Reviewer's report

Title: Bone resorption and environmental exposure to cadmium in children

Version: 1 Date: 15 August 2011

Reviewer: Maryka H Bhattacharyya

Reviewer's report:

RESPONSES TO SPECIFIC QUESTIONS:
1. Is the question posed by the authors new and well defined?
Yes. Others have evaluated relationship between urine cadmium concentration and bone status, but to my knowledge, not in this age group.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
The methods appear to be appropriate, but they need to be described in more detail. Places where this aspect needs attention are identified in specific comments below.

3. Are the data sound and well controlled?
Yes, in general. But places are identified below where improvements should be made in presentation of results, in particular regarding inconsistencies in values presented.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
Yes. Again, suggestions are made that can add to the discussion.

6. Do the title and abstract accurately convey what has been found?
In general, yes. However, the values chosen for presentation of results in the abstract are different from any that are presented in the body of the manuscript, as identified below.

7. Is the writing acceptable?
In general, yes. But the text needs the minor attention of an editor to correct sentence structure and/or word use in places. Some are identified below.

GENERAL COMMENTS: The authors present the results of a study showing a significant direct correlation between urine cadmium concentration (measure of Cd exposure) and urine calcium and DPD concentrations (measures of bone status) in 160 children 8-12 years of age living in Lahore, Pakistan. Although such studies have been conducted in adults, in particular postmenopausal women, a study in this age group is new. One strength of the study is the large
number of children and the clarity of the results. A weakness is the lack of attention to detail applied by the authors regarding presentation of methods and consistency of values reported at different points in the manuscript (see specific comments below).

SPECIFIC COMMENTS:

Major Compulsory Revisions: There were no major compulsory revisions that would affect a decision on publication.

Minor Essential Revisions: The following are essential minor revisions. Each is important but is such that the authors can be trusted to respond appropriately.

Methods: ‘Measurements in Urine’ section:

1. The authors should give the range in times-of-day during which their spot urine samples were collected, because they indicate that time-of-day affects urine DPD concentration, and because they account for time-of-day in their data analyses. Were most samples collected at one time of day?

2. The authors need to indicate if care was taken to account for calcium precipitation when their urine samples were frozen. If not, their urine calcium values are low. Calcium precipitation – e.g., 23% of total calcium for a sample in mid-range of normal -- has been documented, even when urine is frozen at 20°C for one overnight (doi:10.1152/ajprenal.90736.2008). One approach is to acidify the urine before aliquots are taken for calcium analysis. However, the described protocol indicates that a 500 µl aliquot of the thawed urine sample was taken, and then the aliquot was diluted with acid in preparation for analysis. Neglecting calcium precipitation may not have been a critical flaw [because precipitate amount was proportional to urine Ca concentration (above reference)], but this aspect of study protocol should be addressed.

3. The authors should give the limit of detection for cadmium in urine and other characteristics of the Cd analyses, e.g., intra- and interassay coefficients of variation. As is, one COV value is given, but it is not clear which assay this value refers to (probably DPD ELISA). The current approach lacks detail that is needed for this report to stand on its own.

Results:

4. Table 1 and Figure 1. The authors need to address the point that results for urine Cd concentration (Cd-U) in Table 1 appear to disagree with those presented in Figure 1. The median Cd-U value in Table 1 is 0.54 µg Cd/g creatinine, and the 95th percentile value is 0.58 µg Cd/g creatinine. Those results mean that only eight values of the 160 should be >0.58 µg Cd/g creatinine (upper 5%). The latter spread from Table 1 is very different from the spread of Cd-U values shown in Figures 1a and 1b, where many more than eight values are > 0.58 µg Cd/g creatinine. Maybe the 95th percentile value for Cd-U is wrong in Table 1.

5. Table 1. The value of this important dataset would be enhanced by adding two columns to Table 1 that give individual datasets for the males vs. females, in addition to the combined column already presented. For other age ranges, data
for males vs. females have been broken out in published reports. Expansion of this table will allow other investigators to compare gender-based differences across ages, including the new age range presented here.

6. Table 2. The 'b' superscript on the second 'calcium' biomarker line appears to be in error. It is not clear how a correlation between urinary calcium and urinary cadmium can be adjusted for urinary calcium, as indicated by the superscript. Probably the added adjustment for the calcium biomarker line was for DPD.

7. Results 1st para and Table 2. The authors need to address the point that results in the text differ from those in Table 2. The text states that a doubling of urinary Cd corresponds to an increase in urine DPD of 1.86 ng/g creatinine (95% CI: 1.52 to 2.29; p: <0.0001), taking into account urine Ca, along with gender, age, height, weight and socioeconomic class. In Table 2, the values for this same regression are similar but not identical (1.80: 1.44 to 2.22; p: <0.0001).

8. Discussion, 2nd para. Comparison of urine cadmium concentrations in Pakistan (this study) vs. Europe should use values with the same units – either µg Cd/g creatinine or µg Cd/L urine. As is, the authors use different units for the values they compare. Granted the numerical values for these two units are often similar, but readers will not in general know that.

9. Figures 1a and 1b. It is suggested that the data-points be changed so they indentify gender (e.g., by different shapes), to allow the reader to visualize the relationships for the girls vs. boys in the combined dataset. From gender-based differences in means reported here, the girls should be clustered high and the boys low for both Cd U and DPD.

10. Abstract vs. Body of Manuscript: The expressions of results in the abstract need to be changed to match those given in the body of the manuscript. For example, the 71% increase in DPD for a doubling of Cd U given in the abstract is expressed in Table 2 as regression coefficient values of 1.73 and 1.81 for a doubling of urine Cd. Similarly, a 33% increase in urine Ca (p = 0.0006) for a doubling of Cd-U is given in the abstract, while regression coefficients of 1.24 and 1.31 for a doubling of Cd-U (p<0.0001) are given in Table 2.

Discretionary Revisions: Following are several discretionary revisions that came to mind but which the authors can choose to ignore.

11. The authors have the unusual opportunity to evaluate specificity of the cadmium results they report, because they used ICP-MS to analyze many metals in urine. I suggest providing an evaluation of lead in urine vs. bone indicators also, to investigate specificity regarding the cadmium results. These two metals co-exist in nature and both have an effect on bone. Other investigators, though not many, have done this and found a statistically significant correlation for cadmium but not lead. These results strengthen the argument of cause and effect for an experiment where only correlations can be made.

12. Discussion, General: Inclusion of a discussion is suggested regarding why the measures of bone demineralization (urine calcium) and bone resorption (urine DPD) were not themselves significantly related to one another. These markers are both individually related to urine Cd and are each used as indicators...
of Cd-induced bone loss. The hypothesis seems to follow that, when cadmium increases bone demineralization (urine Ca), it would also increase release of collagen cross-links (urine DPD), giving reason to expect a relationship between the two bone markers, but this is not the case.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

My research has been funded by an extramural program of Philip Morris. Cigarettes are a significant source of cadmium exposure. However, any interaction between myself and the company was strictly disallowed by the extramural program administration. No restrictions were placed on publication of results, and no knowledge of results were requested prior to publication.