FOCUS KINETICS

Special Considerations for Metabolites

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Metabolite Kinetics

- More complex than for parent because formation and degradation occur simultaneously
  - Complexity increases with complexity of pathway
    - Number of successive degradation steps
    - Number of metabolites formed at each step
    - Number of precursors
  - Complexity increases with complexity of kinetic models
    - Formation
    - Degradation
Metabolite Curve

- Formation phase
- Maximum
- Decline phase

Time (days) vs Substance (% of applied)
Kinetic Endpoints for Metabolites

• Trigger endpoints
  – Degradation/dissipation $DT_{50}$, $DT_{90}$

• Modeling endpoints
  – Formation rate parameters
    • degradation rate parameters from precursor(s)
      +
    • formation fraction(s)
      +
  – Degradation rate parameters
Modeling of Metabolite Kinetics

• Rate of formation must be considered in addition to rate of degradation

• Formation and degradation are linked, and the parameters can be highly correlated

• Degradation of the precursor(s) must be described properly to be able to describe the degradation of the metabolite
Main Recommendations

• Pathway
  – Conceptual model must reflect actual degradation or dissipation pathway
  – Flows to sink are initially included for formation of other metabolites (identified or not), bound residues and CO₂

• Kinetic model for degradation of precursor(s)
  – SFO Vs. biphasic models
  – Appropriate description at least up to DT₉₀ is necessary
Main Recommendations

• Data weighting
  – Unweighted fit
  – First part of the precursor’s decline curve, covering formation phase of the metabolite is more important than later time points

• Kinetic model for degradation of metabolite
  – SFO Vs. biphasic models (FOMC, DFOP)

• Use stepwise approach
  – Parent first, add metabolites sequentially
Precursor Kinetics

Parent SFO

- DT$_{50}$ Parent: 21 d
- DT$_{90}$ Parent: 69 d
- DT$_{50}$ Metabolite: 8.5 d
- Formation fraction: 1

Parent FOMC

- DT$_{50}$ Parent: 16 d
- DT$_{90}$ Parent: 150 d
- DT$_{50}$ Metabolite: 14 d
- Formation fraction: 0.648
Weighting method

Unweighted fit

- Parent
- Metabolite 1
- Metabolite 2

Weighted fit (fractional)

- Parent
- Metabolite 1
- Metabolite 2

DT_{50} Parent: 12.7 d
DT_{50} Metabolite 1: 41.5 d
DT_{50} Metabolite 2: 133 d

DT_{50} Parent: 17.6 d
DT_{50} Metabolite 1: 47.3 d
DT_{50} Metabolite 2: 369 d
Pathway: including flow to sink

Parent ➔ Metabolite

Parent

Metabolite

Others

Substance (mg/kg)

Time (days)

0 25 50 75 100 125

DT_{50} Parent: 5.8 d
DT_{50} Metabolite: 16 d
Formation fraction: 1

DT_{50} Parent: 3.3 d
DT_{50} Metabolite: 38 d
Formation fraction: 0.466

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Metabolite degradation kinetics

**Metabolite SFO**
- DT$_{50}$ Parent: 0.94 d
- DT$_{50}$ Metabolite: 18.3 d
- DT$_{90}$ Metabolite: 60.9 d

**Metabolite FOMC**
- DT$_{50}$ Parent: 0.94 d
- DT$_{50}$ Metabolite: 15.3 d
- DT$_{90}$ Metabolite: 95.3 d
Implementation of the conceptual model in a kinetic model

• Combine parent kinetics (SFO, FOMC, DFOP or other model), metabolite formation fraction and metabolite kinetics (SFO, FOMC, DFOP or other)
  – Selected kinetic models must be consistent with intended use (trigger Vs. modeling)

• Integrated equations with analytical solution exist for simple cases
  or

• Use sets of differential equations in compartment models with software tool for solving
Compartment Models

Parent: $\frac{dP}{dt} = -FP$

Metabolite 1: $\frac{dM_1}{dt} = FP \cdot ffM_1 - FM_1$

Metabolite 2: $\frac{dM_2}{dt} = FP \cdot ffM_2 - FM_2$

Sink: $\frac{dS}{dt} = FP \cdot (1 - ffM_1 - ffM_2) + FM_1 + FM_2$
Stepwise approach

1) Fit parent substance
2) Add primary metabolite(s), fit with parent parameters fixed to values obtained in 1), check flow to sink and simplify if justified
3) Fit parent and primary metabolite(s) using values obtained in 1) and 2) as starting values
4) Add secondary metabolite(s), fit with parent and primary metabolite(s) parameters fixed to values obtained in 3), check flow to sink and simplify if justified

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n) Final step: fit all substances together using values obtained in n-1) as starting values
Flowsheet for deriving trigger endpoints for metabolites

1. Data entry

2. Run parent only SFO, FOMC

3. If SFO fit acceptable and statistically more appropriate than FOMC, then:
   - Run parent best-fit and metabolite
     - If FOMC and/or DFOP fit acceptable?
       - If yes, then case-by-case decision
       - If no, then run parent best-fit and metabolite FOMC
     - If SFO fit for metabolite acceptable?
       - If yes, then use estimated SFO trigger endpoints (DT50 and DT90 values)
       - If no, then case-by-case decision
     - Use estimated FOMC trigger endpoints (DT50 and DT90 values)

4. If no, then run parent only DFOP

For complex models, perform stepwise, adding metabolites according to proposed pathway.

Note: DT50 and DT90 refer to the half-life and 90% decay time, respectively.
Procedure for Deriving Trigger Endpoints

- Determine parent best-fit kinetic model (SFO, FOMC, DFOP);
  - Check goodness of fit ($\chi^2$ and residuals) and individual parameters (T-test)

- Add metabolites stepwise, using SFO, or if not appropriate, FOMC;
  - Check goodness of fit ($\chi^2$ and residuals) and individual parameters (T-test)

- Derive trigger DT values (best-fit)
Flowsheet for deriving modelling endpoints for metabolites

1. **RUN parent only SFO**
   - Parent SFO acceptable
     - **SFO fit acceptable?**
       - Yes: Use estimated SFO endpoints for fate modelling
       - No: Parent SFO non-acceptable
         - **RUN parent only with appropriate biphasic model**
           - Biphasic fit acceptable?
             - Yes: **RUN parent biphasic and metabolites all-SFO**
               - SFO fit for metabolites acceptable?
                 - Yes: Use estimated SFO endpoints for fate modelling
                 - No: Case-by-case decision (same as 1)
               - No: Case-by-case decision
             - No: Case-by-case decision
           - No: Case-by-case decision
         - No: **RUN parent and metabolites all-SFO**
           - SFO fit for metabolites acceptable?
             - Yes: Use estimated SFO endpoints for fate modelling
             - No: Case-by-case decision
1. Data entry
Procedure for Deriving Modeling Endpoints

• Determine if SFO appropriate for parent ($\chi^2$ and residuals);
  – If parent is biphasic, use higher-tier approach (e.g. PEARLneq, DFOP)

• Add metabolites stepwise, determine if SFO appropriate ($\chi^2$ and residuals);
  – Check individual parameters, may be set to conservative values if estimate not reliable

• Use modeling endpoints (degradation rates and formation fractions) from final fit
Conclusions

• Guidance provided for deriving metabolite kinetic endpoints from studies with parent
  – Trigger endpoints: degradation/dissipation $DT_{50}$ and $DT_{90}$
  – Modeling endpoints: formation and degradation rate

• Harmonized approach for reproducible results independent of software tool used
  – Better acceptance of generated endpoints
  – Facilitates review process