JOB OFFER

The Position

We are looking for a motivated biostatistician to quickly integrate into and complement an existing team of data analysis experts in the Translational Research Sciences department of Roche Pharma (pRED). Tightly integrated with bioinformatics specialists our group develops statistical models for discovery / validation of new targets and biomarkers, monitoring drug effects, as well as elucidating the mechanism behind diseases.

The main focus of the vacant position will be to support pRED scientists (primarily – but not exclusively - in the areas of metabolic/vascular diseases and CNS) in their drug and biomarker discovery programs.
The successful applicant :

- will be responsible for statistical analysis in projects from target identification to proof of concept, with an emphasis on translational medicine projects using high dimensional molecular data,
- will work as a member of interdisciplinary project teams, but also provide consultancy and service on request for specific projects. Typical tasks will include the design of experiments, power analysis and development of statistical analysis plans, general exploratory data analysis and statistical modeling (analysis of *in vivo*-, bioassay- and longitudinal data in particular), analysis of high dimensional data (genetics / genomics proteomics experimental data), documentation and presentation of results,
- will perform training and education of scientists in terms of statistical thinking.

Your Profile

- You have a PhD in Biostatistics or equivalent, with at least 4 years of work experience and have relevant experience in the pharmaceutical / biotechnology industry in research or exploratory development and statistical consultancy.
- You have a strong background in standard statistical analysis methodologies. You are proficient in statistical programming with tools like R/Bioconductor, S-Plus, SAS or SAS/JMP. You have adequate levels of IT, data management and programming skills.
- You are able to provide clear documentation, and enjoy team work.
- Ideally you possess some essential domain knowledge in biology and about the standard measurement technologies (such as PCR, ELISA, microarrays, sequencing) as well as bioinformatics tools and common annotation sources. You need to have the ability to understand the actual requirements of scientists (biologists and biochemists) and translate those into statistical problem formulations, as well as to efficiently communicate results to different target audiences.
- You are motivated to work in interdisciplinary and multi-national teams, requiring fluent spoken English and adequate presentation skills.
- You are ready to develop into new areas and expand existing capabilities, as well as to work independently, be flexible and efficient to deliver on tight time lines.

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Some of the Industry Segments covered by MathWorks solutions

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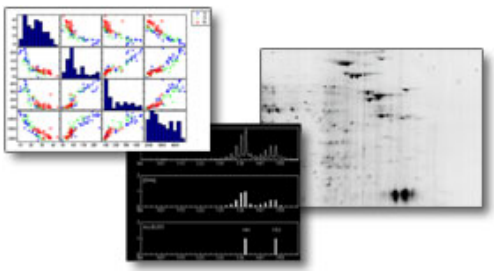
The explosive growth of data about genes, proteins, and drug molecules and their functions and interactions, necessitates efficient mathematical algorithms and software tools to extract meaning from that data. These algorithms and tools must be equally effective with raw data generated through gene sequencing, allele scoring, high-throughput screening assays, and expression array analysis.



From left to right: The MathWorks Statistics Toolbox™ product - Multivariate Analysis of Variance (MANOVA); TrueAllele—an "open system" for high-throughput genotyping; Gel image created by 2-D polyacrylamide-gel electrophoresis. Courtesy of Alan W. Partin, M.D., Ph.D., Johns Hopkins University School of Medicine

Bioinformatics scientists and developers worldwide rely on MATLAB to accelerate scientific discovery and reduce development time. Developers in bioinformatics value the open, component-based architecture of the MATLAB environment, which lets them choose only the tools and extensions needed. In addition, they have direct access to toolbox source code and can modify existing functions and models or add custom functions.

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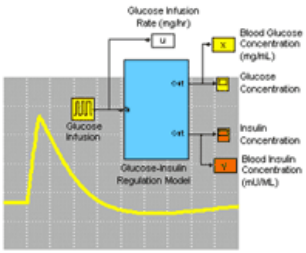

Simulink® model of the physiological control system that regulates blood glucose levels through the secretion of insulin. Courtesy of IEEE Press.

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SAS fournit à ses clients des solutions décisionnelles qui leur permettent de transformer les données réparties dans toute l'entreprise en connaissance, et ainsi, de prendre de bonnes décisions. SAS couvre tout le processus de traitement de la donnée et participe à la création de valeur par la réduction des coûts, la croissance du chiffre d'affaires, l'optimisation des processus internes et la visibilité sur les objectifs et la stratégie. S'appuyant sur une plate-forme décisionnelle intégrée et un ensemble de solutions dédiées, l'offre SAS permet de tirer le meilleur profit des systèmes d'information pour répondre aux problématiques d'entreprise.

Le déploiement de puissantes solutions analytiques et décisionnelles est généralement associé à l'univers des grandes entreprises ; parmi les 100 premières entreprises du classement FORTUNE Global 500® de 2009, 92 sont des clients de SAS. Cependant, aux États-Unis, 80 % des nouveaux clients sont des PME – démontrant clairement que même les entreprises ayant un chiffre d'affaires inférieur à 500 millions de dollars comprennent la valeur ajoutée des solutions analytiques et décisionnelles de SAS.

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SIGMA PLUS diffuse en France depuis 1979 des logiciels statistiques pour le contrôle de la qualité, les plans d'expériences, la validation des méthodes analytiques, les analyses inter-laboratoires, les méthodes multivariées et les simulations Monte Carlo.

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PRINCIPAUX LOGICIELS PRESENTES

STATGRAPHICS Centurion (version 16.1) répond avec ses 170 procédures aux besoins en analyses statistiques descriptives et exploratoires, ajustements de lois, tests, Anova et régressions, contrôle de la qualité, méthodes de survie, plans d'expériences, méthodes de prévision, analyses des données multivariées et simulations Monte Carlo. Ses modules complémentaires Uniwin Plus et Vmail apportent les techniques d'analyse des données « à la française » et d'analyse inter-laboratoires (ISO 5725).

CRYSTAL BALL (version 11.1.2) et **@RISK** (version 5.5) sont des logiciels de simulation et d'optimisation par la méthode Monte Carlo. Analyse de risque lors du développement de nouveaux produits, amélioration des négociations et des prévisions, optimisation de territoires de ventes et démarche Six-Sigma (DMAIC, DFSS) sont quelques-unes des applications possibles de ces outils.

SIMCA-P+ (version 12.0) utilise les méthodes PLS (moindres carrés partiels) pour l'analyse des données multidimensionnelles, le QSAR, les études « omics », l'analyse d'images et pour traiter les problèmes du contrôle multivarié de la qualité pour des procédés continus ou par lots. SIMCA-4000 et SIMCA-BOL sont les versions « on-line » de ce logiciel.

MODDE (version 9.0) permet la définition et l'analyse de plans d'expériences (criblage, surface de réponse, mélange, D-optimal, Taguchi, Doehlert, Rechtschaffner, RED-MUP, …). Des outils pour le « Quality by Design » et l'estimation et la validation du « Design Space » sont proposés.

SYNERGY 2000 (version 8.0) est un logiciel de MSP/SPC en temps réel permettant de déployer les techniques classiques du contrôle de la qualité à tous les niveaux de l'entreprise (opérateurs, ingénieurs et superviseurs).

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Scientific Program

Monday, 27 September 2010		
13:00 – 17:00	Workshop Topics: Fast Sparse Regression and Classification	Intro: Emmanuel Pham Professor Jerome H. Friedman, University of Standford, USA
17:30 – 18:30	Workshop Matworks	
Tuesday, 28 September 2010		
08:30 – 08:45	Welcome	Chair: Emmanuel Pham
08:45 – 09:30	Opening Lecture Improving statistical quality in published research: the clinical experience	Intro: Ludwig Hothorn Professor Martin Bland, Department of Health Sciences, University of York, UK
Session 1	**Assays**	**Chair: Richardus Vonk**
09:30 – 09:50	Statistical analysis of analytical method transfer: proposals for the location-scale approach and tolerance intervals	Cornelia Frömke
09:50 – 10:20	Development and Validation of an in-vivo bioassay	Birgit Niederhaus
10:20 – 10:40	Cut-off determination: can prediction limit be used as an alternative to the 95% percentile?	Marion Berger
10:40 – 11:10	Coffee break	
11:10 – 11:30	Statistical methods for cut-point determination in enzyme-linked immunosorbent assays (ELISA)	Thomas Jaki
11:30 – 11:50	Use of parametric model-averaging in dose-response analysis	Christian Ritz
11:50 – 12:10	Comparison of initial value routines for dose-response models	Anke Schulz
12:10 – 13:10	Lunch	
Session 2A	**Bayesian Statistics**	**Chair: Bruno Boulanger**
13:10 – 13:30	Use of Bayesian inference in ecotoxicology	Sandrine Charles
13:30 – 13:50	Optimization of ligand-binding assay in a QbD environment: Use of Bayesian non-linear hierarchical model to set up precision profile as quality response.	Pierre Lebrun
13:50 – 14:10	A Bayesian approach to predicting precision of a future study in the presence of uncertainty about the variance	John Sherington
14:10 – 14:30	Bayesian modelling of Escherichia coli O157:H7 dose response incorporating age as a covariable	Marie Laure Delignette-Muller
14:30 – 15:00	Coffee break	

Session 3A	**Methodology (I)**	**Chair: Viswanath Devanaryan**
15:00 – 15:20	Probabilistic modeling for risk assessment of residual host cell DNA in biological products	Harry Yang
15:20 – 15:40	Steady-State zone and control chart for process parameters of a powder compactor	Caroline Lévéder
15:40 – 16:00	Design Space – Risk Based Approach	Kevin Lief
16:00 – 16:20	Assessing quality control for repeated bioassay data by parametric and non-parametric prediction intervals	Daniel Gerhard
16:20 – 16:40	/	/
16:40 – 17:00	Coffee break	
Session 4A	**Methodology (II)**	**Chair: Harry Yang**
17:00 – 17:20	How to accept the equivalence of two measurement methods? Comparison and improvements of the Bland and Altman's approach and errors-in-variables regression.	Bernard Francq
17:20 – 17:40	Characterizing in vitro synergy using the ray design methodology	Sophie Ruquet
17:40 – 18:00	/	/
Session 2B	**Toxicology (I)**	**Chair: Ludwig Hothorn**
13:10 – 13:30	Comet Analysis – Issues, Limitations and Recommendations	Jonathan Bright
13:30 – 13:50	Comet Assays – Facts and Figures	Luc Essermeant
13:50 – 14:10	An Overview of the Toxicology SIG	Mike Aylott
14:10 – 14:30	Testing a trend effect for count variables which are bounded by another count variable: application to fertility counts	Jean-Paul Lahmy
14:30 – 15:00	Coffee break	
Session 3B	**Toxicology (II)**	**Chair: Yi Tsong**
15:00 – 15:20	Assessing systemic drug exposure in repeated dose toxicity studies in the case of complete and incomplete sampling	Martin J. Wolfsegger
15:20 – 15:40	Williams-type procedure on monotone trend for normal, non-normal, ordered categorical, proportion and poly-k data, with application in toxicology	Ludwig Hothorn
15:40 – 16:00	A Step-Down Approach to Analyzing Electrocardiogram Endpoints For In Vivo Regulatory Toxicology Studies	Maya L Hanna
16:00 – 16:20	Concentration-response analysis of a stochastic process: Multi-state modelling of in-vitro folliculogenesis and oocyte maturation	Marc Weimer
16:20 – 16:40	Statistical Analysis in ACuteTox: Constructing a classifier for chemical toxicity from in vitro assay concentration-response curves	Annette Kopp-Schneider
16:40 – 17:00	Coffee break	
17:00 – 17:20	Optimization of the irinotecan – efflux transporter inhibitors combination for treating irinotecan resistant tumours	Alexandre Sostelly
17:20 – 17:40	Anova-Type Statistics, a good alternative to parametric methods for analysing repeated data from preclinical experiments	Odile Coudert-Berthion

17:40 – 18:00	Prediction of tumor class from gene expression data using bagged decision trees	Sam Roberts
18:30	**Social event: Dinner (included in the registration fee)**	

Wednesday, 29 September 2010

09:00 – 09:30	Opening Lecture Development of a dose content uniformity test suitable for medium and large sample sizes	Intro: Emmanuel Pham Yi Tsong, Deputy director, Office of Biostatistics, Center for Drug Evaluation and Research, FDA, USA
Session 4	**Translational sciences**	**Chair: Emmanuel Pham**
09:30 – 09:50	K-PD models as a flexible modeling tool in non-clinical statistics	Tom Jacobs
09:50 – 10:20	A Unified Approach to Flexible and Powerful Modeling Of Pre-Clinical Combination Studies	Chris Harbron
10:20 – 10:40	Pharmacokinetic similarity analysis using nonlinear mixed effects models	Anne Dubois
10:40 – 11:10	Coffee break	
11:10 – 11:30	Modeling Dose-response Microarray Data in Early Drug Development Experiments	Dan Lin
11:30 – 11:50	A multivariate analysis of prognostics factors in Chronic Lymphocytic Leukemia	Giuseppe Palermo
11:50 – 12:10	Genomic Biomarkers for Depression: feature-specific and joint biomarkers	Helena Geys
12:10 – 13:10	Lunch	
13:10 – 13:40	Invited Lecture Development of biomarker signatures from high-dimensional array data, and identification of subsets with common patterns between multiple array platforms	Intro: Bruno Boulanger Viswanath Devanaryan, Director Exploratory Statistics, Abbott Laboratories, USA
Session 5	**'Omics**	**Chair: Luc Bijnens**
13:40 – 14:00	Multivariate approaches to develop molecular biomarkers: a reality check.	Willem Talloen
14:00 – 14:20	Dimension Reduction through Variable Selection – A Fibrosis Case Study	Katja Remlinger
14:20 – 14:40	Seeing the Wood for the Trees: Interrogating the Structure of Random Forests	Chris Harbron
14:40 – 15:10	Coffee break	
15:10 – 15:30	Testing for the Differential Expression of Genes at the Probe Level of Affymetrix Microarray data	Tatsiana Khamiakova
15:30 – 15:50	Treatment of Genomic Data in the Context of Macroarrays	Julie Pouget
15:50 – 16:10	Addressing the Challenges of the Analysis of Genomics Data with JMP Genomics	Valerie Nedbal
16:10 – 16:30	**Closing remarks**	**Luc Bijnens & Emmanuel Pham**

SESSION 1

ASSAYS

Chair – Richardus Vonk

Opening Lecture

Improving statistical quality in published research:
the clinical experience

Professor Martin Bland,
Department of Health Sciences, University of York, UK

Statistical analysis of analytical method transfer: proposals for the location-scale approach and tolerance intervals

Cornelia Frömke, Ludwig Hothorn, Michael Schneider

- **Abstract:**

A method transfer is a process, where an analytical process is already validated in one laboratory and this process shall be transferred to a new laboratory. The statistical aim of this transfer is to prove the similarity of the measurements between the two (or more) laboratories. To date, no official guidelines for the statistical analysis of a method transfer exist. The FDA published the Guidance for Industry 'Protocols for the Conduct of Method Transfer Studies for Type C Medicated Feed Assay Methods', however, the statistical analysis is not taken into account. Generally, for the analysis the FDA Guidance for Industry 'Bioanalytical Method Validation' can be referred. Here, a method is validated, if both the trueness (closeness of mean test result obtained by the method to the true value) and the precision (closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix) are less than 15%. In the presentation, confidence intervals are proposed to analyze this location-scale problem. Parametric as well as non-parametric confidence intervals for the difference and the ratio of paired samples are discussed. For the graphical presentation of the data, parametric and nonparametric tolerance intervals for differences and ratios are presented as well.
To illustrate advantages and disadvantages of these approaches, results from simulation studies are shown.

Development and Validation of an in-vivo bioassay

Birgit Niederhaus, Adama Savadogo, Matthias Blumrich, Markus Kohl, Christophe Agut

- **Abstract:**

The main purpose of this presentation is to set out the statistical components of the development of an in-vivo bioassay for a therapeutic protein.
The developed bioassay method was intended for:

- the determination of the potency of the corresponding compound,
- the analysis and comparison of the specific activity after modification of the production process,
- the demonstration of stability of batches.

The bioassay development was done in several subsequent steps.

First, feasibility studies were performed to select the relevant time ranges, to define the statistical end-point together with the relevant concentration range to ensure a linear log(dose)-response relationship.

A subsequent comparability study was designed in which the equivalence of six test batches to a reference standard could be shown for each tested concentration.

A prevalidation study was designed to:

- validate the standard (by dosing the working standard vs. itself)
- establish a variance model for a weighted linear fit
- verify linearity of dose-response relationship fitted and establish equivalence limits for the parallelism of standard and test dose-response lines.

A procedure for routine testing for batch release was established with suitability criteria and analysis of relative potency, just as the method validated in term of trueness and precision.

Cut-off determination: can prediction limit be used as an alternative to the 95% percentile?

Marion Berger, Dave Hoffman

- **Abstract:**

Cut-off assessment is critical as, once determined, it is going to act as a judge declaring whether a sample potentially (in case of screening) or certainly (in case of specificity) contains or not an anti-drug antibody.
Yet, in-study sample analyses are subject to various sources of variability such as the analytical component and the biological component which itself split into the subject to subject variability and the subject variability between the runs. The method validation experiments can help determining the weight of each of these components in the total variability. Then, one can question if all or only some should be kept in the determination of the cut-off.
Based on these findings, another question arises: is the empirical 95% percentile or the 95% percentile estimated from the standard deviation of the distribution of the method validation experiments appropriately integrating the relevant components of the variability? Is it also really ensuring the detection of 5% of the false positive in any next samples analysis? Could the prediction limit approach be considered as an alternative, especially in cases where normality is not rejected? This presentation will propose some responses to these questions.

Statistical methods for cut-point determination in enzyme-linked immunosorbent assays (ELISA)

Thomas Jaki, John-Philip Lawo, Frank Horling, Martin J Wolfsegger, Peter Allacher, Julia Singer

- **Abstract:**

Biotechnology derived therapeutics may induce an unwanted immune response resulting in the formation of anti-drug antibodies (ADA). As a result the efficacy and safety of the therapeutic protein could be impaired. For example, neutralizing antibodies may affect pharmacokinetics of the therapeutic protein or induce autoimmunity. Therefore a drug induced immune response is a major concern and needs to be assessed during drug development.

Appropriate assays need to be developed for the detection and characterization of ADAs. Those assays include screening assays for identifying a positive sample, confirmatory assays to proof the screening results and a functional assay for assessment of the neutralizing capacity of antibodies. A critical step during assay development and validation is to define an appropriate cut off that enables to distinguish between positive and negative samples.

In this talk we will discuss and compare several statistical and heuristic methods for cut-point determination in enzyme-linked immunosorbent assays (ELISA) which are commonly used as screening and confirmatory assays.

Use of parametric model-averaging in dose-response analysis

Christian Ritz
Statistics Group, Department of Basic Sciences and Environment
Faculty of Life Sciences, University of Copenhagen
Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

- **Abstract:**

In the context of benchmark dose estimation, the last decade has seen a lot of interest in model averaging based on parametric dose-response models.

The motivation behind is that model averaging provides a way to take into account at least partly the model uncertainty due to not knowing the precise curve underlying the dose-response data. As a consequence the fact that inference is conditional on the chosen dose-response model is alleviated to some degree. As mentioned initially, this idea has mainly been utilized in the context of benchmark dose estimation in the low-dose range, where typically only very limited data are available. However, the methodology can also be extended to the general problem of estimating EC/ED values that are not necessarily located in the low-dose range, such as EC20/ED20 and EC50/ED50.

This talk will briefly outline the basic concepts involved and illustrate the ideas in a few applications.

Comparison of initial value routines for dose-response models

Anke Schulz
Bayer Schering Pharma AG, Berlin
Global Drug Discovery Statistics

- **Abstract:**

Non-linear models are increasingly important in the drug research and development process, and are in many cases routinely used in an automated fashion. High variation in the data and unknown response behaviour require robust routines for the estimation of the respective parameters, which is usually performed through iteration procedures. The convergence of the iteration process and indeed the final estimation of the parameters tend to be influenced by the initial (starting) values of the process. Different automated initial value routines for use with dose-response models that were found in the literature and some new ideas were evaluated. These procedures constitute a flexible and robust arsenal for obtaining starting values. They will be compared with regard to the accuracy and robustness by simulation studies.

SESSION 2A

BAYESIAN STATISTICS

Chair – Bruno Boulanger

Use of Bayesian inference in ecotoxicology

Sandrine CHARLES[1], Elise BILLOIR[1], Carole FORFAIT[1], Marie Laure DELIGNETTE-MULLER[1,2]

[1] Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France.
[2] Université de Lyon, F-69000, Lyon ; VetAgro Sup Campus Vétérinaire de Lyon, 1 avenue Bourgelat, F-69280 Marcy l'Etoile, France.

Abstract:

Ecotoxicology, in its broad interdisciplinary meaning at the bridge of chemistry, toxicology and modelling, aims at understanding the active role played by an accelerated xenobiotics production in the rising risk of ecosystem contamination, as well as harmful consequences on the long term functioningof ecosystems, and consequently to human health. It attempts to respond to current community expectations or the political demands, as ratifying before market launches of new substances (REACH), or assessing the consequences of « voluntary » pollution (industrial discharges, water purification plants…) on the environment and thus establishing associated management rules. Hence, ecotoxicology must develop tools and methods to identify potentially hazardous compounds, to define concentration thresholds acceptable at the different levels of biological organization (individual, population, community, ecosystem), and to draw up indices of environmental quality, but also to characterize causal links between toxicity and ecological impact. An approach, which is interdisciplinary, integrative, and associated to the development of predictive mathematical and statistical models, is thus clearly needed.

Our group recently introduced Bayesian inference in ecotoxicology to estimate effect model parameters at the individual level [Billoir *et al.*, 2008]; this statistical method has proven very efficient in *simultaneously analyzing* various types of data (like survival, growth or reproduction data), integrating uncertainties related to parameters. The Bayesian approach makes it also possible to combine prior knowledge on parameters and experimental data, and finally provides a joint posterior probability distribution leading to point estimates with credibility intervals for all parameters, that also allows us to detect correlations between parameters.

Our talk will illustrate the use of Bayesian inference in ecotoxicology, through several examples on two model species among aquatic invertebrates (*Daphnia magna and Branchiura sowerbyi*) under heavy metal stressors. We will also illustrate how uncertainties at the individual level can be theoretically extrapolated at the population level.

Optimization of ligand-binding assay in a QbD environment: Use of Bayesian non-linear hierarchical model to set up precision profile as quality response

Pierre Lebrun, Bruno Boulanger

- **Abstract:**

For ligand-binding assay (LBA), different parameters are controlled in routine. In a development phase, we will show how Design of Experiment (DOE) can be used to select these parameters to obtain reliable results in the future use of the ELISA test. Uncertainties of measurements and models should be taken into account in a predictive manner. Bayesian modelling and MCMC simulations are well suited for this purpose and are flexible enough to include random effects on the parameters of non-linear model. We will focus on 4PL regression as it is encountered in most applications. We will also show that deriving a quality response such as a precision profile is clear and direct in the Bayesian setting. The approach is fully compliant with QbD guidelines and a Design Space definition for LBA will be provided.

A Bayesian approach to predicting precision of a future study in the presence of uncertainty about the variance

John Sherington

- **Abstract:**

Dog cardiovascular safety studies are done routinely to test compounds prior to clinical studies. Historical data from such studies are used to produce estimates of likely precision and power for new studies. The data are also used to design quality control charts to see if a current study has achieved the desired precision in line with expectations. However, standard precision/power calculations assume that the underlying variance is constant across studies and known. To allow for the variance to differ between studies, historical data have been used to define a prior distribution for the variance to enable this uncertainty to be taken into account in a Bayesian framework.

Bayesian modelling of *Escherichia coli* O157:H7 dose response incorporating age as a covariable

Marie Laure DELIGNETTE-MULLER [1,2], Séverine JALOUSTRE [1,3,4], Hélène BERGIS[3]

[1] Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France.
[2] Université de Lyon, F-69000, Lyon ; VetAgro Sup Campus Vétérinaire de Lyon, 1 avenue Bourgelat, F-69280 Marcy l'Etoile, France.
[3] Agence française de sécurité des aliments (Afssa), LERQAP, 23 av. du Gal de Gaulle, F-94706, Maisons-Alfort Cedex, France.
[4] AgroSup Dijon, F- 21079 Dijon, France.

Abstract:

E. coli O157:H7 is responsible for a large number of outbreaks and sporadic cases of human infections reported all over the world. Infected humans may develop severe clinical manifestations such as the hemolytic and uremic syndrome (HUS). HUS is the most common cause of acute renal failure in children. It occurs preferentially among young children, and HUS surveillance data show a continuous decrease of HUS incidence as a function of age among children.
Dose response modelling is a critical point in microbial risk assessment. Experimental results observed on human volunteers are rare and generally difficult to extrapolate to the whole exposed population, several factors such as age impacting the host susceptibility to the pathogen. Thus it is important to develop dose-response modelling from human outbreak data. The use of bayesian inference has been recommended to estimate parameters of dose-response models. This approach enables to incorporate various sources of heterogenity in the collected data, and produces posterior distributions of parameters which can be easily incorporated in risk assessment models so as to take into account for uncertainty on parameter estimates.
In this study, bayesian modelling was used to explicitly incorporate the heterogeneity linked to the age of consumers in E.coli O157:H7 dose-response modelling. For this purpose, we used both data collected during a French outbreak linked to consumption of frozen ground beef patties contaminated by E.coli O157:H7, and data from consumer surveys, epidemiological surveillance and microbiological experiments. Age was taken into account as a covariable in a global model of the outbreak, incorporating an exposure module describing variables such as the serving size, the cooking preference and the consumption rate of ground beef as functions of the age of consumers. As a result of bayesian inference using the global model on all the available data, a simple dose-response model incorporating age as a covariable was proposed, that may be easily used in future risk assessment studies.

SESSION 3A

METHODOLOGY (I)

Chair – Viswanath Devanaryan

Probabilistic modeling for risk assessment of residual host cell DNA in biological products

Harry Yang, Lanju Zhang and Mark Galinski
MedImmune, One MedImmune Way, Gaithersburg, MD 20850

- **Abstract:**

Biological products such as viral vaccines manufactured in cells contain residual DNA derived from host cell substrates used in production. It is theoretically possible that the residual DNA could transmit activated oncogenes and/or latent infectious viral genomes to subjects receiving the product, and induce oncogenic or infective events. Regulatory guidance suggests mitigating the risks of oncogenicity and infectivity by decreasing the amount and the size of residual DNA. It also mandates that biological product manufacturers quantify the amount of DNA present in their product and assess the theoretical risks associated with the residual DNA. Although the standard method currently in use is easy to implement and recommended by FDA, it does not into account DNA inactivation step; nor does it account for sizes of individual oncogenes and infective agents. Therefore the risk estimates derived from the method are likely to be overstated as noted and acknowledged by both researchers and regulators. In this presentation, we introduce a probabilistic model to mechanistically study the relationship between the risks of oncogenicity and infectivity, and characteristics of the purification process such as efficiency of enzymatic degradation of DNA, amount of residual DNA in the final dose, and biological nature of the host cells including numbers and sizes of oncogenes and infectious viral DNA and etc. The method overcomes the shortcomings of the standard approach, and provides a much more accurate tool for residual DNA risk assessment. It may also potentially be used to guide manufacturer's effort to improve their manufacturing process and enhance the chance for the final product to meet regulatory standards. The method will be introduced and presented through a real-life example in which the method was successfully utilized in support for a cell-based live, attenuated influenza vaccine biological licensure application. Also illustrated in this talk will be the thought process and collaboration between statisticians and scientists that led to the innovative development of the method.

Steady-State zone and control chart for process parameters of a powder compactor

Henri Da-Cruz[1], Cécile Gabaude-Renou[1], Céline Giroud[2], Pierre Jambaud[1], Caroline Lévéder[1]

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[2] Hardtech

Abstract:

The Pharmaceutical Sciences Department of Sanofi-Aventis R&D has managed - with the support of IS Department - the development of an application that allows to gather on line the process data during the compaction of a powder, for Alexanderwerk compactors.
The main objective of this application is to follow manufacturing parameters on a real time basis and to perform on-line calculations which allow a perfect control of the process.
It allows comparison between manufacturing batches output and it is a strong support for scale-up and for comparing data obtained on equipments of different sizes; The main added-value of this software is to allow a selection of the outputs by having the possibility to select granules manufactured under controlled and stabilized conditions and to reject on line those manufactured under non-stabilized or non-desired process parameters.
The statistical part is dedicated to manufacturing parameters stability area determination and to control of these parameters values. These stability zone and mean control values allow the compactor to select the corresponding desired granules.
Statistical proposals, to determine steady-state zone and to better understand manufacturing process are the followings:
Steady-state determination thanks to 3 indicators (IC CV, first derivative, slope)
Mean Control Chart with predefined targets or with on line mean and standard deviation determination on the stability area
Statistical computations requirements and corresponding are firstly done with SAS programs. Then they have been implemented in C language in order to be processed in real time, and in Labview for results visualization.
Business analyzes have demonstrated this approach allow to save time and product during formulation development, and is helpful to improve scale-up and manufacturing process understanding.

Design Space - Risk Based Approach

Kevin Lief

- **Abstract:**

Describes a risk based approach to assurance of quality, focussing on visualisation of the modelling needed to identify design space. The approach is illustrated through two oral solid dose examples.
Places the modelling approach to identify a design space in the context of the concepts described in ICH Q8 and the Q8 Annexe, with a brief background to building a Design Space. The main focus is on the concepts, surrounding the modelling, needed to identify a design space, which are illustrated through two tableting process examples. The knowledge and design spaces are described and visualised.
The approach accounts for the relationships amongst the critical quality attributes, whilst simultaneously taking into account the relationship between the process parameters and the critical quality attributes.

- Summary and Conclusion

Design space should be described through a risk based approach to assurance of quality.
Assurance of quality cannot be a 100% guarantee (if variability exists in a process).
Design space should use a quantitative model to link critical quality attributes and process parameters.

- References

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Assessing quality control for repeated bioassay data by parametric and non-parametric prediction intervals

Daniel Gerhard

- **Abstract:**

In many bioassays the potency of a reference substance serves as a quality measure of the assay. To monitor the stability of the bioassay, potency estimates are repeatedly collected over a certain period, e.g. nine month. Based on a limited number of new runs of a following re-test phase, the decision is made whether the established reference can be used further or should be renewed.
Simple quality control charts can be used to assess the stability of various bioassays, especially for routine use. To characterise a process under control, prediction interval limits are calculated based on the `historic´ sample of potency estimates, to contain at least a certain amount of observations from the re-test phase.
The software implementation of several parametric and non-parametric prediction intervals in a user-friendly `R` package pred.intervals is presented. The handling of the software is illustrated by a real data example.

- Reference:

Hothorn, Gerhard, Hofmann (2009): Parametric and non-parametric prediction intervals based phase II control charts for repeated bioassay data. *Biologicals*, 37(5): 323-330.

SESSION 4A

METHODOLOGY (II)

Chair – Harry Yang

How to accept the equivalence of two measurement methods? Comparison and improvements of the Bland and Altman's approach and errors-in-variables regression

Bernard Francq, Bernadette Govaerts

- **Abstract:**

The needs of the industries to quickly assess the quality of products and the performance of the manufacturing methods leads to the development and improvement of alternative analytical methods sometimes faster, easier to handle, less expensive or even more accurate than the reference method. These so-called alternative methods should ideally lead to results comparable or equivalent to those obtained by a standard method known as a reference.

To compare two measurement methods, a certain characteristic of a sample can be measured by the two methods in the experimental domain of interest. Firstly, to statistically test the equivalence of measurement methods, the pairs of points (X_i, Y_i), representing the measures given by the reference method and the alternative one can be modelled by an errors-in-variables regression (a straight line). The estimated parameters are very useful to test the equivalence. Indeed, an intercept significantly different from zero indicates a systematic analytical bias between the two methods and a slope significantly different from one indicates a proportional bias. A joint confidence interval can also be used to test the equivalence. Secondly, the differences between paired measures X_i-Y_i can be plotted against their averages and analyzed to assess the degree of agreement between the two measurement methods by computing the limits of agreement. This is the very well known and widely used Bland and Altman's approach.

We review and compare the two methodologies, the Bland and Altman's approach and the errors-in-variables regression, with simulations and real data. Then, we'll propose some improvements like the tolerance interval on the Bland and Altman's approach and how to accept the equivalence by specifying a practical difference threshold on the results given by two measurement methods in the approach of errors-in-variables regression.

Characterizing in vitro synergy using the ray design methodology

Sophie Ruquet, SANOFI-AVENTIS

- **Abstract:**

In this presentation, the ray design methodology is applied to discern antagonism, additivity or synergy between two drugs in vitro on the basis of Loewe additivity index.
A generalized four parameter logistic model is used to analyse the data and calculate confidence intervals of the interaction indices. The analysis is performed with PROC NLMIXED in SAS. The approach is illustrated using data from an oncology study in which the inhibition effect of a combination of two compounds is studied using 96-well plates.

SESSION 2B

TOXICOLOGY (I)

Chair – Ludwig Hothorn

Comet Analysis - Issues, Limitations and Recommendations

Jonathan Bright
Discovery Statistics, AstraZeneca

- **Abstract:**

The Comet assay has been described by Lovell and Omori (2008) as a "quick, sensitive and cheap method for… the investigation of genetic damage associated with exposures to potentially genotoxic agents." It is a part of the risk assessment before a potential new drug goes in to man for the first time.

Data from these studies are analysed in a multitude of ways and conclusions are sometimes contentious e.g. Cederberg (2009)

Building on the work of Wiklund and Agurell (2003), and using both real and simulated data, the most common methods are compared head-to-head in order to assess their limitations and to establish if one of the methods is uniformly superior and hence to recommend it as the industry standard.

Lovell, D. P. and Omori, T. (2008) Statistical issues in the use of the comet assay. *Mutagenesis*, 23, 171-182.
Cederberg, H., Henriksson, J. and Binderup, M. (2009) DNA damage detected by the alkaline comet assay in the liver of mice after oral administration of tetrachloroethylene. *Mutagenesis Advance Access* published November 5, 2009.
Wiklund, S. J. and Agurell, E. (2003) Aspects of design and statistical analysis in the Comet assay. *Mutagenesis*, 18, 167-175.

Comet Assays - Facts and Figures

Luc Essermeant, Sanofi-Aventis
Richardus Vonk, Bayer Schering Pharma AG

- **Abstract:**

The single cell gel electrophoresis assay (comet assay) is a sensitive and short term in-vivo genotoxicity test. The goal of this assay is the assessment of possible treatment-related DNA damage in isolated cells. Such damage results in a comet-like shape of the electrophoresis image (tail). The endpoint of the assay, changes in DNA migration, is denoted by three parameters that describe the form of the comet: tail intensity, tail length, and tail moment. In general, 100 cells are evaluated on two slides per animal. Whilst there tends to be an agreement that the tail intensity is the primary parameter, the recommendations for the statistical analysis of the assay are few and controversial.

This presentation provides an overview on the status of the discussion, which evolves around the summary of the data per slide, and the subsequent analysis of these data. Real data from about 40 assays from validation studies performed by several labs are used to compare several potential summary methods (such as the arithmetic and geometric mean of the values per slide, the median, Q3 and P90) and subsequent analyses, such as trend tests, maximum contrast methods, and pairwise comparisons.

An Overview of the Toxicology SIG

Mike Aylott

- **Abstract:**

The Toxicology SIG (Special Interest Group) was originally formed within PSI (Statisticians in the Pharmaceutical Industry) in 2006, to provide a forum to communicate amongst statisticians working in Regulatory Toxicology. Since then we have run three residential workshops and several webinars to discuss various hot topics in Toxicology. The group is essentially UK-based, though we are particularly keen to extend our influence into Europe. We have five active members in our steering team, from the main Toxicology labs in the UK, and around 40 on our distribution list.
During this talk I aim to give a brief introduction to the area, and a summary of the areas in which we have made progress. These areas include the Micronucleus and Comet assays in Genetic Toxicology, and the analysis of organ weight data in Reproductive Toxicology. Our biggest project to date has been on the analysis of Telemetry data for Cardiovascular dog studies, and in December 2009 we submitted our position paper on the recommended statistical to the Pharmaceutical Statistics journal in December, receiving a positive response from the editor.

Testing a trend effect for count variables which are bounded by another count variable: application to fertility counts

Jean-Paul Lahmy, Aurore Puy

- **Abstract:**

Among parameters of the reproductive toxicological studies, a family of count parameters raises a specific statistical problem. For example, when one analyzes the number of implantations, by dam, the number of corpora lutea has to be taken into account (the number of implantations is limited by the number of corpora lutea), especially because there can have a treatment effect not only on the number of implantations but also on the number of corpora lutea.
The goal of this work is to propose a statistical method for the statistical analysis of this kind of parameters. Without loss of generality, the study is performed on the following parameters: number of corpora lutea → number of implantations → number of foetuses,
but, can also be applied for other parameters like the number of male foetuses in a litter which is bounded by the size of the litter and also parameters of the foetal morphology.
Power and error rates determined by simulation are used to compare selected statistical methods including parametrical and non parametrical methods.

SESSION 3B

TOXICOLOGY (II)

Chair – Yi Tsong

Assessing systemic drug exposure in repeated dose toxicity studies in the case of complete and incomplete sampling

Martin J. Wolfsegger and Thomas Jaki

- **Abstract:**

Repeated dose toxicity studies are performed to characterize the toxicological profile of a test compound following repeated administrations. The findings and interpretations from these systemic exposure studies in animals are essential for designing subsequent studies and evaluating the safety of the test item for humans. Blood samples for assessment of systemic exposure are usually collected on day one and at the end of the study with multiple dosings of the compound in between. Restrictions in blood volume often require an incomplete sampling design, in which each animal contributes sample measurements at some but not all time points. In this manuscript we derive an estimator for the ratio of area under the concentration versus time curves (AUCs), a frequently used measure of exposure to a compound, and a corresponding confidence interval to assess differences in exposure as well as equivalence between first and repeated administration that is applicable in such sparse sampling designs as well as complete data situations. An illustrative example is provided and the statistical properties of the proposed estimator, which incorporates the dependencies of measurements between first and repeated dosings as well as the dependency inherent in repeated sampling for each dosing, is studied asymptotically as well as in simulation.

Williams-type procedure on monotone trend for normal, non-normal, ordered categorical, proportion and poly-k data, with application in toxicology

Hothorn, L.A.

- **Abstract:**

The U.S. National Toxicology Program recommends the use of the Williams (1971) trend test for analysis the one-way layouts in toxicology including a negative control and several dose groups. Bretz (2006) proposed a modified version based on a multiple contrast test concept. Generalizations for normal- but heteroscedastic (Hasler and Hothorn, 2008), skewed, graded histopathological findings (ordered categorical), proportions and poly-k estimates (Hothorn et al. 2010) will be presented. The main focus is on simultaneous confidence intervals allowing an interpretation both in terms of statistical significance and biological relevance. Furthermore, differences-to-control and ratios-to-control hypotheses will be compared. Five real data examples from chronic toxicity studies on rodents will be evaluated using the R programs multcomp, MCPAN, mratios and nparcomp.

- References

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A Step-Down Approach to Analyzing Electrocardiogram Endpoints For *In Vivo* Regulatory Toxicology Studies

Maya L. Hanna
Maya L. Hanna, PharmaTherapeutics Statistics, Pfizer Global Research and Development

- **Abstract:**

In the pharmaceutical industry, the evaluation of Cardiovascular (CV) safety is a mandatory component of all drug safety packages submitted for regulatory review. Electrocardiogram endpoints are collected as part of both short-term (safety pharmacology) and long-term (regulatory toxicology) safety studies in order to assess the CV effects of a drug. These studies help define the overall safety profile of a drug before it is tested in humans. These ECG endpoints reflect features of the heart cycle, including heart rate as well as the length of various intervals within the ECG (e.g., QT, PR, and QRS). Regulatory Toxicology studies follow a simple parallel group design in which animals are randomly assigned to a control group or one of 3 doses of a candidate drug. A common statistical method for determining CV as well as other biological effects of a drug is to conduct 2-tailed Dunnett's adjusted T-tests between the 3 dose groups and the control group. Because ECG endpoints are often collected in studies with large animals, sample sizes tend to be small. In addition, there tends to be significant animal-to-animal variability in these measurements. As a result, the step-down version of Dunnett's test has been used to increase the sensitivity of the analysis. This talk will describe the steps associated with the Dunnett's step-down approach, and through real and simulated cases, evaluate the improvement in sensitivity to detecting dose-related effects on QT_c intervals.

Concentration-response analysis of a stochastic process: Multi-state modelling of in-vitro folliculogenesis and oocyte maturation

Marc Weimer, Rita Cortvrindt and Annette Kopp-Schneider

- **Abstract:**

The mouse follicle bioassay (mFBA) is an in-vitro model for studying the production of mature oocytes. Development of follicles and maturation of oocytes are observed during a time period of 13 days. The observations consist of the developmental and maturation states at a sequence of discrete time points. In toxicology, the assay is used for in-vitro screening of chemical compounds affecting ovarian function and fertility. Of particular interest is the relationship between the concentration of a compound and its effect on folliculogenesis and oocyte maturation.

Multi-state modelling of folliculogenesis and oocyte maturation is a promising approach for the analysis of mFBA data. Several things have to be taken into account. A complete history of the individual process is not available since states are observed at pre-specified time points. Therefore, transition times are interval-censored, and individuals may have undergone several transitions within the same time interval. In addition, observation times are not equally spaced. Finally, the model should allow for the analysis of the relationship between concentration and effect of a compound on the process.

In this talk we examine different multi-state modelling approaches with regard to their applicability to mFBA data. Appropriateness of model assumptions, sample size, and interpretation of results will be discussed. Data of a large number of toxicological screening experiments is available.

Statistical Analysis in ACuteTox: Constructing a classifier for chemical toxicity from *in vitro* assay concentration-response curves

Annette Kopp-Schneider, Sven Stanzel, Marc Weimer
Dept. Biostatistics, German Cancer Research Center, Heidelberg, Germany

- **Abstract:**

The REACH regulation will require producers and importers of chemicals to the European Union to determine the toxicity of all chemicals sold in quantities in excess of 1 ton per annum. The EU FP6 project ACuteTox aims at developing a simple and robust *in vitro* testing strategy for prediction of human acute systemic toxicity which could replace the animal acute toxicity tests used nowadays.
In the first phase of the ACuteTox project, 57 chemical compounds were tested in various *in vitro* test systems (*in vitro* assays), resulting in more than 50 endpoints for evaluation. The main focus of the statistical evaluation of the first phase concentration-response data was the identification of a subset of assays which are promising to classify chemical compounds into five toxicity categories. In the second phase of the project, different classifiers obtained from the first phase are challenged with data from a validation set of 32 compounds evaluated blindly in the subset of selected assays.
Statistical analysis of the concentration-response data produced in ACuteTox was performed using log-logistic models, and a characteristic value such as the EC50 was derived for each experiment. If the experiment showed no clear concentration-response relationship, or if the EC50 estimate exceeded the concentration range tested in the experiment, the estimate was reported as right-censored value.
Often more than one concentration-response experiment was carried out for an endpoint × compound combination. In this situation, a meaningful summary EC50 value had to be computed from all values estimated separately in individual experiments. Summarization of EC50 estimates, especially in situations in which not all the experiments show a clear concentration-response relationship, is not straight forward. Different methods for EC50 summarization will be discussed.
The final goal of the statistical evaluation was the selection of assays and the construction of suitable classification models by use of different classification approaches. The classification task for ACuteTox involves a mixture of uncensored and censored observations in the predictor variables of the classification models and, therefore, classical statistical methods for classification such as CART (see Breiman et al., 1984) or Random Forests (see Breiman, 2001) could not be applied directly to the ACuteTox data. Appropriate modifications of these methods will be presented. Advantages and drawbacks of the algorithms will be discussed and their performance will be illustrated using the validation set of chemicals.

- References

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Optimization of the irinotecan - efflux transporter inhibitors combination for treating irinotecan resistant tumours

Alexandre SOSTELLY[1], Léa PAYEN[2], Benjamin RIBBA[3], Attilio DI PIETRO[4], Pierre FALSON[4],
Gilles FREYER[4,5], Pascal GIRARD[1,6], Michel TOD[1,5]

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[6] INSERM, Lyon, France

Abstract:

ATP Binding Cassette (ABC) transporters are known to play an important role in drug absorption, distribution and anticancer drug resistance. Breast Cancer Resistance Protein (BCRP) is an ABC transporter involved in the efflux of a wide range of substrates such as irinotecan (CPT-11) and its active metabolite SN38. BCRP inhibitors have been recently designed, based on acridone derivatives[1]. For instance, MBLI87 has shown high activity against BCRP efflux in in vivo studies. A proof of concept has been carried out in xenografted mice and has demonstrated the efficiency of this new drug against CPT-11 BCRP mediated resistance[2]. We aim at optimizing the therapeutic regimen and effects of the CPT-11/MBLI87 combination in Severe Combined ImmunoDefficient (SCID) mice with CPT-11 resistant xenografts and comparing the effect of MBLI87 with the BCRP reference inhibitor, gefitinib. Based on the proof of concept data, we developed a non-linear mixed effects tumour growth inhibition model as proposed by Simeoni and colleagues[3] with some modifications. These modifications concerned the simplification of the pharmacokinetic (PK) model, since no PK information was available in those animals. A drug accumulation and a mono-exponential elimination were assumed according to the dose schedule provided to mice. Consequently, drug effects are directly dependent on the amount of drug present in animals. PK parameters were latent variables and their estimation was supported by tumour growth dynamics[4]. An interaction parameter was introduced to quantify the action of MBLI87 or gefitinib on CPT-11 cytotoxic effect. Fixed and random effect parameters of the non-linear mixed effects model were estimated with First Order Conditional Estimate method implemented in NONMEM VI software[5]. Model was fitted successfully according to goodness of fits plots and simulation based diagnostics. A significant synergistic effect was found between MBLI87 and CPT-11 whereas none was found between gefitinib and CPT-11. This new drug is thus able to revert CPT-11 BCRP mediated resistance for a lower dose compared to gefitinib. Model was then used to optimize the design of a dose finding study. Based on model structure and parameter estimates, Monte Carlo simulations of tumour growth profiles were performed to explore the impact of various doses, administration schedules and treatment durations in order to maximize differences between CPT-11 and CPT-11/MBLI87 tumour growth profiles. Objectives of these simulations were to select the most effective therapeutic regimen for the dose finding study in mice. Once this second study will be performed, next step will consist in extrapolating effects of this new drugs combination in humans for preparing the design of a phase I study.

- References:

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Anova-Type Statistics, a good alternative to parametric methods for analysing repeated data from preclinical experiments

Odile COUDERT BERTHION (Keyrus Biopharma); Véronique ONADO (Sanofi-Aventis); Guy MATHIEU (Sanofi-Aventis)

- **Abstract:**

In preclinical and research field, departures from the hypotheses underlying parametric analyses, normal distribution and variances homogeneity, are frequently encountered; this is all the more concerning since the sample size is mall and the data may be repeated.
Anova-Type Statistics (ATS) is a non-parametric method used for analysing longitudinal data from factorial designs without making any assumption on the data distribution and the variances homogeneity. Particularly adapted to small sample sizes, it remains valid even when there is no variability within some factors levels. The ATS method is based on distributions instead of positional parameters. The distributions and the relative factors effects are estimated using the ranks among all observations. This method is implemented in the Mixed procedure of SAS software.
A simulations study has been performed and will be presented that assesses the alpha and beta risks of several statistical analyses methods (ATS, Anova on raw data, on ranks, on normal scores or with covariance matrix modelling) in various situations of variances heterogeneity and/or non-normal distribution in a two-way repeated design. It shows that ATS is highly powerful while conserving a good alpha risk. ATS proves appropriate for analysing data with over/under responding subjects or with variances heterogeneity that can hardly be modelled. Case studies bring materials for advising its use when analysing discrete data.

Prediction of tumor class from gene expression data using bagged decision trees

Sam Roberts, Principal Application Engineer in United-Kigdom
Ascension Vizinho-Coutry, Application Engineer Manager in France

Abstract:

Small, round blue-cell tumors (SRBCTs) belong to four distinct diagnostic categories. The categories have widely differing prognoses and treatment options, making it extremely important that doctors are able to classify the tumor category quickly and accurately. SRBCTs are difficult to distinguish by light microscopy, and are currently diagnosed using a combination of immunohistochemistry, cytogenetics, interphase fluorescence *in situ* hybridisation, and RT-PCR. Currently no single test can precisely distinguish these tumors. Khan *et al.*[1] showed that gene expression data collected using cDNA microarrays holds promise for SRBCT diagnosis as, in contrast to the other techniques mentioned, it allows the simultaneous measurement of multiple markers.
Gene expression studies, however, give rise to very high-dimensional datasets, requiring the use of multivariate statistical methods for analysis.
MathWorks tools provide a very wide variety of such methods (including the neural networks used by Khan *et al.*), allowing users to choose the most appropriate method for their particular application.
Here we apply an ensemble decision tree approach. Ensembles of decision trees have been described as "the best off-the-shelf classifier in the world"[2] for their combination of performance, interpretability, and ease-of-use.

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SESSION 4

TRANSLATIONAL SCIENCES

Chair – Emmanuel Pham

Opening Lecture

Development of a dose content uniformity test suitable for medium and large sample sizes

Yi Tsong, Deputy director, Office of Biostatistics, Center for Drug Evaluation and Research, FDA, USA

K-PD models as a flexible modeling tool in non-clinical statistics

Tom Jacobs, Roel Straetemans, Bruno Boulanger, Luc Bijnens

- **Abstract:**

The revolution of computational power has lead to an important increase in complexity of the models utilized in pharmaceutical development. Statistical and scientific modeling evolved from linear towards generalized linear, mixed effects and nonlinear mixed effects modeling. Typical examples of such nonlinear mixed effects models within pharmaceutical development are PK/PD models (Gabrielson and Weiner, 2001), where a relationship between the response and the underlying drug exposure is established.
PK/PD models are up to now typically applied within a clinical setting to design, simulate and optimize large phase 3 studies. Yet, the principle can be transferred to a nonclinical setting without restriction. Mechanism-based PK modeling is one of its applications (Danhof et. al., 2007).
However, it is not always possible to sample drug concentrations within preclinical pharmacology studies. This can be either due to the size of the animals (e.g. mice) or the potential impact on the pharmacodynamic outcome. However, a latent pharmacokinetic profile can be assumed. Such models are usually referred to as K-PD models (Jacqmin et. al., 2007) to stress the absence of plasma concentrations. The strength of K-PD models is illustrated using three examples.

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A Unified Approach to Flexible and Powerful Modeling Of Pre-Clinical Combination Studies

Chris Harbron
Discovery Statistics, AstraZeneca

- **Abstract:**

The development of drug combination therapies is an area of high interest within the pharmaceutical industry, so a robust evaluation of the potential for compounds to interact synergistically at an early stage within the pharmaceutical pipeline is clearly of great value.

I will describe a recently developed unified approach for assessing synergy which allows a number of different assessments to be made and compared under a common framework, powerfully and flexibly using all the available experimental data and giving a complete description of the studied combination space with statements of confidence. The method is applicable over wide classes of experimental design and response patterns and is backed up with informative graphical displays. The implications for the experimental designs of both in-vitro and in-vivo combination studies will also be discussed.

Pharmacokinetic similarity analysis using nonlinear mixed effects models

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[2] Novartis Pharma AG, Basel, Switzerland.

Abstract:

To show lack of pharmacokinetic (PK) interaction or PK similarity between formulations, tests are usually performed on non compartmental (NC) estimates. Recently, equivalence tests based on nonlinear mixed effects models (NLMEM) have been developed for parallel or crossover trials [1, 2]. NLMEM tests can be performed on the treatment effect of one or several PK parameters, mimicking the standard NC analysis [3]. For crossover trial analysis, we use a statistical model taking into account treatment, period and sequence effects on all PK parameters of the structural model. We also include between-subject (BSV) and within-subject (WSV) variability on all PK parameters. The NLMEM parameters are estimated by the SAEM algorithm implemented in MONOLIX 2.4 [4, 5]. We explain how to perform a Wald test on a secondary parameter of the model like the maximal concentration, and we propose an extension of the likelihood ratio test (LRT) for equivalence. The type I error of the equivalence Wald test and LRT were evaluated by simulations.
When performing a pre-clinical study, it is important to define an appropriate design, which has an important impact on the precision of parameter estimates and on the power of tests. For design evaluation and optimisation, we propose an extension of the evaluation of the Fisher information matrix for NLMEM including WSV, in addition to BSV, and with discrete covariates which may change between periods. We use the predicted standard errors to predict the power of the equivalence Wald test and to compute the number of subject needed. These extensions are implemented in the newly released version PFIM 3.2 [6, 7, 8] and were evaluated by simulations. We illustrate this methodology on PK crossover trials.

Equivalence PK trials analyzed through NLMEM allow one to decrease the number of samples per subject. This may be an important aspect in experiments in which the total number of samples is limited. PFIM can be used to efficiently design these trials.

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Modeling Dose-response Microarray Data in Early Drug Development Experiments

Dan Lin[1,2], Adetayo Kasim[1], Ziv Shkedy[1], Daniel Yekutieli, Dhammika Amatunga, Willem Talloen[3], Hinrich Gőlhmann[3], Luc Bijnens[3]

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[2] I-Biostat, K.U.Leuven, Kapucienenvoer 35, B3000 Leuven, Belgium
[3] Janssen R&D, Janssen N.V. Beerse, Belgium

Abstract:

Dose-response studies are commonly used in pharmaceutical research in order to investigate efficacy and safety of certain compounds. The dose dependency of the response is assessed by investigating the trend of the response or toxicity level in function of dose. We focus on dose-response experiments within a microarray setting. The primary question of interest of such a study is to identify genes that have a monotonic trend with respect to the dose in their expression. To establish the monotonic dose-response relationship, we considered several testing procedures to test the null hypothesis of no dose effect versus ordered alternatives (Lin *et al.* 2007, Lin *et al.* 2010). Resampling-based False Discovery Rate and resampling-based Family-Wise Error Rate are used for controlling the error rate while testing of thousands of genes simultaneously.
The secondary goals of the dose-response study are to determine the minimum effective dose and to identify the shape of dose-response curve. Once a monotone relationship between the gene expression and dose is established, there is a set of R possible monotone models which can be fitted to the data. We focused on classification of dose-response curve shape using information theory for model selection, such as AIC, BIC, and ORIC (Order Restricted Information Criterion) (Lin *et al.* 2009, Lin *et al.* 2010). This method was compared with our second approach where the isotonic means are estimated by using Bayesian models and the posterior probabilities for the set of R models are used for model selection (Lin *et al.* 2010). The methods above were applied to a dose-response microarray experiment with 12 samples (three arrays at each dose level with one control dose and three increasing doses) and consisting of 16,998 genes.

Keywords: Dose-response Relationship; a Monotonic Trend; Microarray; False Discovery Rate; Information Theory for Model Selection; Bayesian Models; Multiple Contrast Test.

- References :

Lin D., Shkedy Z., Yekutieli D., Dhammika A., Bijnens, L., Modeling Dose-response Microarray Data in Early Drug Development Experiments Using R, Springer (To be published in 2010 in the series USE R! of Springer).
Lin D., Shkedy Z., Yekutieli D., Burzykowski T., Goehlmann H. W.H., De Bondt A., Perera T., Geerts T., and Bijnens L. (2007) Testing for Trends in Dose-Response Microarray Experiments: A Comparison of Several Testing Procedures, Multiplicity and Resampling-based Inference. Statistical Applications in Genetics and Molecular Biology, Vol. 6 : Iss. 1, Article 26. Available at: http://www.bepress.com/sagmb/vol6/iss1/art26.
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A multivariate analysis of prognostics factors in Chronic Lymphocytic Leukemia

Giuseppe Palermo[1], Ru-Fang Yeh[3], Laurent Essioux[1], Martin Weisser[2], David Dornan[3] and Guillemette Duchateau-Nguyen[1]

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[2] Roche Diagnostics GmBH, Penzberg, Germany
[3] Hoffman-La Roche Ltd South San Francisco, CA, USA

Abstract:

In this work an exploratory multivariate data analysis was conducted on leukemia samples as available from the REACH clinical trial.
The REACH phase III clinical trial (Roche Study BO17072) was initiated to compare the Progression Free Survival (PFS) of patients treated with Rituximab in addition to fludarabine/cyclophosphamide (FC) chemotherapy to the PFS of patients treated with FC alone. Selected patients in that study were relapsed or refractory patients with CD20 positive Chronic Lymphocytic Leukemia (CLL). The addition of Rituximab to FC chemotherapy yielded a substantial increase in PFS (Robak, 2008).
Five hundred and fifty two patients from 17 countries were randomized to receive either R-FC or FC, with both treatment arms well balanced with regard to age, stage, genomic aberrations and IgVH status. Multiple sub-groups analysis showed that Binet stage as well as mutational status and cytogenetic subgroups would benefit from the addition of rituximab to FC (Robak, 2008).
The objective of this work was to identify a parsimonious set of independent prognostic factors for PFS in the REACH trial using a multivariate analysis and to check if potential outlier/influential observations could bias the outcome. A Cox regression model was applied on a reduced sample size (513 individuals, 276 events of disease progression) with complete data for Treatment (FC or FCR), Age, Gender, Binet Stage, IgVH status, del(11q), del(13q), del(17p) and trisomy 12. CD38 had a very high missing rate (~40%) and could not be used. ZAP70 expression was strongly associated to IgVH status (ROC-AUC=85% for IgVH prediction by ZAP70) and ZAP70 was not included in the model to avoid overadjustment (furthermore ZAP70 had ~25% missing rate). Although genotypes for FC-g-R IIIa gene (SNP 158) and FC-g-R IIa (SNP 131) were available, those polymorphisms were not included in the model as it was already shown that they do not significantly influence PFS independently of either treatment (Dornan, 2009). A modified version of stepwise procedure (Collett, 2003) based on maximum likelihood estimate (MLE) difference between nested models was used to shrink the initial set of predictors. The final multivariate model including treatment (FC vs FCR), Binet stage (C vs A/B), age, del(17p) and IgVH mutational status revealed treatment (HR=0.64 (0.5,0.81);p=0.00023), age (HR=1.02(1.01,1.04);p=0.0037), Binet stage (HR=1.75 (1.36,2.26);p=0.000014), IgVH (HR= 2.14 (1.64,2.8);p=2.8E-08) and del(17p) (HR= 2.82 (1.95,4.08);p=3.3E-08) as independent prognostic factors for PFS. The overall assessment of model fit and testing of the proportional hazard assumption did not show major deviations. Two samples were found as influential observations for the model, but their exclusion did not change significantly the results.

- Reference

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Genomic Biomarkers for Depression: feature-specific and joint biomarkers

Abel Tilahun, Dan Lin, Ziv Shkedy, Helena Geys, Ariel Alonso
Pieter Peeters, Willem Talloen, Wilhelmus Drinkenburg, Hinrich Goehlmann, Evian Gorden, Luc Bijnens, Geert Molenberghs

- **Abstract:**

Recently, pre-clinical microarray experiments have become an increasingly common laboratory tools to investigate the activity of thousands of genes simultaneously and their response to a certain treatment. In some experiments, in addition to the gene expressions, other responses are also available. In such situations, the primary question of interest is to identify whether or not the gene expressions can serve as biomarkers for the responses. In addition to gene expressions, metabolites are potential biomarkers for some responses as well. In the present study, we focus on the identification of genomic biomarkers, based on gene and metabolite expression for depression. One measure of the level of depression is the Hamilton Depression Scale (HDS or HAMD) which is a test measuring the severity of depressive symptoms in individuals. The data for this study are a result of a clinical trial in which both HAMD and gene/metabolites expression were measured. We use three modeling approaches commonly used in the surrogate marker validation theory to select and evaluate a set of genes and metabolites as possible biomarkers for depression, as measured by the HAMD score. In addition to gene and metabolite specific biomarkers, we use supervised principal components analysis and supervised partial least squares regression technique to construct a joint biomarker that uses information from all genes/metabolites in the array.

SESSION 5

‘OMICS

Chair – Luc Bijnens

Invited Lecture

Development of biomarker signatures from high-dimensional array data, and identification of subsets with common patterns between multiple array platforms

Viswanath Devanaryan, Director Exploratory Statistics, Abbott Laboratories, USA

Multivariate approaches to develop molecular biomarkers: a reality check

Willem Talloen and Dhammika Amaratunga

- **Abstract:**

With the completion of the sequencing of the human genome and with the emergence of biotechnologies such as microarrays, we have entered the post-genomic era with much hope to harvest some of the fruits hidden in the genomic text. At the same time, the current difficulties faced by pharma research to discover generally applicable block-buster drugs have lead to think in terms of personalized medicine. Consequently, high hopes are on clinical opportunities for gene-based prediction of illness or drug response using post-genomic tools. The –omics revolution was also warmly welcomed by statisticians as its data properties imposed new and interesting statistical challenges. For example, the quest for biomarkers in the context of personalized medicine has made many statisticians think about classification models that are robust against overfitting for generation of molecular signatures.
Here, we will challenge that this enthusiasm made many researchers forget to think about the practical applicability and the biological, and hence clinical, relevance of these developed classification algorithms. For example, the rationale behind signatures consisting out of many genes is generally overlooked. How these genes should be aggregated into one composite index (i.e., the marker) so as to reflect the underlying biology as well as to remain generalizable will be discussed in this presentation.

Dimension Reduction through Variable Selection – A Fibrosis Case Study

Katja Remlinger, Discovery Analytics, GlaxoSmithKline

- **Abstract:**

Variable selection is an important step in prediction modeling of high dimensional data. Not all variables in the data are informative, and removal of irrelevant noise often improves the prediction performance. If data is of biological origin, cost-efficiency and an improved understanding of the underlying biological process that generated the data are additional reasons for performing variable selection. Once important variables have been selected, it is equally important to assess whether these variables can be validate in subsequent studies. Using biomarker data from a Fibrosis study, we will illustrate the benefits and challenges of the initial variable selection and the subsequent validation steps. We used univariate and multivariate analysis techniques to select variables, and assessed their performance by means of predictive power and validation strength in subsequent studies.

Keywords: Biomarker, Cross Validation, Classification, RandomForest, Selection Bias.

Seeing the Wood for the Trees: Interrogating the Structure of Random Forests

Chris Harbron
Discovery Statistics, AstraZeneca

- **Abstract:**

Since their introduction by Breiman in 2001, Random Forests have gained a wide usage as a robust and effective tool for predictive modelling of multivariate data with many desirable properties. However Breiman always saw Random Forests as far more than a "black box" modelling technique, but also as a way of understanding the relationships within a dataset.

In this talk I will extend the concept of variable importance measures by describing an approach for interrogating a Random Forest model through understanding the structure of its constituent trees. This gives both an understanding of the relationships within the underlying dataset and the properties driving the fitted model. Graphical displays identify the features driving model predictions as well as acting as diagnostics for identifying influential observations.

Breiman, Leo (2001). Random Forests. Machine Learning 45 (1): 5–32.

Testing for the Differential Epxression of Genes at the Probe Level of Affymetrix Microarray data

Tatsiana Khamiakova, Ziv Shkedy, Adetayo Kasim, Suzy Van Sanden, Dan Lin, Hinrich Goehlman and Willem Talloen

- **Abstract:**

Analysis of differentially expressed genes based on Affymetrix data depends on a summarization technique that is used to obtain one expression measurement per array per gene (e.g. GCRMA, MAS5 or FARMS). Summarization in this case will lead to the loss of information. The multivariate measurements of the gene expression can have complex structure and summarization technique does not always tackle this problem in an appropriate way. Summarization can also affect the number of false negative and false positive discoveries in a dataset. In this paper, we propose to model probe level data using linear mixed models, which allows us to model correlation structure in the data and also test for the treatment effect simultaneously without summarization step. The proposed method is illustrated on the dataset with 12 Affymetrix Mouse Whole Genome ms430 arrays, where the size of a probeset can vary. Simulation study was conducted in order to investigate the performance of the proposed methods in terms of power and FDR control.

Treatment of genomic data in the context of macroarrays

Julie POUGET - Société SOLADIS

***Co-Author(s)**:*
Florence PUCH - CHU Grenoble UF de biologie nutritionnelle
François LAPORTE - CHU Grenoble UF de biologie nutritionnelle
François CONESA - Société SOLADIS

- **Abstract:**

DNA macroarrays technology was used to evaluate, as part of a project on antioxidant properties of selenium, the gene expression profiling after supplementation in cell culture media. However, the usual methods used to analyze transcriptomic data could not be totally applied in the context of macroarrays because the number of probes available on each array was insufficient. From exploratory statistics *via* data processing (with normalization as a crucial step) to the modeling methods such as mixed models, a statistical analysis method was developed to remedy the weaknesses of macroarray. The aim of this communication is to present the method developed and used to highlight the genes differentially expressed as a function of time between two conditions and to open the discussion about this approach.

Addressing the Challenges of the Analysis of Genomics Data with JMP Genomics of SAS

Dr. Valérie Nedbal, JMP Pharmaceutical Technical Manager at SAS

- **Abstract:**

Copy number variation (CNV) is proving to play a significant role in common diseases such as cancers and autism. The statistical methods and softwares available for identifying common and rare CNVs are still evolving. There are a number of different methods used in various combinations, including segment analysis, hidden Markov models, comparison of intensity values and assessment of ratios compared to a control group. Each of these methods can be quite useful for specific applications. For example, the combination of hidden Markov algorithms with segment analysis has proven to be useful in identifying chromosomal breakpoints in karyotyping studies.

There can be considerable heterogeneity within diseases where CNV plays a role. Rarely will all samples share a common region of CNV within a single study. Instead, variations may only be shared by a small percentage of samples and as a result, larger sample numbers are required to find these rare shared regions.

The presentation will draw attention to the value of interactive and dynamic visualization of genomics data using cross referencing data analysis. We will show how the statistical tools in JMP Genomics from SAS can be used to find shared regions of CNV, and also correlate SNP intensities within these regions to gene expression differences. Moreover, the example will illustrate CNV-expression-methylation status correlations. For this aim, this presentation will showcase the versatility of statistical applications in Quality Control, Row-by-row modeling like Mixed Models for different data types, Partition Algorithm based on Faster Circular Binary Segmentation, Annotation tools to validate biological findings.

Though, these methods can also be used to perform correlation studies between other types of paired numeric information such as miRNA, ChIP on Chip studies, or SNP genotype frequency values from next-generation sequencing platforms.

POSTERS

Mixed effects models for interspecies pharmacokinetic scaling using historical data

Luc Bijnens[1,2], Abainenamar Doreen[2], Eric Ahoupe Temgoua[2], Geert Molenberhgs[2] and An Van Den Berghe[1]

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[2] I-BioStat, Center for Statistics, Hasselt University, Agoralaan 1, B3590 Diepenbeek and Katholieke Universiteit Leuven, Belgium.

- **Abstract:**

The rule of exponents (Mahmood 2005) extrapolates the pharmacokinetic (PK) findings of animal experiments to the human when no clinical trial data are available. In this project we investigate model building strategies in order to improve predictability. In the current practice covariates are used additionally to simple allometry without testing their value based on the data at hand. The reason for that is that there are usually insufficient data points compared to the number of degrees of freedom required to perform classical likelihood ratio tests. Bijnens et al (2010) suggested the inclusion of historical data of other compounds in a mixed effects model setting in order to improve the predictability of the human data. The advantage of this inclusion is that for other compounds human PK data will be available and in the meta-analytical setting there will be sufficient degrees of freedom for advanced model building methods. The setting can thus be used to look for the most appropriate model to fit the data at hand and can be used to test the significance of physico-chemical covariates, such as solubility, permeability, ionisation constant (pKa), molecular weight etc., that might improve the models. At the same time we are investigating trial design elements in order to improve the efficiency of the assays in order to save resources where possible. During the presentation we will give illustrations based on real life pharmaceutical experimental data.

- References :

Mahmood I. (2005). Interspecies pharmacokinetic scaling – principles and application of allometric scaling. Pine House Publishers Rockville, Maryland; Bijnens L, Van Den Bergh A, Straetemans R, Sinha V, Geys H, Molenberghs G, Verbeke T, Kasim A, De Ridder F and Mackie C (2010). A meta-analytical approach to allometric scaling to include historical data. (Submitted to Statistics for Biopharmaceutical Research).

Multivariate entropy analysis of oxidative stress biomarkers following mobile phone exposure of human volunteers: a pilot study

Paul Fogel - Statistical Consultant, 4 rue Le Goff, 75005 Paris, France

Co-authors:
S. Stanley Young, Anthony Marconi, Albert Tasteyre, René de Sèze, Guy Simoneau, Marc Conti,
Christian Sarbach, Caroline Derome, Valérie Ferriole, Jean-Emmanuel Gilbert, Yolène Thomas

Abstract:

A multi-disciplinary project was conducted to study the possible biological impact of mobile phones emissions. As part of that project, we conducted a pilot study on 24 human volunteers with the treatment being GSM mobile phone exposure. The volunteers were randomized and the study was a double-blind, crossover design. We measured two categories of oxidative stress biomarkers in blood and exhaled air: those assessing oxidative attacks of cell membrane lipids (malondialdehyde, exhaled alkanes, aldehydes and isoprene) and assessing the organism's antioxidant defense systems (superoxyde dismutase, glutathion peroxydase and exhaled halogenated alkanes). We use a multivariate test that measured the entropy of the system, with and without treatment. We found modulation of organization of the biomarkers, less entropy, after a single 30 minutes mobile phone exposure. As the results can be considered startling, these results will need to be confirmed in larger, future studies. The entropy testing approach allows for looking at markers that may increase or decrease significantly, depending on the cell cycle of the individual at the time of the measurement. This test appears to be more robust than simple F tests (ratio of variances with and without treatment), thanks to its multivariate and non-parametric nature.

Simultaneous confidence intervals incorporating historical control data

Andreas Kitsche and Frank Schaarschmidt,
Leibniz University Hannover, Germany

- **Abstract:**

To determine the toxicological effect of potentially hazard substances it is common to perform standardized tests. In a typical bioassay an untreated control group and several increasing dose groups are studied. Considering the large amount of available historical control data from past studies it is reasonable to utilize this additional information for the statistical analysis. For dichotomous response variables it is suitable to compare the binomial proportions, such as the mortality rate or tumor rate, to determine the toxicity. We used an empirical Bayes method in which the prior distribution is estimated from the historical control data. This assumes that the past and current studies are considered to be drawn from a joint distribution. The binomial proportions from the past data are used to estimate the parameter of a beta prior distribution. The inclusion of this knowledge into a current bioassay data leads out to a beta-binomial posterior distribution. To build up the dose-response relationship approximate simultaneous confidence intervals for multiple contrast tests (Williams-type approach) using quantiles of the multivariate normal distribution were applied. Also confidence intervals derived from Markov Chain Monte Carlo sampling were investigated for multiple comparison procedures. This offers several feasible dose-response shapes of interest. The performance of the proposed approaches was investigated in simulation studies on a wide range of potential scenarios. We illustrate our method by the evaluation of a real data set of a toxicological study from the U.S. National Toxicology Program database.

Robustness of an adjuvant formulation manufacturing process

S. Gautheron, C. Hessler, P. Probeck-Queller, L. Dumont, S. Nury and A-L. Drouhin
Sanofi Pasteur, Marcy L'Etoile, 69, France

- **Abstract:**

A Design of Experiment was built to evaluate the robustness of an emulsion preparation towards a 5% variation weight of its five components. Two main responses were studied, Dv90 and polydispersity, and criteria were specified for each of them to demonstrate the robustness of the formulation.
After a model was postulated, the DOE was optimized with the Fedorov algorithm, based on the D optimality criteria. Owing to HLBs contraints, not all the experiments were proposed in the initial set. Finally, 25 distinct formulations were performed, supplemented with 9 experiments including controls and repeats.
A sensibility analysis was addressed to conclude to the robustness of the emulsion with a variation of 5% or 1% in the weight of the different components.

An application of cubic smoothing splines to concentration response curves

David R Wille, Caterina S Virginio

- **Abstract:**

Experiments with multiple doses or concentrations of the same drug or compound occur widely in many areas of discovery research. Frequently, established concentration or dose response models can be used to explore their results but sometimes, due to the nature of the drugs response, or a lack of knowledge of receptor pharmacology, such assumption based approaches are dangerous or not possible.

In this poster, we present an alternative mathematical approach using a class of generic empirical models known as *cubic smoothing splines* which allow curves to be fitted to the data without any prior scientific assumptions on the shape of the concentration responses whilst still allowing their features and properties to be reported and explored statistically in the normal way.

In particular, our innovation centres on the investigation of voltage gated potassium channel positive modulators using the *IonWorks Quattro* platform where complicated non-standard concentration curves are frequently observed and expected. Statistically, this work is interesting because of the application of spline approximation methods to dose response data as well as the use of a number of mathematical devices to compute critical concentration values and the corresponding confidence internals from the resulting models, and their deployment over a web-based platform.

Statistical Software for the Evaluation of *in vitro* Assays in Toxicology: $stat^{4tox}$

$stat^{4tox}$ is a

- Free statistical software package with graphical user interface, available web-based or off-line,
- One integrated GUI: i) test oriented and ii) dose-response modeling for robust EC_{50} estimation
- Methods for continuous data (parametric and non-parametric), counts and proportions,
- With focus on effect size expressed in k-fold change and displaying confidence intervals,
- Focus on one-way layouts, allowing the inclusion of secondary covariates or hierarchical layouts.

Why $stat^{4tox}$?

The contradiction between statistical significance and biological relevance is particularly irritating in toxicology. Eminently, i) testing the point-zero-hypothesis $\mu_i - \mu_0 = 0$ is rarely of biological relevance, and ii) p-values represent a probability of falsification but do not provide interpretation in terms of toxicological (clinical) units, iii) relevance criteria are commonly formulated by k-fold change, i.e. multiplicative models, while most statistical procedures focus on absolute change, i.e. additive models.

What is $stat^{4tox}$ about?

$stat^{4tox}$ (speak: stats-for-tox) focuses on confidence intervals, hence allowing interpretation of both, statistical significance (exclusion of the point of H_0) and biological relevance (distance to the point of H_0). Confidence intervals are provided for both the difference-to-control $\mu_i - \mu_0$ or the ratio-to-control μ_i/μ_0 allowing interpretation in terms of biological relevance via absolute change in the scale of the toxicological measurement unit, or via scale-invariant percentage change, e.g. suitable for k-fold rules.
For two-group comparisons, parametric and non-parametric confidence intervals, and intervals for proportions, such as incidence rates are available.

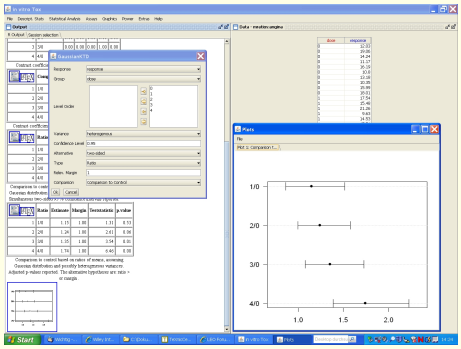
A common design in *in vitro* toxicology is a one-way layout including a negative control, several doses or treatment groups and, optionally, a positive control. $stat^{4tox}$ provides procedures for comparisons of treatments versus a control according to [2], or the comparisons of doses versus control under the assumption of monotonicity according to [5] for normally distributed endpoints, arbitrarily distributed endpoints, counts and proportions, again for the difference- and ratio-to-control [1]. Counts occur frequently in *in vitro* assays, such as number of revertants. Quasi-Poisson models are provided to take the variability between the experimental units (plates, or animals in *in vivo* assays) into account. Similar approaches are available for the variability between proportions. Additional to inferential statistics, descriptive statistics and diagnostic graphics are available, such as a modified version of box-plots. Finally, power calculation for two-sample and comparison-versus-control procedures are provided.

The main paradigm in toxicology is: *be confident in negative results*. Hence, the strict control of the false positive error rate for the experiment (i.e., controlling the FWER) may be not appropriate because of the associated increase of the false negative error rate. $stat^{4tox}$ also provides comparison procedures versus control without multiplicity adjustment. For directional decisions (one-sided hypotheses) a proof of safety is available.
Robust estimation of ED_{50} and its confidence interval for both continuous and proportion data based on vague model information and real data problems. Model selection and model averaging approach were implemented by Christian Ritz, University of Copenhagen.

The statistical methods are based on several packages for the free statistical software R [4]. The validity of the implemented methods has been assessed in simulation studies for medium to small sample sizes - in both *in vitro* and *in vivo* assays. $stat^{4tox}$ with its graphical user interface is the result of a project carried out by the Leibniz University Hannover in collaboration with the European Centre for Validation of Alternative Methods (ECVAM), Institute of Health and Consumer Protection, Joint Research Centre, European Commission.

$stat^{4tox}$:

- provides statistical methods via a user-friendly surface,
- targets toxicologists without a detailed education in biostatistics,
- includes example-based evaluations for the Ames assay, the *in vitro* and *in vivo* micronucleus assay, and the comet assay,
- supports data import for *.xls files in an appropriate format.

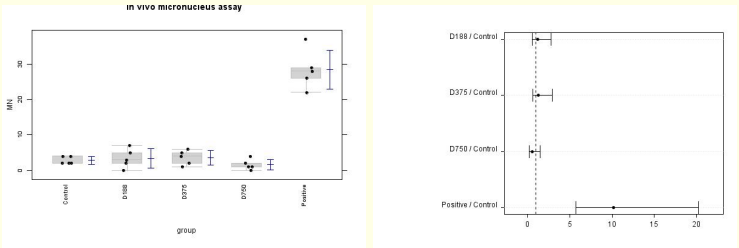


Installation hints

Simply via: http://www.biostat.uni-hannover.de/stat4tox/ Contact: help@biostat.uni-hannover.de

An example

In an in-vivo micronucleus assay, the number of MN are counted in a negative control, several dose groups and a positiv control. Commonly a small number of animals are used. In the example 5 animals per group are used, as can be seen in a jittered box-plot.



A serious statistical problem arises when near-to-zero values in the control occur. One possibility to analyze such data is a Dunnett-type procedure for ratio-to-control using a profile likelihood approach [3]. The related simultaneous confidence intervals (in the right Figure above) reveal a strong effect for the positive control to demonstrate assay sensitivity and no increase in MN in any of the dose groups.

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