Reviewer's report

Title: The aluminum concentrations in central and peripheral areas of malignant breast lesions do not differ from that in normal breast tissues

Version: 1 Date: 10 August 2012

Reviewer: Chris Exley

Reviewer's report:

Major Compulsory Revisions

1. The Introduction, specifically in relation to human exposure to aluminium, is weak and lacking in appropriate and seminal references. In relation to this I personally avoid citing any publications by Priest as he is funded by and is a consultant to the global aluminium industry. This was pointed out by myself to the Journal of Environmental Monitoring (7, 640, 2005) in a Letter to the Editor about the paper the authors have cited [1].

2. Our research [4] has not been accurately cited. We showed that the Al content of the outer breast region (axilla + lateral) was higher than the inner breast region (mid+medial). Additionally, we did not make any reference to whether or not the tissue donors used Al-based antiperspirants. We did not have this information.

3. It is really not the case that previous studies which measured the Al content of breast tissue used inferior analytical methods. For example, we used GFAAS in our study [4]. This emphasis on the improvement in analytical technique should really be removed.

4. The Materials and Methods does not include sufficient information about the measurement of Al in the breast tissues. The authors emphasise their approach to measurement as a key part of their research and if they wish to do so then they must include much more information relating to the tissue Al measurements. By way of example, the authors might wish to consult our recent paper on measuring Al in brain tissue (Metallomics 4, 56-65 2012).

5. The authors might note that we also did not find any significant differences between the mean Al content of the 4 breast regions in our study. However, when the data were analysed to take account of the within-individual trends it is then that significant differences between the outer and inner regions of the breast are found. Perhaps similar statistical analyses as described in our paper [4] could be carried out on this data set?

6. The authors should not continue to make the somewhat spurious claim that their analytical method is superior to previous methods measuring Al in breast tissue.

7. To my knowledge, there has not been any previous attempt to compare the Al
content of ‘diseased’ and ‘healthy’ tissues taken from breast mastectomies? Our research [4] simply looked at the location of the breast tissue sample and not its ‘disease’ state. If the authors want to make this distinction then they need to make it very clear as to the exact locations of each breast tissue sample, as mentioned previously, a diagram might be helpful. I would then suggest that they then apply more sophisticated statistical methods (see our paper for an example) than simply comparisons of mean Al content to try to ascertain whether for each individual there are any trends in the distribution of Al between the sampling regions. For example, the Al content of the tumour regions could be higher in all 150 individuals but because of the heterogeneous nature of Al accumulation in tissues these findings would be obscured by simply looking at mean Al contents.

8. It is clearly wrong and disrespectful to previous research to suggest that there have been no previous ‘precise’ measurements of Al in breast tissue! Please remove this statement or qualify it in some way.

9. This manuscript can claim to have at its disposal the largest number of tissue donors. It is now very important that the data obtained from these donors are validated and analysed using the most appropriate statistical methods.

10. It is not appropriate to compare these data with our data [4]. In the first instance the locations from which the tissues were taken do not seem to match up with those in our study. Secondly we separated the breast fat (oil) from the tissue and measured the two fractions separately. The relationship that we found was with Al associated with the defatted tissue and not with the oil. Incidentally, we have just completed a new study where we did not make this distinction and when we looked at whole tissues we did not see any differences in the regional distribution of Al, as is suggested in this study.

11. The authors need to explain their comment concerning ‘calcium-aluminium compounds’!? What are these? The authors do seem to be a little confused with respect to the biological chemistry of aluminium.

12. Overall, there is much in this manuscript which should eventually be published. However, much more detail, including the use of appropriate controls such as method blanks et c., is required concerning the measurement of Al in the tissues. We also need to be clear as to the exact location from which each tissue sample was taken. The authors should then statistically analyse the Al content of each location for each donor, perhaps in a manner similar to ourselves [4] so that important data can be obtained on whether or not there are any differences in the Al content of the different regions. Mean values for each region are simply not sufficient.

Minor Essential Revisions

Discretionary Revisions

1. A diagram might be helpful in explaining the location of different tissue samples relative to the position of the tumour. I was not able to understand fully
where each sample was taken from.

2. The mean measured Al contents given as mg/kg dry wt. are very similar to those in our study [4] which varied from 1.7 to 2.1 #g/g dry wt. It is really quite easy to convert between nmol and ng, simply divide by the atomic wt. of Al.

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

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

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

**Declaration of competing interests:**

We have a manuscript in preparation which, similarly to this one, has measured the Al content of whole, as opposed to defatted, breast tissues.