**LYONS-WEILER AND RICKETSON**

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Supplementary Material 1 – **UPDATED MARCH 19, 2018**

In 1989, the WHO (Technical Report Series 776), focusing on dietary sources, had recommended no more than 7 mg/kg/week for “aluminum”[12].  In 1996, the AAP Committee on Nutrition cited WHO Report #776 as 1 mg/kg/day, stating “it seems prudent to seek further reductions in the aluminum level of infant formulas” [14].

In 2007, the JECFA established a PTWI of 1 mg/kg/week, also derived from sources from diet in adult mice.

Unfortunately, in 2008, ATSDR, citing one study of dietary exposures, and using two unfounded transformations (because scaling dose toxicity by a factor of three from adult mice to human infants is spurious), reported that an MRL of 1 mg/kg/day reflected intermediate duration oral exposure [15] while the PTWI was updated to 2 mg/kg/week in 2011.

Mitkus et al. (2011) [32], citing ATSDR, used this outdated MRL and cited a gastrointestinal (GI) absorption of 0.78% in their model.  In reality, the GI tract usually absorbs 0.01-0.03% (i.e., 0.0001-0.0003) of aluminum from diet [38].

The modeling used by Mitkus et al. did not properly account for the differences in forms of dietary and parenteral or vaccine sources of aluminum, nor adult/infant weight differences.

Now, in 2018, the CFR/FDA allows 850 mg per dose, placing infants and toddlers well beyond the 1 mg/kg/week for infants.  Days with combined vaccines, or make-up days with multiple aluminum-containing vaccines are especially problematic. Infants in the NICU especially often receive aluminum from a variety of sources.

**PTWI Error**

Unfortunately, that JECFA expression of provisional tolerable doses in terms from weekly intake (PTWI) from all dietary sources including food additives (not daily intake) in 1989 went largely unrecognized in subsequent publications regarding the potential toxicity of aluminum in children. In 1996, the Committee on Nutrition, in their article for aluminum toxicity in infants and children, reported incorrectly that, “a provisional tolerable intake by the Food and Agriculture Organization of the United Nations and the World Health Organization is 1 mg/kg per *day*” (emphasis added). In 2011, the PTWI of 1 mg Al/kg was withdrawn by the JECFA and subsequently established a new PTWI of 2 mg/kg/week, applicable to all aluminum compounds in food, including food additives. These errors propagated into later estimates of safety profiling established by the Minimum Risk Level for aluminum in infants and children (15), and this error has influenced the medical community’s perception of human safe levels of aluminum intake, including but not limited to sources including diet and vaccines.

The total amount of aluminum acquired in adult humans from dietary exposure has been calculated by the ATSDR to be 2 mg/kg per day (Table S1). The human equivalent dose (HED) of 2 mg/kg/day was obtained by converting the amount 62 mg/kg per day in mice scaled to humans. That calculation was obtained by first dividing by the ratio of the body weight/body surface area for a given animal (e.g., for mouse, Km=3) and then dividing by a safety factor of 10 to obtain the 2 mg Al/kg per day in humans (21-23).

**Table S1. ATSDR References for NOAEL and LOAEL**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Population** | **Year Published** | **Route of Exposure** | **NOAEL** | **LOAEL `** | **Reference** |
| Mice | 1989 | Dietary | 62 mg Al/kg | 130 mg Al/kg | Golub et al 1989 |
| Mice | 2001 | Dietary | 26 mg Al/kg | 130 mg Al/kg | Golub et al, 2001 |
| Mice | 2005 | Dietary | 53 mg Al/kg | 103 mg Al/kg | Colomina et al, 2005 |
| Mice | 2000 | Dietary | - | 100 mg Al/kg | Golub et al, 2000 |

In infants and children, however, the amount of aluminum in dietary exposure is, as expected, much less. In children from 6 months to 6 years of age, the highest mean exposure to aluminum is 0.5 mg/kg body weight from all sources including additives (range 0.1-0.35 mg/day from dietary alone) **(Table S2)** (24). A single dose of 0.85 mg at birth (3.34 kg) adjusted by body weight would be 0.250 mg/kg, 50% of the highest mean exposure to aluminum from all sources in a 2-year-old child.

**Table S2. Dietary Intakes od Aluminum in Infants and Children**

|  |  |  |
| --- | --- | --- |
| **Age** | **Aluminum intake (mg/day)** | **Aluminum intake (mg/kg-day** |
| 6-12 months | 0.7 | 0.10 |
| 2 years | 4.6 | 0.35 |
| 6 years | 6.5 | 0.30 |
| 10 years | 6.8 | 0.11 |

A Minimal Risk Level (MRL) of 1 mg Al/kg per day has been widely used, including the ATSDR, to justify that all aluminum content in vaccines is safely at or below 850 micrograms (25-28). The Committee on Nutrition reported inaccurately in an article entitled “Aluminum Toxicity in Infants and Children” that, “the provisional tolerable (*the word “weekly” omitted in the article*) intake recommended by the Food and Agriculture Organization of the United States and the World Health Organization is 1 mg/kg per day”. That unfortunately is a misstatement of the JECFA report that established the PTWI at that point in time as 1 mg/kg per *week*, not 1 mg/kg perday. This is further propagation of the “per week” to “per day” error. Correcting that error by dividing the PTWI by seven days would suggest that the Minimal Risk Level (MRL) per day should have been approximately 0.14 mg Al/kg per day, importantly, inadults. As of 2011, with the newly established PTWI of 2 mg Al/kg, the daily provisional tolerable intake is therefore adjusted upward to 0.286 mg/kg per day.

The ATSDR states:

*“Using a NOAEL/LOAEL approach, the NOAEL of* ***26 mg*** *Al/kg/day identified in the Golub and Germann (2001) study was selected as the point of departure for the MRL (28). An MRL based on this* ***NOAEL*** *should be protective for neurological effects, neurodevelopmental effects, and for delays in maturation. Dividing the NOAEL by an uncertainty factor of 100 (10 to account for the extrapolation from mice to humans and 10 for human variability) and a modifying factor of 0.3 to account for possible differences in the bioavailability of the aluminum lactate used in the Golub and Germann (2001) study and the bioavailability of aluminum from drinking water and a typical U.S. diet results in an MRL of 1 mg Al/kg/day.” (16)*

To determine the MRL in adults, the ATSDR worksheets (17, 18) use the calculation where the NOAEL in humans is equal to the animal dose divided by the Km of the animal used in the study and then divided by the safety factor of 10. Here, the NOAEL of 26 mg Al/kg-day in mice (Mouse Km = 3) converted to the HED would be 26/3=8.67 mg/kg-day. Dividing that by the safety factor of 10 would result in a value for the HED for the MRL in adults to be 0.867 mg Al/kg-day (867 μg Al/kg-day.

It must be emphasized that using the Km ratio is not appropriate for determination of the NOAEL via intramuscular administration, as the local environment in which the vaccine is administered is subject to the local effects in the compartment (intramuscular) and is *not* supported for drugs administered by either the topical, nasal, subcutaneous, or intramuscular routes for the same reason (22). In consideration that the HED calculated by the Km ratio is not considered appropriate through the intramuscular route of exposure, the following equation by the Rule of Exponents is preferred to calculate the HED in adults:

HED=Animal dose NOAEL (mg/kg) x [Animal weight (kg)/Human weight (kg)] (1-BSA exponent 0.67) (22)

The calculated HED, and using the above equation, in a 60-kg adult would be 1.85 mg/kg. Applying the safety factor of 10 would result in the HED (MRL) of 0.185 mg/kg, significantly lower than the previously reported 1 mg Al/kg per day and approximates the JECFA calculation of 0.14 mg/kg per day.

The MRL equivalent in children can then be estimated by multiplying the adult HED by the BW(child)/BW(adult) ratio.

The JECFA established that in a 2-year-old child weighing 12 kg living in the US, the highest mean exposure from dietary sources is reported to be 0.5 mg/kg per day (500 micrograms/kg per day) (13,14). The Provisional Tolerable Weekly Intake (PTWI) from all sources and additives for children is currently established at 2 mg/kg (1000 micrograms/kg). In infants and children, therefore, the provisional tolerable *daily* intake (and the Minimal Risk Level) should be based upon the *daily* calculation from the PTWI. Accordingly, the expected MRL should be 0.29 mg/kg per day (287 μg/kg per day), not 1 mg/kg (1000 micrograms/kg) per day.

This considerable discrepancy between the MRL reported by the JECFA PTWI of 2000 micrograms/kg per week (287 micrograms/kg per day), the FDA dose of 1000 micrograms/kg-day calculation in adults, and the HED Km ratio calculation above *with the safety factor applied* is a cause for grave concern.

Mitkus et al(33) in their study published in 2011 when the PTWI was still at 1 mg/kg, further propagating the day/week error by their calculations of the MRL in infants and children leading to their conclusion that the body burden of aluminum from vaccines and diet throughout an infant’s first year of life is significantly less than the corresponding safe body burden of aluminum (1 mg/kg-day) modeled using the regulatory MRL. In consideration that the regulatory MRL is twice that what it maximally should be in children at 2 years of age (0.5 mg/kg-day), their conclusion is invalid because that corresponding MRL safe daily body burden of aluminum (1 mg/kg-day) using the correct 0.5 mg Al/kg per day reference in children would be 50% less than their calculated MRL using the incorrect 1 mg/kg per day regulatory reference. The accumulated body burden aluminum from vaccines would clearly be much greater than the regulatory MRL. Mitkus et al (33) also averaged exposure over 365 days, ignoring the potential for acute toxicity, genetic and biological variation in tolerances, as well as the effect of repeated doses of aluminum on the brain (neurotoxicity and gliosis).

Any modification to the amount of aluminum in adjuvants would come with a significant cost. Malakoff in 2000 (36) stated:

*“…manufacturers say any effort to replace aluminum with another adjuvant would be costly and complicated. Regulatory and manufacturing requirements, for instance, would make it "a nightmare" to create different formulas for an initial vaccine and its booster, says Nathalie Garcon-Johnson of SmithKline Beecham Biologicals in Rixensart, Belgium.”*

This conclusion comes with an important and even more dire caveat. These calculations are based on accepted models considering any possible (whole-body) adverse events, and on clearance data acquired from adult animals. Safety levels of aluminum are derived from adult micewhose exposure was from a dietary source, not an injection into an anatomic compartment. No safety levels for aluminum in any form has been determined for mice undergoing brain development. Several studies have emerged since the Golub studies in adult mice used to define

the MRL, NOEAL, and LOAEL contained within the ATSDR (31,32, 35).

Tomljenovic et al in 2011 (31) reported a LOAEL of 172.5 µg/kg in humans that would result in a predicted NOAEL of 73.4 µg/kg (LOAEL/2.35). Applying a standard safety factor of 10 would result in a calculated safe dose of 7.34 µg Al/kg. The safety-factor corrected MRL (Tomljenovic et al. (31) closely approximates our initial calculation of the MRL (7.8 μg Al/kg per day) using the JECFA adult reference of 0.14 mg/kg (140 μg Al/kg) per day from all sources and all doses, but less than when recalculated at 0.29 mg/kg per day, the most recent level established by the JECFA during the 74th meeting.

While tissue fates and compartment studies are important, the Priest et al study (34), and the FDA study confused LOAEL and NOAEL, do not address the whole-body adverse event studies. While one can find fast clearance from some compartments, and can study clearance over long periods of time, our concern is the acute empirical toxicity of doses of AL, demonstrated on whole-body studies. When pediatric doses are considered using body weight, vaccines are determined to include doses that exceed regulations based on adult animal dietary studies. Spreading consideration of risk over longer periods can be done mathematically, as in Mitkus et al. (33), but that step begs the questions of acute neurotoxicity. Very long-term accumulations of aluminum appear to play a role in Alzheimer’s Disease, as stunning levels of aluminum concentrations greater than 10 μg/g have been recently found by Dr. Chris Exley and his associates in the brains of afflicted patients (33).

Studies are needed right away to provide estimation of NOAEL of injected doses of aluminum in the context of the biological processes that occur during infant brain development (infant animals) to assess the suitability of aluminum for use in pediatric vaccines. The utility of the CFR/FDA limit for parenteral exposure (45), which does not exclude vaccination, as a reference point for neurotoxicity of aluminum should be tested using developing mice injected with body weight-adjust doses of aluminum representative of single and jointly administered vaccines.

References

See original research article for cited references.