Statistics and Ecotoxicology: Past, Present and Future

Keynote Presentation

by

Prof. David R. Fox

EnviroTox, Darwin 18 April 2011

Introduction

Good morning. It is indeed an honour and privilege to be invited to talk at Envirotox 2011 which is jointly sponsored by the Australasian Society for Ecotoxicology, the Royal Australian Chemical Institute, and SETAC Australasia. I am particularly indebted to conference co-chairs Michelle Iles and Rick van Dam for extending me this invitation and for their months of hard work in bringing this conference to fruition. I also wish to acknowledge the traditional landowners, the Larrakia Aboriginal people.

As a statistician I understand the concept of an 'outlier'. According to Wolfram Research, "an outlier is an observation that lies outside the overall pattern of a distribution. Usually, the presence of an outlier indicates some sort of problem". I sincerely hope that my participation in this conference for *ectoxicologists* is not viewed as indicating some sort of problem! To the contrary – I have an almost evangelical zeal about the potential for statistics to contribute and potentially make a difference to the theory and practice of modern-day ecotoxicology.

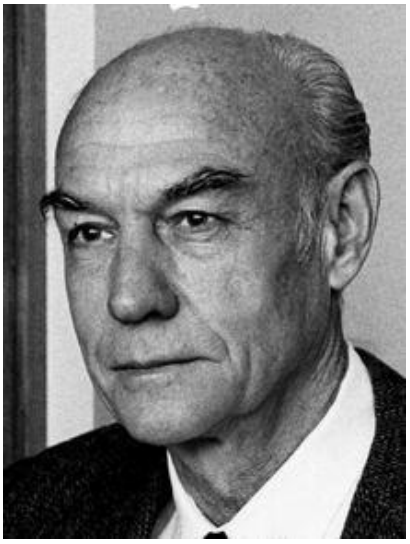
My interest in the statistical aspects of ecotoxicology dates back to the mid-1990s when I was working in CSIRO – firstly as a statistician on the Port Phillip Bay Environmental Study and then as Director of the Effluent Management Study which was undertaken on behalf of Melbourne Water. I remember listening to many study updates at our regular meetings of the scientific committee and being particularly impressed and interested in the work of Jenny Stauber, Graeme Batley and others who with CSI-like skills, committed themselves to the task of solving the particularly heinous crime of '*Hormosira* homicide' down at a lonely beach called Boags Rocks – some 15 km to the south-east of the site of

the infamous disappearance of another species *H. Holtii*. As it turns out, the fate of a prime minister dressed in rubber in the presence of three scantily clad females was a harder case to crack than the identification of the toxic agent for *Hormosoria*.

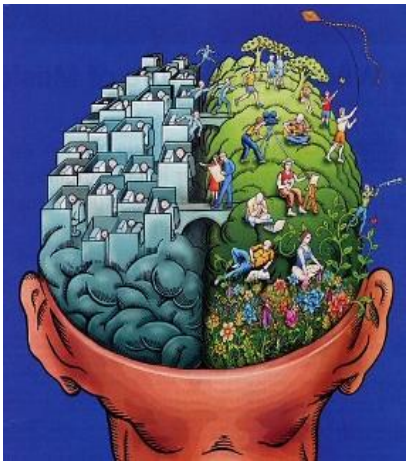
However, I digress. Let me return to my thesis which is: the union of statistics and (eco)toxicology is deserving of formal recognition. Last year I published a *Learned Discourse* in IEAM having the somewhat rhetorical title of "Statistics and Ecotoxciology: Shotgun Marriage or Enduring Partnership?'

In this short presentation I wish to establish the credentials of the bride and groom in this scientific partnership and propose that the parents resist the temptation of a US-style 'quickie' marriage and instead commit to a celebration more befitting of this most worthwhile enterprise.

On its website, your society, the ASE acknowledges the multidisciplinary nature of the endeavour. Specifically, it says “the field of ecotoxicology includes concepts arising from disciplines such as toxicology, biology, analytical, environmental and organic chemistry, physiology, ecology, genetics, microbiology, biochemistry, immunology, molecular biology, soil, water and air sciences, and economics”. Curious - no mention of statistics? Perhaps we can have that changed?

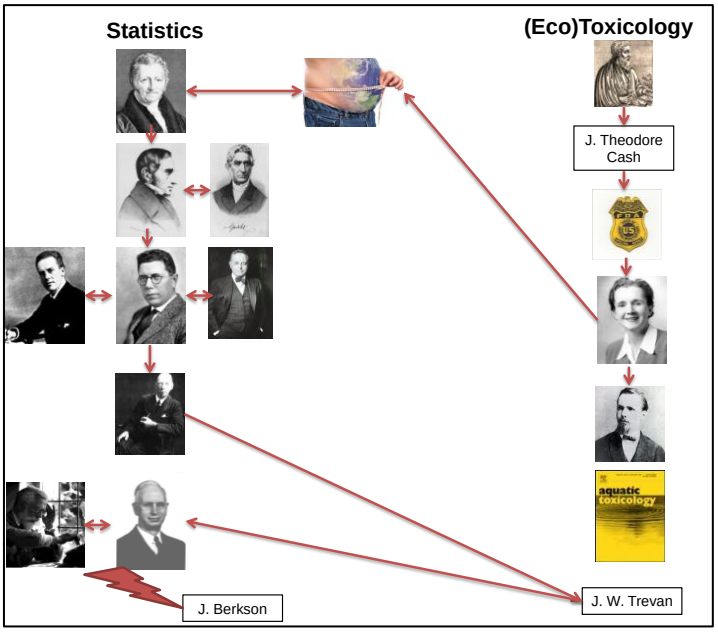


Yesterday was the 17th. anniversary of the death of Roger Wolcott Sperry. Sperry was an American neurobiologist who shared the 1981 Nobel Prize in Medicine for revolutionising our understanding of brain function. Sperry and his colleagues identified the unique capabilities of each hemisphere and demonstrated that the combined effect of bi-hemispheric activity amounted to more than the simple additive effects of the two separate hemispheres. A primal case of a biological synergism that we all rely on - or at least most of us rely on!



Like the brain, ecotoxicology relies on different ‘hemispheres’ of knowledge, expertise, and wisdom to operate effectively, make informed decisions, and to react decisively. In this depiction I see the newlyweds working together, standing on the bridge that links the disciplines, roadmap in hand and a clear sense of direction.

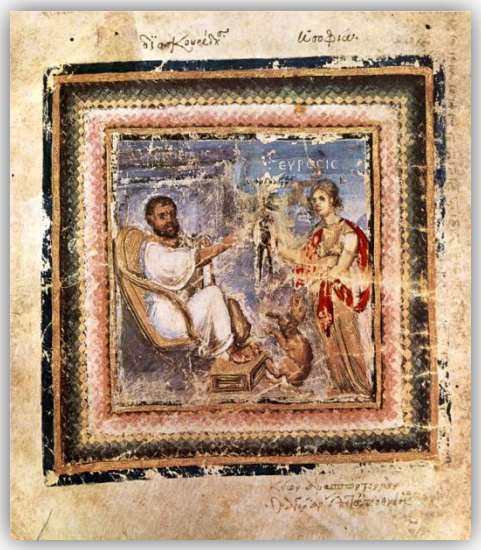
In the remainder of this talk I want to commence by providing an historical sketch of the separate paths of statistics and ecotoxicology; highlighting the intersections of our disciplines and the successes we've enjoyed along the way. I will then briefly touch on what I consider to be some unresolved ecotoxicological issues that will only be solved by strengthening existing 'hemispherical linkages' and developing new ones. Finally, I will conclude with some remarks on what I perceive to be challenges for the future.



The genealogical tree

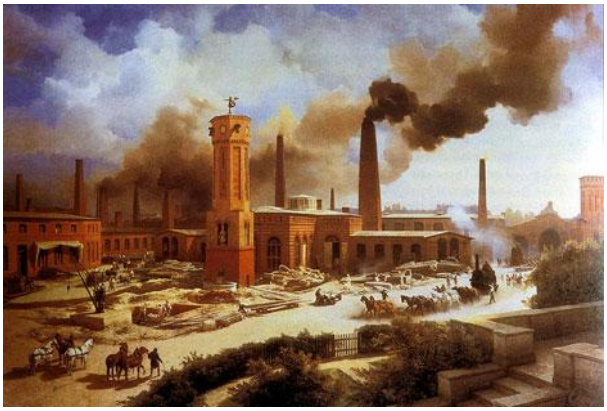
It is probably true to say that humans became conscious of their own mortality shortly after climbing out of the trees. Like all animals, we would have quickly learnt from trial-and-error 'experiments' what was safe for us to eat and drink and what was not. When we actually codified this knowledge is not entirely clear although the works of Pendanius Dioscorides and others certainly establishes that this activity was well underway almost 2,000 years ago.

Dioscorides was a Greek physician, pharmacologist, botanist and author of a five volume encyclopaedia about herbal medicines called *'De Materia Medica'* that is the pre-cursor to modern pharmacopeias. Some 1400 years later, Paracelsus declared that "all substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy".

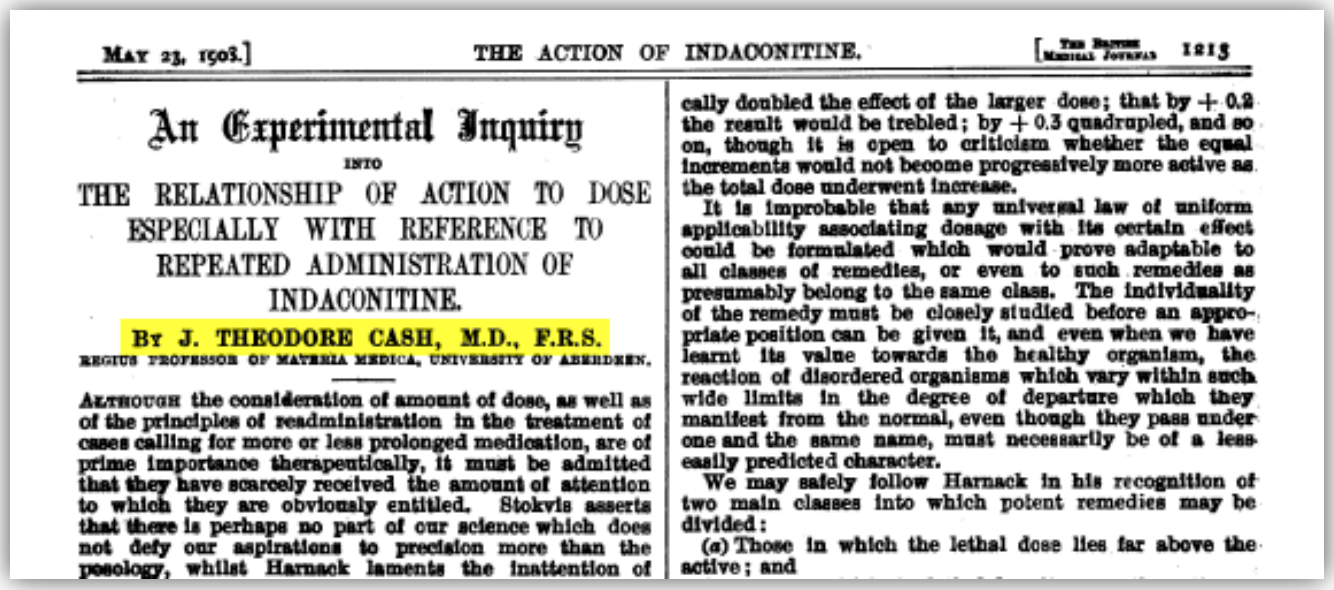


The *De Materia Medica* was circulated in Latin, Greek and Arabic. One of the most important was the illustrated Greek version *'Vienna Dioscorides'* which was discovered in Istanbul in the 1560s.

So let us now time-travel at warp-factor 3 to the *industrio-chemical* revolution of the mid-19th. century. Now, for the first time we have releases of new chemicals and substances hitherto never seen before. Shortly thereafter, scientists began conducting the very first toxicity studies in the 1900s and, importantly we see the first use of 'toxicity.



In 1908, Theodore Cash published a paper in the British Medical Journal, in which he described early dose-response experiments. In his paper he talks of "the



MAY 23, 1903.] THE ACTION OF INDACONITINE. [THE BRITISH MEDICAL JOURNAL 1215

An Experimental Inquiry

INTO

THE RELATIONSHIP OF ACTION TO DOSE ESPECIALLY WITH REFERENCE TO REPEATED ADMINISTRATION OF INDACONITINE.

BY J. THEODORE CASH, M.D., F.R.S.

REGIUS PROFESSOR OF MATERIA MEDICA, UNIVERSITY OF ABERDEEN.

ALTHOUGH the consideration of amount of dose, as well as of the principles of readministration in the treatment of cases calling for more or less prolonged medication, are of prime importance therapeutically, it must be admitted that they have scarcely received the amount of attention to which they are obviously entitled. Stokvis asserts that there is perhaps no part of our science which does not defy our aspirations to precision more than the posology, whilst Harnack laments the inattention of

cally doubled the effect of the larger dose; that by + 0.2 the result would be trebled; by + 0.3 quadrupled, and so on, though it is open to criticism whether the equal increments would not become progressively more active as the total dose underwent increase.

It is improbable that any universal law of uniform applicability associating dosage with its certain effect could be formulated which would prove adaptable to all classes of remedies, or even to such remedies as presumably belong to the same class. The individuality of the remedy must be closely studied before an appropriate position can be given it, and even when we have learnt its value towards the healthy organism, the reaction of disordered organisms which vary within such wide limits in the degree of departure which they manifest from the normal, even though they pass under one and the same name, must necessarily be of a less easily predicted character.

We may safely follow Harnack in his recognition of two main classes into which potent remedies may be divided:

(*a*) Those in which the lethal dose lies far above the active; and

nearest fulfilment of a mathematical relationship seemed to be achieved by working upwards from that amount of any drug which produced the minimum of appreciable action". This dose, he notes was variously referred to as the *"Grenzdose"* or *"limit dose"*. I find this companion development of toxicological nomenclature and acronyms fascinating and something which has survived, if not thrived over the last 100 years. While we now have NOECs, NECs, ECxs, NOAELs, LOECS, LDxs, ICxs etc., back in 1908 Cash was making early inroads with the introduction of the additional terms *"minimal effective dose"* and *"maximal ineffective dose"*.

While these early scientific studies were important in establishing safe doses for medicines they had little immediate application to the more diffuse area of chemicals in the environment. Another ½ century would have to pass before the emergence of the environmental movement. An important development along the way was the establishment in the United States of the FDA in 1930 and the passing of the Food, Drug, and Cosmetics Act in 1938.

Things were about to get a lot tougher for the burgeoning chemical industry. In 1958 an amendment to the Act known as the *Delaney Clause* prohibited the approval of any food additive shown to cause cancer in humans or animals.

In 1962 American marine biologist and conservationist, Rachel Carson released *"Silent Spring"*. This was a turning point, the epiphany that awakened the world to the possibility that chemicals (particularly pesticides such as DDT) in the environment were having both human and ecological impacts. The decade between 1965 and 1975 saw a flurry of intense scientific investigations into the acute and chronic effects of pollutants on aquatic organisms. The results of these studies reduced some of the uncertainty associated with the application of arbitrary *'safety'* or *'assessment'* factors derived from human toxicity studies but, as we shall see, introduced other difficulties with statistical estimation and inference.

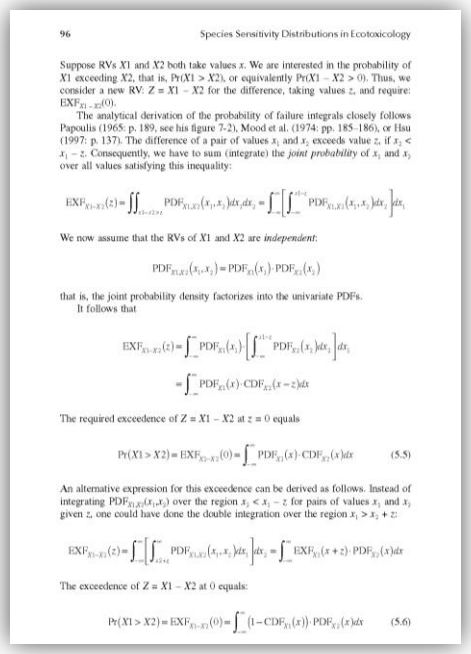
The 1970s commenced with the introduction of the word *eco*toxicology by Rene Truhaut and this decade was to be characterised by research into whole organism responses and effects in biological systems.

The journal *Aquatic Toxicology* was launched in 1981 in response to what it claimed was a world-wide concern about the effects of man-made chemicals on aquatic organisms and ecosystems. Today we see no fewer than 20 sub-disciplines of toxicity although interestingly none concerning the statistical aspects of toxicology and/or ecotoxicology.

The last thirty years has been witness to ever-increasing mathematical and statistical sophistication of the treatment of data and modelling approaches used in ecotoxicology. However, there are still ‘burrs under the saddle’ – the problematic NOEC; the legitimacy of using mixtures of toxicity measures in SSD modelling; SSD modelling itself and the whole statistical underpinning of the method; Bayesian versus Frequentist statistical paradigms; ‘time’ as the missing dimension in concentration-response modelling; and individual versus population effects to name a few.

I now wish to ‘shift gears’ and trace the development of what I call *statistical ecotoxicology.*

The role of statistics in Ecotoxicology



96 Species Sensitivity Distributions in Ecotoxicology

Suppose RVs $X1$ and $X2$ both take values x. We are interested in the probability of $X1$ exceeding $X2$, that is, $\Pr(X1 > X2)$, or equivalently $\Pr(X1 - X2 > 0)$. Thus, we consider a new RV: $Z = X1 - X2$ for the difference, taking values z, and require: $\mathrm{EXF}_{X1-X2}(0)$.

The analytical derivation of the probability of failure integrals closely follows Papoulis (1965: p. 189, see his figure 7-2), Mood et al. (1974: pp. 185–186), or Hsu (1997: p. 137). The difference of a pair of values x_1 and x_2 exceeds value z, if $x_2 < x_1 - z$. Consequently, we have to sum (integrate) the *joint probability* of x_1 and x_2 over all values satisfying this inequality:

$$\mathrm{EXF}_{X1-X2}(z) = \iint_{x1-x2>z} \mathrm{PDF}_{X1,X2}(x_1,x_2)dx_1dx_2 = \int_{-\infty}^{\infty}\left[\int_{-\infty}^{x1-z} \mathrm{PDF}_{X1,X2}(x_1,x_2)dx_2\right]dx_1$$

We now assume that the RVs of $X1$ and $X2$ are *independent*:

$$\mathrm{PDF}_{X1,X2}(x_1,x_2) = \mathrm{PDF}_{X1}(x_1)\cdot\mathrm{PDF}_{X2}(x_2)$$

that is, the joint probability density factorizes into the univariate PDFs.

It follows that

$$\mathrm{EXF}_{X1-X2}(z) = \int_{-\infty}^{\infty}\mathrm{PDF}_{X1}(x_1)\cdot\left[\int_{-\infty}^{x1-z}\mathrm{PDF}_{X2}(x_2)dx_2\right]dx_1$$

$$= \int_{-\infty}^{\infty}\mathrm{PDF}_{X1}(x)\cdot\mathrm{CDF}_{X2}(x-z)dx$$

The required exceedence of $Z = X1 - X2$ at $z = 0$ equals

$$\Pr(X1 > X2) = \mathrm{EXF}_{X1-X2}(0) = \int_{-\infty}^{\infty}\mathrm{PDF}_{X1}(x)\cdot\mathrm{CDF}_{X2}(x)dx \qquad (5.5)$$

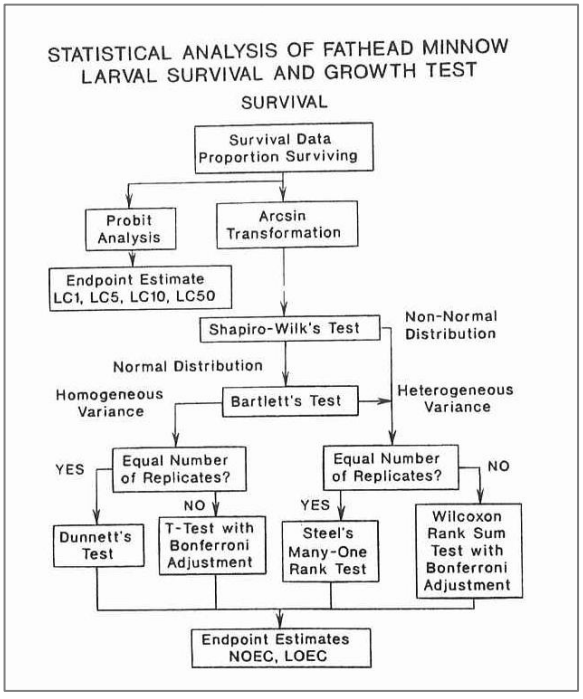
An alternative expression for this exceedence can be derived as follows. Instead of integrating $\mathrm{PDF}_{X1,X2}(x_1,x_2)$ over the region $x_2 < x_1 - z$ for pairs of values x_1 and x_2 given z, one could have done the double integration over the region $x_1 > x_2 + z$:

$$\mathrm{EXF}_{X1-X2}(z) = \int_{-\infty}^{\infty}\left[\int_{x2+z}^{\infty}\mathrm{PDF}_{X1,X2}(x_1,x_2)dx_1\right]dx_2 = \int_{-\infty}^{\infty}\mathrm{EXF}_{X1}(x+z)\cdot\mathrm{PDF}_{X2}(x)dx$$

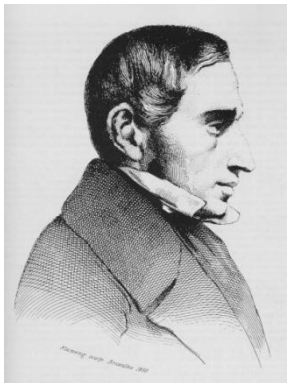
The exceedence of $Z = X1 - X2$ at 0 equals:

$$\Pr(X1 > X2) = \mathrm{EXF}_{X1-X2}(0) = \int_{-\infty}^{\infty}\left(1-\mathrm{CDF}_{X1}(x)\right)\cdot\mathrm{PDF}_{X2}(x)dx \qquad (5.6)$$

We only need look at this page from Posthuma et al or the flowchart for the Fathead Minnow larval survival and growth test to see that statistical methods play a critical role in the assessment of toxicity. Following the flowchart logic, our immediate choice is to either undertake a probit analysis of the survival data or to transform it using the arcsine transformation. The transformed data (which interestingly are now in units of *angular radians*) are then to be checked for their distributional normality using the Shapiro-Wilks test. Should they pass this test, these *angles* are checked for homogeneity of variance using Bartlett's test. A satisfactory result on this test allows us to proceed to the ubiquitous Dunnett's test if we have equal numbers of replicates or, in the case of unequal replicates – the rather formidable-sounding T-test with Bonferroni adjustment. Over on the other side of the flow chart, we are led to either Steel's many-to-one rank test or the Wilcoxon rank sum test with Bonferroni adjustment.



So, how did ecotoxicology become so heavily dependent on statistics? To answer this, we need to go back to the late 60s / early 70s when, in April 1968 a small group of people concerned about the future of humankind met in Rome. The Club of Rome as it became known commissioned a report into the sustainability of economic growth and the ensuing report *'Limits to Growth'* was published in 1972. The '*Limits to Growth'* made predictions using fairly simplistic mathematical models of population growth and resource availability. For us, the important connection is the similarity of these models with the Malthusian growth model – named after the British scholar, Rev. Thomas Malthus who, some 175 years earlier had realised that population growth could not be limitless. The first important link between statistical science and the environmental movement has now been established for the Malthusian growth model is a direct ancestor of the logistic function which was published (posthumously) in 1858 by Francois Verhulst. We will return to the logistic function shortly, but let us continue following the statistical footprint.



Verhulst was a Belgium mathematician. His interest in population modelling commenced while he was at the University of Ghent studying under Adolphe Quetelet. Quetelet was an astronomer, mathematician, statistician, and sociologist and was the first to apply the normal distribution to sociological phenomena. He also gave us the *Quetlet Index of obesity* which we recognise today as the BMI.

Like Malthus, Quetelet was also aware of the limitations of simple exponential growth models and asked Verhulst to look at the problem with a view to modifying

the model so as to provide more realistic population estimates as $t \to \infty$.
Verhulst succeeded in this task and published an equation relating population size to intrinsic growth rate and carrying capacity – he referred to his solution as the logistic function or logistic equation.

For some unknown reason the logistic equation remained a relatively obscure mathematical result until it was rediscovered in 1920 by Raymond Pearl and Lowell Reed.

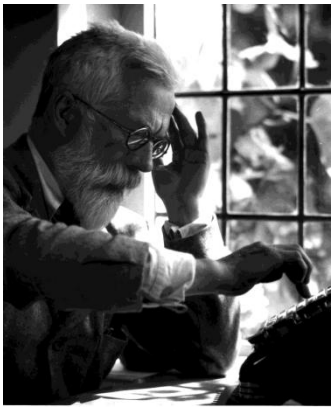
Pearl had just been appointed director of the department of Biometry and Vital Statistics at John Hopkins University and Reed was his deputy. Although a biologist by training, Pearl was deeply interested in statistics and had in fact spent a year in 1905-06 in London with eminent statistician Karl Pearson (one of the pioneers of modern hypothesis testing). Pearl and Reed were, at the time, unaware of Verhulst's work and had independently derived the logistic equation themselves.

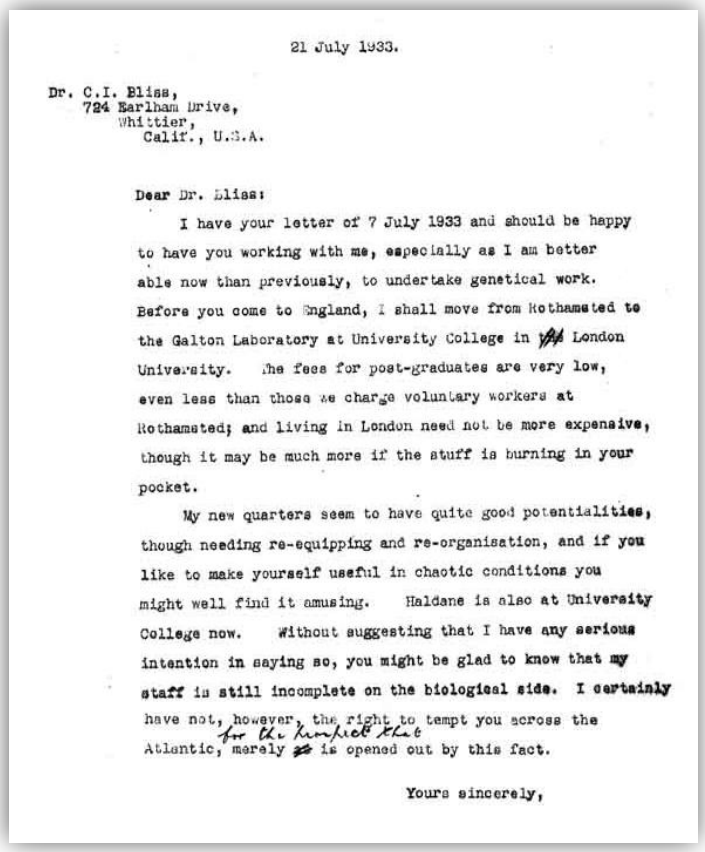
While Pearl and Reed were not mainstream statisticians, Udny Yule certainly was. In his 1925 Presidential address to the Royal Statistical Society, Yule commented on Pearl and Reed's independent discovery of Verhulst's result and cemented the term "logistic" in the statistical lexicon when he remarked that "I have relegated to Appendix II some discussion of the mathematics of the curve, which, following Verhulst, we may term a 'logistic'".

Now the link with toxicology. Two years after Yule's Presidential address to the Royal Statistical Society, English physiologist J.W. Trevan gave a paper to the Royal Society in London in which he wanted to establish "a more accurate definition of such terms as 'minimal lethal dose', minimal effective dose' etc." Trevan suggested that the term 'minimum lethal dose' be dropped altogether and that

toxicity should be stated primarily in terms of the *median lethal dose* which he abbreviated as LD50.

Trevan's paper rapidly gained traction and the 1930s saw the development of a number of data-analytic methods for the estimation of the ED50. A notable development of the time was the collaboration between Ronald Fisher (responsible for the concept of statistical significance) and Chester Bliss which resulted from Fisher's 1933 invitation to Bliss for him to work at the University College, London. Bliss and Fisher's approach to bioassay modelling was to treat the stimulus (dose) as the *covariate* and, because of variability in individual tolerance levels, treat the response as a *random variable*. In this formulation the response is ascribed a probability distribution – in this case the normal.





21 July 1933.

Dr. C.I. Bliss,
724 Earlham Drive,
Whittier,
Calif., U.S.A.

Dear Dr. Bliss:

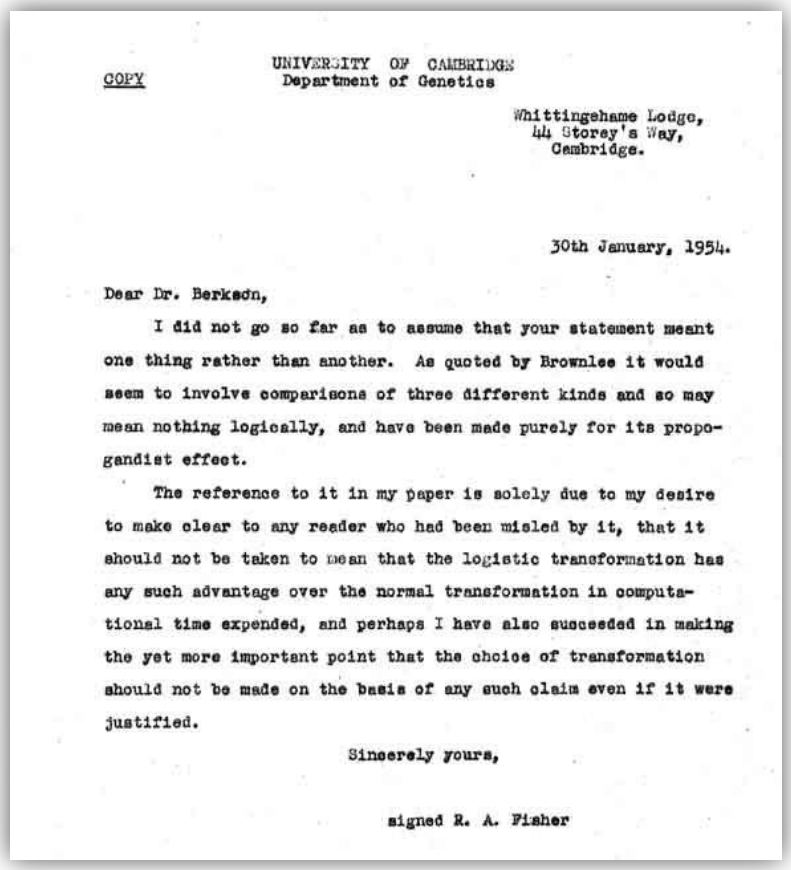
I have your letter of 7 July 1933 and should be happy to have you working with me, especially as I am better able now than previously, to undertake genetical work. Before you come to England, I shall move from Rothamsted to the Galton Laboratory at University College in ~~the~~ London University. The fees for post-graduates are very low, even less than those we charge voluntary workers at Rothamsted; and living in London need not be more expensive, though it may be much more if the stuff is burning in your pocket.

My new quarters seem to have quite good potentialities, though needing re-equipping and re-organisation, and if you like to make yourself useful in chaotic conditions you might well find it amusing. Haldane is also at University College now. Without suggesting that I have any serious intention in saying so, you might be glad to know that my staff is still incomplete on the biological side. I certainly have not, however, the right to tempt you across the Atlantic, merely ~~as~~ for the simple fact is opened out by this fact.

Yours sincerely,

Bliss went on to publish two notes in *Science* and a paper in the *Annals of Applied Biology* titled *"The Calculation of the dosage-mortality curve"*. In his publications in *Science* he introduced the term *probit* as shorthand for *probability unit* while his paper in the Annals laid out the fitting of the probit curve to bioassay data using the recently developed principle of maximum likelihood estimation.

Probit analysis quickly established itself as the de facto modelling approach to describe any relation of a discrete binary outcome to one or more explanatory variables. In 1944 US statistician Joseph Berkson advocated the use of the logistic function as an alternative to probit analysis. He admonished the use of maximum likelihood estimation and instead advocated minimum chi-square as an alternative estimation strategy. In his characteristically antagonistic style and to make his point, Berkson introduced the term *logit* as shorthand for the mathematical transformation of proportions using the logarithm of the odds ratio. Fisher was not amused and much heated debate and controversy ensued. Berkson's suggestions were also eschewed by the biometrics establishment as the logit was viewed as inferior to the probit since it could not be related to an underlying distribution of tolerance levels.



COPY

UNIVERSITY OF CAMBRIDGE
Department of Genetics

Whittingehame Lodge,
44 Storey's Way,
Cambridge.

30th January, 1954.

Dear Dr. Berkson,

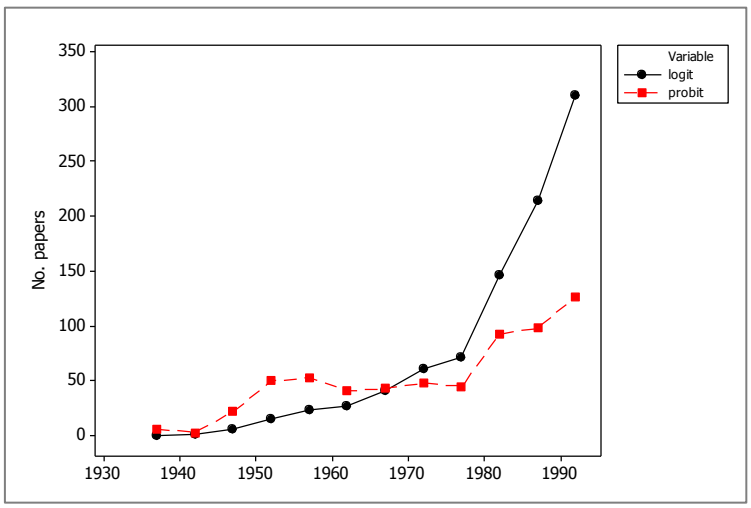
I did not go so far as to assume that your statement meant one thing rather than another. As quoted by Brownlee it would seem to involve comparisons of three different kinds and so may mean nothing logically, and have been made purely for its propogandist effect.

The reference to it in my paper is solely due to my desire to make clear to any reader who had been misled by it, that it should not be taken to mean that the logistic transformation has any such advantage over the normal transformation in computational time expended, and perhaps I have also succeeded in making the yet more important point that the choice of transformation should not be made on the basis of any such claim even if it were justified.

Sincerely yours,

signed R. A. Fisher

Prior to 1980, the computational aspects of statistical modelling were extremely important since both computing power and accessible software were limited. However, it appears that not even Berkson was aware of the significant computational advantages of the logistic equation and by the early 1960s the logit/probit bioassay debate had all but disappeared from the statistics literature. However, a review of the literature shows that since the mid-1970s the use of the logit model seems to be the preferred modelling strategy



even though probit models can now be fitted with ease.

I'd like to conclude this discussion of the origins of statistical ecotoxicology by noting that we appear to have entered an 'interesting' phase of model development. The uptake of the statistical computing package R is breathtaking. Despite Newman's assertion that toxicologists suffer "an overzealous adherence to standard methods" we have witnessed the emergence of a rich suite of tools for toxicity modelling. Christian Ritz claims that "Dose–response modeling is the state-of-the-art methodology underlying modern risk assessment" and his DRC package for R describes more than 20 functions for modelling C-R data. The challenge as I see it will be to educate toxicologists in the *sensible* use of these sophisticated modelling tools. For example, the use of a 5-parameter model to describe more complex responses such as hormesis are meaningless if applied to response data having the typical 5 or 6 concentration levels.

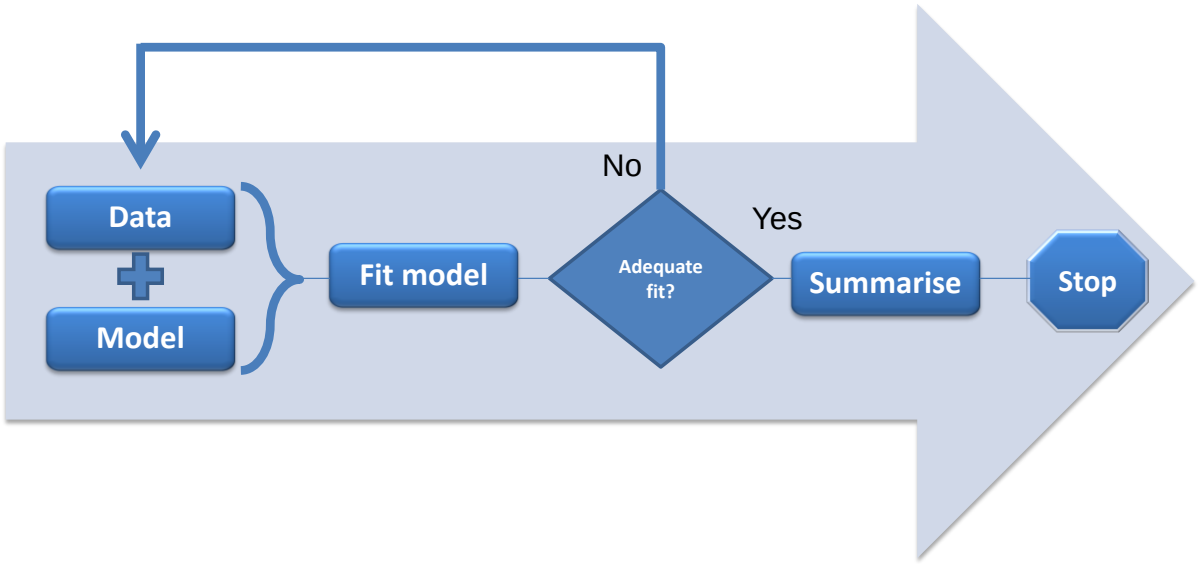
In the 10 or so minutes I have left I would like to touch on some of the problem areas in statistical ecotoxicology – the 'burrs under the saddle' I referred to earlier, and then conclude with some thoughts on future directions.

Burrs under the saddle

As many of you are aware, recently I have been responsible for promoting the *Bayesian paradigm* for the analysis of ecotox. data both through published papers and workshops. If the uptake of Bayesian statistics in other life sciences is any indication, we should expect to see more applications of Bayesian statistics in ecotoxicology. I would nevertheless like to make my position clear: there is no preferred modelling paradigm or framework for statistical inference. The Bayesian model-based approach I use to estimate a *no effect concentration* as a replacement for the bankrupt NOEC is not the only approach and more 'conventional' methods based on frequentist statistics are equally applicable. There are however, certain advantages associated with the Bayesian approach that are not enjoyed by classical approaches. While I don't have time to discuss those points of difference here, the point I do wish to make is that ecotoxicologists should embrace the 'new' paradigm, learn about it, use it judiciously and, above all, avoid rehashing the age-old debates

about the legitimacy of subjective probabilities or the arbitrariness of picking a suitable prior probability density. I have been challenged on this latter point by ecotoxicologists who eschew the notion of a subjective prior yet seem to ignore the inconvenient truth that the blind faith they entrust to the results from ToxCalc is underpinned on a (not insignificant) number of assumptions and somewhat arbitrary modelling choices.

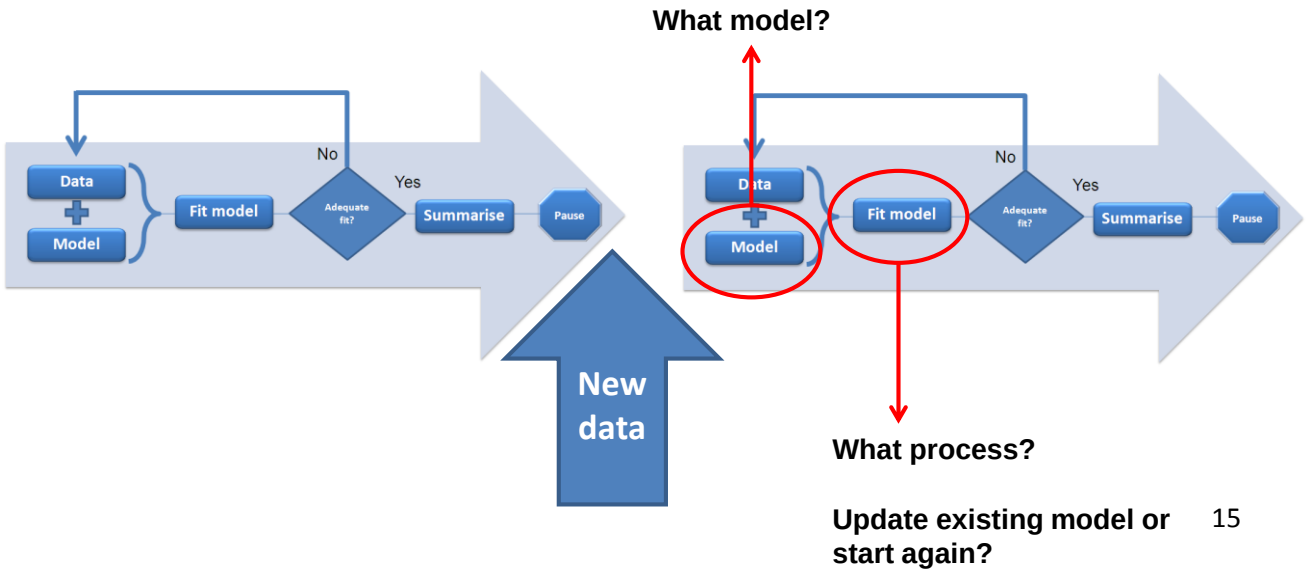
The traditional approach to statistical modelling is illustrated in this slide.



Adapted from Nelder, 1999

Data and a model are brought together to estimate parameters. We use a statistical test or suite of tests to assess the adequacy of the fit. The model may require refinement and/or additional data to be collected. We cycle through this process to develop a parsimonious description of the response-generating mechanism. We summarise the results and then stop.

The situation in ecotoxicology is a little different – the systems we are



describing are invariably dynamic and with the passage of time comes additional data and new insights.

We might repeat the modelling process, but some decisions have to be made about the form of the model and the method of updating our toxicity estimates, triggers, 'safe' concentrations etc.

Other 'difficulties' requiring closer attention are:

1. The *design* of C-R experiments. I'm not talking of the analytical lab. methods – they're well defined. What is less well defined is a contemporary 'roadmap' to assist in making decisions about the number and spacing of concentrations to use; how much replication is required; how to choose a plausible mathematical model – or at the very least, how to eliminate unsuitable ones; how to estimate model parameters and associated uncertainty; how and when to transform data; when or if toxicity measures should be pooled; characterisations of the SSD; and so on.

To be fair, there are a number of statistical guides for ecotoxicology such as those published by Environment Canada and the OECD and Australia and New Zealand are presently working on their own. However, some of this information – particularly in the Canadian document – is so out-dated, and dare I say, flawed, as to render the advice next to useless. This may seem unfairly harsh, however by way of example, the blanket requirement of the Canadian *"Guidance Document on Statistical Methods for Environmental Toxicity Tests*" to always use log-transformed data is both perplexing and unwarranted. Furthermore, the claim that "Canadian investigators …are often reluctant and sometimes actively hostile to the idea of continuing with logarithms for statistical analysis" because, it is suggested, Canadian scientists and technicians have a lack of familiarity with the complexity of logarithms is disingenuous and, frankly insulting. Other advice such as estimating an EC_{50} from a hand-drawn graph and fitting probit curves by eye is nothing short of astonishing. Even Chester Bliss and Ronald Fisher had better algorithms for doing this before the first computer was even invented! But then again, the Canadian document refers to

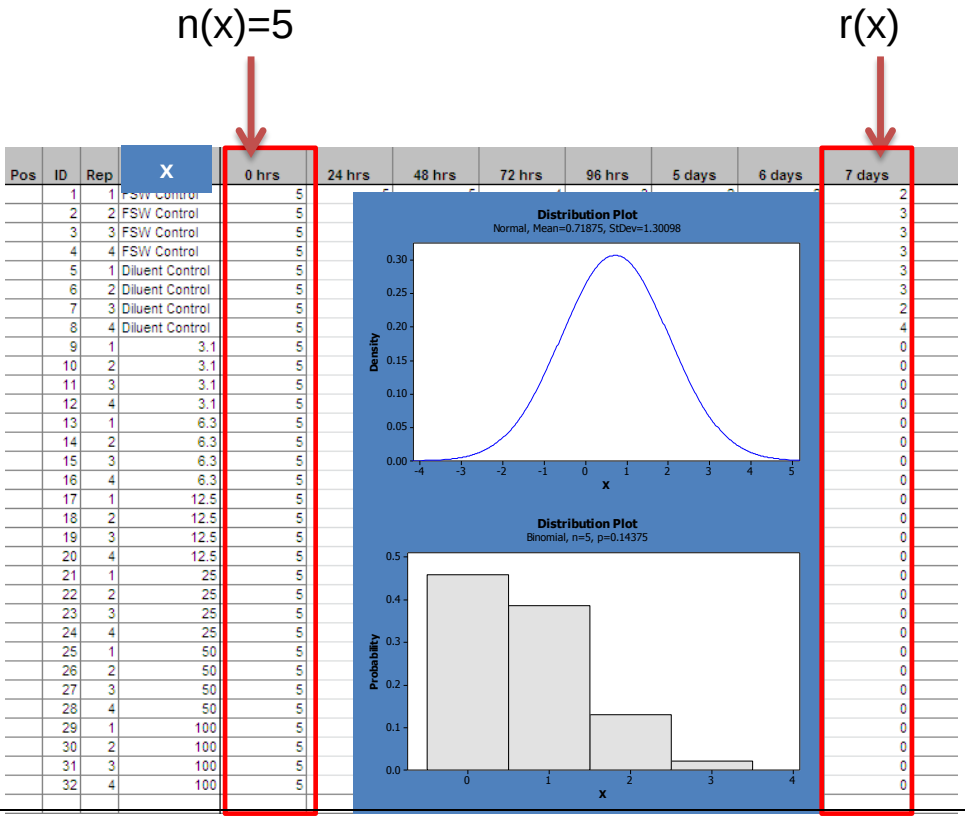
the existence of “modern computers” as an alternative to hand-calculation and furthermore recommends the use of a software tool developed in 1978 and modified for the Windows operating system in 1995! Hardly contemporary stuff!

2. Challenging the assumptions. Contemporary approaches to the identification of a ‘safe’ concentration or dilution of some contaminant rely on a plethora of statistical approaches – from simple (or if you’re Canadian, very difficult) *arcsine* and *logarithmic* transformations of the raw toxicity data to advanced tools for mathematical modelling and statistical inference. We know the NOEC is flawed, but struggle to find suitable alternatives. A common practice, driven by data paucity or claims of superiority, is to use various combinations of NOECs, ECxs, ICxs, or arbitrarily scaled versions of these as inputs into the SSD modelling stage where again we make somewhat arbitrary decisions about the distributional form of the SSD and the data inclusion/exclusion rules. Rather than undertaking more investigations to characterise the difference between say a lognormal SSD and a log-logistic SSD what I believe we need are some well-designed experiments to test the claims that some high fraction of all species is protected provided the environmental concentration of a contaminant is below the threshold or trigger value. Standard C-R test procedures could also benefit from an assessment of the effects of ignoring the time dimension and the consequences of a less than comprehensive assessment of variability. These are the topics of two talks in the *Environmetrics* session after morning tea.

3. Being clear on what we’re doing. If, as I’ve suggested, we move more to model-based inference for deriving toxicity measures, then it is incumbent upon us to use *credible* models. For any modeller, the truth is expressed by the fact that **data = model + error**. Thus our representation of what we observe has two components: a *deterministic component* and a *stochastic component*. The ready accessibility of programs like ToxCalc sometimes means that we spend

either no time or too little time thinking about suitable structures for these model components. ToxCalc provides toxicity estimates that are both empirical (such as a NOEC) and model-based (such as an EC_{10}) although the choices are limited, automated, and not readily apparent. Indeed, one of the selling points boasted on Tidepool's web site is that ToxCalc "automatically chooses the appropriate methods and data transforms". What is not well understood is that programs like ToxCalc base their statistical inference (eg. confidence intervals) on the assumption that the error term follows a normal probability model. Part of ToxCalc's automation is the use of mathematical transformations to beat the data into some semblance of normality when those data fail a test of normality. A more discerning modeller would think about an *appropriate* error structure for the data at hand. For example, toxicologists commonly deal with survival data of the form "*r animals surviving out of n"*.

For example, here we see data relating the number of fish out of an initial *five* surviving at various times as a function of effluent concentration.

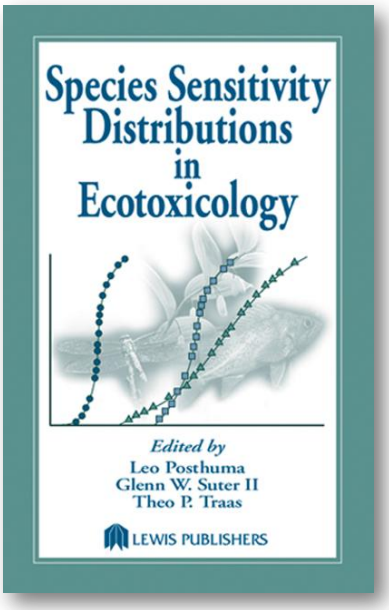


One only needs to think about this for a nanosecond to realise that the response variable (*r)* is *discrete* whereas the normal distribution is a *continuous* probability model. Not to worry, you recall your crusty old statistics professor mumbling something about the law of large numbers or the central limit theorem as the universal life buoy in such cases of disconnect and happily proceed without giving it any further thought. Provided *n* is 'large' (and the statistical version of 'large' is typically *n>30*) you won't drown. But what happens when *n* is small – like 5? Well, in such cases the normal distribution will not provide much buoyancy. A better strategy is to start with an error model that is both commensurate with the scale of measurement *and* which is plausible. The poisson, binomial and negative binomial probability models are usually good candidates.

I will now finish with a few concluding remarks about challenges and opportunities.

Statistical Ectoxicology – Revitalising the marriage

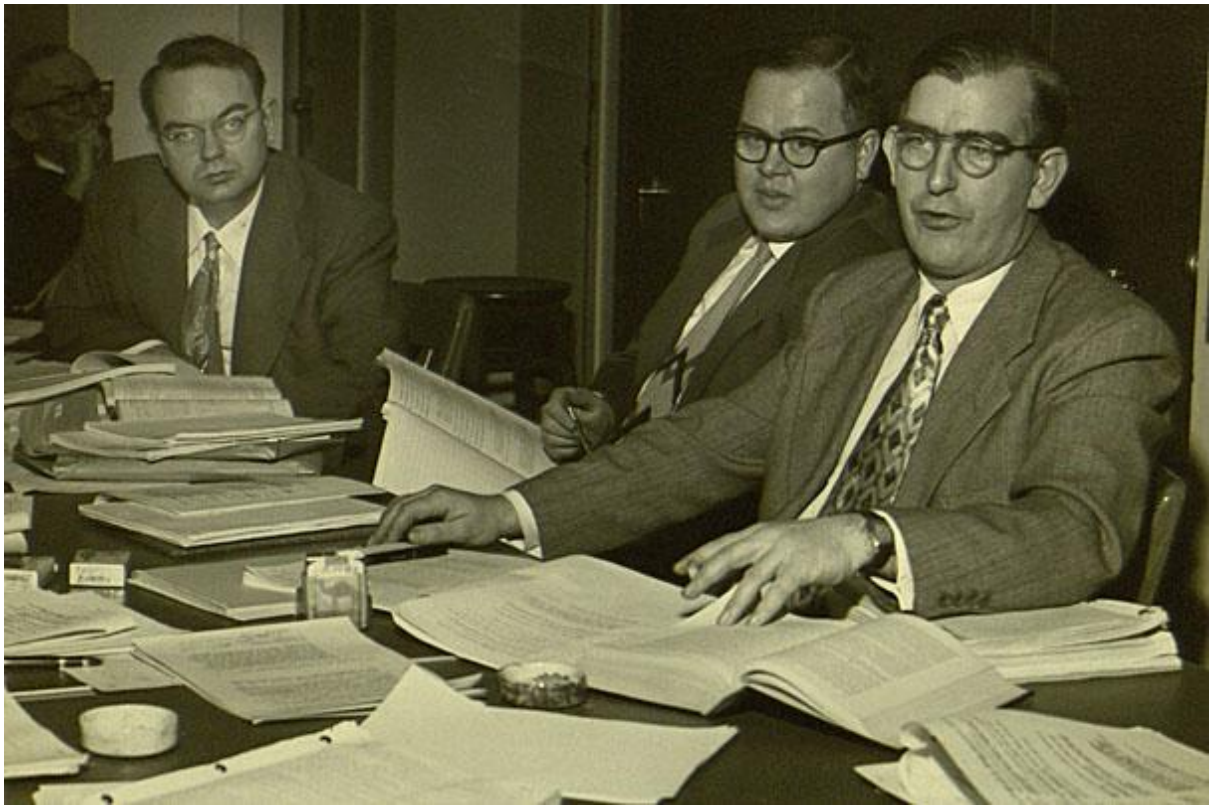
In this short presentation I have attempted to highlight the long, although not always visible, interaction between statistics and ecotoxicology. One only needs thumb through the pages of this text to appreciate the central role of statistics in modern-day ecotoxicology.



I have used the metaphor of *marriage* to characterise the relationship between our disciplines. On reflection – perhaps it <u>was</u> a shotgun marriage. The 'heady' days of the 1960s and 70s witnessed a flurry of activity and interaction that does not appear to have been sustained into the new millennium. I can't be sure, but it seems like cracks have developed which, if true, need to be

stemmed before they become chasms. The marriage needs rejuvenation - we need a new roadmap and we need to reaffirm our vows.

So what does that mean exactly? Well, for me I'd like to see a more *enduring* engagement of statisticians with ecotoxicologists. Over the last 20 years we have seen a de-skilling in quantitative capabilities in many agencies. Biometrics units and the biometricians who worked in them are all but things of the past – casualties of simple-minded economic policies that failed to understand that the benefits of good statistical design and analysis far outweighed the cost of delivering such a service. Interestingly, it was the *real* problems in other disciplines that resulted in some of the most celebrated 'discoveries' in statistics – such as the development of ANOVA by Fisher and Yates at Rothamstead Experimental research station. Here we see another statistical giant – William Cochran (closest to camera) in the presence of Frederick Mosteller (far left) and John Tukey poring over the Kinsey report data in the 1950s (just goes to show, statisticans *do* have an interest in sex!).



Tukey, you might recall gave us such tools as the boxplot, jacknife and, together with James Cooley, a FFT algorithm. He also famously remarked that "we get to play in

everybody's backyard". And that brings me to my point – statisticians are *not* playing in *your* backyard! We have seen the profound impact of statisticians such as Fisher, Bliss, and Berkson on quantitative developments in toxicology. But that was more than 70 years ago. The 1980s and 90s saw a huge amount of work by quantitative biologists and toxicologists into many aspects of SSD modelling but I struggle to name a single statistician who has played a seminal role in these developments.

I will not dwell on the list of challenges for SSDs – Suter has done that already in Chapter 21 of the text by Posthuma et al. Given the importance of the task, the pervasiveness of the results, and the consequences of 'getting it wrong', it is hard to argue against a strengthening of quantitative skills in ecotoxicology. The path already exists – we just have to take the first step. Thank you.