envenomation has been used to launch wildly extrapolative press releases despite over 40 years of using vinegar as a first-response treatment without a clear case of death resulting from its use. Statements such as "*For decades experts have recommended vinegar to treat box jellyfish stings. Now, Queensland researchers have discovered the cure can kill*"[2] are simply not true; there was no death or killing in the Welfare et al study.

Claims that "*Vinegar may kill rather than cure victims of box jellyfish stings ... The remedy, used for decades to treat*

Reply:

We thank Yanagihara and Chen for their comments and for the opportunity to further the discussion.[1] Our statistician has re-examined (and reanalysed) these data, and we have supplied our data to an independent statistician (who supported our subsequent re-analysis) and are more than willing to supply these data to the journal editors should they feel this is necessary. Furthermore, the manuscript was independently reviewed by two reviewers who expressed no concern over our analysis. We are confident of our results.

Yanagihara and Chen have incorrectly assumed that the errors displayed in Figure 2 are SEM. These errors represent the 95% confidence limits (CL) and as such their arguments are invalid. Furthermore, they outline that no statistical significance was provided for the specific W4 vs. W3 comparison. Although no specific statistics were displayed in the article, we do outline that LSD post hoc analysis was conducted and the means and 95% CL (as signified in Figure 2) that were significantly different were listed. This analysis showed that the percentage of venom discharged after the application of voltage (W2) and after the application of vinegar (W4) were significantly different from one another and both were significantly higher than either the initial before-voltage percentage (W1) or after the third washing (W3) after voltage application.

It is further suggested that the samples used (W1 to W4) are internally matched samples and hence ANOVA is inappropriate. They suggest that a simple *t*-test would give different results. To alleviate their concerns, we have re-analysed the data using a paired *t*-test, comparing the level of protein present after the third washing (W3) since voltage was applied to the tentacle and the amount of protein present after vinegar was applied (W4). We paired each sample with itself (which effectively removes the issues surrounding analysis of internally matched samples). This analysis showed that the difference between the matched pairs was significant ($t = 8.938$, df = 2, $P = 0.012$). We further re-analysed these data comparing the mean protein expression after vinegar application (W4) to a standard value (23.2%) which was the mean percentage found after three washings (W3) post voltage application. Once again, the difference was found to be significant ($t = 6.012$, df = 2, $P = 0.027$).

We would argue, however, that the use of *t*-tests, as suggested by Yanagihara and Chen, is inappropriate owing to a possible non-normal distribution of the data. To address this, we further analysed these data using a non-parametric median test to a binomial distribution for data collected after the third washing (W3) post voltage application and data collected after the application of vinegar (W4). We used a one-sample median test to a binomial.[2] This statistical test is non-parametric as no assumption is made about the form of the population distribution except that it is continuous. This analysis once again revealed a significant difference between the treatments ($Z_b = 1.73$, $P = 0.04$) and, as such, the amount of protein after vinegar application is greater than after the washing protocol; that is, the application of vinegar increases the amount of venom expressed. Finally, we have reanalysed our data using a Friedman's test (as suggested by another independent biostatistician consulted by the Editor) and once more found that the application of vinegar increased the presence of toxin ($\chi^2 = 9.0$, df = 3, $P = 0.029$).

We thank Yanagihara and Chen for pointing out an issue of the degrees of freedom listed. We realize there was a transcription error that was not identified by the authors within the proofs. Where it reads ($F = 77.12_{3x82}$), it should read ($F = 77.12_{3x8}$).

Yanagihara and Chen have also expressed concerns about the press releases associated with this paper. We were contacted by the media as a result of the article's abstract release and the cover page of this journal for March 2014 with the heading "*Does vinegar make box jellyfish stings worse?*" Our sole press release (in response to the above) stated our findings and suggested a review of the current guidelines, as we do in the article. We do not have control over what the media publishes. We would point out that in every interview conducted by the authors, it was explicitly stated that first aid for cubozoan envenomings in Australia should continue to follow the ARC guidelines unless these guidelines are changed.

We remind Yanagihara and Chen that the scientific evidence supporting the use of vinegar as first aid is poor and we have seen an increased use of opioid analgesia in patients with Irukandji syndrome, who received vinegar (compared to those who did not).[1,3] Yanagihara and Chen suggest vinegar may enhance venom extraction from a sting site and thus increase survival. This is interesting speculation at best, with no data or evidence to support such an assumption.

In summary, our data show that vinegar promotes further release of venom from *Chironex fleckeri* tentacles that have been electrically discharged. We reiterate that we believe our findings are sufficiently significant for consideration in the development of first-aid guidelines, particularly in the face of a clear absence of any previous evidence supporting vinegar, which has always been assumed to be efficacious and safe.

References

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3 Hartwick R, Callanan V, Williamson J. Disarming the box-jellyfish: nematocyst inhibition in *Chironex fleckeri*. *Med J Aust.* 1980;1(1):5-20.

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