NON-CLINICAL SAFETY IN DRUG DEVELOPMENT

LA SÉCURITÉ NON-CLINIQUE DANS LE DÉVELOPPEMENT DU MÉDICAMENT

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SUMMARY

Non-clinical safety and toxicology are multidisciplinary sciences evaluating the benefit/risk ratio of clinical candidates. To identify the toxicological profile of these new molecules for clinical studies, experimental toxicology studies focus on their adverse effects in selected laboratory animal species, before extrapolating the findings and predict potential adverse effects in man. As the non-clinical safety file is based on stringent global regulatory constraints, core studies are very well defined and integrated in the drug development process. However, in order to minimize the attrition rate and to improve clinical candidate selection based on safety criteria, molecular toxicology methods are also used to show whether non-clinical safety findings can or cannot be extrapolated to man. In some cases, these methods can prove species-specific toxicity. However, molecular toxicology should not be used on its own, but rather as a tool to help the interpretation of in vivo *experimental toxicology data.*

Key words : non-clinical safety, regulatory toxicology, drug development, predictability of adverse effects, extrapolation to human.

RÉSUMÉ

La sécurité non clinique et la toxicologie sont des sciences multidisciplinaires pour l'évaluation du rapport bénéfice/risque des candidats cliniques. Afin d'identifier le profil toxicologique de ces molécules pouvant éventuellement être testées dans des essais cliniques, les études de toxicologie expérimentale se focalisent sur leurs effets indésirables chez des espèces d'animaux de laboratoire sélectionnées, avant de les extrapoler et de prédire les effets indésirables potentiels chez l'homme. Le dossier non clinique est fortement orienté par les contraintes règlementaires globales et, par conséquent, les études principales sont très bien définies et intégrées dans le processus de développement du médicament. Cependant, afin de minimiser le taux d'attrition et d'améliorer la sélection des candidats aux études cliniques selon des critères de sécurité, des méthodes de toxicologie moléculaire sont aussi utilisées pour montrer comment les effets observés en sécurité non clinique peuvent être ou non extrapolés à l'espèce humaine. Dans quelques cas, ces méthodes peuvent prouver que la toxicité est liée à l'espèce. Cependant, la toxicologie moléculaire ne devrait pas être utilisée seule mais pour nous aider à interpréter de façon raisonnée les données de la toxicologie expérimentale *in vivo*.

Mots-clés : sécurité non clinique, toxicologie réglementaire, développement du médicament, prédictibilité des effets indésirables, extrapolation à l'homme.

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INTRODUCTION

Historically toxicology and non-clinical safety were primarily focused on the study of adverse effects of xenobiotics. Nowadays non-clinical safety is mainly driven by global regulatory constraints, and therefore core studies are very well defined and integrated in the drug development process, but these studies go beyond their historical goals up to the study of molecular biology in order to minimize the attrition rate and to improve clinical candidate selection.

SAFETY TESTING AND THE REGULATORY ENVIRONMENT : HISTORY AND TASKS OF TOXICOLOGY

Regulatory and political aspects : Safety Testing in a Global Regulatory Environment

Besides the scientific challenges, drug safety testing is *overwhelmingly* driven by global regulatory constraints, e.g. OECD/ICH guidelines, GLPs, national legislation, animal welfare laws (FDA 2000, Good Laboratory Practice Standards 1999).

There is an increasing pressure from Health Authorities, especially the US-FDA as the World leader in setting the standards. Health Authorities such as the FDA are influenced by politics and public pressure. Guidances are issued for development of drugs in a variety of disease areas where toxicology and non-clinical drug safety play an increasing role.

Scientific and technical aspects

Is toxicology a Science ?

Toxicology is a multidisciplinary science for evaluation of risk/benefit ratio. Toxicology takes methods from other sciences such as : chemistry, pharmacology, pathology, biochemistry and pharmacological chemistry, clinical medicine, forensic medicine, genetics and veterinary medicine.

Toxicology is generally not considered to be a Science *per se*, but applies different scientific methods to answer questions about potential drugs raised by different scientists including clinicians.

Huge safety data packages are not best practice, where the appropriate, relevant and well understood, toxicology can be performed (Diener 1997).

Tasks of Experimental Toxicology

Spectrum of toxicity, Extrapolation and Prediction of adverse effects

In order to identify the toxicological profile of a compound, experimental studies focus on detection of adverse effects in carefully selected laboratory animal species and describing the dose-effect relationship over a broad range of doses.

Results of these studies can be used to predict potential adverse effects in other species, especially humans

Prediction of Safety

Non-clinical safety evaluation is used to identify safe starting doses and subsequent dose escalation schemes in humans. To do this, toxicity and reversibility of toxicity as well as other parameters (e.g. biomarkers) are monitored in target organs and serum. Starting doses are defined with the expectation that adverse effects should be very unlikely to occur Unfortunately, although it can be used to reduce the risk, no tool can prove the existence of a « negative » risk (i.e. prove the absence of any risk).

Risk assessment : consequences and perception

Risk vs. hazards

While predicting safety, one should also consider the acceptable level of risk, which is largely dependent on the seriousness of the disease and public perception and acceptance. Higher risk levels are more easily accepted in certain diseases, like terminal cancer than in attention deficit disorder.

The acceptability of a risk is very dependent on the indication or the use of a drug. Late clinical studies allow epidemiological demonstration of safe exposure. The acceptable numerical risk is tremendously difficult to objectively define (is one case out of 1,000,000 per year negligible ? is one case out of 100,000 per year tolerable ?) and finally depends essentially on analyses of quality of life (cost/benefit ; cost/utility).

Risk Assessment

Risk assessment is performed in several successive steps consisting of hazard identification, hazard characterization, exposure assessment and risk characterization.

The hazard of potential clinical candidates is primarily identified using animal-based and *in vitro* toxicology. The hazard is characterized by dose-response relationships, mechanisms and extrapolation. The exposure is assessed by the mean of plasma (Cmax and AUC) as well as target tissue concentration analysis. The risk is finally characterized by its acceptability, the level of safe exposure, the numerical risk and its effects on quality of life. The acceptability of a risk is very dependent on the indication or the use of a drug (me-too pain killer vs. innovative carcinoma treatment). Late clinical studies allow epidemiological demonstration of safe exposure).

When Safety makes the Drug a Success… (rather than efficacy)

The perception of risk and adverse effects is subjective. For example, the information on adverse effect and warnings often supersede the perception of a drug's efficacy. Safety concerns are the focus of the lay public and some patients in industrialized countries, especially, in « trivial » indications and for me-

too products, where a favorable safety can mean success. Thus, non-clinical safety profiling contributes to lead optimization by identification of the candidate with the most favorable adverse effects profile.

Tragedies Caused by Chemicals

More and more attention is paid to susceptibilities of newborns and foetuses, which is illustrated by two of the most famous tragedies caused by chemicals consisting of the so-called « grey syndrome » and phocomelia. The « grey syndrome » occurred after accumulation of chloramphenicol in newborns due to not fully developed kidneys which led to anaemia and circulatory failure. The famous thalidomide tragedy is often presented as an example to prove the necessity of using more than one species of laboratory animals (Hansen *et al*.1999). This example should be carefully used. It is true that thalidomide is extremely teratogenic in primates (leading to phocomelia) and not teratogenic at all in rodents. However thalidomide is only slightly teratogenic in a few strains of rabbits (including New Zealand White rabbit), which is the most frequently selected non-rodent species selected for teratogenicity studies. Even with the best defined non-clinical safety designs. A 100 % predictability of adverse side effects in the clinic will never be reached.

There are several examples from post-approval adverse side effects leading to drug withdrawals. Some of them are presented in ***table 1***.

Year	Drug	Indication/Class	Causative Side Effet
1991	Enkaid	Antiarrhythmic	Cardivascular
1992	Temafloxacin	Antibiotic	Blood and Kidney
1997	Fenfumarine/ Dexafluramine	Diet pill	Heart valve abnormalities
1998	Posicor (Midefranil) Duract (Bronfemic Na)	Ca-Channel Blocker Rain relief	Lethal drug interactions Liver damage
1999	Trovan Raxar Hismanal Rotashield	Antibiotic Antibiotic Antihistamine Rotavirus vaccine	Liver/Kidney damage QT prolongation Drug-drug interaction Bowel Obstruction
2000	Renzulin Propulsid Lotonex	Type II diabetes Heartburn Irritative Bowel Syndrome	Liver damage Cardio-vascular irregularities Ischemic colitis
2001	Phenylpropanolamine Baychlor	OTC ingredient Cholesterol reducing	Hemorrhagic stroke Rhabdomyolysis

Table 1 : *Examples of post-approval Adverse Side Effects and Related Drug Withdrawals during the last decade.*

SAFETY TESTING : INTEGRAL PART OF DRUG DEVELOPMENT

In order to minimize the attrition rate and to improve clinical candidate selection upon safety criteria, safety testing is integrated in the drug development processes.

Clinical candidate selection

Non-Clinical Safety studies : a Research and Development process for drug candidates

Discovery Research

During discovery research, researchers are focused on the identification of disease. They use molecular modeling and then, for the most promising molecules, chemical synthesis and high throughput screening.

Even at these early stages, some animal efficacy studies as well as early safety testing and prediction already take place.

Discovery Research : Compound Selection or Clinical Candidate Selection (CSS)

During discovery research and compound selection steps, pharmacological studies, analytical work on the active ingredient (A.I.) production and batch planning are performed. Well defined non-clinical safety studies also are performed, including *in silico* approaches [e.g. DEREK (Deductive Estimation of Risk from Existing Knowledge), MCASE (Multiple Computer Automated Structure Evaluation)], bioavailability, safety (overdose limits and morbidity rates, potential of drug-drug interactions and mutagenicity screen) and safety pharmacology (study of side effects), as detailed in the following section.

Drug Discovery : early (Preliminary) Safety Testing

At very early stages, non-clinical safety studies are :
– distribution, metabolism and pharmacokinetics (DMPK),
– toxicology,
– safety pharmacology.

• DMPK

The aim of these studies is to prove that different species are sufficiently exposed to the compound and to define their metabolic profiles. These studies consist namely of the study of metabolism and pharmacokinetics (allowing selection of the most appropriate species) and drug-drug interactions (cytochromes P-450 – « CYPs »).

• Toxicology and safety pharmacology

Historically the first toxicology studies consisted of the use of single dose (acute) studies, which is now more and more subject to controversies, which is why the first general toxicology studies are now dose-range finding (DRF) and maximal tolerated dose (MTD) studies generally performed in rats and dogs for up to 14 days. In parallel *in vitro* mutagenicity tests, including the famous Ames test are performed as well as *in vitro* safety pharmacology studies (for example hERG or Purkinje fiber tests). These studies participate to the selection of clinical candidates.

Clinical candidate characterization : studies preparing Entry in Humans (« Phase 0 »)

After clinical candidate selection, the non-clinical safety studies consist of ADME (absorption, distribution, metabolism and excretion), toxicology studies and safety pharmacology studies.

At this stage, other variables are investigated in parallel including including : dosage, formulation, preparation of first human pharmacokinetic studies.

Toxicity Studies for Entry in Humans

Toxicity studies performed for entry into humans are Good Laboratory Practices (GLP) compliant studies and consist of general toxicology, genotoxicity and safety pharmacology studies (FDA 2000, Good Laboratory Practice Standards 1 999)

General Toxicology

At this stage, general toxicology studies last 2 to 4-week and are performed in rodent and non-rodent animal species. They include toxicokinetics and recovery assessment (treatment-free periods) and are GLP-compliant. Immunotoxicity parameters can also eventually be included.

In addition, acute toxicology studies and local tolerance studies can be performed.

Genotoxicity (GLP-compliant)

Genotoxicity studies are also GLP-compliant and consists of at least two *in vitro* tests (Ames test and mouse lymphoma test or human chromosome aberration). An *in vivo* test can also be performed (*in vivo* micronucleus test in rats or mice)

Safety Pharmacology

The so-called core battery of safety pharmacology studies investigates central nervous system, cardiovascular and respiratory effects.

DMPK (Drug Metabolism/Pharmacokinetic) Studies (ADME)

DMPK studies investigate the exposure to the clinical candidate and its metabolism.

A = Absorption

The absorption is estimated by measuring blood/plasma concentrations during systemic exposure (time-dependent). Single dose pharmacokinetics (per os, intravenously) are performed in several animal species.

D = Distribution

In rodents, the distribution and retention within major organs is estimated by whole body autoradiography. Specifically, mass balance, quantitative whole body autoradiography (QWBA) protein binding, red cell partitioning and brain penetration are determined.

M = Metabolism

Major metabolic pathways are explored through *in vitro* and *in vivo* investigations for demonstration of major metabolic similarity/dissimilarity between the animal species used in toxicity studies and humans. Metabolism is studied (stability and pattern as well as metabolite identification) using *in vitro* studies (microsomes and/or hepatocytes of several species), cytochrome induction (metabolizing enzymes and transporters) and *in vivo* metabolism.

E = Excretion

Rate and routes (most often biliary or urinary) of elimination are also studied.

Screen of different Formulation

At this stage, different formulations and their respective bioavailability are studies and compared.

Non-Clinical Safety Documentation

All these studies are documented and summarized in a well defined way.

Summary Level

Internal summary documents are generally issued by the investigators in order to summarize generated data : usually modular documents detailing background, potential safety-relevant issues, margins of safety as well as an overview of results (summaries of pharmacology, DMPK and toxicity studies).

Other documents also are prepared to be submitted to authorities and consist of Investigators'Drug Brochure (IDB), Expert Reports and Safety Reviews.

Study Level

In addition study reports are written (one report per study, according to GLP). Results can also be reported in 'Letter Reports'

Early Clinical Development : studies following Entry in Humans : phase IIa

The overall objective following first studies performed in human healthy volunteers is to perform the first evaluations of efficacy of the drug in humans with the disease in phase IIa clinical trials (pilot dose ranging, efficacy and safety studies).

Pharmacokinetic and metabolism studies are performed as well. Formulation and active ingredient synthesis are further elaborated.

At this stage, non-clinical safety studies consist of chronic toxicity studies (in life phase), carcinogenicity studies (in life-phase), additional animal ADME studies and eventual mechanistic toxicity studies.

Late Clinical Development: phase IIb/III

The main objective of phase IIb clinical trials is to allow definitive dose finding and selection of minimal effective dose (MED). Pharmacokinetic and metabolism studies are still performed.

Formulation work and active ingredient synthesis are further elaborated to reach the final synthesis under Good Manufacturing Practices and to continue process refinement.

At this stage, non-clinical safety studies consist of final reproduction toxicity studies (segments I and III), chronic toxicity studies (completion), carcinogenicity studies (in life-phase) and eventual mechanistic toxicity studies.

The final market image is defined. A meeting is scheduled with FDA at the end of Phase II.

Entry into life-cycle management (EILC): Full Development

The overall objective is to perform the definitive evaluations to prove safety and efficacy of the drug in order to gain health authority's approval.

Phase III clinical trials are performed. The market formulation is completed.

At this stage, non-clinical safety studies consist of carcinogenicity studies which are under completion.

An environmental assessment has to be prepared in order to issue an Environmental Impact Statement (including disposition of clinical supplies, concentration of active ingredient into environment as a result of its use, use of natural resources/energy and ecotoxicology) and submitted to Regulatory Agencies.

After having put non-clinical safety studies in the perspective of drug development, these studies (toxicity, DMPK and pharmacology studies) will be more detailed.

NON-CLINICAL SAFETY TESTING

Different Toxicological Targets = Different Study Types *(figure 1)*

The different toxicological targets previously discussed justify different study types which are listed below and will be detailed in the next pages:

- single-dose (acute) toxicity or Dose Range Finding (DRF: subacute) studies;
- subchronic and chronic toxicity studies:
  - 1-month; 13-week; 6-month in rodents; 9-month in non-rodents;
- reproductive toxicology studies:
  - pilot and definitive « Segment II » studies in 2 species,
  - « segment I and III » studies;
- mutagenicity studies;
- carcinogenicity studies:
  - pilot and main studies; (alternative testing);
- special studies:
  - irritation (rabbits; dermal, subcutaneous, intravenous),
  - sensitization/phototoxicity (guinea pigs),
  - immunotoxicity (functional tests: T-cell subset classification; T-cell dependent antibody production),
  - mechanistic toxicity studies (in vitro and in vivo).

Animal Species used

In order to correctly define its toxicological profile, taking into consideration possible pharmacokinetic, metabolic and/or physiological differences, clinical candidates have to be evaluated in non-clinical safety studies performed in at least two different species including a non-rodent species. The most often selected species are rats and dogs or those listed below:

– Rodents: rat, mouse;

– Non-Rodents: dog, minipig, non-human primates (Cynomolgus monkey, marmoset, Rhesus monkey.

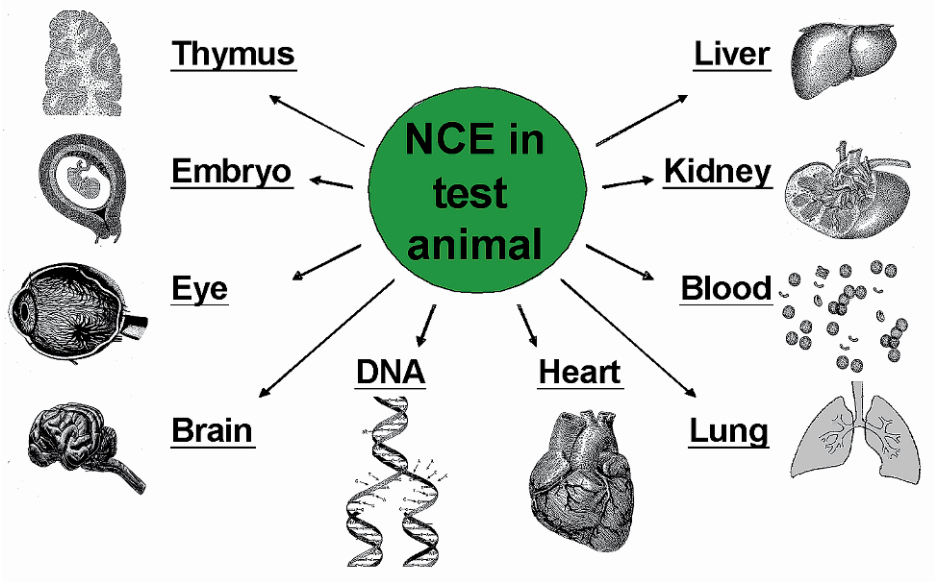

Figure 1: *Target organs in toxicology (NCE: New Clinical Entity).*

Experimental design	Duration (interim kill ; recovery) Dose groups/selection Animal numbers Frequency of dosing Application routes
Clinical investigations	Symptomatology Ophthalmology Electrocardiography Neurology
(Pharmacological investigations)	
Clinical Pathology	Clinical chemistry Hematology Urinalysis
Toxicokinetics	
Pathology	Necropsy, Organ Weights ; Histopathology
Biometrical investigations	

Table 2 : *Integral Parts of Toxicity Studies.*

Repeat-dose (Subchronic) Studies *(tables 2 & 3)*

Objectives

The objectives of the repeat-dose subchronic studies is to determine target organs, to assess reversibility and sex differences, to determine a No Observed Effect Level, to evaluate toxicokinetics, to ensure safety in clinical trials and to take go or no go decisions.

Design

The design consists of repeated dosing for up to 3 months (13-weeks) in two species (rodent and non-rodent) using at least 3 dose-levels : high dose (maximum tolerated dose or MTD), low dose (high therapeutic dose), medium dose (generally geometric mean of the low and high doses), followed by a treatment-free period allowing recovery assessment.

Endpoints

In these studies endpoints are clinical signs, body weight and food consumption, toxicokinetics, clinical pathology (clinical chemistry, hematology) and anatomopathology (macroscopic *post mortem* findings at necropsy, organ weights, histology/pathology).

Reproductive and developmental toxicology studies

Objectives

The objectives of reproductive and developmental toxicology studies is to determine eventual effects on fertility, development, teratology or new born. Potentially pregnant women cannot be included in clinical trials as long as regulatory developmental studies have not been performed (Collins *et al.*1999).

Design (figure 2)

Regulatory authorities have established guidelines and study designs for assessing reproductive risks induced by chemicals. The Food and Drug administration has defined three different segments on development, fertility and general reproductive performance :

– segment I : Fertility and Reproduction Function in Males and Females,
– segment II : Developmental toxicology and Teratology,
– segment III : Perinatal and Postnatal studies.

Study type	OECD Guideline	Duration	Dose Groups	Animals/Group	Groups	Animals/Group	Total No. of animals
			Main study		Recovery		
Rodent							
Range-finding	407	14-day	0,1,2,3,4	5m/5f			50
Subchronic Toxicity	407	28-day	0,1,2,3	10m/10f	0,1,2,3	5m/5f	110
Subchronic Toxicity	408	13-week	0,1,2,3	10m/10f	0,1,2,3	5m/5f	110
Chronic Toxicity	(452)	6-month	0,1,2,3 PK	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	4m/4f	3	2m/2f	36
Subchronic Toxicity	409	13-week	0,1,2,3	4m/4f	3	2m/2f	36
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
Dose selection : Range of effects from a no-effect dose to one wich produces clear effects
m = males, f = females

Table 3 : *Experimental Design According to OECD Guidelines.*

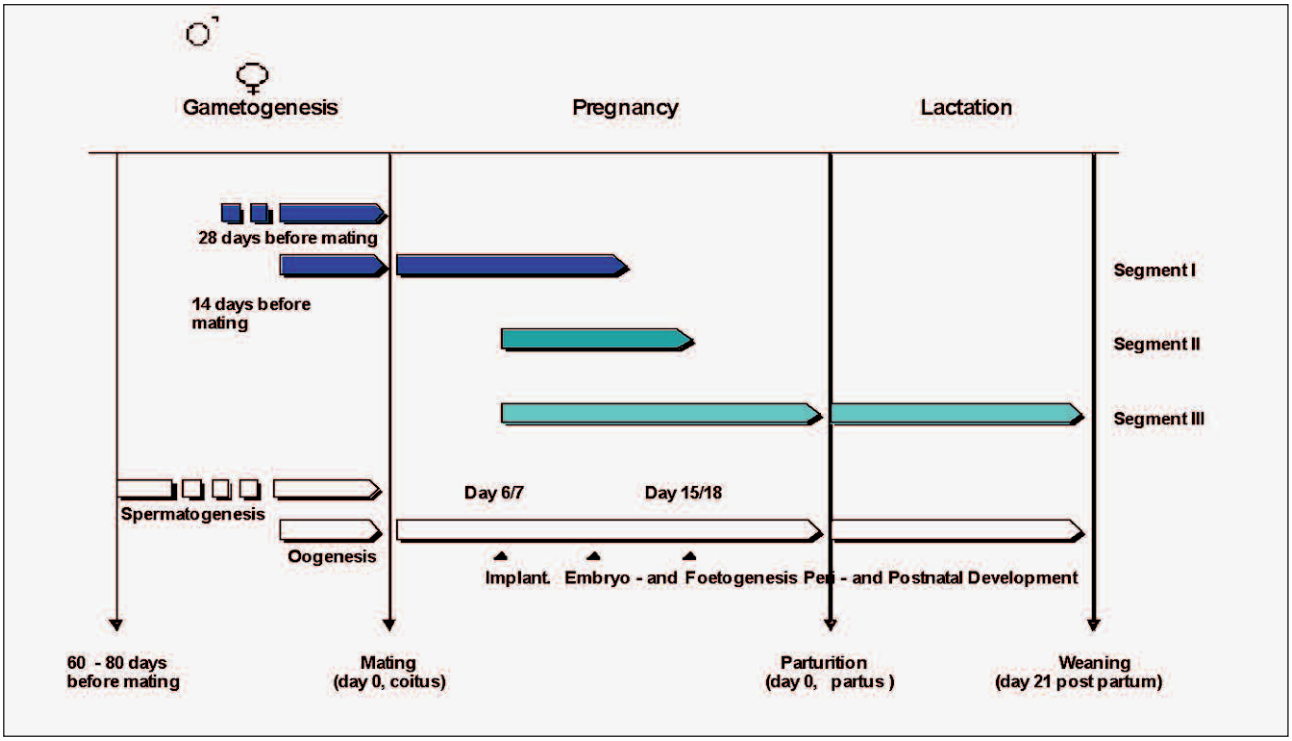

Figure 2: *Reproduction Toxicity – Scheme.*

Endpoints-Females

In female reproductive toxicity studies, endpoints consist of:
– female fertility index,
– gestation index,
– live-born index,
– weaning index,
– sex ratio and percentage per sex,
– viability index.

Endpoints-Males

In male reproductive toxicity studies, endpoints consist of:
- evaluation of testicular spermatid numbers,
- sperm evaluation for motility, morphology and numbers of spermatozoids.

Genotoxicity Studies

Most of these studies are *in vitro* studies but there are also *in vivo* studies (Brusick 1994).

In vitro

The most often used *in vitro* studies are the bacterial reverse mutation test (Ames test), the mouse lymphoma mutation test (ML/tk) and the human chromosome aberration test (HCA).

In vivo

The *in vivo* genotoxicity of choice is the micronucleus test (MNT). This test was historically performed in mice but nowadays more and more often in rats, allowing the use of animals from general toxicology studies.

Carcinogenicity studies

Objective

The objective of carcinogenicity studies is to identify a tumorigenic potential in animals and to assess the relevant risk in humans (Moolenaar 1994)

Design

Carcinogenicity studies are preceded by 90-day range-finding studies with the same 2 rodent species (generally rat and mouse) as those used for the main studies.

The main studies last generally 24 (up to 30) months in rats and 18 (up to 24) months in mouse or hamster. There are 3 dose- and one control groups (high dose = MTD: minimum toxicity, not altering the animal's life-span, no more than 10 % decrease in body weight gain) each comprising 50 animals per sex (EPA 1996).

Endpoints (statistical evaluation; photo documentation)

In these studies, endpoints consist of body weight, food and water consumption, clinical signs (including tumour palpation), clinical biochemistry, haematology, (urinalysis) and gross-, histopathology.

Alternatives for Carcinogenicity Testing

These models are undergoing international validation and consist of initiation/promotion models in rodents, transgenic mouse systems or neonatal rodent tumorigenicity model.

The initiation/promotion model is traditionally a liver model (but being adapted for multiple organs).

Currently, transgenic mouse systems are under validation (p53 +/– deficient model, Tg. AC model, Tg rasH2 model and XPA–/– knock-out mouse model).

The actual status is that there is no single model accepted to replace a carcinogenicity study, but these models are more used to complement standard carcinogenicity studies.

Safety Pharmacology Studies, core Battery

The core battery of safety pharmacology studies involve the central nervous system (Irwin test in rats), the cardiovascular system with telemetry in a non-rodent species, most often dog and/or monkey and electrophysiology *in vitro* studies: often hERG (*in vitro*; human ether a go-go related gene) or Purkinje fiber (*in vitro*; using often rabbit cells) and the respiratory system (plethymsography in rats).

PREDICTABILITY OF ADVERSE EFFECTS AND EXTRAPOLATION OF SAFETY DATA TO MAN

(table 4)

The Information Base for Assessment

Non-clinical assessments are performed in different (at least 2) species. These assessments allow identification of target organs, ideally of mechanisms of toxicity and time and exposure dependent course of adverse effects and their eventual reversibility.

Type Organ Toxicities	Predictability by		Recognition in man
	Standard studies	Special studies	
Idiosyncrasy/Unknow mechanism	0	(+)	+++
Sensitization/Allergies	+	++	+++
Functional disorders	(+)	+	+++
Direct organ toxicities	+	++	+++
Local irritation	++	+++	+++
Mutagenicity	++	++	?
Carcinogenicity	++	++	?
Teratogenicity	++	++	+

Key: *0 = low* *+ = occasionally* *? = questionable* *++=often* *(+)=sometimes possible* *+++=definitely*

Table 4: *Predictability of Adverse Effects.*

Taking into consideration the results of non-clinical safety studies, No Observed Adverse Effect Levels (NOAEL) and/or No Observed Effect Levels (NOEL) are defined in mg/kg and the corresponding exposure is used to compare different species.

The results of these studies permit identification of surrogate markers (for example clinical pathology or clinical investigations: ECG, ultrasound, NMR).

Prior knowledge on « Substance Class » effects is also an important part of the information base to be taken into consideration for assessment.

Criteria Influencing the Assessment

The safety assessment can be influenced by different criteria including the relevance of toxicological findings, the target organs (and their reversibility) and the safety factor (margin of safety - MOS) depending on the NO (A) EL, on inter or intra species differences and variability, and calculations of safety factors (based on « mg/kg/day » versus « toxicokinetic parameters »).

The relevance of toxicological findings depends on the mechanism of action, on the species specificity of the observed effect(s), on the duration of therapy (on the age of patients) and on the experience with related compounds.

EXTRAPOLATION TO HUMAN

The overall aim of non-clinical safety studies is to extrapolate their results to humans.

Although there are arguments defending such extrapolation, the correlations are imperfect and clinical testing remains a necessity.

Nevertheless, effects produced in laboratory animals, when appropriately qualified, are often relevant to humans. The exposure of experimental animals to high doses is necessary to discover possible hazards to humans.

Diverging arguments are linked to the fact that there are species differences in terms of physiology and metabolism (leading to differences in general toxicology) as well as organotrophy (leading to differences in developmental toxicology). In addition, from phase II and beyond, human patients are included in clinical trials, whereas non-clinical safety studies are performed with healthy animals.

Molecular methods can sometimes be a useful tool to interpret how non-clinical safety findings may or may not be extrapolated to humans. But data from such studies must be put in perspective since, in some cases, these methods can uncover species-specific toxicity. For example dioxin, which is toxic chemical that has been shown to bind tightly to a protein in mouse liver, causes liver tumors and testicular damage in rodents. However, unleaded gasoline binds to α2-microglobulin and induces male rat kidney tumors but EPA has accepted that it is without carcinogenic risk for humans.

CONCLUSION : FUTURE REQUIREMENTS AND CHALLENGES

Many experimental approaches were conceived about 50 years ago, when the primary concerns were the lack of toxicity and the determination of a no-effect level. Toxicological screening was performed in few animals (drug-ethanol interaction, neurotoxicity).

Alternatives consist of cytotoxicity, cultures of retina, brain and meningeal cells, hepatocytes or kidney cells or use of molecular methods, proving some cases of species-specific toxicity as described above.

In summary, the actual trend is to move from essentially descriptive activities, complemented by analytical and « measuring science » used in the past to more refined models.

In the future, the aim is to reduce even further animal testing by using improved tools, including : micro-methods for *in vitro* testing, genetically modified cells and animals, toxicogenomics, proteogenomics and metabonomics as well as *in silico* tools.

The usefulness of these or other refined molecular methods is obvious. However, the danger remains that molecular toxicology will be taken at face value, instead of for its predictive value. For example, the LD50, Draize test and 12-month studies have been abandoned and there is an ongoing debate concerning carcinogenicity studies because they are no longer considered important. Notwithstanding improvements, there still remains a tendency to overestimate the importance of molecular toxicology data. Although most classical toxicology studies are still necessary, the trend is to refine studies and to reduce the number of animal used. Thus it is not yet possible to replace most *in vivo* toxicology studies with *in silico* and *in vitro* equivalents. Nevertheless, there has been a tremendous reduction of numbers of animals used in non-clinical studies.

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