Reviewer's report

Title: Early life microbial exposure and fractional exhaled nitric oxide in school-age children: a prospective birth cohort study.

Version: 2
Date: 30 October 2013

Reviewer: Peter Franklin

Reviewer's report:

I am satisfied that the authors have addressed most of the issues I raised, although I still remain concerned about 2 of them: FeNO as a marker of eosinophilic inflammation and the relationship with allergic sensitisation. These were addressed but not satisfactorily. I consider these both important issues but one of them, inflammation, I will categorise as a discretionary revision. This is because there is a general acceptance that FeNO is a marker of eosinophilic airway inflammation (despite ongoing uncertainty).

Major compulsory revision

The authors were able to investigate an objective marker of allergic sensitisation (IgE) that had been measured in some, but not all, of the children. However, the inclusion of allergic sensitisation did attenuate the effect sizes such that they lost significance, although as the authors stated the direction of the effects did not change.

Early exposure to dogs and endotoxin have been associated with reduced allergic sensitisation in later childhood. Allergic sensitisation, with and without disease, has been associated with increased FeNO. Therefore, it is possible that in this study FeNO was a marker of allergic sensitisation and the early exposures are associated with sensitisation and not inflammation per se. Did sensitisation remain significant when included in the models? I am aware that sensitisation was measured at different time points for each of the centres and I agree that this adds a level of complexity. However, no attempt was made to explore the potential implications of these relationships.

Discretionary revision

The authors have acknowledged there is some uncertainty around what FeNO represents but state that it is ‘...most extensively studied biomarker of airway inflammation and its non-invasive nature makes it suitable for epidemiological studies’ (p.17). They continue to explicitly state on a number of occasions (abstracts, Introduction p5, Discussion p16, 18) they that they are measuring FeNO as a biomarker of eosinophilic airway inflammation. I would argue that just because it is the most extensively studied biomarker of inflammation doesn’t necessarily make it a biomarker of inflammation. In the extensive literature around FeNO there is evidence that brings the relationship between FeNO and eosinophilic inflammation into question (eg. it doesn’t exist in the absence of
atopy, it varies depending on the biological compartment where eosinophils are measured, the relationship changes with treatment in a discordant manner). By no means do I discard the possibility that FeNO reflects some aspect of (allergic) airway inflammation but I still feel there is sufficient uncertainty to warrant more cautious language.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests