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Assessment of Genotoxic Impurities in Small Molecule Drug Candidates

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Outline – Genotoxic Impurities (GTIs)

- Background and regulatory framework
- Assessment of impurities for genotoxic potential
- Setting limits for GTIs
- Examples and case study
- Summary

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Background – Small Molecule Impurities

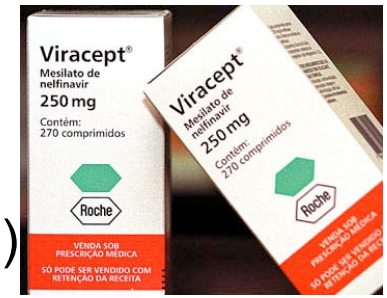
- Impurities occur in essentially all small molecule drug substances (DS, API) and drug products (DP)
- Impurities have the potential to cause adverse effects
- Impurities provide no benefit to patients
- Starting materials and intermediates are common impurities
  - Reactive by nature
    - May be genotoxic
    - Not feasible to completely avoid use
- Need to ensure that level of impurity is sufficiently safe to administer to humans

Why Are We Now More Focused on Genotoxic Impurities?

- Increased regulatory expectations
  - January 2007
    - EMEA CHMP Guideline on the Limits of Genotoxic Impurities
  - February 2008
    - EMEA letter requesting evaluation of sulfonic esters in all marketed products
  - June 2008 and December 2009
    - EMEA Questions & Answers Documents on the CHMP Guideline on the Limits of Genotoxic Impurities
  - December 2008
    - FDA Draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products; Recommended Approaches
- Increased attention from industry
  - Position paper: Müller L et al., *Regul Toxicol Pharmacol 44: 198-211,2006*

Why Are We Now More Focused on Genotoxic Impurities?

- Viracept® (nelfinavir mesylate)
  - HIV protease inhibitor marketed by Roche in EU
  - EMEA suspended marketing in June, 2007
  - Contamination with ethyl methanesulfonate (EMS)
  - Classic genotoxin
  - Formed from reaction between residual ethanol and methyl sulfonic acid counter ion



$$CH_3SO_3H + C_2H_5OH \rightarrow CH_3SO_3C_2H_5$$

  - EU marketing authorization reinstated in October, 2007
    - Contamination problem resolved
    - Roche was requested to conduct toxicity studies to better assess risk to exposed patients

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ICH Impurities Guidance Documents

- ICH Q3A(R2) and ICH Q3B(R2)
  - Impurities in New Drug Substances/Products
  - Limits for reporting, identification and qualification
    - Qualification
      - Process of acquiring and evaluating data that establishes the biological safety of an impurity, eg., toxicology studies
  - "Lower thresholds may be appropriate for unusually toxic impurities"
    - Genotoxins
- ICH Q3C(R4)
  - Residual Solvents
  - Permissible Daily Exposure (PDE)
    - NOAEL/LOAEL + safety factors + interspecies scaling

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ICH Q3A Thresholds for Drug Substance Impurities

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 is unusually toxic

GTI guidances often result in control to levels well below ICH limits

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EMEA Guidance on The Limits of Genotoxic Impurities

- Limit genotoxic impurities in DS and DP must be to levels associated with negligible risk
- Threshold of Toxicological Concern (TTC)
  - Maximal daily intake of a genotoxic impurity at which negligible increased risk for cancer exists
  - Generic limit based on database of several hundred genotoxic rodent carcinogens
  - Use for genotoxic impurities with unknown carcinogenicity
  - At marketing
    - TTC = 1.5 µg/day
    - For pharmaceuticals, risk factor = 1×10^{-5}
  - Clinical development
    - Staged limits based on duration of treatment
    - Risk factor of 1×10^{-6} and additional safety factor of "2"
- A compound-specific limit should be used if sufficient data exist
  - Eg, rodent carcinogenicity data or demonstrated threshold

Also see Müller et al., 2006.

Staged TTC – EMEA Guidance

	Duration of Clinical 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 (_g/day)	120	60	20	10	5	1.5

- Higher levels acceptable for certain conditions
  - Life-threatening conditions
  - Life expectancy < 5 years
  - Human exposure much greater from other sources
- High potency compounds need to be limited to below generic TTC
  - Aflatoxin-like, N-nitroso-, azoxy-compounds
  - Compound-specific limit required

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FDA Draft Guidance on GTIs

- Released for comment in December 2008
- Generally aligned with EMEA guidance
  - Exception
    - Level of 120 µg/day acceptable for less than 14 days rather than just for single dose
- Likely to be withdrawn if GTIs accepted as new ICH topic

Relationship Between Staged TTC, Drug Dose and Impurity Concentration Limit

Duration of exposure	Allowable daily intake[2]	Impurity Concentration Limit (ppm) based on drug daily dose[1]								
		1 mg	5 mg	10 mg	50 mg	0.1 g	0.2 g	0.5 g	1.0 g	2.0 g
>12 months	1.5 µg/day	1500	300	150	30	15	7.5	3	1.5	0.75
≤ 12 months	5 µg/day	5000	1000	500	100	50	25	10	5	2.5
≤ 6 months	10 µg/day	5000	2000	1000	200	100	50	20	10	5
≤ 3 months	20 µg/day	5000	4000	2000	400	200	100	40	20	10
≤ 1 month	60 µg/day	5000	5000	5000	1200	600	300	120	60	30
Single Dose	120 µg/day	5000	5000	5000	2400	1200	600	240	120	60

Many values are below ICH qualification limits (1500 ppm)

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Setting Compound-specific Limits

- Genotoxic, rodent carcinogens
  - Compound-specific limit using cancer risk assessment models
    - Cancer slope factors
      - Linearized multistage model of cancer risk assessment based on rodent carcinogenicity data
    - Regulatory agency limits
      - USEPA, CalEPA
    - Cancer potency database (U of CA-Berkeley)
  - Adjust for appropriate risk factor and duration of treatment
  - May be lower or higher than generic TTC
  - See detailed description of approach in Müller et al., 2006

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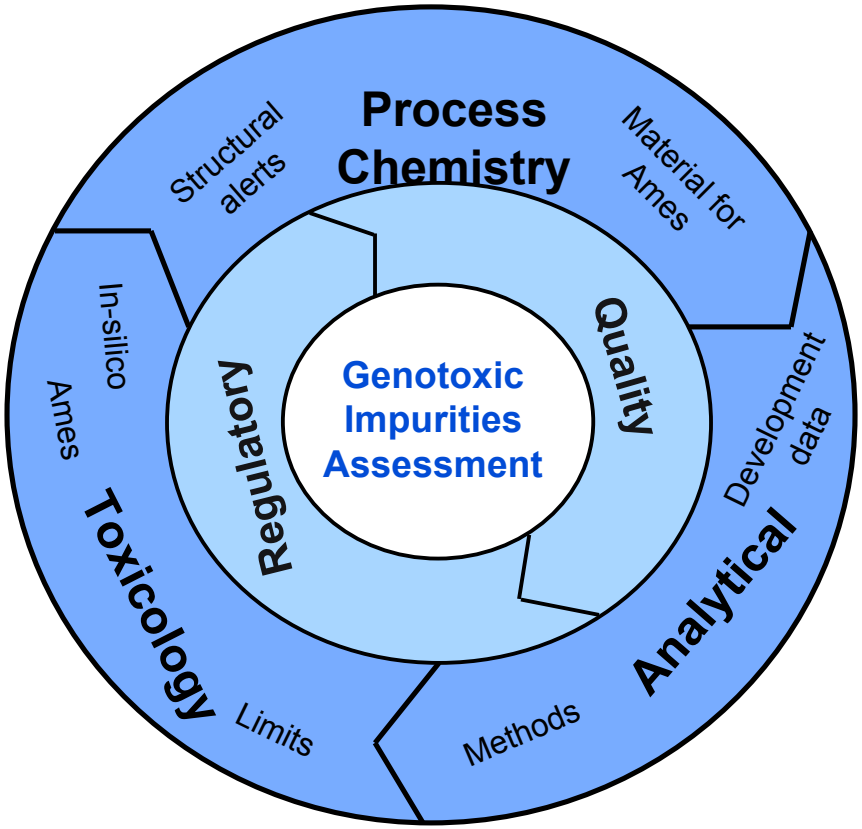
What About Life-threatening Conditions? Do Same Limits Apply?

- Oncology indications as defined in ICH S9, Step 4
  - Advanced, life-threatening disease
  - Studies in patients
  - Genetox on API not needed until registration
  - Genotoxic Impurities
    - Limits based on normal lifetime exposure are not appropriate
    - Higher limits could be justified
  - Minimize delayed access to or withdrawal of potentially effective treatments
- Other oncology indications
  - Long life-expectancy, maintenance therapy, supportive care, healthy volunteer trials
  - Use same approach as for non-oncology indications

Assessment of Potential Genotoxic Impurities

Highly Cross-functional Approach Needed



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GTI Assessment for 1st GMP Process

- Process Chemistry assesses synthetic scheme to identify compounds with structural alerts
  - Starting materials, intermediates, reactants, obvious by-products
  - Simply looking for alerting structures, not SAR
- Process Chemistry and Analytical Chemistry modify the initial list
  - Chemical feasibility of carry over to API (expert judgment)
  - Actual analytical data
- Toxicology performs safety evaluation of high priority compounds
  - Known API impurities and those with high probability to occur
  - Classify compounds as genotoxic or routine impurities
  - Propose limits
- Proposed testing and control strategies developed by cross-functional team
  - Process Chemistry, Analytical Chemistry, Toxicology, Quality, and Regulatory CMC
  - Specifications

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Chemistry Assessment of Process

- Presence of structural alerts
  - Functional groups associated with mutagenicity
    - Expert judgment
    - Eg., aromatic amine
- Chemical feasibility of appearing in API
  - A genotoxic starting material in the first step of a 5-step synthetic process is unlikely to appear in API
  - Genotoxic substance could be destroyed or rejected in subsequent step(s)
- Analytical data
  - Verifies presence of genotoxic impurity
  - Methods, especially early, may not have sensitivity at levels below TTC

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Structural Alerts from National Toxicology Program

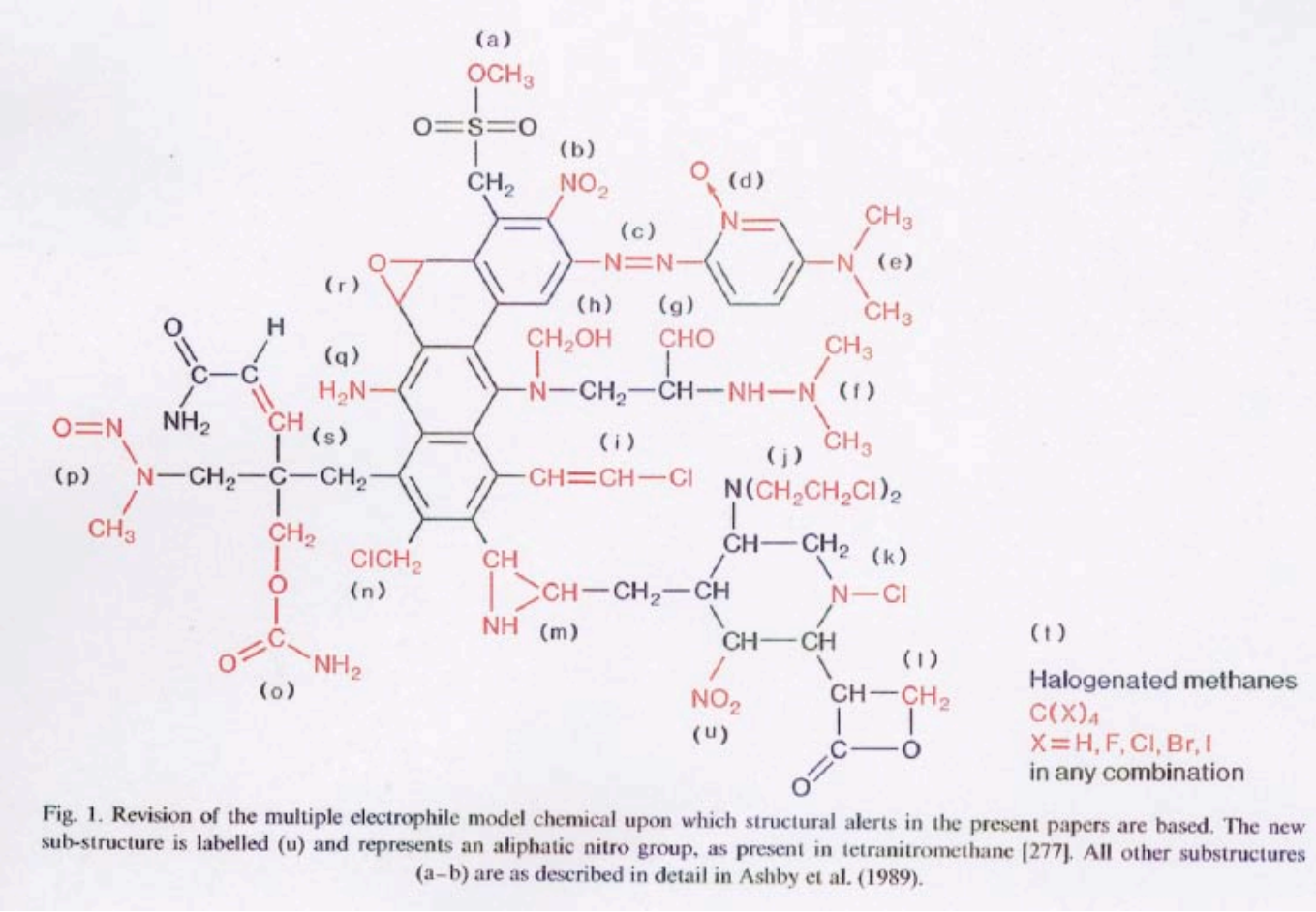

Fig. 1. Revision of the multiple electrophile model chemical upon which structural alerts in the present papers are based. The new sub-structure is labelled (u) and represents an aliphatic nitro group, as present in tetranitromethane [277]. All other substructures (a–b) are as described in detail in Ashby et al. (1989).

Tennant RW et al., *Mutat Res 257:209-227, 1991*.

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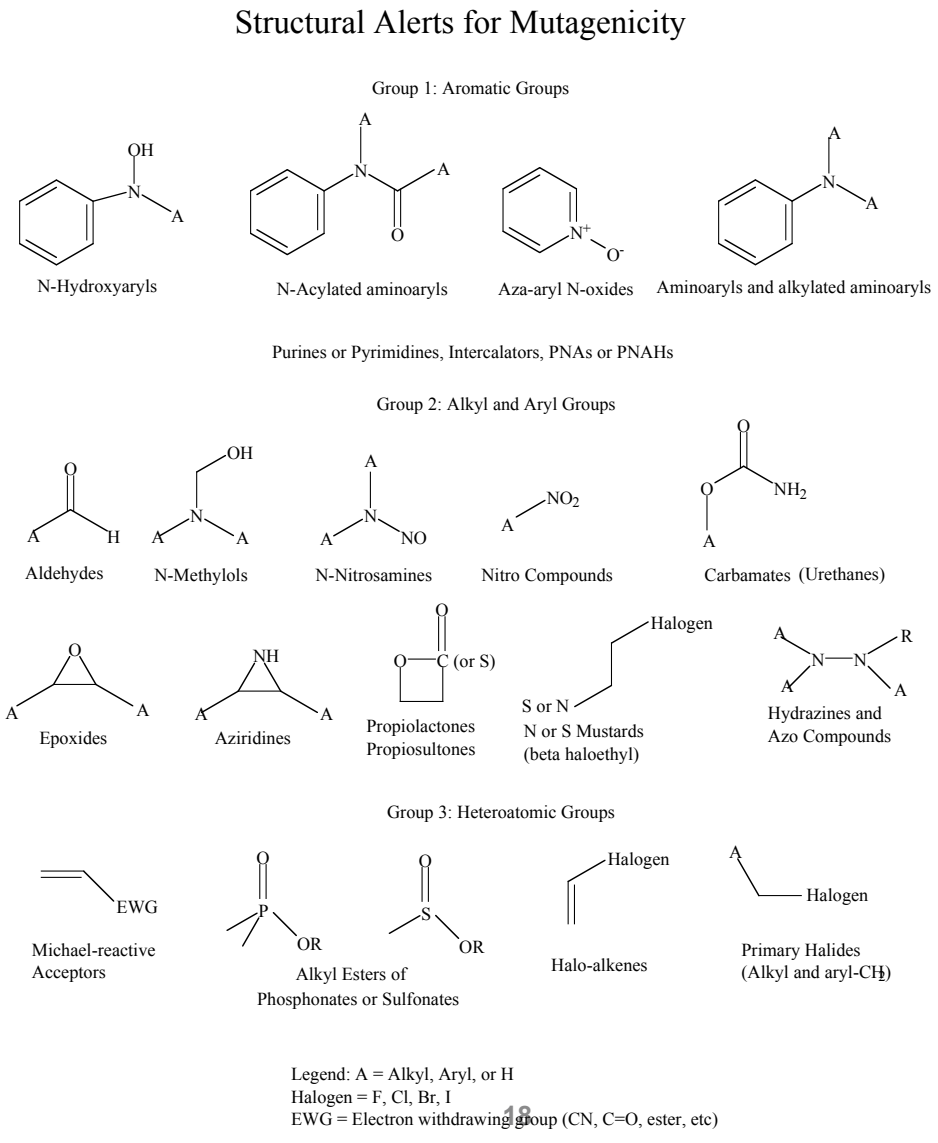
Structural Alerts – Pharmaceutical Impurities



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Safety Assessment of Genotoxicity Potential of Impurities with Known Structure

- Review all available information
  - Material Safety Data Sheet
  - Literature review
  - Comparison of structure to API
  - Regulatory and government agency databases
    - TOXNET at Nat'l Library of Medicine web site
    - CalEPA
    - National Toxicology Program (NTP)
  - Cancer Potency Database from UC Berkeley
- Conduct "In silico" SAR-based prediction of mutagenicity, as appropriate
  - MultiCASE
  - DEREK
  - LeadScope
- Conduct Ames testing, as appropriate
- Classify impurities as routine or gentoxic
- Set limits

Classification of Impurities (Müller et al. 2006)

- Class 1: known genotoxic carcinogens
  - Compound-specific limit
- Class 2: genotoxic but with unknown carcinogenic potential
  - Limit to staged TTC
- Class 3: alerting structure unrelated to API and of unknown genotoxic potential
  - Further evaluation. Limit as appropriate.
- Class 4: alerting structure related to API
  - Treat similarly as API
- Class 5: No alerting structure or indication of genotoxic potential
  - Treat as routine impurity

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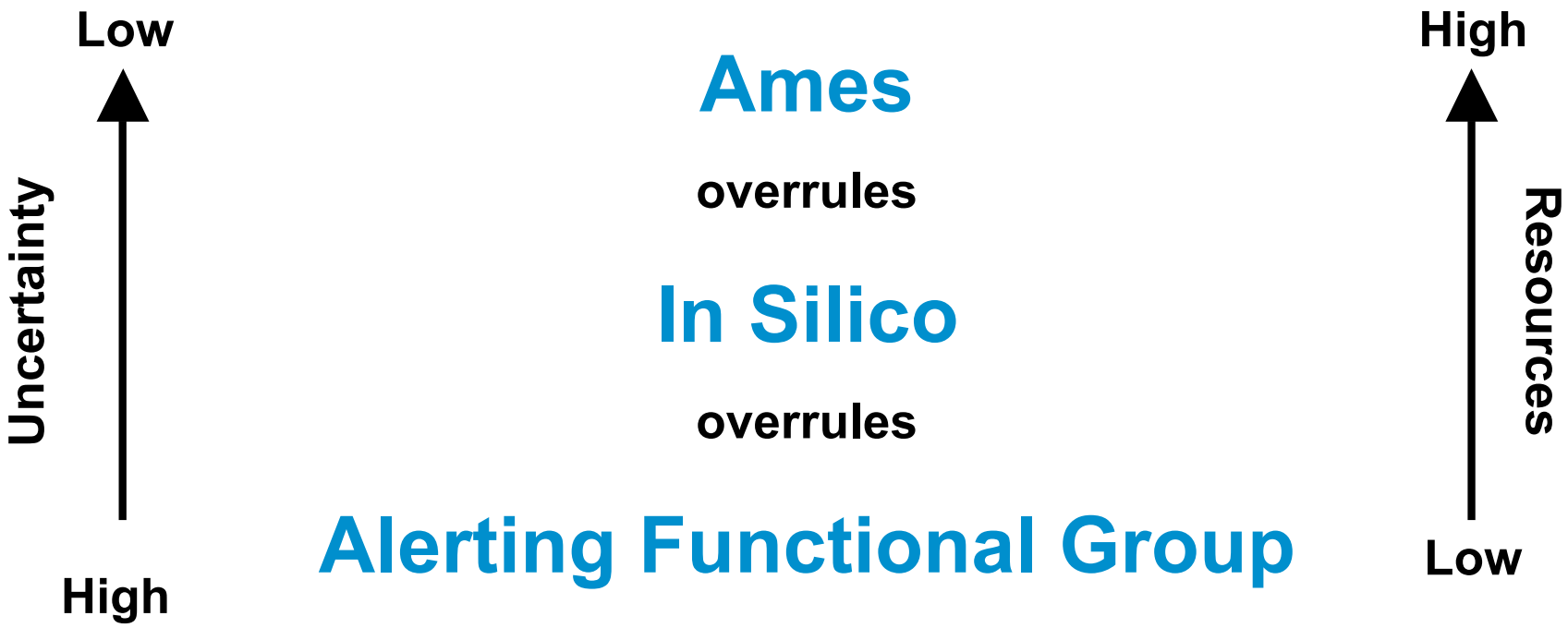
Hierarchy of Genotoxicity Assessments



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Ames Testing of Impurities

- Testing API with existent impurity level is not sufficient to assess gentoxic potential of impurity
  - Need to test impurity at level ≥ 250 µg in Ames test to achieve sufficient sensitivity*
  - 250 µg = 5% at limit dose of 5000 µg in standard Ames test
  - May result in specification for impurity being set at level present as tested
- API spiked with impurity
  - Possible if at least 250 µg of impurity present in standard Ames test
  - Risk of repeating Ames test on previously negative API
- Neat impurity
  - Time and resources to synthesize
  - Will have its own impurity profile

*Kenyon MO et al. *Regul Toxicol Pharmacol 48: 75-86, 2007.*

What to Do With Unidentified Impurities?

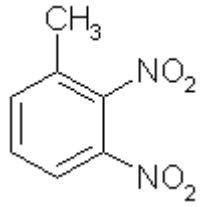
- EMEA GTI Q&A document
  - What is appropriate strategy if new impurity found in Phase III or commercial product?
    - No action generally required if new, unidentified impurity found at levels below ICH identification threshold
    - If structure identified, genotoxic assessment needed
- Draft FDA Guidance on GTI's
  - Clinical Development Stage
    - Evaluate impurities expected to be present based on synthetic pathway
    - If structure identified, genotoxic assessment needed
- However, desirable to minimize exposure to impurities of unknown structure at levels greater than TTC that are subsequently identified and found to be genotoxic

GTI Assessment – Subsequent Routes

- Synthetic process evolves during development
  - Changes require updates of genotoxic impurity assessment
  - Proactive assessment of alternative starting materials
- Assess newly identified impurities
  - Structure may not have been elucidated previously
- Longer duration and/or higher dose ranges in new clinical trials require Toxicology review of staged limits
  - TTC drops with increased duration of treatment
- Production related genotoxic impurities are controlled by GMP cleaning procedures
  - Avoid Viracept®-like situation

Example – Limit Based on Staged TTC

- 2,3-Dinitrotoluene
  - Reactant in process
  - Ames positive*
  - Unknown carcinogenicity
  - Use staged TTC to set limit in API for maximum daily dose of 100 mg



	Duration of Clinical 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 (_g/day)	120	60	20	10	5	1.5
Limit for 100 mg daily d ose (ppm)	1200	600	200	100	50	15

*NTP Database.

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Example: Compound-specific Limit Based on Rodent Carcinogenicity Data

- Hydrazine
  - Genotoxic rodent carcinogen
  - Process impurity
- USEPA IRIS database provides limits for environmental exposure in drinking water
- EPA IRIS RfC in drinking water for carcinogenicity
  - 1x 10^{-5} risk = 0.1 µg/L
  - 1 x 10^{-6} risk = 0.01 ug/L
- Convert to µg/day
  - EPA estimated mean drinking water intake = 2.0 L/day
    - Above RfC's correspond to daily doses of
      - 0.02 and 0.2 µg/day

Hydrazine Limit

	Duration of Clinical 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 (_g/day)	17	17	6	3	1	0.2
Limit for 100 mg daily dose (ppm)	170	170	60	30	10	2

- 10^{-6} risk factor used for durations of ≤12months
- 10^{-5} risk factor used for >12 months
- Note: duration adjustment would support intake of 480 µg/day for single dose but set lower limit for pragmatic reasons

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Case Study – 1st Synthetic Process

- Chronic, non-life threatening indication
- API contained structural alert – aromatic amine
  - Predicted positive by DEREK
  - Negative in standard ICH battery
  - Conclusion: not genotoxic
- Five API impurities contained same structural alert
  - One was metabolite formed by rat microsomes and hepatocytes
    - Would have been tested during genetox on API
  - Treat as routine impurities based on similarity to API
- One impurity contained an additional alert
  - Tested in Ames – negative
  - Treat as routine impurity
- One impurity contained a unique alert and was DEREK positive
  - Tested in Ames – positive
  - Limit to staged TTC
- One byproduct was a predicted genotoxin
  - Uncertain whether would carryover to API
  - Developed assay with sensitivity to staged TTC level
  - Byproduct not detected in penultimate intermediate by this assay
  - Specification not required for this byproduct

Impact on Development Organizations

- Increased resources
  - Toxicology
    - More in silico assessments and genetox tests
    - Safety assessments and limits
  - Process Chemistry
    - Potential change to less optimal routes to minimize GTI's
    - Possible decreased yields due to efforts to reduce concentration of GTI's
  - Analytical Chemistry
    - May need to develop unusually sensitive methods
- Potential program delays
- May need to limit clinical dose if unable to control GTI's to acceptable limits

Summary

- GTI's are of increasing interest to regulatory agencies and industry scientists
- Known GTI's must be limited below established thresholds throughout clinical development
- A proactive, multidisciplinary approach is needed to assess potential for GTI's to affect quality of API
- Assessment of the genotoxic potential of impurities is an iterative process
- A more flexible approach my be feasible for life-threatening indications
- Increased resources from several functional areas are required to address the issue of GTI's

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Resources

- National Toxicology Program Database
  - http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm
- TOXNET Database
  - http://toxnet.nlm.nih.gov
- Univ California Berkeley Carcinogenic Potency Database
  - http://potency.berkeley.edu
- DEREK Software
  - https://www.lhasalimited.org/
- MultiCASE Software
  - http://www.multicase.com/
- Leadscope software
  - http://www.leadscope.com/