ELSEVIER

Toxicon 48 (2006) 662–671

TOXICON

www.elsevier.com/locate/toxicon

Assessment of fitness for purpose of an insect bioassay using the desert locust (*Schistocerca gregaria* L.) for the detection of paralytic shellfish toxins in shellfish flesh

A.C. Cook[a,*], S. Morris[b], R.A. Reese[a], S.N. Irving[a]

[a]*Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, UK*

[b]*Centre for Environment, Fisheries and Aquaculture Science, Burnham on Crouch, Essex, UK*

Received 27 March 2006; accepted 19 July 2006
Available online 26 July 2006

Abstract

We have developed a bioassay using 5th instar desert locusts (*Schistocerca gregaria* L.) for the detection of saxitoxin—the paralytic shellfish poison in shellfish flesh. The bioassay procedure is to inject 10 locusts with a shellfish extract, and assess their conditions at time points up to 2 h post injection, looking for an endpoint of paralysis. From the proportion responding, the equivalent dose of pure saxitoxin could be estimated. Performance characteristics of the bioassay were assessed using shellfish samples spiked with saxitoxin, and we found the bioassay could detect and quantify toxin levels in the range of regulatory relevance. Relative toxicities of selected saxitoxin analogues differed from those reported in mammalian systems. Variation for repeatability conditions was acceptable but variation was higher under reproducibility conditions. This was related to (a) batches of insects from different suppliers, (b) different operators, and (c) different observers assessing the endpoint. We also noted adverse reactions with some shellfish species. These problems may be resolved by further refinement of the method and operator training, before formal validation. Nevertheless, we suggest the method potentially offers a simple, ethically acceptable, broad-specificity functional bioassay, which is desirable in any toxin-monitoring programme.

Keywords: Paralytic shellfish poisoning; PSP; Saxitoxin; Bioassay; Desert locust; *Schistocerca gregaria*

1. Introduction

The UK is required to monitor for harmful algal biotoxins in shellfish under the European Shellfish Hygiene Directive (2002/225/EC), which details appropriate testing methods and sets maximum permissible levels of toxins in shellfish flesh. The test required by statute is the AOAC mouse bioassay (AOAC, 1990) for paralytic shellfish toxins (PSTs) in shellfish flesh. The European reference method is defined as intraperitoneal injection of shellfish extracts into mice. The toxicity is calculated from time to death of the injected mice. This assay is accepted as protecting the consumer from exposure to contaminated shellfish, but raises ethical concerns over the sacrifice of large numbers of mammals and

*Corresponding author. Tel.: +44 1305 206600; fax: +44 1305 206601.
E-mail address: a.c.cook@cefas.co.uk (A.C. Cook).

0041-0101/$ - see front matter Crown Copyright © 2006 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.07.030

has other shortcomings. These include non-specific interference (Schantz et al., 1958; McCulloch et al., 1989), and poor reproducibility and high variability in results (Jellett, 1993; Parks et al., 1986). Most shellfish-producing countries and competent authorities are committed to reducing reliance on mouse bioassays.

Alternative screening methods for shellfish toxins have been developed but not generally applied as replacements for the mouse bioassay. The main constraint on application of new methods in Europe is the requirement for full validation and approval by the EU as well as by national regulatory agencies. This process involves considerable time and expense, with the risk that a method may finally fail to be approved.

Alternative methods include direct chemical analysis, immunoassay, in vitro bioassay and whole-animal invertebrate bioassay (reviewed by Garthwaite, 2000). Immunoassays (e.g. ELISAs) can be extremely sensitive and rapid, but it is difficult to produce antibodies that can detect all toxic analogues and not give false positives by reacting with non-toxic components of the matrix. A range of in vitro bioassays, such as cytotoxicity assays and receptor-binding assays of varying sensitivity, specificity and resolution have been developed, some of which appear to be very promising. However, the use of radioisotopes and the maintenance of tissue cultures are obstacles to the application of these methods.

Chromatographic methods have been developed which are likely to play an increasing role in biotoxin-screening programmes (e.g. Lawrence et al., 2004, 2005). These offer rapid and sensitive quantification of the main PSTs, albeit at a relatively high cost. However, certified reference materials are not available for some toxic analogues, which therefore cannot be effectively screened. Total toxicity of combinations of toxins and their metabolites often found together in shellfish flesh is also difficult to assess using these methods, and hitherto unreported toxins cannot be identified by chemical analysis. For these reasons, biotoxin-monitoring programmes should continue to include a low-specificity functional bioassay.

Previous reports have suggested that insect bioassays could provide alternatives to the current testing methods for PSTs (McElhiney et al., 1998; Ross et al., 1985; Turell and Middlebrook, 1988). Many problems experienced by the mouse bioassay are less likely to affect an insect bioassay. Insect haemolymph typically has high concentrations of amino acids, lipids and proteins that vary with physiological state and temperature. This makes them more tolerant of abrupt changes in haemolymph composition than are vertebrates, and consequently more likely to show greater physiological tolerance of sample matrix constituents which might give false positives in the mouse bioassay. For example, the 'salt effect' (Schantz et al., 1958) is not observed in a housefly bioassay for PSTs (Ross et al., 1985). Another advantage of using insects is that statistical robustness can be increased by using larger numbers of test organisms without the legal and ethical constraints. Insects are less expensive than mice. Despite these advantages of invertebrate bioassay, none has yet been validated.

We describe an insect bioassay for the detection and quantification of PSTs in shellfish flesh and assess its fitness for purpose as an alternative to the standard mouse bioassay. Desert locust, *Schistocerca gregaria*, was selected as it is inexpensive and widely available, its biology and toxicology are well studied, and it is large enough to be easy to handle.

2. Materials and methods

2.1. Calibration using STX and other PST analogues

To test the response of mixed sex 5th instar desert locusts to STX-diHCl certified-reference solution (National Research Council, Canada), dilutions were prepared in acidified distilled water (pH adjusted to 3.5 with dilute HCl). Each insect was injected between the 2nd and 3rd abdominal segments, and assessed at 30, 60, 90 and 120 min post injection as to whether it was able to right itself when placed on its back. All trials were carried out at 20 °C, and the ED_{50}s (ng/animal) were calculated using a probit model (Finney, 1971). Linearity of the relationship between log dose (ng STX-diHCl/g insect) and the probit response was confirmed.

The relative toxicities of some important STX analogues were ascertained to compare with those reported for mammalian systems. Following the method described above, dilutions were prepared and administered from certified reference solutions of neoSTX, decarbamoylSTX, and gonyautoxin 2/3, which came as a mixture epimerised to a stable ratio. ED_{50}s (ng/animal) were calculated for each. These were compared with reported relative mammalian toxicities, including those commonly used by