Reviewer's report

**Title:** Comparative measurement of CNP and NT-proCNP in human blood samples: a methodological evaluation.

**Version:** 1  **Date:** 7 January 2013

**Reviewer number:** 4

These authors sought to determine the stability of endogenous CNP and NTproCNP concentrations during the collection and subsequent blood sample processing. Primarily their study was to determine whether, (1) delayed processing of the blood samples stored at room temperature cause bias, (2) there were any difference in measured concentrations between serum, EDTA plasma or whole blood samples collected for CNP or NT-proCNP analysis, and (3) are these deviations time dependent?

This is well-constructed study that is generally well presented.

**Major Compulsory Revisions:** - none

**Minor Essential Revisions:**

1) Pg 2 (lines 11, 12, 15) and Pg 5 (lines 2, 6, 7, 19), the number of significant figures describing the upper and lower bounds of the data should not exceed 2 significant figures (preferably only 1 significant figure should be presented). For example:- 0.997 +/- 0.379ng/ml should be replaced with 1.0 +/- 0.1 ng/ml.

2) Pg 4 Methods section, please provide assay detection limits, and inter- and intra-assay CVs for both the CNP-22 and NTproCNP assays.

3) Pg 4 Methods section (lines 15 – 17), according to the Phoenix Pharmaceutical “CNP-22 EIA Kit (#EK-012-03)” protocol, plasma samples are to be extracted over C-18 Sep Columns. It would seem unlikely or unwise that whole blood samples would be treated in this manner. If not, how were whole blood samples assayed/extracted. Were whole blood samples assayed directly? Details as to how plasma, serum and whole blood samples were extracted needs to be provided for both the CNP and NT-proCNP analyses.

4) Pg 4 (lines 18-20), Pg 5 line 5). Please use repeated measures ANOVA to compare baseline serum, plasma and whole blood data. Please use an appropriate Post Hoc method to test for differences between the groups (the use of unprotected Student’s t-test is inappropriate when comparing more than 2 groups).

5) Pg 5 final line should read “…the reference range of healthy individuals (3.7-168 pg/ml) reported in other studies (table 1).” 432 pg/ml is incorrect. This figure was derived incorrectly from reference 18 (Olney et al J Clin Endocrinol Metab 2007). The actual data point as describe in Olney et al was 33.8 +/-7.8 pmol/L. NT-proCNP (a peptide containing 50 amino acids) reputedly has a
molecular weight 4985 Da. It is unclear how the present authors made the conversion from pmol/L to pg/ml. The correct formula for the conversion is: Molecular weight multiplied by [concentration in pmol/L] divided by 1000000.

6) Pg 13 Table 1, all data points for NT-proCNP that were converted from pmol/L to pg/ml are incorrect. For example line 16 (Palmer 2009) NT-proCNP column should read 86 pg/ml; Olney 2007 line 24 should read 165pg/ml, line 25 214pg/ml, line 26 168; Prickett 2001 line 30 48 pg/ml, line 31 37 pg/ml.

7) Pg 12 Figure 1 Legend, please provide a description for the asterisk shown on figure 1.

Discretionary Revisions:-

The reviewer is surprised at the large difference observed (approximately 1000 fold) in CNP measurements between data derived using EIA compared to RIA method. Has the EIA method used been properly validated? For example has it been demonstrated that plasma samples and Standards show parallelism when dilute. Has size exclusion HPLC been used to demonstrate that the assay is detecting only appropriately sized molecules and not confounding high molecular weight proteins or other confounders contributing to the “matrix effects” that confound immunoassays.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.