Wakefield GMC  Mercury  DPT  Hepatitis B

# Blockbuster Primate Study Shows Significant Harm from One Birth Dose of a Mercury-Containing Vaccine

[Editor's update: The article is now available for purchase at Science Direct. See link below.]

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A research team led by scientists from the University of Pittsburgh and Thoughtful House reported today that exposure to a birth dose of a hepatitis B vaccine that included an ethyl mercury preservative caused significant delays in the development of several survival reflexes in male rhesus macaque monkeys. The findings were published on line today in the journal *Neurotoxicology*. [See the abstract below and the link to a site where you can purchase the article on Science Direct HERE]

In the first safety study of its kind of the hepatitis vaccine birth dose, the researchers showed that male macaques vaccinated at birth with a hepatitis B vaccine (HBV) took more than twice as long as unexposed macaques to acquire three standardized skills typically used to measure infant brain development. The thirteen vaccinated monkeys each received a dose of Merck's *Recombivax*® hepatitis B vaccine to which a weight-adjusted amount of the ethyl mercury-containing vaccine preservative *thimerosal* had been added (each dose included 2 micrograms of ethyl mercury as opposed to the human infant dose of 12.5 micrograms). Seven unexposed monkeys received either a saline placebo injection or no shot at all.

Over a two week period following birth, the researchers examined the infant macaques daily for their ability to perform nine basic reflexes (four reflexes were tested in two ways, so the paper reports thirteen performance results). Three of nine reflexes showed significant delays in vaccinated macaques while two other reflexes were delayed and "approached significance." As for the three significant reflexes, vaccinated macaques learned more slowly to: 1) turn their head in response to a brush on the cheek (the *root* reflex); 2) open their mouth in response to a brush on the forehead (the *snout* reflex); and 3) suck on a nipple placed in their mouth (the *suck* reflex).

Although the paper is carefully worded and the results reported modestly, these findings are certain to receive intense scrutiny. For while hepatitis B vaccines currently produced in the United States no longer contain thimerosal, the vast majority of American infants born during the 1990s received a vaccine formulation similar to the one the thirteen vaccinated macaques received. In addition, thimerosal-containing HBVs are still routinely administered to newborn infants in developing countries such as Brazil. Consequently, the finding that early exposure to potentially toxic vaccine formulations can cause significant neuro-developmental delays in primates has explosive implications for vaccine safety management. These implications go far beyond the domestic HBV program and raise concerns about HBV formulations sold abroad as well as the domestic influenza vaccine program. Most influenza vaccines, including the vaccines in the upcoming swine flu program, contain thimerosal and are routinely administered to pregnant women and infants.

According to Dr. Andrew Wakefield, Executive Director of Thoughtful House and a co-investigator of the project, "What is particularly concerning is that in spite of the recommendation to remove thimerosal from vaccines a decade ago, millions of people, many of them children and pregnant mothers, are about to get mercury in their shots. Thimerosal is still routinely used in Hepatitis B and numerous other vaccines world-wide. "

The authors are careful to point out several limitations of their analysis. According to the paper, "our study design does not enable us to determine whether it is the vaccine per se, the exposure to [thimerosal], or a combination of both that is causing the observed effects. " In addition, the effects appear in some reflexes to be mediated by other risk factors such as birth weight and gestational age, suggesting that vaccinating premature and/or low birth weight infants may create especially high risk. "Infants of lower birth weight and gestational age were at greater risk" explained Dr. Laura Hewitson of the University of Pittsburgh, one of the principal investigators of the study. "The reflexes affected in this study are controlled by the brainstem, which regulates functions like heart rate, breathing, and intestinal activity, so these findings give us cause for concern, especially for low birth weight and pre-term infants who might be more susceptible to functional brain injury from this vaccine".

Despite their interest in the brainstem, the authors note that the "the mechanism of these effects and the requirement for [thimerosal] is not known and requires further study." At least some of that further study is underway. According to Wakefield, "This study is part of a larger research program looking at the safety of the vaccine schedule from birth to age four years."

In fact, some findings from the group's own further study may already be at hand. Close reading of the published paper reveals that these macaques were followed for

only fourteen days from birth for adverse responses to HBV because "infants received further interventions on Day 14 which would have confounded the independent effects of the HB vaccine." These further interventions were most likely additional vaccinations. Over a year ago, Age of Autism reported on a series of abstracts presented at an autism conference by many of the same authors (see HERE). These abstracts describe a study design in which multiple vaccines were administered, in addition to HBV. The current study design appears similar to the earlier reports, but differs from the previous abstracts in the number of vaccine exposures (one vs. multiple, including MMR) and the number of unexposed macaques (three vs. seven).

**Careful methods, conservative findings**

Basic reporting aside, it's worth digging a bit deeper into some of the details of the study design, which will almost certainly attract further attention. There are numerous elements that go into a primate study like this, elements that can and should influence the scientific acceptance of the results. These include: the quality of the primate lab and research group, the quality of the study design and testing methods, the size of the sample, the authors' statistical interpretations and potential biases.

Primate research expertise. The macaques were bred and housed at the primate nursery of the Pittsburgh Development Center (PDC) at the University of Pittsburgh, where the research team's principal investigator, Dr. Laura Hewitson, is a member of the faculty. The PDC is a part of the Magee Women's Research Institute of the University of Pittsburgh's School of Medicine. PDC's research mission includes *stem cell* development and *infertility* in addition to an *infant development* research program of which the Infant Primate Laboratory headed by Dr. Hewitson is the driving force.

The PDC was founded in 2001 and its primate program is relatively new. But the director of the PDC, Gerald Schatten, came to Pittsburgh from Oregon National Primate Center, one of nine National Primate Research Centers (NPRCs) sponsored by the National Institutes of Health (NIH). In addition, the vaccine study's PDC team has benefited from the direct involvement of two of the most prominent primate researchers in the nation. The late Gerald Ruppenthal was an active collaborator of the PDC and in that capacity also "assisted in the [vaccine] study design, training and implementation of the infant primate developmental measures prior to his death in 2004."

Perhaps most importantly, one of the vaccine study co-authors is Dr. Gene Sackett, who was once director of the University of Washington's Infant Primate Research Laboratory, another one of the nine NIH primate centers (now headed by Dr. Thomas Burbacher). He is now Professor Emeritus at the University of Washington. Along

with Ruppenthal, Dr. Sackett was co-editor of one of the most widely used texts for primate research, *Research Protocol and Technician's Manual*, published in 1992.

In short, while the PDC is a relatively new primate facility, both the PDC primate facility and the vaccine study team have benefited from the active support and participation of the country's leading primate experts.

Study design and testing methods. The study was carefully designed and tightly controlled to prevent bias from entering into any of the assessments. Crucially, the exposure status of the macaques was not known to the team. "Neonatal assessments were performed by [Lisa A. Houser] who was unaware of the study group assignment of each animal, the number of animals in each study group and the number of study groups." Adding rigor to the implementation of the testing, Houser "underwent extensive training" in making these assessments by the leading experts in the field, Gerald Ruppenthal, before his death.

In addition to Ruppenthal's involvement as a trainer, Dr. Sackett's involvement as co-author and data analyst ensured that Houser's data collection was sound. Dr. Wakefield and Dr Hewitson designed the study, but neither was involved in data collection or statistical analysis. Dr. Wakefield's role in the ongoing autism-vaccine controversy may make the design subject to added criticism; at the same time, it provided added incentive to make the design impervious to superficial criticism.

Sample size. At first glance, the number of infant primates involved, thirteen vaccinated macaques and seven unexposed (the paper avoids using the word "unvaccinated" to describe the latter group since four were injected with saline placebo and three received no shot at all) may seem low. Some might offer the concern that this sample is too small to draw any strong conclusions, but this would reflect ignorance of how primate research is typically conducted. Unlike rats and mice, which can be bred in laboratories in relatively large numbers, primates are expensive to raise. And since the purpose of study designs involving infant primates is to infer human exposure risk from the developmental outcomes of our closest animal relatives, the testing protocols are designed to be sufficiently precise so that valid conclusions can be drawn from small numbers of animals.

In other words, the sample is sufficiently large for a study of this type, especially since only two exposure groups were studied. In addition, all 20 macaques described were male, eliminating any confounding effect of gender and further raising the statistical power of the sample.

It's worth comparing the size and composition of these study groups to that of two comparable research projects that have been frequently cited and widely accepted as

sound. In the first of these, published in 2005, Dr. Thomas Burbacher, from University of Washington's NPRC, led a study team that examined the effects of thimerosal and methyl mercury in infant primates. In this study, Burbacher's team examined a different species of macaques in a mixed-gender sample that included seven controls and seventeen animals in each of the two (thimerosal and methyl mercury) exposure groups. The total study involved 41 infant male and female macaques. In a second comparable project, Dr. Burbacher published an influential series of studies on the effect of subclinical methyl mercury exposure, spanning five papers published from 1994-96, in adult macaques. His study sample examined multiple exposure groups with five female macaques in each group and only four unexposed females. The total study involved 27 adult female macaques.

It's worth noting that the seven unexposed macaques reported in today's paper represents an increase in unexposed animals relative to the three animals in the conference abstracts published last year. Last year's control group was similar in size to the 1994-96 Burbacher control group (three infant males vs. four adult females) but may have raised concerns over adequate sample size. The current study's unexposed population is equal in size to Burbacher's infant (but mixed gender) 2005 control group and larger in size than the 1994-96 control group of adult females.

When considering both the confounding effects of gender and the number of animals per exposure group, today's study compares favorably to both of these prior studies, with more gender specific controls than either of the Burbacher studies and more animals of a single gender in the main exposure group than either study as well.

Statistical interpretations. Setting aside the typical standards for primate work, from a purely statistical perspective the raw numbers involved in this vaccine study are still not large. That means that for any difference in developmental outcomes to reach statistical significance, it would have to be large. And despite the modest sample sizes, the statistical differences reported by the researchers are quite robust. Across a wide range of statistical tests the root reflex finding consistently reached significance with 99% confidence, the suck reflex finding reached significance with 98% confidence and the snout reflex finding reached 95% confidence in two out of three analyses and with 94.5% confidence in the third (95% confidence is the level generally accepted as "statistically significant").

These findings are clearly sufficient to make the findings important, but it's worth noting that the authors' interpretation of their data analysis is highly conservative. In addition to significant findings in three of the nine reflexes, it's quite plausible to make the case that two more of the reflexes were delayed by HBV exposure as well. The analysis of these reflexes--the *auditory startle* reflex and the *grasp hand* reflex--

"approached significance" in each case. Two different methodological obstacles contributed to the failure to reach significance.

1. In the case of the auditory startle reflex, the sheer *size of the difference* between the two groups was larger than any of the three reflexes that showed *statistically significant differences*. The vaccinated macaques took a full two days longer on average to acquire the auditory startle reflex than the unexposed group; by contrast, the difference between the averages of the two groups' time to acquire the root reflex was just 1.2 days. The difference in the auditory startle was also understated, since one of the vaccinated macaques *never acquired the reflex* during the entire two week observation period. Because of the modest sample size, however, the relatively larger variation in this group made the result significant with only 89% confidence.

2. In the case of the grasp hand reflex, a result that was significant with 93.5% confidence, both vaccinated and unexposed macaques acquired this reflex relatively quickly. But since *every one of the unexposed macaques acquired the reflex at the first examination*, the average time to acquire it was "left-censored", meaning that it was assigned the minimum possible value of 0.5 days, and almost certainly overestimated the development time of the unexposed sample. If the unexposed group had been measured without any need for "left censoring", then only a modest reduction (under 10%) in the mean time to acquire the grasp hand reflex would have been enough to bring this result into the significant range.

To make the point more simply, it would have taken only modest differences in the management of the data analysis to make over half of the measured reflexes show significant delays instead of a third of them. Pointing this out is not intended as a criticism of the study, however, but rather a demonstration of how conservative the authors were in their interpretation of the results.

Finally, for the three reflexes that were initially found significant, the authors dug deeper and conducted two different kinds of regression analysis to ensure that the differences were not affected by other factors such as premature birth or low birth weight (the study's tables provide a painstakingly intricate array of these regression model outputs). The first of these showed *a consistently strong developmental benefit of not being vaccinated*, with unexposed macaques having a "risk" of developing the three reflexes (where the risk was actually a benefit) that was 3-5 times the "risk" of the vaccinated group (although the snout reflex risk level only reached 94% confidence). The second set of regressions attempted to distinguish whether the effect of vaccination was a *main effect* or the result of *interaction* with the effects of premature birth or low birth weight. *In all models that compared main and interaction*

*effects, the exposure effect was significant in way or the other*: the root and snout reflexes were best explained as main effects (although the root reflect was significant as an interaction effect also), while the suck reflex appeared to be best explained as an exposure risk for low birth weight and/or premature infants.

It's likely that most readers will skim through the results of these regression models, just confirming the fact that the results were significant. But it's important to recognize how carefully the analysts have investigated if other factors besides exposure could explain the developmental delays of the vaccinated macaques. In all cases, the delayed development of these reflexes was clearly traced to vaccination.

Potential biases. One likely tactic of critics of the study will include attempts to nullify the evidence based on the alleged bias of those involved. For one, the study is privately funded and acknowledges some well known autism advocates as financial contributors. These include the Johnson family (Jane Johnson is co-author of *Changing the Course of Autism*, a member of the Board of Directors of Thoughtful House and Director of Defeat Autism Now!), SafeMinds, the Autism Research Institute and Elizabeth Birt. Although all of these groups make clear their research interest is vaccine safety, they are frequently attacked for being "anti-vaccine", an epithet that will almost certainly be hurled again here.

The most aggressive attacks, however, will likely be reserved for the study authors. The basis of these attacks is best anticipated by the following conflict of interest disclosure in the published paper. "Prior to 2005, [Carol Stott] and [Andrew Wakefield] acted as paid experts in MMR-related litigation on behalf of the plaintiff. [Laura Hewitson] has a child who is a petitioner in the National Vaccine Injury Compensation Program. For this reason, [Hewitson] was not involved in any data collection or statistical analyses to preclude the possibility of a perceived conflict of interest."

**Related evidence on the hepatitis B vaccine**

In spite of the active involvement of autism-related organizations and parents in the study's design and funding, this narrow investigation of HBV exposures doesn't bear directly on the question of whether HBVs with (or without) thimerosal contribute to causing autism. The only harm described in these vaccinated macaques is in a tightly defined set of "survival reflexes" during a brief window after birth and exposure. We will have to wait for future reports from the research team to gauge both the nature and the persistence of the development delays in the vaccinated group.

At the same time, the narrow focus of the current study invites a similarly focused comparison to a small set of recent studies, published and unpublished, that explored

the relationship between HBV/thimerosal exposure and autism or other neuro-developmental delays (NDDs). Three separate studies--two recently reported by a pair of scientists from Stony Brook University, the third disclosed via Freedom of Information Act (FOIA) and performed by researchers from the Centers for Disease Control (CDC)—provided compelling additional evidence linking the birth dose of a thimerosal-containing HBV and elevated autism or NDD risk.

In the fall of 2008, Carolyn Gallagher and Melody Goodman, PhD candidate and Assistant Professor of Preventive Medicine, respectively, at the Stony Brook University Medical Center, reported on their analysis of a sample of over 1800 children whose families were surveyed as part of the National Health and Nutrition Examination Survey (NHANES) in 1999-2000. They took advantage of two questions posed in that survey: 1) "Does your child receive Special Education or Early Intervention Services"; and 2) "Has the survey participant ever received the 3-dose series of the hepatitis B vaccine." At the time of the survey, all HBVs administered to children under 10 years of age would have contained thimerosal.

When Gallagher and Goodman analyzed the risk of exposure to the 3 dose HBV series in children (the study didn't query whether this dose included the birth dose, but the CDC's universal birth dose recommendation was in place during the entire study period), they got back a surprising result. There was no apparent risk of HBV exposure in girls (there even appeared to be a protective effect), but the risk of needing special services in fully vaccinated boys was over twice as high as less vaccinated boys and the difference was statistically significant. When they adjusted the odds for confounding factors, they estimated the increased risk for boys needing special services after full HBV exposure to be fully *8.6 times higher* than less vaccinated boys.

Subsequent to this publication, Gallagher and Goodman continued their investigation. Earlier this month, they published an abstract from a poster presentation that reported findings from a more focused investigation. In this analysis they looked at only the birth dose of HBV and boys born before 1999 whose parents reported their child had "received a professional diagnosis of autism." The more precise data for this work was provided by a different survey, the National Health Information Survey (NHIS) and would again have included only thimerosal-containing HBV. They found that "U.S. male neonates vaccinated with hepatitis B vaccine had a 3-fold greater risk of ASD."

*So from two separate data sources, Gallagher and Goodman have produced findings that dovetail almost perfectly with today's primate report. In male infants vaccinated at birth with thimerosal-containing HBV, the risk of immediate developmental delays*

*(macaques), the need for early intervention services (NHANES) and autism (NHIS) is increased anywhere from three to eight fold*.

If this finding is so clear, the obvious question is why the CDC's vaccine safety apparatus hasn't found the same thing.

The answer, of course, is that they have. In their very first examination of the risk of thimerosal exposure in infants, the single most startling finding was this: infants who received the largest exposure of thimerosal in the first month of life showed the highest risk of autism and several other NDDs. Buried deep in a pile of statistical tables that SafeMinds received under FOIA was a risk analysis conducted by CDC analyst Thomas Verstraeten showing statistically significant risk multiples for the most exposed infants. These ranged from 5 times the risk of unexposed infants in the case of sleep disorders to 11.5 times for autism. I summarized these data tables in a report written five years ago. You can find it HERE.

What happened to the CDC findings that are now forcing us to rediscover the risk of thimerosal-containing birth doses of HBV in monkeys? The answer is simple. The CDC team simply censored the data. Infants with the highest levels of thimerosal exposure--those who had received both the HBV and hepatitis B immune globulins--were simply removed from the study sample. In Verstraeten's words, "the following children were excluded from the analysis…Children that received hepatitis B immunoglobulin as these were more likely to have higher exposure and outcome levels."

Why they decided this was a legitimate exclusion is anyone's guess. Unfortunately, it leads us all back to monkeys.

**Future research**

The report makes clear that future research is both needed and forthcoming. The immediate direction of that research is obvious. There is a clear, indeed urgent, need for further publications that describe what happened to the vaccinated macaques and their unexposed counterparts as succeeding vaccines were administered, including the thimerosal-containing DTaP and Hib vaccines and the MMR vaccine. These publications should include not just how the two macaque groups' observable development proceeded, but also how their gastrointestinal tracts were affected by the vaccine exposures and how their brain development was changed. Judging from last year's conference abstracts, the study team's research program has included both brain imaging and gut tissue analysis, so the main obstacle to further dissemination of the research findings appears to be publication.

Between the May 2008 conference abstracts and today's publication, over a year has passed and only a small portion of the promised insight from the original abstracts has been made public. The journal editors at *Neurotoxicology* have taken a courageous stand in publishing what is sure to be unwelcome evidence in some circles. Let's hope we see more from this project team soon.

*Mark Blaxill is Editor-at-Large for Age of Autism and a Director of Safeminds, one of the organizations that sponsored the study.*

**Abstract**: This study examined whether acquisition of neonatal reflexes and sensorimotor skills in newborn rhesus macaques (Macaca mulatta) is influenced by receipt of the single neonatal dose of Hepatitis B (HB) vaccine containing the preservative thimerosal (Th). HB vaccine containing a standardized weight-adjusted Th dose was administered to male macaques within 24 hours of birth (n=13). Unexposed animals received saline placebo (n=4) or no injection (n=3). Infants were raised identically and tested daily for acquisition of 9 survival, motor, and sensorimotor reflexes by a blinded observer. In exposed animals there was a significant delay in the acquisition of three survival reflexes: root, snout and suck, compared with unexposed animals. No neonatal responses were significantly delayed in unexposed animals compared with exposed. Gestational age (GA) and birth weight were not significantly correlated. Cox regression models were used to evaluate the main effects and interactions of exposure with birth weight and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on root and suck when controlling for GA and birth weight such that exposed animals were relatively delayed in time-to-criterion. There was a significant effect of GA on visual follow far when controlling for exposure such that increasing GA was associated with shorter time-to-criterion. Interaction models indicated that while there were no main effects of GA or birth weight on root, suck or snout reflexes there were various interactions between exposure, GA, and birth weight such that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated important influences of birth weight and/or GA on the effect of exposure which, in general, operated in a way that lower birth weight and/or lower GA exacerbated the detrimental effect of vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing HB vaccine exposure, particularly in infants of lower GA or low birth weight. The mechanism of these effects and therequirements for Th is not known and requires further study.