

Refinement and Reduction of the Mouse Bioassay in Shellfish Toxin Testing in the UK

European Partnership for Alternative
Approaches to Animal Testing (EPAA)
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Home Office

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AND TOLERANT SOCIETY

Marine Biotoxins

- Contamination of shellfish by toxic algae
- Several classes of toxin
- Regulatory requirement to test
- Heavy reliance of Mouse Bioassay
 - Paralytic Shellfish Poisoning
 - Lipophilic Toxins (Diarrhetic Shellfish Poisoning)



Relevant Legislation

	Food Hygiene	Protection of Animals Used in Experimentation
Legislation	Regulation EC 2074/2005	Directive 86/609 EEC
Purpose	Consumer safety	Animal welfare
Specifies	Reference methods Methods highly defined: Death as endpoint	Use of practicable alternatives Use of refined methods: Clinical endpoints

Method

PSP (AOAC 1995)	DSP (Yasumoto 1978)
Validated, although not to current standards	Unvalidated
3 mice per sample	3 mice per sample
Acidic: pH 2 to 4	Non-acidic
Injection 1ml of extract via intraperitoneal route	Injection 1ml of extract via intraperitoneal route
Short: 60 minute	Long: 24 hour
Death as endpoint	Death as endpoint
Median death time can be used to determine toxin level	Non-quantitative: death of at least 2 out of 3 positive
Death by respiratory paralysis	Reason for death uncertain

For the MBAs for Marine Biotoxins
can we/ should we apply the 2Ps? **Yes!**



Refinement: Change dosing?

- Route: Oral toxin: oral dosing most ~~appropriate~~
- Injectate
- Volume
 - Dose per kilo ~~X~~
- Content
 - DSP - consistency ~~X~~
 - PSP - pH 2-4 specified in method ✓

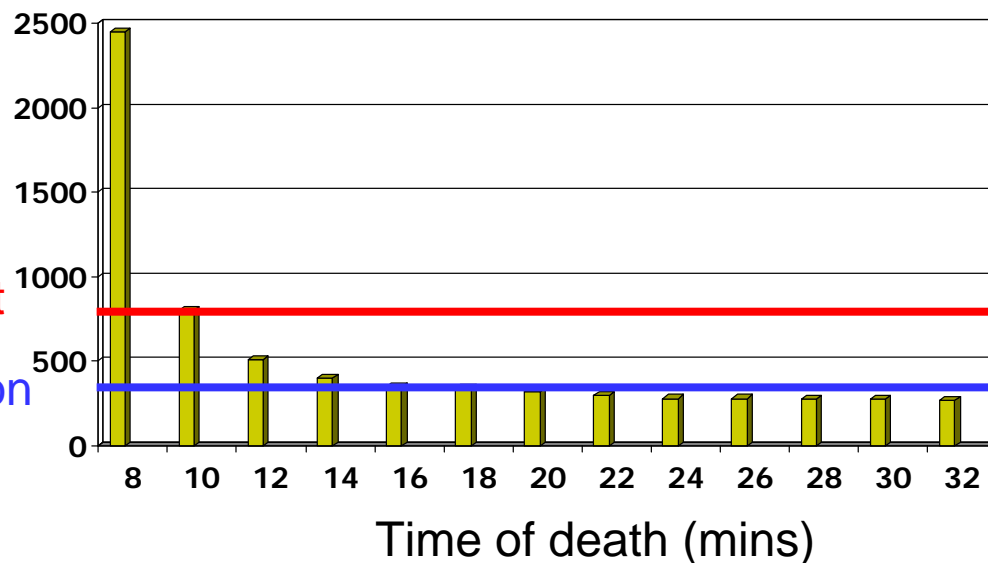
Refinement: Reduce duration?

PSP

Toxin Level
 $\mu\text{g}/\text{kg}$ flesh

Regulatory Limit

Limit of Detection



Duration

Reduced
>65%

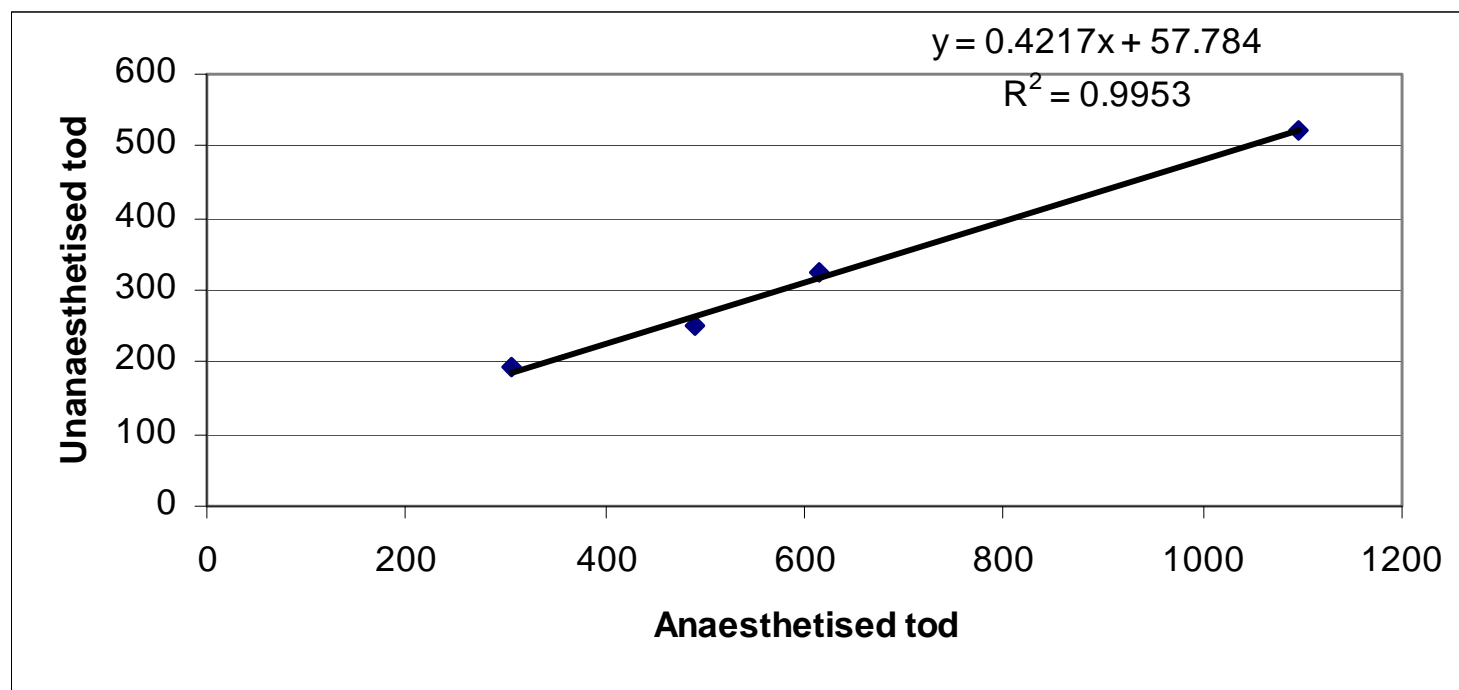
DSP

- In several phases
- Required development of clinical signs predicting death
- If show specific signs by 5 hours, die by 24 hours

Duration

Reduced
> 75%

Refinement: Use anaesthesia?



Median time of death (seconds) for Anaesthetised and Unanaesthetised mice

Holtrop et al (2006)
Toxicol 47: 336-347

- Anaesthesia slows time to death
- Calibration factor to give equivalence

Refinement: Clinical endpoints?

- PSP – death time required: not possible to refine
- DSP – prediction of death acceptable



Combination: Unresponsive, hypothermic, cyanotic, loss of grip reflex



Hypothermia: objective measure

- Use of infra-red laser thermometer
- Hypothesis: detection of conclusive drop in body temp (Warn et al (2003), Lab Anim 37: 126 – 131)
- Results:
 - Initial drop temp all animals
 - Negative samples identifiable by temp rise



Strategies: Reduction

- Overall monitoring strategy
 - Take the right samples at the right time
 - Sample testing strategy
 - Pre-screening methods
- = Reduced sample numbers
- Apply logic principles to existing data
- = Fewer animals per sample

Equivalent or greater level of consumer safety required

Reduction: Pre-screening PSP

- Jellet Rapid Test™
 - HPLC – preferred

 - Negative screen
 - Positive on pre-screen => MBA to quantify

 - Reduction of ~65%
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PSP: mice per sample

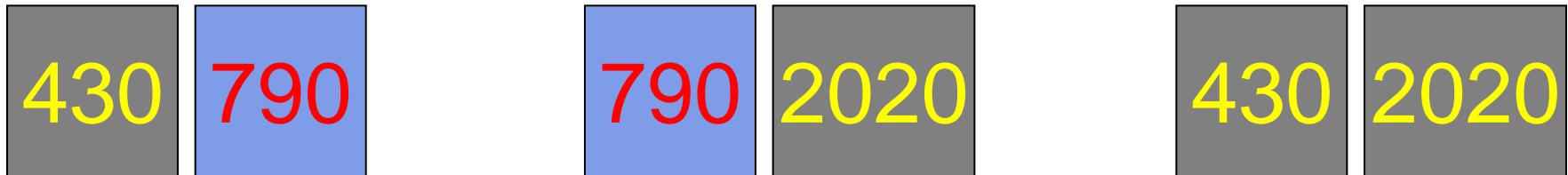
Regulatory Limit = 800

Results from 3 mice for one field sample:



Median

If only 2 mice, possible result combinations:



Taking UPPER value of 2 as safe as median of 3

DSP: mice per sample

- Result based on “2 out of 3”
- Possible outcomes:
 - 3 mice give same result
 - 2 mice give same result + 1 mouse differs

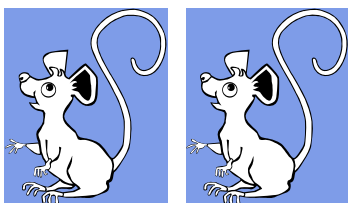


Positive

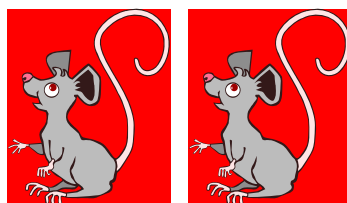


Negative

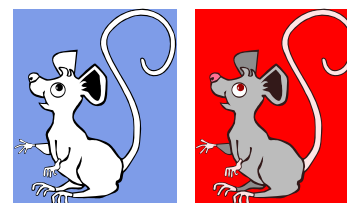
- If use 2 animals scenarios are:



Negative



Positive



Inconclusive

- Options for “inconclusive” results:

- Precautionary principle: “Positive”
- Use an additional animal

- Choice based on

- Risk
- Frequency of occurrence

- Reduction in number of mice per sample for both tests
 - No change in variability
 - Equivalent or improved safety
- So for both MBAs...

“2 will do”

Current U.K. monitoring

PSP

Replacement: Fully quantitative HPLC for some shellfish sp

Pre-screen $\xrightarrow{-ve}$ No further testing **Partial replacement**

Every year in EU
approx **400,000 mice** used in MBAs
If UK approach used this would reduce
to approx **142,000**

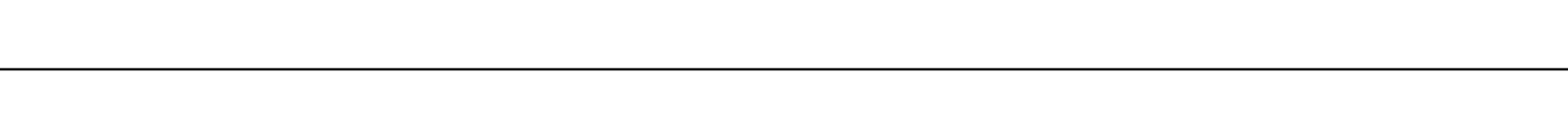
65% reduction in animal use

LCMIS undergoing validation

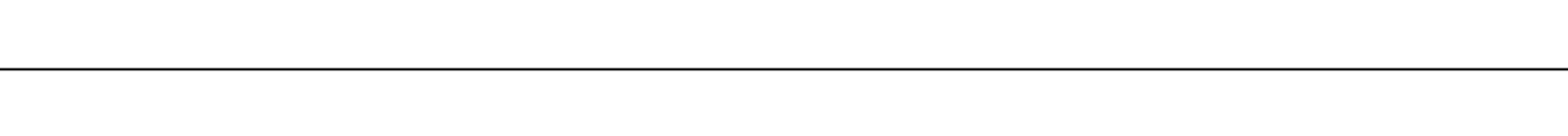
Replacement

Regulatory Difficulties

- **Reliance on poor quality “gold standards”**
 - **Over-specification of methodology**
 - **“Engineering” rather than “performance”**

 - **Still difficult to progress**
 - **Even where equivalence demonstrated**
 - **Refined strategies**
 - **Reduction strategies**
 - **Alternatives**
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Barriers for Progression

- **Science:** Lack of chemical standards
 - **Motivation:** Is there a will?
 - Scientific?
 - Regulatory?
 - **Process:** How can things be changed?
 - Is process in place?
 - Is it functional?
 - Risk assessment? low risk not no risk
 - Expected performance criteria defined?
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The solutions

- Good communication - all stakeholders
- International harmonised discussions/ approach

- Per

>80% Replacement
of all MBAs in UK
expected 2011

Moral:



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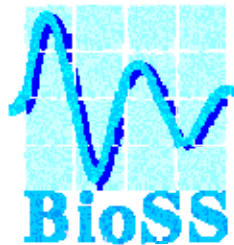


Never give up: never surrender!

Acknowledgements



marinescotland



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DSP MBA – clinical signs

- Positive samples:



- Negative samples:
 - Pain behaviour
 - Weight loss