The use of the mouse lethality assay (MLA; the LD50 method) to measure the potency of botulinum toxin (BoNT) preparations has been established for decades. This test has been applied throughout the history of BoNT research since the earliest publications of, for example, Burke in 1919,1 as well as in the general toxicological testing of new compounds.2 The attention in recent years to the use of the method has only occurred because of the widespread use of BoNT for therapeutic and, particularly, aesthetic (incorrectly and inaccurately called ‘cosmetic’) applications. These uses have prompted many articles on the subject, most especially on why the method should continue to be used when so-called ‘alternatives’ apparently exist.2, 3 Unfortunately, there is still much inaccuracy in what is written about the subject.

Firstly, there has been a complete refusal by the commentators to recognise that the aesthetic use of BoNT is nothing more than personal vanity and “the search for eternal youth” on the part of the individual.3, 4 The ability of BoNT products to exert significant, positive effects on the well-being and health status of the individuals treated is brushed aside and ignored by these commentators.5 For example, effects such as the positive treatment of depression by aesthetic applications of BoNT still have no impact on these views.6–8 Could anyone really call these effects “personal vanity”?3

A replacement for the MLA has been looked for over many years and written about often.9 Contrary to what has been written in many cases, there were no ‘alternatives’ to use as substitutes, since these were either not sufficiently sensitive, not validated against the MLA, or both.10 Despite this situation, which has been clearly documented, the calls for a replacement have often been made.2, 3, 9

Much activity has been undertaken on suitable replacements, together with the refinement or reduction of the method called for by the Three Rs approach.10 Refinement and reduction have both played important, and often overlooked, roles in reducing mouse use in the MLA, yet, these aspects are hardly ever reported. Estimated contributions for these are in the region of 50% less mouse usage for product batch release.11 Still the focus has been on ‘replacement alternatives’ despite the very complex, multi-functional aspects of BoNT that need to be determined, the absence of the correct technology to do this, and the absolute legal need (for regulatory licensing purposes) to have a validated method which cross-correlates with the MLA.11 These activities have long been documented on websites of the main BoNT manufacturers.

Included in the overall process for the identification of a replacement method, a European Working Group (EWG) was established in 2009 which brought the BoNT manufacturers, regulators and scientists together in a European setting, to try to identify what was needed and how this could be achieved. Also, a joint activity between the manufacturers Merz and Ipsen was established,12 in order to combine knowledge and resources on solving the difficult issues at hand.

However, even though the EWG was aware of the Allergan activities toward a cell-based assay method — Allergan had reportedly been working on this for a number of years13 — the EWG was not aware that Allergan would announce their success at having an alternative method approved by the US Food and Drug Administration (FDA) just a few days after an EWG meeting in June 2011. This was a very unprofessional situation, to keep the EWG in the dark, which can only have been due to commercial issues.

The initial Allergan announcement of their first approval gave the impression to everyone that they would implement the cell-based assay immediately. But the Allergan announcements actually stated a three-year time frame for the introduction

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### Comment

**The Botulinum Toxin LD50 Potency Assay — Another Chapter, Another Mystery**

**Andy Pickett**

Observers of the potency assay used for botulinum toxin were greeted last year with the news that one company had an alternative, non-animal alternative in place. But all was not as it seemed from the press release, and over a year later, information is still lacking.