Chapter 5.1

Is *Bacillus thuringiensis* standardisation still possible?
*Update and improvement of Bt titration over 20 years*

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Abstract: The aim of this review is to clarify the situation surrounding titration of bacterial formulations against *Bacillus* reference standards and to discuss the importance of standard procedures. Secondly, with the presence of numerous *B. thuringiensis* Cry toxins, we examine a possible remedy for the absence of classical reference standards. We propose a new way of titrating Bt products combining insect based bioassays and quantitative determination of toxin protein with biochemical methods. Further, we suggest improvement in the standard protocols used for bioassays and the statistical methods applied.

1. INTRODUCTION

The potency of a *Bacillus thuringiensis* (Bt) product indicates the dose-dependent, lethal activity of the product compared to that of an accepted standard Bt. The activity is a result of the interaction of several components of the bacterium of which δ-endotoxins are often very important. Several other toxins and molecules act in concert with δ-endotoxins [29]. In the vegetative phase, some Bt strains produce a strong synergism for δ-endotoxins and a toxic phospholipase [20], and an elimination of some of these enzymes reduced toxicity of Bt [58]. These and possible other toxins and enzymes are only available when the spore...
is alive in the product. A synergising effect of the spore itself was noted by [11, 21]. Insects have defences against bacteria including bactericides called attacines and cecropines. *Bt* is to some extent able to destroy this system by excreting a metallo-proteinase [29, 43]. The target of most *Bt* products is the insect larva. As the development of the immune system of the insect larva is dependent on the instar, toxicity depends on insect species as well as larval instar. Accordingly, toxicity is a complex process and not simply dependent on δ-endotoxin concentration.

In a potency bioassay, mortality is measured against dose or concentration of the *Bt* product and data are usually transformed into log dose-probit mortality to obtain straight lines that are more easily compared [23]. The ratio of LC$_{50}$ values of the standard and of the product determines the potency of the product, expressed in International Toxic Units (ITU) *Aedes aegypti*/ mg product, on a test-insect using a simple formula:

$$LC_{50} \text{ (ppm) reference standard } \times \text{ titre standard (ITU/mg)} \div LC_{50} \text{ (ppm) product}$$

but behind this formula are many preconditions that are often not met as discussed below.

Potency determination allows comparisons between laboratories that use the same standard, despite minor differences in methods of testing or rearing, since these differences should have the same linear influence on the dose response of the product and of the standard. Internationally accepted reference standard *Bt*'s and test protocols can help make potency determinations comparable world-wide. The potency bioassay can be used for research laboratories to compare industrial and experimental formulations and by industry and applicators to check for variability between batches and stability during storage.

Despite the increasing demand for documented quality control in research and production, we believe that standardisation methods for *Bt* products are not progressing. This is partly due to the ease of protein assay methods used to determine concentration of δ-endotoxins, but also due to problems inherent in the bioassays themselves. International test protocols are often not followed, and for many new *Bt* strains they do not exist. The increasing numbers of *Bt* strains used for insect control – whether of natural origins or genetically modified – suggest the need for just as many international standards, but this is not practical. In this chapter we propose a way to compare *Bt* products across laboratories and *Bt* strains by defining certain standard test methods and protocols.