Biological importance of metabolites

Safety and efficacy aspects

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- Biological importance of metabolites
- Safety testing of drug metabolites
- Bioanalytical strategy
- Structural alerts
- Other relevant topics
- General points to consider
- Comments on the draft guidance

When are metabolites important ?

- Discovery : assist in the selection of appropriate compound for development

- Safety : only major human metabolites matter in the testing of animals in support of human safety- structural alerts and reactive metabolites ?

- Efficacy : qualitative (ie identification) and quantitative (ie bioanalytical assessments) evaluations of major human metabolites are necessary for interpretations of clinical response

Major metabolite ?

- For drug interaction : CYP phenotyping for major reactions (primary oxidative metabolites) : sum total 25 to 30 % of compounds clearance.

- For safety testing : recent draft guidelines more than 10 % of the administered dose or circulating drug related material.

Unique metabolite

A unique metabolite is one produced only in humans or formed to a much greater extend in humans compared to animal species used in toxicological studies.

Unique metabolite

The presence of a unique human metabolite(s) can be determined by *in vitro* studies using liver slices, microsomes or hepatocytes from animals and humans, by *in vivo* metabolic profiling in the non-clinical test species.

However, a unique metabolite **may only be recognized** after completion of *in vivo* metabolic profiling in humans.

In vitro tools give a relative good picture of major metabolic reactions but not necessarily major metabolites. Secondary and tertiary phase 1 metabolites are simply not formed in sufficient amounts. In vitro profiles can give a reasonable qualitative picture of circulating metabolites.

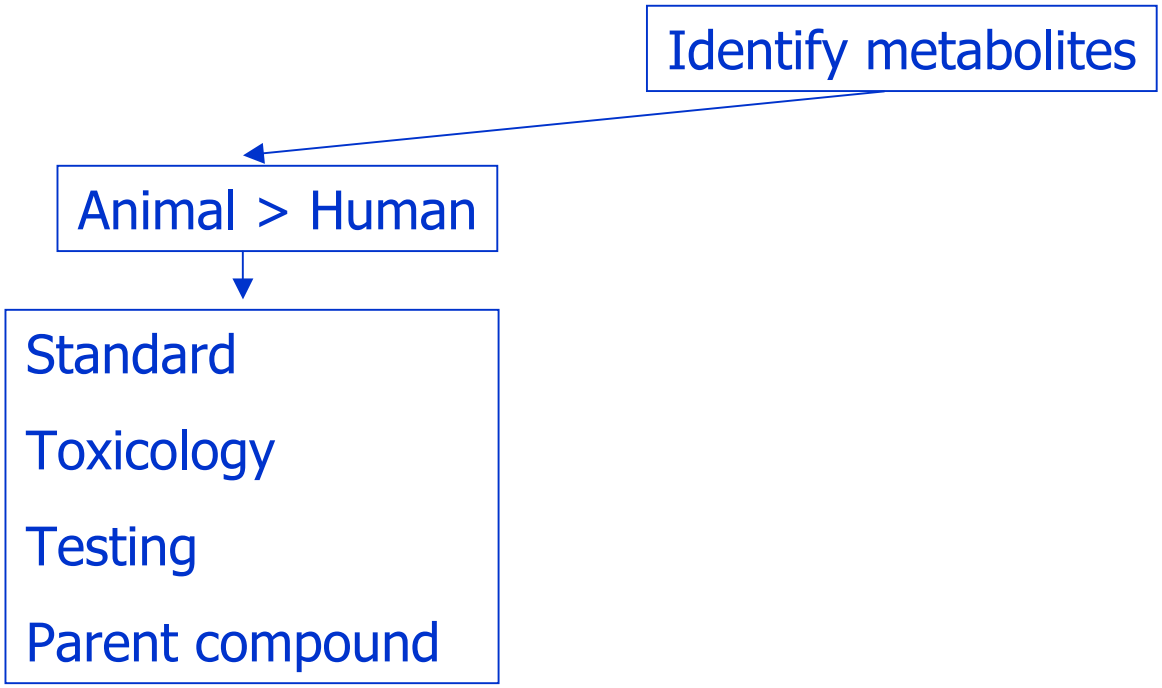
Biological effects (active /toxic metabolites)

- Active metabolites (positive biological effects) refers to target pharmacology
  - Quantitative aspects : participating to the overall pharmacological activity at least 25 % of the total activity
- Toxic metabolites (negative biological effects) implies
  - Extension of pharmacology
  - A different receptor profile
  - Different mechanisms (reactive intermediate, covalent binding, etc.)

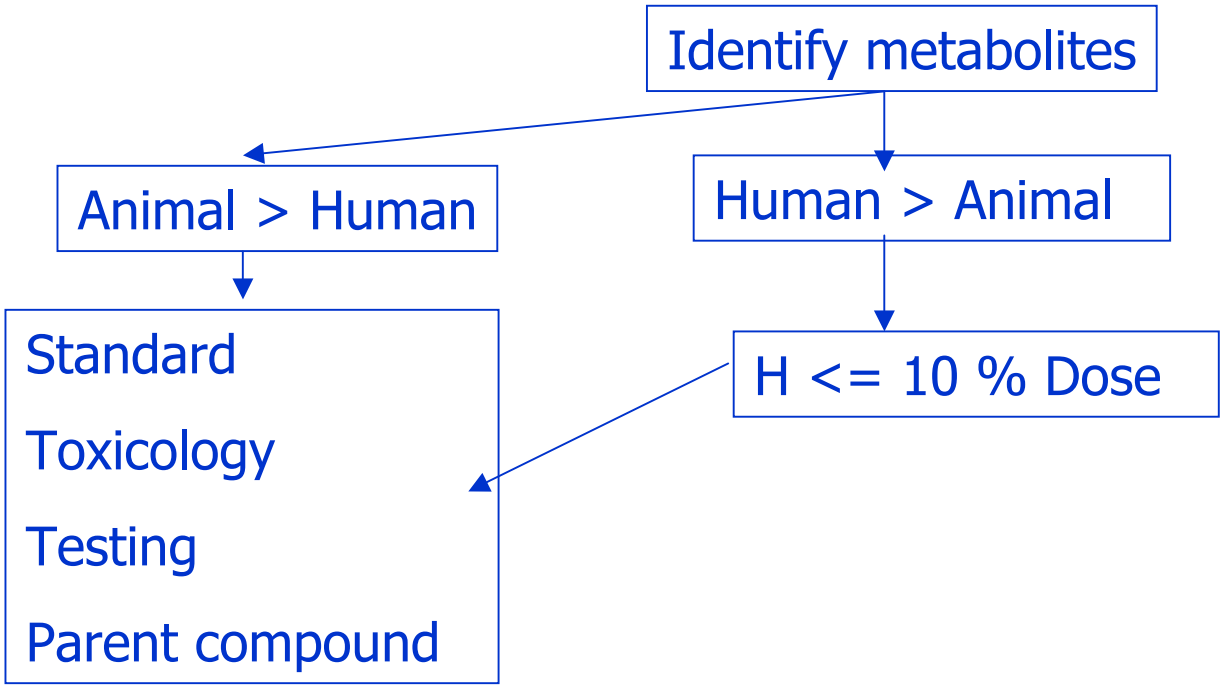
Safety ratio

- Optimal 25 times rule difference in exposure between animals and man ?
- Acceptable exposure multiples ?
- Comparing chemical entity per chemical entity ? Almost impossible throughout species because of differences in rate between species.
- Comparing metabolic routes should be sufficient to support safety

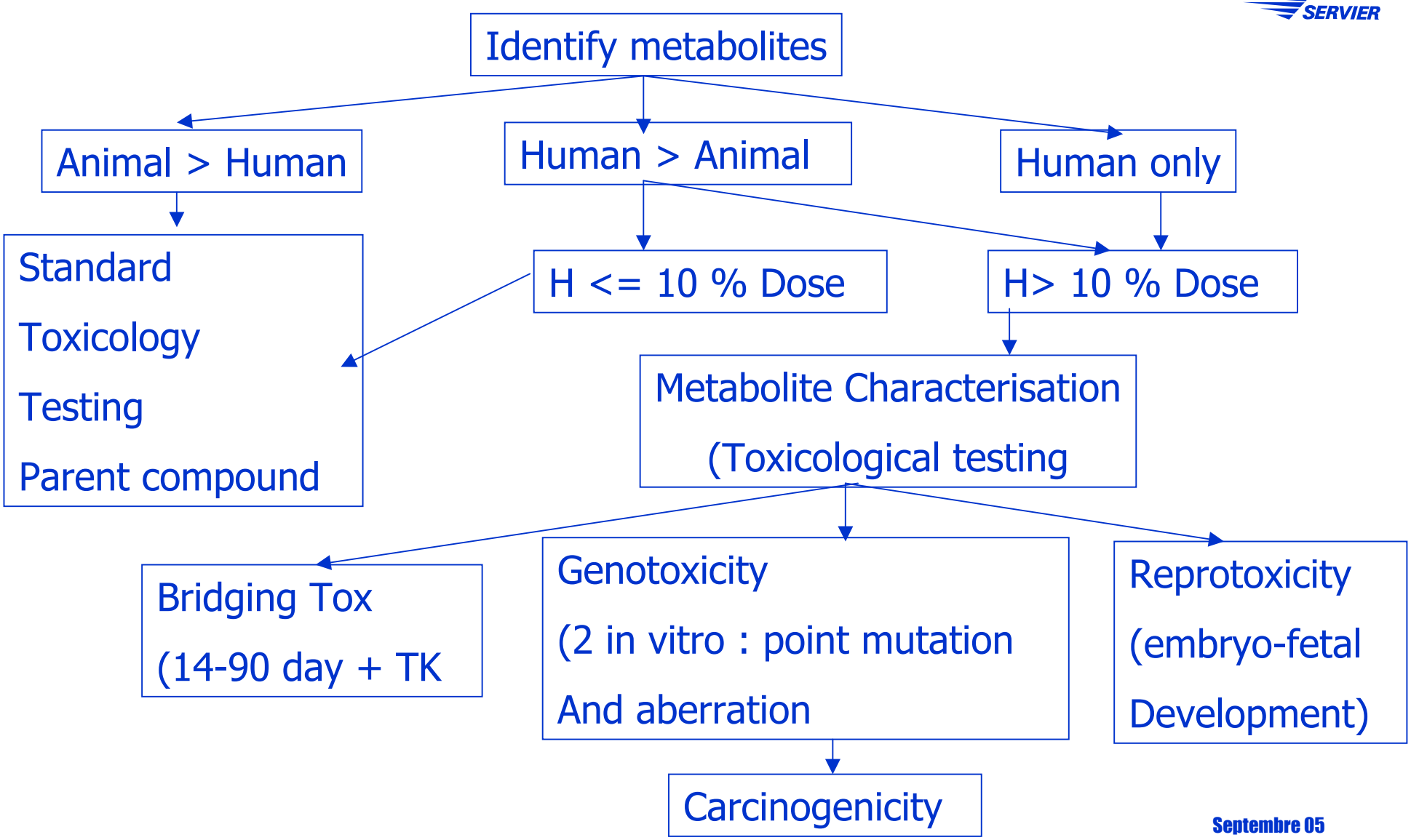
Safety testing of drug metabolite(s)



Safety testing of drug metabolite(s)



Safety testing of drug metabolite(s)



Implications of metabolism data for the safety program

- A case in which a human circulating metabolite which is present in animals at very low concentrations could make conclusions about demonstrated safety in animals difficult to extrapolate to humans
  - potential exposure issue
  - consider use of alternative animal safety species
  - consider further testing on metabolite
- *If* specific toxicological evaluation of the metabolite is warranted, consider:
  - general toxicity testing
  - genotoxicity (mutation and chromosome effects) testing
  - reproductive toxicology studies
  - carcinogenticity testing
    - ← May be warranted only if there was evidence that metabolite caused lesions (not observed with parent) expected to progress to neoplasia

Bioanalytical strategy regarding major metabolites

- If *in vitro* studies suggest a possible major metabolite, identify as early as possible, test simply for activity and toxicity

- As early as possible in the clinical programme, evaluate the metabolic characteristics of the drug
  - First Phase 1
  - Microdosing ?
  - Classical radiolabelled based studies

- Develop/validate assay for relevant human metabolites based on activity and relative quantities

Bioanalytical strategy regarding major metabolites

- Include the metabolite in subsequent analyses in the clinical programmes

- Develop/validate assay for the human metabolite in the toxicological/carcinogenicity species

Other relevant topics

- Metabolites in safety pharmacology
- Which metabolites should be measured in QT studies
- Role of protein binding of metabolites (i.e. on safety margins)
- Metabolites in drug-drug interaction studies

Other relevant topics

- Bioavailability studies (comparison of different groups of subjects/treatments) : include relevant metabolites. The standard BE statistical criteria are used for both parent and metabolite.

- Bioequivalence studies : comparison of different formulations) : based usually on parent drug only more sensitive.

Structural alerts

Guidelines : However, in some cases, it may be appropriate to conduct non-clinical studies **earlier**, for example, if **the metabolites belong** to a **chemical class with known toxicity**, if **the metabolites has positive structural alerts for genotoxicity**, **carcinogenicity** or **reproductive toxicity**, if clinical findings with the metabolite or related products have indicated special clinical safety concerns, such a QT prolongation.

How to screen ? What to do with the data ?

Structural alerts

- Idiosyncratic reactions :
  - adverse reactions that are not related to the pharmacological properties of the drug, occur in a subset of the population at therapeutic doses and that do not occur in the rest of the population despite increasing the dose to otherwise toxic levels.
  - Rare, not reproducible in animals, highly host dependent, frequency (<1/5000)

- Reactive intermediates and covalent binding not necessarily linked
- Covalent binding and cytotoxicity not necessarily from the same metabolite
- Covalent binding and intrinsic cytotoxicity not directly linked
- Reactive intermediates glutathion adducts (acceptable threshold levels ? 50 pmol/mg ?)- change the test without glutathion in excess and in hepatocytes

General Points to Consider

- Case-by-case approach is essential (practical and scientifically sound)
- Emphasis on major circulating human metabolites
- Human ADME should be performed early to define major circulating metabolites
- Only major human metabolites should be considered for monitoring in selected toxicology studies for exposure assessment
- Exclude monitoring conjugated metabolites unless reason to believe they may be chemically reactive (e.g. some acyl glucuronides)
- Exclude monitoring a metabolite that is major in animal but minor in human

General Points to Consider

- Take into account systemic exposure and intrinsic activity of metabolite.
- If a major metabolite has no known target or non-target pharmacological activity, its PK should be defined at least once in humans and tox species following a single dose of parent drug.
- Metabolites defined as major in humans should be considered for monitoring in selected toxicology studies (repeated dose man and animal ?) .
- A metabolite which is significant in human plasma, but very minor in all tox species should be considered for additional study to determine if it poses an unidentified risk to human safety.

General Points to Consider

- Carc studies on an administered metabolite are not recommended *unless there is evidence in separate metabolite toxicity studies that the metabolite caused novel lesions (i.e. different from parent) that might be expected to progress to neoplasia*.
- Make effort to establish that all major circulating human metabolites are present in plasma of at least one animal species used for reproductive and developmental toxicology assessment.