EBF current thinking on the conduct of whole blood stability

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Introduction

1. **EBF-survey on whole blood stability (WBS) showed that the landscape is divided.**
   - Differences in approaches, processes, timing and reporting of experiments between companies
   - Opportunity for recommendations
2. **The survey results were further discussed during an EBF closed meeting in June 2010, finally resulting in "EBF current thinking" presented here**
3. **Goal for today is to stimulate discussions on this topic and use the input to come up with final EBF recommendations in 2011**

EBF Survey (2009): Top Level Conclusions

1. **Regulatory guidance is interpreted ambiguously**
   - ~60% say required
   - ~40% say plasma acceptable surrogate or testing not required
2. **Testing done on routine basis in 50% of the labs, 40% do case-by-case, 10% never**
3. **Main rationale for <u>not</u> performing WBS experiments:**
   - Plasma acceptable surrogate or covered by other DMPK studies (e.g. Blood Cell Distribution study)
4. **Main rationale for performing WBS experiments**
   - Generate scientifically valid data to enable correct guidance on sampling conditions

EBF Survey (2009): Top Level Conclusions

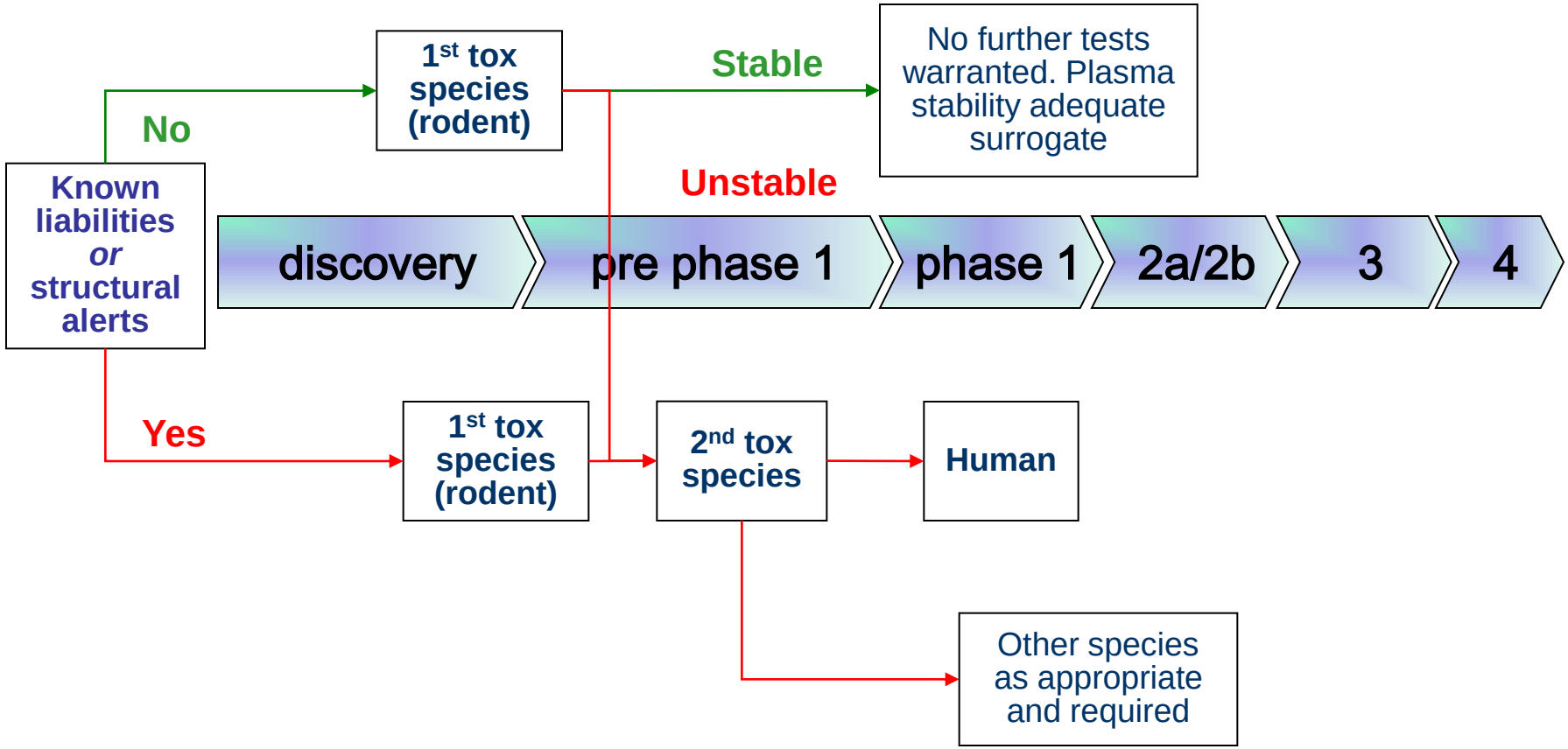
5. **~25% of EBF member companies have experience with blood/plasma stability mismatches, but incidence is low:**
   - Data show incidence in the range of 5% or less
6. **Anticipated reasons for mismatch:**
   - Enzyme activities higher in blood compared to plasma, especially true for esterases and monoamine oxidases
   - Reductive mechanisms involving haemoglobin in blood
7. **If WBS is investigated majority of member companies rely on spiked samples**
   - Fresh blood (≤ 24 hours) without modifiers (e.g. glucose, phosphate buffer) but anticoagulants
   - Two concentration levels, 3-6 replicates
   - Acceptance criteria: ±15% of reference analysis
8. **Limited number of companies also include incurred samples in addition to spiked samples.**
   - More diversity compared to spiked samples

EBF Current Thinking

1. **EBF works toward a recommendation to test blood stability in at least one species**

   - Evaluating only plasma stability may not be sufficient
     - Plasma stability is not always predictive for blood stability
     - Incidence of mismatches (<5% of compounds) is low, but not zero
   - Consider a parallel or a tiered approach
     - Parallel: E.g. main preclinical species and man on same day (could be more efficient)
     - Tiered: Only conduct blood stability in one species. Only extend to other main tox species and man if instability is observed

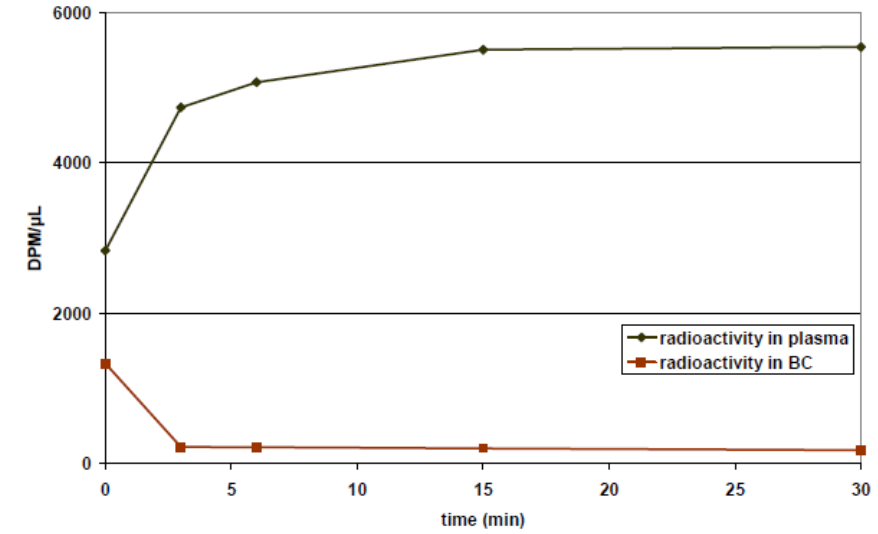
Proposal for Tiered Approach



EBF Current Thinking - cntd

2. Analyse whole blood, and not the plasma fraction

- Circumvent equilibration issue in spiked samples
  - Equilibration is usually not instantaneous
  - Solid estimation requires separate experiment



means of n=2

- Allow distinction between equilibration and instability

EBF Current Thinking - cntd

3. Use a qualified method for blood stability assessment

- Typically, plasma is the matrix of choice for bioanalytical purposes
- However, assay for analysing whole blood cannot be claimed validated due to matrix difference
- As a consequence, WBS data obtained by this procedure reflect qualified data based on relative concentration measurements

4. Reporting

- Blood stability data need to be compiled in a report, e.g. method validation report or other report that is part of the dossier
- Report should reflect the use of a qualified assay for obtaining blood stability data

EBF Current Thinking - cntd

5. Observed instability calls for further investigations

- Instability has a reason, and if the reason is clear then it is more easy to control and manage instability
- Extend tiered approach, include other species, time points, etc.

6. Incurred blood samples may be considered in special cases

- Why? *Metabolites with known liabilities, i.e. metabolites that may convert back to parent drug*
- When to do it? *If those metabolites are present in incurred samples at concentrations that back conversion might influence parent drug concentrations to relevant degree*
- More discussions needed on selection of samples and experimental procedures

Proposal for Experimental Conduct

- Equilibrate blood to 37°C
- Spike fresh blood at 2 concentration levels
  - i.e. ~5x LLOQ and ~75-% ULOQ at 37°C
- Process t=0 aliquots as described below
  - Incubate blood for desired time period and temperature
  - Processing of samples
    - Dilute 1+1 with water for improved handling
    - Precipitate blood aliquots by addition of e.g. acetonitrile containing internal standard
    - Analyse all samples together without calibration curve and QC samples

      Note: Alternatively freeze samples until analytics (next day)
- Compare peak area ratios analyte/IS of incubated samples and t=0
- Report results as % difference from t=0

EBF Current Thinking on WBS - summary

1. Test at least one species, preferably rodent
2. Analyse whole blood – not plasma
3. Recommended to use a qualified assay
4. Data available as part of filing documents
5. Further investigations required once instability is observed
6. Incurred samples might be considered in special cases

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