine, influenza vaccines, diphtheria, pertussis, tetanus (dTP) vaccines, bacillus Calmette-Guérin (BCG) vaccines, measles, mumps, and rubella (MMR) vaccines, pneumococcal vaccines, vaccine adjuvants, autoimmune/inflammatory syndrome induced by adjuvants (ASIA) and other adjuvants such as oil adjuvants and metal adjuvants". A systematic review and meta-analysis of 16 studies [112] concluded that both SLE and RA are associated with vaccination. Kelly-Scumpia et al. (2007) [42] employed aluminum hydroxide to induce a lupus-associated autoantigen and DC maturation, B and T cell activation/proliferation in mice. While renal iron accumulation can occur in SLE [113], patients are also at risk of anemia [114]. Agmon-Levin (2014[47]) found the aluminum hydroxide induced kidney tissue damage, decreased RBCs, caused memory deficits and measurable brain gliosis in mice. Paradoxically, certain aspects of SLE are treatable by oral aluminum hydroxide, specifically soft tissue calcifications that can be problematic can be softened and better removed by surgery [115]. Post-apoptotic ER stress is observed in bone marrow mesenchymal stem cells in patients with SLE [116].

Sjögren's Syndrome

Bagavant et al. (2014) [117] duplicated sialoadenitis (inflammation and malfunction of the salivary gland) in mice using aluminum hydroxide. Amyloid is partly aluminum, and amyloidosis of the parotid gland is not uncommon (see review[118]). Numerous studies have found association of myriad environmental toxins and Sjögren's syndrome, which is consistent both with in-line causality and parallel effect of detoxification deficiency. Multiple chemical sensitivity has been suspected to share a root cause with Sjögren's syndrome since 2006 [119], pointing to a loss of cellular detoxification capacity, as is seen in ASD. Sicca syndrome, which is similar to and sometimes comorbid with Sjögren's syndrome, responds to metal chelation [120].

Food Allergies

Exposure of BALB mice to high aluminum-containing anti-acid powder in a diet of codfish causes IgE seroresponses to subsequent codfish exposure, establishing a causal link between aluminum and food allergies [121]. In a study of the efficacy of quercetin for treating life-threatening anaphylactic reactions to foods, Shishehbor et al (2010) [122] were able to reliably induce immunoglobulin-E mediated peanut allergy in Wistar rats using crude peanut extract, Cholera toxin and injected aluminum hydroxide. Ahrens et al. (2014) [123] found that they could reproducibly induce allergy to ovalbumin, apple, soy, peanut, and pea allergens by sensitizing Brown Norway rats with specific protein extracts, ingested aluminum hydroxide, and Bordetella pertussis. Development of increased IgE levels and food allergies have been reported [124].

Tong et al. (2017) [125] found that iron-focused chelation (the removal of iron from the allergen) reduced the allergenicity of ovotransferrin in the BALB/c mouse model. No studies of the potential utility of aluminum-focused chelation and the reduction of the severity of food allergies have been found. The concurrent massive increase in food allergy and in multiple chemical sensitivity disorders since the expansion of the use of aluminum-containing vaccines has been noted [126). The high prevalence of gluten allergies in ASD is noted, as is the finding that mothers of children with ASD have higher rates of MCS [127].

Glomerulonephritis

Glomerulonephritis is a serious condition that can occur as a frequent condition comorbid with many of these autoimmune conditions. It is a leading cause of death in SLE and can be induced reliably in animals using aluminum hydroxide (Bassi et al., 2012[128]). It has long been known that acute exposure to high doses of aluminum can cause kidney damage, and that detoxification with deferoxamine prevents and reduces kidney damage and glomerulonephritis (e.g. Hood et al., 1984[129]). Pathological deposition of aluminum in the bones accompanies glomerulonephritis and can lead to improper bone development and brittle bone disease. If genetic intolerance of aluminum in vaccine types and doses of aluminum and mercury in vaccine exists such that individuals tend to accumulate larger doses in their tissues, kidney damage could be a serious risk due to cumulative exposures. Excretion of aluminum from bone in the aging during osteoporosis is a likely source of age-related neurodegenerative conditions. Naturally, impaired kidney function can also contribute to the accumulation of other chemicals – especially in the presences of accumulating adjuvants. Metals can themselves be antigenic, making any tissues within which vaccine metals are deposited targets of adaptive immunological attack [130].

Table 2. Autoimmune Diseases Produced by Aluminum Hydroxide in Animal Studies

AA Disease	Aluminum Type	Symptom Manifestations	Citation
allergic asthma	$Al(OH)_3$	asthma	Elsakkar et al., 2016 [40]
	$Al(OH)_3$		Bibi et al., 2014 [79]
allergic rhinitis	$Al(OH)_3$	allergic rhinitis immune suppression	Xi et al., 2014 [49]
	$Al(OH)_3$	allergic rhinitis	Li and Geng, 2015 [70]
	$Al(OH)_3$	allergic rhinitis	Yasar et al., 2016[43]
	$Al(OH)_3$	allergic rhinitis	Yang et al., 2016[48]
bronchial asthma	$Al(OH)_3$	bronchial asthma	
antiphospholipid syndrome	alhydrogel	APS antibodies	Zivković et al., 2013[80]
	$Al(OH)_3$		Zivkovic et al., 2011[81]
arthritis	$Al(OH)_3$	collagen-induced arthritis	Sagawa et al., 2005[50]
	$Al(OH)_3$	severe destructive Lyme arthritis	Croke et al., 2000 [88]
atherosclerosis	$Al(OH)_3$	OVA-specific IgG/ chymase increase	Nishizono et al. 1999 [101]
	$Al(OH)_3$	atherosclerotic lesions	Zhu et al. 2014[41]
chronic prostatitis/ chronic pelvic pain syndrome	$Al(OH)_3$	increased TNF-α and IgG prostatitis	Qi et al., 2012 [45]
gastrointestinal allergy preceding asthma	aluminum potassium sulfate	pulmonary inflammation	Brandt et al., 2006 [46]

systemic lupus erythematosus	$Al(OH)_3$	kidney tissue damage decreased RBCs memory deficits brain gliosis	Agmon-Levin et al., 2014 [47]
	$Al(OH)_3$	DC and lymphocyte	Kelly-Scumpia et al., 2007 [42]
		activation and Sm/RNP autoantigen	
	$Al(OH)_3$	accelerate proteinuria weight loss	Favoino et al., 2014 [223]
motor neuron disease	$Al(OH)_3$	motor deficits motor neuron degeneration	Shaw & Petrik, 2009 [224]
Sjögren's Syndrome	$Al(OH)_3$	salivary gland dysfunction	Bagavant et al., 2014 [117]
food allergy	$Al(OH)_3$	IG-E peanut allergy	Shishehbor et al., 2010 [122]
	$Al(OH)_3$	soy, peanut, pea, apple, ovalbumin	Ahrens et al., 2014 [119]
	multiple vaccines	peanut and egg allergies	Hoyt et al., 2015 [120]

The majority of these studies used ovalbumin in combination with aluminum hydroxide. Ovalbumin, which is found in many vaccines, and the pattern of use in animal models, suggests some cases of vaccine-induced autoimmunity unrelated to egg allergy. These studies typically employ intraperitoneal injection, using doses that range from 10x the amount used in vaccines (e.g., Hepatitis B 250 μg/2kg infant) to much larger doses. In these animal studies, the doses are often acute doses, given once, or twice, with short-term follow-up and do not represent the chronic exposure to low doses reflecting the CDC pediatric schedule.

The high doses, however, may mimic the toxicity of human patients with increased genetic susceptibility due to myriad effects of ER Hyperstress, including cellular detoxification deficiency, who appear to accumulate systemic aluminum at a faster rate than others. Cruz-Tapias et al., [131] noted the widespread use of aluminum hydroxide in animal studies of autoimmune rheumatoid arthritis-like disease, for systemic lupus erythematosus-like disease, autoimmune thyroid disease-like disease, antiphospholipid syndrome, myocarditis and others. To date animal studies have not focused specifically on whether acquired detoxification deficiency accompanies repeated acute aluminum intoxication in dose schedules and administration mimicking vaccine exposures. It can be expected that in a subset of patients with autoimmune disorders, multiple chemical sensitivity is a likely result due to failed cellular detoxification.

Aluminum Dose Toxicity – Non-Linear Due to Aluminum Biochemistry and Volumetric Development

Recently, Ameratunga et al. [132] called for a moratorium on animal studies examining health effects of vaccine-scaled doses in animals, claiming that there was insufficient basis for the ASIA syndrome in humans. Claiming to have applied Bradford's Criteria for causality, and that the use of larger doses of aluminum in subcutaneous immune therapy (SIT) demonstrate safety of aluminum in vaccines, they make a number of errors. They ignore some available science that demonstrates reversibility of the effects of aluminum in mice (e.g.,[133]). The mistakes in their assessments [132,133] include an assumption of linear dose toxicity, which is an incorrect assumption [40]. Amerantunga et al. [132,133] also cite the use of aluminum hydroxide in the treatment of autoimmunity disorders

as evidence of their safety; however, an expert review of such treatments [12] cites aluminum toxicity of forms of aluminum used in vaccines as cause for the ongoing search for new vaccine adjuvants. The US FDA is currently testing alternative forms of adjuvants using appropriate dose escalation studies; such studies should be conducted for all aluminum adjuvants currently in use as well. We also simply cannot afford to and should no longer ignore the roles of animal models as suggested by Amerantunga et al.[132,133], nor the role of genetic susceptibility (individual risk), as this would represent a reversal of the norm of translational research. Such calls for less science are, by definition, anti-science, and are not helpful to the protection of public health via evidence-based preventative medicine.

The use of SIT began with treatment of allergies, but the history of the application of SIT in autoimmune disorder has not been straightforward; there have been many failures due to adverse events [135]. Mechanisms of action of aluminum hydroxide efficacy in the treatment of autoimmune conditions have not been well characterized, and actual long-term health outcomes of the use of aluminum hydroxide in cases of autoimmune conditions have not been sufficiently studied. It is notable that failed treatment with aluminum hydroxide could be medically confounded with exacerbation: the practice is terminated in patients who do not respond well. Long-term adverse health outcomes of SIT interventions that use aluminum have not been systematically studied, such as risk of Alzheimer's disease, or risk of developing secondary autoimmunity [136-137]. The single long-term study found no difference in de novo autoimmunity between autoimmune patients receiving SIT and a non-allergic control group [138], but found an increase in autoimmune disorders in the control group over time. The study was short (3 years), and rare autoantibodies were not assayed. SIT is applied in the exact manner in which many believe vaccines should be administered – with patient monitoring for adverse events on an individual basis, and cessation of the application of the medical procedure upon evidence that the patient has developed an adverse event – both in the treatment autoimmune and allergic diseases [135-136].

The toxicity of any substance is only partly determined by the dose. The body weight of an individual, the duration and repeated exposure, and individual genetics all determine toxicity. Since body weight during development itself is non-linear, and whole-body burden toxicity is due to whole-body metabolism, it is reasonable to expect that the dose-toxicity of any adjuvant in vaccines will also be non-linear. Aluminum, however, has been shown to have an insidious escape of tissue response (sequestration) at very low doses [40]. Not every patient forms a granuloma after injection of aluminum-containing vaccines, but when they do, the aluminum is released slowly, over time. With repeated doses in the vaccine schedule, accumulation and clearance dynamics are not well characterized. However, Flarend et al. (1997) [140] reported only 5.6% of injected radiolabeled aluminum hydroxide was excreted by rabbits after 28 days – which means the available pharmacodynamic modeling that has used much faster serum-clearance rates is overly optimistic in a grand way. Individuals with impaired body and cellular detoxification likely accumulate retained fractions of aluminum at a greater rate than others, and aluminum toxicity includes renal dysfunction. We have a long way to go before we can say we truly understand aluminum clearance and accumulation rates; carefully conducted dose escalation studies reflecting vaccine schedule amounts have not been conducted.

ER Stress and The Unfolded Protein Response in Human Studies of Autoimmunity

Garaud et al. (2011) [61] found that some systemic lupus erythematosus patients had a unique and strong gene expression signature implicating the Unfolded Protein Response regulated by BLIMP1 and concluded that the group of patients likely experienced a different pathophysiological journey to their diagnosis. SLE, like other autoimmune disorders, can have multiple causes – and different primary causes in different patients. In a recent study, Vieira et al (2018) [138] discovered that a genetic mouse model of SLE involved the unusual occurrence of the translocation of one enteric microbe to the liver through the lining of the gut. In their study design, the mouse model (C57BL/6), was depleted of all enteric bacteria. The absence of commensal gut flora prevented the proper healing of the gut lining; they then colonized the gnotobiotic gut with *E. gallinarum*, which could be detected in the

lymph system and the livers of the mice. Th17 expression was increased in the epithelial lining of the gut after re-colonization with *E. gallinarum*. The study used the absence of a healthy flora to induce leaky gut symptoms. Numerous factors can contribute to leaky gut syndrome, contributing to increased risk of autoimmune disorders, including diet and stress [139]. Dietary aluminum has been shown to cause leaky gut in mice [140].

The mice used in the Vieira et al (2018[138]) study have been used extensively in the study of other autoimmune conditions under exposure to aluminum hydroxide, including SLE with a bm12 transfer design [141]. Morokata et al. [142] found that local autoimmune responses are more important than systemic responses due to aluminum hydroxide ovalbumin - induced autoimmunity using these mice. Reddy et al. [143] described a protocol for the induction of asthma in the C57BL/6 mice. Inbar et al. (2017) [144] found behavior anomalies in these mice when aluminum adjuvants or the HPV vaccine Gardasil, which contains an aluminum adjuvant, were injected. Dimitrijević et al. (2012) [145] induced antiphospholipid syndrome in these mice with tetanus vaccine (tetanus toxoid + aluminum hydroxide). The autoantibodies they found were not specific for the linear TLRVYK sequence present in TTd, but instead recognized the shape of the TTd conformation, which is similar to that of b2GPI. The supplier, Jackson Laboratories, warns that the C57BL/6 can have high genetic variability.

Human Studies Fail to Detect Association Due to Mis-design

The role of cross-reactive autoantibodies in autoimmune disease has been known for some time, beginning with risk of cardiac autoimmunity following Streptococcus infection (e.g., [150]). The first expressions of concern over cross-reactivity with pathogen proteins in vaccines occurred in the early 1980's with evidence of cross-reactivity leading to cardiac autoimmunity from Streptococcus vaccine experiments in rabbits [151]. Clearly, not every person that receives vaccines or suffers an infection of agents with proteins that have similarity to human proteins develops autoimmunity; this points to a significant role of genetic variation by which those susceptible have higher structural similarity in self-antigens and antigens found in pathogens.

All of the studies that have demonstrated specific roles of aluminum and mercury in pathophysiological mechanisms of autoimmunity provide the strongest levels of evidence possible. An immense amount of direct causal evidence for the involvement of AL in a wide diversity of autoimmune conditions exists. Yet retrospective whole population association studies consistently fail to find an association at the population level. In autism, epidemiologic studies have failed to detect association of autoimmune disorders due to the use of incorrect study designs that are appropriate for population-wide effects. In the case of atopic disease (AD), an association has successfully been detected between AD and Hib vaccination – in a prospective cohort study.

Epidemiological vaccine safety studies often suffer flawed design by excluding genetic subgroups with comorbid autoimmune conditions such as RA, which represents a serious mistake in design. RA has a very well known HLA-DR class II genetic component in some families; studies such as [94] that are more appropriately designed (via genetic stratification) report much higher odds ratios of association with vaccines.

In the case of autoimmunity, as in ASD, genetic susceptibility to aluminum and mercury-induced ER stress would require studies that are informed by genetic risk. Increased susceptibility caused by mutations in proteins directly involved in pathways involved in each autoimmune disorder would tend to favor dominant patterns of inheritance.

However, more complex inheritance patterns are expected for risk that is due to proteins that confer risk expressed as ER Hyperstress. In ER Hyperstress, the proteins encoded by genes that confer small increases in risk are made more intrinsically disordered and more likely to require special handling by ER processes during folding. Two sources of ER stress – protein variation that leads to initiation of the UPR program and vaccine metals – leads to ER Hyperstress. In the case of ASD, cell death, release of cytokines[146], and the re-release of intracellular metals – explains chronic brain inflammation, multi-organ involvement, and disruption of cellular detoxification leading to the accumulation of other toxins from the environment at a greater rate than seen in neurotypical

individuals. ASD individuals also have a plethora of malformed proteins in their serum – pointing to a potential biomarker for diagnosis [147].

Numerous examples exist in which the Unfolded Protein Response and ER Stress play a central role in autoimmune disorders (Table 3). The question of whether autoimmune disorders are likely due to the expression of misfolded proteins is an important one. First, individuals who are heterozygous for misfolded proteins, which are likely to appear to be neoantigens to the mammalian immune system – expressing both copies will have proteins in tissues made of the properly folded protein with regular expression of the malformed proteins. In some individuals, the recurring cell death will yield a constant supply of malformed proteins. Progressive tissue loss will accompany inflammation. In other individuals, the autoimmunity will target both maternally and paternally inherited proteins when the antigenicity is not sufficiently specific to the malformed proteins.

Table 3. Examples of Unfolded Protein Response/ER Stress in Autoimmune and Autoinflammatory Disorders

Condition	Evidence	Detail	Reference
Amyotrophic Lateral Sclerosis	Review	ER morphology	Jaronen et al, 2014 [151]
		SOD1 accumulation	Doyle et al., 2011 [152]
Gullain-Barre Syndrome	viral hijack	stress granule protein	Hou et al., 2017 [155]
Rheumatoid Arthritis	anti-citrullinated protein antibodies	GADD34 increased UPR signal	Clavarino et al. 2016 [156]
	haploinsufficiency	GRP78 chaperone	Park et al. 2014 [96]
	immunohistochemistry	GRP78 increased	Dong et al. 2009 [157]
Lupus	gene expression	BLIMP1 UPR	Garaud et al. 2011 [61]

Spondyloarthritis – The Exception that Proves the ER Hyperstress Rule?

In contrast to prototypical autoimmune diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), which fit the ER Hyperstress model, spondyloarthritis (SpA), a chronic immune-mediated inflammatory disease of unknown origin does not share genetic risk factors. It also does not share the presence of auto-antibodies and SpA patients do not respond to T-cell or B-cell-targeted therapies. For these reasons, Ambarus et al. (2012) [158] concluded that SpA was likely autoinflammatory, rather than of autoimmune origin.

Intrinsic Protein Disorder and Autoimmunity – Intolerance of Shape-Shifting Mutations

The protein folding and modification processes that occur in the unstressed ER include N-linked glycosylation, disulfide bond formation and proline cis–trans isomerization [156]. Mutations that change any of these protein folding dynamics could lead to ER Hyperstress in any cell in which the altered protein is expressed if that cell is already ER-stressed due to metal intoxication. Antigen folding requires proper execution of the unfolded protein response [157].

If individuals who are at risk of autoimmune disorders following vaccination carry mutations that cause changes to protein intrinsic disorder that manifests the genetic component to ER Hyperstress, the intrinsic disorder of the proteins involved should be lower than that of most proteins in the human proteome. To determine if specific mutations involved in autoimmunity tend to increase disorder, proteins known to be encoded by genes that are screened for risk of ADs were studied. The ADs covered included those summarized in the Eupedia[55] Autoimmune Diseases SNP entries, and included: allergies (general), Ankylosing Spondylitis, Celiac Disease, Crohn's Disease, Grave's Disease, Inflammatory Bowel Disease, psoriasis, Multiple Sclerosis, Rheumatoid Arthritis, Sjögren's

Syndrome, systemic lupus erythematosus, Type 1 Diabetes, Type 2 Diabetes, and ulcerative colitis. A total of 109 protein-AD associations were analyzed. Protein intrinsic disorder was determined for this list of proteins and compared to that of 200 randomly selected proteins from the human proteome with entries in the MobiDb, a curated database of intrinsic disorder, conformational diversity, and interactions in proteins [158].

A great number of case reports exist that outline the clinical progression of a patient without autoimmune disorder who presented with AD after vaccination. Population-level studies that ignore the feasibility of mechanisms of autoimmunity from vaccines also tend to ignore the rates of autoimmune disorders in their study design. Clearly, if vaccines induce autoimmunity, they do in a subset of individuals. The hypothesis that the subset has higher specific risk of autoimmunity from vaccination is not well-tested by study designs that are better suited to test the hypothesis as if the risk was shared across the entire population. The use of whole-population correlational studies in the case of genetic susceptibility will dilute the strength of the association. In that setting, cohort studies and afflicted vs. not can be more powerful. However, the most appropriate study would focus on the rates of AD in individuals with genetic susceptibility who vaccinate compared to those who do not vaccinate. A small sampling of studies of various types that report ADs post-vaccination are presented in Table 4.

Examples from Human Studies

Molecular mimicry can occur between pathogen proteins and human proteins regardless of whether the source is infection or injection. Autoimmunity from vaccines has been reported for numerous conditions, including immune thrombocytopenic purpura and the MMR vaccine [159]. Hepatitis B vaccine 1991–1997 and multiple sclerosis [160]; Narcolepsy and H1N1 influenza vaccine [161]; undifferentiated connective tissue disease and Hepatitis B vaccine [162-163], ovarian failure, Lupus and human papilloma virus [164]. Of these, the HPV vaccine and the current HepB vaccine contain distinct forms of aluminum. Many other studies have reported autoimmunity after aluminum-containing vaccines (Table 4).

Table 4. Autoimmune and other conditions reported after vaccination

Condition	**Adjuvant**	**Vaccine**	**Reference**
cognitive dysfunction	$Al(OH)_3$	various	Couette et al., 2009[168]
glomerulonephritis	$Al(OH)_3$	multiple	Levart, 2013[169]
	$Al(OH)_3$	vaccines	Bassi et al., 2012[128]
Guillain-Barré Syndrome	$Al(OH)_3$	HepB	Bogdanos et al., 2005[170]
		H1N1	Ahmed et al., 2015[10,164]
Hypoinsulinism (Tissue Scurvy)	Various		Innis, 2013[171]
Rheumatoid arthritis (genetic predisposition)	N/A	H1N1	Basra et al., 2012[172] Ray et al., 2011 (cohort study)[97]
Narcolepsy	N/A	H1N1	Ahmed et al., 2015[10] Verstraeten et al., 2016[173]
Vaccine-induced immune thrombocytopenic purpura (VI-ITP)	$Al(OH)_3$	HepB	Meyboom et al., 1995[174]
	n/a	MMR	Cecinati et al., 2013[175] O'Leary et al., 2012[176]
Vasculitis, death	AAHS	HPV	Tomljenovic and Shaw, 2012[177]

Vasculitis	AAHS	HPV	Gomes et al, 2013[178]
Thrombocytopenic purpura	AAHS	HPV	Souayah et al. 2011[179] Pugnet et al., 2009[180]
Demyelinating disease	AAHS	HPV	Alvarez-Soria et al., 2011[181]
Systemic lupus erythematosus	AAHS	HPV	Gatto et al., 2013[167]
Premature ovarian failure	AAHS	HPV	Gatto et al., 2013[167]
increased brain AL	$AL(OH)_3$	adjuvant	Redhead et al., 1992[182]
Undifferentiated connective	$AL(OH)_3$	Hepatitis B	Bruzzese et al., 2013[165]
Tissue disease	$AL(OH)_3$	Hepatitis B	Perricone et al., 2013[166]

Additional examples are reviewed by Tomljenovic and Shaw (2012[174]) and include vasculitides, arthritis/arthralgia, immune thrombocytopenic purpura and multisystem atrophy.

Guillain-Barré Syndrome and Hepatitis B Vaccination

One of the most important and convincing studies in this area [167] was conducted to study common peptide sequences between the small HBV surface antigen (SHBsAg) contained in the HepB vaccine to peptides in known MS (myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG). The study not only found numerous peptide sequences common between SHBsAg and MBP/MOG; they also found that 60% of patients with MS had autoantibodies to both SHBsAg and MOHG. The rate of double reactivity was 2% in the control group.

Ahmed and Steinman (2017) [161] similarly found cross-reactive antibodies against the orexin (hypocretin) receptor and the Pandremix H1N1 vaccine peptide, thereby interfering with the orexin production and resulting in narcolepsy. The cross-reactive antibodies were detected in the blood sera of Pandemrix-vaccinated individuals with narcolepsy than individuals without narcolepsy. Notably, individuals in Finland who developed narcolepsy had the (HLA) DQB1*0602 genotype [13], supporting increased susceptibility to vaccine-induced autoimmunity due to genetic variation.

Aluminum Hydroxide and Ethyl Mercury from Thimerosal Both Activate ER Stress and Activate the UPR

The activity of aluminum as an adjuvant is one of its effects on ER stress. Specifically, aluminum leads to activation of the NLRP3 inflammasome, one route in the UPR to ER stress [181]. Li et al (2008) [184] found that the adjuvanticity of aluminum is dependent on activation of the NLRP3 inflammasome, meaning that ER stress and the UPR are involved with dose. Aluminum also wreaks havoc on neuronal and hepatic cells as an intracellular ROS generator [47] and causes mitochondrial damage and cytoskeletal dysfunction [62]. The route to apoptosis via aluminum ER stress activation of the UPR can occur independent of the p53 pathway [34]. Cellular apoptosis is an unwanted side effect of aluminum hydroxide that leads to the release of incompletely or incorrectly ER-processed proteins. Further, aluminum hydroxide induction of Interleukin-1B (IL-1B) may explain observations of its upregulation in autism, as reported by Goines and Ashwood [185].

This review has focused on the ER Hyperstress mechanism of cellular injury from aluminum adjuvants as a critically important pathophysiological mechanism that can bring about autoimmunity after vaccination. The ER Hyperstress process is the precise manifestation of the G x E interaction between genetics (risk of susceptibility) and environment (exposure to aluminum, mercury and other specific environmental sources of ER stress). ER Hyperstress explains why some individuals are more susceptible to vaccine induced autoimmunity than others; it explains chronic, low-grade inflammation in autoimmune disorders. It is perfectly complementary to and adds a

critical step to explain how individuals could develop autoimmunity via widely-recognized downstream immunological outcomes such as molecular mimicry-induced autoimmunity [25,184,186,187]

Aluminum in Demylinating Disorders

A variety of demyelinating conditions with heterogeneous manifestation are autoimmune in nature that may be attributed to exposures to vaccines with adjuvants via mechanisms including molecular mimicry and ER Hyperstress. These include multiple sclerosis (adult and pediatric), Optic neuritis (ON), Transverse myelitis (TM), Clinically isolated syndrome (CIS), Neuromyelitis Optics (NMO), and Acute Disseminated Encephalomyelitis (ADEM). Fulgenzi et al. (2014) [192] found that aluminum was implicated in 44.8% of cases neurodegenerative cases studied; high levels of iron and aluminum have been found in the urine of patients with MS (Exley et al., 2006) [193]. Numerous case studies have reported efficacy of chelation of aluminum in reducing symptoms of multiple sclerosis [194]; Alzheimer's disease (e.g., chelation with deferoxamine and ascorbic acid [195]; rheumatoid arthritis (EDTA chelation[94]). Special care must be taken to avoid sudden toxicity due to chelation of metals – autoimmunity can result (e.g., [196]).

Therapeutic Effect of Aluminum in Autoimmune Diseases: Numbing Immunity?

The paradoxical use of aluminum in treatments for some symptoms of autoimmune disorders is understandable when a strong skew toward Th1 immunity exists. The boosting of Th2-type immunity in those cases would favor a less inflammatory response [191]. However, balance, not imbalance is sought, and follow-up medical exposures to aluminum hydroxide could lead to exacerbation, or to new autoimmunity. Repeated exposure to high doses of the suspected allergen can tip the immune system toward the infection-like Th1 level; repeated exposure again to the adjuvant (in the form of vaccines) would then amplify Th2. Long-term health outcome studies of patients with autoimmune disorders treated by allergen alone vs. allergen with adjuvant are needed.

Conclusions

Animal studies and human studies alike point to ER Hyperstress as a likely manifestation of genetic x environment interactions in autoimmune disorders. Together, the studies reviewed here provide strong support for the role of aluminum as a contributor to autoimmunity in individuals for whom "small" doses may represent larger effective doses, or for whom exposures to other sources of ER stress are present. In addition to molecular mimicry, which contributes to autoimmunity from vaccines, as is evidenced by the discovery of cross-reactive antibodies, the ER Hyperstress mechanism unleashes a number of additional mechanisms of autoimmunity. The release of semi-processed human proteins due to cell death resolution of the unfolded protein response is one of the most logical sources of problematic self-antigens. The toxicity of aluminum adjuvanted vaccines is seriously underestimated for an unknown percentage of persons and families in the population. Rather than identifying patients who are most susceptible via the induction of myriad conditions of mysterious or unknown origin, screening individuals for risk, using more broadly defined risk factors and specific biomarkers would seem more humane. The use of adjuvants that do not involve mechanisms of action that include ER Stress would leave the unfolded protein response intact, prevent ER Hyperstress, and reduce the global burden of disease from autoimmunity induced by adjuvants.

There are limitations to using animal models to determine general risk of autoimmune disorders. In particular, the chronic doses, while injected, are typically much higher than used in aluminum-containing vaccines, but in some studies the doses are delivered in a saline vehicle. Low doses of aluminum can be more, not less toxic due to failure to initiate a protective response, such as granulomas [40]. Also, intraperitoneal injection is not used in the administration of vaccines; instead, either intramuscular or subdermal injections are used. Further, the various forms of aluminum used in animal models of autoimmunity are variously represented as dissolved powders, often in non-specified liquid with non-specified volumes. Also, the timing of injections is often planned to reflect experimental convenience and have not been replications of the CDC's current recommendations, including regular influenza vaccines, some of which contain thimerosal.

Nevertheless, it is noteworthy that the vast majority of animal studies that employ aluminum hydroxide succeed in creating autoimmune disorders in part by including ovalbumin. The evidence that aluminum hydroxide is involved in autoimmunity at vaccine dose levels in some individuals is overwhelming. The ASIA syndrome has been, by happenstance, replicated in commercial sheep [197]. While the inclusion of alloantigens is central to the strategy of artificial immunity, the option of excluding peptides that match human proteins to avoid molecular mimicry also appears to be have been completely overlooked in spite of the extensive work of by Kanduc and colleagues [198]. Given the dramatic increases in chronic illnesses of "unknown origin" that has occurred since whole-population vaccination programs, discussions of the complexity of autoimmunity must – and does – overtly include a role for vaccines [199].

The localization of the aluminum from vaccines to the brain has been empirically demonstrated – as has its long-term persistence [200]. Neurodegenerative diseases such as Alzheimer's disease are not usually seen as autoimmune disorders, but evidence of intrinsic immune reactions initiated by aluminum is overwhelming. Correlation of elevated Al with degenerative dementia and Alzheimer's disease has been well documented [195]. Excessive dietary AL can also form insoluble aluminum phosphates in the gastrointestinal tract and may lead to hypophosphataemia. Although the role of ER stress has been recognized in Parkinson's disease [58], the importance of metals (including aluminum) is only now coming to light [201]. The disease burden of and cost of industrial and medical uses of aluminum is likely so large as to be immeasurable.

The multifactorial causality of autoimmunity cannot exclude vaccine adjuvants including aluminum based on high doses typically used in animal models. While it is reasonable to conclude from these studies that not all and perhaps most humans will develop intermittent or chronic autoimmunity given that doses of aluminum (mg/kg) in vaccines are so low compared to experimental levels used in non-genetic risk animal models, the low dose non-linear response cannot be ignored. Additionally, the actual CDC pediatric schedule involves repeated vaccinations, separated by months, combined with vaccination of non-aluminum-containing vaccines, repeated over nearly two decades. None of the studies focused on autoimmune disorders were dose escalation studies, or synchronicity studies of multiple antigens/vaccine formulations, and none specifically tested whether ER stress from unfolded proteins combined with stress from aluminum or thimerosal administration, and few employed genetic models of mice based on observed human genetic variants associated with autoimmunity. Exceptions exist. Zhu et al. (2014) [41] successfully induced atherosclerotic lesions using vaccine-representative doses in genetic (apoE null and LDLR null C57BL/6 mice) in an environmental (dietary) background conducive to atherosclerosis.

Studies of autoimmunity that point to infectious pathogens cannot easily dismiss a role of vaccine adjuvants as causal co-factors to infections. Retrospective studies that are designed to test the hypothesis of "association" fall short of testing causality, and are ill-posed to test the ER Hyperstress hypothesis, or any other model that involves a genetically susceptible subgroup.

Nevertheless, grave concerns over the lack of empirically informed aluminum clearance rates, accurate clearance models, and tissue fates have been expressed [31-33]. One of the most important studies focused on injected aluminum pharmacokinetics using rabbits [140] reported very slow body clearance, often misrepresented via use of serum clearance, because 94% of the labeled, injected aluminum had not passed in urine after 28 days. The study investigators misplaced or destroyed brains and bones of some of rabbits, and body clearance and tissue compartment modeling by Mitkus et al. (2011) [199) were not sufficiently informed by relevant empirical measurements, citing oral exposures of dietary forms of aluminum in adult animals as relevant for injected forms of aluminum in human infants and children[31].

A greater question, however, is whether exposure to many doses of injected aluminum has led to impairments that will manifest as a pandemic in countries that use them. The increased rates of ASD, ADHD and autoimmune/autoinflammatory conditions may be the tip the iceberg. Redhead et al. (1992) [182] simulated

vaccination-level exposures to aluminum hydroxide in mice and found elevated levels of aluminum in the brain 2-3 days after injection. Other animal studies focused on neurological, cognitive and behavioral effects [203-205] have consistently reported evidence of neurological and cognitive impairment at vaccine-scaled doses of aluminum adjuvants. In individuals for which cellular detoxification is impaired, either via environmental exposures that impair ER protein folding (like aluminum [37] and thimerosal [206], genetics, or to the combined effects of environmental and genetic ER stress risk leading to ER Hyperstress [34]), the small vaccine doses (compared to animal models) appear to represent unsafe doses for some individuals, families or ethnic groups.

The literature also supports the careful use of aluminum-focused chelation therapies to ease the symptoms of neuroimmune effects of aluminum exposures with added benefits of removal of other metals accumulated as a result of acquired cellular detoxification deficiency (ACDD) caused by ER Hyperstress. Responsible application of chelation techniques requires consideration of limitations [207]. Reduction of tissue aluminum has been reported due to consumption of high-silicic acid mineral waters [208].

Treatments that May Ameliorate Autoimmune Effects

There is a large body of literature on drugs, supplements and treatments that can help reduce inflammation in the human body. Candidates include Vitamin D (reduces inflammation in idiopathic urticaria, astrogaloside in renal failure patients undergoing treatment [209]. Jones et al. (2017) [210] measured a significant increase in the excretion of aluminum in patients who drank silicic acid-rich mineral water. Treatment of patients affected by Al burden with ten EDTA chelation therapies (EDTA intravenous administration once a week) was able to significantly reduce Al intoxication [94].

New findings show that pediatric dosing of aluminum in vaccines, acutely high doses (mg/kg/day) in low birthweight and low body weight infants can occur [31]. Depending on variation in clearance rates, and tissue fates, which are also inadequately characterized for the population as a whole, the repeated chronic exposures likely lead to greater rates of accumulation of aluminum in some individuals. In proper translational research, the dosage toxicity of substances – including carrier molecules in drugs – are subjected to demonstrations of dose-related safety prior to their use in humans. Aluminum was 'grandfathered' in for use in vaccines, and only proteins in vaccines must be tested for safety. Animal dosage escalation studies of injected forms of aluminum are called for because they have not been conducted to date, and the use of argument by analogy from dietary exposures in adult animals as if they apply to human infants is spurious, filled with errors, and unwarranted assumptions [31]. More, not less, animal science is needed to answer specific questions on mechanisms of aluminum adjuvant-induced autoimmunity.

The evidence presented in this literature review is overwhelming: autoimmunity due to the adjuvantive effects of aluminum combined with the release of oddly and incompletely folded and processed proteins is extremely plausible - for those conditions reviewed. Other autoimmune/ autoinflammatory conditions that should be explored for additional evidence, such as cross-reactive antibodies, of the presence of aluminum in the inflamed tissues and the benefit of chelation include primary biliary cirrhosis, scleroderma, CREST syndrome, polymyositis, Telangiectasia, mixed connective disease, and ankylosing spondylitis [211]. The high levels of metal concentrations observed in the cerebral spinal fluid and blood plasma of patients with amyotrophic lateral sclerosis points to failed acquired cellular detoxification syndrome, and because it included aluminum, a potential role for aluminum induced ER stress. And ER stress during placentation is a refined balancing act [212]. Individuals seeking conception might consult with their doctor on the wisdom of injecting aluminum in any form.

ER Hyperstress From Genetic and Environmental Risk in Humans

It is also worth noting that some genetic variants in the ERAP1 gene, which is down-regulated by thimerosal [206] are associated with ankylosing spondylitis (AS) – an autoimmune form of arthritis in which the vertebrae of the

spine can become fused. Individuals with RA are removed from studies of AS, reducing the signal of autoimmunity from vaccination [213]. Variants in ERAP1 are also associated with juvenile idiopathic arthritis [214] and psoriasis [215]. Individuals with LOF-mutations in ERAP1 can be expected to be intolerant of aluminum and mercury from vaccines and other sources, with special challenges to immune system protein production given the role of ERAP1, and the harm to ERAP1 expression in the absence of genetic risk can be considered a form of phenomimicry.

Loebel et al. (2016) [216] found antibodies to adrenergic and muscarinic cholinergic receptors in patients with chronic fatigue syndrome – pointing strongly to CFS as an autoimmune disease. It is reported that the same autoantibodies have been found in individuals suffering from malaise and lethargy post-HPV vaccination [214), but those results have not yet been published.

Metal cellular detoxification is a biological process that involves complex pathways, and thus some individuals will be less tolerant than others to exposures. Both aluminum and mercury bind to sulfhydryl (sulfur-containing) groups of glutathione. Mercury and aluminum both inhibit cellular detoxification mechanisms and signals. Mutations in any of the hundreds of genes involved in cellular detoxification – which may include mutations in proteins involved in fairly basic cellular functions – will confer reduced capacity. More importantly, genetic sensitivity to mercury [218-220] are known. Metals themselves can be antigenic allergens [221]; aluminum sensitivity can be readily determined via a patch test.

Hypersensitivity to aluminum can thwart attempts to immunize with aluminum-containing vaccines [222]. There is also evidence of genetic susceptibility to hypersensitivity to mercury [218], and toxicity of ethylmercury is traced to ER-stress [223]. Aluminum can impair P450 mediated microsomal cellular detoxification [224]. Aluminum in the brain of people with autism [222] likelys play a role in chronic microglial activation and pro-inflammatory IL-6 induction via the same mechanisms. ER stress and the unfolded protein response has proven central to every autoimmune disorder in which it has been studied (e.g. [228-230]). We need to focus on all sources of metals and defects in cellular detoxification, observed in atherosclerosis [231], multiple sclerosis [193] and ALS [232]. The role of ER Hyperstress appears to be also be central in ASD [30]. Epidemiological studies are variable in the outcome, with results that are pliable to manipulations, and those that have found association of autoimmunity [233] have not led to changes in vaccine formulations.

This review points to how any number of genes that encode proteins could carry variation that increases protein intrinsic order, leading to genetic ER stress, combined with environmental ER stress leading to ER Hyperstress in the presence of aluminum and mercury. It is time to respect these minorities' increased familial and individual risk with respect for their differences when considering legislation on vaccine options, and in the medical practice of immunization. In some individuals, a Th2-skewed response is likely in response to injections including aluminum; therefore, aluminum adjuvanted vaccines can no longer expected scientifically to be tolerated well by the entire population. Biomarkers to identify such individuals are needed, and dose-escalation tested adjuvants or approaches to vaccination that approach the immune system more appropriately should be explored.

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Systematic Review of Historical Epidemiologic Studies Influencing Public Health Policies on Vaccination

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Abstract

a. Background. Studies used to inform on public health policies on vaccination are represented are rigorous.

b. Methods. To examine the quality of the science used in high-profile sources on the question of vaccines and autism, an objective evaluation score (OES) was designed that provides a rapid, reproducible assessment of any retrospective or prospective clinical study focused on vaccine safety. The OES was determined for forty-eight "Key Studies" on the question of vaccines and autism association present to the President of United States in 2017, and on the question of the safety of thimerosal us in vaccines present by the US CDC as representative.

c. Results. Of the forty-eight studies, only one had a positive score (+4); the rest had zero or negative scores, with an average OES of -6.61. Moreover, a search of the literature revealed that the list of studies was in fact as biased selection of studies, with the selected studies yielding an average odds ratio of 0.80 (average for ASD-focused studies 0.756); the average odds ratio of studies not cited by these sources was 4.01.

d. Conclusions. Clearly, public health policies focused on low-quality underpowered retrospective correlation studies, which only measure association and do not measure causality, must be replaced with more rigorous, sufficiently powered randomized prospective clinical trials. The safety of vaccines cannot be assumed and used as justification for not performing rigorous science. (Extended Abstract supplied in text.)

Summary: It is widely believed that studies of the question of vaccines and autism are rigorous and have thoroughly tested for a causal link. This study provides an impartial, multi-component objective evaluation score on the key studies listed as demonstrating no link between vaccines and autism, and find that all fall short of ideal and robust.

Extended Abstract

Objective: Numerous agency authorities, resources, and publications cite numerous epidemiological studies as demonstrating a lack of evidence of association between vaccines and autism. Prior analyses have revealed that key studies used by the Institutes of Medicine lack sufficient statistical power to provide confidence that their negative results, i.e., lack of association, could reasonably be due to the combined effects of an inability to detect an association due to low power (attributed to small sample sizes) and to healthy user bias (a form of self-selection in which patients who have previously experienced negative reactions to vaccines opted out of the single vaccine for which association was

sought. In this systematic review, 48 “Key Studies” cited by organizations such at the American Association of Pediatrics, or listed by CDC and two reviews as evidence of no association between “Vaccines” and autism, or allegedly demonstrating the ”safety” of thimerosal-containing vaccines (TCV), are independently and collectively reviewed.

Method: An objective 11-subscore evaluation score (Objective Evaluation Score; OES) was designed that can be applied to any vaccine safety study; the ideal score for any study is +12; the average score for the Key Studies was -6.61. Only two of the forty-eight Key Studies had a non-negative OES.

Results: The studies evaluated fell far short of high-quality science: twenty did not estimate ASD prevalence; twenty-two did not study “Vaccinated vs. Unvaccinated”; thirty-two only examined association of a single vaccine; twenty publications studied thimerosal containing vs. non-thimerosal containing vaccines; at least twenty-one studies had insufficient statistical power; thirty-three had evidence of flawed study design; twenty-eight had flaws in the design of analysis, in some cases leading to false negatives on the question of association; thirty-four made unwarranted conclusions about negative results given the sample size and type of study. All but one study were retrospective studies; none were blinded, prospective randomized clinical trials. All fall into the category of correlational analyses. Due to cohort effects and myriad ingrained flaws, correlation studies fail to provide credible evidence capable of definitively falsifying the hypothesis of causality of vaccines and autism and are at the risk of temporal confounding and cohort effects. Studies of single vaccines obviously fail to provide a sufficient level of evidence permitting a generalization to all vaccines or the CDC pediatric vaccination schedule. Studies that did not measure rates or risks of autism were found to be irrelevant to the question. Studies of TCV vs non-TCV found increased health risks due to non-TCVs, but uniformly attributed the result to a mysterious, previously unknown (ad-hoc) health benefit of thimerosal. The three largest studies fall into the categories of single-vaccine studies or TCV-vaccines. Many studies adjusted for covariates that should have been considered risk factors; none examined interaction terms between vaccines and plausible co-factors. Combined, these studies have been cited by over 6500 other studies or reviews and form the basis of public health policy and practices. The single meta-analysis conducted included low-powered studies that had already been rejected by the IOM as flawed. None of the studies were designed to test the hypothesis that a heterogeneous subgroup of humans carries susceptibility to neurodevelopmental disorders compared to the general population, nor did any of the studies conducted determine whether such individuals could be detected prior to vaccination. The bolus of studies cited by CDC and AAP conspicuously exclude studies that show a positive association of neurodevelopmental disorders and vaccines. Co-signing organizations and recipients of the of the AAP statement letter to POTUS will be interested to know that the average odds ratio of studies cited was 0.80 (average for ASD-focused studies 0.756); the average odds ratio of studies not cited by these sources was 4.01.

Conclusion: Low-powered association studies cannot be used to provide definitive negative evidence for hypotheses of causality, and single-vaccine studies likely suffer from Healthy User Bias. No studies exist that definitively falsify the vaccine/autism link, and the evidence presented as demonstrating no link and the alleged safety of thimerosal is both extremely weak and has not been provided to policy makers in an unbiased manner.

Introduction

Most questions about vaccine safety and efficacy elicit a media response that hearkens the question of whether vaccines contribute to risk of autism (autism spectrum disorder diagnosis), even when the question is not central to the specific question at hand. An example is the recent spate of outbreaks transmission of mumps virus involving individuals who had been fully vaccinated. The question of the

cause of the loss of efficacy is not usually specifically addressed; as mumps is an RNA virus, with much higher baseline mutation rate than DNA viruses and bacteria, the vaccine types and the circulating wild types can be expected to evolve away from other, weakening immunogenicity. The standard modus operandi of media coverage is to cite the study by Wakefield and colleagues, which was a pilot study in which Dr. Wakefield and colleagues posed the hypothesis of a new form of autism involving GI disruption, potentially caused by lingering measles infection. Dr. Wakefield's medical license was stripped by the General Medical Council of the United Kingdom, who nevertheless went on to exonerate Wakefield's colleague, Dr. John Walker-Smith and to quash the suspension of Dr. Walker-Smith's license. The media coverage may also cite studies conducted to exonerate vaccines of any role in ASD risk, but they never cite studies that have found increased risk of ASD following vaccination. Examples of those studies are provided here (Table 1).

Medical associations, led by the AAP, sent President Donald J. Trump a letter in which they cite specific studies they claim demonstrate a lack of association between vaccines and autism (AAP, 2017). The US Centers for Disease Control and Prevention (CDC) also lists studies on their website, which reads "Vaccines Do Not Cause Autism" (CDC, 2018a). ASD rates (four years ago) are 1 in 59 kids by age 8; in boys, the rate was 1 in 34 (CDC, 2018b)[1].

Table 1. Examples of Studies Relevant to the Vaccine/Neurodevelopment Disorder Question Not Cited by AAP or CDC

Avella-Garcia et al., 2016[2]

Bauer et al, 2013[3]

Gallagher and Goodman, 2010[4]

Nevison, 2014[5]

Shultz et al. 2008[6]

Geier and Geier et al., 2003[7]

Geier et al, 2018[8]

Goldman and Yazbak, 2004[9]

In 2010, Shoffner et al., (2010)[11] found that 71% of kids with regressive autism had an episode of fever > 101°F In 33% of these cases, the fever occurred right after vaccination – and none showed regression into autism unless fever had occurred.

The AAP letter, evidently authored by HealthyChildren.org and provided to the AAP and co-signers for signature, and the CDC website both neglect to cite studies in favor of rejection of the null hypothesis of no association (Table 1). The bolus of studies themselves therefore may represent an example of confirmation bias in action; they have "cherry-picked" studies from the literature, leaving out any that lend support to the possibility that vaccine contribute to neurodevelopmental disorders. The US CDC is closely affiliated with vaccine manufacturers, receiving over hundreds of millions of dollars per year via the CDC Foundation. A review of the publications by CDC ACIP committee members reveals universally shared financial conflicts of interest with a single exception.

As reported by ICAN (2017)[10]:

"Since 1995 the CDC Foundation has raised 620 million to pay for 824 programs at the CDC… In 2015 alone, the CDC Foundation raised 157 million for privately funded programs at the CDC, which then obtain the stamp of legitimacy of 1the CDC… Merck, for example, funded an 832,916 program through the CDC Foundation to "expand CDC's ... viral hepatitis prevention and vaccination activities"… As a result, the CDC is reliant on the CDC Foundation for the continued funding of these projects, and even for the services of the staff placed at the CDC by the CDC Foundation, since the CDC is only permitted to use these funds as expressly directed by the CDC Foundation…. This foundation even funds and thus directs CDC 'management training courses.'"

Many of the individual studies cited were funded by vaccine manufacturers, presenting a systematic conflict of interest (Kern et al 2017)[12]. HealthyChildren.org, an unofficial vaccine educator organization, receives funding from the CDC (Doshi, 2017[13]), representing a conflicted source (DeLong, 2012) [14]. CDC also owns numerous patents on vaccines, and ACIP members nearly universally have extensive financial conflicts of interest.

To date, no independent evaluation of the studies cited by AAP and by CDC has been conducted.

Context of the 48 "Key Studies"

The first study to propose that vaccines might be involved in any aspect of ASD (Wakefield et al, 1998[15]) was retracted by the Lancet after some of the authors self-retracted an interpretation. The study has been misrepresented as having concluded that vaccines cause autism. However, in the original study, authors had merely proposed the hypothesis of an MMR/ASD/colitis link and actually concluded: *"We did not prove an association between measles, mumps, and rubella vaccine and the syndrome described"*.

After Dr. Wakefield's initial study, the question of the link between vaccines and autism was raised by the US Congress, which tasked CDC to undertake objective studies to address the question. CDC participated in numerous studies, and subcontracted others. Nearly all studies were retrospective (i.e., correlational) in nature and thus fail to actually test the hypothesis of causality. In 1986, the US Congress passed, and President Ronald Reagan signed it law, the 1986 National Childhood Vaccine Injury act, which stipulated (1) that vaccines be made safer, and (2) that individuals who are genetically susceptible to vaccine injury by found. So far, neither of these mandates have been met. CDC has conducted zero studies on the question of genetic susceptibility to vaccine injury. In the studies CDC did conduct, or fund, all were retrospective studies, i.e., correlational studies, meaning that the hypothesis of causality could not be tested. Some of the studies cited (e.g., McMahon et al., 2008) [16] used data from vaccine safety databases (such as VAERS and the VSD), which entail warnings to users on the inherent limitations of the use of the data sources to infer causality.

At around the same time, the link between thimerosal and autism was addressed by the opportunity afforded by the phasing out of Thimerosal from vaccines, ostensibly (according to CDC) due to an "abundance of caution" (CDC, 1999) [17]. In reality, the amount of mercury being inject to infants had been found to exceed the federal limits of exposure permitted by the EPA (Ball et al., 2001; Hurley et al., 2010[18,19]). Thimerosal is included as a preservative for multi-dose vials of vaccines. "Thimerosal-containing vaccine" vs. "non-thimerosal containing vaccines" were included in the evaluation because they were included in the original listings, although they received a demerit due to their inexact focus on ASD risk. In 2002, for reasons that are unclear, the CDC began recommending that pregnant women and infants as young as 6 months preferentially receive thimerosal-containing annual flu shots at 1-2 doses each season. Gerber and Offit (2009)[20] lamented the shift of the hypothesis of causality of autism due to vaccines from ethyl mercury to "too many vaccines"; however, those observing cases of sudden onset of autism are not responsible for the vaccine schedule, nor for the formulation of vaccines, and aluminum

exposures, a potent neurotoxin, is now known to exceed "safe" levels determined by an unreliable FDA modeling exercise that derived judgment of safe levels of injected forms of aluminum from dietary exposure in adult mice with arbitrary dietary MSL levels (Lyons-Weiler and Ricketson, 2018) [21]. The CDC schedule results in neurotoxic doses of aluminum in the first six months of life (Dórea and Marques, 2010; Lyons-Weiler and Ricketson, 2018) [21,22], with more aluminum exposure from vaccines than from diet (Dórea and Marques, 2010) [22]. Autism can be seen as an acquired syndrome with failed detoxification deficiency (Lyons-Weiler, 2018) [23], meaning susceptible individuals may not fare well with repeated and combined exposures of injected forms of neuro- and immunotoxic metals.

IOM Causality Determinations, Evidence of Bias, Studies of Mechanism Ignored

In 1989, 2004, and 2012, the Institute of Medicine undertook the task (via contract) to evaluate the available scientific literature on issues of vaccine safety, focused specifically on the question of whether the available evidence could, or could not support, a conclusion of causality. Unfortunately, minutes of the meetings leading to the 2012 conclusions revealed that the IOM decided to ignore available animal studies presenting mechanistic plausibility for lack of a "free weekend" (NAS-IOM, 2001)[24].

An absence of sufficient evidence due to a lack of studies was, in the original reports, used appropriately to conclude that insufficient information was available for, or against, a conclusion of causality, but in the latter reports, and in CDC usage of those reports, the conclusion was shifted slightly toward a focus on insufficient evidence to conclude that causality was not an issue. Whereas a lack of evidence for, or against causality previously had been interpreted as "insufficient evidence", in 2012, a new logic was created that allowed the absence of sufficient evidence to emphasized as "no evidence in favor of". This alone is a clear breach of objective analysis and inference.

This shift has had profound and definitive impact on public health policies toward vaccination. The vaccine schedule has expanded, and a cultural phenomenon pitting physicians against parents over the objective reality of vaccine risk has taken hold. There are social consequences for exercising choice both in individuals' personal and professional lives. Where some individuals lose close friends, others have lost employment. In 2014, six healthcare workers at Saint Vincent Hospital in Erie, PA sued their former employer for wrongful termination due to their choice to exercise their rights to refuse the influenza vaccine, and won a settlement that include re-instatement (AHC Media, 2014) [25]. In 2014, a PBS station in Boston, MA, USA "unhired" a scientist, Mish Michaels, who had spoken out while between jobs on vaccine risk. We have to ask ourselves: Is it rational for members of our society to suffer consequences for speaking out about potential risks of a medical procedure of the source of the biased perception on the safety of that procedure is due to activities of those who stand to gain financially from vaccines as a business?

Widespread Cohort Effects: Changes in Schedule

Around the time that thimerosal was being phased out of pediatric vaccines (with exceptions), additional vaccines were being added to the schedule. Many of these were aluminum-containing vaccines (ACVs) and additional booster shots were being added. This, along with the shift in the use of TCVs during pregnancy, introduces a massive and universal cohort effect that impacts any retrospective study. When parents began to observe regression into autism after the DtaP vaccine, parents were accused of moving the goalposts; in reality, the parents were not responsible for the addition of so many new aluminum-containing vaccines, and they began reporting what is now a widespread set of observations: of the back, a high-pitched cry from which the infant could not be consoled, head banging if >2 years old, then a sudden or gradual (over the course of days) of loss of social contact (low eye contact, use of fewer, simpler words, loss of speech) and regression into autism.

Systematic Review Methodology

Search Strategies

Various organizations have published publication lists of studies with short summaries on epidemiological studies reported to address the question of a causal relationship between vaccines and autism. This includes governmental agencies (CDC, 2018) [26], a professional medical association (AAP, 2017[27]), and two additional compendia that selected arbitrary sets of studies from the full literature (Hotez, 2017[28]; Gerber and Offit, 2009[20]). These compendia were compiled into a master list of "Key Studies" for evaluation. This resulting bolus of a widely circulated catalog of studies are used to ostensibly show the massive amount of science that purportedly shows no association between vaccines and autism or other neurodevelopmental disorders. It is assumed that these studies are representative of the full body of literature publish on the question of any connection between vaccines and autism, and on vaccine safety in general. If these studies had been selected without bias, are robust (i.e. free of sources of bias and sufficiently powered), and if the data analyses conducted are appropriate, then the claim that "Vaccines Do Not Cause Autism" listed on CDC's website might be warranted.

As a check on the objectivity and unbiasedness of those lists, a Pubmed and internet search was for studies and reviews that mentioned "autism", "vaccines", and "odds ratio". These studies were analyzed separately from the boluses provided by AAP and CDC.

Inclusion Criteria for this Review

All studies cited by AAP, by CDC and by other sources as demonstrating 'vaccine safety' and addressing the question of whether 'vaccines' cause autism were included and score specifically for their contribution to the question of whether vaccine cause autism. As this was a systematic review of the suitability of studies cited by governmental and medical professional organizations in their statement of their public health policies and public health policy recommendations, all studies cited were subjected to evaluation.

Objective Evaluation Scoring System

Applying a systematic objective evaluation system (Table 2), the studies cited by CDC, AAP and others are evaluated. Independent of study author affiliation, the same scrutiny was applied for all criteria. The criteria were selected to reflect deviation from an ideal study (prospective randomized clinical trial of vaccinated (per schedule) vs. unvaccinated study with the outcome variable of the prevalence of ASD. The studies listed fall short of ideal in a common way (none are prospective RCTs). Based on the aims and scope, study design and design of analysis, it was possible to derive 11 evaluation criteria Table 2). Some of the criteria are obviously not independent (underpowered studies, for example, would also have inappropriate design and may have had an inappropriate conclusion), but all criteria were applied independent of each other to each study because flaws in study design such as low power and the inappropriate use of covariates could have nevertheless been appropriately interpreted.

The scoring system was devised to facilitate rapid evaluation. Scoring proceeded as follows as outlined in Table 2. The final Objective Evaluation Score (OES) was the sum of these subscores for each study is reported. If a particular criterion was irrelevant to a given study, the study was given a score of zero for that criterion. For example, Baird et al. (2008) [29] studied the effects of the number of doses of measles vaccines on serum titer levels in a case/control study, and therefore the criterion on measuring prevalence was irrelevant to that study. Studies of thimerosal-containing vaccines compared to non-thimerosal containing vaccines included by those listing studies were given a subscore of -1 because they universally

fail to be represented by those citing them as a comparison of two periods, suffering from numerous sources of temporal confounding. The first of which thimerosal-period vaccines were given vs. thimerosal use in flu vaccine *plus* a larger number of vaccines overall; the second of which was an expansion of the overall schedule with the additional of numerous aluminum-containing vaccines; the third of which is the potential for interaction between TCVs and ACVs in the latter period.

Under this OES system, an ideal study could achieve a score of +12.

Table 2

Criterion	**Subscore**	**Justification/Example**
Examined ASD Prevalence	+1, -1	Primary hypothesis of interest vaccine injury
Comparison of Vaccinated vs Unvaccinated	+2, 0	Best study aim
Comparison of Groups Based on Single Vaccines	-2, 0	Improper science; susceptible to healthy user/accrual bias
Analysis of Trends Only	-1, 0	Weak science
RCT/Retrospective Correlation Study	+2,-2	Weak science, falls short of testing hypothesis of causality
Sufficient/Insufficient Power	+2,-2	Negative results are expected with small sample sizes
Appropriate/Inappropriate Use of Covariates	+1,-1	Adjusting/correcting for "confounders" that may actually represent risk co-factors. Inappropriate interpretation is prevalent.
Comparison of TCV vs. non-TCVs	-1, 0	Limited scope with respect to primary hypothesis of interest
Appropriate/Inappropriate Design/Aims	+2,-2	Study design must match stated aims
Appropriate/Inappropriate Analysis association "go	+1,-1	Data analysis must not be repeated exercises to make initial away", revealing a pre-determined conclusion ("Analysis-to-Result")
Appropriate/Inappropriate Conclusion	+1,-1	Given limited ability of correlation studies to test causality, sample size, study design and analysis, are conclusions warranted?

Study Design Evaluation

To undertake this review, standard considerations of study design were applied: suitability of design of the study to the stated study aims, representativeness of the population, potential sources of bias, and appropriateness of the study design and the design of analysis (data analysis). The study design evaluation included both whether the stated aims, design, analysis and interpretation matched as well as consideration of statistical power (sample size). For each study, a power analysis was conducted to determine whether the study had sufficiently large sample sizes to detect a biologically plausible positive effect size given the prevalence of ASD at the time period the study examined. When possible, any other limitations of each study were noted.

Evaluation for Study Selection Bias

When available from the primary citation, odds ratios (ORs) or relative risk (RR) reported by the Key Studies were recorded and compared to other studies not cited by the authorities citing the literature. These are presented as funnel plots, which allow for objective visual inspection to determine whether a balanced representation of the literature is evident in a systematic review.

Results

All of the Key Studies were observational analytic studies, which fall short of testing causality. None were prospective randomized clinical trials. These overall results were informative by subclasses of studies, as follows:

Studies That Did Not Study Autism or Neurological Outcomes

In spite of the title of the study, Peltola et al. (1998) [30] could not analyze the prevalence of autism diagnosis because they detect zero cases of autism after 3 million instances of vaccination. This alone suggests a serious ascertainment bias. McMahon et al., 2008 [16] used VAERS data, but like other studies that employ the VAERS resource, *did not carry forward the stated limitations on its use for assessing causality,* and did not report any result relevant to the question of an autism/vaccine link. If the source reported the data are not appropriate for causality assessment, then studies conducted using the data should carry that limitation forward.

Various studies (e.g., Peltola et al., 1998; Black et al., 2002) [30,31] focused exclusively on rates of GI issues in children with, and without ASD rather than ASD association nor causality itself. Black (2002) [31] only studied the relative rates of GI issues prior to a diagnosis of autism. D'Souza et al. (2006) [32] and Hornig et al., (2008) [33] used molecular assays to study whether persistent measles virus (MV) could be detected in the GI tracts of children with autism. While they tested only one proposed mechanism of vaccine involvement in ASD (latent enteric measles virus infection), and other routes by which metals in vaccines might induce autoimmune gastroenteritis (e.g., activation of enteric microglia), and GI issues might contribute directly or reflect (common cause) sensitivity to vaccines or their excipients, these were nevertheless included in the evaluation.

Studies That Only Analyzed Trends

By definition, retrospective studies that compare the incidence of groups receiving a treatment or intervention to groups that have not received the treatment or intervention are, in a way, a consideration of trends because a key assumption is that the incidence of specific outcomes in control group rates reflect baseline rates. Unless baseline incidence data are available, the validity of the between- or across-group comparisons rely on no temporal confounding (cohort effect). In the groups of studies under consideration, two (Kaye et al., 2001; Stehr-Green et al., 2003) [34,35]were analyses of trends with no comparison group, and total of ten studies (Stehr-Green et al., 2003;Fombonne et al., 2001;Lingam et al., 2003;Andrews et al., 2004;Chen et al., 2004;McMahon et al., 2008;Tozzi et al., 2009;Price et al., 2010a;Klein et al., 2011;Taylor et al., 2014) [13,35-43] depended primarily on comparisons of trends.

An analysis of the Fombonne et al. study (2001) [36] by the Cochrane Collaboration (Demicheli et al., 2005) [44] yielded a scathing critique:

"The number and possible impact of biases was so high that interpretation of the results was difficult."

These concerns did not prevent the AAP from listing this study as if it were, indeed, considered a useful study. Similarly, a published critique of Andrews et al. (2004) [38] by Hooker et al. (2014) [45]was not

referenced and the published concerns, which included the inclusion of time as covariate that would be confounded with vaccination practices.

Madsen et al. (2003) [46] found that the incidence of autism diagnosis in children between 2 and 10 years old in the Danish registry from 1971-2000 continued to increase after the removal of thimerosal from vaccines. This is usually interpreted as evidence that thimerosal in vaccines was not contributing to autism rates; however, many vaccines added after the removal of thimerosal contained aluminum hydroxide as an adjuvant. An equally plausible explanation is that autism rates would have decreased, but that the pediatric schedule conferred more risk.

Dales et al. (2001) [47] compared the percentage increase in autism diagnosis in children in California between 2 and 10 years old during the period from 1971-2000. Their rates were recorded as age-specific incidence for first day of first recorded admission with a diagnosis of autism. The found that ASD rates increased 377%, but that MMR vaccination only increased 14%. No formal statistical analysis was reported. Given their data, however, a Fisher's exact test reveals a significant association $p=0.0002$, and therefore it is puzzling why Dales et al. (2001) [47] did not report the association. This is an example of how conclusions might not match results in spite of proper execution. Instead, they reported their opinion that the rate of increase in ASD was higher than the rate of increase in MMR vaccination, which requires a linear relationship between vaccination and ASD. In reality, at highest MMR vaccination rates, the latter populations were also receiving a larger number of vaccines. The study's focus on MMR only in a setting in which other vaccination influences could also exist would require a hyperlinear model. Of course, other factors including changes in diagnosis explain *part,* but not all of the increase (Nevison, 2014) [5]. The limitation of the Dales et al. (2001) [47] study exemplifies the limitation of relying on correlation studies to inform on causality, a limitation shared by in fact all of the studies in this review.

Honda et al. (2005) [48] studied the MMR vaccination rates in Yokohama, Japan from 1988-1996. They found that MMR vaccination in the city of Yokohama declined from 1988 through 1992 and the vaccine was abandoned in 1993 and thereafter. They reported that the cumulative incidence of ASD up to age seven increased significantly between 1988-1996, with the highest increase beginning in 1993.

They concluded that autism rates could not be attributed to the MMR vaccine; however, separate measles, mumps and rubella vaccines were used at the time, i.e., vaccination did not stop. Thus, while the study could arguably be interpreted that the MMR itself might be ruled out as a cause of autism, the study did not test the hypothesis that vaccination against measles, mumps and rubella might individually or together contribute. Also, the number of other vaccines available for use in Japan increased over this time period. It is worth noting that when Japan banned vaccination of children under the age of two, deaths from crib death and meningitis ended (Cherry et al., 1988) [49], and the incidence of infantile mortality plummeted to the lowest in the world.

Madsen et al. (2003) [46], while a very large retrospective study, were found to have removed incidence data from 2001 and manipulated inpatient/outpatient data to create an artificial rise in prevalence in 1994 (Hooker et al., 2014) [45]. One of the authors on the study, Poul Thorsen, is on the HHS's most wanted list for embezzling >1.2 million CDC autism research dollars. Further limiting details of the study include that as an ecological correlation study, its design falls short of the level of evidence required to test causality; studied only one vaccine (MMR). Goldman and Yazbak (2004) [9] pointed to ascertainment bias due to the likelihood that a large portion of children born later than 1997 were too young to have received a diagnosis of autism, and some had not even received their dose of MMR when the data were collected (1998). The average age of diagnosis in the Madsen study was 5 years. This means a large portion of children in the MMR/No Autism group had received the MMR but had not yet been diagnosed.

The "age-adjustment" used by Madsen et al. would not have been able to undo to strong effects of this type of confounding. Their analysis should have used "time since vaccination". This is another example of the extreme weakness of basing public health policies that influence millions of people on mere correlation studies.

McMahon et al., 2008 [16] was a retrospective study focused on rates of reported adverse events in the Vaccine Adverse Events Reporting System (VAER) after preservative-free (PFV), preservative-including (PIV), and preservative unknown (PUV) vaccines in reports from 7/1/2004 to 1/4/2006. Thus, the analysis was restricted to TCV vs. non-TCVs. No power analysis was conducted, and the authors noted the likely effects of underreporting. CDC does not carry forward these limitation in their representation of this study. VAERS is severely limited as a resource for tracking adverse events, not only due to underreporting, which is estimated to be limited to between 1-10% of actual recognized adverse events. Underreporting suggests insufficient power, and likely absence of some adverse events altogether from the records. ASD was not specifically studied; in fact, neurodevelopmental disorders were not explicitly studied.

Stehr-Green et al., 2003 [35] was a retrospective ecological study of autism incidence versus Thimerosal exposure in Denmark, Sweden, and California. Using “graphical ecological analyses”, they compared prevalence of ASD and found it continued to increase in all three countries in spite the alleged discontinuation of thimerosal over the time period. Two independent analyses of the Stehr-Green et al. (2003) [35] study found issues with the interpretation of changes in group designation and with study design (changing entrance criteria in ecological studies) and reporting and interpretation (no all results reported in the final publication, and changes in description of source data (Blaxill, 2004[50]; Hooker et al., 2014) [45].

Andrews et al (2004) [38]was a retrospective study of 109,863 children who were born from 1988 to 1997 and were registered in general practices in the United Kingdom. The author sought to measure a dose correlation of the number of doses of DTP/DT received by 3 and 4 months of age, the cumulative age-specific DTP/DT exposure by 6 months, and autism diagnosis. Finding no correlation, they concluded there was “no evidence that thimerosal exposure via DTP/DT vaccines causes neurodevelopmental disorders”. As a study of adverse events related to a single vaccine, the study was susceptible to healthy user bias, which has been acknowledged to confound such studies since 1992 (Fine and Chen, 1992[51]; Jain 2015[52]). Prior analyses had shown increased risk of developmental disorders with DTP/DT vaccine exposure, which has been seen as further evidence of methodological issues with this study (Hooker et al., 2014) [45].

Baird et al. (2008) [29] conducted a retrospective case/control study focused on whether children with ASD had a higher antibody response to measles virus than individuals with special education needs and neurotypical individuals. All individuals in each sample group where ages 10-12 and from the United Kingdom. All individuals were tested for measles virus and antibody response to measles in serum. Failure to find dose–response relationship between autism symptoms and antibody concentrations could easily have been due to low power due to low sample sizes per group (N=98, 52, and 90 respectively). An alternative study design would have been the rates of high measles antibody rates between the two group.

Fombonne et al. (2001) [3] estimated retrospectively the pervasive developmental disorder prevalence in Montreal, Canada, in cohorts born from 1987 to 1998 to evaluate the potential relationship of trends in pervasive developmental disorder rates to three factors: (1) changes in cumulative exposure to

ethylmercury (thimerosal) and (2) trends in measles-mumps-rubella vaccination use rates and (3) the introduction of a 2-measles-mumps-rubella dosing schedule during the study period.

The Fombonne et al. (2001) [36] study could not have compared thimerosal to non-thimerosal vaccine periods because thimerosal was still in use in the Hepatitis B vaccine until 2001. Thimerosal was also in use (and still is in use) in flu vaccines. Thus, the stated aims are inaccurate, and the conclusions thus cannot be supported by the study. The study actually compared the pervasive developmental disorder prevalence in a period in which thimerosal was used in vaccines and a period in which more vaccines containing aluminum were added while thimerosal was still in use. Regarding the MMR vaccination aims, issues exist with the definition of cohorts because some individuals may have received only one vaccine but not the second; such individuals were scored by the study protocol as out of compliance. Credit to these observations should be attributed to Dr. Paul King. The focus on one vaccine subjects the study to the health user bias, given that children who experienced earlier adverse events from any vaccine may have been excluded from the MMR vaccination program due to parental concern. The study appears to have been sufficiently powered, but statistical power does not overcome source of bias.

Jain et al. (2015) [52] retrospectively examined ASD occurrence in groups defined by MMR vaccine status in a large sample of US children who have older siblings with and without ASD. They found that MMR vaccine was not associated with increased risk of ASD, regardless of whether older siblings had ASD. However, because the only studied one vaccine (MMR), their results are almost certainly due to healthy user bias, which the acknowledge in their text, but for which no steps were taken to avoid.

Uchiyama et al., 2007 [53] studied the rates of MMR vaccine uptake in 904 children with regressive autism from Japan and compared it to the MMR vaccine uptake children without ASD. They found similar rates of MMR vaccine uptake between the two groups. This study is also susceptible to health user bias.

Zerbo et al. (2016) [54] was a retrospective cohort study of 196,929 children enrolled in Kaiser Permanente Northern California from January 1, 2000 to December 31, 2010, at a gestational age of at least 24 weeks. A total of 3,103 had a diagnosis of autism spectrum disorder, in which maternal influenza vaccination during pregnancy was concluded to not be associated with increased autism risk. However, the study initial found an association of ASD with vaccination in the first-trimester (hazard ratio (HR) = 1.2; [95% CI, 1.04-1.39], p=0.01). The significance of the association was lost after correction for multiple comparisons. ASD from vaccination is not a whole-population hypothesis; there appears to be a genetically susceptible subgroup, and given that the signal of ASD risk from vaccination could be both heterogeneous and dilute, the use of multiple hypothesis correction is not a strong reason to reject the association.

The study has also numerous flaws, the most of important of which is that their results are dependent on adjusting for multiple collinear covariates, but rather than interpret the results as pointing to factors that could point to groups susceptible to ASD from vaccination during pregnancy. They should have analyzed the significance of the interaction terms involving vaccination and each covariate. Objective criteria for model selection were not used; rather, the authors preferred the default interpretation of no association with vaccines (overall). Correcting for covariates that are actually risk factors of vaccine injury will mislead.

Hooker (2017) [55] pointed out that the correction for multiple hypothesis testing applied by Zerbo et al. (2016) [54] requires an assumption of independence; it is not meant to be applied when testing significance of different forms of the same hypothesis. Other criteria exist for objective model selection.

Kaiser Permanente North was also a participant of the Simpsonwood meetings at which an initial association of childhood vaccine exposure and risk of any neurodevelopmental disorder, a result that has never been published, and that for which great effort was undertaken via many repeated rounds of analysis specifically to make the association "go away" after very strong risk signal of autism due to exposure to thimerosal in the first year of life (SafeMinds, 2018a,b)[56,57].

The author of the email, Dr. Thomas Verstraeten, has denied (Verstraeten, 2014) [58] that he participated in repeated rounds of analysis to achieve a desired, pre-determined result, but also notes that the study results are not definitive:

"Surprisingly, however, the study is being interpreted now as negative [where 'negative' implies no association was shown between Thimerosal and autism] by many...The article does not state that we found evidence against an association, as a negative study would. It does state, on the contrary, that additional study is recommended, which is the conclusion to which a neutral study must come...A neutral study carries a very distinct message: the investigators could neither confirm nor exclude an association, and therefore more study is required."

This denial is tenuous given the actual text of email, which implored, in the name of objective, CDC collaborators to accept a positive result, while stating that Verstraeten did not wish to appear to be fostering anti-vaccinism. Nevertheless, it is surprising, even given Verstraeten's re-interpretation to to find the original study cited by CDC as exonerating thimerosal as "safe": how does an equivocal result become a strongly supported negative result?

Barile et al. (2012) [60] studied the effects of thimerosal exposure in a retrospective study of 1,047 children ages 7–10 years and their biological mothers in which seven neurological "latent constructs" (evaluation tools) were examined. The used structural equational modeling, but did not report any analyzed interactions between covariates and thimerosal exposure. They found an association between thimerosal exposure and tics in boys, but no association between thimerosal exposure and the other six conditions.

The Barile et al (2012) [60] study is a good example of well-intended handling of covariates potentially gone wrong. They wrote:

"…Failure to adjust for these covariates can lead to the misidentification of positive associations between thimerosal exposure and neurodevelopmental outcomes…"

yet the authors do not seem aware of the value of studying and reporting interactions among covariates and the main treatment effect (in this case, thimerosal exposure) as a source of information on at-risk subgroups.

In fact, they seem to misinterpret the functional significance of covariates when the significance of the main treatment variable is lost after adjustment for a covariate. A good example is their interpretation of the results of Smith and Woods (2010) [61]:

"a recent study found that receiving vaccines on-time (compared to delayed or not at all) was not related to any negative neuropsychological outcomes ***after adjusting for thimerosal exposure*** *(Smith & Woods, 2010). This suggests that the timeliness of vaccination does not appear to adversely affect neuropsychological outcomes of children 7–10 years later after controlling for the level of thimerosal exposure"* (emphasis added, citations theirs).

Destefano et al., (2013) [62] concluded no association between exposure to large numbers of antigens in vaccine after employing extensive multivariate adjustment, as follows:

"Covariates for ASD models included birth weight, maternal age, birth order, duration of breastfeeding, family income, maternal healthcare- seeking behavior (ie, Kotelchuck inadequacy of prenatal care, use of cholesterol screening, use of Pap smear screening), maternal exposures during pregnancy with the study child (ie, alcohol use, folic acid use, viral infection, lead exposure), and early childhood health conditions (ie, anemia at age 6-30 months, pica before age 3 years). Covariates for AD models included birth weight, maternal age, birth order, duration of breastfeeding, family income, maternal healthcare-seeking behavior (ie, Kotelchuck inadequacy of prenatal care, use of cholesterol screening, use of Pap smear screening), maternal exposures during pregnancy with the study child (ie, folic acid use), and early childhood health conditions (ie, anemia at age 6-30 months, pica before age 3 years). Covariates for ASD with regression models included birth weight, maternal age, family income, maternal education level, and maternal exposures during pregnancy with the study child (ie, alcohol use)."

Their adjusted odds ratios hover around 1.0, but it is not reassuring to learn that multiple significant ORs become non-significant after so many adjustments, when clearly these covariates could also be interpreted as risk factors for ASD from exposure to large numbers of vaccines. First, health outcomes that could be *due* to exposures to toxins, including those like aluminum in early vaccination, could well be due to sensitivity to vaccination; both anemia and pica are health outcome that could be caused by exposure to metals in vaccines. Other variables likely interact with vaccination; e.g., lead exposure could very well interact with aluminum in vaccines, if aluminum increases the retention of lead, and birth weight can increase dose toxicity. Many of these covariates are also not independent; maternal age and family income may be related to birthweight; use of folic acid is likely also correlated with access to health care. The study is agnostic to the well-known problems of model overfit and the challenge of using highly collinear covariates.

These results are examples of inappropriate corrections or adjustments for covariates coupled with inappropriate interpretation. A bad practice is emerging in epidemiology in which 'back-door' factors are excluded by statistical adjustment – without sufficient a priori information on the actual functional relationships among covariates. This practice is evidenced by a white paper (Glanz et al., 2018) [64] which provides an example of correcting for confounders to avoid "back-door" influences, which are perceived as confounders. This approach is problematic because the directed acyclic graphs (DAGs) represent a particular set of presumed, or preferred function relationships, which are subjective, debatable and may be incomplete, and not every covariate imaginable can qualify as a confounder; examples include health outcomes that may also be caused by exposure to neurotoxins.

The set of functional relationships among covariates now typically corrected for can also be seen in a different light. Some covariates may increase risk of vaccine adverse event, and thus are not confounders, but co-factors, and the interaction term should be studied. In reality, low income, birthweight and other demographic factors may well be co-factors, i.e., co-predictors of poor health outcomes due to vaccination.

The practice of over-correction for functionally related covariates is rampant in epidemiological studies of vaccine safety. Interaction terms should be studied, instead. When the significance of the main effect is lost after correcting for a covariate, two interpretations are possible; the first is that the other variable accounted for the variation in the outcome independent of the main factor being tested. The other interpretation requires specific analyses of the functional relationships between covariates, between each

covariate and the main effect, specifically the interaction term a covariate of interest and the main exposure.

When the main effect significance is lost after adjusting for a covariate, and the interaction between the primary exposure and the covariate is lost, if the interaction term is ignored, there is a risk of false positive (missed) association that can actually inform on variables that could be used to identify individuals who might not tolerate the primary exposure as well as others. Motivation for adjustment for and interpretation of covariates requires a priori functional understanding, otherwise the analysis falls into a curve-fitting exercise known as "analysis-to-result". Examination of and powering for true interaction terms are both necessary.

This first result of better health in the earlier cohort of TCV-receiving children could be taken to suggest that thimerosal exposure modulates effects of vaccines on neuropsychological outcomes. One of the ways it could modulate it is to be a positively contributing factor. But this interpretation would require an ad-hoc biological mechanism of benefit; i.e., no such hypothesis existed prior to the study. A more direct interpretation would be that the latter vaccines incurred higher risk.

A related risk of a false negative is the arbitrary use of a large number of covariates, which can lead to model overfit. The Barile et al. (2012) [60] study adjusted for many covariates, but did not mention or report interaction terms the for main effect (thimerosal exposure) and covariates. No objective measure of collinearity among the covariates was offered, and the bases of the assumption that covariates were, in fact, confounding variables, were not explicit. The covariates were not studied as potential co-predictors and their interactions with vaccines were not studied. The simultaneous consideration of independent and interaction terms is possible using SEM, including methods are evaluating indicators of a latent interaction (Batista-Foguet et al., 2004; Coenders et al., 2008[65,66]). Formal criteria for model selection available and in wide use at the time included the Akaike information criterion (AIC). The fact that the external generalizability of the models are never tested on independent test sets (i.e., the ability to predict adverse health outcomes using combinations of risk factors and vaccination status) means that model overfit has not been evaluated.

Hviid et al., (2003) [45] was a study retrospective of the rate of autism in children receiving TCVs and non-TCVs. While their sample sizes are very large, the flaws and limitations inherent to the "TCV vs. non-TCV" paradigm apply. Hooker et al. (2014) [45] identified two major sources of ascertainment bias in this study, including the use of "person-years of follow up" instead of numbers of patients per group, which given the available data per group, can induce a bias of underestimating the ASD diagnosis rate and biasing the study towards the null.

The remainder of the studies are not summarized, but instead are represented only via their OES score, and by comment in the Discussion, below.

Studies with Low Statistical Power

Low power can result from large confidence intervals due to small sample sizes rather than from large sample variation reflecting population variation. Actual calculations of statistical power for any positive association for a previously analyzed few studies shows the scale of the studies needed for whole-population association (Table 3). These particular studies were part of the IOM 2012 review, which incredibly cited negative results from a study so small that less than on case of autism would be expected in one group. For example, the samples sizes for the Mrozek-Budzyn et al. (2010) [68] study, which sought association between MMR vaccination and autism, had sample sizes N1=96;N2=198, and the

study was stratified by MMR and measles-only vaccines. The clear limits of small sample sizes leading to negative results due to a lack of power is not communicated by AAP nor by CDC.

Statistical power after the fact (post-hoc statistical power) is legitimate in this case because no effect sizes were estimated from the data and are routinely used to aid in the interpretation of negative results from clinical studies (e.g., Ali et al., 2018)[69]. Full descriptions of analysis of the power of the five studies cited by IOM in 2012 are provided as supplementary material, and they provide a reference point for power required by other studies.

Table 3. Power of Studies Cited by IOM (2012) Supporting No Causal Link Between Vaccines and Autism

		Sample Size				Power > *x*?		
Author	Year	Study Type	Group 1	Group 2	Total N	*x*=0.8	*x*=0.7	x=0.6
Madsen	2003	Tx/Control Cohort	440655	96648	537303	N	Y	Y
Mrozek-Budzyn	2010	Case/Control	96	192	288	N	N	N
Smeeth et al.	2004	Case/Control	1294	4467	5761	N	N	N
Taylor et al.	1999	Case series	233	64	297	Y	Y	Y
Farrington et al.	2001	Case series	233	64	297	Y	Y	Y

NB: Farrington et al. (2001) was a re-analysis of data from Taylor et al. (1999); neither study used an independent control group and are therefore susceptible to temporal intrusion biases.

Using these power calculations as a point of reference, out of the 48 Key Studies, only about half are estimated to have sufficient power to have detected a population-wide association of >1.1 if one, in fact, did exist. Like Mrozek-Budzyn et al. (2010[68], six studies are so small that less than one human subject would be expected to be found to have ASD diagnosis in either one or both study groups assuming prevalence on the order of 1-2% (i.e., Hornig et al. 2008; Fombonne et al. 2001; Black, 2002; D'Souza et al. 2006; Pichichero et al. 2008; Klein et al. 2011)[31,32,33,34,42]. How such studies passed peer review is a complete mystery; all future vaccine safety studies must include a priori power analyses for the least frequent serious adverse event suspected.

Studies that Misinterpret Negative Odds Ratios

In studies that purport to examine the relative risk of ASD from a single vaccine, healthy user bias is very likely to cause any effect to disappear, or for the unvaccinated to have a higher incidence of autism than the vaccinated. Healthy user bias occurs when parents pull their children from the vaccination program due to an earlier negative experience with vaccines. A study focused on a single vaccine, such as MMR, would likely fail to detect an effect – or could find a lower risk of ASD in the vaccinated – if children who would have regressed were pulled from the vaccinated group by alert parents. This is considered a fatal flaw stemming from the use of retrospective epidemiological studies instead of using randomized clinical trials. All of the studies of the effects of single vaccines are thus compromised.

Another potential interpretation of the odds ratios <1 would be that children who received >1 vaccine to achieve the same antigen exposure as the MMR may be experiencing enhanced immune system activation (over-activation). Glickman et al. (2017) [71] provides a confirmed example in which healthy user or healthy family bias affected measurement of vaccine injury in a study.

Combined with Healthy User Bias, the consistent value averaging around OR = 0.8 for most studies may in fact be carrying a vaccine safety signal. Because the MMR was suspected, the overriding focus of the studies appears to have been to attempt to exonerate MMR; the study of the safety of a single multiplex

vaccine in the context of other vaccine options, and vaccine refusal, with not completely unvaccinated control group represents an intractable question.

In the case of TCV vs. non-TCV's, such results have, oddly, been used to propose ad-hoc hypotheses of a "protective" effect of thimerosal. In reality, non-TCV's are often aluminum-containing vaccines, which may point therefore to risks associated with individual exposures to aluminum and to thimerosal due to the expansion of the schedule with ACVs. We should recall that thimerosal was not, and has not, been completely removed pediatric vaccines; it is included in some influenza vaccines. Thus, the unanticipated "health benefits" of TCVs may plausibly have been due to an enhanced toxicity of thimerosal and aluminum hydroxide in children that receive different vaccines with both types of metals in the same office visit. Dosing of aluminum in the pediatric schedule needs to be revisited (Masson et al., 2018[72]; Morris et al, 2017[73]; Lyons-Weiler and Ricketson, 2018[21]).

Studies that Lack Proper Control Groups

Among the study design considerations, the use of appropriate and independent controls is paramount. Chen et al. 2004 [39] used patients with Down's syndrome as a control group, clearly opening the study to any confounding associated with diagnosis of autism. Self-controlled case series studies Taylor et al. (1999) [75] and Farrington et al (2001) [76] may have sufficient power given the use of patients as their own controls, but they are weak in that they only analyzed trends. They are not immune from cohort effects because each patient acts as their own control there is no control over the intrusion of temporal confounding factors. The value of self-controlled case series can be over-stated; the concern over lack of control of incidental temporal confounding is real. DeSoto and Hitlan (2013) [77] outlined the problem of over-matching for the Price et al. (2010) [41] study, pointing to low variation in thimerosal exposure among exposure groups. These concerns are not relayed by the CDC.

In addition to the appropriate interpretation of increase risk of non-TCVs given in combination win thimerosal-containing influenza vaccines, other interpretations of the published results of some of the key studies are also possible:

Ethical and Plausible Alternative Interpretation of Negative Results

The studies included in this systematic review are interpreted by policy makers and vaccine stakeholders who would like their interpretation to influence public health policy. Many of these studies include results that can be interpreted as problematic for vaccine safety. For example, every study that had small sample sizes and large confidence intervals could (and perhaps should) be interpreted as insufficient for a firm negative conclusion (no association) due to low power (e.g, Pichichero et al. 2008[81]. This is problematic because a lack of evidence is not the same as negative evidence when the study has insufficient power to robustly test the hypothesis at hand.

Table 4. Smeeth et al. data (2004) [78] as a contingency table

Smeeth et al.	**ASD**	**No ASD**
MMR	1010	3671
no MMR	285	798

Positive Results Under Appropriate Analysis

Most of the epidemiological studies used odds ratios. Alternative tests of a 2 x 2 contingency, well-applied for the test of independence of vaccination status and ASD status include the Chi-square test, and

Fisher's exact test. Re-analysis of the data from Smeeth et al. (2004) [78], which reported no association, nevertheless results in a significant association (Table 4; Chi-square 11.3402, p = 0.00076). Their analysis included an adjustment for the age at which the patients joined the research database. Their statistical correction was, according to the authors, due to an ascertainment bias on the vaccination status, drawing the entire study into question. The average age of cases under the age of 7 was 4.5 for both cases and controls, and the MMR vaccination rate bias could either have been over- or under-estimated. Without independent means other than the vaccination rates in the controls, the logic for the adjustment was circular and would represent a form of within-study bias resulting from decisions made during the design and execution of the analysis of the data.

Test of Study Selection Bias

In an unbiased bolus of studies, say, used in a meta-analysis, studies would be selected regardless of their findings. To assess study selection bias in the 48 key studies, a Pubmed and an internet search was done for studies that mention "Autism" and "Vaccine" with "Odds ratio" to collect OR values for comparison to those studies selected by AAP and CDC.

Odds ratios and risk ratios are comparable for rare diseases (Rodrigues and Smith, 1999)[79], and thus thy are averaged for studies included and excluded from the Key Studies. Standard deviations were estimated from reported confidence intervals using Chebychev's assumption (which is approximated by SD=CI/3). Standard errors were estimated as (Upper 95% CI-Lower 95% CI-0.05)/1.96. Funnel plots were made to compare these values for included and excluded studies.

Due to concern over bias from fraud in the Destefano et al. (2004) [85] study communicated into the Congressional Record by Congressman William Posey (Posey, 2015)[62], and statements communicated by CDC Senior Research Scientist Dr. William Thompson, the odd ratios for Destefano et al. (2004)[85] were examined separately. A statement of concern was sent to the journal over evidence of changes in the data analysis plan by Destefano et al. (2004)[85] after finding initial positive association results; the statement was ignored without reply.

The Objective Evaluation Score

The calculated OES for each study is provided in the Supplementary Material (S2) and are summarized as Figure 1. Only one study (D'Souza et al., 2006[32]) had a positive score; all others were zero or negative. The average OES score for the studies cited by AAP and CDC was -6.61.

Discussion

The first set of assumptions of statistical hypothesis testing include that the variables are a random variable from a representative set of samples. Many of the studies reviewed define the study groups retrospectively, which has been shown vastly misestimate the timing on the onset of autism (Ozonoff et al., 2018[80]). As these groups are not truly random samples, and thus, the entire practice of the use of epidemiological studies is fraught with risk.

Statistical control of covariates is important when the functional (causal) relationship between covariate pre-empts a main effect. Statistical control has been routinely misapplied, and the results misinterpreted as absolving vaccines overall instead of highlighting potentially increased risk in specific subgroups (low income, young mothers, low birth weight). Many of these variables are highly collinear, and therefore model overfit is possible; each covariate may in fact point to an increased risk of ASD from vaccination, especially if they are biological or medical covariates. Rather than attribute the entire risk of ASD to such covariates, which are often highly collinear (e.g., birthweight, age of gestation, mother's age, mother's

income, race), studies should be designed to test the interaction between covariates and vaccination. The loss of association after "correcting for" such variables requires careful consideration, especially when the study is not sufficiently powered to study interactions among variables as co-predictors with vaccination as risk. One study actually corrected for overall vaccine uptake a covariate, clearly, a contradictory strategy to the agenda to disprove that vaccines cause autism. The practice of the interpretation of the loss of significance of the main effect (vaccines) on adverse events without studying the interaction terms should end.

Prevalence is the rate of a disease or condition in a general population; incidence is the rate of a disease or condition in a population at increased risk. No studies have focused on the incidence of autism in vaccinated and unvaccinated individuals with increased genetic risk of autism. A study of the rates of autism in a prospective trial to provide direct control for covariates that thus far have been misapplied.

Infants whose mothers were not vaccinated during pregnancy who have LOF or COF genetic variation at any of the >850 genes should be randomized into two groups – one which receives vaccines, and the other which receives saline placebos. While some may object to this study as unethical because the unvaccinated would be exposed to risk of childhood diseases, the study would provide true risk rates. Two simultaneously conducted additional arms of individuals with no increased genetic risk of ASD who are vaccinated, and those who are not, would provide additional insight into the role of genetic risk.

Pichichero et al. (2008) [81] was included the AAP's letter to President Trump as evidence of vaccine safety. The study measured blood mercury levels in 216 healthy children prior, 12 hours and 30 days after vaccination with thimerosal-containing vaccines. The study only measured blood levels, which is problematic given the tissue deposition in the brain and other organs was not measured. The study reported that blood mercury half-life 3.7 days, and that it returned to pre-vaccination levels by day 30. The blood-clearance rate is not assuring given that mercury deposited in the brain would not be measured. The observation that blood clearance of thimerosal was faster than for methylmercury –could point to a problem with ethyl mercury due to faster uptake by tissues and organs. An earlier study similarly seems obliquely unaware of the relevant measurements required to understand neurotoxicity levels of injected mercury (Pichichero et al., 2002)[81]. No specific relationship to autism risk was examined in either study. Burbacher et al (2005)[82], a study cited by CDC, found that organic mercury from thimerosal injected into monkeys stayed in the brain longer than that from methyl mercury via oral gavage.

Examples of other studies not included are Gallagher and Goodman (2010) [4], and Nevison (2014) [5]. A growing number of studies that support the hypothesis that Acetaminophen given after vaccination may increase the risk of autism (Avella-Garcia et al., 2016[2]; Bauer and Kriebel, 2013[3]; Saeedan et al., 2018[87];Schultz et al., 2008[6]) were cited neither by AAP nor by CDC. In 2010, Shoffner et al., (2010) [11] found that 71% of kids with regressive autism had an episode of fever > 101°F In 33% of these cases, the fever occurred right after vaccination – and none showed regression unless fever had occurred. Neither CDC nor AAP cite Shoffner et al. (2010) [11].

The cherry-picking, biased use of the literature stands to significantly erode the public trust in the AAP and the CDC. The bias in the ORs of the studies they cite compared to the studies they fail to cite is cause for grave concern.

Interaction Terms Missing from Study Designs

Nearly all studies had flawed designs in that interaction terms for vaccination and other variables were not tested. For example, Price et al. (2010) [41]studied the relationship (using conditional logistic regression) between thimerosal exposure from vaccines in medical records in 256 children with ASD and 752

controls. Patient accrual was conducted by physician consent. Numerous covariates were used; in the covariate-based analyses, the adjusted odds ratio decreased, again potentially pointing to the interaction between thimerosal exposure and covariates – a result not knowable given the limited sample size of the study. The study was powered for odds ratio of the main effect, but not specifically for the interaction terms, which typically requires much larger sample sizes. Interactions among the variables were not explored.

Taylor et al. (2014): Meta-analysis Cites Flawed Studies

Previous serious criticisms of the Taylor et al. (2014) [43] study has fallen on robustly deaf ears. Suissa (cited in Stott et al, 2004) [84] pointed out that when the Madsen et al. data are analyzed using time after vaccination instead of age, the association between MMR vaccination and autism is detected. Stott et al., (2004) [84] demonstrated a logic flaw in Taylor et al.'s conclusion that there was no increase in autism after 1988 because a large number of patients born before 1987 received the MMR as a part of a "catch up program". This makes the dependence of the independent variable (autism rate) difficult to assess with certainty due to unaccounted variance in the independent variable. Stott et al. (2004) [84] pointed out that an analysis of these key data that uses the timing of vaccination, rather than the year of birth as the independent variable, the increase in autism rates become clearly timed with the timing of MMR vaccination.

Incomplete diagnosis in the five-to-seven year age group, and delayed diagnosis in many of the patients in these studies, also draw the Taylor et al's (2014) [43] conclusions into further doubt. Even the largest studies like Madsen et al. (2003) [46] did not – and could not - correct for the fact that many parents of children likely to regress into autism after the MMR may have decided to forego the MMR vaccination, either due to family history, or due to a previous bad reaction on the part of the specific child (healthy user bias). These flaws make confident interpretation of the results of the Madsen study, and any mere retrospective correlation study, impossible.

The Taylor et al. meta-analysis [43] also used studies that were also found to be flawed by the IOM, including the much-scrutinized Destefano et al. (2004) [85] study. That study also should have included two set of positive association for on-time MMR for African American males and for so-called “isolated autism”, both results which were left out because, according to Destefano, the team did not believe them (Frank Destefano to journalist Sharyl Attkisson, *pers. comm.*). The uncritical acceptance of the results of studies, including many that were underpowered, and the obvious fact that Taylor et al. (2014) [43] also ignored a significant body of scientific literature available at the time of their meta-analysis is a serious flaw in their meta-analysis.

Misrepresentation of The Full Available Science by AAP and CDC

To their credit, the authors of many of these studies are forthright about the limitations of their studies. However, neither the AAP nor the CDC are forthright about the acknowledged limitations. They also ignore previously published criticisms of some of the studies they listed. Neither the AAP nor the CDC carry the stated limitations made by authors of the studies over with their listings. The Verstraeten (2014) [58] example is just one of many examples of over-interpretation; certainly, public health policies that influence millions of patients should not be based on negative results from underpowered studies.

More disturbing is the biased selection of studies in the lists provided as representative and definitive on the questions at hand. The average ORs reported in studies cited by APP, CDC and the other sources (overall) is 0.801 (0.756 for ASD-focused studies); by comparison, the average ORs reported in studies not cited is 4.01. To give an idea of the variability, estimated (pseudo) SDs and SEs are provided in

Table 5. It is worth pointing out that many of the reported 95% confidence intervals in studies reporting no association not only overlap with 1.0; many are overlap with 2.0, meaning there is insufficient evidence in those studies on whether the OR is significantly different from both zero risk and double the risk.

Table 5. Study Wide Averages Show Publication Bias

	Overall	
	Cited	*Not Cited*
OR	0.801	4.01
pSD	0.307	3.53
pSE	0.445	5.38

OR = odds ratio; pSD = pseudo standard deviation; pSE = pseudo standard error

The AAP and CDC also have also ignored and continue to fail to transmit published concerns over the integrity of many of the studies they listed (Hooker et al., 2014[45], Kern et al., 2017[12]) and as the current analysis demonstrates, have been selective in the studies AAP cited to POTUS and that both CDC and AAP include in their study lists. Further, the AAP document cites the National Vaccine Injury Compensation Program's Autism Omnibus proceedings, but fails to report the Hanah Poling case, as case that was originally in the Autism Omibus proceeding but removed after a settlement was provided. More importantly, the AAP document fails to report the PACE Law Review by Holland et al. (2011) [86] who found 81 instances in which vaccines were found to found encephalopathy, leading to autism (vaccine-induced encephalopathy-mediated autism) were given awards in the National Vaccine Injury Compensation Program. The omission of this extensive review of relevant case law is further evidence of the biased, one-sided view promulgated by the AAP document provided to the President of the United States.

Our unified and collective goal must be to understand factors that contribute to the increasing rates of ASD and other chronic illnesses of "unknown origin", regardless of where those studies lead us. The results of retrospective ecological correlational epidemiological studies (RECE's) are too malleable to subjective influences. Obviously, the use of retrospective ecological studies on questions of such massive importance to public health is insufficient to test hypotheses of causality. None of the studies actually attempt to determine whether they could have predicted, using genetics, or blood biomarkers, or demographic and medical variables, which children would develop ASD. Our unified goal must be to fulfil the 1986 Congressional mandate to make vaccines safer, and to identify the groups who may be at highest risk of adverse event. In this setting, the only ethical position any medical practitioner or legislator can choose is to respectfully preserve the freedom of vaccine choice by patients and by parents.

Given the extensive and serious limitations in the studies presented as best evidence of no causality, it is time to revisit the question of causality with renewed studies comparing total health outcomes in vaccine-naïve vs vaccine exposed individuals, including neurodevelopmental disorders.

Public health policies based primarily on negative evidence from correlation study predominant paradigms risk harming hundreds of thousands to millions of individuals. To assure public safety and our national well-being, regulatory agencies must be made independent of corporate financial interests.

Recent Studies

No blinded randomized clinical trials have been conducted since the AAP statement was sent to POTUS. A recently published result by Geier et al. (2018) [8] reported increased significant association of autism (odds ratio (OR) = 2.75, $p < 0.02$), developmental delay (OR = 5.39, $p < 0.01$), psychomotor disorder (OR = 2.38, $p < 0.03$), and neurodevelopmental disorder in general (OR = 2.70, $p < 0.001$) associated with the receipt of Thimerosal-containing Hib vaccine than Thimerosal-free Hib vaccine. These are retrospective studies.

Unless and until we see large independently conducted, prospective randomized trials comparing vaccinated vs. unvaccinated, a non-definitive, schizophrenic, broken scientific literature filled with correlations with little information on causality will continue to be developed. Vaccine stakeholders' position rest on a biased selection of unpowered and flawed studies. Sufficiently powered genetic studies that seek associations should include vaccine exposure and study genetic x environmental toxin exposure interaction terms. Strong evidence points to a genetic basis for sensitivity to neurotoxins and immunotoxins in vaccines (as reviewed in Lyons-Weiler, 2018 [23]). Therefore, further exclusively genetic research on autism – and on autoimmune conditions which excludes vaccines as a source of environmental toxins would be an unethical waste of national research resources.

Additional studies scored using OES but not further critiqued are listed in the references (87-93)

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Conflict of Interest Statement: The author has served in the capacity of expert witness in the National Vaccine Compensation Program on various cases, none of which involve autism.

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Grant Final Report

Grant ID: R18 HS 017045

Electronic Support for Public Health–Vaccine Adverse Event Reporting System (ESP:VAERS)

Inclusive dates: 12/01/07 - 09/30/10

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Abstract

Purpose: To develop and disseminate HIT evidence and evidence-based tools to improve healthcare decision making through the use of integrated data and knowledge management.

Scope: To create a generalizable system to facilitate detection and clinician reporting of vaccine adverse events, in order to improve the safety of national vaccination programs.

Methods: Electronic medical records available from all ambulatory care encounters in a large multi-specialty practice were used. Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions were evaluated for values suggestive of an adverse event.

Results: Restructuring at CDC and consequent delays in terms of decision making have made it challenging despite best efforts to move forward with discussions regarding the evaluation of ESP:VAERS performance in a randomized trial and comparison of ESP:VAERS performance to existing VAERS and Vaccine Safety Datalink data. However, Preliminary data were collected and analyzed and this initiative has been presented at a number of national symposia.

Key Words: electronic health records, vaccinations, adverse event reporting

Final Report

Purpose

This research project was funded to improve the quality of vaccination programs by improving the quality of physician adverse vaccine event detection and reporting to the national Vaccine Adverse Event Reporting System (VAERS), via the following aims:

Aim 1. Identify required data elements, and develop systems to monitor ambulatory care electronic medical records for adverse events following vaccine administration.

Aim 2. Prepare, and securely submit clinician approved, electronic reports to the national Vaccine Adverse Event Reporting System (VAERS).

Aim 3. Comprehensively evaluate ESP:VAERS performance in a randomized trial, and in comparison to existing VAERS and Vaccine Safety Datalink data.

Aim 4. Distribute documentation and application software developed and refined in Aims 1 and 2 that are portable to other ambulatory care settings and to other EMR systems.

Scope

Public and professional confidence in vaccination depends on reliable postmarketing surveillance systems to ensure that rare and unexpected adverse effects are rapidly identified. The goal of this project is to improve the quality of vaccination programs by improving the quality of physician adverse vaccine event detection and reporting to the national Vaccine Adverse Event Reporting System (VAERS). This project is serving as an extension of the Electronic Support for Public Health (ESP) project, an automated system using electronic health record (EHR) data to detect and securely report cases of certain diseases to a local public health authority. ESP provides a ready-made platform for automatically converting clinical, laboratory, prescription, and demographic data from almost any EHR system into database tables on a completely independent server, physically located and secured by the same logical and physical security as the EHR data itself. The ESP:VAERS project developed criteria and algorithms to identify important adverse events related to vaccinations in ambulatory care EHR data, and made attempts at formatting and securely sending electronic VAERS reports directly to the Centers for Disease Control and Prevention (CDC).

Patient data were available from Epic System's Certification Commission for Health Information Technology-certified EpicCare system at all ambulatory care encounters within Atrius Health, a large multispecialty group practice with over 35 facilities. Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions are evaluated for values

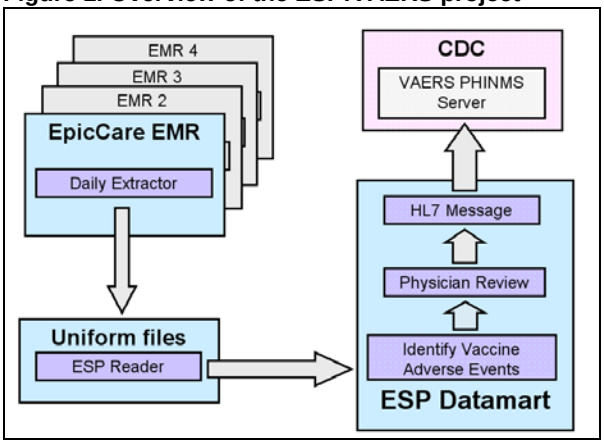
suggestive of an adverse vaccine event. When a possible adverse event was detected, it was recorded, and the appropriate clinician was to be notified electronically.

Clinicians in-basket messaging was designed to provide a preview a pre-populated report with information from the EHR about the patient, including vaccine type, lot number, and possible adverse effect, to inform their clinical judgment regarding whether they wish to send a report to VAERS. Clinicians would then have the option of adding free-text comments to pre-populated VAERS reports or to document their decision not to send a report. The CDC's Public Health Information Network Messaging System (PHIN-MS) software was installed within the facilities so that the approved reports could be securely transferred to VAERS as electronic messages in an interoperable health data exchange format using Health Level 7 (HL7).

Methods

The goal of Aim 1: *Identify required data elements, and develop systems to monitor ambulatory care electronic medical records for adverse events following vaccine administration,* and Aim 2: *Prepare, and securely submit clinician approved, electronic reports to the national Vaccine Adverse Event Reporting System (VAERS),* was to construct the below flow of data in order to support the first two Aims:

Figure 1. Overview of the ESP:VAERS project



Existing and functioning ESP components are shown on the left, and Aims 1 and 2 on the right. ESP:VAERS flags every vaccinated patient, and prospectively accumulate that patient's diagnostic codes, laboratory tests, allergy lists, vital signs, and medication prescriptions. A main component of Aim 1 was to *Develop AE criteria to assess these parameters for new or abnormal values that might be suggestive of an adverse effect.* A reporting protocol & corresponding algorithms were developed to detect potential adverse event cases using diagnostic codes, and methods were tested to identify prescriptions or abnormal laboratory values that might be suggestive of an adverse effect. These algorithms were designed to seek both expected and unexpected adverse effects.

This reporting protocol was approved by both internal & external partners. We initially prepared a draft document describing the elements, algorithms, interval of interest after vaccination, and actions for broad classes of post-vaccination events, including those to be reported immediately without delay (such as acute anaphylactic reaction following vaccination), those never to be reported (such as routine check-ups following vaccination) and those to be reported at the discretion and with additional information from the attending physician through a feedback mechanism. The draft was then widely circulated as an initial / working draft for comment by relevant staff in the CDC and among our clinical colleagues at Atrius. In addition to review by the internal CDC Brighton Collaboration liaison, this protocol has also received review & comment via the CDC's Clinical Immunization Safety Assessment (CISA) Network.

The goal of Aim 2 was the *Development of HL7 messages code for ESP:VAERS to ensure secure transmission to CDC via PHIN-MS*. The HL7 specification describing the elements for an electronic message to be submitted to Constella, the consultants engaged by CDC for this project was implemented. Synthetic and real test data was been generated and transmitted between Harvard and Constella. However, real data transmissions of non-physician approved reports to the CDC was unable to commence, as by the end of this project, the CDC had yet to respond to multiple requests to partner for this activity.

The goal of Aim 3 was to *Comprehensively evaluate ESP:VAERS performance in a randomized trial, and in comparison to existing VAERS and Vaccine Safety Datalink data.*

We had initially planned to evaluate the system by comparing adverse event findings to those in the Vaccine Safety Datalink project—a collaborative effort between CDC's Immunization Safety Office and eight large managed care organizations. Through a randomized trial, we would also test the hypothesis that the combination of secure, computer-assisted, clinician-approved, adverse event detection, and automated electronic reporting will substantially increase the number, completeness, validity, and timeliness of physician-approved case reports to VAERS compared to the existing spontaneous reporting system; however, due to restructuring at CDC and consequent delays in terms of decision making, it became impossible to move forward with discussions regarding the evaluation of ESP:VAERS performance in a randomized trial, and compare ESP:VAERS performance to existing VAERS and Vaccine Safety Datalink data. Therefore, the components under this particular Aim were not achieved.

Aim 4 *Distribution of documentation and application software developed and refined in Aims 1 and 2 that are portable to other ambulatory care settings and to other EMR systems* has been successfully completed. Functioning source code is available to share under an approved open source license. ESP:VAERS source code is available as part of the ESP source code distribution. It is licensed under the LGPL, an open source license compatible with commercial use. We have added the ESP:VAERS code, HL7 and other specifications and documentation to the existing ESP web documentation and distribution resource center http://esphealth.org, specifically, the Subversion repository available at:
http://esphealth.org/trac/ESP/wiki/ESPVAERS.

Results

Preliminary data were collected from June 2006 through October 2009 on 715,000 patients, and 1.4 million doses (of 45 different vaccines) were given to 376,452 individuals. Of these doses, 35,570 possible reactions (2.6 percent of vaccinations) were identified. This is an average of 890 possible events, an average of 1.3 events per clinician, per month. These data were presented at the 2009 AMIA conference.

In addition, ESP:VAERS investigators participated on a panel to explore the perspective of clinicians, electronic health record (EHR) vendors, the pharmaceutical industry, and the FDA towards systems that use proactive, automated adverse event reporting.

Adverse events from drugs and vaccines are common, but underreported. Although 25% of ambulatory patients experience an adverse drug event, less than 0.3% of all adverse drug events and 1-13% of serious events are reported to the Food and Drug Administration (FDA). Likewise, fewer than 1% of vaccine adverse events are reported. Low reporting rates preclude or slow the identification of "problem" drugs and vaccines that endanger public health. New surveillance methods for drug and vaccine adverse effects are needed. Barriers to reporting include a lack of clinician awareness, uncertainty about when and what to report, as well as the burdens of reporting: reporting is not part of clinicians' usual workflow, takes time, and is duplicative. Proactive, spontaneous, automated adverse event reporting imbedded within EHRs and other information systems has the potential to speed the identification of problems with new drugs and more careful quantification of the risks of older drugs.

Unfortunately, there was never an opportunity to perform system performance assessments because the necessary CDC contacts were no longer available and the CDC consultants responsible for receiving data were no longer responsive to our multiple requests to proceed with testing and evaluation.

Inclusion of AHRQ Priority Populations

The focus of our project was the Atrius Health (formerly HealthOne) provider & patient community. This community serves several AHRQ inclusion populations, specifically low-income and minority populations in primarily urban settings.

Atruis currently employs approximately 700 physicians to serve 500,000 patients at more than 18 office sites spread throughout the greater Metropolitan Boston area. The majority of Atruis physicians are primary care internal medicine physicians or pediatricians but the network also includes physicians from every major specialty.

The entire adult and pediatric population served by Atruis was included in our adverse event surveillance system (ESP:VAERS). Atruis serves a full spectrum of patients that reflects the broad diversity of Eastern Massachusetts. A recent analysis suggests that the population served by Atruis is 56% female, 16.6% African American, 4% Hispanic. The prevalence of type 2 diabetes in the adult population is 5.7%. About a quarter of the Atruis population is under age 18.

List of Publications and Products

ESP:VAERS [source code available as part of the ESP source code distribution]. Licensed under the GNU Lesser General Public License (LGPL), an open source license compatible with commercial use. Freely available under an approved open source license at: http://esphealth.org.

Lazarus, R, Klompas M, Hou X, Campion FX, Dunn J, Platt R. Automated Electronic Detection & Reporting of Adverse Events Following Vaccination: ESP:VAERS. The CDC Vaccine Safety Datalink (VSD) Annual Meeting. Atlanta, GA; April, 2008.

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Research Paper

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

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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.

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.

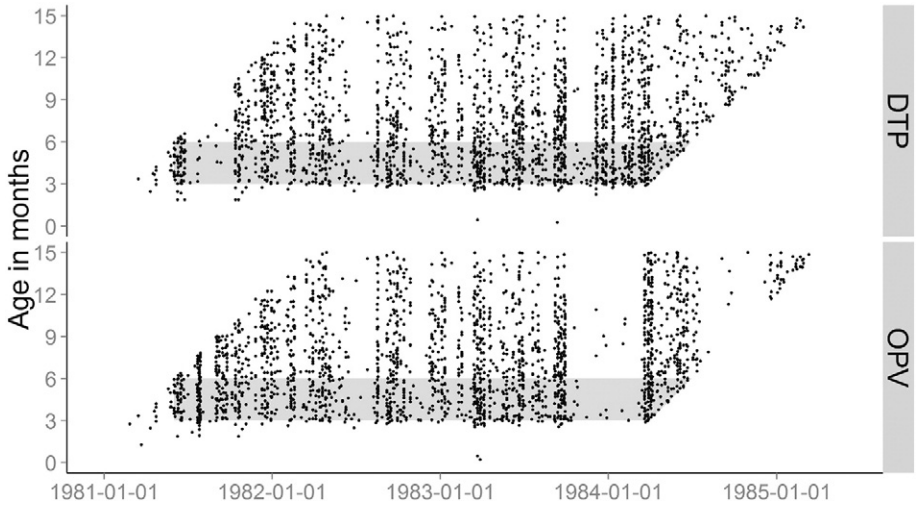

Fig. 1. Each vaccination of the specified type is plotted according age of the recipient and date of vaccination.

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).

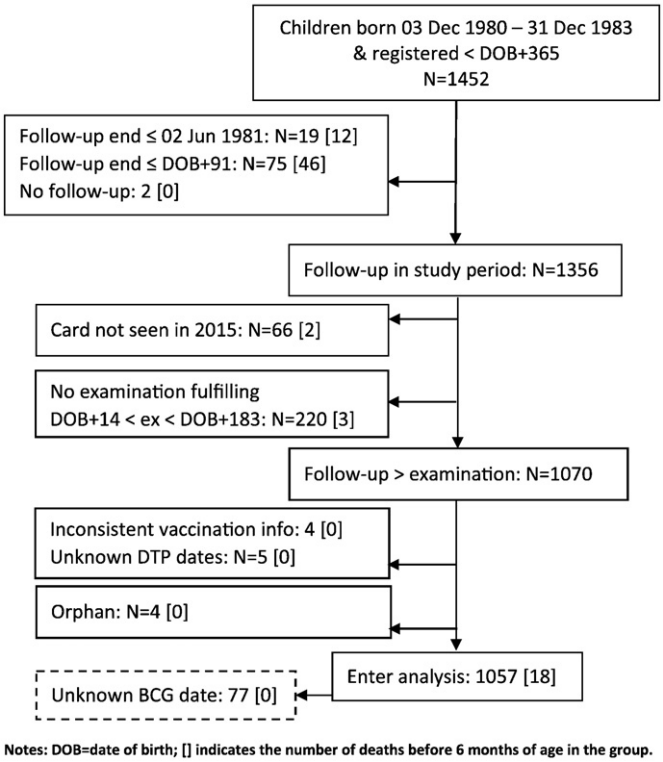

Fig. 2. Flowchart of study population and children included in the analyses. Notes: DOB = date of birth; [] indicates the number of deaths before 6 months of age in the group.

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

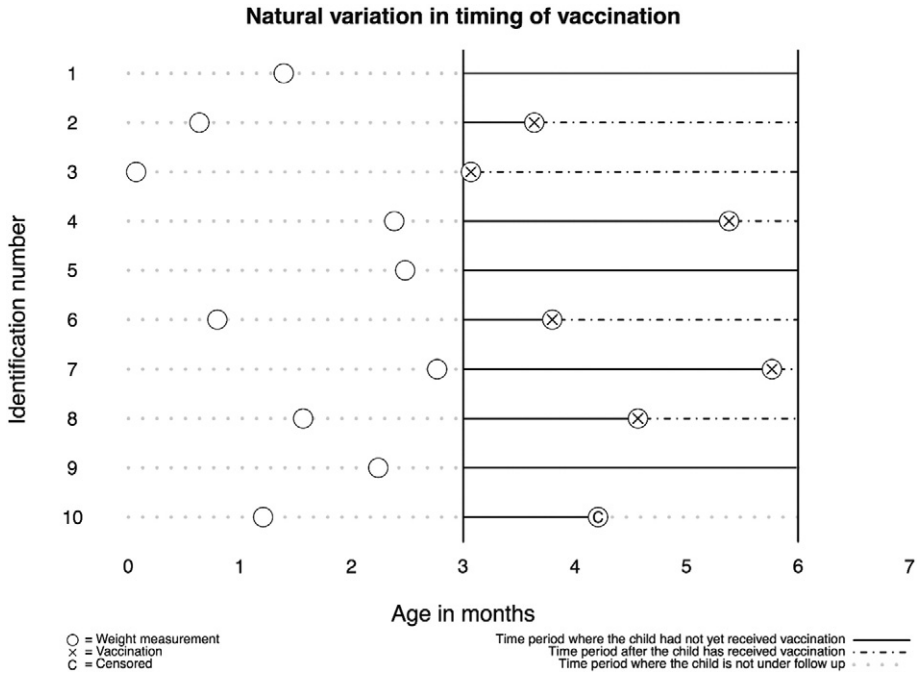

Fig. 3. Natural experiment study design. Note: Children were weighed every third month. After 3 months of age they received DTP and OPV on weighing days if they were healthy. Children who attended but were not vaccinated at a weighing session after 3 months of age were censored in the survival analysis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

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

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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