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REVIEW

The safety of pediatric use of paracetamol (acetaminophen): a narrative review of direct and indirect evidence

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ABSTRACT

Paracetamol (acetaminophen) use during pregnancy and early childhood was accepted as safe in the 1970s, but is now a subject of considerable concern. Careful analysis shows that initial acceptance of the drug was based on the false assumption that drug interactions in babies and adults are the same, and on a complete absence of knowledge regarding the impact of the drug on brain development. At least fourteen epidemiological studies now indicate that prenatal exposure to paracetamol is associated with neurodevelopmental problems. Based on these studies, it can be concluded that prenatal exposure to paracetamol causes statistically significant risks of developmental delays, attention deficit hyperactivity disorder, and a subtype of autism spectrum disorder (ASD) associated with hyperkinetic behavior. In contrast, data regarding postnatal exposure to paracetamol are limited, and several factors impede a classic multivariate analysis of epidemiologic data to resolve the issue. However, circumstantial evidence regarding postnatal exposure to the drug is abundant, and includes at least three otherwise unexplained temporal relationships, data from laboratory animal studies, several miscellaneous and otherwise unexplained correlations, and a lack of alternative suspects that fit the evidence-derived profile. Based on this evidence, it can be concluded without any reasonable doubt that oxidative stress puts some babies and children at risk of paracetamol-induced neurodevelopmental injury, and that postnatal exposure to paracetamol in those susceptible babies and children is responsible for many if not most cases of ASD.

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KEY WORDS: Acetaminophen; Autism spectrum disorder; Vaccination.

Paracetamol, also known as acetaminophen, is almost universally accepted for use in babies and children, with exposure to the drug exceeding 90% in some pediatric populations.[1-3] However, unlike many drugs, some fraction of paracetamol is converted by the body into a highly toxic me-

tabolite.[4] This toxic metabolite, called N-acetyl-p-benzoquinone imine (NAPQI), is rapidly neutralized by conjugation with glutathione, present in abundance in healthy individuals. However, under conditions of oxidative stress, glutathione is depleted, leading to increased production of NAPQI and profound impairment of NAPQI removal.[4] Under these conditions, NAPQI reacts with a wide range of proteins, permanently damaging those proteins and resulting in toxicity to the associated cell. Unfortunately, due to a variety of environmental and genetic factors, many babies and children are exposed to excessive oxidative stress.[5] Factors that increase oxidative stress include infections, treatment with antibiotics, and disorders such as Down syndrome and cerebral palsy, among others. As one example, 18 hours of fasting, the equivalent of missing breakfast and lunch, leads to decreased glutathione, which in turn approximately doubles the toxicity of paracetamol.[6] Thus, while it is expected that most children will metabolize paracetamol efficiently and avoid the most severe adverse outcomes, a substantial number of children, those with excessive oxidative stress leading to glutathione depletion, are expected to be at risk from paracetamol-induced toxicity.[5] A schematic diagram of this model is shown in Figure 1.

Parents and doctors alike have become so comfortable with paracetamol that many administrations of the drug, both at home and in the hospital, are overdoses.[7-11] In addition, several studies have shown that paracetamol is often administered too frequently.[2, 3, 8, 10-13] Further, several investigators have reported "fever phobia"— exaggerated concerns about fever in children and its complications (seizures, brain damage, etc.),[12, 14-17] which leads to use of paracetamol with no potential benefit.[11, 12, 15, 18] For example, an Italian study[19] found that a surprising 74% of all administrations of paracetamol for fever are given to treat fevers less than 38.4 °C, indicating that treatment is being used for mild fevers that pose no health risk to the child.[17, 20, 21] The authors conclude that "*preventive action should be taken regarding the use of paracetamol as antipyretic drug in children in order to reduce the fever phobia and self-prescription…*"[19]. Indeed, even within the higher range of 40 °C to 42 °C, there is no evidence to suggest that typical fevers in children without brain injury present an increased risk for adverse health outcomes such as brain damage.[16, 17, 21]

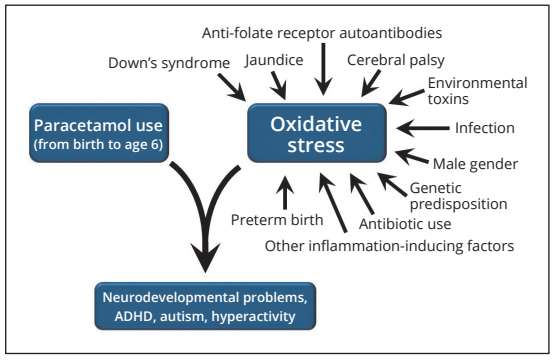

Figure 1.—A model of ASD induction by paracetamol (acetaminophen) combined with oxidative stress. The list of factors associated with oxidative stress is representative of some common factors known to induce oxidative stress, but is not exhaustive. Based on studies in animal models, some long-term neurological effects of early life exposure to paracetamol may act independently of oxidative stress, and are not shown in the diagram. In addition, genetic vulnerability to ASD mediated through mechanisms independent of oxidative stress are plausible, but are not shown in the diagram.

Despite the comfort most caregivers and health care workers have with the drug, a wide range of evidence, reviewed here, indicates that use of paracetamol in the pediatric population is associated with long term neurodevelopmental problems for at-risk children. A recent, exhaustive examination of the literature[22] was conducted by co-authors WP, JCH, LGA and colleagues at Duke University in collaboration with bibliometrics expert Vincent Larivière at the University of Montreal. That analysis demonstrated that the safety of paracetamol use in the pediatric population is an assumption based on numerous studies which show conclusively that the drug does not generally cause liver damage in the pediatric population when used as directed.[22] However, despite the fact that the brain is a primary target organ for the drug's therapeutic effect, none of the studies making claims of safety ever examined the effect of the drug on neurodevelopment.[22] Further, no study making claims of safety ever considered total paracetamol exposure since birth, precluding any effective assessment of the drug on neurodevelopment.[22] In the face of widespread but unfounded assumptions of safety, here we will assess mounting evidence

indicating that use of paracetamol in the pediatric population carries significant risks for neurodevelopment, and that the effects of the drug might be complex, depending on cofactors associated with oxidative stress and metabolism of the drug.

An overview: the effects of prenatal *versus* postnatal exposure to paracetamol differ dramatically

Based on available evidence, the impact of paracetamol on the developing brain is much different in the prenatal period compared to the postnatal period. As pointed out in a recent, widely publicized consensus statement,[23] the long-term effects of paracetamol use during pregnancy have been the subject of considerable study. A number of epidemiologic studies, taken together, demonstrate that paracetamol use during pregnancy has long-term negative effects on brain function in the offspring.[24-37] This conclusion has been corroborated by meta-analyses.[30, 38, 39] The use of paracetamol during pregnancy has been associated with a variety of neurodevelopmental problems, including a subtype of autism spectrum disorder (ASD) associated with hyperkinetic behavior, attention deficit hyperactivity disorder (ADHD), and developmental delays.[24-37] These studies show that, although the large majority of children suffer no obvious or apparent long-term adverse effects from prenatal exposure to paracetamol, a significant fraction of the population is at risk. The mechanism by which paracetamol might profoundly hurt some children while leaving others unharmed has been understood for decades:[40-42] as mentioned in the Introduction, paracetamol becomes toxic in the presence of oxidative stress (Figure 1) due to buildup of a toxic metabolite of paracetamol, N-acetyl-p-benzoquinone imine. As shown in Figure 1, a large number of common environmental and genetic factors can cause oxidative stress, and whether a particular child is injured by paracetamol is likely determined by both 1) the amount of paracetamol, and 2) the amount of oxidative stress present at the time when exposure to paracetamol occurs.

In contrast to the numerous studies of the effects of prenatal paracetamol exposure, studies directly assessing the effects of paracetamol use during the postnatal period are limited. Nevertheless, considerable evidence regarding the effects of postnatal exposure does exist, and it is possible to piece together a case of sufficient strength to conclude without any reasonable doubt that postnatal paracetamol use is indeed hazardous to neurodevelopment for many susceptible children. In particular, we conclude that postnatal use of paracetamol is extremely hazardous in babies and children with oxidative stress, and that use of the drug is responsible for many if not most cases of ASD. This conclusion, as will be discussed in this review, is based on a wide range of circumstantial but compelling evidence. The fact that no alternative explanations can adequately explain the available evidence adds further weight to this conclusion. Most importantly, the conclusion is quite testable, and experiments designed to test the conclusion as well as the expected outcomes are described.

Studies in laboratory animals

The industrial revolution has provided humanity with a number of widely used commercial products that are hazardous to neurodevelopment. Such products include phthalates in plastics,[43] the pesticide DDT,[44] and lead in paint.[45] Studies in laboratory animals have been instrumental in understanding the impact of all of these products on human beings. For example, epidemiological data are still not sufficient to derive quantitative estimates of the hazards of phthalates for human development,[46] leaving studies in animal models as a principal guide to creating regulations that limit exposure of humans to these highly toxic compounds.[46] However, laboratory animals may be, at least in some cases, less sensitive to neurodevelopmental toxicity than are humans. For example, the lowest observed adverse effect level of DDT is 50 mg/kg/day in laboratory rats, but only 10.3 mg/kg/day in humans.[47] As another potential example, experimental studies of the neurodevelopmental toxicity of "low levels" of lead in rats typically employ blood lead levels of 0.2 ug/mL or more,[48, 49] but blood lead levels of about 0.1 ug/mL and possibly as low as 0.05 ug/mL are dangerous in humans.[45]

TABLE I.—*Studies in animal models evaluating the long-term of postnatal paracetamol use. Accepted levels of exposure in the pediatric population are shown for comparison. Despite a wide range of study designs, all studies demonstrate negative long-term effects.*[52-55]

Population or study group	Age at time of exposure	Postnatal exposure during early development (mg paracetamol/ kg body weight)	Effects observed later in life
Humans	From conception onward	Unlimited duration of treatment, 14.7 mg/kg bw, every 4-6 hours, no more than 5 doses per day	To be determined
Mice in Viberg study[52]	Postnatal day 10	1 day of treatment, 30 mg/kg bw, two treatments, 4 hours apart	Decreased learning capability, long-lasting effects on cognitive function, altered adult response to paracetamol
Rats in Suda study[54]	Postnatal days 4-10	7 days of treatment,<14.7 mg/kg bw, every 5 hours	Increased rearing (asocial behavior) when encountering a new rat
Mice in Philippot study[55]	Postnatal days 3, 10, 19	1 day of treatment, 30 mg/kg bw, two treatments, 4 hours apart	Negative effects on adult behavior, cognitive function, and habituation capability (long-term effects only observed following exposure on days 3 and 10, not on day 19 after the period of rapid brain growth)
Rats in Dean study[53]	Postnatal days 7-13	7 days of treatment, 40 mg/kg bw, one dose per day	Long-term modifications to brain development and morphology, decreased social interactions and sensory function in males

Paracetamol is generally thought to be safer in children than it is in adults, but this conclusion is based strictly on the drug's relative lack of ability to cause liver damage in babies and small children.[50] In reality, however, studies in laboratory animals show that paracetamol is actually more deadly in young pups than it is in older animals.[51] At the same time, even at doses that are lethal, young pups suffer no statistically significant liver damage.[51] Thus, based on studies in animal models, the liver is not the target organ for paracetamol-induced toxicity during early development, and liver damage should probably not be used as a measure of paracetamol toxicity in babies and small children. Although the cause of paracetamol-induced death in young laboratory pups has, surprisingly, never been determined, studies using both laboratory mice and laboratory rats have shown adverse, long-term effects on neurodevelopment following exposure to paracetamol shortly after birth (Table I).[52-55] A Swedish study, for example, found that paracetamol exposure shortly after birth almost completely eliminated the ability of laboratory mice to learn a maze later in life (Figure 2A, B).[52, 54] In that study, mice injected with saline shortly after birth learned rapidly, increasing their speed of running a maze by more than 2-fold after only two days of training. But, based on speed of running the maze, mice injected with paracetamol shortly after birth lost more than 90% of their ability to learn a maze compared to their saline injected counterparts ($P<0.0001$). While running the maze on the first day, both the control and paracetamol treated groups had a similar number of errors (15.24 in control, 16.95 in treated). However, the control mice averaged less than one error after two days of training, while the paracetamol-treated mice still averaged more than a dozen errors after the same amount of training ($P=0.0003$).[52] These results demonstrate that paracetamol exposure shortly after birth causes profound, long-term impairment of learning capacity in laboratory mice under the conditions used.

McCarthy's laboratory has demonstrated long-term neurological changes in laboratory rats following early life exposure to paracetamol and to other drugs with similar pharmacological activity.[53] A separate study using laboratory rats was conducted at Duke University by coauthors WP, JPJ and ZK in collaboration with neuro-

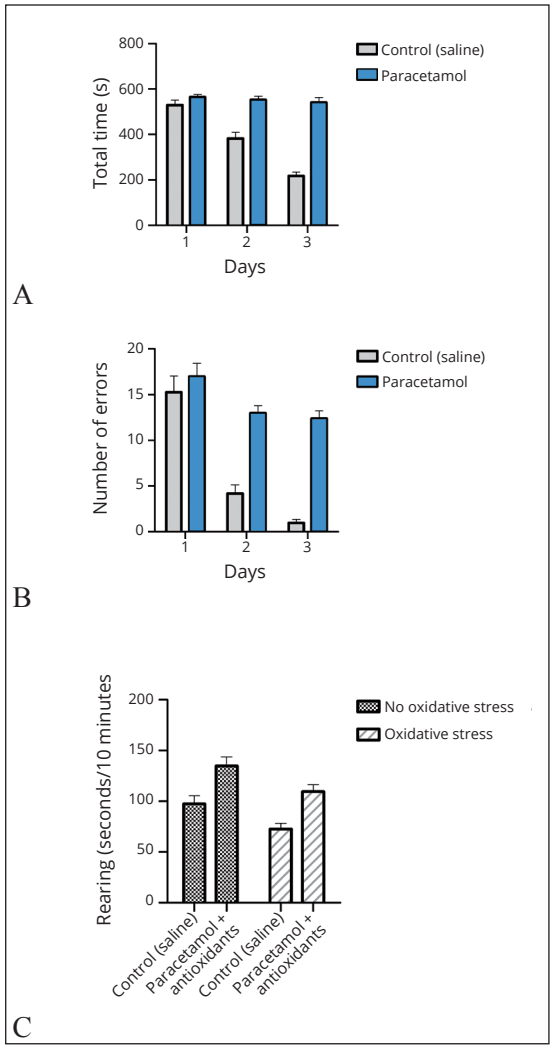

Figure 2.—Early life exposure to paracetamol causes long-term loss of learning ability in laboratory mice (A and B) and long-term increases in asocial behavior in laboratory rats (C).
Laboratory mice were exposed to either 0.9% saline or two doses of 30 mg paracetamol/kg body weight (administered 4 hours apart) on postnatal day 10, and performance was measured at 2 months of age in experiments described by Viberg *et al.*[52] After two days of training, mice injected with saline but not mice injected with paracetamol learned to run the maze (A) faster ($P<0.0001$) and (B) with less errors ($P=0.0003$). The rats were exposed to either saline or paracetamol and antioxidants (cysteine and mannitol) on postnatal days 4 through 10, and rearing, an asocial behavior, (C) was observed on postnatal days 37 through 49 in experiments described by Suda *et al.*[54] Paracetamol and antioxidant treatment had 38.4% more rearing behavior than in control rats in an environment without oxidative stress, and 50.6% more in an environment with oxidative stress ($P<0.0001$). Images from Viberg *et al.*[52] were digitized and quantified, and, along with data from Suda *et al.*,[54] were replotted to generate the Figure. All statistics were obtained through two-way ANOVA tests using GraphPad Prism 9.

scientist Caroline Smith and colleagues.[54] That study recapitulated conditions of paracetamol exposure in human infants and children, and found that paracetamol use shortly after birth resulted in an increase in asocial behavior later in life ($P<0.0001$) (Figure 2C). The magnitude of the paracetamol-induced increase in asocial behavior was concerning: paracetamol exposure shortly after birth led to 38% more asocial behavior than in control subjects. With the addition of oxidative stressors such as mock infections and antibiotics, paracetamol exposure shortly after birth led to 51% more asocial behavior than in control subjects.[54]

The significant effect of early life exposure to paracetamol on learning in mice (Figure 2A, B),[52, 54] brain architecture in rats,[53] and asocial behavior in rats (Figure 2C) provides convincing evidence of the potential for this drug to exhibit detrimental effects in human infants and children. However, because many children given paracetamol are expected to have oxidative stress-inducing conditions such as infection and exposure to antibiotics, and because most studies in laboratory animals use very healthy animals, we expect that some babies and children will be at greater risk than are laboratory animals. For example, the studies described above on laboratory mice[52] were conducted on healthy animals. Similarly, studies in McCarthy's laboratory[53] used healthy laboratory rats, and the studies in rats at Duke University[54] used antioxidants in conjunction with paracetamol, reducing the role of oxidative stress in the induction of neurological changes in that model. Further, standard laboratory rodent diets can be so enriched in antioxidant vitamins that achieving oxidative stress can be difficult. For example, the commonly used ultra-low fiber, high fat and processed sugar "Western Diet" (Envigo rodent diet TD.88137) used in the Duke University study contained 1% weight/volume vitamin mix (Teklad 40060) with a variety of antioxidant B-vitamins. For the sake of comparison, this mass of multivitamin mix is equivalent to more than a dozen multivitamin pills (One-A-Day® Women's 50+: 1.58g/pill) in a typical US diet of 2kg of food per day.

As described above, existing animal studies may not reflect the dangers of exposure to

paracetamol imposed by oxidative stress which occurs in some babies and children living in uncontrolled environments. To make matters worse, long-held industrial standards in the field of drug development employ screening of laboratory animals only for gross abnormalities in behavior such as seizures, paralysis, and drowsiness.[56] Thus, for example, if a drug caused a profound decrease in socialization or lowered intelligence in a laboratory animal, these severe adverse effects would not likely be identified using the typical screening methods currently employed by the pharmaceutical industry and approved by the FDA.

Current FDA guidelines dictate that, under experimental conditions, humans should never receive levels of drug above the "no observed adverse effects level" (NOAEL) in laboratory animals.[57] For example, humans receiving experimental drugs should receive at most 6-fold *less* drug than levels which cause severe adverse events in laboratory rats, and at most 12-fold *less* drug than levels which causes severe adverse effects in laboratory mice.[57] As discussed above, current levels of exposure to paracetamol in children cause long-term neurological changes in laboratory animals, which by definition constitute major adverse events. With this in mind, it is apparent that amounts of paracetamol currently accepted for use in babies and children would not be approved using current FDA guidelines for drug approval, and that the levels of the drug currently administered to babies and children exceed by more than 6-fold the dose that should be administered if experimental trials were to be conducted using current guidelines. That being said, the therapeutic dose of paracetamol covers only a narrow range, and lowering the currently accepted dose only three-fold renders the drug ineffective.[58] Thus, future experimental clinical trials, if conducted, will necessarily be based on the fact that the drug is already in common use, and will necessarily ignore current regulations for safety.

Exposing babies and children to levels of a drug known to have developmental neurotoxicity in laboratory animals may seem egregious, but the situation is potentially much worse than it appears. As discussed above, laboratory animals previously used for pre-clinical testing of paracetamol have been very healthy, and it might be expected that unhealthy animals, with oxidative stress, will be much more sensitive to the adverse effects of the drug. Thus, prior work in healthy laboratory animals may dramatically underestimate the dangers of paracetamol in at-risk babies and children.

Postnatal exposure to paracetamol: limited data in human studies

The first indication that paracetamol was potentially problematic for neurodevelopment came from Stephen Schultz, who had watched his child regress into ASD following vaccination.[59] In 2008, Schultz and a number of distinguished scientists, then at the University of California San Diego and at San Diego State University, published a small survey study that identified a six-fold increased risk of ASD in one to five year old children when vaccines were accompanied with paracetamol (odds ratio [OR]=6.11, 95% confidence interval [CI] 1.42-26.3) but not when the vaccines were given with ibuprofen.[60] The study was immediately criticized for being small and for methodological flaws,[61] but a careful analysis of that study reveals a valid design and extremely concerning results.[62] Although the Schultz study was largely ignored for a decade, a meta-analysis of the long-term effects of postnatal paracetamol exposure was recently published by Alemany *et al.*[30] The analysis included 6 databases, but only the Danish National Birth Cohort (DNBC) database, with more than 61,000 births, contained data on the occurrence of ASD. Correcting for numerous confounding factors, analysis of that database revealed an OR of 1.30 (95% CI 1.02-1.66) for ASD associated with postnatal paracetamol exposure reported by the mother between birth and 18 months.[30] Given that levels of postnatal exposure to paracetamol are approaching 100% for at-risk children in some populations,[1-3] the relative risk (reflected by the odds ratio) is expected to be shockingly close to the absolute risk in those populations. For example, if all at-risk children are exposed to paracetamol, then the relative risk of a 30% increase found by Alemany would mean that 23% of all cases of ASD were caused by the ex-

posures to paracetamol that Alemany considered (0.77+0.23=1.0, and 1.0 is 30% more than 0.77.). Equally concerning is the fact that only 7.7% of the mothers providing data for the DNBC database reported giving paracetamol to their child between birth and 18 months of age. This is an exceptionally low number for children born from 1996-2002, when the children in the DNBC database were born. For example, a study of Danish children born in 2001 found that 65% of the children were exposed to paracetamol by their mothers within a three month period.[63] Thus, it seems possible that the DNBC database underreports paracetamol administration by mothers between birth and 18 months of age. In addition, Alemany's analysis[30] did not consider exposure after 18 months, for example during some childhood vaccinations, and it did not consider administration in the hospital, for example during circumcision.

Here it is important to point out that multivariate analyses typically applied to data sets generally try to eliminate the contribution of factors such as antibiotic treatment and infection that are considered to be "confounding". However, as pointed out above and in Figure 1, these factors that cause oxidative stress are not confounding, *per se*, but rather are co-factors in the induction of neurological injury as well as causes for the exposure to paracetamol. This situation, combined with the very high prevalence of exposure to paracetamol in some populations[1-3] creates an inherent problem for multivariate analyses that can literally cause the true cause of neurological injury to disappear from the results. To illustrate the problem, we created an artificial (virtual, *in silico*) data set in which 2/3 of all ASD was caused in a population of thousands of simulated (virtual, *in silico*) children by a combination of oxidative stress plus paracetamol use. To mimic the real world, factors that cause oxidative stress (such as infection and antibiotic use) were associated with paracetamol use (Figure 3). Despite the fact that 2/3 of all ASD was, by definition, caused by exposure to paracetamol combined

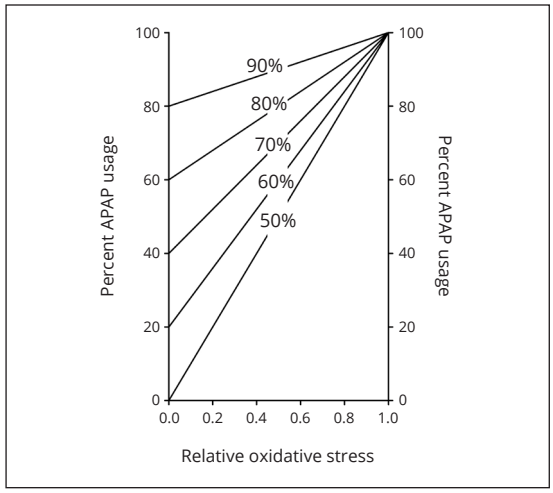

Figure 3.—Assignment of the probability of exposure to paracetamol (APAP) as a function of oxidative stress in 12000 simulated cases. In these simulations, virtual individuals with the highest levels of oxidative stress were assigned a 100% probability of acetaminophen exposure, and individuals with the lowest level of oxidative stress were assigned a probability of acetaminophen exposure equal to 100% - (2×[100% - the average exposure for the population]). Paracetamol use and 10, randomly generated "oxidative stress variables" for the population of 12000 virtual subjects were generated using R version 3.6.1, and the total oxidative stress was taken to be the sum of those 10 variables. The magnitude of oxidative stress was normally distributed, and the total prevalence of exposure to paracetamol for each simulation is shown in the center of the line representing paracetamol use for that simulation.

TABLE II.—*Results of multivariate logistic regression analysis of an artificial data set in which 2/3rds of all autism was induced by oxidative stress plus exposure to paracetamol and 70% of the population was exposed to paracetamol as shown in* Figure 3.

Variable*	adj. OR (95%CI)	p-value (Wald's test)
Oxidative stress variable #1	1.35 (1.24 - 1.46)	< 0.001*
Oxidative stress variable #2	1.34 (1.24 - 1.45)	< 0.001*
Oxidative stress variable #3	1.34 (1.24 - 1.45)	< 0.001*
Oxidative stress variable #4	1.35 (1.25 - 1.46)	< 0.001*
Oxidative stress variable #5	1.40 (1.30 - 1.51)	< 0.001*
APAP use (prevalence 70%)	1.59 (0.96 - 2.64)	0.074

Five out of 10 variables contributing to oxidative stress were included in the analysis in order to mimic a realistic data set in which measures of some but not all of the factors contributing to oxidative stress are available.
*Statistical significance of P<0.05. Statistical analyses of virtual data sets were performed using SAS (SAS Institute, Inc., Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria).
APAP: paracetamol; adj. OR: adjusted odds ratio; CI: confidence interval.

TABLE III.—*Results of multivariate logistic regression analysis of an artificial data set in which 2/3rds of all autism was induced by oxidative stress plus exposure to paracetamol.*

Average APAP use	Range APAP use	adj. OR (95%CI)	P-value (Wald's test)
90%	100-80%	1.54 (0.67-3.53)	0.30
80%	100-60%	1.69 (0.93-3.07)	0.088
70%	100-40%	1.59 (0.96-2.64)	0.074
60%	100-20%	1.80 (1.14-2.82)	0.011*
50%	100-0%	1.84 (1.21-2.79)	0.0042*

The prevalence of paracetamol exposure was varied as described in the Methods between 50% and 90% in the population.
*Statistical significance of P<0.05. Statistical analyses of virtual data sets were performed using SAS (SAS Institute, Inc., Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria).

with oxidative stress in that artificial population, the multivariate analysis failed, showing no significant association between use of paracetamol and ASD as long as 70% or more of children were exposed to paracetamol (Table II, III). Rather, *only* those factors inducing oxidative stress were identified by the multivariate analysis as being associated with ASD (P<0.001 for all factors). When only 50% or 60% of children were exposed to paracetamol in the artificial population, the association between ASD and exposure to paracetamol became statistically significant, but the calculated risk of exposure to paracetamol remained much lower than the real value (Table III). Further, the uncertainty of risk was large, with confidence intervals covering roughly a 10-fold range of risk (Table III), making any practical conclusions difficult to establish. These simulations demonstrate the potential pitfalls of relying on analysis of large data sets when exposure to the causative agent is high and is associated with co-factors in the induction of injury. These simulations may also provide insight into the results obtained by Alemany *et al.*,[30] who, despite assessing a database with more than 60,000 births, found a range of potential risk (95% CI on the OR=1.02-1.66) that is too broad to confidently draw conclusions.

Placebo controlled, prospective analysis of children with and without any exposure to paracetamol followed closely from conception would be, in theory, an ideal way to examine the possibility that paracetamol use in early development contributes to ASD risk, but large numbers of children would be required. For example, a study of 1000 children would likely yield 10 to 20 cases of ASD with dozens of potential confounding factors, resulting in a very low-powered study and potentially making it difficult to draw any firm conclusions. Further, such a study would be very expensive and require the better part of a decade to complete. Fortunately, a variety of other evidence is available (Supplementary Digital Material 1: Supplementary Table I)[5, 22, 24-37, 40-42, 52-55, 60, 64-82] and allows us to conclude that postnatal use of paracetamol is indeed hazardous to neurodevelopment in children with oxidative stress. Although much of that evidence is indirect, it is sufficiently strong to draw conclusions without any reasonable doubt. The conclusion that postnatal use of paracetamol is indeed hazardous to neurodevelopment renders the theoretically ideal study described above unethical and even immoral.

A very small study from the University of Oulu[87] might be considered as evidence that paracetamol is safe when used in preterm babies. The study follows 19 preterm patients and 20 preterm controls given paracetamol or placebo. The authors of that study do not claim that the drug is safe, but rather end their manuscript with the statement that "*Large randomized trial with standardized follow-up protocol should be conducted to detect any potential association with early neonatal paracetamol* (acetaminophen) *treatment and adverse neurological outcomes, like autism spectrum disorders and attention deficit hyperactive disorder."* However, even if the Oulu study had been larger, that study used the IV paracetamol preparation, which contains the glutathione precursor cysteine that serves as an antidote for excess formation of paracetamol's toxic N-acetyl-p-benzoquinone imine metabolite. Thus, we do not expect the IV formulation to induce the most severe neurodevelopmental problems. Unfortunately, the much more commonly used oral formulation does not contain any anti-

dote, so most children taking paracetamol do not have the protection that an antidote might possibly afford. Further, the Oulu study examined the effect of exposure to paracetamol within a window of only 4 days, and did not follow exposure to paracetamol for the duration of neurodevelopment, adding further complexity to any conclusions that might be drawn from the study, even if it had been much larger.

It should be noted here that, even with the addition of cysteine, paracetamol exposure early in life resulted in increases in asocial behavior in laboratory rats later in life (Figure 2C),[52, 54] and it is unknown whether any antidote can fully protect all high-risk babies from the most severe adverse reactions to paracetamol. Thus, the extent to which an antidote for paracetamol toxicity will protect babies and children exposed to the drug is unknown.

Postnatal exposure to paracetamol: overwhelming circumstantial evidence for harm

The histories of ASD and the use of paracetamol in pediatrics reveal a number of temporal connections. First, the incidence of ASD began to rise rapidly in the early 1980s, coinciding with the early rise in the use of paracetamol as physicians became aware of the connection between aspirin and Reye syndrome.[5] Second, as the pediatric community switched from aspirin to paracetamol, a qualitative shift in ASD, with less infantile ASD compared to regressive ASD, was observed during the early 1980s.[64] This shift indicated that some factor had been introduced into the population capable of inducing ASD in children even after neurodevelopment had progressed for years. Third, the prevalence of ASD rose dramatically through the 1990s and 2000s[5] as direct-to-consumer advertising effectively encouraged increased use of pharmaceutical products.[65] Although it is well known that association does not indicate causation, it is also correct that causation cannot occur without association. Further, the multiple temporal associations between ASD and the use of paracetamol described above are extremely concerning. Most importantly, additional lines of evidence, described below, confirm suspicions raised by multiple temporal associations.

Although the increase in prevalence of ASD over the past 40 years is due in part to changing diagnostic criteria, increased awareness, improved funding, and other social factors, these factors do not explain the timing of the increase or the sustainability of the increase. Although social factors, for example, are expected to consistently impact prevalence within a narrow timeframe, it is difficult to envision how such social factors might account for a steady and dramatic increase over a 40-year span. Further and more conclusively, studies comparing children side-by-side demonstrate that some environmental factor or factors is indeed inducing ASD. For example, a study in predominantly affluent ("non-poor") Vietnamese children found that the prevalence of ASD in children of farmers is almost 5-fold greater than the prevalence of ASD in children of government administrators (OR=4.72, 95% CI 2.03-10.97).[83] The authors of the study speculate that liberal use of pesticides, a factor that causes oxidative stress[88] and is common in Vietnam, may underly this observation. Regardless of the underlying reason, this finding demonstrates that some environmental factor or factors can cause a dramatic increase of ASD. As another example, circumcised boys, often exposed to paracetamol at the time of the circumcision procedure, have a prevalence of ASD that is about 50% more than that in uncircumcised boys.[66] This observation points strongly toward a potent environmental factor that induces ASD at the time of circumcision, which typically occurs shortly after birth, when levels of oxidative stress are typically high.[89] The impact of this circumcision-associated induction should not be ignored: if 60% of males are circumcised in a population and 75% of cases of ASD occur in males in that population, then it can be readily calculated that a 50% increase in ASD associated with the procedure means that induction at the time of circumcision accounts for more than 17% of all cases of ASD in that population (17.3% = $[(0.6\times1.5)-(0.6\times1.0)]/[(0.6\times1.5)+(0.4\times1.0)]\times75\%$, where 0.6 and 0.4 are the fraction of circumcised and uncircumcised males respectively, 1.5 and 1.0 are the relative incidence of ASD in circumcised and

uncircumcised males respectively, and 75% is the percentage of ASD occurring in males).

As shown in Supplementary Table I,[5, 22, 24-37, 40-42, 52-55, 60, 64-82] a number of additional factors are consistent with the view that early childhood exposure to paracetamol in the presence of oxidative stress can induce ASD. For example, genetic and autoimmune factors associated with ASD have an influence on paracetamol metabolism.[67] In addition, excessive, population-wide exposure to children's paracetamol in the Korean population[68] is associated with exceedingly high levels of ASD[69, 86] (Supplementary Table I).[5, 22, 24-37, 40-42, 52-55, 60, 64-82] Given no evidence that hazards during prenatal exposure disappear at the time of birth, numerous epidemiological studies showing neurodevelopmental problems with prenatal exposure[24-37] provide additional circumstantial evidence that postnatal exposure to paracetamol is not benign. Studies in animal models, described above, also provide compelling evidence that prenatal exposure to paracetamol is toxic for neurodevelopment. The studies in rats by McCarthy *et al.*,[53] in particular, show that the male brain is more sensitive to the drug than the female brain, potentially contributing to the preponderance of males with ASD even in populations with no practice of circumcision. Further, paracetamol is known to impair social functioning in human adults,[70-72] indicating that the drug does indeed target aspects of brain function known to be altered in ASD. In addition, the induction of ASD by paracetamol exposure under conditions of oxidative stress provides a plausible and much needed explanation for the fact that many parents of children with ASD have attributed the induction of ASD to vaccination,[73, 74] a medical procedure often associated with paracetamol exposure.

In summary, numerous lines of largely independent evidence point toward the conclusion that a variety of neurodevelopmental problems, especially ASD, can be induced by early life, postnatal exposure to paracetamol. The tally shown in Supplementary Table I[5, 22, 24-37, 40-42, 52-55, 60, 64-82] lists 17 lines of such evidence, both direct and circumstantial. Supplementary Table I [5, 22, 24-37, 40-42, 52-55, 60, 64-82] also lists potential objections to the conclusion that paracetamol is toxic during neurodevelopment. Importantly, ten of these objections are either unlikely based on experimental results or verifiably false. Eleven more of the objections have no supporting evidence, and exist solely for the purpose of asserting that paracetamol toxicity in babies and small children is safe ("post-hoc assertions", Supplementary Table I).[5, 22, 24-37, 40-42, 52-55, 60, 64-82] Further, if paracetamol exposure is *not* toxic to neurodevelopment, then a number of observations remain unexplained. The tally shown in Supplementary Table I[5, 22, 24-37, 40-42, 52-55, 60, 64-82]describes six unknown factors that must be invoked to account for all observations, and eight largely independent observations that must be attributed to coincidence.

Prenatal *versus* postnatal exposure to paracetamol and ASD

About half of the evidence presented in Supplementary Table I [5, 22, 24-37, 40-42, 52-55] is consistent with the induction of ASD either before or after birth. However, about half of the evidence in Supplementary Table I[5, 22, 24-37, 40-42, 52-55, 60, 64-82]is consistent *only* with the induction of ASD during the postnatal period, suggesting that many cases of paracetamol-induced ASD occur postnatally. Further, all studies examining the effects of prenatal paracetamol use are potentially confounded by the likely possibility that mothers who rely on paracetamol for personal use will, in turn, rely on the drug for their babies and children. Although accurate calculations of risk are not possible at present, the amount of ASD induced by prenatal exposure to paracetamol can be very roughly estimated from some of the epidemiologic studies evaluating the issue. In particular, Liew *et al.*[33] found roughly a 50% increase (HR=1.51 95% CI 1.19-1.92) in ASD with hyperkinetic disorder, but that subset of ASD accounted for only 31% of total cases of ASD in that study. Further, about 45% of the women in the study never reported using paracetamol, consistent with results in other databases.[30] Given that a significant number of women do not use paracetamol during pregnancy, the absolute risk of paracetamol-induced adverse events is lower than the relative risk. Thus, the absolute amount of ASD induced by paracetamol exposure

during pregnancy, although apparently significant, may be less than 10% to 15% of the total, and is unlikely to be greater than 20%. This level of induction of ASD is insufficient to account for the majority of the increase in prevalence of the disorder starting about 1980. In contrast, postnatal exposure could readily account for much of the increase in the disorder since 1980, potentially accounting for many if not most cases of ASD today.

Alternative explanations?

Objections to the view that early life exposure to paracetamol causes ASD and other neurodevelopmental disorders abound, but none are credible. The view that no environmental factor can possibly have caused the dramatic increase in the prevalence of ASD since 1980 is verifiably false, as discussed above. The contention that the origins of ASD are complex and involve multiple factors, including genetics, has been used as an argument that paracetamol cannot cause many if not most cases of autism. However, this argument is also fallacious: As Figure 1 shows, a number of factors, including genetics, play a role in the induction of ASD by paracetamol. In this model, oxidative stress combined with genetic vulnerability confers enhanced risk to paracetamol exposure early in life. Yet another argument that paracetamol cannot induce ASD can be based on the medical literature that states in hundreds of instances that paracetamol is safe when used as directed in the pediatric population.[22] Indeed, even some experts in the field of research on ASD have publicly promoted the idea that paracetamol is safe in the context of neurodevelopment. However, as described in the Introduction, a systematic analysis of the literature demonstrates that paracetamol was never proven safe for neurodevelopment,[22] despite the fact that the drug targets the brain. Another objection can be derived from the "biomarkers" of ASD that are present at birth; the presence of biomarkers at birth means that the disorder must have been present at birth, and not induced after birth. However, those biomarkers are long-established markers for inflammation,[90, 91] associated with oxidative stress, and thus are biomarkers for susceptibility to paracetamol-induced injury. Another objection might be that one drug cannot possibly be responsible for very different types of disorders such as ASD and ADHD. However, numerous investigators have pointed out connections between ASD and ADHD. For example, the two conditions are related in terms of diagnostic issues, treatment considerations, and risk factors;[92] symptoms of both tend to co-occur in many individuals;[93-95] and studies in animal models, described above, suggest that effects of exposure to paracetamol during early development can be complex.

Importantly, objections to the view that early life exposure to paracetamol induces many if not most cases of ASD are lacking a science-based explanation for the induction of ASD that fits available data.

Discussion

It is now apparent that the use of paracetamol during early development is common practice today because investigators half a century ago made the assumption that babies can be treated as small adults in terms of their reactions to drugs.[22] In this case, since liver damage is the hallmark of paracetamol-induced toxicity in adults, monitoring liver function in babies was assumed to be adequate to evaluate safety of the drug in babies. The profound dangers of this fallacy were known in the 1970s[96] but were apparently not as widely appreciated as they are today.[97] Indeed, studies during the 1980s using laboratory animals demonstrated conclusively that even lethal doses of paracetamol are not associated with extensive liver damage in newborns.[51]

Some of the reasons why widespread use of paracetamol in babies and children has continued for 40 years are perhaps obvious. The diagnosis of ASD is often separated in time from the administration of the drug, imposing some difficulty in determining cause and effect. In cases when both cause and effect happen near simultaneously, the reason for giving the paracetamol, for example vaccination or infection, can be blamed. In addition, the reductionistic focus of the scientific community on molecular and genetic mechanisms has been profoundly helpful, but without the exercise of backing away from the small pieces, the

larger puzzle cannot be solved. Some additional factors that have supported the continued use of paracetamol in the pediatric population are related to human bias. For example, the fact that the average child tolerates the drug well or at least appears to tolerate the drug well can be a source of bias among caregivers. This "anecdotal fallacy" reasons that, for example, "My child had the drug many times and is fine, so therefore the drug must be safe". This fallacy is well known, and is, unfortunately, both compelling and dangerous. Another bias, a "consensus bias", has developed within the community of caregivers with the underlying assumption that the drug is safe because it is commonly accepted and used. This consensus bias was reflected more than a decade ago in objections[61] to the first published evidence suggesting that paracetamol probably causes ASD,[60] and is still evident in recent objections[98] to the overwhelming evidence available today. Another source of resistance to the conclusion that the pediatric use of paracetamol is responsible for widespread and permanent neurodevelopmental disorders may come from anticipated ramifications of this situation for the medical industry. Understandably, if individuals perceive that their careers or reputations may be damaged by a given situation, they are subject to conflicts of interest that might affect their judgement. Further, individuals who have supported the pediatric use of paracetamol in the past may be emotionally compromised when faced with the possibility that they have caused harm, regardless of intention. Unfortunately, clinical reasoning and judgment are susceptible to emotional influence.[99]

The conclusion that postnatal use of paracetamol causes ASD in susceptible children should, if correct, be predictive of experimental outcomes: We predict that paracetamol induced toxicity in laboratory rats exposed to the drug between birth and age 10 days will be observed in the central nervous system. Further, we predict that non-lethal but severe paracetamol-induced toxicity in laboratory rats exposed to the drug between birth and age 10 days will be characterized by profound impairment of social interactions that are reflective of ASD in humans. Given that rats are highly social animals, similar in that regard to a variety of other mammals including humans, the experiments are likely to yield the predicted results if indeed postnatal use of paracetamol causes ASD in susceptible children. Finally, we predict that a reduction in the postnatal use of paracetamol in at-risk babies and children will result in a lower incidence of ASD.

Conclusions

At the present time, it appears that history is repeating itself: thalidomide, another drug that, like paracetamol, is converted into toxic metabolites by the human body,[75] caused developmental problems in thousands of children between 1957 and 1961.[100] It now seems very likely that the widespread use of paracetamol in the pediatric population constitutes a similar tragedy of even greater proportions. The scientific community may disagree on how strong the evidence is. But there should be no disagreement that (a) the evidence is very concerning, (b) physicians and the public should be notified of the current evidence, and (c) the gravity of the issue demands rapid resolution.

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