Recommendations for the Conduction of Preclinical Toxicological Tests for new drugs or drug compounds

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Introduction

Besides comprehensive data from clinical trials, a Marketing Authorization Application (**MAA**) for new drugs must contain all preclinical data encompassing product specification and quality, bioavailability and metabolism, toxicology and safety pharmacology, as well as efficacy and pharmacodynamics. Results of most of the above mentioned preclinical testing must be provided to the regulatory agencies in order to obtain permission to begin clinical testing in humans. Regulatory agencies review the specific tests and documentation that are required to proceed to the next stage of development.

The appropriate schedule for preclinical testing of new drugs strongly depends on the nature of the compound(s), or the intended clinical use, and can therefore not be generalized. In principle, unless tests are bindingly stipulated by an agency, the applicant is free to design his own specific testing programme. In any case, however, the testing strategy must be justified against the regulatory agencies.

As a general rule, the preclinical data must convince the regulators, not the applicant, of the quality, safety, and efficacy of a new drug. Thus, obeying their rules of the game is highly recommended for a successful MAA. Seeking advice and arrangement with the regulatory agencies about the testing programme, as well as designing studies in close accordance (wherever possible) with issued regulatory guidelines and directives, is therefore a prerequisite for any preclinical programme.

Preclinical testing strategy

Step 1

At a first instance, it is worthwhile considering a new active drug substance or a drug compound merely as a chemical, which has to be produced, handled or packed by workers; which may be transported, and which might cause unintended or unavoidable human or environmental exposure. The EU has issued directive 67/548/EEC which defines testing requirements for new chemicals to be placed on the market. The testing requirements are tiered according to the volume placed on the market. The lowest volume triggering the need for testing amounts to 10 kg.

More extensive testing is required when the volume reaches 100 kg, 1 t, 10 t, 100 t and 1,000 t, respectively. Generally, testing requirements at the lower volumes (10 kg to 1 t) focus on acute hazards (immediate or slightly delayed effects after short term exposure) while those at the higher tonnage levels include more expensive studies on the effects of (sub-) chronic exposure, on reproductive toxicity, on carcinogenicity, or on ecotoxicity and biodegradation. The testing package at 1 t is termed ‘base set’ while those triggered by higher tonnage are called Level 1 (100 t) and Level 2 (1,000 t). An almost identical directive has been issued by the US Environmental Protection Agency (EPA).

If a substance is considered as a *chemical*, the two “magical” margins are < 10 kg/year and >1 ton/year. For all *chemicals* produced at volumes not exceeding 10 kg/year, toxicological testing is generally not required. For all chemicals produced at volumes > 1 ton/year at least the base set of toxicity data, as depicted in **table 1**, must be provided. Between both margins the testing strategy must be individually determined and justified against the regulatory authorities on a case-by-case basis. Again, just to mention this universal law, the testing strategy and data must convince the regulators, not the applicant.

In any case, if your active substance or drug compound is produced at volumes > 10 kg/year, which is often the case even during preclinical drug development, you have to consider it invariably as a chemical, which requires testing according to EU directive 67/548/EEC (or the respective US directive). The minimum testing battery, in this case, should encompass at least the fife parameters listed in **table 2**. Take a look at the next available *Material Safety Data Sheet* found in your laboratory to find these parameters listed in section 11 (Toxicology).

Table 1

Base set testing requirements for human health end-points and ecotoxicological end-points based on Annexes VII A, B and C of Directive 67/548/EEC

End-point	EU test method
Acute toxicity	B.1bis: acute toxicity (oral) fixed dose method B.1tris: acute toxicity (oral) – acute toxic class method B.2: acute toxicity (inhalation) B.3: acute toxicity (dermal)
Irritation	B.4: acute toxicity (skin irritation) B.5: acute toxicity (eye irritation)
Corrosivity	B.40: skin corrosion
Skin and respiratory sensitisation	B.6: skin sensitization
Repeated dose toxicity	B.7: repeated dose (28 days) toxicity (oral) B.8: repeated dose (28 days) toxicity (inhalation) B.9: repeated dose (28 days) toxicity (dermal)
Mutagenicity and genotoxicity (not the entire testing battery is required)	B.10: mutagenicity (*in vitro* mammalian chromosome aberration test) B11: mutagenicity (*in vivo* mammalian bone-marrow chromosome aberration test) B.12: mutagenicity mammalian erythrocyte micronucleus test B.13/14: mutagenicity – reverse mutation test using bacteria B.15: gene mutation – *Saccharomyces cerevisiae* B.16: mitotic recombination – *Saccharomyces cerevisiae* B.17: mutagenicity – *in vitro* mammalian cell gene mutation test B.18: DNA damage and repair – unscheduled DNA synthesis – mammalian cells *in vitro* B.19: sister chromatid exchange assay *in vitro* B.20: sex-linked recessive lethal test in *Drosophila melanogaster* B.21: *in vitro* mammalian cell transformation test B.22: rodent dominant lethal test B.23: mammalian spermatogonial chromosome aberration test B.24: mouse spot test B.25: mouse heritable translocation B.39: unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vivo*
Effects on organisms	Acute toxicity for fish Acute toxicity for daphnia Growth inhibition test on algae Bacteriological Inhibition
Degradation	Biotic Abiotic

Table 2

Minimum set testing requirements for human health end-points (market volume < 1 ton)

End-point	EU test method
Acute toxicity	B.1tris: acute toxicity (oral) – acute toxic class method
Irritation / Corrosivity	B.4: acute toxicity (skin irritation) / B.40: skin corrosion B.5: acute toxicity (eye irritation)
Skin and respiratory sensitisation	B.6: skin sensitization
Repeated dose toxicity	B.7: repeated dose (28 days) toxicity (oral)
Mutagenicity and genotoxicity	B.13/14: mutagenicity – reverse mutation test using bacteria B.10: mutagenicity (*in vitro* mammalian chromosome aberration test)

Step 2 (Drugs or drug compounds: safety)

Once a company has identified a promising drug candidate, which can be manufactured with reasonable consistency, the next step in development is to provide evidence to the regulatory agencies that it is safe to administer the product to humans for the first time (Phase I clinical trial). This evidence must be based on a well-designed programme of appropriate preclinical studies. At this stage the preclinical toxicology studies play perhaps their most important role.

Whilst testing chemicals is a very invariable process, testing of drugs must be closely linked to the future clinical application. Testing of acute or subacute oral toxicity, for example, does not make much sense when a drug will be definitively administered via the parenteral route throughout a clinical trial. In this case, however, an appropriate parenteral toxicity testing programme is inevitable. As a general rule, the future mode of clinical application determines the preclinical testing programme. All safety aspects relevant for a certain stage of clinical development must be addressed by preclinical tests before a permission to conduct a clinical trial will be granted by the regulators. Animal safety studies and human clinical trials should be planned and designed to represent an approach that is scientifically and ethically appropriate for the pharmaceutical under development.

The preclinical safety data required before starting a clinical trial normally encompass:

- single dose toxicity
- repeated dose toxicity
- local tolerance
- reproduction toxicity
- genotoxicity
- carcinogenicity
- safety pharmacology
- pharmacokinetics

SINGLE DOSE TOXICITY STUDIES

The single dose (acute) toxicity for a pharmaceutical should be evaluated in two mammalian species prior to the first human exposure. A dose escalation study is considered an acceptable alternative to the single dose design.

REPEATED DOSE TOXICITY STUDIES

The recommended duration of the repeated dose toxicity studies is usually related to the duration, therapeutic indication and scale of the proposed clinical trial. In principle, the duration of the animal toxicity studies conducted in two mammalian species (one non-rodent) should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies.

LOCAL TOLERANCE STUDIES

Local tolerance should be studied in animals using routes relevant to the proposed clinical administration. The evaluation of local tolerance should be performed prior to human exposure. The assessment of local tolerance may be part of other toxicity studies.

REPRODUCTION TOXICITY STUDIES

Reproduction toxicity studies should be conducted as is appropriate for the population that is to be exposed.

Men

Men may be included in Phase I and II trials prior to the conduct of the male fertility study since an evaluation of the male reproductive organs is performed in the repeated dose

toxicity studies. A male fertility study should be completed prior to the initiation of Phase III trials.

Women not of childbearing potential

Women not of childbearing potential (i.e., permanently sterilised, postmenopausal) may be included in clinical trials without reproduction toxicity studies, provided the relevant repeated dose toxicity studies which include an evaluation of the female reproductive organs have been conducted.

Women of childbearing potential

For women of childbearing potential there is a high level of concern for the unintentional exposure of an embryo/fetus before information is available concerning the potential benefits versus potential risks. In the EU, assessment of embryo-fetal development should be completed prior to Phase I trials in women of childbearing potential and female fertility studies prior to Phase III trials. All female reproduction toxicity studies and the standard battery of genotoxicity tests should be completed prior to the inclusion, in any clinical trial, of women of childbearing potential not using highly effective birth control or whose pregnancy status is unknown.

Pregnant women

Prior to the inclusion of pregnant women in clinical trials, all the reproduction toxicity studies and the standard battery of genotoxicity tests should be conducted. In addition, safety data from previous human exposure are generally needed.

CARCINOGENICITY STUDIES

Completed carcinogenicity studies are not usually needed in advance of the conduct of clinical trials unless there is cause for concern. For pharmaceuticals developed to treat certain serious diseases, carcinogenicity testing, if needed, may be concluded post-approval.

GENOTOXICITY STUDIES

Prior to first human exposure, in vitro tests for the evaluation of mutations and chromosomal damage are generally needed. If an equivocal or positive finding occurs, additional testing should be performed. The standard battery of tests for genotoxicity should at least be completed prior to the initiation of Phase II studies.

Genotoxicity (standard battery)

- A test for gene mutation in bacteria (Ames-Test)
- An in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma tk assay.
- An in vivo test for chromosomal damage using rodent hematopoietic cells.

SAFETY PHARMACOLOGY

Safety pharmacology includes the assessment of effects on vital functions, such as cardiovascular, central nervous and respiratory systems, and these should be evaluated prior to human exposure. These evaluations may be conducted as additions to toxicity studies or as separate studies.

TOXICOKINETIC AND PHARMACOKINETIC STUDIES

Exposure data in animals should be evaluated prior to human clinical trials. Further information on absorption, distribution, metabolism and excretion (ADME) in animals should be made available to compare human and animal metabolic pathways. Appropriate information should usually be available by the time the Phase I (Human Pharmacology) studies have been completed.

Once the appropriate toxicology studies have been completed to support a Phase I clinical trial, the results of those toxicology studies (risk) must be weighed against the potential benefit for administration of the product to humans, the seriousness of the disease indication and the proposed patient population. The overall principle here is that more risk is acceptable in the case of serious and life-threatening disease where there is no other treatment available.

Finally, all toxicology studies must be completed in compliance with Good Laboratory Practices (GLP) and the study design should be based on the proposed clinical trial.

Step 3 (Drugs and drug compounds: quality)

This dossier does not focus on quality aspects of pharmaceutical products. Nevertheless, quality is an important aspect even during preclinical trials.

The drug to be tested during all preclinical safety testing and the clinical trials should be exactly defined. Therefore, to fulfil the quality requirements for a successful MAA, an extensive quality assurance system must be implemented into the production process. To achieve this, several analytical procedures, relating to the identification of the product, tests for impurities, and analytical assays regarding the potency/activity of the product, have to be validated and documented. Optimally, the drug should already be produced in compliance with Good Manufacturing Practices (GMP) during the preclinical testing stage.