FOCUS KINETICS

Special Considerations for Metabolites

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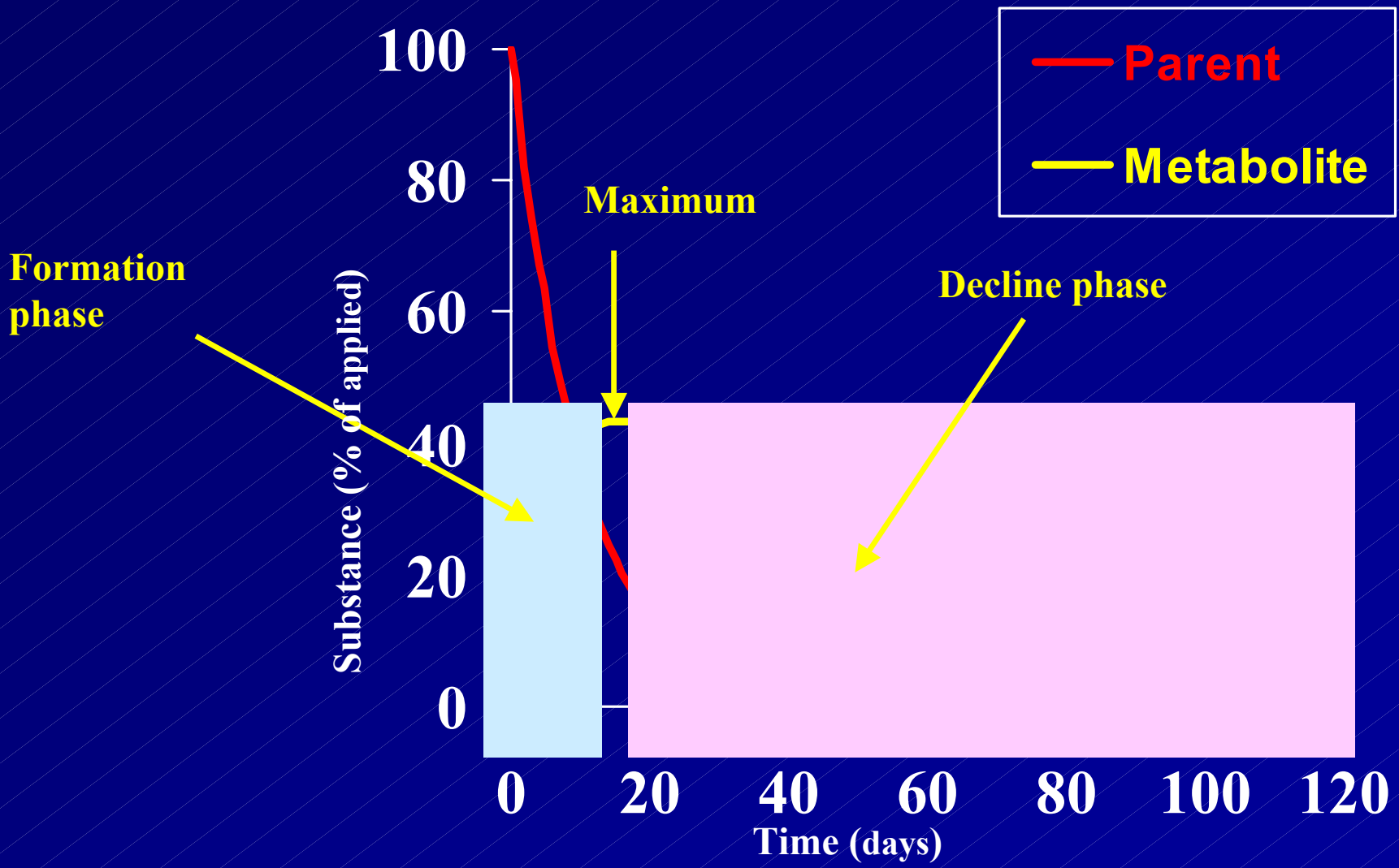
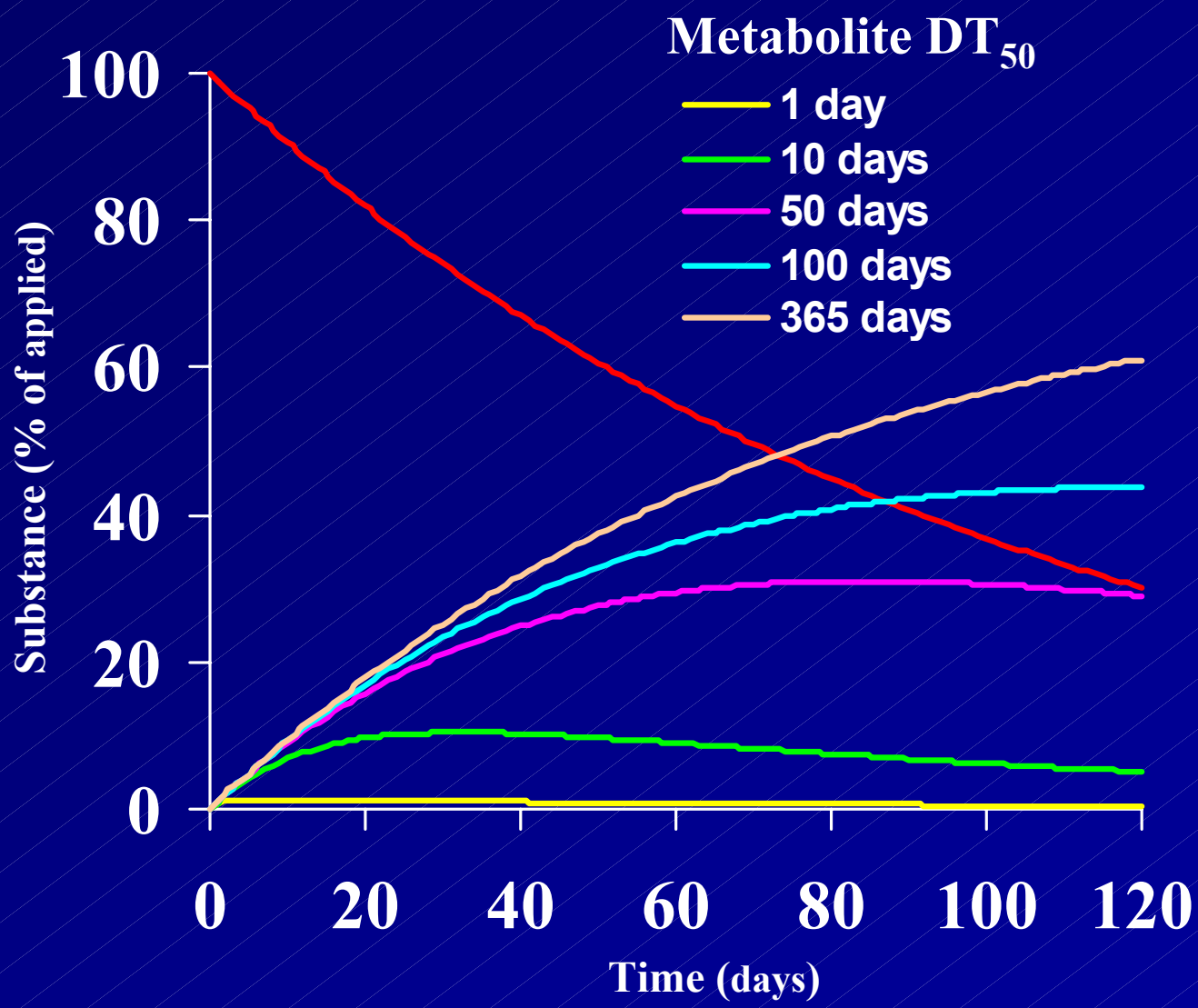
Metabolite Kinetics

- 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



Metabolite Curve



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

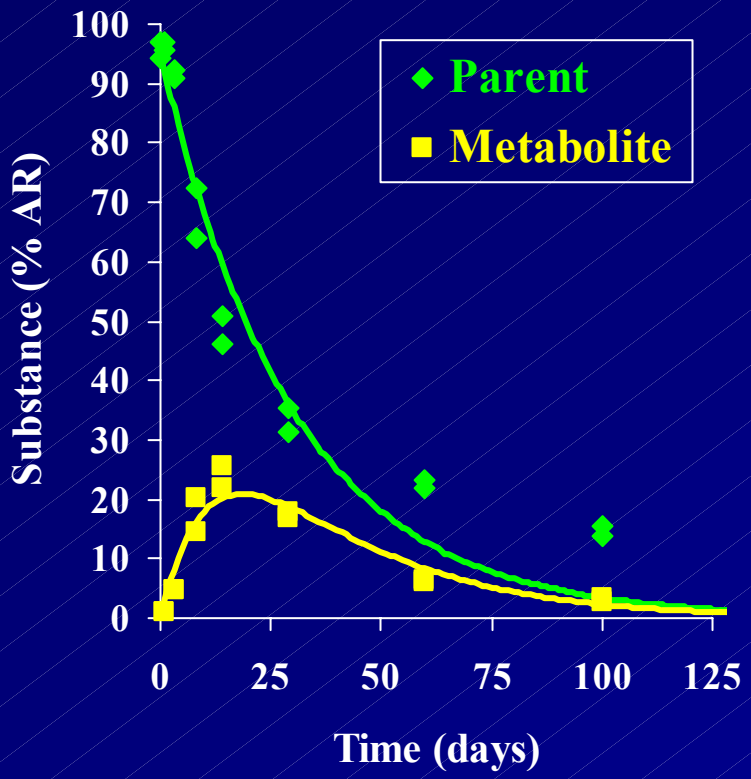
- Pathway
  - Conceptual model must reflect actual degradation or dissipation pathway
  - Flows to sink are initially included for formation of other metabolites (identified or not), bound residues and CO_2
- Kinetic model for degradation of precursor(s)
  - SFO Vs. biphasic models
  - Appropriate description at least up to DT_{90} 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



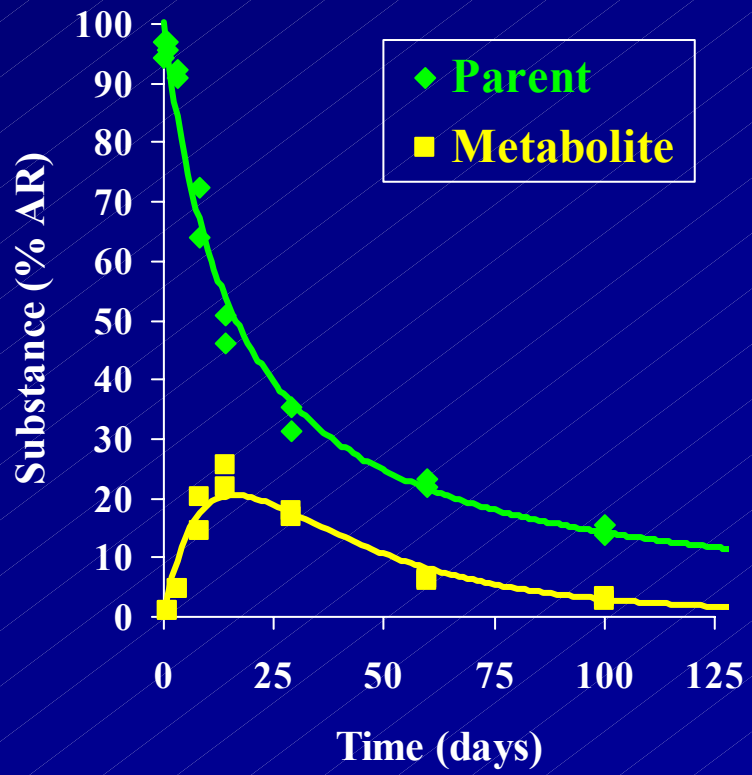
DT_{50} Parent: 21 d

DT_{90} Parent: 69 d

DT_{50} Metabolite: 8.5 d

Formation fraction: 1

Parent FOMC



DT_{50} Parent: 16 d

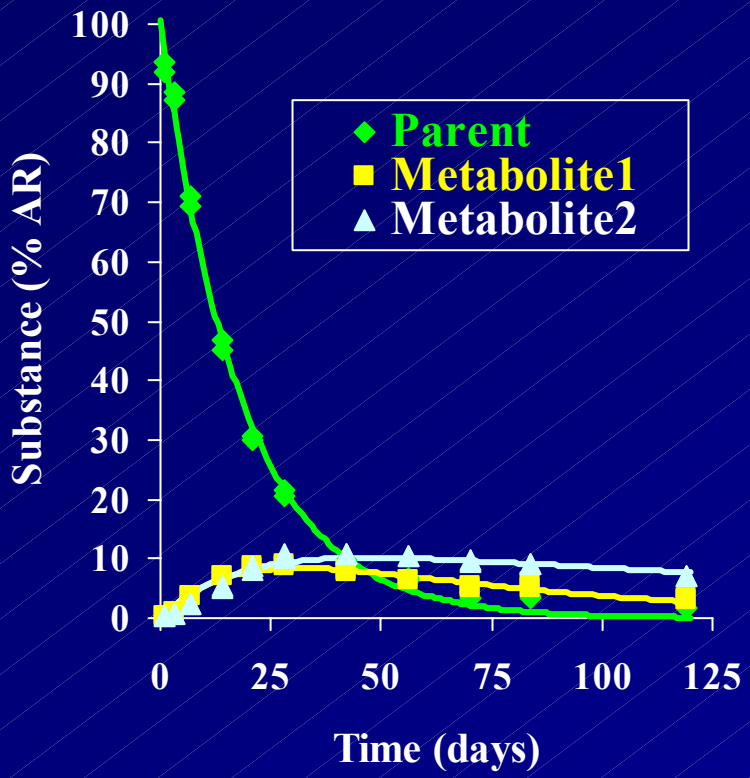
DT_{90} Parent: 150 d

DT_{50} Metabolite: 14 d

Formation fraction: 0.648

Weighting method

Unweighted fit

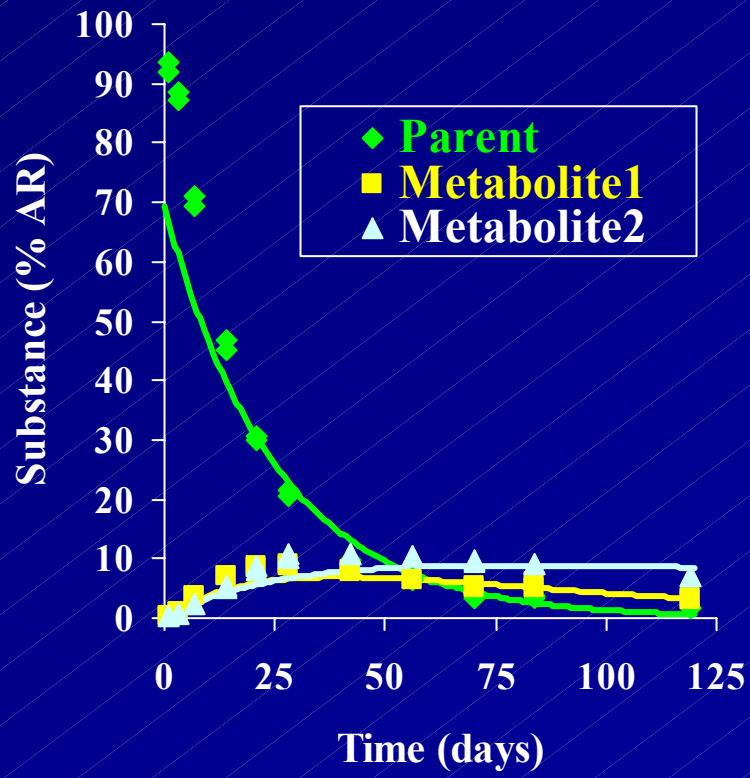


DT_{50} Parent: 12.7 d

DT_{50} Metabolite 1: 41.5 d

DT_{50} Metabolite 2: 133 d

Weighted fit (fractional)



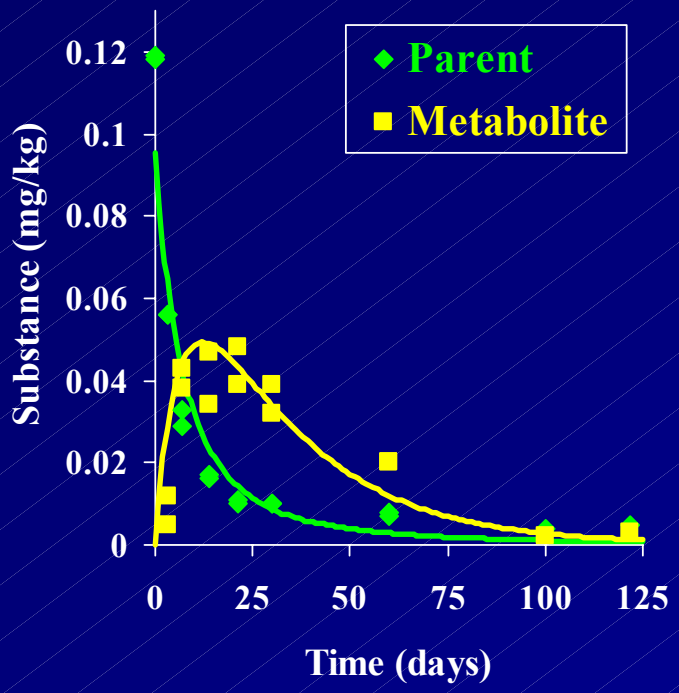
DT_{50} Parent: 17.6 d

DT_{50} Metabolite 1: 47.3 d

DT_{50} Metabolite 2: 369 d

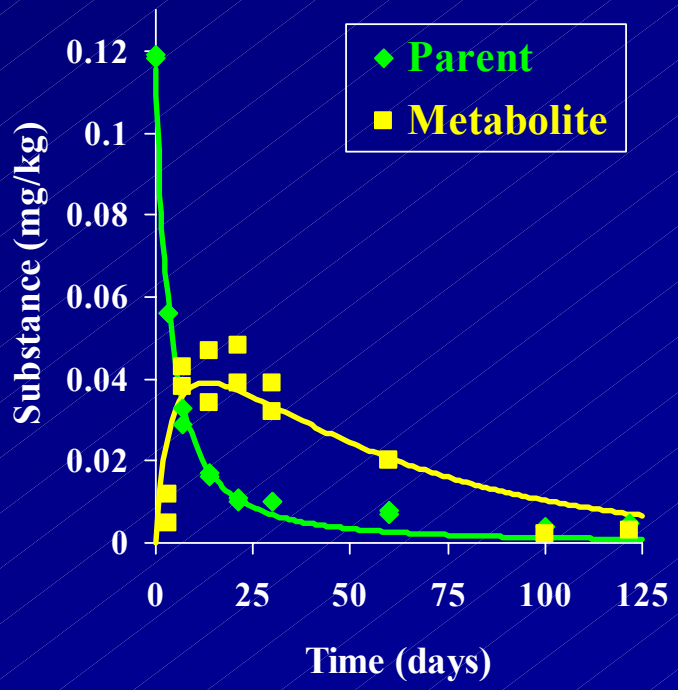
Pathway: including flow to sink

Parent → Metabolite



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

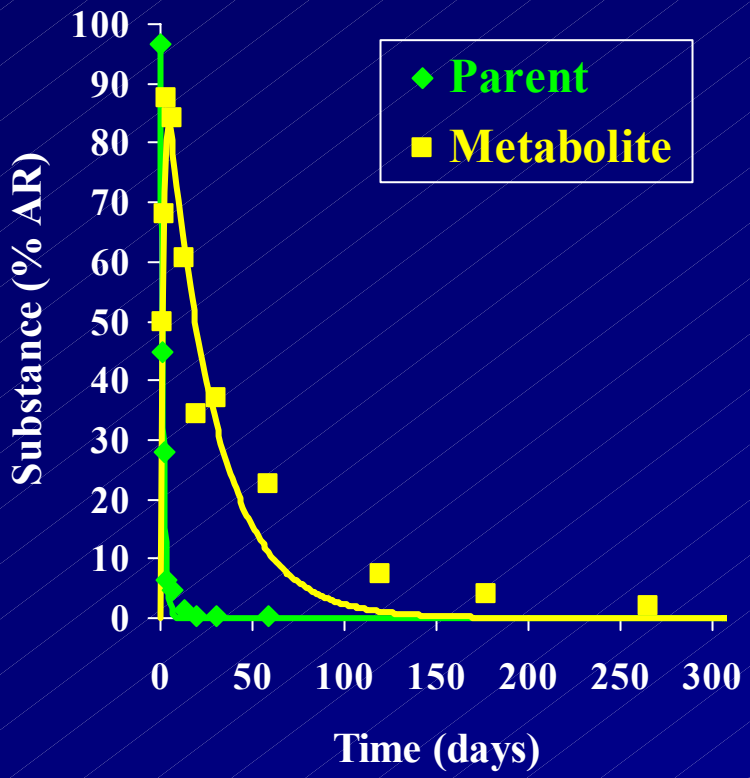
Parent → Metabolite
Parent → Others



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

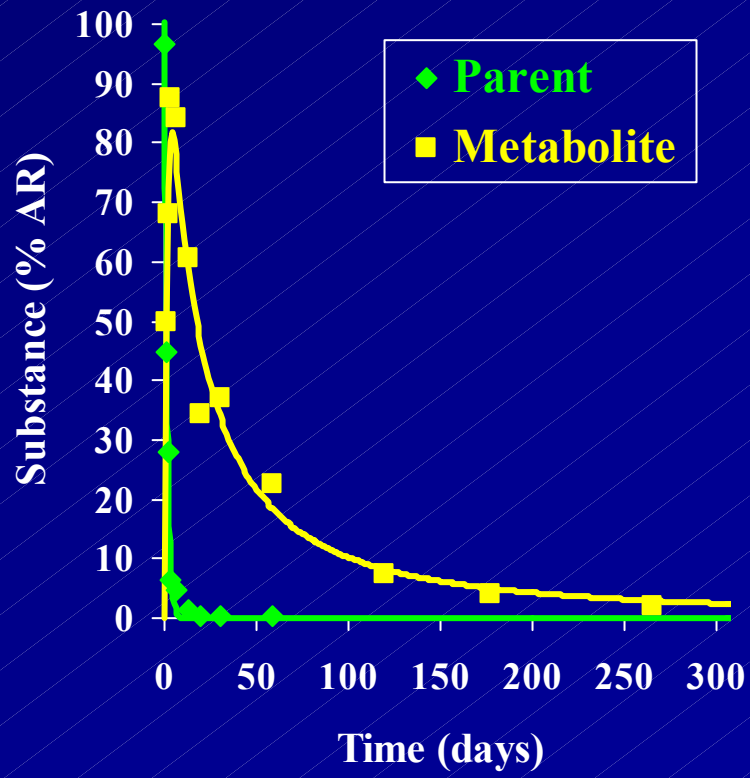
Metabolite degradation kinetics

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

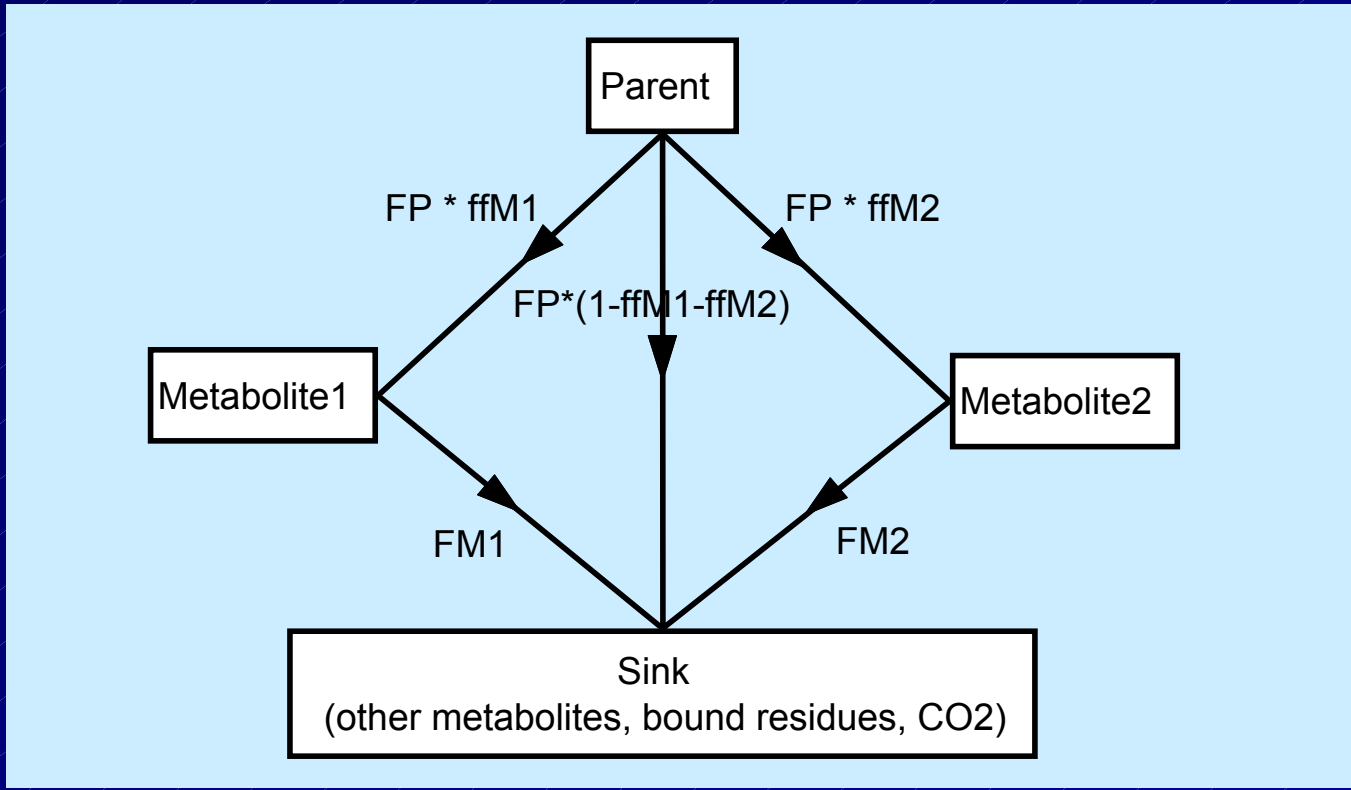
- Combine parent kinetics (SFO, FOMC, DFOP or other model), metabolite formation fraction and metabolite kinetics (SFO, FOMC, DFOP or other)
  - Selected kinetic models must be consistent with intended use (trigger Vs. modeling)

- 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: $dP/dt = -FP$

Metabolite 1: $dM1/dt = FP \cdot ffM1 - FM1$

Metabolite 2: $dM2/dt = FP \cdot ffM2 - FM2$

Sink: $dS/dt = FP \cdot (1 - ffM1 - ffM2) + FM1 + FM2$

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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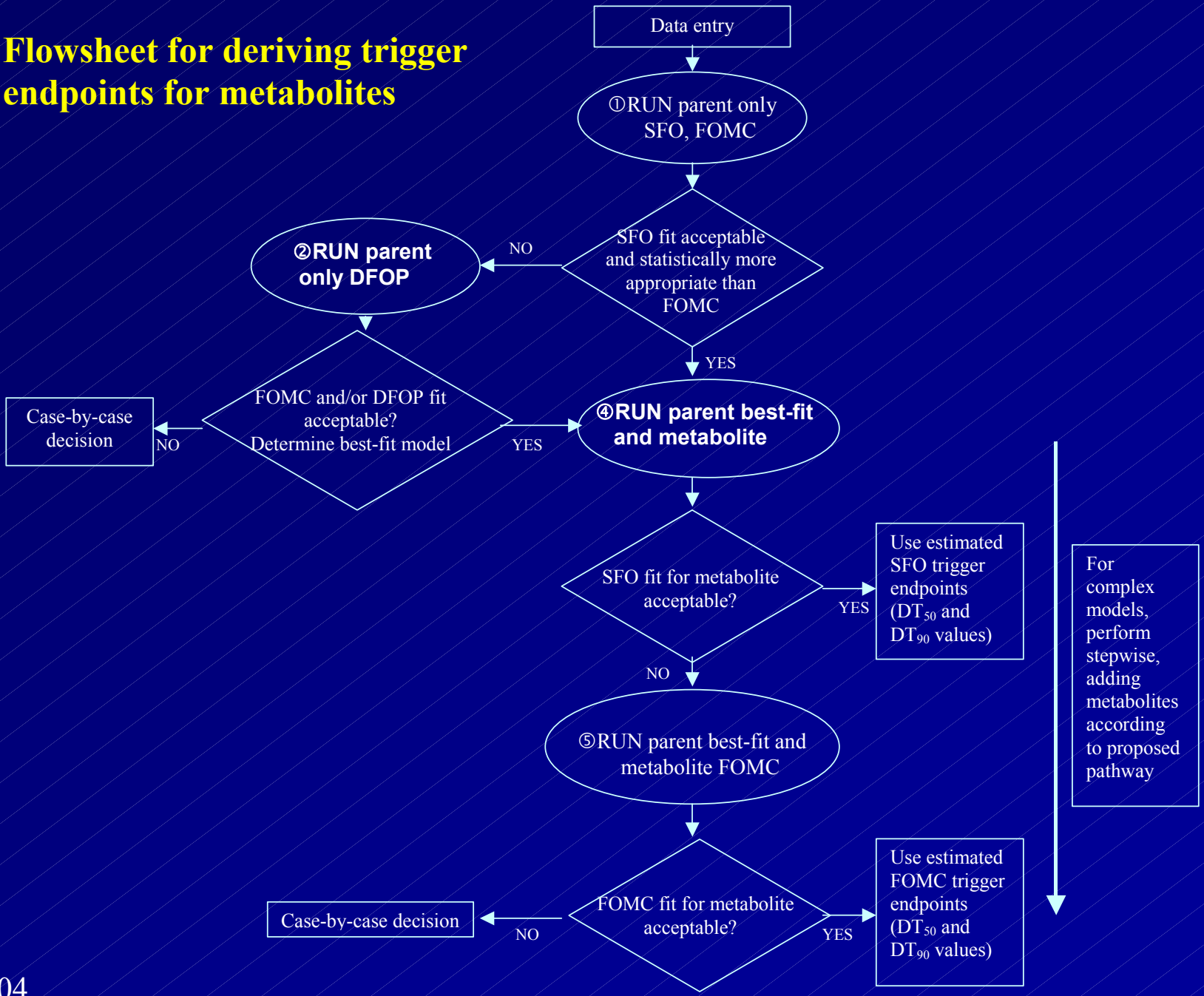
n) Final step: fit all substances together using values obtained in n-1) as starting values

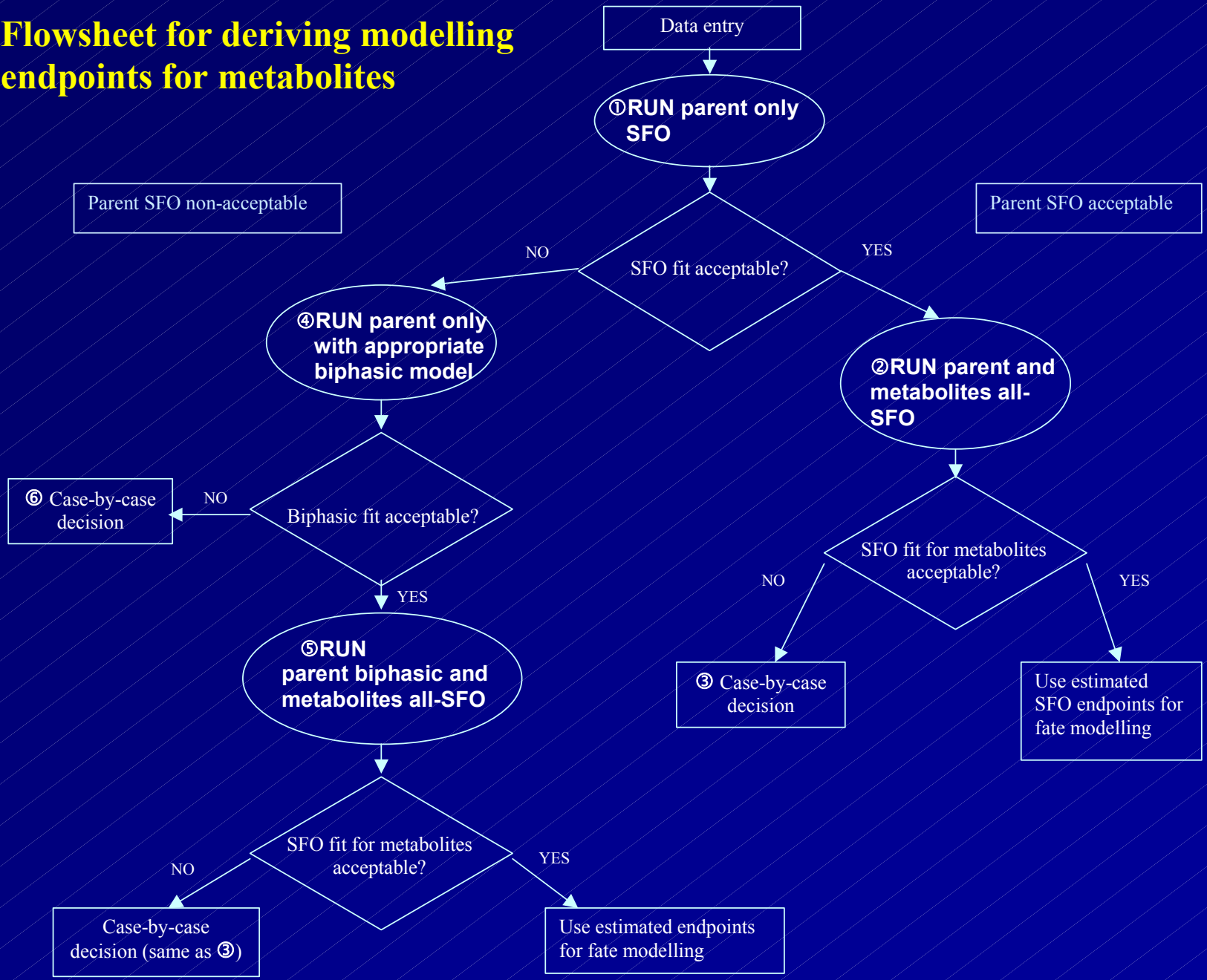
Flowsheet for deriving trigger endpoints for metabolites



Procedure for Deriving Trigger Endpoints

- Determine parent best-fit kinetic model (SFO, FOMC, DFOP);
  - Check goodness of fit (χ^2 and residuals) and individual parameters (T-test)
- Add metabolites stepwise, using SFO, or if not appropriate, FOMC;
  - Check goodness of fit (χ^2 and residuals) and individual parameters (T-test)
- Derive trigger DT values (best-fit)

Flowsheet for deriving modelling endpoints for metabolites



Procedure for Deriving Modeling Endpoints

- Determine if SFO appropriate for parent (χ^2 and residuals);
  - If parent is biphasic, use higher-tier approach (e.g. PEARLneq, DFOP)
- Add metabolites stepwise, determine if SFO appropriate (χ^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