Reviewer's report

Title: A Prospective Cohort Study of Biomarkers of Prenatal Tobacco Smoke Exposure: The Correlation between Serum and Meconium and Their Association with Infant Birth Weight

Version: 1 Date: 20 April 2010

Reviewer: Teresa Gray

Reviewer's report:

This paper compares meconium testing, maternal serum testing and maternal self-report for detecting prenatal tobacco exposure. Meconium testing has been used to document prenatal drug exposure for nearly 20 years; however, the authors add a novel aspect by comparing meconium results to another biological matrix, maternal serum. However, the authors provide too few details about the analytical procedure used to analyze meconium specimens, and as a result, subsequent comparisons and conclusions are difficult to interpret.

Major Compulsory Revisions –
1. Abstract, Methods – This section seems repetitive and should be clarified.
2. Abstract, Conclusions – The conclusion is too vague for the data presented. The authors should limit the statement to say that meconium is a useful biological matrix for measuring environmental tobacco exposure. Given that meconium has been used to identify tobacco exposure for more than 20 years, it probably should not be considered “promising.”
3. Introduction, paragraph 1 – To my knowledge, there are no data to support the authors claim that meconium is metabolically inert, therefore the authors should add a reference or remove this statement. More importantly, recent prospective data demonstrates that drug exposure during the second trimester (as evidenced by maternal urinalysis) is not reliably reflected in meconium, thus the drug detection window of meconium spans just the third trimester. The authors are referred to Clin Pharmacol Ther. 2008 Nov;84(5):604-12.
4. Introduction, paragraph 2 – The authors should specify that the metabolic percentages given are for adult humans as the metabolic capacity of fetuses for nicotine and metabolites are unknown.
5. Methods, Tobacco Smoke Measurements - Why were the self-report periods defined as conception – 20 weeks and 20 weeks – birth? Mid-point of gestation? Also, why were serum specimens collected at 16 weeks, 26 weeks and at birth? What if the women enrolled after 16 weeks; was a serum sample taken at enrollment? How long was meconium collected (i.e. 48 h or until the appearance of milk stool)? Kohler et al demonstrated that nicotine and 3HC concentrations in meconium change in successive passages, so were all meconium specimens collected per child?
6. The LOD reported for the serum assay was 0.015 ng/mL, a value
approximately 100 times lower than what has been reported in recent literature by multiple investigators. Was this LOD established empirically (i.e. with decreasing concentrations of analytes fortified in matrix)?

7. The major limitation of this paper is that the authors do not adequately describe the analytical method used to evaluate meconium. Because the analytical method presumably has not been published, much more detail is necessary. From the given information, it appears that the LC-MS/MS method was not evaluated according to FDA or EU guidelines for bioanalytical methods and has significant deficiencies. The lack of details makes it impossible for the reader to assess the subsequent prevalence findings. The authors should address the following:

1) Which deuterated internal standards were used?
2) More details on the extraction procedure are necessary – particularly the solid phase procedure step. Reference 4 is a review article and does not describe a specific solid phase extraction procedure. Is this the correct reference? Please either give another reference or describe the SPE procedure.
3) What were the conditions for LC-MS/MS analysis, particularly what MS/MS scan mode was employed? Assuming MRM transitions were monitored, how many transitions were analyzed per compound? EU guidelines and most clinical and forensic applications utilize two transitions per analyte and one or two transitions for the internal standard.
4) Because this is a prevalence study, the authors must clearly define how the LOD was determined. Please describe what criteria were required for the LOD (the lowest concentration of an analyte that the bioanalytical procedure can reliably differentiate from background noise) and whether the assay had a separate lower limit of quantification (LOQ, the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.) If the authors do not have a separate LOQ, please explain why. Was the LOD evaluated empirically with a spiked meconium specimen to determine if a peak was discernible from noise? The reported LODs for cotinine and 3HC are nearly 10 times lower than other published meconium methods; how were the authors able to achieve such low limits and maintain adequate identification criteria?
5) The quantification procedure does not appear to follow normal clinical and forensic standards. Were calibration standards used? Here I am defining calibration standards as a series of samples of the same biological matrix to which a known amount of analyte has been added or spiked that is used to construct calibration curves from which the concentrations of analytes in QCs and in unknown study samples are determined. Was multi-point linear regression quantification curve generated? A single point calibration? A historical curve?
6) How did the authors confirm that their “blank” matrices were, in fact, blank? In our experience, finding negative meconium specimens can be rather challenging.
7) Why were the authentic QC materials treated differently than the unknown specimens? The authors use 0.5 g of meconium per assay for the unknown samples, but only 0.125 g of QC meconium per assay. Using 4 times less
meconium for the QCs would affect extraction efficiencies, sensitivity and matrix effects. Also, QC samples were mixed for 2 hours; were unknown samples treated the same way? Were the authors concerned about analyte stability in the extremely alkaline environment?

8) Furthermore, how were the QC concentrations initially determined? Were the low concentrations near the LOD to challenge the accuracy of low concentrations? The RSD for the low nicotine concentration seems high, and while the rest had good precision, what was the accuracy of the concentrations?

8. Statistical analysis - The statistics section is excessively wordy. It is enough to say, for example, the three serum cotinine measurements were averaged and women categorized as nonexposed (<LOD), secondhand exposed (LOD-3 ng/mL) and actively exposed (>3 ng/mL).

9. Why did the authors choose to impute values <LOD for geometric mean determinations? What values were imputed (0.5*LOD?). Also, why did the authors choose to use a geometric mean over the arithmetic mean? Most literature on meconium reports the arithmetic mean or median + range of observed concentrations along with the % positive.

10. Results, Paragraph 1/Table 1 –the authors should add statistical support (p-values, etc) to the comparisons between those with complete and incomplete data.

11. Results, Relationship between self-report and biomarkers of tobacco smoke exposure – The prevalence of tobacco metabolites in meconium and serum was surprisingly high and contrary to other literature. In our experience, nicotine, cotinine and OHCOT have similar prevalence, but the authors found nicotine at much higher rates than cotinine and OHCOT. This is particularly interesting given that the LODs of cotinine and OHCOT are approximately 10X less than nicotine’s. Could the author’s please comment on these findings? Also, Kohler et al. and Gray et al. showed that tobacco biomarkers were not present in meconium of babies whose mothers were nonsmokers or environmentally exposed. While previous research does show higher prevalence and concentrations in non- and passively exposed individuals, the analytical methodology was limited to non-specific immunoassay. Why do the authors think they observed such high rates? The 95% CIs for nicotine, cotinine and 3HC are very small. Do the authors have any theories as to why the concentration ranges were so similar between babies? In paragraph 3, the authors say that SHS-exposed babies have higher meconium concentrations than non-exposed – is this difference statistically significant?

12. Table 2 –It is very difficult to believe that so many unexposed and SHS exposed children had detectable meconium concentration levels, given the targeted population and previous literature demonstrating no tobacco biomarkers in non-exposed and environmentally exposed infants. Also, the geometric means for the non and SHS exposed groups are so close to the LOD and have such narrow CIs (which may be skewed by imputing data), but the authors should comment.
13. Table 3 – It is interesting that there appears to be no meaningful difference in birth weight between SHS and active exposure when defined by meconium or serum levels. One would expect more severe deficits among the actively exposed, but all seemed to be relatively healthy (>3200 g). Why did the authors differentiate SHS and active smokers at a 5 ng/g cutoff for cotinine and 10 ng/g for nicotine and OHCOT?

14. Discussion, paragraph 1 – the authors’ data also suggest that meconium concentrations may also reflect the timing of tobacco use, as concentrations were higher if the mother was deemed SHS-exposed at birth as compared to at 16 weeks.

15. Discussion, Paragraph 2 – again, the authors should comment on why they found much higher prevalence of tobacco biomarkers in meconium compared to other recent investigations (i.e. Pereg, Kohler and Gray), particularly among babies whose mothers were non-smokers or environmentally exposed.

16. Discussion, Paragraph 4 – The authors cite that Gray et al had similar COT and 3HC prevalence, but overall prevalence in their study was much lower (~35-40%) than the current study (~55-70%). Also, the authors attribute differences in prevalence between studies to analytical parameters, but another important component, namely the targeted study population, should be mentioned.

17. Discussion, Paragraph 5 – there are other factors which may influence meconium metabolite concentrations including the amount of meconium deposited in the gut, the time of meconium collection relative to birth (Kohler et al showed that concentrations of NIC and OHCOT varied over sequential meconium passages), extracorporeal urine contamination within the diaper. Also, the authors should provide a reference for the last sentence and summarize what other investigators have found and if this differs from what they observed.

18. Discussion, Paragraph 6 – why did the authors choose serum over another less invasive matrix such as maternal urine? Also, the cited references seem to support the previous paragraph, not the use of serum to identify and classify smoking status.

19. Discussion, Paragraph 7 – The authors conclude that meconium is an adequate matrix for identifying prenatal tobacco exposure, but suggest using serum cotinine concentrations for quantifying prenatal exposures because meconium samples require additional resources. Could the authors please elaborate on the additional requirements? Meconium is more easily and less invasively collected than serum and tobacco biomarkers can be easily extracted from meconium with a simple solid phase extraction procedure without significant specimen pretreatment. The initial meconium digestion and initial liquid liquid extraction performed by the authors could be eliminated without severely compromising detection of tobacco exposed neonates as Gray et al showed that hydrolysis of meconium with enzyme (analogous to the basic digestion performed by the authors) only identified one additional neonate.

20. Discussion, Paragraph 8 – What are the authors referring to by “associations
among active smokers?" Relationships between dose-serum/meconium concentration, dose-infant outcome, concentration-outcome? Please elaborate.

21. Discussion, Paragraph 10 – Since the study population was of relatively high SES, which has lower active and SHS exposure, are the authors surprised at the nearly universal detection of cotinine in at least one serum sample?

22. Discussion, Paragraph 11 – Are the authors suggesting that meconium concentrations can be used to predict the number of cigarettes (i.e. dose) the mother smoked? Because of the analytical deficiencies in the meconium method – namely the determination of LOD - it is very difficult to agree with the authors that nicotine in meconium indicates transient tobacco exposure.

23. Conclusion – “Additional research should determine meconium’s ability to measure gestational exposure to other environmental toxicants.” Please remove as it is not germane to this manuscript.

Minor Essential Revisions
1. Please change gm to g throughout.
2. Abstract, Results – the authors should specify that meconium tobacco smoke metabolite concentrations were inversely related with birth weight. As the sentence is currently constructed it is not clear what is inversely related.
3. Introduction, paragraph 2 – Why do the authors describe active smoking as environmental?
4. Introduction, paragraph– The first sentence requires a reference.
5. Introduction, Paragraph 4, sentence 2 – please change the word “women” to “babies.”
6. Introduction, Paragraph 5 – please move reference 17 to after National Children’s Study, as it seems as though the National Children’s Study is suggesting that the validation be conducted.
7. Methods, study sample – some inclusion criteria are listed twice. Please revise.
8. Methods, Tobacco Smoke Measurements – Please clarify if the number of cigarettes smoked per day in the home was for the mother or her housemates. Why switch between ng/mL and pg/mL when discussing the %CV of the serum assay?
9. Table 1 – the marital status data does not add up correctly – 217+31=248 not 249. Also please add p-values.

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:

I declare that I have no competing interests.