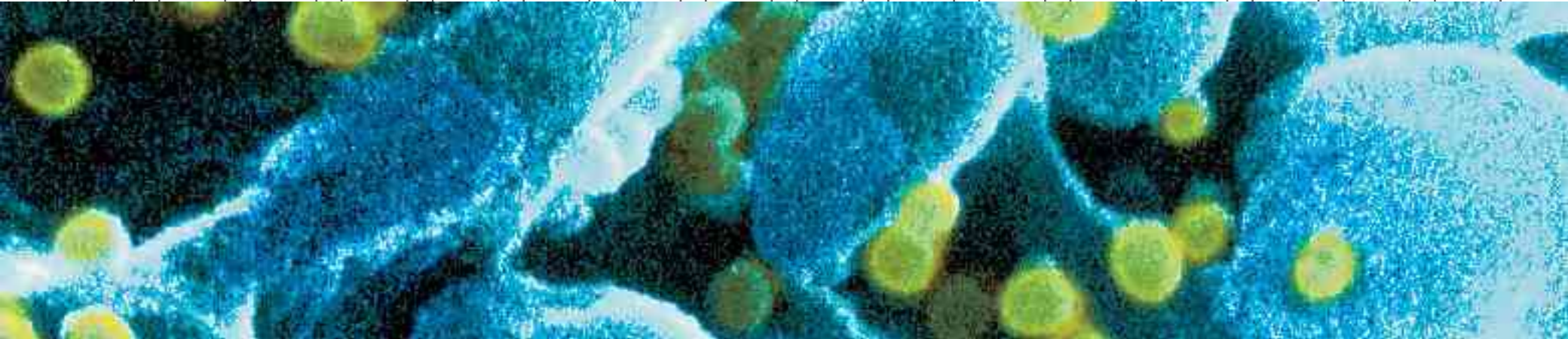


3Rs in Safety Assessment in Pharmaceutical Industry

Prof. Friedlieb Pfannkuch, MD



Introductory Remarks

Personal views – no Company statement

- There is a public interest to ...
 - allow only products in the market with a well characterized safety profile
 - reduce / replace animal testing wherever feasible due to ethical reasons
- There is a generic interest of Pharmaceutical Industry to perform the requested / necessary animal studies in order to profile the safety of drug candidates to avoid ...
 - harm to volunteers and patients
 - liabilities
- Industry's activities are driven by (national, regional, global) regulatory requirements

REPLACEMENT – Current Constraints

Scientific – Regulatory

- Scientific reasons
 - Cells or in vitro systems cannot really provide a reliable picture on a complete biological organism (interactions and functions)
 - There are no alternative methods available which allow responsible safety / risk assessment on endpoints of repeated dosing:
 - Subchronic and chronic toxicity,
 - Reproduction toxicity or
 - Oncogenicity
- Regulatory requirements
 - (Global) regulation request a fixed setting of animal studies for risk assessment and marketing authorization

Phase 0 / I: “Entry-into-human enabling” *Regulatory Toxicity Studies*

- General Toxicology
 - 2- / 4-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery
 - ‘Acute’ (single-dose) toxicology study in rodents
 - For transportation classification – Material Safety Data Sheet; MSDS
 - Local tolerance studies – for parenteral formulations
- Genotoxicity
 - Ames test
 - Mouse lymphoma test / Human Chromosome Aberration
- Safety Pharmacology
 - Core battery for CNS, cardiovascular and respiratory effects

Phase I / II: Early Clinical Development

Regulatory Toxicity Studies

- General Toxicology
 - 13-week toxicity study in rodent and non-rodent animal species, including toxicokinetics and recovery
 - 6-month in rodent and 9-month toxicity study in non-rodent animal species
- Genotoxicity
 - in vivo Micronucleus test in rats / mice
- Reproduction Toxicology
 - Embryo-fetal toxicity (Pilot Segment II) in rats
 - Embryo-fetal toxicity (Segment II) in rats
 - Dose-range finding study in rabbits
 - Embryo-fetal toxicity (Pilot Segment II) in rabbits
 - Fertility (Segment I) in rats
- Special studies
 - (Sensitization / phototoxicity in guinea pigs)
 - (Mechanistic toxicity studies)

Phase III: Entry into ‘life-cycle management’ *Regulatory Toxicity Studies*

- Reproduction toxicity studies
 - Perinatal Development (Segment III)
- Carcinogenicity studies
 - In 2 rodent species or
 - In 1 rodent species and “alternative” test
- Environmental risk assessment

Repeated-dose Toxicity Studies

Animal numbers

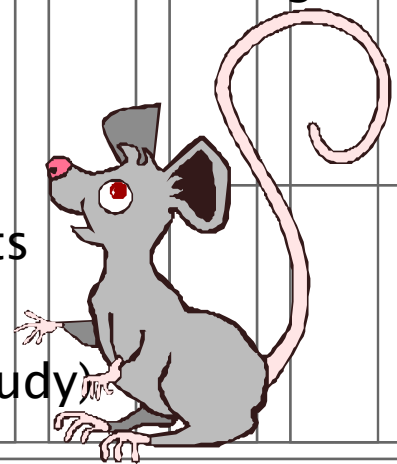
Study type	OECD Guideline	Duration	Dose Groups	Animals / Group	Groups	Animals / Group	Total No. of animals
			Main study		Toxicokinetics and/ or Recovery		
Rodent							
Range-Finding	407	14-day	0, 1, 2, 3	4m / 4f	0, 1, 2, 3	2m / 2f	48
Subchronic Toxicity	407	28-day	0, 1, 2, 3	10m / 10f	0, 1, 2, 3	4m / 4f	112
Subchronic Toxicity	408	13-week	0, 1, 2, 3	10m / 10f	0, 1, 2, 3	6m / 6f	128
Chronic Toxicity	(452)	6-month	0, 1, 2, 3 TK	20m /20f 5m / 5f	0, 1, 2, 3	5m / 5f	200
Non-Rodent							
Range-Finding		14-day	0, 1, 2, 3, 4	1m / 1f			10
Subchronic Toxicity		28-day	0, 1, 2, 3	3m / 3f	0, 3	2m / 2f	32
Subchronic Toxicity	409	13-week	0, 1, 2, 3	3m / 3f	0, 3	2m / 2f	32
Chronic Toxicity	(452)	9-month	0, 1, 2, 3	4m / 4f	0, 1, 2, 3	2m / 2f	48

Dose groups: 0 = Control, 1 = low, 2 = mid, 3 = high

Oncogenicity Studies

Classical approach and Alternatives

- There is poor correlation of tumor incidences in rodents and humans, and predictability of human tumors is not enhanced by rodent data
- Classical approach
 - 2 rodent species (rat; mouse) – 3 dose– and one control group
 - 50 animals / sex / group (400 – 500 animals / study)
- Alternatives
 - Transgenic mice are not overly sensitive, more subject to false negatives than false positives
 - P53+/-: if clearly or equivocally genotoxic
 - Tg.AC: for dermally applied products
 - TgRasH2: for genotoxic or nongenotoxic products
 - Neonatal: if clearly or equivocally genotoxic
 - 15–25 animals / sex / group (210 – 350 animals / study)



REDUCTION – Opportunities / State of the Art

Scientific – Regulatory

- Scientific
 - Early (predictive) safety studies
 - Dose–range finding studies
 - Alternative oncogenicity studies (mentioned before)
- Regulatory
 - International Conference on Harmonization (ICH) Guidelines Revisions
 - New approaches of earlier Entry–Into–Human
 - Exploratory clinical studies (e–IND; microdosing procedures)



Early ('Predictive') Safety Studies

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Discovery: Clinical Candidate Selection (CSS)

Early DMPK Evaluation

- P450 interaction
- Time dependent P450 interaction
- Reactive metabolites
- Microsomal (hepatocyte) stability
- Stability in plasma (first assessment)
- Absorbability
 - e.g. CaCo-2-cell monolayer; PAMPA
- Protein binding
- Transporters
 - P-glycoprotein

in silico Toxicology

...Great help in tailoring safety testing strategy

Paradigm: Structural properties may sheds light on mechanism of metabolic / toxicological action of a compound

- Search for chemically related compounds and associates information (e.g Scifinder)
- Predictive model expert systems for (Quantitative) Structure Activity Relationship – (Q)SAR
 - DEREK
 - for Genotoxicity, Skin sensitization, Irritation, Phototoxicity
 - VITIC database (LHASA; ILSI/HESI, 2004)
 - for Genotoxicity, Carcinogenicity, hERG, Hepatotoxicity, Skin sensitization
 - Multi-CASE
 - for Carcinogenicity, Teratogenicity, Hepatotoxicity in humans
 - Local (Q)SAR tools

- tailored systems offered together with a small program (on the internet) applicable to a certain biological activity e.g

Pilot Toxicity Study in the Dog

Current Approaches

- Ascending dose study
 - Ascending single dose for e.g. 5 days
 - 1 m / 1 f animal
- Fix dose study
 - 14-day
 - No recovery period
 - 1m / 1f animal per control- or dose-group at 3 dose levels (total of 8 animals)
- Age at study commencement: animals not younger than 9 months
- Mode of administration: preferred oral gavage / gelatine capsule or according to clinical program



ICH meeting Nov-2007 in Yokohama (J)

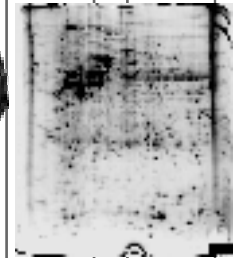
Progress in the right direction ...

- ICH S2: Guidance on Genotoxicity Testing
 - No longer require concurrent positive controls in every in vivo assay
 - Integration of genotoxicity into toxicology assays
 - Reduction in “non-relevant” in vitro results will reduce number of follow-up in vivo assays
 - Advice on choice of second in vivo genotoxicity endpoint includes Comet assay, (decreases emphasis on UDS assay)
- ICH M3: Timing of Pre-clinical Studies in Relation to Clinical Trials
 - 9-month non-rodent studies in almost all cases in all regions
 - 12-month studies only to be used to support replacement of chronic non-rodent and juvenile toxicology study where primary population is pediatric
 - 6-month acceptable in EU
 - Consensus reached on two microdose approaches and sub-therapeutic approach for clinical trials
- Acute Toxicity Testing vs. Dose-range finding approaches

REFINEMENT – Our current focus

Animal Welfare - Scientific

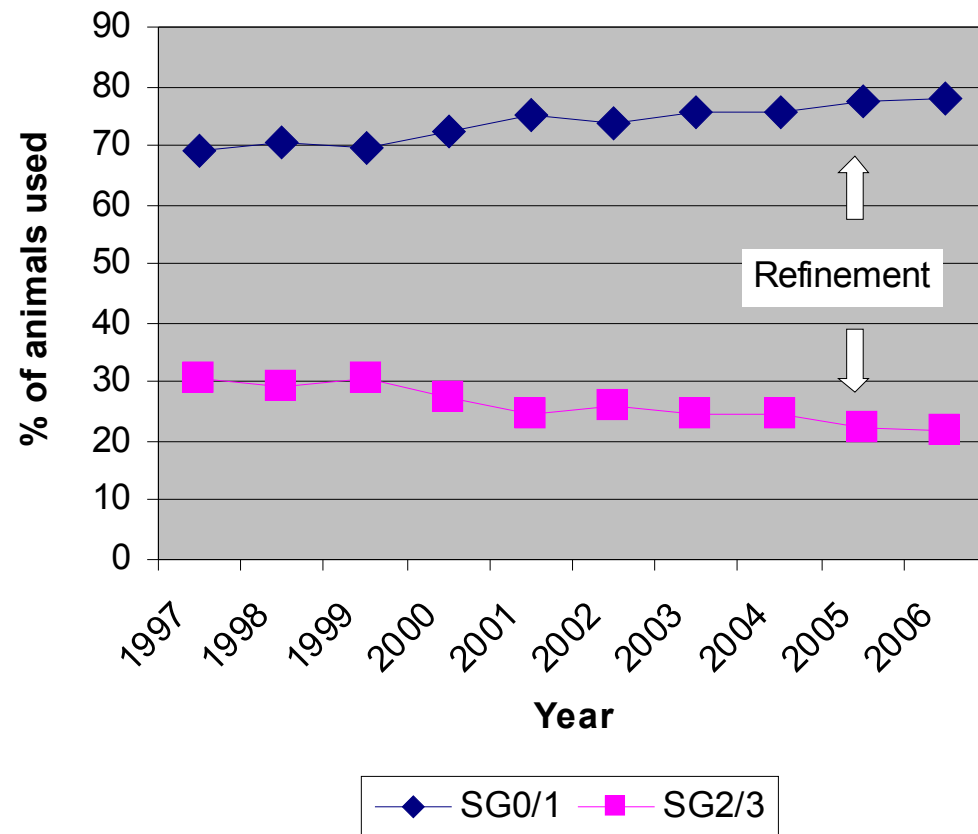
- Animal welfare legislation
 - Improve conditions of animals breeding and safety tests
 - E.g. reduce impact on test animals (degree of severity)
- Use of latest technologies ('omics) -> "PredTox"
 - Toxicogenomics:
 - Gene expression profiling, genome-wide screening of expressed mRNA in a tissues or cell culture: good prediction after single dose studies and data used for mechanistic understanding
 - Proteomics
 - Evaluation of all proteins in a biological sample (e.g. tissue, urine)
 - Metabonomics
 - Metabolic profiling in body fluids (e.g. urine, plasma)
- Development of new, sensitive and specific biomarkers -> "IMI"; C-Path (US)



Animal Welfare Legislation

Adherence without compromises

- Introduction of a wide variety of measure to improve conditions of animals breeding and safety testing, e.g. humane criteria for euthanasia,
- **Reduction of stress / burden**
 - Figure on severity grades (retrograde judgement) of animals studies in Switzerland



New Dimension in Industry Collaboration




















Joint efforts are mandatory

- European Framework Programme 6 – Innovative Medicines for Europe – InnoMed
 - Integrated Project: Predictive Toxicology –> www.innomed-predtox.com
 - More informed decision making earlier in preclinical safety evaluation by combining results from ‘omics technologies together with conventional toxicology methods.
 - Ultimate aim: Design of multiplex assays to rapidly and sensitively detect nephro- and hepatotoxicity
- Innovative Medicines Initiative –> www.imi-europe.org
 - Topics for a “1st. IMI Call” (2008)
 - Predictive Toxicology – PredTox II
 - Qualification of translational biomarkers from non-clinical to early clinical studies
 - Immunogenicity
 - Non-genotoxic carcinogens

Development of expert system for in silico toxicity prediction

The FP6 “PredTox” Consortium



         	        	<table><tr><td>Nycomed (former: Altana)</td></tr><tr><td>Bayer Schering Pharma (former: Bayer)</td></tr><tr><td>Boehringer-Ingelheim</td></tr><tr><td>Johnson & Johnson</td></tr><tr><td>Lilly (B) (Facility closed) - no EU funds</td></tr><tr><td>Merck-Serono (former: Merck KGaA)</td></tr><tr><td>Novartis</td></tr><tr><td>Novo-Nordisk</td></tr><tr><td>Organon (by end-2007: Schering-Plough)</td></tr><tr><td>Roche</td></tr><tr><td>Sanofi-Aventis (D)</td></tr><tr><td>Sanofi-Aventis (F)</td></tr><tr><td>Bayer Schering Pharma (former: Schering)</td></tr><tr><td>Merck-Serono (former: Serono)</td></tr><tr><td>Servier</td></tr><tr><td>University of Wuerzburg</td></tr><tr><td>Univ. College Dublin</td></tr><tr><td>University Hacettepe</td></tr><tr><td>Genedata</td></tr><tr><td>Bio-Rad (former: Ciphergen) - no EU funds</td></tr></table>	Nycomed (former: Altana)	Bayer Schering Pharma (former: Bayer)	Boehringer-Ingelheim	Johnson & Johnson	Lilly (B) (Facility closed) - no EU funds	Merck-Serono (former: Merck KGaA)	Novartis	Novo-Nordisk	Organon (by end-2007: Schering-Plough)	Roche	Sanofi-Aventis (D)	Sanofi-Aventis (F)	Bayer Schering Pharma (former: Schering)	Merck-Serono (former: Serono)	Servier	University of Wuerzburg	Univ. College Dublin	University Hacettepe	Genedata	Bio-Rad (former: Ciphergen) - no EU funds	
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The Primate Issue

How to overcome conflicting requests?

- New compound classes, e.g. therapeutic humanized monoclonal antibodies are currently causing an increased use of primates
 - Relevance of safety results in non-primates questionable
 - Is the request of production of the respective mouse antibodies really the solution?

Discussion ongoing

Conclusions

Replacement - Reduction - Refinement

- Pharmaceutical Industry seriously involved and interested in the 3R's
 - A wide variety of alternative methods are already in place / in use
 - For several relevant sectors of safety evaluation alternatives to animal testing not yet available
- (Global) Regulatory acceptance is of key importance
 - ICH process is the most appropriate platform for 3R challenges
 - Performance of alternative methods as an “add-on” is inappropriate
 - Further develop the basis of clinical trials with less animal data
- Development of the scientific base of safety testing
 - Reliability of extrapolation from animals to humans has to be relevantly improved
 - Collaborative approaches of industry and academia to be strengthened (EU Research Framework Programmes, e.g. FP6 “PredTox”; Innovative

Acknowledgement

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- Dr. Markus Stephan-Gueldner
- Dr. Laura Suter-Dick

Thank you very much for your attention