Toxicity Testing of Impurities and Metabolites

Kurt A. Black, Ph.D, DABT

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Comparative Biology and Safety Sciences

Amgen Inc.

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Outline of Presentation

- Background
- Impurities
  - Drug substance impurities
  - Genotoxic impurities
  - Residual solvents
  - Drug product degradants
- Toxicity testing of drug metabolites
- Scope of presentation limited to small molecule pharmaceuticals

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Background – Impurities and Metabolites

- Primary focus of the toxicology program is to define the safety of the active ingredient
- However, safety aspects of impurities or metabolites can impact potential development and ultimately marketing
  - Viracept® (nelfinavir) removed from the market in Europe due to contamination by a genotoxic impurity
- Goal of this presentation will be to discuss points and strategies to consider during drug development

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Impurities

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Impurities – Background

- Occur in essentially all small molecule active pharmaceutical ingredients (API, drug substance) and drug products
- Provide no therapeutic benefit
- Potential to cause adverse effects
- Need to ensure that level of impurity is sufficiently safe to administer to humans

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Types of API Impurities

- Organic impurities
  - Starting materials and intermediates
  - By-products
  - Degradants
- Residual solvents
- Inorganic impurities
  - Metal catalysts
    - European Medicines Agency (EMEA) Committee for Human Medicinal Products (CHMP) guidance document
  - Heavy metals
    - Pharmaceutical compendia
      - United States and European Pharmacopeia

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Regulatory and Industry Guidance

- International Conference on Harmonisation (ICH) Q3A – impurities in new drug substances
- ICH Q3B – impurities in new drug products
- Genotoxic impurities
  - CHMP guideline on the limits of genotoxic impurities
  - Industry position paper
- ICH Q3C – Impurities: residual solvents

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Highlights of ICH Q3A (API Impurities) (1)

- Thresholds for reporting, identifying, and qualifying impurities
- Qualification of impurities
  - Process of acquiring and evaluating data that establishes the biological safety of an impurity
- Applies at filing of marketing application
- But common practice is to qualify impurities during clinical development
  - Safety of patients in clinical trials

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ICH Thresholds for Drug Substance (API)

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
< 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

[1]The amount of drug substance administered per day

[2]Higher reporting thresholds should be scientifically justified

[3]Lower thresholds can be appropriate if the impurity in unusually toxic

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Highlights of ICH Q3A (API Impurities) (2)

- Level adequately tested in preclinical safety studies would be considered qualified
- Impurities that are significant animal and/or human metabolites are generally considered to be qualified
- A level (%) of a previous impurity at a higher level than previously qualified can be justified based on actual dose administered in previous toxicology studies
  - Compare µg/kg dose of impurity in animals to clinical dose of impurity

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Qualification Studies Recommended in ICH Guidance

- One *in vivo* repeat dose toxicity study
  - Two weeks to 3 months in duration
  - Most appropriate species
- *In vitro* genetic toxicity studies
  - Ames test
  - Chromosome aberration study
- Conduct studies on the API containing a representative amount of the impurity
- Studies using the isolated impurity can also be considered
- Studies must be compliant with Good Laboratory Practices

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Strategies for *In Vivo* Qualification

- Use new lot of API with level to be qualified on ongoing or planned toxicity studies
  - Most efficient approach since incorporates qualification into ongoing program
  - Timeline and compound supply considerations
- Conduct dedicated repeat-dose qualification study in one species
  - May be necessary to minimize delays to clinical program
  - Preferred approach if want to qualify high levels by using API spiked with impurity

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In Vivo Qualification Decision Scheme

- Known impurity present in previous GLP toxicity studies
  - If level (%) higher than in previous batches used in toxicity studies
    - Qualify *in vivo* if
      - Level exceeds ICH qualification limit

      AND

      - Projected clinical dose (µg/kg) of impurity exceeds dose of impurity at NOAEL in previous toxicity studies
- New impurities
  - Qualify *in vivo* if level exceeds ICH threshold

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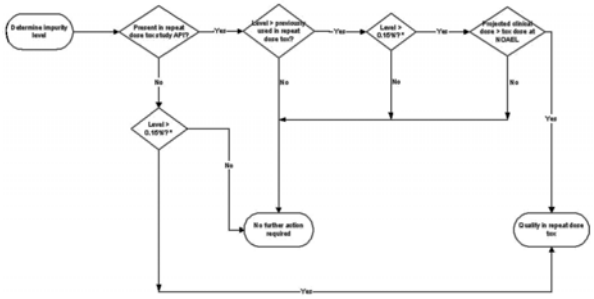
Decision Scheme for *In Vivo* Qualification of API Impurities



* If the impurity level exceeds 1 mg total daily intake then the threshold is also exceeded; For daily dose > 2g, the qualification threshold is 0.05%

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Alternatives to Impurity Qualification

- Reprocess API to reduce impurity to acceptable level
  - Requires additional resources and time
- Limit clinical dose to control exposure to impurity
  - May limit ability to demonstrate efficacy or explore safety and tolerability

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Genotoxic Impurities

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Genotoxic Impurities – Background

- Many starting materials and intermediates used in pharmaceutical synthesis are genotoxic
  - In many cases, not feasible to avoid their use
  - May be present in API as impurities
- Increasing concern about risk posed by exposure to genotoxic impurities

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Regulatory Guidance on Genotoxic Impurities

- ICH Q3A
  - Qualification threshold requiring genotoxicity testing is 0.15% for most drugs
  - Lower thresholds may be appropriate for “unusually toxic” impurities
  - Concerns about risk of exposure to genotoxic impurities present at levels less than qualification limit
- CHMP Guideline on The Limits of Genotoxic Impurities (2006)

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CHMP Guidance on the Limits of Genotoxic Impurities (1)

- Concept of Threshold of Toxicological Concern (TTC)
- TTC = maximal lifetime daily intake of a genotoxic impurity at which acceptable increased risk for cancer exists
  - For pharmaceuticals = 1 x 10^{-5}
  - TTC = 1.5 µg/day
- A compound-specific limit should be used if sufficient data exist
  - Rodent carcinogenicity results
- Applies at marketing application approval or change to an existing approved application

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EMEA Guidance on the Limits of Genotoxic Impurities (2)

- Higher levels acceptable for certain conditions
  - Short-term exposure – not defined in original guidance
  - Life-threatening conditions
  - Life-expectancy < 5 years
  - Human exposure much greater from other sources

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Industry Position Paper on Genotoxic Impurities

- Multi-disciplinary pharmaceutical industry group formed to address issue of genotoxic impurities
  - Müller *et al*., *Regul Toxicol Pharmacol* **44**: 198-211, 2006
- Introduced concept of staged TTC
  - TTC dependent upon duration of exposure
    - Higher limit for shorter duration
  - Apply during all phases of development
- Provided classification scheme and decision tree based on genotoxic potential of impurities

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Classification of Impurities
(Müller *et al*.)

- Class 1: known genotoxic carcinogens
  - Calculate compound-specific limit using cancer risk assessment models and adjust for duration of exposure
- Class 2: known to be genotoxic but with unknown carcinogenic potential
  - Use generic staged TTC
- Class 3: alerting structure, unrelated to parent drug and of unknown genotoxic potential
  - Use generic staged TTC or run Ames test to confirm
- Class 4: alerting structure, related to parent API
  - Assume results for parent extrapolate to impurity
- Class 5: No alerting structure or indication of genotoxic potential
  - Treat as routine impurity
  - Follow ICH guidance to determine qualification need

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CHMP Question and Answer Document (2008)

- Clarified 2006 guidance document
- Endorsed concept of staged TTC during clinical development

	Duration of Exposure					
	Single Dose	>Single dose to ≤1 month	>1 month to ≤3 months	>3 months to ≤6 months	>6 months to ≤12 months	≥12 months or at marketing
Staged TTC	120 µg	60 µg	20 µg	10 µg	5 µg	1.5 µg

- Values are generally lower than those recommended by Müller *et al.*

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Examples – Limits Based on Genotoxic Potential

- Mesityl oxide
  - Not genotoxic
  - Treat as routine impurity according to ICH guidance
- 4-Aminophenol
  - Genotoxic with unknown carcinogenicity
  - Use staged TTC
- Hydrazine
  - Genotoxic rodent carcinogen
  - Calculate compound specific limit staged for duration
  - USEPA IRIS database provides limits for environmental exposure in drinking water
  - Convert to µg/day and stage for duration of exposure

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Assessment of Genotoxic Potential of Impurities

- If structure identified
  - Literature review
  - Expert, *e.g.*, chemist, review for structural alerts
  - *In silico* structure-activity relationship prediction of mutagenicity
    - Multicase, DEREK
  - Potentially conduct genotoxicity testing
- If structure unknown
  - Assume that impurity is potentially genotoxic
  - Potentially test API with impurity level

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Decision Scheme for Genotoxicity Qualification Studies

- If level > than ICH qualification limit
  - Ames test and *in vitro* chromosome aberrations study
- If level ≤ ICH qualification limit
  - Structure known and contains alert
    - Conduct Ames test
  - Structure known and contains no alert
    - No further testing
  - Structure unknown and at level > ICH identification limit
    - Conduct Ames test
  - *In vitro* chromosome aberration study if structural class is associated with clastogenesis or poorly detected in Ames test
- Limiting clinical exposure to ≤ staged TTC is an alternative to conducting qualification studies

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Compound to Use in Genotoxicity Qualification Studies

- API with impurity level
  - May result in specification for impurity set at level present as tested
- API spiked with impurity
  - Need to test impurity at level ≥ 250 µg in Ames test to achieve sufficient sensitivity
    - Kenyon *et al*., 2007
    - 250 µg = 5% at limit dose of 5000 µg in Ames test
  - Risk of repeating previous negative test on API
- Neat impurity
  - Time and resources to synthesize
  - Will have its own impurity profile

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Residual Solvents

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Residual Solvents

- Definition
  - Volatile organic chemicals used in the manufacture of drug substances, products and excipients
  - Not completely removed by manufacturing process
- Necessary component in many pharmaceutical manufacturing processes
- Provide no therapeutic benefit

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ICH Guidance Q3C – Residual Solvents

- Classifies residual solvents according to safety characteristics
- Provides acceptable upper limits for levels of residual solvents
- Guidance document states that it does not apply to clinical development stage
- But industry practice and regulatory agency expectations are that the limits are generally met during this stage

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ICH Q3C Residual Solvents Classes (1)

- Class 1
  - Solvents to be avoided
  - Known or strongly suspected human carcinogens or possess other unacceptable toxicity
    - Benzene
  - Environmental hazards
    - 1,1,1-Trichloroethane
  - Limits are generally at the very low ppm level
- Class 2
  - Solvents to be limited
  - Nongenotoxic rodent carcinogens, teratogens, neurotoxins, other significant toxicities
  - Acetonitrile, ethylene glycol

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ICH Q3C Residual Solvents Classes (2)

- Class 3
  - Solvents with low toxic potential
  - Limits set at 0.5% (5000 ppm)
  - Pragmatic limit based on common manufacturing capabilities
  - Acetone, ethanol
- Solvents with inadequate toxicological data
  - Manufacturer must justify residual levels
  - Isopropyl ether, methylisopropyl ketone

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Limits for ICH Class 2 Solvents (1)

- Permissible Daily Exposure (PDE)
  - Daily intake of a residual solvent in a pharmaceutical product that is considered acceptable (mg/day)
  - Based on results from toxicology studies
- Guidance lists acceptable concentrations at or below which no justification for levels are required
  - Based on PDE and assumed dose of pharmaceutical of 10 g/day
  - Hexane PDE = 2.9 mg/day
  - Hexane concentration limit = 290 ppm

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Limits for ICH Class 2 Solvents (2)

- May be able to justify higher concentrations if daily dose of drug is less than 10 g/day and PDE (mg/day) for solvent not exceeded
  - Could be particularly useful approach early in drug development process
  - May not be universally accepted by all regulatory agencies
  - Efforts should be made to lower levels as much as practical
  - Ultimate goal should be to meet concentration limits

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Drug Product Degradants

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Drug Product Degradants

- Drug product is the formulated dosage form containing the API and excipients
- Drug product impurities
  - Degradants of API
  - Reaction products of API and excipients
  - Reaction products of API and container closure system
- ICH limits for impurities in drug product are higher than those for API (See ICH Q3B)
- Residual solvents
  - Source may be from API, excipients or both
- Difficult to reduce levels in already formulated drug product

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Impurities Summary

- Impurities occur routinely in API
- Provide no benefit
- Qualification is the process of establishing the safety of impurities
- Genotoxic impurities need special consideration
- Reducing an impurity to an acceptable level or limiting its exposure to humans are alternatives to qualification

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Toxicity Testing of Metabolites

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Metabolites – Outline

- Background
- Metabolite considerations during drug candidate selection
- Safety assessment of disproportionate human metabolites
- Case studies
- Summary

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Metabolites – Background

- Drug metabolites are compounds that are formed by enzymatic changes to the drug molecule
- Metabolites of drugs are commonly formed by human and animal systems
- Metabolites may contribute to the pharmacology and/or toxicity of the parent drug
- Important to assess the safety of metabolites during drug development

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Metabolites – Definitions (1)

- Phase 1 metabolite
  - Metabolite formed by direct change to the parent drug molecule
  - Oxidation, reduction, hydrolysis, cleavage
  - Usually catalyzed by various CYP enzymes
- Phase 2 metabolite
  - Metabolite formed by addition of endogenous substance to parent drug molecule or Phase 1 metabolite
  - Drug conjugates
    - Glucuronides, sulfates, *etc*
  - Catalyzed by various transferases

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Metabolites – Definitions (2)

- Active metabolite
  - Metabolite that shares primary pharmacologic action of parent drug
- Reactive metabolite
  - Metabolite that may covalently modify cellular macromolecules
- Unique metabolite
  - Drug metabolite that is formed by only a single species
- Disproportionate human metabolite (FDA 2008)
  - Unique human metabolite
  - Or occurs at higher plasma concentrations in humans than in animals

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Toxicity Assessment of Metabolites

- Truly unique human metabolites are rare
- Toxicity studies on the parent drug are usually sufficient to assess the toxicity of metabolites
- Drug conjugates are usually considered to be non-toxic and separate evaluation is not justified
  - Exception: reactive acyl glucuronides
- Toxicity of some metabolites may need to be assessed separately from parent drug
  - Disproportionate human metabolites
  - Metabolites containing structural alerts for genotoxicity

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Metabolite Considerations During Drug Candidate Selection

- During lead optimization, metabolism of drug molecule in various species is compared
  - Liver microsomes, slices, hepatocytes
  - Limited *in vivo* characterization in animals
- Each human metabolite should be formed in at least one of the animal species selected for the toxicology assessment
  - Presence in only non-rodent species has implications for genotoxicity and reproductive toxicity assessments
- Covalent protein binding assays to assess potential for formation of reactive metabolites

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Toxicity Assessment of Metabolites – Industry Position Paper

- Defined issues and recommended approaches to safety assessment of metabolites during different phases of drug development
- Defined major human metabolite
  - Occurs at >25% of circulating drug-related material after single dose
  - Focus of safety assessment

Baillie TA *et al., Toxicol Appl Pharmacol* **182**: 188-196, 2002

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Metabolites – Regulatory Guidance

- US FDA Guidance on Safety Testing of Drug Metabolites (2008)
  - Provided recommended approaches to assessing safety of metabolites
  - Defined disproportionate human metabolite
    - Occurs only in humans
    - Or present in humans at higher plasma concentration than in animals used in toxicology program

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FDA Guidance – General Considerations

- If level of human metabolite occurs in toxicology species at equivalent or higher levels, studies on parent drug considered sufficient
- Drug conjugates generally do not need further assessment
- Disproportionate metabolites occurring at ≤10% of parent drug exposure at steady state do not need further testing
- If separate testing of metabolite needed, should be completed before large scale clinical trials
- Toxicity studies should be conducted by the same route as for parent drug

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FDA Guidance – Recommended General and Reproductive Toxicity Studies

- General toxicity
  - Duration should follow ICH M3 guidance
  - Systemic exposure at least comparable to that in humans
  - One or two species?
- Reproductive toxicity studies
  - Embryo-fetal development toxicity study
  - Fertility and peri-postnatal toxicity studies on a case by case basis

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FDA Guidance – Recommended Genotoxicity and Carcinogenicity Studies

- Genotoxicity
  - Ames test
  - *In vitro* chromosomal aberrations test
  - If either test positive or equivocal, complete the standard battery
- Carcinogenicity
  - Drugs to be administered ≥6 months
  - Single species

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Considerations for Direct Toxicity Testing of Metabolites

- May be difficult to synthesize metabolite
- Metabolite may not be stable when administered
  - GI tract
- Metabolite may have different pharmacokinetics when administered directly than when formed endogenously
  - Toxicity profile could be different
  - May not be relevant to safety assessment of drug

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Toxicity Assessment of Active Human Metabolites

- Generally, studies on parent drug sufficient to assess safety of active human metabolites
  - If disproportionate, then previous considerations would apply
- If expected to contribute significantly to pharmacologic effect in humans
  - Consider monitoring both parent drug and active metabolite in toxicity studies

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Case Studies – Metabolites

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Metabolite Case Study 1
Acyl Glucuronide

- Phase 1 project
- Acyl glucuronide was major metabolite in human plasma
- Much lower levels in rat plasma, but large amounts formed and excreted in bile
- Levels in monkey plasma approximated human levels
- Conclusion
  - Toxicity studies on parent drug considered sufficient to assess safety of metabolite

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Metabolite Case Study 2 - Major, Active Metabolite

- Phase 1 project
- M1 identified as a major, active metabolite in clinical study
- Not measured in initial GLP toxicity studies
- Subsequently assayed stored, frozen plasma samples from toxicology studies for M1
  - Exposure to M1 higher in animals than in humans
- Conclusions
  - Toxicity studies on parent drug sufficient to assess metabolite
  - Include toxicokinetics of M1 on future toxicity studies

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Metabolite Case Study 3 - Metabolite with Structural Alert (1)

- Lead optimization phase
- Metabolite with structural alert for mutagenicity identified in human and animal systems *in vitro*
- Metabolite circulated in animals at levels higher than parent
- Genotoxicity assessment
  - Metabolite not made by rat S9
    - Ames test on parent did not test mutagenic potential of metabolite
  - Conducted Ames test on metabolite
    - Results = negative
  - Conducted rat bone marrow micronucleus on parent and measured metabolite in plasma
    - Result = negative with high circulating levels of metabolite

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Metabolite Case Study 3 - Metabolite with Structural Alert (2)

- Exposures to metabolite were high in preliminary toxicity studies in rats and monkeys
  - Exposure ~1x to 20x compared to that of parent
- Conclusion
  - Metabolite did not pose a genotoxic risk
  - Toxicology studies on parent drug sufficient to assess safety of metabolite
  - Compound suitable for development
  - Measure plasma metabolite concentrations in toxicology studies

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Toxicology Testing of Metabolites – Summary

- In most cases, toxicity studies of parent drug sufficiently assess safety of metabolites
- It is usually not necessary to quantify exposure to metabolites on toxicity studies of parent
- Safety assessment of disproportionate or active metabolites needs to be addressed on a case by case basis
  - May need to quantify exposure to metabolite in toxicity studies
  - May need to conduct separate studies on metabolite
    - Seek regulatory agency concurrence on strategy

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Summary – Impurities and Metabolites

- Impurities and metabolites of small molecule drugs occur commonly
- It is important to assess the safety of impurities and metabolites during drug development
- Regulatory agency guidances and industry position papers can be used as a framework to design toxicity testing strategies
- Each issue related to impurities and metabolites should be addressed on a case by case basis

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