Canine hair as a model for tracing ethylmercury from Thimerosal-containing vaccines

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Hair-Hg concentration (total or chemically speciated) is an accepted criterion for Hg monitoring related to exposure from fish and/or vaccines. Sedlackova *et al.* (2013) made a timely contribution modelling total Hg in hair of dogs to assess post-vaccine status with Thimerosal-containing vaccines (TCV), thus providing an opportunity to update relevant issues.

Recognizing the need to assay post-vaccine ethylmercury (EtHg), I support the rationale of Sedlackova *et al.* concurring that hair-Hg in dogs' hair should be carried out under assumptions valid for human hair. In this matter there are several aspects deserving attention:

- First, we should ask what the proportion of organic to inorganic Hg is in dogs' hair. It is assumed to be around 80% for methylmercury in humans. During the growing phase of hair (assuming that it is comparable to human), keratinocytes are the main tissue structure that capture Hg (Schoeman *et al.* 2010); under this assumption TCV-EtHg can end up in hair directly (see references 11 and 12 in Sedlackova *et al.*) or as inorganic Hg due to its instability, as suggested by Sedlackova *et al.* Nevertheless, we should also consider that not only do sulfurkeratin proteins in hair possess an affinity for Hg; other hair structures can also accumulate metals (Dórea & Pereira, 1983).
- In relation to post-vaccine change in hair-Hg concentrations, it was no surprise that they did not find significant differences. Because the total exposure of vaccine was not stated, and we

were not informed the rate of hair growth and/ or hair turnover, such crude measures allowed no adjustment amenable to kinetic interpretation. Indeed, in human hair, chemical speciation techniques have shown that the amount of EtHg captured after vaccination is minimal (see references 11 and 12 in Sedlackova *et al.*). Using this analogy, TCV-EtHg may not be quantitatively sufficient to appear in dogs' hair.

- Sedlackova *et al.* used a vaccine against rabies (with 0.01% of Thimerosal), but did not inform if the animals had been previously immunized with the other TCVs they mentioned (rabies; tetanus; leptospirosis; distemper; distemper and parvovirosis; infectious hepatitis; infectious laryngotracheitis; parvovirosis; and parainfluenza). Crucial for the kinetic of vaccine-EtHg, as recognized by the authors, is information on the dosage, i.e., that which we could have from weight of the animals (these data were not presented).
- There is another methodological issue worth reconsidering, i.e., statistical analysis. In the case of time-measure of the same variable a 'repeated measurements analysis' seems more appropriate to test retrospectively the sampling effect of time-related changes in total Hg concentrations. Additionally, because of intra and inter-variability of hair-Hg concentrations, I would also recommend integrating the data as function of time-0, i.e., recalculating all changes as a percentage of that initial value for all dogs individually.

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- For the sake of contextualization, the median values of hair-Hg in Sedlackova *et al.* (23 to 52 ng/g) are in the lower range of those reviewed by Souza *et al.* (2013) in different parts of the world. Fish intake in sledge dog was associated with high values reported in most studies, thus indicating that methylmercury impacted hair-Hg concentrations. Additionally, while in humans the blood:hair ratio is around 250, in dogs these ratios have varied from 50 to 200 (references in Santos *et al.* 2013).
- Last, but not least, the authors discussed their data as a function of vaccine-Hg half-life in blood of human infants; they should consider that once in the human brain, inorganic-Hg half-life seems to be several years five years or longer (Rooney 2013).

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